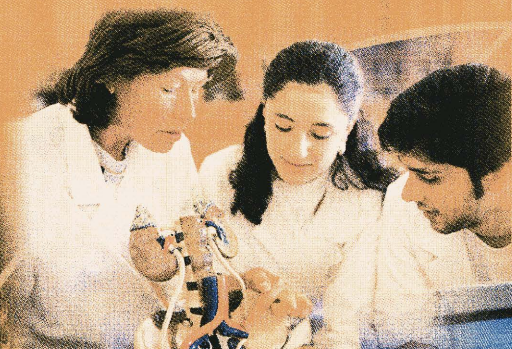


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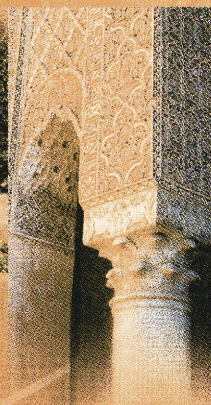
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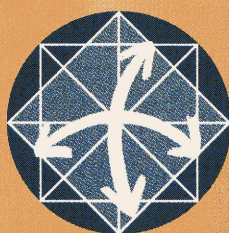
*General Information*



**Euro-Mediterranean  
S&T Cooperation**



*Project Reports  
Edition 1998  
VOLUME I*



CONFIRMING THE INTERNATIONAL  
ROLE OF COMMUNITY RESEARCH



**EUROPEAN COMMISSION**

Edith Cresson, Member of the Commission responsible for Research, Innovation, Education, Training and Youth.

DG XII/E2 – Programme INCO-MED

*Contact: Brian W. Brown*

*European Commission, Rue de la Loi 200,*

*B-1049 Brussels*

*Tel (+32) 2 29 63628*

*Fax (+32) 2 29 66252*

*E-mail: [brian.brown@dg12.cec.be](mailto:brian.brown@dg12.cec.be)*

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# Table of content

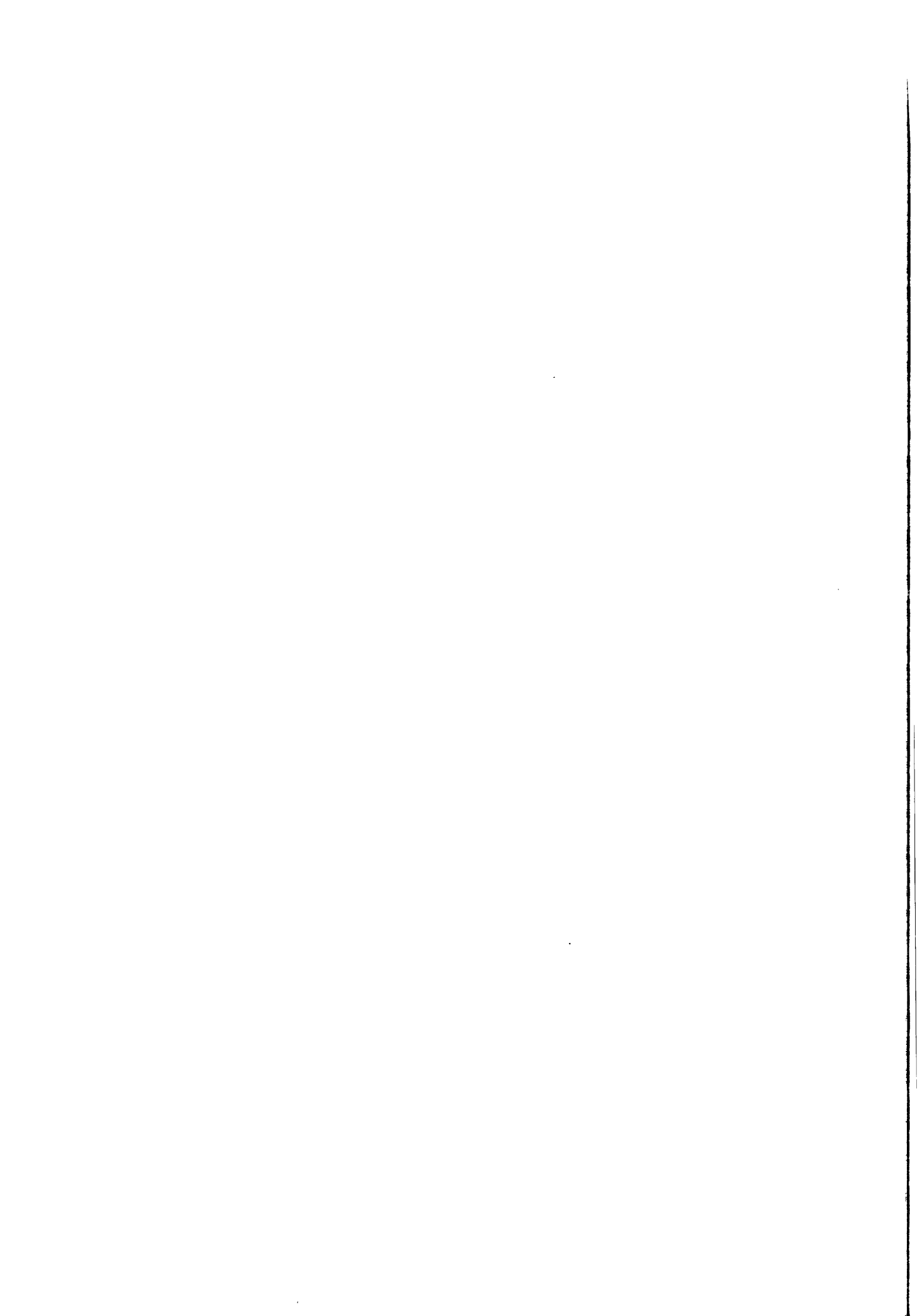
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## Volume I

	<i>Page</i>
<b>Foreword</b> .....	<b>5</b>
<b>Introduction</b> .....	<b>7</b>
<b>General index by topic</b> .....	<b>9</b>
<b>Project summaries:</b>	
Natural resources .....	<b>12</b>
Agriculture.....	<b>308</b>
<b>Index of contracts by topic</b> .....	<b>406</b>
<b>Index of participating scientists</b> .....	<b>430</b>
<b>Index of participating countries</b> .....	<b>452</b>
<b>Index by programmes</b> .....	<b>474</b>

## Volume II

<b>Foreword</b> .....	<b>483</b>
<b>Introduction</b> .....	<b>485</b>
<b>General index by topic</b> .....	<b>487</b>
<b>Project summaries:</b>	
Health .....	<b>490</b>
Additional fields of mutual interest.....	<b>584</b>
<b>Index of contracts by topic</b> .....	<b>740</b>
<b>Index of participating scientists</b> .....	<b>764</b>
<b>Index of participating countries</b> .....	<b>786</b>
<b>Index by programmes</b> .....	<b>808</b>





## FOREWORD

The Mediterranean has always evoked the great image of civilisations and of socio-historical continuities for many centuries. Fragile lands, vulnerable ecosystems and traditional socio-economic systems are increasingly affected by demands of modernisation and developmental efforts. Expanding population, urban concentrations, industrialisation, heavy tourism and competing and conflicting demands on natural resources, require thoughtful planning and management.

Increasingly, therefore, the challenge is one of how to cope with and accommodate a variety of developmental demands in a rather “stressed” environment. Such terms as “vulnerability”, “fragility”, “sustainability” or “carrying capacity” exemplify the underlying principles of systematic research and the need for cooperation within a larger socio-political framework. The ongoing efforts point also out to the need to mobilise resources beyond administrative frontiers and to implement joint concerted action. Scientific and Technological (S&T) cooperation has then become a manifest need of Euro-Mediterranean actions.

The European Union has early emphasised and invested in various facets of S&T development and cooperation with Mediterranean Partner Countries. Numerous bilateral and multilateral agreements, ad-hoc workshops, a Ministerial meeting, conferences and TRTD activities of mutual interest summarise the evolution of the EU policy initiative. They reflect the search for common solutions and the urgency for both corrective and preventive actions regarding a variety of environmental and energy related concerns as well as agricultural policy questions and problems of public health.

Systematic efforts were launched in 1992 with the *Avicenne Initiative* (1992-1994). The underlying dimension of this action has been a problem-solving approach on agreed upon priorities, involving regional schemes and shared concerns which reflect mutual areas of interest. This effort continued under the same premises in the context on the INCO programme (1995-1998) and in particular in the frame of the INCO-DC activity (S&T cooperation with developing countries).

The mechanisms for the above have been a sustained political dialogue institutionalised in the forum of *Monitoring Committee for Euro-Mediterranean S&T Cooperation* where all EU Member States as well as the 12 Mediterranean Partner Countries are represented together with the European Commission. The main input of this Committee, through successive meetings starting as early as 1995, has been recommendations on policy implementation, future actions of regional relevance and priority settings for common RTD activities.

Building on such experience and cumulative research results, the EU has decided to further strengthen the S&T cooperation with Mediterranean partner countries. This commitment was explicitly expressed in the 5<sup>th</sup> Framework Programme (1998-2002), where a distinct activity, INCO-MED, reinforces excellence in research and cooperative approaches to mutual problems. Moreover, the 5<sup>th</sup> Framework Programme is now open for participation to Mediterranean Partner Countries for all specific programmes and activities under relevant terms and conditions.

We are convinced that Research, Technological Development and Innovation are important and necessary keys to urgent socio-economic demands and to contribute to the development of a real Euro-Mediterranean Partnership.

J. GABOLDE  
*Director, International S&T  
Cooperation*



## INTRODUCTION

This catalogue of contracts is the fourth edition of cooperation projects on Science and Technology contracted between the EU and Mediterranean Partner Countries from 1989 to 1998.

Looking back at this period of activity there were a series of successive preparatory actions which led to some 250 joint RTD contracts and more than 50 accompanying measures which reinforced the implementation of the programme. These early efforts include "Science and Technology for Development" (STD3) and "International Scientific Cooperation" (ISC) programmes which had some involvement with Mediterranean case studies.

The creation of the Avicenne Initiative by the European Union in 1992 formally inaugurates the Euro-Mediterranean RTD cooperation activities. Between 1992 to 1994, three particular areas were promoted, namely environmental protection with a particular focus on water related issues, use of renewable energies and health. 71 contracts with a financial European Community contribution of 26 MECU have been concluded during this period.

By 1995 the Euro-Mediterranean cooperation became part of the 4<sup>th</sup> Framework Programme of the EU in the context of the INCO-DC activity. During this time, emphasis was placed on integrated water resources management and related technologies for purification and reuse, coastal zone protection and preservation, forests and drylands, ecosystems related research, marine science, infectious diseases and public health, restoration of the cultural heritage, information and communication technologies and finally production systems in agriculture, research on crop plants, animals and trees. Some 114 joint RTD contacts received a financial contribution of 50 MECU from the INCO-DC programme involving 470 research institutions in both Member States and all 12 Mediterranean Partner Countries. This effort applies on shared cost and concerted RTD actions.

It became obvious that in later years the Euro-Mediterranean S&T cooperation gained importance in commonly selected S&T sectors of regional relevance for the Mediterranean area. The RTD results obtained so far tend to emphasise the need of a coordinated activity on capacity-building in S&T sectors, excellency research and innovation as well as appropriate use of research results by end-users.

The research presented in the volume reflects not only the increased emphasis on holistic approaches, on valid and reliable data and on innovative technological solution. It underscores also the need for integrated approaches and the emphasis on sustainability because of the complexity, interdisciplinarity and vast web of interactions in problems important for the socio-economic development of the Mediterranean.

The project data sheet provided for each contract contains a short description of the objectives of the work, the activities and methodologies, the expected outcome and whenever appropriate the final research results. Finally, four indexes are provided which should help the reader to find projects or partners according to his/her interests.

M. KAYAMANIDOU  
*Euro-mediterranean S&T cooperation*





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# General index by topic

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## Volume I

<b>1. Natural resources</b>	<b>Page</b>
1.1. Basic natural resources	
1.1.1. Water supply and management	12
1.1.2. Water treatment and pollution control	100
1.1.3. Other resources: soil	194
1.2. Environmental research - ecosystems	222
1.3. Renewable energy	270
<b>2. Agriculture</b>	
2.1. Production systems	294
2.2. Crop production	308
2.3. Livestock production	326
2.4. Agriculture related topics	364

## Volume II

<b>3. Health</b>	
3.1. Public health / research	490
3.2. Disease specific research	526
<b>4. Additional fields of mutual interest</b>	
4.1. Information and communication technologies	584
4.2. Biotechnology	652
4.3. Materials and production technologies	688
4.4. Cultural heritage	730



# 1. Natural resources

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## 1.1. Basic natural resources

### *1.1.1. Water supply and management*

**CONTROL OF SALINATION AND COMBATING DESERTIFICATION EFFECTS IN  
THE MEDITERRANEAN REGION. PHASE II**

**Co-ordinator:** Estación Experimental "La Mayora", Spain (Jesus Cuartero)

**OBJECTIVES**

- To develop an integrated methodology for horticultural production under Mediterranean saline conditions.
- To reduce saline water irrigation in function of the crops and, consequently, to reduce salt application to the soils.
- To use salt removing plants in order to naturally reduce remaining salts in the soils.
- Apply this methodology on a pilot plant basis in order to restore small abandoned (because of high salinity) horticultural areas in the Mediterranean region. Revegetation will be maintained by crops and salt removing plants all the year round rather than keeping the soil bare, then desertification will be controlled. The economic viability of the methodology will be tested.

**ACTIVITIES**

- ◇ Wild plant species that grow naturally in salty Mediterranean areas will be collected together with local vegetables usually grown in mild salty areas. Species candidates belong to genus *Atriplex*, *Salicornia*, *Sarcocornia*, *Salsola*, *Beta*, *Halocnemum*, *Chenopodium*, *Suaeda*, *Mesembrianthemum*, *Hordeum*, *Echinocloa*, *Portulaca*, *Limonium*, *Lotus*, *Scorpiurus*, *Medicago*, *Tetragonolobus* and *Moricandia*. Among those species, the criteria for selecting the most suitable salt removing species will be based on the model of Ben-Asher (1993) in which three parameters are determined as potential measures of ion removing from the soil:  $K_m$ , an uptake inhibition factor,  $F_m$ , maximal salt uptake rate, and  $P_p$ , potential productivity (greenhouse work).
- ◇ Adaptation of salt removing species to Mediterranean conditions in real and practical situations will be evaluated growing the salt removing species in experimental saline plots of about 7dS/m. Salt removing species will be growing without irrigation and the salinity of the soil will be monitored every 3 months. For satsumas, irrigation will be carried out through summer according to satsumas water demand. Productivity in fresh and dry basis, and Na and Cl concentration in the shoot at harvesting will be recorded and related to environmental variables (temperature, rainfall, radiation, relative humidity). The balance between the salt in the soil at the beginning and at the end of each growing season and the salt removed by the plants will be established. A non cultivated plot will be the control.
- ◇ Salt and water balance for the different crops (table and processing tomatoes, leafy vegetables and satsuma mandarins) will be calculated taking into account the salt and water applied to the crop, the salt and water leached and the salt concentration in the crop. The production functions of each crop will be formulated on the basis of fruit and shoot productivity and water balance studies in winter and summer conditions with five water salinity levels ranging from 1 to 7 dS/m. Water use efficiency for the different salinities will be determined from the ratios between photosynthesis and transpiration, between dry weight and water consumption, and between yield and water consumption.
- ◇ The modelization of salt accumulation will be made combining the flow equation for salts and water (Ben-Asher, 1993), with uptake rates according to Michaelis-Menten enzymatic reaction. The parameters will be measured by in controlled conditions and under the field conditions of the experimental plots. The modelization will be also done with the data coming from pilot plants.
- ◇ Small abandoned horticultural areas because of salination (pilot plants) will be selected and specific rotation of crops and salt removing species established in these areas to demonstrate the feasibility of our approach in practical situations. Productivity of the crop and of the salt removing species, water application and salinity level of the soil will be monitored. Sheep and goats acceptability of the salt removing species grown will be evaluated. Costs of the growing inputs, hand labour and incomes from the crop and from the salt removing species will be recorded for



evaluating the economic viability of the methodology. Erosion of the soil because of rain and wind, soil salinity evolution, and revegetation of the soil will be also monitored comparing our pilot plants with abandoned areas in order to evaluate the environmental advantages of the proposed methodology.

## **EXPECTED RESULTS**

### *Scientific-technical results*

- ▷ Quantification of salt accumulation capacity of wild species.
- ▷ Salt and water balance for table and processing tomatoes, leafy vegetables and satsuma mandarins in mild salty conditions.
- ▷ Water use efficiency in function of dry weight and yield of table and processing tomatoes, leafy vegetables and satsuma mandarins in mild salty conditions.

### *Deliverables*

- ▷ A list of plants most suitable for desalination of horticultural land in Mediterranean conditions.
- ▷ Data for the refinement of the desalination-resalination model.
- ▷ A refined version of Ben-Asher's model able to explain the salination-desalination process in the pilot plants will be produced.

### *Pilot plants*

- ▷ Pilot plants with the crops and the salt removing species will be established where the productivity of the crop and the salt balance in the soil will be known. These pilot plants will be examples of how abandoned because of salinization areas can be recuperated for sustainable horticultural production and revegetated in Mediterranean conditions.

## **FOLLOW-UP**

### *Work expected to be carried out in 1999*

- ▶ The collection of seeds, graphic material, botanical and economical information of wild species and local vegetable crops grown in salty areas. Spain will receive the information from the rest of the partners. The species to be tested in controlled conditions by Spain and Israel and the species to be grown in experimental salinized plots the rest of the partners, will be chosen, and seeds of those species interchanged, in the first meeting of participants.  
The parameters  $K_m$ ,  $F_m$  and  $P_p$ , and of the  $P_p$  and adaptation in salinised experimental plots, of some salt removing species will be determined in controlled conditions.  
The parameters for the production functions of table tomato, leafy vegetables, satsuma mandarins, and processing tomatoes will be determined in season 1. The experimental conditions, the data to be recorded and the data recording times for every partner will be settled in meeting 1.  
The water use efficiency in function of dry matter and of marketable yield of table tomato, leafy vegetables, satsuma mandarins, and processing tomatoes will be determined in season 1.  
A salinized plot (pilot plant) will be chosen in each country. Climatic (rainfall, relative humidity, maximum and minimum temperature, days of frost, shiny hours, altitude, etc.) and soil data (depth, texture, physical and chemical properties) will be gathered for the selected pilot plants.

### *Work expected to be carried out on years 2000-2002*

- ▶ The parameters  $K_m$ ,  $F_m$  and  $P_p$  in controlled conditions, and of the  $P_p$  and adaptation in salinised experimental plots, of the salt removing species not tested in year 1 will be determined. The results will be collected and decision about the species to be included in the crop rotations and intercropped with satsuma mandarins will be made.  
The parameters for the production functions of table tomato, leafy vegetables, satsuma mandarins, and processing tomatoes in season 2 will be determined. Production functions, salt and water balances, their relations and effects will be established with the data provided by all participants.  
The water use efficiency in function of dry matter and of marketable yield of table tomato, leafy vegetables, and processing tomatoes in season 2 will be determined. The water use efficiency of

satsuma mandarins will be determined year by year the four years of the project. Modelization of salt accumulation and removed will be made.

The representativity and suitability of the pilot plants, and the crop rotation programmes will be discussed.

Crop rotation programmes will be carried out starting with tomatoes and leafy vegetables, and following by salt removing species. Intercropping salt removing species with satsuma mandarins. The performance of the salt removing species both under specific conditions or their overall performance in partners' countries will be evaluated. Besides its suitability to the ecosystem, economic output of the crop and of the salt removing species will be another significant factor in determining the final recommendations. Turkey will receive the data from all participants and will produce a set of recommendations to farmers about how to manage crops and salt removing species.

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## **PARTNERS**

### **CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS**

Estacion Experimental La Majora  
Serrano 117  
28006 Madrid  
**Espana**

Dr Jesus Cuartero  
Tel.: 34-5-2552.656  
Fax: 34-5-2552.677  
E-mail: cuartero@eelm.csic.es

### **UNIVERSIDADE DO ALGARVE**

Unidade De Ciencias E Tecnologias Agrarias  
Campus De Gambelas  
8000 Algarve  
**Portugal**

Dr Jose Beltrao  
Tel.: 351-89.817.080  
Fax: 351-89.817.079  
E-mail: eveiga@ualg.pt

### **EGE UNIVERSITY**

Faculty Of Agriculture Departement Of Horticulture  
35100 Bornova/Izmir  
**Turquie**

Prdr Uygun Aksoy  
Tel.: 90-232-388.1865  
Fax: 90-232-388.1864  
E-mail: aksoy@ziraat.ege.edu.tr

### **BEN-GURION UNIVERSITY OF THE NEGEV**

Res.& Devpt.Authority-Jacob Blaustein Inst. For Desert  
Research  
University Campus  
84493 Sede Boger Campus  
**Israel**

Prdr Jiftah Ben-Asher  
Tel.: 972-7-659.6747  
Fax: 972-7-659.6742  
E-mail: benasher@bgumail.bgu.ac.il

### **NATIONAL RESEARCH CENTRE - EGYPT**

El-Tahrir Street  
12311 Cairo  
**Egypte**

Prdr Mohamed El-Fouly  
Tel.: 202-336.12.30  
Fax: 202-361.08.50  
E-mail: elfouly@intouch.com

**CONCERTED ACTION ON “AGRICULTURE AND URBANISATION IN THE  
MEDITERRANEAN REGION: ENABLING POLICIES FOR SUSTAINABLE USE OF  
SOIL AND WATER”**

**Co-ordinator: Polytechnic of Bari, Department of Architecture and Town Planning, Italy  
(Dino Borri)**

---

**OBJECTIVES**

- The general objective is to connect and co-ordinate the ongoing and future research efforts aiming at a sustainable use of natural resources – in particular soil and water- in the Mediterranean region, in the context of urbanisation processes and their interdependency with agricultural and industrial activities.
- To develop interdisciplinary, multidisciplinary and cross-sectoral scientific approaches to foster scenario building in local-global policies and to verify their feasibility with appropriate private and public stakeholders.
- To prevent the unsustainable use of natural resources, and mitigate the effects of urbanisation on soil and water use in the Mediterranean region.
- To study on how to improve economic productivity of scarce natural resources (soil and water).
- To promote research on ecosystem management involving the partnership of private and public stakeholders.
- To integrate the use of advanced detection technologies with a close and friendly observation of, and participation in, community processes in order to build scenarios based on adequate knowledge, helping concerted and feasible urbanisation/agriculture policies.
- To establish a sustainable data base link through electronic networks and a CD-ROM on the use of natural resources in the Mediterranean region in order to couple economic and biophysical data as a support to eco-systemic research and socio-political awareness.

**ACTIVITIES**

- ◇ The examination of the objectives and the outline of related scenarios will be jointly carried out by policy makers from Mediterranean region and researchers in different disciplines from South Mediterranean region and EU countries, aiming at building policies consistent with: sustainability in use of natural resources, complementarity between urbanisation and agriculture, local economic and cultural identity preservation especially in tourist and coastal zones.
- ◇ After a preliminary stage of exchanging and structuring ideas on relevant local problems, three research meetings will be organized, scenarios will be built, training stages on data collection and analysis will be carried out, and concerted activities will be promoted among partners, highlighting local/global agreements and conflicts on environmental decisions and actions.  
◇The last stage will consist in making data, information, and results of CA exchangeable and comparable among partners, also using for this purpose management information systems.
- ◇ In all stages, meta-analytical, communicative, social learning approaches, and in particular the “soft approaches” recently emerging from the Operations Research field, including problem structuring methods, futures studies and sensitivity analysis, will be used in integration with harder methods and techniques stemming from cognitive science and information technology, including Intelligent and Multimedia Systems, spatio-temporal monitoring systems and GISs .



## RESULTS

### *Scientific-technical results*

- ⇒ increased knowledge of scenario building techniques for sustainable use of soil and water resources among policy makers and other actors;
- ⇒ spreading knowledge about sustainable ways for meeting the emerging demands of urbanization and agricultural production;
- ⇒ contributing to better analyses and monitoring of soil and water quality changes in urban fringes by networking and promotion of general MISs abilities;
- ⇒ promoting models of integrated community-environment framework of self-sustainable socio-economic development;
- ⇒ indirect future change of environmental soil and water decay, due to potential improvement of management quality for ongoing changes of ecosystems in the buffer zone urban land-agricultural land;
- ⇒ indirect future change of agricultural production, according to the new policy guidelines and technology advancements induced by this project in the long run;
- ⇒ tendency to modification of urban landscapes on the urban fringes according to new policy and planning guidelines aimed at protecting rural and natural spaces;
- ⇒ promotion of a stable and inter-active Mediterranean network involving both researchers and public and private stakeholders; management of information needs by Intelligent and Multimedia Systems, spatio-temporal monitoring systems and GISs.

### *Deliverables*

CD-ROM aiming at disseminating the CA main results;  
Short advanced training course on data collection and analysis;  
Database links and Info-Newsletter using electronic networks.

## FOLLOW-UP

- ▶ Work planned for the forthcoming year  
Initiating electronic network for information exchange with other CAs and research teams.  
Organising a one week advanced training course on data collection and analysis carried out.  
Organising cross-sectoral meeting and building scenarios on “Interdependency between Agriculture and Urbanisation”  
Starting the production of database links, WWW dissemination and Info-Newsletter.
- ▶ Work planned for the remaining project period  
Organising cross-sectoral meeting and building scenarios on “The Development of Tourism and Coastal Zone Management” and “Opportunities for Sustainable Growth in the Context of Economic Globalisation and Progressive Emergence of Euro-Med Free Trade Zone”  
Producing database links, WWW dissemination and Info-Newsletter.  
Exchanging of data and tools for defining MISs on CA research topics.  
Organising a Workshop with representatives of local community decision-makers to discuss and test theme scenarios and MISs: documentation on agreed outcomes.  
Producing a CD-ROM for diffusion of CA outcomes on data collection and analyses.

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## **PARTNERS**

### **CENTRE INTERNATIONAL DE HAUTES ETUDES AGRONOMIQUES MEDITERRANEENNES**

Via Orabona, 4  
70125 Bari  
**Italy**

Prof. Dino Borri  
E-mail: borri@poliba.it  
Tel.: 39-80-546.03.47  
Fax: 39-80-546.03.48

### **UNIVERSITY OF ANKARA**

Ernst Reuter Center For Urban Studies  
Faculty Of Political Sciences  
06590 Cebeci  
**Turkey**

Mr. Keles Rusen  
E-mail:  
Tel.: 90-312-3197736  
Fax:

### **INSTITUT DE RECHERCHE SUR LE MAGHREB CONTEMPORAIN**

Rue Mohamed Ali Tahar 20  
1002 Tunis  
**Tunisia**

Mr. Mohamed Elloumi  
E-mail:  
Tel.: 216-1-79.67.22  
Fax: 216-1-79.63.76

### **UNIVERSITE MOHAMMED V**

Centre Etudes Strategiques  
Groupe De Recherche En Economie Inter.  
Boulevard Des Nations Unies  
Bp 721  
Rabat  
**Morocco**

Mr. Driss Ben Ali  
E-mail: grei@mtds.com  
Tel.: 212-7-67.44.82  
Fax:

### **THE PLANNING AUTHORITY ENVIRONMENTAL MANAGEMENT UNIT**

Floriana St Francis Revlin  
Po Box 200  
Valletta  
**Malta**

Mrs. Michelle Borg  
E-mail:  
Tel.: 356-22.90.15.46  
Fax: 356-22.48.46

### **INSTITUT NATIONAL D'AMENAGEMENT ET D'URBANISME**

Hay Ryad  
Secteur 20, Bloc 117  
Rabat  
**Morocco**

Mr. Abdelghani Abouhane  
E-mail:  
Tel.: 212-7-71.21.79  
Fax: 212-7-71.21.79

### **AKADEMILILER SITESI ILKYERLECIM MAHALLESİ**

06370 Ankara  
**Turkey**

Mrs. Nur Sozen  
E-mail: nursozen@tr-net.net.tr  
Tel.: 90-312-3543393  
Fax: 90-426-13.72

### **AGRICOLA UNIVERSIDAD DE ALICANTE**

Depto De Ciencias De La Tierra Y Del  
Medio Ambiente  
Ap. Correus 99  
03080 Alicante  
**Spain**

Mr. Carlos Auernheimer  
E-mail: auernheimer@ua.es  
Tel.: 34-96-590.35.52  
Fax:

### **MINISTERE DE L'EQUIPEMENT ET DE L'HABITAT**

Direction Generale De L Habitat  
Avenue Habib Chrita  
1002 Belvedere Tunis  
**Tunisia**

Mr. Abdelkader Fradi  
E-mail:  
Tel.:  
Fax: 216-1-79.97.41

### **UNIVERSITE MOHAMMED V**

Faculte Des Lettres , Sc. Humaines  
Unite De Formation , Rech. Environm.  
Rabat  
**Morocco**

Mr. Abdellah Laouina  
E-mail:  
Tel.:  
Fax: 212-7-77.20.68

### **UNIVERSITA DEGLI STUDI DI FIRENZE**

Mr. Donato Romano

Dipto Economia Estimativo Agrario  
Forestrale  
P.Le Delle Cascine 18  
50144 Florence  
**Italy**

E-mail: dromano@eco.agr.unifi.it  
Tel.: 39-55-35.20.51  
Fax: 39-55-35.98.70

**ASSOCIACAO TERRAS DENTRO**  
Rua Rossio De Pinheiro  
7095 Alcacovas  
**Portugal**

Mr. Afonso Do O  
E-mail:  
Tel.: 351-66-94.359  
Fax: 351-66-94.002

**UNIVERSITA DI SASSARI**  
Dipto Di Scienze Zootecniche  
Facolta Di Agraria  
Via E. De Nicola  
07100 Sassari  
**Italy**

Mr. Giuseppe Enne  
E-mail: zooagri@ssmain.uniss.it  
Tel.: 39-79-22.93.03  
Fax: 39-79-22.93.02

**UNIVERSITY OF DURHAM**  
Department Of Geography  
Science Laboratories  
South Road  
Dh1 3le Durham  
**United Kingdom**

Mr. David Higgitt  
E-mail: D.L.Higgitt@durham.ac.uk  
Tel.:  
Fax:

**UNIVERSITY OF UMEAA**  
Department Of Political Science  
Faculty Of Social Sciences  
Umea  
**Sweden**

Mr. Abdul Khakee  
E-mail: Abdul.Khakee@pol.umu.se  
Tel.:  
Fax:

**CENTRE INTERNATIONAL DE HAUTES ETUDES  
AGRONOMIQUES MEDITERRANEENNES**  
Rue Newton 11  
75016 Paris  
France

Mr. Antonio Di Giulio  
E-mail: adg@ciheam.org  
Tel.: 33-1-5323.9100  
Fax: 33-1-5323.9102

**UNIVERSITY OF THE AEGEAN**  
Department Of Environmental Studies  
Lab Of Environmental Planning  
Voulgaroktonou Str. 114  
Athens  
**Greece**

Mr. Harry Coccossis  
E-mail: enpl@ru.aegean.gr  
Tel.: 30-1-644.27.27  
Fax: 30-1-644.84.28

**“MED-POL” INNOVATIVE DECENTRALISED ENERGY AND WATER  
MANAGEMENT POLICIES CAN ENCOURAGE THE CREATION OF A MARKET  
ECONOMY AND HELP RURAL DEVELOPMENT**

**Co-ordinator:** Conphoebus srl, Catania, Italy (Beniamino Morgana)

## **OBJECTIVES**

The Concerted Action has the objective of identifying political strategies for the promotion of energy & water production/supply/management at local distributed level. Learning from the set of recent and innovative policy options adopted in several DC in different parts of the world, the project will adapt them to the Third Mediterranean Countries (TMC) regional conditions, evaluate their feasibility interacting with the interested actors, propose a set of common recommendations for the participating TMC and the whole region. The project thesis is that it is possible to achieve a new model for sustainable rural development, where an energy and water de-centralized management is at least in part operated in an innovative way by local SMEs (as opposed to a conventional approach where rural electrification is fully managed by public utilities). The advantage of this approach is not only the improvement of living conditions (home lighting, pumped clean water, wastewater treatment, better education and social life, telecommunication facilities, health, safety, ...), and social conditions (mainly for women and children), but also that the start up of these free market activities can and may be one of the drivers (if supported with credit and infrastructure assistance) to stimulate the improvement of local activities, in agriculture, commerce or handicraft, and finally create the accumulation of small local capitals that will support further investments in a virtuous circle. The analysis will carefully consider the negative findings revealed by WB experiences in existing rural electrification schemes, that where not able to stimulate rural economic development.

## **ACTIVITIES**

The Concerted Action approach will be based on these principles: i) the technological aspects will be assumed as available knowledge (see JOULE projects); ii) the existing experiences on policy options around the world will be gathered, organized and submitted to the evaluation of participants; iii) specific programs will be jointly developed, assisting the authorities already involved in national programs and involving external actors (national and international financing institutions, MED business centres, responsible of other policies, energy utilities, NGOs,...); iv) the final recommendations will be presented at the top national political level. The methodology foresees a strong coordinator's role, six-monthly general meetings with partners' contributions, production of Review and Synthesis Papers, invitation to meetings of external experts representing important actors in the related policies, creation of www conferences to facilitate the share of documents within the network, entrust of small assistance contracts to experts, exchange of scientists.

## **EXPECTED RESULTS**

### *Scientific-technical results:*

- ▷ Innovative policy packages will be jointly developed for energy & water production, supply and management. They will be addressed to the achievement of sustainable development, through locally managed initiatives and adoption of new technologies.

### *Deliverables*

- ▷ The set of recommendations issued by the Concerted Action will be published and distributed.



## **FOLLOW-UP**

The impact on TMC energy/water policies is expected to be relevant (the great Government expectations in this area are demonstrated by their official papers presented in recent Conferences). The social relevance of new rural development strategies cannot be overestimated: to alleviate poverty, inoculate market economy principles, fight social exclusion, avoid harsh urbanization. A sustainable local development, assisted by EU technologies and risk capitals, is coherent with the EURO-MEDITERRANEAN policy objectives.

*Work expected to be carried out within the forthcoming year:*

- ▶ The first expected outcome is the launch of related national programs in participants' Countries. It is also expected that the participants from EU States will involve EU economic operators (utilities, manufacturers of renewable energy components) in Joint Ventures with TMC companies in order to start business (production, installation, distribution, financing) in those areas where national programs for decentralized energy production will be launched. A second expected outcome is the participation of economic operators in one of the Project Meetings.

*Remaining project period*

- ▶ Another important follow-up is the addressing of the participating Research Institute programs towards the further improvement of renewable energy technologies adapted to this local development model.

*Networks*

- ▶ Four EU partners, an international organization and seven TMC institutions are included as partners (other ones will be invited at meetings). The TMC participation tentatively includes, per each Country, a representative of the Institutions in charge of Energy Policy and a specialized Research Institute. The creation of a stable training network between partners is an expected outcome.

*Information exchange within the networks*

- ▶ Seven project meetings and a web site will be the main means for information exchange. Each project meeting will have a specific topic, the presentation of available experiences, and the discussion of expert's studies assigned by the coordinator. Opportunities for technical visits of researchers at the partners' premises and the organization of training sessions by project experts will also be provided.

## **PARTNERS**

### **CONPHOEBUS ISTITUTO DI RICERCHE PER LE ENERGIE RINNOVABILI**

Zona Industriale Passo Martino  
95030 Piano D'arci (Catania)  
**Italia**

Er Beniamino Morgana  
Tel.: 39-95-748.92.05  
Fax: 39-95-29.12.46  
E-mail: conphoeb@tin.it

### **CENTRO DE INVESTIGACIONES ENERGETICAS, MEDIOAMBIENTALES Y TECNOLOGICAS**

Type:Pn Partner Number:002  
Av. Da Complutense 22  
28040 Madrid  
**Espana**

Mr Manuel Varela  
Tel.: 34-1-346.67.47  
Fax: 34-1-346.64.33  
E-mail: varela@ciemat.es

### **CENTRO DE INVESTIGACIONES ENERGETICAS, MEDIOAMBIENTALES Y TECNOLOGICAS**

Av. Da Complutense 22  
28040 Madrid  
**Espana**

Mr Manuel Varela  
Tel.: 34-1-346.67.47  
Fax: 34-1-346.64.33  
E-mail: varela@ciemat.es

### **ROYAL SCIENTIFIC SOCIETY JORDAN**

Renewable Energy Research Centre  
P.O. Box 1438 Al Jubeiha  
11941 Amman  
**Jordanie**

Mr Malek A. Kabariti  
Tel.: 962-6-534.47.01  
Fax: 962-6-534.48.06  
E-mail: kabariti@rss.gov.jo

### **NEW AND RENEWABLE ENERGY AUTHORITY**

Emtedad Abas El-Akad Hay El-Zohor  
Nasr City  
Cairo  
**Egypte**

Er Ibrahim Khedr Fahmi  
Tel.: 20-2-272.58.91  
Fax: 20-2-271.71.73  
E-mail:

### **AGENCE POUR LA MAITRISE DE L'ENERGIE**

Rue 8000 Montplaisir 3 Bp 213  
1002 Tunis Belvedere  
**Tunisie**

Mr Naceur Hammami  
Tel.: 216-1-84.62.41  
Fax: 216-1-78.46.24  
E-mail: naceur.hammami@ame.ati.tn

### **INSTITUTE FOR PROSPECTIVE TECHNOLOGY**

Studies - Ipts  
W.T.C. Isla De La Cartuja S/N  
41092 Sevilla  
**Espana**

Mr I. Spiewak  
Tel.: 34-95-448.83.86  
Fax: 34-95-448.83.39  
E-mail: irving.spiewak@jrc.es

**DEVELOPMENT, APPLICATION AND ANALYSIS OF RAMAN, FLUORESCENCE AND ABSORPTION SPECTROSCOPY USING OPTICAL FIBRE REMOTE SENSING OF CHEMICAL SPECIES IN WATER FOR IN SITU ENVIRONMENTAL POLLUTION STUDIES IN CYPRUS, ISRAEL, ITALY AND THE UNITED KINGDOM**

Co-ordinator: University of Kent, Canterbury, United Kingdom (David Jackson)

## OBJECTIVES

- To establish a database of hazardous pollutants found in water and identify suitable testing locations in the 4 countries involved in the programme.
- To develop a real time, in situ and chemically specific technique based on optical spectroscopy to detect multi-component pollutant mixtures at concentrations down to a few parts per billion.
- To develop or modify an existing (portable) spectrometer which can be used outside the benign laboratory environment for, Raman, fluorescence and absorption spectroscopy.
- To develop high light gathering fibre optic probes and delivery systems compatible with the spectroscopic platform to allow remote spectroscopic measurements in rivers, lakes, land fill sites or the open sea, in areas subject to chemical pollution arising from industrial or agricultural activities.
- To develop chemical concentrators to enhance the sensitivities of the spectroscopic techniques (this is particularly important in the case of Raman spectroscopy) compatible with the fibre optic probes and their aqueous working environment.
- To establish a library of spectra measured in drinking, recreational (lakes or reservoirs), and seawater containing pollutants at known concentration levels.
- To develop neural networks which in conjunction with the library of spectra can be used to identify both the pollutants and their concentrations.
- To train young scientists in various aspects of optical spectroscopy, design of fibre optic systems and probes, computer control of the system, data collection and presentation.
- To demonstrate the fully characterized system to potential users.

## ACTIVITIES

- ◇ To undertake a literature search together with direct contact with local water authorities and similar monitoring agencies to establish acceptable levels of common pollutants in drinking water and in the water returned to a local river extracted for use in an industrial process.
- ◇ Using laboratory equipment currently available to the partners, perform a series of experiments on water samples containing known concentrations of specific pollutants, to establish which of the three spectroscopic techniques offers the necessary sensitivity to detect these pollutants at the specified contamination levels.
- ◇ Purchase (and modify as necessary) or develop a suitable (portable) spectroscopic platform which can operate efficiently at the wavelengths required to make the pollution measurements and be interfaced with the optical fibre probes.
- ◇ Design optical fibre probes to transmit the range of wavelengths necessary for the spectroscopic analysis and the fibre optic probes to transfer the optically generated signals to the spectrometer for analysis.
- ◇ Explore the possibilities of using polymer concentrators or sol-gel films to enhance the sensitivity of the system where the signal to noise is poor for specific pollutants.

- ◇ Establish a database of spectra for water samples with known levels of single contaminants and mixtures and develop neural networks in order to recover this information from unknown samples.
- ◇ Undertake trials with the system at previously identified locations liable to suffer from pollution.

## RESULTS

Local water authorities have been approached however some problems have been encountered in obtaining specific details regarding acceptable levels of known pollutants, possibly due to the political or commercial sensitivity of this information.

Experiments have been performed in collaboration with an international oil company on the detection of crude oil in simulated sea water to emulate typical conditions associated with the environment in the vicinity of the oil rigs in Europe. These experiments have demonstrated that using conventional fluorescent spectroscopy crude oil at the level of a few parts per million in salt water is detectable.

Experiments have also been conducted to measure the presence of hydrocarbons such as benzene and toluene at low concentration levels with Raman spectroscopy, initial results indicate that the sensitivity of the Raman technique is significantly lower at about a hundred parts per million.

Initial studies have been undertaken on the use of Neural Nets to extract the individual components from a simulated absorption spectrum composed of known individual spectra from a variety of liquids with arbitrary weighting. With noise free data the trained nets are able to recover the components very rapidly with good precision. When the simulated data is noisy (again simulated) or contains an unknown spectrum which the net has not been taught to recognise, the performance of the nets is poor.

In order to demonstrate the possibility of performing direct absorption spectroscopy of water samples with current optical fiber technology, two main objectives have been explored:

- 1) Absorption measurements performed on chromium aqueous solutions; chromium is identified as pollutant species since it is frequently present in many environments and because it shows potential to be monitored in the far ultraviolet and visible spectral range;
- 2) Theoretical modelling of an optical fiber probe for long path absorption measurements of liquid samples in the visible spectral range.

New equipment will shortly be commissioned which will allow fluorescence spectra to be measured, in order to obtain better differentiation between pollutants the system incorporates features which allow fluorescence lifetimes and synchronous fluorescence scanning.

Further studies are also planned with an existing Raman system.

Students are working on this project in all 4 partner countries and in due course will submit their work for either a higher degree or a diploma.

## FOLLOW-UP

*Work planned for 1999:*

- ▶ Continue to build a library of spectra of known single pollutants and mixtures.
- ▶ Commence studies of samples taken from selected sites.
- ▶ Refine the neural networks.
- ▶ Design a variety of fibre optic probes for the three spectroscopy techniques to allow direct contact measurements to be made with a remote water sample.
- ▶ Design and fabricate a special optical sample chamber which will have the facility to draw a targeted water sample into it, the chamber will permit all of the three spectroscopic techniques to be used sequentially on the same sample.

*Work planned for the remaining period:*

- ▶ Study the use of chemical concentrators to enhance the sensitivity of the system to enable lower concentrations of pollutants to be determined



- ▶ Undertake field trails at selected locations
- ▶ Arrange demonstrations of system to potential users involved in monitoring water quality and manufacturing companies likely to generate pollute water sources.

## **PARTNERS**

**UNIVERSITY OF KENT**  
School of Physical Sciences  
Canterbury  
Kent CT2 7NR  
**United Kingdom**

David Jackson  
Tel.: +44 1227 823288  
Fax: +44 1227 82 7558

**UNIVERSITY OF CYPRUS**  
School of Natural Sciences  
PO Box 537  
Nicosia 1678  
**Cyprus**

Costas Christofides  
Tel.: +357 2 338 671  
Fax: +357 2 339060

**IROE-CNR**  
Via Panciatichi 64  
Firenze I-50127  
**Italy**

Anna Mignani  
Tel.: +39 055 42351  
Fax: +39 055 4379569

**TEL AVIV UNIVERSITY**  
Interdisciplinary Studies  
PO Box 39040  
Ramat-Aviv  
Tel-Aviv  
**Israel**

Moshe Tur  
Tel.: +972 3 6408125  
Fax: +972 364 10189

**DEVELOPING SUSTAINABLE WATER MANAGEMENT IN THE JORDAN VALLEY**

**Co-ordinator:** Austrian Research Centre, Seibersdorf, Austria (R.Orthofer)

**OBJECTIVES**

- The main objective of this project is to develop the scientific basis for an integral management plan of water resources and their use in the Jordan Valley.
- Development of a GIS-based database containing harmonised sets of population, land use, soil and water data of the Jordan Valley, including consistent sets of maps that project distribution of water sources and users.
- Projection of potential available sources and demands of water until about 2030.
- Assessment of the hydrogeological conditions, including a conceptual model for assessing environmental and health risks of current practices on soils and groundwater.
- Identification of environmental and health risks of water quality degradation.
- Evaluation of water collection, storage, delivery and irrigation practices and development of scenarios for water and soil management, regarding different land use practices.
- Formulation of an optimisation model for water use, considering utilisation conflicts and potentials of conjunctive water uses.

**ACTIVITIES**

- ◇ Collection and harmonisation of existing data on the water supply and demand system.
- ◇ Analysis of current irrigation, water collection, and delivery practices.
- ◇ Application of a set of models for groundwater evaluation, hydrological budgets and groundwater optimisation and allocation, environmental risks of wastewater use and fertiliser application. Model results will be integrated into a GIS in order to provide a spatial resolution and visualisation.
- ◇ Evaluation of model results in view of the expected socio-economic development in the region to provide options for policy makers for the optimisation of management regimes.

**EXPECTED RESULTS**

The outputs of this joint project will provide a scientifically sound basis for better understanding the situation in the Jordan Valley and thus will directly benefit the future development, stability, and sustainability of the region. Final results will provide new knowledge about actual and projected water resources and flows in the regions including supply and demand. Results will allow to identify the impact of surface water diversions, irrigation practices, and water delivery methods on the hydrological budget of the study area thus leading to recommendations for optimising the water usage in the region that will ensure sustainability and environmental protection.

**FOLLOW-UP**

As by mid-1998 project partners have established a homogenous spatial database containing information about topography, land use, water supply and demand, etc. In the second project year (11/98-10/99) emphasis will be given to the use of the collected information for modelling.

During the project's first year a co-operation was established with the Yale University (New Haven, CT, USA) Centre for Earth Observation Southwest Asia Project (CEO-SWAP) on improvement of methods for land-use classification using satellite data.

The project has already stimulated the establishment of new (and the strengthening of existing) networks between scientists in the region and the EU.

## **PARTNERS**

### **AUSTRIAN RESEARCH CENTRE SEIBERSDORF**

Department of Environmental Planning  
Seibersdorf Campus  
A-2444 Seibersdorf  
**Austria**

Rudolf Orthofer  
Tel.: +43-2254-780-3873  
Fax: +43-2254-780-3888  
E-mail: orthofer@arcs.ac.at

### **HEBREW UNIVERSITY JERUSALEM**

Graduate School of Applied Sciences  
Givat Ram  
91904 Jerusalem  
**Israel**

Hillel Shuval; Badri Fattal  
Tel.: +972-2-658.4151  
Fax: +972-2-563.5886  
E-mail: badri@cc.huji.ac.il

### **APPLIED RESEARCH INSTITUTE JERUSALEM**

Caritas Street  
P.O.Box. 860  
Bethlehem  
**Palestine Autonomous Area**

Jad Isaac  
Tel.: +972-2-741.889  
Fax: +972-2-741.889  
E-mail: jad@arij.pl.org

### **ENVIROCONSULT OFFICE**

Salem Al-Hindawi Street, Jude Centre  
P.O.Box 941400  
11194 Amman - Shmeisani  
**Jordan**

Raed Daoud  
Tel.: +962-6-569.9769  
Fax: +962-6-569.7264  
E-mail: eco@go.com.jo

### **JOANNEUM RESEARCH**

Institute of Hydrogeology & Geothermics  
Elisabethstrasse 16/11  
A-8010 Graz  
**Austria**

Hans Zojer  
Tel.: +43-316-876-373  
Fax: +43-316-876-321  
E-mail: hans.zojer@joanneum.ac.at

**RESOURCE MANAGEMENT IN KARSTIC AREAS OF COASTAL REGIONS OF  
THE MEDITERRANEAN (RES MAN MED)**

**Co-ordinator:** CREDCO Ltd., Business Innovation Centre, Sligo, Ireland, (Margaret Savage)

**OBJECTIVES**

- Produce an inventory of environmental resources for selected coastal karstic regions of Malta, Turkey and Lebanon.
- Identify the institutional structures in these countries and carry out comparative analysis and evaluation of policies for resources management.
- Devise integrative methodologies, based on information management tools such as GIS and remote sensing, and to validate these for the selected study areas.
- Formulate sustainable resource management strategies, that can be used to respond effectively to identified development needs.

The fundamental aim of the Res Man Med project is to preserve the karst environment and to satisfy actual and future uses of the karst system.

**ACTIVITIES**

- ◇ Identification of the authorities and bodies who have jurisdiction over environmental resources in each of Turkey, Lebanon and Malta.
- ◇ Identification of the main environmental resources in the study areas, along with an initial assessment of the state of the resources, their importance and the vulnerability of the resources.
- ◇ Identification of the main pressures/problems facing the environmental resources in each of the karstic study areas.
- ◇ Selection of study areas based on agreed criteria.
- ◇ Formulation of a methodology based on GIS and knowledge about relationships within the general karst environment.
- ◇ Creation of a database by collection of agreed data for use in the GIS, followed by entry of data into a suitable format (digitised map, spreadsheet, etc.).
- ◇ Creation of a knowledge database – relationships between resources and pressures.
- ◇ Formulation of a model to use the data and knowledge functions in the database to simulate scenarios.
- ◇ Definition of scenarios for the study areas and testing and validation of the Res Man Med tool based on these scenarios.
- ◇ Development of a methodology guidebook.
- ◇ Demonstration of the tool with relevant decision makers and managers.

**EXPECTED RESULTS**

The expected output of the project will be a tool to help decision makers and this will consist of:

A methodology guidebook

A selective database

Indicative maps which reflect scenarios – the aim of which is to protect the karst system.

Study area system – a working system in each study area.

The tool will be developed for and tested in each of three coastal, karstic areas (one in Lebanon, one in Malta and one in Turkey).

## **PARTNERS**

**CREDCO LTD.,**  
Business Innovation Centre,  
Institute of Technology,  
Ballinode,  
Sligo,  
**Ireland**

Margaret Savage  
Tel.: 00353 71 55395  
Fax: 00353 71 44500  
E-mail: savagem@s4.rtc-sligo.ie

**TARNIUM SARL**  
Chemin des cerisiers  
F-30610,  
Sauve,  
**France**

Dr. Patrick ARNAL  
Tel.: +33 4 66 77 08 38  
Fax : +33 4 66 77 08 39  
E-mail: arnal@tarnium.fr

**CENTRE FOR REMOTE SENSING**  
Sports City Street,  
P.O. Box 11-8281,  
Beirut  
**Lebanon**

Dr. Mohamad Khawlie  
Tel.: 00961 4 409 845 or 846  
Fax: 00961 4 409 847  
E-mail: consult@cnrs.edu.lb

**INTEGRATED RESOURCES**  
Management Co. Ltd.,  
24 Pope Benedict XV Square,  
Seneglea CSP 06,  
**Malta**

Dr Dirk De Ketelaere  
Tel.: 00356 681 340  
Fax: 00365 676 152  
E-mail: irmco@keyworld.net

**INTERNATIONAL RESEARCH AND  
APPLICATION CENTRE FOR KARST**  
Water Resources,  
Hacettepe University,  
Beytepe - Ankara 06532,  
**Turkey**

Professor Gültekin Günay  
Tel.: 00 903 122 352 543  
Fax: 00 903 122 352 862  
E-mail: gunay@jeo.hun.edu.tr

**JOINT RESEARCH CENTRE**  
Institute for Space Applications,  
TP 262,  
Via Fermi,  
I - 21020 Ispra (VA),  
**Italy**

Dr. Jean Francois Dallemand  
Tel.: 0039 332 789 937  
Fax: 0039 332 789 074  
E-mail: jf.dallemand@jrc.it

**GROUNDWATER RECHARGE IN THE EASTERN MEDITERRANEAN (GREM) – A  
COMPARATIVE STUDY ON INTEGRATED EVALUATION TECHNIQUES  
FOR GROUNDWATER RESOURCES**

**Co-ordinator:** University of Würzburg, Department of Hydrogeology, Germany (Peter Udluft)

**OBJECTIVES**

- Quantification of natural recharge in four different hydro-climatic environments in the Eastern Mediterranean in Greece, Cyprus, Israel and Jordan
- Comparison of the suitability of different methods for groundwater recharge estimation in the Eastern Mediterranean
- Development of an integrated model for groundwater resources evaluation including water quality and water quantity.

**ACTIVITIES**

- ◇ Field work is made on groundwater recharge within the four individual study basins using the main estimation techniques water balance modelling, halogen profiles and flow mixing modelling.
- ◇ An extensive database of hydrological parameters has been developed for all study areas: the Aliakmons (Greece), the Troodos Mountain (Cyprus), the Arava valley (Israel) and the Northern Jordan Valley (Salt, Mafraq) in Jordan. Rainfall, runoff, evaporation data as point variables and spatial information on hydro-geological units, vegetation and land-use has been collected. In Jordan halogen profiles at four different locations have been sampled.
- ◇ In Greece and in Cyprus a water balance model was fitted to the hydrological data in order to derive groundwater recharge. First estimates of annual and monthly groundwater recharge have been made for the Aliakmonas basin in Greece. Recession and recharge characteristics of different sub-catchments have been analysed.
- ◇ Stable isotope sampling and collection of water samples for full chemical analysis has been carried out in Greece and Cyprus, Israel and Jordan. The chemical signature of different geological units allowed to define subsurface flow patterns and to derive the hydro-geological flow system.. For the Arava valley the groundwater recharge as 5-years average for each of the hydro-geological units identified has been specified.
- ◇ GIS aided regionalization
  - Digital terrain models have been developed for the study areas in Greece, Cyprus and Jordan. Hydrological flow paths and flow accumulation are derived directly from the digital elevation models.
  - A distributed water balance model within the GIS is developed for Greece and Cyprus. Calculated exposition and slope data are incorporated as distributed information into a water balance model.
  - An important on-going activity is the full integration of the different estimation methods into a Geographic Information System. This part of the study aims at building regional databases on Geographic information systems for mapping and regionalization. Programs for the direct estimation of groundwater recharge are implemented into the GIS system.
- ◇ Flow model development
  - The mixing cell approach, integrating quantitative and qualitative aspects of groundwater resources evaluation, is further developed and applied in the different study basins. In Greece lateral subsurface inflows to the Aliakmonas have been traced back using the mixing cell approach. In Cyprus the mixing cell approach is used to validate groundwater flow models in the coastal area. In Israel the subsurface flow patterns in the Arava valley have been calculated giving insights into the hydrogeological dynamics of the Southern Jordan valley.

- ◇ Comparative studies, exchange of experience
  - Finally, comparative studies on the suitability and performance of several methods in the Eastern Mediterranean environments are made. During the first and second meeting of the project partners in Thessaloniki/Greece and Nikosia/Cyprus experiences on the different groundwater recharge estimation techniques have been exchanged. In each of the study areas the application of several methods based on mutual support allowed to validate and check previous results.

## **EXPECTED RESULTS**

### *Scientific-technical results*

For the Aliakmonas basin groundwater recharge has been calculated for different subcatchments. The differences between the headwater area and the lower floodplains is very distinct. The timing of recharge with high recharge rates in the in the beginning of the winter season due to convective storms (sporadic) and another maximum in spring due to snowmelt (regular) could be demonstrated. Daily rainfall and runoff data has been used as input for the water balance model (Kuells, Zagana & Udluft, 1998). Similar results are observed for the catchments in Cyprus.

The only lake in the Aliakmonas basin has been found to be an important infiltration source – however it could be demonstrated using hydro-chemical evaluations that most of the recharge is actually abstracted downstream of the Kastoria lake by pumping. The water balance at the full catchment scale allows to evaluate the sustainability of the actual water abstraction schemes.

In a first step a preliminary hydro-geological model for the Arava valley (Israel) was built using cluster analysis and available geological data. 5-years averages of groundwater recharge and lateral flows between the different geological units have been derived from hydro-chemical data. The application of the mixing cell model allowed to identify the flow rates by solving a set of linear equations defining the hydro-chemical and hydrological relationships between the water bodies or cells.

### *Deliverables*

In all study areas databases and digital maps of relevant parameters have been compiled that are available within the working group for comparison. Geological maps have been digitised and digital elevation models produced for some of the study areas. These databases will be kept and updated as a base for further studies.

The GIS system has been adapted for direct retrieval of the data. Functionality for the estimation of water balance components is included into the GIS in a way that the program can be used at a later stage as a tool water resources investigations.

The code for the mixing cell model itself has been further developed allowing for a non-steady modelling (time steps of five years).

## **FOLLOW-UP**

Work expected to be carried out in 1999

Within the next year the water balance modelling will be brought from a point or sub-catchment wide approach to a fully distributed model using maps of parameters as input. This model will be applied in Greece and in Cyprus.

A new solver using genetic algorithms will be implemented into the mixing cell model in order to improve the computation time for non-steady runs.

The estimation methods of semi-arid to arid climates will be demonstrated in the study basins and co-operation on this methods will be discussed.

Work expected to be carried remaining project period

Two main tasks will have to be addressed after the second year. The improvement of the GIS software in order to develop an operational tool for groundwater recharge estimation that can be disseminated and used beyond the project group.

And the comparison of different methodologies including the evaluation of the results obtained in the different study catchments.



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## PARTNERS

### UNIVERSITY OF WÜRZBURG

Department of Hydrogeology  
Pleicherwall 1  
97070 Würzburg  
Germany

Peter Udluft  
Tel.: +49-931-312566  
Fax : +49-931-53436  
E-mail: [udluft@mail.uni-wuerzburg.de](mailto:udluft@mail.uni-wuerzburg.de)

### BEN GURION UNIVERSITY OF THE NEGEV

Jakob Blaustein Institute for Desert Research  
M. Sede Boker Campus, 84990  
Israel

Eilon Adar  
Tel.: +972-7-6596-904 (office)  
Fax: +972-7-6596-909 (office)  
E-mail: [eilon@bgumail.bgu.ac.il](mailto:eilon@bgumail.bgu.ac.il)

### UNIVERSITY OF THESSALONIKI

Department of Geology  
54006 Thessaloniki  
Greece

Georges Soulios  
Tel.: +30-31-998-511  
Fax : +30-31-998-506  
E-mail: [oikonomi@geo.auth.gr](mailto:oikonomi@geo.auth.gr)

### GEOLOGICAL SURVEY DEPARTMENT

Nicosia  
CY 1415  
Cyprus

Soterios Afrodisis  
Tel.: +357-2-309-240  
Fax: +357-2-316-773  
E-mail: [costasc@zenon.logos.cy.net](mailto:costasc@zenon.logos.cy.net)

### UNIVERSITY OF JORDAN

11942 Amman  
Jordan

Elias Salameh  
Tel.: +962-6-5355000  
Fax: +962-6-5348932

**MEDITERRANEAN COOPERATION FOR WATER DESALINATION POLICIES IN  
THE PERSPECTIVE OF A SUSTAINABLE DEVELOPMENT**

**Co-ordinator:** National Technical University of Athens (Dr. Dionysis Assimacopoulos)

**OBJECTIVES**

- Assessment of water resources in the Eastern Mediterranean Target Countries (Cyprus, Lebanon, Israel, Palestine and Egypt), assessment of water demand in the same regions for domestic, industrial and agricultural usage and identification of areas under water risk conditions (present/future water shortage, declining water resources, quality/pollution problems);
- Development of a tool under a GIS (Geographical Information System) environment on water demand/availability and advanced desalination solutions. The GIS-tool will record data, analyse water resources/demand, identify areas under risk and evaluate possible RES/desalination solutions to tackle water problems. The system will be able to monitor water problems and support decisions concerning water management;
- Development of regional policies and strategies for water management to assure the water availability and the regional development;
- Development of a training program and a seminar for desalination and advanced desalination solutions.

**ACTIVITIES**

- ◇ Research activities for the development of the GIS-Decision Making Tool  
Specification of the data and maps needed for the development of the GIS-Decision Making Tool.  
Standardisation of the format for data recording and specification of mapping requirements.  
Identification of data availability in the target countries of the project. Collection and recording of data, as well as digitising of the necessary linked maps.  
Design of the GIS database that will accommodate the collected data.  
Design of the GIS-Decision Making Tool. Determination of the methodology to be followed.  
Development of the interface and programming of the program-modules.
- ◇ Research activities for the water demand/supply studies in target countries  
Preliminary studies of the water supply/demand current situation, the water problems and the desalination experience in the target countries

**EXPECTED OUTCOME**

- ▷ A coherent methodological GIS tool for water management based on desalination and using advanced data management methods
- ▷ A coherent methodology for the assessment of profitable use of renewable energy sources to power desalination units in the different areas of the Eastern Mediterranean Basin
- ▷ Identification and recommendation on solutions/policies which will enhance regional water resources and the socio-economic development of the concerned regions
- ▷ Evaluation of advanced desalination technologies and their adaptation to the local environment
- ▷ Promotion of more efficient and economic technologies and techniques, in order to contribute in the reduction of the water supply problem by taking into account the socio-economic determinants of each East Mediterranean area

## RESULTS SO FAR

MEDCO-DESAL project is developing a methodology for analysing the current situation of water demand and supply and examining water management solutions based on RES powered desalination schemes. Results will assist the formulation of regional water management policies. In the first phase of the project's implementation, research focused on the development of the GIS-tool, which will assist the overall analysis. The GIS-tool incorporates the following steps of analysis:

- Identification of available total water resources
- Assessment of water demand in domestic, agricultural and industrial sector
- Identification of areas facing water shortage problems
- Evaluation of the exploitable wind and solar potential to power desalination units in selected areas of target countries
- Siting of advanced desalination systems to serve water needs in selected areas
- Sizing and cost analysis of different RES/desalination schemes

The development of the GIS-tool requires a wide variety of maps and a large amount of different groups of data. All collected data will be introduced in a geographically linked database. The specified maps and data for the implementation of the tool are the following:

- Maps of country, towns, administrative regions, airports, roads, electric grid, archaeological sites, hydrologic regions, springs, rivers, lakes, agricultural areas, industrial areas, altitudes, meteorological stations, wind speed, solar radiation
- Data on precipitation, water run off, surface and ground water resources, temperature, sunshine duration, solar radiation, wind speed, permanent and non-permanent population, water consumption rates, crop irrigated areas, industries

The GIS software that has been selected for the development of the tool is MapInfo and the programming environment is Map Basic and Visual Basic. Self-standing features have been introduced in order to make the application user friendly and easy to handle, while in parallel the capabilities of the MapInfo background environment have been kept. The tool consists of the following modules:

- A module is designed for supporting the application management. The module controls the settings, options and preferences and administrates all features related to directories, files, maps and data-tables.
- A module is designed to perform an advanced geographic survey of all maps and data available in the target countries. The module acts as a "data viewer" sub-tool, which provides general and specialised information and enriches the perception of the situation in hand before proceeding to any resource or economic analysis. The available geo-referenced data on the existing infrastructure, demography, topography, land use, hydrology and meteorology are identified and visualised for the whole country, selected regions or user defined areas.
- A module is designed to carry out the main analysis procedures of the tool. The module consists of three steps of analysis. In the first step the water availability and demand in selected regions of target countries is assessed and areas facing water problems are identified. In the second step the exploitable wind and solar energy potential in problematic areas is assessed and potential sites for the siting of RES/desalination plants are identified. In the third step various RES/desalination schemes for selected candidate sites are examined and cost analysis of proposed RES/desalination plants is performed. The desalination technologies covered are Reverse Osmosis, Vapour Compression and Electrodialysis.

## FOLLOW UP

Within the forthcoming years of the project the following work will be carried out:

- ▶ The developed GIS Decision Making Tool will be disseminated to the target countries to assist the water demand and supply studies, as well as the studies of market potential of desalination systems
- ▶ The actual and expected water demand and supply situation in Cyprus, Lebanon, Israel, Palestine and Egypt will be analyzed and areas under water risk conditions will be identified

- ▶ The existing advanced desalination systems using RES will be evaluated in order to identify standardized technical desalination solutions with adaptability in the target Mediterranean countries
- ▶ The market potential of wind and solar powered desalination schemes (RO, VC and ED systems) will be studied in selected problematic areas of the concerned Mediterranean regions. Water management solutions based on the most viable and competitive RES/desalination projects will be proposed
- ▶ A Training program on desalination and RES/desalination systems and a Mediterranean seminar on policies for wide implementation of water desalination will be realized

## **PARTNERS**

### **NATIONAL TECHNICAL UNIVERSITY OF ATHENS**

Department of Chemical Engineering  
Renewable Energy Sources Unit  
Heroon Polytechniou 9, Zografou Campus  
15780 Athens

**Greece**

Dionysis Assimacopoulos  
E-mail: [assim@chemeng.ntua.gr](mailto:assim@chemeng.ntua.gr)  
Tel.: 30-1-7723218  
Fax: 30-1-7723155

### **CENTRE FOR RENEWABLE ENERGY SOURCES**

Division for the Promotion of Energy  
Technologies and International Co-operation  
19<sup>th</sup> Km Marathonos Ave  
19009 Pikermi

**Greece**

Theocharis Tsoutsos  
Tel.: 30-1-6039900  
Fax: 30-1-6039911  
E-mail: [ttsout@credb.cress.ariadne-t.gr](mailto:ttsout@credb.cress.ariadne-t.gr)

### **SERVICE INGENIERIE DEVELOPPEMENT ET FORMATION**

30 Boulevard Garibaldi  
75015 Paris

**France**

Osman Benchikh  
Tel.: 33-1-47835742  
Fax: 33-1-47835745  
E-mail: [Osman.Benchikh@wanadoo.fr](mailto:Osman.Benchikh@wanadoo.fr)

### **UNESCO**

Engineering and Technology Division  
Rue Miollis 1  
75732 Paris

**France**

Boris Berkovski  
Tel.: 33-1-45683916  
Fax: 33-1-45685820  
E-mail: [b.berkovski@unesco.org](mailto:b.berkovski@unesco.org)

### **ENVIRONMENTAL MANAGEMENT CONSULTANTS**

Environmental Department Court  
Ayias Elenis 8, Elli Court  
1060 Nicosia

**Cyprus**

Ioannis Glekas  
Tel.: 357-2756607  
Fax: 357-2766665  
E-mail: [envmgt@zenon.logos.cy.net](mailto:envmgt@zenon.logos.cy.net)

### **MANSOURA UNIVERSITY**

Mechanical Engineering Department  
35516 El-Mansoura

**Egypt**

Magdy Abou Rayan  
Tel.: 20-3-5970641  
Fax: 20-3-5970641  
E-mail: [mrayan@eic.mans.eun.eg](mailto:mrayan@eic.mans.eun.eg)

### **THE HEBREW UNIVERSITY OF JERUSALEM**

Department of Agricultural Economics and Management  
The Center for Agricultural Economic Research  
P.O. Box 12  
76100 Rehovot

**Israel**

Eli Feinerman  
Tel.: 972-8-9481148  
Fax: 972-8-9466267  
E-mail: [feiner@agri.huji.ac.il](mailto:feiner@agri.huji.ac.il)

### **CONSEIL ET DEVELOPPEMENT S.A.L**

Abraj Center, Damascus Highway  
P.O. Box 6220-143 Hazmieh, Furn El Chebbak

**Lebanon**

Claude Tabbal  
Tel.: 961-1-292594  
Fax: 961-1-292592  
E-mail: [C.and.D@inco.com.lb](mailto:C.and.D@inco.com.lb)

### **BETHLEHEM UNIVERSITY**

Department of Physics  
P.O. Box 9, Bethlehem  
**Palestinian Autonomy**

Hanna Hallak  
Tel.: 972-2-741241  
Fax: 972-2-744440  
E-mail: [hallak@netvision.net.il](mailto:hallak@netvision.net.il)

**MEDWATER: A DECISION SUPPORT SYSTEM FOR WATER MANAGEMENT IN  
THE MEDITERRANEAN REGION**

**Co-ordinator:** National Technical University of Athens, Athens, Greece (Dionysis Assimacopoulos)

### **OBJECTIVES**

The consortium intends to research and develop a prototype management decision support tool based on System Dynamics models for short and intermediate (up to three years) planning horizons for the effective use of available water resources within an integrated resource and supply network. To achieve this broad aim the following objectives are defined:

- To carry out an investigation into the statistical modelling of rainfall and catchment in order to quantify over a given geographic region and time scale the rainfall variation typical of the region.
- To development of a high resolution System Dynamics Model of the water supply and distribution network to support management decision making .
- To extend the Systems Dynamics model to function within an integrated resource management system and to add a GUI to produce a working end user prototype.

The study areas of the project are the islands of Cyprus and Malta.

### **ACTIVITIES**

During the first year work has been undertaken on detailed modelling of rainfall in Cyprus.

- ◇ The analysis has covered rainfall data from 18 stations at the Kouris dam area. The probability distributions of these stations for a period of 80 years has been evaluated and a time series analysis undertaken to examine whether there is any periodicity or overall trends in the data. The results to date indicate no periodicity and a weak decreasing trend in some stations. Work will continue on deriving rainfall estimated for the whole area. In order to improve the modelling of the long term trends a state space model based on Bayesian analysis combined with a dynamic linear model is under development.
- ◇ A key problem identified for Cyprus is the fact that catchment in the Dams has decreased at a greater rate than predicted from changes in rainfall. This is due to increased groundwater abstraction in the catchment area and a detailed investigation has been initiated to quantify the effect for inclusion in the System Dynamics model.
- ◇ A detailed investigation of the water supply networks in Cyprus and Malta has been undertaken for the causal loop model design. This has revealed different but complementary priorities for the management model in the two islands. In Cyprus the management of supply is a key issue with a requirement to include a detailed catchment model and to incorporate on the demand side crop irrigation and groundwater management. On Malta with its increasing dependence on desalination through its existing reverse osmosis plants the central problem is one of managing demand through pricing policy.

## EXPECTED RESULTS

### *Prototype decision support tool*

⇒ Two working System Dynamics models which can be used by water company personnel in Cyprus and Malta as a decision support aid for planning water resource utilization.

### *Scientific result*

⇒ A comprehensive statistical rainfall model which can be used either in conjunction with the System Dynamics models or independently as a long term planning aid for developing improved water catchment strategies in semi arid regions.

## FOLLOW-UP

On completion of the rainfall and catchment models (including a statistical theory of errors) a detailed evaluation will be undertaken to draw conclusions about the applicability of the models developed to other similar geographic regions.

Detailed System Dynamics models will be developed and validated for catchment, irrigation, groundwater management and pricing policy. The models will include all the major features of the supply and distribution networks (Dams, distribution reservoirs, treatment works etc.) and include operational rules and control policies.

The models developed in will be integrated into a prototype decision support tool by adding a GUI. This tool will be evaluated by water company personnel in Cyprus and Malta.

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## PARTNERS

### **NATIONAL TECHNICAL UNIVERSITY OF ATHENS**

Dept Of Chemical Engineering - Systems  
& Applications Of Renew. Energies Unit  
Heroon Polytechniou 9  
Zografou Campus  
15780 Athens  
Greece

Dr. Dionysis Assimacopoulos  
E-mail: [assim@chemeng.ntua.gr](mailto:assim@chemeng.ntua.gr)  
Tel.: 30-1-772.32.18  
Fax: 30-1-772.31.55

### **CENTER FOR RENEWABLE ENERGY SOURCES**

Division For The Promotion Of Energy  
Technologies & International. Coop.  
19th Km Marathonos Ave.  
19009 Pikermi  
Greece

Dr. Theocharis Tsoutsos  
E-mail: [tsout@Cresdb.Cress.Ariadne-T.Gr](mailto:tsout@Cresdb.Cress.Ariadne-T.Gr)  
Tel.: 30-1-603.99.00  
Fax: 30-1-603.99.11 / 04

### **SERVICE INGENIERIE DEVELOPPEMENT ET FORMATION**

30 Boulevard Garibaldi  
75015 Paris  
France

Dr. Osman Benchikh  
E-mail: [Osman.Benchikh@Wanadoo.Fr](mailto:Osman.Benchikh@Wanadoo.Fr)  
Tel.: 33-1-4783.5742  
Fax: 33-1-4783.5745

**INTERACTION BETWEEN MIGRATION, LAND & WATER USE AND RESOURCE  
EXPLOITATION IN THE OASES OF THE MAGHREB**

**Co-ordinator:** University of Amsterdam, Faculty of Environmental Sciences, Amsterdam  
(Leo J. de Haan)

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**OBJECTIVES**

- To study the interaction between migration and changing land & water management with resource exploitation in the oases of the Maghreb.
- To examine the ecological impact of the changes in land & water management and resource exploitation, especially their contribution to desertification processes.
- To design a model for increased investments and improved spin-off of allocation of remittances in sustainable agriculture.

**ACTIVITIES**

- ◇ The project concerns a comparative and interdisciplinary research in four oases in Southern Morocco and Tunisia. It will study the differentiated impact of migration and concomitant socio-economic changes on land & water management and resource exploitation in dryland oases and, consequently, its ecological consequences. The five work packages are:
- ◇ Quantitative socio-economic survey on household level, focusing on demographic structure of the household, migration characteristics, activity patterns, income characteristics and investment patterns, as well as the agricultural exploitation. The impact of migration on decision-making related to resource exploitation among non-migrant groups will be equally studied.
- ◇ Integrated socio-economic interviews with peasants on plot level, focusing on actual land use, irrigation patterns, and land & water conservation measures. In particular on this level the interdisciplinary nature of the research will be realized, by explicitly studying the interaction between socio-economic characteristics and land and water use patterns.
- ◇ General ecological evaluation of the bio-physical environment, comprising climate assessment, assessment of water resources (water quality and quantity), and soil degradation / desertification assessment, through the mapping of land properties (soil, vegetation and land use). The study will apply and develop models of soil degradation (mainly physical and biological degradation, salinization, and wind and water erosion) adapted to oasis environments.
- ◇ Survey on oasis level of the agricultural and hydrological system. This general survey focuses on the collective aspects of land & water management on oasis level, especially concerning the irrigation system and collective soil and water conservation practices.
- ◇ Theoretical modelling based on models developed from the scientific literature, previous research experiences, and the research results, data from all the following work packages will be integrated and hypotheses tested, in order to identify the enabling conditions for sustainable agricultural development in oases.



## **EXPECTED RESULTS**

- ⇒ To identify the main obstacles for investments in agricultural development and for sustainable land & water management, this interdisciplinary project should be able.
- ⇒ To identify the enabling conditions for successful promotion of a sustainable agricultural development of the region as well as for the struggle against desertification.

## **FOLLOW-UP**

### *Work expected to be carried out in 1999*

The beginning of the actual fieldwork in the selected Moroccan and Tunisian oases, which consists of quantitative socio-economic survey on household level, integrated socio-economic interviews with peasants on plot level and a general ecological evaluation of the bio-physical environment.

Besides the actual fieldwork, available documents on the selected oases (maps, aerial photographs, satellite images, reports on ground water resources) will be analysed.

### *Remaining project period*

In the period of March 1999 - March 2000 onward, a survey of the agricultural and hydrological system will be conducted on oasis level. This general survey focuses on the collective aspects of land & water management on oasis level, especially concerning the irrigation system and collective soil and water conservation practices. This data will be collected by own observation and by carrying out open interviews.

Theoretical modelling will start in June 1999. Based on models developed from the scientific literature, previous research experiences, and the preliminary research results, data from all the following work packages will be integrated and hypotheses tested.

## **SELECTED PUBLICATIONS**

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VERBETEN, E. (1998) Irrigation in Arid and Semi-Arid Environments. Report prepared for IMAROM project. May 1998, University of Amsterdam.

## **PARTNERS**

### **UNIVERSITY OF AMSTERDAM**

Faculty of Environmental Sciences  
Nieuwe Prinsengracht 130  
1018 VZ Amsterdam  
**Netherlands**

Leo J. de Haan  
Tel.: +31-20-525 4063  
Fax: +31-20-525 4051  
E-mail: l.j.haan@frw.uva.nl

### **UNIVERSITÉ MOHAMMED V**

Faculté des Lettres et des Sciences Humaines  
B.P. 1040  
Rabat  
**Morocco**

Abdellatif Bencherifa  
Tel.: +212-7-771873  
Fax: +212-7-772068  
E-mail: bencherifa@mail.Alakhawayn.ma

### **UNIVERSITÉ MOHAMMED IER**

Rectorat de l'Université Mohammed Ier  
B.P. 524  
60.000 Oujda  
**Morocco**

Abderrahmane El Harradji  
Tel.: +212-6-74-47-83  
Fax: +212-6-74-47-79  
E-mail: elharradji@lettres.univ-oujda.ac.ma

### **INSTITUT DES RÉGIONS ARIDES MEDENINE**

Route de Djorf km22  
EL FJE  
4119 Medenine  
**Tunisia**

Youssef Moumni  
Tel.: +216-5-640611  
Fax: +216-5-640435

### **ESTACIÓN EXPERIMENTAL DE ZONAS ARIDAS**

C/. General Segura 1  
04001 Almería  
**Spain**

Albert Solé-Benet  
Tel.: +34-50-276400  
Fax: +34-50-277100  
E-mail: albert@eeza.csic.es

**HYDROMED - PROGRAM OF RESEARCH ON HILL RESERVOIRS IN THE SEMI-ARID ZONE OF THE MEDITERRANEAN PERIPHERY**

**Co-ordinator:** ORSTOM - Institut Français de Recherche Scientifique pour le Développement en Coopération, Tunis, Tunisia (Jean Albergel)

**OBJECTIVES**

- To study an optimal management of each type of hill reservoirs. The use of the supplement of water resource by users raises both technical and social questions such as:
  - what is the duration of life of a reservoir (siltation) ?
  - or what are the possible social organization of the users ?
- To define an optimal land use planning upstream and downstream the artificial lake. To protect the reservoir, soil conservation works have to be planed on the slopes of the watershed. The development of lands close to the lake downstream (gravity irrigation) or on slopes (pumping requires adequate management. Fish breeding is a possibility to valorize the new water resource, just as the touristic exploitation of some sites.
- To assess the impact of one or several lakes on the local environment. The hill reservoir is considered as an artificial structure of little size with very few impacts on the environment. This postulate has often occulted researches on real impacts and on ecosystems modifications.

**ACTIVITIES**

- ◇ Synthesis of previous works in each country. This work package aims to establish the state of the art. It includes a bibliographical review, the analysis and synthesis of realizations and projects, the identification of constraints and the choice of pilot sites for relevant experimentation.
- ◇ Water - soil - environment. This work package includes the study of process on pilot sites and the research of indicators on the functioning of watersheds and hill reservoirs.
- ◇ Agronomy, agricultural economy - social management of water and erosion. The following activities are developed: analysis of the new economic practices introduced in traditional production systems; role of the different economic actors in designing, realizing and managing hill reservoirs and watershed works.
- ◇ Sustainability of the hill reservoir and its integration in the durable development of marginal regions. This work package will use data and models crossing from the three preceding packages. Interactive models will be developed: physical resources - human resources - impacts on the environment.

**EXPECTED RESULTS**

- ⇒ The work carried out in this project should provide guidance for small and medium hydraulic works in soil and water conservation in semi-arid hill areas;
- ⇒ It should identify natural and human constraints to the development of hill reservoirs and provide tools for assistance to decision makers at regional and national levels;
- ⇒ It should also highlight the importance of small catchment areas in water resources mobilisation particularly during dry periods;
- ⇒ Starting from the Tunisian example, Hydromed will deepen the reflection on projects of small and medium hydraulic systems and transfer it to the totality of the semi-arid Mediterranean periphery.

### *Scientific-technical results*

According to the first work package the program produced a state of the art in each of the four countries (Lebanon, Morocco, Tunisia, Syria) on watershed management policies and on small and medium hydraulic accomplishment. A complete bibliography is available.

The second work package includes fundamental research aspects on the water cycle and on geohydrochemical associated cycles. The expected scientific results are:

- Simulation of hydrological balance and of water quality in a hill reservoir, depending on the degree of watershed management.
- Models on the impact of slope management onto the water cycle.
- Models on water and solution transfers of the lake to the water table
- Research on indicators of the environment in view of a typology of the Mediterranean small basins, based on explanatory variables of rainy events, on geomorphological characteristics, on types of flows and on changes in the utilization and the occupation of basin lands.

The third work package develops agronomic models and economic studies. The expected scientific results are concerning:

- Water-crop models adapted to limited water resources coupled with the lake hydrological budget.
- Sociological aspects of the erosion/land erosion control.
- Analysis of the new economic practices introduced in traditional production systems.

### *Deliverables, pilot or demonstration plants*

Ten pilot sites have been chosen and equipped. They are the place for demonstration, education and training.

The work carried out in this project is providing guidance for small and medium hydraulic works in soil and water conservation in semi-arid hill areas. It should identify natural and human constraints for the development of hill reservoirs and provide tools for assistance to decision makers at regional and national levels. It should also highlight the importance of small catchment areas in water resources mobilization particularly during dry periods. Starting from the Tunisian example, Hydromed will deepen the reflection on projects of small and medium hydraulic systems and transfer it to the total of the semi-arid Mediterranean periphery.

### **FOLLOW-UP**

To follow-up the project different kinds of activities are carried for information exchange, scientific animation and to take collegiate decisions in Hydromed Network. Therefore an expert workshop was organised in Lund in July, 1997 on "rain water harvesting and management of small reservoirs in arid and semiarid areas".

A project letter is periodically edited (Hydronews). An international symposium is planned for the last year of the project. ACSAD is preparing the reference terms of such a symposium, which will be open to all the Arabic countries.

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ALBERGEL J., SMAOUI M., HABAÏEB H., NASRI S. (1997) : Analyse des régimes hydrologiques des rivières et torrents non pérennes à partir d'observations sur les retenues artificielles. Exemple du semi-aride tunisien. V<sup>ème</sup> assemblée scientifique de l'AISH de Rabat.

RAHAINGOMANANA N., (1998) Caractérisation géochimique des lacs collinaires de la Tunisie semi-aride et régulation géochimique du phosphore. Thèse de doctorat en Hydrologie, Université Montpellier I, 311 p.

## PARTNERS

### ORSTOM - INSTITUT FRANÇAIS DE RECHERCHE SCIENTIFIQUE POUR LE DÉVELOPPEMENT EN COOPÉRATION

Mission de Tunis  
Impasse Cheherazad  
B.P. 434  
1004 Tunis El-Menzah 4  
**Tunisia**

Jean Albergel  
Tel.: +216-1-75 00 09  
Fax: +216-1-75 02 54  
E-mail: albergel@tunis-orstom.rio.org

### INSTITUT NATIONAL DE RECHERCHE DU GÉNIE RURAL ET DES EAUX ET FORETS (INRGREF)

Département de Conservation des Eaux et du Sol  
B.P. 10  
Ariana  
2080 Tunis  
**Tunisia**

Nasri Slah  
Tel.: +216-1-70 90 33  
Fax: +216-1-71 79 51  
E-mail: merzouk@mtds.net.ma

### NATURAL ENVIRONMENT RESEARCH COUNCIL

Institute of Hydrology  
Maclean Building  
Crowmarsh Gifford  
Wallingford  
Oxfordshire OX10 8BB  
**United Kingdom**

Ragab Ragab  
Tel.: +44-1491-69 23 03  
Fax: +44-1491-69 24 24

### CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS

Instituto de Recursos Naturales y Agrobiología de  
Sevilla  
Avenida Reina Mercedes s/n  
P.O. Box 1052  
41080 Sevilla  
**Spain**

Felix Moreno-Lucas  
Tel.: +34-5-462 47 11  
Fax: +34-5-462 40 02

### LUNDS UNIVERSITET

Department of Water Resources Engineering  
P.O. Box 118  
221 00 Lunds  
**Sweden**

Ronny Berndtsson  
Tel.: +46-46 222 89 86  
Fax: +46-46 222 44 35

### INSTITUT AGRONOMIQUE ET VÉTÉINAIRE HASSAN II

Département des Sciences du Sol  
Laboratoire de Physique et Conservation  
Avenue Allal Fassi  
Madinat Al Irfane  
B.P. 6202 - Instituts  
Rabat  
**Morocco**

Abdelaziz Merzouk  
Tel.: +212-7-77 04 36  
Fax: +212-7-77 12 85

**ARAB CENTER FOR THE STUDIES OF ARID  
ZONE AND DRY LANDS**

Water Resources Division

P.O. Box 2440

Damascus

**Syria**

Jean Khouri

Tel.: +963-11-532 30 39 / 532 30 87

Fax: +963-11-532 30 63

**A NEW INTEGRATED GEOPHYSICAL APPROACH FOR THE RATIONAL  
MANAGEMENT AND EXPLORATION OF GROUNDWATER RESOURCES**

**Co-ordinator:** Rijks Geologische Dienst, Haarlem, The Netherlands (James Baker)

**OBJECTIVES**

- First phase fieldwork with performance of measurements using a range of advanced geophysical techniques (PMR, EKS, AMT, AAR, TDEM), together with more traditional techniques (EM, VLF) at selected sites on Cyprus and in Israel.
- Data transformation and integration, with emphasis on integrating the results of different techniques to give a better resolution in the sub-surface exploration for water.
- Second phase field work, in which combinations of geophysical techniques can be rigorously tested in sites in Cyprus and Israel.
- Drilling of selected boreholes to validate results.
- Development of guidelines for the use of combined techniques in specific hydrogeological settings.
- Organisation of a workshop to disseminate the results to potential end-users (policy makers, water suppliers).

**ACTIVITIES**

Field areas were selected in Israel and on Cyprus, which could demonstrate the flexibility of geophysics in solving different water-related problems. On Cyprus the problem is one of a basic water shortage, and a need to find new water reserves in deep aquifers, while in Israel the problem is one of management of existing water resources. Field measurements have been carried out in the selected sites:

In total about 20 proton magnetic resonance (PMR) soundings were performed in Israel in various hydrogeological environments (sandy and clayey formations, fractured chalk). In order to take into account the influence of electro-conductive rocks on PMR interpretation results.

42 audiomagnetotelluric soundings (AMT) were carried out on 37 different sites of the western Mesaoria sedimentary region of Cyprus, and on its southern part, and on the northern border of the Troodos ophiolite range. Data quality of the AMT soundings is medium to poor in the sedimentary zone, and rather good in the ophiolitic zone. In the sedimentary zone, the targets (gravely sand layers, limestones and gypsum layers) are rather difficult to study either because they are too deep (Potami area), or because they are too thin (Meniko area).

A joint interpretation of the TDEM data is required for a full evaluation of these results. It is therefore inappropriate to do more than give interpreted 1-D geoelectric models at all sites and pseudo-2D resistivity cross-sections of the sites where the TDEM soundings have been carried out along the profiles. It can be seen that the quality of both the Israeli data and the inversions are fairly high in the sense that the data are smooth and the misfit between the data and synthetic response is small. Similarly, the quality of the data generated on Cyprus is high.

The Electro kinetic data along the three main profiles made in Cyprus displayed very low amplitudes which were typically a factor of 10 below those associated with aquifer environments in sandstones. The low S/N led to tests of various sensor configurations and the use of large numbers of repeat shots. The data obtained, however, remain difficult to interpret especially at later times. In order to extract reliable information at later times for the study of signal originating at depths from 90 to 200 m, it has been necessary to improve the basic processing and display of the data. This work is currently underway.

The Azimuthal resistivity soundings have, in general, not shown much evidence of fracturing. Divergent offset Wenner measurements have demonstrated that the Lower Pillow Lavas are inhomogeneous. At sites where indications of aligned fracturing have been identified, it has not been found to continue over the distances between sites, even though these were separated by only 20 - 100 m. This is initially

interpreted as indicating localised, shallow, intense fracturing. Given that the fracturing does not appear to be laterally continuous, there is no reason to suppose that it is vertically continuous.

High resolution seismics is being applied in both Israel and Cyprus. The data for three seismic lines for the test site in Israel give good preliminary results. Interpretation is ongoing at the time of writing this report.

9 VLF surveys of 500m and with a line spacing of 50m, and station spacing of 5m near to Klirou, and 5 at Mitsero, Cyprus, close to the AAR sites. Additionally an electrical resistivity survey was performed at Potami, Cyprus, with 28 soundings performed in all. The data have been processed and interpreted to derive the form and structure of different sedimentary layers (marls, sands and gravels) in the target regions, which can be combined with the hydrogeological data to give three dimensional images of the aquifers.

Data interpretation and integration from different techniques has shown that TDEM and PMR form a powerful combination, complemented by high resolution seismics. Where possible, the data have been interpreted in terms identifying sub-surface units, and water quality. Calibration and quantification of the techniques relies on a close co-operation and interdependence between the project partners, and the process of integration of the results is now ongoing. Hydrogeological data from both areas provide important input to this stage.

The second fieldwork campaign is almost complete, and plans for drilling boreholes to validate the data have been made. The Project is on target to finish all tasks as planned.

## **EXPECTED RESULTS**

### *Scientific-technical results*

This is the first time that a complex combination of advanced geophysical techniques have been applied to water evaluation in deep aquifers of the Mediterranean or any other region. Evaluation of results will add greatly to scientific knowledge on the suitability of different combinations in different settings.

### *Deliverables*

The project will provide an integrated, and flexible package of geophysical techniques which can be used for the solution of water exploration or management problems in complex hydrogeological settings.

### *Pilot/demonstration activities*

Ultimately the objective of the work is to establish improved methods of siting productive bore holes (shallow or deep) and to research capabilities of non-invasive methods for monitoring and managing "at risk" aquifers of the Mediterranean region. The effectiveness of the combined technologies needs additional testing and validation at other sites in the Mediterranean.

### *Commercialisation*

The work carried out in this project will lead to improved combinations of innovative geophysical techniques for application in deep aquifers in the Mediterranean region. This evaluation will also lead to a more cost effective approach to water exploration and management studies, by enabling the identification of deeply located water supplies by non-invasive technology, instead of traditional drilling techniques. The partners are aware of the commercial potential, and will wish to test the methodology in new areas of the Mediterranean.



## **PARTNERS**

### **RIJKS GEOLOGISCHE DIENST**

Richard Holkade 10  
P.O. Box 157  
2000 AD Haarlem  
The Netherlands

James Baker  
Tel.: +31-23-530 03 26  
Fax: +31-23-540 17 54  
E-mail: j.h.baker@nitg.tno.nl

### **BUREAU DE RECHERCHES GÉOLOGIQUES ET MINIÈRES**

BP 6009  
45060 Orleans Cedex 2  
France

Alain Beauce  
Tel.: +33- 2-38643434  
Fax: +33-2-38643361  
E-mail: beauce@dr.brgm.fr

### **BRITISH GEOLOGICAL SURVEY**

Keyworth  
Nottingham NG12 5GG  
United Kingdom

David Beamish, Jon Busby  
Tel.: +44-115-9363100  
Fax: +44-115-9363145  
E-mail: dbe@wpo.nerc.ac.uk

### **GEOLOGICAL SURVEY DEPARTMENT MINISTRY OF AGRICULTURE, NATURAL RESOURCES AND ENVIRONMENT**

1415 Nicosia  
Cyprus

Sortiris Kramvis  
Tel.: +357-2-309260  
Fax: +357-2-316873  
E-mail: gsd@cytanet.com.cy

### **GEOINVEST LTD**

P.O.Box 4038  
Aglantzia industrial Area, Building 10  
Nicosia  
Cyprus

Andreas Shiathas  
Tel.: +357-2-330093  
Fax: +357-2-330118  
E-mail: geoinvest@cytanet.com.cy

### **GEOPHYSICAL INSTITUTE OF ISRAEL**

P.O.Box 2286,  
Holon 58122,  
Israel

Mark Goldman, Vladimir Schtivelman  
Tel.: +972-(3)-5576046  
Fax: +972-(3)-5502925  
E-mail: mark@iprg.energy.gov.il

**DEVELOPMENT OF A METHODOLOGY BASED ON NOAA SATELLITES (AVHRR SENSOR) OBSERVATIONS FOR THE CONTROL OF FRESH WATER RESOURCES AND THEIR EVOLUTION**

**Co-ordinator:** Infocarto S.A., Madrid, Spain (Antonio Yague Ballester)

**OBJECTIVES**

- To build a new hydrology related-parameter that estimates indirectly, simply and efficiently the components of the water balance in the Southwest Mediterranean Area :
  - indirectly, because this parameter could substitute the absence of ground meteorological measurements such as rainfall, temperature or insolation;
  - simple, because NOAA satellite observations of AVHRR sensor are easy to be obtained for the whole area;
  - efficiently, because the new parameter calibrated for the study area could estimate the hydrological balance.
- To increase the ability of third Mediterranean countries to manage more efficiently their own natural resources and environment.

**ACTIVITIES**

- ◇ Building a time series of AVHRR data generated with archived data and direct satellite readout, including geometric corrections, resampling mosaicing and radiocalibration of images;
- ◇ Building under common radiometric and geometric criteria series of NOAA/AVHRR vegetation indices for the Iberian peninsula and the Maghreb (Morocco and/or Tunisia) for the period 1990 to 1995;
- ◇ Correlating this series of monthly vegetation indices and/or AVHRR data with existing valid but easy to obtain meteorological measurements of rainfall and temperature in order to extract a new parameter for estimating the water balance from NOAA/AVHRR observations of the area. This includes the building of gridding surfaces of meteorological data;
- ◇ Establishing procedures for calculating a hydrological balance of the test areas requires a characterisation of the land surface for hydrological objectives (irrigation areas, high medium, low infiltration), a transformation of vegetation indices into available water resources and a calibration of this transformation procedure.

**EXPECTED OUTCOME**

- ▷ Validated time series of AVHRR data for the period 1990 to 1995 covering the entire Iberian peninsula, Morocco and Tunisia;
  - ◆ Tested vegetation indices correlated to meteorological data for the same period and area;
  - ◆ Time series of vegetation indices calculated from AVHRR data;
  - ◆ Validated correlations between vegetation indices, calculated from AVHRR and rainfall and temperature data for the Iberian peninsula and the Maghreb;
  - ◆ Validated procedures for calculating a hydrological balance of the test areas from satellite data as simple and efficient tool for water management;
  - ◆ Harmonised data processing and analysing procedures in PO, ESP, MO and TN as basis for future co-operative projects.

## **PARTNERS**

### **INFOCARTO S.A.**

Gobelas 25-3°  
Edificio Link A  
28023 Madrid / La Florida  
**Spain**

Antonio Yague Ballester

Tel.: +34-1-372 85 54

Fax: +34-1-372 81 45

### **GEOMATIC**

Résidence du Nil 30  
Avenue Oqbah-Agdal  
Rabat  
**Morocco**

Hassan Moussaria

Tel.: +212-7-77 03 36

Fax: +212-7-77 00 87

### **UNIVERSIDAD DE EXTREMADURA**

Departamento de Geografia y Ordenacion del Territorio  
Avenida de Los Quijotes S/N  
10004 Caceres  
**Spain**

José Luis Gurria Gascon

Tel.: +34-2-724 96 00

Fax: +34-2-724 88 58

### **GEOGRAFICA LDA**

Geografica - Sistemas de Informacao  
Aeroporto Lisboa  
Rua C., Edificacao 124 - 3° Piso  
1700 Lisboa  
**Portugal**

Joao Romana

Tel.: +351-1-848 13 14

Fax: +351-1-848 35 19

### **DÉPARTEMENT ÉTUDES ET DÉVELOPPEMENT**

B.P. 200  
1080 Tunis  
**Tunisie**

Habib Ben Moussa

Tel.: +216-1-76 13 33

Fax: +216-1-76 08 90

**INTEGRATED MANAGEMENT OF RECLAIMED WASTEWATER RESOURCES IN  
THE MEDITERRANEAN REGION**

**Co-ordinator:** Universidad de Barcelona, Barcelona, Spain (Miquel Salgot De Marçay)

**OBJECTIVES**

- To integrate wastewater management into the global water cycle of the Mediterranean Countries;
- To study the best treatment methods for further reclamation and reuse of wastewater;
- To adjust the reclamation methods to local conditions;
- To define appropriate water quality standards for reclamation and reuse purposes;
- To implement wastewater disposal and reuse approaches, especially those minimising environmental impacts;
- To improve and compare analytical techniques not enough developed in the field of wastewater reclamation and reuse;
- To develop sludge control and reuse among the partners.

**ACTIVITIES**

- ◇ Identification of the best wastewater treatment methods for re-use and disposal. To this end the following aspects will be tackled: Infiltration-percolation techniques, quality assessment and control of reclaimed wastewater, wastewater storage and groundwater recharge, specific reuse of wastewater, and re-use of sludge;
- ◇ Assessment of health and environmental effects through evaluation of the impact of wastewater reuse on man, plants, groundwater and coastal waters;
- ◇ Evaluation of wastewater disposal methods: irrigation and other;
- ◇ Co-ordination and harmonisation of analytical work as carried out by all participants and development of standardised wastewater analytical techniques;
- ◇ Development and evaluation of wastewater disinfection methods.

**EXPECTED OUTCOME**

- ▷ An integrated set of technologies will be identified and tested, either individually and/or in combination, for the reclamation of wastewater and sludge, well adapted to the Mediterranean region;
- ▷ Harmonised analytical methods will be made available to all participants and comparable data sets from 6 Mediterranean and one non-Mediterranean country will be generated;
- ▷ The quality needed to permit wastewaters to be used for recharge of groundwater will be assessed;
- ▷ Specific types of disposal or re-uses of wastewaters and sludges will be compared and analysed under field conditions, thus providing a better understanding of their quality;
- ▷ The existing and potential health risks associated with actual re-use of wastewaters for irrigation or other purposes and the impact of current wastewater re-use on the environment in general and coastal waters in particular will be better understood;
- ▷ Harmonised analyses' methods and, through a co-ordinated effort, analytical techniques for wastewater evaluation will be provided to all participating institutions and other interested parties (through scientific publications).

## RESULTS SO FAR

- ⇒ Wastewater reuse activities in the partner countries have been identified.
- ⇒ Studies are nowadays centred on reclamation technologies, rules and regulations, and impact on several environment media.
- ⇒ Some analytical results are beginning to be available, and several papers are published.
- ⇒ Analytical methods between partners have been compared and some new developments are being spread (nematode eggs, coliphages, suspended solids).
- ⇒ Co-operation is expected with other AVICENNE group on wastewater reuse in order to prepare a network.
- ⇒ Common future tasks, application of the previous ones and more specific, have been identified :
  - wastewater storage;
  - golf course irrigation using wastewater;
  - new methods of wastewater disinfection;
  - pond management;
  - use of soil-plant-aquifer systems for wastewater treatment and disposal.

## SELECTED PUBLICATIONS

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## **PARTNERS**

### **UNIVERSIDAD DE BARCELONA**

Laboratori d'Edafologia  
Departamento de Productes Naturals, Biología Vegetal I  
Edafologia  
Avenida Diagonal 643  
08028 Barcelona  
**Spain**

Miquel Salgot De Marçay  
Tel.: +34-3-402 44 94  
Fax: +34-3-402 18 86

### **TECHNISCHE UNIVERSITÄT HAMBURG- HARBURG**

Arbeitsbereich Gewässerreinigungstechnik  
Eissendorfer Straße 42  
21073 Hamburg  
**Germany**

Uwe Neis  
Tel.: +49-407-718 32 07  
Fax: +49-407-661 80 18

### **UNIVERSIDADE DO ALGARVE**

Unidade de Ciencias e Tecnologias Agrarias  
Campus de Gambelas  
8000 Faro  
**Portugal**

Jose Gil Teixeira Beltrão  
Tel.: +35-1-89 80 01 00  
Fax: +35-1-89 81 84 19

### **LABORATOIRE DE CHIMIE DES EAUX-SOLS- BOUES**

B.P. 10  
2080 Ariana  
**Tunisia**

Akissa Bahri  
Tel.: +216-1-71 78 01  
Fax: +216-1-71 79 51

### **UNIVERSITÉ MONTPELLIER II**

Département Eau Environnement  
Place E. Bataillon  
34095 Montpellier Cedex 05  
**France**

François Brissaud  
Tel.: +33-4-67 14 42 74  
Fax: +33-4-67 52 48 61

### **J. BLAUSTEIN INSTITUTE FOR DESERT RESEARCH**

Water Resources Center  
84993 Kiryat Sde-Boker  
**Israel**

Gideon Oron  
Tel.: +972-57-56 50 70  
Fax: +972-57-55 50 58

### **NATIONAL FOUNDATION AGRICULTURAL RESEARCH**

Institut of Iraklio  
P.O. Box 1841  
71110 Iraklio  
**Greece**

Andreas Angelakis  
Tel.: +30-81-24 58 51  
Fax: +30-81-248 58

**GENERATING NEW TECHNIQUES TO CONTROL DESERTIFICATION AND  
SALINIZATION EFFECTS IN THE MEDITERRANEAN BASIN**

**Co-ordinator:** Universidade Do Algarve, Faro, Portugal (José Gil Beltrão)

**OBJECTIVES**

- To integrate wastewater management into the global water cycle of the Mediterranean countries;
- To study the best treatment methods for further reclamation and reuse of wastewater;
- To adjust the reclamation methods to local conditions;
- To define appropriate water quality standards for reclamation and reuse purposes;
- To implement wastewater disposal and reuse approaches, especially those minimising environmental impacts;
- To improve and compare analytical techniques not enough developed in the field of wastewater reclamation and reuse;
- To develop sludge control and reuse among the partners.

**ACTIVITIES**

- ◇ Identification of the best wastewater treatment methods for reuse and disposal. With this aim the following aspects will be tackled: Infiltration-percolation techniques, quality assessment and control of reclaimed wastewater, wastewater storage and groundwater recharge, specific reuse of wastewater, and reuse of sludge;
- ◇ Assessment of health and environmental effects through evaluation of the impact of wastewater reuse on man, plants, groundwater and coastal waters;
- ◇ Evaluation of wastewater disposal methods: irrigation and other;
- ◇ Co-ordination and harmonisation of analytical work as carried out by all participants and development of standardised wastewater analytical techniques;
- ◇ Development and evaluation of wastewater disinfection methods.

**EXPECTED RESULTS**

*Scientific-technical results*

- ⇒ An integrated set of technologies will be identified and tested, either individually and/or in combination, for the reclamation of wastewater and sludge, well adapted to the Mediterranean region.
- ⇒ Harmonised analytical methods will be made available to all participants and comparable data sets from 6 Mediterranean and one non-Mediterranean country will be generated.
- ⇒ The quality needed to permit wastewater to be used for recharge of groundwater will be assessed.
- ⇒ Specific types of disposal or reuse of wastewater and sludge will be compared and analysed under field conditions, thus providing a better understanding of their quality.
- ⇒ The existing and potential health risks associated with actual reuse of wastewater for irrigation or other purposes and the impact of current wastewater reuse on the environment in general and coastal waters in particular will be better understood.
- ⇒ Harmonised analyses methods and, through a coordinated effort, analytical techniques for wastewater evaluation will be provided to all participating institutions and other interested parties.

## FOLLOW-UP

- ▶ Common tasks developed with the application of the previous results:

Wastewater storage, golf course irrigation using wastewater, new methods of wastewater, disinfecting, pond management, use of soil-plant-aquifer systems for wastewater treatment and disposal, obtaining of private additional funds to work on wastewater disinfection, exchange of students followed at a good pace among the partners.

- ▶ Common future tasks, application of the previous ones and more specific, have been identified:

Development of a mathematical model for sludge management in wastewater treatments plants, development of mathematical model to study disinfection in Infiltration -Percolation technique and Soil/Plant/Aquifer system, seasonal Storage as a tool for reclaimed wastewater management, evaluation of disposal sites by means of the correlation of the results of a survey (MWTPS), automatic measurement of particle size distributions of suspended solids in wastewater in relation with deep bed filtration techniques.

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## **PARTNERS**

### **UNIVERSIDADE DO ALGARVE**

Unidade de Ciências e Tecnologias Agrarias  
Campus de Gambelas  
8000 Faro

**Portugal**

José Gil Beltrão

Tel.: +351-8-980 01 00

Fax: +351-8-981 84 19

### **EGE UNIVERSITY**

Faculty of Agriculture  
Department of Horticulture  
35100 Bornova - Izmir

**Turkey**

Ulygun Aksoy

Tel.: +90-232-388 01 10

Fax: +90-232-388 18 64

### **BEN-GURION UNIVERSITY**

Blaustein Institute for Desert Research  
P.O. Box 653  
84105 Beer Sheva

**Israel**

Jiftah Ben Asher

Tel.: +972-5-756 58 56

Fax: +972-5-756 57 56

### **ESTACION EXPERIMENTAL "LA MAYORA"**

29750 Algarrobo - Costa

**Spain**

Jesus Cuartero

Tel.: +34-5-251 10 00

Fax: +34-5-251 12 52

**CHARACTERISATION OF LARGE WATERSHEDS FOR SURFACE RUN-OFF  
WATER HARVESTING IN SUPPORT OF SUSTAINABLE HUMAN SETTLEMENT  
AND RE-GENERATION OF NATURAL VEGETATION IN ARID AND SEMI-ARID  
REGIONS**

**Co-ordinator:** Synoptics, Wageningen, The Netherlands (Jos Bakker)

## **OBJECTIVES**

- To develop a information tool for decision making for planning in semi-arid rural environment by estimation of runoff during the winter period (November till March) in the semi-arid regions of Israel and Morocco.
- The prototype GIS-environment should enable the combination, analysis and visualization of different types of data sources in order to support management decision of watersheds in these regions.

## **ACTIVITIES**

- ◇ The intensive hydrological study in a small representative watershed for runoff estimation and waterharvesting: In a small Israeli catchment area - measuring ca. 30 ha – channels and plotters were (re-)installed to retrieve actual hydrological information to validate the KINEROS run-off model. From the same area a wealth of historical hydrological data was used to calibrate the model.
- ◇ The extrapolation of knowledge from the small watershed to large watersheds using remote sensing (RS): A larger Israeli watershed was used for field measurements in order to support the remote sensing images for the identification of representative and meaningful segments.
- ◇ Definition of end user requirements and the test of the developed prototype GIS system: A large watershed in Morocco was selected to test the GIS prototype. From this area few hydrological data are known.

## **RESULTS**

The project has been successfully finalised recently. In the frame of the project the results are summarized in the conclusions below. The project aimed to integrate various disciplines to develop a tool to inventorise and evaluate watershed in semi-arid regions for water availability for all kind of uses. The main parameters driving such a prototype are ruled by the bio/geophysical characteristics of the region. These characteristics are generally defined by climate, geomorphology, geology, pedology and management by farmers or local inhabitants.

### *Hydrological conclusions*

A good relationship exists between geomorphology and hydrology in the Negev desert area as well in the Moroccan area, which is the basis for the GIS-prototype for integration of information on large scale applications for waterharvesting.

The rain events occurring in the 1996 and 1997 appeared to have a good basis for the hydrological modelling and the scenario testing.

The calibration of the hydrological for the small watershed by introducing a correction for the influence of the crust to the KINEROS model resulted into the calibrated so-called KINEMAT hydrological model and proved to deliver good results.

The link between the hydrological surface characteristics and geomorphological units as mentioned above is proven and the accompanying slope of each geomorphological units appears to be an important link between the hydrology and remote sensing.

The model parameters belonging to each geomorphological unit are used for the modelling in the large watershed and meant for qualitative analysis for intercomparison of watersheds in the region with the same geomorphological characteristics.

#### *Remote sensing*

Various kind of soils in the Negev can be differentiated by using remote sensing, mostly related to surface color and roughness. The best index for classification between the soils is:  $(\text{Channel 4} - \text{Channel 1}) / (a - (\text{Channel 4} - \text{Channel 1}))$ , where  $a$  is a coefficient.

With the use of multi-spectral data it is possible to distinguish between the following units: The low colluvium belt from the intermediate colluvium belt and upper colluvium belt; the rock; the chalk outcrops; the colluvium in the lower valleys

For present space systems like TM Landsat a differential reflectance between integrated sum of reflectance of the blue and the NIR channels can be used.

For future space systems like Modis, Meris and SeaWiFS the first derivative and changes in slopes can be used.

#### *GIS*

A space-time information model environment has been built in a GIS (Arcview) in order to have an prototype instrument for hydrologists and policy makers for evaluation of watersheds and means for knowledge transfer for education purposes. The prototype is called the KINESAT model, where Hydrological model KINEMAT combined with satellite derived information leads to a hybrid system for evaluation on various scales like (sub) watershed level and regional level between watersheds in semi-arid regions.

The KINESAT prototype has been extended with an editing tool to provide a flexible evaluation instrument in order to include expert knowledge.

A semi-automatic procedure has been developed to have a quick qualitative analysis of watersheds in the Negev region. The prototype needs to be adapted for areas with other relationships between hydrology and geomorphology and its structure provide the flexibility.

The GIS concept as applied in Morocco has been successfully transferred and conceptually adapted and tested for the Moroccan Settat region.

### **FOLLOW-UP**

#### *Benefits/ Perspectives*

One needs to develop improved and efficient procedures for the investigation, modeling and evaluation of hazards (peak flow, duration, etc.) with a final purpose concerning prevention; zoning and cartography of high-risk areas and better information for the use of both the elected officials and the general public; facilitate the study of the impact of land-use changes and human activities on flooding , for a better prevention of risks. The combined use of GIS and RS will permit a better knowledge of the hydrology of river basins:

The use of RS data in hydrological analysis models (DEMs and land use maps) will permit a large number of simulations. This will allow better understanding of the influence of various parameters such as slopes, vegetation, rainfall, soil moisture content, etc., as well as on global hydrological behavior of the watershed.

The use of RS data will allow the study of a large number of basins and a large number of physical parameters that can automatically be derived from a DEM. Thus, the relationship between the hydrological behavior of a basin and its physiographical and topographical characteristics can be observed and studied. Ungauged basins especially can benefit from the development of a knowledge base that can extend the data.

#### *Socio-economic benefits*

Results indicate the feasibility of RS to be practically incorporated with pragmatic hydrological modeling. Both embedded in a GIS environment. This provides fast and real-time collection and definition of spatially and temporarily distributed hydrological parameters such as vegetation cover, and geomorphic units in order to provide a realistic hydrological assessment of runoff in remote basins with scarce hydrological information. It enables a close to real-time assessment of the temporal and the spatial distribution of runoff, allowing the system engineers and designers with better chance for more

reliable planning of surface water harvesting and management of rural water tanks and reservoirs. Scenario building is an important item in this.

The results of this project are in a prototype phase and are of a general nature. For specific fields of application the GIS concept needs to be elaborated and adapted in collaboration with potential user groups.

The methodology studied in this project can be applied to: Forecast floods to protect agricultural lands from flooding and equipment from destruction, improve in flood prevention that will limit the economic impact of disasters, and any development of methodologies and /or tools in this sense will contribute in minimizing the possible damages and their incurred costs, allow rational water management at the scale of events, carry out solutions for the protection of lands from erosion, implement of the operational use of national and transnational flood management structures.

This will allow fixing the rural population against possible immigration due to poverty excess and developing the tourist-sector.

#### *Main beneficiaries*

As this RS-hydrological modeling protocol is mainly aimed for assessing the water harvesting potential in remote basins, and as the assessed water potential is mainly targeted toward surface reservoirs, the beneficiaries are mainly from the rural agricultural sectors. In case up-scaling will be obtained, and this protocol can be adapted to larger basins, where the model might be also applicable to appraise the runoff potential for a given amount of rainfall from larger basins with bigger reservoirs. In this case, the water might also aimed for domestic user in villages and small towns. Specific interested beneficiaries must be found in :

Water resources and environmental agencies, hydraulic administration, meteorological administration, national office of potable water, environmental protections agencies (Ministry of environment), Ministry of interior, Ministry of agriculture and fisheries, royal center for remote sensing, local and regional planning administrations, industrial sector and namely agricultural industry.

#### *Scientific audience*

To reach this group diffusion and dissemination will be through publications, seminars and case studies demonstration. A workshop which will be associated with demonstration and open panel discussion is suggested as major means of dissemination.

The discussions should provide the researchers with actual criticism and applied comments for future directions and improvements of the model. Results are aimed to be published in applied scientific journals accessible to the above-mentioned target audiences. Final goal of the organizing team should be to find appropriate scientific partners that are able to transfer their knowledge into specific fields of applications and organize a project team together with specific User Groups.

#### *User groups*

Most of the target user groups in this field are (semi-)governmental organisations. The approach of this target audience will be through training and local education workshops and seminars on hydrology modeling and computer related tools such as GIS and RS, with emphasis on hands on applications and case studies. During the small-scale workshops in which the core product will be demonstrated. In addition, Synoptics is planning a commercial brochure illustrating the principles and the possible benefits of the newly developed GIS model. In the scope of product promotion a CD-ROM will be fabricated illustrating the potential of the prototype GIS in combination with different data sources.

The workshops for the User Groups should give insight in practical problems arising in watershed management in these areas and to steer the project team in the development of dedicated watershed GIS systems.

## **PARTNERS**

### **SYNOPTICS**

Integrated Remote Sensing & GIS Applications B.V.  
Costerweg 1k  
P.O. Box 117  
6702 AA Wageningen  
**The Netherlands**

Jos Bakker  
Tel.: +31-317-42 12 21  
Fax: +31-317-41 61 46

### **BEN-GURION UNIVERSITY OF THE NEGEV**

Blaustein Institute for Desert Research  
The Remote Sensing Laboratory  
84993 Sede Boker  
**Israel**

Anatoly Gitelson  
Tel.: +972-7-56 58 92  
Fax: +972-7-55 70 42

### **ÉCOLE MOHAMMEDIA D'INGÉNIEURS**

Laboratoire d'Analyse de Systèmes Hydrauliques  
Avenue Iba Siha 765  
P.O. Box 765  
Rabat, Agdal  
**Morocco**

Driez Ouazar  
Tel.: +212-7-67 05 79  
Fax: +212-7-77 88 53

### **BEN-GURION UNIVERSITY OF THE NEGEV**

J. Blaustein Institute For Desert Research  
Water Resources Laboratory  
84993 Sede Boker  
**Israel**

Eilon Adar  
Tel.: +972-7-56 58 92  
Fax: +972-7-55 70 42

**NEW STABILIZATION/SOLIDIFICATION TECHNOLOGIES FOR THE  
PREVENTION OF UNDERGROUND WATER CONTAMINATION FROM  
INDUSTRIAL WASTES**

**Co-ordinator:** Imperial College of Science, Technology & Medicine, London,  
United Kingdom (Chris Cheeseman)

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### OBJECTIVES

- Treatment of hazardous waste which are contaminating local underground water resources in the Mediterranean region by stabilization or solidification, using locally available cementitious/pozzolanic materials and specially prepared absorbents.
- Optimization of the treatment to reduce leaching of hazardous components to levels which make the solidified product effectively inert.
- Production of treated waste products which are safe for subsequent landfill disposal.
- Providing the collaborating institutes with the necessary information, experience and techniques to apply stabilization/solidification technology to other locally generated hazardous wastes.

### ACTIVITIES

- ◇ Selection and testing of industrial wastes which are a potential threat to underground water and suitable pozzolanic additives for waste solidification.
- ◇ Solidification of hazardous waste sludge with selected pozzolanic additives.
- ◇ Systematic optimisation of waste stabilisation so that hazardous components of the waste are concentrated into a form suitable for solidification.
- ◇ Production and testing of a range of solidified wastes using the optimised stabilization process, local cement and pozzolanic materials.
- ◇ Assessment of the leaching of potentially hazardous organic waste components.
- ◇ Optimization of industrial waste treatment by solidification using additions of cheap locally available adsorbent materials.
- ◇ Selection and thermal treatment of locally available carbonaceous materials, to produce adsorbents for leachable organics present in the wastes.
- ◇ Production of inert waste treatment products suitable for subsequent safe landfill disposal, using the optimized adsorbents, stabilization procedures and solidification additives.

### OUTCOME

The investigation has concentrated on three areas in the Mediterranean region: Athens, Greece; Limasol, Cyprus; and Alexandria, Egypt.

Two industrial wastes from each area have been selected which pose a threat to underground waters because of their chemical composition and current disposal practices. These include wastes from tannery works, dye manufacturing, aluminium surface treatment, a mixed industry waste sludge.

Cement for the use as a stabilisation/solidification additive was available in each area. However, after completing a cost analysis, and in the light of the lack of legislative and economic incentives for waste treatment by local industries, the use of this material was likely to be unrealistic. Therefore, work has concentrated on the development of cement-free systems which use locally available, low cost and naturally occurring or waste materials.

A number of binder systems have been optimised to provide suitable compressive strength, acid neutralising capacity and pH environment. Systems include the combination of local lime with bentonite, zeolite, cement kiln dust, power station flyash, or blast furnace slag. Additional alkali activators are being assessed to further improve the system properties.

A systematic methodology has been developed for all collaborators to assess and optimise potential additive materials and to determine limiting waste loadings for these systems. Work has determined maximum safe waste loadings based on interference with binder system, leaching behaviour and toxicity indices.

Carbonaceous wastes have been identified in each area which have potential as feedstock for low-cost activated carbons, including waste tyres, olive stones and grape seeds. The transfer of carbonisation technology to collaborating institutions will provide a means to address problems arising from the leaching of hazardous organic species from waste and solidified waste products in this project and in the future.

So far waste tyres, grape seed wastes and olive stones have been carbonised and activated under a range of conditions. The resulting activated carbons have been tested for their absorptive capacity for phenol and methylene blue. The performance of some of these is comparable or better than commercially available activated carbons.

## **FOLLOW-UP**

We will be applying for funding to take the laboratory-based work completed to date to pilot plant scale, initially in one of the Mediterranean countries collaborating in the project. Work is continuing to study the hydration and effects of wastes on the natural zeolite and bentonite clay systems through a PhD research programme at Imperial College. Further work based on that completed to date is underway at the Institute of Environmental Sciences, Bogazici University, Istanbul, Turkey.

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## **PARTNERS**

**THE IMPERIAL COLLEGE OF SCIENCE,  
TECHNOLOGY & MEDICINE**  
Centre for Environmental Control & Waste Management  
Imperial College Road  
London SW7 2BU  
**United Kingdom**

Chris Cheeseman  
Tel.: +44-171-594 59 70  
Fax: +44-171-823 94 01  
E-mail: c.cheeseman@ic.ac.uk

**UNIVERSITY OF ALEXANDRIA**  
High Institute of Public Health  
Laboratory of Environmental Health  
Horreya Avenue 165  
Alexandria  
**Egypt**

Olfat El -Sebaie  
Tel.: +20-3-421 55 75  
Fax: +20-3-421 84 36

**NATIONAL TECHNICAL UNIVERSITY OF ATHENS**  
Laboratory of General Chemistry  
Chemical Engineering Department  
Iron Polytechniou 9  
15773 Athens  
**Greece**

Maria Loizidou  
Tel.: +30-1-772 42 70  
Fax: +30-1-770 09 89

**MINISTRY OF AGRICULTURE AND NATURAL  
RESOURCES**  
Water Development Department  
Nicosia  
**Cyprus**

Maria Zachariou  
Tel.: +357-2-30 33 03  
Fax: +357-2-44 50 19

**ENALION**  
Environmental Management Centre Ltd  
P.O. Box 1415  
Nicosia  
**Cyprus**

Ioannis Glekas  
Tel.: +357-2-36 74 14  
Fax: +357-2-36 77 06



## RECHARGE CHARACTERISTICS AND GROUNDWATER QUALITY OF THE GRAND ERG ORIENTAL BASIN

Co-ordinator: British Geological Survey, Wallingford, United Kingdom (W. Michael Edmunds)

### OBJECTIVES

The main objective has been to investigate the interface between the palaeowaters underlying much of the area and modern waters with their recharge sustained by the current climatic and hydrological regime. In fact, the estimation of present day recharge remains one of the most difficult problems in hydrogeology.

The project has focussed mainly on geochemical methods as a means of:

- Obtaining an indication of the groundwater age over the timescale accessible by isotopic methods (around 25000 years);
- Obtaining a better idea of the groundwater chemical evolution as it passes along flow lines in the aquifer using reactive natural tracers;
- Extracting information that gives an idea of the former rainfall to the groundwater system using inert tracers and to investigate the concept that aquifers may act as archives of former climatic conditions; and
- To provide basic information on the potability, use and protection of these aquifers.

### ACTIVITIES

The Grand Erg Oriental basin extends over much of Algeria and southern Tunisia and is today an arid region with rainfall mainly below  $100\text{mm}\cdot\text{yr}^{-1}$ . It is underlain by two major aquifer systems, the Continental Intercalaire (CI) and the Complex Terminal (CT), which contain moderately fresh water. The CI extends across the Algerian-Tunisian border and the principal areas of recharge are in the South Atlas Mountains of Algeria and Tunisia and the Dahar mountains of Tunisia. The main discharge area for the CI is in the Chotts area or the Gulf of Gabes, Tunisia and for the CT in the Chott Melrhir of Algeria or the main chotts in Tunisia. Integrated geochemical methods (major and trace elements, stable and radioisotopes) have been used to follow the changes taking place along selected flow paths, to better understand the controls on water quality and the processes of groundwater mineralisation, as well as any discontinuities in the system. Inert natural tracers, such as Cl and  $^2\text{H}$  and  $^{18}\text{O}$  are used together with radiocarbon to determine the extent of modern recharge, the history of recharge, groundwater residence time and information relating to palaeoclimate.

The investigation of the groundwater system in the CI and CT of the whole region has involved separate studies of seven areas, mainly representative aquifer cross-sections. The main CI section is along a west-east line of the flow direction from M'zab to the Gulf of Gabes discharge area. There is a smooth increase in salinity in the CI with an increase in total dissolved solids from  $964$  to  $3377\text{ mg l}^{-1}$ . Sulphate is also an important component of the total mineralisation and ranges in concentration from a minimum of around  $500$  to a maximum of around  $1750\text{ mg l}^{-1}\text{ SO}_4$ . The salinity is mainly derived from evaporites as shown by the Br/Cl ratio and other major and trace elements. Oxidising conditions exist for some  $300\text{km}$  along the flow line from the Atlas mountains, but a redox boundary provides an important control on the chemistry, especially of  $\text{NO}_3$ , Fe and other metals.

The main line studied through the CT aquifer has been in Algeria south to north from the area of low present day rainfall south of Hassi Messaoud to the Chott Melrhir. The data confirm that some of the waters have probably been derived as surface runoff from the M'zab ridge and/or from the Erg Oriental and they discharge to the Chott Melrhir. Most of the groundwaters are from shallow depth and are unconfined or semi-confined over much of the distance. The salinity ranges from  $1875$  to  $6952\text{ mg l}^{-1}$  rather irregularly along the flow line and both Cl and  $\text{SO}_4$  are dominant anions, the Br/Cl ratio and other geochemical trends demonstrating that the salinity is derived from evaporite sources. The groundwaters are aerobic and contain high concentrations of nitrate, probably of natural origin together with significant U and Cr.

The radiocarbon and other isotope results show that in the main CI profile, modern recharge can be recognised in the aquifer to a distance of some 50-100 km from the Atlas recharge area. Water, which recharged during the Holocene or late Pleistocene can be recognised by detectable radiocarbon some 300 km along the west east flow line in Algeria and also in most of the waters discharging in the Tozeur-Nefta area of Tunisia. This indicates two converging lines of flow supplied by palaeorecharge, one from the Atlas and one most likely from the Dahar of southern Tunisia. In the centre of the basin the age of the waters is likely to be well in excess of 50000 yr BP and noble gas ratios indicate that these waters had a recharge temperature some 6° C lower than at the present day.

Estimation of modern recharge has been carried out using unsaturated zone geochemical profiles. Several profiles of the sand were obtained from Tunisia and Algeria to quantify the direct component of recharge using the chloride profile method. Of the three possible tracer methods (tritium, stable isotopes and chloride) the use of chloride proves especially attractive as a low-cost, tracer for recharge estimation in semi-arid regions. Chloride has the advantage over tracers involving the water molecule ( $^3\text{H}$ ,  $^{18}\text{O}$ ,  $^2\text{H}$ ) in that atmospheric inputs are conserved during the recharge process, allowing a mass balance approach to be applied. The profile at Tozeur (20m) is the longest profile obtained in the present study. The mean CI concentration of the interstitial waters is  $322\text{mg l}^{-1}$  and the steady state concentration down the profile indicates recharge is taking place. For the Tozeur region (rainfall of around  $100\text{ mm yr}^{-1}$ ) a value of  $5\text{ mg l}^{-1}$  Cl has been used as the best estimate, based on data from Sfax and a long term recharge estimate based on 2 profiles of  $1.1\text{mm yr}^{-1}$  is obtained. Although "modern" recharge can thus be recognised, these amounts are very small and the overall resources must be regarded as non-renewable.

One of the unsaturated zone profiles at Tozeur also contains a record of the recharge history over the past 600-700 years. An excellent agreement is found between the oscillations in the chloride profile and the instrumental records of wet and dry periods in rainfall at Tunis and Tozeur. An extrapolation to earlier centuries suggests that several wet periods occurred during the timescale back to the 17th century, with drier climate preceding this.

## OUTCOME

### Scientific and technical results

The geochemical approach, with integrated isotopic and geochemical sampling provides a relatively inexpensive means of obtaining a better understanding of the groundwater origins and evolution.

This approach yields information not only on the chemical but also on the physical properties of groundwaters in the basin. The smooth geochemical trends show good agreement with the overall piezometry of the two aquifer systems, although there are instances where deviations from the geochemical trends imply mixing, cross-formational flow or zones of lower transmissivities for example. It has not been possible to follow up these anomalies at a detailed level in the course of the project but they provide a basis for more detailed local interpretation.

In any future drilling it is recommended that full geochemical sampling be carried out to establish the initial conditions (during testing and prior to development) using the regional geochemical models given here in conjunction with other physical and geological data.

The present study highlights the complex nature of the aquifer in the vicinity of the discharge area of the chotts in Tunisia, complicated by geological structure. The present investigation provides several lines of evidence both chemical and isotopic, to characterise the groundwater originating from different flow directions and from different stratigraphic depths. This cannot be done using piezometry and emphasizes the importance of the geochemical monitoring.

Recharge to the basin at the present day from the evidence available is negligible. Where this might be possible (eg in the vicinity of wadis) its impact will only be felt at a local scale.

For management purposes, therefore, the present study reinforces the concept that the freshwater resources of the deep aquifers are palaeowaters and are therefore finite and that a conservation strategy is of supreme importance.

The overall quality of groundwater in the basins from the evidence obtained is unsuitable for direct use for human consumption on a prolonged basis and the water has a restricted value for agricultural use on account of the salinity (risks of salinisation) and scaling of the distribution network. However, this could be improved locally for potable supplies for example using solar desalination.

The areas of natural discharge - as springs or aouans - in the vicinity of the oases are especially vulnerable to contamination mainly by excessive draw-down leading to saline invasion. There is a limit to any development that can be carried out in the immediate vicinity of the oases without severe

environmental damage. Well fields should be sited well away from these areas where possible to avoid irreversible damage to the habitats as well as the biomass.

## **FOLLOW-UP**

The issue of modern recharge and non-renewable water resources is of considerable concern in many Mediterranean countries. The results of the programme have been presented and discussed at a joint meeting between representatives of EC countries and the Arab Region in Damascus in November 1998 with a view to implementation of an action programme. This meeting was supported by the EC together with BGS and ACSAD (Arab Centre for Semi-arid Zones and Drylands).

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## **PARTNERS**

### **BRITISH GEOLOGICAL SURVEY**

Maclea Building  
Crowmarsh Gifford  
Wallingford OX10 8BB  
United Kingdom

W. Michael Edmunds  
Tel.: +44-191-83 88 00  
Fax: +44-191-82 53 88

### **LABORATOIRE DE CHIMIE ISOTOPIQUE & PALEOCLIMATIQUE**

B.P. "W" 3038  
SFAX  
Tunisia

Kamel Zouari  
Tel.: +216-4-740 88  
Fax: +216-4-755 95

### **CENTRE DE DÉVELOPPEMENT DES TECHNIQUES NUCLÉAIRES**

Division Datation et Hydrologie Isotopique  
Boulevard Frantz Fanon 2  
P.O. Box 1017 Alger-Gare  
16000 Algiers  
Algeria

Adrane Moulla  
Tel.: +213-2-63 62 55  
Fax: +213-2-63 23 26

**VULNERABILITY OF GROUNDWATER RESOURCES TO NATURAL  
RADIOLOGICAL HAZARDS IN THE SEMI-ARID TERRAINS OF NORTH-AFRICA  
AND THE MEDITERRANEAN BASIN**

**Co-ordinator:** Natural Environment Research Council, Nottingham, United Kingdom (Barry Smith)

## **OBJECTIVES**

The general objective of this project is to assess the effect of selective remobilisation of trace metals, including naturally occurring radionuclides (U, Th, Ra, etc.) on the quality of shallow groundwater in the semi-arid terrains of Northern Africa and the Mediterranean basin.

Sub-objectives are:

- To transfer and develop expertise in the counterpart's organisation to facilitate the assessment of the vulnerability of socially important, finite, aquatic resources to both man-made and natural hazards. Naturally occurring radionuclides will constitute the hazard under study.
- To determine the spatial and temporal distribution of natural occurring radionuclides and associated major and trace elements in waters of the Amman-Zarka basin and Maroni region and interpret data to define the factors controlling the release and mobility of these contaminants.
- To quantify the degree of radiological and chemical hazard experienced by the local population through potable and agriculture use of water.

## **ACTIVITIES**

A review of existing regional data, preliminary site investigation and formulation of sampling framework for investigating the occurrence of radio-elements. A detailed review of hydrological and geological conditions in the selected study areas with particular emphasis on the identification of potential source terms and migration pathways. The analysis of chemicals and radiochemicals from samples from the Amman-Zarka basin and the Marconi Celestite formation. The chemical analysis of solid and liquid samples, incorporating a wide range of determinants to provide sufficient data for the computation of aqueous phase specification and leachability of chemical species from the solid phase. The radiochemical analysis of samples collected during field excursions will focus on the determination of U and Th series radionuclides. Computer modelling of aqueous phase specification will be used to model aqueous phase speciation of the dominant radiogenic species. The interpretation and identification of controlling factors.

The assessment of hazards based on the estimation of total chemical and radiogenic dose received, calculated from an environmental transfer pathway model and assuming three principal exposure pathways:

- (a) external expose from contaminated land,
- (b) inhalation of soil gas/particulate contamination, and
- (c) ingestion of terrestrial and aquatic foods. Results obtained from study sites will be placed into a regional context by reviewing regional data. Identification of management options.

## **OUTCOME**

From a regional perspective this work has indicated that significantly elevated levels of radiogenic elements and associated trace elements may be encountered in groundwaters used for both irrigation, industry and public supply. Differences observed between Cyprus and Jordan can be partially explained by the proximity of groundwater supplies to formations containing elevated levels of uranium. However, experience has shown that such areas are either poorly defined (lack of data) or inadvertently ignored (lack of interdisciplinary communication) when planning water supply monitoring strategies and/or the exploitation of new resources. The widespread distribution of phosphorites containing uranium throughout the southern Mediterranean states and the potential for other sources of uranium mineralisation such as those found in Jordan and Cyprus indicate that there is a clear need to encourage the development of national monitoring programmes and awareness of natural radioactivity and its potential implications to groundwater quality throughout the southern Mediterranean states.

Work undertaken in this project has highlighted the need for the provision and incorporation of the geochemical and environmental data produced into integrated, well managed, national data banks. The development and use of these in water resources planning should therefore be actively promoted.

## **FOLLOW-UP**

Technical collaboration funding from UK Department for International Development: Feasibility analysis and laboratory scale testing of remedial methodologies for the removal of naturally occurring radionuclides and potentially toxic elements from Jordanian Groundwaters.

Co-funded trials with suppliers of reverse osmosis, phytoremediation and modified locally produced clays.

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## **PARTNERS**

### **NATURAL ENVIRONMENT RESEARCH COUNCIL**

British Geological Survey  
Analytical Geochemistry Group  
Keyworth  
Nottingham NG12 5GG  
**United Kingdom**

Barry Smith  
Tel.: +44-1602-36 31 00  
Fax: +44-1602-362 00

### **MINISTRY OF WATER AND IRRIGATION**

Environmental Isotope Laboratory  
Water Authority of Jordan  
Laboratory of Water Monitoring Department  
P.O. Box 2412  
Amman  
**Jordan**

Raja Gedeon  
Tel.: +962-6-71 13 61  
Fax: +962-6-71 22 75

### **MINISTRY OF AGRICULTURE AND NATURAL RESOURCES**

Geological Survey Department  
Nicosia  
**Cyprus**

Sotiris Afrodisis  
Tel.: +357-2-30 23 38  
Fax: +357-2-36 79 11

**DEVELOPMENT OF A METHODOLOGY BASED ON NOAA SATELLITES (AVHRR SENSOR) OBSERVATIONS FOR THE CONTROL OF FRESH WATER RESOURCES AND THEIR EVOLUTION**

**Co-ordinator:** Infocarto S.A., Madrid, Spain (Antonio Yague Ballester)

**OBJECTIVES**

- To build a new hydrology related-parameter that estimates indirectly, simply and efficiently the components of the water balance in the Southwest Mediterranean Area :
  - indirectly, because this parameter could substitute the absence of ground meteorological measurements such as rainfall, temperature or insolation;
  - simple, because NOAA satellite observations of AVHRR sensor are easy to be obtained for the whole area;
  - efficiently, because the new parameter calibrated for the study area could estimate the hydrological balance.
- To increase the ability of third Mediterranean countries to manage more efficiently their own natural resources and environment.

**ACTIVITIES**

- ◇ Building a time series of AVHRR data generated with archived data and direct satellite readout, including geometric corrections, resampling mosaicing and radiocalibration of images;
- ◇ Building under common radiometric and geometric criteria series of NOAA/AVHRR vegetation indices for the Iberian peninsula and the Maghreb (Morocco and/or Tunisia) for the period 1990 to 1995;
- ◇ Correlating this series of monthly vegetation indices and/or AVHRR data with existing valid but easy to obtain meteorological measurements of rainfall and temperature in order to extract a new parameter for estimating the water balance from NOAA/AVHRR observations of the area. This includes the building of gridding surfaces of meteorological data;
- ◇ Establishing procedures for calculating a hydrological balance of the test areas requires a characterisation of the land surface for hydrological objectives (irrigation areas, high medium, low infiltration), a transformation of vegetation indices into available water resources and a calibration of this transformation procedure.

**EXPECTED OUTCOME**

- ▷ Validated time series of AVHRR data for the period 1990 to 1995 covering the entire Iberian peninsula, Morocco and Tunisia;
- ▷ Tested vegetation indices correlated to meteorological data for the same period and area;
- ▷ Time series of vegetation indices calculated from AVHRR data;
- ▷ Validated correlations between vegetation indices, calculated from AVHRR and rainfall and temperature data for the Iberian peninsula and the Maghreb;
- ▷ Validated procedures for calculating a hydrological balance of the test areas from satellite data as simple and efficient tool for water management;
- ▷ Harmonised data processing and analysing procedures in PO, ESP, MO and TN as basis for future co-operative projects.



## **PARTNERS**

### **INFOCARTO S.A.**

Gobelos 25-3°  
Edificio Link A  
28023 Madrid / La Florida  
**Spain**

Antonio Yague Ballester

Tel.: +34-1-372 85 54

Fax: +34-1-372 81 45

### **GEOMATIC**

Résidence du Nil 30  
Avenue Oqbah-Agdal  
Rabat  
**Morocco**

Hassan Moussaria

Tel.: +212-7-77 03 36

Fax: +212-7-77 00 87

### **UNIVERSIDAD DE EXTREMADURA**

Departamento de Geografía y Ordenación del Territorio  
Avenida de Los Quijotes S/N  
10004 Cáceres  
**Spain**

José Luis Gurria Gascon

Tel.: +34-2-724 96 00

Fax: +34-2-724 88 58

### **GEOGRAFICA IDA**

Geografica - Sistemas de Informacao  
Aeroporto Lisboa  
Rua C., Edificacao 124 - 3° Piso  
1700 Lisboa  
**Portugal**

Joao Romana

Tel.: +351-1-848 13 14

Fax: +351-1-848 35 19

### **DEPARTEMENT ETUDES ET DEVELOPPEMENT**

B.P. 200  
1080 Tunis  
**Tunisia**

Habib Ben Moussa

Tel.: +216-1-76 13 33

Fax: +216-1-76 08 90

**HYDROMED: DEVELOPMENT OF A COMPUTERISED METHODOLOGY FOR  
THE EVALUATION OF THE VULNERABILITY OF AQUIFIERS IN THE  
SOUTHERN MEDITERRANEAN BASIN AND ITS VALIDATION ON TWO SAMPLE  
AREAS IN TUNISIA AND MALTA**

**Co-ordinator: Institute D'Appolonia SpA., Genova, Italy (Marco Giovanni Cremonini)**

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## **OBJECTIVES**

- To determine the aquifer pollution potential of a given area and summarise this into an index (set of indices), suitable for instance:
  - to evaluate the impact of a pollution source on the aquifer such as industries, storage area, livestock, etc.;
  - to compare different locations of potential pollution sources;
  - to select priorities for restoration and remediation actions in territorial planning.
- To validate the methodology in two sample areas in Tunisia and Malta in order to assess the potential for a general application of the procedure in other parts of the southern Mediterranean region.

## **ACTIVITIES**

- ◇ Selection of the study areas on the basis of existing data, followed by a hierarchical evaluation of potential study areas. The area located in Tunisia should be representative of large temperature North-African alluvial basins facing the Mediterranean sea, while the Malta case study should provide insight in the use of the methodology within smaller basins located on Mediterranean islands, also accounting for karstic characteristics;
- ◇ On-site surveys in the two most suitable areas taking into account local characteristics (aquifer confinement, depth of ground-water table, boundary conditions of the hydro-geological system, availability of information, etc..) as well as groundwater use and land use;
- ◇ Development of the methodology for aquifer vulnerability evaluation through definition of the critical factors for study areas and their evaluations; the selected critical factors are: surface acclivity, net recharge, soil cover characteristics, vadose zone properties, depth to water, aquifer media properties, aquifer hydraulic conductivity;
- ◇ Collection of climatological, morphological/hydrological, surfaces oil and geological/hydrogeological data;
- ◇ Remote sensing interpretation and isotopic analysis of groundwater samples;
- ◇ Development of a computerised (GIS based) model of the overall methodology;
- ◇ Application of the methodology by:
  - computerised storage of all data that refers to the critical factors;
  - evaluation and cartographic mapping of critical factors;
  - development of a computerised hydrogeological vulnerability map for each area.
- ◇ Presentation of results (methodology and case studies) in reports and scientific publications;
- ◇ At the conclusion of the project, a workshop will be organised to disseminate the information about project development and results and to promote the diffusion of the knowledge of the methodology in countries of similar nature in the Mediterranean basin.

## RESULTS

- ⇒ A computer supported methodology has been developed aimed at the assessment of the vulnerability of aquifers in the Mediterranean basin, based on readily available data sets and building on the information about the relative importance of different data. In order to evaluate the vulnerability, the study zone is analysed under a raster (grid) scheme and divided into subareas or cells. This raster scheme allows the assessment of the aquifer vulnerability on a regional perspective, rather than on a site specific point of view.
- ⇒ Through collection, digitisation and storage of data, an organised database for each study area has been performed. These databases are being used to validate the described computer GIS based methodology.
- ⇒ A confidence analysis of the results is also being conducted, with reference to the reliability of the input data. A confidence index is assigned quantifying the level of confidence (based on the following three elements: data last update, source reliability and degree of completeness) of the input data.
- ⇒ In addition, the methodology takes into account the run-off concentration and rapid infiltration, which are typical phenomena in karstic and arid endoreic basins within the Southern Mediterranean regions.

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LGI. 1996. Database for Study Areas. WPR-D1-07/REV0/LGI, prepared for Economic Union.

## PARTNERS

**INSTITUTE D'APPOLONIA S.P.A.**  
Via S. Nazaro 19  
16146 Genova  
**Italy**

Marco Giovanni Cremonini  
Tel.: +39-10-362 81 48  
Fax: +39-10-362 10 78

**UNIVERSITE DE TUNIS**  
Laboratoire des Ressources Minérales  
Campus Universitaire  
1060 Tunis  
**Tunisia**

Ayed Added  
Tel.: +216-1-51 26 00  
Fax: +216-1-88 54 08

**UNIVERSITE DE NICE-SOPHIA ANTIPOLIS**  
Laboratoire Géochimie Isotopique  
Parc Valrose  
06108 Nice Cedex 2  
**France**

Bernat Michel  
Tel.: +33-4-93 52 98 79  
Fax: +33-4-93 52 99 65

**UNIVERSITY OF MALTA**  
Mediterranean Institute  
University Heights  
MSD 06 MSIDA  
**Malta**

Dirk De Ketelaere  
Tel.: +356-33 39 03  
Fax: +356-34 56 55

**DEVELOPMENT OF WATER RESOURCES MANAGEMENT TOOLS FOR  
PROBLEMS OF SEAWATER INTRUSION AND CONTAMINATION OF  
FRESHWATER RESOURCES IN COASTAL AQUIFIERS**

**Co-ordinator:** Universiteit Gent, Gent, Belgium (William De Breuck)

**OBJECTIVES**

- Development of an information system aiming at the evaluation, the analysis and the management of water resources;
- Development of efficient and user-friendly numerical models to serve as reliable tools in decision-making strategies for the management of groundwater resources, particularly focusing on the complex problems of saltwater intrusion.

**ACTIVITIES**

- ◇ Characterisation of coastal aquifers via geophysical prospecting, borings and piezometer installations and measurements, well logging, pumping tests, hydrogeochemical observations;
- ◇ Development of numerical methods for coupled groundwater flow and transport models based on innovative algorithms (Eulerian-Lagrangian approach. Eulerian based finite elements, boundary element modelling, finite difference method);
- ◇ Development of user interface systems for models and data applying techniques such as GIS and expert systems and the inverse calibration method and sensitivity analysis;
- ◇ Implementation of numerical models in high performance computing (HPC) environments;
- ◇ Application of developed numerical models to characterised coastal aquifers.

**RESULTS SO FAR**

- ⇒ Coastal aquifers in NE and NW Morocco, Tunisia and Israel are being characterised. The inventory of available data has been finalised.
- ⇒ The geophysical prospecting campaigns are being continued. Borings and piezometer installation, as well as piezometric and hydrogeochemical observations have been started.
- ⇒ To continue: the aquifer structure and the hydrogeological characteristics of the chosen test areas are gradually being established.
- ⇒ The model development task advances smoothly. An important part of the work during the first project year has been devoted to the study of numerical algorithms (linear solvers, linearization strategies). Several computer codes based on the finite element approach (sharp interface model, model based on the Modified Eulerian Lagrangian scheme, model with hexahedral finite elements) and the boundary element approach have been developed. The testing of the model performance by running bench-marks has been executed. The results for the developed models show good agreement with analytical solutions.

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## PARTNERS

### UNIVERSITEIT GENT

Laboratory for Applied Geology & Hydrogeology  
Krijgslaan 281  
9000 Gent  
**Belgium**

William De Breuck  
Tel.: +32-9-264 46 47  
Fax: +32-9-264 49 88

### UNIVERSITE MOHAMMED I

Faculté des Sciences  
Département de Géologie  
Route Sidi Maafa  
Oujda  
**Morocco**

Mimoun Boughriba  
Tel.: +212-6-68 90 51  
Fax: +212-6-68 90 52

### ECOLE MOHAMMADIA D'INGENIEURS

Laboratoire d'Hydrogéologie et de Géologie de l'Ingénieur  
Avenue Ibn Sina  
B.P. 765  
Rabat - Agdal  
**Morocco**

Benyounes Haddouchi  
Tel.: +212-7-77 26 47 / 77 19 05  
Fax: +212-7-77 88 53

### UNIVERSITA DI CAGLIARI

Dipartimento di Ingegneria del Territorio  
Piazza D'Armi  
09123 Cagliari  
**Italy**

Giovanni Barrocu  
Tel.: +39-70-28 12 45  
Fax: +39-70-27 52 81

### INSTITUT NATIONAL AGRONOMIQUE DE TUNISIE

Département de Génie Rural, des Eaux et Forêts  
Avenue Charles Nicolle 43  
1082 Tunis  
**Tunisia**

Noureddine Ennabli  
Tel.: +216-1-28 09 50  
Fax: +216-1-79 93 91

### VRIJE UNIVERSITEIT BRUSSEL

Dienst Hydrologie  
Pleinlaan 2  
1050 Brussels  
**Belgium**

Florimond De Smedt  
Tel.: +32-2-641 35 47  
Fax : +32-2-641 30 22

## MANAGEMENT OF KARST WATER RESOURCES

**Co-ordinator:** Universität Karlsruhe, Karlsruhe, Germany (Heinz Hötzl)

### OBJECTIVES

The main objective of the research project is a reconnaissance study of the travertine karst aquifer from Antalya, Turkey for an optimal water exploitation and a reasonable management of the available water resources. In order to get the necessary data basis for the Antalya karst aquifer the evaluation of the general water balance, the estimation of the storage capacity as well as of the hydrodynamic behaviour of the travertine aquifer are the specific goals. In a final synoptic view the elaborated effective and sensitive investigation procedure for getting the essential aquifer parameters will be established for a reasonable management of this specific karst aquifer. These results will be generalised to provide a representative investigation method for the investigation and optimal exploitation of Mediterranean karst aquifers.

### ACTIVITIES

- ◇ Detailed hydrogeological mapping of the test site with emphasis on the geological and tectorial structures as well as on the karstmorphological features;
- ◇ Superimposing all obtained and evaluated data on a digital terrain model for a three dimensional representation of the surface and subsurface structures;
- ◇ Defining thickness and porosity of the travertine deposits, extent and location of preferential groundwater flow paths, location of submarine springs and quantification of their discharge;
- ◇ Long term measurements of the various groundwater budget components with an automatic observation equipment;
- ◇ Application of environmental and artificial tracers for localisation of the recharge atlas of the various springs as well as the calculation of the subsurface water storage;
- ◇ Interpretation of tracer data by means of mathematical transport models based on the dispersion equation and adapted to their migration behaviour and to the karst aquifer;
- ◇ Interdisciplinary interpretation of all data obtained during the project.

### OUTCOME

The detailed hydrogeological mapping of the test site was performed parallel to the evaluation and interpretation of the remote sensing data. Main emphasis of both the field mapping as well as of the satellite interpretation was put on geological and tectonical structures and karstmorphological features. Detailed hydrogeological maps in various scales are now available. By superimposing all obtained and evaluated data a digital terrain model is available and enables a three dimensional presentation of the surface and subsurface structures. A geophysical survey commonly performed from AGK and UKAM in November 1995 proved the existence of submarine karstwater outlets in the Antalya Bay as well as preferential underground flow pathes in selected travertine areas.

Frequent registration of various groundwater budget components (precipitation, infiltration, evaporation, evapotranspiration, recharge, discharge) started after an inspection and a necessary smaller repair of the existing observation equipment in April 1995. Calculations of the groundwater balance based on the existing long-term and the current registrations proved a significant lack of the recharge volume of the travertine aquifer taking into account only the surface infiltration into the travertine area and the re-infiltration of the discharge of the Kirkgözler springs. A remarkable interflow between the two aquifers exists.

Frequent sampling campaigns of selected springs, wells and sinkholes as well as of rainwater in the investigation area were started in spring 1995. Hydrochemical analyses were, isotope samples were analysed. Results for both the isotopic and hydrochemical investigations reveal the great similarity between the springs discharging from the Mesozoic carbonate aquifer and the springs and wells discharging from the travertine. A first tracing experiment with uranine, a fluorescent dye was carried

out at the end of the dry season in November 1995 and proved connections between the chosen sinkhole in Mesozoic limestones with some travertine springs of the upper plateau.

## **FOLLOW-UP**

- ▶ Long-term observations for the various groundwater budget components and for hydrochemical investigations will be regularly carried out. Further isotope samplings are planned to clarify the seasonal effects. A second tracing experiment will be undertaken at the end of the rainy season, depending on the flow conditions;
- ▶ The last step of the project will be the interdisciplinary interpretation of all data obtained during the research in form of a final report and in scientific publications. Besides the complete reconnaissance study of the karst aquifer of Antalya this report will provide general recommendations in regard to an optimal investigation strategy of karst aquifers in the Mediterranean region.

## **SELECTED PUBLICATIONS**

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## **PARTNERS**

**UNIVERSITÄT KARLSRUHE**  
AGK Department of Applied Geology  
Kaiserstraße 12  
Postfach 6980  
76128 Karlsruhe  
Germany

Heinz Hötzl - Barbara Reichert  
Tel.: +49-721-608 30 96  
Fax: +49-721-60 62 79

**HACETTEPE UNIVERSITY**  
International Research and Application  
Center for Karst Water Resources (UKAM)  
Beytepe Campus  
06532 Ankara  
Turkey

Gultekin Gunay - Levent Tezcan  
Tel.: +90-312-311 94 42  
Fax: +90-312-310 55 52

**HEBREW UNIVERSITY OF JERUSALEM**  
Department of Soil and Water Sciences  
P.O. Box 12  
76100 Rehovot  
Israel

Ronit Nativ  
Tel.: +972-8-48 12 97 / 48 13 40  
Fax: +972-8-47 51 81

**LAGUNIS : STUDY OF THE MANAGEMENT OF NATURAL WATER RESOURCES  
BY LAGOONING ALONG THE MAGHREB'S MEDITERRANEAN COAST TAKING  
INTO ACCOUNT THE QUALITY OF WATER DISCHARGES**

**Co-ordinator:** Cabinet d'Etudes Techniques Industrielles et d'Innovations Scientifiques (CETIIS),  
Aix-en-Provence, France (Pierre-Marie Lehucher)

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### **OBJECTIVES**

- To assess the spatio-temporal variability of water discharges and associated polluting agents from the Northern African watersheds, including urban waste-water;
- To improve the knowledge of rainfalls and climatology in this area;
- To estimate the efficiency of lagoons all along the Maghreb's Mediterranean coast as a water management system, and determine the best place for potential settlement of lagoons;
- To provide decision makers with a methodology to evaluate and foresee the lagooning impact;
- To provide economical parameters for a coastal management purpose.

### **ACTIVITIES**

- ◇ Adaptation and integration of models into a GIS:
  - assessment of methods and inventory of data;
  - adaptation of existing models and integration into a GIS;
  - development of specialised applications.
- ◇ Experiments and data gathering:
  - selection of basins to which models may be adapted;
  - acquisition and collection of data;
  - field studies of prototype lagoons' efficiency.
- ◇ Exploitation of models:
  - introduction of data into the GIS and data management;
  - data handling, parameterisation and use of models for pollution flow estimates;
  - study of extreme inflows (and polluting loads) over the year.
- ◇ Estimation of the lagooning potential:
  - study of the efficiency and the expected cost of lagoons;
  - calculation of the zones with the highest potential for lagooning;
  - assessment of the potential water resources and water balance.

### **OUTCOME**

- ▷ The efficiency to use lagoons for the water management under typical Mediterranean conditions will be assessed, including waste water flows from urban zones;
- ▷ Areas where lagoons could be particularly efficient will be identified and mapped;
- ▷ A GIS based decision support system will be developed which could help to assess the potential applicability of this concept under any local conditions. This would be of major interest for water supply managers in the Mediterranean countries.



## **PARTNERS**

### **CABINET D'ÉTUDES TECHNIQUES INDUSTRIELLES ET D'INNOVATIONS SCIENTIFIQUES (CETIIS)**

Aix Métropole Bâtiment D  
Avenue Malacrida 30  
13100 Aix-en-Provence  
**France**

Pierre-Marie Lehucher  
Tel.: +33-4-42 93 47 07  
Fax: +33-4-42 26 52 19

### **ECOLE NATIONALE DE L'INDUSTRIE MINÉRALE**

Laboratoire d'Ingénierie des Procédés et d'Environnement  
B.P. 753 Agdal  
Rabat  
**Morocco**

Mohammed Behaj-Soulami  
Tel.: +212-7-77 13 60  
Fax: +212-7-77 10 55

### **IDEE**

Rue Fontaine de l'Hopital 1  
B.P. 59  
34431 Saint Jean-de-Vedas Cedex 1  
**France**

Jacques Trichereau  
Tel.: +33-4-67 69 91 28  
Fax: +33-4-67 69 91 22

### **UNIVERSITÉ DE NICE - SOPHIA ANTIPOLIS**

Laboratoire d'Analyses Spatiales  
Boulevard Edouard Hériot 98  
B.P. 209  
06204 Nice Cedex 3  
**France**

Jean-Pierre Laborde  
Tel.: +33-4-93 37 54 61  
Fax: +33-4-93 37 54 30

### **OFFICE NATIONAL DE L'ASSAINISSEMENT**

Rue de la Monnaie 32  
1001 Tunis  
**Tunisia**

Abderrahman Guennoun  
Tel.: +216-1-34 32 00  
Fax: +216-1-35 04 11

**WATER RESOURCES MANAGEMENT IN URBAN AND PERI-URBAN AREAS OF  
THE MEDITERRANEAN REGION: AMMAN AND RABAT**

**Co-ordinator: CERFE, Roma, Italy (Francesco Ambrogetti)**

**OBJECTIVES**

- To identify the most suitable ways to improve water resources management at urban level. Two urban areas of the Mediterranean region, Amman in Jordan and Rabat in Morocco, will be taken as test sites;
- To establish a comprehensive assessment of the current water supply and consumption in Amman and Rabat in terms of:
  - technical parameters, such as quality and quantity of water supply in comparison to household and industrial demand;
  - social aspects such as consumer's involvement in water management questions, situation of water supply organisations including staff related aspects, institutional and legislative constraints on water resource planning and land-use management, health risks and environmental impact on water pollution in urban areas.
- To develop an improved water quality control through technological means and/or enhanced involvement of consumers;
- To contribute to an improvement of water supply through reduced pollution input, better water treatment, innovative water harvesting and overcoming institutional and legislative barriers;
- To assess the potential applicability of findings from Amman and Rabat to other Mediterranean cities.

**ACTIVITIES**

Preparatory studies to provide basic existing data on water supply and socio-economic parameters.

- ◇ Survey A: Water use and quality. It will be carried out by analysing water samples taken from five groups of consumers and by providing questionnaires to representative groups of users on aspects of perception of water supply and health related parameters. Interpretation of survey results in comparison to health statistics;
- ◇ Survey B: Technologies for water quality monitoring available at international level and being accessible for the current water supply managers in Amman and Rabat;
- ◇ Survey C: Regulatory and economic instruments for water quality monitoring at municipality and national level;
- ◇ Survey D: Mapping water pollution sources in Amman and Rabat;
- ◇ Survey E: User's involvement, establishing the involvement of water consumers in urban and peri-urban areas in the surveying, monitoring and protection of water resources.
- ▷ Based on the set of surveys foreseen in this project and the hard data on water quality generated through water quality analysis and mapping, a good overview will be provided of the actual water supply situation in two urban centres of the Mediterranean region;
- ▷ Due to the co-ordinated work in two different cities, generalisations are expected to be drawn of relevance for larger parts of that region;
- ▷ For the two cities analysed in detail, much improved planning informations will be provided to the responsible authorities.

## **PARTNERS**

### **CERFE**

Via Montezebio 32  
00195 Roma

**Italy**

Francesco Ambrogetti

Tel.: +39-6-323 24 73 / 323 25 05

Fax: +39-6-32 21 21 18

### **OFFICE NATIONAL DE L'EAU POTABLE**

Laboratoire Qualité des Eaux

Rue Patrice Lumumba 6B

Rabat Chellah

**Morocco**

H. Abouzaid

Tel.: +212-7-75 96 00 / 01

Fax: +212-7-75 23 77

### **UNIVERSITY OF JORDAN**

Department of Architecture

Amman

**Jordan**

Kamel Mahadin

Tel.: +962-6-84 35 55

Fax: +962-6-84 85 58

### **INTERNATIONAL REFERENCE CENTRE FOR COMMUNITY WATER**

P.O. Box 93190

2509 AD The Hague

**The Netherlands**

Han Hejnen

Tel.: +31-70-33 14 13

Fax: +31-70-381 40 34

**IMPROVEMENT OF THE TECHNIQUES AND PARAMETERS OF SURFACE  
IRRIGATION IN THE OASIS OF NORTH AFRICA**

**Co-ordinator: Wageningen Agricultural University, Wageningen, The Netherlands**  
**(Gerrit van Vuren)**

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**OBJECTIVES**

- Calibration of formulas for the Estimation of evapotranspiration for the desertic conditions in North Africa.
- Determination of crop coefficients for a multiple level crop canopy as practised in the oasis.
- Elaboration of models for dimensioning surface irrigation systems under oasis conditions.
- Salt balance studies in the oasis.

**ACTIVITIES**

- ◇ The work is conducted on two sites in Tunisia and one site in Morocco where surface irrigation is represented by basin techniques on sandy soil.
- ◇ Assessment of crop water requirements by measurements of water retention and transpiration at a level where hydrological stress is avoided.
- ◇ Determination of the consumptive use in a hydrological parcel, serving as experimental plot, through the direct measurement of water content and controlled inflow.
- ◇ Calculation of the potential evapotranspiration (ETP) as a function of climatic parameters applying the Penman-Monteith formula. The formula for the ETP calculation in the oasis will be calibrated by measuring ETP inside and outside the oasis.
- ◇ Calculating global crop coefficients from water consumption and ETP.
- ◇ Assessment of the salt balance of the irrigation and drainage water through direct measurement of the electrical conductivity.
- ◇ Modelling of surface irrigation techniques for oasis conditions.
- ◇ Design of a monitoring and irrigation system on the basis of the developed package with particular emphasis on an adequate allocation of water to the individual parcels as well as to the appropriate management at the level of the parcel.

**OUTCOME**

The results have led to reasonable indications on crop coefficients for the different agricultural systems in the oasis. These results have been presented to development agencies in Tunisia during a 1 day seminar. The proceedings of this seminar are available from the project coordinator. The improved understanding will provide a better decision basis for water management.

The developed package of models will provide a tool for water managers to apply the generated knowledge to their advantage.

Overall it is expected that the project will contribute to a more efficient use of the scarce water resources of the oasis in North Africa.

**SELECTED PUBLICATIONS**

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## **PARTNERS**

**WAGENINGEN AGRICULTURAL UNIVERSITY**  
Département de l'Irrigation & de la Conservation du Sol  
Nieuwe Kanaal 11  
6709 PA Wageningen  
**The Netherlands**

Gerrit van Vuren  
Tel.: +31-317-48 27 69  
Fax: +31-317-48 47 59

**INSTITUT AGRONOMIQUE DE TUNISIE**  
Département de Génie Rural Eaux et Forêts  
Avenue Charles Nicole 43  
1082 Tunis  
**Tunisia**

Mohamed Mechergui  
Tel.: +216-1-28 62 70  
Fax: +216-1-79 93 91

**INSTITUT AGRONOMIQUE & VÉTÉRINAIRE  
HASSAN II**  
Département de l'Hydraulique  
B.P. 6202  
Rabat-Instituts  
10101 Rabat  
**Morocco**

Mohamed Bazza  
Tel/Fax: +212-7-77 30 38

**UNIVERSIDADE TECHNICA DE LISBOA**  
Departement de Engenharia Rural  
Tapada Da Ajuda  
1399 Lisboa  
**Portugal**

José Luis Monteiro Teixeira  
Tel.: +351-1-363 81 61  
Fax: +351-1-363 50 31

## **WATER RESOURCE MANAGEMENT IN AN INTERDISCIPLINARY PERSPECTIVE**

**Co-ordinator:** Wagner Advies B.V., Noordwolde, The Netherlands (Hendrik Reysoo)

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### **OBJECTIVES**

- To generate scientific and practical benefits in the field of water resource management through an interdisciplinary approach in which the complex interrelationships and interdependencies of technological, organisational, sociological and cultural aspects are integrated;
- To contribute to the transfer of technology in the field of waste water treatment and management in a trilateral way;
- To integrate the gender perspective in all aspects of water resource management with special emphasis on the technical and decision making aspects;
- To organise training on location;
- To incorporate remote sensing techniques (a.o. GIS) to evaluate and monitor the sanitation infrastructure and aspects of environmental impact;
- To implement the transfer of knowledge of and by consultants;
- To transform the results from the projects to a conceptual model for interdisciplinary approaches to water resource management in order to add scientific and practical benefits.

### **ACTIVITIES**

The project consisted of two phases:

- ◇ Phase 1 (year 1): to investigate the possibilities of interdisciplinary co-operation in order to develop interdisciplinary concepts applicable to the category of problems in the field of water resource management. This phase investigation was done through organising two intensive workshops;
- ◇ Phase 2 was programmed to actually conduct the several interdisciplinary projects designed in phase 1.

### **RESULTS**

- ⇒ The two workshops were executed.
- ⇒ From the start of the EGMONE phase 1 project, we have assumed that water resource management (WRM) would profit from an interdisciplinary perspective. We therefore have tried in a collective effort (W1, and in a moderate way W2) to define the contours of that interdisciplinary perspective and the added-value of this perspective to WRM.
- ⇒ This happened to be an extremely difficult exercise, because of the following:
  - as a multidisciplinary team, we succeeded in highlighting important contributions of each (mono-discipline to WRM);
  - we did not manage to work out the added-value of the various disciplines in an interdisciplinary approach to WRM;
  - as the different participating experts were located at different levels of the model, we did not communicate at the same level of planning (macro, meso, micro), level of abstraction, level of disciplinary jargon, etc.;
  - at some levels of the model to be developed, there should be a plea for an integrated (multi-disciplinary) approach (that might merge in an interdisciplinary perspective);
  - we have to bring together experts who are willing to cross the boundaries of their own disciplines, and who are not hindered by institutional constraints such as professional status or statutory mandates.
- ⇒ Although the two workshops were successful and the input regarded as relevant to each of the individual partners, it was concluded not to organise a follow-up of the phase 1.

## **PARTNERS**

### **WAGNER ADVIES B.V.**

Kerkhoflaan 2  
9784 PD Noordwolde  
**The Netherlands**

Hendrik P. Reysoo  
Tel.: +31-50-527 65 57  
Fax: +31-50-527 51 31  
E-mail: [office@wagner-advies.nl](mailto:office@wagner-advies.nl)

### **NATIONAL RESEARCH CENTRE**

Department of Drinking Water  
Tahrir Street 11  
Cairo Dokko  
**Egypt**

Anwar El-Deeb  
Tel/Fax: +20-2-70 09 31

### **PROF. H.C. VAN HALL INSTITUTE**

Agricultural and Environmental Sciences  
Hereweg 99  
P.O. Box 17  
9700 AA Groningen  
**The Netherlands**

Sameh Sayed  
Tel.: +31-50-25 58 90  
Fax: +31-50-27 48 85

### **CERAD**

P.O. Box 6471  
Rue Al Batras 21, Appt 4  
Rabat-Instituts  
10101 Rabat-Agdal  
**Morocco**

Fenneke Reysoo  
Tel.: +212-7-77 59 94  
Fax: +212-7-67 12 48

<p align="center"><b>OBSERVATION AND MODELLING OF THE CIRCULATION IN THE ZONE NORTH-TUNISIA/SARDINIA/SICILY -SALTO-</b></p>
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**Co-ordinator:** Université Pierre et Marie Curie, Paris VI, France (Michel Crepon)

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**OBJECTIVES**

- To study the oceanic circulation between the Strait of Sicily and the Sardinia channel with intensive in-situ observations that will be analysed by means of inverse and prognostic models. Emphasis is put on the seasonal variation of the circulation and the evaluation of fluxes.

**ACTIVITIES**

- ◇ Measurement of temperature, salinity, oxygen and nutrients in bi-monthly and monthly hydrological sections and in current-meters' records during a two year period. Satellite infrared and seacolor images will complete the data set;
- ◇ Processing of these observations with inverse models dealing with variational methods developed by the SALTO team;
- ◇ Study of the circulation with three-dimensional models. The regional model will be an extension of the circulation model of the western Mediterranean sea developed by the EUROMODEL team. This model will be run with a locally very fine grid mesh of the order of 3 km. It will provide adequate boundary conditions to coastal models devoted to water management. This problem will be addressed with methods of coupling of models with different grid meshes.

**OUTCOME**

- ▷ As the SALTO project gathers 6 teams and as it is an extension of the EUROMODEL and MERMAID MAST Programs and of the PRIMO IOC experiment, the results generated by SALTO complement the incomplete understanding of the oceanographic circulation in the Mediterranean sea. This provides an improved basis for the assessment of the cross-Mediterranean interactions transferred by the shared water body of the Mediterranean sea.



## **PARTNERS**

**UNIVERSITÉ PIERRE ET MARIE CURIE - PARIS VI**  
Laboratoire d'Océanographie Dynamique et de Climatologie  
Place Jussieu 4  
Tour 14, 2ème Etage  
Case 100, UPMC  
75252 Paris  
**France**

Michel Crepon  
Tel.: +33-1-44 27 72 74  
Fax: +33-1-44 27 38 66

**INSTITUT NATIONAL DES SCIENCES DE LA MER**  
Molysmologie et Chimie Marine  
Villa 4, Plage Ouests Sidi-Ferruch  
Wilaya de Tipaza  
**Algeria**

Mostfa Boulahdid  
Tel.: +213-2-39 19 13  
Fax: +213-2-39 35 38

**UNIVERSITÉ D'AIX MARSEILLE**  
Centre d'Océanologie de Marseille  
B.P. 330  
83507 La Seyne-Sur-Mer  
**France**

Claude Millot  
Tel.: +33-4-94 30 48 84  
Fax: +33-4-94 30 13 72

**CONSIGLIO NAZIONALE DELLE RICERCHE**  
Istituto Studio Dinamica Grandi Masse  
Staz. Oceanographia  
C.P. 316  
19100 La Spezia  
**Italy**

Mario Astraldi  
Tel.: +39-187-53 63 01  
Fax: +39-187-97 05 85

**INSTITUT NATIONAL S&T D'OCÉANOGRAPHIE  
ET DE PÊCHE**  
Rue 2 Mars 1934 n°2  
2025 Salammbô  
**Tunisia**

Cherif Sammari  
Tel.: +216-1-73 05 48  
Fax: +216-1-73 26 22

**CETIIS**  
Boulevard Paul Vaillant-Couturier 24  
94200 Ivry-Sur-Seine  
**France**

Laurent Mortier  
Tel.: +33-1-49 59 04 54  
Fax: +33-1-49 59 04 49

## INTER-LABORATORY STUDY OF ORGANIC POLLUTANTS IN GROUNDWATER

**Co-ordinator:** Fraunhofer Institut für Toxikologie und Aerosolforschung,  
Hannover, Germany (Karsten Levsen)

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### OBJECTIVES

- To develop new methods for the determination of pesticides in aqueous samples, in particular in groundwater. These methods should be applied to determine pesticides in water samples in Germany, Cyprus and Israel;
- To develop a new method for sampling of groundwater that allows the determination of vertical concentration profiles and in several cases also the migration of pollutants in groundwater.

### ACTIVITIES

- ◇ Work was concentrated on two compound classes, i.e. pesticides and explosives, which are important classes of pollutants found in groundwater. The determination of pollutants in aqueous samples comprises the extraction of the analyte and its instrumental determination. Within the project the classical liquid/liquid extraction was compared with solid phase extraction. Moreover, the new method of solid phase microextraction (SPME) was applied for the first time to the determination of pesticides in aqueous samples. It was demonstrated that this method is simple and sensitive and has a good reproducibility. Moreover, only small water samples are necessary. A further new approach for sample extraction was the "online" coupling of the extraction with its instrumental determination (vide infra);
- ◇ For the instrumental determination of both pesticides and explosives, a large variety of powerful techniques was explored and validated. Basic work was done by gas chromatography, also coupled to mass spectrometry. However, this method is only amenable to thermally stable compounds, where many pesticides but also explosives are thermally labile and cannot be determined with this method. Thus, HPLC methods were developed both for the determination of pesticides and explosives. For the determination of carbamates HPLC with post-column derivatization and fluorescence detection was used, while other pesticides (in particular phenylurea herbicides) but also explosives (in particular nitramines) were determined by HPLC with UV detection. This HPLC analysis was coupled on-line to the solid phase extraction leading to a very effective and fast instrumental method for the analysis of pesticides, which requires only small water samples and which can be automatized readily;
- ◇ The HPLC method alone is not sufficiently selective to detect pesticides or explosives in aqueous samples, if they are present as very complex mixture. Thus, coupling of HPLC with mass spectrometry using the thermospray ionisation was employed for their analysis. This method has a sufficient sensitivity, a very good specificity and has proven to be rugged. This sophisticated method has subsequently coupled also on-line to solid phase extraction.

### OUTCOME

Within the project, a large variety of different analytical methods for the analysis of pollutants in groundwater (here pesticides and explosives) were developed and validated. Apart from internal quality control, also external quality control measures by interlaboratory comparisons were carried out demonstrating that the three partners are able to analyse trace amounts of pesticides in aqueous samples reliably. The methods were applied to analyse a large number of groundwater samples for pesticides in Germany, Cyprus and Israel, but also for explosives in Germany. While the pesticide level in groundwater was usually low in Cyprus and Germany, in specific areas of Israel rather high pollution levels were encountered. A new sampling device (multilayer sampler) was developed within the project and tested in Israel and Germany. In Israel, this device was used to determine vertical concentration gradients of two pesticides in groundwater, while in Germany this new sampling unit was applied to the determination of explosives in groundwater demonstrating that a very detailed information on the vertical distribution is available by this sampling technique.

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## PARTNERS

**FRAUNHOFER INSTITUT FÜR TOXIKOLOGIE  
UND AEROSOLFORSCHUNG**  
Nikolai-Fuchs-Straße 1  
30625 Hannover  
Germany

Karsten Levsen  
Tel.: +49-511-534 02 18  
Fax: +49-511-535 01 55  
E-mail: levsen@ita.fhg.de

**STATE GENERAL LABORATORY**  
Kimonos 44  
138 Nicosia  
Cyprus

Stella Canna Michalidou  
Tel.: +357-2-30 50 63  
Fax: +357-2-31 64 34

**THE WEIZMANN INSTITUTE OF SCIENCE**  
Department of Environmental Sciences & Energy Research  
76100 Rehovot  
Israel

Jehuda Yinon  
Tel.: +972-8-34 25 22  
Fax: +972-8-34 41 24

**IRRIGATION WATER MANAGEMENT AND SALINISATION:  
INTERCOMPARISON OF SIMULATION MODELS IN ARGENTINA AND EGYPT**

**Co-ordinator:** Winand Starring Centre of Integrated Land Soil and Water Research,  
Wageningen, The Netherlands (Massimo Menenti)

### OBJECTIVES

- To compare two models (SIWARE and TUNIN), which describe the regional aspects of salt and water balance, and two models (BIWASA and SWAP), which describe coupled salt and water flow in the soil profile, in terms of accuracy and sensitivity to input data and system schematisation.
- To assess the practical applicability of these models in the context of such different water administration practices as in Argentina and Egypt.

### ACTIVITIES

- ◇ Intercomparison experiment of models BIWASA and SWAP  
Identical measurements were collected at two test sites, Zankalon in Egypt and Lavelle in Argentina, for model validation and further evaluation.
- ◇ Field and laboratory experiments were carried out to obtain soil hydrological properties. Results of simulation experiments were compared with field measurements to assess accuracy. Simulation experiments were made to assess sensitivity of model output to errors in input data and to system schematisation.
- ◇ Intercomparison experiment of models SIWARE and TUNIN  
The two models were considered as being comparable on the basis of conveyance efficiency, when they would give the same results in terms of water delivered to the on-farm distribution system. Accuracy of models results was evaluated using measurements of drainage discharge, depth of shallow groundwater table and salt concentration in drainage water and soil.
- ◇ Analysis of water management practices, with special emphasis on salinisation-prone areas, in Spain, Argentina and Egypt
- ◇ Simulation models of salt and water balance in irrigated areas are potentially useful tools to support management of water and land. The physical, technical and administrative context of irrigation in Argentina, particularly in Mendoza, Egypt and Spain was reviewed to identify constraints and opportunities for the practical use of simulation models. The study provided a basis to assess the practical applicability of these models within the context of irrigation management in the three countries considered in this investigation.

### OUTCOME

#### *Scientific-technical results*

The on-farm model BIWASA, developed at Cairo University simulates water and solute flow in unsaturated - saturated porous media based on the Richards' equation which is solved using a finite-element integration scheme in two-dimensions. The model had never been applied to real situations before and tested with observations. The model was applied first using the data collected at the Zankalon experimental farm.

The on-farm model SWAP, developed at the Winand Starring Centre in Wageningen simulates, as BIWASA, water and solute flow in unsaturated- saturated porous media based on the Richards' equation. It includes diffusion, convection and dispersion of solute and calculates the amount of adsorbed salts. Only one dimension (depth) is considered, however, and the equation is solved using a finite difference integration scheme. This model has been applied to study several cases world-wide. One main required improvement was the calculation of a related coefficient, in the function describing root water uptake, to the sensitivity of crops for salinity.

The regional model SIWARE, developed by the Winand Staring Centre in Wageningen and the Drainage Research Institute in Cairo describes water management processes in an irrigation and drainage system.

The regional model TUNIN was developed by the Winand Staring Centre in Wageningen and the Centro Regional Andino of INCYTH in Mendoza, Argentina. The model describes water conveyance in the irrigation system using so called path-efficiencies, i.e. coefficients and / or functions determined by measuring volumes of water per unit area at primary, secondary etc. offtakes.

Since actual data collection to apply TUNIN to the case of the Nile Delta was not feasible within the scope of the investigation, the SIWARE input data and the calculated discharges were used to build the TUNIN input data. Major improvements were required to develop a new version of TUNIN which could be compared with the observations of groundwater table depth and of drainage discharge and which could be applicable to the case of the Nile Delta. Notwithstanding a crude schematisation of hydrological processes, the modelled results were rather close to the observed values. The original version of TUNIN was coded in outdated FORTRAN IV, so the code was adapted to FORTRAN 77, streamlined and documented. Two user interfaces were developed to handle input and output in an efficient way. The first one was programmed using FoxPro and the second one, which includes a graphic module to display data in map form, in DELPHI. The latter one does also allow for data conversion from a general purpose data base to TUNIN input data format. Water conveyance in the irrigation system was calculated by TUNIN in the Nile Eastern Delta. The yearly averaged path-efficiencies were subsequently used to re-calculate the water volumes at all nodal points in the SIWARE network. The results between the two models were rather good, indicating that water conveyance and allocation calculated as in SIWARE (i.e. through detailed modelling of sub-processes) was rather similar to the result obtained with the simpler TUNIN procedure (conveyance determined by the path-efficiencies and allocation proportional to irrigated area).

#### *Analysis of water management practices*

The relevance of the models described above to irrigation practice depends on how well they fit in current irrigation management practices. Simulation models may be applied to fine-tune e.g. irrigation schedules to control the concentration of dissolved salts in the soil. Practical application of such model-based guidelines, however, requires a significant level of flexibility in irrigation management and in the irrigation and drainage systems. A review has been made on such practices in Argentina, Egypt and Spain to evaluate the practical relevance of models. This analysis was based on written materials, including legislation and administrative regulations (from the past and current time) on irrigation, and interviews both collected during repeated field visits in Egypt and Argentina. For Mendoza, Argentina, particular attention was given to the enforcement of the original legislation (1884) on water allocation, proportional to the actual irrigated area. With time, the area having water rights (a permanent attribute of farms) replaced the notion of actual irrigated area, with the consequence of water being often allocated on the basis of outdated figures. More recently the original notion was re-established, giving the possibility to the Irrigation Water Board to withhold water to farmers not actually using their land. This has also the consequence of requiring a significantly more flexible planning and operation of the irrigation system. The analysis of current legislation and future trends showed a clear evolution towards more accurate planning and operation of irrigation systems. The new legislation on water use in Egypt does include already the principle of allocation based on actual crop water requirements, while in Mendoza proposed new legislation on water prescribes water allocation on the basis of crop water requirements, soil type and hydrological conditions (e.g. shallow water table). The review did indicate useful improvements in the models described above, where the major restriction of their operational use is the current rigidity of planning and operation of irrigation and drainage systems.

#### *Deliverables*

1. new software developed within the project has been distributed to all partners.
2. data base Tunuyan
3. Proceedings of FAO Expert Consultation Meeting

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## **PARTNERS**

### **WINAND STARING CENTRE OF INTEGRATED LAND SOIL & WATER RESERACH**

Marijkeweg 11/22  
P.O. Box 125  
6700 AC Wageningen  
**The Netherlands**

Massimo Menenti  
Tel.: +31-317-47 43 24  
Fax: +31-317-42 48 12

### **INSTITUTO NACIONAL DE CIENCIA Y TECNICA HIDRICAS**

Centro Regional Andino  
Belgrano 210 - Cas, Correo 6  
5500 Mendoza  
**Argentina**

Jorge L. Chambouleyron  
Tel.: +54-61-28 69 93  
Fax: +54-61-28 82 51

### **INSTITUTO NACIONAL REFORMA Y DESARROLLO AGRARIO (IRYDA)**

Paseo de La Castellana 112  
28071 Madrid  
**Spain**

Julian Martínez Beltrán  
Tel.: +34-91-347 15 11  
Fax: +34-91-411 37 70

### **UNIVERSITY OF CAIRO**

Department of Irrigation and Hydraulics  
Giza  
Cairo  
**Egypt**

Mohamed Halim Salem  
Tel.: +20-2-570 28 22  
Fax: +20-2-72 70 09

### **DRAINAGE RESEARCH INSTITUTE**

Kanater 1362/5  
Cairo  
**Egypt**

Shaden Abdel Gawad  
Tel.: +20-2-218 93 83  
Fax: +20-2-218 91 53

<p align="center"><b>FIBEROPTIC LASER SENSOR: A DIAGNOSTIC TOOL FOR ENVIRONMENTAL PROTECTION</b></p>
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**Co-ordinator:** Fraunhofer Institut für Physikalische Messtechnik, Freiburg, Germany (Maurus Tacke)

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### **OBJECTIVES**

- Development of selective sensors for hydrocarbons in water;
- Development, investigation and test of systems that employ mid infrared fiberoptic evanescent wave spectroscopy.

### **ACTIVITIES**

- ◇ Special fibres for the mid infrared were made and improved for transmission of the radiation of tuneable diode lasers, as required for the high sensitivity sensor application;
- ◇ Polymer fibre coatings were optimised. The polymer coating isolates the mid infrared radiation from the extremely absorbing water and allows the hydrocarbons to diffuse into the cladding, thus generating spectrally specific fingerprint absorption;
- ◇ An optical system was made with a diode laser as radiation source;
- ◇ Measurement strategies were investigated and optimised. The laser was tuned very rapidly, and thus probes the spectral features with high speed and sensitivity;
- ◇ Specific properties of the system components were evaluated, and the system performance was analysed.

### **RESULTS**

- ⇒ Selective and sensitive sub ppm detection of selected hydrocarbons in water was demonstrated. The experimental sensitivity allowed detection of 50 ppb tetrachloroethylene (TeCE) in water.
- ⇒ The fibres were analysed and optimised for their sensor performance.
- ⇒ Polymers were analysed. The 50 ppb result was obtained with polyisobutylene (PIB) coating.
- ⇒ A laboratory sensor system was built, that made use of our new „pulse modulation“ laser technique.



## **PARTNERS**

**FRAUNHOFER INSTITUT FÜR PHYSIKALISCHE  
MESSTECHNIK**  
Heidenhofstraße 8  
7800 Freiburg  
**Germany**

Maurus Tacke  
Tel.: +49-761-885 71 25  
Fax: +49-761-885 72 24

**TEL AVIV UNIVERSITY**  
Department of Physics and Astronomy  
Ramat Aviv  
69978 Tel Aviv  
**Israel**

Abraham Katzir  
Tel.: +972-3-640 83 01  
Fax: +972-3-641 58 50

**UNIVERSITY OF TECHNOLOGY**  
Institute for Analytical Chemistry  
Laboratory for Chemical Analysis &  
Ir-Spectroscopy  
Getreidemarkt 9  
1060 Vienne  
**Austria**

Robert Kellner  
Tel.: +43-1-588 01- 48 31  
Fax: +43-1-586 78 13



# 1. Natural resources

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## 1.1. Basic natural resources

### *1.1.2. Water treatment and pollution control*

**OCCURRENCE OF TOXIC CYANOBACTERIA WATERBLOOMS: IMPACT ON  
AQUATIC ENVIRONMENTS AND POTENTIAL HUMAN HEALTH RISK;  
ENVIRONMENTAL, PHYSIOLOGICAL AND GENETIC MECHANISMS INVOLVED  
IN TOXINS PRODUCTION**

**Co-ordinator:** Université Paul Sabatier, Toulouse, France (Alain Dauta)

## OBJECTIVES

- To develop a set of methodologies
- To determine the toxicity of blooms in water bodies
- To assess the toxicity risk in order to avoid human health problems
- To initiate a monitoring process of a crisis period, in relation to:
  - the strain of cyanobacteria implicated (genetic definition),
  - steps of the growth (ecophysiology) and the environmental conditions (ecology).

## ACTIVITIES

- ◇ Ecology of cyanobacterial strains: i)- made in situ in reservoirs or rivers, ii) mainly done on strains isolated from natural blooms occurring in water supplies, iii) data provided by thorough ecophysiological studies taking mainly in account environmental factors, iv) and use of data from literature. The modern molecular biological methods have made possible to compare cyanobacteria ◇ Toxin obtained directly from i) in-situ sampling (water supplies), ii) mass cultures under controlled conditions (directly connected with ecophysiological factors knowledge).
  - ◇ A whole study of toxins (effects, toxicity threshold, toxicity tests,...) to define a range of toxicity effects, levels and strength. Intoxication by food chain must be considered. Chronic intoxication by sublethal levels of hepatotoxins needs to be further investigated and a maximum acceptable concentration for oral consumption proposed considering the environmental characteristics and data base of each country.
- ◇ Study of the chemical structure and of the ways of action of the various toxins (chemical modelling). The chemical stability of each toxin in a water body needs further investigation. In this case, studies on tropical conditions need to be considered. In order to be able to measure the toxic compounds one should be able to identify them. New toxic compounds should be purified for the structure determination and toxicological analysis. Standards of different microcystins are needed for quantification. The purification of peptide toxins will be performed by well established HPLC and TLC methods. Chemical structure will be performed by NMR. Mode of action of the different toxins will be assayed on several enzymes known to be their potential target: acetylcholinesterase and phosphatase.

## EXPECTED RESULTS

The applied objective is to propose a set of various tests specially devoted to the early detection of toxic cyanobacterial blooms and to the determination of the probability of toxicity evolution in the case of massive blooming.

The scientific work will enhance the knowledge on cyanobacteria, with strain collection, physiological description and data of molecular taxonomic characterisation (in situ and in laboratory), toxin production and toxic effects, toxin standards, databank of cyanobacterial 16sRNA gene sequences, data of molecular characteristics of the strains and knowledge of distribution of microcystin genes among isolates. A predictive model to assess the risk of toxin production, integrating information from both field and laboratory data. All the data and publications will be available on a Web site, with also a protocol for toxic risk assessment, guidelines for toxins studies (identification and toxic effect) and classification of toxins.

## **PARTNERS**

### **UNIVERSITE PAUL SABATIER**

Centre d'Ecologie des Systèmes Aquatiques Continentaux  
UMR C5576 CNRS / UPS  
118, route de Narbonne  
31062 TOULOUSE CEDEX 04  
**France**

Alain Dauta

Tel.: +33-5-6155 67 26  
Fax: +33-5-61 55 60 96  
E-mail: dauta@cict.fr

### **UNIVERSITE MOULAY ISMAIL**

Faculté des Sciences, Département de Biologie  
B.P. 4010 Beni M'Hamed  
MA 50 003 MEKNES  
**Maroc**

Mustapha Derraz

Tel.: +212-5-53 88 70  
Fax: +212-5-53 68 08  
E-mail: mderraz@yahoo.com

### **UNIVERSIDAD AUTONOMA DE MADRID**

Facultad de Ciencias Departamento de Biología  
Departamento de Biología Cantoblanco  
E-28049 Madrid  
**Spain**

Antonio Quesada

Tel.: +34-91-397 8181  
Fax: +34-91-397 8344  
E-mail: quesada@bosque.sdi.uam.es

### **ISRAEL OCEANOGRAPHIC & LIMNOLOGICAL RESEARCH**

Marine Biology Department,  
National Institute of Oceanography  
Tel Shikmona P.O. Box 8030  
31 080 HAIFA  
**Israel**

Assaf Sukenik

Tel.: +972-4-851 5202  
Fax: +972-4- 851 1911  
E-mail: assaf@ocean.org.il

### **ARISTOTLE UNIVERSITY OF THESSALONIKI**

Department of Botany  
P.O. Box 109  
GR - 540 06 Thessaloniki  
**Greece**

Tom Lanaras

Tel.: +30-31-998 383  
Fax: +30-31-998 389  
E-mail: lanaras@pegasus.bio.auth.gr

### **UNIVERSITY OF HELSINKI**

Department of Applied Chemistry and Microbiology  
Biocenter Viikki  
Viikinkaari 9, P.O. Box 56  
FI - 00014 Helsinki  
**Finland**

Kaarina Sivonen

Tel.: +358-9-70859270  
E-mail: ksivonen@LadyBird.Helsinki.fi

### **FUNDAÇÃO BIO-RIO**

Núcleo de Pesquisas em Produtos Naturais  
Universidade Federal do Rio de Janeiro  
21941 - 590 Ilha do Fundão - RJ  
**Brasil**

Sandra Azevedo

Tel/Fax: +005521 270-2683  
E-mail: SAZEVEDO@nppn.ufjf.br

**TECHNICAL DEVELOPMENT & DEMONSTRATION OF CLOSED-LOOP PROCEDURES IN ELECTROPLATING AND METAL CHEMISTRY USING SOLAR ENERGY OR WASTE HEAT TO AVOID WASTEWATER AND TO MINIMIZE SOLID WASTE WHICH CAN BE UTILIZED**

**Co-ordinator:** Gesellschaft Fuer Umweltvertraegliche Verfahrensinovat Mbh, Teltow, Deutschland  
(Hans-Wilhelm Lieber)

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## **OBJECTIVES**

*The main objectives are defined as follows:*

- Reduction of the specific consumption of chemicals for pretreatment, surface finishing and posttreatment in electroplating shops.
- Introduction of multiple-step, counter-current rinsing systems to reduce the specific water consumption for rinsing.
- Avoidance of wastewater to be drained.
- Minimization of the quantities of solid waste which should be utilized instead of dumping.
- Use of solar heat and waste heat, respectively, for water recirculation.
- Putting this mode of operation into practice.

## **ACTIVITIES**

*The key activities involve:*

- ◇ Determination and reduction of specific losses of chemicals due to drag-out and a limited lifetime of process solutions.
- ◇ Introduction of improved rinsing systems for minimum water consumption.
- ◇ Selection and tests of suitable solar collectors and equipments for volatilization and heat recovery systems, respectively.
- ◇ Introduction of closed-loop procedures into practice, evaluation and optimization of operating conditions in different plating shops.

## **EXPECTED OUTCOME**

*The principal results will comprise:*

- ▷ A reduced specific consumption of water and chemicals, bringing about cost savings and reduced volumes of waste.
- ▷ The avoidance of wastewater, leading to improved water management for compliance with environmental regulations.
- ▷ Suggestions and experience in utilization of solar energy and waste heat, respectively, for closed-loop procedures.
- ▷ Practical experience in low-waste technology in electroplating by application of the best available technology.
- ▷ Recommendations for the promotion of effective environmental protection in electroplating, metal chemistry and related technologies.

## **PARTNERS**

### **GESELLSCHAFT FUER UMWELTVERTRAEGLICHE VERFAHRENSINNOVAT MBH**

Potsdamer Strasse 18a  
14513 Teltow

**Deutschland**

Pr Hans-Wilhelm Lieber  
Tel.: 49-3328-430.120  
Fax: 49-3328-430.123  
E-mail: tzt-guv@t-online.de

### **NATIONAL TECHNICAL UNIVERSITY OF ATHENS**

Chemical Engineering Department  
9 Heroon Polytechniou Zografou Campus - Zografou  
15773 Athens

**Greece**

Pr Maria Loizidou  
Tel. 30-1-772.31.06  
Fax. 30-1-984.39.17  
E-mail. mloiz@chemeng.ntua.gr

### **ENVIRONMENTAL MANAGEMENT CONSULTANTS**

8 Ayias Elenis P.O. Box 1559  
1510 Nicosia

**Chypre**

Dr Ioannis Glekas  
Tel. 357-2-456.607  
Fax. 357-2-456.665  
E-mail.envmgt@zenon.logos.cy.net

### **HIGH INSTITUTE OF PUBLIC HEALTH ENVIRONMENTAL HEALTH**

165 El Horlya Avenue  
Alexandria

**Egypte**

Pr Olfat El-Sebaie  
Tel. 20-3-421.55.75  
Fax. 20-3-421.84.36  
E-mail.

## SUSTAINIBILITY AND OPTIMISATION OF TREATMENT AND USE OF WASTEWATER IN AGRICULTURE

**Co-ordinator:** Faculté Universitaire Des Sciences Agronomiques, Gembloux, Belgium  
(Dimitri Xanthoulis)

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### OBJECTIVES

- *Wastewater treatment to obtain a effluent which can be reused in agriculture:* the first aim is the development of new technologies in the treatment of wastewater from small settlements, villages and towns to produce treated water for irrigation.
- *Irrigation techniques which are compatible with sustainable agricultural practices:*  
To produce irrigated crops with wastewater treated by technology provided by objective 1 and by other traditional existing systems (stabilisation ponds, rapid infiltration). Environmental consideration and socio-economic profitability will be taken into account.  
The use of wastewater may have potentially adverse impacts on both environment and the public health, largely depend on the wastewater characteristics, the degree of purification, the method and location of use and the crop irrigated. Scientifically sound planning and effective management of the irrigation or fertilisation regime, according to water and nutrient requirement of selected crops, can minimise these disadvantages to the level of environmental insignificance.

### ACTIVITIES

- ◇ *Wastewater treatment to obtain a effluent which can be reused in agriculture*

The proposed project will investigate natural vegetation from the Mediterranean region that could be used in wastewater treatments. Three wastewater treatment plants will be installed and optimised: Free Water Surface system, Epuvalisation and Long Term Storage in maturation ponds. Such technologies should be considered as: low cost, appropriate for the existing living standards, adaptable to the Mediterranean environment and appropriated for the production of advanced treated effluent for reuse.

The first aim is the development of new technology in the treatment of wastewater from municipal and industrial origin to produce treated water for irrigation.

#### *Free water surface system (FWS)*

Evaluation of existing indigenous species and construction of an experimental FWS wetland unit.  
Operation of the unit. Measurement of specific contaminants (nitrogenous species and pathogens) in the influent, effluent recycled and effluent for reuse (irrigation of vineyards).  
Investigate the removal mechanisms for specific contaminants in relation to: a) The species used, b) hydraulic load rates, c) plant parts removal, and d) the type of the organic material added.

#### *Long term storage (LTS)*

The optimisation of the storage time in ponds of wastewater, on water quality will be studied.  
We will verify the conformity of the crops quality with the E.U. quality standards for imported products.

#### *Epuvalisation (EPU)*

Improve the purified wastewater by a performant tertiary treatment and make them available for a non restrictive agricultural use.  
Produce plants which can be used as house plants (cyperus, phoenix, washingtonia, bamboo), as seaside windbreaks or for greenhouse flowers.

- ◇ *Irrigation techniques which are compatible with sustainable agricultural practices*

Use of treated wastewater of appropriate quality with 3 different irrigation techniques which are surface, sprinkler and drip irrigation to irrigate crops.  
Plots installation and study of the effect of treated wastewater on soil, groundwater and crops.



Economic evaluation of the use of treated wastewater with the 3 irrigation techniques in comparison with the use of fresh water and traditional irrigation techniques. Environmental consideration and socio-economic profitability will be taken in account.

The experiments will take place in open fields conditions and in greenhouses with different water quality, irrigation systems, crops and soil. The trials will be organised to optimise nitrogen fertilisation, use of treated wastewater for complementary irrigation and use of treated wastewater in intensive agriculture within acceptable level of risk concerning environment and public health.

#### *Use of treated wastewater in intensive agriculture within acceptable level of risk concerning environment and public health*

On these main treatments we will check in the growing plants the balance between in coming and out going nutrients, we will measure the depth of nutrient percolation and accumulation in the soil and we will try to estimate the proper amount of irrigation with effluents in the presence of variable plants that will keep the sustainability of the soil and plants for human and fodder.

#### *Complementation irrigation for cereal crops*

The trial will try to answer the question: is it possible to use purified wastewater in a complementary irrigation system for cereals (winter wheat, barley, maize) during drought periods?

Three types of water will be used: rain water (without irrigation) as test, wastewater purified by infiltration filtration and wastewater purified by epuvalisation. As test, we will apply the usual recommended fertilisation whereas for the wastewater treatments, no fertiliser will be applied.

#### *Optimisation of the use of nitrogen in the wastewater*

Follow up of the drainage waters and of their composition by the use of lysimeters.

Establish the quantitative balances of salts and of nitrates and quantify nitrate leaching and estimate the contamination risk of groundwater. The lysimeter results will be compared with mass balance results based on the quantification of nitrogen inputs and outputs in the plant-soil system.

#### *Impact of waste water on soil structure*

### **EXPECTED RESULTS**

#### ▷ *Scientific - technical results*

This study will give us detailed information on the best way for wastewater treatment and exact amounts of water and nutrients needed to safe use effluents in producing human edible and non edible crops. A continuous use of effluents year round by agriculture crop can help in effluents storage pools management and increase the fresh water available to the growing human population in the area.

This study will allow us to develop good management practices aimed at minimising environmental and health impact of the wastewater reuse considering: The water use efficiency of the tested crops;

Nutrient uptake from TWW source; Leaching depth of TWW component; Degree of coli contamination in harvested crops; Nutrient balance in soil and plants; The effect of TWW on soil stability; High value crops; The optimal water regime for barley, wheat and maize; Optimal yield for barley, wheat and maize achieved adopting optimal water regime; Required quality of the crop products following WHO guidelines; Rationalise the supplement irrigation taking into count the impact of the TWW on the soil quality, groundwater contamination and crop qualitative and quantitative yield; Wastewater treatment system well adapted to the Mediterranean region; Development of decentralising system for WW management i.e. cost effective technology suitable for wastewater reclamation and reuse in Mediterranean region.

#### ▷ *Deliverables*

- Rehabilitation of treatment plans and installation of epuvalisation systems (EPU).
- Free Water Surface System (FWS) installation and optimisation.
- Long Term Storage system (LTS) installation and optimisation.
- Selection of indigenous aquatic plant species.
- Experience in greenhouse and in field - intensive agriculture irrigated with treated wastewater.
- Mathematical model for salts and nitrates transfer.

- Collected data on the effect of treated wastewater on soil, groundwater and crops.
- Guidelines for futures activities.

## **PARTNERS**

### **FACULTE UNIVERSITAIRE DES SCIENCES**

#### **AGRONOMIQUES**

Unite d'Hydraulique Agricole  
2 Passage Des Deportes  
5030 Gembloux

**Belgium**

Prof. Doct. Dimitri Xanthoulis

E-mail: xanthoulis@fsagx.ac.be

Tel.: 32-81-62.21.86

Fax: 32-81-62.21.81

### **AGRICULTURAL UNIVERSITY OF ATHENS**

Dept Of Land Reclamation & Agric. Eng.  
75 Iera Odos Str.  
11855 Athens

**Greece**

Prof. Doct. George Mavroyannopoulos

E-mail: naxos@auadec.aua.ariadnet-t.gr

Tel.: 30-1-52.94.008

Fax: 30-1-52.94.015

### **NATIONAL FOUNDATION FOR AGRICULTURAL RESEARCH INSTITUT OF IRAKLIO**

Institute Of Iraklio  
Dept Of Water Resources & Environment  
Po Box 1841  
71 110 Iraklio

**Greece**

Dr. A.N. Angelakis

E-mail: angelak@cc.uoh.gr

Tel.: 30-81-24.58.51

Fax: 30-81-24.58.58

### **INSTITUT NATIONAL DE RECHERCHE GENIE RURAL, EAUX, FORETS**

Labo De Physique Du Sol, Physiol. Veg.  
& Microbiologie  
Rue Hedi Karray  
Bp 10  
2080 Ariana

**Tunisia**

Mr. Zouhaier Chaabouni

E-mail:

Tel.: 216-1-71.96.30

Fax: 216-1-71.79.51

### **INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II**

Complexe Horticole D'agadir  
Bp 773  
Iavh Ii Agadir  
Agadir

**Morocco**

Prof. Redouane Choukr-Allah

E-mail: chagadir@mtds.com

Tel.: 212-8-24.49.51

Fax: 212-8-24.22.43

### **AGRICULTURAL RESEARCH INSTITUTE SOIL AND WATER USE SECTION**

Po Box 2016  
1516 Nicosia

**Cyprus**

Dr. Ioannis Papadopoulos

E-mail: ari@athena.cc.ucy.ac.cy

Tel.: 357-2-30.51.01

Fax: 357-2-31.67.70

### **THE HEBREW UNIVERSITY OF JERUSALEM- REHOVOT**

Fac. Of Agricultural, Food & Env. Sc.  
Dept Of Field Crops, Veg. & Genetics  
Po Box 12  
76100 Rehovot

**Israel**

Prof. Doct. Uzi Kafkafi

E-mail: kafkafi@agri.huki.ac.il

Tel.: 972-8-948.11.05

Fax: 972-8-946.96.57

### **AL QUDS UNIVERSITY COLLEGE OF SCIENCE & TECHNOLOGY**

Dept Of Chemistry  
Po Box 20002  
Jerusalem

**Palestinian Autonomy**

Dr. Mustapha Khamis

E-mail:

Tel.: 972-2-74.97.53

Fax: 972-2-74.69.60

**INTEGRATED WASTEWATER REUSE BY SOLAR-CATALYTIC TREATMENT:  
A PILOT STUDY IN THE TEXTILE INDUSTRY**

**Co-ordinator:** Technische Universitaet Clausthal, Clausthal-Zellerfeld, Germany (Alfons Vogelpohl)

**OBJECTIVES**

- Application of a sustainable and economical process to promote the conservation of natural resources.
- Water saving and reduction of environmental pollution with a process using solar energy.
- Environmentally friendly, long term and sustainable enhancement of the industrial capacity with a production integrated process.
- Solar purification of real textile wastewaters with the goal of wastewater re-utilization.
- Application and further development of a cheap and simple process with materials and equipment available in the Third Countries.
- Cost reduction in wastewater treatment and water supply.
- Drawing the attention of plant managers, authorities and the population to the sustainable use of water.
- Improvement of the solar-catalytic wastewater treatment with the perspective of a future application in sun poorer countries.
- Proof of the environmental and health toleration of the process.
- Upgrading and optimization of existing pretreatment plants (mechanical, chemical and biological treatment plants) with respect to European standards.
- Elimination of recalcitrant compounds with a solar process insured by strict quality control.
- Quality control for the prevention of toxic intermediate reaction products by chemical analyses (e.g. COD/BOD5 ratio) and bio-tests.
- Recycling and reuse of textile industry wastewater for process water supply. Test in the production process.
- Optimization of the reactor design (e.g. mass transfer, residence time distribution, evaporation, design, investment costs).
- Optimization of the catalyst (e.g. performance, stability, aging) and their fixation.
- Scale-up of a Thin Film Fixed Bed Reactor to semi-technical application and comparison of the performance with bench-scale reactors.
- Demonstration of the performance, the safety in operation and the feasibility by a pilot test under real conditions.
- Design instructions for technical scale plants and concepts for logistics and reuse of the treated wastewaters (e.g. storage, transport, place for reuse).
- Business management analysis for the determination of the fields, the time and the countries of application.

**ACTIVITIES**

*Optimization of the pretreatment plants*

- ◇ The existing wastewater pretreatment plants of the two textile factories in Algeria and Tunisia will be upgraded and optimized with the objective to reduce the consumption of chemicals and energy as well as to reach European effluent standards.

### *Laboratory studies*

- ◇ The solar-catalytic treatment shall lead to a water quality which is as high as possible. The improvement of the catalysts and their fixation on materials which is cheaper than 10 Euro/m<sup>2</sup> as well as their test and adaptation to the reactor-system in bench-scale plants are essential for an economic application of the solar-catalytic techniques. Intermediate products which might be generated during the oxidation will be detected and assessed by quality control via analyses and bio-tests.

### *Pilot studies*

- ◇ The design of the pilot plants will involve the knowledge of all partners and especially economic aspects, the feasibility and the adaptation to the requirements in Third Countries. The pilot tests will start with the effluents of the treatment plants where the performance of the solar-catalytic treatment is known. Different wastewater flows will be investigated afterwards. The reuse of wastewater with or without treatment will be proved. All applicable technologies will be compared with the solar-catalytic treatment. Design instructions and a business management analysis will indicate the full-scale applications at the end of the project.

## **EXPECTED RESULTS**

With this proposal positive effects on the environment and the water resources will be realized. The proposed research will introduce new and more efficiently adapted technologies of wastewater treatment and reuse in these countries. The interest of managers, politicians and the public in this technology will be aroused through the use of a pilot scale plant and the use of real wastewaters. Long term water saving due to recycling is expected.

### *Pilot studies*

- ▷ The pilot study will provide informations on the long term stability, the safety of operation and the design instructions of solar catalytic wastewater treatment. Additionally, possibilities for the reuse of water in the textile industry in Developing Countries will be investigated.

### *Commercialisation*

- ▷ The final business management analysis will assess the applicability of the solar catalytic wastewater treatment and recycling.

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## **PARTNERS**

### **TECHNISCHE UNIVERSITAET CLAUSTHAL**

Inst. Fur Thermische Verfahrenstechnik  
Adolph-Roemer-Strasse 2a  
38678 Clausthal-Zellerfeld  
**Germany**

Alfons Vogelpohl  
E-mail: Vogel@ltv.Tu-Clausthal.De  
Tel.: 49-5323-722.355  
Fax: 49-5323-723.570

### **INSTITUT FUER SOLARENERGIEFORSCHUNG GMBH**

Hameln / Emmerthal, Abteilung  
Photochemie & Dunnschichttechnik  
Sokelantsrasse 5  
30165 Hannover  
**Germany**

Detlef Bahnmann  
E-mail: Isfh.Bahnmann@Oln.Comlink.Ap  
Org  
Tel.: 49-511-358.500  
Fax: 49-511-3585.010

### **INSTITUT NATIONAL DE LA RECHERCHE SCIENTIFIQUE ET TECHNIQUE**

Laboratoire Environnement  
43 Avenue Charles Nicolle  
Cite Mahrajene  
1082 Tunis  
**Tunisia**

Ahmed Ghrabi  
Tel.: 216-1-788.436  
Fax: 216-1-430.934

### **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE**

Ecole Centrale De Lyon-Er539  
Lab. Photocatalyse, Catalyse, Envir.  
36 Avenue Guy De Collongue  
B.P. 163  
69131 Lyon  
**France**

Pierre Pichat  
E-mail: Pichat@Cc.Ec-Lyon.Fr  
Tel.: 33-4-7218.6495  
Fax: 33-4-7833.0337

**DEVELOPMENT AND OPTIMIZATION OF A NEW PROCESS FOR  
DESALINATION OF SEA WATER BY MEANS OF SOLAR ENERGY**

**Co-ordinator: Ruhr-Universitaet Bochum, Bochum (Nrw), Deutschland (Efat Chafik)**

## **OBJECTIVES**

The main objectives are defined as follows:

- Development of a new sea water desalination process using air which will be heated by solar energy and then humidified by injection of sea water into the heated air flow. This heating/humidifying procedure will be repeated to allow an enhancement of vapor concentration in air up to about 20 wt%. Such a high concentration is usually attainable by spraying water in air heated up to 500°C. The present process achieves the same effect at operating temperature not in the range of 80°C.
- Economical optimization of the new process by developing cost effective solar collectors as well as efficient humidifier- and dehumidifier designs.
- Errection, test and optimization of a pilot plant using the new desalination process to produce 1m<sup>3</sup> water daily. This pilot plant will be located in Tunisia on the mediterranean coast. The desalination process will be optimized during a one-year test-run.

## **ACTIVITIES**

The key activities involve:

- ◇ Theoretical and experimental preinvestigations
  - Development and optimization of solar collectors as well as the humidifier system and the dehumidifier.
  - Elaboration of an energy-optimized flow sheet of the desalination process under consideration of heat recovery.
  - Testing of the new desalination process by experiments on one-stage-setup including fan, collector, humidifier and dehumidifier. These tests will also deliver the optimum operating parameter to be used in the pilot plant.
- ◇ Engineering, planning, Installing and starting up of the pilot plant
  - Development and optimization of the process flow sheet using computer simulation technique.
  - Planning and construction of the pilot plant.
  - Field tests on single plant equipments. Start up of the pilot plant.
- ◇ Long term run and measurements on pilot plant
  - A longterm run of the plant for a duration of one year should give results on water production rate under different conditions. Production rate and energy consumption will be optimized.

## **EXPECTED OUTCOME**

- ⇒ This project should provide reliable data on the new solar desalination process. Technical and economical parameter of this process will be won. Reports on types and design guidelines of the optimized and recommended collectors, humidifiers and dehumidifier will be delivered. The experience gained during the longterm run of the desalination pilot plant will be documented to give design and optimizing rules of the new solar desalination process. Guidelines for a medium size demonstration plant will be delivered.

## **PARTNERS**

**RUHR-UNIVERSITAET BOCHUM**  
Inst. Of Thermo- And Fluid Dynamics Dept Of Mechanical  
Engineering  
Universitaet Strasse 150  
44721 Bochum (Nrw)

Dr Efat Chafik  
Tel.: 49-234-700.64.23  
Fax: 49-234-709.41.62  
E-mail:chafik@vtp.ruhr-uni-bochum.de

**Deutschland**

**AGRICULTURAL UNIVERSITY OF ATHENS**

Dept Of Food Science & Technology

Iera Odos 75

11855 Athens

**Greece**

Dr Stavros Yanniotis

Tel.: 30-1-529.4703

Fax. 30-1-5294703/3460885

E-mail: Yannioti@Auadec.Aua.Gr

**INSTITUT NATIONAL DE LA RECHERCHE**

**SCIENTIFIQUE ET TECHNIQUE**

Laboratoire De Dessalement Des Eaux

Route Touristique De Soliman P.O. Box 95

2050 Hammam-Lif

**Tunisie**

Dr Mohamed Maalej

Tel.: 216-1-43.00.44/53

Fax: 216-1-43.09.34

**PALESTINIAN ENERGY & ENVIRONMENT**

**RESEARCH CENTER (PEC)**

Rafidia Street P.O. Box 085

Nablus - West Bank

**Palestinian Autonomy**

Dr Husein Hamed

Tel.: 972-9-38.48.03/04

Fax: 972-9-38.43.88

E-mail: Pec@Planet.Edu

**DEVELOPMENT OF A SIMPLE TECHNOLOGY IN DRINKING WATER  
TREATMENT FOR NITRATE AND PESTICIDE REMOVAL**

**Co-ordinator:** Universitaet Stuttgart, Institut Fuer Siedlungwasserbau, Stuttgart, Germany  
(Wolf-Rudiger Mueller)

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**OBJECTIVES**

- To develop an inventory of the actual situation on-site concerning nitrate and pesticide occurrence in groundwater for selected regions of Morocco and Turkey.
- To develop of a simple, easy to use technology for nitrate and pesticide removal in drinking water treatment.
- Part of the population of EU and non-EU-countries is living in rural areas. Especially small communities and hamlets risk not to provide a safe water to their inhabitants. The intense use of fertilizers and pesticides in agriculture is a main source of deterioration of ground- and surfacewater. The EEC-directive on quality of water for human consumption (1980) requires a max. admissible concentration in water of 11.3 mg/L N-NO<sub>3</sub>, 0.1 mg/L N-NO<sub>2</sub> and 0.1 mg/L pesticide per single substance, 0.5 mg/L as total pesticide content.
- A web for analytical quality control between the partners will be installed, which may lead to a nucleus for national proficiency testing of pesticides.

**ACTIVITIES**

- ◇ To test the different solid substrates in semi-batch and continuous flow reactors.
- ◇ Heterotrophic denitrification with Natural Organic Solid Substrates (NOSS), e.g. straw, bark of woods, hydrolyzed birchwood, Glycyrrhiza glabra.
- ◇ Chemolithotrophic denitrification using sulfur.
- ◇ Heterotrophic denitrification with water insoluble BioDegradable Polymers (BDP), e.g. Poly-β-Hydroxy-Butyric acid (PHB), Poly-ε-Caprolactone (PCL), Bionolle, a synthetic thermoplastic aliphatic polyester, based on polyethylene and polybutylene succinates (PESU, PBSU).
- ◇ Optimizing the reactor packing containing NOSS. Improvement of the BDPs for denitrification by blending with nutrient salts containing phosphorous, iron and trace elements.
- ◇ The examination of pesticide elimination in denitrifying reactors. How these conditions affect fungal activity and pesticide re-moval will be investigated. Furthermore the action of PHB, PCL and other biodegrad-able polymers as potential sinks for pesticides will be examined by sorption experiments. There are hints that in analogy to polyethylene (PE) a strong sorptive capacity exists.
- ◇ The elaboration of an easy-to-use Standard Operation Procedure (SOP) for pesticide analysis. The method will use a solid phase extraction process and GC and HPLC-methods for their separation and detection.
- ◇ The isolation and determination of bacteria and fungi, which essentially govern the denitrification and pesticide removal process. The interaction of fungi and bacteria, enhancement or inhibition have consequences on the reactor configuration.



## EXPECTED RESULTS

### ⇒ *Scientific-technical results*

A pillar of this project is the pesticide analysis. Based on standard methods, considering the means of the partners an easy-to-use SOP for selected pesticides in the different partner countries will be elaborated. In order to install and to assure Good Laboratory Practice (GLP), the partners will exchange information, samples, and standards on a regular basis. Metabolites of the investigated pesticides will be analyzed. The sorption behavior of the pesticides selected in relation to the BDP used will be elucidated.

As the project is of precompetitive character it allows to scrutinize the best way towards a realization of potentially low and "easy-to-use" technologies for biological systems in drinking water treatment with respect to ni-trate and pesticide removal.

New perspectives are expected when realizing heterotrophic denitrification with BDPs. No sorption data for pesticides have been reported, however a strong analogy to PE a sorption capacity can be postulated. In a reactor this will lead in a first step to a physico-chemical removal, the second step desired may be the induction of a cometabolism. These terms "cometabolism" or "cooxidation" are used to describe the concomitant oxidation of a nongrowth substrate during growth of a micro-organism on a utilisable carbon and energy source. Thus we examine the effects under aerobic and subsequent anoxic conditions whether biodegradation of the polymers leads to a pesticide degradation.

The microbial examination will concentrate on the isolation and determination of the bacteria and fungi, essentially governing the denitrification and the pesticide removal process. The interaction of fungi and bacteria, enhancement or inhibition may have consequences on the reactor configuration.

A deepened knowledge of the microbial flora in the reactors is of primary importance, not only to exclude the presence of opportunistic pathogens for men and animals e.g. different *Pseudomonas* and *Acinetobacter* species but also with respect to the action of fungi .

Denitrifying conditions allow the growth of several fungi, which may be useful for pesticide elimination or which are undesired molds, so mycotoxin contamination is possible. Therefore use of a simple and sensitive bioassay for assessing the potential risk of mycotoxins in water will be examined.

The experiments will be carried out in lab-scale semi-batch and continuous flow test units. The results obtained must yet be substantiated in view of technical applications in semi- and technical plants, which however is not within the scope of this project.

## **PARTNERS**

### **UNIVERSITAET STUTTGART**

Institut Fuer Siedlungwasserbau,  
Wasserguete And Abfallwirtschaft  
Bandtaele 2  
70569 Stuttgart  
**Germany**

Doct. Ing. Wolf-Rudiger Mueller  
E-mail: W-R.Mueller@Iswa.Uni-Stuttgart.De  
Tel.: 49-711-685.5411  
Fax: 49-711-685.3729

### **RIJKSUNIVERSITEIT GENT**

Vakgroep Biochemie, Fysiologie En  
Microbiologie - Lab.Voor Microbiologie  
K.L. Ledeganckstraat 35  
9000 Gent  
**Belgium**

Prof. Dr. Ing. Jean Swings  
E-mail: Jean.Swings@Rug.Ac.Be  
Tel.: 32-9-264.51.16  
Fax: 32-9-264.53.46

### **UNIVERSITAET KARLSRUHE**

Engler-Bunte-Institut  
Bereich Wasserchemie  
Richard Willstaetter Allee 5  
76128 Karlsruhe  
**Germany**

Prof. Doct. Fritz Frimmel  
E-mail: Fritz.Frimmel@Ciw.Uni-Karlsruhe.De  
Tel.: 49-721-608.2580  
Fax: 49-721-699.154

### **ORGANISATION NATIONALE DE L'EAU POTABLE**

Dir. Du Laboratoire De La Qualite De  
L'eau - Station Du Traitement Des Eaux  
6 Bis, Rue Patricia Lumumba  
100002 Rabat  
**Morocco**

Mr. Lahoussaine Echihabi  
E-mail: Onepdlq@Mtds.Com  
Tel.: 212-7-75.88.92  
Fax: 212-7-75.23.77

### **UNIVERSITE ABDELMALEK ESSAADI**

Faculte Des Sciences De Tetouan  
Dept. De Chimie  
B.P. 2121  
93000 Tetouan  
**Morocco**

Prof. Doct. Abdelhamid Ouassini  
E-mail: Ouassini@Tangeroise.Net.Ma  
Tel.: 212-9-97.24.23 (Ext. 43)  
Fax: 212-9-99.45.00

### **EGE UNIVERSITY**

Engineering Faculty  
Environmental Research Group  
Ege University Kampusu  
35100 Izmir - Bornova  
**Turkey**

Prof. Dr. Ing. Ibrahim Alyanak  
E-mail: Ialy@Textil.Ege.Edu.Tr  
Tel.: 90-232-388.7600  
Fax: 90-232-374.1401

**A SYSTEM APPROACH TO WASTEWATER BIOTREATMENT FOR THE PROTECTION OF MEDITERRANEAN COASTAL AREAS ("BIOWATSYST")**

**Co-ordinator:** Istituto Agronomico Mediterraneo, Valenzano (Bari), Italy (Atef Hamdy)

**OBJECTIVES**

- To monitor, prevent and remediate coastal pollution due to wastewater disposal through the use of multipurpose subsurface-flow constructed wetlands (SFW), inserting them into the productive system of rural societies and, in a more general way, into the surrounding environment;
- To gain information useful to help in the optimisation of the design and management of constructed wetlands;
- To assess the effectiveness of SFW as a low-tech, low-cost solution to domestic wastewaters treatment;
- To demonstrate the possibility of reusing treated waters for irrigation purposes and aquifer recharging;
- To conduct an economic-social-environmental analysis to evaluate the global impact of wetland construction and management.

**ACTIVITIES**

The project is based on the construction of biodepurating systems in six representative places, each system comprising six constructed cells with annexed spreading basins for aquifer recharging.

The six cells which can be split in two, so totalling twelve sub-cells, will offer the possibility of exploring a variety of conditions useful in the process of optimisation, namely detention time, filling media, depurating plants, water depth, etc.

Phytodepuration efficiency will be evaluated comparing physical, chemical and microbiological characters of inflowing and treated waters.

The results of system monitoring in the technical, economic and environmental aspects will be analysed to issue recommendations and guidelines and a decision support tool will be released.

**EXPECTED RESULTS**

The main expected outcome is a set of information and recommendations addressing operators and decision-makers, explaining and demonstrating how to take advantage of domestic wastewaters instead of polluting watercourses and seacoasts.

Further outcomes are methods for optimising the design and management of constructed wetlands. Aspects of interest are also: the biomass production and use as an organic compost or to generate biopower; the observation of effects of treated wastewaters used for irrigation purposes and aquifer recharging; the demonstration action addressing extensionists and operators at large.

## **PARTNERS**

### **ISTITUTO AGRONOMO MEDITERRANEO**

Via Ceglie 23  
70010 Valenzano (Bari)  
**Italy**

Prof. Atef Hamdy  
E-mail: Hamdy@Iamb.It  
Tel.: 39-80-780.6236/6222  
Fax: 39-80-780.6206

### **CENTRO DE INVESTIGACIONES ENERGETICAS, MEDIOAMBIENTALES Y TECNOLOGICAS**

Depto De Energias Renovables  
Avda Complutense 22  
28040 Madrid  
**Spain**

Dr. Juan E. Carrasco  
E-mail: Carrasco@Ciemat.Es  
Tel.: 34-1-346.6682  
Fax: 34-1-346.6680

### **NATIONAL TECHNICAL UNIVERSITY OF ATHENS**

Department Of Chemical Engineering  
Organic And Anvironmental Technologies  
Zografou Campus  
15700 Athens  
**Greece**

Prof. Emmanuel Koukios  
E-mail: Koukios@Chemeng.Ntua.Gr  
Tel.: 30-1-772.3191  
Fax: 30-1-772.3192

### **INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II**

Wastewater Treatment & Reuse Unit  
B.P. 6202 Rabat-Institut  
Rabat  
**Morocco**

Prof. Bouchaib Elhamouri  
E-mail: Hamouri@Maghreb.Net.Ma  
Tel.: 212-7-777.564  
Fax: 212-7-777.564

### **UNIVERSITY OF JORDAN**

Water & Environment Res.& Study Centre  
Amman  
**Jordan**

Prof. Muhammad Shatanawi  
E-mail: Juwater@Amra.Nic.Gov.Jo  
Tel.: 962-6-843.555  
Fax: 962-6-830.190

### **SUEZ CANAL UNIVERSITY**

Centre For Environment Studies And  
Consultation  
Ismailia  
**Egypt**

Prof. Ahmed Dewdar  
E-mail:  
Tel.: 20-64-327.125  
Fax: 20-64-325.208

**CONTROL OF BACTERIAL REGROWTH IN WATER SUPPLY DISTRIBUTION SYSTEMS IN WATER SHORT EUROPEAN AND MEDITERRANEAN COUNTRIES**

**Co-ordinator:** University of Newcastle upon Tyne, UK (Lilian M. Evison)

**OBJECTIVES**

- To study changes in bacterial numbers throughout drinking water distribution systems in temperate and semi-arid countries, particularly under water short conditions, in order to understand and model the interactions between physical conditions (e.g. mixing, storage, retention time, temperature), chemical conditions (e.g. disinfectant concentration, pH, organic matter concentration, redox potential, nutrients, chlorophyll etc.) and bacterial regrowth.
- To evaluate the potential for opportunistic pathogens such as *Aeromonas*, *Pseudomonas*, *Yersinia*, *Shigella*, *Salmonella* & *E.coli* 0157 to regrow in distribution systems, and to make epidemiological studies of populations exposed to such organisms.
- To study changes in bacterial numbers when water is stored in distribution system reservoirs, and particularly in household storage tanks in regions with intermittent water supplies.
- To study the effect of infiltration into systems subject to intermittent flow, in particular to evaluate the presence of opportunistic pathogens.
- To evaluate disinfection strategies for distribution systems and household storage tanks.
- To develop and validate a model for control of bacterial regrowth in distribution networks, by refining an existing hydraulic model using the inputs outlined above.

**ACTIVITIES**

- ◇ Determination of kinetics of growth and death in suspended culture of coliforms, typical heterotrophic plate count isolates and pathogens such as *Aeromonas*, *Pseudomonas*, *Yersinia*, *Salmonella*, *Shigella* and *E.coli* 0157 at pH, TOC, temperature and disinfectant regimes found in distribution systems.
- ◇ Kinetics of biofilm growth and death under laboratory conditions.
- ◇ Investigation of biofilm growth and sloughing in laboratory rig under various hydraulic conditions
- ◇ Using data mentioned above, develop model of bacterial growth in distribution system conditions
- ◇ In Jordan, Lebanon, Palestine, Portugal and UK investigate correlations between physical, chemical, and bacterial regrowth in water distribution systems, under summer and winter conditions
- ◇ Where HPC counts increase, seek presence of opportunistic pathogens.
- ◇ In Jordan, Lebanon, and Palestine, where intermittent water supplies occur, investigate behaviour of bacterial numbers in household storage tanks up to 7 days and longer, to determine potential health implications.
- ◇ Determine effect of infiltration on bacterial numbers in mains subject to intermittent flow
- ◇ Carry out epidemiological study in areas found to be supplied with mains water subject to regrowth conditions.

**EXPECTED RESULTS**

- ▷ Model development  
Development of EPANET hydraulic models in each country for at least one distribution system, calibrated for conservative tracers and disinfectants, a conceptual model of bacterial regrowth in distribution, a validation of models of bacterial regrowth in each country.

- ▷ **Distribution system studies**  
Collected physical, chemical and bacterial data will indicate the conditions under which mains systems are liable to bacterial regrowth.
- ▷ **Water storage tank studies**  
These will identify conditions under which household storage tanks pose a threat to health, in Mediterranean countries.  
In temperate climates, the conditions of storage in mains reservoirs giving increases in bacterial numbers will be identified, especially in the summer season.  
Effective control strategies will be identified for bacterial regrowth in household storage tanks, and mains reservoirs.
- ▷ **Kinetics of bacterial regrowth**  
Growth kinetics of suspended and attached systems will be determined for coliforms, plate count organisms and opportunistic pathogens.  
Disinfection kinetics will be identified for suspended and attached systems, and organisms listed above.  
Effect of hydraulics on attached/ suspended bacterial interactions will be identified.
- ▷ **Epidemiological study**  
In Palestine, studies should establish a correlation between water quality and enteric illness in populations exposed to water with high plate counts and the presence of opportunistic pathogens.
- ▷ **Development of control strategies**  
Based on the forgoing information it is anticipated that each collaborator will be able to predict which water supplies are at risk from bacterial regrowth, and to identify disinfection/ operational regimes to control this problem before adverse health effects occur.  
The experiences found in the Mediterranean countries will help more temperate countries to anticipate the effects of global warming.

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## **PARTNERS**

### **UNIVERSITY OF NEWCASTLE UPON TYNE**

Dept Of Civil Engineering  
Claremont Road  
Cassie Building  
Nel 7ru Newcastle-Upon-Tyne  
**United Kingdom**

Mrs. Lilian Evison  
E-mail: L.M.Evison@Ncl.Ac.Uk  
Tel.: 44-191-222.79.31  
Fax: 44-191-222.66.69

### **LABORATORIO NACIONAL DE ENGENHARIA CIVIL**

Av. Brasil 101  
1799 Lisboa Codex  
**Portugal**

Dr. Sergio Teixeira Coelho  
E-mail: Stcoelho@Lnec.Pt  
Tel.: 351-1-848.21.31 (Ext. 2836)  
Fax: 351-1-847.86.14

### **JORDANIAN MINISTRY OF WATER AND IRRIGATION**

Water Authority  
P.O. Box 2412  
Amman  
**Jordan**

Engineer Ahmed Uleimat  
E-mail:  
Tel.:  
Fax: 962-6-82.52.75

### **LEBANESE AMERICAN UNIVERSITY**

P.O. Box 13-5053  
Beirut  
**Lebanon**

Dr. Fuad Hashwa  
E-mail: Fhashwa@Lau.Edu.Lb  
Tel.: 961-3-24.11.06  
Fax: 961-9-94.48.51

### **AN-NAJAH NATIONAL UNIVERSITY**

P.O. Box 7  
Nablus  
**Palestinian Autonomy**

Dr. Yahya Faydi  
E-mail: Faydi@Najah.Edu  
Tel.: 972-9-37.00.42  
Fax: 972-9-38.79.82

<b>DESALINATION OF SEA-WATER USING RENEWABLE ENERGY SOURCES</b>
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**Co-ordinator:** Dimman Consulting Ltd, Pilea-Thessaloniki, Greece (Dimitri Paschaloudis)

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### OBJECTIVES

- To accumulate meteorological data in the Mediterranean area for desalination purposes.
- To design a desalination method using the vapour compression method, under low pressure where,
  - the compressor is powered by wind energy and
  - solar energy is used for heating the feed. The scheme to be used will be of multiple effects.
- To design another desalination method using the Multiple Effect Evaporation method, where the external heating in the top effect is achieved by solar trough collector.
- To construct two pilot units using the two mentioned methods, and install them in two different countries (Greece and Jordan).
- To monitor the operation of the pilot units and measure the necessary parameters.
- To make a feasibility study for future construction of larger units in various sites of the Mediterranean.

### ACTIVITIES

- ◇ Optimization of the operational parameters for the two methods (temperature, pressure, concentration ration etc.).
- ◇ Design of the key components for the two methods (evaporator/condenser, compressor, windmill, solar collectors, auxiliary heat exchangers). Three critical points will be taken under consideration: a) Low construction cost, b) Low maintenance cost, c) Minimum control needs.
- ◇ Construction and installation of the two pilot units. The installation areas are in Makedonia, Northern Greece, and in Aqaba, Southern Jordan. These sites have been chosen due to good meteorological conditions and available facilities for experimental operation.
- ◇ Installation of a data acquisition system for monitoring the operation of the units. Development of the corresponding software.
- ◇ Presentation of the operation results. These will correlate the quality and quantity of the produced water with the operational parameters, and also include the financial analysis of the operation.
- ◇ Scale and site analysis for future industrial application.

### EXPECTED RESULTS

- ▷ *Scientific-technical results:* Applied improvements in the multiple effect evaporation and vapor compression method will be elaborated. The improvements consist of a new method for pressure gradient regulation, the use of cheap materials of construction, the efficient use of wind energy and the self stabilization of the system under transient conditions.
- ▷ *Pilot plants:* Two pilot plants using the two different methods are to be constructed in Greece and Jordan.
- ▷ *Deliverables:* A complete set of measurements concerning the fresh water production (quality and quantity) of the pilot plants.
- ▷ *Commercialisation:* A feasibility study for the commercialization of the two methods at higher (industrial) capacity will show the ability of using the methods in various places of the Mediterranean. In a further (beyond the obligations of this project) step there is a high interest to apply the method on a Greek island.



## **FOLLOW-UP**

- ▶ The remaining parts of the two pilot plants will be fully installed. After that a test operation will show the correct connection of all the components. Meanwhile the measurement instruments will be completely connected with the plants.
- ▶ The operation of the plants will last (for the remaining of the project) 6-8 months. During this period the measurements will show the quality and quantity of product fresh water vs. the input of solar and wind energy.
- ▶ In the final step a cost analysis will show the cost of the product and the prospective for construction of plants of higher capacity.

## **PARTNERS**

### **DIMMAN CONSULTING LTD**

M. Alexandrou Street 115  
55535 Pilea-Thessaloniki  
**Greece**

Dimitri Paschaloudis  
Tel.: +30-31-32 50 09  
Fax: +30-31-32 50 17  
E-mail: Orman@Vergina.Eng.Auth.Gr

### **ARISTOTLE UNIVERSITY OF THESSALONIKI**

Department Of Chemical Engineering  
Laboratory Of Physical Chemistry  
P.O. Box 427  
54006 Thessaloniki  
**Greece**

Prodromos Bekiaroglou  
Tel.: +30-31-99 62 21  
Fax: +30-31-99 62 2  
E-mail: Orman@Vergina.Eng.Auth.Gr

### **HIGHER TECHNICAL INSTITUTE**

Mechanical Engineering Department  
P.O. Box 2423  
Nicosia  
**Cyprus**

Demetrios Lazarides  
Tel.: +35-72-49 44 54  
Fax: +35-72-49 49 53  
E-mail: ioan@cytanet.com.cy

### **ROYAL SCIENTIFIC SOCIETY JORDAN**

Renewable Energy Research Centre  
P.O. Box 925819  
11110 Amman  
**Jordan**

Malek Kabariti  
Tel.: +962-6-84 47 01  
Fax: +962-6-84 48 06  
E-mail: kabariti@rss.gov.jo

### **CONPHOEBUS CAMPO PROVE**

Institut Di Ricerche Per Le Energie Rinn.  
Passo Martino  
Zona Industriale  
C.P. 95030  
Piano D'arci - Catania  
**Italy**

Roberto Lo Cicero Vaina  
Tel.: +39-95-29 14 07  
Fax: +39-95-29 12 46  
E-mail: Robloc@Mbox.Vol.It

### **ELECTRICIDADE DE PORTUGAL**

Projectos Engenharia E Tecnologia  
Avenida Infante Santo 17-6  
1300 Lisboa  
**Portugal**

Antonio Anastacio Batista  
Tel.: +351-1-395 59 00  
Fax: +351-1-60 25 31  
E-mail: Antonio-Batista@Edinfor.Pt

**WASTE WATER RECYCLING SUPPLIED BY RENEWABLE ENERGIES  
IN THE NEAR EAST**

**Co-ordinator:** Fachhochschule Aachen, Jülich, Germany (Apostolos Neskakis)

**OBJECTIVES**

- To identify the potential for the use of renewable energies for waste water treatment.
- To work out the technical problems of purification plants supplied with renewable energies depending on: the waste water amount and pollution, the demand of water for reuse, the present state in energy supply, the potential of the renewable energies (especially solar and wind energy). This includes the design of an optimal energy management for these systems.
- To determine the environmental and socio-economic impacts resulting from waste water treatment including water reuse and a power supply with renewable energies. With a cost benefit analysis it will be possible to compare the external costs of conventional systems and the systems proposed in this project.
- To analyse these objectives in detail for the Near East region and to give criteria for the transfer of the proposed systems to the West Mediterranean region where similar problems exist.

**ACTIVITIES**

- ◇ Analysis of the amount and pollution of waste water, the existing situation in waste water treatment and the demand of water for drinking and irrigation including the future development.
- ◇ Analysis of the present state in energy supply for municipal and rural areas including future plans and determination of the potential of solar and wind energy.
- ◇ Work out of purification methods taking into consideration the waste water amount and pollution and the water demand for irrigation.
- ◇ Adaptation of these purification plants to the renewable energy supply.
- ◇ Determination of sites where these purification plants are feasible.
- ◇ Consideration of environmental and socio-economic impacts and cost benefit analysis for purification plants supplied by renewable energies.
- ◇ Work out of criteria for the installation of purification plants in the Near East.
- ◇ Concepts for consultation of local and national authorities and for the transfer to the West Mediterranean countries.

**RESULTS**

⇒ *Scientific-technical results:*

An analysis of the energy demand of waste water treatment plants will be carried out, using of renewable energies and optimised combination of renewable energies and waste water treatment plants.

The result of this analysis show that two different systems are of special interest: the aerobic treatment by a trickling filter and the anaerobic treatment by a series of ponds. Both technologies have a low energy demand.

For pond systems for more than 1500 capita the production of biogas becomes a very interesting option. The biogas is produced with covered anaerobic stabilisation ponds (CASP) as a first treatment step. Particularly the hot and dry climate in the Mediterranean region allows the use of this technology because higher temperatures in the pond lead to a higher production of biogas. In the case of a biogas production a co-generation unit can be driven to supply the WWTP itself and also to supply other loads with electrical energy.

By simulation of the complete electrical energy system the feasibility of a combination of a renewable power supply with a WWTP was investigated. It reveals that for small systems, if no other loads exist, an autonomous energy supply by a photovoltaic system is feasible. For bigger systems nearly always the grid connection is the more favourable solution so that water treatment and energy supply become two de-coupled systems, which can be optimised one by one. The

simulation results show that for a covered pond with production of biogas a further use by a co-generation unit about 50 % of the electrical energy produced can be supplied to other consumers (about 20 % if a denitrification step is included).

⇒ *Socio-economic analysis, cost benefit analysis:*

In a further step the environmental and socio-economic impacts were analysed. One of the most important socio-economic impacts of controlled waste water treatment is the improvement of the public health, because fresh water production and sewage disposal are well ordered.

The identification of villages where the application of waste-water treatment plants supplied by renewable energies is of special interest is done by a detailed analysis of the socio-economic situation in rural villages in Jordan, Palestine and Israel.

The benefits from a WWTP with a renewable power supply have to be compared with the costs. This is done by a cost benefit analysis, which was carried out with the so-called UNIDO method.

## **PARTNERS**

### **FACHHOCHSCHULE AACHEN**

Solar Institut Jülich  
Ginsterweg 1  
52428 Jülich  
**Germany**

Apostolos Neskakis  
Tel.: +49-2461-68 92 00  
Fax: +49-2461-68 92 35  
E-mail: neskakis@fh-aachen.de

### **RHEINISCH-WESTFÄLISCHE TECHNISCHE HOCHSCHULE AACHEN**

Forschungsinstitut für Inter. Techn. und Wirtschaftliche Zusammenarbeit  
Ahornstraße 55  
52056 Aachen  
**Germany**

Arno Sommerfeld  
Tel.: +49-241-88 94 70  
Fax: +49-241-88 82 84  
E-Mail : solar@fiz.rwth-aachen.de

### **TECHNOLOGICAL EDUCATION INSTITUTE**

Department of Electronics  
Electronics Laboratory  
P. Kelaidi 24  
73136 Hania  
**Greece**

Ioannis Kaliakatsos  
Tel.: +30-821-99 188  
Fax: +30-821-95 691  
E-mail: nvc@tarra.ced.tuc.gr

### **UNIVERSIDAD DE LAS ISLAS BALEARES**

Departamento de Fisica  
Carretera de Valldemossa km 7.5  
07071 Palma de Mallorca  
**Spain**

Eugenio Garcia-Moreno  
Tel.: +34-71-17 32 31  
Fax: +34-71-17 34 26  
E-mail: dfsegmo@ps.uib.es

### **INSTITUTO TECNOLOGICO DE CANARIAS**

Centro Investigacion en Energia y Agua  
C/ Cebrian 3.5  
35003 Las Palmas de Gran Canaria  
**Spain**

Roque Calero Perez  
Tel.: +34-28-45 20 20  
Fax: +34-28-45 20 07  
E-mail: rcalero@cistia.es

### **ROYAL SCIENTIFIC SOCIETY JORDAN**

Renewable Energy Research Centre  
Royal Scientific Society Street  
P.O. Box 925 819  
11110 Amman  
**Jordan**

Malek Kabariti  
Tel.: +962-6-84 47 01  
Fax: +962-6-84 48 06  
E-mail: kabariti@rss.gov.jo

### **AN-NAJAH NATIONAL UNIVERSITY**

Water & Environment Studies Center  
Umar Ibn Al-Khatib Street  
P.O. Box 7  
Nablus  
**West Bank & Gaza Strip**

Marwan Haddad  
Tel.: +972-9-38 31 24  
Fax: +972-9-38 79 82  
E-mail: wesc@najah.edu

**DEVELOPMENT OF ENVIRONMENTALLY FRIENDLY PHOTOACTIVABLE COMPOUNDS FOR TREATMENT OF MICROBIAALLY POLLUTED WATER**

**Co-ordinator:** Università di Padova, Dipartimento di Biologia, (Giulio Jori)

**OBJECTIVES**

- To develop a pilot plant for the decontamination of microbially polluted water by a photochemical technique. This is based on the use of visible light from filament lamps, or possibly sunlight, and porphyrin-type photosensitizers, which are intrinsically atoxic to living organisms. The technique requires a simple and cheap technology and is characterized by a low environmental impact.
- To identify porphyrin photosensitizers (or analogues thereof) which exhibit an efficient and non-specific phototoxic action against a broad number of microbial species, including Gram-positive and Gram-negative bacteria, yeasts and mycoplasmas.
- To define irradiation protocols that (a) induce an efficient water sterilization by using the lowest possible amount of photosensitizer and light dose; (b) are easily applicable on a large scale, even in the presence of turbid water; (c) do not generate toxins which can cause undesired damage to fishes; and (d) are non-mutagenic to microbial cells in order to avoid the selection of photoresistant species.
- To develop a procedure for recycling the porphyrin compound that acts as a photosterilizing agent in order to achieve a quantitative recovery of the porphyrin and reutilize it in the pilot plant, thereby minimizing the costs of the overall operation.

**ACTIVITIES**

The building of the pilot plant represents the final step in the project, preliminary studies are being carried out in order to define the most suitable organization of the irradiation equipment, as well as the level of emitted power and fluence rate which allows for a uniform illumination of a large water volume. Options under scrutiny include the irradiation of the photosterilization chamber by a set of filament bulbs having a nominal output of some hundred Watts or sequences of light-emitting diodes incorporated in plastic fibers that are suspended at different levels in the aqueous medium.

Porphyrins of different chemical structure and physico-chemical properties are screened for their photoinactivating efficiency against typical representatives of Gram-negative bacteria (*Vibrio anguillarum*, *Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*), yeasts (*Candida albicans*) and mycoplasmas (*Mycoplasma hominis*, *Acholeplasma laidlawii*). The structural factors to be investigated include the extension of the aromatic macrocycle, the nature of the metal ion coordinated to the tetrapyrrolic ring, the substituents located in the peripheral positions of the macrocycle, and the axial ligands occupying the fifth and sixth coordination position of the metal ion. The cell response to photosensitization is determined by a clonogenic assay.

For those porphyrins, which exhibit the largest photosensitizing activities, the photoinactivation of the isolated and pooled pathogenic microbial strains is further studied as a function of selected experimental parameters, such as the photosensitizer dose, the pre-irradiation incubation time, the total light dose and the irradiation fluence rate. Under conditions yielding the most efficient biological response, suitable analytical approaches are performed in order to identify the primary and secondary targets of the photoprocess, as well as to assess any possible mutagenic effect.

The aqueous solutions of the porphyrin selected in the above described investigations are filtered through different types of columns (e.g. activated charcoal, zeolites, sand) in order to define conditions for a quantitative removal and subsequent desorption of the dye. This would allow a cyclic reutilization of the photosensitizing agent.

**EXPECTED RESULTS**

*Scientific-technical results*

- ▷ To establish a data base on the relationships between the chemical structure of porphyrins (and their analogs) and their efficiency as photosensitizers for the inactivation of microbial cells.
- ▷ To identify a selected group of porphyrins with optimal efficiency for the visible light-induced sterilization of water from aquaculture systems.
- ▷ To develop photosterilization protocols tailored to the control of the microbial population in a variety of environmental conditions.

#### *Applied results*

- ▷ To implement an irradiation equipment based on visible light-emitting sources which is appropriate for the uniform illumination of large water volumes.
- ▷ Development of a porphyrin formulation, that is useful for the photosensitized sterilization of water, and is characterized by low cost, high photostability upon visible light-irradiation, and easy and quantitative removal from an aqueous medium.

#### *Pilot plant*

- ▷ A pilot plant for photosterilization of microbially polluted water obtained from aquaculture systems will be developed and validated.

### **FOLLOW-UP**

The activities to be carried out within the forthcoming year will be essentially focused on the definition of an experimental procedure for the quantitative recycling of the photosensitizing agents and the stepwise development of the pilot plant.

Exploratory investigations will be carried out in order to ascertain whether the overall procedure, which has been developed for the photodecontamination of microbially polluted water from aquaculture systems, can be extended to the treatment of water from industrial plants, water for irrigation and drinkable water.

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## **PARTNERS**

### **UNIVERSITÀ DEGLI STUDI DI PADOVA**

Dipartimento di Biologia  
Laboratorio Fotobiologia Medica e Ambientale  
Via Trieste 75  
35121 Padova

**Italy**

Giulio Jori  
Tel.: +39-49-827 63 33  
Fax: +39-49-827 63 44  
E-mail: jori@civ.bio.unipd.it

### **UNIVERSIDAD AUTONOMA DE BARCELONA**

Departamento de Química  
Laboratorio de Química Orgánica  
08193 Bellaterra, Barcelona

**Spain**

Jose L. Bourdelande  
Tel.: +34-3-581 19 83  
Fax: +34-3-581 12 65  
E-mail: iqor2@cc.uab.es

### **MAX PLANCK INSTITUT**

Institut für Strahlenchemie  
Photochemistry Photobiology  
Postfach 10 13 65  
Stiftstraße 34-36  
45413 Muelheim A.D. Ruhr

**Germany**

Silvia Elsa Braslavsky  
Tel.: +49-208-306 36 81  
Fax: +49-208-306 39 51  
E-mail: braslavsky@mpi-muelheim.mpg.d400.ge

### **UNIVERSIDAD DE BUENOS AIRES**

Inquimae  
Laboratorio de Fotoquímica  
Ciudad Universitaria  
Pabellon II  
1428 Buenos Aires

**Argentina**

Enrique Arnoldo San Roman  
Tel.: +54-1-782 88 43  
Fax: +54-1-782 04 41  
E-mail: esr@nahuel.ql.fcen.uba.ar

### **BAR-ILAN UNIVERSITY**

Department of Life Sciences & Physics  
Photobiology and Photophysics  
52900 Ramat Gan

**Israel**

Zvi Malik  
Tel.: +972-3-531 82 04  
Fax: +972-3-535 18 24  
E-mail: ehren@physnet.ph.biu.ac.il

### **CENTRE DE RECHERCHES DU GÉNIE RURAL**

Laboratory Waste Water & Sewage Sludge Paras.  
Rue Hedi Karray 10  
2080 Ariana

**Tunisia**

Zoubeir Alouini  
Tel.: +216-1-71 96 30  
Fax: +216-1-71 79 51

### **FACULTÉ DES SCIENCES DE MEKNES**

Département de Chimie  
B.P. 4010  
Beni M'Hamed  
Meknes

**Morocco**

Mohammed Zaher Benabdallah  
Tel.: +212-5-53 88 70  
Fax: +212-5-52 73 14



**DEVELOPMENT OF A TECHNOLOGICALLY SIMPLE, LOW ENERGY COST  
METHOD OF TREATING WASTEWATER FOR REUSE IN AGRICULTURE**

**Co-ordinator:** HR Wallingford Ltd, Wallingford Oxon, United Kingdom (Tom E. Brabben)

## OBJECTIVES

The purpose of the project was to test the feasibility of using a simple plastic cover to harness the free energy of the sun to treat wastewater for unrestricted reuse in agriculture.

- To test the effectiveness of the treatment technique in meeting the recommended WHO health guidelines for the reuse of wastewater for unrestricted irrigation.
- To investigate the feasibility of trapping the free energy of the sun to produce the required critical time temperature combinations in wastewater.
- To investigate at temperatures above “normal” but below critical kill temperatures, investigate the influence of other factors which in combination with enhanced temperature may assist in producing the required bacterial die-off in the temperature range, 40-50°C.
- To establish the die-off time-temperature combination for helminth eggs found in wastewater.

## ACTIVITIES

- ◇ Establish experimental ponds in Jordan, Portugal, and Tunisia; to determine the most appropriate and cost effective method of trapping the heat from the sun, under a floating plastic cover, in order to kill faecal coliforms.
- ◇ Determine the most appropriate method of isolating helminth eggs from wastewater in Jordan, to test their viability. Hold a workshop on “Pathogen assessment for wastewater reuse” and practical training on helminth identification and viability testing.
- ◇ In Tunisian and British laboratories establish the critical time-temperature combinations to achieve pathogen die-off.
- ◇ Test the effectiveness of the treatment technique in Jordan, Portugal, and Tunisia by; Monitoring faecal coliform numbers and viable helminth eggs in the effluent from the covered ponds;
- ◇ Investigating the beneficial influence of other factors;
- ◇ Monitoring long term viability of the helminth eggs in the soil by the application of treated wastewater to grass plots in Portugal.
- ◇ Identify practical constraints to the using the technique on a larger scale.

## RESULTS

### *Field investigations*

- ⇒ The Jordanian experience showed that the plastic covers can raise the temperature and assist in bacterial removal. The covers only enhance temperatures during the summer months. Covering the ponds has some disadvantages in limiting the light penetration, which in turn affects the growth of algae and dissolved oxygen production (DOP). The reduction in algae growth and DOP has a detrimental effect on faecal coliform removal. The overall efficiency of using plastic covers is probably neutral.
- ⇒ The plastic covers can be easily ruptured and damaged by strong winds and heavy rainfall. Dust readily accumulates on the covers and is difficult to remove successfully. The opinion is that such low cost covers should be replaced every six months, which would increase the capital costs of the system.
- ⇒ The main conclusion from the Portuguese partners is that the pond effluent is not suitable for unrestricted irrigation. The pond efficiency for pathogen removal was low. This was due to the short retention time in the pond and most importantly because the plastic cover did not induce the desired liquid warming.

- ⇒ In the Tunisian experiment, the use of a bubble plastic cover did not result in a sufficient increase of temperature to produce faecal coliform die-off. The other parameters such as pH and dissolved oxygen were more favourable to faecal coliform elimination. The climatic conditions during the field experiments (even those conducted during summer) were less favourable than usual.

#### *Mathematical simulation*

- ⇒ The mathematical studies, undertaken by HR Wallingford, indicate that a daytime temperature rise of 10 to 15 degC is possible under the plastic cover. However, given the likely initial water temperatures in the pond, this will not be enough to ensure die-off in periods of less than six hours. Since the LDPE plastic effectively results in no day to day 'base' gain in water temperature, die-off temperatures would still not be reached after a number of days.
- ⇒ Significant warming of small wastewater ponds (facultative or maturation) is only possible when extra-terrestrial irradiance is greater than 400 W/m<sup>2</sup>, when air temperatures are high and when there is a sufficient number of sunny days, such as in the Mediterranean region during the summer (April to August).
- ⇒ Warming achieved in the experimental ponds is probably due to a reduction of convection at night and not due to the "greenhouse" effect.
- ⇒ To make the best use of trapped solar radiation, specially designed plastic films or coatings should be used. The extra capital costs of the film and supporting structure may be balanced by the need to replace the low cost bubble plastic every six months.

#### **FOLLOW-UP**

In remote rural locations or in peri-urban situations, not served by wastewater collection and treatment services there is a high chance that surface water sources may contain a significant percentage of wastewater products. Water storage, for up to seven days, in a farm pond before it is used could enhance the biological quality of the water and avoid most health risks. Covering the pond with "greenhouse" plastic films (such as "luminal-4") could raise the temperature of the pond water to kill-off harmful bacteria. These higher quality "greenhouse" plastics would allow perhaps an 18 degC rise during daytime which, if combined with high initial water temperatures of over 30°C, could achieve appropriate die-off times. This would reduce the time needed to three or four days and reduce the amount of land used for pond construction. Unrestricted irrigation could then be possible which would allow cultivation of high-value salad crops in and around urban centres. Two ponds, arranged in parallel, each with a surface area of 22 m<sup>2</sup> (7.5m by 3m) and 1m deep would be sufficient to drip irrigate 1ha of vegetable crops each week.

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## **PARTNERS**

### **HR WALLINGFORD LTD**

Wallingford Howbery Park  
Overseas Development Section  
Oxon OX10 8 BA  
**United Kingdom**

Messrs Thomas E Brabben, Allister Thomson, & Adam

Dickinson  
Tel.: +44 (0) 1491 822490  
Fax: +44 (0) 1491 826352  
E-mail: t.brabben@hrwallingford.co.uk

### **UNIVERSITY OF NEWCASTLE-UPON-TYNE**

Newcastle-upon-Tyne  
Department of Civil Engineering  
NE1 7RU  
**United Kingdom**

Mrs Lilian Evison & Dr Tom Curtis  
Tel.: +44 (0) 191 222 6690  
Fax: +44 (0) 191 222 6669  
E-mail: Tom.Curtis@newcastle.ac.uk

### **LABORATÓRIO NACIONAL DE ENGENHARIA CIVIL**

Núcleo de Engenharia Sanitária  
Departamento de Hidráulica  
Avenida do Brasil, 101  
PT-1799 LISBOA Codex  
**Portugal**

Dr Maria Helena Marecos do Monte  
Tel.: +351 (0) 1 848 21 31/7  
Fax: +351 (0) 1 847 86 14  
E-mail: Hmarecos@lnec.pt

### **UNIVERSITY OF JORDAN**

Water & Environment Research & Study Center  
Amman  
**Jordan**

Dr Muhammad R Shatanawi & Prof. Manar Fayyad  
Tel.: +962 (0) 6 5355000 Ext 2332  
Fax: +962 (0) 6 5355560  
E-mail: juwater@amra.nic.gov.jo

### **WATER AUTHORITY OF JORDAN**

PO Box 2412  
Amman  
**Jordan**

Eng. Abdel Wahab Matar  
Tel.: +962 (0) 6 680100  
Fax: + 962 (0) 6 679143

### **INSTITUT NATIONAL DE RECHERCHES EN GENIE RURAL, EAUX, ET FORÊTS**

Lab. Microbiologie  
Rue Hédi Karray - TUNIS – 1004  
BP No 10 - 2080 ARIANA  
**Tunisia**

Dr Monia Trad Rais  
Tel.: +216 (0) 1 718055  
Fax: +216 (0) 1 717951

**UTILISATION DES EAUX USÉES EN IRRIGATION, APPROCHE GLOBALE DU  
TRAITEMENT DES EFFLUENTS COMPARAISON DE DIFFÉRENTS SYSTEMES D'  
IRRIGATION SUR DIVERSES CULTURES ET LEURS ASPECTS INSTITUTIONNEL  
ET ORGANISATIONNEL**

**Co-ordinateur:** Faculté des Sciences Agronomique de Gembloux, Belgique (Dimitri Xanthoulis)

## OBJECTIFS

Le projet a abordé trois thèmes particuliers et complémentaires. Ces trois thèmes sont :

→ *Le traitement des eaux*

Dégager des techniques pour réduire le niveau de contamination des eaux en métaux lourds et micro-organismes pour les rendre utilisables en agriculture, traitement tertiaire des eaux et des boues activées et élimination des métaux par fixation dans les algues et macrophytes, optimisation du stockage des eaux usées et leur traitement par les plantes.

Rechercher les techniques les plus adaptées pour réduire la pollution des eaux à un niveau compatible avec leur utilisation en agriculture et en vue d'éliminer tout risque de maladie infectieuse chez l'homme.

→ *L'irrigation*

Evaluer l'impact de l'irrigation avec les eaux usées sur les plantes, le sol, la qualité sanitaire des productions végétales et les rendements.

→ *Les aspects institutionnels*

Identifier les voies à adopter par les exploitants, autorités sanitaires et agricoles pour optimiser l'utilisation des eaux usées d'une manière efficace, durable et économiquement rentable.

## ACTIVITES

### *Le traitement des effluents*

◇ Filtration sur sable

Mise en place de colonnes de filtration sur sable. Étude de l'efficacité du système et de la qualité des eaux ainsi traitées après épuration classique en station d'épuration.

◇ Essais de stockage

Aménagement de bassins de stockage des eaux usées traitées. Étude de l'impact du stockage sur la qualité des eaux.

◇ Élimination des métaux lourds

Exploration des capacités de fixation des métaux lourds par des cultures d'algues en laboratoire et dans un Chenal Algal à Haut Rendement (CAHR). Identification des algues, mise au point des techniques d'immobilisation et de récolte, étude de la biosorption des ions métalliques via l'immobilisation par les algues en laboratoire et contrôle in situ de la biosorption.

Étude des métaux lourds contenus dans les eaux usées, leur comportement dans le sol, les eaux et les plantes.

### *Irrigation au moyen des eaux usées*

◇ Sous climat tempéré

Étude économique de la réutilisation des eaux usées en irrigation de cultures maraîchères. L'étude de l'influence de l'irrigation avec des eaux usées sur l'émergence de facteurs de dégradation des récoltes (*Sclerotinia*). Suivi de la fertilisation azotée et de la dynamique résultante de l'azote.

- ◇ Sous climat méditerranéen  
Étude de l'irrigation avec des eaux usées de différentes cultures (les agrumes, les cultures à haute valeur ajoutée sous serre, les cultures forestières, les fourrages, cultures légumières). Étude des impacts sur la consommation en eau des plantes, sur les productions, sur la qualité sanitaire des produits, sur la santé, sur le sol et le matériel d'irrigation.

*Les aspects institutionnels et organisationnels de la réutilisation des eaux usées*

- ◇ Détermination du rôle des différents organismes impliqués dans la prise de décision dans le but de jeter les bases d'une législation sur l'utilisation des eaux usées et élaboration d'une structure institutionnelle regroupant les différents organes de décision. Adaptation au cas d'un périmètre irrigué à Ouarzazate.

## RESULTATS

*Le traitement des effluents*

- ⇒ Filtration sur sable  
Les expérimentations permettent de conclure que :
  - plus la couche de sable est importante, meilleur est le rendement d'élimination des MES (matières en suspension);
  - le taux d'élimination, abstraction faite des autres paramètres hydrauliques et épuratoires, diminue lorsque le débit augmente;
  - la chloration des eaux épurées a un effet négatif sur le rendement de la filtration car les germes responsables du colmatage biologique sont éliminés;
  - la différence en rendement en fonction de la granulométrie n'étant pas importante, il est plus pratique de choisir un sable de diamètre inférieur à 2000 µm.
- ⇒ Essais de stockage  
Les résultats obtenus lors des différents essais en bassins de stockage d'effluents secondaires indiquent que la décontamination naturelle de ces effluents est plus rapide lorsque la profondeur des bassins est plus faible. Pour une température des eaux se situant entre 23 et 28°C, un abattement des coliformes fécaux de 3 U.log est réalisé en trois jours lorsque la profondeur du bassin est inférieure à 1,5m. Lorsque cette profondeur atteint 4 m, 7 à 10 jours de stockage sont nécessaires pour réaliser le même abattement. Un stockage prolongé des effluents secondaires (durant 2 à 5 mois) n'affecte pas leur qualité et le niveau de contamination bactérienne continue à évoluer dans le sens d'une baisse jusqu'à l'élimination totale des germes témoins de contamination fécale.
- ⇒ Élimination des métaux lourds  
Une étude de la fixation des métaux lourds par des microalgues produites dans un Chenal Algal à Haut Rendement (CAHR) a permis la détermination des genres algaux et leurs concentrations relatives au cours de l'année et montré que la colonisation du chenal par les algues dépend de la saison. Les genres *Micractinium* et *Chlorella* dominant alternativement la population algale. Les résultats obtenus confirment la grande capacité des algues qui se développent dans le CAHR à fixer les métaux lourds et permettent d'entrevoir une nouvelle approche d'élimination biologique des éléments minéraux en traces et des métaux lourds contaminant l'eau par piégeage des métaux toxiques, en particulier le Cu et le Cd.  
Plusieurs voies de valorisation de ces microalgues comme, par exemple, l'extraction de colorants naturels chlorophylliens et le marché des engrais et des protéines pour l'alimentation animale, permettent le dégagement d'une valeur ajoutée très appréciable.

*Irrigation au moyen des eaux usées*

- ⇒ Sous climat tempéré, les travaux réalisés sur l'évolution de la teneur en azote des sols irrigués au moyen des eaux usées montrent des rendements nettement plus réguliers sous irrigation. D'autre part, on voit que l'irrigation bien conduite et sur sol couvert n'a aucune influence sur la migration des nitrates et par conséquent sur le lessivage des reliquats azotés. Mieux, elle contribue à une utilisation bien plus efficace de ceux-ci. L'irrigation avec les eaux usées ne contribue pas à l'enrichissement en azote du sol puisque les apports supplémentaires sont compensés par un prélèvement plus efficace des plantes sous irrigation.

L'étude de l'influence de l'irrigation avec des eaux usées sur le potentiel infectieux du sclérotinia montre que, quel que soit le type d'eau, l'irrigation peut être un facteur déterminant de la dynamique de contamination des carottes au champs du fait de l'humidité du sol maintenue volontairement la plus constante possible. Il est cependant trop tôt pour affirmer que le fait d'utiliser des eaux usées pour l'irrigation de ces cultures soit à la base du problème.

D'un point de vue économique, l'étude menée sur les aspects économiques de la réutilisation des eaux usées en irrigation a montré les différents avantages que procure la récupération et réutilisation des eaux de traitement dans le cadre d'une culture maraîchère industrielle. Cela permet de résoudre le problème des rejets - et par là même le problème des taxes auxquelles sont soumis ces rejets - et engendre des résultats agronomiques plus que positifs.

Cette étude a également montré qu'un réseau d'irrigation apporte des solutions aux contraintes inhérentes au fonctionnement d'une station d'épuration traditionnelle pour un investissement nettement moins élevé tout en apportant une plus-value non seulement à l'usine mais également aux agriculteurs car ce système d'irrigation est plus intéressant qu'un système individuel utilisant l'eau d'un puits.

- ⇒ Sous climat méditerranéen, plusieurs études ont été réalisées sur l'irrigation de cultures au moyen d'eau usée. Ce principe a été testé sur différentes cultures comme les agrumes, les cultures à haute valeur ajoutée sous serre, les cultures forestières, les fourrages et différents types de cultures légumières.

De manière globale, toutes ces études confirment le bien fondé de cette pratique qui permet des résultats intéressants tant d'un point de vue agronomique qu'économique tout en garantissant, si l'irrigation est pratiquée de manière adéquate, une qualité sanitaire satisfaisante.

Les essais réalisés sur agrumes ont montré une efficacité de l'eau usée (exprimée en poids de matière produite par litre d'eau consommée) accrue par rapport à l'eau de puits, et a aussi démontré l'efficacité de l'irrigation souterraine par jarre qui, non seulement, permet d'obtenir les mêmes résultats, mais a également un impact favorable sur la protection de l'environnement et des cultures d'un point de vue sanitaire.

L'irrigation de surface aux eaux usées traitées désinfectées par stockage en bassins engendre également une efficacité de l'eau consommée nettement supérieure à celle de l'eau de nappe.

La qualité sanitaire des racines, fruits se développant en contact avec le sol ou fruits se développant à une certaine distance du sol est équivalente à celle obtenue en cas d'irrigation par les eaux de nappe.

- ⇒ Le suivi de la qualité des eaux épurées par le système infiltration-percolation et ses effets sur le comportement d'une culture de tomate nous ont permis de tirer plusieurs conclusions.

La qualité sanitaire des eaux usées traitées correspond à la catégorie A des normes de l'OMS et peuvent donc être utilisées pour l'irrigation des cultures maraîchères, moyennant la prise de précautions adéquates.

La richesse des eaux usées permet une réduction des intrants en fertilisants et garantit des rendements quantitatifs et qualitatifs supérieurs à ceux obtenus avec une irrigation à l'eau de puits complémentée en fertilisants.

La réutilisation des eaux usées épurées pour l'irrigation des cultures à haute valeur ajoutée tel le melon et l'œillet s'avère être une opportunité qui a donné des résultats satisfaisants tant sur le plan de la production agricole, d'un point de vue quantitatif et qualitatif, que sur celui de la préservation de l'environnement, en réduisant les quantités d'effluents déversées dans les cours d'eau et en conservant les ressources hydriques conventionnelles.

La comparaison des résultats obtenus sur aubergine et sur sorgho avec l'eau de puits et l'eau usée traitée, toutes deux complémentées avec différentes doses d'azote, donne des résultats intéressants.

L'eau usée non complémentée donne en moyenne des rendements en aubergine de 10% supérieurs à l'eau de puits complémentée avec 150 unités d'azote. De même, sur sorgho, l'eau usée non complémentée donne des résultats similaires à l'eau de puits complémentée avec 150 unités d'azote.

- ⇒ Dans un autre registre, les résultats des essais menés sur cultures forestières ont montré que l'irrigation localisée avec des eaux usées épurées permet une croissance et un développement plus rapide des différentes essences et peuvent être utilisées sans danger pour l'irrigation de plants destinés à la reforestation et à la production de biomasse.

L'irrigation au moyen des eaux usées n'apporte aucune différence significative au niveau du sol en ce qui concerne le pH, le phosphore, l'ammoniaque, les nitrates, le calcium et les métaux lourds. Par contre ces essais ont révélé une augmentation de la teneur du sol en potassium en oligo-éléments et en salinité. Il a été noté une amélioration notable de la qualité physique des sols sous irrigation avec les eaux usées.

- ⇒ En ce qui concerne l'impact de la réutilisation des eaux usées traitées sur la qualité microbiologique des cultures, il a été démontré que l'irrigation localisée est tout à fait appropriée pour irriguer des cultures légumières lorsque l'eau répond aux critères de qualités définis par l'OMS.
- La consommation de fourrages irrigués avec des eaux usées traitées n'a pas d'impact négatif sur la santé des animaux si les directives de l'OMS sont respectées.

#### *Les aspects institutionnels et organisationnels de la réutilisation des eaux usées*

- ⇒ Certains pays du bassin méditerranéen se sont dotés de textes et de recommandations complets et bien définis concernant les eaux usées et, en particulier, la réutilisation de celles-ci. D'autres pays sont dotés de lois sur l'Eau en général, parfois assez anciennes, mais les différents aspects de la réutilisation des eaux usées n'y sont qu'effleurés dans le sens où la réutilisation n'est pas codifiée par des textes et des normes précis mais au travers de textes très généraux relatifs soit aux rejets des eaux urbaines, soit à la ressource en eau, soit à la protection de l'environnement, ce qui rend la législation assez inefficace.
- Enfin, dans les autres pays du bassin méditerranéen, l'émergence de la réutilisation des eaux usées comme ressource en eau agricole amène ces pays à travailler à la modification de lois souvent incomplètes ou obsolètes. En effet, la réutilisation des eaux usées est rarement abordée dans les textes de loi et les décrets, si ce n'est:
- ⇒ L'étude faite au Maroc sur la mise en place d'un périmètre irrigué avec des eaux usées traitées a révélé qu'il est indispensable de tenir compte de tous les éléments du cadre dans lequel se situe cette mise en place tant en ce qui concerne la situation socio-économique, agro-économique et sanitaire de la région, que des différents statuts juridiques et des autorités régionales et nationales compétentes en la matière. L'application de la législation sur le terrain n'est pas toujours chose aisée, et il est primordial qu'il y ait un contact et une collaboration directs entre les utilisateurs de l'eau usée et les responsables de sa gestion par l'instauration de comités d'utilisateurs en liaison constante avec les autorités locales de manière à permettre une bonne circulation des informations.

## **PERSPECTIVES**

Bien que les travaux réalisés dans le cadre de ce projet ont permis d'obtenir des résultats concluants, il serait du plus grand intérêt d'élargir le champs de cette recherche en se penchant sur différents points de manière à couvrir un plus large éventail de sujets complémentaires à la réutilisation des eaux usées en irrigation.

*Les aspects qu'il serait souhaitable d'envisager sont les suivants :*

- ▷ Etude de la commercialisation des produits agricoles issus de l'irrigation avec des eaux usées en fonction des normes internationales de qualité microbiologique pour l'exportation;
- ▷ Etablissement d'un code des bonnes pratiques agricoles spécifique à la zone méditerranéenne en matière de réutilisation des eaux usées, en y incluant les thèmes suivants:
  - Le traitement des eaux usées destinées à l'irrigation
  - La gestion des eaux usées en irrigation
  - L'utilisation des eaux usées et la santé humaine
  - Les méthodes d'irrigation, les cultures et les pratiques utilisant les eaux usées
  - La qualité des eaux usées en irrigation
  - La protection des nappes et la qualité des produits;
- ▷ Les différents aspects économiques de la réutilisation des eaux usées dans le bassin méditerranéen, et en particulier le coût de l'eau usée traitée en comparaison avec le coût de l'eau utilisée en irrigation traditionnelle.

Un autre point qui nous semble important de souligner est la nécessité de créer des projets de démonstration destinés à mettre en évidence les acquis de la recherche et les technologies utilisées, ce qui constituerait une étape préalable à la mise en place de projets en vraie grandeur. Ces unités de démonstration pourraient inclure non seulement les systèmes d'épuration primaire et secondaire, mais également les systèmes d'épuration tertiaire permettant de satisfaire aux normes en matière de qualité microbiologique des eaux usées destinées à l'irrigation.

Ces projets pilotes pourraient être associés à des périmètres d'irrigation pourvus de parcelles de démonstration comprenant les trois grands systèmes d'irrigation à savoir: l'irrigation au goutte à goutte, l'irrigation par aspersion et l'irrigation gravitaire.



## **PARTENAIRES**

### **FACULTÉ DES SCIENCES AGRONOMIQUES DE GEMBOUX**

FSAGX  
Passage des Déportés 2  
5030 Gembloux  
**Belgium**

Dimitri Xanthoulis  
Tel.: +32-81-62 21 86  
Fax: +32-81-62 21 81

### **INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE HASSAN II**

Complexe Horticole d'Agadir - Chaga  
B.P. 773  
Agadir  
**Morocco**

Redouane Choukr-Allah  
Tel.: +212-8-24 01 55 / 24 10 06  
Fax: +212-8-84 39 77 / 24 22 43

### **ECOLE SUPÉRIEURE DES INGÉNIEURS DE L'ÉQUIPEMENT RURAL**

Route de Testour  
9070 Medjez El Bab  
**Tunisia**

Bechir Ben Thayer  
Tel.: +216-8-45 74 95 / 45 67 73  
Fax: +216-8-45 76 81

### **AGRICULTURAL UNIVERSITY OF ATHENS**

Iera Odos 75  
11855 Athens  
**Greece**

Spyros Kyritsis  
Tel.: +30-1-529 40 01  
Fax: +30-1-529 40 23

### **INSTITUTO SUPERIOR DE AGRONOMIA**

Seccao Autonoma de Chimica Agricola  
Tapada Da Ajuda  
1399 Lisboa Codex  
**Portugal**

Joachim Quelhas Dos Santos  
Tel.: +351-1-36 37 98 70  
Fax: +351-1-36 37 89 70

### **AGRICULTURAL RESEARCH INSTITUTE**

ARICYP  
2016 Nicosia  
**Cyprus**

Ioannis Papadopoulos  
Tel.: +357-2-30 51 01  
Fax: +357-2-31 67 70

### **INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE HASSAN II**

Complexe de Ouarzazat  
B.P. 6202  
Rabat-Instituts  
Rabat  
**Morocco**

Bouchaib El Hamouri  
Tel.: +212-7-77 75 64  
Fax: +212-7-77 81 10 / 77 58 38

### **ECOLE SUPÉRIEURE DES INGÉNIEURS DE L'ÉQUIPEMENT RURAL**

CRGR  
Rue Hedi Karray  
B.P. 10  
2080 Tunis  
**Tunisia**

Zouheir Chaabouni  
Tel.: +216-1-71 78 01 / 71 96 30

**PURIFICATION AND RE-USE OF DOMESTIC WASTE WATER USING LOW-COST  
ECO-BIOTECHNOLOGICAL METHODES**

**Co-ordinator:** Wageningen Agricultural University, Wageningen, The Netherlands (Gatze Lettinga)

## **OBJECTIVES**

The overall objective is to contribute to the development of low-cost eco-biotechnological methods for the treatment and re-use of domestic wastewaters in Mediterranean countries. The specific scientific objectives are:

- Assessment of the most efficient hydrolysis/pre-treatment step for the degradation of suspended solids in raw sewage;
- Development of most appropriate reactor configuration for the anaerobic treatment of domestic wastewater under local conditions;
- Research on the most appropriate post-treatment process to remove or recover the remaining pollutants and decimate the number of pathogenic bacteria;
- Assessment of the re-use potential of treated wastewater for irrigation and/or fertilisation under local environmental conditions.

## **ACTIVITIES**

- ◇ Anaerobic pre-treatment of raw domestic sewage (The Netherlands/Spain)  
Experiments for assessing the hydrolysis rate of the suspended solids under various conditions in a Hydrolysis-UASB reactor under low temperature conditions.  
Mathematical model development in order to predict the performance of a hydrolysis reactor at various temperatures and for domestic waste waters with different compositions/ concentrations. The model will be used for making proper designs for these reactor systems.
- ◇ Anaerobic treatment of domestic sewage in the Mediterranean - Middle East region (Egypt/Jordan/Palestine)  
Characterisation of the Middle East sewage at the various locations.  
Adaptation of current reactor designs to local conditions. Adaptations are required because of large seasonal temperature fluctuations (winter: 12-15°C, summer: 24-28°C), high concentrations of organic pollutants (up to 2000 mg COD/l), high concentration of suspended solids, particularly for community-on-site treatment of domestic waste water, high concentration of pathogenic bacteria, parasites and viruses, Start-up, operation and evaluation of anaerobic reactor systems. The work in Hebron is focused on the feasibility of anaerobic treatment for olive mill effluents.
- ◇ Development of most appropriate post-treatment methods (Egypt/Jordan)  
Assessment on the feasibility of pond systems for post treatment. Research includes application of duckweed ponds, algae ponds and fishponds.  
Research on compact post-treatment systems for effluent polishing: rotating biological contactors (RBC).  
Assessment of fate of residual pollutants and pathogenic bacteria in the above post-treatment systems.
- ◇ Assessment of re-use potential of the purified waste water (Jordan / Egypt)  
Characterisation of final effluent. The effluent quality determines whether unrestricted irrigation or restricted irrigation should be applied. Data will be compared with international or local guidelines (due to time constraints and changes in research proposal, little attention could be paid to this item).

## OUTCOME

### *Scientific-technical results*

#### **Netherlands:**

The first stage of the investigated bench-scale two-stage anaerobic up-flow system is entrapping up to 85% of the incoming suspended solids (SS) at hydraulic retention times (HRT) of about 4 h. The degree of solids removal, linearly correlates with the SS loading rate. However, the treatment efficiency of the reactor is disturbed by the build-up of a floating layer, particularly at higher temperatures. The reactor systems are operated at 15°C and 25°C and fed with Dutch sewage thickened with primary sludge to simulate concentrated on-site sewage (total COD » 2000 mg/l). The calculated hydrolysis constants of the entrapped sludge are rather low (about 0.005 day<sup>-1</sup>), which can be attributed to the large quantity of primary sludge additions.

The solids retention time (SRT) can be considered as the key-parameter for achieving a sufficiently high degree of hydrolysis/acidification with or without the presence of methanogenesis. Linear correlations were found between the hydrolysis of polysaccharides and the SRT for a SRT between 3 and 8 days. Lipid hydrolysis becomes only significant at SRTs > 8 days and protein hydrolysis at SRTs > 8-10 days. Therefore, 10 days is so far considered to be the minimum SRT for the first stage of the two-step anaerobic system at 25°C. Based on the current results a kinetic model was developed for calculating the required HRT at a design SRT. The latter is dependent on the sewage temperature, rate of hydrolysis, entrapment of solids, reactor sludge concentration, etc. The complexity of the hydrolysis process, however, does not allow the development of a complete model yet. Nonetheless, rough estimates can be made of the required size of either a single- or a two-stage reactor system for sewage treatment and thus, a rough economic evaluation.

#### **Spain:**

Research is performed in a two-stage UASB system (4.3 + 3 m<sup>3</sup>) which was started up under winter conditions. Reactors are fed with sewage pumped from the university sewage collector (COD concentrations: 400-500 mg/l). COD removal rates increased with increasing temperature and under summer conditions up to 80% COD removal occurred in the first stage. It was observed that during long term continuous operation, the first stage serves as an efficient hydrolyses and acidification step for the entrapped suspended solids (SRT < 40 days). The average concentration of volatile fatty acids, the main products of acidification, is distinctly higher in the effluent of the first reactor module compared to the influent. Interestingly, the second stage sludge exhibits a higher methanogenic activity than the sludge from the first stage. Yet, adaptation of methanogenic sludge under ambient (winter) conditions requires a long time. Seeding the anaerobic sewage treatment reactors with a high quality methanogenic granular sludge, reduces the required start-up period.

#### **Egypt:**

The investigations concerned research on one and two modular UASB-systems at bench scale (40 L) for pre-treatment, and algae and duckweed ponds and a Rotating Biological Contactor (RBC) for post-treatment. Also a two modular 2.2 m<sup>3</sup> UASB-reactor was put in operation. The average sewage COD concentration in Cairo is: 700 mg/l, with maximum values up to 1280 mg/l. The assessed biodegradability is about 90 %. The obtained results with the single step system are very promising: 79 % COD removal (at HRT = 8 h) and a rather satisfactory removal of various pathogens was achieved (particularly Helminth eggs). Also the two modular reactor performed very well: up to 67 % COD-removal at only HRT = 5 h in the first module (29 L), which was distinctly shorter than those applied so far in the reactors operated in Jordan and Spain. In the second module (15 L) another 50% COD removal was achieved, resulting in 83 % overall COD removal for the total system. The results appeared to be very excellent despite the Summer - Winter fluctuating temperature conditions.

The 2.2 m<sup>3</sup> reactor system was started in April 1997 (spring time conditions). The applied HRT during the start-up was 18 and 13 h, for the first and second module, respectively, and average CODt-removal efficiencies amounted up to 72,6 %, which mainly proceeded in the first module. Hereafter, the HRT was reduced to 20 h, while the average CODt-removal improved to 82 % (summertime). In the third period, at HRT = 15 h (November-December), the average CODt-removal still was 79 %. The lowest temperatures were found to be 16-17°C (December - February). In the final Summer period CODt-removal rates increased to 85% at HRTs of only 8 h for the total system.

Also the results obtained in the bench scale post-treatment systems were very encouraging. Following the RBC-system a CODt removal up 91% was achieved for the entire anaerobic-aerobic system. Algae ponds (HRT=10 days) were less efficient in removing remaining COD (83% for the total system), but they performed quite satisfactorily for pathogen removal, i.e., 99.99 % faecal coliform reduction. Duckweed (*Lemna*) ponds were more effective in residual COD removal than algae ponds but performed slightly worse in removing pathogens. The average pathogen removal capacity of the total system (anaerobic + ponds) was about 5 log units, indicating that the effluent is suitable for restricted irrigation.

#### **Jordan:**

A two stage 100 m<sup>3</sup> research reactor (UASB type) is constructed at the side of Al Samra waste stabilisation ponds near Amman. The assessed average sewage COD<sub>total</sub> amounted 1109 mg/l (up to 77 % biodegradable), and the temperature varied between 15 - 25 °C, with extreme values during a few days as low as 10 °C and as high as 27 °C. The average COD value was based on grab samples taken on several days at more or less the same time. The pollution strength of the sewage daily fluctuated between 1000 and 2000 mg COD/l. For evaluating the reactor performance based on grab samples, correction factors must be used. Various sludge types from the pond system were tested on its specific methanogenic activity. The tests show that sludge from the anaerobic ponds at 4 m depth may serve as an inoculum for the bio-reactor, but the maximum methanogenic activity is fairly low < 0.05 kg COD.kg-1 VSS. day<sup>-1</sup>.

Due to delays in construction works the reactor is still under start-up conditions. Several start-up trials were performed but serious problems manifested in feeding the reactor. Lack of funds made it impossible to install a proper feed supply system in time. Despite that, already an average COD-reduction up to 70 % is achieved at HRTs of 10-20 h, which looks rather promising. In addition to the anaerobic wastewater treatment investigations, also preliminary studies were started concerning the re-use of conventionally treated domestic wastewater and on post-treatment using duckweed ponds. However, results on the later systems can only be discussed once the pre-treatment step is adequately operated.

#### **Palestine:**

The investigations were primarily directed to the anaerobic treatment of olive oil mill effluents. The experimental work concerned biodegradability studies and the characterisation of effluents of 15 mills, viz. at the beginning, the mid and the end of the harvesting season. It was found that the characteristics vary substantially over the season, but also between the various mills. The survey therefore is very useful for assessing the feasibility of anaerobic wastewater treatment and its possible implementation. Based on a questionnaire conducted, it was found that presently most mill effluents (estimated at a total volume of 300,000 m<sup>3</sup>) remain untreated, and simply are discharged in open valleys and fields. Biodegradability studies show that anaerobic conversion of olive mill effluents needs a sufficiently long adaptation period. Cow dung may serve as inoculum but more adapted seed material is preferred. Continuous flow studies were conducted in 15 l laboratory scale UASB reactors. However, still no results are available, which can be attributed to the unstable political situation in Palestine and particularly Hebron. Due to political tensions, the Israelian authorities closed Hebron University for about 10 months. Agricultural test fields only became available at the end of 1997 (the site is still under construction).

## **FOLLOW-UP**

The Avicenne partners recently started a 4 years educational project on "Capacity building on wastewater valorisation for agricultural production in the Middle East area by using low-cost technologies". The project is funded by the Dutch Government and coordinated by Wageningen Agricultural University. The main goals are 'knowledge transfer' and 'institutional development' at the counterparts, including PhD and MSc training and organisation of specialised courses.

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## **PARTNERS**

### **WAGENINGEN AGRICULTURAL UNIVERSITY**

Department of Environmental Technology  
Bomenweg 2  
P.O. Box 8129  
6703 HD Wageningen  
The Netherlands

Gatze Lettinga  
Tel.: +31-317-48 33 39 / 48 32 43  
Fax: +31-317-48 21 08

### **UNIVERSITY OF JORDAN**

Water and Environment Research and Study Center  
P.O. Box 1300  
Amman  
Jordan

Muhammad R. Shatanawi  
Tel.: +962-6-84 35 55 (ext. 2332)  
Fax: +962-6-830 11 90 / 840 50

### **NATIONAL RESEARCH CENTRE**

Water Pollution Control Department  
Tahreer Street, Dokki  
12622 Cairo  
Egypt

Fatma El-Gohary  
Tel.: +20-2-337 14 99  
Fax: +20-2-337 09 31

### **HEBRON UNIVERSITY**

Faculty of Sciences  
P.O. Box 40  
Hebron  
Westbank and Gaza Strip

Awni Khatib  
Tel.: +972-2-992 92 93  
Fax: +972-2-992 93 03

### **CEHA-WHO**

Amman  
Jordan

A. Gur  
Tel.: +962-2-992 92 93 / 92 09 96  
Fax: +962-2-668 65 91

### **UNIVERSIDAD DE VALLADOLID**

Facultad de Ciencias  
Prado de la Magdalena S/N  
47005 Valladolid  
Spain

Fernando Fernandez-Polanco  
Tel.: +34-83-42 31 72  
Fax: +34-83-42 30 13

**CONTINUOUS PRODUCTION OF LIGNINOLYTIC ENZYMES BY WHITE ROT FUNGI FOR DETOXIFICATION OF RECALCITRANT POLLUTANTS**

**Co-ordinator:** Wageningen Agricultural University, Wageningen, The Netherlands (J. A. Field)

**OBJECTIVES**

- White rot fungi are unique organisms capable of oxidising lignin, which is a heterogeneous aromatic biopolymer. These fungi possess a complex extracellular enzyme machinery composed of peroxidases, hydrogen peroxide producing oxidases, low molecular weight cofactors (Mn, organic acids, secondary metabolites). This enzyme system is responsible for the initial oxidation of lignin. Since these reactions are non-selective, many other aromatic compounds such as polycyclic aromatic hydrocarbons (PAH), dioxins, azo dyes, etc. can be oxidised by white rot fungi. Consequently, white rot fungi are being considered for applications in the clean-up of organopollutant contaminated sites and in wastewater treatment of different industries;
- The objectives of the project are to determine the crucial physiological factors for increased peroxidase production in two white rot fungal strains, *Bjerkandera sp.* strain BOS55 and *Phanerochaete chrysosporium*. The overproduction of the main two peroxidases of these strains, namely lignin peroxidase (LiP) and manganese-dependent-peroxidase (MnP) was attempted by identifying and manipulating important physiological signals responsible for their expression. This knowledge was used to sustain their continuous production by fungi immobilised in bioreactors.

**ACTIVITIES**

- ◇ In *Bjerkandera sp.* strain BOS55, both LiP and MnP could be overproduced in nitrogen-sufficient liquid cultures. MnP was stimulated the most by applying 100 to 500  $\mu\text{M}$  of Mn, a pH of 5.2 and supplementing the medium with organic acids (i.e. 5 mM glycolate). LiP production was greatly enhanced in the absence of Mn due to the inhibitory effect of Mn on veratryl alcohol biosynthesis, a secondary metabolite needed to stabilise LiP activity;
- ◇ In *Phanerochaete chrysosporium*, overproduction of LiP was observed in nitrogen-sufficient un-submerged cultures where the mycelium is immobilised on polyurethane foam blocks. The mechanism of LiP induction was found to be related to oxygen radicals, the highest LiP production was observed under an oxygen atmosphere. Mn reduced LiP production since it stimulated Mn-dependent superoxide dismutase that reduced the exposure of the fungus to oxygen radicals. A unique enzyme was discovered that dephosphorylates LiP isozymes, creating new LiP isozymes;
- ◇ Continuous production of ligninolytic enzymes by *Phanerochaete chrysosporium* in bioreactors with mycelium immobilised on foam blocks or as pellets was feasible for long periods of time (more than 140 days). A key design feature required for long term operation is the use of a pulsed air reactor that prevents conglomeration of mycelium pellets and subsequent clogging of the reactor. The ideal condition for the continuous production of LiP was the use of an oxygen gas-phase instead of air. The ideal condition for MnP was the use of a medium that contained 5000  $\mu\text{P}$  of Mn.

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## PARTNERS

### WAGENINGEN AGRICULTURAL UNIVERSITY

Division of Industrial Microbiology

P.O. Box 8129

6700 EV Wageningen

The Netherlands

J. A. Field

Tel.: +31-317-48 47 49

Fax: +31-317-48 49 78

### MIGAL-GALILEE TECHNOLOGICAL CENTER

Department of Mycology & Microbiology

South Industrial Zone

10200 Kiryat Shmona

Israel

Dan Levanon

Tel.: +972-6-95 35 11

Fax: +972-6-94 49 80

### UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

Chemical Engineering Department

15706 Santiago de Compostela

Spain

Juan M. Lema

Tel.: +34-81-56 31 00

Fax: +34-81-59 50 12

**MEMBRANE RECOVERY OF METAL POLLUTANTS FROM WASTEWATERS OF THE FERTILIZERS INDUSTRY (MERMEP)**

**Co-ordinator:** Universidad de Cantabria, Santander, Spain (Inmaculada Ortiz Uribe)

**OBJECTIVES**

- Analysis of the viability of the membrane processes (non-dispersive solvent extraction, supported liquid membrane, emulsion liquid membrane, hybrid liquid membranes and ion exchange) for the reduction and recovery of Cd and U in the waste waters of the fertilisers industry, studying two different options: -reduction of the metallic content in the phosphoric acid (30% P<sub>2</sub>O<sub>5</sub>) -reduction of the metallic content in the waste waters;
- Study of the selective recovery of Cd and U in the presence of Cu, Pb, V and Zn;
- Comparison of the membrane technologies and design of the integrated process for the recovery of metal pollutants from the waste waters of the fertilisers industry.

**ACTIVITIES**

- ◇ Definition of initial conditions; during the first progress meeting and as a result of the collaborative work of the five groups it was decided the analysis of the separation processes in their application to the removal of Cd and U from wet phosphoric acid, considering as main advantages the treatment of lower liquid volumes as well as the obtention of a phosphoric acid free of cadmium, that could be safely used in the manufacture of fertilizers.
- ◇ Analysis of the feasibility of the five membrane technologies in their application to the removal of cadmium and uranium from phosphoric acid solutions. Concerning the removal of cadmium three different types of extractants have been analysed in the project i) a thiophosphinic acid with commercial name, CYANEX 302, that allows the removal of cadmium after a previous step of removal of copper, since the latter acts as a poison for the extractant; it can also be used impregnated on the ion exchange resins. ii) ALIQUAT 336, a quaternary ammonium salt, able to remove cadmium even in the presence of copper, and iii) polyvinyl sulfonic acid, PVSA, a high molecular weight polymer soluble in aqueous phases and of possible use with industrial acid . Concerning the removal of uranium, it was checked that the unique feasible technology was ion exchange obtaining the optimum results in the separation process with the resin Purolite S940.
- ◇ The recovery of cadmium and uranium after their separation from the phosphoric acid was analysed in a stripping step, using a selective stripping agent, i.e., sodium carbonate for recovery of uranium, a high concentration hydrochloric acid solution for the recovery of cadmium except when ALIQUAT 336 is employed, extractant that allows the use of tap water as stripping agent.
- ◇ The analysis of the experimental results led to the obtention of the mass transfer mathematical models together with the characteristic parameters useful in the design of the considered technologies and allowing the comparison of the different processes.
- ◇ Quantitative comparison of the technologies for the removal of 50 mg/l of cadmium and 200 mg/l of uranium when a throughput of 1m<sup>3</sup>/h of wet phosphoric acid is used, defining equipment characteristics (membrane area, equipment volume...) and operation variables (amount and type of stripping agent, membrane lifetime...).



- ◇ Definition of the flow sheet diagram of an integrated process considering two different alternatives for the removal of cadmium and uranium from wet phosphoric acid that are possible from the technological point of view. The selection between the two options should consider additional aspects, such as risk analysis, scale-up and continuous operation and generation of waste effluents in order to be able to make a proper selection of the technological option for the removal of U and Cd from wet phosphoric acid.

## OUTCOME

### *Scientific-technical results*

The feasibility of the considered membrane technologies for the removal of cadmium and uranium from phosphoric acid has been checked throughout the project, concluding the capacity and conditions for the removal of cadmium by non-dispersive solvent extraction and hybrid liquid membranes. The removal of cadmium after a previous separation step eliminating copper by emulsion liquid membranes and supported liquid membranes, and the removal of uranium by solid ion exchange has been proven.

### *Deliverables*

The mass transfer models and design parameters have been determined for all the studied technologies allowing the quantitative comparison of the separation efficiency in the application to 1m<sup>3</sup>/h of phosphoric acid containing 50mg/l of cadmium and 200 mg/l of uranium.

As a result of the joint research work it has been also defined the flow sheet diagram of an integrated membrane process that gives a technological alternative to the removal of heavy metals and uranium from phosphoric acid, and that could serve as starting point for the scaling-up and design of an industrial process.

### *Pilot plant*

During the project it was constructed an automatically controlled pilot plant based on two Hollow Fiber modules with an effective area of 19.3 m<sup>2</sup> each and able to treat 0.25 m<sup>3</sup> per hour of phosphoric acid. The pilot plant can be used i) for the development of the non-dispersive solvent extraction technology, NDSX, to the separation and recovery of the cadmium and other metals using ALIQUAT 336 as selective extractant and water as backextraction agent, ii) for the development of the Supported Liquid Membrane process, SLM for the staged removal of copper and cadmium from the acid, and iii) for the development of the Hybrid Liquid Membrane technology, HLM, for the removal of Cd, Cu and Zn using PVSA as selective agent and hydrochloric acid as stripping agent. In order to remove uranium the pilot plant needs to be implemented with the corresponding ion exchange columns for continuous operation.

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## **PARTNERS**

### **UNIVERSIDAD DE CANTABRIA**

Departamento de Quimica  
Avenida de Los Castros  
39005 Santander  
**Spain**

Inmaculada Ortiz Uribe  
Tel.: +34-42-20 15 85  
Fax: +34-42-20 15 91

**IMPERIAL COLLEGE OF SCIENCE,  
TECHNOLOGY & MEDICINE**  
Chemical Engineering Department  
London SW7 2AZ  
**United Kingdom**

Susana E. Ortiz  
Tel.: +44-171-594 55 87  
Fax: +44-171-594 56 04

### **EGE UNIVERSITY**

Chemical Engineering Department  
35100 Bornova Izmir  
**Turkey**

Nalan Kabay  
Tel.: +90-232-388 40 00 (ext. 486)  
Fax: +90-232-374 14 01

### **HEBREW UNIVERSITY OF JERUSALEM**

Casali Institute of Applied Chemistry  
Givat Ram Campus  
91904 Jerusalem  
**Israel**

Aharon Eyal  
Tel.: +972-2-53 59 93  
Fax: +972-2-58 45 33

### **ATOMIC ENERGY AUTHORITY**

Hot Laboratory Centre  
13758 Cairo  
**Egypt**

Jacqueline A. Daoud  
Tel.: +20-2-355 08 59  
Fax: +20-2-354 09 82

**CONTROLLING CONTAMINANTS AFFECTING USE AND RE-USE OF WATER,  
SEWAGE AND SLUDGE IN JORDAN AND SYRIA**

**Co-ordinator: Technical University of Denmark, Lyngby, Denmark (Hans Mosbæk)**

**OBJECTIVES**

- To obtain an updated overview of existing methods and procedures for monitoring of contaminants in the water cycle and on wastewater and sludge re-use practices;
- To establish knowledge of the geochemical fate of pesticides and of the composition of sludge and waste water in the study area;
- To obtain results which will help Syrian and Jordanian water authorities to assess the state of springs and aquifers, in Syria with particular emphasis on pesticide residues and in Jordan on the re-use of sludge and waste water;
- To develop and formulate in close co-ordination with the concerned governmental agencies various options for proper management of wastewater and sludge.

**ACTIVITIES**

- ◇ Comprehensive review and analysis of existing reports and related literature to document existing and past efforts, identify needs and avoid duplication of efforts in the field of pesticide control and waste water and sludge re-use;
- ◇ Evaluation of regional and international waste water and sludge re-use practises and identification of relevant constraints and conditions with potential effects on the intended resource re-use in the study area;
- ◇ Development of appropriate analytical capabilities in two Arab research institutions, including equipment acquisition and installation, personal training and testing of analytical procedures;
- ◇ Development of computer models for understanding and the geochemical fate of pesticides.

The first action has started in Jordan and Syria and is almost finished. Equipment has been installed in Jordan for measurements of metals and training of the staff is in progress. Measurement of heavy metal concentrations in sludge treated soil and crops are in progress in order to find the significance of the metal content of sludge use for agricultural use.

Considering the literature review it is decided to focus on insecticides in Syria. Development of analytical procedures and training will be within this area.

**OUTCOME**

- ⇒ The fate of pesticides in the aquifer of Damascus will be assessed and modelled, thus providing a useful decision base to water authorities;
- ⇒ The significance of heavy metal pollution of sludges and treated wastewaters of the Jordan valley region will be analysed and management options outlines;
- ⇒ Adequate capacities for water supply analysis in terms of water quality and water quantity will be developed in Syria and Jordan allowing the concerned water authorities to base management decisions on available expertise of local institutions, possessing appropriate models of the water sources and the capacity to employ them.

## **PARTNERS**

### **TECHNICAL UNIVERSITY OF DENMARK**

Department of Environmental Engineering  
Building 115  
2800 Lyngby  
**Denmark**

Hans Mosbaek  
Tel.: +45-45 93 28 50  
Fax: +45-45 93 39 08

### **HIGHER INSTITUTE OF APPLIED SCIENCES AND TECHNOLOGY**

Environmental Studies Laboratory  
P.O. Box 31983  
Damascus  
**Syria**

Fouad Abosamra  
Tel.: +963-11-77 46 39  
Fax: +963-11-23 77 10

### **UNIVERSITY OF JORDAN**

Faculty of Engineering & Technology  
Civil Engineering Laboratories  
Environmental Engineering Section  
Amman  
**Jordan**

Fawzi Rayyan  
Tel.: +962-6-84 355 / 2743  
Fax: +962-6-84 85 58

**DEFLUORATION OF WATERS IN THE NORTHERN PARTS OF THE NORTH-  
AFRICAN SAHARA**

**Co-ordinator:** Université de Rennes, Rennes, France (Bernard Bariou)

**OBJECTIVES**

- Mastering techniques for the defluorination of Saharan waters;
- Estimation of the treatment costs of different technological options;
- Identification of the most cost effective process.

**ACTIVITIES**

- ◇ Mapping of the area of the northern Sahara affected by fluoridised waters through literature and field studies;
- ◇ Testing of different defluorisation.

**RESULTS**

- ⇒ An analytical study of the water distributed in certain zones of the Sahara "septentrional" was carried out. In a number of cases, the water contained too many sulphate ions and calcium ions to be treated directly by procedures such as inverse osmosis or double channel electro dialysis.
- ⇒ After calcium precipitation, inverse osmosis allowed more than 90% of the original amount of water to be recovered.
- ⇒ Four channel electro dialysis also gave good results but used a lot of brine. A combination of double channel and four channel electro dialysis is currently under study.
- ⇒ Other techniques such as electrochemical reactor-absorption allowed a good elimination of fluor, however, the total salinity remained high.

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## **PARTNERS**

### **UNIVERSITÉ DE RENNES I**

Laboratoires des Procédés de Séparation  
IUT, Département Chimie  
Rue du Clos Courtel  
B.P. 1144  
35000 Rennes  
**France**

Bernard Bariou  
Tel.: +33-2-99 36 26 51  
Fax: +33-2-99 63 78 23

### **UNIVERSITE CATHOLIQUE DE LOUVAIN**

Faculté des Sciences Appliquées  
Unité des Procédés  
Voie Minckelers 1  
1348 Louvain-La-Neuve  
**Belgium**

Robert Leenaerts  
Tel.: +32-10-47 23 22  
Fax: +32-10-47 23 21

### **ÉCOLE NATIONALE POLYTECHNIQUE D'ALGER**

Laboratoire des Biotechnologies  
Avenue Pasteur 10  
EL Harrach  
16000 Alger  
**Algeria**

Nabil Mameri  
Tel.: +213-2-76 59 29  
Fax: +213-2-76 09 66

### **SOCIÉTÉ ALGERIENNE DE CANALISATION D'OUVRAGES ET CHARPENTES (SASCOC S.A.R.L.)**

Département de Recherche & Développement  
Avenue du 5 juillet 121  
Bordj El Kiffan - Alger  
**Algeria**

Mouloud Mameri  
Tel/fax: +213-2-86 66 32

### **UNIVERSITÉ DE KENITRA**

Département de Chimie  
14000 Kenitra  
**Morocco**

A. El Midaoui  
Tel.: +212-7-37 22 01  
Fax: +212-7-37 27 70

**TREATMENT OF WATER AND EMUENTS BY TIO<sub>2</sub> PHOTOCATALYSIS FOR  
REMOVAL OF ORGANOSYNTHETIC CONTAMINANTS AND METALS**

**Co-ordinator:** Universität Bremen, Bremen, Germany (Wolfram Thiemann)

**OBJECTIVES**

- The environment around us is increasingly contaminated with the residues from human activities. The main sources for this contamination are the ongoing process of world-wide industrialisation with its adverse side-effect of disposing waste products into air, soil, and waters as well as the intensification of agricultural production for satisfying the nutritional demand for the ever-increasing human population with its use of a large spectrum of fertilisers and pesticides leaking into soil and groundwater resources. In order to safeguard the terrestrial environment for the survival of future generations there has been an urgent need for a recycling of wastes through an effective waste treatment that should not only be efficient but at the same time affordable in economic terms. Existing waste treatment technologies have been worked out and applied successfully mainly in the wealthier regions of the industrially more developed countries of the world. The less fortunate countries which make up by far the largest part of the world in terms of area and population number, present in general a rather bleak scenario in terms of environmental quality: Effective waste treatment procedures are literally not existent or at best in an embryonical state in these areas, due to the fact that the existing technologies are not affordable;
- Hence our objective to introduce a technology of processing wastes with two main goals: It should be cheap - by applying regenerative energy sources such as solar radiation, which is free and abundant in particular in southern areas which make up the "poor" areas - and particularly effective for removing even the most refractory pollutants that escape the conventional biological treatment technologies as described in the literature. It seems that the TiO<sub>2</sub> mediated photochemical oxidation with sunlight is an ideal technology suitable for large-scale application of treating waste waters in these poorer areas of the world which fulfilled the above conditions of low capital investment, easy maintenance, and high efficiency;
- The three involved contractors have undertaken this joint research project to study the guidelines along which a large-scale application of solar radiation driven photo-oxidative treatment of wastewater should be developed.

**ACTIVITIES**

- ◇ In Israel, the catalytic effect of TiO<sub>2</sub> particles is investigated to make use even of the visible spectrum of the sun radiation in order to break highly refractory pesticides from agriculture;
- ◇ In Turkey, "black" light source is studied to remove both toxic metals by adsorption to TiO<sub>2</sub> and organic compounds by simultaneous photocatalyzed oxidation processes;
- ◇ In Germany, the mechanism of photooxidation in combination with H<sub>2</sub>O<sub>2</sub> is studied for the case of selected carbamate class pesticides.

## RESULTS SO FAR

- ⇒ In the Israel laboratory, efficient solar photocatalysis of nitro phenols has been observed, and also of the insecticide 2,4-dinitrocresol. The studies were conducted in solution of 1L, under polythene cover to prevent losses due to evaporation. Distinction between two different reaction patterns of longwave ( $> 300$  nm) photooxidation has been witnessed: In the first case oxygen has a pronounced effect on the rate of photooxidation, as exemplified by metribuzin (4-amino-6-tert-butyl-4,5 dihydro-3-methylthio-1,2,4 triazine-5-one), while the influence of hydrogen peroxide is quite moderate. The photolytic process in this case would apparently start via a reaction of the excited pesticide with an oxygen molecule, generating the  $\text{HO}_2$  free radical. In another case, exemplified by bromacil (5-bromo-3-sec-butyl-6-methyluracil), oxygen does not have a pronounced effect on the rate of photooxidation, which, however, is considerably enhanced by hydrogen peroxide. Here the excited substrate molecule would transfer excitation to hydrogen peroxide, generating hydroxyl radicals that propagate a chain reaction and carry out the oxidation process. The effect of scavenger on the rate of photodegradation also supports the existence of two different reaction routes. These measurements, carried out with isopropanol, indicate a strong retardation in the photodegradation of bromacil (rate slowed down by more than tenfold by 0,01 M isopropanol), while in the case of metribuzin the same level of scavenger slows down the reaction rate only tenfold.
- ⇒ These are yet early conclusions that deserve further studies. However, the recognition that several different direct photooxidation mechanisms could be implicated in the treatment of water pollution should be of significant practical importance, indicating the need to tailor the exact photooxidation to the specific pollutant.
- ⇒ The results obtained indicate the practical value of solar photocatalytic oxidation in the purification of highly polluted agricultural rinse waters. Further, the results of direct photooxidation of pesticides revealed the specific role of oxygen in some of the non-catalytic reactions, while in other non-catalytic processes the unique role of hydrogen peroxide could be demonstrated. These results are also of interest in the clarification of the photooxidation mechanism.
- ⇒ In the laboratory of Istanbul, preparation of  $\text{TiO}_2$  impregnated with some selected cations of the first transition series: {Cr(III), Mn(II), Fe(III), Fe(II), Co(II) and Ni(II)} using dark and photocatalytic impregnation methods has been demonstrated, and the activity of these samples in photodegradation of 4-chlorophenol in aerated aqueous solutions have been investigated. The effect of sulphate anions on the degree of 4-chlorophenol photooxidation to  $\text{CO}_2$  has also been studied. The addition of alcohols, which scavenge the holes of the illuminated  $\text{TiO}_2$  has been examined by using methanol as a model hole scavenger. BET areas for Co(II) and Ni(II) impregnated  $\text{TiO}_2$  samples have been completed.
- ⇒ The photocatalytic oxidation of aqueous solutions of phenol, p-nitrophenol, 4-chlorophenol, aniline, hydroquinone and pyridine has been studied in gas recycling reactor in the presence and in the absence of  $\text{H}_2\text{O}_2$ . Further, the initial kinetics of  $\text{CO}_2$  formation in the photocatalytic oxidation of malonic, succinic, and adipic acids has also been investigated as target pollutants. Activation energies and quantum yields are obtained and the results are published.
- ⇒ In the Bremen group the case of photooxidation of carbendazim with UV source was investigated systematically for the effect of various initial pesticide concentrations (from 0,3 to 3,0 mg/l dissolved in water), of  $\text{H}_2\text{O}_2$  concentration (ranging from 0 to 500 mg/l) and of pH variation (from pH 4 to 10).



⇒ Depending on light intensity we observed a first order decomposition kinetics as a function of both UV dose and carbendazim concentration. At a H<sub>2</sub>O<sub>2</sub> concentration of 200 mg/l decomposition rate was at maximum, whereas oxidation rate is at maximum at neutral conditions (pH 7) while in the presence of H<sub>2</sub>O<sub>2</sub> the pH dependence vanished. The overall rate of the photooxidation followed the equation,

$$r = \frac{k_1 \cdot c_{\text{Carb}} \cdot c_{\text{H}_2\text{O}_2}}{k_2 \cdot c_{\text{Carb}} + k_3 \cdot c_{\text{H}_2\text{O}_2}}$$

where the highest intensity is included in the rate constant K<sub>1</sub>.

⇒ Similar experiments were performed in the case of the carbamate pesticide pirimicarb. pH, temperature, initial concentration of substrate, and H<sub>2</sub>O<sub>2</sub> concentration influence was investigated in detail, while in this case acidic conditions yielded maximum efficiency, lower initial concentrations of substrate, higher H<sub>2</sub>O<sub>2</sub> levels and higher temperatures (max. observed at 66°C) proved to be most effective but reached saturation levels at the conditions applied.

## FOLLOW-UP

The ongoing research will go into the following directions :

- ▶ Extension of the pesticides to be photo-oxidised to pirimicarb, ethiofencarb, carbofuran, and their intermediates;
- ▶ Further elucidation of the detailed mechanism by the use of scavengers and electron spin resonance (ESR) methods to identify the crucial radical active species;
- ▶ Application of laboratory results to in-field-experiments.

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## **PARTNERS**

### **UNIVERSITÄT BREMEN**

FG Physikalische Chemie im FB2  
Postfach 330 440  
28334 Bremen  
**Germany**

Wolfram Thiemann  
Tel.: +49-421-218 23 71  
Fax: +49-421-218 49 18

### **THE VOLCANI CENTER**

Agricultural Research Organization  
Institute of Plant Protection  
P.O. Box 6  
50250 Bet Dagan  
**Israel**

Lea Muszkat  
Tel.: +972-3-968 34 40  
Fax: +972-3-960 41 80

### **BOGAZICI UNIVERSITY**

Department of Chemistry  
Bebek  
80815 Istanbul  
**Turkey**

Yuksel Inel  
Tel.: +90-1-263 15 40  
Fax: +90-1-265 97 78

**PURIFICATION AND RECYCLING OF WASTEWATER BY SOLAR-CATALYTIC AND BIOLOGICAL TREATMENT IN ALGERIA, SYRIA AND TUNISIA**

**Co-ordinator:** Technische Universität Clausthal, Clausthal-Zellerfeld, Germany (Alfons Vogelpohl)

**OBJECTIVES**

- To examine and optimise, under real conditions, the solar catalytic treatment of waste water (municipal, textile industry) in order to reach a water quality as high as possible (drinking water);
- To assess, for cases where sufficient high water quality standards cannot be realised, other possibilities for re-using lower quality water.

**ACTIVITIES**

- ◇ Adaptation of existing biological treatment plants at ITV and INRST for the purposes of this project;
- ◇ Optimisation of biological treatment for textile industry effluents;
- ◇ Production of biologically pre-treated waste water for the experiments with solar-catalytic plants;
- ◇ Construction of 4 solar Thin Film Reactors (TN, AL, SY, DE);
- ◇ Evaluation of the theoretical performance of these reactors in terms of water quality and economic feasibility and verifying these findings in small scale experiments using either biologically pre-treated waste water or crude waste water;
- ◇ Assessment of the necessity of a biological pre-treatment;
- ◇ Optimisation of operating conditions and catalysts for biological pre-treated wastewaters and for original waste water;
- ◇ Catalyst optimisation and fixation including verification of environmental impact of the processes;
- ◇ Techno-economic assessment of the designed processes;
- ◇ Solar-catalytic treatment of textile, hospital and municipal wastewater;
- ◇ Disinfection experiments;
- ◇ Construction and test of a self-sufficient PV- TFFBR.

**OUTCOME**

The solar plants have been successfully installed and operated in Algeria, Syria and Tunisia, and the biological treatment of municipal and textile wastewater has been optimised. Studies on the hydrodynamics of the Thin Film Fixed Bed Reactor (TFFBR) and on the relation between the hydrodynamics and the reaction rate velocity lead to improve degradation rates of model and real wastewater under artificial as well as under solar illumination. A biological pre-treatment improves the overall performance of the system, as only non-biodegradable components are treated photocatalytically. A reduction of indicator bacteria such as *E. coli* and *streptococcus faecalis* by solar light has been detected. A new fixation technology by heating-sprinkling simplifies the production of long-life catalyst panes for the TFFBR. Novel catalysts and commercially available titanium dioxides have been tested with textile wastewater under solar conditions.

The improvement of the TFFBR leads to a technology that is close to a transfer into a technical scale and which will be competitive to other wastewater treatment techniques in the near future.

A reduction of chlorinated organic compounds was observed during the photocatalytic treatment of hospital and textile factory effluents.

The comparison of several commercially available  $\text{TiO}_2$  powders showed that Degussa  $\text{P}_2\text{O}_5$  is the most active catalyst for many applications. Platinization of  $\text{TiO}_2$  improves the reaction velocity for the treatment of model compounds in suspended systems only.

A business management analysis proofed that treatment and recycling of textile wastewater in Tunisia and Algeria is possible with specific costs of less than 1 US\$/(m<sup>3</sup> /h).

## **FOLLOW-UP**

The feasibility and the design criteria for a solar-catalytic wastewater treatment and recycling, especially in the textile industry, were demonstrated with the TFFBR. Therefore, the scale-up of this technology is necessary for a successful transfer to practical applications. This will be done in the frame of the INCO-DC programme, within the project "Integrated Wastewater Reuse by Solar-Catalytic Treatment: A Pilot Study in the Textile Industry". The pilot plants will be designed for wastewater flows of 1 m<sup>3</sup>/h and will be installed in textile factories in Tunisia and Algeria. Within this project:

- treatment of wastewater from different origins will be investigated (e.g. washing, dyeing);
- reuse of treated water will be tested;
- existing pretreatment plants will be upgraded;
- catalysts and fixation technologies will be further improved;
- quality control will secure the safety of the process.

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## **PARTNERS**

### **TECHNISCHE UNIVERSITÄT CLAUSTHAL**

Institut für Thermische Verfahrenstechnik  
Leibnizstraße 15  
38678 Clausthal-Zellerfeld

**Germany**

Alfons Vogelpohl

Tel.: +49-5323-72 23 55

Fax: +49-5323-72 35 70

E-mail : freudenh@itv.tu-clausthal.de

### **INSTITUT FÜR SOLARENERGIEFORSCHUNG GMBH**

Sokelantstraße 5  
30165 Hannover

**Germany**

Detlef Bahnemann

Tel.: +49-511-35 85 00

Fax: +49-511-358 50 10

### **DAMASCUS UNIVERSITY**

P.O. Box 2415  
Damascus

**Syria**

Fouad Salch

Tel/Fax: +963-11-44 84 38

### **INSTITUT ALGÉRIEN DU PÉTROLE**

35000 Bourmedes

**Algeria**

Abdenour Si-Salah

Tel.: +213-2-81 18 60

Fax: +213-2-82 40 00

### **INSTITUT NATIONAL DE RECHERCHE SCIENTIFIQUE ET TECHNIQUE**

B.P. 95  
2050 Hamman Lif

**Tunisia**

Ahmed Ghrabi

Tel.: +216-1-43 02 15

Fax: +216-1-43 09 17

**MED-NPS CONTROL ON SURVEILLANCE OF NON-POINT SOURCES (NPS)  
MEDITERRANEAN (MED) POLLUTION VIA GIS: A CORINNE & EURUSTAT  
DATABASE EXTENTION**

**Co-ordinator:** Epsilon International S.A., Athens, Greece (Despina Kallidromitou)

**OBJECTIVES**

- To establish a network of Mediterranean scientific organizations that will define, develop and apply state-of-the-art GIS, remote sensing and mathematical modeling methods in an integrated database for the control and surveillance of the Mediterranean coastal water resources and the estimation of NPS pollution impact.
- To conduct a series of GIS applications aimed at mapping current pollution in the Mediterranean countries involved, that will lead to the development of a coastal water management environmental plan for the regions concerned.
- To develop a database of parameters governing non-point source pollution in the Mediterranean basin that will support the EUROSTAT, CORINE and NATURA 2000 programmes .
- To introduce an integrated concept for the study of Mediterranean basins and coastal zones interactively and encourage Med-coastal nations to adopt Integrated Coastal Zone Management (ICZM) plans as recommended in "AGENDA 21" of the UN Conference on Environment and Development (UNCED, Rio 1992).

**ACTIVITIES**

- ◇ Project Management and networking: Effective project management and establishment of networking system (via e-mail and INTERNET) for data transfer between partners and the EC.
- ◇ NPS pollution parameters and database: Selection of the NPS model, Identification, definition and quantification of eight (8) dominant water quality parameters such as total suspended solids, total nitrogen, total phosphorus, BOD, fecal coliform, total organic carbon etc. Design of relational database compatible to EC CORINE, NATURA 2000 and EUROSTAT Databases.
- ◇ Use of Remote Sensing /GIS-Methodology: Landuse recognition and classification via Remote Sensing, Test of methodology in selected watersheds using satellite data and field data; total climatic and water quality data analysis for the watersheds. Land use pattern recognition, classification and simulation in watershed and coastal zone via digital satellite image processing.
- ◇ Integration of GIS and NPS Model. GIS MED-NPS thematic mapping & mathematical modeling, Land use mapping from images using ARC/INFO (raster & vector, GRID module) on PC and UNIX workstation; application and calibration of NPS pollution and models; calculation of total NPS runoff loads from watersheds and pollution prediction.
- ◇ Database development, data collection and delivery: Development of a numerical database on ARC/INFO GIS. All data have been stored in this Dbase such as land-use types (Remote sensing analysis of SPOT, TM, ERS-1 images), runoff parameter, water quality parameters etc. The above parameters have been vectored to be input in the database..
- ◇ Applications in Italy, Greece, Turkey & Egypt
- ◇ Workshops, training, networking, dissemination Seminars and workshop organization in different countries involved and information dissemination.
- ◇ Control measures, surveillance and closure Formulation of strategies and control measures for sustainable use of water resources, design of future extension of database, presentation of results to the EC.

**OUTCOME**

*Scientific - technical results*

- ▷ Analysis in an integral way large catchment basins and Mediterranean coastal water.

- ▷ Introduction of a GIS raster and vector analysis and software in developing countries and in particular the Mediterranean.
- ▷ Establishment of a link for a major trans-Mediterranean concerted action in GIS and database development networking to be fully compatible and also transferred to the EC CORINE and EUROSTAT programmes
- ▷ Extention of research of the participating organizations in advanced non-point source pollution runoff modeling, to be utilized in conjunction with GIS, as conducted in a very limited number of EC countries (know-how)
- ▷ Introduction of a multi-disciplinary and multi-structural effort of scientists, organizations, and technologies, to be co-ordinated towards producing a product (maps, software, database) of added value for the partners and the EC (CORINE, EUROSTAT, NATURA 2000) fulfilling also UN strategic targets towards conservation and sustainable use of water and coastal resources.
- ▷ Catalogization of the environmental information for future use and planning
- ▷ Establishment links of organizations aimed at protecting the environment
- ▷ Contribution to the other integrated Mediterranean management actions (MAP-UNEP, MEDNET etc.) and EC programmes (MEDSPA, MED CAMPUS, MED URBS).
- ▷ The linkage of GIS/RS is a powerful tool to handle and provide the large amounts of detailed input data that the NPS models require, reducing uncertainty caused by spatial averaging. In addition the GIS approach has significant advantages in efficiently exploring scenarios and managing the data volumes involved.
- ▷ RS/GIS techniques can assist industries and business in facilities siting and management while greatly enhancing proper identification of potential NPS pollution sites. The models produced in this project can help determine priority areas for future management, enabling all participants to plan and allocate resources and to improve the quality of surface waters more effectively.
- ▷ The management scenarios demonstrated that reducing fertilizer application rates and availability levels would bring approximately proportional improvements in water quality related to NPS pollution.
- ▷ An analysis using the spatial capability of the model showed that the initial project definition of a critical area was a good surrogate for some pollutants, but not for others. The critical areas were displayed using the GIS.

### *Deliverables*

Software packages and the database with all data concerning the test areas (Alphone watershed, Italy, Nif basin, Turkey, El Qasr west of Alexandria, Egypt, Sperchios River catchment, Greece) with visualized results of modeling and production of ARC/INFO relational thematic maps (Runoff, Land use, basin boundaries delineation, water quality). Data have been made available with the CORINE, NATURA 2000 and EUROSTAT Databases.

### **FOLLOW-UP**

*Strategies and Control measures* - Each partner has formulated strategies and control measures for sustainable use of water resources in its country, has designed a future extension of the database.

*A further co-operation among partners*--The partners have further teamed up in other international tenders and research programmes, as for example COPERNICUS and TACIS submissions and national authority submissions. The referenced project has been the spark of this co-operation that has resulted to almost a permanent and capable team, as frequently demanded by the EC tenders of RTD programmes. The team covers scientists for various disciplines and can tackle issues of RTD and private enterprise consulting services.

*Promotion of the state-of-the-art of sciences in the non-point source pollution modeling of river basins*--The results of the project have promoted the state-of-the art in sciences and technologies related to environmental mathematical river basin modeling coupled to GIS technologies relationally distributed (data bases). Various scientists worldwide and the final concept of environmental models have requested the final results of the project and GIS interface has been innovative by all means, in addition to creating a new era of mathematical decision tools.

*Classification of the results under a methodological framework to be further investigated by the partners*--The partners and the project have classified the results in a way that individual mathematical modeling routines can be replaced as time progresses and new scientific evidence demands the substitution of the modules. This framework will stay in tact for a number of years, since, according to the recent literature surveys (e.g. <http://www.envirocom.edu>) the NPS-GIS model is among the very respected in the literature.

*Production of scientific s/w packages that are under investigation for further development*--Though the aim of the AVICENNE programme is not to force scientists to produce products, the NPS-GIS RTD results are almost at the stage to be further investigated for a further upgrade and commercialisation prior to an additional validation verification stage, to be undertaken by the partners. The s/w build under Windows, and supported by a database is attractive enough to impress the European market of scientific s/w environmental mathematical modeling under a standard and open GIS platform.

*Conductance of additional applied research for the governments of the participating countries*--All partners has utilised the project results to conduct additional RTD for their governments. Examples of this have been the results of the Sperxios river in Greece for the Region of Central Greece, and many others. Publications have resulted form these publications.

*Conductance of demonstration presentations to the authorities of the countries of concern*--All partners have assumed presentations national and international committees and their government authorities, towards safeguarding additional funds for RTD tasks.

#### *Feedback evaluation*

Feedback was requested from the cities and users. Their 3 main comments are:

1. MED-NPS is a unique system with extensions in other sectors
2. MED-NPS project has established control measures, which will in the future to benefit the countries that involved.
3. MED-NPS must be further funded.

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## **PARTNERS**

### **EPSILON INTERNATIONAL S.A.**

Kifisias Avenue 16  
15125 Athens  
**Greece**

Despina Kallidromitou  
Tel.: +30-1-680 07 00  
Fax: +30-1-684 24 20  
E-mail: [epsilon@prometheus.hol.gr](mailto:epsilon@prometheus.hol.gr)

### **NATIONAL TECHNICAL UNIVERSITY OF ATHENS**

Division of Water Resources  
Hydraulics and Maritime Engineering  
Iron Polytechniou  
15773 Athens  
**Greece**

Marc Bonazountas  
Tel.: +30-1-777 29 24  
Fax: +30-1-779 89 02

### **COASTAL RESEARCH INSTITUTE**

El-Pharaanastreet 15  
21514 Alexandria  
**Egypt**

Omran Frihy  
Tel.: +20-3-482 12 08  
Fax: +20-3-482 95 82

### **BETA STUDIO S.R.L.**

Via Monte Sabotino 2/A  
35020 Ponte San Nicolo' - PD  
**Italy**

Sergio Fattorelli  
Tel.: +39-49-896 11 20 / 10  
Fax: +39-49-896 10 90

### **DOKUZ EYLÜT UNIVERSITY**

Institute of Marine Sciences & Technology  
SSK Tesisleri D Blok Kat : 2  
35260 Konak - Izmir  
**Turkey**

Orhan Uslu  
Tel.: +90-232-425 43 38  
Fax: +90-232-483 48 49



**DATA PROCESSING FOR A MEDITERRANEAN AUTOMATED ENVIRONMENTAL MONITORING NETWORK (MEDNET)**

**Co-ordinator:** Institut Français pour l'Exploitation de la Mer (IFREMER), Nantes, France  
(Jean-Paul Berthomé)

**OBJECTIVES**

- The overall objective of this project is to undertake research in relation to the establishment of a Mediterranean environmental monitoring network. The project is based on a legacy data model of a distributed database, client/server system acting as a platform for the flow of data in delay mode/near real-time between coastal monitoring pylons/buoys, localised control stations and a central control centre. This platform is supported by the Microsoft SQL Database Server that operates under Microsoft Windows NT and incorporates the management, processing and archival of data as well as the access by end-users through a user-friendly interface. These elements will set the ground for the future setting up a real-time environmental monitoring network for the Mediterranean with a main control station in Malta and established as an extension of the RAVEL network in France;
- In particular, it is intended to study the features that such a network must possess in order to meet the requirements of the Mediterranean environment with regard to sophisticated data storage and data management techniques which will enable detailed archival patterns, easy and rapid access, and user-defined analysis protocols.

**ACTIVITIES**

- ◇ Pre-study phase  
Identification of test sites and specialised scientific fields for the experimental application of the monitoring network to be designed together with the identification of potential users and their needs. Planning of data management structures and identification of major technological problems to be tackled.
- ◇ Construction of the basic unit
  - Development and validation of the concept for a monitoring platform equipped with different sensors that automatically transmits physico-chemical parameters to a local control station. The platform will be designed and tested for long deployments, ease of servicing and for continuous acquisition of data at all weather conditions.
  - Experiments will be conducted in order to test and improve the data management system and the data transmission to the control station in Brest.
- ◇ Operation of the basic unit
  - This will allow to verify the feasibility of the chosen approach and to test all system components under real life conditions. It will also generate enough data to carry out experiments with regard to the user interface to be developed in this project.
  - Development of algorithms for the automated detection of thresholds/jumps in data sets.

**OUTCOME**

- ▷ Monitoring platforms will be designed and tested under real life conditions with regard to the possibility to integrate them into an automated environmental monitoring system for the Mediterranean Sea;
- ▷ Data handling procedures and protocols will be developed and tested as well as the necessary technologies that allow the efficient and fast processing and exchange of the data generated;
- ▷ The project will therefore provide a sound basis for the development of a larger Mediterranean network for the monitoring of the state of the environment.

## **PARTNERS**

### **INSTITUT FRANÇAIS POUR L'EXPLOITATION DE LA MER (IFREMER)**

Direction Environnement Littoral Nantes  
Rue de l'Île d'Yeu  
B.P. 1105  
44311 Nantes Cedex 3

**France**

Jean-Paul Berthomé  
Tel.: +33-2-40 37 40 00  
Fax: +33-2-40 37 40 01

### **MINISTRY OF AGRICULTURE AND NATURAL RESOURCES**

Department of Fisheries  
Aeolou 13  
Nicosia  
**Cyprus**

Andreas Demetropoulos  
Tel.: +357-2-30 32 79  
Fax: +357-2-36 59 55

### **MORS - GROUP**

Department of Environnement  
Rue Galvani 3  
91300 Massy

**France**

Michel Combier  
Tel.: +33-1-64 47 19 19  
Fax: +33-1-64 47 19 32

### **MALTA COUNCIL FOR SCIENCE AND TECHNOLOGY**

Marine Sciences Network  
West Street 112  
Valetta  
**Malta**

Aldo Drago  
Tel.: +356-24 11 76  
Fax: +356-24 11 77

### **EURO-MEDITERRANEAN CENTRE IN MARINE CONTAMINATION HAZARDS**

Foundation for International Studies  
St Paul Street  
Valetta  
**Malta**

Anton Micallef  
Tel.: +356-24 07 46 / 23 41 21/2  
Fax: +356-23 05 51

**MULTIDISCIPLINARY STUDY OF DRINKING AND AGRICULTURAL WATER  
TREATMENT USING ULTRAVIOLET LIGHT SOURCES SUPPLIED BY SOLAR  
ENERGY IN RURAL ENVIRONMENTS**

**Co-ordinator:** Université Paul Sabatier -Toulouse III, Toulouse, France (Jean-Jacques Damelincourt)

**OBJECTIVES**

- To improve the biological quality of rural water sources through phototreatment, allowing the water to be stored without deterioration;
- To prove the scientific/technical and economic feasibility and reliability of a small solar energy based water treatment with ultraviolet light where the system components are largely derived from present knowledge and technology through (a) modelling (b) experiments;
- To optimize the "solar energy-power storage-discharge power supply mode-emitted radiation spectrum" conversion chain for photo-biological action on certain biological pollutants;
- To develop a complementary ultraviolet radiation treatment, making wastewater fit for irrigation in market gardening and seed production;
- To develop an effective communication network between participants from different disciplines.

**ACTIVITIES**

- ◇ Definition of identifiers for the efficiency of different treatments with regard to certain biological pollutants to be measured following a standardized protocol;
- ◇ Different ultraviolet lamps and circuits will be modelled and these models will be coupled. Lamps will subsequently be developed and tested for their efficiency;
- ◇ Existing solar panels will be used but tests will be made on the products of Tunisian laboratories;
- ◇ Modelling the entire system;
- ◇ Proving, scientifically and economically, the feasibility, workability and reliability of an ultraviolet water treatment unit, build on the basis of modelling results. To this end, small test facilities, which can supply drinking water to a dwelling, and a medium power pilot scale test facility for study of end-treatment of waste water will be set up.

**OUTCOME**

The realisation of a pilot version of the electronic power supply for high pressure UV lamps is accomplished. This power supply has been tested with some high pressure lamps operating up to 3 kW fabricated during the previous project stage. The system's operating stability has been demonstrated and the influence of external parameters (as power, frequency and duty cycle) on the lamp characteristics (UV production and electric parameters) is now in study.

The Self-Consistent Collisional Radiative Code developed during the previous stage of the project has been used in order to determine the optimal lamp parameters (rare gas filling pressure and type, arc current). Our objective was to obtain these optimal values for a low pressure lamp producing a large quantity of UV radiation density at 254nm, with an acceptable efficacy, at every cold spot temperature among 10 and 40 °C.

A new computer code has been developed in order to describe the thermal exchange between the low-pressure lamp and its surrounding (air and water). In this model we take into account free convection of air between the lamp wall and the external quartz tube as well as thermal conduction. This code permits to calculate the cold spot temperature as function of the lamp external characteristics and the water temperature in the proximity of the external quartz tube for a given system configuration.

A radiative transfer computer code is now under realisation. This code will be able to predict with a high accuracy the radiation output from a discharge lamp if the plasma characteristics are known. At this stage it is almost difficult to conceive a "global" code for both high and low pressure discharges. Thus the work of one institute is devoted to the low-pressure version whereas another institute works on the high pressure. The elaboration of a numerical code describing the photo-reactor is also under work.

Several low pressures Hg-rare gas lamps are under realisation. These lamps will use in order to test the validity of numerical codes that we developed. Then the optimal lamps, as predicted by the calculation will equip the experimental integrator that is now under construction.

An experimental apparatus (integrator) for the ultraviolet flux measurements is almost accomplished in Tunisia. This apparatus, especially adapted for the low-pressure lamp geometry, will permit the experimental characterisation of these lamps before integration in the final system photo-reactor.

Finally, an experimental protocol for the biological quality testing of the water has been established by.

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## **PARTNERS**

### **UNIVERSITÉ PAUL SABATIER - TOULOUSE III**

Laboratoire des Décharges dans le Gaz  
Route de Narbonne 118  
31062 Toulouse Cedex 4

**France**

Jean-Jacques Damelincourt

Tel.: +33-5-61 55 68 63

Fax: +33-5-61 55 63 32

### **ECOLE NORMALE SUPÉRIEURE DE L'ENSEIGNEMENT TECHNIQUE**

Avenue Taha Hussein 5  
1008 Tunis

**Tunisia**

Slaheddine Gherissi

Tel.: +216-1-49 30 46

Fax: +216-1-39 11 66

### **INSTITUT NATIONAL DE LA RECHERCHE SCIENTIFIQUE ET TECHNIQUE**

B.P. 95  
2050 Tunis-Hammamlif

**Tunisia**

Mohamed Annabi

Tel.: +216-1-43 02 15

Fax: +216-1-43 09 17

### **UNIVERSITÉ ABDELMALEK ESSAADI**

Faculté des Sciences de Tetouan

B.P. 21

M'Hannech II

Tetouan

**Morocco**

Adel Asselman

### **CENTRE D'INGÉNÉRIE ET DE RECHERCHE TECHNOLOGIQUE EN ÉLECTRONIQUE MODERNE (CIRTEM)**

B.P. 661  
31319 Labège

**France**

Christian Saubion

Tel.: +33-5-62 24 26 00

Fax: +33-5-62 24 26 24

### **ETABLISSEMENT GUY DARIC S.A.**

Rue du Goulet 26  
93302 Aubervilliers

**France**

Maurice Dechaume

Tel.: +33-1-43 52 37 00

Fax: +33-1-43 55 27 863

**DISINFECTION OF DRINKING WATER & TREATED DOMESTIC WASTE WATER  
BY CHLORINE AND CHLORINE DIOXIDE**

**Co-ordinator:** University of Crete, Heraclion, Greece (Euripides Stephanou)

### OBJECTIVES

- Develop and apply analytical methods for the determination of organic (trihalomethanes, haloacetic acids and trihaloacetonitriles) and inorganic (especially  $\text{BrO}_3^-$ ,  $\text{ClO}_2^-$  and  $\text{ClO}_3^-$ ) disinfection by-products (DBPs), and their precursors (especially organic carbon TOC and  $\text{Br}^-$ ) in drinking water and treated wastewater.
- Characterize the organic and ionic chemical composition of raw drinking water and wastewater effluents after biological treatment.
- Determine the DBPs in drinking waters of the Mediterranean area and to establish a qualitative and quantitative relationship with their precursors.
- Perform kinetic experiments with real samples and model compounds in order to better understand the formation of DBPs, and
- Compare the potential of disinfection and of the DBPs formation between chlorine and chlorine dioxide by performing experiments with biologically treated effluents.

### ACTIVITIES

#### *DEACUC activities:*

- ◇ Development of analytical methods for the determination of organic (e.g. trihalomethanes, trihalogenated acetic acids etc.) and inorganic (e.g. oxy-anions) disinfection by-products in drinking and wastewater.
- ◇ For the organic DBPs a closed-loop-stripping extraction (CLSE) technique with gas chromatography and electron capture detection (GC-ECD) was developed and applied while for the inorganic DBPs ion chromatography (IC) was mainly used.
- ◇ Determination of DBPs in drinking and treated wastewater of different Greek cities
- ◇ Wastewater-disinfection investigations.  
The determination of surfactants, with high-pressure liquid chromatography (HPLC), in detergent formulation used in Greece and determination of surfactants in all stages of treatment of a wastewater plant. In addition ions and dissolved organic carbon (DOC) in all stages of treatment of a wastewater plant were also analyzed.
- ◇ Reaction kinetic experiments of disinfectants with surrogate and real samples.  
Experiments on the disinfection of wastewater with chlorine and chlorine dioxide by controlling the disinfectant-dose and disinfection-time effect on microbial activity and disinfection by-products formation potential. In addition experiments on the disinfection reaction with chlorine and chlorine dioxide with model compounds (e.g. linear alkyl benzene sulphonates (LAS)) were performed.

#### *TECHNION activities:*

- ◇ Wastewater-disinfection investigations.  
TECHNION paid particular effort to the investigation of the effects of the use of chlorine dioxide as disinfectant in laboratory and pilot scale. The combined use of chlorine and chlorine dioxide was explored. Experiments on the disinfection of wastewater with chlorine and chlorine dioxide by controlling the disinfectant-dose and disinfection-time effect on microbial activity and disinfection by-products (total organic halides (TOX) and chlorite and chlorate ions) formation potential. In addition the use of ferrous ions to the minimisation of the concentration of chlorite ions has been studied.
- ◇ Reaction kinetic experiments of disinfectants with surrogate and real samples.  
TECHNION studied thoroughly the interaction of chlorine dioxide with another important category of surfactants, namely the non-ionic surfactants. These studies were carried out in wastewater and as well as in synthetic aqueous solutions.

#### *SGL activities:*

- ◇ Application of analytical methods for the determination of organic recalcitrant residues (e.g. non-ionic and anionic detergents) in wastewater with gas chromatography mass spectrometry (GC/MS)
- ◇ Inter-comparison between DEACUC and SGL was also performed.
- ◇ Determination of DBPs and of their precursors (dissolved organic carbon and bromide ions) in drinking and treated wastewater of Nicosia (Cyprus).
- ◇ Wastewater investigations.
  - The determination of surfactants in all stages of treatment of a wastewater plant.
  - Examination of the reaction kinetic experiments of disinfectants with surrogate and real samples.
  - Experiments on the disinfection of wastewater with chlorine and chlorine dioxide by controlling the disinfectant-dose and disinfection-time effect on disinfection by-products formation potential. Experiments on the disinfection reaction with chlorine and chlorine dioxide with model compounds (e.g. linear alkyl benzene sulphonates (LAS)), were also performed.

## **OUTCOME**

### *Scientific-technical results*

- ⇒ The analytical methodology for the determination of DBPs in drinking water, Closed Loop Stripping Extraction (CLSE) of analyses and GC-ECD, GC-MS analysis was further improved. Detection limits for both non-polar (trihalomethanes (THMs)) and polar (haloacetic acids (HAA) and haloacetonitriles (HAN)) DBPs were dropped to the lower ppt levels. In order to extend our study to the determination of all possible inorganic disinfection by-products we improved and modified the analytical protocol based on ion chromatography. We now have an analytical protocol allowing the reliable determination of a large range of ions. Among these ions we can determine chlorate, chlorite and bromate.
- ⇒ The analysis of chlorination DBPs in drinking waters of Greek cities (e.g. Heraclion, Athens, Thessaloniki, Patras, Ioannina and others) and Nicosia, gave the following results: In Athens for the period 1994 to 1995 the bromo and bromo/chloro THMs were in higher concentrations than chloroform. In 1996 the chlorinated species and especially chloroform and bromodichloromethane predominated over the more brominated ones. The high degree of bromine incorporation is to be related with ground water quality degradation observed especially in coastal cities. Ground water from different wells showed a seasonal trend, for the years 1995 to 1996, for chloride and bromide ion concentrations. These ions showed the same seasonal trend (i.e. chloride and bromide concentrations increased from June to October and then decreased from November to March). All ground water samples exhibited a stoichiometric relationship between chloride and bromide level, which was near-by the same as in seawater, indicating an intrusion of the later to these wells. The bromide ion concentrations observed in coastal cities (e.g. Heraclion) ground waters and the average free available chlorine (FAC) doses used (high molar ratio  $[\text{Br}^-]/[\text{Cl}^-]$ ), are the determining factors of the high bromine incorporation in these drinking waters. TOC concentrations were low enough (0.2 to 0.5 ppm) and do not seem to be a determining factor on the THM speciation and concentration. In Cyprus a seasonal trend (increase) was observed for the chloro/bromo THMs, during the autumn and early winter period. This trend was not observed for Heraclion drinking waters where  $\text{CHBr}_3$  was by far the most abundant THM. This increase was not observed for  $\text{CHCl}_3$ . Raw waters were examined in Cyprus also in respect to their  $\text{Br}^-$  concentration. Bromide ion concentrations in Cyprus were in the same order of magnitude as Heraclion, but not a stoichiometric correlation was established between chloride and bromide concentrations. Thus the presence of bromide in Cyprus raw waters is apparently not connected (at least for the area of Nicosia) with seawater intrusion as in Heraclion. The presence of bromide in these raw waters should be further investigated. The TOC content of Cyprus raw waters was in the same order of magnitude as in Heraclion. The one-year mean concentrations determined in the above cities (in Greece and Cyprus) comply the USEPA and WHO requirements for maximum contaminant levels (MCLs). Some bottled waters of Greece in which ozone is used as a disinfectant were characterised by relatively high concentration of bromate ions (35-170 ppb), when bromide ions were present in the raw water before disinfection. These waters originate mainly from coastal areas.

### *Deliverables*

- ⇒ Quantification of the process of formation of organic and inorganic disinfection by-products (DBPs) during the disinfection of drinking and wastewater with chlorine and chlorine dioxide.
- ⇒ Determination of the main disinfection by-products in biologically treated effluents after their disinfection with chlorine and chlorine dioxide.
- ⇒ Assessment the possibility of reuse of disinfected effluents.
- ⇒ Choice of a disinfectant for drinking water or wastewater in relation to their content in DOC and ions (e.g. bromide).
- ⇒ Analytical chemical protocols for the determination of organic and inorganic DBPs in both drinking water and wastewater treatment plant effluents.

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## **PARTNERS**

### **UNIVERSITY OF CRETE**

Department of Chemistry  
Division of Environmental Chemistry  
Environmental Chemical Processes Laboratory  
71409 Heraclion  
Greece

Euripides G. Stephanou  
Tel.: +30-81-39 32 00 / 39 32 77 / 39 33 13  
Fax: +30-81-21 09 51 / 23 68 56  
E-mail: estef@cc.uh.gr

### **STATE GENERAL LABORATORY**

Kimonos 44  
138 Nicosia  
Cyprus

Michael Constantinos  
Tel.: +357-2-30 50 62  
Fax: +357-2-31 64 34

### **TECHNION - ISRAEL INSTITUTE OF TECHNOLOGY**

Environmental and Waste Resources Engineering  
32000 Technion City  
Haifa  
Israel

Nava Narkis  
Tel.: +972-4-829 23 59 / 829 27 78  
Fax: +972-4-822 88 98

**NATURAL CLAYS, MODIFIED OR SHARPENED, AS CATALYSERS FOR  
PURIFYING NATURAL OR INDUSTRIAL WATER, MATERIALS AND  
MECHANISMS**

**Co-ordinator:** Centre National de la Recherche Scientifique, Orléans, France (Faiza Bergaya)

**OBJECTIVES**

- To study the elimination of pollutants from a water stream through catalytic oxidation and to increase the knowledge about the mechanisms of oxidation in a very diluted aquatic milieu, two processes are to be analysed:
- catalytic oxidation of organic pollutants dissolved in water, in order to realise a re-utilisation of the treated water either in industry or for human consumption;
  - selective and catalytic oxidation of secondary industrial products (dissolved in water) in order to realise, on the one hand, their recycling and, on the other hand, to provide sufficiently clean water that can be re-used.

**ACTIVITIES**

- ◇ Preparation of catalytic materials: mixed systems (Al-Fe); synthesising bridged clays where the kегgin ions are exchanged against other ions such as Fe, Cu, CO, Mn; preparation of bridges clays on basis of zirconium, cerium or titanium or mixtures thereof where one of these ions is to be replaced by Fe, Co, Cu or Mn; intercalation of polyanions based on molybdenum or vanadium in anionic clays;
- ◇ Physico-chemical characterisation of materials: chemical composition, specific surface, volume of pores, interlamal distance, composition of the intercalated aggregates;
- ◇ Determination of catalytic properties: laboratory scale experiments to select the most performing materials, extrapolation of results to pilot scale. These experiments are also used to analyse the catalytic mechanism in terms of relation between activity/selectivity of the oxidation and the nature of the catalyst, in particular the bridging aggregate aiming at the production of a isopreous material.

**EXPECTED OUTCOME**

- ▷ Several alternative materials are developed and tested in view of their catalytic performance and the most promising are tried at pilot scale. This will provide important information on the overall potential of this technology to be used for wastewater treatment;
- ▷ Basic knowledge will be generated on the mechanism determining the performance of catalysts under the tested conditions, i.e. in very diluted aquatic milieu;
- ▷ The potential of this technology will be assessed to realise a water treatment efficiency allowing for a recycling of the water to industry or to human consumption and, in case of industrial effluents, also enabling a recycling of valuable elements.

## **PARTNERS**

### **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE**

Rue de la Ferrollerie 1B  
45071 Orléans Cedex 02

**France**

Faiza Bergaya  
Tel.: +33-2-38 51 53 72  
Fax: +33-2-38 69 70 31

### **ÉCOLE SUPÉRIEURE DE TECHNOLOGIE DE FES**

Laboratoire de Chimie  
Route D'Immouzer  
B.P. 2427

Fes

**Morocco**

Abdellah Kherbeche  
Tel.: +212-5-60 05 85 / 86  
Fax: +212-5-60 05 88

### **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE**

Laboratoire de Chimie de l'Eau  
URA 1468 - ESIP  
Avenue du Recteur Pineau 40  
860022 Poitiers Cedex

**France**

Marcel Dore  
Tel.: +33-5-49 45 39 16  
Fax: +33-5-49 45 37 68

### **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE**

Laboratoire de Catalyse  
URA 350 - ESIP  
Avenue du Recteur Pineau 40  
860022 Poitiers Cedex

**France**

Jöel Barrault  
Tel.: +33-5-49 45 39 99  
Fax: +33-5-49 45 33 49

### **INSTITUT NATIONAL DES HYDROCARBURES & DE LA CHIMIE**

Avenue de l'Indépendance 8  
35000 Bourmedes

**Algeria**

Djoughra Messad  
Tel.: +213-2-82 34 20  
Fax: +213-2-82 63 73

**CHARACTERISATION OF WATERS IN NORTH-WEST TUNISIA POLLUTED BY  
HEAVY METALS AND DEVELOPMENT OF SEPARATION TECHNIQUES  
ADAPTED TO THEIR TREATMENT**

**Co-ordinator:** Institut National Polytechnique de Lorraine, Vandoeuvre, France (Pierre Blazy)

**OBJECTIVES**

- To develop a methodology to analyse the mechanism of the pollution of surface waters with heavy metals;
- To establish a manual for the impact assessment of mining (and industrial) sites on water pollution;
- To study de-pollution technologies adapted to the treatment of surface waters polluted with heavy metals originating from mining or other industries in order to assess their applicability;
- To apply this methodology and these technologies to the characterisation and treatment of the polluted waters in north-west Tunisia (Oued Medjerda and his sources) contaminated with metals such as As, Hg, Cd, Pb, Zn, Mn, Cu, Fe in order to verify their efficiency.

**ACTIVITIES**

- ◇ Characterisation of the pollution : establishing a hydrological balance and an inventory of the metal content of the Oued Medjerda and his upstream rivers in order to quantify the contribution of the different pollution sources;
- ◇ Analysis of the mechanisms of the pollution of the Oued Medjerda : definition and application of a methodology which allows to understand the mechanisms of mobility of heavy metals from mineral phases of the sites and neighbouring regions in order to understand the kinetics of the solution of heavy metals and the contribution of the different mineralogical phases to the pollution of the waters of the Oued Medjerda;
- ◇ Elimination of heavy metals from the water of the Oued Medjerda : search for appropriate solutions for the de-pollution of the water of the Oued Medjerda and its effluents following a conventional and a non-conventional approach;
- ◇ Techno-economic evaluation of the approaches.

**OUTCOME**

- ⇒ Reliable map of the heavy metal pollution in the north western part of Tunisia;
- ⇒ Understanding of the pollution mechanism and the relative contribution of natural (demineralisation processes) and man made pollution sources;
- ⇒ Comparative assessment of different de-pollution techniques and techno-economic assessment of the most promising ones;
- ⇒ Appropriate methodology for the characterisation of pollution and technologies for the de-pollution of rivers and effluents in the mining region of N-W Tunisia, allowing to separate important elements;
- ⇒ Manual for the impact assessment of mining sites with regard to water pollution.

## **PARTNERS**

### **INSTITUT NATIONAL POLYTECHNIQUE DE LORRAINE**

Ecole Nationale Supérieure de Géologie  
Centre de Recherche sur la Valorisation des Minéraux  
Laboratoire Environnement Minéralurgie  
LEM CNRS UA 235  
Rue Doyen Marcel Roubault  
B.P. 40  
54501 Vandoeuvre Cedex  
**France**

Pierre Blazy  
Tel.: +33-3-83 59 63 31  
Fax: +33-3-83 55 21 53

### **ECOLE NATIONALE DE L'INDUSTRIE MINÉRALE**

Département Mines et Traitement  
Hadj Ahmed Cherkaoui - Agdal 753  
10000 Rabat  
**Morocco**

Bouchaib Marouf  
Tel.: +212-7-77 13 60  
Fax: +212-7-77 10 55

### **COMPAGNIE MINIÈRE DU NORD OUEST**

Rue du Kenya 4  
1002 Tunis - Belvédère  
**Tunisia**

Amor Oueslati  
Tel.: +216-1-28 12 60  
Fax: +216-1-78 26 07

**DEVELOPMENT OF SENSORS FOR ON-SITE MONITORING OF THE EXTENT OF POLLUTION IN WATER RESOURCES**

**Co-ordinator:** University of Newcastle-Upon-Tyne, Newcastle-Upon-Tyne, United Kingdom  
(Calum J. McNeil)

**OBJECTIVES**

- To develop a system for monitoring the extent of pollution of water resources based on low-cost, highly sensitive amperometric biosensor instrumentation that utilises biological recognition components (chelators and antibodies) in intimate contact with electrode surfaces for signal transduction and pollutant determination. The pollutants that will be considered are heavy metal ions, pesticides and bacteria;
- To develop a user-friendly, compact and reliable instrument for field use.

**ACTIVITIES**

- ◇ Design, synthesis and testing of metal-specific complexing agents suitable for immobilisation on printed disposable electrodes for voltammetric analysis of heavy metal pollution;
- ◇ Development of electrochemical immunosensors for measurement of pesticide and bacterial contamination. The biological recognition elements (antibodies) were to be immobilised at or near the surface of disposable electrodes and two approaches to electrochemical detection of enzyme label activity assessed and optimised. Horseradish peroxidase to be used as the enzyme label for bacterial immunosensors and glucose oxidase for pesticide immunosensors;
- ◇ Manufacture of generic disposable printed electrodes, which could be employed in all aspects of the project simply by changing the surface recognition components;
- ◇ Development of low-cost instrumentation.

**OUTCOME**

- ⇒ The major objective of identification and synthesis of compounds that displayed suitable electrochemistry to be used as specific chelators for heavy metal ions resulted in the development of electrodes for Cu, Cd, Al and Pb. These are presently being incorporated into the disposable electrodes by both screen printing and ink-jet printing techniques and are available for in-field testing.
- ⇒ A second major objective has seen the development of a one-step, separation-free, electrochemical immunosensor system for the bacteria *Escherichia coli c600*, *Salmonella typhimurium* and *Bacillus subtilis*. These have been demonstrated and have been adapted for use in conjunction with disposable screen-printed electrodes. This approach has been published in *Biosensors and Bioelectronics*.
- ⇒ In a third major objective, a new immunosensor system for one-step immunoassay of pesticides such as atrazine has been developed. The immunosensor employs a competitive immunoassay with glucose oxidase-pesticide conjugates and direct electron transfer to horseradish peroxidase immobilised at an electrode surface.
- ⇒ Electrochemical biosensor instrumentation has been constructed and is undergoing evaluation in Malta.

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## **PARTNERS**

### **UNIVERSITY OF NEWCASTLE UPON TYNE**

The Medical School  
Department of Clinical Biochemistry  
Framlington Place  
Newcastle Upon Tyne NE2 4HH  
**United Kingdom**

Calum J. McNeil  
Tel.: +44-191-222 69 31  
Fax: +44-191-222 79 91

### **TEL AVIV UNIVERSITY**

Department of Molecular Microbiology & Biotechnology  
Ramat Aviv  
69978 Tel Aviv  
**Israel**

Judith Rishpon  
Tel.: +972-3-640 90 80  
Fax: +972-3-640 94 07

### **UNIVERSITY OF MALTA**

Department of Biomedical Sciences  
Msida  
**Malta**

Joseph Victor Bannister  
Tel.: +356-31 66 55  
Fax: +356-31 05 77

**SURVEILLANCE OF POLLUTION IN THE MEDITERRANEAN SEA: MARINE ORGANISMS AS UBIQUITOUS MARKERS - NOVEL APPROACH**

**Co-ordinator:** Johannes Gutenberg Universität, Mainz, Germany (Werner E. G. Müller)

## **OBJECTIVES**

- To study and characterize the stress proteins and the MFO system of four common Mediterranean organisms (the gullhead seabream *Sparus aurata*, the colonial sea squirt *Botryllus schlosseri*, the siliceous sponge *Geodia cydonium*, and mytilid mussels) under defined field and laboratory conditions.
- To study the effects of pollutants on cell cultures raised from tunicates and sponges.
- To develop an array of probes (oligonucleotides and antibodies) for fast and simple detection of stress protein levels.
- To establish a combined bioassay method to survey different levels and sources of pollution in the Mediterranean Sea.

## **ACTIVITIES AND OUTCOME**

### *Scientific-technical results*

In general, the biomarker concept is involved with the use of either biochemical, cellular or physiological parameters. We concentrated mainly on the molecular aspects with respect to the whole organisms. We used hsp70 from the marine sponge *Geodia cydonium*, cytochrome P450 and metallothioneines from fish.

The first objective of our study was to characterise the MFO system of common Mediterranean organisms. We completely fulfilled this objective, since metallothioneines and cytochrome P450 were cloned from a common sparid fish, which is found in all Mediterranean habitats (papers submitted for publication and may be obtained upon request [because of lack of time to get the reprints from the partners]) and the hsp70 from a Mediterranean sponge (reprints [references] included [see below]).

Then we established a bioassay to survey different levels of pollution in the Mediterranean Sea using Haifa Bay as a model system. In Haifa, we followed four different stations from a more polluted to a less polluted areas. We found that the fish cytochrome P450 and metallothioneines (response to mercury pollution) are responding to different levels of pollution not only in our laboratory experiments but also in the field. With the sponge we could establish that the gene expression of hsp70 is upregulated not only under physiological conditions but also and - much more dramatic - by environmental anthropogenic load.

In addition, we established the maintenance of first primary cells from the sponge *Suberites domuncula*. They were used to determine environmental stressors on the expression of hsp70 and also of the MDR system (reprints [references] included [see below]).

### *Demonstrations*

Field studies were already performed during the course of the research and even now, thereafter. We are routinely monitoring fish in Haifa Bay for the expression of metallothioneine and cytochrome P450. Furthermore, the expression studies of hsp70 have been done and are still in progress in the Rovinj area (Northern Adriatic) to evaluate anthropogenic stress on marine animals.

We still need the help of grant supporting agencies to fine-tune our methods. For example, in the field we have recently found that the levels of cytochrome P450 and metallothioneines change in accordance with water temperature and/or the reproduction season of the fish. Therefore, there is a need for further experimentation to evaluate all facts of our techniques. Concerning hsp70, this biomarker turned out to be very reliable in the field for the detection of chronic stress caused by low concentrations of cadmium and zinc. Further studies are needed to test the variation of hsp70 with the physiological status of the animals (sponge) especially under various field conditions.



### *Commercialization*

At this stage of the research, we may suggest our method(s) to be adapted as routine test systems for monitoring of anthropogenic stress in the marine environment. We chose common Mediterranean animal species so that other scientists along the coasts of the Mediterranean Sea can easily apply the methods established by us for their own needs.

### **SELECTED PUBLICATIONS**

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### **PARTNERS**

#### **JOHANNES GUTENBERG-UNIVERSITÄT**

Institut für Physiologische Chemie

Düsbergweg 6

55099 Mainz

**Germany**

Werner E.G. Müller & Karl Spath

Tel.: +49-6131-39 59 10

Fax: +49-6131-39 52 43

#### **MIDDLE EAST TECHNICAL UNIVERSITY**

Department of Biology

Inoenue Bulvari

06531 Ankara

**Turkey**

Emel Arinc & Sevuck

Tel.: +90-312-210 10 00 / 31 05 / 31 06

Fax: +90-312-210 12 89

#### **NATIONAL INSTITUTE OF OCEANOGRAPHY**

Tel Shikmona

P.O. Box 8030

31080 Haifa

**Israel**

Baruch Rinkevich & Moshe Levi

Tel.: +972-4-51 52 02

Fax: +972-4-51 19 11

#### **MANSOURA UNIVERSITY**

Faculty of Pharmacy

Department Pharmacognosy

35516 Mansoura

**Egypt**

Farid Badria & Argul

Tel.: +20-50-34 62 53

Fax: +20-50-34 79 00

## **TOXIC SOLID WASTE TERRESTRIAL AND AQUATIC IMPACT PROBLEMS AND SOLUTIONS**

**Co-ordinator: University of Patras, Patras, Greece (S. P. Varnavas)**

### **OBJECTIVES**

- To assess the environmental impact of sulphide mining activities in view of the leaching of sulphide related elements such as Fe, Cu, Zn, Pb, As, Cd from mine tailing.

### **ACTIVITIES**

- ◇ Geochemical mapping of the distribution of sulphide related elements such as Fe, Cu, Zn, Pb, As, Cd around the mine tailings;
- ◇ Chemical and mineralogical analysis of mine tailings in order to determine the input of metals in the surrounding environment;
- ◇ Soil analysis with regard to heavy metal content, water movement and absorption of metals in sediments;
- ◇ Study of the uptake of metals by plants (root vegetables), growing in the neighbourhood of the tailings and modelling of heavy metal distribution in soils and streams;
- ◇ Modelling the behaviour of metals in streams (water and sediments);
- ◇ Sampling of sea water, sediments and beach sands at coasts close to mining activities.

### **OUTCOME**

- ⇒ The project has provided a comprehensive picture of the environmental situation around mining sites in the Mediterranean region.
- ⇒ Together with the deepened understanding of the related processes, this has provided a better base for any management decisions and land use activities in these areas, including agricultural or horticultural use of contaminated soils.
- ⇒ A model to calculate the transport of metals from mineral waste in the Limni Mine area, Cyprus to the Chrysochou bay has been developed. Model calculations show that the bulk of copper transported to the bay is particulate, and chemically inert. Cadmium on the other hand is transported mainly in chemically active form. The model is capable of reproducing reasonably well the measured sediment composition of the Limni stream. Model calculations further show that the water composition with respect to dissolved constituents at the mouth of the Limni stream is fairly constant, mainly due to the very high alkalinity of the headwater (7-7.5 meq/l), which is capable of neutralising large quantities of acid mine drainage water. Therefore, the pH of the Limni water at the point where it enters the sea is always (in the calculation) among 7.9 and 8.35. In the period following a (simulated) rainfall event of 200 mm in the mountains east of the Limni mine, about 2 tons of copper are transported to the Chrysochou bay from the Limni stream alone.
- ⇒ The soils occurring in the vicinity of the Limni mine mineral waste in which wheat and peanut are cultivated are markedly polluted with Cu, Zn, Cd, Mo, Se, As and Pb.
- ⇒ Significant toxic metal enrichments were found in cultivated plants.
- ⇒ Of the metals related to the toxic solid waste Cu, Si, Zn and Fe accumulate in a higher degree in the roots than in the remaining parts of the plant. By contrast, Zn and Mn accumulate in a higher degree in the upper part of the plant than in the roots.
- ⇒ It is revealed that large quantities of mineral waste were dispersed on the beach being a constant source of toxic metals to the marine environment. Copper and Zn are markedly enriched at the seawater beach interface whereas As is mostly enriched at the upper zone of the beach.
- ⇒ Pyrite content in the Limni Mine beach reaches 65.4%, while at 200m offshore, it reaches the value of 24%.
- ⇒ The acid mine drainage waters of the tailings contain significant amounts of Fe, Cu, Zn and S indicating the continuous release of these elements from the mining tailings during the wet season and their dispersion in the surrounding area.

- ⇒ The coastal seawaters are enriched in dissolved Fe, Zn and Cu relative to eastern Mediterranean seawaters indicating that the released Fe, Cu and Zn from the mining tailings reach the marine environment.
- ⇒ In the seafloor sediments, significant amounts of Fe, Mn, Cu, Zn, Pb and Ni are present in the acetic acid soluble fraction indicating that they are loosely held in the sediments. It is therefore implied that significant amounts of the above metals have recently accumulated on the seafloor.
- ⇒ A large part of the bay is influenced by the chemical weathering of the waste since it is covered by sediments rich in metals identical to those encountered in the waste (i.e. Cu, Pb, Co, Mn, Zn).
- ⇒ The stream sediment analysis showed that the streams Argaki Limni and Argaki Karioulasi are the main paths through which significant amounts of metals such as Cd, Cu, Ni, Cr, Zn are dispersed away from the waste mounds.
- ⇒ Significant enrichments of Cd, Cu, Ni, Cr and Zn were located in the stream sediments very close to the coast.
- ⇒ The EDTA leaching analysis have shown that the above metals are easily removable from the sediments. Therefore, they enter the surrounding soils during the wet season in the soluble form. In addition, they may enter the marine environment both incorporated in the transported sediments and in the dissolved form.

### **FOLLOW-UP**

Since it has been revealed that the chemical weathering of the mineral waste affected a large part of the surrounding mining area including areas where food production takes place, it is proposed to carry out research towards:

- ▶ The remediation of all areas where food production takes place such as the soils and the coastal zone and offshore area;
- ▶ The prevention of further weathering of mineral waste, the degree of which increases very rapidly;
- ▶ The detail determination of the quantities of toxic metals taken by the population with their food;
- ▶ The dispersion of toxic metals by dust to the urban environment, marine and agricultural environment;
- ▶ The determination of the influence of the bad quality of seawater related to the weathering of mineral waste on the seafood and the fish.

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Modelling the heavy metal behavior in the Limni Mine area, Cyprus (to be submitted to the Environmental Technology).

Seawater-toxic solid waste interaction experiments. The Limni Mine Cyprus Case (to be submitted to the Science of the Total Environment).

Distribution of toxic metals in plants in the Limni Mine Area, Cyprus (to be submitted Fresenius EnvironmentalBulletin).

Impact of the mining waste on the Limni Mine, Cyprus, coastal and offshore environment (to be submitted to the Marine Pollution Bulletin).

## **PARTNERS**

### **UNIVERSITY OF PATRAS**

Department of Geology  
261 10 Patras  
**Greece**

S.P. Varnavas  
Tel.: +30-61-99 19 72  
Fax: +30-61-99 19 96

### **INSTITUTE FOR SOIL FERTILITY RESEARCH**

Oosterweg 92  
P.O. Box 30003  
9750 Haren  
**The Netherlands**

W. Salomons  
Tel.: +31-50-33 77 77  
Fax: +31-50-33 73 93

### **ISRAEL OCEANOGRAPHIC AND LIMNOLOGICAL RESEARCH**

Telshikmova  
P.O. Box 8030  
31080 Haifa  
**Israel**

Abraham Golik  
Tel.: +972-4-51 52 02  
Fax: +972-4-51 19 11

### **TECHNISCHE UNIVERSITÄT HAMBURG-HARBURG**

Eissendorferstraße 40  
2100 Hamburg 90  
**Germany**

Ulrich Förstner  
Tel.: +49-40-77 18 30 08  
Fax: +49-40-77 18 23 15

### **MINISTRY OF AGRICULTURE, NATURAL RESOURCES AND ENVIRONMENT**

Department of Fisheries  
Aeolou 13  
Nicosia  
**Cyprus**

L. Loizides, G. Zodiatis  
Tel.: +357-2-30 32 79  
Fax: +357-2-36 59 55

**DEVELOPPEMENT D'UN PROCÉDE BIOLOGIQUE POUR LE TRAITEMENT DES EAUX USEES PROVENANT DES MOULINS A OLIVES. ETUDES MOLECULAIRES DU SYSTEME LIGNINOLYTIQUE DE PHANEROCHAETE CHRYSOSPORIUM**

**Coordinateur:** ORSTOM-Université de Provence, Marseille, France (J.L. Garcia)

### OBJECTIFS

- Dépollution d'un résidu liquide fortement polluant issu de l'industrie oléicole méditerranéenne, les margines;
- Mise au point d'un procédé de dépollution biologique biphasique aérobie/anaérobie pour les margines;
- Connaissance du système ligninolytique de *Phanerochaete chrysosporium* utilisé dans l'étape aérobie.

### ACTIVITES

- ◇ Traitement des margines par *Phanerochaete chrysosporium*: rôle des lignines et traitement de l'effluent issu de l'étape aérobie, par fermentation méthanique. Isolement et étude des bactéries anaérobies dégradant les composés aromatiques des margines;
- ◇ Etude moléculaire du système ligninolytique de *Phanerochaete chrysosporium* : expression du gène chez *Saccharomyces cerevisiae* et *Aspergillus sp.*, recherche de gènes de ligninase chez *Streptomyces*.

### RESULTATS OBTENUS

- ⇒ Les nombreux résultats obtenus nous ont amenés à orienter essentiellement nos travaux vers le procédé de traitement des margines (olive mill wastewaters) et à montrer que ce traitement devait être réalisé en plusieurs étapes.
- ⇒ Choix d'une première étape anaérobie  
Les résultats microbiologiques ont montré l'existence de très forts taux de dégradation des margines non prétraitées en réacteurs de type batch, ce qui implique obligatoirement une dégradation anaérobie des composés aromatiques présents. Ces résultats montrent en outre, que cette dégradation est également efficace sur des composés polyphénoliques de hauts poids moléculaires, ce qui représente des voies biochimiques de dégradation potentiellement inconnues. Malgré tout, les taux de dégradation par voie anaérobie de ces margines non prétraitées restent faibles en continu (<50%) du fait que les cinétiques de dégradation anaérobie de tels composés restent très lents (comparables aux cinétiques des bactéries syntrophes obligatoires), ce qui rend obligatoire une étape d'oxydation initiale.
- ⇒ Choix d'une première étape aérobie  
Cette étape d'oxydation initiale des margines est réalisée grâce à *Phanerochaete chrysosporium*, champignon filamenteux possédant les complexes enzymatiques nécessaires pour dépolymériser et/ou minéraliser les composés phénoliques. Le principal objectif de cette étape a donc été de tester la dépolymérisation des polyphénols contenus dans les margines brutes. Après avoir testé plusieurs types de réacteurs et de supports, les résultats actuels ont montré qu'avec du mycelium immobilisé ou en réacteur de type colonne à bulles, le taux de dégradation pouvait atteindre plus de 50% et même jusqu'à 80% dans des conditions bien précises (margines plus diluées). Dans ce dernier cas, on observe une décoloration importante des margines.

- ⇒ Etape de méthanisation  
Le procédé méthanogène le plus efficace pour traiter les margines déjà prétraitées s'est révélé être le filtre anaérobie. Cette étape de méthanisation a permis un taux de dégradation de 70% de la DCO résiduelle, ce qui représente un taux de traitement global de plus de 80% en deux étapes.
- ⇒ Les deux premières étapes les plus efficaces ont donc ainsi pu être identifiées :  
1ère étape : oxydation des margines brutes et notamment des polymères phénoliques par *Phanerochaete chrysosporium* ;  
2ème étape : fermentation méthanique en filtre anaérobie de ce substrat prétraité.  
Ces différentes étapes ont été jusqu'à présent expérimentées en fermenteurs de laboratoire.
- ⇒ Les travaux sur la microbiologie des bactéries actives au cours de cette 2ème étape ou étape de méthanogénèse ont été effectués. Des bilans de dégradation de différents composés aromatiques connus comme présents dans les margines, ont permis de montrer que la plupart de ces composés étaient dégradables par les consortiums bactériens présents.
- ⇒ Concernant la partie génétique du projet, celle-ci reste essentiellement liée à des problèmes d'expression du système ligninolytique de *Phanerochaete chrysosporium* sur les types bactériens testés. La recherche d'une expression efficace se poursuit, grâce notamment à l'obtention de transformants d'*Aspergillus* sur plusieurs des constructions élaborées.

## PERSPECTIVES

- ▷ Les résultats obtenus à partir du procédé pilote à 2 étapes testé au laboratoire vont permettre la construction d'une petite unité de démonstration dans une huilerie de Sfax, Tunisie. Cette unité sera alimentée à partir des margines produites au cours de la campagne 96-97. La troisième étape ou étape de post-traitement sera définie à l'issue de ces travaux sur cette unité ;
- ▷ A partir des travaux effectués sur la microbiologie des bactéries actives au cours de la 2ème étape, quatre souches de bactéries mésophiles ont pu être isolées : deux souches méthanogènes et deux souches dégradant les composés aromatiques syringate et coumarate en anaérobiose stricte. Les voies métaboliques utilisées sont en cours d'évaluation.

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## PARTENAIRES

### UNIVERSITE DE PROVENCE

Laboratoire Orstom de Microbiologie des Anaérobies  
Case 925  
Avenue de Luminy 163  
13288 Marseille Cedex 9  
France

J.-L. Garcia, M. Labat  
Tel.: +33-4-91 82 85 72  
Fax: +33-4-91 82 85 70

### CENTRE DE BIOTECHNOLOGIE DE SFAUX

B.P. W  
3038 Sfax  
Tunisie

S. Sayadi, A. Gargouri  
Tel.: +216-427 41 10  
Fax: +216-427 59 70

**TREATMENT OF DRINKING WATER AND INDUSTRIAL WASTE  
CONTAMINATED BY HEAVY METALS WITH MEMBRANE TECHNOLOGY**

**Co-ordinator:** Ecole Nationale Supérieure de Chimie Montpellier, Montpellier, France  
(Louis Cot & André Larbot)

### OBJECTIVES

- To establish membrane processes for the treatment of water and liquid industrial waste. The approached problems are specific for the Magreb: natural water for drinking and industrial liquid waste from metal industries and from tanneries, the latter being rich on chrome;
- While for the natural waters the objective is to eliminate pollutants in order to reach drinking water quality standards, the objective for industrial waste treatment includes the need to recycle the contaminating substances, in particular if they are rather valuable. This implies the need to include an inorganic pre-treatment;
- For drinking water the coupling of different membrane technologies is envisaged (microfiltration (MF) for eliminating bacteria and sediments, ultrafiltration (UF) or nanofiltration (NF) for the cleaning from macromolecules and organic molecules, electro dialysis for the separation of dissolved salts). For the tangential filtration new membranes will be developed and it is intended to produce newer, cheaper membranes from *marocain* clays.

### ACTIVITIES

- ◇ Production of new ceramic membranes for nanofiltration, ultrafiltration and microfiltration from *marocain* clays;
- ◇ Physicochemical characterisation of membranes of ion permeability and of ceramic membranes;
- ◇ Study of the interactions between the solid phases as a basis for selecting the appropriate membrane for a given purpose. This interaction is particularly unknown for *marocain* clays;
- ◇ Preindustrials, including electro dialysis, electro-electro dialysis or tangential filtration;
- ◇ Trials of reconcentration through acid electro dialysis of acid waste streams containing metallic salts;
- ◇ Assessment of the economics of such a technology.

### OUTCOME

#### *Membrane synthesis, modification and selection*

- ⇒ The membranes for the pressure-driven processes are inorganic membranes developed from new filters made from Moroccan clays and from cordierite as support materials. Monochannel and multichannel configurations have been elaborated by extruding a ceramic paste; the pore diameters vary from 2.5 to 6.5  $\mu\text{m}$  and the porous volume is about equal to 40 %. The materials used for preparing the membranes are: Moroccan clay, zirconia, titania, hafnia and potassium titanyl phosphate ( $\text{KTiOPO}_4$ ) with pore diameters varying from 1.8 nm to 0.2  $\mu\text{m}$ .
- ⇒ Microfiltration membranes have been prepared the powder suspension method; ultra and nanofiltration membranes by the sol-gel method.
- ⇒ For the treatment of waste acids, the electro dialyser must be equipped with anion exchange membranes (AEM) having a low proton leakage. These AEM will be associated with cation exchange membranes (CEM) highly permeable to protons but not permeable to bivalent cations.

- ⇒ In this research, we have studied effluents of galvanisation plants and of tanneries. The first effluent contains  $Zn^{2+}$  ions in the presence of  $Cl^-$  ions. As these two ions form complexes, therefore the AEM must not be permeable to the anionic complexed species ( $ZnCl_3^-$ ,  $ZnCl_4^{2-}$ ) which, in the concentration range are present in the media.
- ⇒ The new CEM are obtained by adsorbing, at the surface of the membrane, layers of a positively charged polyelectrolyte : the poly-ethylene-imine (PEI). The CEM selected for the treatment of tannery effluents, must be highly resistant to poisoning by  $Cr^{3+}$  ions because this multicharged ion gives strong interactions with the fixed ion exchanging sites of the membrane material.

*Characterisation of the membrane materials and membrane properties*

- ⇒ With inorganic membranes, interactions between salt solutions and membrane materials play an important role for the selectivity of the membranes and must be studied. The total charge of the membrane material can be determined with the help of electrophoretic measurements. The powdered material was put in salt solutions at different pH. The mobility of the particles was measured under an electric field. The pH at which the mobility is equal to zero is called Iso Electric Point. For higher pH the material is negatively charged, for lower pH the material is positively charged. This point explains the difference in the selectivity for various membranes. For instance, in the pH range 2.5 to 10,  $KTiOPO_4$  membrane is negatively charged. That means that cations will be attracted and anions will be repulsed by the membrane. The respective charge of cations and anions will determine higher or lower rejection rate.
- ⇒ As a result of ED membrane characterisation, the following table collects the data obtained with a commercial CEM membrane after surface modification by adsorbing PEI from a solution of this polyelectrolyte. The selectivity to proton with respect to bivalent cations is quantified by measuring the  $Ni^{2+}$  transport number through the membrane between two solutions containing a mixture of acid (1N ) with a nickel salt (1N). We can see that the transport number of the bivalent cation is reduced from 0.28 to 0.11 by the adsorption of PEI. The increase of the electrical resistance remains acceptable.

MEC	CRA	CRA 100	CRA 500	CRA 1000	CRA 1500	CRA 2000	CRA 3000	CRA 5000
tNi	0,28	0,11	0,10	0,13	0,12	0,11	0,11	0,11
Rms	1,5	6,2	9,5	6,4	8,6	6,4	8,2	7,2

Tableau I -  $t_{Ni}$  : transport number of a bivalent ion (Nickel ) in membrane modified by surface deposition of PEI.  $R_{ms}$  : electrical resistance (in  $\Omega.cm^2$ ) of the membrane in sodium chloride 10g/l.

- ⇒ For the treatment of effluents containing zinc and chloride ions, a complete study of the behaviour of some membranes has been performed in these media; the main characteristics have been measured : water content, electric resistance, ions amount inside the membrane, self-diffusion and electrotransport fluxes.

*Results of process experiments*

- ⇒ The inorganic membranes have been introduced into modules for treating several kinds of waters. Waste water was analysed in differents points of rivers near the city of FES. Rejections of industries like dairy, oil mill or tannery, which are responsible for a large part of the pollution, were also analysed.
- ⇒ Numerous filtrations of water and industrial rejections were performed using the filters prepared during this work. In a first time, a laboratory pilot was used ( the surface of the membrane was about  $30 cm^2$ ), then, in a second time filtration tests were conducted with a bigger pilot that can use about  $1 m^2$  membrane surface. Using the 3 types of microfiltration membranes we can observe:
  - a complete rejection of suspended substances by measuring the turbidity;
  - a decrease by a factor of 10 of the chemical and biochemical oxygen demands.



- ⇒ The rejection rate for (i) sulphate and chloride ions is equal to 30%, (ii) for monovalent cations is equal to 50%. Divalent cations are not rejected by the membrane. This can be due to the complexity of the medium to be filtered.
- ⇒ An interesting point is the rejection of heavy metals and particularly chromium in case of tannery. The rejection rate is equal to about 73 and 92% when the alone clay support and titania microfiltration membrane coated on this support were used respectively.
- ⇒ All these results show the interest of membrane processes for the treatment of waste water and industrial rejections.
- ⇒ On the other hand, some electro dialysis experiments have been performed with solutions of HCl + ZnCl<sub>2</sub>, using AFN as an anion exchange membrane and several cation exchange membranes : it could be shown that better electro dialysis performances are obtained with surface modified membranes. For instance, when the treated solution contains the acid and the zinc salt at the same concentration (1N), the final concentrations in the concentrate are 2.5 M HCl and 0.15 M Zn<sup>2+</sup>.
- ⇒ In the case of industrial effluents containing chromium, the problem is not easy to solve because of the complexity of the solutions to be treated. So, we first studied, by electro dialysis, synthetic solutions containing chromium sulphate in presence of chloride ions.
- ⇒ Experiments have been made with industrial effluents from tanneries. The obtained results' evidence that the elimination of chromium using this technique is possible. However, some problems must be taken into consideration:
  - water dissociation that can occur and is strongly catalysed by chromium ions.
  - the competition between Na<sup>+</sup> and Cr<sup>3+</sup> that is very in favour of monovalent cations during the transfer through the cation exchange membranes; so, it will be necessary to do preliminary electro dialysis in order to desaline completely the chromium solutions to be treated.
- ⇒ Finally, some experiments were performed by a technique that couples together electro dialysis and ion exchange, using textiles (sulfonic or carboxylic) instead of resins (EDI). When such a textile conditioned in the acid form is equilibrated with a chromium nitrate solution, the ionic exchange occurs, especially with sulfonic textiles that can be then regenerated by immersion into a solution 1 M HCl. So, the experiments can be performed with a five compartments classical electro dialyser in which the central compartment is filled with the textile that fixes chromium ions. The first results that have been obtained are very encouraging, especially for dilute solutions because, in these cases, electro dialysis is not convenient and must be used only for concentrated solutions.

### *Conclusion*

- ⇒ The researches performed in this program have induced significant improvements in the treatment of waste waters by membrane technology. New membranes have been elaborated and characterised : inorganic membranes for pressure driven filtrations and surface modified membranes for electro dialysis. Pilot experiments performed with these membranes have confirmed their interest for large scale applications of the processes.

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## **PARTNERS**

**ÉCOLE NATIONALE SUPÉRIEURE DE CHIMIE DE MONTPELLIER**  
Laboratoire de Physicochimie des Matériaux  
Rue de l'Ecole Normale 8  
34296 Montpellier Cedex 5  
France

Louis Cot & André Larbot  
Tel.: +33-4-67 14 43 44  
Fax: +33-4-67 14 72 36

**UNIVERSITÉ DES SCIENCES ET DE LA TECHNOLOGIE HOUARI BOUMEDIENE (USTHB)**  
Laboratoire de Valorisation des Minerais  
B.P. 32  
Alia – Bab Ezzorian  
16111 Alger  
Algeria

Hacene Kerdjoudj  
Fax: +213-2-75 13 71

**CENTRE DE DÉVELOPPEMENT DES MATÉRIAUX**  
B.P. 29  
Draria W de Tipaza  
Alger  
Algeria

Azzedine Lounis  
Fax: +213-2-36 87 56

**UNIVERSITÉ DE FES**  
Laboratoire de Chimie Minérale Appliquée  
B.P. 1796  
Atlas Fes  
Fes  
Morocco

Mohamed Rafiq  
Tel.: +212-5-64 23 82  
Fax: +212-5-64 25 00

**UNIVERSITÉ DE MONTPELLIER II**  
Laboratoire de Physico-Chimie des Systèmes Polyphases  
Route de Mende 1919  
B.P. 5051  
34033 Montpellier  
France

Claude Gavach  
Tel.: +33-4-67 61 34 09  
Fax: +33-4-67 04 28 20

**TELEDETECTION BY LASER (LIDAR) OF ORGANIC AND INORGANIC  
POLLUTION FROM MOBILE GROUND STATIONS ADAPTED TO THE  
CONDITIONS IN SOUTHERN MEDITERRANEAN COUNTRIES**

**Co-ordinator:** Centre National de Recherche Scientifique, Orsay, France (Guy Taieb)

### **OBJECTIVES**

- To make an inventory of the atmospheric pollution problems existing in Tunisia and in Algeria: the origin of the pollution, the nature of the pollutants, the geographical distribution of them;
- To make an up to date bibliography and a preliminary laboratory work on the qualitative and quantitative detection of species chosen using the results of the inventory;
- To determine the specifications of a station suitable for teledetection in the climatic, economic and social conditions of North Africa;
- To set up the technical document necessary for the realisation of the LIDAR.

### **ACTIVITIES**

- ◇ Updating all the information concerning the main atmospheric pollution problems in their countries, where it appeared that industrial and petrol production were the main sources of deterioration of the environment;
- ◇ Bibliographic work concerning the data on the subject and the state of the art of the analytic methods of detection, and conducting preliminary experiments on the chosen species;
- ◇ Determination of specifications of the teledetection station in co-operation between the Industrial LD and the other partners, using all the qualifications;
- ◇ Elaboration of a final technical document;
- ◇ Some main decisions for the construction of the LIDAR have been made during the processing of the project : the domain of wavelength are chosen in the near infra red, between  $3\mu$  and  $4\mu$ , and the first species to be detected is the methane  $CH_4$ . The excitation sources are laser diodes excited by a pulsed or continuous power supply. For the laboratory experiments, a  $CO_2$  laser (IP) to detect  $O_3$  is used, and a Titanium-Sapphire pumped by a pulsed YAG (LPPM) is used to create by stimulated Raman scattering the  $3.3\mu$  wavelength to detect  $CH_4$ . Electronic devices have been realised in LPPM for the CW diode excitation source (for FST), and electronic devices have been designed in LPPM for the pulsed diode excitation (for CDTA);

### **RESULTS**

- ⇒ The final technical document describing the LIDAR station using both CW and pulsed laser diode source, which was the main aim of the project.
- ⇒ The final scientific document including the data on pollution, results on indoor measurements on ammonia and ozone.
- ⇒ One electronic device, made at FST and at LPPM with the industrial partner collaboration, for CW infra-red laser diode excitation source.
- ⇒ The conception of one electronic device, realised at CDTA and at LPPM with the industrial partner collaboration, for pulsed infra-red diode excitation source.
- ⇒ The setting up of an infra-red pulsed laser source using stimulated Raman emission of HD, excited par a Ti-Sa laser (almost ready) in LPPM.
- ⇒ The setting up of a LIDAR detection using a pulsed  $CO_2$  laser for monitoring hydrocarbures and ozone in Madrid district, at IP (almost ready).

### **FOLLOW-UP**

- ▶ Two Algerian engineers have spent some months in LPPM this year (1997) to achieve the electronic devices included in the LIDAR and to work on the stimulated Raman source;

- ▶ A Tunisian engineer has spent some weeks in LPPM this year (1997) to work with J.-L. Duvent on the electronic acquisition system;
- ▶ An Algerian engineer is at the moment in Madrid building up the telescope for far field teledetection;
- ▶ A PhD student is working at the moment in Algiers on the teledetection of methane;
- ▶ The second step, which is the realisation of the LIDAR station, has already started by the realisation of excitation sources, optical detection device and fast electronic for acquisition and analysis of data.

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## PARTNERS

### CENTRE NATIONAL DE RECHERCHE SCIENTIFIQUE

Université de Paris Sud  
Laboratoire de Photophysique Moléculaire (LPPM)  
Bâtiment 213  
91405 Orsay  
France

Guy Taieb  
Tel.: +33-1-69 15 67 76  
Fax: +33-1-69 15 67 77

### CILAS

Route de Nozay  
91460 Marcoussis  
France

Jean-Louis Duvent  
Tel.: +33-1-64 54 48 00  
Fax: +33-1-64 54 48 18

### UNIVERSIDAD COMPLUTENSE DE MADRID

Institut Pluridisciplinaire  
Laboratoire des Faisceaux Moléculaires et des Lasers  
Paseo Juan XXIII 1  
28040 Madrid  
Spain

Angel Gonzalez Urena  
Tel.: +34-1-394 32 65  
Fax: +34-1-394 32 65

### CENTRE DE DEVELOPPEMENT DES TECHNOLOGIES AVANCEES (CDTA)

Laboratoire des Lasers et Applications  
Chemin Gacem el Madania 128  
El Madania  
B.P. 245  
Alger  
Algeria

Amine Mokhtari  
Tel.: +213-2-67 73 25  
Fax: +213-2-66 26 89

### UNIVERSITE DE TUNIS II

Faculté des Sciences (FST)  
Département de Physique  
Laboratoire de Physique Atomique et Moléculaire  
Campus Universitaire  
1060 Tunis  
Tunisia

Zorah Ben Lakhdar  
Tel.: +216-1-87 26 00  
Fax: +216-1-88 50 73

**RECHERCHES ET ÉTUDES POUR LA VALORISATION ET L'ASSAINISSEMENT  
DES EAUX USÉES PAR ÉPUVALISATION**

**Coordinateur:** Faculté des Sciences Agronomiques de Gembloux, Belgique (Dimitri Xanthoulis)

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### OBJECTIFS

Le programme général proposé visait l'étude, sous divers climats, de la dépollution et de l'assainissement des eaux usées domestiques et des effluents d'élevage tout en ne perdant pas de vue le côté valorisation du système (productions végétales pouvant servir à la nutrition animale, à la production de semences, à l'obtention de matières humifères par compostage ou lombricompostage mais, possibilité de réutilisation des eaux usées assainies pour réaliser une irrigation et améliorer ainsi les rendements agricoles).

Suivant les conditions d'utilisation et les pays concernés, les buts recherchés peuvent être différents: assainissement de quantités appréciables d'eau pour les pays déficitaires, supplément de production, élimination des nitrates et des phosphates causant l'eutrophisation des cours d'eau.

### ACTIVITES

De manière générale, l'ensemble des partenaires s'est penché sur l'étude des performances physico-chimiques et microbiologiques de l'épuvalisation et l'adaptation de la technique aux conditions économiques et environnementales locales. Étude des rendements obtenus en épuvalisation avec des plantes déjà connues et introduction de nouvelles espèces. Étude des filières de valorisation possible et des possibilités d'amélioration du système.

De manière plus précise, les différents partenaires ont abordé les points suivants:

- ◇ Agadir (Maroc)  
Comparaison des rendements obtenus entre l'épuvalisation et la technique d'infiltration-percolation utilisée à la station de Ben Sergao. Étude de la qualité sanitaire des produits.
- ◇ Dakar (Sénégal)  
Étude de l'adaptation de plantes tropicales et de l'utilisation locale des productions et des sous-produits.  
Étude des réactions des consommateurs vis-à-vis de plantes qui ont été cultivées sur eaux usées.
- ◇ Lisbonne (Portugal)  
Étude sur les aspects de la nutrition chimique en épuvalisation, sur le problème des métaux lourds et sur la résistance des plantes à la salinité.
- ◇ Gembloux (Belgique)  
Étude des rendements obtenus en épuvalisation sur différents types d'effluents (eaux usées traitées en station d'épuration et lisier bovin) et avec différents types de plantes. Étude et comparaison de l'efficacité du principe d'épuvalisation en circuit ouvert et en circuit fermé.  
Le rôle de l'asbl Epuvaleau est de faire la démonstration de l'efficacité de la méthode et de la faire connaître en multipliant les collaborations afin de progresser plus rapidement vers une généralisation de l'utilisation de cette nouvelle technique.

## RESULTATS

### *Performances culturelles des espèces utilisées*

- ⇒ En Belgique, les espèces les plus performantes sont le céleri, l'iris et le cresson. Il peut être utile, en tenant compte des diverses caractéristiques végétales, d'utiliser des séquences d'espèces différentes pour améliorer la dépollution globale.
- ⇒ Au Sénégal, l'Institut Sénégalais de Recherches Agricoles - Unité Régionale de Recherches des Niayes qui a testé de nombreuses séquences d'espèces diverses, a mis en évidence la meilleure efficacité de 4 espèces : canna, céleri, cresson et cypéris et l'utilité de procéder à des éclaircissements réguliers et à une diminution de la densité de plantation qui assure une plus rapide formation du matelas racinaire dans les goulottes.
- ⇒ Au Maroc, (Institut Agronomique et Vétérinaire Hassan II complexe Horticole d'Agadir) l'épuration, testée sur plusieurs espèces, dont la tomate, a permis d'abaisser le flux des matières testées (DCO, NH<sub>4</sub>, NO<sub>3</sub>, PO<sub>4</sub> et MES) à des taux intéressants. Ceci permet de suggérer que le système d'épuration peut servir de traitement tertiaire après l'infiltration-percolation, qui produit des effluents très riches en nitrates. L'épuration utilisant le cypéris, s'est montrée efficace pour l'abaissement des paramètres microbiens et chimiques de pollution. Sur la séquence canna-tomate, l'influence du pH a été mise en évidence. En effet, celui-ci augmente lors du passage dans la goulotte et peut ainsi modifier le comportement d'une espèce très sensible à ce facteur. La tomate est dans ce cas, et son rendement est donc aussi influencé par sa localisation dans la goulotte.
- ⇒ Au Portugal, (Instituto Superior de Agronomia - Seccao autonoma de Quimica Agricola), les études ont porté sur des effluents porcins dilués de 1/20 à 1/30. Les résultats observés montrent qu'il est possible d'obtenir des rendements élevés en terme d'épuration d'agents polluants, en particulier des éléments minéraux (dissous ou minéralisés à partir de la matière organique déposée ou retenue par le système racinaire des plantes), ainsi que des solides en suspension. Les bons résultats obtenus, en particulier avec la tomate, le tabac et le céleri, et la possibilité de produire des plantes commercialement intéressantes justifie l'intérêt de la continuation des études de ce système.

### *Performances du système d'épuration.*

- ⇒ Abatement de la pollution physico-chimique:  
Pour l'ensemble des travaux réalisés chez divers partenaires, les abattements de la pollution physico-chimique sont évalués par les mesures en MES, DBO<sub>5</sub>, DCO, N Kjeldahl, NH<sub>4</sub>, et PO<sub>4</sub>; mais aussi pour les métaux lourds (Pb, Cd, Ni, Cr, Cu, Zn) et les cations (Na, Ca, K, et Mg.). Il apparaît que ces abattements sont toujours très intéressants mais éminemment variables en fonction de la qualité de l'effluent à traiter et, dans une moindre mesure, du type d'écoulement utilisé. L'espèce employée intervient également ainsi que l'utilisation adéquate de décanteurs intermédiaires disposés entre les éléments de goulottes ou en aval de celles-ci. Les meilleurs résultats obtenus pour 40 mètres de goulotte, en traitement tertiaire d'une station d'épuration fonctionnant correctement, atteignent près de 90% pour la DCO, 80% pour le NH<sub>4</sub> et le NO<sub>3</sub>, 70% pour le PO<sub>4</sub>. Les abattements moyens observés après 50 mètres de goulotte dépassent les 80% pour les MES et le DBO<sub>5</sub>. Avec l'utilisation de décanteurs appropriés, de courtes goulottes de 23 mètres ont permis d'atteindre des abattements intéressants : 50% de DCO, 56% de NO<sub>3</sub>, 34% de NH<sub>4</sub> et 24% de PO<sub>4</sub>. Des résultats exprimés en quantité de matière éliminée par mètre de goulotte et par jour montrent des abattements moyens de l'ordre de 10,3g/m.jour pour la DCO, de 1,3g/m.jour pour le NH<sub>4</sub>, de 11,6g/m.jour pour le NO<sub>3</sub> et de 1,7g/m.jour pour le PO<sub>4</sub>.

- ⇒ Métaux lourds  
L'efficacité du système d'épuvalisation sur l'élimination des métaux lourds a notamment montré des abattements de l'ordre de 99,8% pour le Cd et le Pb et la prépondérance de l'élimination par rétention dans les sédiments par rapport à l'absorption par les plantes qui, suivant les essais, oscille entre 10 et 23% pour le cadmium et ne dépasse pas 10% pour le plomb.
- ⇒ Abattement de la pollution microbiologique et helminthologique  
Les abattements en coliformes fécaux et totaux et en streptocoques fécaux sont compris, tant en Belgique qu'au Sénégal, entre 80 et 99,99% ce qui indique que l'épuvalisation est très efficace sous des climats nettement différents.  
L'épuration microbiologique réalisée permet ainsi de se rapprocher de la qualité requise pour les eaux de baignade et d'atteindre celle nécessaire à une irrigation fertilisante.  
L'épuration bactériologique par épuvalisation au Maroc est cependant moins performante que celle obtenue par la technique de l'infiltration-percolation. La combinaison des deux systèmes pourrait conduire à d'excellents résultats permettant une réutilisation optimale des eaux usées.  
L'élimination des helminthes a été plus efficace par l'utilisation de goulottes d'épuvalisation dans lesquelles du gravier avait été ajouté, ainsi qu'une plantation de cypérus.
- ⇒ Qualité sanitaire des produits  
L'analyse des fruits a montré que la qualité bactériologique de ceux-ci est nettement en deçà des seuils limites et ne présente aucun danger pour la consommation.

## **PERSPECTIVES**

Les perspectives d'application de l'épuvalisation à des effluents très chargés en matières organiques ne peuvent se concevoir qu'après un pré-traitement et une clarification. L'épuvalisation est donc surtout efficace comme traitement tertiaire destiné à améliorer les rejets en vue de diminuer l'eutrophisation des cours d'eau. Elle est aussi très utile pour réduire les concentrations en métaux lourds et pour réaliser un très bon assainissement des eaux usées en vue d'une réutilisation pour l'irrigation fertilisante notamment.

L'utilité de l'épuvalisation est aussi démontrée par le fait qu'elle peut être valorisante de diverses façons: production d'aliments pour la nutrition animale, de graines, de compost et lombricompost.

Il faut souligner le grand avantage que présente une installation d'épuvalisation de pouvoir être placée sur des terrains impropres à toute culture.

L'épuvalisation sera aussi particulièrement appréciée dans les pays chauds où la disponibilité en eau diminue constamment et où la production d'humus entre dans les préoccupations des agriculteurs.

## **PARTENAIRES**

- FACULTÉ DES SCIENCES AGRONOMIQUES DE GEMBLoux**  
FSAGX  
Passage des Déportés 2  
5030 Gembloux  
**Belgium**
- Dimitri Xanthoulis  
Tel.: +32-81-62 21 86  
Fax: +32-81-62 21 81
- INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE HASSAN II**  
Complexe Horticole d'Agadir - Chaga  
B.P. 773  
Agadir  
**Morocco**
- Redouane Choukr-Allah  
Tel.: +212-8-24 01 55 / 24 10 06  
Fax: +212-8-84 39 77 / 24 22 43
- ECOLE SUPÉRIEURE DES INGÉNIEURS DE L'ÉQUIPEMENT RURAL**  
Route de Testour  
9070 Medjez El Bab  
**Tunisia**
- Bechir Ben Thayer  
Tel.: +216-8-45 74 95 / 45 67 73  
Fax: +216-8-45 76 81
- AGRICULTURAL UNIVERSITY OF ATHENS**  
Iera Odos 75  
11855 Athens  
**Greece**
- Spyros Kyritsis  
Tel.: +30-1-529 40 01  
Fax: +30-1-529 40 23
- INSTITUTO SUPERIOR DE AGRONOMIA**  
Seccao Autonoma de Chimica Agricola  
Tapada Da Ajuda  
1399 Lisboa Codex  
**Portugal**
- Joachim Quelhas Dos Santos  
Tel.: +351-1-36 37 98 70  
Fax: +351-1-36 37 89 70
- AGRICULTURAL RESEARCH INSTITUTE ARICY P**  
2016 Nicosia  
**Cyprus**
- Ioannis Papadopoulos  
Tel.: +357-2-30 51 01  
Fax: +357-2-31 67 70
- INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE HASSAN II**  
Complexe de Ouarzazat  
B.P. 6202  
Rabat-Instituts  
Rabat  
**Morocco**
- Bouchaib El Hamouri  
Tel.: +212-7-77 75 64  
Fax: +212-7-77 81 10 / 77 58 38
- ECOLE SUPÉRIEURE DES INGÉNIEURS DE L'ÉQUIPEMENT RURAL**  
CRGR  
Rue Hedi Karray  
B.P. 10  
2080 Tunis  
**Tunisia**
- Zouheir Chaabouni  
Tel.: +216-1-71 78 01 / 71 96 30



# 1. Natural resources

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## 1.1. Basic natural resources

### *1.1.3. Other resources: soil*

**USE OF MYCORRHIZAL AND RHIZOBIAL SYMBIOSES FOR THE SUSTAINABLE DEVELOPMENT OF FOREST RESOURCES IN THE MEDITERRANEAN REGION (MYRISME)**

**Co-ordinator:** Centro Internacional de Altos Estudios Agronomicos Mediterraneo, Instituto Agronomico Mediterraneo de Zaragoza, Spain (Jose Miguel Barea)

### OBJECTIVES

- The study of microsymbiont biodiversity in the rhizosphere of the test plants growing in their natural habitats. This concerns arbuscular and ectomycorrhizal fungi, *Rhizobium* sp. and rhizobacteria, and refers to traditional and molecular techniques of identification, tagging and tracking.
- The screening of microbial efficient strains for host/environment compatibility.
- The constitution of mycorrhizal fungi and rhizobial banks.
- The optimization of both the microbial inoculation techniques and the nursery production of inoculated seedlings.
- The evaluation of the effects of controlled inoculation of the seedlings in established experimental reforestation plots under natural conditions. This includes monitoring both plant performance and microbial population dynamics.
- The target plants and the symbiosis types are: *Pinus halepensis* and *Cedrus atlantica* (both ectomycorrhizal hosts), *Argania spinosa* (arbuscular mycorrhizal host) *Acacia raddiana* and *Anthyllis cytisoides* (both arbuscular mycorrhizal and rhizobial hosts).

### ACTIVITIES

- ◇ Analysis of microsymbiont diversity, screening of mycorrhizal fungi and rhizobial strains adapted to the potential trasplantation sites, and establishment of microbial banks. A novel molecular biology approach will be adapted or developed.
- ◇ Optimization of inoculated seedling production upon selecting appropriate host/symbiont/environment combinations. Modern ecophysiological techniques, a molecular biology approach and isotopic methods will be applied to ascertain seedling quality (microbial optimal symbiont establishment- and physiological-based on the analysis of mechanisms of drought tolerance and adaptation to calcareous soils, as well as the measurement of N<sub>2</sub>-fixation and N transfer).
- ◇ Monitoring of plant/microbial performance upon outplanting in the experimental field plots, molecular microbial ecology techniques, ecophysiology-related methodologies and isotopic- <sup>15</sup>N and <sup>13</sup>C based-approaches will be used.

### EXPECTED RESULTS

The project should provide recommendations for the development of acceptable models for managing forest resources constituting alternatives to deforestation and resource degradation. It should define appropriate strategies and specific technologies for implementing the following particular aspects:

- ⇒ To set available collection of effective ecosystem-adapted microsymbionts;
- ⇒ To deliver a work-package (ecophysiology, biochemistry and molecular biology approaches) for microbial identification, tagging and tracking;
- ⇒ To produce plant material endowed with optimized myco-rhizosphere (improvement of quality in nursery production of target seedlings) available for transplant purposes;
- ⇒ To establish experimental plots as pilot models available for technology transfer.

## **FOLLOW-UP**

At the end of the first year, ecosystem-adapted microsymbionts will have been isolated and, in a certain extent, characterized. Compatibility among the substrates and protocols commonly used in nursery production and the establishment of effective mycorrhizal and rhizobial symbioses will have been also determined.

At the end of the second year, characterization of the ecosystem-adapted microsymbionts will have been completed, their efficiency studied and seedlings of the different target plants endowed with an optimized myco-rhizosphere should be available for the reforestation experiments under natural conditions.

At the end of the third year, the ecophysiological quality of the seedlings obtained by controlled microbial inoculation should have been studied and the performance of the plantlets produced and of the introduced microsymbionts during the reforestation experiments determined.

## **PARTNERS**

### **CENTRO INTERNACIONAL DE ALTOS ESTUDIOS AGRONOMICOS MEDITERRANEOS**

Instituto Agronomico Mediterraneo de  
Zaragoza  
Ctra. Montanana 117, Apartado 202  
50080 Zaragoza

**Spain**

Jose Miguel Barea  
Tel.: +34-976-57.60.13  
Fax: +34-976-57.63.77  
E-mail: iamz@iamz.ciheam.org

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Labo de recherches sur les symbiotes  
des racines  
Place Viala, 2  
34060 Montpellier CEDEX 01

**France**

Daniel Mousain  
Tel.: +33-4-9961.2269  
Fax: +33-4-6754.5708  
E-mail: symbiote@ensam.inra.fr

### **CENTRE NATIONAL DE LA RECHERCHE FORESTIERE SERVICE DE SYLVICULTURE**

Charia Oma Ibn al Khattab, B.P. 763  
10050 Agdal-Rabat

**Morocco**

Mohamed Abourough  
Tel.: +212-7-67.38.30  
Fax: +212-7-67.11.51

### **INSTITUTION DE RECHERCHE ET DE L'ENSEIGNEMENT SUPERIEUR AGRICOLES**

Institut National de Recherches Engenie Rural, Eaux Et Forets  
(INGREF)

B.P. 2  
2080 Ariana-Tunis

**Tunisia**

Hafedh Nasr  
Tel.: +216-1-23.00.39  
Fax: +216-1-71.79.51

**DESERTIFICATION IN THE MEDITERRANEAN DRYLANDS: DEVELOPMENT OF  
A MONITORING SYSTEMS BASED ON PLANT ECOPHYSIOLOGY (DEMOS)**

**Co-ordinator:** Università degli Studi di Trieste, Department of Biology, Trieste, Italy  
(Sebastiano Salleo)

## OBJECTIVES

The situation of the Mediterranean is very representative of fragile ecosystems with uncertain equilibria. The Mediterranean region, being a transitional zone between the arid tropics and the northern temperate areas, is characterised by marked seasonality with hot dry summer. Frequent fires and relatively poor soils produced particular vegetation types, as *maquis* and *garrigue*. More, historical intensive landuses lead to overexploitation and degradation of natural sources, so that any future climate change will cause major environment degradation and will enhance the drift towards desertification. The need of sensitive and reliable environmental monitoring methods is evident.

The main objective of the project is the control of the desertification processes in some edge ecosystems of Turkey and Lebanon (overgrazed pastures, overcultivated or abandoned lands, coastal vegetation, etc.), in particular:

- To develop a control and monitoring system of the desertification processes in the Mediterranean areas of the DC Countries based on the measurement of the ecophysiological parameters of vegetation (i.e. those linked to the water balance and the carbon absorption) in some ecosystems particularly vulnerable to desertification (edge systems, overgrazed pastured, overcultivated or abandoned lands, etc.);
- To create and develop information models, correlating the ecophysiological parameters with the environmental variables (soil, climate) to produce indices for the synthetic evaluation of the state of the environment (vegetation, water balance) and the associated hazard of progressive desertification;
- To integrate the information models and the associated indices with advanced methods of land analysis (landscape-oriented models, Geographic Information Systems, remote-sensing) for assessing the dynamic evolution of these parameters with time at area unit scale, defining classes of risk per area/type;
- To define standard methodological guidelines for the land degradation assessment of the Mediterranean ecosystems under desertification detecting the major vulnerability areas, thus creating a device to help the local authorities and institutions in making decisions for planning interventions of land restoration or management.

## ACTIVITIES

### ◇ Land analysis and evaluation.

- Evaluation of the areas, selection of the sample sites;
- Floristic and vegetation analysis, climatic and soil data collection of the selected areas;
- Detection of vegetation types for standard plots, individuating the indicator species to monitor desertification processes;

### ◇ Ecophysiological studies.

- Systematic sampling of the ecophysiological parameters by field techniques, according seasonal and daily cycles, of: (a) leaf conductance, (b) leaf water potential, (c) leaf relative water content, (d) ultrasound acoustic emissions, (e) leaf water potential at turgor loss point, (f) soil water content, (g) photosynthesis rate;
- Analysis of the parameters and functions per standard plot, as leaf water potential, xylem cavitation, photosynthetic efficiency of light and water, CO<sub>2</sub> absorption rate, production efficiency, evapotranspiration, etc.

#### ◇ **Modelling and information systems.**

- Analysis of correlations of ecophysiological parameters with the environmental variables, development of response functions, definition of models and environmental risk indices;
- Integration of models-indices with GIS, correlation of the indices with remote-sensing analysis, production of dynamic land maps, synthetic evaluation of the models, simulations of land scenarios, definition of possible land management planning guidelines.

### **EXPECTED RESULTS**

The most important results should be the definition of new methodologies for land monitoring and evaluation, the production of information devices (data sets, indices, functions and models, software, thematic maps) to detect in reliable time the evolution and the entity of the desertification processes in the Mediterranean drylands, and a consistent transfer of acquired knowledge and technology to the countries most affected by desertification, carrying out parallel activities to disseminate the results (workshops, lectures, training courses, papers, etc.)

#### *Scientific results*

- The development of new methodologies for monitoring desertification, based on ecophysiological and biochemical parameters;
- The gain of knowledge on the desertification mechanisms and processes (new experimental field data and derived environmental functions);

#### *Deliverables*

- The creation of tools for land management for local authorities (indicated models and GIS maps for assessment and simulation of scenarios, guidelines for land management, etc.).

#### *Technology transfer, dissemination, formation*

- Foundation of new networks as observatories of desertification;
- Preparation of regional-scaled rehabilitation plans;
- Research of regulations to harmonize land restoration and population needs at legislation level;
- Technology transfer and formation of qualified scientific personnel in the Mediterranean DCs.

### **FOLLOW-UP**

Field surveys will continue up to summer-autumn 2000, on seasonal basis, three times per vegetative season (spring, summer, autumn). The surveys will involve researchers from all countries, and in some sites, will be carried out by DC's staff only.

Data analysis, modelling analysis and GIS production will be performed mainly by the partners from Italy and Greece, starting from the end of 1998 after a representative amount of experimental data will have been collected.

Dissemination will be performed through the whole project duration. Other opportunities for formation, training and in-field courses for DCs researchers will be proposed by the partners from Italy and Greece, mainly regarding experimental techniques, data and modelling analysis and GIS use.

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## **PARTNERS**

### **UNIVERSITA DEGLI STUDI DI TRIESTE**

Department of Biology  
Via L. Giorgieri 10  
34100 Trieste  
**Italy**

Sebastiano Salleo  
Tel.: +39-40-676.3878  
Fax: +39-40-568.855  
E-Mail: [ecoqua@univ.trieste.it](mailto:ecoqua@univ.trieste.it)

### **MEDITERRANEAN AGRONOMIC INSTITUTE OF CHANIA**

Dept. of Environment and Renewable Resources  
PO BOX 85  
73100 CHANIA  
**Greece**

Andreas Doulis  
Tel.: +30-821-81151  
Fax: +30-821-81154

### **EGE UNIVERSITY**

Science Faculty  
Centre for Environmental Studies  
E-BLOK  
35100 Bornova, Izmir  
**Turkey**

Munir Ozturk  
Tel.: + 90-232-388.0110/2445  
Fax: +90-232-388.1036

### **LEBANESE UNIVERSITY**

Faculty of Agricultural Sciences  
Laboratory of Plant Physiology  
PO BOX 135368 (Chouran)  
Beirut  
**Lebanon**

Mouin Hamze  
Tel.: +961-1-785.428  
Fax: +961-1-785.427

**MEDCHANGE – EFFECTS OF LAND USE AND LAND MANAGEMENT PRACTICES  
CHANGES ON LAND DEGRADATION UNDER FOREST AND GRAZING  
ECOSYSTEMS**

**Co-ordinator:** Universidade de Aveiro, Depto. de Ambiente e Ordenamento, Aveiro, Portugal,  
(Celeste de Oliveira Alves Coelho)

**OBJECTIVES**

- To investigate the changes in land degradation, with increasing forestry and grazing activities in the western Mediterranean region (North Africa -Morocco and Tunisia and the Iberian peninsula);
- To assess the current and likely future changes in land-use and land management practices that the contemporary socio-economic trends national/regional policies, the 2010 free trade zone and the EU CAP Aid Scheme for Forestry may induce in areas vulnerable to land degradation and desertification.
- A holistic research approach combining both environmental and socio-economic dimensions will be adopted in order to:
  - improve policies for sustainable development;
  - evaluate the effects of natural conditions and human activities on water depletion, soil degradation and vegetation health;
  - produce models, both conceptual and semi-quantitative, that describe the relationship between hydrology, vegetation, land use and socio-economic constraints;
  - establish optimal practices for land management, in order to achieve greater sustainability through a reconciliation of potentially conflicting interests;
  - contribute to the definition of criteria for evaluation and mitigation of land degradation;
  - stimulate the exchange of knowledge and expertise;
  - benefit the common Mediterranean producing practices on agro-forestry systems;
  - prepare joint educational activities (seminars, workshops, reports, etc.);
  - disseminate results among rural populations and government officials.

**ACTIVITIES**

- ◇ Evaluation of current responses and prediction of likely future responses in terms of land-use and land management practices to (a) national/regional government policies (soil-water conservation) and (b) contemporary secular socio-economic trends. This includes:
  - Establishing exactly what the policies (soil, water, forestry, agriculture and protected areas) encompass;
  - An assessment of current land-use and recent land-use trends in the study regions, using national/regional demographic and agricultural statistics;
  - Structured interviews with forestry, agricultural and local government administrators and officials concerning current/future land-use and land management problems as well as impacts of international, national/regional policies, especially the impacts of 2010 free trade zone and EU CAP Aid Scheme for Forestry;
  - A questionnaire survey of land-users as to their views of current/future land-use and land management practice trends and problems. Their responses to national initiatives and policy, secular socio-economic changes and climatic variability will also be studied. The interviews/questionnaires both with officials and land-users will also investigate their suggestions regarding modifications to the national policies and their reactions to policy alternatives and conservation measures and techniques.
- ◇ Gathering of data on hydrology, erosion, soils and vegetation for the different land-use/land management practice types within each of the study areas. This involves the following steps:
  - Identification and specification of the land use/land management practice types within the study areas. This will be assessed by means of field work and cartographic document analysis;

- Sampling of the key surface and soil properties that influence erosion and hydrology;
  - Rainfall simulation experiments to establish susceptibility to erosion and overland flow dynamics. A rainfall simulation apparatus will be used;
  - Analysis of existing rainfall and river flow records to establish basin-scale hydrological characteristics of the principal land-use types;
  - Measuring and modelling hydrology and sediment yield to investigate the response of river basins in areas undergoing land degradation to specific changes in climate and land use;
- ◇ Data gathered in the previous groups, will be used to predict erosion, hydrological and soil sustainability consequences of the land-use/land management practice changes. Attention will be given to those land uses predicted to ensue from the national/ regional soil-water conservation strategies and land management practice alternatives. This group encompasses:
- Simulation of foreseeable changes, taking into consideration the option's typical physical and human limitations;
  - Creating a database (on the basis of existing maps, aerial photographs) with basic spatial information on: (e.g. relief, aspect, slope angle, position in slope, slope length discharge network, land use, ...);
  - Combination of the database with the results of the investigation on socio-economic and physical aspects in a GIS model for each of the studied regions.
  - Identification of problematic areas and definition of strategies to achieve sustainable land use.
- ◇ To propose recommendations on possible modifications to the national/ regional policies that will minimise adverse environmental consequences.

## EXPECTED RESULTS

### *Scientific-technical results*

- ⇒ To contribute towards sustainable development in rural areas affected by drought and subject to over exploitation of natural resources, by integrating local populations views as an efficient part of land and water conservation strategies.
- ⇒ Building scenarios for feasible alternative land-use/land management practices, under different socio-economic conditions, with the help of GIS techniques.
- ⇒ To contribute to the establishment of thresholds for sustainability and degradation limits for the present status, and of mitigation measures proposed by the various groups acting in the field (land users, government officials, researchers).
- ⇒ Allow a better collaboration between scientists with interests in political, socio-economical and physical aspects of desertification processes and mitigation measures.
- ⇒ To reinforce information transfer between scientists, local government officials, managers and rural populations to benefit each other from a better statement of managed systems, forced by interrelationships between 1) hydrological factors, 2) vegetation and land use and 3) socio-economic constraints.

### *Deliverables*

- ⇒ Data-bases regarding socio-economic aspects (based on surveys and interviews), and physical aspects (i.e. rainfall/runoff, soil hydrological processes, soil properties, and vegetation status).
- ⇒ To prepare a handbook on methods and techniques used to collect data on physical and socio-economic aspects which can be used elsewhere in the Mediterranean or in other areas.
- ⇒ Training program on the monitoring and modelling of hydrology, erosion and vegetation status.
- ⇒ Training program on GIS and remote sensing.

## FOLLOW-UP

Work planned for the forthcoming year:

With respect to the physical/natural dimension, the assessment of rainfall, catchment runoff, overland flow, vegetation status, soil properties assessment will continue. In addition, rainfall simulation experiments will be carried out.

With respect to the socio/economic dimension, the reviewing of policies will proceed, as well as the analysis of socio-economic statistics. Interviews to the local key actors will continue, and a



questionnaire will be made to the people in the study areas In the forthcoming year, digitalisation and modelling with GIS will commence. A training course will take place on this subject.

**Work planned for the remaining part of the project:**

In the final year, an attempt will be made to incorporate the knowledge on socio-economic and physical aspects into integrated GIS-based models. A major objective is the dissemination of results. This involves the edition of a manual of methods and techniques applied in the project. In addition, seminars/workshops will be organised in Northern Africa, that are open to government officials, administrators, planners, land-users and their associations, and both junior and senior scientists and research students.

**PARTNERS**

**UNIVERSIDADE DE AVEIRO**

Depto. de Ambiente e Ordenamento  
Campus Univeritario  
3810 Aveiro

**Portugal**

Celeste de Oliveira Alves Coelho

Tel.: +351-34-370.831

Fax: +351-34-29.290

E-mail: Coelho@dao.ua.pt

**UNIVERSIDAD DE BARCELONA**

Dep. de Geografia i Analisi Geografia  
Regional - Fac. Geografia i Historia  
Baldiri i Reixac S/N  
08028 Barcelona

**Spain**

Maria Sala

E-Mail: sala@trivium.gh.ub.es

Tel.: +34-3-440.92.00

Fax: +34-3-449.85.10

**UNIVERSIDAD POLITECNICA DE MADRID**

Depto. Ingeniera Forestal  
Lal. de Hidrologia  
Ciudad Universitaria  
28040 Madrid

**Spain**

Marta Gonzalez del Tanago

Tel.: +34-1-336.71.18

Fax: +34-1-543.95.57

**UNIVERSITE MOHAMMED V**

Faculte Lettres et Sciences Humaines  
Unite Formation & Rech. Environ.  
BP 1040  
Rabat

**Morocco**

Abdellah Laouina

Tel.: +212-7-77.18.93

Fax: +212-7-77.20.68

**INSTITUT NATIONAL AGRONOMIQUE DE TUNISIE**

Dept. Genie Rural Eau et Foret  
Avenue Charles Nicolle43  
1082 Tunis

**Tunisia**

Ali Hamza

Tel.: +216-1-28.94.31

Fax: +216-1-79.93.91

**CLIMATE IMPACT ON WATER RESOURCES AND DRYLAND AGRICULTURE  
(CLIWARDA)**

**Co-ordinator:** Winand Starling Centre of Integrated Land Soil & Water Research, Wageningen,  
The Netherlands (Massimo Menenti)

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### OBJECTIVES

- To establish a cooperation mechanism among leading research organizations, active in representative drylands of four continents. The network members contributed to develop innovative solutions facing similar scientific and development issues to manage natural resources in the regions where they operate and to introduce in these regions new concepts and technologies. The network participants intend to establish a baseline, against which the impact of forecast climatic variability may be assessed, both by looking at the effects of past climate variability on farming systems (with special reference to irrigated agriculture) and on water resources, and by taking into account the history of land use, of farming systems and of socio-economic conditions.
- To collect and analyse information about the variability of hydrological conditions in the recent past (e.g. 300 years), and about the impact of this variability on agricultural production in order to tackle the following issues:
  - The influence of the climate variability in the last few centuries affecting water resources in drylands,
  - To monitor if effective changes have occurred in the agricultural production system caused by the variability in climate and water supply;

If such changes occurred, the assessment of the vulnerability of irrigated agriculture in drylands facing future climate variability.

### ACTIVITIES

Irrigated agriculture in drylands can adapt to diminished water supply by concentrating irrigation water use in a smaller area and raising unit yields and/or market value. To assess the impact of climate variability on production systems, separate issues have been addressed:

A. Within which limits can unit yields be increased and how long does it take to achieve widespread and higher yields?

B. Does the variability of water supply produce permanent effects, e.g. soil salinisation, which do not allow full recovery of land productivity during wetter periods?

C. To what extent a different balance between irrigated and rainfed agriculture would improve the sustainability of the production system and reduce negative impacts on ecosystems?

The test areas which have been studied by the participating institutions and about which a wealth of data and studies exist already are: in Argentina, the watersheds of Rio Mendoza and Rio Atuel; the rims of the Taklimakan desert and the Hei He and Shiyang He watersheds in north-western China; in India, the basins of the rivers Luni and Yamuna; the oases in the Western Desert of Egypt and the eastern Nile Delta; in Niger, rainfed agricultural lands. The following main scientific issues are analysed: 1) climate in the test areas during the recent past; 2) actual and reconstructed hydrologic records in the test areas; 3) analysis of historic archives to characterize past farming systems; 4) land degradation in the test areas: observed processes and expected trends; 5) development and application of regional hydrologic modelling; 6) computational decision support tools for water management; 7) down-scaling of climate scenario's; 8) appraisal of trends in unit yield; 9) socio-economic and institutional constraints on dryland resource management; 10) consensus approach for impact assessment.

## RESULTS

Investigations completed during the last 20 years led to establish a representative set of tree-rings chronologies for a large region including Argentina and Chile. Chronologies were used to reconstruct North Patagonia mean summer temperature, winter precipitation at Santiago de Chile, precipitation patterns in sub-tropical north-western Argentina, the position of the southeastern Pacific anticyclone and the streamflow of some rivers. Millennium-long chronologies are used to assess the effect of climatic forcing factors. Historical climatic and hydrological information stretching over the last 450 years did provide useful insights on landscape changes and hydrological conditions in the central and north western regions of Argentina and for Central and South-West regions of Bolivia. Drier, wetter and colder periods were identified and fluctuations of streamflow in the Rio Mendoza were documented. Studies did also address the impact of environmental changes on human activities.

*Evolution of farming systems and its response to climate.* The on-going studies in China involve the environment change of inland river basins, especially, the lower reaches. The interpretation of historical documents and the data analysis of natural resource use some skills and techniques which have been developed in the past decade, assessment of evolution of farming systems. It is still difficult to make a model of evolution of farming systems because there are few quantitative data in the historical archives. Statistical models for predicting regional-scale meteorological events have been developed. Historical information and multi-century-long records of climatic and hydrology variables have provided useful insight on landscapes change in and region of Argentina. It can be used to distinguish impact of climate variability from socio-economic activities.

Desertification is closely related with management problems of soil and water, such as competition for water use between agriculture industry etc., increasing complexity of laws and regulations. It is necessary to use computational decision support tool to better manipulate the available resources.

In recent years, much attention has been given to climatic change in the Taklimakan Desert. It is still a controversial issue as to whether increasing temperature, glacier shrinkage, a higher snow line, or the decrease in precipitation leading to sustained drought, may eventually cause severe desertification and a southward extension of the Taklimakan Desert, which would threaten the existence of a vast area containing oases on the southern edges. The conclusion is that during the next 200 years, the climate at the southern Taklimakan Desert will tend to become warmer. Although there were periodic fluctuation, the general trend seems to point toward a drier climate. This coincides with the temperature change and with the curve of annual run-off of the Keriya River.

*Expected trends of land degradation in the test areas.* Land degradation speeds up because of the increasing pressure of human population and drier trend of climatic changes. Sandy desertification is a main kind of land degradation in North China. which mainly caused by excessive human impact through wind erosion. In last four decades, although the sandy desertification has shown to be controlled in some areas, but it is still increasing in whole of arid and semi-arid region of the North China. Accordingly, the form and intensity of land use determine to the large extent the development and control of sandy desertification in the arid and semi-arid region.

*Glaciers and snow hydrology.* Semi-empirical models able to monitor and model snowmelt process were used to study climate variability in the past and to estimate future trends for estimated river flow. The final simulation scenario shows three melt seasons fairly well simulated with the same parameter set. Their differences are determined by the temperature series, SCA curves, and a slight variation of the maximum value of the melt factor.

*Hydrology of irrigated lands.* The model SIMGRO was applied to calculate irrigation efficiency for the Tunuyan Inferior irrigated area. Project efficiency (ep) is defined as the ratio between the actual evapotranspiration and the total volume of water used for irrigation (surface and groundwater). The 1D-SWAP model predicts the water salt balance and was applied for irrigated and rainfed vineyard in Lavalle Mendoza. The model gave good results for the simulation of soil water storage and for the salinity profile.

A groundwater model flow3 was applied in the desert area of the Hexi Region to study different hydrological processes such as water recharge and flow, salt movement, evaporation of groundwater, etc. The impact of seasonal freezing and thawing processes on soil salinisation was studied showed that the salt content tends to increase during freezing and tends to decrease during thawing. The objective of the soil reclamation is to restrain the salinisation and to prevent the soils from alkalization. The agricultural measures should be different in the shallow- and deep-buried zones of phreatic water. In the former, the major task is to remove the excess chlorides, and in the latter, the major task is to control the

leaching process of salt, in the same basin, even in the same irrigation areas, the irrigation application rate should be different.

The results of isotopic age determination showed that the groundwater was recharged presently with calibration age of  $^{14}\text{C}$ , while in the period of nuclear explosion with age of T. Furthermore, the T was used as a tracer element for the investigation of the groundwater flow system.

In some areas such as in desert heartland and in lower reaches of the inland river basin, saline water with salinity content over 3 or 5 g/l is an important water source. In Taklimakan Desert heartland, a pilot experiment of green ecosystem construction has been done since 1993. The experiment demonstrated that the salt-resisting species of vegetable, grass and some other sand fixing species chosen from the dry lands could grow well when irrigated with groundwater of Cl.  $\text{SO}_4$ - Na . Mg type and 3.4 - 4.5 g/l of salinity. Because of infiltration water, the groundwater level rise leads to land salinisation in oasis areas. According to 1990 investigation, the salinised land area in the irrigation district reached 2,867 ha, of which severely salinised cultivated land is 1,533 ha and abandoned cultivated land is 1,000 ha. All these salt- affected land were high-yield fields with better water condition before the irrigation project was settled. According to the studies in Ningxia irrigation area, irrigation backwater belongs to nontoxic but harmful water, it can be mixed with Yellow River water in a proper proportion and used to irrigate crops.

The "Integration Research on Water Resources Sustainable Development Capacity of Shiyanghe River Basin" and "Integration Research on Rational Water and Land development and Environment Protection of Shulehe River Basin" deal with the regional water resources planning of the Hexi project, focussing on social, economical, and environmental aspects. The main task is first to improve Shiyanghe water resources planning decision support system (SYWRDSS) and then to develop Shulehe water resources planning decision support system (SLWRDSS) for each region, and then, use DSS to analyse the future of these regions in different developing alternatives.

Based on the previous version of SYWRDSS, all the new DSS include water resources simulation model, water requirement prediction models for crop, industry and living daily use, conjunctive allocation surface water and groundwater model, multi-objective optimization model, multi-criteria evaluation model and a database management system. Moreover, the new version of DSS will develop some new models such as macro-economic analysis model, ecosystem simulation model and salinity simulation model in SLWRDSS and will verify the database management system and groundwater simulation model as GIS oriented ones. Up to now, most of base data and GIS maps are processed and some models are under improving and developing.

The socio economic and institutional constraints to agricultural development in drylands have been shown to be many and varied. The improvement of sub-Saharan African agricultural productivity to a level which can provide food security for the whole population will require agricultural restructuring and investment in both fixed and turn over capital. These measures have huge social and land tenurial hurdles to overcome and will take a long time to implement. The problem lies in inadequate economic policies and management orientated mainly to short term political gains rather than to long term national benefits, apart from the difficulties imposed by successive droughts, wars and external economic factors. The risks involved in irrigation are clearly higher than the risks of pastoralism, and the development programmes within irrigated agriculture have yet to demonstrate that they can offer viable alternatives to pastoralism. When compared to results, capital and other costs have been extravagant. The whole issue for continued support for irrigated agriculture especially in the dry arid and semi arid lands areas clearly needs to be reconsidered, partly because the potential for increasing crop production maybe much higher within rainfed farming backed by the use of water harvesting techniques.

The agricultural reform and new inputs alone are not enough to raise the level of agricultural productivity, even if it is supposed that agriculture's needs can be fully understood. Agricultural output cannot be significantly increased without change in the African economics. Without confidence in the export sector, without some stability and efficiency in the operation of the farmers markets and without food prices which encourage farmers to grow more food there will be no interest in furthering agricultural development beyond the immediate needs of the farmers themselves.

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## PARTNERS

### WINAND STARING CENTRE OF INTEGRATED LAND SOIL & WATER RESERACH

Marijkeweg 11/22  
P.O. Box 125  
6700 AC Wageningen  
The Netherlands

Massimo Menenti  
Tel.: +31-317-47 43 24  
Fax: +31-317-42 48 12

### INSTITUTO NACIONAL DE CIENCIA Y TECNICA HIDRICAS

Centro Regional Andino  
Belgrano 210 - Cas, Correo 6  
5500 Mendoza  
Argentina

Jorge L. Chambouleyron  
Tel.: +54-61-28 69 93  
Fax: +54-61-28 82 51

### INSTITUTO NACIONAL REFORMA Y DESARROLLO AGRARIO (IRYDA)

Paseo De La Castellana 112  
28071 Madrid  
Spain

Julian Martinez Beltran  
Tel.: +34-1-347 15 11  
Fax: +34-1-411 37 70

### UNIVERSITY OF CAIRO

Department of Irrigation and Hydraulics  
Giza  
Cairo  
Egypt

Mohamed Halim Salem  
Tel.: +20-2-570 28 22  
Fax: +20-2-72 70 09

### DRAINAGE RESEARCH INSTITUTE

Kanater 1362/5  
Cairo  
Egypt

Shaden Abdel Gawad  
Tel.: +20-2-218 93 83  
Fax: +20-2-218 91 53

**NITROGEN FIXATION AND YIELD OF GRAIN LEGUME IN SALINE  
MEDITERRANEAN ZONES (FYSAME)**

**Co-ordinator:** Institut National de la Recherche Agronomique, Montpellier, France  
(Jean-Jacques Drevon)

### OBJECTIVES

- To select chickpea (*Cicer arietinum*) and common bean (*Phaseolus vulgaris*), and their specifically adapted *Rhizobium* strains for symbiotic nitrogen fixation (SNF) tolerance to NaCl salinity.
- To assess the yield of selected symbioses through multilocal field trials in salinized soils, or soils irrigated with saline water, in Northern Africa and Southern Europe.
- To progress in understanding biochemical mechanisms involved in SNF tolerance to salinity.

### ACTIVITIES

- ◇ At the agronomic level, to survey saline zones for prospection of macrosymbiotic lines and microsymbiotic strains, and to assess selected symbioses through multilocal-pluriannual field trials;
- ◇ At the biodiversity level, to screen macrosymbionts for SNF tolerance to salinity, and for intraspecificity with microsymbionts, through cross inoculation trials with and without salinity;
- ◇ At the physiological level, to define optimal procedures for the above screenings, and to investigate SNF structures and functions associated with salt tolerance;
- ◇ At the genetic level, to utilize molecular biology tools to characterize the genetic diversity of symbiotic partners, and tag the genetic determinants of SNF tolerance to NaCl.

### EXPECTED RESULTS

Overall, the work in FYSAME should strengthen the links between basic investigations in SNF and finalized agronomic research to improve nitrogen fixation and yield of legumes in adverse edapho-climatic conditions in the mediterranean basin. It is expected that an improvement of grain-legume SNF, particularly under salinized environment, in combination with improved technical practices, will contribute to increase the mean yield of grain-legumes from actually 0.7 to 1 t/ha. The subsequent increase in competitiveness of legume crops might benefit their role in rotations with other crops for sustainable agriculture, national food demand and stabilization of farmers in rural zones.

#### *Scientific-technical results*

Within FYSAME, agronomic surveys in 1997 show that nodulation with native rhizobia was low in Ben Slimane (Morocco) with chickpea cv Rizky and common bean cv Coco, in Castelnaudary (France) with common bean cv Linex, and in Jendouba, Nabel, Bizerte and Beja (Tunisia) with chickpea cv Amdoun and common bean cv Coco. In Tunisia, a decay in nodulation at early flowering was repeatedly observed. In Ben Slimane and Castelnaudary, the yield was SNF-limited since it was significantly increased from 0.8 to 1.3 t/ha for chickpea and from 2 to 2.5 t/ha for common bean, by nitrogen fertilization (120 kg/ha). The rhizobial inoculation of common bean with local *Rhizobium* isolate, and *R. tropici* CIAT 899 as an international reference, increased the yield from 3.6 to 4.1 t/ha in the experimental station of Dijon-France with cv Extasy, but not with cv Rugally (ca. 3.7 t/ha), neither in the 4 experimental stations of northern Tunisia with cv Coco (ca. 0.7 t/ha) nor at Castelnaudary with cv Linex (2.5 t/ha).

The biodiversity of the rhizobia nodulating common bean was investigated on 152 *Rhizobium* isolates from Cap Bon and Bizerte in Tunisia. These isolates were found to belong to 4 *Rhizobium* species already known to nodulate common bean in France, namely *R. etli*, *R. leguminosarum* bv *phaseoli*, *R. giardinii* and *R. gallicum*, and to other bacterial species which were not previously found in common bean nodules, namely *Sinorhizobium fredii* and *S. medicae*. The geographical distribution of the above *Rhizobium* spp. varied, which might be linked with environmental conditions. From 14 native rhizobia belonging to these above species, at least one rhizobium was more efficient than *R. tropici* CIAT 899

although this difference varied with cv Coco or Belna, and NaCl concentrations. In Morocco, 10 *Rhizobium* isolates from the sandy soil Loukkos perimeter, distributed at least in 4 local serotype-groups and had large differences for nitrogen fixation ability with similar infectious potential. Two of them were more efficient than *R. tropici* CIAT 899.

None of the common bean local rhizobia from Morocco and Tunisia tolerated more than 0.2 M NaCl. More diversity in salt tolerance was found with chickpea local rhizobia, the most tolerant strain, Rch60, being not affected by as much as 0.5 M NaCl. This strain was used to further investigate the osmoprotection mechanisms. The addition of glycine-betaine, or its precursor, choline, was found to increase the Rch60 growth with NaCl. Whereas NaCl stimulated significantly the glycine betaine uptake, the choline uptake was strongly inhibited, except when the strain was grown with exogenous choline. Only high affinity uptake activities were observed for glycine betaine and choline.

The exploration of the macrosymbiont diversity for SNF tolerance to NaCl was started with a collection of (i) 220 chickpea genotypes, including 200 Moroccan local populations and 20 lines from foreign collections grown in greenhouse with 25 mM NaCl in nitrogen-free sand benches inoculated with a mixture of 4 Moroccan salt-resistant *Rhizobium* strains, and (ii) 140 common-bean genotypes, including locally-grown cultivars and genotypes from CIAT (Cali) and other foreign collections grown in field with local cultivation techniques. Seven chickpea genotypes were selected as tolerant to salt concentration based on their performance for nitrogenase acetylene reducing activity, plant color, dry weight, nodule mass, and total nitrogen as a direct measurement of SNF in these experimental conditions. Dry weight, nodule mass and plant color were all closely associated to total nitrogen. Seven common bean genotypes were selected from their biomass and grain-yield performances in the field where a large diversity in adaptation to local environment was found. Seeds of the selected genotypes were multiplied for further physiological and agronomic characterizations, and exploration of their interactions with local rhizobia.

The common bean genotype BAT 477, previously selected by CIAT for its tolerance to drought, was used to further investigate the mechanisms of SNF inhibition by NaCl in greenhouse either in sand or liquid culture. The SNF potential of BAT 477 was evidenced during 1977 to be much higher and more tolerant to NaCl than that of Coco, widely grown in Maghreb. Whatever the genotype, the common-bean growth was less sensitive to salinity when it depended upon mineral nitrogen than on SNF. Also, the SNF tolerance of BAT477 decreased under P deficiency, a limitation that may be found in many salinized soils where common-bean is grown. Salinity increased the nodule phosphoenol pyruvate carboxylase and malate dehydrogenase activities, substantiating the conclusion that the inhibition of nitrogenase activity by NaCl was not due to a lack of photosynthates. The respiratory cost of SNF ( $\text{CO}_2$  evolved or  $\text{O}_2$  uptaken per  $\text{N}_2$  reduced) and the nodule conductance to  $\text{O}_2$  diffusion, were lower and less affected by salinity in BAT477 than in Coco. By comparing Coco with Dark, the tolerance was correlated with better  $\text{K}^+/\text{Na}^+$  nodule selectivity, root  $\text{Na}^+$  retention and lower anionic deficiency.

To investigate the genetic determinants of salt tolerance, the *in situ* hybridization of nodules embedded in a metacrylic resin was developed using a radioactive probe of a symbiotic gene coding for a nodule-specific carbonic anhydrase. Also, 5 salt tolerant mutants were obtained from a non-tolerant wild-type strain of *Rhizobium* sp. (*Phaseolus*). Their acquired salt tolerant stability, and lipopolysaccharides and protein profiles were verified.

#### *Deliverables*

- Cultivars of common bean and chickpea, with highest SNF and yield potential among locally cultivated lines under moderate salinity;
- Genotypes with increased SNF adaptation to moderate salinity to be used as progenitor to increase SNF potential of locally adapted cultivars;
- *Rhizobium* strains selected among native rhizobia with molecular characterization and highest SNF efficiency specifically with selected macrosymbionts;
- Diagnosis method to localize zones and types of soils where the expression of the SNF-dependant yield potential of grain legume might be maximized by use of adapted symbiotic partners;

## FOLLOW-UP

- ▶ At the agronomic level: (i) to perform surveys and inoculation trials on winter-sown chickpea in 1 zone of Morocco and Tunisia, on spring-sown chickpea in 2 zones of Morocco, and on common-bean in 2 zones of Tunisia and one zone of Morocco, with 5 to 10 fields and one inoculation / N nutrition trial per zone chosen with reference to the 1997 results; (ii) to explore the possibility to supply *Rhizobium* isolates from these regions to the microsymbiont biodiversity studies;
- ▶ At the biodiversity levels: (i) to focus the study of local diversity of native rhizobia for chickpea and common-bean on the FYSAME reference regions; (ii) to screen another set of chickpea genotypes for SNF potential and tolerance to salinity in order to select genotypes for winter-sowing; (iii) to explore with the greenhouse procedure the diversity in SNF potential and tolerance to salinity of the common-bean selected and multiplied from field observations; (iv) to study the symbiotic interreaction of selected rhizobia from various species with 6 selected genotypes of common-bean and chickpea from 1997 results, including contrasting pairs for progress physiological characterization; (v) to propose physiological criterias to assess the growth, nitrogen fixation and nutrients utilisation efficiency of contrasting symbioses under salted medium
- ▶ At the cell and molecular level: (i) to search for osmoprotecting compounds among amino acids ; (ii) to continue the study of short- and long-term effects of salts on plant and nodule C and N metabolisms; (iii) to test whether the specific sensitivity of SNF to salinity is a consequence of Na<sup>+</sup> accumulation in nodules and disturbance of the root nutrient transports using split-root system and girdling methods, (iv) to correlate the nodule permeability to O<sub>2</sub>, and nitrogenase-linked respiration, with gene expression in the nodule cortex.

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## **PARTNERS**

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Laboratoire de Recherche sur les Symbioses des Racines  
Place Pierre Viala 2  
34060 Montpellier Cedex 1  
**France**

Jean-Jacques Drevon  
Tel.: +33-4-67 61 23 32  
Fax: +33-4-67 54 57 08  
E-mail: drevonjj@ensam.inra.fr

### **INSTITUT AGRONOMIQUE & VÉTÉRINAIRE HASSAN II**

Agronomie et Amélioration des Plantes  
Laboratoire de Génétique des Légumineuses  
Avenue Maa Elainain  
B.P. 6202 Rabat-Instituts  
10101 Rabat  
**Morocco**

Mohammed Sadiki  
Tel/Fax: +212-7-77 48 69

### **UNIVERSIDAD DE GRANADA FACULTAD DE CIENCIAS**

Departamento Biología Vegetal  
Campus de Fuentenueva  
18071 Granada  
**Spain**

Carmen Lluch  
Tel.: +34-58-24 33 82  
Fax: +34-58-24 32 54  
E-mail: clluch@goliat.ugr.es

### **DIRECTION GÉNÉRALE DE LA PRODUCTION AGRICOLE**

Direction des Grandes Cultures  
Laboratoire de Production d'Inoculum  
Rue Alain Savary 30  
1002 Tunis  
**Tunisia**

Bouaziz Sifi  
Tel.: +216-1-68 16 52  
Fax: +216-1-78 02 46

**SUSTAINABLE DOMESTICATION OF INDIGENOUS FRUIT TREES:  
INTERACTIONS BETWEEN SOIL AND BIOTIC RESOURCES IN SOME  
DRYLANDS OF SOUTHERN AFRICA**

**Co-ordinator:** Università Degli Studi di Torino, Grugliasco, Italy (Ermanno Zanini)

## OBJECTIVES

Indigenous fruit trees may play an important role in the agriculture of the areas subjected to periodical drought. In fact indigenous trees are adapted to harsh environment such as very poor fertility soils and dry climates and may give a yield also during the years that give rise to a failure of traditional crops. These trees grow in the wild, but at least some species appear to have a significant economic potential, both as fresh fruit and as transformation product.

The general objective of this project is to verify the potential of the trees toward domestication and to find the best conditions for their growth and production. The domestication of these trees is intended to be sustainable as we would like to attain it by a better knowledge of all the factors of success of the trees in the wild such as that a transfer of information from the natural situation to the agronomic practices will be possible. The dry environment is regarded as much as possible as a whole and the inter-relationships between the trees and their abiotic counterparts, such as the soils, will be also investigated.

The specific objectives of the project are:

- To assess the ecological and physiological requirements of *Sclerocarya birrea*, *Ricinodendron rautanenii*, *Strychnos cocculoides*, *Strychnos spinosa*, *Vangueria infausta*, *Grewia flava* and *Azanza garckeana* in the drylands of Namibia and Botswana.
- To select superior phenotypes of these species
- To assess the nutritional value of the fruits or their derivatives
- To assess the efficiency of the root system in exploiting soil resources
- To assess the dependence of these fruit trees on mycorrhizal symbiosis for growth and drought tolerance
- To evaluate appropriate agronomic practises
- To evaluate the efficacy and limits of locally available organic fertilisers, like seaweeds
- To evaluate the effect of the trees and of the techniques used for cultivation on the physical and chemical characteristics of the soils

## ACTIVITIES

- ◇ Knowledge of the trees and their habitat in natural conditions:
  - Soil investigation: soil survey, pedological assessment, classification, fertility status, structural stability studies.
  - Collection of biological information from the trees: root architecture, distribution of fine and coarse roots, evaluation of the above-ground compartments
  - Mycorrhizal status: collection and isolation of spores, assessment of the type and degree of mycorrhizal associations
  - Fruit characterisation: nutrient value of the fruits, analysis of the seed oil contents
  - Trees characterisation: search for superior phenotypes based on the indigenous knowledge
- ◇ Knowledge of the fitness of the trees in controlled situations:
  - Mycorrhizal associations: inoculation with mycorrhized roots or spores and assessment of the infection, finger printing of the symbiosis
  - Tree growth: Seed germination trials in controlled conditions, survival tests
  - Plant physiology: monitoring of root growth in rhizotrones, assessment of nutrient needs
  - Agronomic practises: assessment of the effect of seaweed as fertilisers or stimulators, techniques for water harvesting and catchment
  - Soil plant relationships: assessment of the effect of plants on soil characteristics.

## EXPECTED RESULTS

### Scientific - technical results

- ⇒ A better knowledge of the biotic and abiotic resources of an environment which has been poorly investigated up to now that will be disseminated through scientific publications as well as through technical booklets.
- ⇒ The assessment of methodologies that are useful for further studies in sandy environments such as adaptations of methods for soil chemical analyses (scientific publications)
- ⇒ The assessment of the possible hosts for co-cultivation of the Kalahari truffle with the fruit tree species
- ⇒ The evaluation of the potential of Namibian seaweeds as growth stimulators

### Deliverables

- Germination protocols that will help to overcome the difficulties of germination of wild trees
- Booklets for farmers and horticultural organisations about the needs of fruit trees
- Superior phenotype material
- Mycorrhized plant material if the mycorrhizal association is proved to be essential in increasing the fitness of the fruit trees

### Pilots and demonstration plants

Nine areas in Botswana were selected for survival tests of the plants. These demonstration plants have also the purpose to train school pupils in the management of the trees as they are actively involved, together with their teachers in the data recording.

## FOLLOW-UP

- ▶ Nutritional evaluation of the fruits and their derivatives;
- ▶ Evaluation of mycorrhizal associations (inoculation of host plants and assessment of the infection, finger printing of the symbiosis);
- ▶ Physiological effects of the mycorrhizae (evaluation of the fitness of the association towards different factors);
- ▶ Evaluation of the soil plant relationship, especially regarding soil structure;
- ▶ Survival tests;
- ▶ Assessment of the suitable agronomic practises (rainwater harvesting and conservation, seaweeds extracts as fertilisers and stimulators);

## SELECTED PUBLICATIONS

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## **PARTNERS**

### **UNIVERSITÀ DEGLI STUDI DI TORINO**

Dipartimento Valorizzazione e Protezione AgroForest.  
Divapra - Chimica Agraria  
Via Leonardo Da Vinci 44  
10095 Grugliasco (Torino)

**Italy**

Ermanno Zanini  
Tel.: +39-11-670 85 18  
Fax: +39-11-403 18 19  
E-mail: zanini@valnet.it

### **UNIVERSITÄT GOETTINGEN**

Institut für Forstbotanik  
Buengenweg 2  
37077 Goettingen

**Germany**

Douglas Lawrence Godbold  
Tel.: +49-551-39 95 91  
Fax: +49-551-39 27 05  
E-mail: dgobol@gwdg.de

### **VELD PRODUCTS RESEARCH**

P.O. Box 2020  
Gaborone

**Botswana**

Frank William Taylor  
Tel/Fax: +267-34 70 47  
E-mail: veldprod@info.bw

### **UNIVERSITY OF NAMIBIA**

Department of Biological Sciences  
Storch Street 13  
P.O. Box 13301  
9000 Windhoek

**Namibia**

Erika Maass  
Tel.: +264-61-206 37 48  
Fax: +264-61-206 37 91  
E-mail: emaas@unam.na

### **BEN-GURION UNIVERSITY OF THE NEGEV**

Institute for Applied Research  
Mycorrhizal Laboratory  
Hashalom 1  
84105 Beer-Sheva

**Israel**

Varda Kagan-Zur  
Tel.: +972-7-46 19 74  
Fax: +972-7-47 29 84  
E-mail: zur@bgumail.bgu.ac.il

**ANALYSIS OF PESTICIDES IN SOIL AND WATER BY IN SITU BIOSENSOR AND  
MODELLING OF PESTICIDE REMOVAL BY BIOREMEDIATION METHODS**

**Co-ordinator:** Cranfield Biotechnology Centre, Cranfield University, Cranfileld, United Kingdom  
(N.Magan)

### **OBJECTIVES**

- Assessment of existing sensors and development of new sensors for quantifying pesticides and pesticide metabolites in terrestrial and aquatic environments
- Determination of natural degradation rates of these pesticides and their ultimate biodegradability by conventional analytical techniques
- Application of biosensors to in situ and in vitro monitoring of pesticide levels
- Development of bioremedial strategies using microbial inoculants to enhance natural degradation rates in soil
- Modelling, as determined by the developed methodology, of pesticide mobility under natural and bioremedial regimes, and comparison with existing models.

### **ACTIVITIES**

The work at Cranfield University concentrated on

- ◇ Effect of temperature and water potential on the natural breakdown rates of the pesticides dieldrin, simazine and trifluralin in soil over periods of 2-3 months;
- ◇ Screening of novel temperate and tropical basidiomycetes for efficacy as bioremedial inoculants
- ◇ Detailed studies of the effect of bioremedial fungal additions on rates of breakdown of these mixtures of pesticides on the UK red list in soil in relation to temperature and water potential and single and mixed inocula using straw as an inoculant carrier.

At University of Malta an immunosensor device has been fabricated which can sensitively detect atrazine/simazine down to ppb levels. Tests were carried out in water and soil to evaluate the instrument.

Collaborative trials were carried out to evaluate and compare HPLC and immunosensor systems for accuracy and sensitivity. Data in the final phase of the project has been inputted into modelling systems of the bioremediation found in this study.

### **OUTCOME**

Scientific - technical results

#### *Cranfield University*

The natural rates of degradation of dieldrin, simazine and trifluralin in water and soil in relation to temperature and water potential were determined

In vitro studies demonstrated that certain temperate and tropical fungi (*Polystictus versicolor* and *Trametes socotrana*) were effective at producing enzymes and significantly degrade the three pesticides. A gradient HPLC method was developed for the simultaneous analyses of all three pesticides in the same run.

In situ studies showed that depending on the temperature, *P. versicolor* and *Tsocotrana* were particularly effective when inoculated in soil on a straw-based carrier over periods of 2-3 months. Up to 80% for trifluralin, 30% for simazine improvement in the natural rates of breakdown, but very little enhanced degradation of dieldrin was observed.

It was demonstrated that enhanced degradation was achieved best at 25 and 30°C, with very slow enhancement at 15/20°C.

Water potential had a variable effect on enhanced degradation. *P. versicolor* operated best at intermediate water potentials, while. *Tsocotrana* was best at maximum water potential.

### *University of Malta:*

Assessment of existing sensors for atrazine and related pesticides was examined in detail the this showed that there was a definite need for a rapid, simple, immunosensor type systems for field use.

An am 1 perometric detection system was optimised using hydrogen peroxidase activity after a competitive binding assay for the pesticides.

This enabled the design of an immunosensor system for the rapid detection of atrazine/simazine

The sensitivity of the assay was improved by using novel paramagnetic particles on which the IGGs are coated onto the magnetic particles. After washing steps the tube is placed in a magnetic separation rack.

After washing the magnetic particles are resuspended and the assay is continued in the traditional way.

Samples of water/soil spiked with pesticides (atrazine, simazine) using the optimised assay showed that at the level of 0.5 to 1.5 ppb the immunosensor was able to detect accurately the concentrations when compared with other analytical techniques.

The detailed data. is being examined for input into modelling systems. Investigations will focus on using the data in "Physicochemical Evaluation: The environment (PETE)" as a potentially useful system for this purpose. Outputs could include sorption and mobility data, water solubility and volatility, and half-lives in soil as a function of temperature and water potential.

### *Key deliverables*

Novel bioremediation systems for field use are being examined for commercial exploitation at Cranfield University. The rapid novel immunosensor system for detection of the pesticides is being examined for commercial applications and field use in the University of Malta.

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## **PARTNERS**

### **CRANFIELD UNIVERSITY**

Cranfield Biotechnology Centre

Bedfordshire MK 43 OAL

**United Kingdom**

Naresh Magan

Tel.: +44-1234-75 01 11

Fax: +44-1234-75 09 07

E-mail: N.Magan@Cranfield.ac.uk

### **UNIVERSITY OF MALTA**

Department of Biomedical Science

Msida MSD06M

**Malta**

Joe V. Bannister

Tel.: +356-31 66 55

Fax: +356-31 05 77

**AN INVESTIGATION INTO THE EFFECTS OF SLUDGE AMENDMENT OF SOILS  
ON AGRICULTURAL PESTICIDE TRANSPORT**

**Co-ordinator:** Institut für Wasser-, Boden- und Lufthygiene, Berlin, Germany (Norbert Litz)

**OBJECTIVES**

- Investigation of the effects of sludge amendment to soil on pesticide transport affected by sludge-derived detergents, organic matter and dissolved organic matter;
- Influence of organic matter, dissolved organic matter and incubation of amended soils on adsorption and desorption of pesticides (Atrazine, Simazine, Terbutylazine and Ametryne);
- Influence of sludge amendment to soil on heavy metal content and degradation of pesticides (Atrazine), PAHs (polyaromatic hydrocarbons), PCB (polychlorinated biphenyls), OCP (organochlorine pesticides) and detergents (LAS = linear alkyl benzenesulfonate and homologues and Nonylphenol);
- Influence on sludge amendment to soil on growth yield and soil properties.

**ACTIVITIES**

- ◇ Sorption studies under laboratory conditions with triazine herbicides (Ametryne, Atrazine, Simazine Terbutylazine) with samples from an Egyptian clay soil and Israeli loess soil, sewage sludge and compost;
- ◇ Breakthrough studies under laboratory conditions with Ametryne, Atrazine and Terbutylazine with compost and sewage sludge amended fine sand and loess: fine sand columns;
- ◇ Breakthrough studies under laboratory conditions with triazine herbicides (Ametryne, Atrazine, Terbutylazine) with amended soils: Fresh versus incubated systems;
- ◇ Movement of Terbutylazine in test microcosms;
- ◇ Atrazine degradation under laboratory conditions in sludge amended soils;
- ◇ Field experiments in Egypt (a sandy Gypsum Soil and a Vertisol) to investigate the effects on sludge-amendment to soil on crop production and soil properties;
- ◇ Field experiments in Egypt (a sandy Gypsum Soil and a Vertisol) to investigate degradation and movement of Atrazine, LAS including homologues and Nonylphenol after sewage sludge amendment;
- ◇ Field experiments in Egypt (a sandy Gypsum Soil and a clay rich Vertisol) to investigate the influence of sludge amendment on the heavy metal content;
- ◇ Influence of sewage sludge amendment on PAH (poly aromatic hydrocarbons), PCB (polychlorinated biphenyls) or OCP (organochlorine pesticides) content in soil.

**OUTCOME**

*Laboratory experiments:*

- ⇒ Different aspects of sorption of Atrazine, Simazine, Ametryne and Terbutylazin to organic matter was investigated in compost and sewage sludge.

*Field experiments:*

- ⇒ The application of sewage sludge and accumulative effects were tested. The behaviour of heavy metals, organic compounds and some pesticides like Atrazine was investigated. The influence of the application of sewage sludge on plant growth was investigated.

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## PARTNERS

### INSTITUT FÜR WASSER-, BODEN- UND LUFTHYGIENE

Umweltbundesamt

Postfach 33 00 22

14191 Berlin

Germany

Norbert Litz

Tel.: +49-30-89 03 13 37

Fax: +49-30-89 03 28 30

### SOIL AND WATER RESEARCH INSTITUTE ARC

Giza

Egypt

Nabil el-Mowehlhi

Tel.: + 20-2-72 55 49

Fax: + 20-2-72 06 08

### THE VOLCANI CENTER

Agricultural Research Organization

Institute for Soils & Water

P.O. Box 6

50250 Bet Dagan

Israel

Ellen Graber

Tel.: +972-3-968 33 43

Fax: +972-3-997 71 93



**REHABILITATION DES TERRES DEGRADEES AU NORD ET AU SUD DU SAHARA. UTILISATION DE LEGUMINEUSES PERENNES ET DES MICRO-ORGANISMES ASSOCIES POUR L'ETABLISSEMENT DE FORMATIONS PLURISTRATES**

**Coordinateur:** Orstom, Paris, France (Michel Grouzis)

### OBJECTIFS

- De comparer les caractéristiques des micro-organismes et d'acquérir une bonne connaissance du fonctionnement de la symbiose dans son milieu naturel;
- De clarifier les interactions biochimiques de la rhizosphère;
- De déterminer les mécanismes d'adaptation à l'aridité des plantes hôtes et des associations symbiotiques;

### ACTIVITES

- ◇ L'étude de la diversité, orientée sur la place des légumineuses pérennes herbacées et ligneuses dans les systèmes écologiques et de production et l'analyse de la diversité des micro-organismes en fonction des espèces et des biotopes;
- ◇ Les aspects fonctionnels axés sur la recherche des caractères d'adaptation à l'aridité. Dans différentes conditions expérimentales (*in vitro*, en conditions contrôlées et *in situ*), les caractéristiques biochimiques, physiologiques et écophysiologiques des micro-organismes symbiotiques et des plantes hôtes sont déterminées, pour mettre en évidence leurs réponses adaptatives à l'aridité. Les caractéristiques de croissance des isolats et leurs réponses au stress hydrique et à la salinité, l'écophysiologie de l'hôte, la phénologie de la nodulation, la vitesse de croissance du pivot racinaire et l'impact de l'inoculation avec des souches sélectionnées, constituent les points de ce niveau d'étude;
- ◇ L'application à la réhabilitation des terres, qui constitue le troisième volet du programme, rassemble les modalités de mise en place de formations pluristrates (propriétés germinatives, modalités d'élevage et de transplantation) et expérimente aux champs les acquis des résultats antérieurs. Parcelles témoins et parcelles enrichies en légumineuses pérennes sont évaluées sur leurs capacités de production et leur impact sur la fertilité des sols.

### RESULTATS OBTENUS

Au Sénégal, les légumineuses spontanées (281 espèces - 76 genres) représentent 16% des 2100 taxons. Avec respectivement 32 % et 41 % des taxons, les phanérophytes et les thérophytes sont les types biologiques dominants. Globalement l'importance des légumineuses décroît du sud au nord du pays. De nombreuses légumineuses sont utilisées dans l'artisanat, l'alimentation humaine et animale et en agroforesterie.

En Tunisie, les légumineuses (232 espèces - 40 genres) relèvent en grande partie de l'élément phytogéographique méditerranéen. On ne compte que 33% d'espèces pérennes, mais l'abondance relative des pérennes par rapport à l'ensemble tunisien devient plus importante dans le sud (41%). 65 % des espèces se retrouvent dans les bioclimats arides. De plus, les légumineuses présentent globalement un niveau moyen d'appétibilité plus élevé (60% présentent un intérêt pastoral) que celui de l'ensemble des taxons (26 %).

Des prospections/récoltes de plantes, de nodules et de sols ont été effectuées en Tunisie et au Sénégal. Sur la base de l'étude du profil protéique, une grande diversité a été mise en évidence parmi les 660 souches isolées de différentes légumineuses d'origine variée [Sénégal, Tunisie, Afrique de l'Ouest, de l'Est, Amérique (Brésil)], ainsi que de représentants de différentes espèces reconnues de *Rhizobium*, *Bradyrhizobium*, *Azorhizobium* et *Sinorhizobium*. Vingt-deux groupes principaux SDS-PAGE ont été mis en évidence parmi les souches à croissance rapide. Il n'apparaît aucune relation entre diversité taxinomique, la plante d'isolement et l'origine géographique.

Au Sénégal, l'analyse de 150 souches originaires des zones semi-arides et isolées à partir de 55 espèces de petites légumineuses appartenant à 17 genres, montre que la plupart des isolats appartiennent au genre *Bradyrhizobium* (120 isolats) et se répartissent en six groupes électrophorétiques déjà décrits. Les 30 isolats restants, à nodulation plus spécifique, sont classés parmi les rhizobiums à croissance rapide et constituent 4 groupes électrophorétiques distincts, qui pourraient représenter des espèces nouvelles.

En Tunisie, l'analyse de 108 souches issues de 42 espèces de légumineuses a permis de distinguer 27 groupes électrophorétiques dont 17 nouveaux. Des techniques plus discriminatoires (PCR/RFLP, hybridation ADN/ADN) sont nécessaires pour la caractérisation de ces souches de *Rhizobium*.

Les réponses physiologiques à la chaleur, à la salinité et à l'aridité (sécheresse simulée) d'une cinquantaine de souches tunisiennes et sénégalaises ont été analysées. Il apparaît que les souches sont en général capables de se développer à des températures supérieures à 40°C. Contrairement aux souches de Tunisie, celles du Sénégal semblent supporter des teneurs en sels élevées (> ou = à 1 % NaCl). A potentiel osmotique égal, le stress salin inhibe davantage la croissance des micro-organismes, car à l'effet osmotique s'ajoute l'effet toxique des ions. Il y a une plus grande tolérance des souches de Tunisie aux antibiotiques par rapport à celles du Sénégal.

Des travaux spécifiques ont été conduits sur *Acacia tortilis* subsp *raddiana*, espèce commune du nord et du sud du Sahara.

On observe une forte variabilité intersites de la phénologie chez cette espèce. Au Sénégal cette variabilité semble être liée étroitement à la réserve hydrique du sol, contrairement à la Tunisie où le cycle de vie est indépendant des précipitations et par conséquent des réserves en eau. Le déroulement des différentes phases se réalise en saison estivale, qui en climat méditerranéen est sèche. Le facteur thermique y semble plus déterminant.

L'étude du comportement de l'espèce en milieu naturel fait apparaître de nettes différences entre *Acacia raddiana* du nord et du sud du Sahara:

- les variations saisonnières de la tension de base sont nettement plus élevées au Sénégal;
- la contrainte hydrique interne maximale est nettement plus élevée pour les *Acacia* du Sénégal;
- l'équilibre entre tension de sève et état hydrique du sol ne se réalise pas en Tunisie;
- dans les deux pays cette espèce développe une régulation stomatique.

Ces différences semblent être liées à une plus forte saisonnalité de la feuillaison au Sénégal et à la proximité d'une nappe phréatique en Tunisie.

Au Sénégal, les réponses stomatiques de deux espèces d'*Acacia* et d'*Indigofera* aux facteurs du milieu (Rayonnement photosynthétiquement actif (PAR), Température de l'air, Déficit de Pression de Vapeur) et aux caractéristiques intrinsèques à la plante (tension de sève), ont été établies à partir d'observations *in natura*, ce qui a permis de modéliser le fonctionnement stomatique de ces espèces.

La comparaison du comportement des populations d'*Acacia raddiana* du Sénégal et de Tunisie en conditions contrôlées a montré les spécificités de chacune d'entre elles sur le plan de la croissance des parties aériennes et souterraines et de la mise en place des nodosités. Les travaux ont permis d'avancer l'hypothèse d'une séquence obligatoire d'étapes de fonctionnement de divers « puits » lors de la reprise de la vie active après une saison sèche, à savoir : apparition de racines, débourrement, développement du feuillage et de racines secondaires et enfin nodulation.

La caractérisation de l'environnement biochimique et microbiologique dans lequel se déroulent l'infection microbienne et la fixation de l'azote a été réalisée par l'évaluation d'un certain nombre d'indicateurs (pH, P assimilable, C-CO<sub>2</sub>, C biomasse microbienne, qCO<sub>2</sub> (mg CO<sub>2</sub>.h<sup>-1</sup>. µg C-biomasse) et l'activité de quelques enzymes (protéase, phosphatase, glutaminase). Cette analyse a été conduite sur une diversité de sols (3 au Sénégal, 2 en Tunisie) et d'espèces (3 au Sénégal, 2 en Tunisie). En général les activités biochimiques et biologiques des sols des zones semi-arides du Sénégal et arides de Tunisie sont très différentes. Il semble que la situation tunisienne permet une activité élevée de la biomasse microbienne avec une production de nutriments et une dynamique plus élevée. Au Sénégal, une nodulation abondante a été montrée *in situ* au cours de la saison des pluies sur les racines d'*Acacia tortilis* adultes, suggérant une active fixation d'azote pendant cette période. L'état hydrique du sol joue un grand rôle dans les variations de l'activité rhizosphérique en milieu naturel.

Les propriétés germinatives d'une trentaine d'espèces de légumineuses dont neuf espèces d'*Acacia* ont été étudiées en Tunisie et au Sénégal, en vue de réunir les modalités d'établissement d'espèces utilisables dans les opérations de réhabilitation.

En général la température optimale de germination est plus élevée pour les taxons du Sénégal (30-35°C) que de Tunisie (20°C). Dans leur grande majorité les espèces sont indifférentes à la lumière. On observe généralement (9/10 des espèces du Sénégal, 2/3 des espèces de Tunisie) une inhibition tégumentaire

facilement levée par une scarification mécanique ou chimique (immersion dans l'H<sub>2</sub>SO<sub>4</sub>). Les espèces tolèrent généralement des conditions de stress hydrique au stade germination notamment certains *Indigofera*. A potentiel osmotique égal les *Acacia* sont plus sensibles au stress salin qu'hydrique. On éprouve une grande difficulté à mettre en relation la tolérance au stress au stade germination avec l'écologie de l'espèce.

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## PARTENAIRES

### ORSTOM

Département Milieux et Activité Agricole  
Rue Lafayette 213  
75480 Paris 10  
France

Michel Grouzis  
Tel.: +33-1-48 03 77 15  
Fax: +33-1-48 03 08 29

### INSTITUT DES REGIONS ARIDES MEDENINE

B.P. 158-159  
4119 Medenine  
Tunisia

Noureddine Akrimi  
Tel.: +216-5-64 06 61  
Fax: +216-5-64 04 35

### UNIVERSITA DEGLI STUDI DELLA TUSCIA

Dipartimento di Agrobiologia ed Agrochimica  
Via S. Camillo de Lellis  
01100 Viterbo  
Italy

Stefano Grego  
Tel.: +39-7-61 35 72 46  
Fax: +39-7-61 35 72 42

### INSTITUT SENEGALAIS DE RECHERCHES AGRICOLES

Direction des Recherches sur les Productions Forestières  
B.P. 2312  
Route des Pères-Maristes Hann  
Dakar  
Sénégal

Pape Ndiengou Sall  
Tel.: +221-8-32 32 19  
Fax: +221-8-32 96 17

### CENTRE D'ECOLOGIE FONCTIONNELLE ET EVOLUTIVE

Centre L. Emberger  
B.P. 5051  
34033 Montpellier Cedex 1  
France

Fernand Warembourg  
Tel.: +33-4-67 61 32 00  
Fax: +33-4-67 41 21 38



# **1. Natural resources**

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## 1.2. Environmental research - ecosystems

**BASES FOR THE INTEGRATED SUSTAINABLE MANAGEMENT OF  
MEDITERRANEAN SENSITIVE COASTAL ECOSYSTEMS**

**Co-ordinator:** Consorzio Nazionale Interuniversitario per le Scienze del Mare, Unità Locale di Ricerca  
Firenze, Italy (Felicita Scapini)

## **OBJECTIVES**

The project will focus on sandy coastal environments and associated areas. The general objective of the project is the derivation of a management strategy for the sustainable use and development of coastal areas in the Mediterranean, and to provide a manual of recommended guidelines for its application in a general context.

- To produce baseline information on specific habitats and ecosystems in the study areas.
- To evaluate the present state of health of the ecosystems identified in 1. Suitable biological indicators will be identified and studies in order to assess their value as indicators of the adaptability of the ecosystems to change.
- To identify and characterise socio-economic constraints and potentialities regarding the economic and cultural value of each study-area.
- To develop sustainable management strategies for resource use and enhancement in the study areas.

## **ACTIVITIES**

A number of sites have been identified in each of the countries of the southern Mediterranean coast participating in the project. The sites, representative of the diversity found in the region, share a number of common issues, namely: a) intense and ever increasing human pressure, b) degradation of water quality due to an increase in sewage and waste waters, c) loss of important habitats, thus endangering numerous species of regional interest, d) coastal erosion, e) need for sustainable uses.

Sites will be characterised in terms of geography, ecology and present and potential use, by an interdisciplinary team of partners. Data base will be developed to be used by both the appropriate scientific and management agencies.

Identification of suitable biological indicators at different scales (e.g. molecular level, population level, ecosystem integrity). In this context biodiversity will be assessed, identifying the more important species, populations and assemblages of international, national and local interest, including endemics. Key species (readily available and wide-spread in the Mediterranean area) will be chosen for in-depth studies of population dynamics, eco-physiology and genetics. From these data bioassays will be developed and proposed for an early identification of human impact on diversity.

These sites will be analysed on the basis of socio-economic parameters of the study-area, with specific reference to tourism, in order to provide information to enable the correlation of activity and management practices with biological activity and diversity.

The results will be used for:

- Development of models describing in an integrated way ecosystems in the identified sites.
- Development of management strategies for resource use and enhancement in the study areas. This will include the valuation of any projects already proposed for the study areas for the socio-economic and environmental impacts.
- Constraints for the application of existing and proposed plans will eventually be evaluated.

## **EXPECTED RESULTS**

### *Scientific-technical results*

- ⇒ The project will provide an integrated knowledge of coastal areas relevant to the Mediterranean region, their socio-economic use and potentialities. The exchange of expertise and co-operative works will allow the direct transfer of techniques relevant to the areas of study, improving existing capabilities.

### *Applied results*

⇒ Guidelines and plans for sustainable use of the chosen areas will be developed from the scientific data base. Existing techniques will be tuned to local needs and new ones proposed as tools to monitor the effectiveness of the management plans.

The results of the project will be useful to the respective planning authorities of southern Mediterranean countries involved in the management of coastal environments. Other countries may follow afterwards, and indeed maximise on their returns, by implementing integrated planning and management techniques modelled on a sound scientific basis, and in considering the most beneficial trade-offs between the natural environment and socio-economic development. The project may act as a pilot study to be applied to other scenarios in both developing and developed countries in the region.

### *Deliverables*

- A guideline book for the survey and management of sandy coastal areas will be published and diffused in the region.
- Thematic maps of the sites will be constructed of the study sites to be used by planners and managers.
- Eco-assay technology and models as tools to evaluate responses to changes will be developed.

## **FOLLOW-UP**

### *Work to be carried out within the first year*

- ▶ A baseline survey at the chosen sites will be carried out by all the groups, to achieve a geographical, ecological and socio-economic description of the areas. This will permit to characterise each study-area and evaluate constraints and potentialities. Key species will be selected and the bioassay techniques tuned in the laboratories involved. Guidelines will be proposed for the formulation of management plans of the sites.

### *Follow-up for the remaining period*

- ▶ The activities of each task (geography, ecology, socio-economy, management) will continue for enhancement, repetition and consolidation of the results. Bioassays techniques will be tuned and tested. Management plans for the study sites will be proposed, each with a life-span and methods for evaluation. The second year general meeting, venue in Morocco, will be devoted to ecology; the third year general meeting, venue in Tunisia, will concern coastal management of Mediterranean sensitive ecosystems. At the end of the project a workshop on sand coastal areas integrated management will be organised in Italy involving scientific authorities and managers of the Mediterranean and other regions of the world.

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## **PARTNERS**

### **CONISMA-CONSORZIO NAZIONALE INTERUNIVERSITARIO SCIENZE DEL MARE,**

Unita' Locale Di Ricerca Di Firenze  
Dipartimento Biologia Animale e Genetica  
via Romana 17  
50125 Firenze

**Italy**

Felicita Scapini  
Tel.: +39-55-2288217  
Fax: +39-55-222565  
E-mail: scapini@dbag.unifi.it

### **AGENCE DE PROTECTION ET D'AMANAGEMENT DU LITTORAL**

Les berges du lac à zone nord, 3eme etage  
BP/2045 Cedex Tunis  
Tunisia

Habib Ben Moussa  
Tel.: +216-1-861812  
Fax: +216-1-861391

### **UNIVERSITE MOHAMMED V INSTITUT SCIENTIFIQUE**

Departement de zoologie et ecologie animale  
Avenue Ibn Battota, B.P. 703 Agdal  
10106 Rabat

**Morocco**

Abdellatif Bayed  
Tel.: +212-7-774548  
Fax: +212-7-774540  
E-mail: gecpm@maghrebnet.net.ma

### **UNIVERSITY OF MALTA INTERNATIONAL ENVIRONMENT INSTITUTE**

Old University Building  
St. Paul Street  
Valletta VLT 07

**Malta**

Patrick J. Schembri  
Tel.: +356-32902789/+356-240741  
Fax: +356-230551  
E-mail: psch@cis.um.edu.mt

### **IMAR – INSTITUTO DO MAR**

Department of Zoology  
Faculty of Sciences and Technology  
University of Coimbra  
3000 Coimbra

**Portugal**

João Carlos Marques  
Tel.: +351-39-36386  
Fax: +351-39-23603  
E-mail: jcmimar@cygnus.ci.uc.pt

### **UNIVERSITY OF BIRMINGHAM**

Centre for Urban and regional Studies and School of  
Biological Sciences  
Edgbaston  
Birmingham B15 2TT

**United Kingdom**

Alison Caffyn  
Tel.: +44-121-4147492  
Fax: +44-121-4143279  
E-mail: caffyna@css.bham.ac.uk

### **FACULTE DES SCIENCES DE TUNIS**

Laboratoire de bioecologie et systematique évolutive  
Campus Universitaire  
1060 Belvédère - Tunis

**Tunisia**

Faouzia Charfi  
Tel.: +216-1-872600  
Fax: +216-1-885480



**IMPACT ASSESSMENT AND ECONOMIC EVALUATION OF WATER  
HARVESTING TECHNIQUES IN DRY MEDITERRANEAN ZONES (WAHIA)**

**Co-ordinator:** Wageningen Agricultural University, Wageningen, Nederland (Prdr Leo Stroosnijder)

## **OBJECTIVES**

The project will develop a methodology for impact assessment and economic evaluation of water harvesting techniques in dry Mediterranean areas. Water harvesting techniques can contribute to the conservation of land and water resources and can help increasing or at least maintaining agricultural production in semi-arid and regions. By analysing the various effects of three different water harvesting techniques in dry zones in Tunisia and Morocco, the project will find out under which physical and socio-economic conditions these techniques indeed contribute to an efficient, equitable and sustainable development.

## **ACTIVITIES**

- ◇ To make an inventory of water harvesting techniques in selected dry Mediterranean zones (Work package 1);
- ◇ To identify and quantify the physical effects of selected traditional and modern water harvesting techniques within their micro-catchments (on the water balance, soil physical and chemical status, flooding hazard, ground water storage and water quality; Work package 2);
- ◇ To screen existing and develop new modules and models for assessing the economic impact of water harvesting methods (Work package 3);
- ◇ To undertake an impact assessment of these techniques, whereby the various physical effect are translated in productivity and other economic parameters, and to make an economic evaluation of these selected water harvesting techniques (Work package 4);
- ◇ To make an assessment of the potential role of water harvesting techniques with regard to the mobilisation and management of water resources in dry Mediterranean zones (Work package 5).

## **EXPECTED RESULTS**

The impact assessment methodology will be presented as a tool for decision making in water resource management in these dry zones. In this tool attention will also be given to guidelines for the users of these water-harvesting techniques. The developed methodology will be used by the agricultural development services (soil and water conservation, water resources, etc.) and other agencies responsible for planning and management of natural resources and watershed projects in the dry areas. This will have a direct and indirect impact on the well being of the population. Besides, it could also be extended to other countries with similar ecological conditions.

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## **PARTNERS**

### **WAGENINGEN AGRICULTURAL UNIVERSITY**

Nieuwe Kanaal 11  
6709 A Wageningen  
**Nederland**

Prdr Leo Stroosnijder  
Tel.: 31-317-48.24.46  
Fax: 31-317-48.47.59  
E-mail: [leo.stroosnijder@users.tct.wau.nl](mailto:leo.stroosnijder@users.tct.wau.nl)

### **INSTITUT DES REGIONS ARIDES MEDENINE**

Route De Djorf Km 22.5  
4119 Medenine  
**Tunisie**

Mr Mohamed Ouessar  
Tel.: 216-5-64.06.61  
Fax: 216-5-64.04.35  
E-mail: [houcine.khatteli@ira.rnrt.tn](mailto:houcine.khatteli@ira.rnrt.tn)

### **UNIVERSITE IBNOU ZOHR AGADIR**

Faculte Des Lettres Departement De Geographie  
B.P. 29  
8000 Agadir  
**Maroc**

Dr Lahcen Ait Tirri  
Tel.: 212-8-22.08.78  
Fax: 212-8-22.16.20

### **RIJKSUNIVERSITEIT GENT**

Vakgroep Bodembeheer En Hygiene  
Coupure Links, 653  
9000 Gent  
**Belgium**

Prdr Donald Gabriels  
Tel.: 32-9-264.60.50  
Fax: 32-9-264.62.47  
E-mail: [donald.gabriels@rug.ac.be](mailto:donald.gabriels@rug.ac.be)

### **MINISTRY OF AGRICULTURE**

Ground Water Resources Service  
**Tunisie**

Mr M.A. Mammou

**IMPROVED MANAGEMENT OF AGROFORESTRY PARKLAND SYSTEMS  
IN SUB-SAHARAN AFRICA.**

**Co-ordinator:** University Of Wales, Bangor, United-Kingdom (Dr Zewge Teklehaimanot)

**GENERAL OBJECTIVES OF THE PROJECT**

- To promote security and self-sufficiency in food production in Sub-Saharan Africa (SSA) a) by enhancing the productivity of traditional agroforestry parkland systems, and b) by maximising the economic values and optimising the marketing of parkland tree products (with particular emphasis on *Parkia biglobosa* and *Vitellaria paradoxa*).
- To help reverse the trend of environmental degradation and combat desertification in SSA by a) conserving the biodiversity of parklands, and b) promoting the sustainable use of indigenous woody plant resources.
- To use and develop the expertise of North and South research teams through the use of advanced technologies and enhance North/South collaboration to achieve a sustainable improvement in agricultural production and natural resource management in SSA.

**SPECIFIC OBJECTIVES AND GOALS**

- To assess indigenous parkland management strategies and evaluate the influence of biological, technological, socio-cultural and economic factors, including gender and tenure issues, on current and future strategies of parkland management with particular emphasis on the major tree species therein - *V. paradoxa* and *P. biglobosa*;
- To measure the impact of parkland establishment and management practices on plant resource diversity and develop strategies for restoring parkland biodiversity and sustainability;
- To assess the impact of parkland establishment and management practices on the genetic diversity and its organisation in *V. paradoxa* and *P. biglobosa* to devise strategies for conserving their genetic resources;
- To study the reproductive biology and breeding systems of *V. paradoxa* and *P. biglobosa* in relation to their genetic diversity to make identification of horticulturally superior genotypes feasible and to recommend appropriate management of these;
- To investigate the effect of pruning of *V. paradoxa* and *P. biglobosa* on yield of associated crops as an improved management technique of agroforestry parkland systems;
- To evaluate and select *V. paradoxa* and *P. biglobosa* trees with superior attributes (early fruiting, resistance to parasitism and drought, fruit yield, pulp size and sweetness, nut size, fat content and quality) through ethnotaxonomy and subsequent analysis of collections from across the range of both species;
- To improve the performance of *V. paradoxa* and *P. biglobosa* by a) reducing their juvenile phase, and b) investigating the ecology and biology of their parasites and developing their control methods;
- To investigate the chemistry of *V. paradoxa* oil, and identify unique compounds in parkland fruits with commercial potential;
- To study the market structure and methods of storage and processing of *V. paradoxa* and *P. biglobosa* products and identify limitations and up-to-date development (quality, demand, innovation) potentials; and
- To test and develop improved methods of fruit storage, processing and post-processing village technologies to improve and enhance quality of *V. paradoxa* products.

**ACTIVITIES**

Traditional agroforestry parkland systems - where annual crops are grown in fields with scattered, "protected" trees - are one of the most widespread system of land use in Sub-Saharan Africa (SSA). Recent evidence, however, suggests that parklands have been degrading, at the system, species and

genetic level. This project, therefore, proposes to initiate an integrated and multi-disciplinary approach in order to reverse this trend. Initially, the indigenous knowledge and management practices of parkland systems will be studied so that due prominence is given to local people's knowledge and practices in the research and future improvement of parklands. Possibilities of improving parklands productivity will be investigated by applying pruning to *Vitellaria paradoxa* and *Parkia biglobosa*. The impacts of different parkland management practices on plant resource diversity will be assessed at systems and species and genetic levels. Methods of improving *Vitellaria* and *Parkia* trees will be investigated through selection of superior genotypes and by developing methods of enhancing their growth and fruit yield. The chemical composition of *Vitellaria* and *Parkia* fruits will also be investigated to identify compounds with commercial potential. Current marketing and processing practices *Vitellaria* and *Parkia* products will be assessed to identify constraints.

## **EXPECTED RESULTS AND IMPORTANCE FOR SUB-SAHARAN AFRICA**

Methods of enhancing the agricultural and economic productivity of parklands in Sub-Saharan Africa, while conserving and restoring biodiversity, will be developed. This is based on better yields of annual crops through better tree-crop combinations and revised management of trees, generation of greater value from tree products through development and reintroduction of improved cultivars of *Vitellaria* and *Parkia*, and by improved methods of storage and processing, and by identification of unique compounds of fruits with commercial potential. A unique database of marketing information system will also be developed for improvement of existing methods, develop new market channels and recommend appropriate price structure such that farmers, merchants, processors and consumers are all reasonably satisfied, without either the merchants or the processors making an excessive profit at the expense of the farmers. The major goal of the proposed project is the improvement of traditional agroforestry parklands, which has fundamental bearings on food security and sustainable natural resource management in SSA. The benefits intended through the present proposal are restoration of tree resources to parklands, and increased supplies of products from *Vitellaria* and *Parkia*. The domestication of *Vitellaria* and *Parkia* in parklands through improved agroforestry practices will notably enable farmers' families to gain nutritionally and generate cash income from the sale of the fruits. The existence of regional and international markets for *Vitellaria* and *Parkia* products offers remarkable opportunities for the development of agriculturally based industries in SSA.

## **PARTNERS**

### **UNIVERSITY OF WALES**

Deiniol Road  
L157 2uw Bangor  
United Kingdom

Dr. Zewge Teklehaimanot  
E-mail: Z.Teklehaimanot@Bangor.Ac.Uk  
Tel.: 44-1248-382.639  
Fax: 44-1248-354.997

### **BEN-GURION UNIVERSITY OF THE NEGEV**

Inst. For Agric. And Applied Biology  
P.O. Box 653  
84105 Beer-Sheva  
Israel

Dr. Zeev Wiesman  
E-mail: Wiesman@Bgumail.Bgu.Ac.II  
Tel.: 972-7-464.1905  
Fax: 972-7-647.2984

### **CENTRE NATIONAL DE SEMENCES FORESTIERES**

01 B.P. 2682  
Ouagadougou 01  
Burkina Faso

Mr.Sibidou Sina  
Tel.: 226-30.12.33  
Fax: 226-30.12.32

### **INSTITUT D'ECONOMIE RURALE (IER)**

P.O. Box 258  
Bamako  
Mali

Dr. Modibo Sidibe  
E-mail: Ier.Mali@Cgnet.Com  
Tel.: 223-22.37.75  
Fax: 223-22.37.75

### **COOP. OFFICE FOR VOLUNTARY ORGANISATIONS**

Of Uganda  
P.O. Box 6908  
Kampala  
Uganda

Mr. Henry Lam  
E-mail: Shea@Covol.Org  
Tel.: 256-41-341.110  
Fax: 254-41257.803

### **ICRAF - INTERNATIONAL CENTRE FOR RESEARCH IN**

Dr. Edouard Bonkoukou

**AGROFORESTRY**

P.O. Box 320  
Bamako  
Mali

E-mail: E.Bonkougou@Cgnet.Com  
Tel.: 223-22.33.75  
Fax: 223-22.86.83

**CIRAD**

Campus International De Baillarguet  
B.P. 5035  
34032 Montpellier  
France

Dr. Helene I. Joly  
E-mail: Joly.H@Cirad.Fr  
Tel.: 33-4-6759.3771  
Fax: 33-4-6759.3732

**ALBERT-LUDWIGS-UNIVERSITAET FREIBURG**

Heinrich-Von-Stephan-Strasse 25  
79085 Freiburg  
Germany

Prof. Michel Becker  
E-mail: Fopoml@Ruf.Uni-Freiburg.De  
Tel.: 49-761-203.37.07  
Fax: 49-761-203.37.29

**WAGENINGEN AGRICULTURAL UNIVERSITY**

Costerweg 50  
P.O. Box 9101  
6700 Hb Wageningen  
Netherlands

Prof. L.J.G. Van Der Maessen  
E-mail: Jos.Vandermaesen@Algem.Pt.Wau.Nl  
Tel.: 31-317-483.170  
Fax: 31-317-484.917

**PROPAGE**

13 Rue Paul Martin  
34000 Montpellier  
France

Dr Armelle Saint Sauveur  
E-mail: Asauveur@Pratique.Fr  
Tel.: 33-467521772  
Fax: 33-467521772

**INSTITUT DE L'ENVIRONNEMENT ET DE RECHERCHES AGRICOLES (INERA)**

03 Bp 7047  
Ouagadougou 03  
Burkina Faso

Dr. Sibiri Jean Ouedraogo  
Tel.: 226-334098  
Fax: 226-314938

**UNIVERSITY OF IBADAN**

Department Of Crop Protection And Environmental Biology  
Oyo Road  
Ibadan  
Nigeria

Prof. J. Adebayo Odebiyi  
Tel.: 234-2-8101100  
Fax: 234-2-8103043

**UNIVERSITÉ PIERRE ET MARIE CURIE**

Laboratoire De Cytologie Expérimentale Et Morphogenèse Végétale  
4 Place Jussieu  
Bâtiment N2 - Case Courrier 150  
F-75252 Paris Cedex 05  
France

Dr Georges Sallé  
E-mail: Cemu@Snv.Jussieu.Fr  
Tel.: 33-1-44274898  
Fax: 33-1-44274582

**ISTITUTO SPERIMENTALE PER LA ELAIOTECNICA (ISE)**

Contrada Fonte Umato, No 37  
65013 Citta S. Angelo  
Perscara  
Italy

Prof. Giorgio Bianchi  
E-mail: Elaiotec@Unich>It  
Tel.: 39-8595212  
Fax: 39-85959518

**AARHUS OLIEFABRIK A/S**

M.P. Bruuns Gade 27  
O. Box 50  
Dk-1800 Aarhus  
Denmark

Mr Johan Bisgaard  
Tel.: 45-87306000  
Fax: 45-87306042

**GLOBAL, PHYSIOLOGICAL AND MOLECULAR RESPONSES TO CLIMATIC STRESSES OF THREE MEDITERRANEAN CONIFERS**

**Co-ordinator:** Institut National De La Recherche Agronomique (Inra), Gazinet, France  
(M. Arbez, C. Plomion)

**OBJECTIVES**

The main objectives for Pinus of the halepensis - brutia section, Pinus pinaster and Mediterranean Cedars, are:

- to characterise the genetic variability in drought and frost conditions. Results will allow varietal tests, optimal allocation of seeds sources to sites of afforestation programmes, as well as setting up consensus core collections for conservation objectives. The variability will be analysed with both adaptive traits and molecular markers (isozymes, RAPD, AFLP, SSR) in populations covering the range of ecological conditions encountered in the different species. A simultaneous analysis of variability based on quantitative traits and the molecular markers will allow to identify disequilibria between marker loci and phenotypic traits.
- to investigate the ecophysiological and molecular mechanisms involved in drought and frost responses in order to define accessible criteria allowing early screening of tolerant genotypes in breeding and/or conservation programmes. We will focus on the definition of ecophysiological early tests for drought and frost tolerance (and/or avoidance), as well as the detection of proteins (boiling-stable drought-associated proteins) and DNA probes associated with drought response.
- to finally use the gathered information for application in afforestation, breeding and gene conservation programmes.

**ACTIVITIES**

The project is based on:

- ◇ Characterisation of the genetic diversity involving phenotypic evaluation of adaptive traits (growth, phenology, drought and cold tolerance, disease and pest resistances) through early tests in nursery or controlled chamber, as well as provenance and progeny field tests with common data bank and integrated multi-site interpretation.
- ◇ Molecular characterisation of the genetic diversity combining different aspects:
  - genetic geographic diversity on the whole repartition area of the studied species
  - evolution with time of the transferred populations, faced to a new and/or harsher habitat (Pinus halepensis, Pinus pinaster).

The project will use and compare different methods of investigating neutral diversity (isozymes, RAPD, nuclear and chloroplastic SSR, AFLP). The evaluations will be crossed with the variation patterns observed with the adaptive traits, to detect any linkage disequilibrium between quantitative traits and molecular markers.

- ◇ Study of ecophysiological and molecular response to drought.
  - This section of the project deals with measurement of different ecophysiological parameters able to define the mechanisms providing drought tolerance (water potential, leaf conductance, transpiration rate, seed germination on calibrated PEG solutions).
  - The study is extended to the molecular analysis of drought tolerance in pine trees, through identification of drought-associated boiling-stable proteins and water stress inducible genes. The sequencing of the relevant proteins and cloning of the corresponding genes will generate DNA probes for early selection of drought resistant genotypes.

Ecophysiological and molecular responses to drought of the same plant material will be compared.

## **EXPECTED OUTCOME**

### *Scientific:*

- ⇒ A joint analysis of the genetic diversity, based on variation in relevant multigenic adaptive traits, as well as polymorphism of neutral genes, combined with a better understanding of the basic mechanisms of frost and drought tolerance, will improve information and techniques to better select and conserve the forest genetic resources.

### *Techniques:*

- ⇒ Use of new powerful molecular marking methods, for evaluating genetic diversity, early selection, and discriminate varietal tests.  
Pooling data on adaptive traits and molecular marker diversity to settle consensus core collections, to scientifically base gene conservation programmes of Mediterranean conifers, and to organise dynamic ex situ gene conservation procedures.

## **PARTNERS**

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Centre De Recherches De Bordeaux  
Station De Recherches Forestieres  
B.P 45  
33611 Gazinet Cedex  
**France**

Dr Michel Arbez,  
Dr. Christophe Plomion  
E-mail: Arbez@Pierroton.Inra.Fr  
Plomion@Pierroton.Inra.Fr  
Tel.: 33.5.57.97.90.16  
Fax: 33.5.58.68.02.23

### **CENTRE DE RECHERCHES D'AVIGNON**

Unite De Recherches Forestieres Mediterraneennes  
Avenue Vivaldi  
84000 Avignon  
**France**

Dr Michel Bariteau  
E-mail: Bariteau@Avignon.Inra.Fr  
Tel.: 33.4.90.89.33.25  
Fax: 33.4.90.89.98.73

### **THE HEBREW UNIVERSITY OF JERUSALEM-REHOVOT**

Faculty Of Agriculture  
Dept Of Horticulture  
The Otto Warburg Centre  
Po Box 12  
76100 Rehovot  
**Israël**

Prof. Arie Altman  
E-mail: Altman@Agri.Huji.Ac.IL  
Tel.: 972.8.948.10.77  
Fax: 972.8.946.82.63

### **MIDDLE EAST TECHNICAL UNIVERSITY**

Departement Of Biological Sciences  
Plant Genetic & Tissue Culture Lab  
Inonu Bulvari  
06531 Ankara  
**Turkey**

Prof. Zeki Kaya  
E-mail: Zeki.Kaya@Metu.Edu.Tr  
Tel.: 90.312.210.51.17  
Fax: 90.312.210.12.89

### **INSTITUT AGRONOMIQUE & VETERINAIRE HASSAN II**

Dept. D'agronomie Et D'amelioration Des Plantes, Labo De Genetique  
B.P 6201 Rabat - Instituts  
10101 Rabat  
**Morocco**

Dr. Abdellah Ouassou  
E-mail: Ouassou@Acdim.Net.Ma  
Tel.: 212.7.681.112  
Fax: 212.7.680.112

### **CENTRE NATIONAL DE LA RECHERCHE FORESTIERE**

Charia Omar Ibn El Khattab  
Bp 763  
1050 Agdal Rabat  
**Morocco**

Dr Hassan Sbay  
E-mail: Hsbay@Hotmail.Com  
Tel.: 00.212.7.75.31.45  
Fax: 00.212.7.67.11.51

### **INSTITUT SUPERIEUR AGRICOLE DE BEAUVAIS**

Centre De Ressources Regionales En Biologie Moleculaire  
Ilot Des Poulies  
33 Rue St Leu  
80039 Amiens Cedex  
**France**

Dr Arnould Savoure  
Dr Ghislaine Grenier  
E-mail: Arnould.Savoure@U-Picardie.Fr  
E-mail: Ghislaine.Grenier@Isab.Fr  
Tel.: 33.3.22.82.7976  
Fax: 33.3.22.82.7976

### **CONSIGLIO NAZIONALE DELLE RICERCHE**

Instituto Miglioramento Genetico Delle Piante Forestali  
Via Atto Vannucci 13  
50134 Firenze  
**Italy**

Dr. Giovanni Vendramin  
E-MAIL: Vendramin@Imgpf.Fi.Cnr.It  
Tel.: 39.55.46.14.53/46.10.71

Dr Marco Michelozzi  
E-mail: Michelozzi@Imgpf.Fi.Cnr.It  
Tel.: 39.55.46.10.71  
Fax: 39.55.48.66.04

### **AGRICULTURAL RESEARCH ORGANIZATION**

The Volcani Centre  
Dept Of Field Crops & Nat. Resources  
Po Box 6  
50250 Bet Dagan  
**Israel**

Dr. Gabriel Schiller  
E-mail: Vcgabi@Volcani.Agri.Gov.II  
Tel.: 972.3.968.3678  
Fax: 972.3.966.9642



**SOUTHWEST ANATOLIA FOREST RESEARCH INSTITUTE**

Po Box 264  
07002 Antalya  
Turkey

Dr. Fikret Isik  
E-mail: Fikret.Isik@Rocketmail.Com  
Tel.: 90.242.345.0438  
Fax: 90.242.345.0442

**INSTITUT NATIONAL DE RECHERCHE GENIE RURAL, EAUX,  
FORETS**

Laboratoire De Genetique Forestiere  
Po Box 2 2080 Ariana- Tunis  
Tunisia

Dr Larbi Khouja  
Tel.: 216.1.230.039  
Fax: 216.1.717.951

**INRF**

Arboretum De Bainem  
Bp 37  
Cheraga Alger  
Algérie

Dr Abdelkader Harfouche  
E-mail: Inrf@Ist.Cerist.Dz  
Tel.: 00.213.02.94.13.99  
Fax: 00.213.02.56.07.65

**A DECISION SUPPORT SYSTEM FOR MITIGATION OF DROUGHT IMPACTS IN THE MEDITERRANEAN REGIONS**

**Co-ordinator:** Institute of Hydraulics Hydrology and Water Management, University of Catania, Italy  
(Giuseppe Rossi)

**OBJECTIVES**

- The main objective of the research is the development of a Decision Support System (DSS), aiding decision makers in the operation of water supply systems under drought conditions, determining the most effective delivery policies for irrigation. In particular, the core of DSS will include three connected modules aiming to:
  - Identify the regional drought events and determine their characteristics (duration, cumulative deficit, etc.);
  - Determine the most effective irrigation scheduling under drought conditions by using an appropriate crop-water simulation model;
  - Define the operation rules for storage facilities of the water supply system and individuate a set of drought mitigation measures.
- A second objective is the identification of main requisites and institutional framework for developing an appropriate scheme of a Drought Watch System in Mediterranean countries.

**ACTIVITIES**

- ◇ Identification of drought characteristics, including the selection of the methodology to characterise severe droughts affecting large-scale water supply systems and the analysis of the variation of drought characteristics in Mediterranean areas on the basis of the selected case studies.
- ◇ Modelling irrigation management under drought conditions, aiming to select, improve, and/or develop the models to be used for determining appropriate irrigation scheduling at different levels of information availability with reference to different management objectives.
- ◇ Modelling of water system operation and evaluation of drought mitigation actions, oriented to define the operation rules of the water supply system and the emergency measures, necessary for minimising negative drought impacts in different conditions as identified in the selected case studies.
- ◇ Evaluation of the overall performance of operation models and of the specific performance during drought periods.
- ◇ Development of the software package for a Decision Support System, conceived as a tool to be easily used by decision-makers in selecting the most suitable options in irrigation management, on the basis of the results of the different interconnected models.
- ◇ Definition of the design requisites for a Drought Watch System, aiming to allow an early drought warning and a timely implementation of drought mitigation measures, on the basis of adequate hydrometeorological and water resource monitoring networks.

**EXPECTED RESULTS**

- ⇒ The development of appropriate procedures to identify drought, to define irrigation scheduling and to simulate water supply system under drought conditions, to be connected in a Decision Support System;
- ⇒ The analysis of the applicability of the proposed procedures and of the DSS package to some irrigation districts selected in the Mediterranean semi-arid countries involved in the Project (Portugal, Tunisia, Italy, Jordan and Syria);

⇒ The definition of criteria and organisational structure for developing a Drought Watch System in the Mediterranean semi-arid regions.

The main outcome of the project should be a comprehensive approach, developed with the contribution of significant experiences of different countries, to the problem of coping with drought in semiarid Mediterranean regions which present a high vulnerability to water deficit especially in agricultural sector.

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## PARTNERS

**ISTITUTO DI IDRAULICA IDROLOGIA E  
GESTIONE DELLE ACQUE**  
FACOLTÀ INGEGNERIA-UNIVERSITÀ DI CATANIA  
V.le Andrea Doria, 6  
95125 Catania  
**Italy**

Giuseppe Rossi  
Tel.: (+39-95) 737 24 07  
Fax: (+39-95) 33 92 25  
E-mail: grossi@k200.cdc.unict.it

**INSTITUTO SUPERIOR DE AGRONOMIA (DER)  
UNIVERSIDADE TÉCNICA DE LISBOA**  
Tapada da Ajuda  
1399 Lisboa Codex - Lisbon  
**Portugal**

Luis Santos Pereira  
Tel.: (+351-1)360 20 80/362 15 75  
Fax: (+351-1)362 15 75  
E-mail: lspereira@isa.utl.pt

**INSTITUT NATIONAL DE RECHERCHE EN GENIE  
RURAL EAUX ET FORETS**  
Rue Hedikhanay  
Tunis 1004  
BP N. 10  
Ariana 2080  
**Tunisia**

Abdelaziz Zairi  
Tel.: (+216-1)70 90 33 / 71 96 30  
Fax: (+216-1)71 79 51  
E-mail: bergaouim@yahoo.com

**WATER AND ENVIRONMENT RESEARCH  
AND STUDY CENTER**  
University Of Jordan  
Salt Road  
Amman  
**Jordan**

Muhammed Shatanawi  
Tel.: (+962-6)535 50 00  
Fax: (+962-6)535 55 60  
E-mail: juwater@amra.nic.gov.jo

**CHANGES IN ARID MEDITERRANEAN ECOSYSTEMS ON THE LONG  
TERM THROUGH EARTH OBSERVATION**

**Co-ordinator:** Joint Research Centre Ispra, Space Applications Institute, Ispra, Italy (Jacques Megier)

**OBJECTIVES**

- The objective of the project is to develop a comprehensive method for monitoring desertification in the south of the Mediterranean basin, which provides information useful for the operational management of arid lands and which involves all the affected countries.
- The main purpose is to discriminate, at local scale, (and after elimination of seasonal fluctuations) areas where soil and vegetation are degrading, where they are stable, where they are recovering (e.g. after restoration action has been taken). In addition, the understanding of the relationships between those changes and land use will be a major objective.
- This aim is an answer to the need for reliable and detailed data on the condition and evolution of arid zones as has been strongly expressed by officials in charge of environmental policies. Desertification in the northern shore of the Mediterranean is already a concern at the European level.
- The southern shore is far more affected because of the dryer climate, the inherent fragility of the ecosystems and high demographic pressure; a strong political will to tackle this phenomenon exists at national and international levels (confer the international Convention to Combat Desertification).

**ACTIVITIES**

- ◇ The general concept is to integrate all available data on the studied environments. This will include data collected on the ground as well as data acquired by earth observation programmes.
- ◇ The scientific approach relies heavily on the experience and results already gained by different partners, it includes:
  - Identification of ground indicators of local ecological changes (degraded condition, stable, restored,...),
  - Determination of those that can be remotely sensed,
  - Selection of the most adequate high resolution satellite data,
  - Refinement and the design of processing algorithms and data output.
- ◇ This « bottom-up » approach will then be applied to historical records of data to identify long-term changes. These local changes will be analysed in a regional eco-climatic context using medium resolution imagery acquired from the NOAA-AVHRR. Combining these results with socio-economic data will allow the recognition of changes in relation to land use. Future scenarios will be derived from an experiment on modelling changes.
- ◇ The activities over the three years of the project are divided in several main components:
  - data and information inventory,
  - local ecological changes detection,
  - change assessment in the regional and global
  - human dimension of the changes,
  - analysis and interpretation of the changes.

**EXPECTED RESULTS**

- ⇒ Remotely sensed indicators of ecological changes for the production of maps suited to land management,
- ⇒ Assessment of ecological changes over the test sites during the last 20 years,
- ⇒ Geographic Information System with ecologically homogeneous zones of Northern Africa,
- ⇒ Change interpretation according to land use and experimental modelling of change forecast,

⇒ Definition of a comprehensive processing chain dedicated to land management, which will be able to accommodate future enhancements (new types of data and algorithms).

## **PARTNERS**

### **JOINT RESEARCH CENTRE ISPRA**

Space Applications Institute  
Mapping And Modelling Unit  
Via E. Fermi 1  
21020 Ispra  
**Italy**

Jacques Megier  
E-mail: Jacques.Megier@Jrc.It  
Tel.: 39-332-789.333  
Fax: 39-332-789.469

### **ORSTOM**

Dept Milieux Et Activites Agricoles  
Rue Lafayette 213  
75480 Paris Cedex 10  
**France**

Antoine Cornet  
Tel.: 33-1-4803.7645  
Fax: 33-1-4035.1713

### **CONSIGLIO NAZIONALE DELLE RICERCHE**

Istituto Di Agrometeorologia E Analisi  
Ambientale Applicata All'agricoltura  
P. Le Delle Cascine 18  
50144 Firenze  
**Italy**

Fabio Maselli  
Tel.: 39-55-354.895/7  
Fax: 39-55-350.833

### **NATIONAL AUTHORITY FOR REMOTE SENSING & SPACE SCIENCES**

Environmental Studies & Land Use Div.  
23 Joseph Browns Tito St.  
El-Nozha El-Gedida  
Cairo  
**Egypt**

Abd-Alla Gad  
Tel.: 20-2-417.80.35 / 36  
Fax: 20-2-290.37.82

### **OFFICE REGIONAL DE MISE EN VALEUR AGRICOLE DE OUARZAZATE**

Bp 29 O.R.M.V.A.O  
4500 Ouarzazate  
**Morocco**

Ahmed Zouhri  
Tel.: 212-4-88.26.02  
Fax: 212-4-88.34.42

### **CENTRE NATIONAL DE TELEDETECTION**

Service Etudes Et Developpement  
Bp 200  
1080 Tunis Cedex  
**Tunisia**

Hedi Sahnoun  
Tel.: 216-1-76.13.33  
Fax: 216-1-76.08.90

**DEVELOPING OF REMOTE SENSING TECHNIQS FOR EVALUATING THE  
SPATIAL AND TEMPORAL DISTRIBUTION OF HYDROLOGICAL PARAMETERS  
IN ARID BASINS  
(FLAUBERT: FLOOD IN ARID UNITS BY EARTH REMOTE TECHNIQS)**

**Co-ordinator: Centre D'etude Des Environnements Terrestre & Planet, Velizy, France  
(Dr Daniel Vidal-Madjar)**

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## **OBJECTIVES**

The project is built up to provide better insights in understanding surface hydrology in arid and semi-arid regions such as Mediterranean (Israel, Morocco, Tunisia) from various remote sensing of earth technics, with an emphasis on active microwaves one (space-borne radars).

The main parameters controlling surface water cycle in arid and semi-arid regions are relative distribution of bare rock area versus colluvial and alluvial sections, inclination of hill slopes, spatial and temporal distribution of vegetation, soil moisture and soil roughness. In arid regions, the maintenance of conventional watershed gauged network is both a difficult and expensive task. Because of characteristic high resolution combined with possible global coverage, space-borne synthetic aperture radar can provide a unique tool for monitoring hydrological parameters in large watersheds. The resulting high resolution map could improve hydrological modelling for which the bottle neck is due to the lack of spatially distributed physical parameters and frequent updating.

The general objective of this project is the evaluation of accurate quantitative spatially distributed hydrological parameters, such as soil roughness and moisture content, or vegetation density, by remote sensing (optical and active microwaves) in arid and semi-arid regions to improve prediction of run-off, infiltration and erosion by their assimilation in rainfall-runoff models.

## **ACTIVITIES**

The project represents an effort to bridge the significance of soil properties derived from high resolution remote sensing with existing hydrological modelisations. Derivation of hydrological surface indices from remote sensing and their implementation in spatialised rainfall-runoff models will be first developed on relatively small units very well instrumented, test watersheds in Neguev Desert. The extension will be applied in larger scale over two real « size » basins in Morocco and Tunisia.

The validation of the significance of these indices will be performed at two levels:

- ◇ at the level of the extraction of hydrological parameters from satellites data, by comparison within specific sites in the basins with ground and surface remote measurements,
- ◇ at the level of predictions of the runoff models, by hydrometric measurements of out flows at the outlet of the entire basins and some subunits.

## **EXPECTED OUTCOME**

The expected output are the followings:

- ▷ generalisation of SPOT/Landsat-TM methods of classification for image segmentation function of roughness and vegetation density,
- ▷ evaluation of SAR data to derive indices of soil roughness and moisture,
- ▷ development of ad hoc hydrological models able to make use of remote sensing data.

## **PARTNERS**

### **CENTRE D'ETUDE DES ENVIRONNEMENTS**

#### **TERRESTRE & PLANET**

Cnrs-Dept.Observation De La Terre  
10-12 Avenue De L'europe  
78140 Velizy

**France**

Dr Daniel Vidal-Madjar

Tel.: 33-1-44.96.43.80

Fax: 33-1-44.96.49.65

E-mail: Daniel.Vidal-Madjar@Cnrs-Dir.Fr

### **CONSIGLIO NAZIONALE DELLE RICERCHE**

Istituto Di Ricerca Sulle Onde Elettromagnetiche  
Via Panciatichi 64  
50127 Firenze

**Italia**

Dr Simonetta Paloscia

Tel.: 39-55-4235.220

Fax: 39-55-4235.290

E-mail: Paloscia@Iroe.Fi.Cnr.It

### **UNIVERSIDAD DE VALENCIA**

Facultad De Fisica Depto.De Termodinamica  
C/Dr. Moliner 50  
46100 Burjassot (Valencia)

**Espana**

Dr Jose Moreno

Tel.: 34-6-398.3112

Fax: 34-6-364.2345

E-mail: Jose.Mreno@Uv.Es

### **BEN GURION UNIVERSITY**

Blaustein Inst. For Desert Research Desert Hydrology Unit  
Sede Boker Campus  
84990 Sede Boker

**Israel**

Dr Eilon Adar

Tel.: 972-7-6596.901/904

Fax: 972-7-6596.909

E-mail: Eilon@Bgumail.Bgu.Ac.II

### **BEN GURION UNIVERSITY**

Blaustein Inst. For Desert Research Remote Sensing  
Laboratory  
Sede Boker Campus  
84990 Sede Boker

**Israel**

Pr Anatoly Gitelson

Tel.: 972-7-6596.858

Fax: 972-7-6596.909

E-mail: Gitelson@Bgumail.Ac.II

### **UNIVERSITE MOHAMMED V**

Ecole Mohammadia D'ingenieurs (Emi) Labo Analyse De  
Systemes Hydrauliques  
Avenue Ibn Sina Po Box 765  
Agdal (Rabat)

**Maroc**

Pr Driss Ouazar

Tel.: 212-7-67.05.79

Fax: 212-7-77.88.53

E-mail: Ouazar@Emi.Ac.Ma

### **ECOLE NATIONALE INGENIEURS TUNIS**

Labo De Teledetection & Systemes D'information A  
Reference Spatiale  
Bp 37 - Campus Universitaire  
1002 Tunis Belvedere

**Tunisie**

Dr Mohammed Rached Boussema

Tel.: 216-1-872.627

Fax: 216-1-872.729

**SUSTAINABLE HALOPHYTE UTILISATION IN THE MEDITERRANEAN  
AND SUBTROPICAL DRY REGIONS**

**Co-ordinator:** Universität Osnabrück, Osnabrück, Germany (Helmut Lieth)

**OBJECTIVES**

- Demonstration of ecological sustainability and economic feasibility of halophytic crops irrigated with saline water up to seawater salinity; final goal: cashcrop halophytes.
- Development of quick checks for the salinity tolerance of species from different climatic regions to be used in European and third Mediterranean countries.
- Development of sustainable growth conditions in inland and coastal sites.
- Testing for selected varieties of conventional Mediterranean crops the feasibility of lower saline irrigation.
- Demonstration of the feasibility of saline irrigated ecosystems for land reclamation, dune stabilisation, landscape management, greenification of housing areas, wasteland, roadsides and roofs, horticulture, agriculture, sea food, saving of fresh water, tertiary waste water treatment and CO<sub>2</sub> sequestration
- Testing the suitability of salinity tolerant species e.g. for animal feed, pharmacology and cosmetics.
- Collection of suitable species and information about optimal irrigation techniques with saline water in salt affected soils, coastal and desert areas.
- Demonstrating climatic impacts on halophytes growing in different salinities.
- Modelling the economic potential of halophyte utilisation and saline irrigation systems.

**ACTIVITIES**

- ◇ Establishment of saline irrigation systems at various locations in the Mediterranean region:
  - Terias site/Catania, Sicily;
  - Hammam Lif site/Hammam Lif, Tunisia;
  - Agadir site/Agadir, Morocco;
  - More locations are planned for future proposals.
- ◇ Stimulation of saline irrigation projects at feasible locations outside of the CA group.
- ◇ Further developments of the Mediterranean and global networks.

**EXPECTED RESULTS**

*Achieved:*

- ⇒ Scientific-technical results  
The ecological sustainability for several halophytes under seawater and lower salinity irrigation has been shown in different environments around the Mediterranean region.  
Quick check methods for salinity tolerance ranges on physiological, laboratory and small field scale have been elaborated and are now in operation at several partners institutions.
- ⇒ Pilot and demonstration plants  
Establishment of research demonstration sites:
  - at Terias, Sicily/Catania seawater and brackish water irrigation testing systems;
  - at Agadir, IAV Hassan II/Morocco for halophyte propagation and quick check analysis;
  - at Hammam Lif/Tunisia salt water irrigation tanks and plots for research;
  - at Agadir Beach Club seawater irrigation demonstration site for halophyte plantation;
  - at University of Giessen/Germany salinity quick check system under controlled environment;
- ⇒ Commercialisation



- Contacts have been established with small firms to apply halophytes and saline irrigation on
- roof greenification
  - golf course turf

*Expected:*

⇒ All results achieved so far justify the establishment of small field scale application. Feasible pilot farms modelled after the Terias site will be described for future funding. Pilot sites are needed in the East Mediterranean region for various reasons. More contacts for commercial application are expected through the two networks.

## **FOLLOW-UP**

*Research oriented*

- ▶ Crop growth trials of different varieties irrigated with saline water of different salt concentrations and crop physiological parameters will be established. At University of Osnabrück/Germany a research project on salinity stress physiology has been initiated. Salt accumulation and its distribution in the soil under successive irrigation with saline water will be investigated. The economic feasibility will be proofed with a model.

*Application oriented*

- ▶ The demonstration sites will be further developed in Terias/Catania and Agadir/Morocco. Small farms in Dakhla will be developed in South Morocco, Hammam Lif/Tunisia and Ras Sudr/Egypt depending on availability of funds.

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## **PARTNERS**

### **UNIVERSITÄT OSNABRÜCK**

Institute for Environmental Systems Research  
Artilleriestraße 34  
49069 Osnabrück  
**Germany**

Helmut Lieth - Michael Matthies  
Tel.: +49-451-969 25 47  
Fax: +49-451-969 25 70  
E-mail: [helmut.lieth@usf.uni-osnabrueck.de](mailto:helmut.lieth@usf.uni-osnabrueck.de)

### **TIERÄRZTLICHE HOCHSCHULE HANNOVER**

Botanisches Institut  
Ecophysiological Investigations  
Bunteweg 17D  
30559 Hannover  
**Germany**

Bernd Huchzermeyer  
Tel.: +49-511-95 38 83  
Fax: +49-511-953 85 84

### **INSTITUT AGRONOMIQUE MÉDITERRANÉEN DE BARI**

International Centre for Advanced Mediterranean  
Agronomic Studies  
Via Cherio 9  
70010 Valenzano / Bari  
**Italy**

Atef Hamdy  
Tel.: +39-80-780 61 11  
Fax: +39-80-780 62 06  
E-mail: [iamdir@vm.csata.it](mailto:iamdir@vm.csata.it)

### **STUDIO SARDO DI CATANIA**

Agricultural Research  
Via Dr. Consoli 13  
95124 Catania / Sicilia  
**Italy**

Vito Sardo  
Tel.: +39-95-31 73 03  
Fax: +39-95-731 19 92

### **UNIVERSIDADE DE LISBOA**

Faculdade de Ciencias  
Departamento de Biologia Vegetal  
Campo Grande Edificio Cé, 4 Piso  
1700 Lisboa  
**Portugal**

Isabel Cacador  
Tel.: +351-1-757 31 41  
Fax: +351-1-759 77 16

university of the aegean  
Department of Environmental Sciences  
Karantoni 17  
81100 Mytilini  
**Greece**

Nikos Margaritis  
Tel.: +30-251-200 73  
Fax: +30-251-237 83

### **INSTITUT AGRONOMIQUE & VÉTÉRINAIRE HASSAN II**

Complexe Horticole d'Agadir  
P.O. Box 773  
Agadir  
**Morocco**

Redouane Choukr-Allah - Sherif Harrouni  
Tel.: +212-8-22 57 53  
Fax: +212-8-24 22 43  
E-mail: [chagadir@mtds.com](mailto:chagadir@mtds.com)

**UNIVERSITY OF KARACHI**  
Department of Botany  
75270 Karachi  
**Pakistan**

Ajmal Khan  
Tel.: +92-21-47 90 04  
Fax: +92-21-496 97 13  
E-mail: [ajmal@www.fascom.com](mailto:ajmal@www.fascom.com)

**INSTITUT NATIONAL DE LA RECHERCHE  
SCIENTIFIQUE ET TECHNIQUE**  
Nutrition Mineral Laboratory  
P.O. Box 95  
2050 Hamman Lif  
**Tunisia**

Moktar Hajji - Abdelly Chedly  
Tel.: +216-1-43 09 17  
Fax: +216-1-43 09 34

**DESERT RESEARCH CENTRE**  
Mineral Nutrition  
El Mataria  
Cairo  
**Egypt**

Hassan El Shaer - Mahmoud El Kadi  
Tel.: +20-2-243 54 49  
Fax: +20-2-245 78 58

**ENVIRONMENT RESEARCH AND WILDLIFE**  
Development Agency  
P.O. Box 9903  
Abu Dhabi / Sweihan  
**United Arab Emirates**

Benno Boer  
Tel.: +971-3-74 75 55  
Fax: +971-3-74 76 07  
E-mail: [narc@emirates.net.ae](mailto:narc@emirates.net.ae)

**DAMAGE OF CORAL REEFS BY RECREATIONAL ACTIVITIES: RESTORATION STRATEGIES AND THE DEVELOPMENT OF NOVEL MARKERS FOR ENVIRONMENTAL STRESS**

**Co-ordinator:** Johannes Gutenberg Universität, Mainz, Germany (Werner E.G. Müller)

**OBJECTIVES**

- To carry out a long term quantitative account of species diversity in 4 different areas along northern Red Sea coral reefs (Hurghada, Ras Muhammad, Nueiba, Eilat). In each place, heavily visited and less visited sites will be studied.
- To establish an active restoration protocol by following the success of sexual and asexual coral recruits in situ.
- To establish a ubiquitous reef-stress bioassay (HSP70) and to assess the stress loads from tourist activities in different reef localities.
- To provide the scientific community and decision-making authorities with data for best assessment towards the active conservation of Red Sea coral reefs.

**ACTIVITIES**

*Haifa Bay*

An area adjacent to one of the major industrial areas of Israel, and a considerable number of industries discharge their effluents into the bay either directly or through the Kishon river. It is also an area that is used as a commercial fishing ground.

*The Northern Adriatic (Rovinj)*

A highly polluted area including zones heavily polluted and others that are less affected by human activities. The gradient of pollution at the marine sites near Rovinj ranges from non-impacted sites through gradually more impacted sites, and areas heavily impacted by the wastes of a local cannery.

*The Aegean coast (Izmir Bay)*

This is with a total length of 2,800 km, Turkey's longest coastline. Wastewater discharges into the Aegean occur at a total of fifteen points along its Turkish coastline, including seven rivers, six tourist enterprises, other sources of household wastes of various sizes, and one industrial zone. The total pollution load of these sources is equivalent to that of a population of around 10 million.

- ◇ The selected organisms
  - Botryllus schlosseri (Pallas) is a cosmopolitan encrusting colonial tunicate (Protochordata);
  - The siliceous sponge Geodia cydonium (Jameson) is a common cosmopolitan organism;
  - The mytilid mussels [Mytilus galloprovincialis, Mytilaster minimus] are cosmopolitan bivalves;
  - The gilthead seabream (Sparus aurata; Sparidae) is an economically important marine fish.

All four organisms were found to be useful as model organisms for biochemical monitoring of environmental pollutants.

- ◇ Stress proteins and HSP70

Many cells from bacteria to human respond ubiquitously to stress with the synthesis of a small group of highly conserved proteins, termed heat shock proteins (HSPs). HSPs are divided into five families, designated by their apparent molecular weight (in kDa) on SDS-polyacrylamide gels. The present proposal deals with the HSP70 family which are phylogenetically highly conserved proteins.

The study will be done simultaneously on all four animals collected from clean, relatively non-polluted areas (laboratory studies) and on animals collected from polluted or semi polluted areas for evaluation of the levels of different heat shock proteins (hsps) in their tissues (field studies). Additionally, laboratory studies on hsp synthesis will be performed in vitro on the Botryllus cell line recently developed. In the laboratory studies different stressors and combinations of different stressors will be used such as heat shock, heavy metals, water soluble fractions of oil,

sedimentation, detergents, carcinogens, different drugs, aerial exposure, mechanical damages, polychlorinated biphenyls and more. In these experiments, the study of stress proteins will be conducted through three routes (metabolic labelling, hsp-expression using specific antibodies as probes, measurement) of hsp-mRNA level using cDNA probes, to obtain the rate of expression of the hsp-gene on the level of transcription/post-transcription.

- ◇ Study on MFO systems in fish and mussels  
This study will be done simultaneously on the gilthead seabream and the mussel *M. galloprovincialis*. The methods being used are partly similar to those used in the section of the hsp (such as using of monoclonal antibodies, immunoprecipitation, cloning of MFO genes, determination of the MFO mRNA expression rates by Northern blotting) and will not be repeated here.
- ◇ Establishing bioassays with tissue cultures  
Cell cultures derived from tunicate blood cells and sponge tissues will be used. While permanent cell lines from tunicates are already established in the groups, until now only short term cultures are available from sponges. Permanent lines will be obtained from *G. cydonium* following the successful procedures applied previously with *B. schlosseri*.

## RESULTS

Until now biomarkers for the assessment of the health of coral reefs have not yet been reported. Recently we published that heat-shock proteins (HSP) are suitable markers in sponges to monitor the degree of environmental stress on these animals.

In our study the HSP with a molecular weight of 90 kDa has been selected to prove its potential usefulness as biomarker under controlled laboratory conditions and in the field. The studies have been performed with the octocoral *Dendronephthya klunzingeri* from which the cDNA coding for HSP90 was cloned first. The cDNA, termed HS9DEKL, obtained was 2558 nt in length and contained a potential open reading frame coding for 733 aa; the complete size of the cDNA (2.7 kb) was confirmed by Northern blot analysis. The deduced Mr of the putative coral HSP90, HS9DEKL, is 86,309. Homology searches revealed that the *D. klunzingeri* HSP90 is grouped together with the related metazoan polypeptides. The evolutionary rate of the coral HSP90 is low (kaa-value of  $0.158 \times 10^{-9}$ ). The expression of the HSP90 gene is upregulated by thermal stress; treatment of the animals for 2 hours at 4°C below or above the ambient temperature resulted in a >4.5-fold higher steady-state level of the respective mRNA. Also animals taken from stressed locations in the field showed an increased expression. On translational level the amount of HSP90 protein in *D. klunzingeri* was found to be strongly increased under thermal stress, exposure to the polychlorinated biphenyl (congener 118), but not after treatment with cadmium. Field studies revealed that samples taken from a non-stressed area have a low level of HSP90, but those collected from locations at which the corals are under physical stress (destruction through storm or land filling) show a high expression of HSP90. It is concluded that the chaperone HSP90 is a suitable biomarker to monitor environmental stress on corals.

- ⇒ Asexual coral recruits  
The potential role of coral transplantation in coral reef management has been studied and discussed by several authors working in coral reefs worldwide. We succeeded to establish in Hurghada a plant to raise asexual coral recruits.
- ⇒ Deliverables and demonstration plant  
Antibodies and cDNA are available for detecting of stress genes in corals, as well as the technology to raise asexual coral recruits.

## FOLLOW-UP

In the forthcoming year recruits in recreational areas will apply the molecular tools in the field application of the molecular tools, to lead to a restoration of the coral reefs.

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## **PARTNERS**

### **JOHANNES GUTENBERG-UNIVERSITÄT**

Institut für Physiologische Chemie  
Düsbergweg 6  
55099 Mainz  
Germany

Werner E.G. Müller  
Tel.: +49-6131-39 59 10  
Fax: +49-6131-39 52 43

### **UNIVERSITY OF AMSTERDAM**

Department of Zoology, and Netherlands Institute for Sea  
Research,  
P.O. Box 59,  
1790 AB Den Burg, Texel  
The Netherlands

R.P.M. Bak

### **NATIONAL INSTITUTE OF OCEANOGRAPHY**

Tel Shikmona  
P.O. Box 8030  
31080 Haifa  
Israel

Baruch Rinkevich  
Tel.: +972-4-51 52 02  
Fax: +972-4-51 19 11

### **NATIONAL INSTITUTE OF OCEANOGRAPHY & FISHERIES**

Cairo  
Egypt

H.K. Badawi

**CHANGE, STRESS AND SUSTAINABILITY: AQUATIC ECOSYSTEM RESILIENCE  
IN NORTH AFRICA (CASSARINA)**

**Co-ordinator:** University College London, London, United Kingdom (Simon Patrick)

## OBJECTIVES

CASSARINA is a multidisciplinary project concerned with the assessment of the past and present status of a group of nine wetland lakes in these three North African countries. The project has several parts. Part 1 involves the examination of dated sediment cores to reveal records of past environmental change since ca 1900. Lake contamination and disturbance, together with evidence about changes in the composition and structure of the aquatic ecosystems over a variety of time scales will be obtained from palaeo-environmental analysis of sediment cores. Part 2 comprises of modern base-line surveys in each lake for water quality and biological attributes and the implementation of a co-ordinated and validated scheme of monitoring each site over a period of one year. These approaches will not only allow the current ecological status of valued sites to be assessed with regard to past conditions but also indicate the degree of recent environmental change experienced by each lake.

## ACTIVITIES

Two successful workshops have now been held (in London and Tunis) to enable the co-ordination and harmonisation of partner group tasks and to make agreements about methodologies. Reports concerning these aspects have now been written and circulated to project members.

Part 1 field activities are now complete with sediment cores being collected from the nine primary sites during 1997 and early 1998. Monitoring of each lake began early 1988 and is on going with three monthly visits by each group to each site for the collection of water samples, zooplankton, fish and aquatic vegetation.

## EXPECTED RESULTS

### *Scientific-technical results*

The analysis of lake sediment cores in Part 1 of the project is a laboratory intensive activity and is currently on going. However, radiometric dating of some cores has been carried out and reveal a high rate of sediment accumulation (ca. 2 cm per year at one Moroccan site). Microfossil (animal and plant remains) and geochemical (metals and pesticides) analysis of recent sediments can show the nature, timing and magnitude of past changes in natural communities and indicate the causal factors. Several sites show major biological change whilst one site exhibits little disturbance. We will apply these palaeo-environmental measures to assess ecosystem resilience to environmental change at each site since ca. 1900.

In Part 2 the monitoring of modern ecosystem attributes (water chemistry, zooplankton, algae, fish, aquatic plants) at each site will reveal the extent of seasonal variation and will provide base-line species data for each site. Since the monitoring programme is currently under way and results are only available for the first period of 1998, little can be said at this stage about the nature of biological and chemical changes variation currently occurring at the sites.

### *Deliverables*

For Part 1 of the project we expect to be able to produce recent environmental histories for each site during 1999. Results from Part 2 will concern changes in ecosystem attributes, particularly in algae, zooplankton and aquatic higher plants. By combining these parts we envisage the following:

- ⇒ A comparison of modern ecological information with corresponding historical data obtained from microfossil and geochemical analysis of radiometrically dated lake sediment cores to time trends, in both site disturbance and in the ecosystem species responses.
- ⇒ To establish sampling and analytical protocols combined with selection of permanent lake transect points to encourage co-ordinated quality controlled long-term future monitoring of sites by appropriate bodies in each country.

- ⇒ The programme intends not only to enable recent environmental changes to be identified and measured over several decades but also provide a quality controlled data set and methodological framework for accurate assessment of future environmental change and the formulation of appropriate future management strategies.

## **FOLLOW-UP**

### *Workplan for 1998*

A workshop is convened for early December in Rabat, Morocco. The purpose of this meeting will be to evaluate and discuss the initial results of the monitoring programme conducted over the previous year. Final exchanges of samples will take place and initial results of the sediment core analyses will be presented.

In addition to the aims of the original CASSARINA proposal, we have discovered following extensive fieldwork on all three countries that several aspects of environmental monitoring probably can best be undertaken by employment of remote sensing techniques. This is especially important for the larger CASSARINA lakes such as the three Egyptian coastal lakes where ground survey of the very extensive but threatened reed beds is not feasible on a routine basis. With this consideration in mind we are currently promoting the value of using relevant ground survey data about the CASSARINA sites for validation of satellite generated images.

### *Workplan for 1999*

In the first part of this year we plan to finish sediment core analyses and to continue with laboratory analysis of the samples collected by the monitoring programme. In May 1999 we have planned a final workshop in El Minia (Egypt) to examine final results and diagram quality and to plan final report writing and papers for publication. Copies of the CASSARINA EXCEL database of measurement results from all the monitored sites will be checked and distributed to each partner group.

Important forward planning activities will also be undertaken at this workshop:

- ▶ To discuss how to continue the monitoring programme into the next millennium.
- ▶ To consider how to extend monitoring programme within and beyond North Africa, for example by the addition of other countries to the network and/or the inclusion of flowing waters for example.
- ▶ To evaluate the potential for integrating CASSARINA survey results with information from satellite observation.

It is planned to set up an exploratory pilot study to compare satellite images of emergent vegetation in the SW sector of Lake Manzala (this Egyptian coastal lake is the largest CASSARINA site) with species distributions derived from ground survey. Results available in 1999 should enable us to decide the feasibility of using these techniques in further work programmes.

Several CASSARINA sites are recognised as internationally important bird reserves and all are threatened by inimical environmental changes. Despite the seemingly ever increasing pressures on natural biological resources, we believe it of paramount importance that conservation and effective site management is undertaken to ensure the survival of at least some biologically valuable North African wetland lakes. This can however only be achieved by producing reliable and adequate scientific data on the past, present and future status of selected sites.



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## PARTNERS

### UNIVERSITY COLLEGE LONDON

Environmental Change Research Centre  
Bedford Way 26  
London WC1H 0AP  
United Kingdom

Simon Patrick  
Tel.: +44-171-436 92 48  
Fax: +44-171-380 75 65  
E-mail : spatrick@geog.ucl.ac.uk

### UNIVERSITÉ MOHAMMED V

Département de Zoologie et d'Ecologie  
Charia Ibn Batouta  
10106 Rabat  
Morocco

Mohamed Ramdani  
Tel.: +212-7-77 45 48  
Fax: +212-7-77 45 40

### UNIVERSITÉ TUNIS II

Faculté des Sciences  
Département de Biologie  
Laboratoire d'Ecologie Animale  
Campus Universitaire  
1060 Tunis  
Tunisia

Mohammed Mejeddine Kraiem  
Tel.: +216-1-51 26 00  
Fax: +216-1-88 54 80

### EL MINIA UNIVERSITY

Department of Botany  
61111 El Minia  
Egypt

Abdel Ahamed Fathi  
Tel.: +20-86-32 08 14  
Fax: +20-86-33 26 01

### UNIVERSITY OF BERGEN

Institute of Botany  
Allegaten 41  
5007 Bergen  
Norway

Hilary Birks  
Tel.: +47-55 21 33 45  
Fax: +47-55 31 22 33  
E-mail : birks@bot.uib.no

**GENETIC AND PHYSIOLOGICAL APPROACH TO THE DEVELOPMENT OF NEW BACTERIAL POLYMERS AND BIOMASS WITH IMPROVED PROPERTIES FOR HEAVY METAL ACCUMULATION**

**Co-ordinator:** Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol, Belgium (Ludo Diels)

**OBJECTIVES**

- To characterize of the heavy metal binding capacity and specificity of *Acinetobacter lwoffii* 1437, *Alcaligenes eutrophus* CH34 and ER121.
- To isolate of metal binding polymers (polysaccharides, proteins) from ER121
- To clone of genes encoding metal binding proteins
- To construct a vector system to insert metal binding polymers in outer membrane proteins.
- To construct recombinant *Pseudomonas putida* strains with metal binding polymers (metallothioneins, new isolated proteins) integrated in the outer membrane proteins.
- To compare metal recovery by the different biomasses in three reactor types (packed bed column, membrane bioreactor, chemostat)

**ACTIVITIES AND RESULTS**

The strain *Alcaligenes eutrophus* ER121 shows the formation of some outer membrane proteins and EPS in the presence of Zn metal ions. It was observed via electrophoretic mobility measurements and electron microscopy that the outer membrane proteins could play a role in the binding of metals like Cd, Zn, Cu, Co and Ni. The role of the polysaccharides was clearly indicated in the binding of Ag. Probably in some cases both polymers played a complementary role.

At least two proteins could be identified, a 18 kDa and a 38 kDa protein. Some fragments of the 18 kDa and a 38 kDa protein were partly sequenced. They showed some homology with a Cd homeobox and with ATPases indicating a possible role they can play in efflux or related systems. Via PCR on the 38 kDa protein a 700 bp DNA-fragment could be isolated and sequenced. This sequence indicated some homology with Zn-fingerproteins, RNA-polymerases and proteins involved in phosphate transport and regulation. Again some metal protein interaction functions could be deduced from these sequences. Further work is going on to sequence the full gene of the two proteins.

Fermentor experiments indicated the production of EPS with a hexose equivalent concentration of at least 100 mg/l. The ratio between fructose and glucose was 2/1 with a minor presence of N-acetylglucosamine and uronic acids. Experiments are going on in order to indicate the role the EPS play in the metal binding and biosorption process. Biosorption experiments will indicate the biosorption capacity of these polymers. From sand column experiments it could be concluded that the formation of polymeric substances in presence of Zn ions was very substantial. The production resulted in a complete blocking of the columns.

Expression on the surface of *E. coli* of a peptide of two hexa-His chains with the ability to chelate divalent ions caused a more than ten-fold increase of  $Cd^{2+}$  accumulation by the cells. The chelation ability of the proteins by itself does not fully account for the observed enhancement of the metal bioaccumulation properties of the cell.

Apart of increasing metal adsorption, insertion of a hexa-histidine chain at both of the two permissive sites of the LamB protein (around amino acid residues 153 and 253) appeared to result in a structure flexible enough to reach out and form a coordination sphere with the  $Ni^{2+}$  atoms coating an agarose-NTA resin. Interactions established through such "metal-ion bridges" were strong enough to cause specific adhesion of cells expressing the LamB-His hybrids to the solid support, i.e. cells displayed a kind of tropism towards surfaces coated with metal ions.

The LamB protein has shown to be an extremely versatile vector to expose peptides. Expression of LamB-His hybrids for metal biosorption or metallotropism may, therefore, be adapted to a variety of bacteria suitable for environments where desolubilization of toxic metals is desired. The results presented in this work should encourage the genetic engineering of bacterial surface structures with

heavy-ion specificity for the use as biosorption agents in reclamation of toxic, radioactive or precious metals from diluted wastes.

LamB fusions were constructed in which the whole MT sequences are anchored by their N-terminal and C-terminal ends to the permissive site 153 of the protein. Such a double-anchor appears to result in an increased stability and maintenance of the topology of the hybrid and the properties of the two separate proteins.

LamB-MT fusions multiply by more than one order of magnitude the natural ability of *E. coli* cells to bind  $Cd^{2+}$ , a trait that can be unequivocally traced to the expression and surface-presentation of the metal-binding polypeptide.

Work is in progress, with optimal preliminary results, to express LamB-MT hybrids in environmentally robust strains of *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*) and *P. putida*, in view of the potential for increasing bioadsorption of cations from sites and liquid wastes polluted with heavy metals.

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## PARTNERS

### VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK

Biotechnology Department  
Boeretang 200  
2400 Mol  
Belgium

Ludo Diels  
Tel.: +32-14-33 31 11  
Fax: +32-14-32 03 72

### UNIVERSITY OF TEL AVIV

George S. Wise Faculty of Life Sciences  
Molecular Microbiology & Biotechnology Department  
69978 Ramat-Aviv - Tel Aviv  
Israel

David L. Gutnick  
Tel.: +972-3-640 98 36  
Fax: +972-3-640 94 07

### CENTRO DE INVESTIGACIONES BIOLÓGICAS CSIC

Velasquez 144  
28006 Madrid  
Spain

Victor de Lorenzo  
Tel.: +34-1-561 18 00  
Fax: +34-1-562 75 18

**M.U.R.EX.: MEDITERRANEAN URBAN REJECTS EXPERIMENT**

**Co-ordinator:** Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare (ICRAM),  
Roma, Italy (Ezio Amato)

**OBJECTIVES**

- To develop the knowledge and instruments that will help to improve the management of the coastal waters quality in the Maghreb with regard to the fate of pollutants released into the sea by urban sewage;
- To identify the spatial and temporal fate in the coastal zone of the anthropogenic pollutants bound to the suspended particulate matter of the investigated sewage;
- To set out the distribution of the pollutants concentrations in the marine sediments affected by the investigated sewage;
- To appraise the toxic strength of the polluted marine sediments.

**ACTIVITIES**

- ◇ Along the Mediterranean coastline, selection of one test site for each of the Maghreb countries participating in the project (Algeria, Morocco and Tunisia) through the analysis of the available data about the characteristics of the considered sewage and the concerned marine environments;
- ◇ Development of sampling and measure instruments and methodologies suitable for the sampling of the superficial sediment layer and for the study of the sewage pollutants transfer towards the marine sediments;
- ◇ Mapping of the distribution on the seabed of the tracer ( $Hf_{181}$ ) utilised to mark the suspended particulate matter released by each of the investigated sewage in order to detect the dispersion of the pollutants in the marine coastal area and to establish the sampling design;
- ◇ Sampling of superficial sediment layer in the located seabed in order to analyse the concentrations of some anthropogenic pollutants (heavy metals, PAH, PCB, anionic surfactants, pathogenic micro-organisms, etc.) and to carry out bioassays;
- ◇ Exploitation of the achieved outcomes through scientific workshops and papers and the drawing up of a methodological guide.

**RESULTS**

- ⇒ The sites selected for the research are located along the coast of Sousse (Tunisia), Al Hoceïma (Morocco) and Algiers (Algeria).
- ⇒ An in situ radioactivity detector and a superficial sediment layer sampler have been developed and employed during the campaigns already carried out in Sousse (Tunisia) and Al Hoceïma (Morocco).
- ⇒ The campaigns carried out in the waters of Sousse (Tunisia) and Al Hoceïma (Morocco) have enabled the collection of samples and data whose analyses are in progress.

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## **PARTNERS**

### **ICRAM - ISTITUTO CENTRALE PER LA RICERCA SCIENTIFICA E TECNOLOGICA APPLICATA AL MARE**

Unita Organica Ambiente  
Via L. Respighi 5  
00197 Roma  
**Italy**

Ezio Amato  
Tel.: +39-6-807 75 51 / 807 22 76  
Fax: +39-6-808 83 26

### **INSTITUT FRANÇAIS DE RECHERCHE POUR L'EXPLOITATION DE LA MER (IFREMER)**

Laboratoire Chimie des Contaminants et Modélisation  
B.P. 330  
83507 La Seyne-sur-mer Cedex  
**France**

Louis-Alexandre Romana  
Tel.: +33-4-94 30 49 02  
Fax: +33-4-94 06 55 29

### **COMMISSARIAT À L'ENERGIE ATOMIQUE (DAMRI/SAR)**

Service d'Applications des Radioéléments  
Cen Saclay B.P. 52  
91193 Gif-sur-Yvette  
**France**

Patrick Brisset  
Tel.: +33-1-69 08 20 03  
Fax: +33-1-69 08 60 30

### **UNIVERSITÀ DI VENEZIA**

Dipartimento Scienze Ambientali  
Calle Larga S. Marta 2137  
30123 Venezia  
**Italy**

Bruno Pavoni  
Tel.: +39-41-529 85 22  
Fax: +39-41-52 98 85 84

### **CENTRE NATIONAL DE L'ÉNERGIE, DES SCIENCES ET DES TECHNOLOGIES NUCLEAIRES**

Département Techniques Nucléaires  
Rue Tansift - Agdal 65  
Rabat  
**Morocco**

Rachad Alami  
Tel.: +212-7-77 87 04  
Fax: +212-7-77 99 78

### **OFFICE NATIONAL DE L'ASSAINISSEMENT**

Laboratoire Central  
Rue de La Monnaie 32  
Tunis République  
1001 Tunis  
**Tunisia**

Amel Jrad-Fantar  
Tel.: +216-1-34 32 00  
Fax: +216-1-35 04 11

### **CENTRE DE DEVELOPPEMENT DES TECHNIQUES NUCLEAIRES**

Division Datation et Hydrologie Isotopique  
Boulevard Frantz Fanon 02  
B.P. 1017 Alger Gare  
16000 Alger  
**Algeria**

Saïd Tobbeche  
Tel.: +213-2-63 62 55  
Fax: +213-2-63 23 26

**A COMPLEX INVESTIGATION AND PREDICTION OF ATMOSPHERIC  
POLLUTION TRANSFER IN THE MEDITERRANEAN AREA**

**Co-ordinator:** Aristotelian University of Thessaloniki, Thessaloniki, Greece (Stavros G. Nychas)

**OBJECTIVES**

- To study the dispersion of industrial pollutants in the atmosphere and to assess their transport over long distances;
- To develop a methodology for the prediction of air borne mesoscale transport phenomena.

**ACTIVITIES**

- ◇ Wind tunnel simulation of releases from industrial facilities and their mixing in the atmospheric turbulent boundary layer;
- ◇ Development of an adequate phenomenological model of pollution transfer in quasi - two-dimensional atmospheric turbulence. The model will be based on the analogy between turbulent mixing in the stratosphere and the flow of a conductive fluid under the influence of a magnetic field;
- ◇ Adaptation of the mathematical description of these phenomena to the Mediterranean geophysical conditions, allowing for a high resolution application over a range of several hundred kilometres;
- ◇ Definition of a transfer coefficient as an essential parameter of the models, through experiments and theoretical analysis;
- ◇ Investigation of atmospheric air pollution scenarios, defining concentration profiles of air pollution for various meteorological conditions and distributions of pollution sources.

**RESULTS**

- ⇒ Basic knowledge about the processes involved in the atmospheric dispersion of air pollution.
- ⇒ Description of the initial stages of pollutant dispersion.
- ⇒ Improved ability to predict mesoscale pollution transfer events over the Mediterranean region.
- ⇒ Computer-based methodology, including modelling, for the development and assessment of pollutant scenarios, providing an improved basis for industrial pollution management measures in the region.

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## **PARTNERS**

### **ARISTOTLE UNIVERSITY OF THESSALONIKI**

Department of Chemical Engineering  
University Campus  
Box 453  
54006 Thessaloniki  
**Greece**

Stayros G. Nychas  
Tel.: +30-31-99 15 32  
Fax: +30-31-20 61 38  
Email: nychas@vergina.eng.auth.gr

### **BEN-GURION UNIVERSITY OF THE NEGEV**

P.O. Box 653  
84105 Beer-Sheva  
**Israel**

H. Branover  
Tel.: +972-7-28 04 51  
Fax: +972-7-28 04 67  
Email: eidel@bgumail.bgu.ac.il

### **HIGHER TECHNICAL INSTITUTE**

P.O. Box 2423  
Nicosia  
**Cyprus**

G. Iordanou  
Tel.: +357-2-49 44 54  
Fax: +357-2-49 49 53

**BENTHIC FORAMINIFERA AS INDICATORS OF HEAVY METAL POLLUTION - A  
NEW METHOD OF BIOLOGICAL MONITORING OF THE MEDITERRANEAN SEA**

**Co-ordinator:** Università Degli Studi Di Milano, Milano, Italy (Maria Bianca Cita)

**OBJECTIVES**

- To understand the effects of man made pollution of the Mediterranean sea with heavy metals on foraminifera in order to use these as bioindicators for monitoring marine pollution;
- To understand the environmental evolution of the Iskenderun, Atlit and Haifa Bays in the last 10.000 years due to natural and anthropogenic causes.

**ACTIVITIES**

- ◇ Field studies including sampling and treatment of sediment and living foraminiferal samples, in-situ measurements of environmental parameters (i.e. temperature, salinity, pH), coring in strategic areas closed to pollution sources;
- ◇ Laboratory analyses of water and sediment samples (grain size and chemical analyses of sediment from grab and core samples for heavy metals concentration), chemical analyses of sea water for nutrients determination (i.e. phosphates and nitrates), organic carbon content, microstructures, anthropogenic pollution evidence in the sediment and in the benthic foraminifera;
- ◇ Laboratory treatment of foraminifera, taxonomic studies, biochemical analyses of foraminiferal tests, crystallographic analyses of foraminiferal tests;
- ◇ Culturing experiments over six months period to grow selected species at various controlled quantities of specific trace metals;
- ◇ Statistical evaluation of all collected data.

**RESULTS**

- ⇒ Four marine sampling cruises have been successfully carried out. A large number of specialists from many fields, including botany, ecology, ecotoxicology, geology, palaeontology, chemistry and oceanography have worked together to analyse the samples collected at the 229 stations (each station was sampled at two different seasons). An extensive data base has been compiled for the two study regions. This data base includes environmental chemical-physical parameters of the sea water and sediments, benthic foraminiferal population characteristics and concentration and distribution of heavy metals.
- ⇒ To achieve the primary goal of the project, the following tasks have been accomplished:
  - taxonomic composition, assemblage structure, relative abundance, richness, diversity and ecology of benthic foraminifera;
  - influence of oceanographic and sedimentological parameters on foraminiferal distribution;
  - influence of primary productivity on foraminiferal distribution;
  - influence of heavy metals on foraminiferal distribution;
  - influence of heavy metals on test morphology and biochemistry;
  - influence of heavy metals on defence mechanisms of foraminifera.
- ⇒ After three years of research, the following achievements can be drawn:
  - Benthic foraminifera are very sensitive to wastes disposed into their environment;
  - Benthic foraminifera have several defence mechanisms that protect them from toxins. The stunting decrease in population density and the deformation of the tests are all outward manifestations that different levels of these biological barriers have been breached;
  - Heavy metals are responsible for tests' deformities. Deformed tests are characterised by elevate concentration of Mg compared with non-deformed tests;
  - The Turkish coast-line suffered more contamination than the Israeli coasts, however in both areas a considerable concentration of Chernobyl-derived radiocesium has been detected. High



concentration of heavy metals such Cr, Cu, Pb, Cd and Zn were detected in the sediments recovered nearby the iron-steel complex in Iskenderun Bay;

- The Israeli northern shelf is potentially dangerous for endemic disease of biota because of very high concentration of Cd and As.

⇒ To achieve the secondary goal of the project, the following tasks have been accomplished:

- relation between substrata and distribution of benthic foraminifera in sediments from cores;
- relation between environmental parameters and distribution of benthic foraminifera in the surface sediments compared with their distribution in sediments from the cores;
- age determination of sediments in the cores by <sup>14</sup>C dating;
- general geology of the area in the collisional context of the eastern Mediterranean area.

## **FOLLOW-UP**

Further work will be focused on culture experiments for monitoring the response of foraminifera to various levels of selected trace metals. This research will enable to reveal biological defence mechanisms of foraminifera as well as to determine which metal at what degree of pollution induces various deformations of foraminiferal tests. The work in the incoming year will also include tests on the influence of heavy metal pollution on foraminiferal tests with geochemical, morphological, morphometrical and crystallographic analyses and the continuation of investigations on cores.

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## **PARTNERS**

### **UNIVERSITA' DEGLI STUDI DI MILANO**

Dipartimento di Scienze Della Terra  
Via Mangiagalli 34  
20133 Milano

**Italy**

Maria Bianca Cita  
Tel.: +39-2-23 69 82 48  
Fax: +39-2-70 63 82 61

### **TEL AVIV UNIVERSITY**

Institute for Nature Conservation Research  
Ramat Aviv  
69978 Tel Aviv

**Israel**

Valentina Yanko  
Tel.: +972-3-640 77 72  
Fax: +972-3-640 73 04

### **ISTANBUL UNIVERSITY**

Mühendislik Fakültesi  
Jeoloji Mühendisliği Böl  
34850 Avcılar Istanbul

**Turkey**

Engin Meric  
Tel.: +90-212-591 19 98  
Fax: +90-212-591 19 97

**INTEGRATION OF SATELLITE LAND SURFACE ASSESSMENT WITH SOCIO-ECONOMIC PARAMETERS FOR GLOBAL DESERTIFICATION MONITORING IN THE ARID MEDITERRANEAN ZONE (TUNISIA AND EGYPT)**

Co-ordinator: ORSTOM, Paris, France (Richard Escadafal)

### OBJECTIVES

- To contribute to the development of a global understanding of the desertification phenomena as a basis for strategies to combat it;
- To design an approach integrating the biophysical ground observations with remote sensing to detect changes occurring at the land surface;
- To link socio-economic information with the remotely sensed land surface changes using a GIS, towards the definition of global desertification indicators.

### ACTIVITIES

- ◇ Existing data inventory:  
retrieving and reformatting existing maps (topographic, geological, ecological), meteorological data reports, aerial photographs, thesis, socio-economic data, ...
- ◇ Field campaigns:  
acquisition of ground data on land use, land surface condition (soil and vegetation), measurement of radiometric properties of vegetation and soil at different levels of desertification have been done on both test areas over a hundred of sites, accurately localised using the Global Positioning System (GPS) or detailed maps.
- ◇ Spectroradiometric measurement processing:  
a specific software has been developed (SpecPro) to perform spectra averaging, noise correction and resampling; the final output is accurate reflectance values of the target (plant / soil) in the bands of current existing remote sensing satellites (Landsat, Spot).
- ◇ Ground data organisation:  
a user-friendly data base has been developed (SpecBase, implemented under Windows environment) to handle all ground-collected information: precise localisation, soil and vegetation description, laboratory analysis, spectral reflectance as well as field photographs.
- ◇ Remote sensing images inventory and acquisition:  
different images' sources have been used to build an historical archive of Landsat MSS starting in 1972, whereas Spot data were acquired to document recent years.
- ◇ Satellite data base organisation:  
images from the various sources have been put under a standard format and geometrically corrected to be superimposable.
- ◇ Establishment of a GIS:  
acquisition of reference maps and digitalisation under Arc/Info, update of administrative boundaries.
- ◇ Socio-economic data:  
incorporating existing data into the GIS has been tested.
- ◇ Satellite data inter-calibration:  
using reflectances measured on the ground on specific reference target, images recorded at different dates have been radiometrically rectified to allow inter-comparing them.
- ◇ Change detection techniques:  
two techniques of change detection have been developed, one in Tunisia on rangelands with medium scale imagery (Landsat MSS), and one in Egypt on land use with the more detailed Spot imagery.
- ◇ Long term change detection:  
time series of images over twenty years built over the Tunisian test have been tested for the assessment long term trends in landscape changes.

## OUTCOME

### ⇒ **Identification relevant land surface parameters:**

From the field investigations and from the analysis of the radiometric measurements gathered in the database, it has been established that the main land features related to desertification are :

- the soil surface aspect (soil material composition, colour and structure) such as presence of sand sheets, of soil crust, of microphytes;
- vegetation abundance and phenological state.

### ⇒ **Spectral characterisation of degraded land:**

Simulation experiments and real data have shown that the classical satellite-based monitoring technique using vegetation indices, is not suited to the steppic vegetation encountered in the area. Building on the results of field work, specific indices have been developed, characterising the land surface brightness and colour. The surface colour index is a powerful tool to distinguish sand sheets or crusted soils from surfaces in good condition.

### ⇒ **Appropriate technique for remote sensing of desertification:**

Land degradation cannot be assessed from one image, only the difference with a previous state can indicate if a change has occurred and in which direction. Corrections techniques have to be carefully applied to satellite images, both for the geometry and the radiometry, to allow comparison and computation of changes. As an example, on the Tunisian site an increase of colour index values indicates a sand sheets' extension.

### ⇒ **Application/Documenting land surface changes in the test areas:**

- In the centre of the Nile Valley (Egypt): between 1990 and 1994 a dramatic extension of saline and bare soil has been observed whereas urban land used increased by 60%, but mainly gained from desert area (non irrigated);
- In the rangeland (Tunisia): following land surface changes over 7 images from 1972 to 1993 clearly showed the dramatic extension of mobile sand (sand sheets and dunes) with the development of cultivation until the mid 80's (drier years) and a fast recovery of the areas treated with sand fixing barriers and enclosure after the more humid recent years.

These results demonstrate the feasibility of satellite monitoring of desertification progression or decrease under the effect of actions undertaken to combat it. This is a step towards the concept of a « desertification watch » satisfying the needs for information driven by the implementation of the CCD («International Convention to Combat Desertification»).

### ⇒ **Training / Collaboration with universities:**

During the project several students have been offered training periods in the institutes involved :

- three students from ENIT (Tunis), on spectral signature analysis, processing and modelling (see Marackchi, Masmoudi and Niang in the publication list),
- two Ph.D.s. in collaboration with the university of Paris 6, on sand movement in oasis environment, and on assessment of radar images' potential for desertification monitoring (thesis to be defended in early 1997).

## FOLLOW-UP

As an answer to the need for a desertification monitoring tool capable of delivering information for environmental management at a regional level, a new program has been designed involving partners from all countries of the southern part of the Mediterranean basin. Building upon the results of our tests in Egypt and Tunisia it has been conceived in close collaboration between ORSTOM (France) and the Space Applications Institute (Joint Research Centre of the European Commission), particularly the EMAP unit of this institute, dealing with desertification monitoring in Mediterranean countries of Europe. This has led to the preparation, in association with the Sahara and Sahel Observatory (OSS, Paris).

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## **PARTNERS**

### **ORSTROM**

Département "Milieu et Activité Agricole"

Rue La Fayette 213

75480 Paris Cedex 10

**France**

Richard Escadafal

Tel.: +39-332-78 93 39

Fax: +39-332-78 94 69

E-mail: richard.escadafal@jrc.it

### **CENTRE NATIONAL DE TELEDETECTION**

B.P. 200

1080 Tunis Cedex

**Tunisia**

Chokri Turki

Tel.: +216-1-76 13 33

Fax: +216-1-76 08 90

### **NATIONAL AUTHORITY FOR REMOTE SENSING AND SPACE**

Kasr El Eini Street 101

Cairo

**Egypt**

Hussein Younes

Tel.: +20-2-354 01 73

Fax: +20-2-355 71 10

**TRANSPORT AND TRANSFORMATION OF AIR POLLUTANTS FROM EUROPE  
TO THE EAST MEDITERRANEAN REGION (T-TRAPEM PROJECT)**

**Co-ordinator:** University of Athens, Athens, Greece (Georgios Kallos)

**OBJECTIVES**

To identify and quantify the transport and transformation of polluted air masses travelling over the eastern section of the Mediterranean area in order to assess the cross Mediterranean north-south pollution transfer.

**ACTIVITIES**

- ◇ Continuous monitoring of the background levels of the primary SO<sub>2</sub>, NO<sub>x</sub> and the secondary pollutant O<sub>3</sub> at selected sites in the SW Mediterranean Coast of Turkey and at the central Israeli coastline;
- ◇ Monitoring of the sulphate and nitrate particulates on an intermitted basis in Turkey and Israel;
- ◇ Performance of additional measurements in Israel of other chemical species (CH<sub>2</sub>O, O<sub>3</sub>, NO<sub>2</sub>, HNO<sub>2</sub>, NO<sub>3</sub>) with the aid of a DOAS instrument;
- ◇ Performance of aircraft flights over the Eastern Mediterranean region with emphasis on the Aegean Sea in order to measure the lower tropospheric concentrations of SO<sub>2</sub>, NO<sub>y</sub>, O<sub>3</sub> and particulates.
- ◇ Performance of atmospheric modelling and transport simulations with the aid of an advanced regional/mesoscale modelling system (3-D simulations).

**RESULTS**

- ⇒ The aim of this project was to study the transport of air pollutants in the Eastern Mediterranean region. Despite the fact that some work had been conducted in the past in order to understand the levels and transport of pollutants to the Western Mediterranean, almost no information was available for the Eastern part of it. In the framework of this project, continuous measurements, experimental campaigns, including research aircraft flights, and model simulations have been conducted during the period 1993-1996 in order to better understand the transport and transformation phenomena in the Eastern Mediterranean. The suggestion that the urban and industrial plumes can travel large distances keeping their characteristics for some days and therefore can affect the air quality in remote locations, urban or rural, has been validated in the frame of this study from surface and airborne measurements as well as from extensive numerical modelling. The surface observations made at the coast of Israel in combination with the airborne measurements in the Eastern Mediterranean Sea showed that this area is influenced by elevated levels of sulphate and nitrate species. Israel is especially affected by high particulate sulphate values.
- ⇒ From the airborne measurements performed over the Greek territory and more specifically over the Aegean Sea, the Attica peninsula and East Peloponnese, it appears that the air masses are funnelled southwards across the Aegean Sea and the highest levels are observed close to Crete and especially near Peloponnese. This area was found to be influenced by the urban plume of Athens for several days every year. The area South and Southeast of Peloponnese is also affected by the long range transport from the Central Mediterranean and Italy. The airborne measurements along the Northern and Southern Aegean showed that the transport across the Aegean Sea (from north to south) should occur during several days per year. So, polluted air masses from large urban areas like Istanbul or from industrial areas around the Black Sea should be transported across the Aegean Sea towards the Middle East and Africa. Observation of the composition of the pollutant in the air masses reaching the Israeli coast indicates that the primary pollutants were entrapped thousands of kilometres away. The travel time elapsed between pollution entrapment and arrival at the Israeli coast is sufficient to allow the formation of sulphates and nitrates and the deposition and/or transformation of the primary pollutants.

- ⇒ Extensive numerical modelling has been performed in order to investigate the paths of air pollutants from Europe to the Eastern part of the Mediterranean. One path of transport of air pollutants towards the Eastern Mediterranean is across the straight South of Italy and Greece. This path might be followed either during the warm or the cold period of the year but mainly during spring as it is associated with the prevalence of westerly flow. When this westerly flow over the central part of the Mediterranean is weak and the lower troposphere is quite stable, the plumes of Italy can travel through the gap between Peloponnese and Crete. When it is stronger or from NW directions, the main path of the plumes passes from the South of Crete towards the eastern part of the Mediterranean. The second path of transport is across the Aegean Sea and seems to occur more frequently because of the synoptic conditions and the developed regional-scale circulations. It is the dominant mechanism during summer.
- ⇒ Model results also showed the significant role that mesoscale local circulations of various types on the dispersion of such plumes and on the definition of air quality at local scales. The time scale for transport of air pollutants from sources located in Eastern Europe towards the Eastern Mediterranean has been shown to be approximately two to three days, while for the transport from the western to the eastern part of the Mediterranean is in general longer.

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## PARTNERS

### NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

Department of Applied Physics  
 Meteorology Laboratory  
 Ippocratus 3  
 10680 Athens  
 Greece

Georgios Kallos  
 Tel.: +30-1-363 62 79  
 Fax: +30-1-360 50 80  
 E-mail: kallos@etesian.meteolab.ariadne-t.gr

### HEBREW UNIVERSITY OF JERUSALEM (UJERUS)

School of Applied Sciences  
 Environmental Sciences  
 Givat Ram Campus  
 91904 Jerusalem  
 Israel

Menachem Luria  
 Tel.: +972-2-63 68 41 / 58 41 56  
 Fax: +972-2-63 72 60 / 66 68 04  
 E-mail: luria@vms.huji.ac.il

### MIDDLE EAST TECHNICAL UNIVERSITY

Department of Environmental Engineering  
 06531 Ankara  
 Turkey

Gurdal Tuncel  
 Tel.: +90-4-210 10 00 / 26 45  
 Fax: +90-4-286 86 25

**USE OF PARASITE SPECIES COMPOSITION INDICES OF FISHES TO MEASURE THE  
DEGREE OF ENVIRONMENTAL DETERIORATION DUE TO POLLUTANTS AND OTHER  
MAN-MADE ADVERSE EFFECTS**

**Co-ordinator: Universita' Degli Studi Di Roma "La Sapienza", Roma, Italy (Lia Paggi)**

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## **OBJECTIVES**

The objective of the proposed research was to develop a biological methodology to measure the environmental quality of an estuarine - lagoonaire habitat by an analytical study of the parasite assemblage of an ubiquitous estuarine fish host - the grey mullets (*Liza aurata*, *Liza ramada* and *Liza saliens*). This should allow to detect pollution and other detrimental environmental effects. The methodology was based on a working hypothesis that the ratio between heteroxenous (developing via several hosts) and monoxenous (depending on single host for their development) parasites (in the habitat as a whole or on a single host population) can provide a measurable bioassay of the habitat: parasites with heteroxenous development can only complete their development in diverse - stable - habitats.

## **ACTIVITIES**

Sampling programs, parasites' collection and diagnosis, and data registrations were carried out by a co-ordinated standard methodology. All obtained data from each country were compiled into one comprehensive data base and were jointly processed by the team members. Species diversity indices of parasites in sampled young of the year mullets in selected polluted and seemingly pollution-free estuarine and lagoonaire habitats were measured qualitatively and quantitatively.

## **RESULTS**

A bio-indicator based methodology for the investigative assessment of the state of the environment in estuaries and lagoons was developed and tested at a range of sites around the Mediterranean sea (Italy, Greece, Israel, Egypt). Different indicators were used in order to respond to specific questions. In order to standardise the methodologies of fish examination and parasite species identification a comprehensive protocol was defined. Data were included in a data-base, using Excel programme. Taxonomic identification has led to the detection of many parasite species including bacteria, protozoans, helminths and crustaceans. Collected data were processed for biometric and ecological parameters such as prevalence, abundance and intensity of infections, calculation of ratios of heteroxenous to monoxenous species, similarity indices among localities and statistical processing of confidence level. A novel approach was applied for the evaluation of species richness as a function of sampling size. The occurrence of parasitic species proved to be significantly correlated to biogeographical aspects and to the different size classes within host samples. Differences in the species richness, biodiversity and evenness values were obtained analysing parasite communities from habitats showing different environmental conditions. The calculation of ratios of heteroxenous to monoxenous species has proved to be very fruitful as a discrimination tool of polluted and not polluted areas. Adverse environmental conditions were expected to affect the total diversity of aquatic habitats, resulting in a lack of intermediate hosts that are necessary for the completion of life cycle of heteroxenous parasitic species, i.e. within helminths, molluscs for digeneans and crustaceans for cestodes, nematodes and acanthocephalans. In some regions rare species play a major role in contributing to the similarity between habitats, whereas in other regions dominant species appear to markedly characterise habitats. The study of species richness as a function of sampling size has provided different curves in polluted and not polluted areas. The results obtained indicate that a biological methodology to measure the environmental quality of an estuarine - lagoonaire habitat using parasite species composition indices is suitable and advantageous.

### *Concluding remarks*

The studies of parasitic organisms provide a number of advantages with respect to those carried out on free-living organisms. As animals with complex life cycles, parasites should be considered as excellent integrators of environmental conditions and hence are useful as indicators for environmental change or ecosystem health. Since parasites are obligate symbionts, their hosts represent precise and unambiguous boundaries within which matters related to spatial and temporal distribution can be measured and compared in a nested system. For these reasons the study of biodiversity in parasite communities could represent a model for the analysis of diversity in endangered aquatic ecosystems. The potential of studies on parasite ecology should stimulate scientists to explore new approaches in the study of the structure of parasite communities, and set up ad hoc indices rather than applying those used in free-living species ecology.

### **PARTNERS**

#### **UNIVERSITA' DEGLI STUDI DI ROMA**

"la sapienza"  
Istituto di Parassitologia  
Piazzale Aldo Moro 5  
00185 Roma  
**Italy**

Lia Paggi  
Tel.: +39-6-446 38 68  
Fax: +39-6-49 91 46 44

#### **INSTITUTE OF MARINE BIOLOGY OF CRETE**

P.O. Box 2214  
71003 Iraklion Crete  
**Greece**

Anastasius Eleftheriou  
Tel.: +30-81-24 20 22  
Fax: +30-81-24 18 82

#### **UNIVERSITA' DEGLI STUDI ROMA**

"tor vergata"  
Dipartimento Di Sanita "Pubblica e Biologia Cellulare"  
Via Di Tor Vergata 135  
00133 Roma  
**Italy**

Paola Orecchia  
Tel.: +39-6-72 59 60 40  
Fax: +39-6-72 59 68 18

#### **MEDICAL RESEARCH INSTITUTE**

Department of Parasitology  
El Horreya Avenue 165  
Alexandria  
**Egypt**

Laila Abou Basha  
Tel.: +203-421 54 55  
Fax: +203-421 64 59

#### **HEBREW UNIVERSITY OF JERUSALEM**

Department of Animal Sciences  
P.O. Box 1276  
100 Rehovot  
**Israel**

Ilan Paperna  
Tel.: +972-8-48 19 45  
Fax: +972-8-46 57 63



**ARE THE HCFC/HFC/HBC COMPOUNDS ENVIRONMENTALLY ACCEPTABLE ALTERNATIVES**

**Co-ordinator:** Universität Heidelberg, Heidelberg, Germany (Jürgen Wolfrum)

**OBJECTIVES**

- Elucidation of primary reaction mechanisms of atmospheric reactions of metastable oxygen atoms (O(1D)) and hydroxyl radicals (OH) with halogenated hydrocarbons (HCFCs, HFCs, HBCs);
- Investigation of the primary processes in the UV and VUV photodissociation of halogenated hydrocarbons (HCFCs, HFCs, HBCs);
- A more detailed understanding of the actual molecular reaction and dissociation mechanisms. Detailed information about the reaction/dissociation rates and pathways that will allow the environmental impact of the degradation of halogenated hydrocarbons in the atmosphere to be assessed.

**ACTIVITIES**

- ◇ Carrying out laboratory studies to investigate the gas-phase reaction dynamics and kinetics of the reactions of metastable oxygen atoms (O(1D)) and hydroxyl radicals (OH) with selected halogenated hydrocarbons. Measurements of product quantum state distributions and reactive cross sections, reaction rates and branching ratios in order to obtain information about the underlying reaction mechanisms;
- ◇ Laser-based laboratory studies on the UV and VUV photodissociation dynamics of halogenated hydrocarbons. Measurements of primary product distributions and branching ratios at different wavelengths. Extension of the photolysis wavelength ranges down to the atmospherically important Lyman-alpha spectral line. Development of an experimental method for the measurement of absolute halogen and hydrogen atom photolysis quantum yields;
- ◇ Comparison of the experimental results with calculations based on ab initio molecular structure data and/or statistical models for different mechanisms for reaction (complex forming versus abstraction) and dissociation (two-body versus three-body).

**EXPECTED OUTCOME**

- ▷ The measured quantum state distributions will give insight into the underlying reaction/photodissociation mechanism in the initial step of the atmospheric degradation of the HFCs/HCFCs/HBCs. The thermal rate coefficients and the absolute reactive cross section will allow to determine the rate of removal of these compounds by O(1D)/OH radicals in the atmosphere.

## RESULTS SO FAR

⇒ The kinetics of the reaction of O(1D) with CHF<sub>2</sub>Cl (HCFC-22) have been studied using the laser photolysis-laser induced fluorescence (LP/LIF) pump-and-probe technique. Thermal rates for the gas-phase reaction were measured in a flow system. O(1D) atoms were generated via laser photodissociation of N<sub>2</sub>O. Using a calibration method where the photodissociation of H<sub>2</sub>O<sub>2</sub> was employed to produce well-defined OH radical concentrations the branching ratio for the OH producing channel was determined. In addition, reaction dynamics studies were carried out under low pressure conditions where translationally excited O(1D) atoms with a well-defined collision energy distribution, were allowed to react with CHF<sub>2</sub>Cl and the absolute reaction cross section as well as the population distribution of the fine structure quantum states of the OH product radicals were measured under single-collision conditions using LIF. The OH product radical spin-orbit states were found to be equally populated. Also the OH Lambda-doublet distributions showed no preference for either one of the two components. A comparison of the nascent OH internal product quantum state distributions with the results of RRKM calculations, in which it was assumed that the reaction proceeds via a CHF<sub>2</sub>Cl-OH transition state, clearly showed the non-statistical character of the measured OH vibrational and rotational distributions. The analysis of the measured rotational state distributions suggests that the reaction O(1D) + CHF<sub>2</sub>Cl actually proceeds via two different mechanisms : one in which the oxygen atom inserts into the C-H bond, leading to CHF<sub>2</sub>ClOH-complex formation followed by an OH producing unimolecular decomposition and a second one in which the oxygen atom directly abstracts a H atom during its approach to the CHF<sub>2</sub>Cl reagent. Experiments in which H atom product formation is investigated are currently under way in order to quantify the relative contribution of the complex formation pathway in more detail. UV and VUV photodissociation dynamics studies have so far been carried out for chlorinated methanes (CH<sub>n</sub>Cl<sub>4-n</sub>, n= 1-4) and CHF<sub>2</sub>Cl (HCFC-22) and CHF<sub>2</sub>ClCH<sub>3</sub> (HCFC-142b) after excitation at 193.3 nm.

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## **PARTNERS**

### **UNIVERSITÄT HEIDELBERG**

Physikalisch-Chemisches Institut  
Im Neuenheimerfeld 253  
69120 Heidelberg  
Germany

Jürgen Wolfrum

Tel.: +49-6221-54 84 63 / 54 42 48

Fax: +49-6221-54 42 55

E-mail [volpp@sun0.urz.uni-heidelberg.de](mailto:volpp@sun0.urz.uni-heidelberg.de)

### **BEN GURION UNIVERSITY OF THE NEGEV**

Physics Department  
P.O. Box 653  
84105 Beer Sheva  
Israel

Salman Rosenwaks

Tel.: +972-7-46 11 70

Fax: +972-7-31 340

E-mail [zamik@36uvm.il](mailto:zamik@36uvm.il)

### **BEN GURION UNIVERSITY OF THE NEGEV**

The Institutes for Applied Research  
P.O. Box 653  
84105 Beer Sheva  
Israel

Bar Ilana

Tel.: +972-7-46 15 71/0

Fax: +972-7-31 340

E-mail [llana@36uvm.il](mailto:llana@36uvm.il)



# 1. Natural resources

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## 1.3. Renewable energy

**WASTEWATER DISTILLATION BY SUN ENERGY (WADISUN) – TREATMENT OF HIGHLY CONTAMINATED WASTE WATER BY SIMPLE AND LOW COST PROCEDURES**

**Co-ordinator: AquaAmbiente S.A., Mem Martins, Portugal (Joao Bordado)**

### **OBJECTIVES**

- To develop a low cost technology with minimal capital investment for wastewater treatment, based on sun energy and targeted on thermal disinfection and distillation of effluent. The aim is to produce rather clean water (at least suitable for irrigation) and solid residues which can be either used, i.e. as soil conditioner, or treated as genuine waste;
- To develop a low cost smell sequestering agent based on selected dried vegetable material (renewable raw material) for treating the residues from the distillation process;
- To develop drying and vitrification processes for the further treatment of the resulting solid residues.

### **ACTIVITIES**

- ◇ Recording and comparison of practiced and available solar energy based waste water treatment procedures with regard to assessing their technical and economic efficiency;
- ◇ Laboratory tests, choice of material, planning of pilot plants, material procurement construction of the pilot plant, putting into operation;
- ◇ Testing, at laboratory and pilot scale, the thermal treatment of different effluents : from olive oil mills, liquid manure, leachate;
- ◇ Comparative assessment of the different treatment procedures tested and identification of the most appropriate process;
- ◇ Elaboration of a feasibility study for the construction of a large-scale plant based on the process identified before.

### **OUTCOME**

- ▷ Available procedures for treating waste water by means of solar energy, including those at development stage, are recorded and comparatively assessed with regard to their technical and economic efficiency;
- ▷ A small transportable pilot plant will be built in Portugal and pilot plants are also constructed and validated in Tunisia and Spain, each pilot plant being specifically prepared to deal with one specific effluent;
- ▷ An appropriate, cost efficient, solar energy based waste water treatment procedure will be identified, tested at laboratory and pilot scale under different conditions and for different effluents, and described in detail;
- ▷ A feasibility study will be carried out for the developing of an industrial scale WADISUN plant based on the most appropriate process.

## **PARTNERS**

### **AQUAAMBIENTE S.A.**

Tratamento de Águas e Soluções para o Ambiente  
Estrada Nacional 249

Km 12

2726 Mem Martins Codex

**Portugal**

Joao Bordado

Tel.: +351-1-926 81 70 / 9

Fax: +351-1-926 97 59

### **DEPARTAMENTO DE ORDINACION DEL TERRITORIO URBANISMO Y MEDIO AMBIENTE CAMINOS CANALES Y PUERTOS**

Avenida Universitaria S/N

28040 Madrid

**Spain**

Aurelio Hernandez Muñoz

Tel.: +34-1-336 66 87

Fax: +34-1-336 66 86

### **FORSCHUNGSINSTITUT FÜR WASSER- UND ABFALLWIRTSCHAFT**

An der RWTH Aachen (FIW)

Mies Van Der Rohe Straße 17

52056 Aachen

**Germany**

Eric D. Werner

Tel.: +49-241-80 68 25

Fax: +49-241-87 09 24

### **ISTANBUL TECHNICAL UNIVERSITY**

Environmental Engineering Department

80626 Istanbul

**Turkey**

Akmet Samsunlu

Tel.: +90-212-285 34 16

Fax: +90-212-285 37 86

### **UNIVERSITY OF ISTANBUL**

Faculty of Pharmacy

Department of Analytical Chemistry

Université-Beyazit

Istanbul

**Turkey**

Imre Seda T

Tel.: +90-212-526 29 09

Fax: +90-212-519 08 12

### **ECOLE NATIONALE D'INGÉNIEUR DE TUNIS**

B.P. 37

Le Belvédère

1002 Tunis

**Tunisia**

Mohamed Safi

Tel.: +216-1-51 47 00

Fax: +216-1-51 07 29

**COMPARATIVE ASSESSMENT OF TECHNOLOGIES FOR SOLAR DETOXIFICATION  
AND DISINFECTION OF CONTAMINATED WATER**

**Co-ordinator:** Deutsche Forschungsanstalt für Luft-und Raumfahrt e. V., Köln, Germany  
(Karl-Heinz Funken)

## OBJECTIVES

The objective of the joint research project is to develop the basis for an efficient technology of solar water detoxification and disinfection that makes use of dye-sensitised and catalytic photoreactions. For both the photocatalytic detoxification and the photosensitised disinfection, a light source is required. In the Mediterranean countries, the sun is a versatile non-polluting radiation source. Eventually, the application of solar radiation should make the process independent from expensive artificial light sources and power supply. A careful evaluation over a range of conditions and with a range of contaminants shall help to choose the most appropriate technology that should be reliable under the conditions found in the respective regions.

## ACTIVITIES

- ◇ To determine the influence of key parameters (such as pH-value, temperature, concentration of dissolved oxygen, presence of quenching substances) on the chemical destruction of pollutants and on the killing rates of micro-organisms;
- ◇ To determine the most promising conditions for the solar photocatalytic mineralization and dye-sensitised disinfection in the prototype reactors;
- ◇ To develop approaches for the fixation of both photocatalysts and dyes and to fabricate prototype samples;
- ◇ To design, construct and test photoreactor prototypes for the solar detoxification and disinfection of polluted water according to three technological approaches : highly concentrated radiation (solar furnace applications and fixed focus applications), Heterogenized titanium dioxide in CPC collectors and Heterogenized dyes in falling film reactors;
- ◇ To determine technical and engineering requirements for scale-up.

## RESULTS

- ⇒ Oxidative degradation of the following pesticides was tested with homogeneous and heterogeneous photocatalysts: Bromacil, EPTC, Tribufos, Atrazine, butiphos, Lindane. Tests were run both with lamps for achieving basic information and with direct sunlight that covered a range from 1 sun to 3000 - 5000 suns in a solar furnace. Photocatalysts were TiO<sub>2</sub> (slurry and film) for OH radical generation, dyes such as methylene blue and rose bengal (homogeneous and fixed) for singlet oxygen generation, and ferric chloride/hydrogen peroxide for OH radical production. It was found that singlet oxygen was effective against some of the pesticides but reacted very slowly or not at all with the others. All pesticides were degraded with OH radical generating agents, both TiO<sub>2</sub> and Photo-Fenton. E. coli were effectively destroyed by solar irradiation with methylene blue or ferric chloride catalyst. Dyes were fixed to supports successfully, thus there is a chance that after the water treatment process the dye does not need to be removed. Three prototypes of reactors were constructed and actually they are under on-sun-tests.
- ⇒ A simple model was developed for estimating the economics of solar detoxification and disinfection. It appears that the cost of treatment with methylene blue is much less than the cost of the Fenton system which in turn promises to be much cheaper than heterogeneous phototreatment with titanium dioxide catalysts. However, these systems have different capabilities that must also be considered, and a direct comparison is not easy to achieve. The cost of heterogenized dyes and titanium dioxide catalysts have not yet been evaluated.



## **FOLLOW-UP**

There will be further tests with other micro-organisms. Attempts will also be made to measure the efficiency of use of the sunlight in selected cases to better define the expected range of costs. The knowledge gained of these processes to ideas to design and operate engineering-scale test systems and to extend the applicability to solve further water problems.

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## **PARTNERS**

### **DEUTSCHE FORSCHUNGSANSTALT FÜR LUFT- UND RAUMFAHRT E.V. (DLR)**

Hauptabteilung Energietechnik  
Linder Höhestraße  
51170 Köln  
**Germany**

Karl-Heinz Funken  
Tel.: +49-2203-601 32 20  
Fax: +49-2203-669 00

### **UNIVERSITY OF MALTA**

Institute for Energy Technology  
Marsaxlokk ZTN 10  
Triq il-Port Ruman  
MSD 04 MSIDA  
**Malta**

Mario Fsadni  
Tel.: +356-65 06 75  
Fax: +356-65 06 15

### **MARMARA RESEARCH CENTRE-TÜBITAK (MRC-TÜBITAK)**

P.O. Box 21  
41470 Gebze-Kocaeli  
**Turkey**

Siddik Icli  
Tel.: +90-262-641 23 00  
Fax: +90-262-641 23 09

### **INSTITUTO DE MAQUINA HERRAMIENTA**

Development & Technology Transfer Centre  
San Roke Auzoa 1  
P.O. Box 48  
20870 Elgoibar  
**Spain**

Ion Gonzáles  
Tel.: +34-9-43 74 41 32  
Fax: +34-9-43 74 41 53

### **BOMIN SOLAR RESEARCH GMBH**

Industriestraße 8 - 10  
79541 Lörrach  
**Germany**

Jürgen Kleinwächter  
Tel.: +49-7621-94 95 10 (19)  
Fax: +49-7621-94 95 26

### **ASOCIACION CENTRO TECNOLOGICO GAIKER**

Parque Tecnológico  
Edificio 202  
48016 Zamudio (Bizkaia)  
**Spain**

Inigo Muguruza  
Tel.: +34-94-452 23 23  
Fax: +34-94-452 22 36

### **CENTRO DE INVESTIGACIONES ENERGETICAS MEDIOAMBIENTALES Y TECNOLOGICAS**

Instituto de Energias Renovables  
Avenida Complutense 22  
28040 Madrid  
**Spain**

Manuel Romero Alvarez  
Tel.: +34-1-346 63 44  
Fax: +34-1-346 60 37

### **INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE HASSAN II**

Food Engineering Department  
B.P. 6202 (R-I)  
Rabat  
**Morocco**

Faouzi A. Senhaji  
Tel.: +212-7-77 74 43  
Fax: +212-7-77 58 38

### **THE WEIZMANN INSTITUTE OF SCIENCE**

Solar Research Facilities Unit  
P.O. Box 26  
76100 Rehovot  
**Israel**

Amnon Yogev  
Tel : +972-8-934 29 34  
Fax: +972-8-934 41 17

**SIMULTANEOUS NITROGEN ELIMINATION AND ENERGY PRODUCTION FOR RECLAMATION OF WASTEWATERS AND PRODUCTION OF RAW MATERIALS**

**Co-ordinator:** Universidad de Barcelona, Barcelona, Spain (Joan Mata-Alvarez)

**OBJECTIVES**

- To study the optimal aeration pattern for optimal nitrification and denitrification.
- To study the cycles of ion-exchange and biological regeneration of zeolites for ammonia recovery and to establish optimal procedures to carry out the operation.
- To study the production of Volatile Fatty Acids from highly COD loaded wastewaters. To optimise this production and its conversion to useful products such as biogas or its application to biological nutrient removal process.
- To set up different integrated process to remove/recover N and P from wastewaters with an increased energy production.

**ACTIVITIES**

Partner 1, at the University of Barcelona, has studied the nitrification and denitrification process, at high concentrations of ammonia and nitrate respectively. The process has been optimised in what refers to the cycles of aeration and non-aeration in order to obtain a better yield and better settlement characteristics. In a parallel study, technology has been developed to improve the removal of COD from highly loaded wastewater by removing the ammonia using zeolites (process studied mainly by Partner 4). The COD removal process has improved the production of biogas by around 30%. The zeolite bio-regeneration process has also optimised by the results obtained during the previous activity. Finally an integrated process has been set up using a SBR for simultaneous N and P removal using the VFA produced by the fermentation of wastes (accordingly with the process developed by Partner 3).

Partner 2 at the INRA-Narbonne has studied the simultaneous removal of nitrogen and energy production in a novel integrated process at laboratory level. Wastewater in a first step produces volatile fatty acids (VFA), (as in the studies of Partner 3). These VFA are subsequently used to remove nitrogen (via denitrification) and to obtain energy through biogas in a second methanization stage. The process has been studied with simulated and real wastewaters.

Partner 3 at the University of L'Aquila has studied the production of VFA from wastewaters and the optimisation of the fermentation process. The studies have been carried out at a pilot plant level. VFA are used for the biological nutrient removal of wastewater in different reactor configurations. A design of a demonstration plant has also been carried out, using data from the pilot plant.

Partner 4 at Technion, Haifa has studied and optimised at laboratory level the recovery of ammonium from wastewaters through an ion-exchange operation, using zeolites as a carrier. These zeolites are biologically regenerated via a nitrification process using the same carrier for nitrifying biomass immobilisation. Ion-exchange behaviour of other anions such as potassium, magnesium, etc. has also been studied to favour the recovery of ammonium. Resulting nitrate-rich waters can be used in agricultural applications for fertilisation purposes.

Partner 5 at University of Tétouan has devoted his studies to the development of an botanical inventory in a given zone of Morocco and to develop a basic technology to use macrophytes to treat wastewaters polluted with some metal ions.

## OUTCOME

### *Scientific–technical results*

A procedure was established to recover ammonia from wastewaters and, at the same time to remove nitrogen from wastewater through a ion-exchange process using zeolite (chabazite and clinoptilolite) as a carrier. “Adsorbed” ammonia is recovered using a nitrification process. Final effluent is a water loaded with nitrates which can be used as a rich fertiliser. Wastewater free from ammonia is much better treated anaerobically with a significant increase in energy production, as was demonstrated using pig manure as substrate. For nitrification and denitrification, optimum aeration patterns have been established. These patterns can be used for clinoptilolite/chabazite regeneration. Finally the use of VFA for removal of nutrients has also been studied using a SBR system with results similar to the ones that can be obtained using a conventional C-source such as acetate.

A novel integrated process using an anaerobic reactor and an aerobic step has been set up to remove N and carbon in an optimised way. The results have been tested treating pig manure. The treatment includes an anaerobic section in which VFA are produced. These are later used to denitrify the produced nitrates and once denitrification has finished they are methanized. Thus it is possible to remove simultaneously nitrogen and to produce energy with low operation costs treating for instance pig manure. The procedure could also be applied to any wastewater highly loaded with ammonia and COD.

In another study, a novel process has been developed to overcome the drawbacks associated with the simultaneous carbon and nitrogen removal by separating these two operations and concentrating the ammonium from wastewater into a small volume reactor in which high nitrifying biomass concentrations and optimal conditions for nitrification (temperature, oxygen, pH etc.) can be maintained easily. Recovery of nitrogen takes place through ion exchange, using zeolites (chabazite). Regeneration of zeolites takes place by nitrification, using accelerated patterns of aeration. Experiments to determine the affinity sequence of the major cations present in sewage to the chabazite yielded the following  $K^+ > Ca^{2+} > NH_4^+ > Na^+ > Mg^{2+}$ . Breakthrough curves showed that in the presence of these counter ions in the sewage a breakthrough value of 4 mg/l is achieved after 220 bed volumes, at which point the ammonium occupies only about 20% of the chabazite's capacity. The process was tested with simulated and real secondary effluents in a bench scale plant.

An inventory of botanical species has been carried out in a zone of interest in Morocco. Finally some studies have been carried out to test the metal tolerance of two species *Lemma Gibba* and *Lemma Minor*. Both are capable to accumulate Cu and Cr, especially the last.

### *Pilot plant*

In a pilot plant level, a procedure has been established to recover volatile fatty acids (VFA) from wastewaters through fermentation process involving the organic fraction of municipal wastes. More than 40 g/l as VFA and light alcohols and lactic acid have been obtained, representing a very high yield. In the same pilot plant, denitrification is then achieved with a remarkable rate from 0.01 to 0.06 kgN-NO<sub>3</sub>/kg VSS.d. and with nitrate uptake rates using methanol of 0.28 kg N-NO<sub>3</sub>/kg VSS.d. P removal also takes place with a 3% in the Waste Sludge. However there is room enough to improve the biological removal of P using VFA as the sole carbon source.

Results of this pilot plant have been used to design a demonstration unit at the wastewater treatment plant of Treviso (Italy).

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## PARTNERS

### UNIVERSIDAD DE BARCELONA

Departamento d'Enginyeria Quimica I Metallurgica  
Martí i Franquès 1, Pta. 6  
08028 Barcelona

**Spain**

Joan Mata-Alvarez

Tel.: +34-3-402 13 05

Fax: +34-3-402 12 91

E-mail: [jmata@medicina.ub.es](mailto:jmata@medicina.ub.es)

### TECHNION - ISRAEL INSTITUTE OF TECHNOLOGY

Faculty of Agricultural Engineering  
Technion City  
32000 Haifa

**Israel**

Michael Green

Tel.: +972-4-29 26 20

Fax: +972-4-22 15 29

### UNIVERSITÀ DI VENEZIA

Dipartimento di Scienze Ambientali  
Calle Larga, S. Marta 2137  
30123 Venezia

**Italy**

Franco Cecchi

Tel.: +39-41-529 85 33

Fax: +39-41-529 86 45

### INSTITUT NATIONAL DE LA RECHERCHE AGROALIMENTAIRE

Laboratoire de Biotechnologie, de l'Environnement des I.A.A.  
Boulevard Général de Gaulle  
B.P. 429  
11104 Narbonne

**France**

René Moletta

Tel.: +33-4-68 32 89 06

Fax: +33-4-68 32 89 10

### UNIVERSITÉ ABDELMALEK ESSAADI

Faculté des Sciences  
Département de Chimie  
B.P. 2121  
Tetouan

**Morocco**

Amina Azmani

Tel.: +212-9-97 24 23

Fax: +212-9-99 45 00

**SELECTIVE COATING FOR SOLAR HEATING AND COOLING: PREPARATION  
AND CHARACTERISATION**

**Co-ordinator:** Université Paul Sabatier, Toulouse, France (Jean-Pierre Traverse)

**OBJECTIVES**

- To optimise optical and thermal properties for coatings to be used in the most popular conversion systems as well as in more prospective ones;
- To improve durability of the materials, bearing in mind the possibility of high temperature degradation of selective absorbers;
- To develop non polluting Cr VI -industrially feasible, relatively not expensive,-versatile processes of preparation;
- Preparation at pilot scale of coatings for assessment in solar panel under real conditions (degradation of the efficiency, effect of the external parameters)

**ACTIVITIES**

- ◇ Preparation of solar selective films;
- ◇ Preparation of silicon oxynitride films;
- ◇ Pigmented polyethylene foil and Te, PbS, ZnS films;
- ◇ Characterisation of the films;
- ◇ Computerised simulation and modelization.

**RESULTS**

- ⇒ Improvement of the homogeneity of the Te films, found PbS to be a good shield material and have started the test of the cooling devices
- ⇒ Photothermal Radiometry (PTR) has been applied in order to investigate the IR emission of thin silicon nitride films.
- ⇒ PTR was also applied and demonstrated as a non-destructive technique for the evaluation of solar selective coatings. The photothermal technique can easily be implemented in industry for in-line characterisation during production.
- ⇒ FTIR Spectroscopy was also used to provide a qualitative description of the behaviour of the films in the IR range that shows favourable properties of these coatings to be used in passive cooling applications.
- ⇒ It has been shown that the theoretical one dimensional model that is used to fit the experimental PTR results, is allowing the extraction of some key parameters of the solar absorbing coatings.
- ⇒ Optical refractive indices were collected and by using regression techniques, complex dispersion equations were formulated and stored in the programme library.

**FOLLOW-UP**

Solar energy in the Mediterranean area is abundant. This leads to the development and extensive use of solar heating of water. However the non-selectivity of solar absorbers significantly reduces their efficiency. There is also a lack of durable selective materials for high temperature applications, such as active cooling and electricity generation. On the other hand passive cooling is still in its infancy while it could be used for cold storage of foods, climatization, desalination and water vapour condensation.

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## PARTNERS

### UNIVERSITÉ PAUL SABATIER

Laboratoire Matériaux et Energy  
Route de Narbonne 118  
31062 Toulouse Cedex

France

Jean-Pierre Traverse

Tel.: +33-5-61 55 61 13

Fax: +33-5-61 55 61 05

### UNIVERSITY OF CYPRUS

Department of Natural Sciences  
P.O. Box 537  
1678 Nicosia

Cyprus

Constantinos Christofides

Tel.: +357-2-33 86 71

Fax: +357-2-33 90 60

E-mail: costasc@earth.ns.ucy.ac.cy

### UNIVERSIDAD AUTONOMA DE MADRID

Departamento de Fisica Aplicada  
C-XII  
28049 Cantoblanco

Madrid

Spain

Jose Manuel Martinez-Duart

Tel.: +34-1-397 45 09

Fax: +34-1-397 39 69

### WEIZMANN INSTITUTE OF SCIENCE

Department of Materials Interfaces  
76100 Rehovot

Israel

David Cahen

Tel.: +972-8-934 22 46

Fax: +972-8-934 41 39

### BETLEHEM UNIVERSITY

Physics Department

P.O. Box 9

Bethlehem

West Bank & Gaza Strip

Hanna Hallak

Tel.: +972-2-74 12 41

Fax: +972-2-74 44 40

**ASSESSMENT OF BIOMASS BASED ELECTRICITY AND HEAT GENERATION IN  
DECENTRALISED AREAS IN EGYPT AND MOROCCO FOR JOB CREATION AND  
IMPROVEMENT OF LIVING STANDARDS**

**Co-ordinator:** Engineering and Computer Applications S.A., Athens, Greece (Vasilios Vassilatos)

### **OBJECTIVES**

To assess the contribution which biomass based electricity and heat generation could make in decentralised areas in Egypt and Morocco to the creation of jobs and hence an improvement of living standards.

### **ACTIVITIES**

- ◇ Assessment of existing biomass supply in the region through geographical tabulation of the biomass potential in Egypt and Morocco, focus on agricultural wastes/by-products;
- ◇ Identification of the potential to establish dedicated biomass plantations in so called energy farms (ecological and socio-economic constraints);
- ◇ Collection of socio-economic data for the decentralised areas of these countries;
- ◇ Evaluation of the available biomass-to-energy conversion technologies and identification of those best adapted to the given biomass supply situation identified before (biomass quantity and quality to be taken into account as well as environmental impact);
- ◇ Site selection in the analysed regions for a potential application of biomass technologies and estimation of the potential impact on job creation and improvement of living standards.

### **OUTCOME**

- ▷ Maps of the theoretical and realisable potential biomass supply in Morocco and Egypt, taking due account of the socio-economic context;
- ▷ Identification and techno-economic assessment of the most suitable biomass conversion technologies (electricity production);
- ▷ Estimation of the impact on income and job generation of biomass based energy production in decentralised regions of Egypt and Morocco, also considering the improvement of living standard made possible by the availability of bio-energy;
- ▷ These “products” will be of general value as similar situations are likely to exist in all Mediterranean countries.



## **PARTNERS**

### **ENGINEERING AND COMPUTER APPLICATIONS**

**S.A.**  
Kallistratou 89  
15771 Athens  
**Greece**

Vasilios Vassilatos  
Tel.: +30-1-775 46 58 / 775 16 53  
Fax: +30-1-775 99 77 / 804 11 09

### **MINISTRY OF ELECTRICITY AND ENERGY**

New and Renewable Energy Authority  
Abbasia  
Cairo  
**Egypt**

Mohmoud Zannoun  
Tel.: +20-2-273 31 76  
Fax: +20-2-273 71 73

### **ZENTRUM NEUER TECHNOLOGIEN**

Engelhardstraße 12  
81369 München  
**Germany**

Herbert Grimm  
Tel.: +49-89-720 12 73  
Fax: +49-89-720 12 40

### **CENTRE FOR THE DEVELOPMENT OF RENEWABLE ENERGIES**

Rue El Machaar El Haram  
B.P. 509  
40000 Marrakech  
**Morocco**

Ali Fassi Fihri  
Tel.: +212-4-30 98 14  
Fax: +212-4-30 97 95

### **SIDEF**

Rue Jules Breton 2 Bis  
75013 Paris  
**France**

Osman Benchikh  
Tel.: +33-1-43 37 60 67  
Fax: +33-1-43 36 02 81

**INTEGRATED CONCEPT FOR THE FERMENTATION OF WASTEWATER  
SLUDGE AND ORGANIC WASTE FOR THE PRODUCTION OF RENEWABLE  
ENERGY AND THE USE OF THE FERMENTED PRODUCTS AS FERTILIZER AND  
FOR SOIL IMPROVEMENT**

**Co-ordinator:** Universität Stuttgart, Institut für Siedlungswasserbau, Stuttgart, Germany  
(Klaus-Fischer)

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## **OBJECTIVES**

The objective is the basis for an integrated concept in which sewage sludge and organic waste are treated by fermentation technology, using biogas, to produce both a form of renewable energy and a hygienic, plant-tolerable soil conditioner. This latter can provide a partial replacement for artificial fertilisers, act as stabiliser of soil structure and prevent the growing problem of soil erosion. Implementing this concept, local fossil fuels for the generation of electricity or heat can be replaced, reducing the impact on health of the local population and returning large parts of the valuable organic material of organic waste to the natural material chain. A further objective of the project is leading to innovative impulse and to create jobs in the region. Social tolerable and environmental sense are precondition for reaching the objectives of the project.

## **ACTIVITIES**

- ◇ Analysis of housing and social structures of two cities (in Turkey and Egypt) or parts of cities;
- ◇ Determination of specific waste quantities.
- ◇ Identification of different agricultural residues and useful plants.
- ◇ Waste analyses of five resort hotels with half-board service and more than 400 beds.
- ◇ Use of aquatic plants grown in ponds for the treatment of wastewater and harvesting the plants for fermentation.
- ◇ Testing of different mixtures of biowaste, hydrophytes and wastewater sludge for joint fermentation.
- ◇ Final selection of test sites, taking into account social compatibility, ecological and infrastructural impact, and local markets for energy, heat and fermentation product.
- ◇ Collection of data on the available biowastes and wastewater sludge at the selected test sites (two campaigns each, volume and composition).
- ◇ Experimental water treatment with hydrophytes in new testing ponds nearby an existing water treatment plant in Selcuk, harvesting and fermentation of the hydrophytes and assessment of the water cleaning efficiency of these plants.
- ◇ Analyses of the samples taken (water content, ash content, ammonium, nitrate, nitrite, C-concentration, N-concentration, P-content, organic pollutants, heavy metals).
- ◇ Calculation of specific gas production rates.
- ◇ Fermentation experiments at laboratory scale (volume and quality of biogas, hygienic parameters of the fermentation product).
- ◇ Fermentation experiments at pilot scale with the most promising substrates, identified before (one pilot plant to be used by all participants).
- ◇ Evaluation of the technological options to use fermentation in relation to the analysed sites in view of optimised production of biogas and fermentation product and assessment of the potential to install fermentation plants.

## **OUTCOME**

In the beginning of the project several questions relating to waste and wastewater treatment, regenerative energy production and hygiene improvement in connection with anaerobic fermentation in Mediterranean countries were asked. Structural contrasts between densely populated and rural areas as well as corresponding infrastructure problems had been taken into consideration. These are considerably further exacerbated locally by tourism and intensive farming. Therefore environment and residents of

most regions suffer under insufficient wastewater and waste disposal, power production practises and hygiene problems. In order to prevent environmental damage in these regions, new approaches in environmental technologies favouring adapted "low-tech" and "low-cost" technologies have to be developed.

### *Methodology*

To meet answers for regionally defined main subject areas – urban districts, useful plants, tourism regions and pond based wastewater treatment – several methodologies were used to produce scientific reliable results. On the one hand analyses of the current situation in model regions on the basis of inquiries, information and literature were carried out. On the other hand several practical tests, data collections, measurements, laboratory-scaled and pilot-scaled fermentation trials were performed.

### *Organic waste, quantities, fermentation*

In the **rural areas** of the Aegean region and the governorate Sharkia in Egypt determined useful plants are cotton seeds, cereal seeds, cotton branches and wastewater from olive oil production. All these residues are of no value for farmers and are usually disposed on dump sites or beside the fields. Large amounts of several kinds of residues and mostly good results of fermentation test, make fermentation very useful. Especially for smaller towns in rural areas additional energy supply as well as natural fertiliser are really welcome.

Sorting analyses of **urban household waste** to obtain specific biowaste quantities [kg/capita\*day] were performed as on-site analysis in urban testing areas. Hereby income structures for the model cities Karsiyaka and Zagazig were taken into consideration. Sorting analyses to receive quantities of biowaste generation were successful and brought interesting results as planning basis for anaerobic fermentation. While Zagazig shows similar biowaste production level as cities in Germany, Karsiyaka generated double to triple biowaste quantities than Zagazig. Learning about waste collection, municipal infrastructure was very interesting and essential for any further planning of biowaste use.

For **Karsiyaka** and also for **Greater Izmir Metropolitan Area**, the only practicable possibility for fermentation is use as decentralised plants. Traffic situation and environmental aspects make transportation within this city nearly impossible. Especially in Karsiyaka gececondus (shanty towns) an additional energy source is welcome. But before introducing fermentation, biowaste collection at source must be organised. The organisation should be private and directly responsible, in order to guarantee a continuous functioning of the system. The running of several small decentralised fermentation plants would create new jobs in waste collection, transportation, and technical monitoring of the biogas reactors.

For **Zagazig** co-fermentation of biowaste and sludge from wastewater treatment would be an important step for health improvement. Anaerobic treatment of the sludge together with biowaste will prevent infectious risks for farmers which are using the unhygienic sludge for agriculture. Another positive aspect would be energy generation urgently needed in this area. Before introducing anaerobic fermentation, separate collection of biowaste must be introduced. This should be organised in direct responsibility by private companies or Zabbaleen people who are most experienced concerning waste disposal.

Model regions for **tourism** were the Aegean and Mediterranean coastal region in Turkey and South Sinai in Egypt. All examined hotels showed very high biowaste quantities, which are easily to collect. Specific biowaste quantities range from 0,6-2,9 kg/guest\*day. The main fraction of biowaste are leftovers (about 50-60%). At present, waste is mostly dumped and burnt on open dump sites near the resorts. Consequences are health problems and irreversible pollution of the environment. Since many hotels have small wastewater treatment plants, but do not know how to dispose the sludge, fermentation is the most suitable way to solve waste disposal problems while generating renewable energy.

In the agriculture and tourism region of Ephesus/Selcuk (Turkey) a **pond based water treatment** pilot plant was erected where water hyacinths achieved the best results. The water hyacinths had been harvested once a month for feeding the pilot biogas reactor at EGE-University in Izmir. The pilot wastewater treatment plant worked satisfactory although the plant is not optimised as for planning and technical side. Affects on the length-wise flow in ponds by water hyacinths must be taken into consideration and appropriate equipment installed.

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## **PARTNERS**

### **UNIVERSITÄT STUTTGART**

Institut für Siedlungswasserbau  
Postfach 10 60 37  
70049 Stuttgart  
**Germany**

Klaus Fischer

Tel.: +49-711-685 54 27

Fax: +49-711-685 37 29

### **ENVIRONMENTAL QUALITY INTERNATIONAL**

3B Vahgat Ali Street Zamalek  
Cairo  
**Egypt**

Kamal T. Ewida

Tel.: +20-2-340 00 52

Fax: +20-2-341 33 31

### **PAMUKKÜLE UNIVERSITY**

Mühendislik Fakültesi  
20017 Denizli  
**Turkey**

Ibrahim Alyanak

Tel.: +90-258-266 21 32

Fax: +90-258-266 20 12

<p style="text-align: center;"><b>TESTING AND COST REDUCTION OF PHOTOVOLTAIC WATER PUMPING SYSTEMS</b></p>
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**Co-ordinator: IT Power Ltd, United Kingdom (A. Derrick)**

## **OBJECTIVES**

The general objective of the project was to co-ordinate the work of the participating institutes in the field of testing and developing photovoltaic water pumps and to establish a strong network of excellence in this field, including institutes from the EU and Third Mediterranean Countries (TMCs).

The area chosen as being currently of most interest in PV pumping is the small to medium size range, i.e. systems of less than 500W. There is huge potential in this market sector if system costs for a given hydraulic duty can be reduced far enough. One way to achieve this is through high overall sub-system efficiency, resulting in reduced PV array costs for a given water output. Manufacturers have only recently begun to address these areas, with the introduction of systems using positive displacement pumps and high efficiency motors in size ranges of a few hundred watts. However, it was believed that there is still much room for improvement, and it was the objective of this project to co-ordinate the various participating institutes in identifying common goals and paths towards low costs and increased efficiency.

Specific objectives of the programme were:

- To form a strong network of institutes from EU and TMCs already active in PV applications research to exchange information and experience, and collaborate in the field of PV pumping;
- To standardise PV pump laboratory and field test procedures between institutes;
- To carry out round-robin testing of the most advanced commercially available PV pumping systems. This will act both to cross-calibrate individual laboratories and to provide useful test data on several commercial systems;
- To analyse loss paths in the present generation of PV pumps and to co-ordinate goals to move towards a new generation of higher-efficiency PV pumping systems.

## **ACTIVITIES**

### ◇ Networking and Information Exchange

Regular meetings of all participants were held. In addition, exchange visits between researchers of participating institutes took place. In terms of information exchange a network was created to:

- Distribute up-to-date information and papers on PV pumping;
- Freely exchange test results and other pump research work;
- Facilitate exchange visits by researchers to each other's institutes;
- Allow institutes with less experience in pump testing to benefit from working with those that have more experience;
- Promote collaborative links between participating institutes.

It was regarded as vital that the information gathered and results produced from the Concerted Action are made available to the widest possible audience to which they will be of use. To this end, the final project meeting incorporated an open seminar which was attended by representatives of manufacturers, researchers, users, etc. who were interested in the project results. In addition a state-of-the-art compilation of recent papers and publications was produced at the end of the project which contains the most relevant material circulated amongst the participants during the project.

- ◇ **Development of Test Procedures for Pump Testing**  
As part of the programme, test procedures for both laboratory and field testing of PV pumps were developed. Draft procedures had been circulated to all participants and comments were used to revise the documents. The tests described below were carried out according to these test procedures.
- ◇ **Testing of PV Pumps**  
A programme of round-robin laboratory and field testing was carried out. This served several useful purposes:
  - Independent test results on the systems tested were provided;
  - The testing was used to cross-calibrate the participating laboratories;
  - The testing provided valuable experience in PV pump testing to those institutes which are less well equipped.

In all 11 pumps were procured, 6 of which were included on a round-robin laboratory test at several different institutes, and the other 5 were used for field testing. Most of the pump systems chosen are designed for arrays of under 500 Wp, use positive displacement pumps, use DC motors, have a design head of 20-50m, and/or are new products or are in some way unusual. Some more conventional diaphragm and centrifugal pumps have been included to allow comparison.
- ◇ **Improvement of System Efficiency and Cost Reduction**  
The test results from the pump tests were used to analyse loss mechanisms in the present generation of PV pumps. The goal of this work was to move towards the design of a new generation of higher-efficiency 'third generation' PV pumping systems. In addition, an economic analysis was carried out with the aim of reducing the overall system cost for a given water demand.

## **OUTCOME**

- ▷ Collaboration on testing, regular meetings and exchange visits led to a strong network between many of the participants.
- ▷ Test procedures were developed, circulated, revised and agreed by participants. These test procedures are now considered by the IEC (International Electrotechnical Commission) for inclusion in a standard on PV pumping systems.
- ▷ Pump tests were carried out and the results were analysed. As expected, the test results showed a significant difference in the performance of the different pumping systems.
- ▷ From the results of the tests, it was determined where avoidable losses occur and goals for future research to reduce these losses were set.
- ▷ Other issues such as economic aspects of PV pumping systems or their durability were addressed.

## **FOLLOW-UP**

The network, which was established under the AVICENNE Concerted Action, is still proving very useful for the participants. Information exchange between the partners is taking place. This ranges from assisting each other with useful information to the exchange of results from research projects.

Various partners co-operate on different projects. For instance, IT Power are co-operating on a desalination project with RERC (Jordan) and the partner from the West Bank. IT Power are also working with the University of Reading.

A paper was presented at the 14th European Photovoltaic Solar Energy Conference held in Barcelona, Spain, from 30 June to 4 July 1997. (R Oldach, A Derrick, G R Whitfield: Concerted Action on the Testing and Cost Reduction of PV Water Pumping Systems under the EC AVICENNE Programme)

## **PARTNERS**

### **IT POWER**

The Warren, Bramshill Road,  
Eversley, Hants, RG27 OPR  
UK

A. Derrick  
Tel.: +44 118 973 0073  
Fax: +44 118 973 0820

### **BIRZEIT UNIVERSITY**

Electrical Engineering Dept.,  
Birzeit University, PO Box 14, Birzeit  
West Bank

K. A. Nigim  
Tel.: +972 7 862930  
Fax: +972 7 822026

### **CENTRE DEVELOPPEMENT DES ENERGIES RENOUVELABLES (CDER)**

Route de l'Observatoire,  
BP 62  
16340 Bouzareah, , Algiers  
Algeria

A. Hamidat  
Tel.: +213 2 94 13 56  
Fax: +213 2 94 18 61

### **CIEMAT (PVLabier)**

Avenida Complutense 22,  
28024 Madrid,  
Spain

M. Alonso-Abella  
Tel.: +34 1 346 6743  
Fax: +34 1 346 6037

### **CENTRE FOR RENEWABLE ENERGY RESOURCES (CRES)**

19th km Marathon Avenue, Pikermi, 190 09,  
Attika  
Greece

C. Protogeropoulos  
Tel.: +30 1 603 9900  
Fax: +30 1 603 9905

### **GERMAN AEROSPACE RESEARCH ESTABLISHMENT (DLR)**

Linder Höhe,  
51140 Köln  
Germany

Wolfhart Bucher  
Tel.: +49 2203 601 2936  
Fax: +49 2203 66900

### **NETHERLANDS ENERGY RESEARCH FOUNDATION (ECN)**

PO Box 1,  
1755 ZG Petten,  
Netherlands

N. van der Borg  
Tel.: +31 224 564401  
Fax: +31 224 563214

### **HACETTEPE UNIVERSITY**

Physics Eng Dept., Beytepe  
Ankara, 06532  
Turkey

D. Inan  
Tel.: +90 312 2352551  
Fax: +90 312 4268544

### **UNIVERSITÄT DER BUNDESWEHR MÜNCHEN**

Werner-Heisenberg-Weg 39,  
85577 München  
Germany

O. Mayer  
Tel.: +49 89 6004 3493  
Fax: +49 89 6004 3858

### **NEW & RENEWABLE ENERGY AUTHORITY (NREA)**

27 Project Building, Building No 20, 8th Region, Nasr City,  
Cairo  
Egypt

R. Botros  
Tel.: +202 271 3173  
Fax: +202 271 7173

### **UNIVERSITY OF READING (ENERGY GROUP)**

Dept. of Engineering, Whiteknights PO Box 225,  
Reading, RG6 2AY  
UK

J. Burton / J. Lobo-Guerrero  
Tel.: +44 118 931 8565  
Fax: +44 118 931 3327

### **UNIVERSITY OF READING (CYBERNETICS)**

Dept of Cybernetics, Whiteknights, PO Box 225,  
Reading, RG6 2AY  
UK

G. Whitfield  
Tel.: +44 118 931 8223  
Fax: +44 118 931 8220

**RENEWABLE ENERGY RESEARCH CENTRE (RERC)**

Royal Scientific Society,  
PO Box 1438, Aljubiha 11941

**Jordan**

**TECHNISCHE UNIVERSITEIT EINDHOVEN (TUE)**

PO Box 513,  
5600 MB Eindhoven,

**Netherlands**

M. Amr

Tel.: +962 6 844701

Fax: +962 6 844806

P. Smulders / W. van Helden

Tel.: +31 40 247 3191

Fax: +31 40 246 4151



<b>THERMOCHEMICAL UPGRADING OF BIOMASSES TO GASEOUS AND LIQUID FUELS AND FEEDSTOCKS</b>
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**Co-ordinator:** Nederlands Meetinstituut, Eindhoven, The Netherlands (Adriaan M.H. van der Veen)

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## OBJECTIVES

- To investigate and to improve methods for the thermochemical conversion of biomass into gaseous and liquid fuels and feedstocks. These processes are of relevance to developing countries as well as to the EU-member states. The use of biomass reduces the need for crude oil and other fossil fuels, and thus has a positive effect on the environment.

## ACTIVITIES AND RESULTS

The experiments were carried out in Chile, China and United Kingdom. There were catalyst samples and biomass samples supplied by the co-ordinator.

- ⇒ Two stages of the conversion of biomass are studied in the United Kingdom, in fact the hydrolysis of biomass and the hydrocracking of pyrolytic oils from the hydrolysis stage:
- ⇒ **Hydrolysis of biomass:** it involves well-established experimental pyrolysis systems, sparingly a fixed bed "hot-rod" reactor and a high pressure wire-mesh reactor. The experiments focus on optimisation of the experimental conditions, such as temperature, pressure, gas flow rate and residence time;
- ⇒ **Hydrocracking of pyrolytic oils from the hydrolysis stage:** it involves a packed catalyst fixed-bed reactor to be placed in series with the fixed bed "hot-rod" reactor. Experiments are ongoing, using a variety of catalysts in order to find the best operating conditions.
- ⇒ Atmospheric pressure gasification is carried out in Chile. The Chilean partner also supplied two biomass samples as well as a series of catalysts (dolomite and calcite), that underwent different treatments. The facilities in Chile are run with the same materials as those supplied by the co-ordinator to the other partners;
- ⇒ The main objective of the third part of the experimental work in China is the assessment of the process of converting biomass using an atmospheric fluidised bed reactor for energy production. After modification of the existing test facilities, experiments with Chinese biomass samples, as well as experiments with the Chilean biomass samples are run.

## **PARTNERS**

### **NEDERLANDS MEETINSTITUT**

P.O. Box 151  
6470 ED Eysgelshoven.

**The Netherlands**

Adriaan M.H. van der Veen

Tel.: +31-45-535 20 11

Fax: +31-45-546 56 53

E-mail: avanderveen@nmi.nl

### **UNIVERSIDAD DE CONCEPTION**

Departamento de Ingenieria Quimica

Casilla 53-C

Correo 3

Concepcion

**Chile**

A. Gordon

Tel.: +56-41-23 49 85 (ext. 2534)

Fax: +56-41-24 08 26

E-mail: agordon@merlin.dig.udec.cl

### **TSINGHUA UNIVERSITY**

Department of Thermal Engineering

1000084 Beijing

**China**

Mao Jiangxiong

Tel.: +86-10-62 59 57 01

Fax: +86-10-62 55 12 24

E-mail: mx@mail.tsinghua.edu.cn

### **IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE**

Department of Chemical Engineering and Chemical

Technology

Prince Consort Road

London SW7 2BY

**United Kingdom**

R. Kandiyoti

Tel.: +44-171-589 51 11 (ext. 4460)

Fax: +44-171-584 11 70

# **1. Natural resources**

## **1.1. Basic natural resources**

<b>1.1.1. Water supply and management</b>	<b>1.1.2. Water treatment and pollution control</b>
<p>Contract number: IC18-CT98-0289 Contract number: IC18-CT98-0268 Contract number: IC18-CT98-0266 Contract number: IC18-CT97-0171 Contract number: IC18-CT97-0161 Contract number: IC18-CT97-0151 Contract number: IC18-CT97-0143 Contract number: IC18-CT97-0142 Contract number: IC18-CT97-0138 Contract number: IC18-CT97-0134 Contract number: IC18-CT96-0091 Contract number: IC18-CT96-0122 Contract number: AVI2-CT93-076 Contract number: AVI2-CT93-008 Contract number: AVI-CT93-143 Contract number: AVI2-CT93-126 Contract number: AVI2-CT93-099 Contract number: AVI2-CT93-073 Contract number: AVI2-CT93-062 Contract number: AVI-CT93-0015 Contract number: AVI-CT93-0005 Contract number: AVI-CT93-072 Contract number: AVI2-CT93-058 Contract number: AVI2-CT93-020 Contract number: AVI-CT93-0009 Contract number: AVI-CT93-0013 Contract number: CI1-CT93-0362 Contract number: AVI-CT93-0003 Contract number: AVI-CT92-0004 Contract number: TS3-CT92-0061</p>	<p>Contract number: IC18-CT98-0293 Contract number: IC18-CT98-0273 Contract number: IC18-CT98-0272 Contract number: IC18-CT98-0267 Contract number: IC18-CT98-0265 Contract number: IC18-CT97-0167 Contract number: IC18-CT97-0163 Contract number: IC18-CT97-0136 Contract number: IC18-CT96-0039 Contract number: IC18-CT96-0099 Contract number: IC18-CT96-0076 Contract number: AVI-CT94-0012 Contract number: AVI-CT94-002 Contract number: AVI-CT94-0009 Contract number: CI1-CT94-0086 Contract number: AVI-CT94-0014 Contract number: AVI-CT94-0013 Contract number: AVI2-CT93-112 Contract number: AVI2-CT93-081 Contract number: AVI2-CT93-074 Contract number: AVI-CT94-0007 Contract number: AVI2-CT93-054 Contract number: AVI2-CT93-092 Contract number: AVI2-CT93-083 Contract number: AVI2-CT93-019 Contract number: AVI-CT93-0006 Contract number: AVI-CT93-0002 Contract number: AVI-CT93-0001 Contract number: AVI-CT92-0016 Contract number: AVI-CT92-0015 Contract number: AVI-CT92-0014 Contract number: AVI-CT92-0011 Contract number: TS3-CT92-0126 Contract number: CI1-CT92-0104</p>
<b>1.1.3. Other resources: soil</b>	
<p>Contract number: IC18-CT97-0197 Contract number: IC18-CT97-0153 Contract number: IC18-CT97-0147 Contract number: IC18-CT96-0069 Contract number: IC18-CT96-0081 Contract number: IC18-CT96-0035 Contract number: AVI-CT93-0067 Contract number: AVI-CT92-0006 Contract number: TS3-CT92-0047</p>	

**1.2. Environmental research**

Contract number: IC18-CT98-0270  
Contract number: IC18-CT98-0269  
Contract number: IC18-CT98-0261  
Contract number: IC18-CT97-0200  
Contract number: IC18-CT97-0169  
Contract number: IC18-CT97-0155  
Contract number: IC18-CT97-0154  
Contract number: IC18-CT96-0055  
Contract number: IC18-CT96-0034  
Contract number: IC18-CT96-0029  
Contract number: CI1-CT94-0083  
Contract number: AVI2-CT93-087  
Contract number: AVI-CT92-0017  
Contract number: AVI-CT92-0007  
Contract number: AVI-CT92-0008  
Contract number: AVI-CT92-0005  
Contract number: AVI-CT92-0001  
Contract number: CI1-CT94-0096

**1.3. Renewable energy**

Contract number: AVI-CT94-0015  
Contract number: AVI-CT94-0011  
Contract number: AVI-CT94-0008  
Contract number: AVI-CT94-0006  
Contract number: AVI-CT94-0005  
Contract number: AVI-CT94-0004  
Contract number: TS3-CT92-0093

## **2. Agriculture**

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### 2.1. Production systems

**PROCESSING OF AGRICULTURAL WASTES BY WHITE-ROT FUNGI FOR  
PRODUCTION OF FODDER FOR SMALL RUMINANTS**

**Co-ordinator:** Georg-August-Universität Göttingen, Göttingen, Germany (Aloys Hüttermann)

**OBJECTIVES**

- The objective of the project is to increase the supply of meat in Egypt by providing fodder for small ruminants by upgrading agricultural wastes by white-rot fungi.

**ACTIVITIES**

- ◇ Screening of strains of white-rot fungi which has higher efficiencies of straw conversion and which are suitable for on-farm technologies;
- ◇ Improving the methods for pre-treatment of straw for fungal upgrading;
- ◇ Production of fodder with high enough *in situ* digestibility for sheep and goats that it is economically feasible;
- ◇ Establishment of a pilot plant on a farm in Egypt with feeding of goats and sheep.

**EXPECTED OUTCOME**

- ▷ *Scientific-technical results*
  - Improved strains of white-rot fungi, which are suitable for low-tech processes;
  - Better understanding of the enzymology of lignin degradation in straw;
  - Low-tech methods of growing the fungi in a cubic meter scale;
  - Results of the *in vitro* and *in vivo* digestibility studies and feeding trials with sheep.

- ▷ *Pilot plant operation*

The project will provide new methods for an economically feasible upgrading of straw of cotton, corn, and wheat for fodder for small cattle, sheep and goats. The economy of the new processes will be tested in a pilot operation, which will be set up in a village in the Nile Delta region.

**FOLLOW-UP**

- ▶ Upscaling of the fungal treatments and measuring the changes in enzyme activities and digestibility as a means for controlling the process;
- ▶ Continuation of the feeding trials;
- ▶ Preparing the pilot plant in the Nile Delta.

## **PARTNERS**

### **UNIVERSITÄT GÖTTINGEN**

Institut für Forstbotanik  
Abt. Technische Mykologie,  
Büsgenweg 2  
37077 Göttingen  
**Germany**

Aloys Hüttermann  
Tel.: +49-551-393481  
Fax: +49-551-392705  
E-mail: ahuette@gwdg.de

### **CENTRAL LABORATORY FOR FOOD AND FEED**

El Gamaa Street 9  
Giza - Cairo  
**Egypt**

Akila Hamza  
Tel.: +20-2-5713245  
Fax: +20-2-5732280  
E-mail: clff@nile.enal.sci.eg

### **THE HEBREW UNIVERSITY OF JERUSALEM – REHOVOT**

Faculty of Agriculture  
Dept. of Microbiology and Plant Pathology  
P.O.Box 12  
76100 Rehovot  
**Israel**

Yitzak Hadar  
Tel.: +972-8-9481315  
Fax: +972-8-9488785  
E-mail: hadar@agri-huji.ac.il

**BIODIVERSITY AUDIT, PROPAGATION AND SUSTAINABLE EXPLOITATION OF  
CEDARS (*CEDRUS SPP*) IN THE MEDITERRANEAN REGION**

**Co-ordinator:** The University of Reading, Agricultural Botany Department, United Kingdom  
(Peter D S Caligari)

### OBJECTIVES

The goal of the project is to establish methodologies for identifying cedar genotypes and multiplying commercially elite material within a framework of an environmentally sensitive production system and with due regard to safeguarding and enriching the natural biodiversity. This will be achieved by establishing an integrated system across a circum-Mediterranean network of expertise, which will allow cedars to be profitably utilised, but in a truly sustainable way.

- Molecular markers will be used to assess and analyse the genetic variability in cedar provenances across the Mediterranean region.
- Appropriate strategies for the management and environmentally sensitive exploitation of cedar germplasm in each of the study areas will be formulated.
- Genotypes suitable for use as landscape trees and as timber trees or suitable for use in arid and semi-arid areas will be evaluated, identified and multiplied.
- Regeneration and multiplication protocols, particularly *in vitro* systems will be developed, appropriate for each cedar species.
- Nurseries will be established for cedar production and a forest enrichment strategy for the region. It will be planned to forge links between countries in the region and to establish a preliminary network for the free exchanges of results and expertise. This will lead to the establishment of a long-term circum-Mediterranean network for cedar silviculture future.

### ACTIVITIES

- ◇ Assessment of genetic diversity between and within the natural cedar provenances in the Mediterranean region in relation to species, countries, stands and individuals as revealed by a number of molecular marker systems (RAPDs, ISSR). To develop a partial marker map for cedar species and provide the potential for trait tagging.
- ◇ Representative tree material will be sampled to allow the successful development of marker systems and to provide the population data needed to analyse the biodiversity present.
- ◇ Agreed traits will be defined by a combination of measurement and visual assessment. Local material will be surveyed and recorded on an agreed plan. This will provide complementary information to that from molecular markers and simultaneously opens the potential to tag significant variation in these traits to our molecular markers.
- ◇ *In vitro* systems of propagating cedar will be developed. These will include micropropagation, somatic embryogenesis and cultures with growth promoting bacteria (*A. rhizogenes*) and VA-Ectomycorrhizal fungi. Protocols for slow growth cultures and cryopreservation will also be developed to allow the practical *in vitro* conservation of germplasm to be planned.
- ◇ Complete propagation protocols will be devised which include the practical development of planting into amenity and other commercial sites but with a clear conservation strategy. The commercial plans will need to be an integral part of this activity.



## RESULTS

- ⇒ The assessment of genetic diversity between and within the natural cedar provenances in the Mediterranean (molecular level, morphological level) depends on the careful selection of individuals within each population and on the uniformity of collection strategy between countries. Following discussions at the first meeting, a consensus sampling and morphological assessment protocol was adopted (see appendices 1 and 2).
- ⇒ The sampling has started in Turkey and Morocco and is virtually finished in Lebanon. Morphological and geographical were recorded on each tree. Turkey and Lebanon were visited by the participant from the UK to ensure uniformity of collection strategy and to discuss modification of the strategy in the light of operational difficulties.
- ⇒ Several DNA extraction protocols used on fresh and desiccated cedar leaves material yield large amount of qualitative DNA. RAPD primers have already been screened and the first ISSR primers tested produced promising high level of polymorphism.
- ⇒ Propagation systems have been investigated. In vitro cultures have been established from juvenile and adult explants of *Cedrus atlantica* and *C. libani*. Callus cultures have been induced from seedling explants and immature seeds.

## FOLLOW UP

- ▶ The best DNA extraction protocol will be adopted for the project after comparison of the reliability of the PCR products obtained. DNA will be extracted from the first natural population of cedars sampled and the biodiversity of those ones will be assessed. DNA to be extracted from megagametophyte in order to develop a linkage map from haploid tissues. RAPD markers to be used for analysis of genetic stability of long-term cultures of cedar.
- ▶ Sampling of natural populations and planted stands in Lebanon to be completed. Sampling of Turkish and Moroccan region to be followed.
- ▶ Morphological data collection to be continued on the remaining population to be sampled. On the light of the first data collected, new characters might be added.
- ▶ *In vitro* propagation cultures will be initiated in the fall when immature embryos become available. Established in vitro cultures of cedar will be employed for minimal growth cultures in vitro, shoot tip encapsulation and shoot tip cryopreservation.

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## **PARTNERS**

### **THE UNIVERSITY OF READING**

Dept. of Agricultural Botany  
PO Box 221  
RG6 6AS Whiteknights, Reading  
**United Kingdom**

Peter Caligari  
Tel.: + 44 118 931 6684  
Fax: + 44 118 931 6657  
E-mail: p.d.s.caligari@reading.ac.uk

### **THE AMERICAN UNIVERSITY OF BEIRUT**

Faculty of Agriculture and Food  
PO Box 11-0236  
Beirut  
**Lebanon**

Salma N Talhouk  
Tel.: + 961 01 350 000  
Fax: + 1 212 478 1995  
E-mail: ntsalma@aub.edu.lb

### **INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II**

Complexe Horticole d'Agadir  
BP 18/s  
Agadir  
**Morocco**

Lahcen Kenny  
Tel.: + 212 8 241 006  
Fax: + 212 8 242 243  
E-mail: chagadir@mtds.com

### **UNIVERSITAT DE VALENCIA**

Dept. de Biologia Vegetal, Fac de Farmàcia  
Avgd Vicent Andrés Estelles, s/n  
46100 Burjassot, Valencia  
**Spain**

Juan Segura  
Tel.: + 34 6 386 4927  
Fax: + 34 6 386 4926  
E-mail: juan.segura@uv.es

### **AKDENİZ ÜNİVERSİTESİ**

Dept. of Horticulture, Ziraat Fakültesi  
Bahçe Bitkileri Bölümü  
07059 Antalya  
**Turkey**

Mustafa Pekmezci  
Tel.: + 90 242 227 4560  
Fax: + 90 242 227 4564  
E-mail: naci@agric.akdeniz.edu.tr

**SUSTAINABLE MANAGEMENT OF RENEWABLE MARINE RESOURCES: A  
COMPARATIVE STUDY OF MANAGEMENT SYSTEMS AND MARKETS IN  
NORTHWEST AFRICAN CEPHALOPOD FISHERIES**

**Co-ordinator:** University of Portsmouth, Portsmouth, United Kingdom (Pierre Failler)

## **OBJECTIVES**

- The project studied the regulation of the various production systems that exist in the context of the Moroccan, Mauritanian and Senegalese cephalopod fisheries. Bringing together researchers from both natural and social sciences, the project aimed to document and analyze the dynamics of the interactions which exist between the productive and management systems. The project took a comparative approach analyzing the dynamics particular to each country's situation and using these as the basis for discussion of methods to enhance sustainable management of the resources. The ultimate aim of the project was to identify ways in which current management systems might be improved so as to safeguard the natural resources and the exploitation systems based upon them.

## **ACTIVITIES**

*The project has proceeded via 3 major work phases:*

- ◇ In the **first phase**, researchers developed a multi-disciplinary synthesis of the concept of sustainable management. This synthesis was constructed on discipline-based views of the concept (law, economics, sociology, biology) as well as the viewpoint of those involved with the management of the fishery (ministries). The aim was to develop and promulgate the concept of sustainable management in these fisheries;
- ◇ The **second phase** sought to define the cephalopod fishing system by taking a historical perspective of the dynamics of the fishery. This was an essential phase since clearly successful management relies upon identifying a discrete management unit. However, this was often easier said than done, especially when a wider perspective than the fish stock itself is taken;
- ◇ The **third phase** sought to evaluate effects that have been induced by different management systems with respect to the concept of sustainable management.

## **OUTCOME, BENEFITS**

- ▷ The cephalopod fisheries of Northwest Africa represent the most valuable of the fishery resources of the region. In common with other fisheries, it is the most valuable stocks which are most in need of management. If management is to be sustainable then there is a need to take into account not only the physical condition of the fish stock but also the economical, social and legal circumstances under which the fishery is prosecuted. To date most research effort in the region has concentrated on the fish stock problem. The major benefit of this project has been to widen the scope of research, thereby extending the knowledge based on which management systems might be developed or refined;
- ▷ The results of the project have a number of impacts at the regional level. At the management level, each of the Administrations have a broader range of management options. In addition interactions between the fisheries especially at the market level has been made explicit, laying the base for concerted management measures;
- ▷ The project was also of interest at the European level since many EU fishermen depend on the cephalopod resources of these regions and stand to benefit from sustainable management. The EU is also a large consumer of cephalopods and EU consumers stand to gain by having access to more stable supplies, possibly at lower prices;
- ▷ At a training level, the project has involved a significant exchange of expertise. Partners have developed both within their own disciplines, especially in the social sciences, and by being

confronted with different disciplinary approaches. The project has lead to significant integration of North-South research institutes.

## **FOLLOW-UP**

This project will be followed-up by a workshop on "Intentionally oriented fisheries : modes of governance and development " (to be held in Lisbon, May 1999), and by a Concerted Action Research Program. Both of these will focus on the institutional aspects of fishery governance and the influences of the international market on fishery development as well.

Sustainable management of renewable marine resources : a comparative study of management systems in Northwest African cephalopod fisheries

## **PARTNERS**

### **UNIVERSITY OF PORTSMOUTH**

Centre for the Economics & Management of Aquatic Resources  
Locksway Road  
Portsmouth P04 8JF  
**United Kingdom**

Pierre Failer  
Tel.: +44-1705-844 085  
Fax: +44-1705-844 037  
E-mail: pierre.failerp@pbs.port.ac.uk

### **ORSTOM**

Laboratoire Halieutique & Ecosystèmes Aquatiques  
Ressources Marines Vivantes  
Avenue Agropolis 911  
B.P. 5045  
34042 Montpellier Cedex 1  
**France**

Joseph Catanzano  
Tel.: +33-1-46 48 22 56  
Fax: +33-1-46 48 22 76  
E-mail: joseph.catanzano@ifremer.fr

### **CENTRE NATIONAL DE RECHERCHES OCÉANOGRAPHIQUES & DE PECHEs**

Département de Recherche Halieutique  
P.O. Box 22  
Nouadhibou  
**Mauritania**

Ismaila Thiam  
Tel.: +222-245.124  
Fax: +222-245.081

### **CENTRE DE RECHERCHES OCÉANOGRAPHIQUES DE DAKAR-THIAROYE**

Gestion des Ressources Aquatiques Naturelles  
P.O. Box 2241  
Dakar  
**Senegal**

Moustapha Deme  
Tel.: +221-34 05 36  
Fax: +221-34 27 92  
E-mail: sarr@isra.isra.sn

### **INSTITUT SCIENTIFIQUE DES PÊCHES MARITIMES**

Rue de Tiznit 2  
Casablanca  
**Morocco**

Lamine Mounir  
Tel.: +212-2-26 78 11  
Fax: +212-2-26 69 67  
E-mail: inrh@mail.cbi.net.ma

<p style="text-align: center;"><b>MODELLING BENTHIC DISTURBANCE AND RECOVERY IN WARM WATER MARICULTURE</b></p>
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**Co-ordinator:** Universität Kiel, Kiel, Germany (Harald Rosenthal)

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**OBJECTIVES**

- To model environmental consequences of intensive marine fish farming in warm, oligotrophic waters in the Mediterranean region, i.e. the hypertrophication of the underlying sediments;
- To characterise and model the recovery of the disturbed benthos at the impacted site once the fish farm has been removed.

**ACTIVITIES**

- ◇ Quarter-annual measurements at Cypriot and Israeli fish farms, focusing on the changes occurring at the study sites:
- ◇ as a result of fish farming activity: this will include imaging documentation, sediment samples, macrofauna samples, sediment trap measurements, flux chamber experiments and fish farm management data;
- ◇ after cessation of such activity : the measurements will focus on the dynamics of the recovery and theoretic simulation will be applied too.
- ◇ Considering these measurements, predictive models, on both organic matter accumulation and on recovery dynamics, will be compiled, applying approaches of Findlay and Watson (1994), Gowen and Bradbury (1987), Gowen et al (1991, 1994) and others. As most work has been done for cold waters, the existing models have to be adapted to warm, oligotrophic waters.

**EXPECTED OUTCOME**

- ▷ Quantitative information on the environmental impact of intensive fish farming in warm, oligotrophic waters typical for the Mediterranean region;
- ▷ Improved understanding of the pollution and recovery processes linked to net pen fish farming and the related disturbance of the benthic and identification of appropriate indicators for the actual state of the marine environment under stress;
- ▷ Information on the ability and speed of the ecosystem to recover after the fish farming activity has ceased;
- ▷ Finally, the model will provide a useful tool for environmentally friendly fish farming in the Mediterranean sea.

## **PARTNERS**

**UNIVERSITÄT KIEL**  
Institut für Meereskunde  
Düsternbrooker Weg 20  
24105 Kiel  
**Germany**

Harald Rosenthal  
Tel.: +49-431-597 39 17  
Fax: +49-431-56 58 76

**ISRAEL OCEANOGRAPHIC & LIMNOLOGICAL  
RESEARCH LTD**  
National Centre for Mariculture  
P.O. Box 1212  
88112 Eilat  
**Israel**

Dror Angel  
Fax: +972-7-637 57 61

**MINISTRY OF AGRICULTURE, NATURAL  
RESOURCES & ENVIRONMENT**  
Department of Fisheries  
Eolou Street 13  
Nicosia  
**Cyprus**

Andreas Demetropoulos  
Fax: +357-2-36 59 55

## **RAINFALL FORESCASTS AND STRATEGIC IRRIGATION MANAGEMENT**

**Co-ordinator:** Società di Ricerca e Servizi di Ingegneria (ISMES) S.P.A., Bergamo, Italy  
(Stefano Clementel)

### **OBJECTIVES**

The overall objective of the project was to develop and test a methodology for the optimal utilisation of meteorological rain forecast in the North of the Nile delta for a better understanding and protection against extreme hydrometeorological events and for the optimal use of available resources for irrigation purposes.

*Specific tasks of the project are:*

- Set-up an integrated methodology for rainfall forecast and strategic irrigation management;
- Develop operating policies for the irrigation network of the Northwest Mediterranean Coast Region that optimise the contribution of the rainfall in agricultural land expansion;
- Layout of a hydrometeorological centre for the coastal strip between Alexandria and El Allum on the Egyptian border with Libya.

### **ACTIVITIES**

*The project covered various aspects of:*

- ◇ territorial characterisation of a 'Pilot area' on the Nile delta;
- ◇ hydro-meteorological modelling with application of meteorological limited area model (LAM) to the area of the Nile delta;
- ◇ development of multi-objective analysis models for irrigation management optimisation;
- ◇ preliminary design of HW and SW of a hydro-meteorological centre on the Nile delta.

Finally the methodology have been applied to hydro-meteorological data of the last ten years on the northern part of the delta and the possibility of optimisation of water management during rainy period was quantified.

Activities 2) and 3) are organised in four modules with the following main objectives:

- Rainfall forecast in the Delta irrigated area based on GCM (Global Circulation Model) and LAM (Local Area Model) results,
- Fresh water availability from the Nile river at Delta barrage,
- Optimisation of water allocation management,
- Irrigation planning in the Western Delta.

Numerical tools that allow the analysis of the different processes in the Nile Delta area compound each module.

The first module uses the ECMWF (European Centre for Medium range Weather Forecast) model and the LAM (Limited Area Model) to forecast the rainfall events in the Delta area. The second module, the HYBAD model, has been developed in order to compute the hydrological balance in the Delta area. The third module, the model WAO, is used for the optimisation of the water temporary storage in the storage sinks. Finally the fourth module, the model WEAP, leads to an optimal irrigation planning of the new irrigated areas.

The models used in the different modules of the project are closely interconnected and the output of one simulation is part of the input for the following process.

### **OUTCOME**

*Scientific-technical applied results.*

***Application of meteorological Limited Area Models (LAM) to the Nile Delta***

- ⇒ Two different time periods has been selected for the study. Both periods are located in the winter season when the Mediterranean perturbations affect the Nile Delta with heavy showers.
- ⇒ The first period, ranging from 18 to 23 November 1994, is characterised by significant and intense precipitation. The second period, ranging from 3 to 14 December 1996 has been selected for the presence of days of intense rainfall alternated with drought periods. In order to produce the meteorological forecasting two different models have been used for the selected periods: the ECMWF (European Centre of Medium Range Weather Forecast) global model, that allows to reproduce the phenomena on the synoptic scale (800-1000 km), and the MEPHYSTO Limited Area Model (LAM), which runs in operative way at the Italian National Electric Board.
- ⇒ Since forecasting reliability decrease with time, only to forecast at time +24 and +48 (tomorrow and the day after) where considered. The LAM model works also at +24 and +48 with a grid of 30 x 30 Km.
- ⇒ A statistical analysis of precipitation records, obtained from the Egyptian Meteorological Authorities in the period 1973-1996, has pointed out the principal rainfall events occurred in the Nile Delta.
- ⇒ The precipitation predicted by the models has been compared with the data recorded at the Egyptian stations. Furthermore, the fields of the upper atmosphere has been examined to understand the onset of rainfall events and to provide a definition of large scale weather regimes associated with rainfall over the Egyptian territory.
- ⇒ The results are encouraging: both models simulate the synoptic flow quite well and the precipitation events are time-centred. The use of the LAM in the Mediterranean part of Egypt, where the flow regimes mostly pertain to the large-scale range, seems not to add a relevant contribute to the forecast, particularly when light precipitation occurred.
- ⇒ Moreover the lack of tuning of the LAM model parameters to run at lower latitudes, (the LAM is calibrated for Italian area, and Italian climate) could have affect the forecasting quality, in particular for light rain. The ECMWF model seems to produce good precipitation forecasting mainly when the moist processes are governed by dynamical forcing.

### **Hydrological Balance of the Nile Delta**

- ⇒ During the period from October to March some rainfall events usually occurs in the already irrigated area of the Nile Delta; part of this water is lost through the drainage system to the sea. Whenever a forecasting system of two-three days rainfall is implemented, a fraction of the irrigation water could be stored for successive use or distributed within the irrigation network considering the location and intensity of the forecasted rain.
- ⇒ First of all the response of the Delta area to rainfall events was studied, based on historical data set of thirteen rain gauges and on the corresponding discharges to the sea through out the main Nile branches of Damietta and of Rosetta.
- ⇒ The analysis of the hydrological balance in the Nile Delta area leads to a correlation expression between the water income to the Delta irrigated areas - from the Nile plus the rainfall - and the water consumption in the system plus the outcome to the sea. The correlation expression, even if derived from very general assumptions concerning rainfall distribution, irrigation needs, infiltration and evaporation values in the Delta area, can be use as a first estimation of the water volume from rainfall that can be used for storage or direct irrigation purposes in new areas at the Western coast. This correlation expression is proposed as a *preliminary estimator* to derive the upper limit of water volume available for new purposes at Delta Barrage, at rainfall event scale, by minimising the discharges to the Mediterranean Sea.
- ⇒ The application of the HYBAD model permits to calculate the daily volumes of the available fresh water from the Nile River at Delta Barrage that can be used for new purposes.

### **Water Allocation Management**

- ⇒ This activity s leads to the formulation of the water allocation optimisation model for water-supply-distribution in a pilot area of Western Delta, and to the implementation of such model using the numerical tool Solver from Microsoft Excel.
- ⇒ Four alternatives are available as harvesting places for the management of the excess water. These zones are: Delta Barrage pool, Maryout or Edko lakes, ground water and channel cross section at the Extension El Hamman Canal.



- ⇒ A “water supply and distribution system planning scheme” has been developed in order to provide the decision-makers with a set of optimal policies with regard to the selection of supply sources and the associated transmission lines in meeting the demands at various locations in the systems.
- ⇒ The available water comes from the Nile River and rainfall, and the water demand considers irrigation, minimum flow in the Nile for navigation and water quality purposes.

#### *Pilot Area*

- ⇒ The methodology has been applied to a Pilot Area using historical data of the last ten years. The Pilot Area is located on the West Delta near the North coast, and includes the irrigated area of El Nasr Canal after Pump Station N5 and the Extension of Bahig Canal. The first irrigated area is 27300 hectares wide and the second one 7560 hectares.

#### *Design of a hydro-meteorological centre*

- ⇒ A technical documentation have been produced containing the technical specification of hydrological and meteorological equipments, of the telecommunication system including both Meteor Burst system and a solution based on a Ku band satellite communication system.
- ⇒ Specification are given also for a reference configuration of the main computer system HW and SW; is based on a HP 9000 series 800 system; it is, of course, a basic reference, considering the extremely rapid evolution of commercial HW. The need for factory training and on site training, maintenance and spare parts and special tools are also been considered.

#### ***Promotion of Achieved Results: Establishment of a Hydro-meteorological Centre for the Coastal Zone***

The expected benefit from a Hydro-meteorological Centre with a multi-discipline staff team have been analysed. Its priorities have to be set towards establishing global and integrated organisation and approach to Rainfall Forecast and Water Resources Management. Through this approach, emphasis has to be put on capacity building, monitoring network and a management system with hardware and software components.

The Centre can best be viewed as special interdisciplinary and integrated institution studying and collecting hydro-meteorological data, generating a great variety of potential scenarios relating to the development of North Coast Region and increasing environmental and socio-economic awareness at the policy-making level. Several outputs can be brought from the Centre:

- daily material on weather forecast in the area under consideration especially in the days of high / medium potential for rainfall;
- daily water management scheme during the above named period in which use is made of different types of water and utilised for different purposes;
- models and knowledge of various factors affecting future agricultural development;
- short-term plans for the improvement of infrastructure in order to optimise the use of water obtained from different sources and used for different purposes;
- long-term strategies of water use in the study area, which takes into consideration all the possible scenarios, which increases the system efficiency;
- Environmental Impact Assessment of each of future scenarios which allows for all the possible responses to changes that are going to take place due to the introduction of the new processes;
- new technologies which achieve a better resource utilisation towards water conservation;
- dissemination of information and knowledge gained to decision-makers and practitioners.

The Centre should be organised for forecast dissemination to potential interested users.

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## PARTNERS

- |   |  |
|---|--|
| <b>SOCIETÀ DI RICERCA E SERVIZI DI INGEGNERIA (ISMES) S.P.A.</b><br>Viale Giulio Cesare 29<br>24124 Bergamo<br><b>Italy</b> | Stefano Clementel<br>Tel.: +39-35-30 75 30<br>Fax: +39-35-30 29 99         |
| <b>WATER MANAGEMENT RESEARCH INSTITUTE</b><br>Delta Barrage WRC Building<br>Cairo<br><b>Egypt</b>                           | Dia-El-Din Ahmed El-Quosy<br>Tel.: +20-2-218 95 63<br>Fax: +20-2-218 95 61 |
| <b>CAIRO UNIVERSITY</b><br>Faculty of Engineering<br>Department of Irrigation & Hydraulics<br>Cairo<br><b>Egypt</b>         | Ibrahim El-Assouty<br>Tel.: +20-2-375 75 70<br>Fax: +20-2-36 33 65         |

## **2. Agriculture**

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### **2.2. Crop production**

**OPTIMISING MARGINAL RESOURCES IN INTENSIVE HORTICULTURAL PRODUCTION IN SOUTHERN TURKEY AND NORTHERN EGYPT**

**Co-ordinator:** University of Wales, Aberystwyth, United Kingdom (Michael Anthony Hall)

**OBJECTIVES**

- Development of suitable systems for horticultural production on marginal land in the context of the problems of water availability and quality, fluctuating temperatures and soil characteristics; to select appropriate plant varieties adapted to the prevailing conditions and through studies on post-harvest physiology to develop methods to enhance fruit stability and quality and hence transportability and exportability;
- Development of appropriate greenhouse / plastic shelter climate control, to include testing and evaluation of low cost systems, modelling and simulation of internal microclimate and its management;
- Assessment of water requirements of the crops to include measurement and modelling of crop transpiration and irrigation scheduling; investigations on crop physiology under the known adverse conditions to include work on gaseous exchanges and crop yield prediction;
- Development of soil and soilless culture techniques to include sand-based culture;
- Selection of appropriate plant varieties to include work on plant growth and development and on fruit quality in response to the established environmental factors and transportability;
- Development and testing of appropriate ways and means to involve local farmers and local SMEs in the development of intensive horticultural production.

**ACTIVITIES**

- ◇ Testing and evaluation of low cost systems for the improvement of internal microclimate; modelling of energy and mass balance enabling simulations of internal microclimate and evaluations of performance of greenhouse equipment;
- ◇ Preparation of a blueprint for crop management;
- ◇ Studies on the water requirements of the crops, development of irrigation scheduling;
- ◇ Studies on the effect of the environment on crop physiology in relation to fruit quality and post-harvest stability;
- ◇ Development of chemometric techniques to assess fruit quality;
- ◇ Studies on substrate suitability;
- ◇ Selection of appropriate cultivars.

**RESULTS**

*Scientific-technical results*

- ⇒ Laboratory measurements have been made of air speed inside a reduced scale greenhouse using laser velocimetry and in situ measurements have been carried out in a two span greenhouse and a plastic tunnel using sonic anemometry to determine the influence of vent opening. In addition, modelling of the air flow around and inside the greenhouse has been accomplished using computational fluid dynamics.
- ⇒ The relative effects of roof and side ventilation have been explored and the effect of different glass colourings on yield have been investigated. Comprehensive weather data have been collected and a model for predicting plastic house temperature from outside air temperature

compared. This is now in the validation stage. Similar measurements on humidity and radiation are being analysed.

- ⇒ A sprinkler system for frost protection has been tested, climatic data has been collected and is being analysed and growth parameters are being assessed.
- ⇒ A programme for irrigation scheduling has been developed and validation is in progress.
- ⇒ A wide range of possible cultivars sweet pepper have been investigated in relation to nutrient uptake and different climatic conditions, and the relationship between the above parameters and the occurrence of blossom and root (BER) is being investigated.
- ⇒ Trialling of substrate material has been ongoing and comparisons are being made with the nutrient film technique.
- ⇒ A wide range of characterised and uncharacterised tomato cultivars have been investigated in relation to pollen yield and viability as well as growth parameters. Shelf-life tests have been carried out on a wide range of cultivars for subsequent input into the fruit quality assessment programme.
- ⇒ Test systems have been developed for trialling Egyptian and Turkish cultivars under defined conditions in relation to saline conditions. At the same time germination of specific cultivars under saline conditions is being investigated.

#### *Deliverables*

- A prototype wetness duration sensor has been developed, laboratory tests have proved satisfactory and in situ tests are underway;
- A model-based algorithm for irrigation scheduling has been developed and its validation will be performed in 1998. A prototype automated irrigation controller has been produced which will be trialled in 1998;
- A generic crop model is under development and a preliminary version will be available and tested in the coming year;
- Dialogue has been established between local horticultural industry and scientists.
- Chemometric techniques (pyrolysis mass spectroscopy) coupled with neural networks have been developed for the assessment of fruit quality;
- A web page for the project is on the internet at: <http://www.aber.ac.uk/hej93/index.html>

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## **PARTNERS**

### **UNIVERSITY OF WALES**

Institute of Biological Sciences  
Edward Lloyd Building  
Aberystwyth SY23 3DA  
**United Kingdom**

Michael Anthony Hall  
Tel.: +44-1970-62 23 13  
Fax: +44-1970-62 23 50  
E-mail: mah@aber.ac.uk

### **MINISTRY OF AGRICULTURE**

Agricultural Research Center  
El-Bosaily Protected Cultivation  
P.O. Box 296  
12411 Imbaba (Giza)  
**Egypt**

Ayman Abou-Hadid  
Tel.: +20-2-220 13 86  
Fax: +20-2-221 68 51

### **EGE UNIVERSITY**

Faculty of Agriculture  
Department of Horticulture  
35100 Bornova-Izmir  
**Turkey**

Yuksel Tuzel  
Tel.: +90-232-388 01 10  
Fax: +90-232-388 18 64

### **INSTITUT NATIONAL DE LA RECHERCHE**

#### **AGRONOMIQUE**

Centre de Recherche d'Avignon  
Unité de Bioclimatologie d'Avignon  
Domaine Saint Paul  
Site Agroparc  
84914 Avignon Cedex 9  
**France**

Alain Baille  
Tel.: +33-4-90 31 60 79  
Fax: +33-4-90 89 98 10  
E-mail: mba@avignon.inra.fr

### **WYE COLLEGE**

Department of Agriculture, Horticulture & Environment  
Ashford TN25 5AH  
**United Kingdom**

Stanley Walter Burrage  
Tel.: +44-1233-81 24 01  
Fax: +44-1233-81 30 17

### **INSTITUTO VALENCIANO DE INVESTIGACIONES AGRARIAS**

Departamento Horticultura  
Apartado Oficial  
Carretera de Moncada a Naquera Km 4.5  
46113 Moncada  
**Spain**

Pedro-Florian Martinez  
Tel.: +34-6-139 10 00  
Fax: +34-6-139 02 40  
E-mail: pfmarti@inia.ivia.es

**IMPROVEMENT OF THE WATER USE EFFICIENCY OF WHEAT UNDER DRY  
AND SALINE CONDITIONS IN THE MAGHREB**

**Co-ordinator:** Faculté des Sciences Agronomiques, Gembloux, Belgium (Roger Paul)

### OBJECTIVES

Selection of wheat varieties with high water use efficiency for the semi-arid and arid zones of the Maghreb.

### ACTIVITIES

- ◇ Study and collection of Maghrebian ecotypes of wheat;
- ◇ Field and greenhouse experiments for identification of those ecotypes with the highest water use efficiency under dry and saline conditions;
- ◇ Second selection between the best ecotypes identified in the first phase based on physiological parameters;
- ◇ Laboratory and greenhouse studies of the physiological mechanisms determining water use efficiency;
- ◇ Field and greenhouse trials with antitranspirants and analysis of their effects on physiological processes relevant for water use efficiency and salinity tolerance.

### OUTCOME

#### *Water use efficiency (WUE)*

- ▷ According to the varieties of wheat selected, water consumption may be reduced by half (in optimal conditions). The Tunisian varieties of *Triticum durum* Khiar and Razzak are interesting, and their choice will depend on the regional agrometeorologic conditions. Razzak is less resistant to drought than Khiar.
- ▷ The Algerian variety MBB shows the best WUE. The second Algerian variety, Oued Znati, is the most resistant to drought but uses about twice more water. This example illustrates the interest not to be focused only on drought resistance for the choice of varieties in arid and semi-arid regions.
- ▷ As this project is aiming at decreasing to the maximum the water losses by evapotranspiration of the cultures, Oued Znati must be eliminated. But, it remains an interesting support for the genetic improvement, taking into account its drought resistance.

#### *Salinity resistance*

- ▷ At germination stage  
The Moroccan varieties of *Triticum aestivum*, Nesma and Achar, have good resistance to salinity and drought at the germination stage.  
For *T. durum* varieties, Khiar and Razzak can support saline stress fairly, with a slight superiority for Khiar in conditions of drought.  
In spite of its weaker germination rate, MBB is the most powerful variety in the event of moderated saline stress, but it answers negatively severe saline stress and drought.
- ▷ After germination.  
Resistance to salinity increases when  $\text{Na}^+$  transport to the shoots decreases. The selected varieties preserve a fair K/Na ratio in their shoots, in spite of salinity. MBB proves to be the most resistant at the young stage.

### *WUE and other parameters*

- ▷ A significant positive correlation between the relative water content (RWC) of the leaves and the WUE was shown on a large number of varieties. A high and stable RWC could reduce plant transpiration and save water.
- ▷ Nevertheless, this limitation of the gaseous exchanges must be a compromise, in order not to disrupt photosynthesis, and the yield in the long run.  
The importance of the cuticular factors was also highlighted for WUE.

### *Potentiality of use of antitranspirants*

- ▷ The spraying of linseedoil facilitates the maintenance of the waterstatus in plants exposed to drought.  
This technique appears interesting, in particular when episodes of severe water deficits take place at the stage of the grain filling.

### **FOLLOW-UP**

- ▶ The AVICENNE CT93-0007 contract was the basis for:  
The EMMA (Environmental Management of Marginal Areas) network, including universities and research centers from Algeria, Belgium, France, Morocco, Palestine and Syria. The network is in charge of a EEC MED-CAMPUS project.
- ▶ The CIERSACO (Centre Interdisciplinaire d'Etude de la Résistance aux Stress Abiotiques chez les Céréales et les Oléagineux), in Meknès (Morocco), funded by the Belgian Cooperation (AGCD).

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## **PARTNERS**

### **FACULTE DES SCIENCES AGRONOMIQUES**

Unité d'Enregistrement et de Recherche de Biologie  
Végétale

Avenue de la Faculté d'Agronomie 2A

5030 Gembloux

**Belgium**

Roger Paul

Tel.: +32-81-62 24 59

Fax: +32-81-61 41 20

### **FACULTE DES SCIENCES DE MEKNES**

Département de Biologie

Beni M'Hamed

B.P. 4010

Meknes

**Morocco**

Samir El Jaafari

Tel.: +212-5-55 88 70

Fax: +212-5-53 68 09

### **INSTITUT NATIONAL DE LA RECHERCHE**

#### **AGRONOMIQUE DE TUNIS (INRAT)**

Laboratoire de Physiologie Végétale

Rue Hedi Karray

2049 Ariana - Tunis

**Tunisia**

M'Barek Ben Naceur

Tel.: +216-1-23 00 24

### **UNIVERSITE DE CONSTANTINE**

Institut des Sciences de la Nature

Route Ain El Bay

25000 Constantine

**Algeria**

Abdelmalek Chorfi

Tel.: +213-4-69 45 00

Fax: +213-4-69 09 16

**IMPROVING THE GROWTH OF TROPICAL NITROGEN-FIXING FOREST TREES  
IN THE GENERA ACACIA AND CASUARINA THROUGH TISSUE CULTURE AND  
GENETIC TRANSFORMATION**

**Co-ordinator: ORSTOM GeneTrop, Montpellier, France (Emile Duhoux)**

**OBJECTIVES**

- To develop techniques for the micropropagation of superior clones of *Acacia* sp. and *Casuarina* and to establish techniques for efficient regeneration of whole plants from somatic cells;
- To achieve genetic transformation of *Acacia* and *Casuarina* using either the natural vector *Agrobacterium* or direct gene transfer techniques (high velocity microprojectiles);
- To identify constitutive or tissue-specific expression vectors for *Acacia* and *Casuarina*; using the  $\beta$ -glucuronidase gene under the control of different known regulatory sequences and to study the stability of transgenes expression;
- To introduce a metallothionein gene into *Casuarina* using the most appropriate vector and to regenerate transgenic plants.

**ACTIVITIES**

- ◇ Micropropagation;
- ◇ Regeneration procedure;
- ◇ Identification of a selection marker for the genetic transformation of *Acacia* and *Casuarina*;
- ◇ Transformation vectors to follow shoot meristem formation;
- ◇ *Agrobacterium*-mediated DNA transfer;
- ◇ Direct DNA transfer by high velocity microprojectiles;
- ◇ Analysis of transformed tissues;
- ◇ Expression vectors for *Acacia* and *Casuarina*;
- ◇ Introduction of important trait.

*The key activities were :*

- ◇ Micropropagation techniques were optimized via axillary shoot formation. Methods of whole plant regeneration which were suitable for genetic transformation were identified (adventitious regeneration from juvenile explants or induction of shoot primordia by *Rhodococcus fascians*).
- ◇ Genetic transformation of *Acacia* and *Casuarina* spp. was investigated. Explants were inoculated with a collection of disarmed strains of *Agrobacterium tumefaciens* and the efficiency of gene transfer was monitored by the expression of the GUS histochemical marker. Gene transfer based on the introduction of DNA coated on high velocity microprojectiles was attempted in *Acacia*.
- ◇ Chimeric genes containing different constitutive and tissue specific promoters were introduced into *Casuarina* and the level and specificity of expression was determined using the GUS reporter gene.

**OUTCOME**

*Scientific results*

- ▷ Identification of superior clones of *A. mangium* with good organogenic potential;
- ▷ Micropropagation of *A. mangium* and *A. mearnsii*;
- ▷ Micropropagation of *C. glauca* using shoots from mature trees;
- ▷ Using thidiazuron, differentiation of buds on *A. mangium* calli derived from hypocotyls;
- ▷ Regeneration of rooted plants from cotyledons of *A. crassicarpa*;
- ▷ Identification of selection markers for *Acacia* and *Casuarina*;

- ▷ Induction of tumors after inoculation of *A. mangium*, *A. mearnsii* and *A. crassicarpa* by wild-type *A. tumefaciens* strains;
- ▷ Transfer of the  $\beta$ -glucuronidase gene into *A. mangium* using a disarmed strain of *Agrobacterium tumefaciens*;
- ▷ Transient expression of the *uidA* gene in *Acacia mangium* calli, cotyledons and leaves, following particle bombardment;
- ▷ Regeneration of transgenic *A. verticillata* and *Casuarina glauca* plants expressing the  $\beta$ -glucuronidase gene under the control of constitutive or tissue-specific promoters;
- ▷ Expression of the 35S promoter in transgenic *A. verticillata* and *Casuarina glauca*;
- ▷ Nodulation by *Frankia* of transgenic *A. verticillata* and *Casuarina glauca*;
- ▷ Isolation of a cDNA from *C. glauca* encoding a metallothionein gene;
- ▷ Transformation of *Allocasuarina* and *Nicotiana tabacum* with a metallothionein gene;
- ▷ Characterization of *Rhodococcus fascians* genes involved in leafy gall formation and evaluation of its potential for regeneration programs.

#### *Patents in progress*

- ▷ *Rhodococcus fascians* induced fasciation: implications for regeneration and propagation of plants (Universiteit Gent);
- ▷ Promoter and cDNA of a metallothionein gene from *Casuarina* (ORSTOM).

#### **FOLLOW-UP**

- ▶ The genetic transformation procedure developed for Casuarinaceae trees is currently used in several laboratories for academic research. Transgenic *Casuarina* provide valuable tools in exploring the regulation of plant genes involved in the symbiotic process with the actinomycete *Frankia* (promoter analysis, over and under-expression of plant symbiotic genes). The characterization of the *Casuarina* metallothionein gene is continuing and its potential for applied projects of afforestation in heavy-metals polluted areas will be evaluated in model plants (ORSTOM Laboratory).
- ▶ Genetic transformation of tropical *Acacia* will continue via a three-year-collaboration between Brazil and France (ORSTOM/CNPq). The non lethal reporter gene GFP will be used to screen for transformation events.
- ▶ A completely novel method based on the infection of plant tissue by *Rhodococcus fascians* has been developed to micropropagate and/or regenerate plants. This technique will be further tested on different target species and the bacterial genes involved in the fasciation process will be characterized (Universiteit Gent).

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## **PARTNERS**

### **ORSTOM GENE TROP**

Physiologie Cellulaire et Moléculaire des Arbres  
Avenue Agropolis 911  
B.P. 5045  
34032 Montpellier Cedex 1  
**France**

Emile Duhoux  
Tel.: +33-4-67 61 10 12  
Fax: +33-4-67 63 82 65  
E-mail: duhoux@orstom.rio.net

### **UNIVERSITE MOHAMMED V**

Faculté des Sciences  
Laboratoire de Microbiologie  
IBN Battouta Street  
B.P. 1014  
10000 Rabat  
**Morocco**

Abdelkrim Maltouf Filali  
Tel.: +212-7-77 18 16  
Fax: +212-7-77 54 61

### **INNOPRISE CORPORATION SENDIRIAN BERHAD**

Plant Biotechnology Laboratory  
P.O. Box 60793  
91017 Tawau  
**Malaysia**

O. Monteuis  
Tel.: +60-89-77 53 28  
Fax: +60-89-76 23 14

### **RIJKSUNIVERSITEIT GENT**

Faculty of Science  
Laboratorium voor Genetica  
K.L. Ledeganckstraat 35  
9000 Gent  
**Belgium**

Marc Van Montagu  
Tel.: +32-9-264 52 05  
Fax: +32-9-264 53 49

### **UNIVERSIDADE FEDERAL DO RIO DE JANEIRO**

Instituto de Biologia  
Departamento de Genética  
Avenida 24 S/N CEP 21941-590  
C.P. 08042, Ilha Do Fundao  
Rio de Janeiro  
**Brazil**

Dulce Eleonora De Oliveira  
Tel.: +55-21-290 03 91  
Fax: +55-21-260 79 20

### **TEAGASC**

Kinsealy Research & Development Centre  
Agriculture & Food Development Authority  
Malahide Road  
Dublin 17  
**Ireland**

Gerard Douglas  
Tel.: +353-1-846 06 44  
Fax: +353-1-846 05 24

**DEVELOPMENT OF SELECTION AND CLONAL PROPAGATION TECHNIQUES  
FOR MULTIPLICATION OF ELITE YIELD AND ANTHRACNOSE TOLERANT  
CASHEW (ANACARDIUM OCCIDENTALE L.)**

**Co-ordinator:** Instituto de Investigação Científica Tropical, Oeiras, Portugal  
(Carlos-José Rodrigues Junior)

### OBJECTIVES

- To identify elite yield and anthracnose-tolerant cashew trees in wild and domesticated stands to be clonal propagated or to be used as "plus" trees in further breeding programmes;
- To introduce elite yield and anthracnose-tolerant germplasm into Brazil, Guinea-Bissau and Morocco;
- To strengthen research and training links between Moroccan, West African and European countries with Brazilian centres where traditional approaches to cashew breeding and selection have been underway in the natural genetic diversity of the *Anacardium* genus;
- To strengthen research and training links between Morocco, Guinea-Bissau and Brazil with European countries where new approaches like cashew in vitro propagation and biochemical and molecular fingerprint characterisation are being carried out.

### OBJECTIVES

- To expand the cashew germplasm collection and develop propagation techniques required for the safe introduction of appropriate elite cashew germplasm (yield potential and if possible anthracnose tolerance) into Morocco and Guinea-Bissau. In the particular case of Morocco, the objective is to study the edapho-climatic conditions and even the management requirements for the introduction of this crop in Moroccan agro-systems.
- To increase the general knowledge about clonal propagation techniques.
- To extend and strengthen research and training links between African countries and Brazilian centres where traditional approaches to cashew breeding and selection have been under way.
- Within the scope of these general objectives, field and greenhouse work, as well as in vitro propagation, anthracnose and genetic fingerprinting studies and other activities were carried out.

### ACTIVITIES

- ◇ *Field work*
  - Amplification of the cashew and *Colletotrichum gloeosporioides* germplasm.
  - Clonal propagation of cashew in Morocco and Guinea-Bissau
  - Progeny fields in Guinea-Bissau
  - Introduction of cashew in Morocco
- ◇ *Greenhouse work*

The greenhouse cashew germplasm collection at CIFIC (Portugal) was increased with new genotypes from Brazil, Guinea-Bissau and other cashew-growing countries. This diversity was available for clonal propagation experiments, to evaluate cashew tolerance to the anthracnose and to be used in the in vitro culture. Healthy mother plants were maintained for provision of large amounts of shoot explants. Soft, young shoots were used as sources of leaf, stem and nodal explants for the micropropagation and genetic fingerprinting studies. Some of this material was also maintained in the Wye College (U.K), for in vitro work.
- ◇ *In vitro work*
  - Apical and axillary node culture
  - Somatic embryogenesis from rejuvenated shoots and seedlings tissues
  - Micrografting
  - Production of rootstocks by micropropagation
  - Thin cell layer technique

- ◇ *Development of techniques for screening tolerant cashew to anthracnose*
- ◇ *Genetic fingerprinting of cashew*

## **OUTCOME**

### ▷ *Field work*

The enlargement of the germplasm collections at UFAL and CIFIC was carried out with cashew genotypes (wild, semi-spontaneous and improved clones, and other Anacardiaceae) aiming at yield potential and anthracnose tolerance in the expeditions made to North-East of Brazil. Seed, budwood and cuttings were collected. Samples of the same material were sent to Wye College and IAV (Morocco). Potential zones for the cashew introduction in Morocco were the strips Rabat-Salékenitra and Azila-Tanger-Sebta. Studies were made on the effect of storage on seed germination, transplanting conditions and cultural methods for cashew plantation. In Guinea-Bissau 4 progeny fields were established in 4 representative sites with offspring of 45 elite trees previously selected locally and of some additional introductions in a total of 51 genotypes.

### ▷ *Greenhouse work*

A cashew gene resource population was established at CIFIC (Portugal) including more than 270 plants from 85 progenies collected in Guinea-Bissau, Brazil, Tanzania, India, China and Mozambique. At UAPS (Brazil) 50 new introductions were made collected in Brazil. Both collections were used as source of explants for micropropagation and anthracnose studies. Some cashew mother plants were also maintained at the Wye College.

### ▷ *In vitro propagation work*

Using greenhouse-raised cashew mother plants, factors affecting bud sprouting, shoot elongation and node development from shoot nodes of elite selections from Brazil, Guinea-Bissau and Tanzania were investigated. Sprouting decreased strongly with the increasing age of mother plants and best bud sprouting performances were achieved from explants of 1 month old plants. The main obstacle encountered in the further development of the callus tissue was the accumulation of phenolic compounds and the severe browning of tissues. The work carried out on TLC suggests that the use of a liquid medium may reduce accumulation of phenolic compounds and that anti-oxidants other than ascorbic acid such as cystein or polyclar should be tested.

### ▷ *Cashew anthracnose studies*

Both in the laboratory tests and in the field observations no plants totally resistant could be found. As much as a 50-fold difference in the amount of template DNA gave identical RAPD profiles suggesting that within this range of DNA concentrations amplification profiles were relatively insensitive to template DNA concentrations. Random 10-mer primers, which gave well resolved RAPD profiles, could be identified and were used for analysing the DNA samples. Striking polymorphism and reproducible and distinct differences were readily visible within accessions from Brazil, Mozambique and Guinea-Bissau and within a collection of 20 elite Tanzania clones.

## **FOLLOW-UP**

- ▶ The continuation of these activities on cashew breeding is being carried out at the IICT (Portugal) on projects financed by its own funding. Specific work areas are receiving funding from the Institute for the Portuguese co-operation and the Praxis program. This work is continuing in the following areas:
  - ▶ Studies on cashew and *C. gloeosporioides* association in terms of the characterisation of the pathogenic agent and the identification of cashew genotypes tolerant to this disease;
  - ▶ Molecular characterisation of a *C. gloeosporioides* collection isolated from cashew and other horticultural crops;
  - ▶ Within the scope of the cashew-breeding program in development in Guinea-Bissau, to follow-up the evaluation of the progeny fields established in that country on 1996.

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## **PARTNERS**

### **INSTITUTO DE INVESTIGAÇÃO CIENTIFICA TROPICAL QUINTA DO MARQUÊS**

Centro De Investigação das Ferrugens do Cafeeiro  
Quinta Da Marques  
2780 Oeiras

**Portugal**

Carlos-José Rodrigues Junior

Tel.: +351-1-442 33 23

Fax: +351-1-442 08 67

### **UNIVERSITY OF LONDON**

Agriculture, Horticulture, Environment

Wye College

Wye, Ashford, Kent TN25 5AH

**United Kingdom**

Sinclair Mantell

Tel.: +44-1223-81 24 01

Fax: +44-1223-81 33 20

### **UNIVERSIDADE FEDERALE DE ALAGOAS**

Departamento de Química

Laboratório de Pesquisa em Recursos Naturais

Cidade Universitária

Tabuleiro dos Martins

57000 Maceió

Alagoas

**Brazil**

Eurico Eduardo Pinto de Lemos

Tel.: +55-82-324 12 38

Fax: +55-82-324 13 45

### **MINISTERIO DE DESENVOLVIMENTO RURAL E AGRICULTURA**

Direcção dos Serviços Florestais e Caça

C.P. 71

Bissau

**Guinea-Bissau**

Cipriano Cassama

Tel.: +245-22 17 80

Fax: +245-22 10 71

### **INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II**

Departement d'Horticulture

Laboratoire de Biotechnologie Végétale

B.P. 6202

Rabat

**Morocco**

Abdelhadi Abousalim

Tel.: +212-7-77 17 45

Fax: +212-7-77 58 38



**STRUCTURAL ASPECTS OF WHEAT GLUTENINS AND THE MECHANISMS OF THEIR ASSEMBLY TO FORM THE GLUTEN MATRIX**

**Co-ordinator:** University of Bristol, Bristol, United Kingdom (P.R. Shewry)

**OBJECTIVES**

- To understand the mechanisms of wheat gluten protein synthesis, transport, folding and assembly in relation to the formation of protein bodies and the structure and functional properties of gluten.
- To determine the pathway of HMW subunit and gamma-gliadin trafficking and deposition and how this is affected by the addition of targeting sequences or the presence of specific structural features, notably disulphide bonds.
- To determine the roles of other proteins, notably binding protein (BiP), protein disulphide isomerase (PDI) and thioredoxin, on the translocation of gluten proteins across the ER membrane and their folding, assembly and deposition within the secretory pathway.

**ACTIVITIES**

- ◇ Preparation of protein bodies from developing endosperm cells to determine the presence of gluten proteins and their interactions with other proteins.
- ◇ Purification of gluten proteins from wheat to determine their interactions with thioredoxin in vitro.
- ◇ Expression of gluten proteins in yeast and E.coli to determine their interactions with chaperonins and protein disulphide isomerase.
- ◇ Immunogold labelling of electron micrographs of tissue sections from developing endosperm cells to determine the presence of gluten proteins and other proteins in protein bodies.
- ◇ Expression of wild type and mutant gluten proteins in transgenic tobacco plants to determine their trafficking and targeting and how these processes are affected by the addition or deletion of specific signal sequences or structural features.
- ◇ The use of in vitro transcription/translation and cross-linking to determine the interactions of gluten proteins during their translocation across the ER membrane.

**RESULTS**

- ⇒ Immunogold labelling of tissue sections confirmed the presence of gluten proteins in vacuolar-derived protein bodies, the distribution of tonoplast marker proteins indicating that the protein contents may have arisen by autophagy.
- ⇒ Expression of HMW subunits in transgenic tobacco resulted in accumulation in dense protein bodies. In contrast, a gamma-gliadin was unstable, probably due to proteolytic digestion in a post-ER compartment. Accumulation of the gamma-gliadin occurred when a C-terminal retention sequence was added.
- ⇒ Analysis of protein bodies showed that BiP was present in complex with other proteins while a specific interaction with BiP also occurred when a gamma-gliadin was expressed in yeast. Similarly, a purified gamma-gliadin was shown to act as a substrate for thioredoxin in vitro. The level of gamma-gliadin expressed in yeast was also affected by co-expression with BiP or PDI, but the in vivo significance of this is not known.
- ⇒ In order to study the trafficking and deposition of gamma-gliadin in a homologous background, a gamma-gliadin cDNA has been labelled with a C-terminal c-myc oncogene tag, allowing detection using a commercially-available monoclonal antibody (9E10), and transferred to wheat under the control of the HMW glutenin subunit promoter.

- ⇒ The gamma-gliadin pGEM template has been used to set up protein translocation assays in a cell free system containing pancreatic canine microsomal membranes. The translocation of the gamma-gliadin was observed, as determined by signal peptide cleavage and protease-protection assays. The use of stalled translocation-intermediates was attempted to examine the interaction between the gamma-gliadin-translocating nascent chain and chaperones such as BiP and PDI. Initial attempts utilized long nascent chains of greater than 20 kD in length. However, these polypeptides failed to produce crosslinks, indicating that such protein-protein interactions occur when the nascent chain initially enters the ER lumen. Further studies will therefore be carried out using shorter nascent chains based on the gamma-gliadin repetitive domain.
- ⇒ Expression of gamma-gliadin in yeast resulted in interaction with the yeast BiP within the ER, as demonstrated by immunoprecipitation with BiP and gamma-gliadin antibodies.

### **SELECTED PUBLICATIONS**

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### **PARTNERS**

#### **UNIVERSITY OF BRISTOL**

Department of Agricultural Sciences  
IACR-Long Ashton Research Station  
Bristol BS18 9AF  
**United Kingdom**

P.R. Shewry

Tel.: +44-1275-39 21 81 / 54 93 84  
Fax: +44-1275-39 42 99

#### **WEIZMANN INSTITUTE OF SCIENCE**

Department of Plant Genetics  
P.O. Box 26  
76100 Rehovot  
**Israel**

Gad Galili

Tel.: +972-8-948 12 11  
Fax: +972-8-934 41 81

## GENETIC AND PHYSICAL MAPPING OF THE TOMATO I2 LOCUS

Co-ordinator: Weizmann Institute of Science, Rehovot, Israel (Robert Fluhr)

### OBJECTIVES

- To develop and apply reverse genetic and megabase cloning technologies in tomato with the final goal of physically isolating the I2 locus, a gene of dominant character that encodes resistance to Fusarium wilt disease;
- One main focus was to provide a high resolution map in the context of the I2 locus that could be used a platform to launch a map-based cloning project.

### ACTIVITIES AND RESULTS

- ◇ Fusarium wilt is an economically important disease of tomatoes, caused by the soil-born fungus *Fusarium oxysporum* f. sp. *lycopersici*. There are three host-specific races of this pathogen. The dominant tomato gene I2 confers resistance to race 2. The I2 Fusarium resistance gene was mapped genetically to chromosome 11 of tomato, between the RFLP markers TG105 and TG36, 0.4 centiMorgan (cM) from TG105 (1). A mean value of 43 kb for each cM was assigned in the vicinity of I2. We have generated new RFLP markers in the region by chromosome walking from TG105 towards I2 on lambda clones, and by subcloning a 350 kb long YAC clone (YAC 8) (2) that contains TG105. These RFLP markers were mapped physically on YAC 8 by PFGE. High resolution was carried out on special populations and sets the stage for final cloning of the I2 locus (3).
- ◇ A cDNA clone, D14, that was isolated by YAC 8, turned out to be 53% similar to xanthine dehydrogenase from mammals and flies. Antibodies rose against a part of the protein encoded by D14 recognise cross reacting material of MW 80 kD, that is highly enriched in nodules of legumes, and seems to be induced by various environmental and pathogenic stress conditions.

### FOLLOW UP

- ▶ Aldehyde oxidase (AO) and xanthine dehydrogenase (XD) are a group of ubiquitous hydroxylases, containing a Molybdenum cofactor (MoCo) and two iron sulphur groups. Plant AO and XD activities are involved in nitrogen metabolism and hormone biosynthesis, and their corresponding genes have not yet been isolated. Here we describe a new gene from tomato, which shows the characteristics of a MoCo containing hydroxylase (4). It shares sequence homology with xanthine dehydrogenases and aldehyde oxidases from various organisms, and similarly contains binding sites for two iron-sulphur centres and a molybdenum binding region. However, it does not contain the XD conserved sequences thought to be involved in NAD binding and in substrate specificity, and is likely to encode an AO-type activity. This gene was designated tomato aldehyde oxidase 1 (TAO1). TAO1 belongs to a multigene family, whose members are shown to map to clusters on chromosomes 1 and 11. MoCo hydroxylase activity is shown to be recognised by antibodies rose against recombinant TAO1 polypeptides. Immunoblots reveal that TAO1 cross reacting material is ubiquitously expressed in various organisms, and in plants it is mostly abundant in fruits and rapidly dividing tissues;
- ▶ Members of a new multigen family, complex I2C, were isolated by map-based cloning from the I2 F.o. f sp *lycopersici* race 2 resistance locus (5). The genes show structural similarity to the group of recently isolated resistance genes that contain a nucleotide binding motif and leucine rich repeats. Importantly, the presence of I2C antisense transgenes abrogated race 2 but not race 1 resistance in otherwise normal plants. Expression of the complete sense I2C-1 transgene conferred significant but partial resistance to F.o. f sp *lycopersici* race 2. All members of the I2C gene family have been mapped genetically and are dispersed on three different chromosomes. Some of the I2C members cosegregate with other tomato resistance loci. Comparison within the leucine-

rich repeated region of I2C gene family members shows that they differ from each other mainly by insertions or deletions.

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### **PARTNERS**

#### **WEIZMANN INSTITUTE OF SCIENCE**

Department of Plant Genetics  
76100 Rehovot

**Israel**

Robert Fluhr

Tel.: +972-8-948 12 11

Fax: +972-8-934 41 81

#### **AGRICULTURAL UNIVERSITY OF WAGENINGEN**

Department of Molecular Biology  
P.O. Box 8128  
6700 Et Wageningen

**The Netherlands**

Pim Zabel & Maarten Koornneef

Tel.: +31-317-48 20 36

Fax: +31-317-48 35 84

## **2. Agriculture**

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### 2.3. Livestock production

**DESERTIFICATION RISK ASSESSMENT IN SILVOPASTORAL MEDITERRANEAN ECOSYSTEMS: BASES TOWARDS A SUSTAINABLE MANAGEMENT OF NATURAL RESOURCES (DRASME)**

**Co-ordinator:** Consejo Superior De Investigaciones Cientificas, Zaragoza, Espana  
(Dr Concepcion L. Alados)

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## **OBJECTIVES**

The aim is to predict the management consequences for both livestock and plant community on silvopastoral Mediterranean ecosystems in order to reduce desertification process and to improve natural resources of drought prone areas by maintaining biodiversity and ecological infrastructure of rural economy. In order to do that it will be achieved the following main objectives:

To develop combined population dynamics-GIS modelling approach to investigate the consequences of water deficit and grazing management impacts on the Mediterranean ecosystem's stability.

To model community structure and dynamic quantifying the interaction of grazing intensity with plant interactions and weather in order to know the stability and sustainability of the ecosystem.

To estimate the tolerance to grazing of plants by novel bio-sensors capable of measuring population health conditions.

To integrate farm systems and wildlife sub-products into the rural economy and related social aspects of silvopastoral systems, providing tools for monitoring the sustainability of silvopastoral ecosystems.

## **ACTIVITIES**

The key activities involved are:

The spatial patterns of grazing management will be first analysed through the application of geostatistical methods. The study will compile several spatial variables of landscape characteristics and several derived variables describing landscape structure and its relation with grazing impact and weather in the Mediterranean.

Once locations have been selected from previous analyses, we will collect field data for population dynamics analysis model. The results can then be used to make predictions of changes in population community structure concomitant to grazing impact. Additionally, we will estimate health conditions of plant species by developmental instability analyses. Once evaluated stress indexes of selected species, they will be stored into the GIS database and included as a layer in order to be analysed together with the remaining variables.

Finally, in order to maintain the rural economy it will be analytically evaluated the relation between the ecological variables and human related variables with special emphasis on the socio-economic value of wildlife sub-products. In order to make this model available to the managers a commercial version will be developed.

## **EXPECTED OUTCOME**

This project should determine the influence of grazing in plant community dynamic of drought prone areas, and its consequences on the ecosystem stability. The project should set-up a database of Mediterranean bio-indicators of environmental quality and sustainable management particularly applied to grazing systems in order to prevent desertification. It should provide tools for monitoring sustainability of silvopastoral system by developing commercial software of progressive sophistication that may be adapted to the needs of farmer and managers.

## **PARTNERS**

### **CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS**

Ipe  
Avd. Montanana 177 Campus Aula Dei  
50080 Zaragoza  
**Espana**

Dr Concepcion L. Alados  
Tel.: 34-976-57-58-83  
Fax: 34-976-57-58-84  
E-mail: alados@ipe.csic.es

### **UNIVERSIDADE DE COIMBRA**

Prdr Helena Freitas  
Dept. De Zoologia Imar-Instituto Do Mar  
3000 Coimbra  
**Portugal**

Tel.: 351-39-22-897  
Fax: 351-39-20-780  
E-mail: hfreitas@cygnus.ci.uc.pt

### **ARISTOTLE UNIVERSITY OF THESSALONIKI**

Pr Vasilios P. Papanastasis  
Lab. Of Range Science  
54006 Thessaloniki  
**Greece**

Tel.: 30-31-99-89-33  
Fax: 30-31-99-88-86  
E-mail: vpapan@for.auth.gr

### **UNIVERSITY OF CUKUROVA**

Pr Huseyin Ozbek  
Faculty Of Agriculture Soil Science Department  
Ziraat Fakultesi Toprak Boluumu  
01330 Adana  
**Turquie**

Tel.: 90-322-338-6643  
Fax: 90-322-338-6643  
E-mail: zerdekes@pamuk.cu.edu.tr

### **INSTITUT AGRONOMIQUE ET VETERINAIRE HASSAN II**

Pr Ahmed El Aich  
Dept. Des Productions Animales  
6202 Rabat  
**Maroc**

Tel.: 212-7-77-43-27  
Fax: 212-7-77-43-27  
E-mail: ahelaich@syfed.ma.ma.refer.org

**DEVELOPMENT OF BIOCAPSULED FEED FOR LARVAL FISH BASED ON  
NUTRITIONALLY ENRICHED NEMATODES**

**Co-ordinator:** Bio Integrated Technology s.r.l., Pantalla di Todi, Italy (Bertold Fridlender)

**OBJECTIVES**

- The goal of the project is the enhancement of larval fish production by using nutritionally enriched nematodes as a suitable feed base. This innovative live feed will be of adequate size for the use even by the very small larvae emerging during the first days of culture.
- The target is to obtain a product with shelf-life of several months that due to its characteristics will result in increased production efficiency and reduced costs of operation in the aquaculture fish production.
- Systems for the Bio-loading of nematodes during their growth cycle will be developed.
- Specific chemoattractants that can enhance food consumption will be identified.
- A large scale economically feasible "in vitro" production system will be developed. Selected nematodes in which nutritional enhancers can be added will be used for this purpose.
- A formulation of biocapsulated nematodes including specific chemoattractants will be established. The formulation should be capable to provide nutritional value to the fish larvae as well as storage and transportation stability.

**ACTIVITIES**

- ◇ Testing of selected free-living nematodes as fish larvae food source for different species.
- ◇ Identification and selection of nutritional and growth promoting materials to be use in bio-loading of nematodes.
- ◇ Determination of the effect of nutritional additives on nematodes growth.
- ◇ Testing of the effect of bio-loaded nematodes on larval fish growth and survival.
- ◇ Selection of specific chemoattractants for different fish species, and studies of their effect on larval growth and feeding efficacy.
- ◇ Liquid culture fermentation processes for nematodes production.
- ◇ Studies of nematode persistence in different formulation materials.
- ◇ Physico-chemical characteristics of biocapsulated nematodes.
- ◇ Stability and nutritional capabilities of formulated products.

**EXPECTED RESULTS**

*Scientific – technical results*

- ⇒ Definition of the use of nematodes as an alternative fish food source.
- ⇒ Identification of fish nutritional enhancers that will improve nematode consumption by fish larvae.
- ⇒ Methodology for the introduction of specific nutritional elements to nematodes.
- ⇒ Establishment of the effect of attractants in growth and development of fish larvae.
- ⇒ Protocol for the use of nematodes as a source of fish food.
- ⇒ Cost efficient liquid fermentation process for specific nematodes species.
- ⇒ Biocapsulated formulation for nematodes.

*Deliverables*

- ⇒ Protocol for the production of nematodes as food for fish larvae.
- ⇒ Standard Operation Procedure for liquid culture fermentation of specific nematodes.
- ⇒ Standard Operation Procedure for the formulation of biocapsulated nematodes to be used in the fish industry.



## **FOLLOW-UP**

### *First year*

- ▶ Study of the effect of additives on nematodes growth.
- ▶ Identification of food fish attractants and their effect on selected fish species.
- ▶ Rearing of nematodes in liquid cultures in flasks up to 1 liter.
- ▶ Identification of inert materials to be used in the biocapsulated formulation.

### *Second and third year*

- ▶ Definition of additives for nematodes growth.
- ▶ Optimization of the bio-loading system.
- ▶ Determination of the additives on fish growth.
- ▶ Selection of Attractants and studies on fish growth.
- ▶ Rearing of nematodes in 40 and 500 liter fermenters.
- ▶ Optimization of the formulation process.

## **PARTNERS**

### **BIO INTEGRATED TECHNOLOGY S.R.L.**

Agrifood Technology Park of Umbria  
Fraz. Pantalla di Todi  
06050 Todi - PG  
**Italy**

Bertold Fridlender  
Tel.: +39 075 895 72 42  
Fax: +39 075 888 776  
E-mail: bfm@bit.it

### **AGRICULTURAL RESEARCH ORGANIZATION**

Volcani Center Dept. of Aquaculture  
P.O. Box 6  
50-250 Bet Dagan  
**Israel**

Sheenan Harpaz  
Tel.: +972 3 968 33 88  
Fax: +972 3 960 56 67  
E-mail: harpaz@agri.huji.ac.il

### **UNIVERSITÄT HOHENHEIM**

Inst. Animal Prod. in Tropics/Subt. Dept of Animal  
Nutrition/Aquaculture  
Schloss Hohenheim  
70599 Stuttgart  
**Germany**

Klaus Becker  
Tel.: +49 711 459 3158  
Fax: +49 711 459 3702  
E-mail: kbecker@uni-hohenheim.de

### **UNIVERSITI PUTRA MALAYSIA**

Dept. Agronomy/Horticulture  
Faculty of Agriculture - A.B.R.G.  
43400 Serdang  
**Malaysia**

Mohd Salleh  
Tel.: +60 3 94 86 101  
Fax: +60 3 943 59 73  
E-mail: msalleh@agri.upm.edu.my

### **SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER**

Dept of Aquaculture Binangonan Freshwater Station  
1940 Rizal Binangonan  
**Philippines**

Corazon Santiago  
Tel.: +63 2 372 39 80  
Fax: +63 2 372 39 83  
E-mail: bfs-seafdec@phil.gn.apc.org

**SUSTAINABLE DEVELOPMENT OF AFRICAN CONTINENTAL FISHERIES: A  
REGIONAL STUDY OF POLICY OPTIONS AND POLICY FORMATION  
MECHANISMS FOR THE LAKE CHAD BASIN**

**Co-ordinator:** University of Portsmouth, Portsmouth, United Kingdom (Arthur Neiland)

**OBJECTIVES**

- To contribute to the sustainable development of the continental fisheries of the Lake Chad Basin.
- To strengthen the relationship between the fisheries sector, research institutes, and both national and international administrations in the region.
- To encourage the greater participation of fisheries stakeholders (fishers and fisher organisations) in the policy formation process.
- To enhance the capabilities of the research institutes involved in the project.
- To strengthen the links between regional research institutes in the Lake Chad Basin through South-South and North-South collaboration in the project.

**ACTIVITIES**

- ◇ The project will carry out multi-disciplinary research in order to provide a better understanding of the key researchable constraints to improvements in fisheries policy formation: poorly established policy-formation mechanisms; lack of relevant data and information; and limited institutional capacity. In turn, this will provide a basis for developing a range of approaches by which the constraints can be reduced or removed, leading to an overall improvement in policy-formation, with particular reference to fisheries management systems. A series of meetings and training seminars will build local capacity in multi-disciplinary assessment techniques and for policy formation, and establish the targets for subsequent work periods.
- ◇ The project will use a range of research methodologies, from both natural and social sciences, which reflect the multi-disciplinary research and information needs of the work. Wherever possible, complementary and parallel methodologies will be employed to provide clarification and verification of attributes under investigation (triangulation, quadrangulation).

**EXPECTED OUTCOME**

The project will provide:

- ▷ a better understanding of the operation and context of the fisheries of the Lake Chad Basin;
- ▷ the identification of options for improved fisheries policy formation;
- ▷ a series of recommendations to governments for regional co-operation and policy interventions;
- ▷ the creation of greater local capacity for information gathering and analysis;
- ▷ the creation of greater local capacity for policy formation.

## **PARTNERS**

**UNIVERSITY OF PORTSMOUTH**  
CEMARE  
Locksway road  
PO4 8JF Portsmouth, Hants  
**United Kingdom**

Arthur Neiland  
Tel.: +44 1705 844 214  
Fax: +44 1705 844 037  
E-mail: arthur.neiland@port.ac.uk

**ORSTOM**  
213, rue Lafayette  
75480 Paris Cedex 10  
**France**

Jacques Quensiere  
Tel.: +33 1 48 03.77 77  
Fax: +33 1 40 35 37 18

**NATIONAL INSTITUTE OF FRESHWATER  
FISHERIES RESEARCH**  
P.M.B. 6006  
New-Bussa  
**Nigeria**

E.O. Ita  
Tel.: +234 31 67 04 44  
Fax: +234 31 67 07 79

**MINISTERE DE L'ELEVAGE, DES PECHEES  
ET DES INDUSTRIES ANIMALES**  
Yaoundé  
**Cameroon**

J.C. Njock  
Tel.: +237 22 37 75  
Fax: +237 22 14 05

**ANALYSIS AND MANAGEMENT OF ORGANIC MATTER AND NITROGEN IN  
AQUACULTURAL PONDS FOR A MINIMAL WASTE PRODUCTION AND  
OPTIMAL EFFICIENCY**

**Co-ordinator:** Wageningen Agricultural University, Wageningen, Netherlands (Johan Verreth)

## **OBJECTIVES**

The general objective of the present project is to develop a management system for feed driven fish/shrimp ponds that minimizes the accumulation of dischargeable products in the system. The working hypothesis is that this can be achieved when the concentration of organic carbon, nitrogen and phosphorous in the system are balanced to each other. This will result in a maximal conversion of these nutrients into bacterial biomass, which in turn may be harvested by the fish.

The specific objectives of the project are geared towards the collection of the empirical data needed to construct a model for organic carbon and nitrogen fluxes in feed driven fish ponds and to use and validate this under different management procedures. This aims at (1) quantifying the nitrogen and organic carbon flows in a feed driven fish/shrimp pond system under different (quantitative and qualitative) levels of nutrient input, (2) investigating nitrogen and organic carbon conversions in the water column and in the pond sediments along the algal and bacterial food chain up to the fish/shrimp, (3) modelling the flows of organic matter, carbon and nitrogen in feed driven ponds, and (4) using the model(s) for designing and testing feed compositions and pond management procedures that should result in the general objective.

## **ACTIVITIES**

Project activities concentrate on (1) physical and biological characterization of the flocculent layer, (2) the kinetics of organic matter breakdown and the influence of C:N ratio's on breakdown velocity, (3) the sedimentation to and resuspension from the flocculent layer of organic matter, (4) diffusion rates of nutrients at the flocculent layer – water interface, (5) the contribution of autotrophic and heterotrophic production to fish/shrimp production, (6) the indirect stimulation of the algae growth either through the ammonia excretion by fish/shrimp or by ammonia and phosphorous diffusing into the water column from the flocculent layer, (8) up-scaling the results to pilot farm conditions and verification of modelling results.

## **EXPECTED RESULTS**

Already achieved:

A first working model was written that models amounts of N-species present in feed driven fresh water ponds during the fish production cycle.

Other milestones and deliverables planned:

- ⇒ Estimates of parameter values for development of the flocculent layer;
- ⇒ Estimates of parameter values for the development of an oxygen profile layer;
- ⇒ Estimates of parameter values for kinetics/stoichiometric description of organic matter breakdown layer;
- ⇒ Estimates of parameter values for the effect of organic matter decomposition on the oxygen profile in ponds layer.

## **FOLLOW-UP**

At the end of the project, the expected outcomes are (1) a detailed knowledge and understanding of the nutrient exchange processes at the soil-water interface in stratified and stagnant ponds, (2) an operational and validated dynamic simulation model describing nutrient conversion in intensive fish and shrimp ponds, and (3) an outline in which direction practical pond management should develop to make intensive fish/shrimp farming more environmentally friendly.

At present, field experiments are ongoing in Israel, Mexico and Costa Rica, with the main goal to measure fluxes of N-species between the flocculent layer and the water column.

The Netherlands and Costa Rica are jointly writing a model that predicts N-species concentrations in ponds, with special attention to the flocculent layer. A first version (draft) of this model is presently being tested.

Small incubation units to monitor fluxes between the flocculent layer and the water column are being constructed in the Netherlands. The next year, these units will be used to calibrate various sub-units of the model.

## **PARTNERS**

### **WAGENINGEN AGRICULTURAL UNIVERSITY**

Fish Culture and Fisheries Group  
Dept. of Animal Sciences  
Marijkweg 40 – P.O. Box 338  
6700 AH Wageningen  
Netherlands

Johan Verreth  
Tel.: +31 317 48 33 07  
Fax: +31 317 48 39 37  
E-mail: [johan.verreth@alg.venv.wau.nl](mailto:johan.verreth@alg.venv.wau.nl)

### **TECHNION – ISRAEL INST. OF TECHNOLOGY**

Faculty of Agricultural Engineering  
Lab. Management of Environm. Studies  
Technion City  
32000 Haifa  
Israel

Yoram Avnimelech  
Tel.: +972 4 829 24 80  
Fax: +972 4 822 15 29  
E-mail: [agyoram@tx.technion.ac.il](mailto:agyoram@tx.technion.ac.il)

### **UNIVERSIDAD NACIONAL HEREDIA**

Escuela de Ciencias Biologicas  
P.O. Box 86-3000  
86-3000 Heredia  
Costa Rica

Ricardo Jimenez-Montealegre  
Tel.: +506 237 64 27  
Fax: +506 237 64 27  
E-mail: [rjimenez@irazu.una.ac.cr](mailto:rjimenez@irazu.una.ac.cr)

### **CENTRO DE INVESTIGACION EN ALIMENTACION Y DESARROLLO**

Unidad Mazatlan en Acuicultura y M.A.  
Sabalo Cerritos s/n Estero del Yugo AP. 711  
82010 Mazatlan  
Mexico

Omar Calvario-Martinez  
Tel.: +52 69 88 01 57  
Fax: +52 69 88 01 59  
E-mail: [calvario@servidor.unam.mx](mailto:calvario@servidor.unam.mx)

**DEVELOPMENT OF IMPROVED STRATEGIES FOR THE CONTROL OF FABA  
BEAN NECROTIC YELLOWS VIRUS IN FOOD LEGUME CROPS OF WEST ASIA  
AND NORTH AFRICA**

**Co-ordinator:** Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig, Germany  
(Heinrich Josef Vetten)

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### **OBJECTIVES**

- To conduct field studies of FBNYV build-up and spread in Egypt and Syria to obtain a better understanding of the factors involved in virus survival and spread under field conditions;
- To develop screening methods that will permit the precise determination of virus resistance levels in the existing faba bean, chickpea and lentil germplasm;
- To determine the incidence, distribution and variability of FBNYV in countries of West Asia and North Africa (WANA) by providing diagnostic tools to collaborators of the existing ICARDA network;
- To characterise the FBNYV genome by infectivity tests, by determining FBNYV gene functions and variability as well as by testing in vitro engineered replicase-associated protein (Rep) variants of FBNYV in a single cell-based replication assay for their capacity to inhibit the replication of wild-type FBNYV;
- To use in vitro engineered Rep genes for generating transgenic faba bean plants and determine the levels of virus resistance in these lines.

### **ACTIVITIES**

- ◇ Epidemiological and ecological studies to obtain a better understanding of the incidence, distribution and variability of FBNYV in WANA countries and the factors involved in FBNYV epidemics under field conditions.
- ◇ Development of improved screening methods for locating and evaluating virus resistance in the existing faba bean, chickpea and lentil germplasm under greenhouse and field conditions.
- ◇ Analysis of the variability of FBNYV genes with special emphasis on the variation of Rep genes and on the presence and absence of distinct Rep-encoding components in geographically diverse isolates of FBNYV.
- ◇ Infectivity tests: biolistic bombardment or agroinoculation of faba bean seedlings with various combinations of cloned viral DNA components will be conducted for determining the functions of individual genome components and the integral parts of the FBNYV genome.
- ◇ An assay for studying FBNYV replication will be established for testing defective interfering Rep protein functions.
- ◇ Gene cassettes suitable for the transformation of faba bean will be constructed, and transformed faba bean lines will be generated and evaluated.

### **EXPECTED RESULTS**

- ⇒ The wide occurrence of chickpea chlorotic dwarf geminivirus (CCDV) in faba bean was demonstrated for most of the major production areas in the Sudan. In addition, evidence was provided that at least one luteovirus also affects legume crops causing yellowing and stunting symptoms indistinguishable from those caused by CCDV (and FBNYV). However, the presence of FBNYV has not yet been detected in the Sudan, even not in the northern areas that are close to Egypt where FBNYV has been causing severe epidemics for several years. In collaborative work between the Sudanese partners and ISV, France, the genome of CCDV was sequenced, and work on infectivity tests with cloned CCDV DNA is in progress. Results from field and laboratory experiments will lead to a better understanding of CCDV spread and to developing strategies for CCDV control.

- ⇒ Some progress has been made in studies on a reliable method for identifying FBNYV-resistant genotypes and on ecological factors influencing FBNYV spread in Egypt and Syria. The commencement of these sub-projects was delayed due to problems in identifying suitable students in WANA countries.
- ⇒ Analysis of the genome of a FBNYV isolate from Syria (Sy) revealed the presence of ten circular ssDNA species each of about 1 kb. All ten components have one major open reading frame (ORF) in the virion sense and a non-coding region containing a highly conserved sequence possibly forming a stem-loop (SL) structure. The SL also contains the conserved nonanucleotide sequence (AGTATTACC) which, as in geminiviruses, may be the origin of a rolling-circle replication mechanism. The 10 circular DNA components (C1-C10) found associated with the FBNYV genome potentially encode four distinct replication-associated proteins (Rep) and six non-Rep proteins. The presence of four Rep and six non-Rep components in the FBNYV-Sy genome is further supported by sequence and PCR data on another virus isolate from Egypt (FBNYV-Eg) as well as by findings in Japan on milk-vetch dwarf virus (MDV), a close relative of FBNYV. Although FBNYV and MDV differ in two Rep components, their genomes appear strikingly similar not only in the total number of identified components but also in the number and types of homologous non-Rep components present in each genome. The presence of four genome components potentially encoding distinct Rep proteins in two FBNYV isolates and in MDV is a most puzzling phenomenon and contrasts with geminiviruses which possess only one (begomoviruses) or two (mastreviruses) Rep genes. Tentative evidence suggests that the Rep protein encoded by FBNYV-C2 may play a pivotal and indispensable role in FBNYV replication, whereas the three other Rep components (C1, C7 and C9) may actually be non-integral parts (satellite DNAs) of the FBNYV genome, as already suggested for some Rep components of BBTV. The four Rep and six non-Rep components found associated with FBNYV infections may comprise the complete viral genome. However, the possibility that the FBNYV genome contains further, not yet identified components cannot be ruled out at present. In order to identify all integral parts of the FBNYV genome, attempts are made to conduct infectivity tests with cloned components for reproducing a disease whose causal agent is indistinguishable in all its biological properties from field isolates of FBNYV. If infectivity can be demonstrated, this will facilitate studies to determine not only the role of the additional Rep components but also the functions of three (C3, C6, C8) of the six non-Rep components. Definite and tentative functions have been assigned only to C5, C4, and C10 which respectively encode the capsid protein, a movement protein, and a protein involved in cell cycle regulation.
- ⇒ Biochemical and mutational analyses of the FBNYV Rep components 1 and 2 at ISV, France, have shown that the FBNYV Rep proteins share several enzymatic functions with the geminivirus Rep proteins. Therefore, Ti plasmid-based expression vectors for the Rep1 and Rep2 proteins mutated in their ATP binding sites were constructed and have been supplied to AGERI, Egypt, for generating transgenic *Vicia faba* lines. While AGERI is still working on establishing a transformation and regeneration protocol for faba bean, these constructs have been used at ISV for producing transgenic *Nicotiana benthamiana* and *Medicago truncatula*. As *M. truncatula* is a leguminous host of FBNYV, future studies on transgenic *M. truncatula* plants will provide evidence whether, by analogy to geminiviruses, the dominant-negative Rep approach is also effective in controlling FBNYV in faba bean. In view of this control strategy the German partners have started studying FBNYV variability by determining the presence and absence of the additional (non-integral) Rep components in geographically diverse FBNYV isolates as well as the extent of variation present in the (indispensable) C2 Rep component of all these isolates. Results of these analyses are considered essential for assessing the durability of the resistance conferred by the mutated Rep2 protein in transgenic faba bean.

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## PARTNERS

### **BIOLOGISCHE BUNDESANSTALT FÜR LAND- UND FORSTWIRTSCHAFT**

Institut für Biochemie und Pflanzenvirologie  
Messeweg 11-12  
38104 Braunschweig  
Germany

Heinrich Josef Vetten  
Tel.: +49-531-299 37 20  
Fax: +49-531-299 30 06  
E-mail: [hj.vetten@bba.de](mailto:hj.vetten@bba.de)

### **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE**

Institut des Sciences Végétales  
Avenue de la Terrasse  
91198 Gif-Sur-Yvette Cedex  
France

Bruno Gronenborn  
Tel.: +33-1-69 82 38 33  
Fax: +33-1-69 82 36 95  
E-mail: [bruno.gronenborn@isv.cnrs-gif.fr](mailto:bruno.gronenborn@isv.cnrs-gif.fr)

### **AGRICULTURAL GENETIC ENGINEERING RESEARCH INSTITUTE**

Molecular Plant Pathology  
Gamaa Street 9  
12619 Giza  
Egypt

Magdy Madkour  
Tel.: +20-2-572 78 31  
Fax: +20-2-568 95 19  
E-mail: [madkour@ageri.sci.eg](mailto:madkour@ageri.sci.eg)

### **INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREA**

Germplasm Program  
Virology Laboratory  
P.O. Box 5466  
Aleppo  
Syrian Arab Republic

Khaled Makkouk  
Tel.: +963-21-21 34 33  
Fax: +963-21-21 34 90  
E-mail: [makkouk@cgnnet.com](mailto:makkouk@cgnnet.com)

### **UNIVERSITY OF GEZIRA**

Department of Plant Pathology  
P.O. Box 20  
Wad Medani  
Sudan

Gasim Dafalla  
Tel.: +249-051-3001



**INTEGRATED CONTROL OF TICKS AND TICK BORNE DISEASES  
(ICTTD CONCERTED ACTION PROJECT)**

**Co-ordinator:** Utrecht University, Utrecht, The Netherlands (Frans Jongejan)

## OBJECTIVES

- The overall objective of this concerted action is to exploit the results obtained within several research projects on tropical tick-borne diseases of livestock supported by the INCO-DC programme of the EU and to make a positive contribution towards increased livestock productivity and therefore improved living conditions in developing countries.

## ACTIVITIES

### *Vaccine development*

- ◇ Theileria annulata recombinant vaccine laboratory trials were conducted in 1997 by researchers involved in INCO-DC project IC18-CT95-0003 and IC18-CT95-0004, whereby ICTTD provided logistical support through meetings, shipment of material and exchange visits.
- ◇ Cowdria ruminantium inactivated vaccines developed by INCO-DC project IC18-CT95-0008 entered a critical stage, whereby ICTTD assisted in the planning of field trials in selected African livestock production systems.
- ◇ A workshop was held in September 1998 in Athens, Greece, on protective immune mechanisms to ticks and tick-borne diseases.

### *Development of improved ELISA and PCR diagnostics*

- ◇ The project organised a laboratory workshop on PCR-based detection and identification of pathogens in tropical ticks in Utrecht in February 1997. The possibility to store ticks in ethanol before PCR is a great advantage and makes transport of samples cheap and easy. Detailed recommendations concerning DNA extraction, specificity and sensitivity were given and have been published in ICTTD Newsletter no. 6.
- ◇ Serodiagnosis of cowdriosis is currently based on a specific fragment of the Major Antigenic Protein 1 (MAP1) of Cowdria ruminantium in an indirect ELISA. Validation of the MAP1b ELISA test kit has been carried out on a large scale in Guadeloupe and Zimbabwe and another 7 laboratories in West Africa (Burkina-Faso, Ivory-Cost, Gambia, Ghana, Guinea-Conakry, Niger and Senegal) and 8 labs in Eastern and Southern Africa (Botswana, Kenya, Madagascar, Mozambique, South Africa, Sudan, Swaziland and Tanzania) have also received the ELISA test kit through the ICTTD project and are generating valuable epidemiological data on cowdriosis which will be discussed at one of the forthcoming workshops of the project.

## EXPECTED OUTCOME

- ▷ The ICTTD project is creating a large international Network of researchers on Tropical Ticks and Tick-borne Diseases. It is expected that such a powerful global Network will contribute to solving some of the main problems associated with these diseases.

## RESULTS SO FAR

- ⇒ The ICTTD project has started with 24 laboratories, located in 8 European and 8 African and Caribbean countries and 42 participating scientists. Participation of associate members has been encouraged which has resulted in an overwhelming response and so far more than 100 new members have been registered.
- ⇒ The project has so far been divided into 8 different actions with action leaders. These actions deal with vaccine development for cowdriosis and theileriosis, serodiagnosis and molecular characterisation of tick-borne pathogens, a ruminant anaplasmosis and babesiosis working group

and an action on dermatophilosis. Two further actions on integrated tick control and on integrated tick-borne disease control will start at a later date.

- ⇒ Several short term exchange visits and small workshops and meetings have been sponsored by the project.
- ⇒ The project has published two issues of a new Newsletter on Integrated Control of Ticks and Tick-Borne Diseases. The first, announcing issue, has appeared in March 1996 and the second in October 1996. The Newsletter contains abstracts of recently published papers, contributions from individuals and co-editors on EC-DC collaborative projects and contains various announcements related to tropical ticks and tick-borne diseases. The ICTTD Newsletter is distributed free of charge (750 copies).
- ⇒ Finally, a project homepage has been created on the Internet, which also contains an electronic version of the ICTTD Newsletter: <http://www.ruu.nl/tropical.ticks>.

#### **FOLLOW-UP**

- ▶ Four additional issues of the ICTTD Newsletter will be published in 1998 and 1999 and distributed free of charge to approximately 750 subscribers, who are mainly from developing countries.
- ▶ Additional workshops will be held in 1999 on integrated molecular and serological diagnosis of tick-borne pathogens.
- ▶ Sustain the large international Network on ticks and tick-borne diseases that has been created.
- ▶ Electronic version of the ICTTD Newsletter available at <http://www.ruu.nl/tropical.ticks>

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## **PARTNERS**

### **UTRECHT UNIVERSITY**

Faculty of Veterinary Medicine  
Department of Parasitology & Tropical Veterinary Medicine  
Yalelaan 1  
P.O. Box 80165  
3508 TD Utrecht  
**The Netherlands**

Frans Jongejan  
Tel.: +31-30-253 25 67  
Fax: +31-30-254 07 84

### **UNIVERSITY OF GLASGOW**

Department of Veterinary Parasitology  
Bearsden Road  
Glasgow G61 1QH  
**United Kingdom**

Andrew Tait  
Tel.: +44-141-339 88 55  
Fax: +44-141-330 56 03  
E-mail: gvne01@udcf.gla.ac.uk

### **UNIVERSITY OF EDINBURGH**

Centre for Tropical Veterinary Medicine  
Easter Bush  
Roslin Midlothian EH25 9SG  
**United Kingdom**

Keith Sumption  
Tel.: +44-131-650 99 14  
Fax: +44-131-445 50 99  
E-mail: keiths@lab0.vet.ed.ac.uk

### **UNIVERSITY OF EDINBURGH**

Institute of Cell, Animal & Population  
Biology - Division of Biological Sciences  
King's Building  
West Mains Road  
Edinburgh EH9 3JT  
**United Kingdom**

Patricia Preston  
Tel.: +44-131-650 54 77  
Fax: +44-131-667 32 10  
E-mail: pat.preston@ed.ac.uk

### **UNIVERSITY OF YORK**

Department of Biology  
Heslington  
York YO1 5DD  
**United Kingdom**

Francis R. Hall  
Tel.: +44-1904-43 28 64  
Fax: +44-1904-43 28 60  
E-mail: frhl@york.ac.uk

### **ROSLIN INSTITUTE**

Roslin - Midlothian EH25 9PS  
**United Kingdom**

Elizabeth Glass  
Tel.: +44-131-440 27 26  
Fax: +44-131-440 04 34  
E-mail: liz.glass@bbsrc.ac.uk

### **ROYAL VETERINARY COLLEGE**

Dermatology Unit  
Hawkshead Lane  
North Mymms  
Hertfordshire AL9 7TA  
**United Kingdom**

David H. Lloyd  
Tel.: +44-1707-66 62 84  
Fax: +44-1707-65 20 90  
E-mail: david.lloyd@ps.com

### **CIRAD**

Campus de Montferrier  
Baillarguet  
B.P. 5035  
34032 Montpellier Cedex 1  
**France**

Albert Bensaid  
Tel.: +33-4-67 59 37 01  
Fax: +33-4-67 59 37 97  
E-mail: bensaid@cirad.fr

### **FORSCHUNGSZENTRUM BORSTEL**

Institut für Experimentelle Biologie und Medizin  
Parkallee 1 - 40  
23845 Borstel  
**Germany**

Jabbar S. Ahmed  
Tel.: +49-4537-18 84 28  
Fax: +49-4537-18 84 04

### **UNIVERSIDAD DE EXTREMADURA**

Facultad de Veterinaria  
Laboratory of Parasitology & Parasitological Diseases  
Avenida de la Universidad  
10071 Caceres  
**Spain**

Miguel Angel Habela  
Tel.: +34-27-25 71 00  
Fax: +34-27-25 71 10  
E-mail: mahabela@ba.unex.es

### **UNIVERSIDADE NOVA DE LISBOA**

Centro de Malaria e Outras Doencas Tropicais  
Rua da Junqueira 96

Virgilio Do Rosario  
Tel.: +351-1-362 24 58  
Fax: +351-1-362 24 58

1300 Lisbon  
**Portugal**

E-mail: cmdt1@feunl.fe.unl.pt

**INSTITUT PASTEUR DE TUNIS**  
Place Pasteur 13  
P.O. Box 74  
1002 Tunis-Belvédère  
**Tunisia**

Ali Bouattour  
Tel.: +216-1-78 30 22  
Fax: +216-1-79 18 33

**ECOLE NATIONALE DE MEDECINE  
VETERINAIRE**  
Department of Clinical Sciences  
Laboratory of Parasitology  
Sidi Thabet  
2020 Ariana  
**Tunisia**

Mohammed Aziz Darghouth  
Tel.: +216-1-55 22 00  
Fax: +216-1-55 24 41

**INSTITUT AGRONOMIQUE ET VETERINAIRE  
HASSAN II**  
Département de Parasitologie  
Avenue Allal El Fassi  
P.O. Box 6202  
10101 Rabat  
**Morocco**

Malika Kachani  
Tel/Fax: +212-7-77 34 64

**INSTITUT SENEGALAIS DE RECHERCHES  
AGRICOLES**  
Laboratoire National de l'Elevage et de Recherches  
Vétérinaires  
P.O. Box 2057  
Dakar-Hann  
**Senegal**

Arona Gueye  
Tel.: +221-8-32 51 46  
Fax: +221-8-32 41 46

**CENTRE INTERNATIONAL DE RECHERCHE &  
DEVELOPPEMENT**  
P.O. Box 454  
Bobo-Dioulasso  
**Burkina Faso**

Frederic Stachurski  
Tel.: +226-97 22 87  
Fax: +226-97 23 20  
E-mail: toure@ouaga.orstom.bf

**UNIVERSITY OF KHARTOUM**  
Faculty of Veterinary Science  
Department of Parasitology  
P.O. Box 32  
Shambat  
Khartoum North  
**Republic of Sudan**

Abdulla A. Latif  
Tel.: +249-11-61 10 70  
Fax: +249-11-77 49 27

**UNIVERSITY OF ZIMBABWE**  
Faculty of Veterinary Science  
Department of Paraclinical Veterinary Studies  
Mount Pleasant Drive  
P.O. Box MP 167  
Harare  
**Zimbabwe**

Nancy Kock  
Tel.: +263-4-30 32 11  
Fax: +263-4-33 34 07  
E-mail: dow@esonet.zw

**ONDERSTEEPOORT VETERINARY INSTITUTE**  
Protozoology Division  
Onderstepoort  
Private Bag X05  
Pretoria  
**South Africa**

Theo De Waal  
Tel.: +27-12-529 92 12  
Fax: +27-12-529 94 34  
E-mail: theo@moon.oivi.ac.za

**APPLICATION OF RECOMBINANT DNA TECHNOLOGY TO VACCINATION  
DIAGNOSIS AND EPIDEMIOLOGY OF TROPICAL THEILERIOSIS**

**Co-ordinator:** University of Glasgow, Glasgow, United Kingdom (Andrew Tait)

**OBJECTIVES**

- To develop ELISA based diagnostic assays using recombinant antigens which are specific to the sporozoite, macroschizont and merozoite / piroplasm stages of the parasite;
- To undertake vaccine trials using available candidate recombinant antigens with the aim of developing a sub-unit vaccine. These studies include the objective of determining the optimum delivery system;
- To investigate the ability of recombinant candidate vaccine antigens to potentiate the protection provided by attenuated cell line vaccines;
- To fully analyse the diversity of the candidate antigens within an endemic region (Tunisia);
- To extend and develop the study of the population genetic structure of *Theileria annulata* in Tunisia and Morocco;
- To investigate the potential of attenuated vaccines to revert to virulence after tick transmission;
- To assess the effect of attenuated cell line vaccination on transmission rates of the parasite by the tick vector and evaluate the effect on genotypic variation.

**ACTIVITIES**

- ◇ The expression of recombinant sporozoite and merozoite antigens in *E. coli* and their evaluation in microtitre plate based ELISAs against a panel of defined sera to determine their species specificity and sensitivity. The cloning, sequencing and recombinant expression of an immunodominant macroschizont specific antigen gene and evaluation of its potential as an ELISA based diagnostic;
- ◇ The expression of the candidate vaccine antigens in different viral and bacterial expression systems to produce sufficient material for small scale vaccination trials in cattle. The immune response (humoral and cellular) will be evaluated as well as a range of clinical parameters that will be measured after challenge. Different delivery and adjuvant systems will be tested and their role in eliciting an immune response / protection evaluated;
- ◇ Inclusion of recombinant antigens (SPAG and Tams-1) with an attenuated cell line vaccine to determine whether these antigens can reduce the initial reaction to vaccination and potentiate protective immunity;
- ◇ In addition, recombinant antigens (SPAG and Tams-1) will be included with an attenuated cell line vaccine to determine whether these antigens can reduce the initial reaction to live cell vaccination and potentiate the activity of the cell line vaccine.
- ◇ The collection of a series of parasite samples from the endemic region in Tunisia and the evaluation of the level of sequence and antigenic diversity in the sporozoite and merozoite antigen genes using a combination of PCR, RFLP, recombinant expression and Western blot techniques;
- ◇ The Tunisian cell line vaccine will be evaluated for its ability to express 'virulence' markers (merozoite production and host metalloproteinase expression) and the clinical signs produced by vaccination. The effect of tick transmission of the vaccine on the virulence of the resulting sporozoites will then be determined by animal infection;
- ◇ The effect of cell line vaccination of cattle in the field on the rate of transmission of the parasites by ticks will be evaluated by the measurement of tick infection rates both pre and post

vaccination. Additionally, the effect of vaccination on the genotypes' diversity of the parasite will be evaluated by PCR based genotyping of the parasite population in ticks.

- ◇ The effect of cell line vaccination of cattle in the field on the rate of transmission of the parasites by ticks will be evaluated by the measurement of tick infection rates both pre- and post-vaccination. Additionally, the effect of vaccination on the genotypic diversity of the parasite will be evaluated by PCR based genotyping of the parasite population in ticks pre- and post-vaccination.

## EXPECTED OUTCOME

- ▷ The work carried out in this project will lead to full evaluation of the potential for developing a sub-unit vaccine based on existing recombinant antigens and determine whether a search for further antigens is required. A second outcome will be the development of sensitive diagnostic ELISA tests that potentially distinguish between vaccinated and challenged animals and can be used for routine diagnosis, epidemiological studies and post vaccination analysis. The ability of current cell lines to revert to virulence will be determined and, if they are shown to retain low virulence, this will provide assurance on the safety of large scale vaccination which, coupled with analysis of post-vaccination tick transmission rates, will allow a better understanding of the long term effects of vaccination on disease incidence. The outcomes obtained should lead to a better control, understanding and diagnosis of the disease that will provide significant benefit to countries where the disease is endemic.

## OUTCOME

### *Recombinant antigen based ELISA's*

- ⇒ A series of ELISA's have been developed using recombinant antigens that, in vivo, are specific to different life cycle stages (sporozoite, macroschizont, merozoite/piroplasms). These tests, for use in diagnosis and epidemiology, are at different stages of development. The sporozoite ELISA has been shown to be *T.annulata* specific and give a sensitivity of 74% when tested with bovine sera from an endemic region. A macroschizont ELISA is in the early stages of development but initial analysis shows it to be species specific. Three merozoite/piroplasm antigens are currently being evaluated; one of these (Tarh-1) is specific to *T.annulata* infections and can discriminate between vaccinated animals and those infected by sporozoites.

### *Immunisation trials using recombinant antigens*

- ⇒ Previous experiments had shown that the merozoite surface antigen, Tams-1, gave protection when delivered in ISCOM's. However, this outcome was not obtained in a repeat experiment. A further immunisation experiment has been undertaken testing both SPAG-1 and Tams-1 separately and combined using either ISCOM's or the adjuvant RWL. The data obtained show that SPAG can elicit a 'protective' response when delivered with the appropriate adjuvant although, on challenge, animals still suffer a clinical response. The responses using ISCOM's as a delivery system are poor and the question of whether Tams-1 is a useful component of a vaccine requires further experiments. Currently further immunisation trials are underway using candidate schizont antigens.

### *Antigenic and sequence diversity of candidate vaccine antigens*

- ⇒ The main focus of research has been on the analysis of Tams-1 and SPAG-1 variation using a combination of sequence, RFLP and immunological analysis of different alleles of these genes from Tunisian (and other) isolates. In addition this analysis has been extended to related species of *Theileria*. Analysis of the sequence variation of Tams-1 has shown that this molecule is highly polymorphic. The variation across the molecule can be divided into blocks of conserved and blocks of highly variable sequence with evidence for selection for variation in the latter regions. Expression of representative alleles followed by Western blot analysis has shown that this variation also occurs at the immunological level.
- ⇒ An extensive phylogenetic analysis of the sequences of Tams-1 from a range of *Theileria* species has been completed. The results show that *Babesia equi* is closely related to *Theileria* and that the *Theileria* species can be divided into three groups: 1. *T.mutans* 2. *T.annulata/T.lestouardi/T.parva/T.taurotragi* and 3. *T.buffeli/T.sergenti*. The latter group does

not show any clustering of isolates from the two species suggesting that they are a single species with high levels of divergence between isolates. Analysis of a Chinese 'non-annulata' isolate by Tams-1 and r-DNA sequence analysis places this isolate in the *T. buffeli/sergenti* phylogenetic cluster.

⇒ The remaining activities in the programme are ongoing and the outcome of these will be known by the end of the contract.

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## **PARTNERS**

### **UNIVERSITY OF GLASGOW**

Faculty of Veterinary Medicine  
Department of Veterinary Parasitology  
Bearsden Road  
Glasgow G61 1QH  
**United Kingdom**

Andrew Tait  
Tel: +44-141-339 88 55 (ext. 5750)  
Fax: +44-141-330 56 03

### **UNIVERSITEIT VAN UTRECHT**

Department of Parasitology  
Laboratory of Veterinary Medicine  
Yalelaan 1  
P.O. Box 80.165  
3508 TD Utrecht  
**The Netherlands**

Frans Jongejan  
Tel: +31-30-53 25 68  
Fax: +31-30-54 07 84  
E-mail: f.jongejan@vetmic.dg

### **UNIVERSITY OF YORK**

Department of Biology  
Heslington  
P.O. Box 373  
York YO1 5YW  
**United Kingdom**

Francis Roger Hall  
Tel: +44-1904-43 28 64  
Fax: +44-1904-43 28 60  
E-mail: frhi@unix.york.ac.uk

### **INSTITUT PASTEUR DE TUNIS**

Laboratoire d'Hématologie et d'Immunopathologie  
Place Pasteur 13  
1002 Tunis - Belvédère  
**Tunisie**

Leila Ben Miled  
Tel: +216-1-78 30 22 (ext. 206)  
Fax: +216-1-79 18 33

### **INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE HASSAN II**

Département de Parasitologie  
B.P. 6202  
10101 Rabat  
**Morocco**

Malika Kachani  
Tel: +212-7-77 36 26  
Fax: +212-7-77 34 64

### **UNIVERSITY OF BERN**

Institute of Animal Pathology  
Langgasstraße 122  
3012 Bern  
**Switzerland**

Dirk Dobbelaere  
Tel: +41-31-631 23 94  
Fax: +41-31-631 26 35

### **ÉCOLE NATIONALE DE MÉDECINE VÉTÉRINAIRE**

Department of Clinical Sciences  
Laboratory of Parasitology  
2020 Sidi Thabet  
**Tunisia**

Mohamed Aziz Darghouth  
Tel: +216-1-55 24 60  
Fax: +216-1-55 24 41



## THE AFRICAN HORSE SICKNESS VIRUS EPIDEMIC IN MOROCCO

Co-ordinator: Institute for Animal Health, Woking Surrey, United Kingdom (Philip Mellor)

### OBJECTIVES

This project was designed to gain a clear understanding of the controlling epidemiological factors involved in the persistence of African horse sickness virus (AHSV) in Morocco by:

- Identifying the major insect vector(s) and providing detailed information on its/their distribution, seasonal incidence and population densities;
- Elucidating the role of vaccinated equines and non-horse equines in the maintenance of the virus;
- Assessing the stability of the AHS vaccine virus in use in Morocco, subsequent to its passage through vector insects;
- Investigating the environmental conditions (particularly temperature) necessary for AHSV virogenesis to take place in vector insects;
- Developing appropriate epidemiological models for AHS.

### ACTIVITIES AND RESULTS

- ◇ Vector surveillance and satellite imagery
- ⇒ *Culicoides imicola* is widely distributed throughout Morocco. It is most abundant in the Northwest and at Marrakech; there is an intermediate abundance at Berkane, Errachidia and Taroudant; and the insect is rare at the remaining sites. At only one site (Settat) was *C. imicola* apparently absent. The species was found at altitudes ranging from 15 - 1275 m and in climatic conditions ranging from subhumid to Saharan. At some sites (Marrakech, Sablons and Sidi Moussa) *C. imicola* is present in adult phase throughout the year. Sparingly, there was a strong relationship between abundance and the proportion of the year in which adult activity was detected. There was a peak of abundance of *C. imicola* at most sites in late summer and early autumn. The only exceptions to this rule were in the extreme Northwest where the peak was less pronounced and in June/July at Arbaoua and in March at Tangier.
- ⇒ *Culicoides imicola* activity levels were low at mean air temperatures of less than 15°C or above 30°C; and at minimum relative humidities above 40% (probably days of rain). There was also a significant negative correlation between maximum wind speed and the number of *C. imicola* captured. Sparingly, analyses of activity data suggest that *C. imicola* is less active on days when it is dryer and more windy than usual but are more active on days when it is moister and calmer than usual. Activity seems to be independent of whether the day is much cooler or hotter than usual (within the constraints of the < 15°C and > 30°C levels mentioned above).
- ⇒ The potential AHSV vectors, *C. obsoletus* and *C. pulicaris*, are both widely distributed in Morocco but are generally less abundant than *C. imicola*. Greatest densities of *C. obsoletus* were in the North of Morocco, particularly at Tangier, while those of *C. pulicaris* were more north-eastern. Both species occur in adult phase throughout the year. *Culicoides obsoletus* was most abundant from February to June (peak in April); *C. pulicaris* also showed a peak in April.
- ⇒ During the 1989-91 epizootic in Morocco, investigations showed that all cases of AHS were reported between the months of July and December and there was a large peak in the number of reported cases in the month of October. Furthermore, outbreaks were relatively rare in the areas of Morocco where *C. pulicaris* is common. These data suggest that in Morocco neither *C. obsoletus* or *C. pulicaris* contributed significantly to the AHS epizootic.
- ⇒ Analyses of data identified a strong correlation between the mean abundance of *C. imicola* and NDVI<sub>min</sub>. Using NDVI<sub>min</sub> 4 out of 5 of the observed *C. imicola* "abundant" sites and 9 out of 11 of the observed "rare/absent" sites were correctly predicted. Furthermore, using the same technique,

19 out of 26 *C. imicola* collecting sites in Iberia were also correctly predicted. It is therefore clear that the relationship between NDVI<sub>min</sub> and *C. imicola* extends beyond the Moroccan context.

- ◇ AHSV in the African donkey and mule, and the European donkey
- ⇒ ELISA and virus neutralisation results show that detectable AHSV antibodies develop in vaccinated mules and donkeys later than in horses. Furthermore, virus isolation procedures failed to detect the presence of a vaccine virus viraemia in any of 8 vaccinated mules and donkeys. However, it was shown that some AHS vaccinated and protected horses do develop a viraemia on reinfection, with virulent virus even when this is of the same serotype as the vaccine and even in the face of circulating antibody.
- ⇒ When non-vaccinated mules and donkeys were infected with virulent AHSV4, viraemia detected by virus isolation or by PCR, and extending for as long as 16 dpi in donkeys (measured by virus isolation) and up to 51dpi in mules (measured by PCR) was recorded. During this work, no signs of clinical disease were observed in any of the 16 African donkeys and mules, and 4 European donkeys.
- ◇ AHS vaccine virus in vector *Culicoides*
- ⇒ AHS vaccine virus as used in Morocco was passaged through vector *Culicoides* via oral and intrathoracic routes. Progeny virus from the first and second insect passages was then inoculated separately into a total of 8 AHS naive horses. None of the experimental horses developed signs of clinical disease. Neither did either of 2 vaccine control horses that had received standard doses of the vaccine. However 1 of the 2 control horses did develop a viraemia due to the vaccine virus that extended from 8 to 17 days post vaccination.
- ◇ Effect of temperature on AHSV virogenesis in vector *Culicoides* and *Culicoides* survival
- ⇒ *Culicoides* survival rates at 10, 15 and 20°C were very similar and 80-90% of midges remained alive after 14 days. At 25°C after the same time period, survival was reduced to 40%. The rate of AHS virogenesis in vector *Culicoides* and the infection rate were also related to temperature. At 25°C a maximum mean virus titre of 10<sup>4.3</sup> TCID<sub>50</sub> per midge was reached by 9dpi and the infection rate was among 60-80%. At 20°C virogenesis was slower and the maximum titre was reached only after 23 days. The infection rate was also reduced to 50-70%. At 15°C there was an overall decline in virus titre with time although in a few individuals virogenesis up to 10<sup>4.0</sup> TCID<sub>50</sub> per midge did still occur. However, the infection rate at this temperature decreased dramatically to 0-15% by 9dpi. At 10°C there was no detectable virogenesis and all midges tested negative by 13dpi. However, when surviving midges that had been maintained at 10°C for 35 days were returned to 25°C for 3 days, the infection rate increased from an apparent zero to 15.5%.
- ◇ Epidemiological modelling
- ⇒ A simulation model was developed to investigate what factors affect the likelihood of an epizootic after the introduction of AHSV into an area. Epizootics were most likely to occur in late summer or autumn and were usually rapid with the time from first to last case being < 4 weeks. If no control measures were taken, most of the horses in a herd were infected by the virus following introduction. The model also predicted a very low prevalence of infected midges even at the peak of an epizootic.
- ⇒ With respect to vaccination strategies, protecting donkeys as well as horses increased the effectiveness of vaccination. Prevention of 50% of epizootics required > 75% coverage of horses and donkeys, or > 90% of horses alone. Protection after the introduction of the virus was rarely successful in preventing outbreaks though it reduced the number of animals affected during an outbreak. If horses alone were protected, the number of donkeys was the most significant factor determining the level of protection needed to prevent an epizootic. If both hosts were protected, the abundance of other hosts as a source for vector bloodmeals was the most significant factor.
- ◇ Training in *Culicoides* biology and identification
- ⇒ Moroccan participants and interested parties were provided with a comprehensive training course comprising both theoretical and practical components and involving both laboratory and field based studies.

## **PARTNERS**

### **INSTITUTE FOR ANIMAL HEALTH**

Pirbright Laboratory  
Ash Road - Pirbright  
Woking Surrey GU24 0NF  
**United Kingdom**

Philip Mellor  
Tel.: +44-1483-23 24 41  
Fax: + 44-1483-23 24 48  
E-mail: mellor@bbstc.ac.uk

### **MINISTERE DE L'AGRICULTURE ET DE LA MISE EN VALEUR AGRICOLE**

Direction de l'Élevage  
Division de la Santé Animale  
Quartier Administratif  
Rabat  
**Morocco**

A. Tber - A. Fikri  
Tel/Fax: +212-7-76 44 04

### **UNIVERSIDADE DA MADEIRA**

Colegio dos Jesuitas  
Largo do Colegio  
9000 Funchal  
**Portugal**

Ruben Capela  
Tel.: +351-91-22 24 17  
Fax: +351-91-23 02 43

### **UNIVERSIDADE DE LISBOA**

Instituto de Ciencias  
Departamento de Zoologia  
Rua Ernesto de Vasconcelos  
1700 Lisboa  
**Portugal**

Tel.: +351-1-757 31 41

**MECHANISMS OF PATHOGENESES AND IMMUNITY IN TROPICAL  
THEILERIOSIS: THEIR RELEVANCE TO VACCINE DEVELOPMENT AND  
DISEASE CONTROL**

**Co-ordinator:** University of Edinburgh, Edinburgh, United Kingdom (Roger Spooner)

**OBJECTIVES**

- Study the epidemiology of bovine Tropical Theileriosis in the Mediterranean Basin;
- Expand the cell line vaccination programme for Morocco and study the effects of vaccination on epidemiology;
- Study the pathogenesis of disease to answer several fundamental problems that have been identified in our attempts to produce immunity with dead parasite material;
- To expand the collaboration with Morocco and Turkey built up under STD1 and 2 and extend this to Germany;
- Organise a co-ordination meeting of workers from Theileriosis endemic areas and Europe in Turkey in 1994 and a tick and tick borne diseases meeting in China in 1996.

**ACTIVITIES**

- ◇ Tropical Theileriosis is arguably the major constraint to cattle production from Morocco to China. TS3-CT92-0143 addressed important aspects of epidemiology, pathogenesis, immunity and control;
- ◇ Built on the work already started in Morocco under STD2 on the epidemiology and economic importance of the disease and extended this to Turkey. It also involved collaboration with TS3-CT91-0019 which studied the epidemiology of Theileriosis in Tunisia;
- ◇ Continued the development and commercialisation of a novel formulation of a cell line vaccine;
- ◇ Collaboration between the Roslin Institute, Centre for Tropical Veterinary Medicine (C.T.V.M) and Institute of Cell Animal and Population Biology, Edinburgh, together with the Institute of Experimental Biology and Medicine, Borstel, Germany concentrating on the pathogenesis of the disease.  
*This had three main components:*
  - A study of the immune response to *Theileria annulata* in vivo using particularly lymphatic cannulation;
  - An investigation into the pathology of the disease using histopathology and immunohistochemistry and the role of cytokines in the pathogenesis of disease;
  - Study of the response of pure-bred *Bos indicus* to infection.
- ◇ Support to the Centre for Tropical Veterinary Medicine to provide parasite and animal facilities for this and other EU theileriosis projects;
- ◇ A co-ordination meeting of all workers in the field of theileriosis was organised in Turkey in 1994;
- ◇ An International Symposium on Ticks and Tick borne diseases was held in China in 1996.

## RESULTS

### *Epidemiology*

- ⇒ In Morocco a detailed epidemiological survey over 4 years was carried out in one of the major dairying regions before and after vaccination. This was supplemented by a national survey in all the main dairy production areas in 1995. In the Sidi Bennour region Theileriosis was and is the major cause of loss in imported black and white cattle. Disease is prevented by vaccination and the tick infection rates are not significantly affected by vaccination. The disease is present at a significant level (over 50%) in all of the main dairy areas. It is the main cause of death in dairy cattle. Economic importance is estimated at approximately \$15 million per annum. The only vector is *Hyalomma detritum*.
- ⇒ An ELISA test was developed in Morocco for the diagnosis of Tropical Theileriosis and two new enzymes based tests for anti theilerial antibodies were developed in Roslin and Borstel Germany. The Roslin test, using peroxidase, has been used for the nation-wide epidemiological studies in Morocco. It has significant advantages over the indirect fluorescent antibody test, particularly because of the permanence of the preparations, ease of reading and the fact that a fluorescent microscope is not required. It has now replaced IFAT in Morocco.
- ⇒ In Turkey the epidemiology of Theileriosis was studied in three areas, Eastern Anatolia, the Marmara region and the Mediterranean Coast. The ticks transmitting the disease were found to be *Hyalomma anatolicum anatolicum* and *Hyalomma detritum* in most regions although in the Mediterranean region *H. anatolicum excavatum* transmitted the disease. The seasonal activity of larval, nymph and adult stages was identified. The incidence of the disease was 67% in Eastern Anatolia, 22% in the Mediterranean coast and 2% in the Marmara region.

### *Vaccination*

- ⇒ In Morocco a low dose fresh cell line vaccine has been successfully tested in extensive field trials. It was shown that it was equally effective one week after preparation when stored at ambient temperature thus showing that producing frozen vaccine is unnecessary. It used 104 infected cells compared with the existing concentrations of 106 or more used in other countries. It thus significantly reduced the cost of vaccine production. Vaccination reduced the incidence of the disease by 98% in endemic areas. It did not change tick infection rates significantly on the farms where it was used. It is currently undergoing commercialisation.
- ⇒ Some studies on the high cell dose frozen vaccine in Turkey were carried out. It also produced high levels of sero conversion and was effective in the areas where it was used.

### *Pathogenesis and immunity*

- ⇒ A very collaborative research programme involving the Roslin Institute, Centre for Tropical Veterinary Medicine, and Institute of Cell, Animal and Population Biology in Edinburgh and the Forschungszentrum in Borstel Germany we have:
  - shown that parasite evades the immune response in lethal infections through the stimulation of high levels of IFN-gamma and disabling of cytotoxic T cells which lose CD2;
  - provided evidence for a major role for macrophages in the pathology of the disease, as hosts for schizonts and in granulomatous reactions, and in protective immune mechanisms, via nitric oxide synthesis;
  - shown that following lethal sporozoite infection, germinal centres in lymph nodes were totally destroyed whereas following cell line immunisation there was a normal immune response with germinal centres;
  - identified peptides on the surface of macroschizont infected cells that are recognised by cytotoxic T cells from immune animals. These antigens are potential candidates for a molecular vaccine;
  - shown using lymphatic cannulation that parasite transfer was essential for immunity. We also showed that reimmunisation with the same cell line did not boost immunity because the vaccine was rejected like a graft;

- identified novel antigens and cytokine profiles that may correlate with the ability of cell lines to produce disease. This may enable us to attenuate cell lines without prolonged culture;
- shown that Sahiwal (*Bos indicus*) was more resistant to *T. annulata* infection than calves of West European breeds (*Bos taurus*).

#### **FOLLOW UP**

- ▶ In IC18-CT95-0004 the basic immunology of Theileriosis and generation of immunity by vaccination is being followed up. Also epidemiology post vaccination and the development of better diagnostic tests are continuing and they are being used for epidemiological studies in Turkey and Tunisia;
- ▶ It is hoped that the meeting in Xi'an will lead to much expanded collaboration with China.

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## PARTNERS

### AGRICULTURAL & FOOD RESEARCH COUNCIL

Roslin Institute  
Midlothian EH25 9PS  
Scotland  
United Kingdom

Roger Spooner  
Tel.: +44-131-440 27 26  
Fax: +44-131-440 04 34

### INSTITUT AGRONOMIQUE ET VÉTÉRINAIRE

HASSAN II  
Rabat Instituts  
Département de Parasitologie  
B.P. 6202  
Rabat  
Morocco

Ouhelli Hammou  
Tel.: +212-7-73 626  
Fax: +212-7-75 838

### UNIVERSITY OF EDINBURGH

Institute of Cell, Animal & Population Biology Division of  
Biological Sciences  
Kings Buildings  
West Mains Road  
Edinburgh EH9 3JT  
Scotland  
United Kingdom

Patricia Preston  
Tel.: +44-131-650 54 77  
Fax: +44-131-667 32 10

### UNIVERSITY OF EDINBURGH

Centre of Tropical Veterinary Medicine  
Easter Bush  
Roslin  
Edinburgh EH25 9RG  
Scotland  
United Kingdom

C.G. Duncan Brown  
Tel.: +44-131-445 20 01  
Fax: +44-131-445 50 99

### UNIVERSITY OF ANKARA

Faculty of Veterinary Medicine  
Department of Protozoology & Entomology  
Irfan Bastug Cad Diskapi 06220  
Ankara  
Turkey

Fahri Sayin  
Tel.: +90-312-43 17 03 15  
Fax: +90-312-43 16 44 72

**INCREASE IN GOAT PRODUCTION: REPRODUCTION AND NUTRITION**

**Co-ordinator:** Istituto Sperimentale Italiano "L. Spallanzani", Milano, Italy (Giuseppe Enne)

**OBJECTIVES**

The project is composed of two integrated and parallel phases of technical scientific and socio-economic research. Both should lead, either to the potentiation of animal reproduction capability and a better understanding of the nutritional aspects or to improvements in the breeding system yield.

These objectives are developed, according to the proposal, through the following tasks:

→ **Socio-economic research**

While scientifically evaluating the progressive application of new techniques it is important to gain some overview of the situation as a whole, especially from the socio-economic point of view and to analyse the behaviour and problems faced by breeders in areas of Northern Africa.

The main problem is to understand the socio-economic and structural impacts of the transition from a system of extensive production to a semi-intensive one. These impacts concern both breeding methods and ways of life. The problem may be addressed through a geographic analysis of breeding, a demographic and socio-economic analysis of breeder's families and an analysis of correlations between family undertakings and external environment factors.

→ **Registration of performance**

The goals of this task are: the evaluation of reproduction and production parameters through the measurement and the collection of the phenotypic characteristics of the goat population in Morocco, Tunisia and Italy. The monitoring of productive and managerial conditions will use a common scheme in order to allow a comparison among hundreds of animals bred in different ecological conditions, the scheme will include; the study of possible correlations among body condition score, metabolic profiles and milk qualitative characteristics; the characterisation of forage essences from different areas of North-African regions and the phenotypic characterisation of different milks collected from several autochthonous breeds.

→ **Nutrition and milk quality**

The objectives are: to evaluate the nutritional value of the forages used; to detect the relationship between nourishment and milk quality through blood and milk samples and to establish a phenotypic milk characterisation through electrophoretic methodology.

→ **Endocrinological studies**

This will be carried out to try to improve the methodologies. In particular an attempt will be made to establish new immunoenzymatic methods for hormone detection to reduce the problems connected with the use and disposal of radioisotopes. Co-operation with maghrebian scientists will be enhanced to help upgrade local researchers and transfer to them knowledge of the most recent developments in the field.

→ **Andrology research**

The aim is to accurately determine the number and the characteristics of spermatozoa in different autochthonous breeds of North Africa and Europe. In the past, and especially in developing countries, techniques of buck semen collection, macroscopic and microscopic evaluation as well as motility assessment were often not standardised.

→ **Reproduction technique**

This will focus on Artificial Insemination (A.I.) and will be strongly supported by the Italian scientists. The aim of this task is to improve the knowledge of reproduction techniques with special reference to frozen semen. Frozen semen techniques for goat semen are not well developed.



## ACTIVITIES

- ◇ As part of the socio-economic research a geographic analysis of breeding distribution is being carried out together with a demographic and socio-economic evaluation of breeder's families and any correlations between family undertakings and the external environmental factors;
- ◇ Concerning the registration of performance reproductive and productive data, blood and milk collection and evaluation of body score have been carried out. The genetic standardisation of different milks collected from the various autochthonous breeds has been performed;
- ◇ The effective degradability of forages has been evaluated and in situ rumen ADF and NDF digestion kinetics of common forages have been determined;
- ◇ Haematological and plasma metabolites have been used to evaluate the physiological status, fulfilment of alimentary plans and to confirm health status;
- ◇ The Moroccan and the Italian scientists, have supported Tunisian researchers in detecting steroid hormones by RIA. Different plasma pools with high and low progesterone levels have been established to facilitate inter-assay and intra-assay evaluation among the Moroccan, Tunisian and Italian laboratories;
- ◇ The development and the application of an enzyme immunoassay for progesterone has been completed;
- ◇ The biochemical evaluation and the physical examination of semen in different autochthonous breeds has been performed;
- ◇ The Italian scientists have supported the other researchers in the artificial insemination techniques using frozen semen.

## RESULTS

- ⇒ Appropriate innovations in goat production systems can undoubtedly contribute to the rural development and to the well being of the rural population but the degree of success is strictly dependent upon the capacity of integration within the socio-economic framework.
- ⇒ Observations on body weight and BCS show correlations with the quality of available food.
- ⇒ Data obtained during the analysis of milk production suggest that an increase in total production is possible, but the limitation appears to be food availability in all seasons. Local breed average milk production is lower (-30%) than Maltese or even more the Saanen and Alpine. The forages characterisation revealed a middle quality with a high level fibre content.
- ⇒ In comparative trials (goats, sheep and cows) assessments of nutritional values of some forages confirm that it is not possible to apply forage degradability results obtained from experiments with sheep and bovine species to goats.
- ⇒ The polymorphism of a S1 caseins locus confirms a higher frequency of dominant alleles in African goats, these alleles are associated with higher protein content and better yield in the cheese transformation process.
- ⇒ The results obtained using the ELISA methodology confirmed the main objective of defining several physiological conditions (oestrus, ovulation, dioestrus and pregnancy) but, comparative trials with other show that this method is not quantitative. This is certainly due to the matrix effect of different plasmas that interfere with the assay and this interference may only be overcome by solvent extraction of hormones.
- ⇒ Andrological studies permitted the characterisation of the seminal material of local breeds. Much effort was devoted to improving seminal washing methods to permit the successful freezing of semen. The availability of frozen semen opens many possibilities for the genetic progress of local breeds.

## **FOLLOW UP**

- ▶ Acquisition of synchronisation and superovulation protocols for local breeds;
- ▶ Acquisition of cryoconservation methods for semen;
- ▶ Application of large scale AI with frozen semen and evaluation of transcervical methods for sheep;
- ▶ Database on the Genotypic characteristics of indigenous breeds, for use by researchers and technicians;
- ▶ Design of selected programs on the basis of genetic quality and quantity production characteristics;
- ▶ Evaluation of a natural progestinic product;
- ▶ Elaboration of indigenous feeding strategies based on the identification of gap period nutrition;
- ▶ Knowledge of structure, organisation and management of livestock under smallholder farming conditions;
- ▶ Verification of the mechanisms of marketing and selling of products by the farmers;
- ▶ Simulated definition of possible socio-economic scenarios after productivity improvement.

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## PARTNERS

### ISTITUTO SPERIMENTALE ITALIANO

“I. spallanzani”  
Via Capolago 16  
20133 Milano  
Italy

Giuseppe Enne  
Tel.: +39-2-76 11 11 01 / 76 11 09 35  
Fax: +39-2-76 11 11 08  
E-mail: valentin@icil64.cilea.it

### INSTITUT AGRONIQUE ET VETERINAIRE

HASSAN II  
Rabat Instituts  
Département de Reproduction Animale et D'I.A.  
B.P. 6202  
Rabat  
Morocco

Lahsen Derqaoui  
Tel.: +212-7-77 86 61  
Fax: +212-7-77 81 10

### ECOLE SUPERIEURE D'AGRICULTURE

7030 Mateur  
Tunisia

Rouissi Hamadi  
Tel.: +216-2-65 290  
Fax: +216-2-65 088

### RESEARCH AND DEVELOPMENT

Department of AN.KA  
Rue Artesianou E5  
Kolokotroni B.P. 178  
43100 Karditsa  
Greece

Dimitris Katsaros  
Tel.: +30-2-372 32 00  
Fax: +30-2-372 54 03

### INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE

Laboratoire de Recherches de Technologies Laitières  
Rue de St. Brieuc 65  
35042 Rennes  
France

J.L. Maubois  
Tel.: +33-2-99 28 50 00  
Fax: +33-2-99 28 53 60

### INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE

Laboratoire d'Etude du Métabolisme Azotée  
63122 Theix  
France

Claude Champredon  
Tel.: +33-4-73 62 40 00  
Fax: +33-4-73 62 44 50

### CENTRE INTERNATIONAL DE HAUTES ETUDES AGRONOMIQUES MEDITERRANEENNES

Rue Newton 11  
75116 Paris  
France

Tel.: +33-1-47 20 70 03  
Fax: +33-1-47 20 10 47

**DEFINITION AND CRITERIA FOR THE USE OF IMMUNODIAGNOSIS IN THE FIELD FOR PREVAILING PARASITE DISEASES OF EXTENSIVELY FARMED LIVESTOCK**

**Co-ordinator:** Institut National de la Recherche Agronomique, Nouzilly, France (Chantal Boulard)

**OBJECTIVES**

- To characterise antigens for an early diagnosis of three endoparasites of ruminants : Fasciola hepatica, Hypoderma bovis and Oestrus ovis;
- To produce these antigens as pure as possible to develop kits of immunodiagnostics;
- To set up a field immuno-test;
- To start an immuno-epidemiological survey of each of these parasites.

**ACTIVITIES**

- ◇ Definition of the antigenic pattern of each parasite, using sequential sampling or sera, following experimental monoinfestation, by western blotting. Analysis of antigenic community between these parasites;
- ◇ Characterisation of the kinetics of the antibodies during a natural infestation and effect of a chemotherapy treatment on kinetics will be studied by ELISA;
- ◇ Antigen production will be dependant on the work previously done on each parasite. Liquid chromatography, monoclonal antibodies or molecular biology will be used to produce the protein;
- ◇ Depending on the field technique chosen, the antigen concentration, serum dilution and condition of use of the other reagents will be ascertained;
- ◇ In order to use these fields tests in optimum conditions, monthly blood samplings will be carried out during the first two years of the programme, to determine the ideal period to start an immuno epidemiological survey of each disease;
- ◇ During the last year of the programme, a large epidemiological survey will be carried out with the developed test by each participating group.

**RESULTS**

- ⇒ In this project, five teams from Algeria, France, Mexico and Portugal have collaborated to develop new tools of field diagnosis for three zoonotic parasitic diseases, fasciolosis, hypodermosis and oestrosis, presenting economical incidences for the livestock productions and public health problems.
- ⇒ Antigens for immunological diagnosis have been characterised for their specificity by experimental infestations with these parasites and experimental infestations with parasites found in the same biotope.
- ⇒ Methods of production of specific semi-purified ones and recombinant proteins for F. hepatica and H. lineatum are described. Field immunological diagnosis technics of low cost have been started (ELISA and DIG-ELISA). Methodology of sampling (sera or milk, individual or pooled) at period defined by the biological cycle of the parasite in each country has also been established. These means of investigation for these three diseases have conducted our groups to develop large immuno-epidemiological surveys. Where the studies were undertaken, therapeutic or biological measures of control have been suggested to the farms or to the veterinary services and advertisements to the medical doctors.
- ⇒ Nevertheless, if the members of this group have clearly stated the mode of interpretation of these immunological results, training of public and animal health technician and practitioners need to be developed to interpret the meaning, the advantages and the limits of utilisation of such technics.

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## PARTNERS

**INSTITUT NATIONAL DE LA RECHERCHE  
AGRONOMIQUE**  
Station de Pathologie Aviaire et de Parasitologie  
Centre de Recherche de Tours-Nouzilly  
Domaine de L'Orfraise  
37380 Monnaie (Nouzilly)  
**France**

Chantal Boulard  
Tel.: +33-2-47 42 77 57  
Fax: +33-2-47 42 77 74

**ECOLE NATIONALE VETERINAIRE**  
Service de Parasitologie  
Maladies Parasitaires  
Case Postale 3013  
44087 Nantes  
**France**

Alain Chauvin  
Tel.: +33-2-40 68 77 00  
Fax: +33-2-40 68 77 78

**ECOLE NATIONALE VETERINAIRE**  
Laboratoire de Parasitologie  
Avenue Pasteur  
B.P. 161  
16200 El-Harrach (Alger)  
**Algeria**

Fifi Oussaid  
Tel.: +213-2-76 67 81

**INSTITUTO NACIONAL DE INVESTIGACIONES  
FORESTALES Y AGROLAS PECUARIAS**  
Laboratorio Parasitologia Veterinaria  
Division Trematodiasis Cenicid  
Carretera Federal Cuernavaca km 11.5  
Cuahtla  
62500 Cuernavaca, Estado de Morelos  
**Mexico**

Froylan Ibarra Velarde  
Tel.: +52-73-19 02 02 (ext. 192860)  
Fax: +52-73-20 43 62

**INSTITUTO NACIONAL DE SAÚDE**  
Departamento de Parasitologia  
Avenida Padre Cruz  
4000 Porto  
**Portugal**

Maria Sampaio Silva  
Tel.: +351-2-57 15 09  
Fax: +351-2-200 53 23

**UNIVERSIDAD DE SANTIAGO DE COMPOSTELA**  
Facultad de Veterinaria  
Parasitologia y Enf. Parasitarias  
Avenida de Madrid 81  
27002 Lugo  
**Spain**

Pablo Diez-Baños  
Tel.: +34-82-25 23 61  
Fax: +34-82-25 21 95

**CHARACTERISATION OF PROTECTIVE ANTIGENS AND THEIR GENES FROM  
THEILERIA ANNULATA: APPLICATION TO SUB-UNIT VACCINES,  
EPIDEMIOLOGY AND DIAGNOSIS**

**Co-ordinator:** University of Glasgow, Glasgow, United Kingdom (Andrew Tait)

**OBJECTIVES**

- To isolate and characterise the genes encoding surface antigens and invasion associated organelle proteins from two stages of the parasite (sporozoite and merozoite);
- To define the epitopes on these molecules which elicit an immune response from cattle and to test whether these antigens can elicit a protective immune response;
- To define the level of parasite diversity within an endemic area (Tunisia) and examine its relevance to immunisation with candidate protective antigens.

**ACTIVITIES**

- ◇ The mapping of bovine T & B cell epitopes on the recombinant sporozoite surface antigen SPAG-1 and the isolation and characterisation of further sporozoite surface antigens;
- ◇ The characterisation of the surface and rhoptry polypeptides of the merozoite and the cloning and sequencing of the genes encoding them;
- ◇ The development of an in vitro red blood cell merozoite invasion assay and the assessment of the invasion blocking activity of antibodies raised against recombinant merozoite antigen genes;
- ◇ The immunisation of calves with such recombinant antigens and the testing of their role in protection to parasite challenge;
- ◇ The collection of parasite samples from different regions of Tunisia and the assessment of the level of parasite diversity with particular reference to sporozoite and merozoite surface antigens;
- ◇ The study will focus on the collection of parasites from Tunisia, the isolation and characterisation of the merozoite and sporozoite surface antigens and the assessment of their diversity in the endemic area. The antigen genes will be sequenced and expressed in bacterial systems, sera to the recombinant antigens will be developed and used to test their activity in blocking assays and the recombinant antigens used to immunise calves.

**ACTIVITIES**

- ◇ The mapping of bovine T & B cell epitopes on the recombinant sporozoite surface antigen SPAG-1 and the isolation and characterisation of further sporozoite surface antigens;
- ◇ The characterisation of the surface and rhoptry polypeptides of the merozoite and the cloning and sequencing of the genes encoding them;
- ◇ The analysis of PCR based methods for the detection of Theileria annulata in infected animals and ticks;
- ◇ The immunisation of calves with candidate sporozoite and merozoite recombinant antigens and the testing of their role in protection to parasite challenge;
- ◇ The collection of parasite samples from different regions of Tunisia and the assessment of the level of parasite diversity with particular reference to sporozoite and merozoite surface antigens;
- ◇ The study will focus on the collection of parasites from Tunisia, the isolation and characterisation of the merozoite and sporozoite surface antigens and the assessment of their diversity in the endemic area. The antigen genes will be sequenced and expressed in bacterial systems, sera to the recombinant antigens will be developed and used to test their activity in blocking assays and the recombinant antigens used to immunize calves.

## EXPECTED OUTCOME

- ▷ The work undertaken will lead to an evaluation of the use of recombinant surface antigens to generate a protective immune response and as reagents for development of diagnostic reagents. The epidemiological studies will define the levels of parasite diversity within a region and generate data on the level of diversity in the candidate vaccine antigens. These studies will lay the groundwork for the potential development of a sub-unit vaccine and generate the reagents for future epidemiological and immunological studies of the disease.

## OUTCOME

These are summarised under the headings described in the activities (see above).

### ***Bovine T & B cell epitopes of the sporozoite surface antigen (SPAG).***

- ⇒ Using a series of bovine sera (raised by sporozoite infection or immunisation with recombinant SPAG), the regions of the SPAG molecule contain B-cell and invasion neutralising epitopes have been determined. A neutralising B-cell epitope in the C-terminus of SPAG was identified and a series of B-cell epitopes in other regions of the molecule mapped but shown not to produce antibodies capable of inhibiting sporozoite invasion of peripheral blood lymphocytes. A series of T-cell epitopes were defined using sequence algorithms but were not defined functionally. A collaborative analysis (with scientists at ILRI, Kenya) of the homologous candidate vaccine antigen from *Theileria parva* has shown that the two antigens contain cross-reacting epitopes at the C-terminus and that the neutralising epitopes were common to both antigens. These results led to the conclusion that the C-terminus of the sporozoite antigen is the most immunologically relevant region and therefore a suitable region for inclusion in a molecular vaccine. Furthermore the cross reactivity of sera to this region between *T. parva* and *T. annulata* raise the possibility that a single antigen could be used to protect against both parasites.

### **Characterisation of rhoptry and merozoite surface antigens**

- ⇒ A single copy merozoite surface antigen gene (Tams-1) was cloned from a merozoite c-DNA library and from sequence analysis shown to be homologous to a similar molecule isolated from *T. buffeli*. Expression and immunolocalisation studies have shown this protein to be primarily expressed in the merozoite stage, localised on the parasite surface and strongly recognised by bovine immune sera. Using a hyperimmune rabbit anti merozoite serum, a series of c-DNA clones were isolated from an expression library and two of these further characterised by sequence and expression analysis. One of these was shown to encode a 117kd rhoptry protein (Tarh-1) while the second was shown to encode a large (>400kd) merozoite surface antigen (Tams-2). Experiments aimed at determining whether these proteins bound to the surface of red cells were unsuccessful, however, as a result of their strong recognition by bovine immune sera, both are good candidates for use in diagnostic ELISA's.

### **Analysis of PCR based diagnostic methods**

- ⇒ The availability of *Theileria annulata* gene sequences for both the Tams-1 and r-RNA genes allows the design of oligonucleotides for use in PCR amplification of these genes from animal and vector material infected with the parasite. A PCR based approach to diagnosis has a number of advantages over existing methods: it can be totally species specific, it is very sensitive and can be used directly on blood or tissue samples. Based on the available Tams-1 sequences from a number of different *Theileria* species, oligonucleotide primers were designed and shown to allow PCR amplification of a 720bp fragment of the Tams-1 gene. Analysis using DNA from six different *Theileria* species showed that the amplification was *T. annulata* specific and by serial dilution of infected blood of known parasitaemia, the PCR method could detect a parasitaemia of 0.004%. Analysis of cattle blood samples from the field showed that the PCR method detected infections more effectively than Giemsa or immunofluorescence assays. A similar PCR based assay was developed for detecting *T. annulata* in infected ticks based on the ribosomal DNA sequence. While further analysis is required to fully determine species specificity and sensitivity, the results showed that infections can be detected readily from simple extracts of tick material.
- ⇒ Additional research has been undertaken to improve serological diagnosis using recombinant *Theileria* antigens in an ELISA format. Using the Tarh-1 antigen, a research based, species

specific test has been developed which can discriminate between vaccinated animals and those infected by sporozoite challenge. However, once vaccinated animals are challenged antibody is detected by this assay.

#### **Immunisation with recombinant antigens**

- ⇒ Two candidate vaccine antigens have been tested by calf immunisation experiments followed by parasite challenge. The results suggest that both antigens show some level of protective effect but further research is required to reach the goal of full protection. The C-terminal portion of the sporozoite surface antigen, SPAG-1, was engineered into a vector expressing the hepatitis core antigen which, from published data, is able to provide T-cell epitopes when assembled as a polymeric particulate structure. Immunisation with these particles containing the C-terminus of SPAG, elicited a high antibody titre and these antibodies were able to very effectively block sporozoite invasion of lymphocytes in vitro. Challenge of the immunised animals with a sporozoite LD<sub>50</sub> (compared to non-immunised controls) resulted in clinical theileriosis, however by a number of criteria (white blood cell count, PCV, recovery from infection, time of appearance of schizonts) the immunised group were less affected by challenge than the control group. The results support the inclusion of SPAG as a component of a sub-unit vaccine but highlight the need for the inclusion of antigens expressed at other life cycle stages.
- ⇒ On the basis of immunogenicity, abundance and localisation on the surface of the merozoite, Tams-1 was considered as a vaccine candidate for blocking the invasion of red blood cells by the parasite. An immunisation trial was undertaken using Tams-1 delivered in three different forms: in ISCOM's, as DNA and expressed in Salmonella (oral delivery) followed by challenge with a blood stabilate of the parasite. The group of animals immunised with Tams-1/ISCOMS, while becoming infected, did not show clinical theileriosis suggesting that they had been protected. These experiments need to be repeated and treated with some caution given the small numbers of animals used and the nature of the challenge infection.

#### **Parasite diversity in an endemic region (Tunisia)**

- ⇒ Two types of analysis were undertaken, firstly an assessment of the population diversity of *T.annulata* isolates within and between farms and regions using a series of phenotypic and genotypic markers and secondly a preliminary analysis of the levels of diversity in the two candidate vaccine antigens. The results of the first analysis show that *T.annulata* is highly polymorphic and that no (or limited) sub-structuring of the population occurs between different regions within Tunisia. Almost all isolates from infected animals contained mixtures of different parasite genotypes. These results suggest that parasite diversity is an issue in relation to sub-unit vaccine design and that genetic exchange plays a significant role in generating diversity. The preliminary analysis of diversity in Tams, SPAG and Tarh-1 has been limited to using RFLP analysis of a small set of isolates. Four alleles of Tams-1, 3 alleles of SPAG-1 and 2 alleles of Tarh-1 were detected. Further analysis is required to determine whether variation in immunologically relevant portions of these genes occurs.

#### **FOLLOW UP**

The research completed in this project has formed the basis for a further research contract from the EU to follow up the initial findings on new diagnostics and vaccines. Research has continued on the development of a sub-unit vaccine and the evaluation of two candidate antigens is nearly complete. Several further antigens have been identified and are currently being evaluated. A series of stage specific ELISA's are being developed providing effective tools for both epidemiological analysis and diagnosis.



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## PARTNERS

### UNIVERSITY OF GLASGOW

Wellcome Unit Molecular Parasitology  
Department of Veterinary Parasitology  
Bearsden Road  
Glasgow G61 1QH  
United Kingdom

Andrew Tait  
Tel.: +44-141-339 88 55 (ext. 5750)  
Fax: +44-141-330 56 03

### ÉCOLE NATIONALE DE MÉDECINE VÉTÉRINAIRE

PRN de Biologie Médicale  
Laboratoire d'Hématologie et Immunopathologie  
Place Pasteur 13  
B.P. 74  
Tunis  
Tunisia

L. Ben-Miled  
Tel.: +216-1-78 96 08  
Fax: +216-1-79 18 33

### UNIVERSITEIT VAN UTRECHT

Department of Parasitology  
Institute of Infectious Diseases  
Yalelaan 1  
P.O. Box 80165  
3508 TD Utrecht  
The Netherlands

A. Cornelissen  
Tel.: +31-30-53 48 82  
Fax: +31-30-54 07 84

### UNIVERSITY OF EDINBURGH

Centre for Tropical Veterinary Medicine  
Easter Bush - Midlothian  
Edinburgh  
United Kingdom

C. Brown  
Tel.: +44-131-650 34 72  
Fax: +44-131-662 40 61

### UNIVERSITY OF YORK

Department of Biology  
Heslington  
P.O. Box 373  
York YO1 5YW  
United Kingdom

Francis Roger Hall  
Tel.: +44-1904-43 28 64  
Fax: +44-1904-43 28 60  
E-mail: frhi@unix.york.ac.uk



## **2. Agriculture**

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### 2.4 Agriculture related topics

**SAFETY ASSESSMENT OF THE RELEASE OF TRANSGENIC CROPS: SPREAD OF HERBICIDE-RESISTANCE GENES FROM WHEAT AND FOXTAIL MILLET TO WEEDY SPECIES**

**Co-ordinator:** Institut National De La Recherche Agronomique, Dijon, France (Dr Henri Darmency)

**OBJECTIVES**

The main objective is to provide science-based informations on the possibility and probability of transgene escape from biotechnologically derived herbicide-resistant wheat to wild plants, via spontaneous interspecific hybridization. This includes to evaluate the likelihood of introgression in laboratory and fields studies at locations where the wild relatives are indigenous in China, France, Israel and Spain, and to investigate if different wheat transformants, or different modes of inheritance of transgenes, are less prone to transfer the gene.

A further objective is to evaluate the impact of the transfer of herbicide resistance genes into a wild population (i.e. weediness) and to address the biosafety implications of such movements and the extent (if any) of negative impact on farming activity and on the environment.

**ACTIVITIES**

The key activities can be grouped as follow:

- The development of genetically different types of herbicide-resistant wheat from a large number of transformations and their characterization using introgression-substitution lines to identify on which genome is located the resistance gene. Herbicide resistance is both a very desired trait in agronomy and a good marker to detect gene escape and experiment a clear-cut selection pressure.
- The identification of grasses that can cross with wheat in specified ecological conditions and countries. This includes field study of flowering time and weed characteristics of wheat related grass species, the estimate of the possibility of intercrossing of wheat and the wild relatives by hand-crossing and embryo rescue technique, and spontaneous interspecific hybridizations in the greenhouse and the garden. The rate of spontaneous mutations in cell culture of wild plants will be compared to gene escape from transgenic wheat.
- The study of growth, developmental fitness and natural reproduction of the different hybrids compared to those of the parents. This is followed by monitoring the introgression between wheat and wild relatives by means of chromosome identifying tools and DNA markers among hybrid progeny and further generations.
- The collection of data on the spread of resistance genes after a large scale release of a resistant crop and the behaviour of resistance genes when inherited in different ways and under different regimes of selection pressure. This part apply to herbicide resistant millet, a summer cereal crop of similar mating system which rotates with winter wheat in the same field in China. It is used as advanced model because resistant varieties are already available.

**EXPECTED OUTCOME**

This project should provide recommendations to breeders for wheat engineering in order to prevent gene escape by hybridization between wheat and related grass weeds in the field. It should determine the likelihood of the spread of resistance genes into a weed population and propose guidelines about the actual gene flow and its management to make risk/benefit balance more informed and make risks as low as possible.

## **PARTNERS**

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Laboratoire De Malherbologie  
B.P. 1540  
21034 Dijon  
**France**

Dr. Henri Darmency  
Tel.: 33-3-8063.3186  
Fax: 33-3-8063.3262  
E-mail: darmency@epoisses.inra.fr

### **WEIZMANN INSTITUTE OF SCIENCE**

P.O.B. 26  
76100 Renovot  
**Israel**

Pr. Jonathan Gressel  
Tel.: 972-8-934-34-81  
Fax: 972-8-934-41-81  
E-mail: pgress2@Wiccmil.Weizmann.Ac.II

### **INSTITUTO NACIONAL DE INVESTIGACION AGRARIA Y AGROINDUSTRIAL**

Area Proteccion Vegetal Garretera  
Carretera La Coruna Km 7.5  
28040 Madrid  
**Espana**

Dr. Maria-Christina Chueca  
Tel.: 34-1-347-68-48  
Fax: 34-1-357-22-93  
E-mail: chueca@Inia.Es

### **HEBEI ACADEMY OF AGRICULTURAL AND FORESTRY SCIENCES**

Hepingxilu N0 598  
050051 Shijiazhuang  
**China**

Dr. Haibo Wang  
Tel.: 86-311-7042-853  
Fax: 86-311-7042-490

### **CHINESE ACADEMY OF AGRICULTURAL SCIENCES**

Institute Of Crop Breeding/Cultivation  
Baishiqiao Road N0 30 Haidan District  
100081 Beijing  
**China**

Dr. Tianyu Wang  
Tel.: 86-10-6217-6667  
Fax: 86-10-6217-4142

**THE STUDY OF ATRIPLEX HALIMUS AS A GENETIC RESOURCE  
INSILVOPASTORAL USE AND IN THE RESTORATION OF DAMAGED  
RANGELANDS IN ARID AND SEMI-ARID MEDITERRANEAN ZONES**

**Co-ordinator:** Université De Paris-Sud XI, France (P. Dutoit)

### OBJECTIVES

- Save and exploit the genetic resources that *Atriplex halimus* represents for silviculture and land improvement in southern Mediterranean countries.
- Contribute to an improved availability of forage in the steppe regions of arid Mediterranean zones through the use of *Atriplex halimus*.
- Protect these particularly fragile ecosystems from the risk of desertification and to restore damaged zones for the protection of native fauna.

### ACTIVITIES

- ◇ Ecosystem choice and characterisation: the location will be decided in an on going manner for those regions chosen from an edaphoclimatic viewpoint in Algeria, Morocco, and Tunisia.
- ◇ Selection:  
*In the natural environment:* The native populations of *Atriplex halimus* are highly heterogeneous, in particular within North Africa and Spain. From within this biological diversity, individuals responding to certain selection criteria will be chosen and protected.  
*In controlled environments:* Studies at an early stage of plants produced through seeding in artificial conditions will lead to the development of selection criteria.
- ◇ Development of physiological and molecular methods allowing the characterisation and selection of individuals tolerant to drought, high salinity and cold, as well as being capable of soil retention and natural regeneration through regrowth and seed production, and equally as a source of forage and firewood.
- ◇ Studies of stress resistance mechanisms related to abiotic constraints, on young plants as well as on plant and tissue cultures, in controlled environments.
- ◇ Study of the forage value (qualitative and quantitative), through research on individuals presenting a good palatability for the ovine and cameline races.
- ◇ Study of the reproduction system in view of producing hybrid seeds and establishing the most appropriate re-population strategies.
- ◇ Crossbreeding and seed production.
- ◇ Development of in-vitro micro-cutting and somatic embryogenesis techniques for the large-scale production of individuals selected for their respective qualities.
- ◇ Re-population of damaged zones and potential species sites with individual clones originating from either the natural population or selected crossbreeding.
- ◇ Study within the natural environment of the behaviour of in-vitro clones and individuals resulting from selected crossbreeding.

### EXPECTED OUTCOMES

- ▷ Constitution of a plant collection comprising individuals selected from the natural habitat or from in-vitro cultures, and individuals issued from seeds obtained in controlled crossbreeding experiments.
- ▷ Reintroduction of plants resulting from selected clones or produced seeds, into potential *Atriplex halimus* sites of the Maghreb countries.

## **PARTNERS**

### **UNIVERSITÉ DE PARIS.SUD XI**

Laboratoire D'écotechnologie  
Lab. De Botanique  
Tour El -2 Éme Étage  
92296 Chatenay-Malabry Cedex  
**France**

P. Dutuit  
Tel.: 33-1-46 83 54 19  
Fax: 33-1-46 83 54 19  
E-mail: Pierre.Dutuit@Cep.U-Psud.Fr

### **UNIVERSITÉ CATHOLIQUE DE LOUVAIN**

Laboratoire De Cytogénétique  
Place Croix Du Sud 4-5  
1348 Louvain-La-Neuve  
**Belgique**

J.M. Kinet  
Tel.: 32-10.47 20 50  
Fax: 32.10-47 34 35  
E-mail: Kinet@Bota.Ucl.Ac.Be

### **FACULTÉ DES SCIENCES DE TUNIS**

Lab. De Biologie Végétale  
Campus Universitaire  
Tunis  
**Tunisie**

S. Bouzid  
Tel.: 216-1-872 600  
Fax: 216-1-885 480

### **UNIVERSITÉ CADI AYYAD**

Faculté Des Sciences Semlalia  
Laboratoire D'écologie Végétale  
Bp: S15  
40 000 Marrakech  
**Maroc**

A. Benchaabane  
Tél.: 212 4 30 36 77  
Fax: 212 4 30 36 77

### **UNIVERSITÉ DES SCIENCES & TECHNIQUES**

Institut D'agronomie,  
Dépt. De Phytotechnie  
Bp270  
Blida  
**Algerie**

F. Benrebiha  
Tel.: 213-3-41 58 50  
Fax: 213-3-41 78 13

### **CENTRO DE INVESTIGACIÓN Y DESARROLLO AGROALIMENTARIO (CIDA)**

Consejería De Medio Ambiente  
Agricultura Y Agua Estacion Sericola  
La Alberca  
30150 Murcia  
**Espagne.**

E. Correal  
Tel.: 34 68 84 01 50  
Fax: 34 68 84 48 02

**IMPROVING FRENCH BEAN CULTIVATION UNDER SEMI-ARID CONDITIONS  
BY CONSTRUCTING ACID AND SALT TOLERANT RHIZOBIAL N<sub>2</sub>-FIXING  
SYMBIONTS FOR PLANT INOCULATION-PHIMED**

**Co-ordinator:** Rheinisch-Westfaelische Technische Hochschule, Aachen, Germany  
(Ursula B. Priefer)

### OBJECTIVES

Work will be aimed towards improving the opportunities to cultivate one of the most important leguminous crops, French bean (*Phaseolus vulgaris*), in arid and semi-arid lands by developing more stress tolerant rhizobial symbionts.

The main objectives are defined as follows:

- To understand the reasons of French bean nodulation failure in Egyptian soils;
- To gain a detailed knowledge of the physiological and molecular mechanisms of stress tolerance in rhizobia (especially drought, salt and pH stress);
- To transfer the results into the construction of more tolerant strains and to prepare a commercial application by formulating agricultural inocula.

### ACTIVITIES

- ◇ The taxonomic classification of *Phaseolus* symbionts isolated from acidic soils and their physiological and biochemical characterisation;
- ◇ The development of molecular probes to screen Egyptian soils for the presence of *Phaseolus* symbionts and to monitor their persistence under various stress conditions;
- ◇ The isolation and characterisation of rhizobial genes involved in drought, pH and osmotic stress tolerance;
- ◇ The identification of rhizobial promoters and genes induced under specific stress conditions;
- ◇ The application of the gained knowledge in the establishment of an effective, more stress tolerant interaction between *Phaseolus* and its rhizobial symbiont.

### EXPECTED RESULTS

- ⇒ An understanding of nodulation failure in Egyptian soils and recommendations to solve this problem;
- ⇒ Knowledge about the biochemical and genetic background of stress tolerance in *Phaseolus* symbionts;
- ⇒ Stress tolerant *Phaseolus* symbionts potentially applicable as commercial inocula;
- ⇒ Coupled with the use of more tolerant plant cultivars, this approach may ultimately lead to a wider utilisation and an increased production of French bean in marginal lands using less input of synthetic nitrogen fertiliser.



## **PARTNERS**

### **RHEINISCH-WESTFAELISCHE TECHNISCHE HOCHSCHULE AACHEN**

Oekologie des Bodens  
Worringer Weg 1  
52056 Aachen  
**Germany**

Ursula B. Priefer  
Tel.: +49 241 80 6644  
Fax: +49 241 8888 637  
E-mail: [priefer@biol.rwth-aachen.de](mailto:priefer@biol.rwth-aachen.de)

### **GENETIC ENGINEERING AND BIOTECHNOLOGY RESEARCH INSTITUTE**

Mubarak City for Scientific Research and  
Technological Applications  
Manchiat El-Olama  
Dekheela, PO Box 5  
Alexandrai  
**Egypt**

Hassan Moawad  
Tel.: +20 2 360 1036  
Fax: +20 2 360 1036  
E-mail: [ruhmo@rusys.eg.net](mailto:ruhmo@rusys.eg.net)

### **CONSIGLIO NAZIONALE DELLE RICERCHE**

Intern.Inst. of Genetics and Biophysics  
Via Gugleilmo Marconi 12  
80125 Napoli  
**Italy**

Roberto Defez  
Tel.: +39 081 593 2428  
Fax: +39 081 593 6123  
E-mail: [defez@iigbna.iigb.na.cnr.it](mailto:defez@iigbna.iigb.na.cnr.it)

### **UNIVERSITE MOHAMMED V**

Faculté des Sciences  
Lab. de Microbiologie et de Biologie Moléculaire  
Av. Ibn Battouta, B.P. 1014  
Rabat  
**Morocco**

Abdelkarim Filali-Maltouf  
Tel.: +21 2 7 77 5461  
Fax: +21 2 7 77 5461  
E-mail: [filali@fsr.ac.ma](mailto:filali@fsr.ac.ma)

**STABLE YIELDS IN MEDITERRANEAN BARLEY: APPLICATION OF  
MOLECULAR TECHNOLOGIES IN IMPROVING DROUGHT TOLERANCE AND  
MILDEW RESISTANCE**

**Co-ordinator: SCRI, Invergowrie, Dundee, Scotland, UK (Brian P. Forster)**

**OBJECTIVES**

- The general objective is to compare three molecular breeding approaches for their efficiencies in developing better adapted barley for droughted, low input agricultural conditions of Mediterranean rim countries;
- Identification of genetic markers and physiological traits which are retained, and which are lost in the various approaches;
- Verification, if these markers and physiological traits correspond to those inherited from cultivars, landraces or wild germplasm;
- Identification of the similarities/contrasts in lines selected in the three N. African field trials;
- Identification, if genes/physiological traits associated with drought tolerance also associated with yield penalties, if undesirable linkages can be broken, how important are genetic background effects;
- Identification of the potential for widening the gene pool in molecular breeding of barley to include landraces and wild species;
- Identification of correlation between drought stress genes relate to the breakdown of mildew resistance;
- Identification of the genetic control of drought relief induced mildew susceptibility;
- Verification, if more efficient molecular breeding strategies can be developed.

**ACTIVITIES**

Approach 1 (Advanced Backcross Quantitative trait Analysis, ABQA) exploits existing material developed from crosses between wild barley donors and a European recipient cultivar. The material developed will be grown in N. African field trials (Morocco, Tunisia and Egypt) with selections made for performance under drought. The best lines will be backcrossed and doubled haploids (DHs) produced which will also be tested for drought tolerance. Quantitative trait loci (QTL) analysis will be performed to see which molecular markers (RAPDs, RFLPs, AFLPs, and SSRs) are associated with selected traits. The aim is to overcome problems associated with wild gene introgression into crop plants, e.g. linkage drag, background genetic effects, and errors associated with the detection of QTLs.

Approach 2 (Backcross Conversion, BC) has similar aims but uses a different approach. Here wild barley lines are exploited which are known to carry molecular genetic markers for water stress. These are to be crossed onto contemporary N. African cultivars and DHs produced from the hybrids. Selections will be made on the basis of genotype (genetic markers) and field tested in N. African trials.

Approach 1 & 2 can then used to compare the expression of wild barley genes in two contrasting genetic backgrounds, European and N. African. This approach 3 uses landrace barley rather than wild barley as a source of genes for drought tolerance and concentrates on selection for physiological traits associated with drought tolerance.

A fourth approach: Drought Relief Induced Mildew Susceptibility (DRIMS) is included. This aims to locate genes controlling DRIMS and to investigate the expression of this trait in the material developed.

## **EXPECTED RESULTS**

### *Scientific-technical results:*

- ⇒ Identification of genetic markers and physiological traits associated with tolerance to drought;
- ⇒ Identification of QTLs associated with drought relief induced mildew susceptibility;
- ⇒ The development of high-density genetic maps in target genomic regions;
- ⇒ Comparison of gene introgression from wild barley into N. African and European barley cultivars;
- ⇒ Comparison of controlled environment experimental data with field performance;
- ⇒ The effects of QTLs and their manipulation in molecular breeding will be tested;
- ⇒ Development of appropriate molecular breeding strategies.

### *Deliverables*

- ⇒ Establishment of molecular genetic facilities in three N. African laboratories;
- ⇒ Assessment of contrasting methods in exploiting genetic resources for drought and drought relief induced mildew susceptibility;
- ⇒ Production of doubled haploids and the identification of superior lines which can be utilised in breeding programmes;
- ⇒ Publication and availability of marker systems associated with drought and drought relief induced mildew susceptibility.

## **FOLLOW-UP**

The N. African partners already have in place extension programmes which will be used to disseminate the results of the work on a practical basis to local farmers and breeders.

The genetic markers and methods developed will be published and made available to local MED users and international research organisations such as ICARDA.

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## PARTNERS

### CELL AND MOLECULAR GENETICS DEPT.

Scottish Crop Research Institute  
Invergowrie, Dundee, DD2 5DA  
United Kingdom

Brian P. Forster, Roger P. Ellis, Adrian C. Newton  
Tel.: +44 1382 56 27 31  
Fax: +44 1382 56 24 26  
E-mail: bforst@scri.sari.ac.uk

### DIPARTIMENTO DI AGRONOMIA

Universita degli Studi di Bologna  
Via Filippo Re 6  
40126 Bologna  
Italy

Roberto Tuberossa  
Tel.: +39 051 35 15 36  
Fax: +39 051 35 15 11  
E-mail: tuberossa@pop.agrsci.unibo.it

### INRA MONTPELLIER

Genetique et Amelioration des Plantes  
ENSAM Montpellier  
2 place Viala  
34060 Montpellier cedex  
France

Dominique This  
Tel.: +33 4 67 61 22 52  
Fax: +33 4 67 04 54 15  
E-mail: this@msdos.ensam.inra.fr

### BARLEY RESEARCH DEPARTMENT

Field Crops Research Institute  
8 El-Gamaa Street  
Giza, 12619  
Egypt

Ahmad S. El-Gamal  
Tel.: +202 573 65 15  
Fax: +202 572 23 42

### DEPT. D'AGRONOMIE ET D'AMELIORATION DES PLANTES

Ecole Nationale d'Agriculture de Meknes  
PBS40, Meknes  
Morocco

Hakima Bahri  
Tel.: +212 5 30 02 39  
Fax: +212 5 30 02 38  
E-mail: bb.ena@aim.net.ma

### LABO. DE PHYSIOLOGIE VEGETALE

INRAT, 2049  
Rue Hedi Karray  
Ariana, Tunis  
Tunisia

Moncef Ben Salem  
Tel.: +216 1 230 024  
Fax: +216 1 752 897

**PROSPECTION, CHARACTERIZATION AND ASSESSMENT OF APRICOT  
GENETIC RESSOURCES IN THE MEDITERRANEAN REGION FOR THE  
PRODUCTION IN ARID AND SEMI-ARID AREAS**

**Co-ordinator:** Instituto Agronomico Mediterraneo de Zaragoza, Zaragoza, Spain  
(Jean-Marc Audergon)

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## **OBJECTIVES**

The objectives are to characterise, to preserve and to identify the apricot genetic resources in the Mediterranean Region (Turkey, Spain, Italy, France, Morocco, Tunisia).

An integrated and multidisciplinary approach divided in 4 intermediate goals is defined:

- Prospection, health control and preservation of genetic resources (rootstocks or varieties).
- Characterisation of the collected material together with the identification of varieties and a phylogenetic study.
- Assessment of their interest as cultivars or potential parents.
- Valorisation of genetic resources, setting up a database linked to the IBGRI and national collections (under construction in the European Union).

## **ACTIVITIES**

Field studies on existing orchard and/or collection genetic resources in the Mediterranean Region. The study will emphasise identification of present apricot cultivars (seedling or grafted plants). It will focus on an identification of apricot genetic variability, giving due attention to access a representative sample of the apricot tree population in each production area of the countries associated in the project.

Characterisation of the selected apricot populations. This study will be conducted simultaneously at the field level and at laboratory level. It will emphasise the sanitary status and the genetic identification of the related plant. The genetic identification will be conducted by morphological traits, by agronomic performances including floral biology, fruit quality, susceptibility to diseases, and by molecular characterisation.

Constitution of Apricot gene bank collections in each country. It will emphasise analysis of the genetic variability at the different level of approach, giving due attention to summarise in a collection a representative set of genotypes. Representativity will be analysed in term of genetic polymorphism (genetic distance), and in term of agronomic performances (selection of adapted varieties or parents to be included in breeding programmes).

## **EXPECTED OUTCOME**

- ▷ Constitution, maintenance of apricot gene bank collections in the different countries;
- ▷ Analysis of the structure of apricot Mediterranean variability from a phylogenetic point of view;
- ▷ Analysis of the relationship between morphological, agronomic traits and molecular markers;
- ▷ Evaluation of the agronomic performances under study in relation to their potential use in applied apricot breeding programmes;
- ▷ Identification of new varieties adapted to arid and semi-arid Mediterranean conditions.

## **FOLLOW-UP**

At the end of the first year, the first clones issued of the prospections will be identified, grafted and placed under sanitary status evaluation; the characterisation of the plants materials in collections in Europe should be made.

At the end of the second year, a list of the overall plant material identified by the partners should be ready; morphological descriptors should be completed for the European countries.

At the end of the third year, marker characterisation of the European plants should be achieved, and phenotypic characterisation of the plants materials from Mediterranean countries will be nearly achieved.

At the end of the fourth year, the relationship between morphological traits/agronomic traits/markers should be determined for the European plant materials; a phylogenic analysis of the apricot genetic variability will be engaged and collections should be ready and complete in all the countries.

## **PARTNERS**

### **INSTITUTO AGRONOMOICO MEDITERRANEI DE ZARAGOZA**

Int. Centre for Advanced Mediterranean Agronomic Studies  
Apartado 202  
50080 Zaragoza  
**Spain**

Jean-Marc Audergon  
Tel.: +34 976 576 013  
Fax: +34 976 576 377  
E-mail: iamz@ciheam.mizar.csic.es

### **INSTITUTO VALENCIANO DE INVESTIGACIONES AGRARIAS**

Carretera de Moncada a Naquera Km 5  
46113 Moncada  
**Spain**

Maria José Asins  
Tel.: +34 6 139 10 00  
Fax: +34 6 139 02 40  
E-mail: mjasins@ivia.es

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Station de Recherches Fruitières Méditerranéennes  
Domaine de St. Paul  
84914 Avignon Cedex 9  
**France**

Jean-Marc Audergon  
Tel.: +33 4 90 31 60 31  
Fax: +33 4 90 31 62 91  
E-mail: jma@avignon.inra.fr

### **SCUOLA SUPERIORE DI STUDI UNIVERSITARI E DI PERFEZIONAMENTO S. ANNA**

Chaire Arboricole Fruitière  
Via G. Carducci 40  
56100 Pisa  
**Italy**

Susanna Bartolini  
Tel.: +39 50 883 307  
Fax: +39 50 883 215

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Avenue de la Victoire  
BP 415  
Rabat  
**Morocco**

Mohamed Laghezali  
Tel.: +212 5 520 300  
Fax: +212 5 520 30 07

### **INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE**

Rue Hedi Karray  
2049 Ariana  
**Tunisia**

Mohamed Mlika  
Tel.: +216 1 230 024  
Fax: +216 1 752 897

### **EGE UNIVERSITY**

Faculty of Agriculture  
Dept. of Horticulture  
35100 Bornova/Izmir  
**Turkey**

Ruhinaz Gulcan  
Tel.: +90 232 388 01 10  
Fax: +90 232 388 18 64

**DATE PALM (*PHOENIX DACTYLIFERA* L.): IMPROVEMENT AND DEVELOPMENT OF PALM GROVES VIA TISSUE CULTURE AND MOLECULAR BIOLOGY TOOLS**

**Co-ordinator:** Laboratoire de Recherches en Physiologie Végétale (L.R.P.V.), Angers, France  
(Robert Letouzé)

### OBJECTIVES

- To protect and maintain the existing top quality varieties which are threatened of extinction.
- To provide sufficient disease resistant plant material for the immediate needs of the farmers.
- To develop molecular biology tools allowing the identification of the cultivars, before, during, and after the micropropagation process.

### ACTIVITIES

#### *Tissue culture programme:*

- ◇ Regeneration and mass micropropagation through somatic embryogenesis from offshoot tissue important varieties and selected clones.
- ◇ Development of new techniques based on the use of inflorescence tissues as a suitable new source of plant material to propagate selected clones that have no offshoots.
- ◇ Adaptation of the tissue culture system to clones selected for their resistance to Bayoud.
- ◇ Germplasm collection to high quality varieties which are highly sensitive to Bayoud to preserve them from a complete extinction.

#### *Molecular biology programme:*

- DNA extraction and collection.
- Optimization of the rapid DNA extraction techniques from different kind of plant material (leaves from adult trees, tissues at different stages of *in vitro* culture)
- Optimization of RAPD conditions for date palm.
- Screening of primers for their ability to generate polymorphism.
- Application of RAPD fingerprinting for testing the level of genetic stability of plants obtained from different micropropagation techniques (Somatic embryogenesis, organogenesis).

### EXPECTED RESULTS

- ⇒ Establishment of an efficient micropropagation procedure which can be used for a large scale multiplication of date palm cultivars.
- ⇒ Establishment of DNA markers that can be used for cultivar identification, genetic variability evaluation and assisting the ongoing classical breeding program.
- ⇒ Fast expansion of the existing palm groves and preservation of the oasis ecosystem.
- ⇒ Reduction of the rate of desertification by restabilising the oasis ecosystem.
- ⇒ Reduction of the outmigration from oasis to cities and fast access to nutritional self-sufficiency and sustainable agricultural production.



## SELECTED PUBLICATIONS

LETOUZÉ R., DAGUIN F., SATOUR P. and MARIONNET F. (1998) - Somatic embryogenesis and mass micropropagation of date palm (*Phoenix dactylifera* L.), characterization and stability of regenerated plantlets by RAPD markers. The First International Conference on Date Palm - Al-Ain-UAE.

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ANJARNE M., BOUGERFAOUI M., CHEIKH R. and AIT CHITT M. (1995) - Production de vitroplants de palmier dattier par la technique d'organogenèse *in vitro* : l'expérience marocaine. Journées Internationales sur le palmier dattier dans l'Agriculture des Oasis, Elche (Espagne) : 25-27.

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## PARTNERS

### LABORATOIRE DE RECHERCHE EN PHYSIOLOGIE VEGETALE DES PAYS DE LA LOIRE (L.R.P.V.)

Boulevard Lavoisier 16  
49045 Angers Cedex 01

France

Robert Letouzé

Tel.: +33 2 41 48 21 10

Fax: +33 2 41 73 93 09

E-mail: Letouze@unimedia.fr

### WYE COLLEGE

Unit For Advanced Propagation Systems

Universty of London

TN25 5AH Ashford, Kent

United Kingdom

Sinclair Mantell

Tel.: +44 1233 81 24 01

Fax: +44 1233 81 30 17

E-mail: s.mantell@wye.ac.uk

### INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE

Laboratoire de Recherche en Biotechnologie Végétale

Centre Régional de la Recherche Agronomique

BP 533, 40 000 Marrakech

Morocco

Mohamed Anjame

Tel.: +212 4 44 78 82

Fax: +212 4 44 63 80

### GENERAL ORGANIZATION OF SEED MULTIPLICATION (G.O.S.M.)

Ministry of Agriculture

Tissue Culture Laboratory

El Midane

P.O. Box 5857

Aleppo

Syrian Arab Republic

Marwan Homedan

Tel.: +963 21 64 49 02

Fax: +963 21 64 49 01

**SALTMED: A SYSTEMS APPROACH TO A SUSTAINABLE INCREASE IN IRRIGATED VEGETABLE CROP PRODUCTION IN SALINITY-PRONE AREAS OF THE MEDITERRANEAN REGION**

**Co-ordinator:** Plant Stress Unit, University of Sussex, UK (Anthony Yeo)

**OBJECTIVES**

→ *General*

To increase productivity and sustainability of irrigated vegetable cropping in salinity-prone land. The project focuses on tomato as the vegetable crop and on Egypt and Syria as two countries with representative salt-affected irrigation problems (coastal and inland, respectively). The project has relevance to all salt-affected irrigated systems (the whole Mediterranean Region including EU States in southern Europe, central and eastern Europe, the Middle East, and one hectare in five world-wide).

→ *Specific*

Provide Guidelines for farmers of salinity-prone irrigated land. These Guidelines will provide an optimal balance between crop salt tolerance, economic yield, and sustainable management of irrigation and drainage, achieved through theoretical and practical modelling and through measurements in both experimental and field sites.

Enhance the salt tolerance of tomato through the application of physiological criteria to established conventional breeding programmes with the help of molecular markers. The exploitation plans include the development of an economic tomato cultivar with enhanced tolerance of salinity.

**ACTIVITIES**

- ◇ Define the current situation in the field in the target sites and define the target salt tolerance for the crop. Utilise these data in a model for soil water/salt balance and crop growth, calibrate and verify this model by laboratory and field experiment. In pilot experiments with participating local farms determine relationships between crop salt tolerance and the quantity and quality of water needed to irrigate that crop. Produce Guidelines for farmers.
- ◇ Prioritise the characters to be used in producing a tomato with increased salt tolerance and economic viability and locate donor parental genotypes. Ensure the heritability of chosen characters and then include them in a breeding programme facilitated by the incorporation of DNA marker technology. Evaluate the tolerance and impact on irrigation/drainage management of this genetic material in the field at the target sites and initiate cultivar development.  
Two target sites are chosen to cover different hydrological conditions. These are in the Nile Delta in Egypt (marine contaminated coastal aquifer) and in Syria (inland salinity from long-term irrigation in the Euphrates basin). The project requires the acquisition of field data and the development of plant breeding material.  
It is recognised that crop salt tolerance is only one facet of sustainable irrigated agriculture. Unless the flexibility this should give to water management is utilised to reduce inputs it will not arrest the continuing development of salinisation. However, irrigation management is itself constrained by the salt sensitivity of most crops. It is planned to integrate crop tolerance and water management by refining a model for soil water/salt balance and crop growth. The integration of information of the impacts of management and salt tolerance on crop production will allow a soundly-based framework for decision-making on the relative effects of changing genotype within crop, changing crop, and changing irrigation management. Involvement of farmers and extension workers will enable post-project continuation in the application and refinement of these principles and of genetic improvement towards cultivar release in the respective target environments.
- ◇ Modelling at field sites

The mathematical model will extend the crop-water-yield function (CWYF) model describing soil water movement by Richard's equation (Darcian flow) and solute flow by a convection-dispersion equation. It will take account of osmotic as well as gravitational and pressure potentials. A function to describe the relation between salt concentration and crop water uptake will be developed which has been the weakest part of models to date. The basic model will be refined using data collected from the target sites and then tested in pilot experiments. Incorporating crop performance data the model will then be developed into Guidelines. Local farmers will participate in the development and testing of the model.

Data on salt and water application; leaching and leaching requirement; salt content of soil, drainage water and water tables will be collected in the field. Plant growth, ion contents and yield will be recorded along with physiological parameters including gas exchange.

- ◇ Laboratory studies of plant salt/water balance and seedling pre-adaptation  
Plants will be grown in sand or soil in benches through which excess irrigation can drain and be collected. The data will enable calculation of (1) the optimal rate of irrigation with waters of different salinity for different genotypes and (2) the effect of the tolerance of the genotype on water use. This will be an input to the field model but the initial plant information will be more accurately obtained in controlled conditions.  
If seedling pre-treatment increases the tolerance at later stages then this will be a management adjunct that can reduce inputs at later stages in the crop cycle. Stress treatments will be applied at the seedling stage to discover if this will enhance the tolerance of the mature plant to such stress (literature reports a 'developmental window' in which pre-treatment is effective).
- ◇ Elite breeding lines of tomato  
Donor parents will be taken from amongst local varieties with good reputation and from tolerant wild relatives. Selection criteria will be based on advanced physiological and molecular methods to incorporate specific traits from (*e.g.*) *L. pimpinellifolium* which is more salt tolerant than the domesticated species, but is much better from an agronomic viewpoint than the species (ecological extremes such as *L. cheesmanii*, which have been tried-and-failed in the past). Methods will include measurements of component traits of salt tolerance including ion relations and succulence in the selection of parents and will use and further develop DNA-based marker assisted methods to follow these traits in subsequent breeding populations.  
Plant breeding is a long-term proposition and the deliverables within the project time-frame can only be elite breeding material - though it is intended that this will subsequently be developed towards cultivar status in national programmes. To establish the utility of elite material, the heritability of physiological traits will be studied using genotypes differing broadly in the expression of the character (parents and families composed by the two parents, F<sub>1</sub> and F<sub>2</sub>, and back crosses in both directions will be prepared). All these crosses are possible with *L. esculentum* and *L. pimpinellifolium*. Genetic analysis will be enhanced by the use of molecular markers.

## EXPECTED RESULTS

- ⇒ The main deliverables by the end of the fourth year are:  
Published Guidelines for farmers  
Developed elite breeding lines  
A workshop
- ⇒ The expected results are:  
That the model will provide a decision support system that can provide specific guidelines for farmers for defined crops and situations, so enhancing the sustainable use of saline water in irrigation;  
That the plant improvement programme will provide material that will enhance breeding for salt tolerance in tomato;

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## **PARTNERS**

### **UNIVERSITY OF SUSSEX**

Plant stress unit  
School of Biological Sciences  
Brighton BN1 9QG  
**United Kingdom**

Dr. Anthony Yeo  
Tel.: 44-1273-606 755  
Fax: 44-1273-678 433  
E-mail: a.r.yeo@sussex.ac.uk

### **NATURAL ENVIRONMENT RESEARCH COUNCIL**

Institute of Hydrology  
Hydrological Processes Division  
Wallingford OX10 8BB  
**United Kingdom**

Dr. Ragab Ragab  
Tel.: 44-1491-692.303  
Fax: 44-1491-692.424  
E-mail: r.ragab@ua.nwl.ac.uk

### **CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS**

(CSIC: EELM and CEBAS)  
Estacion "La Mayora"  
29750 Algarrobo Costa  
**Spain**

Dr. Jesus Cuartero  
Tel.: 34-5-255.26.56  
Fax: 34-5-255.26.77  
E-mail: cuartero@eelm.csic.es

### **MENOUFIYA UNIVERSITY**

Faculty of Agriculture  
Mostafa Kamel St.- PO Box 32516  
Shebin-El-Kom  
**Egypt**

Dr. Nabeel Malash  
Tel.: 20-48-22.81.87  
Fax: 20-2-76.94.95  
E-mail: n.malash@shebin.eun.eg

### **MINISTRY OF AGRICULTURE AND AGRARIAN REFORM**

Soil Directorate  
PO Box 113  
Douma, Damascus  
**Syrian Arab Republic**

Dr. Abdelrahman Gaibeh  
Tel.: 963-11-532.30.99  
Fax: 963-11-532.31.00

### **ARAB CENTRE FOR THE STUDIES OF ARID ZONES AND DRY LANDS**

(ACSAAD)  
PO Box 2440  
Damascus  
**Syrian Arab Republic**

Dr. Abdelrahman Gaibeh  
Tel.: 963-11-532.30.39  
Fax: 963-11-532.30.63

**YIELD STABILITY AND RESISTANCE OF FABA BEAN TO MAJOR PATHOGENS  
IN WESTERN MEDITERRANEAN BASIN (FRYMED)**

**Co-ordinator:** Institut National de la Recherche Agronomique, Le Rheu, France (Georges Caubel)

### OBJECTIVES

To generate scientific and practical results for durable resistance in faba bean to fungal diseases and nematodes by:

- identification of sources of resistance in faba bean to fungal diseases and nematodes;
- assesment of the resistant genotypes through multilocal trials;
- investigation of the extend of pathogen variability in relation to resistant genotypes using molecular technologies;
- understanding molecular and biochemical mechanisms of resistance to these diseases.

### ACTIVITIES

FRYMED is based on a multidisciplinary approach developed by pathologists, plant breeders, agronomists and molecular biologists in order to select faba bean for resistance to fungi and nematodes, using the following tasks:

- ◇ at the plant level, to screen local and introduced germplasms for resistance to *Botrytis*, *Ascochyta*, stem (*Ditylenchus*) and root lesion (*Pratylenchus*) nematodes; evaluate the performance of the resistant genotypes in different cropping systems in the sites identified in the faba bean major growing areas of the participating countries;
- ◇ at the pathogen level, to assess the variability and characterise the populations of the pathogens: collection, biological characterisation of pathogenicity, biochemical and molecular characterisation of pathogen populations;
- ◇ at the physiological and genetic level, to analyse the mechanisms of resistance and the genetic control of resistance through molecular biology tools.

### EXPECTED RESULTS

Selecting resistant varieties of faba bean, a grain legume crop, is an appropriate strategy for contributing to the development of sustainable agriculture in the rain fed and marginal lands.

The work will enhance our scientific understanding of the variability of pathogens in Maghreb/Europe, to identify markers correlated to the virulence, and to characterise faba bean germplasm collections for resistance to major pathogens in order to identify resistance sources suitable for the Mediterranean environment. Identification of biochemical and molecular markers of resistance will improve the efficiency of breeding programmes.

Local transfer of results to farmers and extension services will rely on the multilocal trials, which will be conducted in relation with extension and development services. Technical package for Integrated Disease Management in the Mediterranean region will be transferred through co-operation extension networks.

The final result is to increase and stabilise faba bean yield by developing resistant and adapted cultivars. In addition to the improvement of soil fertility, this system protects and preserves the environment. This may contribute stabilising small farmers in marginal zones of the Maghreb, improving their income, and enhancing the role of grain legumes as major components in the sustainable cropping systems.

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## PARTNERS

### INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE

Laboratoire de Zoologie  
Domaine de la Motte BP 29  
35653 Le Rheu Cedex  
France

Georges Caubel  
Tel.: +33 2 99 28 51 58  
Fax: +33 2 99 28 51 50  
E-mail: caubel@rennes.inra.fr

### INSTITUT AGRONOMIQUE ET VÉTÉRIINAIRE HASSAN II

Dépt. d'Agronomie et d'Amélioration des Plantes  
Lab. de Génétique et Sélection des Légumineuses  
B.P. 6202 Rabat-Institut  
Rabat  
Morocco

Mohamed Sadiki  
Tel/Fax: +212 7 77 48 69  
E-mail: sadiki@mail.sis.net.ma

### INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE DE TUNISIE

Laboratoire des Légumineuses à graines  
Rue Hedi Karray  
2049 Ariana  
Tunisia

Mohamed Kharrat  
Tel.: +216 1 23 00 24  
Fax: +216 1 75 28 97

### ISTITUTO SPERIMENTALE PER LA PATOLOGIA VEGETALE

Section Epidemiology and Resistance  
Via C.G. Bertero, 22  
00156 Roma  
Italy

Angelo Porta Puglia  
Tel.: +39 06 82 07 03 55  
Fax: +39 06 86 80 22 96  
E-mail: ispave@flashnet.it

### INSTITUT NATIONAL AGRONOMIQUE

Dépt. de Botanique et de Pathologie végétale  
Avenue Pasteur  
Hassen Badi - El Harrach  
Alger  
Algeria

Zouaoui Bouznad  
Tel.: +213 2 76 19 87 98  
Fax: +213 2 52 35 47  
E-mail: enasa@ist.cerist.dz

<p style="text-align: center;"><b>INTEGRATED CONTROL OF CROWN GALL IN MEDITERRANEAN COUNTRIES (CG-MED)</b></p>
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**Co-ordinator:** Université Claude Bernard – Lyon 1, Villeurbanne, France (Xavier Nesme)

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## OBJECTIVES

The crown gall caused by *Agrobacterium tumefaciens* harbouring a Ti plasmid is presently the main bacterial disease of crops produced in nurseries in Mediterranean countries. The long term objective of the project is to decrease the prevalence of crown gall in nurseries below a reasonable threshold by using new sanitary practices based on:

- Utilisation of epidemiological and ecological data to establish in common a set of standardised procedures to detect, identify and monitor the fate of the causative agent.
- Improvement and evaluation of methods of biological and genetic controls.

## ACTIVITIES

### *Disease prevention by utilisation of epidemiological and ecological data*

- ◇ Studies on new agrobacteria  
Isolation and pathogenicity testing of *Agrobacterium* spp.  
Characterisation of new pathogenic *Agrobacterium*.
- ◇ Identification, detection, and quantification of pathogenic agrobacteria.  
Molecular markers to identify and detect pathogenic *Agrobacterium*.  
Detection and quantification in plant and soil.  
Development of tools for phytosanitary inspection and certification.
- ◇ Origin and fate of agrobacterial populations  
Persistency of agrobacteria in tumours plants and soil.  
Stability and dispersion of pTi  
Procedures to lower the pathogenic populations in plant and soil reservoirs.

### *Biological and genetic controls*

- ◇ Efficiency of biocontrol agents.  
Method to screen the biocontrol activity in planta.  
Efficiency of available biocontrol agents.  
Finding and testing new biocontrol agents.
- ◇ Feasibility of genetic and physiologic control  
Searching for intrinsic resistance among cultivars.  
Efficiency of compounds extracted from weeds.

## EXPECTED RESULTS

### *Scientific-technical results*

Isolation, identification, detection, and quantification of pathogenic agrobacteria from various crops in the different countries to study the origin and the fate of agrobacterial populations within and between different crops and countries.

Accurate characterisation of bacterial strains by PCR, RFLP, AFLP, sequencing and DNA hybridisation will be conducted at both chromosomal and pTi levels by using ribosomal, and vir and T-DNA regions, respectively. This should lead to the discovery of new agrobacteria and new pTi encoding for the biosynthesis of new opine compounds.

The persistency of agrobacteria in plants and soil including studies of stability and dispersion of the pTi in various bacterial species from the soil microflora will be experimentally evaluated.

Evaluation of procedures (sanitary practices, solarisation, heat treatments, etc.), which could lower pathogenic populations in plant and soil reservoirs.

Development of biologic, genetic and physiologic controls, which includes: testing efficiency of available biocontrol agents (K84, K1086); screening of new biocontrol agents in vitro and in planta; searching for intrinsic resistance among major fruit tree cultivars and rootstocks; evaluating the control efficiency of compounds extracted from selected weeds.

#### *Deliverables*

- the most important collection of *Agrobacterium* spp. isolated in standardised conditions in six countries around the Mediterranean Sea, which will furnish exceptional data about the epidemiology of *Agrobacterium* for the present project as well as for the future.
- the discovery of strains belonging to new types of agrobacteria and pTi with putative potential for biotechnology including the discovery of novel opine compounds. Among them there will be new agrobacterial species from which sequencing of the 16S gene will improve international sequence data banks.
- new molecular markers specific of both agrobacteria and pTi.
- new tools for phytosanitary inspection.
- procedures to lower pathogenic populations.
- the extended and integrated use of efficient biocontrol agent on major crops.
- the knowledge of the resistance to crown gall of commercially most important plant material.
- if they succeed, original prospective researches will deliver interesting new ways of control including new biocontrol agents, and phytotherapy.

#### *Training and exchange of scientists*

A first common training course of one week took place during the first general meeting. During the course partners learned and agreed about the molecular techniques and procedures necessary for the project. The molecular techniques taught are extraction of DNA, PCR methodology, PCR-RFLP analysis of both chromosomal and Ti plasmid regions, and demonstration of computer assisted data analysis.

Longer individual training (one month or more) in experienced laboratories also occurred for partners from Morocco, Tunisia and Algeria, and other scientist exchanges are intended to occur.

#### *Pilot or demonstration plants*

The project involved scientists directly concerned by the control of the crown gall disease in their respective country. Their concerted action at the regional scale will provide a strong view of the epidemics threat of crown gall, providing them a skilful experience in the control of the disease. As most partners are involved or co-operate with local Plant Protection Services, these organisations will be aware of the progress of the project. Partners are strongly requested to inform main nurserymen, national and local nurseryman associations and other concerned people of the progress of the work by taking opportunity of their own field experiments.

#### **FOLLOW-UP**

After 6 months of programme, researches concerned mainly season-depending tasks, such as strain isolations, or long term experiments, such as host plant production for genetic and biological experiments, soil solarisation, or the setting up of molecular probes, because those tasks are time consuming and have direct bearing for other tasks.



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## PARTNERS

### UNIVERSITÉ CLAUDE BERNARD LYON I

Ecologie Microbienne du Sol

UMR CNRS 5557

43 bd. du 11 novembre 1918

69622 Villeurbanne Cedex

France

Xavier Nesme

Tel.: +33 4 72 44 82 89

Fax: +33 4 72 43 12 23

E-mail: nesme@biomserv.univ-lyon1.fr

### ISTITUTO VALENCIANO DE INVESTIGACIONES AGRARIAS (IVIA)

Departamento. De Proteccion Vegetal y Biotecnologia

Carretera Moncada a Naquera Km 5

46113 Moncada, Valencia

Spain

Maria M. Lopez Gonzalez

Tel.: +34 96 139 10 00

Fax: +34 96 139 02 40

E-mail: mlopez@master.ivia.es

### UNIVERSITY OF JORDAN

Dept. of Plant Protection

11492 Amman

Jordan

Hamed Khlaif

Tel.: +962 6 84 35 55

Fax: +962 6 83 30 59

E-mail: research@mra.nic.gov.jo

### INSTITUT NATIONAL AGRONOMIQUE DE TUNISIE (INAT)

Lab. Phytopathologie

43 av. Charles Nicolle

1082 Tunis Maharajene

Tunisia

Ali Boubaker

Tel.: +216 1 28 71 10 - ext. 419

Fax: +216 1 79 93 91

### INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE DU MAROC (INRAM)

Centre Régional du Haouz – Présahara

Lab. Phytobactériologie

BP 533

40000 Marrakech

Morocco

Abdelhadi Benjama

Tel.: +212 4 44 78 64

Fax: +212 4 44 63 80

E-mail: benjama@cybernet.net.ma

### UNIVERSITA' DE NAPOLI FEDERICO II

Dipto. di Arboricoltura, Botanica e Patologia Vegetale

via Università' 100

80055 Portici

Italy

Astolfo Zoina

Tel.: +39 81 775 51 42

Fax: +39 81 775 51 14 / 549 68 10

E-mail: zoina@cds.unina.it

### UNIVERSITÉ DE TLEMCCEN

Faculté de Foresterie

Tlemcen

Algeria

Zoulikha Krimi

Tel.: +213 7 20 58 17 / 49 82

Fax: +213 7 26 43 17

E-mail: tlemcen@ist.cerist.dz

### CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)

Institut des Sciences Végétales

avenue de la Terrasse

91198 Gif-sur-Yvette

France

Yves Dessaux

Tel.: +33 1 69 82 36 90

Fax: +33 1 69 82 36 95

E-mail: dessaux@isv.cnrs-gif.fr

**ETUDE DE LA DIVERSITE BIOLOGIQUE DE L'ATRIPLEX HALIMUS POUR LE  
REPERAGE IN VITRO ET IN VIVO D'INDIVIDUS RESISTANTS A DES  
CONDITIONS EXTREMES DU MILIEU ET CONSTITUTION DE CLONES**

**Coordinateur:** Université de Paris-Sud XI, Chatenay-Malabry, France (Pierre Dutuit)

**OBJECTIFS**

- Contribuer aux efforts d'amélioration de la production des steppes fourragères à base d'Atriplex halimus, afin de permettre une augmentation de la production animale des régions arides méditerranéennes;
- Protéger ces écosystèmes particulièrement fragiles.

**ACTIVITES**

- ◇ Le repeuplement de zones dégradées à l'aide de populations homogènes résistantes obtenues par le clonage in vitro d'individus choisis au sein de la diversité biologique de populations naturelles ou créées;
- ◇ Le développement des techniques in vitro a nécessité l'équipement d'unités de culture autonomes pour le clonage par microbouturage ou par embryogenèse somatique de quantités importantes d'individus aux qualités de tolérance à la sécheresse, à la salinité et possédant de bonnes performances fourragères.

Le projet comporte plusieurs étapes :

*Sélection in-situ :*

De nombreuses campagnes de repérage ont été menées dans des régions aux caractéristiques édaphoclimatiques précises au Chili, en Tunisie, Algérie et au Maroc;  
Les populations naturelles d'Atriplex halimus sont hautement hétérogènes. De cette diversité biologique plusieurs individus répondant à des critères de résistance à la sécheresse, à la salinité, mais aussi à des critères de productivité fourragère et de bonne adaptation générale sont sélectionnés et mis en défens. Ils servent pour les prélèvements d'échantillons destinés à la culture in vitro.

*Clonage in vitro :*

- Etude de la micropropagation;
- Etude des contraintes;
- Sélection in vitro;
- Mise au point de tests;
- Embryogenèse somatique;
- Semences artificielles.

*Etude du comportement des clones en écosystèmes naturels :*

Cette étape consiste au retour aux conditions naturelles des clones produits in vitro.

**RESULTATS ATTENDUS**

*Repérage sur le terrain*

La sélection in-situ prévue dans le programme a pris beaucoup de retard, les dispositifs initiaux de mise en défens n'ayant pas été assez performants. Dans ce domaine l'effort s'est rapidement porté vers la recherche de collaborations avec des organismes aptes à effectuer cette mise en défens.

En Tunisie, le Commissariat Régional de Développement Agricole (CRDA), a permis d'initier, dans la région de Kairouan, sur un parcours, une zone de mise en défens.

Pour pallier les difficultés rencontrées par les collègues algériens, une collaboration avec le Maroc, a permis d'ouvrir, dans la région de Marrakech, de nouvelles zones de prospection.

### *Etude de la biologie florale*

Les observations effectuées en serre et in situ ont permis de montrer la complexité des structures de reproduction. Les travaux ont porté sur l'ontogénie des structures florales et inflorescencielles. Nous avons pu mettre en évidence, en plus des fleurs mâles ou femelles, qui caractérisent cette espèce, l'existence de structures hermaphrodites dont il faut préciser maintenant si elles peuvent être fonctionnelles.

### *Etude du polymorphisme de l'espèce*

Les travaux réalisés jusqu'à présent, tant sur le terrain qu'au laboratoire, ont mis en évidence le remarquable polymorphisme de l'*A. halimus*. Ce polymorphisme se manifeste tant au niveau de la morphologie des structures végétatives qu'au niveau des structures reproductrices. Ainsi, nous avons observé que la forme des feuilles d'*A. halimus* peut correspondre à celle d'autres espèces du même genre. Elle varie également avec la provenance de l'individu et, sur un même pied, elle est différente selon l'état physiologique de la plante ou la position de la feuille sur un axe. Ainsi, le rabattage d'un pied, qui stimule le développement de bourgeons axillaires, entraîne très souvent l'apparition de feuilles hastées-dentées alors qu'auparavant la plante formait des feuilles, entières, ovales ou lancéolées, formes typiquement attribuées à l'espèce *halimus*.

- Variabilité interspécifique et intraspécifique

Cette variabilité a été mise en évidence dans diverses conditions à plusieurs niveaux d'observation :

- floral et foliaire.
- protéique et enzymatique
- efficacité de la transpiration en conditions de stress hydrique
- phytomasse en situation de compétition entre différents génotypes
- fluorescence de la chlorophylle
  - \* dans l'efficacité du PSII en contrainte sodique et calcique
  - \* dans la caractérisation de plusieurs espèces et provenances
- efficacité de l'utilisation de l'eau.

- Variabilité dans la descendance maternelle et repérage des caractères discriminants

L'analyse statistique multivariée a permis la mise en évidence d'une variabilité morphogénétique et la mise au point d'une méthode de repérage des caractères les plus discriminants.

### *Le clonage in vitro et cultures cellulaires*

- Le bourgeonnement axillaire, a été favorisé par l'adjonction de substances de croissance. En partant d'apex, des taux de multiplication d'environ 3 ont été enregistrés en présence de BAP à 0,5 mM.
- La micropropagation in vitro, à partir de pousses feuillées des vitroplants issus de semis ou d'individus adultes, fait apparaître des taux d'enracinement variant respectivement de 70 à 60 % des axes mis en culture.
- De nombreuses tentatives, à partir de cultures de tissus et de cellules, ont été faites pour stimuler l'embryogenèse somatique, sans avoir atteint des résultats significatifs à ce jour. Seules des manifestations d'organogénèse ont été obtenues.

### *Résistance aux stress*

- Stress hydrique

Des feuilles de plantes soumises à des arrosages espacés présentent une chute de potentiel osmotique enregistrée qui est associée à une accumulation de proline. Par contre la teneur en proline n'augmente pas dans des cals dont le potentiel osmotique est réduit par la présence de polyéthylène glycol dans le milieu de culture.

- Stress salin

Au niveau de la plante entière le caractère halophile de l'espèce a tout d'abord été confirmé. En effet, la croissance de la partie aérienne de jeunes plantes est stimulée en présence de NaCl à 150 mM. Des concentrations plus élevées ont, au contraire, un effet inhibiteur sur toutes les portions de la plante et particulièrement lors des premiers stades de développement. En outre, les traitements combinés des graines par l'acide gibbérellique et la kinétine, ont permis de limiter l'effet inhibiteur du sel.

Le potentiel osmotique du suc cellulaire extrait des feuilles, des racines et des cals diminue avec l'augmentation de la salinité dans le milieu de culture. Au niveau racinaire, le potentiel osmotique reste proche de celui de la solution nutritive. Par contre, au niveau des feuilles, il est toujours nettement plus négatif. Les cals, issus de feuilles et de racines, se comportent de la même manière avec des potentiels osmotiques toujours inférieurs à ceux du milieu externe.

Au niveau des racines et des feuilles, l'accumulation du sodium, évidente aux concentrations de sel les plus faibles dans le milieu, ne se poursuit pas au-delà de 300 mM pour la partie aérienne et de 450 mM pour la partie racinaire.

Des cultures de tissus, initiées à partir de plantules en germination, vont soit proliférer, à un rythme lent certes, soit dégénérer progressivement sur un milieu contenant une concentration de sel de 300 mM.

- **Autres stress**

Dans son environnement naturel, *A. halimus* est soumis à divers stress, autres que salin et hydrique. Parmi ces contraintes, la compétition entre génotypes, la présence de métaux lourds dans le sol et le parasitisme par d'autres espèces végétales (orobanche, cystanche), ont été abordés en fin de contrat afin d'en apprécier la portée dans les objectifs de sélection. Les résultats sont encore fragmentaires.

#### *Les marqueurs de résistance aux stress*

- **Recherche de différents composés**

Des solutés inorganiques et organiques (proline, glucides, acides aminés), des activités peroxydasiques, etc. ont été mises en relation avec des variations des conditions d'environnement auxquelles sont soumises les plantes d'*A. halimus*. Il reste cependant à préciser si les composés étudiés sont impliqués dans des mécanismes de résistance ou si leur accumulation est le symptôme de dégâts subis par la plante stressée.

- **Recherche de marqueurs moléculaires par PCR-RAPD** - la technique d'amplification de l'ADN nucléaire d'*A. halimus* est actuellement au point. Il reste cependant à établir avec certitude la correspondance entre des phénotypes particuliers de résistance ou de sensibilité à différents stress et des profils de PCR-RAPD déterminés.

#### *Aspects sylvopastoraux et fourragers*

Les études ont montré que les espèces, provenances ou individus les plus intéressants ne sont pas nécessairement les plus palatables. En effet, une palatabilité excessive favorise le surpâturage. Une palatabilité moindre est préférable.

Ces études ont également montré que la phytomasse fourragère provenant des *Atriplex* est particulièrement riche en matières azotées mais pauvre en énergie. Ce déficit énergétique peut être comblé par les fourrages herbacés ce qui a justifié une étude des effets des *Atriplex*, sur la strate herbacée associée dans le but de sélectionner les arbustes qui favorisent la productivité et la qualité nutritionnelle fourragère de la strate herbacée.

Les tiges ligneuses des *Atriplex* sont également utilisées pour satisfaire les besoins des petits éleveurs et agriculteurs en combustible, une pratique qui contribue, elle aussi, à accélérer le processus de désertification de parcours déjà détériorés par le surpâturage et la céréaliculture.

Les rendements en bois et les capacités calorifiques du bois de chauffe provenant des arbustes de différents individus d'*Atriplex halimus* ont été analysés comparativement à ceux d'individus d'autres espèces. Ces études ont en particulier montré que le rendement en bois est étroitement corrélé au port de la plante (hauteur et diamètre). Quant à la capacité calorifique, elle ne présente pas suffisamment de variations entre individus et provenances pour justifier son utilisation comme critère de sélection.

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## PARTENAIRES

### UNIVERSITE DE PARIS SUD XI

Equipe d'Ecotechnologie  
Laboratoire de Botanique  
Tour E1 - 2ème étage  
92296 Chatenay-Malabry Cedex  
France

Pierre Dutuit  
Tel: +33 1 46 83 54 19  
Fax: +33 1 46 83 54 19  
E-mail: pierre.dutuit@cep.u-psud.fr

### UNIVERSITE CATHOLIQUE DE LOUVAIN

Laboratoire de Cytogénétique  
Place Croix du Sud 4-5  
1348 Louvain-La-Neuve  
Belgium

Jean Marie Kinet  
Tel: +32 10 47 20 50  
Fax: +32 10 47 34 35  
E-mail: kinet@bota.ucl.ac.be

### UNIVERSITE DES SCIENCES & TECHNIQUES BLIDA

Institut d'Agronomie  
Département de Phytotechnie  
B.P. 270  
Blida  
Algeria

Fatima Benrebiha  
Tel: +213 3 41 58 50  
Fax: +213 3 41 78 13

### FACULTE DES SCIENCES DE TUNIS

Laboratoire de Biologie Végétale  
Campus Universitaire  
Tunis  
Tunisia

Sadok Bouzid  
Tel: +216 1 87 26 00  
Fax: +216 1 88 54 80

### UNIVERSIDAD DE CHILE

Departamento de Production Animale  
Faculté des Sciences Agraires & Forestières  
Santa Rosa  
11 315 La Pintana/Santiago  
Chile

Sergio Lailhacar  
Tel: +56 2 678 57 32  
Fax: +56 2 678 58 02

**ISOLATION AND IDENTIFICATION OF PHEROMONES AND THEIR MODE OF ACTION  
IN AFRICAN LOCUSTS**

**Co-ordinator:** Universität Hannover, Hannover, Germany (Gerhard H. Schmidt)

**OBJECTIVES**

- To perform chemical analysis of pheromones in African locusts and comparable grasshoppers;
- To identify the mainly volatile and air borne compounds and prepare them for behavioural studies;
- To analyse the less volatile substances on the cuticle of the locust after solution in pentane;
- To find out the mode of action of the identified compounds in relation to their pheromone effect.

**ACTIVITIES**

- ◇ Identification of the headspace volatiles from adults, hoppers and faeces by using CLSA stripping method and GC + MS;
- ◇ Applying an olfactometer to various adult and hopper stages to find out, whether the moving air-column contains stage specific volatiles, and to identify them by means of the analysed compounds;
- ◇ Performing research on the mode of action of the analysed volatiles by special rearing experiments and antennograms;
- ◇ Using special less volatile identified compounds as cuticular layer to find out whether there is a substance functioning as a contact pheromone;
- ◇ Treatment of immature adult females of locusts with the main headspace compounds of mature males demonstrating their influence on reproduction process;
- ◇ Investigations of the olfactory signalling by patch-champ techniques;
- ◇ Generation of monoclonal antibodies which interact with distinct subpopulations of cells in locust antenna;
- ◇ Carry out special experiments showing the influence of locust pheromones on the endocrine system;
- ◇ Evaluation of botanicals interfering the pheromone action chain to reduce reproduction and on this way population density.

**RESULTS**

- ⇒ Identification of headspace compounds  
Headspace analysis of *Schistocerca gregaria* and *Locusta migratoria* were carried out extensively, 27 compounds were identified having mostly aromatic structure. Some of them are sexually specific. Benzylcyanide was found only in adult yellow males and for the first time in insects.

At the beginning of maturation gregarious males of *S. gregaria* produce 1, 2, 3-trimethoxybenzene, (Z)-5-octenoid acid, trans-3-ethyl-1,4-dimethyl-pyrrolidin-2,5-dione (I) and veratrole; the compound (I) could be synthesised and its configuration was identified by NOE-experiment. Adult gregarious females emit the same compounds as the nymphs that mainly produce phenol and guaiacol in both sexes. In *S. gregaria* and *Aiolopus thalassinus* (F.) males' methyl benzoate was released. In extracts of faeces obtained by steam distillation 4-vinylguaiacol was found in high concentrations that may be an artefact, because the compound was not detectable in headspace extract of faeces, in which phenol, guaiacol and benzylcyanide were found.

- ⇒ Analysis of less volatile cuticular surface compounds  
Only a weak dimorphism was found in the composition of hydrocarbons, 128 various alkanes were identified in *S. gregaria*, *L. migratoria* and *A. thalassinus*. It seems not likely that there is an alkane functioned as pheromone in locusts. Additionally, in *S. gregaria* 20 different long-chained branched and straight-lined ketones were identified, from which 18 compounds are new as natural products. The 2-alkanones 3,7-dimethylpentacosan-2-one, 3,7-dimethylheptacosan-2-one and 3,7,1,5-trimethylheptacosan-2-one are sexual and stage specific. In contrast to that 6-alkanones were found in both sexes of adults. In nymphs, mainly 5-alkanones were identified. Besides the ketones, in *S. gregaria* various 2,5-disubstituted tetrahydrofurans were identified in the polar fraction of the cuticle. Also long-chained bishomolog nitrogen compounds of unknown structure were detected.
- ⇒ Molecular basis of pheromone reception  
Due to the important functional role of c-AMP activated potassium channels in olfactory neurones of locusts, attempts were made to identify the channel protein using molecular cloning approaches. Screening a c-DNA library from locust antennae with appropriate molecular probes led to the isolation of highly reactive clones. It contains several sequence motifs that are characteristic for cyclic nucleotide-binding domain within partially hydrophobic region near the C-terminus, a pore-forming region and a residual voltage-sensor motif.
- ⇒ Olfactometric responses to volatile compounds  
Strong positive responses were observed for sexually mature males of *L. migratoria*, when offered either extracts concentrated over Poropak or air impregnated with volatiles emitted by females. The reverse was also observed for sexually immature females responded to mature males. The identified compound ethyl-acetophenone indicated a strong and prolonged arrestant effect in sexually mature males. In *S. gregaria* mature females responded significantly to guaiacol. Veratrole showed an inhibition effect. For the responses of mature males' guaiacol has to be combined with veratrole or benzylcyanide. For immature males' guaiacol, benzaldehyde and benzylcyanide in binary or tertiary combination gave high responses. Immature females responded only to the combination of four compounds. According to these observations, the aggregation pheromone of *S. gregaria* will be a blend of headspace compounds.
- ⇒ Elektrophysiological reactions of the antennae to volatiles  
Electroantennographically, all components of headspace tested evoked a significant response depending on their concentrations, showing that there are receptors on the antennae which percept the chemicals. A strong reaction to the mixture was observed in immature females and males, thus there may be an additive or synergistic effect of the compounds. From the singly tested compounds' benzylcyanide had the strongest antennographical reaction in adult locusts, especially in immature males.
- ⇒ Benzylcyanide and colour change in *S. gregaria* males  
Two different populations were tested for colour change in males and the influence of benzylcyanide on copulation and egg-laying were studied. Benzylcyanide was produced only from yellow males. For copulation and egg deposition a colour change was not necessary. The colour change did not depend on the maturation process. Benzylcyanide was unable to induce yellow coloration in immature and mature males. But the maturation process in males and females increased rapidly if a yellow male was induced. For the induction of yellow coloration, i.e. production of benzylcyanide, a body contact with a yellow male was necessary for rapid coloration.

- ⇒ Pheromones and reproduction in *S. gregaria*  
In unmated females egg-pod production increased in the presence of males by means of a male volatile chemical factor. Mislaid egg-pods were highest in females without antennae and in virgin females near to males. A high number of eggs per pod were correlated to a low production rate of egg-pods. The egg-laying interval was shorter than in virgin females; in the latter it was shortened in the presence of males producing volatile pheromones.
- ⇒ Effect of Melia extract on larval development, maturation and egg production in *S. gregaria*  
Oral applications of Melia extract prolonged the nymphal development, delayed the maturation of the adults, thus no oviposition took place. Males did not become yellow and their fat body was reduced. In oocytes no formation of chorion was observed.
- ⇒ Population differences in *S. gregaria*  
In *S. gregaria* normally 4 to 5 generations can be obtained in gregarious populations if continuous breeding takes place through one year. A population found in Tenerife reproduced only twice per year with a higher number of eggs per egg-pod, but with low hatching rate. The lowest number of eggs/pod was correlated with the fasted reproduction. No difference was found in nymphal development. The period of preoviposition was much longer in the population from Tenerife than from other places. Yellow coloration of mature males occurred only under crowded conditions.

#### **FOLLOW-UP**

- ▶ What is the real blend of volatiles acting as pheromone ?
- ▶ Are there different blends of volatiles having various pheromone effects ?
- ▶ How induce the body contact with yellow males the production of benzylcyanide in conspecific mature males and what is the meaning of the yellow coloration ?
- ▶ Do the less volatile compounds on the cuticle, i.e. long-chain ketones, tetrahydrofurans, di-n-alkylethers and long-chained bishomolog nitrogen compounds have any pheromone character ?
- ▶ Heterologous expression of the c-AMP activated channel type from locusts may be allowed to fully characterise for the first time a cyclic nucleotide gated potassium channel to understand the structure/function relationship between cyclic nucleotide gated channel types;
- ▶ What are the real pheromones influencing reproduction and gregarisation ?
- ▶ Besides behavioural mechanisms, there may be a genetic factor influencing aggregation and reproduction differing in various populations.



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## PARTNERS

**UNIVERSITÄT HANNOVER**  
Lehrgebiet Zoologie - Entomologie  
Herrenhäuser Straße 2  
30419 Hannover  
Germany

Gerhard H. Schmidt  
Tel.: +49-511-762 55 40  
Fax: +49-511-762 53 81

**UNIVERSITÄT HOHENHEIM**  
Institute of Zoophysiology  
Garbenstraße 30  
70593 Stuttgart  
Germany

Heinz Breer  
Tel.: +49-711-459 22 67  
Fax: +49-711-459 37 26

**UNIVERSITÄT HAMBURG**  
Institut für Organische Chemie  
Martin Luther King Platz 6  
20146 Hamburg  
Germany

Wittko Francke  
Tel.: +49-40-41 23 28 66  
Fax: +49-40-41 23 38 34

**UNIVERSIDADE NOVA DE LISBOA**  
Faculdade de Ciências e Tecnologia  
Departamento Ciências E Engenharia Do Ambiente  
Quinta Da Torre  
2825 Monte de Caparica  
Portugal

Maria Rosa Paiva  
Tel.: +351-1-295 44 64  
Fax: +351-1-294 24 41

**UNIVERSITY OF CAIRO**  
Faculty of Agriculture  
Economic Entomology & Pesticides Department  
Cairo University Street  
Giza  
Egypt

Kamal Othman  
Tel.: +20-2-72 49 66  
Fax: +20-2-62 39 28

**UNIVERSITÉ CADI AYYAD**  
Faculté des Sciences Semlalia  
Département de Biologie  
B.P. S 15  
Marrakech  
Morocco

Abdeljalil Bakri  
Tel.: +212-4-43 46 49  
Fax: +212-4-43 67 69

**EXPLORATION OF NEW FINDINGS IN INSECT ENDOCRINOLOGY AND  
PHYSIOLOGY FOR DEVELOPING NOVEL WAYS OF LOCUST CONTROL**

**Co-ordinator:** Katholieke Universiteit Leuven, Leuven, Belgium (Arnold De Loof)

**OBJECTIVES**

- To develop the new field of locust peptide neurotransmitters and hormones to such a stage that the organic chemists and molecular biologists can engage in the development of practical applications for locust control;
- To establish laboratories of excellence in the field of locust research in North Africa.

**ACTIVITIES**

- ◇ Establishing methods for mass rearing *Schistocerca* under laboratory conditions as a common effort of all participating labs to be able to collect enough material to do the hormone extractions;
- ◇ Isolation and identification of peptides which control the biosynthesis of juvenile hormone (JH) (allatostatins and allatotropins), reproduction and digestion;
- ◇ Determining the active core of the peptides;
- ◇ Gaining data about the similarities and differences between *Locusta* and *Schistocerca*: *Locusta* has been a very good experimental insect but *Schistocerca* is a much more important pest.

**OUTCOME**

- ▷ Identification of novel neuropeptides  
The following peptides have been identified in *Schistocerca*: 8 different allatostatins, salivation peptide, a few other myotropins, the releasing factor for adipokinetic hormone (the first releasing factor ever found in any invertebrate species), neuroparsins and ovary maturing parsin (a gonadotropin).
- ▷ Digestion  
The presence of an ovarian factor, which inhibits trypsin biosynthesis in the gut, could be demonstrated and partially purified. Because of the very small amount of peptide which could be purified from several thousands of ovaries, only an incomplete sequence was obtained. Many of the myotropins which were identified, have effects on gut motility. Some are stimulators, others paralyse the gut. The allatostatins are likely to have a function in digestion, as the allatostatin gene is also expressed in the gut. A search for inhibitors of protease inhibitors yielded the sequences of 4 trypsin/chymotrypsin inhibitors plus indications of the existence of additional ones. These inhibitors are present in high concentrations in the ovary, not in the gut itself. Several more inhibitors remain to be fully identified. Extracts of *Cestrum* and olive leaf extracts inhibit feeding.
- ▷ Molecular biology  
The cDNA coding for the allatostatins was sequenced. All peptides are present in one gene. This analysis showed that the gene contains another 2 related allatostatins, bringing the total number of identified allatostatic peptides to 10. Methods were elaborated for cloning and identifying locust neuropeptide receptors. Two G-protein coupled receptors were identified this way. The cDNA of the trypsin/chymotrypsin inhibitors was cloned and the developmental expression of the gene was documented.

- ▷ Juvenile hormone biosynthesis  
A novel inhibitor of juvenile hormone biosynthesis was found in the fungus *Penicillium*. The identified locust allatostatins are not potent inhibitors of JH biosynthesis. The biochemical study of JH biosynthesis yielded novel insights.
- ▷ Physiological experiments  
Many results were obtained, especially on the effects of ovary maturing parsin, the neuroparsins and several myotropic peptides.
- ▷ The active core of peptides  
The regions carrying the biological activity of ovary maturing parsin and of the myotropins were identified. All these cores were synthesised and tested for biological activity.
- ▷ Gregarisation  
An important discovery in the study of gregarisation was that isolating the eggs soon (preferably within a few minutes) after egg laying resulted in a high percentage of solitary animals. This treatment is supposed to remove the maternal gregarisation factor. This is important for future studies on phase polymorphism.

### **FOLLOW UP**

This project substantially contributed to developing locusts as model animals in insect neuroendocrinology and neurobiology. It resulted in a FAIR project, which aims at developing insect signalling peptides as potential insecticides. At this moment, tobacco (as model system) has already been transformed with the synthetic genes coding for the insect peptides proctolin and trypsin modulating oostatic factor. The methods for cloning G-protein coupled receptors are being introduced in the laboratories of phytopharmaceutical companies as screening methods for novel insecticides. The methods developed for the isolation of locust neuropeptides allowed to identify, in 1998, the melanisation hormone of locusts. This hormone is the first peptidic marker of gregarisation and is likely to open the "black box" of the endocrinology of phase polymorphism. Further research also resulted in the isolation of additional locust protease inhibitors.

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## PARTNERS

### KATHOLIEKE UNIVERSITEIT LEUVEN

Laboratory for Developmental Physiology

Naamsestraat 59

3000 Leuven

**Belgium**

Arnold De Loof

Tel.: +32 16 28 39 12

Fax: +32 16 28 39 02

### UNIVERSITE DE BORDEAUX I

Laboratoire de Neuroendocrinologie

Avenue des Facultés

33405 Talence

**France**

A. Girardie

Tel.: +33 5 56 84 87 51

Fax: +33 5 56 84 87 50

### UNIVERSIDAD DE VALENCIA

Facultad Ciencias Biologicas

Departamento Biologia Animal

C/Lauria 6

46100 Burjasot (Valencia)

**Spain**

Rafael Martinez-Pardo

Tel.: +34 6 386 46 01

Fax: +34 6 386 43 72

### INSTITUT NATIONAL D'AGRICULTURE DE TUNISIE

Lab. de Physiologie & Physiopathologie des Insectes

Avenue Charles Nicolle 43

1082 Tunis Mahrajene

**Tunisia**

Mohamed Habib Ben Hamouda

Tel.: +216 1 28 09 50 (ext. 280 046)

### UNIVERSITE MOHAMMED V

Laboratoire de Zoologie & de Biologie Général

Avenue Ibn Battouta

B.P. 1014

Rabat

**Morocco**

Z. Atay-Kadiri

Tel.: +212 7 77 54 61

**LA PREVISION MENSUELLE ET SAISONNIERE DU CYCLE DES  
PRECIPITATIONS SUR LE BASSIN MEDITERRANEEN (EMALSIFA)**

**Coordinateur:** Groupement d'Intérêt Public MEDIAS-FRANCE, Toulouse, France  
(Jean-Pierre Verdou)

## OBJECTIFS

- De préciser, à partir d'études rétrospectives, l'effet de mémoire que la structure océanique et ses anomalies exercent sur la circulation atmosphérique et la succession des types de temps et des régimes de précipitations ;
- D'évaluer *à posteriori*, à partir d'archives d'observations *in situ*, la qualité de différents essais de prévisions fournis aux trois autres participants par Météo-France ;
- De réaliser en temps quasi réel, et à partir de 1996, des prévisions à échéances mensuelle et saisonnière sur le Bassin Méditerranéen ;
- D'évaluer ces prévisions en les comparant localement avec les données locales et d'effectuer une évaluation socio-économique auprès d'utilisateurs potentiels.

## ACTIVITES

Pour les phases A et B:

L'**ONM-Algérie** a étudié le comportement du champs de température de surface de l'océan (TSO), recherché des analogues, étudié des corrélations entre les pluies mensuelles et saisonnières et la TSO et produit des prévisions statistiques; puis a évalué *à posteriori*, la qualité des résultats du modèle ARPEGE-Climat fournis par Météo-France.

La **DMN-Maroc** a étudié les relations statistiques entre TSO et différents champs météorologiques et entre l'Oscillation Nord Atlantique et les précipitations afin de développer des prévisions statistiques à longue échéance, puis vérifié le potentiel de prévisibilité des précipitations fournies par le modèle ARPEGE-Climat.

L'**INM-Tunisie** a approfondi l'étude statistique des corrélations entre précipitations et champs météorologiques pour produire des prévisions statistiques, a calculé des scores de vérifications statistiques afin d'évaluer la prédictibilité des précipitations à travers le modèle ARPEGE-Climat.

**Météo-France** a participé aux analyses de corrélation entre la TSO et les précipitations sur la région, fourni les essais de prévisions passés et étudié la prévisibilité des précipitations par la méthode du modèle parfait.

Pour la phase C de juin 1996 décembre 1997

Météo-France a utilisé les sorties du modèle ARPEGE adaptées, pour produire en temps réel des indices de précipitation régionale tandis que les participants élaboraient des prévisions par les analyses statistiques. Une évaluation scientifique et socio-économique de ces prévisions saisonnières de pluie a été effectuée pour déterminer la qualité et la faisabilité des "bulletins ELMASIFA"

- ARPEGE-Climat: modèle numérique de prévisions météorologiques de Météo-France
- CEPMMT: Centre européen pour les prévisions météorologiques à moyen terme
- TSO: Température de Surface de l'Océan

## RESULTATS

Des scores d'un intérêt non négligeable, même s'ils ne peuvent être comparés à ce que l'on obtient en prévision météorologique à courte échéance, ont été obtenus lorsque la température surface de l'océan global est spécifique à un modèle de circulation générale d'après les observations. Les coefficients de corrélation en hiver valent de 0.3 à 0.5. Il faut remarquer que les scores de précipitations sont bien meilleurs lorsqu'on utilise comme vérification des mesures *in-situ* fournies par les services météorologiques des pays concernés que lorsqu'on utilise des analyses ou des reconstitutions météorologiques. L'utilisation de températures de surface de l'océan prévues par un algorithme statistique conduit à des scores plus faibles et proches de ceux d'une méthode de prévision statistique directe (c-à-d sans utiliser de modèle de circulation générale) des précipitations. Toutefois, lorsque les

anomalies sont persistantes, comme cela a été le cas pendant la deuxième moitié de la phase de prévision en temps réel avec El Nino 1997-98, les précipitations à l'échelle saisonnière sont bien prévues. Les perspectives d'amélioration du système passent donc par une meilleure prise en compte des conditions océaniques, soit en raffinant les méthodes statistiques de prédiction de sa température, soit en faisant appel à un modèle couple.

### *Perspectives*

Les résultats encourageants obtenus, malgré des scores encore modestes ont incité les participants à signer un accord de coopération pour que l'expérience se poursuive après la fin du contrat européen. Déjà, une délocalisation du calcul numérique à DMN-Maroc permet d'élaborer depuis février 1998 des indices de prévision ELMASIFA qui sont fournis aux autres participants. Ainsi la combinaison de la prévision du modèle ARPEGE et de la prévision statistique élaborée au Maghreb permet la réalisation, par chacun des pays, chaque mois pour les quatre mois suivants, des "bulletins ELMASIFA" qui sont validés par les scientifiques et des usagers intéressés. La poursuite de l'expérience permettra d'affiner et de conforter ces premiers résultats.

L'évaluation socio-économique par des utilisateurs potentiels (Services de l'Agriculture et Service de l'Hydraulique) a permis de constater que cette expérience pouvait avoir déjà des retombées sur les économies des pays de la région et que ces premiers résultats étaient une aide incontournable à la décision pour la gestion des travaux agricoles et des ressources en eau.

Cette expérience ELMASIFA et la méthodologie développée, peuvent être étendues assez facilement. sous réserve d'accords de coopération, à d'autres pays africains ou méditerranéens intéressés par la prévision saisonnière des précipitations.

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## **PARTENAIRES**

### **MEDIAS-FRANCE**

Avenue Edouard-Belin 18  
B.P. 2102  
31055 Toulouse Cedex  
**France**

Jean-Pierre Verdou  
Tel.: +33-5-61 28 29 46  
Fax: +33-5-61 28 29 05  
E-mail: verdou@medias.cst.cnes.fr

### **INSTITUT NATIONAL DE LA METEOROLOGIE**

Direction des Systèmes Météo de Base sous Direction de  
Développement et des Méthodes de Prévisions  
B.P. 156  
2035 Tunis-Carthage  
**Tunisia**

Chadly Ben M'Hamed  
Tel.: +216-1-78 24 00  
Fax: +216-1-78 46 08  
E-mail: chedly@hp-meteo.inm.tn

### **MAROC-METEO**

Direction de la Météorologie Nationale  
Centre National du Climat et des Recherches  
Météorologiques  
B.P. 8106 Casa-Oasis  
Casablanca  
**Morocco**

Abdallah Mokssit  
Tel.: +212-2-90 43 80  
Fax: +212-2-90 48 96

### **METEO-FRANCE**

Centre National de Recherches Météorologiques  
Avenue G. Coriolis 42  
31057 Toulouse Cedex  
**France**

Michel Deque  
Tel.: +33-5-61 07 93 82  
Fax: +33-5-61 07 96 10  
E-mail: michel.deque@meteo.fr

### **OFFICE NATIONALE DE LA METEOROLOGIE**

Centre Climatique National  
B.P. 153  
Dar el Beida  
16100 Alger  
**Algeria**

Mohamed Kadi  
Tel.: +213-2-50 89 47  
Fax: +213-2-50 88 49  
E-mail: onm-ccn@ist.cerist.dz

**ANALYSED CLIMATOLOGY OF RAINFALL OBTAINED FROM SATELLITE AND SURFACE DATA FOR THE MEDITERRANEAN REGION (ACROSS) - A VERSION FOR THE EASTERN MEDITERRANEAN REGION**

**Co-ordinator:** University of Genova, Genova, Italy (Franco Siccardi)

## **OBJECTIVES**

The major objective of this research is the improvement of the rainfall forecasting in the eastern Mediterranean region. To this end the following detailed objectives are set:

- To develop and demonstrate a methodology for total area or contouring, evaluation and interpretation of rainfall over-land and over-water anomalies in the target region as basis for ongoing climate-hydrological analysis and forecasting;
- To prepare a unified climatology of rainfall over that region;
- To describe the observed rainfall distribution through climatological analysis of satellite data (passive microwave);
- To identify and map the most significant departures from the long term average rainfall, as well as the characteristic space and time scales of extremes;
- To investigate the major effects of significant rainfall anomalies through related analyses of passive microwave data (land surface types) for the microwave period among 1978 and 1994.

## **ACTIVITIES**

- ◇ Collecting ground-based rainfall data from WMO and local networks, development and calibration of techniques for the processing of SMMR and SSM/I images for rainfall and surface characteristics, acquisition and preparation of satellite images, development of graphical tools for data analysis and presentation;
- ◇ Application of techniques for SMMR data analysis and development of methods for the integration with land and sea surface data via GIS;
- ◇ Description and presentation of results through climatological atlases and time series of climatological data associated with surface characteristics;
- ◇ Open end of project seminar on remote sensing applied to weather/rainfall forecast in the Mediterranean region, bringing together all groups working on this subject and in particular all relevant projects currently funded under the AVICENNE programme.

## **RESULTS**

- ⇒ The work undertaken during the first year of the project activity was mainly devoted to the acquisition of the basic data resources needed for the eventual development of dedicated research studies, and to the generation of initial overwater products based on SMMR satellite data (1978-87). In particular the collection, collation and analysis of raingauge data for suitable and available stations in the study area were addressed, in order to produce maps of average rainfall for months, seasons, years, plus maps, graphs and statistics for rainfall variability and departures from the norm. At the same time the collection and geo-registering of SMMR (1978-1987) and SSM/I (1987-1994) images prior to their preliminary analysis for over water rainfall within the study area to complement the above mentioned products, and so complete a regional picture for the Eastern Mediterranean. The definition of suitable graphical tools for presentation of project results was also addressed and the acquisition of ancillary data completed. The two data-sets were implemented within a relational database and a hydrologically oriented Geographical Information Systems (GIS).
- ⇒ The 30-seconds Digital Elevation Model of the Mediterranean region, obtained from USGS, was selected as the basic information over which both satellite and raingauge data are represented. A large number of raingauge and meteo-climatic stations were identified in the study area and the acquisition of data for the period 1978-1994 started. In particular daily rainfall series from the



NOAA / NCDC dataset were acquired and complemented with sparse data from the national networks (provided by partner institutions) in order to achieve the information density of about one rain gauge per 625 km<sup>2</sup> (25 x 25 km grid).

⇒ Microwave satellite images were also collected for the period 1978-1994, both from SSMR and SSM/I sensors. In particular data from the SSMR were obtained in the form of Temperature Calibrated Tapes (TCTs) for the entire instrument operation period from 25 October 1978 to 20 August 1987 from the US National Space Science Data Centre. SSM/I images for the period 1987-1994 were obtained from the US Defence Meteorological Satellite Programme (DMSP) via the Marshall Space Flight Centre of NASA.

## **PARTNERS**

### **UNIVERSITY OF GENOVA**

Institute of Hydraulics

Montallegro 1

16145 Genova

**Italy**

Franco Siccardi

Tel : +39-103-53 24 97

Fax : +39-103-53 24 81

Email : franck@idra.unige.it

### **UNIVERSITY OF BRISTOL**

Department of Geography

Remote Sensing Unit

Bristol BS8 1SS

**United Kingdom**

Eric Barrett

Tel : +44-1272-30 37 45

Fax : +44-1272-30 37 46

Email : e.c.barret@bris.ac.uk

### **MIDDLE EAST TECHNICAL UNIVERSITY**

Civil Engineering Department

06531 Ankara

**Turkey**

Dogan Altinbilek

Tel : +90-312-210 10 00 / 24 01

Fax : +90-312-210 12 62

### **UNIVERSITY OF JORDAN**

Department of Geology

Amman

**Jordan**

Elias Salameh

Tel : +962-6-84 35 55 (ext. 2331, 2345)

Fax : +962-6-84 68 41

### **INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREA (ICAZDA)**

P.O. Box 5466

Aleppo

**Syria**

Theib Y. Oweis

Tel : +963-21-21 34 77 / 22 51 12 / 22 50 12

Fax : +963-21-21 34 90 / 22 51 05

**MICROBIAL ACTIVITY IN THE RHIZOSPHERE IN RELATION TO THE IRON NUTRITION OF PLANTS**

**Co-ordinator:** Universität Hohenheim, Stuttgart, Germany (H. Marschner & V. Römheld)

**OBJECTIVES**

The overall goal of this project is to study the utilisation of different Fe siderophores and phytosiderophores by higher plants in short- and long-term experiments with particular consideration of the rhizosphere and root apoplasmic space.

**ACTIVITIES**

- ◇ Extraction, characterisation and quantification of microbial siderophores in different calcareous soils;
- ◇ Uptake of different Fe siderophores and Fe phytosiderophores by dicot plants and grasses in short term experiments;
- ◇ Utilisation of Fe siderophores by graminaceous plant roots after exchange chelation with plant-born phytosiderophores;
- ◇ Importance of the root apoplasm as transient iron storage for Fe acquisition in general and in particular for exchange chelation;
- ◇ Diurnal rhythm in biosynthesis and release of phytosiderophores in sorghum and different maize mutants in comparison with barley;
- ◇ Possible transport of Fe through mycorrhiza hyphae into higher plants.

**RESULTS**

- ⇒ Hydroxamate siderophores are the most abundant siderophores in calcareous soils under agricultural cultivation. Beside an easily water extractable pool of siderophores firmly bound siderophores to the soil matrix can be determined. Hydroxamates of both pools are enriched in the rhizosphere compared with the bulk soil. A slow transformation between both pools can be detected.
- ⇒ By short term uptake studies it could be shown that the microbial siderophore rhizoferrin can be easily used by dicots (strategy I plants) and by grasses (strategy II plants). Dicots utilise Fe rhizoferrin via the Fe deficiency-induced reductase system and grasses via the phytosiderophore system.
- ⇒ The mechanism of utilisation involves the process of an exchange chelation between Fe rhizoferrin and phytosiderophores as shown by *in-vitro* and *in-vivo* experiments. Studies on the kinetics have shown a reaction time of  $t_{1/2} = 0.5$  h. The presence of isolated cell walls (root apoplast) had no further stimulating effect.
- ⇒ It could be demonstrated that in grasses plant-borne phytosiderophores can efficiently utilise apoplasmic bound Fe and also Fe in the root-soil interface (rhizosphere) of a calcareous soil.
- ⇒ Maize and barley have the same rate of synthesis of phytosiderophores. However, in contrast to barley, maize and also sorghum do not store phytosiderophores in roots for the short-term release during a few hours once per day, but release phytosiderophore continuously at a constant rate over the whole day. Obviously, maize and sorghum do not have the typical vesicles as found in barley. Beside the maize mutant  $ys_1/ys_1$  that is defect in the specific Fe phytosiderophore transporter, we have characterised another maize mutant  $ys_3/ys_3$ . This mutant is defect in the secretion mechanism for phytosiderophores.
- ⇒ A transport of Fe through mycorrhiza hyphae into roots could be proofed by use of special techniques with separated hyphae compartments.

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## PARTNERS

### UNIVERSITÄT HOHENHEIM

Institut für Pflanzenernährung

Fruwirthstraße 20

70599 Stuttgart

Germany

H. Marschner & V. Römheld

Tel.: +49-711-459 37 14/2344

Fax: +49-711-459 32 95

### HEBREW UNIVERSITY OF JERUSALEM

Faculty of Agriculture

76100 Rehovot

Israel

Y. Chen and Y. Hadar

Tel.: +972-8-948 12 11

Fax: +972-8-946 23 84

## 2. Agriculture

<p>2.1. Production systems</p> <p>Contract number: AVI2-CT93-123 Contract number: IC18-CT97-0186 Contract number: IC18-CT97-0177 Contract number: AVI2-CT93-091 Contract number : IC18-CT96-0064</p>	<p>2.3. Livestock production</p> <p>Contract number: IC18-CT98-0392 Contract number: IC18-CT98-0333 Contract number: IC18-CT95-0009 Contract number: IC18-CT98-0331 Contract number: IC18-CT95-0003 Contract number: IC18-CT97-0202 Contract number: IC18-CT96-0121 Contract number: TS3-CT91-0019 Contract number: TS3-CT92-0106 Contract number: TS3-CT92-0151 Contract number: TS3-CT92-0143 Contract number: TS3-CT92-0119</p>
<p>2.2. Crop production</p> <p>Contract number: IC18-CT96-0082 Contract number: AVI-CT93-0007 Contract number: TS3-CT93-0221 Contract number: TS3-CT94-0278 Contract number: CI1-CT94-0087 Contract number: CI1-CT91-0932</p>	<p>2.4. Agriculture related topics</p> <p>Contract number: IC18-CT98-0301 Contract number: IC18-CT98-0310 Contract number: IC18-CT98-0300 Contract number: IC18-CT98-0391 Contract number: IC18-CT98-0390 Contract number: IC18-CT98-0311 Contract number: IC18-CT98-0308 Contract number: IC18-CT97-0198 Contract number: IC18-CT98-0313 Numéro de contrat: TS3-CT94-0264 Contract number: TS3-CT93-0249 Contract number: TS3-CT93-0208 Contract number: TS3-CT92-0015 Contract number: AVI2-CT93-080 Numéro de contrat: AVI-CT93-0010 Contract number: CI1-CT93-0006</p>

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## **Index of contracts by topic**

---

1. Natural resources	Page
1.1. Basic natural resources	
1.1.1. Water supply and management	
<i>Contract number: IC18-CT98-0289</i>	20
“Med-Pol” innovative decentralised energy and water management policies can encourage the creation of a market economy and help rural development	
<i>Co-ordinator: Conphoebus Scrl, Catania, Italy</i>	
<i>Contract number: IC18-CT98-0268</i>	16
Agriculture and urbanisation in the Mediterranean region: enabling policies for sustainable use of soil and water	
<i>Co-ordinator: Centre International de Hautes Etudes Agronomiques Méditerranéennes, Bari, Italia</i>	
<i>Contract number: IC18-CT98-0266</i>	12
Control of salination and combating desertification effects in the Mediterranean region: phase II	
<i>Co-ordinator: Estación Experimental “La Mayora”, Spain</i>	
<i>Contract number: IC18-CT97-0171</i>	23
Development, application and analysis of raman, fluorescence and absorption spectroscopy using optical fibre remote sensing of chemical species in water for in situ environmental pollution studies in Cyprus, Israel, Italy and the United Kingdom	
<i>Co-ordinator: University of Kent, United Kingdom</i>	
<i>Contract number: IC18-CT97-0161</i>	27
Developing sustainable water management in the Jordan Valley	
<i>Co-ordinator: Austrian Research Centre, Seibersdorf, Austria</i>	
<i>Contract number: IC18-CT97-0151</i>	29
Resource management in karstic areas of coastal regions of the Mediterranean (Res Man Med)	
<i>Co-ordinator: Credco Ltd., Ireland</i>	
<i>Contract number: IC18-CT97-0143</i>	31
Groundwater recharge in the Eastern Mediterranean (GREM) – a comparative study on integrated evaluation techniques for groundwater resources	
<i>Co-ordinator: University of Würzburg, Department of Hydrogeology, Germany</i>	
<i>Contract number: IC18-CT97-0142</i>	34
Mediterranean cooperation for water desalination policies in the perspective of a sustainable development	
<i>Co-ordinator: National Technical University of Athens, Greece</i>	
<i>Contract number: IC18-CT97-0138</i>	38
Medwater: a decision support system for water management in the Mediterranean region	
<i>Co-ordinator: National Technical University of Athens, Dept of Chemical Engineering-Systems, Greece</i>	

	Page
<p><i>Contract number: IC18-CT97-0134</i>  Interaction between migration, land &amp; water use and resource exploitation in the oases of the Maghreb  <i>Co-ordinator: University of Amsterdam, Faculty of Environmental Sciences, The Netherlands</i></p>	40
<p><i>Contract number: IC18-CT96-0122</i>  A new integrated geophysical approach for the rational management and exploration of groundwater resources  <i>Co-ordinator: Rijks Geologische Dienst, Haarlem, The Netherlands</i></p>	47
<p><i>Contract number: IC18-CT96-0091</i>  Hydromed - program of research on hill reservoirs in the semi-arid zone of the Mediterranean periphery  <i>Co-ordinator: Orstom, Tunis, Tunisie</i></p>	43
<p><i>Contract number: AVI-CT93-143</i>  Characterisation of large watersheds for surface run-off water harvesting in support of sustainable human settlement of natural vegetation in arid and semi-arid areas  <i>Co-ordinator: Synoptics, Wageningen, The Netherlands</i></p>	58
<p><i>Contract number: AVI2-CT93-126</i>  Development of a methodology based on NOAA satellites (AVHRR sensor) observations for the control for freshwater resources and their evolution  <i>Co-ordinator: Infocarta S.A., Madrid, Spain</i></p>	50
<p><i>Contract number: AVI2-CT93-099</i>  Hydromed: development of a computerised methodology for the evaluation of the vulnerability of aquifers in the Southern Mediterranean Basin and its validation on two sample areas in Tunisia and Malta  <i>Co-ordinator: Institute d'Appolonia Spa., Genova, Italy</i></p>	74
<p><i>Contract number: AVI2-CT93-076</i>  Integrated management of reclaimed wastewater resources in the Mediterranean region  <i>Co-ordinator: Universidad de Barcelona, Barcelona, Spain</i></p>	52
<p><i>Contract number: AVI2-CT93-073</i>  Development of water resource management tools for problems of seawater intrusion and contamination of fresh water resources in coastal aquifers  <i>Co-ordinator: Universiteit Gent, Gent, Belgium</i></p>	76
<p><i>Contract number: AVI2-CT93-072</i>  Management of karst water resources  <i>Co-ordinator: Universität Karlsruhe, Karlsruhe, Germany</i></p>	78
<p><i>Contract number: AVI2-CT93-062</i>  New stabilization/solidification technologies for the prevention of underground water contamination from industrial wastes  <i>Co-ordinator: Imperial College of Science, Technology &amp; Medicine, London, United Kingdom</i></p>	62

	Page
<i>Contract number: AVI2-CT93-058</i>	80
Lagunis: study of the management of natural water resources by lagooning along the Maghreb's Mediterranean coast taking into account the quality of water discharges	
<i>Co-ordinator: Cabinet d'Etudes Techniques Industrielles et d'Innovations Scientifiques, Aix en Provence, France</i>	
<i>Contract number: AVI2-CT93-020</i>	82
Water resources management in urban and peri-urban areas of the Mediterranean region: Amman and Rabat	
<i>Co-ordinator: Cerfe, Roma, Italy</i>	
<i>Contract number: AVI-CT93-0015</i>	65
Recharge characteristics and groundwater quality of the Grand Erg Oriental Basin	
<i>Co-ordinator: British Geological Survey, Wallingford, United Kingdom</i>	
<i>Contract number: AVI-CT93-0013</i>	86
Water resource management in an interdisciplinary perspective	
<i>Co-ordinator: Wagner Advies B.V., Noordwolde, The Netherlands</i>	
<i>Contract number: AVI-CT93-0009</i>	84
Improvement of the techniques and parameters of surface irrigation in the oasis of North Africa	
<i>Co-ordinator: Wageningen Agricultural University, Wageningen, The Netherlands</i>	
<i>Contract number: AVI2-CT93-008</i>	55
Generating new techniques to control desertification and salinization effects in the Mediterranean Basin	
<i>Co-ordinator: Universidade do Algarve, Faro, Portugal</i>	
<i>Contract number: AVI-CT93-0005</i>	69
Vulnerability of groundwater resources to natural radiological hazards in the semi-arid terrains of North Africa and the Mediterranean Basin	
<i>Co-ordinator: Natural Environment Research Council, Nottingham, United Kingdom</i>	
<i>Contract number: AVI-CT93-0003</i>	88
Observation and modelling of the circulation in the zone North-Tunisia/Sardinia/Sicily (Salto)	
<i>Co-ordinator: Université Pierre et Marie Curie, Paris VI, France</i>	
<i>Contract number: AVI-CT92-0004</i>	90
Interlaboratory study of organic pollutants in groundwater	
<i>Co-ordinator: Fraunhofer Institut für Toxikologie und Aerosolforschung, Hannover, Germany</i>	
<i>Contract number: TS3-CT92-0061</i>	92
Irrigation water management and salinisation: intercomparison of simulation models in Argentina and Egypt	
<i>Co-ordinator: Winand Staring Centre of Integrated Land Soil &amp; Water Research, Wageningen, The Netherlands</i>	
<i>Contract number: CII-CT93-0362</i>	96
Feberoptic laser sensor: a diagnostic tool for environmental protection	
<i>Co-ordinator: Fraunhofer Institut für Physikalische Messtechnik, Freiburg, Germany</i>	



	Page
1.1.2. Water treatment and pollution control	
<i>Contract number: IC18-CT98-0293</i>	100
Occurrence of toxic cyanobacteria waterblooms: impact on water environments and potential human health risk. Environmental, physiological and genetic mechanisms involved in toxins production. <i>Co-ordinator: Université Paul Sabatier - Toulouse III, Toulouse, France</i>	
<i>Contract number: IC18-CT98-0273</i>	102
Technical development & demonstration of closed-loop procedures in electroplating and metal chemistry using solar energy or waste heat to avoid water and to minimize solid waste which can be utilized <i>Co-ordinator: Gesellschaft für Umweltverträgliche, Teltow, Deutschland</i>	
<i>Contract number: IC18-CT98-0272</i>	104
Sustainability and optimisation of treatment and use of wastewater in agriculture <i>Co-ordinator: Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgium</i>	
<i>Contract number: IC18-CT98-0267</i>	107
Integrated wastewater reuse by solar-catalytic treatment: a pilot study in the textile industry <i>Co-ordinator: Technische Universität Clausthal, Clausthal-Zellerfeld, Germany</i>	
<i>Contract number: IC18-CT98-0265</i>	110
Development & optimization of a new process for desalination of sea water by means of solar energy <i>Co-ordinator: Ruhr-Universität Bochum, Bochum (Nrw), Deutschland</i>	
<i>Contract number: IC18-CT97-0167</i>	112
Development of a simple technology in drinking water treatment for nitrate and pesticide removal <i>Co-ordinator: Universitaet Stuttgart, Stuttgart, Deutschland</i>	
<i>Contract number: IC18-CT97-0163</i>	115
A system approach to wastewater biotreatment for the protection of Mediterranean coastal areas (BIOWATSYST) <i>Co-ordinator: Istituto Agronomico Mediterraneo, Valenzano (Bari), Italy</i>	
<i>Contract number: IC18-CT97-0136</i>	117
Control of bacterial regrowth in water supply distribution systems in water short European and Mediterranean countries <i>Co-ordinator: University of Newcastle Upon Tyne, United Kingdom</i>	
<i>Contract number: IC18-CT96-0039</i>	120
Desalination of sea-water using renewable energy sources <i>Co-ordinator: Dimman Consulting Ltd, Pilea-Thessaloniki, Greece</i>	
<i>Contract number: IC18-CT96-0099</i>	123
Waste water recycling supplied by renewable energies in the Near East <i>Co-ordinator: Fachhochschule Aachen, Jülich, Germany</i>	

	Page
<p><i>Contract number: IC18-CT96-0076</i>            Development of environmentally friendly photoactivatable compounds for treatment of microbially polluted water  <i>Co-ordinator: Università degli Studi di Padova, Padova, Italy</i></p>	126
<p><i>Contract number: AVI-CT94-0014</i>            Membrane recovery of metal pollutants from wastewaters of the fertilizers industry (MERMEP)  <i>Co-ordinator: Universidad de Cantabria, Santander, Spain</i></p>	144
<p><i>Contract number: AVI-CT94-0013</i>            Comparative assessment of technologies for solar detoxification and disinfection of contaminated water  <i>Co-ordinator: Deutsche Forschungsanstalt für Luft - und Raumfahrt E.V., Köln, Germany</i></p>	272
<p><i>Contract number: AVI-CT94-0012</i>            Development of a technologically simple, low energy cost method of treating wastewater for reuse in agriculture  <i>Co-ordinator: Hr Wallingford Ltd, Wallingford Oxon, United Kingdom</i></p>	129
<p><i>Contract number: AVI-CT94-0002</i>            Use of wastewater for irrigation, a global approach blending water treatment, irrigation with various systems on various crops and institutional/organisational aspects  <i>Co-ordinator: Faculté des Sciences Agronomiques de Gembloux, Gembloux, Belgium</i></p>	132
<p><i>Contract number: AVI-CT94-0009</i>            Purification and re-use of domestic waste-water using low-cost eco-biotechnological methods  <i>Co-ordinator: Wageningen Agricultural University, Wageningen, The Netherlands</i></p>	138
<p><i>Contract number: AVI-CT94-0007</i>            Purification and recycling of wastewater by solar-catalytic and biological treatment in Algeria, Syria and Tunisia  <i>Co-ordinator: Technische Universität Clausthal, Clausthal-Zellerfeld, Germany</i></p>	155
<p><i>Contract number: AVI-CT93-0006</i>            Med-Nps control and surveillance of non-point source (NPS) Mediterranean (MED) pollution via GIS: a Corinne &amp; Eurostat database extension  <i>Co-ordinator: Epsilon International S.A., Athens, Greece</i></p>	157
<p><i>Contract number: AVI-CT93-0002</i>            Development of sensors for on-site monitoring of the extent of pollution in water resources  <i>Co-ordinator: University of Newcastle-Upon-Tyne, Newcastle-Upon-Tyne, United Kingdom</i></p>	174
<p><i>Contract number: AVI-CT93-0001</i>            Surveillance of pollution in the Mediterranean sea: marine organisms as ubiquitous markers - novel approach  <i>Co-ordinator: Johannes Gutenberg Universität, Mainz, Germany</i></p>	176

	Page
<p><i>Contract number: AVI-CT92-0016</i>            Toxic solid waste terrestrial and aquatic impact problems and solutions  <i>Co-ordinator: University of Patras, Patras, Greece</i></p>	178
<p><i>Contract number: AVI-CT92-0015</i>            Data processing for a Mediterranean automated environmental monitoring network (Mednet)  <i>Co-ordinator: Institut Français pour l'Exploitation de la Mer (IFREMER), Nantes, France</i></p>	161
<p><i>Contract number: AVI2-CT93-112</i>            Controlling contaminants affecting use and re-use of water, sewage and sludge in Jordan and Syria  <i>Co-ordinator: Technical University of Denmark, Lungby, Denmark</i></p>	147
<p><i>Contract number: AVI2-CT93-081</i>            Defluoration of waters in the northern parts of the North-African Sahara  <i>Co-ordinator: Université de Rennes, Rennes, France</i></p>	149
<p><i>Contract number: AVI2-CT93-074</i>            Treatment of water and emuents by TiO<sub>2</sub> photocatalysis for removal of organosynthetic contaminants and metals  <i>Co-ordinator: Universität Bremen, Bremen, Germany</i></p>	151
<p><i>Contract number: AVI2-CT93-054</i>            Multidisciplinary study of drinking and agricultural water treatment using ultraviolet light sources supplied by solar energy in rural environments  <i>Co-ordinator: Université Paul Sabatier - Toulouse III, Toulouse, France</i></p>	163
<p><i>Contract number: AVI2-CT93-092</i>            Disinfection of drinking water &amp; treated domestic wastewater by chlorine and chlorine dioxide  <i>Co-ordinator: University of Crete, Heraclion, Greece</i></p>	166
<p><i>Contract number: AVI2-CT93-083</i>            Natural clays, modified or sharpened, as catalysers for purifying natural or industrial water, materials and mechanisms  <i>Co-ordinator: Centre National de la Recherche Scientifique, Orléans, France</i></p>	170
<p><i>Contract number: AVI2-CT93-019</i>            Characterisation of waters in north-west Tunisia polluted by heavy metals and development of separation techniques adapted to their treatment  <i>Co-ordinator: Institut National Polytechnique de Lorraine, Vandoeuvre, France</i></p>	172
<p><i>Contract number: CII-CT94-0086</i>            Continuous production of ligninolytic enzymes by white rot fungi for detoxification of recalcitrant pollutants  <i>Co-ordinator: Wageningen Agricultural University, Wageningen, The Netherlands</i></p>	142
<p><i>Contract number: CII-CT92-0104</i>            Développement d'un procédé biologique pour le traitement des eaux usées provenant des moulins à olives. Etudes moléculaires du système ligninolytique de <i>phanerochaete chrysosporium</i>  <i>Co-ordinator: Orstom-Université de Provence, Marseille, France</i></p>	181

	Page
<p><i>Contract number: TS3-CT92-0126</i>  Recherches et études pour la valorisation et l'assainissement des eaux usées par épuvalisation  <i>Co-ordinateur: Faculté des Sciences de Gembloux, Gembloux, Belgium</i></p>	189
<p><i>Contract number: AVI-CT92-0011</i>  Teledetection by laser (LIDAR) of organic and inorganic pollution from mobile ground stations adapted to the conditions in Southern Mediterranean countries  <i>Co-ordinator: Centre National de Recherche Scientifique, Orsay, France</i></p>	187
<p><i>Contract number: AVI-CT92-0014</i>  Treatment of drinking water and industrial waste contaminated by heavy metals with membrane technology  <i>Co-ordinator: Ecole Nationale Supérieure de Chimie, Montpellier, France</i></p>	183
<p>1.1.3. Other resource: soils</p>	
<p><i>Contract number: IC18-CT97-0197</i>  Use of mycorrhizal and rhizobial symbioses for the sustainable development of forest resources in the Mediterranean region (Myrisme)  <i>Co-ordinator: Centro Internacional des Altos Estudios Agronomicos Mediterraneos, Zaragoza, Spain</i></p>	194
<p><i>Contract number: IC18-CT97-0153</i>  Desertification in the Mediterranean drylands: development of a monitoring systems based on plant ecophysiology (Demos)  <i>Co-ordinator: Università delgi Studi di Trieste, Department of Biology, Trieste, Italy</i></p>	196
<p><i>Contract number: IC18-CT97-0147</i>  Medchange – effects of land use and land management practices changes on land degradation under forest and grazing ecosystems  <i>Co-ordinator: Universidade de Aveiro, Depto. de Ambiente e Ordenamento, Aveiro, Portugal</i></p>	199
<p><i>Contract number: IC18-CT96-0069</i>  Climate impact on water resources and drylands agriculture (Cliwarda)  <i>Co-ordinator: DLO Winand Staring Centre for Integrated Land, Soil and Water, Wageningen, The Netherlands</i></p>	202
<p><i>Contract number: IC18-CT96-0081</i>  Nitrogen fixation and yield of grain legume in saline Mediterranean zones (Eysame)  <i>Co-ordinator: Institut National de la Recherche Agronomique, Montpellier, France</i></p>	206
<p><i>Contract number: IC18-CT96-0035</i>  Sustainable domestication of indogenous fruit trees: interactions between soil and biotic resources in some drylands of southern Africa  <i>Co-ordinator: Università degli Studi di Torino, Grugliasco, Italy</i></p>	210
<p><i>Contract number: AVI-CT93-0067</i>  Analysis of pesticides in soil and water by in situ biosensor and modelling of pesticide removal by bioremedial methods  <i>Co-ordinator: Cranfield University, Bedfordshire, United Kingdom</i></p>	213

	Page
<p><i>Contract number: AVI-CT92-0006</i>  An investigation into the effects of sludge amendment of soils on agricultural pesticide transport  <i>Co-ordinator: Institut für Wasser-, Boden- und Lufthygiene, Berlin, Germany</i></p>	215
<p><i>Contract number: TS3-CT92-0047</i>  Réhabilitation des terres dégradées au nord et au sud du Sahara. Utilisation de légumineuses pérennes et des micro-organismes associés pour l'établissement de formations pluristrates  <i>Co-ordinator: Orstom, Paris, France</i></p>	217
<p>1.2. Environmental research - ecosystems</p>	
<p><i>Contract number: IC18-CT98-0270</i>  Bases for the integrated sustainable management of Mediterranean sensitive coastal ecosystems  <i>Co-ordinator: Consorzio Nazionale Interuniversitario per le Scienze del Mare, Unità Locale di Ricerca Firenze, Italy</i></p>	220
<p><i>Contract number: IC18-CT98-0269</i>  Impact assessment and economic evaluation of water harvesting techniques in dry Mediterranean zones (Wahia)  <i>Co-ordinator: Wageningen Agricultural University, Wageningen Nederland</i></p>	225
<p><i>Contract number: IC18-CT98-0261</i>  Improved management of agroforestry parkland systems in Sub-Saharan Africa.  <i>Co-ordinator: University of Wales, Bangor, United-Kingdom</i></p>	227
<p><i>Contract number: IC18-CT97-0200</i>  Global, physiological and molecular responses to climatic stresses of three Mediterranean conifers  <i>Co-ordinator: Institut National de la Recherche Agronomique (INRA), Gazinet, France</i></p>	230
<p><i>Contract number: IC18-CT97-0169</i>  A decision support system for mitigation of drought impacts in the Mediterranean regions  <i>Co-ordinator: Institute of Hydraulics Hydrology and Water Management, University of Catania, Italy</i></p>	234
<p><i>Contract number: IC18-CT97-0155</i>  Changes in arid Mediterranean ecosystems on the long term through earth observation (CAMELEO)  <i>Co-ordinator: Joint Research Centre Ispra, Space Applications Institute, Ispra, Italy</i></p>	236
<p><i>Contract number: IC18-CT97-0154</i>  Developing of remote sensing technics for evaluating the spatial and temporal distribution of hydrological parameters in arid basins: Flaubert (flood in arid units by earth remote technics)  <i>Co-ordinator: Centre d' Etude des Environnements Terrestre et Planet, Velizy, France</i></p>	238
<p><i>Contract number: IC18-CT96-0055</i>  Sustainable halophyte utilisation in the Mediterranean and subtropical regions  <i>Co-ordinator: Universität Osnabrück, Osnabrück, Germany</i></p>	240

	Page
<p><i>Contract number: IC18-CT96-0034</i></p> <p>Damage of coral reefs by recreational activities: restoration strategies and the development of novel markers for environmental stress</p> <p><i>Co-ordinator: Johannes Gutenberg Universität, Mainz, Germany</i></p>	244
<p><i>Contract number: IC18-CT96-0029</i></p> <p>Change, stress and sustainability: aquatic ecosystem resilience in North Africa (Cassarina)</p> <p><i>Co-ordinator: University College London, London, United Kingdom</i></p>	247
<p><i>Contract number: AVI2-CT93-087</i></p> <p>M.U.R.E.X: Mediterranean urban rejects experiment</p> <p><i>Co-ordinator: Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare (ICRAM), Roma, Italy</i></p>	252
<p><i>Contract number: AVI-CT92-0007</i></p> <p>Benthic foraminifera as indicators of heavy metal pollution - a new method of biological monitoring of the Mediterranean sea</p> <p><i>Co-ordinator: Università degli Studi di Milano, Milano, Italy</i></p>	256
<p><i>Contract number: AVI-CT92-0001</i></p> <p>Use of parasite species composition indices of fishes to measure the degree of environmental deterioration due to pollutants and other man-made adverse effects</p> <p><i>Co-ordinator: Università degli Studi di Roma "La Sapienza", Roma, Italy</i></p>	263
<p><i>Contract number: AVI-CT92-0017</i></p> <p>A complex investigation and prediction of atmospheric pollution transfer in the Mediterranean area</p> <p><i>Co-ordinator: Aristotelian University of Thessaloniki, Thessaloniki, Greece</i></p>	254
<p><i>Contract number: AVI-CT92-0008</i></p> <p>Integration of satellite land surface assessment with socio-economic parameters for global desertification monitoring in the arid Mediterranean zone (Tunisia and Egypt)</p> <p><i>Co-ordinator: ORSTOM, Paris, France</i></p>	258
<p><i>Contract number: AVI-CT92-0005</i></p> <p>Transport and transformation of air pollutants from Europe to the East Mediterranean region (T-Trapem project)</p> <p><i>Co-ordinator: University of Athens, Athens, Greece</i></p>	261
<p><i>Contract number: CII-CT94-0096</i></p> <p>Are the HCF/HFC/HBC compounds environmentally acceptable alternatives</p> <p><i>Co-ordinator: University Heidelberg, Heidelberg, Germany</i></p>	265
<p><i>Contract number: CII-CT94-0083</i></p> <p>Genetic and physiological approach to the development of new bacterial polymers and biomass with improved properties for heavy metal accumulation</p> <p><i>Co-ordinator: Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol, Belgium</i></p>	250

	Page
<b>1.3. Renewable energy</b>	
<i>Contract number: AVI-CT94-0015</i>	270
Wastewater distillation by sun energy (WADISUN) - treatment of highly contaminated waste-water by simple and low cost procedures <i>Co-ordinator: Aquaambiente S.A., Mem Martins, Portugal</i>	
<i>Contract number: AVI-CT94-0013</i>	272
Comparative assessment of technologies for solar detoxification and disinfection of contaminated water <i>Co-ordinator: Deutsche Forschungsanstalt für Luft - und Raumfahrt e. V., Köln, Germany</i>	
<i>Contract number: AVI-CT94-0011</i>	275
Simultaneous nitrogen elimination and energy production for reclamation of wastewaters and production of raw materials <i>Co-ordinator: Universidad de Barcelona, Barcelona, Spain</i>	
<i>Contract number: AVI-CT94-0008</i>	278
Selective coating for solar heating and cooling: preparation and characterisation <i>Co-ordinator: Université Paul Sabatier, Toulouse, France</i>	
<i>Contract number: AVI-CT94-0006</i>	280
Assessment of biomass based electricity and heat generation in decentralised areas in Egypt and Morocco for job creation and improvement of living standards <i>Co-ordinator: Engineering and Computer Applications S.A., Athens, Greece</i>	
<i>Contract number: AVI-CT94-0005</i>	282
Integrated concept for the fermentation of waste-water sludge and organic waste for the production of renewable energy and the use of the fermented product as fertiliser and for soil improvement <i>Co-ordinator: Universität Stuttgart, Stuttgart, Germany</i>	
<i>Contract number: AVI-CT94-0004</i>	285
Concerted action for the testing and cost reduction of photovoltaic water pumping systems <i>Co-ordinator: I.T. Power Ltd, Eversley Hants, United Kingdom</i>	
<i>Contract number: TS3-CT92-0093</i>	289
Thermochemical upgrading of biomasses to gaseous and liquid fuels and feedstocks <i>Co-ordinator: Nederlands Meetinstituut, Eindhoven, The Netherlands</i>	
<b>2. Agriculture</b>	
<b>2.1. Production systems</b>	
<i>Contract number: IC18-CT97-0186</i>	294
Processing of agricultural wastes by white-rot fungi for production of fodder for small ruminants <i>Co-ordinator: Georg-August-Universität Göttingen, Göttingen, Germany</i>	

	Page
<i>Contract number: IC18-CT97-0177</i>	296
Biodiversity audit, propagation and sustainable exploitation of cedars ( <i>cedrus</i> SPP) in the Mediterranean region	
<i>Co-ordinator: The University of Reading, Agricultural Botany Department, United Kingdom</i>	
<i>Contract number: IC18-CT96-0064</i>	299
Sustainable management of renewable marine resources: a comparative study of management systems and markets in Northwest African cephalopod fisheries	
<i>Co-ordinator: University of Portsmouth, Portsmouth, United Kingdom</i>	
<i>Contract number: AVI2-CT93-123</i>	301
Modelling benthic disturbance and recovery in warm water mariculture	
<i>Co-ordinator: Universität Kiel, Kiel, Germany</i>	
<i>Contract number: AVI2-CT93-091</i>	303
Rainfall forecasts and strategic irrigation management	
<i>Co-ordinator: Società di Ricerca e Servizi di Ingegneria (ISMES) S.P.A., Bergamo, Italy</i>	
<b>2.2. Crop production</b>	
<i>Contract number: IC18-CT96-0082</i>	308
Optimising marginal resources in intensive horticultural production in Southern Turkey and Northern Egypt	
<i>Co-ordinator: University of Wales, Aberystwyth, United Kingdom</i>	
<i>Contract number: TS3-CT94-0278</i>	314
Improving the growth of tropical nitrogen-fixing forest trees in the Gerera acacia and casuarina through tissue culture and genetic transformation	
<i>Co-ordinator: ORSTOM Genetrop, Montpellier, France</i>	
<i>Contract number: AVI-CT93-0007</i>	311
Improvement of the water use efficiency of wheat under dry and saline conditions in the Maghreb	
<i>Co-ordinator: Faculté des Sciences Agronomiques, Gembloux, Belgium</i>	
<i>Contract number: TS3-CT93-0221</i>	317
Development of selection and clonal propagation techniques for multiplication of elite yield and anthracnose tolerant cashew ( <i>Anacardium Occidentale</i> L.)	
<i>Co-ordinator: Instituto de Investigação Científica Tropical, Oeiras, Portugal</i>	
<i>Contract number: CII-CT94-0087</i>	321
Structural aspects of wheat glutenins and the mechanisms of their assembly to form the gluten matrix	
<i>Co-ordinator: University of Bristol, Bristol, United Kingdom</i>	
<i>Contract number: CII-CT91-0932</i>	323
Genetic and physical mapping of the tomato 12 locus	
<i>Co-ordinator: Weizmann Institute of Science, Rehovot, Israel</i>	



	Page
<b>2.3. Livestock production</b>	
<i>Contract number: IC18-CT98-0392</i>	326
Desertification risk assessment in silvopastoral Mediterranean ecosystems: bases towards a sustainable management of natural resources (DRASME)	
<i>Co-ordinator: Consejo Superior De Investigaciones Cientificas, Zaragoza, Espana</i>	
<i>Contract number: IC18-CT98-0333</i>	328
Development of biocapsuled feed for larval fish based on nutritionally enriched nematodes	
<i>Co-ordinator: Bio Integrated Technology S.R.L., Pantalla di Todi, Italy</i>	
<i>Contract number: IC18-CT98-0331</i>	330
Sustainable development of African continental fisheries: a regional study of policy options and policy formation mechanisms for the Lake Chad Basin	
<i>Co-ordinator: University of Portsmouth, Portsmouth, United Kingdom</i>	
<i>Contract number: IC18-CT97-0202</i>	332
Analysis and management of organic matter and nitrogen in aquacultural ponds for a minimal waste production and optimal efficiency	
<i>Co-ordinator: Wageningen Agricultural University, Wageningen, Netherlands</i>	
<i>Contract number: IC18-CT96-0121</i>	334
Development of improved strategies for the control of faba bean necrotic yellows virus in food legume crops of West Asia and North Africa	
<i>Co-ordinator: Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig, Germany</i>	
<i>Contract number: IC18-CT95-0009</i>	337
Integrated control of ticks and tick-borne diseases (ICTTD concerted action project)	
<i>Co-ordinator: Utrecht University, Utrecht, The Netherlands</i>	
<i>Contract number: IC18-CT95-0003</i>	341
Application of recombinant DNA technology to vaccination diagnosis and epidemiology of tropical theileriosis	
<i>Co-ordinator: University of Glasgow, Glasgow, United Kingdom</i>	
<i>Contract number: TS3-CT92-0119</i>	358
Increase in goat production: reproduction and nutrition	
<i>Co-ordinator: Istituto Sperimentale Italiano "L. Spallanzani", Milano, Italy</i>	
<i>Contract number: TS3-CT92-0151</i>	345
The African horse sickness virus epidemic in Morocco	
<i>Co-ordinator: Institute for Animal Health, Woking Surrey, United Kingdom</i>	
<i>Contract number: TS3-CT92-0106</i>	356
Definition and criteria for the use of immunodiagnosis in the field for prevailing parasite diseases of extensively farmed livestock	
<i>Co-ordinator: Institut National de la Recherche Agronomique, Nouzilly, France</i>	

	Page
<i>Contract number: TS3-CT92-0143</i>	348
Mechanisms of pathogenesis and immunity in tropical Theileriosis: their relevance to vaccine development and disease control	
<i>Co-ordinator: University of Edinburgh, Edinburgh, United Kingdom</i>	
<i>Contract number: TS3-CT91-0019</i>	358
Characterisation of protective antigens and their genes from theileria annulata: application to sub-unit vaccines, epidemiology and diagnosis	
<i>Co-ordinator: University of Glasgow, Glasgow, United Kingdom</i>	
<b>2.4. Agriculture related topics</b>	
<i>Contract Number: IC18-CT98-0390</i>	366
The study of Atriplex halimus as a genetic resource in silvopastoral use and in the restoration of damaged rangelands in arid and semi-arid Mediterranean zones	
<i>Co-ordinator: Université de Paris-Sud XI, France</i>	
<i>Contract number: IC18-CT98-0391</i>	364
Safety assessment of the release of transgenic crops: spread of herbicide-resistance genes from wheat and foxtail millet to weedy species	
<i>Co-ordinator: Institut National de la Recherche Agronomique, Dijon, France</i>	
<i>Contract number: IC18-CT98-0313</i>	368
Improving French bean cultivation under semi-arid conditions by constructing acid and salt tolerant rhizobial N <sub>2</sub> -fixing symbionts for plant inoculation (PHIMED)	
<i>Co-ordinator: Rheinisch-Westfälische Technische Hochschule, Aachen, Germany</i>	
<i>Contract number: IC18-CT98-0311</i>	370
Stable yields in Mediterranean barley: application of molecular technologies in improving drought tolerance and mildew resistance	
<i>Co-ordinator: SCRI, Invergowrie, Dundee, Scotland, UK</i>	
<i>Contract number: IC18-CT98-0310</i>	373
Prospection, characterisation and assessment of apricot genetic resources in the Mediterranean region for the production in arid and semi-arid areas	
<i>Co-ordinator: Instituto Agronomico Mediterraneo de Zaragoza, Zaragoza, Spain</i>	
<i>Contract number: IC18-CT980308</i>	376
Date palm ( <i>Phoenix dactylifera</i> L.): improvement and development of palm groves via tissue culture and molecular biology tools	
<i>Co-ordinator: Laboratoire de Recherches en Physiologie Végétale (L.R.P.V.), Angers, France</i>	
<i>Contract number: IC18-CT98-0301</i>	378
Saltmed: a systems approach to a sustainable increase in irrigated vegetable crop production in salinity-prone areas of the Mediterranean region	
<i>Co-ordinator: Plant Stress Unit, University of Sussex, UK</i>	
<i>Contract number: IC18-CT98-0300</i>	381
Yield stability and resistance of faba bean to major pathogens in Western Mediterranean basin (FRYMED)	
<i>Co-ordinator: Institut National de la Recherche Agronomique, Le Rheu, France</i>	

	Page
<i>Contract number: IC18-CT97-0198</i>	383
Integrated control of crown gall in Mediterranean countries (Cg-Med)	
<i>Co-ordinator: Université Claude Bernard – Lyon I, Villeurbanne, France</i>	
<i>Contract number: TS3-CT93-0208</i>	394
Exploration of new findings in insect endocrinology and physiology for developing novel ways of locust control	
<i>Co-ordinator: Katholieke Universiteit Leuven, Leuven, Belgium</i>	
<i>Contract number: TS3-CT94-0264</i>	386
Etude de la diversité biologique de l'Atriplex halimus pour le repérage in vitro et in vivo d'individus résistants à des conditions extrêmes du milieu et constitution de clones	
<i>Co-ordinator: Université de Paris-Sud XI, Chatenay-Malabry, France</i>	
<i>Contract number: TS3-CT93-0249</i>	390
Isolation and identification of pheromones and their mode of action in African locusts	
<i>Co-ordinator: Universität Hannover, Hannover, Germany</i>	
<i>Contract number: TS3-CT92-0015</i>	—
Selection and characterisation of natural isolates of lactic acid bacteria in African cheeses with specific reference to salt tolerance, bacteriophage resistance and impact on product quality	
<i>Co-ordinator: Universität Hohenheim, Stuttgart, Germany</i>	
<i>Contract number: AVI2-CT93-080</i>	400
Analysed climatology of rainfall obtained from satellite and surface data for the Mediterranean region (across) - a version for the Eastern Mediterranean region	
<i>Co-ordinator: University of Genova, Genova, Italy</i>	
<i>Contract number: AVI-CT93-0010</i>	397
Monthly and seasonal forecasts of rainfall cycle over the Mediterranean basin (ELMASIFA)	
<i>Co-ordinator: Groupement d'intérêt Public Medias-France, Toulouse, France</i>	
<i>Contract number: CII-CT93-0006</i>	402
Microbial activity in the rhizosphere in relation to the iron nutrition of plants	
<i>Co-ordinator: Universität Hohenheim, Stuttgart, Germany</i>	
<b>3. Health</b>	
<b>3.1. Public health / research</b>	
<i>Contract number: IC18-CT98-0352</i>	490
Feasibility and effects of shifting the mix of tertiary care, primary care and preventive and promotion in dealing with cardiovascular disease in Lebanon and Turkey	
<i>Co-ordinator: American University of Beirut, Medical Center, Beirut, Lebanon</i>	

	Page
<p><i>Contract number IC18-CT98-0349</i>  Hospital near-miss enquiries as a strategy to improve the quality of obstetric care in Benin, Ivory Coast and Morocco  <i>Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom</i></p>	493
<p><i>Contract number: IC18-CT98-0346</i>  The practice of health care reform: lessons for the future  <i>Co-ordinator: Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium</i></p>	496
<p><i>Contract number: AVI-CT94-0010</i>  Advanced disinfection and health care aspects of wastewater reclamation and re-use agriculture in Mediterranean regions  <i>Co-ordinator: Community of Mediterranean Universities, Bari, Italy</i></p>	499
<p><i>Contract number: AVI-CT94-0003</i>  Maximising maternal health strategies to reduce maternal morbidity and mortality in the primary health care sector  <i>Co-ordinator: London School of Hygiene and Tropical Medicine, London, United Kingdom</i></p>	503
<p><i>Contract number: AVI2-CT93-031</i>  Evaluation &amp; improvement of maternal and child preventive resources &amp; services of the Palestinians in the Gaza strip and of the Bedouin Arabs in the Negev (Israel)  <i>Co-ordinator: London School of Hygiene And Tropical Medicine, United Kingdom</i></p>	507
<p><i>Contract number: AVI-CT93-0012</i>  Prise en charge de problèmes de santé chroniques et leur implication dans l'organisation des soins de santé  <i>Co-ordinator: Prins Leopold Instituut voor Tropische Geneeskunde, Antwerpen, Belgium</i></p>	509
<p><i>Contract number: AVI-CT93-0011</i>  Spatial, medical, epidemiological, economical and socio-cultural key factors and treatment of chronic health problems in Maghreb cities  <i>Co-ordinator: Institut Français de Recherche Scientifique pour le Développement en Coopération, Paris, France</i></p>	512
<p><i>Contract number: TS3-CT94-0282</i>  Effet de l'ingestion des produits laitiers fermentés sur la capacité immunitaire des sujets bien nourris et malnourris  <i>Co-ordinator: Instituto de Nutrición y Bromatología, Madrid, Spain</i></p>	514
<p><i>Contract number: TS3-CT92-0144</i>  An applied interdisciplinary research project to investigate the utilisation and perception of health care systems by infants and their families  <i>Co-ordinator: Centre International de l'Enfance, Paris, France</i></p>	517
<p><i>Contract number: TS3-CT92-0112</i>  Identification des conditions d'amélioration de la référence/contre-référence dans les districts de santé  <i>Co-ordinator: Institut National d'Administration Sanitaire, Rabat, Morocco</i></p>	519
<p><i>Contract number: TS3-CT92-0088</i>  Health and the current economic crisis in Brazil: the impact on the health and care of mothers and children  <i>Co-ordinator: Escuela Andaluza de Salud Publica, Granada, Spain</i></p>	523

	Page
3.2. Disease specific research	
<i>Contract number IC18-CT98-0367</i>	526
CD's for DC's: development of the compact disc (CD) as a novel, cost-effective & versatile platform for immunoassays for infectious diseases	
<i>Co-ordinator: Glasgow University, Institute of Biomedical and Life Sciences, Glasgow, United Kingdom</i>	
<i>Contract number: IC18-CT98-0354</i>	531
Cystic echinococcosis (hydatidosis) in the Eastern Mediterranean and Middle East- diagnostic tools for public health and epidemiology	
<i>Co-ordinator: University of Salford, Department of Biological Sciences, Salford M5 4wt, UK</i>	
<i>Contract number: IC18-CT96-0036</i>	533
A proposal to assess the impact on families and state of traumatic injury related disability among adults in Lebanon and the Occupied Territories	
<i>Co-ordinator: University of Cambridge, Cambridge, United Kingdom</i>	
<i>Contract number: IC18-CT95-0023</i>	536
Development and immunological evaluation of vaccine for canine visceral leishmaniasis	
<i>Co-ordinator: Royal Tropical Institute, Amsterdam, The Netherlands</i>	
<i>Contract number: IC18-CT95-0004</i>	538
<i>Theileria annulata</i> macrophage-infected cells in vaccination and disease	
<i>Co-ordinator: University of Edinburgh, Edinburgh, United Kingdom</i>	
<i>Contract number: AVI-CT94-0001</i>	540
Monitoring water for contamination by schistosomes: development and field testing of new technologies and approaches	
<i>Co-ordinator: Universität Heidelberg, Heidelberg, Germany</i>	
<i>Contract number: AVI2-CT93-107</i>	542
<i>Cryptosporidium</i> oocyst wall protein (COWP): a tool for studying parasite host-cell interactions & designing prevention measures against <i>cryptosporidium</i> infection	
<i>Co-ordinator: Università di Roma La Sapienza, Roma, Italy</i>	
<i>Contract number: AVI-CT93-0004</i>	548
Environmental control of schistosomiasis in irrigation schemes of the Mediterranean region	
<i>Co-ordinator: Prins Leopold Instituut voor Tropische Geneeskunde, Antwerp, Belgium</i>	
<i>Contract number: AVI-CT92-0018</i>	550
Rodent ecology for the epidemiology and control of cutaneous leishmaniasis in North Africa and West Asia	
<i>Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom</i>	
<i>Contract number: AVI-CT93-0014</i>	544
Health systems and the prevention of genetic diseases: application to hemoglobin disorders	
<i>Co-ordinator: Hospital Henri Mondor, Creteil, France</i>	

	Page
<i>Contract number: AVI-CT93-0008</i> Entamoeba histolyca: parasite and host determinants of tissue invasion <i>Co-ordinator: Universität Tübingen, Tübingen, Germany</i>	546
<i>Contract number: AVI-CT92-0013</i> Variabilité Génétique de l'infantum, agent de la Leishmaniose viscérale: corollaires épidémiologiques <i>Co-ordinator: ORSTOM, Montpellier, France</i>	552
<i>Contract number: AVI-CT92-0010</i> A new vector serving antigen preparation for diagnosis, vaccination and epidemiological surveillance of L. infantum and L. major <i>Co-ordinator: Vrije Universiteit Brussel, Brussel, Belgium</i>	555
<i>Contract number: AVI-CT92-0009</i> Molecular genetics of familial Mediterranean fever <i>Co-ordinator: Son Dureta Hospital, Palma De Mallorca, Spain</i>	557
<i>Contract number: AVI-CT92-0003</i> Epidemiology, diagnosis and control of leishmaniasis in the Mediterranean region <i>Co-ordinator: Royal Tropical Institute, Amsterdam, The Netherlands</i>	559
<i>Contract number: AVI-CT92-0002</i> Molecular epidemiology of hemoglobin, molecular biology of globin gene expression and prevention of thalassemia <i>Co-ordinator: University of Cagliari, Cagliari, Italy</i>	562
<i>Contract number: TS3-CT93-0253</i> Comparative evaluation of classical and molecular tools for the diagnosis and for eco-epidemiological investigations of leishmaniasis <i>Co-ordinator: Institut Pasteur de Tunis, Tunis-Belvédère, Tunisie</i>	565
<i>Contract number: TS3-CT93-0244</i> Molecular mechanisms of genetic variability in the expression of major hemoglobinopathies: prognostic value of genetic factors and therapeutic perspectives <i>Co-ordinator: Hopital Robert Debré, Paris, France</i>	569
<i>Contract number: CII-CT94-0126</i> Phototherapeutic potential of cell-directed (bacterio) chlorophyll conjugates <i>Co-ordinator: Universität München, München, Germany</i>	572
<i>Contract number: CII-CT94-0122</i> Molecular genetics of apoe, ace & agt & their effects on cardiovascular disease & carotid stenosis <i>Co-ordinator: Imperial College of Science, Technology And Medicine, London, United Kingdom</i>	574
<i>Contract number: CII-CT93-0005</i> Dopaminergic involvement in latent inhibition as an animal model of attentional dysfunction in schizophrenia <i>Co-ordinator: University of London, London, United Kingdom</i>	577

	Page
4. Additional fields of mutual interest	
4.1. Information and communication technologies	
<i>Contract number: IC18-CT96-1791</i>	588
Short term achievement of a corpus-based multilingual basic arabic lexical DB and related resource-productive tool-box (DIINAR-MBC)	
<i>Co-ordinator: Université Lumière-Lyon II, France</i>	
<i>Contract number: IC18-CT96-1301</i>	585
A Euro Mediterranean project for the development of upgraded science and engineering education in Southern Mediterranean universities through the use of telematics technologies	
<i>Co-ordinator: U.N.E.S.C.O. Cairo, Egypt</i>	
<i>Contract number: IC18-CT96-1263</i>	591
IRS-based document localisation (IDOL)	
<i>Co-ordinator: EPOS Etudes et Programmation en Optimisation et Software, France</i>	
<i>Contract number: IC18-CT95-1139</i>	596
High-performance computing for financial planning under uncertainty (HPC-Finance)	
<i>Co-ordinator: University of Cyprus, Nicosia, Cyprus</i>	
<i>Contract number: IC18-CT95-0905</i>	594
ARAMED: extension and integration of arabic lingware components in a unification-based MR system for the field of medical terminology and classification	
<i>Co-ordinator: Universität des Saarlandes, Saarbrücken, Germany</i>	
<i>Contract number: IC18-CT95-0895</i>	599
Peace by high performance computing (HPC)	
<i>Co-ordinator: Parsytec Computer GMBH, Aachen, Germany</i>	
<i>Contract number: IC18-CT95-0809</i>	602
Gaia: a multi-media tool for natural resources management and environmental education	
<i>Co-ordinator: Environmental Software and Services, Gumpoldskirchen, Austria</i>	
<i>Contract number: IC18-CT95-0363</i>	605
Telesun - a world wide multimedia teleteaching system for universities	
<i>Co-ordinator: Université Joseph Fourier, Grenoble, France</i>	
<i>Contract number: IC18-CT95-0175</i>	607
Arabic english french software localisation tool (AREF)	
<i>Co-ordinator: Bull S.A., Les Clayes-sous-Bois, France</i>	
<i>Contract number: AVI2-CT93-091</i>	627
Rainfall forecasts and strategic irrigation management	
<i>Co-ordinator: Società di Ricerca e Servizi di Ingegneria (ISMES) S.P.A., Bergamo, Italy</i>	
<i>Contract number: AVI2-CT93-080</i>	630
Analysed climatology of rainfall obtained from satellite and surface data for the Mediterranean region (ACROSS) - a version for the Eastern Mediterranean region	
<i>Co-ordinator: University of Genova, Genova, Italy</i>	

	Page
<i>Contract number: C11-CT93-0004</i> Semi-shift-invariant operations for optical computing <i>Co-ordinator: The Weizmann Institute of Science, Rehovot, Israel</i>	633
<i>Contract number: ITDC 135-82159</i> Parallel computing applied to geographic information system <i>Co-ordinator: Faculté des Sciences de Tunis, Tunis, Tunisia</i>	613
<i>Contract number: ITDC 204-82166</i> Unstructured domain mapping for distributed memory architectures <i>Co-ordinator: Bilkent University, Ankara, Turkey</i>	611
<i>Contract number: EC-ISR-93003</i> Shape and motion <i>Co-ordinator: Aalborg University, Aalborg, Denmark</i>	614
<i>Contract number: EC-MED-35</i> Exploiting genetic algorithms by optimising industrial site clustering and telecommunication network <i>Co-ordinator: Cap Volmac B.V., Utrecht, The Netherlands</i>	616
<i>Contract number: ITDC-94-201-82164</i> Distributed object oriented numerical software (DOONS) <i>Co-ordinator: Université Mohamed I, Oujda, Morocco</i>	609
<i>Contract number: EC-ISR 90</i> Bottom-up analysis of logic programming languages: theory, practice and applications <i>Co-ordinator: Università di Pisa, Pisa, Italy</i>	619
<i>Contract number: KIT Nr. 204</i> Surface modelling system <i>Co-ordinator: Universität Stuttgart, Stuttgart, Germany</i>	622
<i>Contract number: TEDIPP</i> Electronic data interchange <i>Co-ordinator: Edfrance, Paris, France</i>	626
<i>Contract number: KIT Nr. 12</i> Compucyprus <i>Co-ordinator: University of Leeds, Leeds, United Kingdom</i>	624
<i>Contract number: ICA-17</i> Cooperation in VLSI - circuit design training <i>Co-ordinator: InterActional Microelectronic Center (IMEC), Leuven, Belgium</i>	635
<b>4.2. Biotechnology</b>	
<i>Contract number: TS3-CT92-0096</i> Protein components of chemoreceptor organelles and of the cuticle of nematodes: identification and cloning of the genes, production as recombinant proteins and analysis of the immune response elicited <i>Co-ordinator: Istituto Internazionale de Genetica e Biofisica, Napoli, Italy</i>	680



	Page
<i>Contract number: CII-CT94-0131</i>	652
New approaches to localise and sustain drug release in the colon <i>Co-ordinator: University of Nottingham, Nottingham, United Kingdom</i>	
<i>Contract number: CII-CT94-0130</i>	654
Microtubule-associated proteins as diagnostic determinants and therapeutical targets for neuroblastoma tumors <i>Co-ordinator: Max-Planck-Institut für Hirnforschung, Frankfurt/Main, Germany</i>	
<i>Contract number: CII-CT94-0108</i>	656
Biosynthesis of new pyrimidine derivatives in actinomycin-producing streptomyces formed as a response to stress their role and function <i>Co-ordinator: Technical University of Berlin, Berlin, Germany</i>	
<i>Contract number: CII-CT94-0106</i>	658
Interspecific & intergeneric protoplast fusion in red algae <i>Co-ordinator: University of Tübingen, Tübingen, Germany</i>	
<i>Contract number: CII-CT94-0097</i>	660
Structure-function studies of enzymes involved in chitin degradation <i>Co-ordinator: European Molecular Biology Laboratory, Hamburg, Germany</i>	
<i>Contract number: CII-CT94-0080</i>	663
Assembly and degradation of the Cytochrome B-F complex in chloroplasts of higher plants <i>Co-ordinator: University of Cambridge, Cambridge, United Kingdom</i>	
<i>Contract number: CII-CT94-0779</i>	664
Characterisation and development of bioadhesive controlled drug delivery systems based on modified polysaccharides <i>Co-ordinator: University of Gent, Gent, Belgium</i>	
<i>Contract number: CII-CT94-0105</i>	668
Structural studies and computer simulations of substrate and inhibitor binding to Acetylcholinesterase <i>Co-ordinator: Université Libre de Bruxelles, Bruxelles, Belgium</i>	
<i>Contract number: CII-CT94-0085</i>	670
Biogenesis and stability of the photosynthetic apparatus in Gymnosperms under normal and stress conditions <i>Co-ordinator: Université de Liège, Liège, Belgium</i>	
<i>Contract number: CII-CT93-0074</i>	672
Molecular analysis of the Gibberellin-regulated gene expression in petunia flowers <i>Co-ordinator: Free University of Amsterdam, Amsterdam, The Netherlands</i>	
<i>Contract number: CII-CT93-0066</i>	675
New electrically conducting organic materials: design, synthesis and characterisation <i>Co-ordinator: Universität Tübingen, Tübingen, Germany</i>	
<i>Contract number: CII-CT93-0003</i>	677
Development of a packed bed bioreactor using twisted ribbons of polystyrene for the cultivation of mammalian cells <i>Co-ordinator: Weizmann Institute of Science, Rehovot, Israel</i>	

	Page
<i>Contract number: CII-CT91-0932</i> Genetic and physical mapping of the tomato I2 locus <i>Co-ordinator: Weizmann Institute of Science, Rehovot, Israel</i>	
<i>Contract number: CII-CT91-0907</i> Distribution comparison between coliphages and anaerobic bacteria phages in water sources <i>Co-ordinator: University of Barcelona, Barcelona, Spain</i>	683
<b>4.3. Materials and production technologies</b>	
<i>Contract number: IC18-CT95-0507</i> Integrated gas flow and gas sensors by using porous silicon micromachining <i>Co-ordinator: Institute of Microelectronics, Athens, Greece</i>	688
<i>Contract number: IC18-CT95-0410</i> Quality control in the Middle East (QCIME) <i>Co-ordinator: IT Consult GmbH, Lilienthal, Germany</i>	690
<i>Contract number: AVI-CT92-0012</i> The development of environmental emission control catalysts <i>Co-ordinator: Brunel University, Middlesex, United Kingdom</i>	712
<i>Contract number: CII-CT94-0125</i> Structure and chemistry of air pollutants on metal surfaces <i>Co-ordinator: University of Cambridge, Cambridge, United Kingdom</i>	693
<i>Contract number: CII-CT93-0070</i> Electrochemical control of silicon surfaces for electronic and solar applications <i>Co-ordinator: Centre National de la Recherche Scientifique, Meudon, France</i>	702
<i>Contract number: CII-CT93-0069</i> Irradiation-induced enhancement of critical currents in high-TC superconductors <i>Co-ordinator: Centre National de la Recherche Scientifique, Palaiseau, France</i>	714
<i>Contract number: CII-CT93-0313</i> Epitaxial growth of wide gap semiconductors (Al, Ga) N for optoelectronics <i>Co-ordinator: Centre de Recherche sur l'Hétéroépitaxie et ses Applications (CRHEA-CNRS), Valbonne, France</i>	697
<i>Contract number: CII-CT93-0312</i> Development of large area gaseous imaging photomultipliers for applications in nuclear medicine <i>Co-ordinator: Ecole Polytechnique, Palaiseau, France</i>	699
<i>Contract number: CII-CT93-0311</i> Structure and properties of multicomponent L1 <sub>0</sub> intermetallics at ambient and high temperatures <i>Co-ordinator: Technion-Israel Institute of Technology, Haifa, Israel</i>	701
<i>Contract number: CII-CT93-2027</i> Microwave properties of high TC thin films and supraconductor-insulator composites <i>Co-ordinator: Ecole Normale Supérieure, Paris, France</i>	695

	Page
<i>Contract number: CII-CT93-0065</i>	707
Environmental challenges addressed with new electrode materials: diamond and diamond-like carbon films	
<i>Co-ordinator: Centre National de la Recherche Scientifique, Meudon, France</i>	
<i>Contract number: CII-CT92-0095</i>	716
Spatial light modulators for analog optical computing, in particular conoscopic holography	
<i>Co-ordinator: Hebrew University of Jerusalem, Jerusalem, Israel</i>	
<i>Contract number: CII-CT92-0096</i>	711
The confluence of confocal near-field microscopy: zooming with light to 50 nm resolution	
<i>Co-ordinator: University of Amsterdam, Amsterdam, The Netherlands</i>	
<i>Contract number: CII-CT92-0063</i>	717
Effects of implantation on growth, defect formation and doping of diamond	
<i>Co-ordinator: Technion-Solid State Institute, Haifa, Israel</i>	
<i>Contract number: CII-CT91-0931</i>	719
Study of SI-GE layers epitaxially grown on SI by ion beam sputter deposition	
<i>Co-ordinator: Université Paris XI, Orsay, France</i>	
<i>Contract number: CII-CT91-0923</i>	723
Improvement of combustion processes by swirling flows and turbulent recirculating flames	
<i>Co-ordinator: Tel Aviv University, Tel Aviv, Israel</i>	
<i>Contract number: CII-CT91-0927</i>	721
Quantitative radiographic, tomographic, holographic methods for nondestructive measurements of structural integrity of high strength engineering ceramics	
<i>Co-ordinator: Technical University of Denmark, Lyngby, Denmark</i>	
<i>Contract number: CII-CT90-0542</i>	725
Electro-optical studies of ferroelectric liquid crystals	
<i>Co-ordinator: Technische Hochschule Darmstadt, Darmstadt, Germany</i>	
<i>Contract number: CII-CT89-0442</i>	727
Optical bistability in molecular systems	
<i>Co-ordinator: Universität Regensburg, Regensburg, Germany</i>	

	Page
4.4. Cultural heritage	
<i>Numéro de contrat: IC18-CT98-0386</i>	730
Euro-Med-Glacures: Etude physique et préservation des céramiques glacurées de l'espace Méditerranéen architecture-archéologie	
<i>Coordinateur: Université Michel de Montaigne – Bordeaux III, France</i>	
<i>Contract number: IC18-CT98-0385</i>	732
Integration of traditional and new techniques for the protection and conservation of historical and cultural built heritage in earthquake-prone areas.	
<i>Co-ordinator: Istituto di Ricerca sul Rischio Sismico, Italia</i>	
<i>Contract number: IC18-CT98-0384</i>	735
Study, characterisation and analysis of degradation phenomena of ancient, traditional and improved building materials of geologic origin used in construction of historical monuments in the Mediterranean area.	
<i>Co-ordinator: Universidad Autonoma de Barcelona, Espana</i>	

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## **Index of participating scientists**

---

A-Najjar-Khateeb, S.	AVI*CT94-0003
Abd-Alla, M.	AVI*CT93-0008
Abdel Gawad, S.	TS3*CT92-0061
Abdel-Gawad, S.	IC18-CT96-0069
Abdulwahab, M	AVI*CT94-0012
Abed, Y.	AVI2-CT93-031
	AVI*CT94-0003
Abosamra, F.	AVI2-CT93-112
Abou Basha, L.	AVI*CT92-0001
Abou-Hadid, A.	IC18-CT96-0082
Abou Rayan	IC18-CT97-0142
Abouhani, A.	IC18-CT98-0268
Abousalim, A.	TS3*CT93-0221
Abourough, M.	IC18-CT97-0197
Abouzaid, H.	AVI2-CT93-020
Abranches, P.	IC18-CT95-0023
	TS3*CT93-0253
	AVI*CT92-0003
Ackers, J.	AVI*CT93-0008
Adam, Z.	CI1*CT94-0080
Adar, E.	AVI*CT93-143
	IC18-CT97-0143
	IC18-CT97-0154
Added, A.	AVI2-CT93-099
Afrodisis, S.	AVI*CT93-0005
	IC18-CT97-0143
Agranat, A.J.	CI1*CT92-0095
Aguirre, R.	IC18-CT95-0809
Ahmed, J.S.	IC18-CT95-0009
	IC18-CT95-0004
Aich, A.E.	IC18-CT98-0392
Aïtchafa, D.	AVI*CT92-0013
Ait Tirri, L	IC18-CT98-0269
Akrimi, N.	TS3*CT92-0047
Aksoy, U.	AVI2-CT93-008
	IC18-CT98-0266
Alihonou, E	IC18-CT98-0349
Al-Homoud, A. S.	IC18-CT98-0385
Al Nahal, N.	AVI*CT94-0003
Al Qutob, R.	AVI*CT94-0003
Aladili, N	AVI*CT94-0003
Alados, C. L.	IC18-CT98-0392
Alami, R.	AVI2-CT93-087
Albergel, J.	IC18-CT96-0091
Abdez-Hafez, S.	IC18-CT98-0354
Alonso-Abella, M.	AVI*CT94-0004
Alouini, Z.	IC18-CT96-0076
Altinbilek, D.	AVI2-CT93-080
Altman, A.	IC18-CT97-0200
Alvar, J.	AVI*CT92-0013
Alvarez, J.M.	AVI*CT94-0011
Alvarez, M.R.	AVI*CT94-0013
Alyanak, I.	AVI*CT94-0005
	IC18-CT97-0167

Amato, E.	AVI2-CT93-087
Ambendet	TS3*CT92-0112
Ambrogetti, F.	AVI2-CT93-020
Amr, M.	AVI*CT94-0004
Angastiniotis, M.	AVI*CT92-0002
Angel, D.	AVI2-CT93-123
Angelakis, A.	AVI2-CT93-076
Anjarne, M.	IC18-CT98-0308
	IC18-CT98-0272
Annabi, M.	AVI2-CT93-054
Arbez, M.	IC18-CT97-0200
Argul	AVI*CT93-0001
Arinc, E.	AVI*CT93-0001
Armon, R.	CI1*CT91-0907
Arnal, P.	IC18-CT97-0161
Ashford, R.W.	AVI*CT92-0018
Asscher, M.	CI1*CT94-0125
Asselman, A.	AVI2-CT93-054
Assimadi, K.	TS3*CT92-0144
Asins, M. J.	IC18-CT98-0310
Assimacopoulos, D.	IC18-CT97-0142
	IC18-CT97-0138
Astraldi, M.	AVI*CT93-0003
Aswad, N.	AVI*CT94-0003
Atay-Kadiri, Z.	TS3*CT93-0208
Audergon, J-M.	IC18-CT98-0310
Auernheimer, C.	IC18-CT98-0268
Avnimelech, Y.	IC18-CT97-0202
Avriel, M.	IC18-CT95-1139
Awar, M.	IC18-CT98-0346
Axiak, V.	AVI*CT94-0010
Aykanat, C.	ITDC 204-82166
Azevado, S.	IC18-CT98-0293
Azmani, A.	AVI*CT94-0011
Bacci, A.	AVI*CT94-0003
	IC18-CT98-0349
Badawi, H.K.	IC18-CT96-0034
Badria, F.	AVI*CT93-0001
Bahnemann, D.	AVI*CT94-0007
	IC18-CT98-0267
Bahri, A.	AVI2-CT93-076
Bahri, H.	IC18-CT98-0311
Baille, A.	IC18-CT96-0082
Bak, R.P.M.	IC18-CT96-0034
Bakala	TS3*CT92-0112
Baker, J.	IC18-CT96-0122
Bakker, J.	AVI*CT93-143
Bakri, A.	TS3*CT93-0249
Ballester, A.Y.	AVI2-CT93-126
Bannister, J.V.	AVI*CT93-0002
	AVI*CT93-0067
Barea, J. M.	IC18-CT97-0197
Bariou, B.	AVI2-CT93-081
Bariteau, M.	IC18-CT97-0200

Barrault, J.	AVI2-CT93-083
Barrett, E.	AVI2-CT93-080
Barrocu, G.	AVI2-CT93-073
Barros, F.	TS3*CT92-0088
Bartolini, S.	IC18-CT98-0310
Bashour, H.	AVI*CT94-0003
Batista, A.A.	IC18-CT96-0039
Bayed, A.	IC18-CT98-0270
Bazza, M.	AVI*CT93-0009
Bazzicalupo, P.	TS3*CT92-0096
Beamish, D.	IC18-CT96-0122
Beauce, A.	IC18-CT96-0122
Bechtel, F.	IC18-CT98-0386
Becker, J.	CI1*CT93-0066
Becker, K.	IC18-CT98-0333
Behaj-Soulami, M.	AVI2-CT93-058
Bekiaroglou, P.	IC18-CT96-0039
Belazzoug, S.	AVI*CT92-0010
Belmaker, I.	AVI2-CT93-031
Belouali, R.	TS3*CT92-0144
Beltrao, J.G.	AVI2-CT93-008
	IC18-CT98-0266
Ben Ali, D.	IC18-CT98-0268
	IC18-CT98-0266
Ben Asher, J.	AVI2-CT93-008
Bencherifa, A.	IC18-CT97-0134
Ben Hamouda, M.H.	TS3*CT93-0208
Ben Ismael, R.	AVI2-CT93-107
	AVI*CT92-0018
	TS3*CT93-0253
Ben Lakhdar, Z.	AVI*CT92-0011
Ben M'Hamed, C.	AVI*CT93-0010
Ben Miled, L.	IC18-CT95-0003
Ben Moussa, H.	AVI2-CT93-126
Ben Naceur, M.	AVI*CT93-0007
Ben Salem, M.	IC18-CT98-0311
Ben Sassi	TEDIPP
Ben Thayer, B.	AVI*CT94-0002
Ben-Miled, L.	TS3*CT91-0019
Benabdallah, S.	EC-MED-35
Benchaabane, A.	IC18-CT98-0390
Benchikh, O.	AVI*CT94-0006
	IC18-CT97-0142
	IC18-CT97-0138
Benjama, A.	IC18-CT97-0198
Benrebaha, F.	TS3*CT94-0264
	IC18-CT98-0390
Bensaid, A.	IC18-CT95-0009
Benslimane, A.	TS3*CT92-0096
Bergaya, F.	AVI2-CT93-083
Berkovski, B.	IC18-CT97-0142
Berndtsson, R.	IC18-CT96-0091
Bernstein, J.	CI1*CT93-0066
Berrada, M.	TS3*CT94-0282



Bertan, M.	AVI*CT93-0012
Berthomé, J.P.	AVI*CT92-0015
Bertocchi, M.	IC18-CT95-1139
Bilgin, Y.	AVI*CT93-0012
	IC18-CT98-0352
Birks, H.	IC18-CT96-0029
Blazy, P.	AVI2-CT93-019
Bodo, J.M.	TS3*CT93-0244
Boelee, E.	AVI*CT93-0004
Boer, B.	IC18-CT96-0055
Boissonnat, J.D.	KIT Nr. 204
Bonazountas, M.	AVI*CT93-0006
Bontemps, N.	CI1*CT93-2027
Bonzel, H.	CI1*CT94-0125
Bordado, J.	AVI*CT94-0015
Borelli, E.	IC18-CT98-0384
Borg, M.	IC18-CT98-0268
Borri, D.	IC18-CT98-0268
Botros, R.	AVI*CT94-0004
Bouattour, A.	IC18-CT95-0009
Boubaker, A.	IC18-CT97-0198
Boughriba, M.	AVI2-CT93-073
Boulahdid, M.	AVI*CT93-0003
Boulard, C.	TS3*CT92-0106
Bourdeaud'hui, E.	ICA-17
Bourdelande, J.L.	IC18-CT96-0076
Boussema, M. R.	IC18-CT97-0154
Bouزيد, S.	TS3*CT94-0264
	IC18-CT98-0390
Bouznad, Z.	IC18-CT98-0300
Brabben, T.	AVI*CT94-0012
Brakenhoff, G.J.	CI1*CT92-0096
Branover, H.	AVI*CT92-0017
Braslavsky, S.E.	IC18-CT96-0076
Breer, H.	TS3*CT93-0249
Breskin, A.	CI1*CT93-0312
Brianso-Penalva, J.	IC18-CT98-0384
Brissaud, F.	AVI2-CT93-076
Brisset, P.	AVI2-CT93-087
Bouabdelli, M.	IC18-CT98-0384
Brown, C.	TS3*CT91-0019
Brown, C.G.D.	TS3*CT92-0143
Bruckstein, A.	EC-ISR-93003
Bucher, W.	AVI*CT94-0004
Burchard, G.D.	AVI*CT93-0008
Burrage, S.W.	IC18-CT96-0082
Burton, J.	AVI*CT94-0004
Butler, J.	AVI*CT94-0010
Cacador, I.	IC18-CT96-0055
Cahen, D.	AVI*CT94-0008
Caffyn, A.	IC18-CT98-0270
Calero Perez, R.	IC18-CT96-0099
Calvario-Martinez, O.	IC18-CT97-0202
Campbell, B.	IC18-CT95-0809

Campbell, O.	AVI*CT94-0003
Cao, A.	AVI*CT92-0002
Caligari, P.D.S.	IC18-CT97-0177
Capela, R.	TS3*CT92-0151
Capurro, A.	IC18-CT95-0809
Cariolou, M.	CI1*CT94-0122
Carrasco, J. E.	IC18-CT97-0163
Cassama, C.	TS3*CT93-0221
Cassar, J.	IC18-CT98-0384
Catanzano, J.	IC18-CT96-0064
Cauber, G.	IC18-CT98-0300
Cavallis, C.	IC18-CT95-0410
Cecchi, F.	AVI*CT94-0011
Chaabouni, Z.	AVI*CT94-0002
	IC18-CT98-0272
Chambouleyron, J.L.	TS3*CT92-0061
Champredon, C.	TS3*CT92-0119
Charfi, F.	IC18-CT98-0270
Chari, A.H.	CI1*CT93-0070
Chauvin, A.	TS3*CT92-0106
Chavel, P.	CI1*CT93-0004
Chazalviel, J.N.	CI1*CT93-0070
Chedly, A.	IC18-CT96-0055
Cheeseman, C.	AVI2-CT93-062
Cheman, M.N.	ITDC 204-82166
Chen, Y.	CI1*CT93-0006
Chiaromonti, C.	TEDIPP
Chorfi, A.	AVI*CT93-0007
Choukr-Allah, R.	AVI*CT94-0002
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	TS3*CT92-0126
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Christofides, C.	AVI*CT94-0008
	IC18-CT95-0507
	IC18-CT97-0171
Chueca, M-C	IC18-CT98-0391
Cita, M.B.	AVI*CT92-0007
Clementel, S.	AVI2-CT93-091
Coccossis, H.	IC18-CT98-02
Codish, M.	EC-ISR 90
Coelho, C. O.	IC18-CT97-0147
Coelho, S. T.	IC18-CT97-0136
Cohn, A.G.	KIT Nr. 12
Cokkinos, D.	CI1*CT94-0122
Combier, M.	AVI*CT92-0015
Constantinos, M.	AVI2-CT93-092
Cornelissen, A.	TS3*CT91-0019
Cornet, A.	IC18-CT97-0155
Correal, E.	IC18-CT98-0390
Cot, L.	AVI*CT92-0014
Craig, P. S.	IC18-CT98-0354
Cremonini, M.G.	AVI2-CT93-099
Crepon, M.	AVI*CT93-0003
Crisanti, A.	AVI2-CT93-107

Crowley, J.	EC-ISR-93003
Cuartero, J.	AVI2-CT93-008
	IC18-CT98-0266
	IC18-CT98-0313
Dafalla, G.	IC18-CT96-0121
Dakkak, A.	AVI*CT92-0010
Dalla Fontana, G.	IC18-CT96-0069
Dallemand, J. F.	IC18-CT97-0161
Damelincourt, J.J.	AVI2-CT93-054
Danish, A.	IC18-CT95-0410
Danlos, L.	IC18-CT95-0905
Danon, Y.	AVI*CT92-0009
Daoud, J.A.	AVI*CT94-0014
Daoudi, E.M.	ITDC 94-201-82164
Daoulati, A.	IC18-CT98-0386
Darghouth, M.A.	IC18-CT95-0009
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	IC18-CT95-0004
Darmency, H.	IC18-CT98-0391
Dauta, A.	IC18-CT98-0293
Davidov, D.	CH1*CT93-2027
	CH1*CT90-0542
Davis, S.S.	CH1*CT94-0131
De Breuck, W.	AVI2-CT93-073
De Hann, L.	IC18-CT97-0134
De Ketelaere, D.	AVI2-CT93-099
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De Loof, A.	TS3*CT93-0208
de Lorenzo, V.	CH1*CT94-0083
De Oliveira, D.E.	TS3*CT94-0278
De Namor, A. D.	IC18-CT98-0384
De Maisonneuve, P.	IC18-CT98-0385
De Smedt, F.	AVI2-CT93-073
De Waal, T.	IC18-CT95-0009
Debieche, M.	IC18-CT98-0386
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Dechaume, M.	AVI2-CT93-054
Deeb, M.	AVI*CT94-0003
Defez, R.	IC18-CT98-0313
del Mar Garcia Calvente, M.	TS3*CT92-0088
Dellagi, K.	AVI*CT93-0014
Deme, M.	IC18-CT96-0064
Demetropoulos, A.	AVI*CT92-0015
Demoen, B.	EC-ISR 90
Dempster, M.	IC18-CT95-1139
Deque, M.	AVI*CT93-0010
Derqaoui, L.	TS3*CT92-0119
Derraz, M.	IC18-CT98-0293
Derrick, A.	AVI*CT94-0004
Dessaux, Y.	IC18-CT97-0198
Devaney, E.	TS3*CT92-0096
Dewdar, A.	IC18-CT97-0163
Diaz Mora, E.	IC18-CT95-0809
Diels, L.	CH1*CT94-0083

Diez-Baños, P.	TS3*CT92-0106
Di Giulio, A.	IC18-CT98-0268
Djолоv, G.	IC18-CT95-0809
Doo, A.	IC18-CT98-0268
Do Rosario, V.	IC18-CT95-0009
Dobbelaere, D.	IC18-CT95-0003
Dore, M.	AVI2-CT93-083
Dou, Y.	IC18-CT96-0069
Doulis, A.	IC18-CT97-0153
Douglas, G.	TS3*CT94-0278
Drago, A.	AVI*CT92-0015
Drevon, J.J.	IC18-CT96-0081
Duhoux, E.	TS3*CT94-0278
Duivenbooden, N.V.	IC18-CT96-0069
Dujardin, B.	TS3*CT92-0112
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Dupacova, J.	IC18-CT95-1139
Dutuit, P.	TS3*CT94-0264
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Duvent, J.L.	AVI*CT92-0011
Echihabi, L.	IC18-CT97-0167
Edmunds, W.M.	AVI*CT93-0015
Einav, S.	IC18-CT95-0895
	CII*CT91-0923
Ellis, R. P.	IC18-CT98-0311
Eizenberg, M.	CII*CT91-0931
Eklundh, J.O.	EC-ISR-93003
El Alem, A.	AVI*CT94-0003
El Gamal, A. S.	IC18-CT98-0311
El Hamouri, B.	AVI*CT94-0002
	AVI*CT94-0010
	IC18-CT97-0163
El Harradji, A.	IC18-CT97-0134
El Jaafari, S.	AVI*CT93-0007
El Jani, B.	CII*CT93-0313
El Kadi, M.	IC18-CT96-0055
El Midaoui, A.	AVI2-CT93-081
El Shaer, H.	IC18-CT96-0055
El Soda, M.	TS3*CT92-0015
El-Assouty, I.	AVI2-CT93-091
El-Deeb, A.	AVI*CT93-0013
El-Gohary, F.	AVI*CT94-0009
El-Fouly, M.	IC18-CT98-0266
El-Mowehlhi, N.	AVI*CT92-0006
El-On, J.	IC18-CT98-0354
El-Quosy, A.	AVI2-CT93-091
El-Raey, M.	IC18-CT95-0809
El-Sebaie, O.	AVI2-CT93-062
Eleftheriou, A.	AVI*CT92-0001
Elion, J.	TS3*CT93-0244
Elloumi, M.	IC18-CT98-0268
Engell, J.	CII*CT91-0927
Ennabli, N.	AVI2-CT93-073
Enne, G.	TS3*CT92-0119

Escadafal, R.	IC18-CT98-0268
Essatara, M.	AVI*CT92-0008
Etman, M.	TS3*CT94-0282
Evangelista, E.	CII*CT93-0070
Evison, L.	CII*CT93-0311
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Ewida, K.T.	AVI*CT94-0005
Eyal, A.	AVI*CT94-0014
Failer, P.	IC18-CT96-0064
Falaschi, M.	EC-ISR 90
Fathi, A.A.	IC18-CT96-0029
Fattorelli, S.	AVI*CT93-0006
Faydi, Y.	IC18-CT97-0136
Fedra, K.	IC18-CT95-0809
Feldon, J.	CII*CT93-0005
Feinerman, E.	IC18-CT97-0142
Felice, A.	AVI*CT92-0002
Fernandez-Polanco, F.	AVI*CT94-0009
Ferrinho, P.	IC18-CT98-0346
Field, J.A.	CII*CT94-0086
Figueiredo, M. O.	IC18-CT98-0384
Fihri, A.F.	AVI*CT94-0006
Fikri, A.	TS3*CT92-0151
Filali, A.M.	TS3*CT94-0278
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Fischer, K.	AVI*CT94-0005
Fitzgerald, G.	TS3*CT92-0015
Fitzner, B.	IC18-CT98-0384
Fluhr, R.	CII*CT91-0932
Forster, B. P.	IC18-CT98-0311
Förstner, U.	AVI*CT92-0016
Fradi, A.	IC18-CT98-0268
Franck, F.	CII*CT94-0085
Francke, W.	TS3*CT93-0249
Fridlender, B.	IC18-CT98-0333
Freitas; H.	IC18-CT98-0392
Frihy, O.	AVI*CT93-0006
Frimmel, F.	IC18-CT97-0167
Fsadni, M.	AVI*CT94-0013
Funken, K.H.	AVI*CT94-0013
Gabriels, D.	IC18-CT98-0269
Gad; A. A.	IC18-CT97-0155
Gaibeh, A.	IC18-CT98-0313
Galili, G.	CII*CT94-0087
Gao, Q.	IC18-CT96-0069
Garcia, F.	IC18-CT95-0809
Garcia, J.L.	CII*CT92-0104
Garcia-Moreno, E.	IC18-CT96-0099
Gargouri, A.	CII*CT92-0104
Gavach, C.	AVI*CT92-0014
Gayraud, R-P.	IC18-CT98-0386
Gedeon, R.	AVI*CT93-0005
Gherissi, S.	AVI2-CT93-054

Ghrabi, A.	AVI*CT94-0007 IC18-CT98-0267
Gibart, P.	CI1*CT93-0313
Ginzburg, I.	CI1*CT94-0130
Girardie, A.	TS3*CT93-0208
Giro, R.	TS3*CT93-0244
Gitelson, A.	AVI*CT93-143 IC18-CT97-0154
Glaser, I.	CI1*CT93-0004
Glass, E.	IC18-CT95-0009 IC18-CT95-0004
Glekas, I.	AVI2-CT93-062 IC18-CT97-0142
Godbold, D.L.	IC18-CT96-0035
Goldfarb, D.	AVI*CT92-0012
Goldman, M.	IC18-CT96-0122
Golik, A.	AVI*CT92-0016
Golosovsky, M.	CI1*CT93-2027
Gomes, A.	IC18-CT98-0346
González, I.	AVI*CT94-0013
González, M. M. L.	IC18-CT97-0198
Goossens, M.	AVI*CT93-0014
Göral, V.	AVI*CT93-0008
Gordon, A.	TS3*CT92-0093
Görge, H.	TS3*CT92-0112
Gorman, A.	CI1*CT94-0126
Gorocho, O.	CI1*CT93-0070
Graber, E.	AVI*CT92-0006
Grandinetti, L.	IC18-CT95-1139
Grangaud, J.P.	TS3*CT92-0144
Granlund, G.	EC-ISR-93003
Granum, E.	EC-ISR-93003
Gray, J.	CI1*CT93-0005
Gray, J.C.	CI1*CT94-0080
Green, M.	AVI*CT94-0011
Gressel, J.	IC18-CT98-0391
Grego, S.	TS3*CT92-0047
Grimm, H.	AVI*CT94-0006
Gronenborn, B.	IC18-CT96-0121
Grouzis, M.	TS3*CT92-0047
Gryseels, B.	AVI*CT93-0004
Guariso, G.	IC18-CT95-0809
Guennoun, A.	AVI2-CT93-058
Gueye, A.	IC18-CT95-0009
Guizani, I.	AVI*CT92-0013
Gulcan, R.	IC18-CT98-0310
Gunay, G.	AVI2-CT93-072 IC18-CT97-0161
Gur, A.	AVI*CT94-0009
Gurria Gascon, J.L.	AVI2-CT93-126
Guter, N.	AVI*CT92-0012
Gutnick, D.L.	CI1*CT94-0083
Haase, W.	CI1*CT90-0542
Habela, M.A.	IC18-CT95-0009

Hadar, Y.	CII*CT93-0006
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Haddad, M.	IC18-CT96-0099
Haddouchi, B.	AVI2-CT93-073
Hadjichristophorou, M.	AVI2-CT93-123
Hajji, M.	IC18-CT96-0055
Halevy, A.	CII*CT93-0074
Halim Salem, M.	TS3*CT92-0061
Halim, A.A.	AVI*CT94-0003
Hall, F.R.	IC18-CT95-0009
	IC18-CT95-0003
	TS3*CT91-0019
Hall, M.A.	IC18-CT96-0082
Hallak, H.	AVI*CT94-0008
	IC18-CT97-0142
Hamad Wafa, A.A.	AVI*CT93-0004
Hamadi, R.	TS3*CT92-0119
Hamburger, J.	AVI*CT94-0001
	IC18-CT98-0354
Hamdy, A.	IC18-CT96-0055
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Hamers, R.	AVI*CT92-0010
Hamidat, A.	AVI*CT94-0004
Hammami, N.	IC18-CT98-0289
Hammou, O.	TS3*CT92-0143
Hampp, R.	CII*CT94-0106
Hamze, M.	IC18-CT97-0153
Hamza, A.	IC18-CT97-0147
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Hanack, M.	CII*CT93-0066
Harfouche, A.	IC18-CT97-0200
Harrison, P.G.	AVI*CT92-0012
Harrouni, S.	IC18-CT96-0055
Harpaz, S.	IC18-CT98-0333
Hashwa, F.	IC18-CT98-0136
Hegazi, N.	IC18-CT95-0905
Heitor, M.V.	CII*CT91-0923
Hejnen, H.	AVI2-CT93-020
Hermenegildo, M.	EC-ISR 90
Higgit, D.	IC18-CT98-0268
Himonas, C. A.	IC18-CT98-0354
Hoelmann, A.	IC18-CT96-0099
Homedan, M.	IC18-CT98-0308
Hötzl, H.	AVI2-CT93-072
Hours, B.	AVI*CT93-0011
Huang, J.	IC18-CT95-0809
Huchzermeyer, B.	IC18-CT96-0055
Huibers, F.	AVI*CT93-0004
Hüttermann, A.	IC18-CT97-0186
Ibrahimi, S.	AVI2-CT93-107
Icli, S.	AVI*CT94-0013
Ilana, B.	CII*CT94-0096
Iman, H. I.	IC18-CT98-0384
Inan, D.	AVI*CT94-0004

Inclan, U.	IC18-CT95-0809
Inel, Y.	AVI2-CT93-074
Inglebert, M.	IC18-CT95-0175
Ioannides, M.	KIT Nr. 204
Iordanou, G.	AVI*CT92-0017
Isik, F.	IC18-CT97-0200
Issa, M.	IC18-CT95-0175
Ita, E. O.	IC18-CT98-0331
Jackson, D.	IC18-CT97-0171
Jaffe, C.L.	IC18-CT95-0023
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Jiangxiong, M.	TS3*CT92-0093
Jimenez-Montealegre, R.	IC18-CT97-0202
Jofre, J.	CI1*CT91-0907
Jongegan, F.	IC18-CT95-0009
	IC18-CT95-0003
Jordan, A.	IC18-CT95-0895
Jori, G.	IC18-CT96-0076
Jourdane, J.	AVI*CT94-0001
Jrad-Fantar, A.	AVI2-CT93-087
Kabariti, M.	IC18-CT96-0039
	IC18-CT96-0099
	IC18-CT98-0289
Kabay, N.	AVI*CT94-0014
Kacem, R.B.	IC18-CT95-0175
Kachani, M.	IC18-CT95-0009
	IC18-CT95-0003
Kadi, M.	AVI*CT93-0010
Kadouri, A.	CI1*CT93-0003
Kafkafi, U.	IC18-CT98-0272
Kagan-Zur, V.	IC18-CT96-0035
Kakas, A.C.	KIT Nr. 12
Kaliakatsos, I.	IC18-CT96-0099
Kaliras, P.	IC18-CT98-0367
Kalish, R.	CI1*CT93-0065
	CI1*CT92-0063
Kallidromitou, D.	AVI*CT93-0006
Kallos, G.	AVI*CT92-0005
Kalogeras, N.	IC18-CT98-0385
Kandiyoti, R.	TS3*CT92-0093
Kassas, M.	AVI*CT94-0003
Katriel, J.	CI1*CT89-0442
Katsaros, D.	TS3*CT92-0119
Katzir, A.	CI1*CT93-0362
Kautek, W.	CI1*CT93-0065
Kaya, Z.	IC18-CT97-0200
Kegels, G.	AVI*CT93-0011
Keller, U.	CI1*CT94-0108
Kellner, R.	CI1*CT93-0362
Kewny, L.	IC18-CT97-0177
Kerdjoudj, H.	AVI*CT92-0014
Kes, P.H.	CI1*CT93-0069
Khallaayoune, K.	AVI*CT93-0004
Khamis, M.	IC18-CT98-0272



Khan, A.	IC18-CT96-0055
Kharrat, M.	IC18-CT98-0300
Khakee, A.	IC18-CT98-0268
Khatib, A.	AVI*CT94-0009
Khawlie, M.	IC18-CT97-0161
Khedr Fahmi, I.	IC18-CT98-0289
Kherbeche, A.	AVI2-CT93-083
Khlat, M.	IC18-CT96-0036
Khliat, H.	IC18-CT97-0198
Khogali, M.	AVI*CT93-0012
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Khouja, L.	IC18-CT97-0200
Khoury, J.	IC18-CT96-0091
Khosrof, S.	IC18-CT98-0385
	IC18-CT98-0384
Kimchie, S.	IC18-CT96-0099
Kinet, J.M.	TS3*CT94-0264
	IC18-CT98-0390
King, D.A.	CI1*CT94-0125
Kirsch, J.	CI1*CT94-0130
Kittler, J.	EC-ISR-93003
Kleinwächter, J.	AVI*CT94-0013
Kock, N.	IC18-CT95-0009
Koidl, P.	CI1*CT92-0063
Konczykowski, M.	CI1*CT93-0069
Koornneef, M.	CI1*CT91-0932
Köseoglu, G.	ICA-17
Kost, J.	CI1*CT94-0779
Kotea, N.	TS3*CT93-0244
Koudijs, A.	EC-MED-35
Kouklos, E.	IC18-CT97-0163
Kramvis, S.	IC18-CT96-0122
Krimai, Z.	IC18-CT97-0198
Krishnamoorthy, R.	TS3*CT93-0244
Kusel, J.	IC18-CT98-0367
Kutiefan, L.	IC18-CT98-0385
Kyritsis, S.	AVI*CT94-0002
Laabid, A.	TS3*CT92-0112
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Labat, M.	CI1*CT92-0104
Labed, L.	IC18-CT95-0175
Laborde, J.P.	AVI2-CT93-058
Laghezali, M.	IC18-CT98-0310
Lailhacar, S.	TS3*CT94-0264
Laouina, A.	IC18-CT98-0268
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Lanarhs, T.	IC18-CT98-0293
Lapidot, A.	CI1*CT94-0108
Larbot, A.	AVI*CT92-0014
Latif, A.A.	IC18-CT95-0009
Lazarides, D.	IC18-CT96-0039
Leblebici, D.	ICA-17
Leenaerts, R.	AVI2-CT93-081
Lehucher, P.M.	AVI2-CT93-058

Lema, J.M.	CI1*CT94-0086
Lemonnier, D.	TS3*CT94-0282
Lerberge	
Letouze, R.	IC18-CT98-0308
Lettinga, G.	AVI*CT94-0009
Leupold, D.	CI1*CT94-0126
Levanon, D.	CI1*CT94-0086
Levi, G.	EC-ISR 90
Levi, M.	AVI*CT93-0001
Levin, L.A.	CI1*CT93-0311
Levsen, K.	AVI*CT92-0004
Lévy-Clément, C.	CI1*CT93-0065
Lewando-Hundt, G.	AVI2-CT93-031
	AVI*CT94-0003
Lewis, A.	CI1*CT92-0096
Liberti, L.	AVI*CT94-0010
Lieth, H.	IC18-CT96-0055
Lindegaard-Andersen, A.	CI1*CT91-0927
Litz, N.	AVI*CT92-0006
Lloyd, D.H.	IC18-CT95-0009
Lluch, C.	IC18-CT96-0081
Lo Cicero Vaina, R.	IC18-CT96-0039
Lobo-Guerrero, J.	AVI*CT94-0004
Loizides, L.	AVI*CT92-0016
Loizidou, M.	AVI2-CT93-062
Lopez-Manzanares, F. V.	IC18-CT98-0385
Lorenz, N.	TS3*CT92-0112
Lounis, A.	AVI*CT92-0014
Luria, M.	AVI*CT92-0005
Luzzatto, L.	TS3*CT93-0244
Maass, E.	IC18-CT96-0035
Madkour, M.	IC18-CT96-0121
Madsen, H.	AVI*CT93-0004
Magan, N.	AVI*CT93-0067
Maggetti, M.	IC18-CT98-0386
Mahadin, K.	AVI2-CT93-020
Mahasneh, I.	IC18-CT95-0895
Mahjoub, Z.	ITDC 135-82159
Majali-Mahasneh, S.	AVI*CT94-0003
Makkouk, K.	IC18-CT96-0121
Malash, N.	IC18-CT98-0313
Malik, Z.	IC18-CT96-0076
Mameri, M.	AVI2-CT93-081
Mameri, N.	AVI2-CT93-081
Mammou, M. A.	IC18-CT98-0269
Manneback, P.	ITDC 94-201-82164
Maniatis, Y.	IC18-CT98-0386
Mannis, A.	IC18-CT95-0809
Mantell, S.	TS3*CT93-0221
	IC18-CT98-0308
Marcos, A.	TS3*CT94-0282
Marder, J.B.	CI1*CT94-0085
Marecos Da Monte, H.	AVI*CT94-0012
Margaris, N.	IC18-CT96-0055

Marouf, B.	AVI2-CT93-019
Marschner, H.	CI1*CT93-0006
Marques, J. C.	IC18-CT98-0270
Maselli, F.	IC18-CT97-0155
Martinez Beltran, J.	TS3*CT92-0061
Martinez, P.F.	IC18-CT96-0082
Martinez-Duart, J.M.	AVI*CT94-0008
Martinez-Pardo, R.	TS3*CT93-0208
Mata-Alvarez, J.	AVI-CT94-0011
Matthies, M.	IC18-CT96-0055
Maubois, J.L.	TS3*CT92-0119
Mavroyannopoulos, G.	IC18-CT98-0272
Mawajdeh, S.	AVI*CT94-0003
Mayer, O.	AVI*CT94-0004
Maza, J.A.	IC18-CT96-0069
Mazharsolook, E.	IC18-CT95-0410
Mbaye, A.	TS3*CT92-0126
Mc Lean, J.	ICA-17
McNeil, C.J.	AVI*CT93-0002
Mebrahtu, A.M.	TS3*CT92-0015
Mechergui, M.	AVI*CT93-0009
Megier, J.	IC18-CT97-0155
Mejdeddine Kraiem, M.	IC18-CT96-0029
Mellor, P.	TS3*CT92-0151
Menenti, M.	TS3*CT92-0061
	IC18-CT96-0069
Meric, E.	AVI*CT92-0007
Merzouk, A.	IC18-CT96-0091
Messad, D.	AVI2-CT93-083
Meyer, F.	CI1*CT91-0931
Micallef, A.	AVI*CT92-0015
Micallef, J.	ICA-17
Michaelidou, S.C.	AVI*CT92-0004
Michelozzi, M.	IC18-CT97-0200
Michel, B.	AVI2-CT93-099
Mignani, A.	IC18-CT97-0171
Millot, C.	AVI*CT93-0003
Mine, P.	CI1*CT93-0312
Mirelman, D.	AVI*CT93-0008
Mlika, M.	IC18-CT98-0310
Moawad, H.	IC18-CT98-0313
Mokhtari, A.	AVI*CT92-0011
Mokssit, A.	AVI*CT93-0010
Mol, J.N.V.	CI1*CT93-0074
Moletta, R.	AVI*CT94-0011
Monteiro Teixeira, J.L.	AVI*CT93-0009
Monteuuis, O.	TS3*CT94-0278
Moreno, J.	IC18-CT97-0154
Moreno-Lucas, F.	IC18-CT96-0091
Morgana, B.	IC18-CT98-0289
Mortier, L.	AVI*CT93-0003
Mosback, H.	AVI2-CT93-112
Moser, J.G.	CI1*CT94-0126
Motosh, N.	IC18-CT95-0895

Mottaleb, A.	CII*CT93-0070
Moua'Zen, M.	IC18-CT98-0386
Moulla, A.	AVI*CT93-0015
Moumni, Y.	IC18-CT97-0134
Mounir, L.	IC18-CT96-0064
Mousain, D.	IC18-CT97-0197
Moussa, H. B.	IC18-CT98-0270
Moussaria, H.	AVI2-CT93-126
Mueller, W-R.	IC18-CT97-0167
Muguruza, I.	AVI*CT94-0013
Müller, W.E.G.	AVI*CT93-0001
	IC18-CT96-0034
Muñoz, A.H.	AVI*CT94-0015
Murli, A.	IC18-CT95-1139
Muszkat, L.	AVI2-CT93-074
Nadifi, S.	AVI*CT93-0014
Nafa, K.	TS3*CT93-0244
Narkis, N.	AVI2-CT93-092
Nassiopoulos, A.	IC18-CT95-0507
Nasr, H.	IC18-CT97-0197
Nativ, R.	AVI2-CT93-072
Neiland, A.	IC18-CT96-0064
	IC18-CT98-0331
Neis, U.	AVI2-CT93-076
Neskakis, A.	IC18-CT96-0099
Nesme, X.	IC18-CT97-0198
Newton, C.	IC18-CT98-0311
Nicolaides, A.	CII*CT94-0122
Nigim, K.A.	AVI*CT94-0004
Nitayarumphong, S.	IC18-CT98-0346
Njock, J. C.	IC18-CT98-0331
Nychas, S.G.	AVI*CT92-0017
Nzingoula, S.	TS3*CT92-0144
Oppenheim, A.	CII*CT94-0097
Orecchia, P.	AVI*CT92-0001
Oron, G.	AVI2-CT93-076
Orthofer, R.	IC18-CT97-0161
Ortiz Uribe, I.	AVI*CT94-0014
Ortiz, S.E.	AVI*CT94-0014
Oskam, L.	IC18-CT95-0023
	AVI*CT92-0003
Othman, K.	TS3*CT93-0249
Ouassini, A.	IC18-CT98-0167
Ouassou, A.	IC18-CT97-0200
Ouazar, D.	AVI*CT93-143
Ouessar, M.	IC18-CT98-0269
Oueslati, A.	AVI2-CT93-019
Oussaid, F.	TS3*CT92-0106
Oweis, T.Y.	AVI2-CT93-080
Ozer, A. M.	IC18-CT98-0386
Ozbek, H.	IC18-CT98-0392
Özcel, A.	IC18-CT95-0023
	AVI*CT92-0003
Ozturk, M.	IC18-CT97-0153

Paggi, L.	AVI*CT92-0001
Paiva, M.R.	TS3*CT93-0249
Paloscia, S.	IC18-CT97-0154
Papadopoulos, I.	AVI*CT94-0002
	IC18-CT98-0272
Paperna, I.	AVI*CT92-0001
Paschaloudis, D.	IC18-CT96-0039
Patrick, S.	IC18-CT96-0029
Paul, R.	AVI*CT93-0007
Pavoni, B.	AVI2-CT93-087
Pearce, D.	KIT Nr. 12
Pease, C.	IC18-CT95-0905
Peduto, P.	IC18-CT98-0386
Pekmezci, M.	IC18-CT97-0177
Peleg, S.	EC-ISR-93003
Penzkofer, A.	CI1*CT89-0442
Pereira, L. S.	IC18-CT97-0169
Perry, C.C.	AVI*CT92-0012
Peter, L.	CI1*CT93-0070
Petrini, V.	IC18-CT98-0385
Pichat, P.	IC18-CT98-0267
Pinto de Lemos, E.E.	TS3*CT93-0221
Plomion, C.	IC18-CT97-0200
Preston, P.	IC18-CT95-0009
	TS3*CT92-0143
Priefer, U. B.	IC18-CT98-0313
Prieto, M.R.	IC18-CT96-0069
Pritschow, G.	KIT Nr. 204
Protogeropoulos, C.	AVI*CT94-0004
Puglia, A. P.	IC18-CT98-0300
Quazar, D.	IC18-CT97-0154
Quelhas Dos Santos, J.	AVI*CT94-0002
	TS3*CT92-0126
Quensiere, J.	IC18-CT98-0331
Quesada, A.	IC18-CT98-0293
Qunzhu, Z.	IC18-CT96-0069
Rafiq, M.	AVI*CT92-0014
Ragab, R.	IC18-CT96-0091
	IC18-CT98-0313
Ragaie, H.F.	CI1*CT93-0070
Rahimy, C.	TS3*CT93-0244
Ramdani, M.	IC18-CT96-0029
Ramzy, R.	AVI*CT94-0001
	IC18-CT98-0367
	IC18-CT98-0354
Rayyan, F.	AVI2-CT93-112
Razum, O.	AVI*CT93-0012
	IC18-CT98-0352
Ready, P.D.	TS3*CT93-0253
Rebella, C.M.	IC18-CT96-0069
Reichert, B.	AVI2-CT93-072
Remon, J.P.	CI1*CT94-0779
Reoulengar, G.	TS3*CT92-0112
Reysoo, F.	AVI*CT93-0013

Reysoo, H.P.	AVI*CT93-0013
Rietbrock, C.	ITDC 204-82166
Rinkevich, B.	AVI*CT93-0001
Rinkevich, B.	IC18-CT96-0034
Rishpon, J.	AVI*CT93-0002
Rodrigues Junior, C.J.	TS3*CT93-0221
Roig, B.J.	AVI*CT92-0009
Romana, J.	AVI2-CT93-126
Romana, L.A.	AVI2-CT93-087
Romano, D.	IC18-CT98-0268
Römheld, V.	CI1*CT93-0006
Ronsmans, C.	IC18-CT98-0349
Rosenthal, H.	AVI2-CT93-123
Rosenwaks, S.	CI1*CT94-0096
Rosier, J.	IC18-CT98-0384
Rossi, G.	IC18-CT97-0169
Rubinstein, A.	CI1*CT94-0131
Ruppel, A.	AVI*CT94-0001
	IC18-CT98-0367
Rusen, K.	IC18-CT98-0268
Sbay, H.	IC18-CT97-0200
Sadananda, R.	IC18-CT95-0809
Sadiki, M.	IC18-CT96-0081
	IC18-CT98-0300
Safi, M.	AVI*CT94-0015
Sagiv, Y.	EC-ISR 90
Sahnoun, H.	IC18-CT97-0155
Sala, M.	IC18-CT97-0147
Salameh, E.	AVI2-CT93-080
	IC18-CT97-0143
Salch, F.	AVI*CT94-0007
Salgot De Marçay, M.	AVI2-CT93-076
Sall, P.N.	TS3*CT92-0047
Salleh, M.	IC18-CT98-0333
Salleo, S.	IC18-CT97-0153
Salomon, Y.	CI1*CT94-0126
Salomons, W.	AVI*CT92-0016
Sammari, C.	AVI*CT93-0003
Samsunlu, A.	AVI*CT94-0015
San Roman, E.A.	IC18-CT96-0076
Sandini, G.	EC-ISR-93003
Sansur, M.	IC18-CT96-0036
Santiago, C.	IC18-CT98-0333
Sanchez Viesca, A. F.	IC18-CT98-0346
Sardo, V.	IC18-CT96-0055
Saubion, C.	AVI2-CT93-054
Sauthoff, G.	CI1*CT93-0311
Savage, M.	IC18-CT97-0161
Savoure, A.	IC18-CT97-0200
Sayadi, S.	CI1*CT92-0104
Sayed, S.	AVI*CT93-0013
Sayin, F.	TS3*CT92-0143
	IC18-CT95-0004
Scapini, F.	IC18-CT98-0270

Scheer, H.	CI1*CT94-0126
Schembri, P. J.	IC18-CT98-0270
Schiller, G.	IC18-CT97-0200
Scherz, A.	CI1*CT94-0126
Schmidt, G.H.	TS3*CT93-0249
Schnur, L.	AVI*CT92-0003
Sclarovsky, S.	CI1*CT94-0122
Seda T, I.	AVI*CT94-0015
Seferis, K.	IC18-CT95-0410
Segovia, M.	AVI*CT92-0010
Segura, J.	IC18-CT97-0177
Sen, K.	IC18-CT96-0036
Senhaji, F.A.	AVI*CT94-0013
Seoane-Prado, C.	CI1*CT93-0066
Sevuek	AVI*CT93-0001
Shaaban, D.A.	AVI*CT94-0003
Shamir, J.	CI1*CT91-0927
Sharma, K.	IC18-CT96-0069
Shatanawi, M.R.	AVI*CT94-0009
	IC18-CT97-0163
	IC18-CT97-0169
Shewry, P.R.	CI1*CT94-0087
Shiathas, A.	IC18-CT96-0122
Shihab-Eldin	IC18-CT98-0385
	IC18-CT98-0384
Shrestha, S.	IC18-CT95-0809
Shuval, H.	AVI*CT94-0010
Si-Salah, A.	AVI*CT94-0007
Sibai, A.	IC18-CT96-0036
Sicard, P.	IC18-CT95-0363
Siccardi, F.	AVI2-CT93-080
Sifi, B.	IC18-CT96-0081
Silva, M.S.	TS3*CT92-0106
Singh, V.P.	IC18-CT96-0069
Sirat, G.	CI1*CT92-0095
Sivan, A.	CI1*CT94-0106
Sivonen, K.	IC18-CT98-0293
Slah, N.	IC18-CT96-0091
Smith, B.	AVI*CT93-0005
Smith, D.	TS3*CT93-0253
Smulders, P.	AVI*CT94-0004
Sole-Benet, A.	IC18-CT97-0134
Somerfeld, A.	IC18-CT96-0099
Somers, H.	IC18-CT95-0175
Somper, J.	IC18-CT95-0809
Soulios, G.	IC18-CT97-0143
Sozen, N.	IC18-CT98-0268
Spath, K.	AVI*CT93-0001
Spier, R.	CI1*CT93-0003
Spiewak, I.	IC18-CT98-0289
Splizer, S.	CI1*CT89-0442
Spooner, R.	TS3*CT92-0143
	IC18-CT95-0004
Spronk, J.	IC18-CT95-1139

Sqetz, A.L.	IC18-CT95-0507
Stachurski, F.	IC18-CT95-0009
Stassinopoulos, G.	IC18-CT95-0895
Steele, P.	IC18-CT95-0809
Stephanou, E.G.	AVI2-CT93-092
Stern, A.	CI1*CT93-0311
Stroosnijder, L.	IC18-CT98-0269
Sukenik	IC18-CT98-0293
Sumption, K.	IC18-CT95-0009
Sussman, J.L.	CI1*CT94-0105
Süssmuth, R.	TS3*CT92-0015
Swings, J.	IC18-CT97-0167
Tabbal, C.	IC18-CT97-0142
Tacke, M.	CI1*CT93-0362
Taieb, G.	AVI*CT92-0011
Tait, A.	IC18-CT95-0009
	IC18-CT95-0003
	TS3*CT91-0019
Talaat, H.	IC18-CT95-0507
Talhouk, S. N.	IC18-CT97-0177
Talianker, M.	CI1*CT93-0311
Tan, M.A.	ICA-17
Tanago, M. G.	IC18-CT97-0147
Tavernier, S.	CI1*CT93-0312
Taylor, F.W.	IC18-CT96-0035
Tber, A.	TS3*CT92-0151
Teixeira Beltrao, J.G.	AVI2-CT93-076
Tenne, R.	CI1*CT93-0065
Tezcan, L.	AVI2-CT93-072
	IC18-CT98-0352
Theocharis, C.R.	AVI*CT92-0012
Thiam, I.	IC18-CT96-0064
Thiemann, W.	AVI2-CT93-074
This, D.	IC18-CT98-0311
Tibayrenc, M.	AVI*CT92-0013
Tobbeche, S.	AVI2-CT93-087
Tokgoz, G.	AVI*CT92-0009
Tomson, G.	IC18-CT98-0346
Tonella, G.	IC18-CT95-0809
Toraldo, G.	IC18-CT95-1139
Toulmin, C.	IC18-CT96-0069
Tourancheau, B.	ITDC 94-201-82164
Touri, A.	IC18-CT98-0385
Trad, M.	AVI*CT94-0012
Traverse, J.P.	AVI*CT94-0008
Trebst, A.	CI1*CT94-0085
Trichereau, J.	AVI2-CT93-058
Trilla-Arrufat, J.	AVI*CT94-0010
Tsoutsos, T.	IC18-CT97-0142
	IC18-CT97-0138
Tuberossa, R.	IC18-CT98-0311
Tuncel, G.	AVI*CT92-0005
Turki, C.	AVI*CT92-0008
Tur, M.	IC18-CT97-0171



Tursz, A.	TS3*CT92-0144
Tuzel, Y.	IC18-CT96-0082
Udluft, P.	IC18-CT97-0143
Uleimat, A.	IC18-CT97-0136
Ullman, S.	EC-ISR-93003
Urena, A.G.	AVI*CT92-0011
Uslu, O.	AVI*CT93-0006
van der Beek, K	CI1*CT93-0069
Van Der Borg, N.J.C.M.	AVI*CT94-0004
van der Veen, A.M.H.	TS3*CT92-0093
van Helden, W.	AVI*CT94-0004
Van Lerberghe, W.	AVI*CT93-0012
	IC18-CT98-0352
	IC18-CT98-0349
	IC18-CT98-0346
Van Montagu, M.	TS3*CT94-0278
Van Overstraeten, R.	ICA-17
Van Vuren, G.	AVI*CT93-0009
Varela, M.	IC18-CT98-0289
Varnavas, S.P.	AVI*CT92-0016
Vassilatos, V.	AVI*CT94-0006
Vaughan, P.	TS3*CT92-0088
Velarde, F.I.	TS3*CT92-0106
Vendramin, G.	IC18-CT97-0200
Verdou, J.P.	AVI*CT93-0010
Verreth, J.	IC18-CT97-0202
Vetten, H.J.	IC18-CT96-0121
Vidal-Madjar, D.	IC18-CT97-0154
Vladimirou, H.	IC18-CT95-1139
Vogele, T.	IC18-CT95-0809
Vogelpohl, A.	AVI*CT94-0007
	IC18-CT98-0267
Von Goldammer, E.	EC-MED-35
Vorgias, C.E.	CI1*CT94-0097
Vorst, T.	IC18-CT95-1139
Wahab Amer, A.	IC18-CT96-0069
Wahab, A.A.	IC18-CT95-0895
Wang, H.	IC18-CT98-0391
Wang, J.	IC18-CT96-0069
Wang, T.	IC18-CT98-0391
Warembourg, F.	TS3*CT92-0047
Weiner, I.	CI1*CT93-0005
Welffens-Ekra	IC18-CT98-0349
Werner, E.D.	AVI*CT94-0015
Whitfield, G.	AVI*CT94-0004
Wilchek, M.	CI1*CT93-0003
Wilquin, H.	IC18-CT98-0385
Winkelbauer, L.	IC18-CT95-0809
Wittig, T.	IC18-CT95-0410
Wodak, S.	CI1*CT94-0105
Wolfrum, J	CI1*CT94-0096
Xanthoulis, D.	AVI*CT94-0002
	TS3*CT92-0126
	IC18-CT98-0272

Yanko, V.	AVI*CT92-0007
Yeshurun, Y.	CII*CT93-0069
Yeshurun, Y.	EC-ISR-93003
Yeo, A.	IC18-CT98-0313
Yetis, O.	ICA-17
Yinon, J.	AVI*CT92-0004
Yogev, A.	AVI*CT94-0013
Younes, H.	AVI*CT92-0008
Zabel, P.	CII*CT91-0932
Zacharioudodou, M.	AVI2-CT93-062
Zaher Benabdallah, M.	IC18-CT96-0076
Zhao, C.	IC18-CT95-0809
Zairi, A/	IC18-CT97-0169
Zanini, E.	IC18-CT96-0035
Zannoun, M.	AVI*CT94-0006
Zayyoun, M.	AVI*CT93-0011
Zenios, S.A.	IC18-CT95-1139
Zerhouni, D.	AVI*CT93-0011
Zodiatis, G.	AVI*CT92-0016
Zoina, A.	IC18-CT97-0198
Zouari, K.	AVI*CT93-0015
Zouhri, A.	IC18-CT97-0155

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## **Index of participating institutions by country**

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## ALGERIA

Agence Nationale Architecturale Sites et Monuments	IC18-CT98-0386
Agence Nationale Architecturale Sites et Monuments	IC18-CT98-0385
Centre de Développement des Energies Renouvelables	AVI*CT94-0004
Centre de Développement des Matériaux	AVI*CT92-0014
Centre de Développement des Techniques Nucléaires	AVI*CT93-0015
Centre de Développement des Techniques Nucléaires	AVI2-CT93-087
Centre de Développement des Technologies Avancées	AVI*CT92-0011
Centre Hospitalo-Universitaire Mustapha	TS3*CT93-0244
Ecole Nationale Polytechnique d'Alger	AVI2-CT93-081
Ecole Nationale Vétérinaire	TS3*CT92-0106
Hopital Parnet	AVI*CT92-0010
Hopital Parnet	AVI*CT92-0013
INRF	IC18-CT97-0200
Institut Algérien du Pétrole	AVI*CT94-0007
Institut National des Hydrocarbures & de la Chimie	AVI2-CT93-083
Institut National des Sciences de la Mer	AVI*CT93-0003
Institut National Agronomique	IC18-CT98-0300
Ministère de la Santé	TS3*CT92-0144
Office National de la Météorologie	AVI*CT93-0010
Société Algérienne de Canalisation d'Ouvrages et Charpentes	AVI2-CT93-081
Université de Constantine	CI1*CT93-0070
Université de Constantine	AVI*CT93-0007
Université des Sciences et de la Technologie Houari Boumediene	AVI*CT92-0014
Université des Sciences et Techniques Blida	TS3*CT94-0264
Université des Sciences et Techniques Blida	IC18-CT98-0390
Université de Tlemcen	AVI*CT93-0011

## AUSTRIA

Environmental Software and Services	IC18-CT95-0809
University of Technology	CI1*CT93-0362

## BELGIUM

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Faculté des Sciences Agronomiques de Gembloux	TS3*CT92-0126
Faculté des Sciences Agronomiques de Gembloux	AVI*CT94-0002
Faculté des Sciences Agronomiques de Gembloux	IC18-CT98-0272
Faculté Polytechnique de Mons	IC18-CT98-0385
Faculté Polytechnique de Mons	ITDC-94-201-82164
IMEC	ICA-17
Katholieke Universiteit Leuven	EC-ISR 90
Katholieke Universiteit Leuven	TS3*CT93-0208
Prins Leopold Instituut voor Tropische Geneeskunde	AVI*CT93-0004
Prins Leopold Instituut voor Tropische Geneeskunde	AVI*CT93-0011
Prins Leopold Instituut voor Tropische Geneeskunde	AVI*CT93-0012
Prins Leopold Instituut voor Tropische Geneeskunde	TS3*CT92-0112
Prins Leopold Instituut voor Tropische Geneeskunde	TS3*CT92-0144
Prins Leopold Instituut voor Tropische Geneeskunde	IC18-CT98-0352
Prins Leopold Instituut voor Tropische Geneeskunde	IC18-CT98-0349
Prins Leopold Instituut voor Tropische Geneeskunde	IC18-CT98-0346
Rijksuniversiteit Gent	TS3*CT94-0278
Rijksuniversiteit Gent	IC18-CT98-0269
Université Catholique de Louvain	AVI2-CT93-081
Université Catholique de Louvain	TS3*CT94-0264

Université Catholique de Louvain	IC18-CT95-0363
Université Catholique de Louvain	IC18-CT98-0390
Université de Liège	CI1*CT94-0085
Universiteit Gent	CI1*CT94-0779
Universiteit Gent	AVI2-CT93-073
Universiteit Gent	IC18-CT97-0167
Université Libre de Bruxelles	CI1*CT94-0105
Vlaamse Instelling voor Technologisch Onderzoek	CI1*CT94-0083
Vrije Universiteit Brussel	AVI*CT92-0010
Vrije Universiteit Brussel	AVI2-CT93-073
Vrije Universiteit Brussel	CI1*CT93-0312

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## **BRAZIL**

Universidade de Rio de Janeiro	IC18-CT98-0293
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## **CAMEROON**

Ministere de L'Elevage, des Pêches et des Industries Animales	IC18-CT98-0331
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## **CHINA**

HEBEI Academy-Shijiazhuang	IC18-CT98-0391
Chinese Academy of Agricultural Sciences	IC18-CT98-0391

## **COSTA RICA**

Universidad Nacional Heredia	IC18-CT97-0202
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## **CYPRUS**

Agricultural Research Institute	AVI*CT94-0002
Agricultural Research Institute	IC18-CT98-0272
Cyprus Institute of Neurology and Genetics	CI1*CT94-0122
Enalion	AVI2-CT93-062
Environmental Man' Con'	IC18-CT97-0142
Geoinvest Ltd	IC18-CT96-0122
Geological Survey Department	IC18-CT97-0143
Higher Technical Institute	KIT Nr 204
Higher Technical Institute	IC18-CT96-0039
Higher Technical Institute	AVI*CT92-0017
Hospital Archbishop Makarios III	AVI*CT92-0002
Ministry of Agriculture, Natural Resources & Environment	AVI*CT92-0016
Ministry of Agriculture, Natural Resources & Environment	AVI*CT92-0015
Ministry of Agriculture, Natural Resources & Environment	AVI2-CT93-123
Ministry of Agriculture, Natural Resources & Environment	AVI*CT93-0005
Ministry of Agriculture, Natural Resources & Environment	IC18-CT96-0122
Ministry of Agriculture, Natural Resources & Environment	AVI2-CT93-062
State General Laboratory	AVI*CT92-0004
University of Cyprus	KIT Nr 12
University of Cyprus	AVI*CT94-0008
University of Cyprus	AVI*CT92-0012

University of Cyprus  
University of Cyprus  
University of Cyprus

IC18-CT95-0507  
IC18-CT95-1139  
IC18-CT97-0171

## DENMARK

Aalborg University  
Danish Bilharziosis Laboratory  
Technical University of Denmark  
Technical University of Denmark  
Technical University of Denmark

EC-ISR-93003  
AVI\*CT93-0004  
AVI2-CT93-112  
CI1\*CT91-0927  
CI1\*CT91-0927

## EGYPT

Agricultural Genetic Engineering Research Institute  
Ain-Shams University  
Ain-Shams University  
Ain-Shams University  
Ain-Shams University  
Ain-Shams University  
Ain-Shams University  
Atomic Energy Authority  
Central Laboratory for Food and Feed  
Climate Water and Environment Research  
Coastal Research Institute  
Desert Research Centre  
Drainage Research Institute  
Egyptian Consulting and Trading Co  
Egyptian Fertility Care Society  
Electronic Research Institute  
Electronic Research Institute  
El-Hussein University Hospital  
El Minia University  
Environmental Quality International  
Fields Crops Research Unit  
Mansoura University  
Mansoura University  
Medical Research Institute  
Minister of Electricity and Energy  
Ministry of Agriculture  
Ministry of Health  
Ministry of Health  
Mubarak City for Scientific Research  
National Authority for Remote Sensing & Space  
National Authority for Remote Sensing & Space  
National Institute of Oceanography & Fisheries  
National Research Centre  
National Research Centre  
National Research Centre  
New & Renewable Energy Authority  
New & Renewable Energy Authority  
Pyramid Paper Mills S.A.E.  
Soil and Water Research Institute ARC  
Standardata Egypt  
UNESCO Cairo  
UNESCO Cairo  
University of Alexandria

IC18-CT96-0121  
CI1\*CT93-0070  
CI1\*CT93-0070  
IC18-CT95-0507  
AVI\*CT94-0001  
IC18-CT98-0367  
IC18-CT98-0354  
AVI\*CT94-0014  
IC18-CT97-0186  
IC18-CT96-0069  
AVI\*CT93-0006  
IC18-CT96-0055  
TS3\*CT92-0061  
IC18-CT95-0895  
AVI\*CT94-0003  
IC18-CT95-0895  
IC18-CT95-0905  
AVI\*CT93-0008  
IC18-CT96-0029  
AVI\*CT94-0005  
IC18-CT98-0311  
AVI\*CT93-0001  
IC18-CT97-0142  
AVI\*CT92-0001  
AVI\*CT94-0006  
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AVI\*CT93-0004  
IC18-CT98-0313  
IC18-CT97-0155  
AVI\*CT92-0008  
IC18-CT96-0034  
AVI\*CT93-0013  
AVI\*CT94-0009  
IC18-CT98-0266  
AVI\*CT94-0004  
IC18-CT98-0289  
IC18-CT95-0410  
AVI\*CT92-0006  
IC18-CT95-0410  
IC18-CT98-0385  
IC18-CT98-0384  
TS3\*CT92-0015

University of Alexandria	AVI2-CT93-062
University of Alexandria	IC18-CT95-0809
University of Cairo	TS3*CT92-0061
University of Cairo	IC18-CT96-0069
University of Cairo	TS3*CT93-0249
University of Cairo	AVI2-CT93-091
University of Cairo	IC18-CT98-0384
University of Menoufiya	IC18-CT98-0301
Water Management Research Institute	AVI2-CT93-091

## FINLAND

University of Helsinki	IC18-CT98-0293
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## FRANCE

Bull S.A.	IC18-CT95-0175
Centre d'Etude des Environnements Terrestres & Planétaires	IC18-CT97-0154
Bureau de Recherches Géologiques et minières	IC18-CT96-0122
Cabinet d'Etudes Techniques Industrielles et d'Innovations Scientifiques (CETTIS)	AVI2-CT93-058
Cabinet d'Etudes Techniques Industrielles et d'Innovations Scientifiques (CETTIS)	AVI*CT93-0003
Centre d'Ecologie Fonctionnelle et Evolutive	TS3*CT92-0047
Centre d'Ingénierie et de Recherche Technologique en Electronique Moderne (CIRTEM)	AVI2-CT93-054
Centre International de Hautes Etudes Agronomiques Méditerranéennes	TS3*CT92-0119
Centre International de Hautes Etudes Agronomiques Méditerranéennes	IC18-CT98-0268
Centre International de l'Enfance	TS3*CT92-0144
Centre National de la Recherche Scientifique	CI1*CT93-0313
Centre National de la Recherche Scientifique	CI1*CT93-0069
Centre National de la Recherche Scientifique	IC18-CT96-0121
Centre National de la Recherche Scientifique	CI1*CT93-0004
Centre National de la Recherche Scientifique	AVI2-CT93-083
Centre National de la Recherche Scientifique	CI1*CT93-0070
Centre National de la Recherche Scientifique	AVI*CT92-0011
Centre National de la Recherche Scientifique	CI1*CT93-0065
Centre National de la Recherche Scientifique	CI1*CT93-0070
Centre National de la Recherche Scientifique	AVI2-CT93-083
Centre National de la Recherche Scientifique	IC18-CT98-0267
Centre de Recherche d'Avignon	IC18-CT97-0200
CILAS	AVI*CT92-0011
CIRAD	IC18-CT95-0009
Commissariat à l'Energie Atomique	AVI2-CT93-087
Ecole d'Architecture de Paris la Seine	IC18-CT98-0385
Ecole Nationale Supérieure de Chimie de Montpellier	AVI*CT92-0014
Ecole Nationale Supérieure de Télécommunication	CI1*CT92-0095
Ecole Nationale Vétérinaire	TS3*CT92-0106
Ecole Normale Supérieure	CI1*CT93-2027
Ecole Normale Supérieure	ITDC-94-201-82164
Ecole Polytechnique Palaiseau	CI1*CT93-0070
Ecole Polytechnique Palaiseau	CI1*CT93-0312
EDIFRANCE	TEDIPP
ENSAM Montpellier	IC18-CT98-0311
EPOS	IC18-CT95-0175
Etablissement Guy Daric S.A.	AVI2-CT93-054
Hopital Robert Debré	TS3*CT93-0244
Hopital Robert Debré	TS3*CT93-0244

Hopital Tenon	TS3*CT93-0244
Hospital Henri Mondor	AVI*CT93-0014
IDEE	AVI2-CT93-058
INSERM-GERM	TS3*CT94-0282
Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER)	AVI2-CT93-087
Institut Français pour l'Exploitation de la Mer	AVI*CT92-0015
Institut National de la Recherche Agroalimentaire	AVI*CT94-0011
Institut National de la Recherche Agronomique	TS3*CT92-0119
Institut National de la Recherche Agronomique	TS3*CT92-0119
Institut National de la Recherche Agronomique	IC18-CT96-0081
Institut National de la Recherche Agronomique	TS3*CT92-0106
Institut National de la Recherche Agronomique	IC18-CT96-0082
Institut National de la Recherche Agronomique	IC18-CT97-0197
Institut National de la Recherche Agronomique	IC18-CT97-0200
Institut National de la Recherche Agronomique	IC18-CT98-0391
Institut National de la Recherche Agronomique	IC18-CT98-0310
Institut National de la Recherche Agronomique	IC18-CT98-0300
Institut National d'Etudes Démographiques	IC18-CT96-0036
Institut National de Recherche en Informatique	KIT Nr 204
Institut National des Sciences Appliquées et de Technologies	IC18-CT95-0363
Institut National Polytechnique de Grenoble	EC-ISR-93003
Institut National Polytechnique de Lorraine	AVI2-CT93-019
L.R.P.V.	IC18-CT98-0308
Medias-France	AVI*CT93-0010
Météo-France	AVI*CT93-0010
MORS - Group	AVI*CT92-0015
ORSTOM	AVI*CT92-0008
ORSTOM	TS3*CT92-0047
ORSTOM	AVI*CT93-0011
ORSTOM	AVI*CT92-0013
ORSTOM	IC18-CT96-0064
ORSTOM	IC18-CT97-0155
ORSTOM	IC18-CT98-0331
ORSTOM	TS3*CT94-0278
Service Ingenierie	IC18-CT97-0142
TARNIUM S.A.R.L	IC18-CT97-0161
SIDEF	AVI*CT94-0006
U.N.E.S.C.O	IC18-CT97-0142
Université d'Aix Marseille	AVI*CT93-0003
Université de Bordeaux I	TS3*CT93-0208
Université de Bordeaux I	IC18-CT98-0386
Université de Nice - Sophia Antipolis	AVI2-CT93-058
Université de Nice - Sophia Antipolis	AVI2-CT93-099
Université de Limoges	IC18-CT98-0384
Université de Paris VII	IC18-CT95-0905
Université Paris XI	CII*CT91-0931
Université de Paris-Sud XI	TS3*CT94-0264
Université de Paris-Sud XI	IC18-CT98-0390
Université de Perpignan	AVI*CT94-0001
Université de Provence	CII*CT92-0104
Université de Rennes I	AVI2-CT93-081
Université Joseph Fourier	IC18-CT95-0363
Université Montpellier II	AVI2-CT93-076
Université Montpellier II	AVI*CT92-0014
Université Michel de Montaigne	IC18-CT98-0386
Université Paul Sabatier - Toulouse III	AVI2-CT93-054
Université Paul Sabatier - Toulouse III	AVI*CT94-0008
Université Paul Sabatier - Toulouse III	IC18-CT98-0293



**GERMANY**

Biologische Bundesanstalt für Land- und Forstwirtschaft	IC18-CT96-0121
Bomin Solar Research GmbH	AVI*CT94-0013
Bomin Solar Research GmbH	IC18-CT98-0267
Centrum Neue Technologien	AVI*CT94-0006
Deutsche Forschungsanstalt für Luft- und Raumfahrt e.V.	AVI*CT94-0013
DFKI - Compulog-Net	KIT Nr 12
Eberhard-Karls-Universität Tübingen	AVI*CT93-0008
European Molecular Biology Laboratory	CI1*CT94-0097
Fachhochschule Aachen	IC18-CT96-0099
Federal Institute for Materials Research & Testing	CI1*CT93-0065
Forschungsinstitut für Wasser und Abfallwirtschaft	AVI*CT94-0015
Forschungsinstitut Borstel	IC18-CT95-0004
Forschungszentrum	CI1*CT94-0125
Forschungszentrum Borstel	IC18-CT95-0009
Fraunhofer Institut IAF	CI1*CT92-0063
Fraunhofer Institut für Physikalische Messtechnik	CI1*CT93-0362
Fraunhofer Institut für Toxikologie und Aerosolforschung	AVI*CT92-0004
German Aerospace Research Establishment	AVI*CT94-0004
GTZ	TS3*CT92-0112
Hovelmann & Bidinger	IC18-CT96-0099
Institut für Kybernetik und Systemtheorie	EC-MED-35
Institut für Solarenergieforschung GmbH	AVI*CT94-0007
	IC18-CT98-0267
Institut für Wasser-, Boden- und Lufthygiene	AVI*CT92-0006
IT consult GmbH	IC18-CT95-0410
Johannes Gutenberg-Universität	AVI*CT93-0001
Johannes Gutenberg-Universität	IC18-CT96-0034
Max-Born-Institut für Nichtlineare Optik & Kurzzeitspektroskopie	CI1*CT94-0126
Max Planck Institut	IC18-CT96-0076
Max Planck Institut for Iron Research	CI1*CT93-0311
Max Planck Institut für Hirnforschung	CI1*CT94-0130
Parsytec Computer GmbH	ITDC 204-82166
Parsytec Computer GmbH	IC18-CT95-0895
Rheinisch-Westfälische Technische Hochschule Aachen	IC18-CT96-0099
Rheinisch-Westfälische Technische Hochschule Aachen	IC18-CT98-0313
Rheinisch-Westfälische Technische Hochschule Aachen	IC18-CT98-0384
Ruprecht-Karls-Universität Heidelberg	AVI*CT93-0012
Ruprecht-Karls-Universität Heidelberg	AVI*CT94-0001
Ruprecht-Karls-Universität Heidelberg	IC18-CT98-0352
Ruprecht-Karls-Universität Heidelberg	IC18-CT98-0367
Ruhr-Universität Bochum	CI1*CT94-0085
Technische Hochschule Darmstadt	CI1*CT90-0542
Technical University of Berlin	CI1*CT94-0108
Technische Universität Clausthal	AVI*CT94-0007
Technische Universität Clausthal	IC18-CT98-0267
Technische Universität Hamburg-Harburg	AVI2-CT93-076
Technische Universität Hamburg-Harburg	AVI*CT92-0016
Tieraerztliche Hochschule Hannover	IC18-CT96-0055
Türkisch-Deutsche Gesundheitsstiftung E.V.	AVI*CT93-0012
Türkisch-Deutsche Gesundheitsstiftung E.V.	IC18-CT98-0352
Universität Bremen	AVI2-CT93-074
Universität der Bundeswehr München	AVI*CT94-0004

Universität des Saarlandes	IC18-CT95-0905
Universität Düsseldorf	CI1*CT94-0126
Universität Goettingen	IC18-CT96-0035
Universität Goettingen	IC18-CT97-0186
Universität Hamburg	TS3*CT93-0249
Universität Hannover	TS3*CT93-0249
Universität Heidelberg	CI1*CT94-0096
Universität Hohenheim	TS3*CT92-0015
Universität Hohenheim	CI1*CT93-0006
Universität Hohenheim	TS3*CT93-0249
Universität Hohenheim	IC18-CT98-0333
Universität Karlsruhe	AVI2-CT93-072
Universität Karlsruhe	IC18-CT97-0167
Universität Kiel	AVI2-CT93-123
Universität München	CI1*CT94-0126
Universität Osnabrück	IC18-CT96-0055
Universität Regensburg	CI1*CT89-0442
Universität Stuttgart	KIT Nr 204
Universität Stuttgart	AVI*CT94-0005
Universität Stuttgart	IC18-CT97-0167
Universität Tübingen	CI1*CT94-0106
Universität Tübingen	CI1*CT93-0066
Universität Wurzburg	IC18-CT97-0143

## GREECE

Agricultural University of Athens	AVI*CT94-0002
Agricultural University of Athens	IC18-CT98-0272
Aristotle University of Thessaloniki	AVI*CT92-0017
Aristotle University of Thessaloniki	IC18-CT96-0039
Aristotle University of Thessaloniki	IC18-CT98-0392
Aristotle University of Thessaloniki	IC18-CT98-0354
Centre for Renewable Energy Sources	AVI*CT94-0004
DEMOKRITOS National Centre for Research	IC18-CT98-0386
Dimman Consulting Ltd	IC18-CT96-0039
Engineering & Computer Applications S.A.	AVI*CT94-0006
Epsilon International S.A.	AVI*CT93-0006
Institute of Marine Biology of Crete	AVI*CT92-0001
Institute of Ghania	IC18-CT97-0153
Institute of Microelectronics	IC18-CT95-0507
Intelltech S.A.	IC18-CT95-0410
National & Kapodistrian University of Athens	AVI*CT92-0005
National Foundation Agricultural Research	AVI2-CT93-076
National Foundation Agricultural Research	IC18-CT98-0272
National Technical University of Athens	IC18-CT95-0895
National Technical University of Athens	AVI*CT93-0006
National Technical University of Athens	AVI2-CT93-062
National Technical University of Athens	IC18-CT97-0138
National Technical University of Athens	IC18-CT97-0163
National Technical University of Athens	IC18-CT98-0385
Onassis Cardiac Surgery Centre	CI1*CT94-0122
Research and Development	TS3*CT92-0119
Technological Education Institute	IC18-CT96-0099
University of Crete	AVI2-CT93-092
University of Patras	AVI*CT92-0016
University of the Aegean	IC18-CT96-0055
University of the Aegean	IC18-CT98-0268

University of Thessaloniki

IC18-CT97-0143

## **GUATEMALA**

Ministerio de Salud Publica y Asistencia Social

IC18-CT98-0346

## **INDIA**

Anna University, Madras

IC18-CT98-0367

## **IRELAND**

CREDCO

IC18-CT97-0161

TEAGASC

TS3\*CT94-0278

University College Cork

TS3\*CT92-0015

## **ISRAEL**

Agriculture Research Org

IC18-CT97-0200

Agriculture Research Org

IC18-CT98-0333

Bar-Ilan University

IC18-CT96-0076

Bar-Ilan University

CII\*CT93-0069

Ben-Gurion University

AVI2-CT93-008

Ben-Gurion University

AVI\*CT93-143

Ben-Gurion University

AVI2-CT93-076

Ben-Gurion University

AVI\*CT93-143

Ben-Gurion University

CII\*CT93-0311

Ben-Gurion University

EC-ISR 90

Ben-Gurion University

CII\*CT94-0779

Ben-Gurion University

AVI2-CT93-031

Ben-Gurion University

CII\*CT93-0066

Ben-Gurion University

CII\*CT94-0106

Ben-Gurion University

IC18-CT96-0035

Ben-Gurion University

AVI\*CT92-0017

Ben-Gurion University

CII\*CT94-0096

Ben-Gurion University

CII\*CT94-0096

Ben-Gurion University

IC18-CT98-0266

Ben-Gurion University

IC18-CT97-0143

Ben-Gurion University

IC18-CT97-0154

Ben-Gurion University

IC18-CT98-0354

Hebrew University of Jerusalem

AVI\*CT94-0014

Hebrew University of Jerusalem

AVI\*CT92-0001

Hebrew University of Jerusalem

CII\*CT92-0095

Hebrew University of Jerusalem

EC-ISR 90

Hebrew University of Jerusalem

AVI\*CT94-0010

Hebrew University of Jerusalem

CII\*CT94-0125

Hebrew University of Jerusalem

AVI2-CT93-072

Hebrew University of Jerusalem

CII\*CT92-0096

Hebrew University of Jerusalem

CII\*CT93-0006

Hebrew University of Jerusalem

CII\*CT94-0080

Hebrew University of Jerusalem

CII\*CT94-0085

Hebrew University of Jerusalem

CII\*CT93-0074

Hebrew University of Jerusalem

CII\*CT90-0542

Hebrew University of Jerusalem

AVI\*CT92-0003

Hebrew University of Jerusalem

AVI\*CT94-0001

Hebrew University of Jerusalem	CI1*CT94-0097
Hebrew University of Jerusalem	IC18-CT95-0023
Hebrew University of Jerusalem	EC-ISR-93003
Hebrew University of Jerusalem	CI1*CT93-2027
Hebrew University of Jerusalem	AVI*CT92-0005
Hebrew University of Jerusalem	CI1*CT94-0131
Hebrew University of Jerusalem	IC18-CT98-0354
Hebrew University of Jerusalem	IC18-CT97-0186
Hebrew University of Jerusalem	IC18-CT97-0200
Hebrew University of Jerusalem	IC18-CT97-0142
Hebrew University of Jerusalem	IC18-CT98-0272
Israel Oceanographic & Limnological Research	AVI*CT92-0016
Israel Oceanographic & Limnological Research	AVI2-CT93-123
Israel Oceanographic & Limnological Research	IC18-CT98-0293
Migal-Galilee Technological Center	CI1*CT94-0086
National Institute of Oceanography	IC18-CT96-0034
National Institute of Oceanography	AVI*CT93-0001
Technion-Israel Institute for Technology	CI1*CT89-0442
Technion-Israel Institute for Technology	CI1*CT91-0907
Technion-Israel Institute of Technology	CI1*CT91-0927
Technion-Israel Institute of Technology	CI1*CT93-0311
Technion-Israel Institute for Technology	CI1*CT91-0931
Technion-Israel Institute of Technology	AVI2-CT93-092
Technion-Israel Institute of Technology	AVI*CT94-0011
Technion-Israel Institute of Technology	EC-ISR-93003
Technion-Israel Institute of Technology	IC18-CT95-1139
Technion-Israel Institute of Technology	IC18-CT96-0099
Technion-Israel Institute of Technology	CI1*CT93-0065
Technion-Israel Institute of Technology	IC18-CT97-0202
Technion-Israel Institute of Technology	CI1*CT92-0063
Technion-Solid State Institute	CI1*CT94-0122
Tel Aviv University	IC18-CT95-0895
Tel Aviv University	AVI*CT92-0009
Tel Aviv University	EC-ISR-93003
Tel Aviv University	CI1*CT91-0923
Tel Aviv University	CI1*CT93-0362
Tel Aviv University	AVI*CT93-0002
Tel Aviv University	CI1*CT94-0083
Tel Aviv University	CI1*CT93-0005
Tel Aviv University	AVI*CT92-0007
Tel Aviv University	IC18-CT97-0171
The Institute for Petroleum Research & Geophysics	IC18-CT96-0122
The Volcani Center	AVI2-CT93-074
The Volcani Center	AVI*CT92-0006
The Weizmann Institute of Science	EC-ISR-93003
The Weizmann Institute of Science	CI1*CT94-0126
The Weizmann Institute of Science	CI1*CT94-0126
The Weizmann Institute of Science	AVI*CT92-0004
The Weizmann Institute of Science	AVI*CT94-0008
The Weizmann Institute of Science	CI1*CT93-0003
The Weizmann Institute of Science	AVI*CT93-0008
The Weizmann Institute of Science	CI1*CT94-0130
The Weizmann Institute of Science	CI1*CT94-0108
The Weizmann Institute of Science	CI1*CT91-0932
The Weizmann Institute of Science	CI1*CT94-0087
The Weizmann Institute of Science	CI1*CT94-0105
The Weizmann Institute of Science	CI1*CT93-0004
The Weizmann Institute of Science	CI1*CT93-0312

The Weizmann Institute of Science  
The Weizmann Institute of Science  
The Weizmann Institute of Science  
The Weizmann Institute of Science

AVI\*CT94-0013  
CII\*CT93-0065  
AVI\*CT92-0012  
IC18-CT98-0391

## ITALY

Istituto de Agrometeorologia e Analisi	IC18-CT97-0155
Beta Studio S.R.L.	AVI*CT93-0006
Centro Ricerche sur Calcolo Parallelo e Supercalcolatori	IC18-CT95-1139
CERFE	AVI2-CT93-020
Community of Mediterranean Universities	AVI*CT94-0010
Conphoebus Campo Prove	IC18-CT96-0039
CONPHOEBUS Istituto di Ricerche per le Energie Rinnovabili	IC18-CT98-0289
Consiglio Nazionale delle Ricerche	AVI*CT93-0003
Consiglio Nazionale delle Ricerche	IC18-CT98-0289
Joint Research Centre	IC18-CT97-0161
IROE-CNR	IC18-CT97-0171
Institut Agronomique Mediterranéen de Bari	IC18-CT96-0055
Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare	AVI2-CT93-087
Institute d'Appolonia S.P.A.	AVI2-CT93-099
Istituto Internazionale de Genetica e Biofisica	TS3*CT92-0096
Istituto per l'Infanzi	AVI*CT94-0003
Istituto per l'Infanzi	IC18-CT98-0349
Istituto Sperimentale Italiano "L. Spallanzani"	TS3*CT92-0119
Politecnico di Milano	IC18-CT95-0809
CÓNISMA	IC18-CT98-0270
Istituto di Ricerca sulle Ondi Elettromagnetichi	IC18-CT97-0154
Istituto Sperimentale per la Patologia Vegetale	IC18-CT98-0300
Istituto di Ricerca sul Rischio Sismico	IC18-CT98-0385
Intern Centre for Preservation	IC18-CT98-0384
Società di Ricerca e Servizi di Ingegneria	AVI2-CT93-091
Studio Sardo di Catania	IC18-CT96-0055
Universita degli studi della Tuscia	TS3*CT92-0047
Universita degli studi de Trieste	IC18-CT97-0153
Universita degli studi di Ancona	CII*CT93-0311
Universita degli studi di Bologna	IC18-CT98-0311
Universita degli studi di Firenze	IC18-CT98-0268
Universita degli studi di Milano	AVI*CT92-0007
Universita degli studi di Padova	IC18-CT96-0076
Universita degli studi di Padova	IC18-CT96-0069
Universita degli studi di Roma "La Sapienza"	AVI*CT92-0001
Universita degli studi di Roma "La Sapienza"	AVI2-CT93-107
Universita degli studi di Roma "Tor Vergata"	AVI*CT92-0001
Universita degli studi di Torino	IC18-CT96-0035
Universita della Calabria	IC18-CT95-1139
Universita di Cagliari	AVI2-CT93-073
Universita de Catania	IC18-CT97-0169
Universita di Napoli	IC18-CT95-1139
Universita di Pisa	EC-ISR 90
Universita di Pisa	IC18-CT98-0310
Universita di Sassari	IC18-CT98-0268
Universita di Udine	EC-ISR 90
Universita di Venezia	AVI2-CT93-087
Universita di Venezia	AVI*CT94-0011
University of Bergamo	IC18-CT95-1139
University of Cagliari	AVI*CT92-0002

University of Genova  
University of Genova  
University of Salerno

AVI2-CT93-080  
EC-ISR-93003  
IC18-CT98-0386

## IVORY COAST

Centre Hospitalier Universitaire de Yopougon

IC18-CT98-CT98-  
0349

## JORDAN

Al Al-Bayt University  
CEHA-WHO  
Family Health Group  
Jordan University of Science & Technology  
Ministry of Water and Irrigation  
Ministry of Water and Irrigation  
Ministry of Water and Irrigation  
Renewable Energy Research Centre  
Royal Scientific Society Jordan  
Royal Scientific Society Jordan  
Royal Scientific Society Jordan  
University of Jordan  
University of Jordan  
University of Jordan  
University of Jordan  
University of Jordan  
University of Jordan  
University of Jordan  
University of Jordan  
Yarmouk University

IC18-CT95-0895  
AVI\*CT94-0009  
AVI\*CT94-0003  
AVI\*CT94-0003  
AVI\*CT93-0005  
AVI\*CT94-0012  
IC18-CT97-0136  
AVI\*CT94-0004  
IC18-CT96-0039  
IC18-CT96-0099  
IC18-CT98-0289  
AVI2-CT93-020  
AVI2-CT93-080  
AVI2-CT93-112  
AVI\*CT94-0009  
AVI\*CT94-0003  
IC18-CT98-0385  
IC18-CT97-0143  
IC18-CT97-0163  
IC18-CT97-0169  
IC18-CT98-0354

## LEBANON

American University of Beirut  
American University of Beirut  
American University of Beirut  
American University of Beirut  
American University of Beirut  
American University of Beirut  
American University of Beirut  
Conseil et Développement  
Centre for Remote Sensing  
Integro Middle East  
Lebanese University – Ministry of Public Health

IC18-CT96-0036  
AVI\*CT94-0003  
AVI\*CT93-0012  
AVI\*CT94-0003  
IC18-CT98-0352  
IC18-CT97-0177  
IC18-CT97-0136  
IC18-CT97-0142  
IC18-CT97-0161  
IC18-CT95-0175  
IC18-CT97-0153

## MALAYSIA

University Putra Malaysia

IC18-CT98-0333

## MALTA

Malta Council for Science & Technology  
Euro-Mediterranean Centre in Marine Contamination Hazards  
University of Malta

AVI\*CT92-0015  
AVI\*CT92-0015  
AVI\*CT94-0010



Institut National d'Administration Sanitaire	TS3*CT92-0112
Institut National d'Administration Sanitaire	TS3*CT92-0144
Institut National d'Administration Sanitaire	AVI*CT93-0011
Institut National d'Administration Sanitaire	IC18-CT98-0349
Institut Pasteur	TS3*CT92-0096
Institut Pasteur	AVI*CT93-0014
Institut Pasteur	AVI2-CT93-107
Maroc-Météo	AVI*CT93-0010
Ministère de la Culture	IC18-CT98-0385
Ministry of Public Health	IC18-CT98-0346
Ministère de l'Agriculture et de la Mise en Valeur Agricole	TS3*CT92-0151
Office National de l'Eau Potable	AVI2-CT93-020
Office National de l'Eau Potable	IC18-CT97-0267
Université Abdelmalek Essaadi	AVI*CT94-0011
Université Abdelmalek Essaadi	AVI2-CT93-054
Université Abdelmalek Essaadi	IC18-CT97-0167
Université Cadi Ayyad	TS3*CT93-0249
Université Cadi Ayyad	IC18-CT98-0390
Université Cadi Ayyad	IC18-CT98-0384
Université de Fes	AVI*CT92-0014
Université de Kenitra	AVI2-CT93-081
Université Ibnou Zohr Agadir	IC18-CT98-0269
Université Mohammed I	AVI2-CT93-073
Université Mohammed I	ITDC-94-201-82164
Université Mohammed V	IC18-CT96-0029
Université Mohammed V	TS3*CT94-0278
Université Mohammed V	TS3*CT93-0208
Université Mohammed V	IC18-CT97-0147
Université Mohammed V	IC18-CT98-0270
Université Mohammed V	IC18-CT97-0134
Université Mohammed V	IC18-CT97-0154
Université Mohammed V	IC18-CT98-0313
Université Moulay Ismail	IC18-CT98-0293

## MOZAMBIQUE

Centro de Investigacao para a Saude & Desenvolvimento	IC18-CT98-0346
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## NIGERIA

National Institute of Freshwater Fisheries Research	IC18-CT98-0331
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## NORWAY

University of Bergen	IC18-CT96-0029
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## PHILIPPINES

Southeast Asian Fisheries Development Centre	IC18-CT98-0333
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## PORTUGAL

Associação Terras Dentro	IC18-CT98-0268
AquaAmbiente S.A.	AVI*CT94-0015
Electricidade de Portugal	IC18-CT96-0039
Geografica Lda	AVI2-CT93-126



Instituto de Higiene e Medicina Tropical	AVI*CT92-0003
Instituto de Higiene e Medicina Tropical	TS3*CT93-0253
Instituto de Investigaç�o Cient�fica Tropical Quinta do Marqu�s	TS3*CT93-0221
Instituto Nacional de Sa�de	TS3*CT92-0106
Instituto Investigaç�o Cient�fica Tropical	IC18-CT98-0384
Instituto Superior de Agronomia	AVI*CT94-0002
Instituto Superior de Agronomia	TS3*CT92-0126
Instituto Superior T�cnico	CII*CT91-0923
Laboratorio Nacional de Engenharia Civil	AVI*CT94-0012
Laboratorio Nacional de Engenharia Civil	IC18-CT97-0136
Universidade da Madeira	TS3*CT92-0151
Universidade de Lisboa	IC18-CT96-0055
Universidade de Lisboa	TS3*CT92-0151
Universidade Nova de Lisboa	IC18-CT95-0009
Universidade Nova de Lisboa	TS3*CT93-0249
Universidade Nova de Lisboa	IC18-CT95-0023
Universidade Nova de Lisboa	IC18-CT97-0169
Universidade Nova de Lisboa	IC18-CT98-0346
Universidade do Algarve	AVI2-CT93-076
Universidade do Algarve	AVI2-CT93-008
Universidade do Algarve	IC18-CT98-0266
Universidade Technica de Lisboa	AVI*CT93-0009
Universidade de Aveiro	IC18-CT97-0147
Universidade de Coimbra	IC18-CT98-0270
Universidade de Coimbra	IC18-CT98-0392

## SPAIN

Asociacion Centro Tecnologico	AVI*CT94-0013
Centro Internacional Agronomicos Mediterraneo	IC18-CT97-0197
Centro de Investigaciones Biologicas	CII*CT94-0083
Centro de Investigaciones Energeticas Medioambientales y Tecnologicas	AVI*CT94-0013
Centro de Investigaciones Energeticas Medioambientales y Tecnologicas	IC18-CT98-0289
	IC18-CT97-0163
CIEMAT	AVI*CT94-0004
CIDA	IC18-CT98-0390
Consejo Superior de Investigaciones Cientificas	IC18-CT96-0091
Consejo Superior de Investigaciones Cientificas	IC18-CT98-0266
Consejo Superior de Investigaciones Cientificas	IC18-CT98-0392
Consejo Superior de Investigaciones Cientificas	IC18-CT98-0301
Dpt de Ordinacion del Territorio Urbanismo y Medio Ambiente Caminos Canales y Puertos	AVI*CT94-0015
Escuela Andaluza de Salud Publica	TS3*CT92-0088
Estacion Experimental "La Mayora"	AVI2-CT93-008
Estacion Experimental de Zonas Aridas	IC18-CT97-0134
Infocarta S.A.	AVI2-CT93-126
Instituto de Maquina Herramienta	AVI*CT94-0013
Instituto de Nutrici�n y Bromatolog�a	TS3*CT94-0282
Institute for Prospective Technology	IC18-CT98-0289
Instituto de Salud Carlos III	AVI*CT92-0013
Instituto Nacional Reforma y Desarrollo Agrario	TS3*CT92-0061
Instituto Tecnologico de Canarias	IC18-CT96-0099
Instituto Valenciano de Investigaciones Agrarias	IC18-CT96-0082
Instituto Valenciano de Investigaciones Agrarias	IC18-CT98-0310
Instituto Zaragoza Agronomico	IC18-CT98-0310
Son Dureta Hospital	AVI*CT92-0009
Universidad Autonoma de Barcelona	AVI*CT94-0008
Universidad Autonoma de Barcelona	IC18-CT96-0076

Universidad Autonoma de Barcelona	AVI*CT94-0010
Universidad Autonoma de Barcelona	IC18-CT97-0147
Universidad Autonoma de Barcelona	IC18-CT98-0384
Universidad Complutense de Madrid	CI1*CT93-0066
Universidad Complutense de Madrid	AVI*CT92-0011
Universidad Complutense de Madrid	IC18-CT97-0147
Universidad Complutense de Madrid	IC18-CT98-0293
Universidad de Barcelona	AVI*CT94-0011
Universidad de Barcelona	CI1*CT91-0907
Universidad de Barcelona	AVI2-CT93-076
Universidad de Barcelona	IC18-CT98-0384
Universidad de Cantabria	AVI*CT94-0014
Universidad de Extremadura	AVI2-CT93-126
Universidad de Extremadura	IC18-CT95-0009
Universidad de Granada	IC18-CT96-0081
Universidad de las Islas Baleares	IC18-CT96-0099
Universidad de Murcia	AVI*CT92-0010
Universidad de Santiago de Compostela	CI1*CT94-0086
Universidad de Santiago de Compostela	TS3*CT92-0106
Universidad de Valencia	TS3*CT93-0208
Universidad de Valencia	IC18-CT97-0154
Universidad de Valencia	IC18-CT97-0177
Universidad de Valencia	IC18-CT98-0385
Universidad de Valladolid	AVI*CT94-0009
Universidad Politécnica de Madrid	EC-ISR 90

## SWEDEN

Korolinska Institute	IC18-CT98-0346
Linköping University	EC-ISR-93003
Linköping University	IC18-CT95-0507
Lunds Universitet	IC18-CT96-0091
Royal Institute of Technology	EC-ISR-93003
UMEAA Universitet	IC18-CT98-0268

## SWITZERLAND

University of Fribourg	IC18-CT98-0386
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## SYRIA

Ministère de la Culture	IC18-CT98-0385
Arab Center for the Studies of Arid Zone and Dry Land	IC18-CT96-0091
Damascus University	AVI*CT94-0007
Damascus University	AVI*CT94-0003
GOSM	IC18-CT98-0308
Higher Institute of Applied Science and Technology	AVI2-CT93-112
International Center for Agricultural Research in the Dry Area	AVI2-CT93-080
International Center for Agricultural Research in the Dry Area	IC18-CT96-0121
International Center for Agricultural Research in the Dry Area	IC18-CT98-0301
Ministry of Agriculture & Agrarian Reform	IC18-CT98-0301
Musée National de Damas	IC18-CT98-0386

## THAILANDE

Ministry of Public Health	IC18-CT98-0346
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## THE NETHERLANDS

Cap Volmac B.V.	EC-MED-35
Erasmus Center for Financial Research	IC18-CT95-1139
Erasmus Universiteit Rotterdam	IC18-CT95-1139
Free University of Amsterdam	CII*CT92-0096
Free University of Amsterdam	CII*CT93-0074
Free University of Amsterdam	IC18-CT97-0134
Koninklijk Instituut voor de Tropen	AVI*CT92-0003
Institute for Soil Fertility Research	AVI*CT92-0016
International Reference Centre for Community Water	AVI2-CT93-020
Nederlands Instituut voor Onderzoek der Zee	IC18-CT96-0034
Nederlands Meetinstituut	TS3*CT92-0093
Netherlands Energy Research Foundation	AVI*CT94-0004
Prof. H.C. Van Hall Institute	AVI*CT93-0013
Rijks Geologische Dienst	IC18-CT96-0122
Royal Tropical Institute	IC18-CT95-0023
Synoptics	AVI*CT93-143
Technische Universiteit Eindhoven	AVI*CT94-0004
University of Leiden	CII*CT93-0069
University of Leiden	AVI*CT93-0004
Utrecht University	TS3*CT91-0019
Utrecht University	IC18-CT95-0003
Utrecht University	IC18-CT95-0009
Wageningen Agricultural University	AVI*CT94-0009
Wageningen Agricultural University	AVI*CT93-0004
Wageningen Agricultural University	CII*CT91-0932
Wageningen Agricultural University	CII*CT94-0086
Wageningen Agricultural University	AVI*CT93-0009
Wageningen Agricultural University	IC18-CT98-0269
Wageningen Agricultural University	IC18-CT97-0202
Wagner Advies B.V.	AVI*CT93-0013
Winand Staring Centre of Integrated Land Soil & Water Research	IC18-CT96-0069
Winand Staring Centre of Integrated Land Soil & Water Research	TS3*CT92-0061

## TUNISIA

Agence pour la Maîtrise de l'Energie	IC18-CT98-0289
Centre de Biotechnologie de Sfax	CII*CT92-0104
Centre de Recherche du Génie Rural	IC18-CT96-0076
Centre National de l'Informatique	TEDIPP
Centre National de Télédétection	IC18-CT97-0155
Centre National de Télédétection	AVI*CT92-0008
Compagnie Minière du Nord Ouest	AVI2-CT93-019
Département Etudes et Développement	AVI2-CT93-126
Direction Générale de la Production Agricole	IC18-CT96-0081
Ecole Nationale d'Ingénieurs de Monastir	IC18-CT95-0363
Ecole Nationale d'Ingénieurs de Tunis	IC18-CT97-0154
Ecole Nationale d'Ingénieurs de Tunis	AVI*CT94-0015
Ecole Nationale de Médecine Vétérinaire	IC18-CT95-0003
Ecole Nationale de Médecine Vétérinaire	IC18-CT95-0004
Ecole Nationale de Médecine Vétérinaire	IC18-CT95-0009
Ecole Nationale de Médecine Vétérinaire	TS3*CT91-0019
Ecole Normale Supérieure de l'Enseignement Technique	AVI2-CT93-054
Ecole Supérieure d'Agriculture	TS3*CT92-0119
Ecole Supérieure des Ingénieurs de l'Equipement Rural	AVI*CT94-0002
Faculté des Sciences de Tunis	ITDC 135-82159

Faculté des Sciences de Tunis	IC18-CT98-0270
Faculté des Sciences de Tunis	IC18-CT98-0390
Faculté des Sciences de Tunis	TS3*CT94-0264
Institut Agronomique de Tunisie	AVI*CT93-0009
Institut des Régions Arides Medenine	TS3*CT92-0047
Institut des Régions Arides Medenine	IC18-CT98-0269
Institut des Régions Arides Medenine	IC18-CT97-0134
Institut National Agronomique de Tunisie	AVI2-CT93-073
Institut National Agronomique de Tunisie	IC18-CT98-0310
Institut National d'Agriculture de Tunisie	TS3*CT93-0208
Institut National de la Météorologie	AVI*CT93-0010
Institut National de la Recherche Agronomique de Tunis	IC18-CT98-0300
Institut National de la Recherche Agronomique de Tunis	AVI*CT93-0007
Institut National de Recherche du Génie Rural et des Eaux et Forêts	IC18-CT96-0091
Institut National de Recherche du Génie Rural et des Eaux et Forêts	AVI*CT94-0012
Institut National de Recherche du Génie Rural et des Eaux et Forêts	IC18-CT97-0169
Institut National de Recherche du Génie Rural et des Eaux et Forêts	IC18-CT98-0272
Institut National de Recherche du Génie Rural et des Eaux et Forêts	IC18-CT97-0197
Institut National de Recherche du Génie Rural et des Eaux et Forêts	IC18-CT97-0147
Institut National de Recherche Scientifique et Technique	IC18-CT96-0055
Institut National de Recherche Scientifique et Technique	AVI*CT94-0007
Institut National de Recherche Scientifique et Technique	AVI2-CT93-054
Institut National de Recherche Scientifique et Technique	IC18-CT98-0267
Institut National s&t d'Océanographie et de Pêche	AVI*CT93-0003
Institut National s&t du Patrimoine	IC18-CT98-0386
Institut National s&t du Patrimoine	IC18-CT98-0385
Institut National s&t du Patrimoine	IC18-CT98-0384
Institut Pasteur de Tunis	AVI*CT92-0018
Institut Pasteur de Tunis	AVI2-CT93-107
Institut Pasteur de Tunis	TS3*CT93-0253
Institut Pasteur de Tunis	AVI*CT92-0013
Institut Pasteur de Tunis	IC18-CT95-0009
Institut Pasteur de Tunis	AVI*CT93-0014
Institut Pasteur de Tunis	IC18-CT95-0003
Institut Régional des Sciences Informatiques & des Télécommunications	EC-MED-35
Institut Régional des Sciences Informatiques & des Télécommunications	ITDC 135-82159
Laboratoire de Physiologie Végétale	IC18-CT98-0311
Laboratoire de Chimie des Eaux-Sols-Boues	AVI2-CT93-076
Laboratoire de Chimie Isotopique & Paléoclimatique	AVI*CT93-0015
Ministry of Agriculture	IC18-CT98-0269
Office National de l'Assainissement	AVI2-CT93-087
Office National de l'Assainissement	AVI2-CT93-058
ORSTOM - Institut Français de 'Recherche Scientifique pour le Développement en Coopération	IC18-CT96-0091
Tunisian Institute for Computational & Telecommunications Research	IC18-CT95-0175
Université de Monastir	CI1*CT93-0313
Université de Tunis	AVI2-CT93-099
Université Tunis II	IC18-CT96-0029
Université Tunis II	AVI*CT92-0011

## TURKEY

Akdeniz University	IC18-CT97-0177
Ankara University	AVI*CT92-0009
Ankara University	TS3*CT92-0143
Ankara University	IC18-CT95-0004
Bilkent University	ICA-17
Bilkent University	ITDC 204-82166

Bogazici University	AVI2-CT93-074
Cukurova University	IC18-CT98-0392
Dicle University	AVI*CT93-0008
Dokuz Eylul University	AVI*CT93-0006
Ege University	AVI*CT94-0014
Ege University	AVI2-CT93-008
Ege University	IC18-CT96-0082
Ege University	AVI*CT92-0003
Ege University	IC18-CT95-0023
Ege University	IC18-CT98-0266
Ege University	IC18-CT97-0167
Ege University	IC18-CT97-0153
Ege University	IC18-CT98-0310
Hacettepe University	AVI*CT93-0012
Hacettepe University	AVI2-CT93-072
Hacettepe University	AVI*CT94-0004
Istanbul Technical University	IC18-CT97-0161
Istanbul Technical University	IC18-CT98-0352
Istanbul Technical University	AVI*CT94-0015
Istanbul Technical University	ICA-17
Istanbul University	AVI*CT94-0015
Istanbul University	AVI*CT92-0007
Marmara Research Centre-Tübitak	AVI*CT94-0013
Middle East Technical University	AVI2-CT93-080
Middle East Technical University	AVI*CT93-0001
Middle East Technical University	AVI*CT92-0005
Middle East Technical University	IC18-CT97-0200
Middle East Technical University	IC18-CT98-0386
Pamukküle Üniversitesi	AVI*CT94-0005
The Marmara Scientific & Research Center	ICA-17
The Middle East Technical University	ICA-17
Anadolı Forest Research Institute	IC18-CT97-0200

## UNITED KINGDOM

Agricultural & Food Research Council	TS3*CT92-0143
British Museum of Natural History	TS3*CT93-0253
Brunel University	AVI*CT92-0012
Cranfield University	AVI*CT93-0067
Hammersmith Hospital	TS3*CT93-0244
HR Wallingford	AVI*CT94-0012
Imperial College of Science, Technology & Medicine	TS3*CT93-0253
Imperial College of Science, Technology & Medicine	TS3*CT92-0093
Imperial College of Science, Technology & Medicine	CII*CT94-0122
Institute for Animal Health	TS3*CT92-0151
International Centre for Conservation Education	IC18-CT95-0809
International Institute for Environment and Development	IC18-CT96-0069
Key Industrial Software Systems Ltd	IC18-CT95-0410
Liverpool School of Tropical Medicine	AVI*CT92-0018
London School of Hygiene & Tropical Medicine	AVI*CT93-0008
London School of Hygiene & Tropical Medicine	TS3*CT92-0088
London School of Hygiene & Tropical Medicine	AVI2-CT93-031
London School of Hygiene & Tropical Medicine	AVI*CT94-0003
London School of Hygiene & Tropical Medicine	AVI*CT94-0003
London School of Hygiene & Tropical Medicine	IC18-CT98-0349
L.T. Power Ltd	AVI*CT94-0004
Natural Environment Research Council	IC18-CT96-0122

Natural Environment Research Council	AVI*CT93-0015
Natural Environment Research Council	AVI*CT93-0005
Natural Environment Research Council	IC18-CT96-0091
Natural Environment Research Council	IC18-CT98-0301
Roslin Institute	IC18-CT95-0004
Roslin Institute	IC18-CT95-0009
Royal Veterinary College	IC18-CT95-0009
Rutherford Appleton Laboratory	ICA-17
Scottish Crop Research Institute	IC18-CT98-0311
The Imperial College of Science, Technology & Medicine	AVI2-CT93-062
The Imperial College of Science, Technology & Medicine	AVI*CT94-0014
UMIST/Centre for Computational Linguistics	IC18-CT95-0175
University College London	IC18-CT96-0029
University of Bath	CI1*CT93-0070
University of Birmingham	IC18-CT98-0270
University of Bristol	CI1*CT94-0087
University of Bristol	AVI2-CT93-080
University of Cambridge	CI1*CT94-0125
University of Cambridge	CI1*CT94-0080
University of Cambridge	IC18-CT96-0036
University of Cambridge	IC18-CT95-1139
University of Durham	IC18-CT98-0268
University of Edinburgh	TS3*CT91-0019
University of Edinburgh	TS3*CT92-0143
University of Edinburgh	IC18-CT95-0004
University of Edinburgh	IC18-CT95-0009
University of Edinburgh	TS3*CT92-0143
University of Edinburgh	IC18-CT95-0009
University of Glasgow	IC18-CT95-0003
University of Glasgow	TS3*CT92-0096
University of Glasgow	IC18-CT95-0009
University of Glasgow	TS3*CT91-0019
University of Glasgow	IC18-CT98-0367
University of Kent	IC18-CT97-0171
University of Leeds	KIT Nr 12
University of London	TS3*CT93-0221
University of London	CI1*CT93-0005
University of London	IC18-CT98-0308
University of Manchester	CI1*CT94-0126
University of Newcastle Upon Tyne	AVI*CT94-0012
University of Newcastle Upon Tyne	AVI*CT93-0002
University of Newcastle Upon Tyne	IC18-CT97-0136
University of Nottingham	AVI*CT92-0012
University of Nottingham	CI1*CT94-0131
University of Portsmouth	IC18-CT96-0064
University of Portsmouth	AVI*CT94-0010
University of Portsmouth	IC18-CT98-0331
University of Reading	AVI*CT94-0004
University of Reading	AVI*CT94-0004
University of Reading	IC18-CT97-0177
University of Salford	IC18-CT98-0354
University of Surrey	EC-ISR-93003
University of Surrey	CI1*CT93-0003
University of Surrey	IC18-CT98-0384
University of Sussex	IC18-CT98-0301
University of Wales	IC18-CT96-0082
University of York	TS3*CT91-0019
University of York	IC18-CT95-0003

University of York  
Wye College

IC18-CT95-0009  
IC18-CT96-0082

## **WEST BANK AND GAZA STRIP**

Aid to the Aged (ATTA)	IC18-CT96-0036
Al Nada Centre for Women's Health & Nutrition	AVI*CT94-0003
Al Quds University – College of Science & Technology	IC18-CT98-0272
An-Najah National University	IC18-CT96-0099
Bethlehem University	AVI*CT94-0008
Bethlehem University	IC18-CT97-0142
Bethlehem University	IC18-CT97-0136
Birzeit University	AVI*CT94-0004
Government Public Health Services	AVI*CT94-0003
Hebron University	AVI*CT94-0009
Ministry of Health	AVI2-CT93-031
Ministry of Health	AVI*CT94-0003
Rimal Clinic	AVI*CT94-0003
United Nations Relief Works Administration	AVI*CT94-0003





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Index by programmes and contract numbers

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## Index by programmes and contract numbers

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### Contract number

### co-ordinator

#### International Scientific Co-operation (ISC)

CI1*CT89-0442i	Penzkoferi	A.
CI1*CT90-0542i	Haasei	W.
CI1*CT91-0907i	Jofrei	J.
CI1*CT91-0923i	Einavi	S.
CI1*CT91-0927i	Lindegaard-Andersen	A.
CI1*CT91-0931i	Meyeri	F.
CI1*CT91-0932i	Fluhri	R.
CI1*CT92-0063i	Kalishi	R.
CI1*CT92-0095i	Agranati	A. - J.
CI1*CT92-0096i	Brakenhoffi	G. - J.
CI1*CT92-0104i	Garciai	J. - L.
CI1*CT93-0003i	Kadouri	A.
CI1*CT93-0004i	Chaveli	P.
CI1*CT93-0005i	Grayi	J.
CI1*CT93-0006i	Marschneri	H.
CI1*CT93-0065i	LévyClémenti	C.
CI1*CT93-0066i	Hanacki	M.
CI1*CT93-0069i	Konczykowski	M.
CI1*CT93-0070i	Etmani	M.
CI1*CT93-0074i	Moli	J. - N. - V.
CI1*CT93-0311i	Levini	L. - A.
CI1*CT93-0312i	Minei	P.
CI1*CT93-0313i	Gibarti	P.
CI1*CT93-0362i	Tackei	M.
CI1*CT93-2027i	Bontempsi	N.
CI1*CT94-0080i	Grayi	J.
CI1*CT94-0083i	Dielsi	L.
CI1*CT94-0085i	Francki	F.
CI1*CT94-0086i	Fieldi	J. - A.
CI1*CT94-0087i	Shewryi	P. - R.
CI1*CT94-0096i	Wolfrumi	J.
CI1*CT94-0097i	Vorgiasi	C. - E.
CI1*CT94-0105i	Wodaki	S.
CI1*CT94-0106i	Hamppi	R.
CI1*CT94-0108i	Kelleri	U.
CI1*CT94-0122i	Nicolaidesi	A.
CI1*CT94-0125i	Kingi	D. - A.
CI1*CT94-0126i	Scheeri	H.
CI1*CT94-0130i	Kirschi	J.
CI1*CT94-0131i	Davisi	S.
CI1*CT94-0779i	Remoni	J. - P.
EC-ISR 90i	Levi	G.
EC-ISR-93003i	Granumi	E.
EC-MED-35i	Koudijisi	A.

#### Science and Technology for Development 3 (STD 3)

TS3*CT91-0019i	Taiti	A.
TS3*CT92-0015i	Süssmuthi	R.
TS3*CT92-0047i	Grouzisi	M.
TS3*CT92-0061i	Menenti	M.

TS3\*CT92-0088  
 TS3\*CT92-0093  
 TS3\*CT92-0096  
 TS3\*CT92-0106  
 TS3\*CT92-0112  
 TS3\*CT92-0119  
 TS3\*CT92-0126  
 TS3\*CT92-0143  
 TS3\*CT92-0144  
 TS3\*CT92-0151  
 TS3\*CT93-0208  
 TS3\*CT93-0221  
 TS3\*CT93-0244  
 TS3\*CT93-0249  
 TS3\*CT93-0253  
 TS3\*CT94-0264  
 TS3\*CT94-0278  
 TS3\*CT94-0282

Del Mar Garcia Calvente M.  
 Van der Veen A. - M. - H.  
 Bazzicalupo P.  
 Boulard C.  
 Laabid A.  
 Enne G.  
 Xanthoulis D.  
 Spooner R.  
 Tursz A.  
 Mellor P.  
 De Loof A.  
 Rodrigues Junior C. - J.  
 Krishnamoorthy R.  
 Schmidt G. - H.  
 Ben-Ismael R.  
 Dutuit P.  
 Duhoux E.  
 Marcos A.

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 AVI\*CT92-0002  
 AVI\*CT92-0003  
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 AVI\*CT92-0005  
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 AVI\*CT92-0007  
 AVI\*CT92-0008  
 AVI\*CT92-0009  
 AVI\*CT92-0010  
 AVI\*CT92-0011  
 AVI\*CT92-0012  
 AVI\*CT92-0013  
 AVI\*CT92-0014  
 AVI\*CT92-0015  
 AVI\*CT92-0016  
 AVI\*CT92-0017  
 AVI\*CT92-0018  
 AVI\*CT93-0001  
 AVI\*CT93-0002  
 AVI\*CT93-0003  
 AVI\*CT93-0004  
 AVI\*CT93-0005  
 AVI\*CT93-0006  
 AVI\*CT93-0007  
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 AVI\*CT93-0011  
 AVI\*CT93-0012  
 AVI\*CT93-0013  
 AVI\*CT93-0014  
 AVI\*CT93-0015  
 AVI\*CT93-0067  
 AVI\*CT93-0143  
 AVI\*CT94-0001  
 AVI\*CT94-0002  
 AVI\*CT94-0003  
 AVI\*CT94-0004  
 AVI\*CT94-0005  
 AVI\*CT94-0006  
 AVI\*CT94-0007  
 AVI\*CT94-0008

Paggi L.  
 Cao A.  
 Oskam L.  
 Levsen K.  
 Kallos G.  
 Litz N.  
 Cita M. - B.  
 Escadafal R.  
 Roig B. - J.  
 Hamers R.  
 Taieb G.  
 Harrison P. - G.  
 Tibayrenc M.  
 Cot L. & A. - L.  
 Berthomé J. - P.  
 Varnavas S. - P.  
 Nychas S. - G.  
 Ashford R. - W.  
 Müller W. - E. - G.  
 McNeil C. - J.  
 Crepon M.  
 Gryseels B.  
 Smith B.  
 Kallidromitou D.  
 Paul R.  
 Burchard G. - D.  
 van Vuren G.  
 Verdou J. - P.  
 Hours B.  
 Van Lerberghe W.  
 Reysoo H. - P.  
 Goossens M.  
 Edmunds W. - M.  
 Magan N.  
 Bakker J.  
 Ruppel A.  
 Xanthoulis D.  
 Lewando-Hundt G.  
 Derrick A.  
 Fischer K.  
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 Vogelpohl A.  
 Traverse J. - P.

AVI*CT94-0009	Lettinga	G.
AVI*CT94-0010	Liberti	L.
AVI*CT94-0011	Mata-Alvarez	J.
AVI*CT94-0012	Brabben	T.
AVI*CT94-0013	Funken	K. - H.
AVI*CT94-0014	Uribe	I. - O.
AVI*CT94-0015	Bordado	J.
AVI2-CT93-008	Beltrão	J. - G.
AVI2-CT93-019	Blazy	P.
AVI2-CT93-020	Ambrogetti	F.
AVI2-CT93-031	Lewando-Hundt	G.
AVI2-CT93-054	Damelincourt	J. - J.
AVI2-CT93-058	Lehucher	P. - M.
AVI2-CT93-062	Cheeseman	C.
AVI2-CT93-072	Hötzl	H.
AVI2-CT93-073	De Breuck	W.
AVI2-CT93-074	Thiemann	W.
AVI2-CT93-076	Salgot De Marçay	M.
AVI2-CT93-080	Siccardi	F.
AVI2-CT93-081	Bariou	B.
AVI2-CT93-083	Bergaya	F.
AVI2-CT93-087	Amato	E.
AVI2-CT93-091	Clementel	S.
AVI2-CT93-092	Stephanou	E. - G.
AVI2-CT93-099	Cremonini	M. - G.
AVI2-CT93-107	Crisanti	A.
AVI2-CT93-112	Mosbaek	H.
AVI2-CT93-123	Rosenthal	H.
AVI2-CT93-126	Yague Ballester	A.

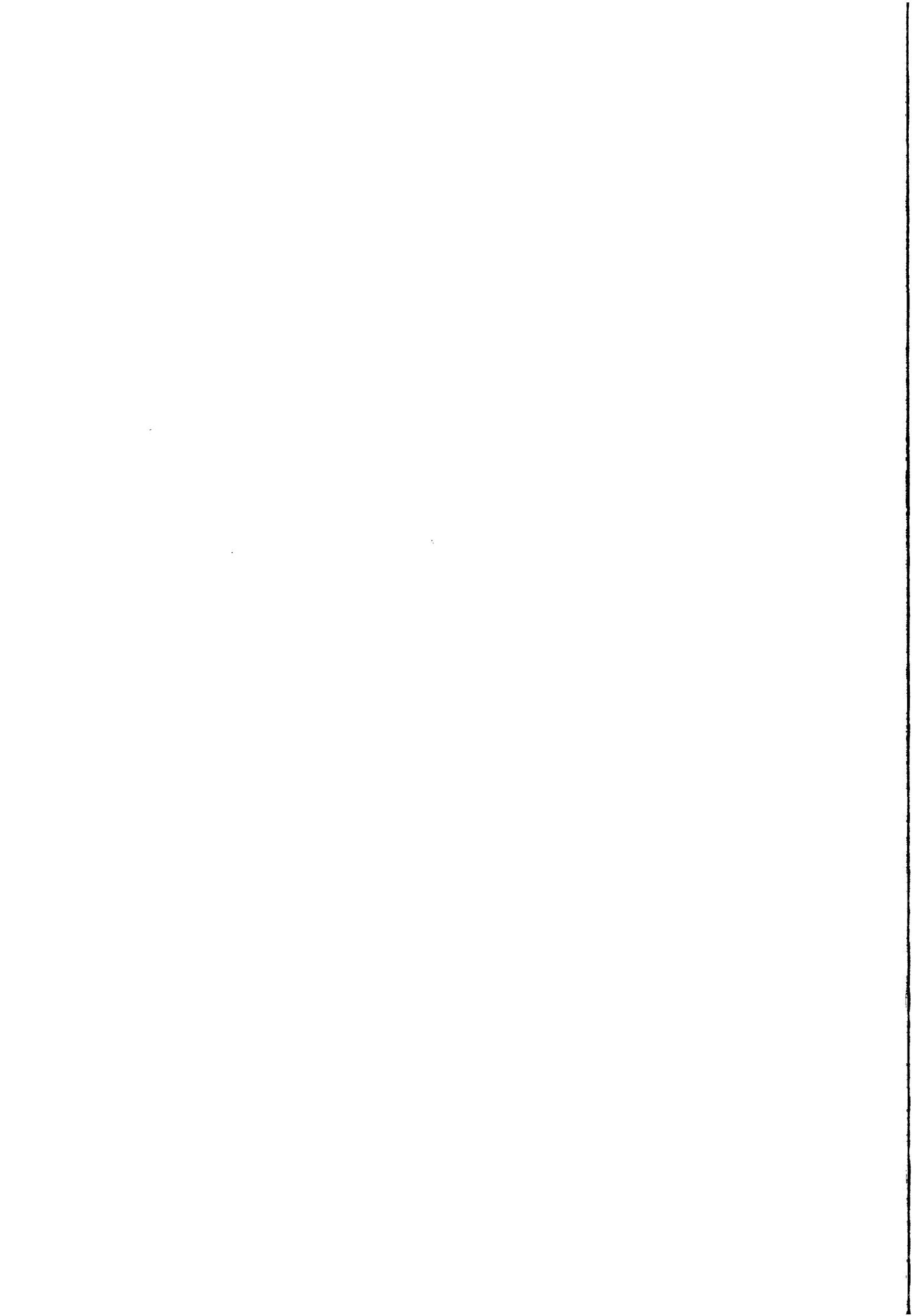
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IC18-CT95-0004	Spooner	R.
IC18-CT95-0009	Jongejan	F.
IC18-CT95-0023	Oskam	L.
IC18-CT95-0363	Sicard	P.
IC18-CT95-0410	Wittig	T.
IC18-CT95-0507	Nassiopoulos	A. - G.
IC18-CT95-0809	Fedra	K.
IC18-CT95-0895	Jordan	A.
IC18-CT95-0905	Pease	C.
IC18-CT95-1139	Zenios	S. - A.
IC18-CT96-0029	Patrick	S.
IC18-CT96-0034	Müller	W. - E. - G.
IC18-CT96-0036	Sen	K.
IC18-CT96-0039	Paschaloudis	D.
IC18-CT96-0055	Lieth	H.
IC18-CT96-0064	Neiland	A.
IC18-CT96-0069	Menenti	M.
IC18-CT96-0076	Jori	G.
IC18-CT96-0082	Hall	M. - A.
IC18-CT96-0091	Albergel	J.
IC18-CT96-0099	Neskakis	A.
IC18-CT96-0121	Vetten	H. - J.
ITDC 135-82159	Mahjoub	Z.
ITDC 204-82166	Aykanat	C.
KIT Nr. 204	Pritschow	G.
IC18-CT95-0175	Belhadj Kacem	R.
IC18-CT96-0081	Drevon	J. - J.
IC18-CT96-0122	Baker	J.
ICA-17	Bourdeaud'hui	E.
ITDC-94-201-82164	Daoudi	EI M.
KIT Nr. 12	Cohn	A. - G.

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 IC18-CT96-1791  
 IC18-CT96-1301  
 IC18-CT96-1263  
 IC18-CT96-0035  
 IC18-CT97-0134  
 IC18-CT97-0136  
 IC18-CT97-0138  
 IC18-CT97-0142  
 IC18-CT97-0143  
 IC18-CT97-0147  
  
 IC18-CT97-0151  
 IC18-CT97-0153  
 IC18-CT97-0154  
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 IC18-CT97-0163  
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 IC18-CT97-0169  
 IC18-CT97-0171  
 IC18-CT97-0177  
 IC18-CT97-0186  
 IC18-CT97-0197  
 IC18-CT97-0198  
 IC18-CT97-0200  
 IC18-CT97-0202  
 IC18-CT97-0214  
 IC18-CT97-0228  
 IC18-CT97-0252  
 IC18-CT97-0256  
 IC18-CT98-0261  
 IC18-CT98-0265  
 IC18-CT98-0266  
 IC18-CT98-0267  
 IC18-CT98-0268  
 IC18-CT98-0269  
 IC18-CT98-0270  
 IC18-CT98-0273  
 IC18-CT98-0272  
 IC18-CT98-0289  
 IC18-CT98-0293  
 IC18-CT98-0300  
 IC18-CT98-0301  
 IC18-CT98-0308  
 IC18-CT98-0310  
 IC18-CT98-0311  
 IC18-CT98-0313  
 IC18-CT98-0331  
 IC18-CT98-0333  
 IC18-CT98-0346  
 IC18-CT98-0349  
 IC18-CT98-0352  
 IC18-CT98-0354  
 IC18-CT98-0367  
 IC18-CT98-0384  
 IC18-CT98-0385  
 IC18-CT98-0386  
 IC18-CT98-0390  
 IC18-CT98-0391  
 IC18-CT98-0392

Chiaramonti C.  
 Drevon J-J  
 Dichy J  
 Shihab-Eldin A  
 Belhadijkalem R  
 Zanini E.  
 De Haan L.  
 Evison L.  
 Fletcher E. - J.  
 Assimacopoulos D.  
 Udluft P.  
 De Oliveira Alves C.  
 Coelho  
 Savage M.  
 Salleo S.  
 Vidal-Madjar D.  
 Megier J.  
 Orthofer R.  
 Hamdy A.  
 Bontoux L.  
 Mueller W. - R.  
 Rossi G.  
 Jackson D.  
 Caligari P. - D. - S.  
 Hutterman A.  
 Barea J. - M.  
 Nesme X.  
 Arbez M.  
 Verreth J.  
 Jaidi L.  
 Renganathan E.  
 Gicquel B.  
 Guizani I.  
 Teklehaimanot Z.  
 Chafik E  
 Cuartero J.  
 Vogelpohl A.  
 Borri D  
 Stroosnijder L.  
 Scapini F  
 Lieder H-W  
 Xanthoulis D.  
 Morgana B.  
 Dauta A.  
 Caubel G.  
 Yeo A.  
 Letouze R.  
 Audergon J. - M.  
 Forster B P  
 Priefer U.  
 Neiland A  
 Fridlender B.  
 Van Lerberghe W.  
 Ronsmans C.  
 Khogali M  
 Craig P.  
 Kusel J-R  
 Brianso-Penalva J L  
 Petrini V  
 Bechtel F  
 Dutuit P  
 Darmency H  
 Alados C L



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