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**Contribution to environmental risk management
of pesticides in agricultural areas of
North Vulnerable Area of the Tejo.**

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Dissertação para obtenção do Grau de Mestre em
Engenharia do Ambiente

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ABSTRACT

This study was made to provide a better knowledge of the hazard of groundwater resources to pesticides, in agricultural ecosystems, mainly in maize and potato irrigated areas inserted in North Vulnerable zone of Tejo.

The studies were developed in areas of high vulnerability to groundwater contamination. Surface and groundwater resources are intimately connected, with changes in one affecting the other. Due to the importance of the integration of ground and surface water, sampling was performed in Almonda river and “Dique dos Vinte” marsh.

The registered pesticides for the main crops in the region were selected and considered to environmental modeling (model of Mackay, Bacci & Gaggi, GUS and EPRIP indices).

Of the 26 pesticides and metabolites tested, three were detected in the both natural resources: atrazine, alachlor, metolachlor, reaching a maximum concentration of 0.28, 1.73 and 0.21 µg/L respectively.

Ecotoxicological tests were performed with the *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Chironomus riparius* aquatic organisms. Toxicity effects were observed in the both natural resources. *P. subcapitata* was the most sensitive organism followed *D. magna*.

This study highlights the need for a more conscientious management of the factors which determine the contamination of water resources with pesticides.

Key-words: groundwater, contamination, pesticides, Vulnerable Zone

RESUMO

Este estudo teve como objectivo proporcionar um melhor conhecimento do perigo dos pesticidas para as águas subterrâneas, em ecossistemas agrícolas, principalmente em áreas irrigadas de milho e batata inseridos na zona norte Vulnerável do Tejo.

Os estudos foram desenvolvidos em áreas de elevada vulnerabilidade da água subterrânea à contaminação. As águas subterrâneas e superficiais encontram-se intimamente ligadas, as mudanças numas afectam as outras. Devido à importância de uma abordagem integrada dos mesmos, foi realizada a amostragem no rio Almonda e na alverca "Dique dos Vinte".

Os pesticidas homologados para as principais culturas na região foram seleccionados e considerados para modelagem ambiental (modelo de Mackay, Bacci & Gaggi, GUS e EPRIP índices).

Dos 26 pesticidas e metabolitos testados, três foram detectados em ambos os recursos: atrazina, alacloro e metolacloro, atingindo uma concentração máxima de 0,28, 1,73 e 0.21 $\mu\text{g} / \text{L}$, respectivamente.

Testes ecotoxicológicos foram desenvolvidos com os organismos aquáticos *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Daphnia magna* e *Chironomus riparius*, tendo-se observado efeitos tóxicos em ambos os recursos. A *P. subcapitata* foi o organismo mais sensível seguindo-se a *D. magna*.

Este estudo evidencia a necessidade de uma gestão mais consciente dos factores que determinam a contaminação dos recursos hídricos com pesticidas.

Palavras-chave: águas subterrâneas, contaminação, pesticidas, Zona Vulnerável

RESUMO ALARGADO

Considerando que a actividade agrícola ocupa cerca de 48% do território nacional e que o sector agrícola é o maior consumidor de água subterrânea (4210 hm³) (64%) (MADRP, 2008), o presente estudo tem como objectivo contribuir para uma avaliação integrada da exposição dos recursos hídricos subterrâneos a pesticidas e analisar a influência dos potenciais factores chave na mesma, em ecossistemas agrícolas inseridos na Zona Vulnerável Norte do Tejo. Esta região apresenta elevada importância nacional a nível agrícola, estando localizada na região do Vale do Tejo que integra actualmente cerca de 28,3 milhares de explorações agrícolas que ocupam cerca de 7% da SAU (superfície agrícola utilizada). De modo, a obter uma ferramenta que possa auxiliar a tomada de decisão por parte dos técnicos e agricultores e contribuir para uma gestão agro-ambiental apropriada, ou seja, gerir o risco através da sua avaliação e caracterização permitindo a discussão de medidas a tomar de modo a alterar os principais factores de risco.

Nas áreas em estudo, em geral localizadas em zonas de elevada vulnerabilidade da água subterrânea à contaminação (do ponto de vista hidrogeológico) seleccionaram-se dois tipos de ecossistemas agrícolas, batata e milho, em locais com prática de rega, apontados como de elevada importância na economia agrária da região, onde se colheram as amostras de água subterrânea. A amostragem de águas subterrâneas foi realizada em quatro datas distintas de modo a analisar a evolução dos pesticidas presentes.

No sentido de melhor compreender a dinâmica e a pressão a que esta zona está sujeita foram colhidas amostras de águas superficiais no rio Almonda e na alverca Dique dos Vinte. Os sedimentos podem funcionar como um sumidouro para os elementos químicos presentes no ambiente aquático, uma vez que estes são um grande repositório de muitos dos produtos químicos mais persistentes que são introduzidas nas águas superficiais, considerou-se assim importante a sua avaliação toxicológica.

Os pesticidas seleccionados para o estudo correspondem aos homologados para as principais culturas da região, e foram submetidos a uma primeira abordagem preditiva através de modelação para determinar a sua afinidade para a água (modelo de fugacidade de Mackay), o seu potencial de lixiviação (índice GUS e Bacci & Gaggi), e avaliados no que diz respeito ao seu risco para três diferentes sistemas ambientais (terrestre epígeo, hipogeo e águas superficiais) através do índice EPRIP, de forma a fazer um “rastreamento” dos mesmos, possibilitando uma selecção *á priori* daqueles que apresentam maior potencial de

contaminação da água, nomeadamente da subterrânea e maior risco para os organismos não visados.

Subsequentemente e já numa abordagem de avaliação da exposição das águas aos pesticidas, as amostras foram analisadas, sendo a extracção realizada com base na técnica SPME (solid-phase microextraction) e os resultados obtidos por GC-MS (Gas chromatography-mass spectrometry). Foram analisadas 24 amostras de água subterrânea das quais 16% apresentaram níveis de, pelo menos um, dos compostos superiores a 0.1µg/L, valor paramétrico nas águas para consumo humano (D.L. n° 243/2001). Nas águas superficiais as amostras analisadas (100%) apresentam níveis de, pelo menos um, dos compostos mais elevados que 0.1µg/L. Em ambos os tipos de amostras foram doseados os herbicidas alacloro, atrazina e metolacloro, atingindo um nível máximo de concentração de 0,28; 1,73 e 0,21 µg/L respectivamente.

Com a finalidade de alargar o espectro de análise no caso das águas subterrâneas, efectuou-se uma análise qualitativa dos compostos orgânicos por GC-MS. Detectou-se a presença de alguns pesticidas sendo que nas 6 amostras analisadas foi identificado o nematodocida dicloropropeno, bem como a presença de compostos orgânicos voláteis, que provavelmente existirão na formulação dos mesmos. Verificou-se a co-ocorrência de compostos orgânicos voláteis e de misturas de pesticidas, o que poderá ser tema para futuras investigações, porque como evidenciado em vários estudos a co-ocorrência de compostos pode influenciar as propriedades individuais de cada um, por exemplo a toxicidade da mistura poderá ser superior à dos compostos individuais mesmo quando presentes individualmente em concentrações superiores do que as em mistura (Stackelberg *et al.*, 2001).

Finalmente a realização de estudos ecotoxicológicos permitiu avaliar a toxicidade aguda e crónica. Das 24 amostras de água subterrâneas submetidas a testes de toxicidade, 83% apresentaram uma percentagem de efeito (imobilização) para a *Daphnia magna* (48 h) \geq 50% e em 30% foi observada uma percentagem de inibição do crescimento da *Pseudokirchneriella subcapitata* (ALgaltookitTM) superior a 50%. As amostras de água superficial não revelaram toxicidade para a *Vibrio fischeri* (Microtox[®]), foram também realizados testes de toxicidade para *D. magna* (21 dias) e *P. subcapitata* (72h, “microplate technique”), sendo que em ambos os casos a amostra colhida num ponto do rio Almonda (designada como AR) revelou ser mais tóxica. No que diz respeito à avaliação da toxicidade do sedimento para o *Chironomus riparius* (7 dias) este não apresentou toxicidade. Integrando os valores de toxicidade e exposição, concluiu-se que, para os níveis de efeito e concentração de pesticidas obtidos não seria possível estabelecer uma relação entre os níveis considerados.

Os resultados obtidos confirmam a vulnerabilidade dos recursos hídricos, nomeadamente os subterrâneos a este tipo de contaminação. Considera-se assim de extrema importância o reforço da investigação na área dos pesticidas, a monitorização contínua, e a integração multidisciplinar de metodologias e acções com vista à gestão do risco, especialmente em zonas de elevada vulnerabilidade hidrogeológica e com forte pressão agrícola contribuindo para uma utilização sustentável dos pesticidas.

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ACRONYMS

- ADI – Acceptable daily intake
- DGADR – “Direcção Geral de Agricultura e desenvolvimento rural”
- DRARO – “Direcção regional de Agricultura da Região Oeste”
- DT₅₀ – Dissipation time
- EPPNA – “Equipa de projecto do Plano Nacional da Água”
- EU – European Union
- EC – European community
- EC₅₀ – median effective concentration
- EEC – European Economic Community
- EPA – Environmental Protect Agency
- FAO – Food and agriculture organization
- GC – Gas chromatography
- GC-MS – Gas Chromotography coupled with mass spectrometry
- GLs – Guide levels
- GR – Growth rate
- GUS – Groundwater ubiquity score
- H – Henry’s law constant
- HAS – Recommended health levels
- HQ – Hazard coefficient
- IC₅₀ – Concentration wich causes 50% inhibition
- IC_p – Inhibition concentration for a specified effect
- IMAR – “Instituto do Mar”
- INAG – “Instituto Nacional da Água”
- K_{AW} – Partition coefficient air/water
- K_{OC} – Organic carbon sorption coefficient
- K_{OW} – Partition coefficient octanol/water
- LD₅₀ – median lethal dose
- LOD – Level of detection
- LOEC – Lowest observed effect concentration
- MAC – Maximum allowable levels
- MATC - Maximum allowable toxicant concentration
- MRA – Maximum application rate
- MRLs – Maximum residue levels
- NOEC – No observed effect concentration
- NOEL – No observed effect level
- NWWA – National association well water
- OECD – Organization for economic co-operation and development
- PA – Polyacrilate
- PDMS – Polymethyl siloxane

- PEC – Predicted environment concentration
- PED – Predicted environment distribution
- PNA – “ Plano Nacional da Água”
- PNEC – Predicted no effect concentration
- UAS – Utilized agricultural area
- SPME – Solid-phase microextraction
- TDI – total daily intake
- TER – Toxicity exposure ratio
- U.S.E.P.A – Protection agency of the United States of America
- VOC’s – Volatile organic compounds
- WHO – World health organization
- % - percentage
- > - Greater than
- <- Less than
- = - equal to

1. INTRODUCTION

The first recorded use of chemicals to control pests dates back to 2500BC. Nevertheless, it was only in the last 50 years that chemical control has been widely used (Pretty, 2005). Because pesticides are designed to kill, prevent, or mitigate pests, they may pose a risk to users or the environment. However, when used properly, pesticides also provide benefits (USEPA, 2006).

Since the early 1960's, when the negative environmental impacts of pesticide use became a topic of societal debate, an increasingly refined and detailed regime of measures was implemented in order to reduce the environmental impacts of pesticides (Hond *et al.*, 2003). Yet, only approximately one decade later, in the 70's and in the 80's, emerged the concern about the groundwater contamination with pesticides.

The groundwater is a precious limited natural resource that constitutes an important source of freshwater, in particular for human consumption, but also plays important ecological functions (Bachmant, 1994; Mandl *et al.*, 1994; INAG, 2001). Groundwater protection and preservation is a priority for several reasons:

- once contaminated, groundwater is harder to clean than surface water and the consequences can last for decades;
- as groundwater is frequently used for the abstraction of drinking water, for industry and for agriculture, groundwater pollution can endanger human health and threaten those activities;
- groundwater provides the base flow for many rivers (it can provide up to 90% of the flow in some watercourses) and can thus affect the quality of surface water systems;
- groundwater acts as a buffer through dry periods, and is essential for maintaining wetlands.

In order to protect water quality, particularly of groundwater, and prevent risks to human health and environment, several directives have been implemented within the European Community. There are several key directives, like Directive 98/83/CE on the water quality for human consumption. The Directive 2000/60/CE, known as the Water Framework Directive, which establishes a framework of action in the field of water policy and includes a list of priority substances, which includes several pesticides, and its "daughter directive", Directive 2006/118/CE, relative to the protection of groundwater against pollution and deterioration. Moreover, under Directive 91/414/EEC on the placement of products on the market, it was established that it should not be granted authorization to the active substances

whose concentration in groundwater is likely to exceed a value of 0.1 µg/L (originally defined as part of Directive 80/778/EEC, as maximum allowable concentration for individual pesticides in the water for human consumption. In 2001, the 6th Framework Program of Action on the Environment provided the development of a Thematic Strategy on the Sustainable Use of Pesticides, which aims to reduce the impact of pesticides on human health and the environment, i.e., to achieve a more sustainable use of pesticides and reducing risks not neglecting the protection of crops.

Managing the risk or effects of pesticides requires far more information than it is possible to afford, to directly measure for all the places and all the times, and all the pesticides of interest. Strategies and/or tools are therefore required to focus monitoring and risk assessment programs in a cost-effective manner, and to predict pesticide concentrations and effects. To assess the risk of pesticides in aquatic ecosystems, information is required on the environmental fate of pesticides and their concentrations in the environment (exposures) and on their potential adverse effects (i.e., toxicity) on organisms. The overall ecological risk can then be determined based on the general principle that risk is a function of toxicity and exposure (Figure 1.1) (DPI, 2007).

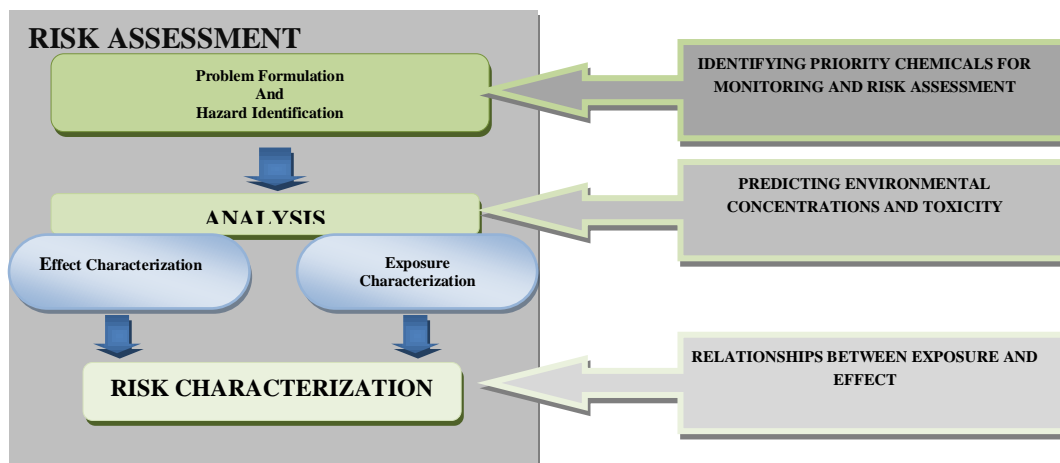


FIGURE 1.1: Risk assessment steps (DPI, 2007).

The studies of the contamination of water bodies with pesticides have started in Portugal in 1989, in agricultural areas in the counties of Chamusca and Golegã (Cerejeira, 1993; Cerejeira *et al.*, 1995 a, b), and included modeling studies and laboratory and field investigations, both in soils and waters. The assessment of the hazard of pesticides in water was later extended to other agricultural areas of the counties of "Ribatejo e Oeste", "Beira litoral" and "Alentejo" regions (Batista *et al.*, 2000b, 2001, 2002, 2006a,b, 2007; Batista, 2003; Cerejeira *et al.*, 1997, 2000a,b, 2003, 2005; Mendes *et al.*, 2008; Paralta *et al.*, 2001;

Pereira, 2003; Pereira *et al.*, 2000, 2007; Silva *et al.*, 2006, 2008, 2009; Silva-Fernandes *et al.*, 1999, 2005).

The ultimate goal of the present study was to contribute for the risk management of pesticide use in agricultural areas located in the North Vulnerable Zone of the Tejo. In order to help agricultural experts and farmers in the region, to make better management of factors that influence the occurrence of contamination by pesticides in groundwater resources, for a sustainable agriculture and reduction of environmental impacts. To this end, the main objective of the present study was to provide information on the environmental hazard of the pesticides that are used in the selected crops within the North Vulnerable Zone of the Tejo, in what concerns: (i) fate through modeling, (ii) real environmental concentrations, and (iii) effects.

The pesticides to be evaluated were selected based on those that were registered for the main crops of the study area. To evaluate the pesticides potential environmental behavior, their physical-chemical properties have been selected, to submit this assessment through modeling. Considering this information, it's also important to study the pesticides potential for leaching. Choose the toxicological and ecotoxicological characteristics in order to assess the potential risk to three different systems for using a risk index, likewise the exposure of groundwater and surface water sampling and their potential toxic effects on non-target organisms.

The present study is organized in seven chapters. Following this introductory chapter, Chapter 2 focuses the importance and the need to preserve the groundwater resources, and underlines the need for a Sustainable Agriculture and use of pesticides. Chapter 3 focuses the behavior of pesticides in the environment, specifically in the groundwater resource. In chapter 4, the concepts of ecotoxicology and hazard assessment are presented. In chapter 5, the evaluation of the pesticides impact on the quality of the hydrical resources (focus in groundwater resources) in the study zone is performed by presenting: the general characterization of the study area; the evaluation of environmental impact of the registered pesticides for the study area, through predictive approaches and analytical methodologies to evaluate the exposure of groundwater to pesticides and through toxicity testing to evaluate the potential adverse effects of the used pesticides on non-target aquatic organisms. In chapter 5 the results of the different approaches followed are presented and discussed. Chapter 6 points out some measures to minimize the phenomena of contamination of groundwater with pesticides. Finally, Chapter 7 sets out the final conclusions of the study and future developments.

2. IMPORTANCE OF GROUNDWATER QUALITY PRESERVATION

2.1. Environmental significance (contribution to surface water and importance to drinking-water quality)

Groundwater is generally pure from the microbiological point of view (Vighi & Funari, 1995). In many countries this resource is the main source of water for different purposes, particularly for public supply (Bachmat, 1994, Mandl *et al.*, 1994). Thus, even in countries where surface water is abundant, there good quality of the groundwater, its good accessibility and relatively low operating costs are factors that promote the increased exploitation of the groundwater resource (Wills & Yeh, 1987; Vighi & Funari, 1995). However, the quality of this resource can be seriously threatened by contamination or excessive exploitation (Mandl *et al.*, 1994).

There are two principal reasons of concern for the contamination of groundwater by pesticides (Wilson, 2003). Firstly, the concentration of pesticides in groundwater may limit its use for drinking. Secondly, the pesticide contaminated groundwater may be a source of pesticides for the surface waters that support aquatic ecosystems, which are also important drinking water supplies (Wilson, 2003).

It is now assumed as a worldwide scale problem that the traditional separation done on the studies about groundwater and surface water is not a realistic approach. It has been recognized such type of simplistic approach is more related to the difficulties associated with the development of quantitative relationships between these components of the land branch of the hydrological cycle than with the lack of need to manage an integrated manner the groundwater and surface water (Monteiro *et al.*, 2007). Another point to be taken into account is the fact that there is a relationship between the rivers and groundwater, i.e., in a given time the river can recharge the aquifers, having in this way an influence of the groundwater (INAG, 1999). Presently, the Water Framework Directive (Directive 2000/60/EC), as well as various institutional and technical documents from countries in other regions of the world, make calls and argue about the practical relevance and the inevitability of water management, that have to be done integrating the management of water resources (groundwater and surface water).

The combination of surface water feature(s), such as a river, estuary or wetland, and the groundwater system(s) that can directly interact in terms of movement of water can be designed as a **Connected Water resource** (Winter *et al.*, 1998). In this resources the flow of water between the surface water feature and the aquifer is termed the *seepage flux*, i.e., is the

magnitude and direction of water movement at the interface between surface water and groundwater systems. The flow of water is generally made through porous medium, such as sediments. The rule is that positive seepage flux values signify flow of groundwater to the surface water feature (also referred to as discharge or outflow) and negative flux values designate the flow from the surface water feature to the aquifer (also referred to as recharge, inflow) (Figure 2.1) (Winter *et al.*, 1998). The magnitude and the direction of the seepage flux is regulated by the Darcy's Law, though the flow of water between the water surface and the underlying aquifer is controlled by different factors, such as: (i) the hydraulic properties and features of the aquifer, (ii) the geological material unrevealing the aquifer from the surface water feature and (iii) the difference between the surface water level and the groundwater level. If the stream level is higher than the groundwater level measured within the aquifer, then the stream has the potential to lose water to the aquifer (Figure 2.2), leading to the occurrence of negative seepage flux because the shallow watertable is lower than the stream stage. In opposition, for groundwater to discharge into a stream channel, the elevation of the groundwater surface near the stream must be higher than the elevation of the river stage (Figure 2.3) (Winter *et al.*, 1998).

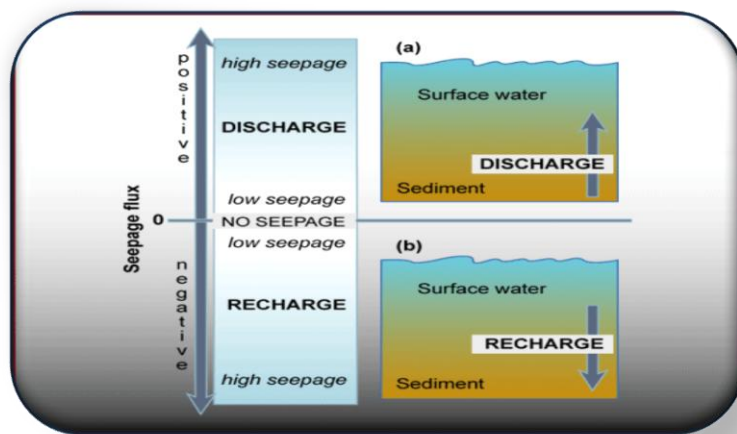


FIGURE 2.1: Vertical directions of seepage flux (Winter *et al.*, 1998).

The interactions of surface and groundwater can be modified in time and space in response to natural factors (e.g. climate factors) or in response to anthropogenic factors such as the catchment and management of the stream-aquifer, for example by direct discharges (e.g. sewage treatment plants), or seasonal return flows from drainage of irrigation areas. The extraction of groundwater using a pump can be sufficient to lower the watertable and decrease or reverse the hydraulic gradient towards the stream (Winter *et al.*, 1998). In some cases, groundwater pumping can reverse flowpaths near the stream, i.e., induce the recharge as the

stream becomes losing (Figure 2.4) (Winter *et al.*, 1998). Understanding the spatial and temporal interactions of land use, the associated potential sources of contamination, and the intrinsic susceptibility of a groundwater resource are key factors determining the geochemical system, and ultimately, the vulnerability of groundwater to contamination (Focazio *et al.*, 2003).

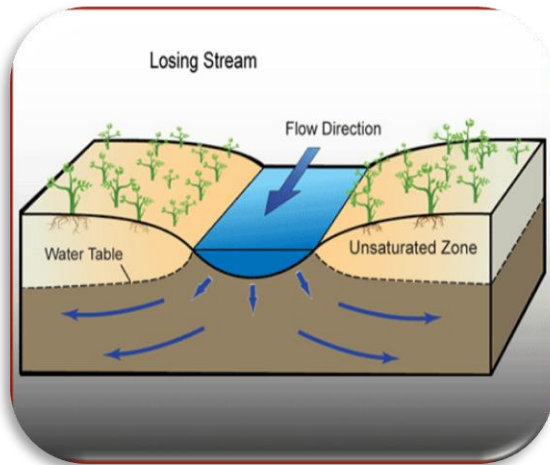


FIGURE 2.2: A losing stream where stream levels are higher than the surrounding watertable (Winter *et al.*, 1998)

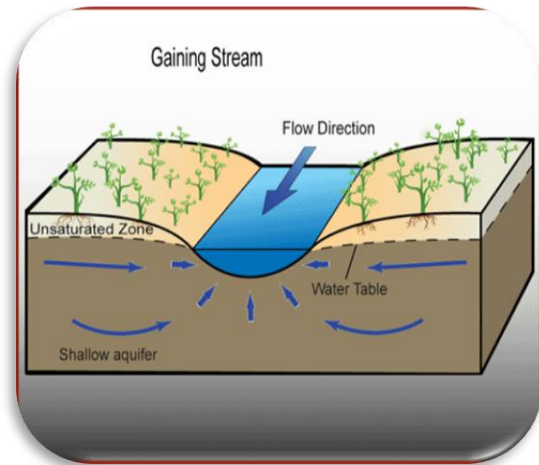


FIGURE 2.3: A gaining stream where stream levels are lower than the surrounding watertable (Winter *et al.*, 1998)

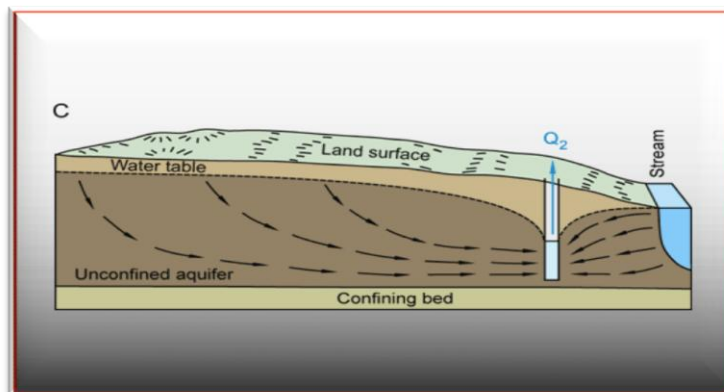


FIGURE 2.4: Effects of groundwater pumping on river-groundwater interaction showing a induced recharge conditions (Winter *et al.*, 1998).

In the study area this considerations are important not only because of the existence of a relation between surface and groundwater but according the PNA (“Plano Nacional da Água”) for the important dependence of the alluvium systems with the water courses, with are connected, revealing take into account the operation of those hydraulic seasonal hydrological systems (INAG, 2001).

2.2. Persistence of pesticides in groundwater and effects on human health

The pesticides when present in groundwater have generally low concentrations, and their potential effects on the environment and the human health stem from their potential stay in the same for long periods of time, and the possible existence of mixtures of compounds, including pesticides whose effects are still unclear (Batista, 2003; Hond *et al.*, 2003). The main potential effects of pesticides on human health resulting from the consumption of pesticide contaminated waters are carcinogenesis, mutagenesis, teratogenesis, neurotoxicity, changes in immunological and reproductive functions and endocrine deregulations (Trautman *et al.*, 1998; Hock & Brown, 1999). Side effects are dependent on the inherent toxicity of the pesticides beyond the period of exposure to the substance, and the speed with which it is metabolized and excreted from the body (Trautman *et al.*, 1998).

The World Health Organization (WHO) and the environmental protection agency of the United States of America (USEPA) have procedures for assessing the levels of pesticides in water consumption considered safe for human health. In 1984, the WHO set guide values (GLS) for maximum exposure to pesticides through water intake considered safe over a lifetime, values which are were being reviewed consecutively (WHO, 1987, 1993, 1996, 1998). The USEPA set recommended levels of health (Has) for many pesticides and for different periods of exposure, which vary from one day to lifelong (USEPA, 2004, 2006). According to that WHO, the establishment of these guide values should not be regarded as a justification for the degradation of water quality for human consumption by achieving those limits (WHO, 1998). The maximum allowable concentrations (MAC) established by the European Community Directive 80/788/EEC on the quality of water for human consumption reflect the principle that due to pesticides not be present in drinking water. Although some authors consider these values too restrictive and severe (Cohen, 1990), others (Mandl *et al.*, 1994) consider the approach consistent with the principles of precaution and compatible with sustainable development.

2.3. Legislative aspects – pesticides and water

2.3.1. Placing of plant protection products on the market

The regulations for pesticide registration in Portugal were initiated in 1967 with the implementation of D.L. 47 802 on July 19 establishing that pesticides for agricultural use could only be marketed through a prior registration process. The biggest change in the

evaluation of the impacts of pesticides on the environment, however, only took place with the introduction of risk analysis in its three components: assessment, management and communication of risk, and no doubt with the Directive 91/414/EEC, concerning the placement of products on the market, which was established in the European community requirements and rules for the risk analysis of pesticides. This directive, which came into force on July 26, of 1993, and was transposed to the Portuguese law through the D.L. 284/94 of November 11, presents new requirements, determining the need to follow the procedures and criteria for evaluation and decision.

2.3.2. Towards a thematic strategy on the sustainable use of pesticides

The increased spiraling of agriculture in many parts of the world has generally been accompanied by higher pesticides consumption. The most recent Food and Agriculture Organization (FAO) projections suggest that annual growth rates in agricultural production in developing countries will be slowing down over the next decades (FAO, 2006). The new challenge for agriculture is to find an economically viable way of producing a sufficient amount of safe and nutritious food while reducing negative impacts on the environment (BIAC, 2004).

Sustainable agriculture integrates three main goals: environmental health, economic profitability and social and economic equity. Sustainability rests on the principle that the needs of the present must be met without compromising the capacity of future generations to meet their own needs. Consequently, stewardship of both natural and human is of prime importance. Agriculture has a potential impact on the environment, especially on air, soil, water, biodiversity and landscapes. The overall environmental aim of sustainable agriculture is to optimize the use of natural resources while at the same time maximizing the efficiency of input use and preserving environmental integrity (Feenstra, 1997). Therefore, the basic long-term challenge for agriculture is to produce food and industrial crops efficiently, profitably and safely, and to meet a growing world demand without degrading natural resources and the environment.

In January 2001, the 6th Environmental Action Program set at the European Union level decided to proceed with the development of a Thematic Strategy for Sustainable Use of Pesticides. The sustainable use of agricultural pesticides is defined by the European Commission, as the use of pesticides without irreversible effects on natural systems and does not cause acute or chronic health effects in humans, animals and the environment. Thus, the

maximum reduction of pesticides use, the restriction of the use or the replacement of the most dangerous and the adoption of the precautionary principle in decisions of approval of pesticides are key issues. Optimizing pesticide use is a very broad phrase that can be interpreted in a number of different ways. The interaction between the needs of agriculture, environmental protection and concerns for human health is complex and needs to be optimized (Hond *et al.*, 2003) (Figure 2.5).



FIGURE 2.5: Interaction of the principal factors in pest-control practices (Hond *et al.*, 2003)

The Integrated Protection Principle is stringently related with the concept of sustainable agriculture. Also the integrated production is related with the same principle (Figure 2.6). The integrated production was developed as a farming system capable of meeting the requirements of the long-term sustainability. For the practice of integrated production, there are a number of key elements which enable the balance between economic production and environmental responsibility (ANIPLA, 2008). According the Organization for Economic Co-operation and Development (OECD, 1999), many countries are developing main types of indexes that provide a view of various linkages between pesticide use, risk, management and other agro-environmental indicators that convey a general idea about the risk, use and management.

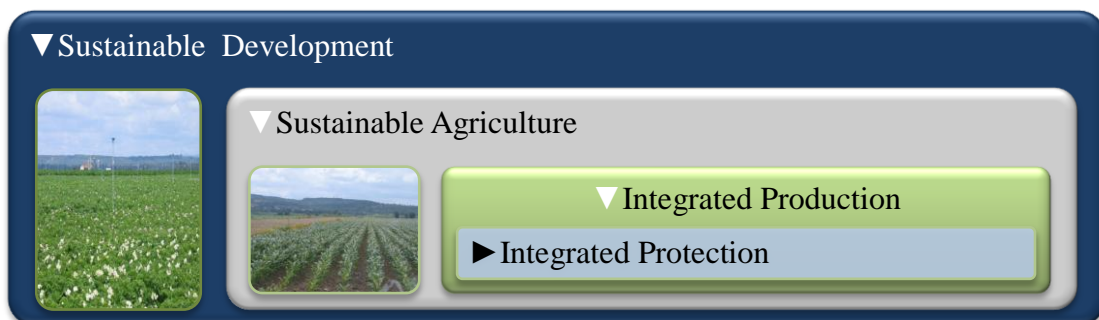


FIGURE 2.6: Levels of integration between Sustainable Development and Integrated Protection (ANIPLA, 2008).

2.3.3. Water protection and management (Water Framework Directive, Quality of drinking water, Protection of groundwater against pollution, Quality of surface water)

The Water Framework Directive (2000/60/EC) of 23 October came up with the aim of establishing a framework for the protection of surface water and groundwater to avoid further deterioration, protect and improve the water quality status. Under the directive, member states pledged to achieve the following objectives for groundwater:

- 1) take the measures necessary to prevent or limit the discharge of pollutants into groundwater and prevent the deterioration of all bodies of water;
- 2) protect, enhance and restore all bodies of groundwater, ensure a balance between abstraction and refills of water;
- 3) implement measures necessary to reverse any significant and sustained upward trend of the concentration of pollutants that results of the impact of human activity in order to gradually reduce groundwater pollution.

Another important aspect of the Directive relates to the need for carrying out detailed studies on water quality, ensuring the development of programs for monitoring the status of water in each hydrographic region.

With the Decision No 2455/2001/EC of the European Parliament and the Council, of November 20, 2001, a list of priority substances was defined, which would be reviewed every four years. This list includes 33 groups of substances including 13 pesticides - alachlor, atrazine, chlorfenvinphos, diuron, endosulfan, HCH, hexachlorobenzene, isoproturon, lindane, pentachlorophenol, simazine and trifluralin, which should be subject to a progressive reduction of discharges, emissions and losses. The European Commission presented, in September 2003, a proposal for a directive of the European Parliament and Council on the protection of groundwater to prevent and control the pollution of this resource. Regarding pesticides and their metabolites, the proposal sets the value of 0.1 µg/L as a quality standard for groundwater.

The “daughter directive”, Directive 2006/118/CE of the European Parliament and the Council, of December 12, 2006, relative to the protection of groundwater against pollution and deterioration implements criteria for the assessment of the good chemical status of groundwater. According to Article 2, the applicable linear for a good groundwater chemical status should be based on protection of water mass with particular attention to the impact on,

and its interrelation with, the surface waters and associated ecosystems and wetlands directly dependent.

Quality standards defined as criteria under Directive 2000/60/EC are congruent with the Council Directive 91/414/EEC on the placing of plant protection products on the market, and Council Directive 98/83/EC on the quality of water intended for urban consumption. The Proposal of 17 July 2006 for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC established environmental quality standards so as to limit the quantity of certain chemical substances that pose a significant risk to the environment or to health in surface water in the European Union.

3. PESTICIDES AND GROUNDWATER CONTAMINATION

3.1. Processes that govern pesticides behaviour and fate in groundwater

The assessment of the potential environmental fate and behavior as well as of their toxic effects before substances are released into the environment, and the monitoring and analysis of the frequency of detection after their use, is an extremely important component in evaluating the safety of a particular compound.

Pesticides, after being applied in agricultural ecosystems, have a complex environmental behavior resulting from various physical, chemical, biological processes and partition properties which determine their transport and processing (Mackay & Paterson, 1981; Calamari & Vighi, 1991; Cerejeira, 1993; Bacci, 1994; Batista, 2003). Handling practices can also influence the fate process (Waldron, 1997).

Knowledge on the fate of pesticides, requires an understanding of three major processes: transformation; transfer; and transport (Calamari & Vighi, 1991). Transformation refers to biological and chemical processes that change the structure of a pesticide or completely degrade it. Transfer refers to the way in which a pesticide is distributed between solids and liquids (e.g., between soil and soil water), or between solids and gases (as between soil and the air it contains). And finally, transport is the movement from one environmental compartment to another, such as the leaching of pesticides through soil to groundwater, volatilization into the air or runoff to surface water (Withford *et al.*, 2001). Thus, after entering the environment a pesticide may suffer changes that eliminate or transform into more or less toxic substances than the original molecule (Cerejeira, 1993). However, the fate processes can be beneficial as they can move a pesticide to the target area or destroy its potentially harmful residues.

Despite their importance for agriculture, in fighting the enemies of cultures, the introduction of pesticides into the environment has created environmental problems and on human health, sometimes only detectable in the long term (Cerejeira, 1993). In general, aquatic ecosystems are more vulnerable to the presence of pesticides. The pesticides occurrence in groundwater depends on an intricate combination of different factors, such as quantities applied, modes of application, environmental characteristics and intrinsic properties of the compounds (Vighi & Funari, 1995). The most hydrogeological vulnerable areas are those where the groundwater level is relatively superficial and soil has characteristics that allow vertical entrainment of pesticides through preferential transport (Cerejeira, 1993; Bauld, 1994).

The leaching of pesticides from the most superficial layer to deeper layers of soil is the process of transportation generally responsible for the presence of pesticides in groundwater (Cohen, 1990; Smith, 1990; Bauld, 1994). The pesticides with greater susceptibility to leaching are those that being somewhat volatile have a high solubility in water, low absorption capacity in the soil and a high persistence.

In the past, it was generally accepted that the processes involved in the dissipation of pesticides was paralleled by their disappearance, so that the quantities that appear in the groundwater would be negligible. However, the appearance of the first areas with contaminated groundwater led to concerns about this assumption (Rao *et al.*, 1985; Mandl *et al.*, 1994). The persistence of pesticides in groundwater is generally high because in this compartment the environmental conditions are not favorable to dissipation (CEC, 1992; Mandl *et al.*, 1994). This limitations to dissipation are associated with the characteristics of this particular environment, namely the anerobiose characteristics, the low content of colloidal particles of clay, the inexistence of organic matter, and the absence of light and low microbial activity that slow the deterioration of the molecules (Mandl *et al.*, 1994). Moreover, recovering groundwater once polluted it is very difficult (Smith, 1990; Batista, 2003).

It should be considered that a pesticide may become detectable in groundwater only after numerous agricultural applications over a period of time (Vighi & Funari, 1995). In addition, the level of pesticides in groundwater may suffer a recharge through the surface water (typically more exposed to contamination), in very permeable areas where the pesticides dissolved in surface water can soon reach the aquifers. A highly publicized case was that of atrazine detected (at levels above 0.1µg/L) in groundwater near the city of Bergamo, Italy. According with Beretta *et al.* (1998) (in CEC, 1992), this contamination was largely due to the groundwater recharges by the river Bembo, which was receiving industrial discharges with a high content of atrazine.

3.2. National and international studies on groundwater exposure assessment to pesticides

The first news on the detection of pesticides in groundwater worldwide occurred in the 60's (Barros, 1996). The detection of pesticide residues in water to drink from groundwater towels was responsible for the launch of studies to clarify the transport of these chemicals from soil into groundwater (Cerejeira, 1993). In these studies, one of the most detected pesticides in groundwater was atrazine (Funari *et al.*, 1995; Barbash & Resek, 1997; Sanchez

- Camazano *et al.*, 2005). In the Iberian Peninsula, Sanchez-Camazano *et al.* (2005) studied the inputs of atrazine and alachlor herbicides into surface and groundwater from irrigated zones dedicated to corn cultivation in Castilla-León (Spain); the concentration ranges of pesticides detected varied from 0.04 to 3.45 µg/L for atrazine and 0.05 to 4.85 µg/L for alachlor.

In Portugal, studies on groundwater contamination with pesticides have already been started in the late 80's, in areas with corn in the agricultural area of “Lezíria do Tejo”, where the dynamics of the herbicide atrazine were studied in particular (Cerejeira, 1993; Cerejeira *et al.*, 1995a, b, 1998). Given the obtained results, further studies were designed (Moura, 1996) and in 1995 and in later years an extension of that assessment to other agricultural ecosystems of “Ribatejo e Oeste” was made. From 1996-1998, under the project PAMAF 4024, studies began to cover almost all areas in the entire land of DRARO (Direcção Regional de Agricultura da Região Oeste”), also extending the range of products evaluated (Batista *et al.*, 1998, 2001; Cerejeira *et al.*, 1999 a, b, 2003; Silva-Fernandes *et al.*, 1999). Between 1998 and 2000, the studies have been intensified in the area of “Ribatejo e Oeste” and were also extended to “Beira Litoral”, in accordance with a previously identified vulnerability (Batista 2003, Batista *et al.*, 2000a, b, 2002; Paralta *et al.*, 2001). The project Agro 24 was developed from 2002 to 2004, in the area of the “Baixo Sado e Tejo”, was been assessed the public and private borrowings located in agricultural areas of agricultural ecosystems (Silva *et al.*, 2005; Silva *et al.*, 2006). Between 2004 to 2006, the project Agro 530 took place, aiming to complement a decision support system in the vulnerable zone of the Tejo (Barros, 2005; Mendes *et al.*, 2006; Rei, 2005). Within the framework of all these studies, several works were published at the national and international level (Batista *et al.*, 1999 a, 2000 b, 2001, 2002; Cerejeira *et al.*, 1998 a, 2000 b, 2003; Silva-Fernandes *et al.*, 1999). Table 2.1 synthesizes the results of the above mentioned studies on the evaluation of the exposure of groundwater to pesticides.

TABLE 2.1: Summary of the results of the main studies developed in Portugal, between 1995 and 2006, in the field of action of the detection of pesticides in groundwater.

Project/Region/Years		N° of wells	Percentage of wells with pesticide levels:	
			< LD	>0.1 µg/L
Chamusca, Golegã and Riachos (1991-1992) ^{a, b and c}	HI	110	15	40
	HPS	14	25	50
Chamusca, Golegã (1995) ^d	HI	2	0	100
	HPS	2	0	50
Salvaterra de Magos, Coruche (1995) ^e	HI	6	0	50
	HPS	4	100	0
PAMAF 4024 (1998-2000) ^f	HI	131	21	28
	HPS	44	50	9
Ribatejo and Oeste, Beira Litoral (1998-2000) ^g	HI	168	33	24
	HPS	73	59	10
Agro 24 (2002-2004) ^h	HI	96	20	14
	HPS	107	45	6
Agro 530 (2004-2006), Results from 2004 ⁱ	HI	72	70	22
	HPS	17	88	6

< LD- lower than detection limit; HI – holes for irrigation; HPS-holes for public supply (a) Cerejeira (1993); (b) Cerejeira *et al.* (1995 a); (c) Cerejeira *et al.* (1995 b); (d) Moura (1996); (e) Batista (1996); (f) Silva-Fernandes *et al.* (1999); (g) Batista *et al.* (2000 b); (h) Silva *et al.* (2005); (i) Cerejeira *et al.* (2005).

4. THE ECOTOXICOLOGY AND HAZARD ASSESSMENT

In 1969 Trufhaut defined ecotoxicology as a branch of toxicology that studies the effects of toxic chemicals in living organisms in an ecosystem as well as the process of transfer of compounds and their interaction with the environment (Rand & Petrocelli, 1985). One of the crucial concepts in ecotoxicology is that of hazard assessment, that derives from a comparison among the effects that a chemical substance can produce on living organisms or on ecosystems and the level of exposure, allowing to assess the potential danger of a substance before the product is introduced on the market. The effects could be quantitatively represented by for example a safe concentration (NOEL; No Observed Effect Level) and the exposure by a predicted environmental concentration (PEC) (Figure 4.1) (Vighi & Funari, 1995).



FIGURE 4.1: Hazard assessment, adapted from Vighi & Funari (1995)

The effective exposure to a pesticide that is dependent on its conditions of application and precautions for those who use pesticides, influence the probability of incidence and severity of adverse effects that may occur in the human population or in the environmental compartment (Amaro *et al.*, 2006).

According the aquatic risk assessment and mitigation dialogue group (ADG), the ecotoxicological effects resulting from the use of pesticides may be direct or indirect and have most often been measured through laboratory acute and chronic toxicity tests or by observing field effects on organisms.

Aquatic toxicology is part of the ecotoxicology science, which is multidisciplinary in scope and interdisciplinary in practice.

The aquatic environment vulnerability for pesticides depends on several factors, including (1) physical and chemical properties of the pesticide and its transformation products; (2) concentration and total loading of the pesticide entering the ecosystem; (3) inputs period and type; (4) ecosystem properties that enable it to resist changes, that could result from the pesticides presence and (5) ecosystem location, relative to point source of pesticides. Because aquatic ecosystem involve complex interactions of physical, chemical and

biological factors, it is difficult to understand the system response to pesticides, unless the relationships among components of the system are well defined (Rand, 1995).

Typical endpoints are the mortality, behavior and growth. The values of LC_{50} (median Lethal Concentration) and EC_{50} (median Effective Concentration) are associated to the previous endpoints. LC_{50} represents the concentration that causes death of 50% of the organisms during the test; EC_{50} is the concentration that causes effects on 50% of the organisms during the test (Kamrin, 1997). This concentration is designated as “exposure” in the role of ecotoxicology. Exposure evaluation can be done using mathematical models, considering the Predict Environmental Concentration (PEC) or through monitoring studies. It also can include the Predicted Environmental Distribution (PED) evaluation, in order to assess the molecules behaviour in the environment (Pereira, 2003).

5. ENVIRONMENTAL IMPACT ASSESSMENT OF PESTICIDES IN THE NORTH OF THE TEJO VULNERABLE ZONE

5.1. Material and methods

5.1.1. Study area characterization

5.1.1.1. Location – North of the Tejo Vulnerable zone

The study place selection was based into two fundamental criteria which were the high vulnerable contamination zones and the areas that reflected the area of agricultural ecosystems more representative of that sector.

Thus the study area (Figure 5.1) covers 4 areas located respectively in the agricultural counties of Torres Vedras (Riachos) e Golegã (“Campo”) where groundwater samples were taken. It was also included in the study area the locals of surface water sampling included in “Campo” of Golegã. The study area is included in the alluvial north zone of the river Tejo (Figure 5.2), included in “Almonda” sub basin).

In administrative terms the study area is then located in the region known as the Lisbon and Tejo valley, included in the district of Santarém, located in the center of the country in the fertile valley of the river Tejo, being considered one of the areas with greatest agropecuary potential and with unique natural conditions for developing the agricultural sector. It constitutes a usable area of about 7% of national territory.



FIGURE 5.2: Counties covered by ZVT (Tejo Vulnerable zone)



FIGURE 5.1: Study área: QP -“ Quinta dos Pinheiros”, Riachos ; DV – “Dique dos Vinte”, Alverca da Golegã; BD-“ Barracão do Duque”, P -“Praias”, L -“Lezíria” and RA – Rio Almonda in “Campo da Golegã”.

5.1.1.2. Hydrogeological unit, aquifer system, soils types, hydrogeological vulnerability assessment of the study area

a) Hydrogeological unit and aquifer system

According to “Instituto Nacional da Água” INAG (1999), the study area belongs to the Hydrogeological Unit of “Bacia Tejo Sado”, sub-unit “Bacia Terciária do Tejo”, which is a sedimentary basin of great dimensions, filled by sediments of tertiary and quaternary origin which includes the largest aquifer in the national territory. The study area in “Riachos” and in “Golegã” are included in two of these units respectively called “Transição” (Quaternary terraces and sand surface) and in the alluvial flood plain of the River “Tejo”, called “Campo”.

The study area is consequently placed in the aquifer known as “Tejo alluvium”. This system is of high importance, covering a total area of 1113 km², where the aquifer dominant formations are the Alluvium (Holocene) and “Terraços Fluviais” (Pleistocenic).

Under the hydrographic basin plan of the river Tejo was assessed that there was a relation between groundwater and surface waters, i.e., at some time the river may recharge the aquifer (INAG, 1999). In the case of the aquifer system, where the study area is included, taking into account their characteristics its evaluation was positive (Table 5.1), which is the possibility of the river at some point to recharge the aquifer (INAG, 1999); there is the possibility of negative seepage flux previously described in chapter (2.1).

TABLE 5.1: Classification of aquifer systems on the type of aquifer, flow middle, relation River / groundwater. Adapted from hydrographic basin plan of the river Tejo (INAG, 1999)

Hydrological system	Aquifer type	Flow middle	Relation river/groundwater
“Tejo Alluvium”	Free, confine or semi-confined	Porous	Yes

b) Soils

According to the soil classification charter of Portugal and the FAO scheme for the soil classification chart for Europe, the types of soils that predominate in the areas covered by the study area are the Eutric Fluvisols (Je) and the Calcaric Fluvisols (Jc) in the “Campo” (Moura, 1996) and Vertisols (V) in the area of “Riachos”.

c) Hydrogeological vulnerability assessment of the study area

The vulnerability of groundwater can be understood as the greater or lesser capacity of attenuation of the upper layers of the aquifer to the passage of pollutants (Mendes *et al.*, 2006). Thus the vulnerability of groundwater to pollution is not a parameter or character that

can be measured on the field. This concept is based on the perception that certain areas are more susceptible to contamination than others, regarding the mitigation of natural processes and the constitution of lithological formations where the phenomenon of pollution may occur (Ribeiro, 2004).

The transport of contaminants to water is conditioned by several factors such as weather conditions, soil type, vegetation cover, topography and hydrological factors. In the same way the action of man and particularly agricultural practices can contribute to greater vulnerability to contamination, especially in areas of intensive farming as the case of vulnerable area of Tejo (Carvalho, 2005).

The vulnerability to contamination of the aquifer depends, among other things, of the nature of the contaminant in question, such as pesticides, since, according to the characteristics of these substances, their behavior in the vadose zone will be different (Lobo - Ferreira & Oliveira, 1995).

Then to achieve the objectives required at legislative level in relation to this issue, there is a need to provide the techniques and methodologies for assessing the vulnerability of aquifers and the risk of pollution of these resources (Ribeiro, 2004). Given the range of options in the choice of valuation methods, the success of its implementation will depend not only on accuracy but also the availability of all data (Ribeiro, 2005).

Usually more expeditious methods of implementing a large scale into account only with the lithology factor, which makes it a very subjective matter. An example of an index based on lithological rankings is the one obtained by EPPNA (“Equipa de projecto do Plano National da água”) that establishes correlation between the vulnerability and permeability of aquifers or hydrogeological formations, in order to determine the potential of the same in mitigating the possible contamination (INAG, 2001).

In addition to the above mentioned methods are also parametric methods that are based on a set of parameters representative to assess the degree of vulnerability to contamination, and then the final outcome of their application is the elaboration of vulnerability maps. The DRASTIC and IS indexes are examples of parametric methods (Carvalho, 2005).

✓ **Vulnerability in the study zone**

According to the map of vulnerability of groundwater for Portugal developed by Lobo-Ferreira & Oliveira (1995) (Figure A3.1, annex 3), obtained by the method DRASTIC, checked that the study area shows high vulnerability of the groundwater contaminants.

In previous works was used the method DRASTIC, DRASTIC pesticide and IS (Batista, 2003; Paralta *et al.*, 2001) for the Ribatejo zone, some of them were analyzed in this study to determine the areas of greatest vulnerability aquifer in the area of study.

According to Lobo-Ferreira (2003) the comparison of different indices classes can be made using a reclassification of methods (Table 5.2).

TABLE 5.2: Comparison of different indices classes using a reclassification of methods (Lobo-Ferreira, 2003).

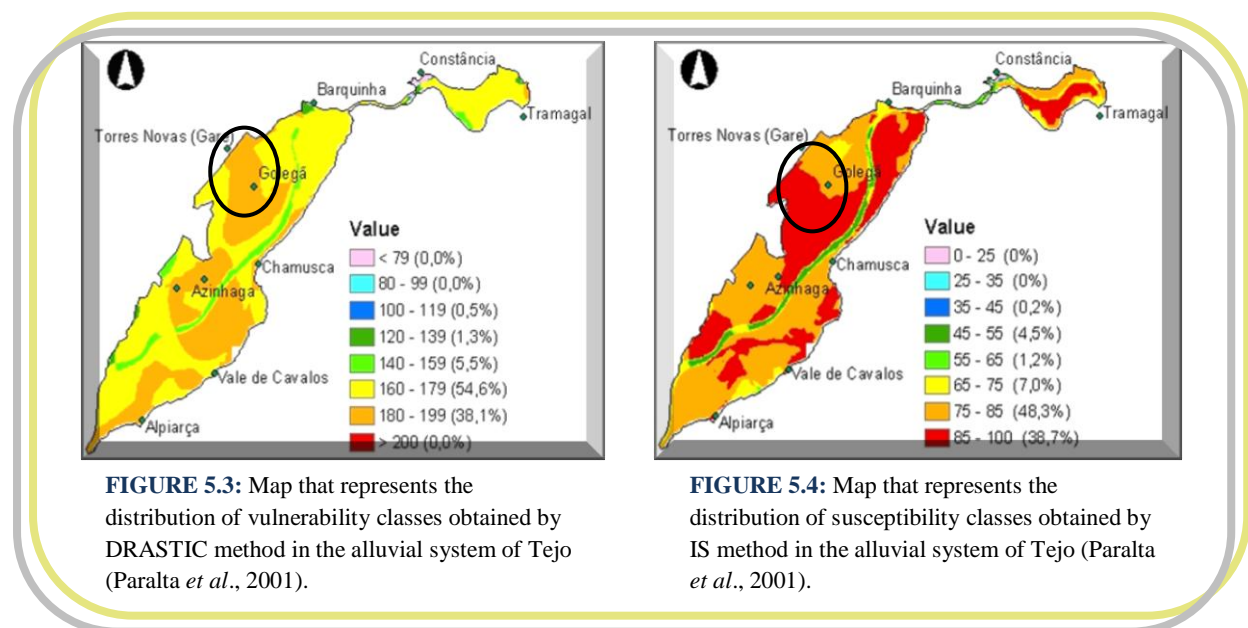
Vulnerability	Class	EPPNA	DRASTIC	IS
Very high	A	V1,V3	>199	85-100
High	B	V2,V4	160-199	65-85
Medium	C	V5,V6	120-159	45-65
Low	D	V7,V8	<120	0-45

- a) The DRASTIC index (Figure 5.3) for the study area varies from 160-199, with most of the area included in 160-199 class, the Class B, this represents a high vulnerability for the study area. The Drastic index pesticide (Figure A3.2, annex 3) in the study area varies from 155-205, with most of the area included in the class 185-205, which has a higher vulnerability.
- b) According to the susceptibility index (Figure 5.4), for the area of study, the score varies between 75%-100%, with most of the area in 85-100 class, the class A, this represents a very high vulnerability to the study area. The reason for the “Campo” zone of Golegã is indicated by IS as the zone with higher susceptibility to contamination, is mainly because of the occupation of the soil in that area by intensive agricultural zones, and irrigation perimeters, which the index attributes an higher classification.
- c) The vulnerability index (EPPNA) (Figure A3.3, annex 3), for the study area varies between V3 and V4 classes, with the most part of the area included in V3 class, A class (very high vulnerability).

Comparing the three methods (according to the table elaborated by Lobo-Ferreira (2003) concludes that area of study shows a high vulnerability to contamination. Comparing the maps obtained by 4 methods for assessing vulnerability concluded that the study zone is characterized as having high susceptibility to contamination by agriculture. It is noted that the empirical nature of the vast majority of methods for assessing the vulnerability arises when a

degree of subjectivity, which comes from different interpretations made by different technicians (Ribeiro, 2005).

As a preliminary conclusion it must be said that these results should always be subject to a careful analysis, taking into account that a classification as an area of high vulnerability can lead blindly to the immediate ban of activities that operate there. Similarly classified as an area of low vulnerability cannot mean that there is no risk of contamination. This decision may result in approval for implementation of activities without restrictions, which may be the short term; potential contaminants. Thus decision-making should be based on knowledge of monitoring field as well as other information such as number and type of contaminant and assessment of mixtures of contaminants.



5.1.1.3. Agricultural activities

In regional terms the "Ribatejo – Oeste", where the study area is located, owns 11.6% of the national UAS (utilized agricultural area) (INE, 2001).

According to the last general census of Agriculture for the area, dating back to 1999, agricultural activity is directed towards the production of cereals for grain (70 609 ha) and mainly maize for grain (36 110 ha), wheat (14 224 ha) and rice (8 766 ha) .Within the cultivation of a temporary nature are also widespread horticultural crops (28 910 ha) of which potato (9 379 ha) had already high importance to date. In addition to these permanent crops we can also find vineyards (52 081 hectares), orchards of fresh fruit (26 614 ha) (INE, 2001).

In this analysis of the structure of the agricultural area of study we have not yet taken into account the data provided by AGROTEJO (farming association of “Norte do Vale do Tejo”). According to the data of the year 2006 AGROTEJO represents about 19.000 ha (distributed for 573 explorations), of which 35% of the is occupied for cereals, occupying the maize cultivation a substantial part of that area (4211.87 hectares, approximately 23% of total) and horticultural crops, 11% of the total. The potato crop has been increasing in production with regard to the potato industry.

In general, and according to previous data, irrigated crops are those with greatest expression in terms of production. In this work and after analyzing the figures mentioned above the following crops were considered: rice, plum, cherry, apricot, peach, beet, corn, potato, oats, rye, barley and wheat. With a particular attention to the cultures of potato and maize (Figure 5.5), due to its importance in this area.

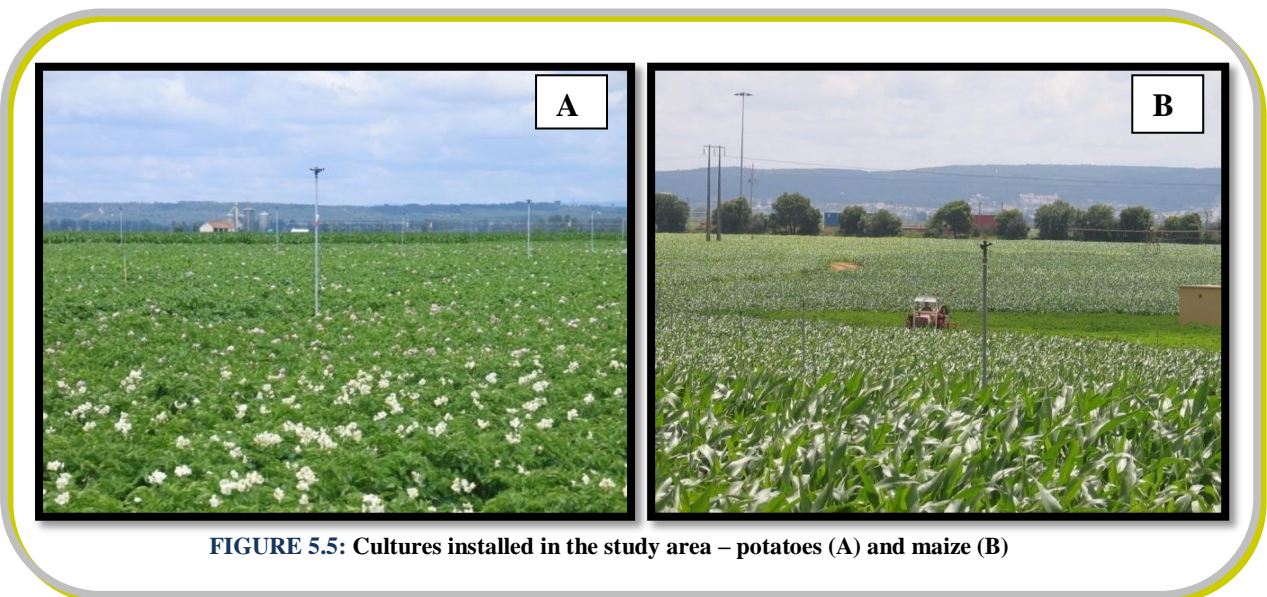


FIGURE 5.5: Cultures installed in the study area – potatoes (A) and maize (B)

5.1.1.4. Use of pesticides

Recently, an ongoing pesticides sales increase has been observed in Portugal. This tendency has been influenced by the positive conditions for the development of diseases and weeds in vine, potato, tomato, vegetables and maize crops. The intensity of plant protection product consumption among the EU 27 Member States was highest in Portugal (predominantly in the use of fungicide products) (EUROSTAT, 2008).

So, in 2007, approximately 14 T were sold, having the fungicides the highest sales %. (Figure 5.6).

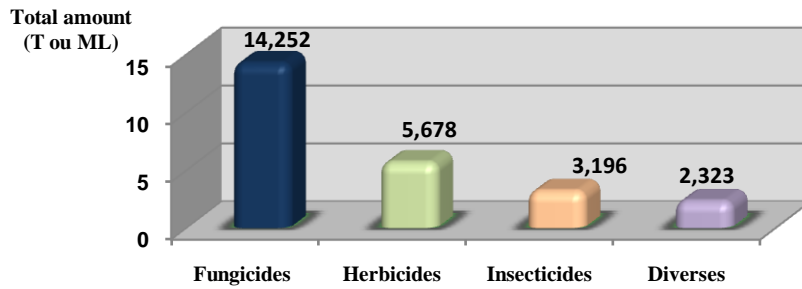


FIGURE 5.6: Quantities of sold pesticides in Portugal, year 2007, expressed in T or ML (adapted from ANIPLA: Associação Nacional da Indústria para a Protecção das plantas, 2008).

Once the obtained data were not as exact and exhaustive as the main goal, it was only possible to define the principals pesticides used in the area in study in terms of quality.

The table 5.3 resumes the pesticides that are used more frequently in qualitative terms, for the maize and potato crops in the AGROTEJO and AGROMAIS influenced area.

As mentioned above, the maize and potato crops are the most important in this region.

Table 5.3: Pesticides used in maize and potato crops in Golegã.

	INSECTICIDES	FUNGICIDES	HERBICIDES
POTATO	chlorpyrifos chlorpyrifos-methyl ethoprophos lambda-cyhalothrin thiacloprid thiamethoxam	cymoxanil dimethomorph fluazinam mancozeb metalaxyl-M zoxamide	deltamethrin
MAIZE	carbofuran chlorpyrifos endossulfon imidacloprid lambda-cyhalothrin		atrazine bentazone mesotrione nicosulfuron S- metolachlor sulcotrione

5.1.2. Pesticide selection to study

With basis on the cultures selected for the study, a review was made of the registered pesticides for the same, totalizing 138 pesticides (DGADR, 2008). It was carried out the lifting of the values of physical-chemical properties and environmental partition of the study pesticides (Table A1.1, annex 1), that influences its affinity for the water compartment, its potential for contamination of groundwater as well as the ecotoxicological and toxicological end-points (Table A1.2 and A1.4, annex 1). For a reference manual (Tomlin, 2006) and a credible database on pesticides “FOOTPRINT PPDB” (accessible: <http://sitem.herts.ac.uk/aeru/footprint/en/index.htm>) has been used. That is a comprehensive database holding data on environmental fate and ecotoxicological properties for a large number of pesticides and their metabolites, including all those registered in Europe.

5.1.3. Predictive approach's for the environmental impact of the selected pesticides

5.1.3.1. Physic-chemical and partition properties characterization

Certain attributes of chemicals in the environment can be measured directly, particularly its concentrations. Other attributes cannot be measured directly, such evaporation rates, persistence and distance travelled. They can only be estimated by using models (OECD, 2001).

The physical-chemical properties and of environmental partition considered most important and at the same time, have a considerable influence on the environmental performance of pesticides are listed below.

A. Vapor pressure

The vapor pressure (P) is the pressure exerted by the vapor of a substance in equilibrium with its pure phase (liquid or solid) at a given temperature. This parameter gives us, then, a measure of its affinity for the compartment air in its pure phase.

Generally chemicals that are highly volatile are easily lost to the atmosphere and are less likely to leach to groundwater, except if they are also highly soluble and, thus, carried with the water flow (Waldron, 1997).

B. Water solubility

The maximum amount of a substance that is dissolved in a certain amount of pure water at a given temperature (usually at room temperature, 20 ° C or 25 ° C) corresponds to its solubility in water (Sw).

Pesticide chemicals that dissolve readily in water are highly soluble. Such pesticides have a greater probability to be transported by runoff to the surface waters, or to leach, from the soil to groundwater (Waldron, 1997; Kanwar, 1996).

Although several pesticides do not leach because they are adsorbed on the soil particles or organic matter even though they may have a relatively high solubility (Waldron, 1997). So in a first approach the affinity for the water compartment can be quantified by the solubility of the substance (Vighi & Di Guardo, 1995).

C. Henry's law constant and air-water partition coefficient

As losses of pesticides by volatilization depend on the partition of the substance between gas and water, the K_{aw} and H can be taken as indices of affinity for the room air (Bacci, 1994; Mackay *et al.*, 1997; Vighi & Di Guardo, 1995). Values of Henry's Law Constant above $10 \text{ Pa m}^3 \text{ mol}^{-1}$ are always indicative of a very high affinity for the air, while values less than $10^{-4} \text{ Pa m}^3 \text{ mol}^{-1}$ show an affinity for this compartment low or negligible (Vighi & Di Guardo, 1995).

D. Half -life and persistence

The resistance to degradation of pesticides is translated by its persistence. Persistence is routinely expressed as a half-life of the compound (DT_{50}), half-life can be defined as the time required for half of the applied pesticide to be completely degraded, or broken down (Batista, 2003; Waldron, 1997).

The pesticide is subject to various degradation processes such as the reactions of hydrolysis, oxidation-reduction, photolysis and biodegradation. The persistence of the pesticide is dependent on these processes of degradation and the constant speed of degradation of reactions, ranging therefore, with pesticide intrinsic characteristics and the environmental compartment considered (Mackay *et al.*, 1997).

The half-life of the substance is affected by factors as the temperature, luminosity intensity and nature of microbial community so that there is not an exact and unique half- life (Mackay *et al.*, 1997).

In that way the persistence of the pesticide influences the potential for contamination. For example, the longer the compound lasts before it is broken down, the longer it is subject to the forces of leaching, by this way the degradation affects the potential for a pesticide to reach groundwater. However, some pesticides of low persistence, such as aldicarb, have been found in groundwater and several highly persistent pesticides, such as the chlorinated hydrocarbons, have not been found in groundwater because of their low solubility and strong adsorption to soil particles (Waldron, 1997).

E. Organic Carbon Sorption Coefficient

This coefficient (K_{oc}) is in generally assumed as an index of soil affinity and represents the sorption coefficient for the organic carbon of the soil. Is calculated by measuring the ratio (K_d) of sorbed solution pesticides concentrations after equilibration of a

pesticide in a water/soil slurry, and then dividing by the weight fraction of organic carbon present in the soil (F_{oc}).

$$K_{oc} = K_d / F_{oc} \quad [1]$$

This coefficient is strictly related to octanol/water partition coefficient (K_{ow}) (Vighi & Di Guardo, 1995). It is an important parameter in the environmental evaluation of bioaccumulation in plants from air. In general values below 4 represent very low affinity for plants and values above 8 indicate high bioaccumulation potential (Vighi & Di Guardo, 1995).

F. Octanol/ water partition coefficient

This coefficient (K_{ow}) quantifies the lipophilicity of a substance and can be defined as the ratio between the concentration of the chemical, on equilibrium, in the phase of octanol (C_o) and in the aqueous phase (C_w) (Vighi & Di Guardo, 1995). Values of K_{ow} are expressed, usually in the logarithmic form ($\log K_{ow}$).

It is used to estimate the ability of that substance to cross the biological membranes and to bioaccumulate in the organisms, i.e., as a measure of its affinity for the biota (Vighi & Di Guardo, 1995).

The following classification due to its simplicity is often used (Vighi & Di Guardo, 1995; Calamari & Vighi, 1987):

- ➔ $\log K_{ow} > 3.5 \rightarrow$ bioaccumulating substances
- ➔ $3 < \log K_{ow} < 3.5 \rightarrow$ low bioaccumulation potential
- ➔ $\log K_{ow} < 3 \rightarrow$ nonbioaccumulating substances

5.1.3.2. Mackay fugacity model –Level I

Once a substance is released to the environment from an anthropogenic activity, it becomes important to comprehend where a substance will reside in the environment, how much of it will reside there and for how long (Webster, 2004).

The environmental models integrate the physico-chemical properties of molecules partition and environmental parameters. This application allows the evaluation of environmental distribution and final fate of pesticides that with toxicological data, contribute for hazard evaluation of these products (Cerejeira, 1993). So the knowledge of physical and chemical properties, including environmental partition of pesticides is essential to develop the

predictive ability of its environmental impact before its introduction in environment (Batista, 2003).

The evaluation of predicted environmental distribution (PED) of registered pesticides in Portugal for the most representative cultures in the study region has been performed based in the calculation of Mackay Fugacity Model level I, using its least version (version 3.00, 2004, Trent University, Canada, obtained in <http://www.trentu.ca/cemc/VBL1.html>) that allows the PED's calculations of substances to various environmental compartments, being focused, in this work water compartment.

The multicompartimental Mackay Fugacity Model is based in the partition analyse of one substance between the various environmental compartments and in the concept of fugacity (f) that represents the trend of one chemical substance to escape of one phase to another in equilibrium conditions (Mackay *et al.*, 1997).

In the Mackay Fugacity Model a normalized universe World Unity with a surface of 100 000 Km² has been adopted. It's constituted by six environmental compartments – air, water, soil, sediments, suspense solids and aquatic biota – each one with defined characteristics. The model can be applied at different levels of complexity (level I) there shall be an evaluation of distribution of the chemical substance between the various environmental compartments, considering a closed system and is in equilibrium and steady state and it is assumed that no are degradation and occurs one unique emission of the chemical substance in the system.

At level I Mackay Fugacity Model assumes static conditions, with distribution planned to be achieved in balance, not considering all aspects kinetic and degrading that characterize the behavior of a substance in the environment (Batista, 2003). Level I assumes a simple, evaluative, closed environment with user-defined volumes and densities for the following seven homogeneous environmental media (or compartments): air, water, soil, sediment, suspended particles, fish and aerosols (Mackay, 2001).

Three types of chemicals are treated in this model: chemicals that partition into all media (Type 1- excluding ionizing chemicals), involatile chemicals (Type 2), and chemicals with zero or near-zero solubility (Type 3) (Mackay, 2001). This model (in Level I) requires the following physical and chemical properties of chemicals to the calculation of the PED's : molar mass (g/mL), using the simulation temperature (20°C), solubility in water (g/mL), vapor pressure (Pa), log K_{ow} and melting point (°C) that are previously selected and collected. The results of the pesticides affinity to the different compartments obtained by the use of Mackay Fugacity Model are in table A2.1, in annex 2.

5.1.3.3. Leaching indexes – Bacci and Gaggi, GUS

In order to evaluate the potential for leaching of chemicals, various indexes of leaching have been developed. In general these substances based on their potential for contamination of groundwater can be ordered in a ranking (Bacci, 1994). The indexes GUS and Bacci & Gaggi are two examples of this.

The **leaching index GUS** (Groundwater Ubiquity Score) developed by Gustafson (1989) considers the persistence (DT_{50}) and the mobility of pesticides (K_{oc}), resulting of the following calculation:

$$GUS = \log_{10}DT_{50soil} (4 - \log_{10}K_{oc}) \quad [2]$$

It's an empiric model, where the number four is an arbitrary value, limited by the fact that only considers two properties of substances (DT_{50} and K_{oc}) (Bacci, 1994).

It permits the classification of the chemical substances in function of groundwater potential of contamination, if: **GUS > 2.8** the substances are considerate as **leaching**; **GUS < 1.8** the substances are considerate as **non leaching**; **1.8 < GUS < 2.8** the substances are considerate as **transition substances** (Gustafson, 1989).

The model can be applied to polar and non polar molecules, and given its simplicity the GUS index continues to be one of the most widely used indexes of leaching (Funari *et al.*, 1995; Vighi & Di Guardo, 1995).

The leaching index of Bacci & Gaggi (1993) have another approach derived from the model of Surface Soil Mackay, calculated the fraction of leaching of pesticides, assuming certain conditions of soil standard.

This index allows to the application to polar and non polar substances, by selecting the appropriate partition coefficient, i.e., the partition coefficient between the mineral mater and water (K_{pm}) and the partition coefficient of organic carbon-water (K_{oc}) respectively (Batista, 2003). For calculated this index the following properties are used molar mass, temperature used in simulation, solubility in water, vapor pressure, K_{oc} or K_{pm} , Dt_{50soil} . For the polar substances is considered $K_{pm} = 0.02 * K_{oc}$. The program GWBASIC is used for calculate the index. If the index is between **1 and 1E-01** the substances are considerate as **leaching substances**; between **9.9E-02 and 1E-02** as **transition substances** and if the index is **<1E-02** as **non leaching substances** (Bacci, 1994).

Based on the previously selected physical-chemical properties and partition coefficients the results of the potential of leaching obtained by calculating the indexes of GUS and Bacci & Gaggi are present on the table A2.2, annex 2.

5.1.3.4. Rating systems for pesticide risk classification on different ecosystems

In the last few years, different strategies in risk management have been proposed with diverse purposes; however, at present, the criteria used to decide the acceptability of environmental risks is commonly based on the concept of toxicity exposure ratio (TER). This ratio must be calculated for each of the environmental compartments at risk (groundwater, surface water, soil) to establish critical thresholds as a trigger for the need of further information (Finizio *et al.*, 2000).

Pesticide indicators can provide a useful tool for the evaluation of domestic policies and international obligations related to pesticides use in agricultures, and can also convey a general idea about pesticide use, risk, management and the impact of pesticides on human health and environment (Hond *et al.*, 2003).

The EPRIP index is according to Finizio *et al.* (2000) the result of sponsored project ANPA (Agenzia Nazionale Protezione Ambiente of Italy) for setting up different rating indexes for pesticides for different environmental scenarios.

The information used in the construction of these indexes has based on the Annex V of Directive 91/414/EEC. The EPRIP contemplates three distinct environments (surface waters, terrestrial hypogean, and epygean systems) and indexes are based on exposure indicators (rate of application, environmental distribution, bioaccumulation, and soil persistence) and on the effects (i.e., EC₅₀, NOEL) that these substances can exert on nontarget organisms considered representative of the three environmental systems, according to Directive 91/414/EEC (e.g., algae, *Daphnia*, fish for surface water), this values are selected considering the context of a worst- case scenario.

It was considered two different time space scales for each system, the short term (refers to a risk posed by a pesticide, immediately after application) and the long term scale. Because of the large number of parameters involved in the characterization of environmental risks and the inability to produce quantitative values (for exposure or effects) this system returns only qualitative information.

According the Finizio *et al.* (2000) the main problem encountered is the lack of data on the side effects of these substances (i.e., microorganisms, beneficial arthropods) or the big

difference (in some cases more than one order of magnitude) among literature values for the same toxicological end point or for physical-chemical properties, so when literature data were not available default or estimate values have been applied.

The values of the toxicological end points and physic –chemical properties used to apply this classification method have been compiled by adequate literature, particularly in Tomlin (2006) and completed within the possible through reliable databases available on the Internet (FOOTPRINT PPDB), was also used the values of the predicted environmental distribution obtained by applying the model of fugacity of Mackay Level I.

The indexes for the three systems are more extensively explained in annex 4 as well the tables of the obtained scores.

5.1.4. Groundwater and sediments sampling in the study area

In the area covered by the study, six private water wells which held the sampling of groundwater were selected. These places of sampling (Figure 5.7) were followed on four sampling dates (Table 5.4) for assessing the dynamics of pesticides. Were also selected two sampling sites for surface water (Figure 5.7). The date of harvest on August 13 was coincident with the last date of groundwater sampling in order to try understanding the dynamics between surface water and groundwater at the site, one of these sites was also selected to sediment sampling.

Water sampling was performed, after pumping water from the wells and stabilizing the temperature. Then they were stored in glass bottles (1L) properly identified and transported to the laboratory in chilled conditions for proceeding to the extraction and analysis of residues of pesticides. Surface waters were also collected and stored in bottles properly labeled and transported under refrigeration to the laboratory for proceeding to the extraction and analysis of residues of pesticides and toxicity tests. The sampling of sediment (Figure 5.7) was carried out in the first 2 cm in depth which is placed in glass flasks and duly transported to the laboratory also in terms of cooling to proceeding to toxicity tests

TABLE 5.4: Sampling location and dates (in 2008)

Sampling location		Date											
		June 5 th			June 27 th			July 22 nd			August 13 th		
		W	G	Sed	W	G	Sed	W	G	Sed	W	G	Sed
Almonda River	AR												✓
"Alverca do Campo"	DV										✓		✓
"Campo da Golegã"	BD		✓			✓			✓			✓	
	P		✓			✓			✓			✓	
	L		✓			✓			✓			✓	
"Quinta dos Pinheiros"	QP1		✓			✓			✓			✓	
	QP2		✓			✓			✓			✓	
	QP3		✓			✓			✓			✓	

S- surface water sampling; G – groundwater sampling; Sed- sediment sampling;
 DV – “Dique dos Vinte”; BD – “Barracão do Duque”; P – “Praias”; L – “Lezíria”; QP1,2 and 3 – “Quinta dos Pinheiros”,
 wel 1,2 and 3.
 ✓ Sampling

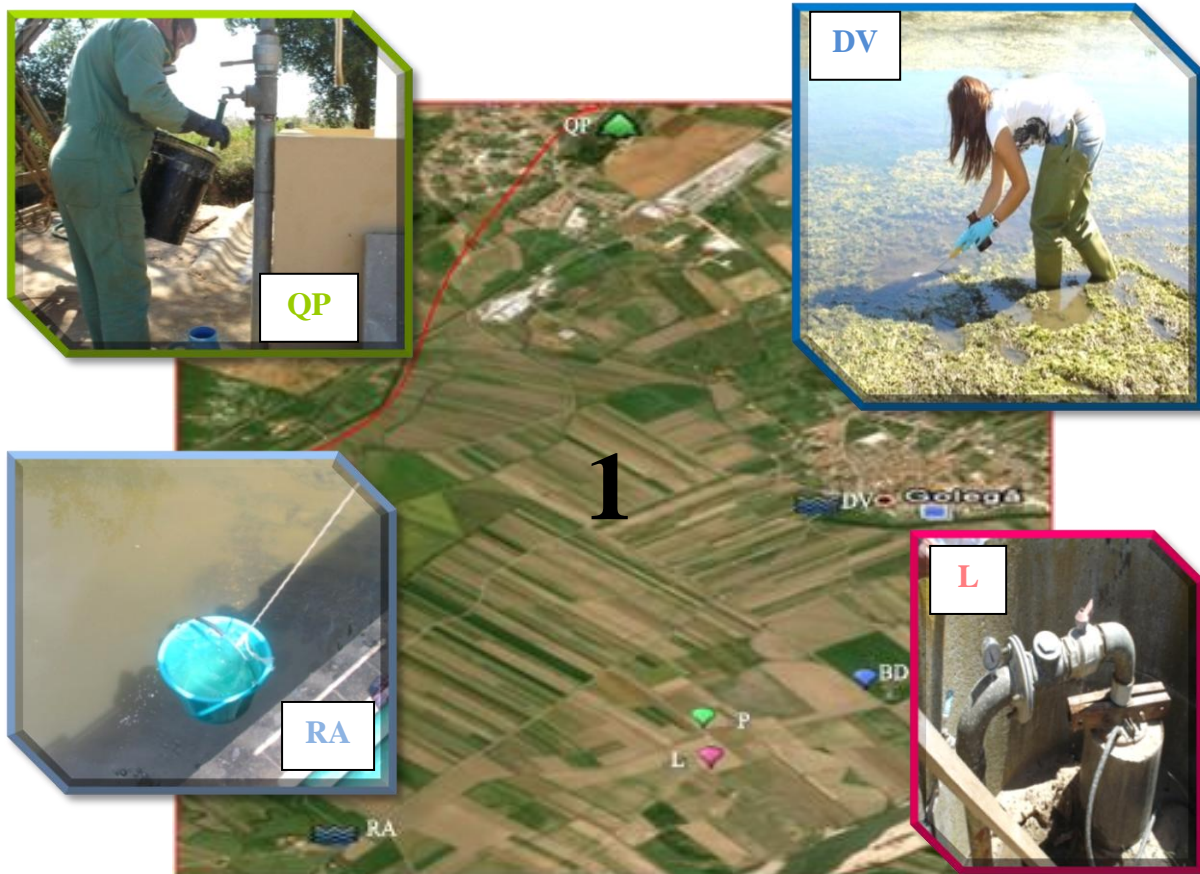


FIGURE 5.7: Localization of the places of sampling (1), groundwater sampling in “Quinta dos Pinheiros” (QP), sampling of sediment in “Alverca do Campo”, marsh (DV), surface water sampling in Almonda river (RA), groundwater sampling in “Lezíria” (L)

5.1.5. Analytical methodology to groundwater exposure assessment to pesticides – solid phase microextraction (SPME) and gas chromatography coupled to mass spectrometry (GC – MS)

The introduction of SPME (solid-phase microextraction) methodology was initially referenced in 1989 and in 1994 when appeared the first applications on pesticide determination. Most applications of SPME in determination of pesticides residues involve the extraction of water samples, not only because its environmental relevance but because this technique straits rightly in aqueous matrices extraction. Revealing itself as a helpful tool in analysis of residues pesticides in quantitative and qualitative determinations (Beltran *et al.*, 2000).

The solid-phase microextraction (SPME) is a simple and efficient technique, that of consists of two separated steps; absorption and desorption. Appears as a solvent-free extraction (Alpendurada, 2000; Beltran *et al.*, 2000; Dietz *et al.*, 2006; Eisert *et al.*, 1996) that have no needs of complex equipment for concentrating volatile or nonvolatile compounds in liquid samples or headspace (Alpendurada, 2000; Beltran *et al.*, 2000).

In the first step, the analytes extraction is performed, based on the partitioning of analytes between the analyte and a fused silica fibre coated with a stationary phase, which can be a liquid polymer, a solid sorbent, or a combination of both. That can be made by the direct exposition of a polymeric phase of a coated fiber to the sample or by the exposition of a coated fiber to its headspace, then occurs the analytes partition to the stationary phase where we belongs adsorbed (Grote *et al.*, 1999; Prosen & Krajl, 1999; Wong & Wan, 1996). Agitation is normally used to achieve faster equilibration because it enhances the diffusion of analytes toward the fibre (Alpendurada, 2000).

In a second step the process of desorption occurs, the fiber bearing the concentrated analytes is transferred to the analytical instrument where desorption, separation and quantification of analytes is performed (Alpendurada, 2000; Dietz *et al.*, 2006; Eisert *et al.*, 1996; Stashenko *et al.*, 2004). In a GC, the desorption of analytes to the fiber is realized at high temperatures (Grote *et al.*, 1999).

An SPME fiber can be directly analyzed by GC or LC, thus minimizing any potential analyte losses due to multi-step processes. Presently this technique continues to be headed towards GC, because this combination enables the minimization of any potential analyte losses due to multi-step processes (Stashenko *et al.*, 2004).

The SPME device based on a reusable microsyringe was commercialized for Supelco for the first time in 1993, simultaneously with the coated fibers used for extraction that were

initially polymethylsiloxane (PDMS), and polyacrilate (PA), that differs in its polarity and thickness (Beltran *et al.*, 2000). Currently, several coatings are commercially accessible, like Carbowax-divinylbenzene and Carboxen-PDMS (Alpendurada, 2000; Beltran *et al.*, 2000; Prosen & Kralj, 1999). Some of these materials are also employed as stationary phases in gas chromatographic (GC) columns.

Typically the SPME extraction is complete when the analytes concentration has reached equilibrium between the fiber coated and the sample matrix. This means that once equilibrium is established, the extracted amount is constant within the limits of experimental error and that is independent of further increase in extraction time (Lord & Pawliszyn, 2000). The efficiency of extraction in equilibrium it's dependent of polymeric nature and also of this thickness (Urruty & Montury, 1998).

The final results of SPME can be affected by extraction and desorption conditions (Beltran *et al.*, 2000; Prosen & Kralj, 1999; Urruty & Montury, 1998). So for the development of a particularly method to the determination of pesticides using SPME is necessary to the optimizing the variables that influences both phases of process. This variable includes, fiber type, extraction time, temperature and time of desorption and extraction phases and ionic strength for the extraction step and others (Beltran *et al.*, 2000).

To establish a compromise between extraction times and extracted quantity, normally the extraction times shorter than the equilibrium are selected due to the experimental considerations compromise (Batista, 2003; Beltran *et al.*, 2000).

The temperature can influence the results in two different ways, for high temperatures the diffusion coefficients in water are greater and the extraction time is shorter but consequently partition coefficients decrease (Batista, 2003).

Another important extraction parameter is the salting out effect obtained by adding ionic salts to the sample. This procedure makes the organic compounds less soluble and the partition coefficients can be increased several times. Although most authors agree on the positive effect of the addition of ionic salts (e.g. NaCl) to the sample over extraction efficiency of most compounds, some discrepancies have been found and it's impossible to establish a direct relation between extraction efficiency and salt addition. Additionally it has been reported that high salt concentrations can led to negative effects on fiber stability (Beltran *et al.*, 2000).

Further the SPME to be a very simple technique and solvent-free this process has other vantages, is a precise method and has a good sensibility, needs small samples water volumes and can be easily modify and used in different matrices (Barceló, 2000; Vrana *et al.*, 2005).

The methodology of extraction (SPME) and dosage by gaseous chromatography coupled with mass-spectrometry (GC-MS) adopted in this work and routinely used in the Ecotoxicology Laboratory ISA/DPPF/SAPI for extraction of pesticides residues from water samples (26 pesticides and metabolites were tested).

I. Extraction of pesticides from the groundwater samples by SPME:

1° - Sample preparation

- Weigh 1g of NaCl and add to a 10ml water sample
- Place the 10ml glass, containing the water sample, in the GC injector (Combi Pal CTC Analytics AG model);

2° - Extraction

- The first point was the water samples pesticides residues extraction, in the injector port, resorting to fibers (of SPME) with Carbowax/divinilbenzeno (CW/DVB) coating.
- Absorption of analytes: by dipping the SPME fiber in the sample, during 60 minutes, with stirring with the energy of 250 rpm.

II. Desorption, dosage and quantification by gaseous chromatography coupled with mass-spectrometry (GC)

The equipment used was one Gas Chromatograph “Varian Chrompack CP-3800” coupled with a mass-spectrometer “Saturn 2000 GC/MS” by Varian, with the followed chromatography conditions:

→GC Injector: split/slitless, with valve opening past 5 minutes;GC Injector temperature: 240°C;Column: J&W DB-5MS 30m x 0,25mm Low Bleed/MS, with 0,25µm of film thickness; oven is range of temperatures: 50°C in the beginning (1 minute), range 10°C/min till 170°C, range of 1°C/min till 180°C, range of 5°C/min till 220°C (6 minutes), range of 15°C/min till 240°C/min (4 minutes); Carrier Gas: Helium C-60 (GASIN, Perafita, Porto); Flow of the carrier gas: 12 Psi (Ψ);Detector: “Ion Trap”;Ionization mode : by electronic contact (EI); specter obtained a 70 eV, em “full scan” de m/z 70 a m/z 350;“Axial modulation voltage”: 4.0;Temperatura do “Manifold”: 40°C;“Ion Trap” temperature: 190°C; Transfer line temperature: 230°C.

The compounds in question were identified by comparing their mass spectrum and retention times with the mass spectrum and retention times obtained for the reference standard

solutions. In the identification and quantification of pesticides were considered the main ionic fragments of pesticides analyzed. Dosage was made based on the area of the peaks achieved, and through the driveway of calibration, obtained from standard solutions containing the mixture of pesticides in question, of 0.05µm/L; 0.1µm/L; 0.25µm/L; 0.5µm/L; 1µm/L e 5µm/L.

5.1.6. Bioassays to toxicity assessment on water and sediments

The aquatic environment is one of the major areas of concern since many chemicals and waste materials may ultimately find their way into water courses. Besides, aquatic life is often very sensitive to pollution and damage can have important consequences for major food chains. International authorities are continuing to develop testing guidelines to provide a framework for assessing the toxic effects of chemicals on aquatic organisms (OECD, 1998 a).

The concept of a concentration-response, or more classically, a dose-response relationship is “the most fundamental and pervasive one in toxicology” and assumes that there is a cause and effect relationship between the dose of a toxicant (or concentration for toxicants in solution) and a measured response (Casarett & Doull, 2007). The diversity of effect endpoints and the different possible routes of exposure for the test organisms (e.g., electron transport, growth, immobilization, mortality, and reproduction) were considered important for screening effluent toxicity (Tarkpea *et al.*, 1999).

Although all the technological changes implemented to reduce toxicity, the use of pesticides in the agriculture continues often to present toxicity to a range of groups of aquatic organisms. In order to fully evaluate the environmental impact of pesticides, both physicochemical and toxicological analyses, should be performed (EPA, 1993).

In order to assess cause-and-effect relationships between pesticides and biological responses, a battery of tests was conducted with organisms that occupy several functions in the ecosystems, particularly Toxicity tests with *Vibrio fischeri* (bacteria; decomposer), *Pseudokirchneriella subcapitata* (planktonic microalgae; primary producer), *Daphnia magna* (planktonic cladoceran; primary consumer; filter feeder) and *Chironomus riparius* (benthic midge larvae; deposit feeder) was used.

5.1.6.1. Lethal toxicity tests

5.1.6.1.1. Daphtoxkit FTM magna

This technique is based on the determination of the immobilization rate of the young *Daphnia magna* when placed in toxic solutions (EN ISO 6341). This method can be applied to natural samples, superficial waters, groundwaters, supplying water, interstitial and residual waters, and chemical water solutions. *D.magna* is a component of freshwater zooplankton. It refers to group Arthropoda, Branchiopoda, Daphniidae. Daphnia are small arthropods of 1-5 mm in size that live in freshwaters and some species also in the sea. Often they are found in large amounts. They feed on plankton and organic detritus. Ontogenesis of individual is direct without larval stage (Adema, 1978).

The daphnia magna test was performed following the methodology described in the Standard Operational Procedure (SOP, 1996a) for Daphtoxkit FTM bases on ISO 6341, presented in annex 5. The test was performed to 100% samples concentration.

The immobilization test is performed by resorting to the young *Daphnia magna* obtained by egg eclosion (Daphtoxkit FTM) that are exposed to the test samples throughout 48h. From the dose-response relation, it is possible to calculate the effective sample concentration capable of immobilize 50% of the tested organisms (EC₅₀) (Environment Canada, 2000).

5.1.6.1.2. Toxicity test using luminescent bacteria *Vibrio fischeri*

In 1978 the luminescent bacteria toxicity test was developed commercially as Microtox (Environment Canada, 1992a).

Throughout the last years, the Microtox toxicity test has been used increasingly to assess the toxicity of environmental samples like water or sediments, industrial waste samples and several groups of individual organic compounds (Ruíz *et al.*, 1997; Boluda *et al.*, 2002). Moreover it's now used around the world and the results of using it are well illustrated in a extensive scientific literature (Environment Canada, 1992a).

One kind of attractive features of this bioassay is the rapid response to toxicants, the uses of small samples volumes (Environment Canada, 1992a; Ruíz *et al.*, 1997). For this reasons this test is particularly useful for exploration or monitoring (Environment Canada, 1992a).

Another advantage of this test is that it could be used like a pretest or toxicity screening test due to its ease of handling, short exposure time and reproducibility of the interlaboratory results. If toxicity is not detected than more expensive chemical analyses can be avoided (Ruíz *et al.*, 1997).

The Microtox test is based on the measurement of light production inhibition by organisms. A specific strain of the marine bacterium *Vibrio fischeri* is used in this test to determinate the toxicity of samples (Boluda *et al.*, 2002; Environment Canada, 1992a; Ruíz *et al.*, 1997). The *P. phosphoreum* now known as *Vibrio fischeri* is a bacterium that usually lives in the oceans, and produces blue-green light by enzymatic reactions, on a continual basis sufficient oxygen is available. The production of light is a result of normal metabolic processes, and the light is under specific conditions measured with a standard photodetection device. The toxicity is measured by the reduction of bioluminescence and expressed as the IC₅₀ (concentration causing 50% inhibition) after the specific periods of exposure (Boluda *et al.*, 2002; Environment Canada, 1992a). Bioluminescence is inhibited in proportion to the metabolic effect by any toxic action of substances in the sample which are presumed to affect metabolic processes of the bacteria (Environment Canada, 1992a).

It is plausible that in freshwater the toxicity of certain substance might be different and the Microtox results might consequently be less suitable. When compared with lethality tests that used fathead minnows, trout, and daphnia, Microtox was frequently less sensitive to inorganic toxicants and pesticides (Environment Canada, 1992a).

Another point to be considered is that the availability of organic chemicals and also their potential toxicity to bacteria can be affected by the interactions between pesticides and different components of the sample.

The *V. fischeri* Lehmann & Neumann test was performed according to the Microtox basic test protocol (www.azurenv.com/mtox.htm), presented in annex 5.

5.1.6.1.3. Growth inhibition tests using the freshwater alga *Pseudokirchneriella subcapitata*

The algae *Pseudokirchneriella subcapitata* (formely named *Raphidocelis subcapitata* and *Selenastrum capricornutum*) growth inhibition test can be applied into a vast game of samples like: surface waters, groundwaters, supply waters, interstitial and residual waters, and chemical water solutions, generally is used in bioassays to determine the toxicity of freshwater samples (ISO/DIS 8692).

P. subcapitata, is a green unicellular alga, nonmotile, crescent-shaped (40 to 60 μm^3), that can be found in atrophic and oligotrophic environments and is ubiquitous in most freshwaters (Blaise *et al.*, 2000; Environment Canada, 1992b).

The turbidity, color, salinity and pH of samples can interfere in the organisms response to the exposure (ISO/DIS 8692).

The costs and disadvantages of “conventional” ecotoxicological tests allowed the development of cost effective microbioassay called “Toxkit tests”. This kind of tests using dormant stages as immobilized forms of different aquatic organisms is presently applied in a large number of laboratories (Cerejeira *et al.*, 1998 b).

In the present work the Algaltoxkit™ (SOP, 1996 b) (microbioassay) was used to analyse the toxicity of groundwater samples, and the microplates technique (is a “conventional” technique) to analyse the surface waters. The two tests are performed and used routinely in the Ecotoxicology Laboratory DPPF/ISA and in “Instituto do Mar” (IMAR), “Departamento de Zoologia da Universidade de Coimbra” respectively.

Both tests have a duration of 72-h of exposure. The mean specific growth rate (GR) per day was estimated [3]. The recommended statistical endpoint is the inhibiting concentration for a specified effect (IC_p).

$$GR = \frac{\ln N_n - \ln N_1}{t_n - t_1} \quad [3]$$

Where:

N_n = measured number of cells/mL at time t_n ; N_1 = measured number of cells/mL at time t_1 ; t_n =time of n^{th} measurement after beginning of test; t_1 =time of first measurement after beginning of test.

A) Microplates Technique

The microplates technique used in the growth inhibition tests of freshwater *P. subcapitata*, confers a number of advantages over the standardized alga bottle tests, in this way became to substitute the most traditionally alga bottle tests used to assess the substances phytotoxicity . The microplate technique can be used as a screening test increasing in this way the efficiency in the processing of samples, compared with the classic alga bottle test (Environment Canada, 1992b).

The algal *P. subcapitata* should be in a phase of exponential growth (4 at 7 days old culture), are exposed in a static system beneath defined conditions to various concentrations

of a test substance or a dilution series of a mixture or effluent sample, throughout 72-h. The sample tested is considered toxic if the difference among the alga growth in an appropriated control and the growth of alga exposed to the sample are statistically significant, that is when dose-dependent inhibition of alga growth occurs (Environment Canada, 1992b).

It's required that two conditions are satisfied for the results of the growth inhibition tests to be acceptable: the cell concentration in the control cultures should have increased by a factor of at least 16 within three days (72-h) and the coefficient of variation for the control replicate should be < 20% (OECD, 1984; Environment Canada, 1992b).

In fact the microplate technique has a group of advantages, the test requires small sample volumes, a small volume of algae, and is necessary less space to the incubation than in the alga bottle test. On the other hand with any standardized toxicity test this procedure has some limitations, filtering the sample previously might influence the toxicity, that can be significantly reduce other limitation is that for high concentrations of dissolved organic material the results can be confound (Environmental Canada, 1992b).

The 72-h *P. subcapitata* growth test was carried out following OECD (1984) and EEC (1989) guidelines, presented in annex 5. Both samples (AR (Almonda River) and DV ("Dique dos Vinte", marsh) were subjected to a dilution gradient of 6.25, 12.5, 25.0, 50.0, and 100% (v/v).

B) AlgaltoxitTM

In this test concentrates of immobile algae in a matrix were used allowing to be immediately used and placed under refrigerator conservation. The test is based on the exposure of the algae suspension during 72-h at the end of the test the optical densities at 670 nm in spectrophotometer are measured and used to calculate the growth inhibition % microalgae (ISO /DIS 8692). The 72-h *Pseudokirchneriella subcapitata* growth test required that three conditions were satisfied so that the results could be accepted: the cell concentration in the control cultures should be increased by a factor of at least 67 times within three days (72-h) and the coefficient of variation for the control replicate should be < 20%. Also the pH of the control shouldn't changes more than 1.5 units since the beginning of the test.

A reference solution of potassium dichromate or 3,5- dyclorofenol can be used. The preparation of the reference solution should be done periodically for performance validation of the operator and verification of the physiologic conditions of the EC₅₀ (72 h) the organism

of the potassium dichromate was used and should be located in the interval indicated by the firm for each batch of individuals (ISO/DIS 8692).

The *P. subcapitata* test was performed following the methodology prescribed in the Standard Operational Procedure for Algaltoxitest™ (SOP, 1996b) is based on ISO /DIS 8692, presented in annex 5. The test was performed to 100% samples concentration.

5.1.6.2. Sublethal toxicity tests

5.1.6.2.1. *Daphnia magna* reproduction test

One reason why *Daphnia magna* is a commonly used test animal in aquatic toxicology is that it is easily cultured in the laboratory and Daphnids are of ubiquitous occurrence and form an important link in food chains (Adema, 1978).

To evaluate the toxicity of pollutants and waste water on the aquatic invertebrates the standard method of *Daphnia magna* 21-days survival and reproduction test has been used (ASTM, 1981; OECD, 1998b; U.S.E.P.A., 1996). The aim of the test is to assess the effect of the chemical on the reproduction output of *D.magna*. At the end of 21-d of exposure in order to determine the lowest observed effects concentration (LOEC) and hence the no observed effect concentration (NOEC), the reproduction output of the animals is compared to that of the controls. In addition the data can be analyzed using a regression model in order to estimate the concentration that would cause a x % reduction in reproductive output, i.e. EC_x (e.g. EC₅₀, EC₂₀ or EC₁₀) (OECD, 1998b).

With all tests this method presents same disadvantages, the condition of a 21-d exposure period makes this a costly test (Adams *et al.*, 1985).

Adams *et al.* (1985) compared the *Daphnia magna* 21-d and 14-d survival and reproduction test methods with 30 tests of 21-d chronic toxicity and 18 tests of 48-hr acute toxicity. The results showed that the MATC (Maximum Allowable Toxicant Concentration) after 21-d exposure might be exactly estimated with the reproduction and survival data of 14-d tests. So they suggested that the standard protocols for *D.magna* chronic test should be changed to a requirement of only a 14-d period of exposure instead of 21-d.

The test was carried out during 21 days according to the principles described on OECD (1998) guidelines, the procedure is presented in annex 5. Both samples (AR (Almonda River) and DV (“Dique dos Vinte”, marsh) were subjected to a dilution gradient of 6.25, 12.5, 25.0, 50.0, and 100% (v/v).

5.1.6.2.2. Test for survival and growth in sediment using the larvae of freshwater Midges - *Chironomus riparius*

The sediment can be like a sink to the chemicals elements presents in the aquatic environment, since being a major repository for many of the more persistent chemicals that are introduced to surface waters at the same time that provides habitat for many aquatic organisms (Environmental Canada, 1997).

Even where the criteria for water quality are not exceeded there is a growing evidence of environmental deterioration including adverse effects or aquatic organisms that are in or /close to the contaminated sediment (Environmental Canada, 1997). However it is also possible that sediment may be contaminated with high levels of certain substances and that is not evident at this point in communities of benthic or epibenthic organisms and no harm to exposed aquatic life can be demonstrate (Environmental Canada, 1997).

The importance of having a standard sediment bioassay is explained for the variability of sediments, which results in the variable partitioning of chemicals between water and sediment (Burton *et al.*, 1990).

Ecotoxicological testing of sediments began in the late 1970s and there were no standard methods for conducting sediments toxicity tests until the early 1990s (Bat *et al.*, 1999). Currently there are various types of standardized tests for sediments. The environmental protection agency of U.S.A. (U.S.E.P.A., 2000) normalized the bioassays for assessing the quality of sediments with benthic organisms that includes: *Hyalella azetca*, *Chironomus riparius* and *Lumbriculus variegates*. So, sediments toxicity tests are actually cost-effective tools for determining whether contaminants in sediment are harmful for the aquatic organisms. Is clearly that is necessary to know the ecological needs and sensitivity of the organisms for contaminates to may get actions to protect aquatic habitat in relation to potential damage that can result from the presence of contaminants in sediment (Environmental Canada, 1997).

Chironomids are widespread and abundant (holartic) nonbiting flies. Ecologically, Chironomids (Diptera) form an important group of organisms. These insects have four live stages: egg, larvae, pupa and adult. The greatest part of their time life cycle the larva step is spent in sediment, larval growth occurs in four instars of about 4 to 7 days each. This stage is followed by a pupal stage and then emergence to the adult stage (Bat *et al.*, 1999; Environmental Canada, 1997).

The chironomids commonly referred to as midges have many features that are advantages for their use in toxic sediments tests, like their widespread distribution, ecological importance, ease of culture and rapid growth, sensitivity to contaminated sediments and short life cycle. Beyond this the midge larvae are robust and can support a wide range of conditions (Environmental Canada, 1997).

There are several procedures and conditions that should be followed in the realization of the test to accurate the validity criterias. The cultures used in tests must have low mortality and must appear health.

The test for survival and growth in sediment using the larvae of freshwater midges *C. riparius*, includes two options to measure the sediment toxicity (the option (a) was performed with the sediment sampled in “Dique dos Vinte”, marsh (DV)):

(a)- A static toxicity test, which the overlying water is not renewed, the accepting loss for evaporation and with continuous aeration;

(b)- A non-static toxicity test, with an intermittent renewal of the overlying water and generally no aeration.

At the end of the exposure period (10-day normally) the number of dead and alive midge larvae on each control and test sample are recorded as well as their weight (Environmental Canada, 1997). The *C. riparius* Meigen growth test was carried out following the OECD (2004) and ASTM (2002b) guidelines presented in annex 5, but the larvae have 11-days at the end of the test. The sample was subjected to a dilution gradient of 12.5, 25.0, 50.0, and 100% (m/m).

5.2. Surface water and sediment data analysis

For data analysis the program Statistica 7 which provides two analyses was used: an analysis of variance (Anova); and a multiple comparison of treatment means with the control mean (Dunnett’s Procedure) (Zar, 1996).

For all tests, the measured organism responses in the different treatments (water or sediment dilutions) were examined for significant differences using one-way analysis of variance (ANOVA) or nested ANOVA.

When significant differences were found, the Dunnett’s test was performed to test for significant differences between the control and the tested dilutions, to determine the no-observed-effect dilution (NOEC) and the lowest-observed-effect dilution (LOEC). Although

the Microtox software (Microtox Omni Software 1.18; Azur Environmental) did not provide replicate data to perform an ANOVA, a dilution causing a luminescence inhibition lower or higher than 10% was considered as the NOEC and LOEC value, respectively. The median effective dilutions (EC_{50}) and respective 95% confidence limits (CL) were calculated either through the recommended software (Microtox) or by fitting organism responses to a logistic model using the least squares method (OECD, 1998a, d).

5.3. Results and discussion

5.3.1. Predicted environmental distribution (PED) through Mackay fugacity model – Level I calculation and Leaching potential through Bacci and Gaggi, GUS leaching indexes

For the registered pesticides to crops considered in this study, data on physical - chemical properties necessary for the calculation of the Mackay model of fugacity and of indexes leaching GUS and Bacci & Gaggi was obtained. The data was processed with the intention of classifying and sorting the pesticides which, under standard conditions show greater affinity to the water compartment and greater potential for leaching.

Pesticides are considered to have a medium to very high affinity for the water compartment when $PED_{water} \geq 40\%$, quiet adopted by various authors (Table 5.5), and high potential for leaching into the groundwater when the index $GUS > 2.8$ (Gustafson, 1989) and/or index Bacci & Gaggi ≥ 0.1 (Bacci, 1994) (Table 5.6). Of the 138 pesticides in study the data obtained only permits the calculation of Mackay model for 83 and 43% of these presents very high affinity for water compartment. Furthermore, herbicides are the pesticides group with more affinity to water compartment, when compared to insecticides and fungicides groups (Table 5.5).

TABLE 5.5: Sorting and classification of pesticides registered for selected crops belonging to the study region, depending on their affinity for the water compartment on the basis of the results of the model Mackay

PESTICIDE TYPE	AFFINITY TO WATER COMPARTMENT			
	Very High ($\geq 80\%$)		High ($\geq 60\% - 80\%$)	Medium ($\geq 40\% - 60\%$)
Insecticides	acetamiprid	dimethoate	malathion	phosmet
	aldicarb	pirimicarb		
	beta - cyflutrin	pymetrozine		
	carbofuran	trichlorfon		
	dazomet			
Fungicides	bitertanol	imidacloprid	captan	azoxystrobin
	carbendazim	mancozebe	dimethomorf	cyazofamid
	carboxin	thiabendazole	fenamidone	myclobutanil
	carbendazim	triacetazolo		
	dodine	ziram		
	fosetyl - aluminium			
Herbicides	2,4 -D	MCPA	benoxacor	linuron
	bromoxynil	mesosulfuron -methyl	diuron	mesotrione
	chloridazon	metamitron	ethofumesate	S - metolachlor
	clopyralid	nicosulfuron		
	cicloxydim	sulcotrione		
	dicamba	tribenuron -methyl		
	glufosinate -ammonium			
	lenacil			

The herbicides group shows a great potential for leaching; 25 pesticides shows potential for leaching according both indexes where 10 are herbicides (40%) (Table 5.6).

TABLE 5.6: Sorting and classification of pesticides approved for selected crops belonging to the study region, depending on their leaching potential calculated by the indexes of GUS and Bacci & Gaggi

PESTICIDE TYPE	LEACHING POTENTIAL			
	GUS > 2.8		Bacci & Gaggi ≥ 0.1	
Insecticides	abamectin	pirimicarb	abamectin	methiocarb
	aldicarb	pymetrozine	aldicarb	pirimicarb
	carbofuran	tebufenozide	acetamiprid	pymetrozine
	clorpyrifos	trichlorfon	carbofuran	trichlorfon
	dimethoate		clorpyrifos	
			dimethoate	
Fungicides			fosetyl - aluminium	
			flufenoxuron	
	bifenthrin	metalaxyl -M	bifenthrin	myclobutanil
	bitertanol	propamocarb - hydrochloride	bitertanol	penconazole
	copper hydroxide	thiabendazole	carbendazim	propamocarb - hydrochloride
	dodine		fenbuconazole	thiabendazole
	fenbuconazole		fenpropidin	ziram
	flusiazole		metalaxyl -M	
iprovilcarb				
Herbicides	2,4 - D	ethofumesate	2,4 - D	ethofumesate
	amitrole	fluazifop -p -butyl	benoxacor	fluazifop -p -butyl
	bentazone	glufosinate - ammonium	bromoxynil	glufosinate - ammonium
	chlorotulorun	glyphosate (trimethylsulfonium)	bentazone	glyphosate (trimethylsulfonium)
	clopyralid	mesosulfuron -methyl	chlorotulorun	linuron
	dicamba	rinsulfuron	clopyralid	mancozeb
	diuron		cicloxydim	mesosulfuron -methyl
			dicamba	paraquat
			diuron	S - metolachlor
			dodine	tribenuron - methyl

It was concluded that herbicides: clopyralid, dicamba and mesosulfuron-methyl, showed greater affinity for the water compartment with results from $PEd_{water} = 100\%$ and GUS index > 2.8 and Bacci & Gaggi > 0.1.

5.3.2. Pesticide risk classification on different ecosystems (hypogean and epygean soil, surface water systems)

The pesticides in study are classified for each one of the indexes of EPRIP according to the classes of risk: negligible, low, medium, high and very high set in Finizio *et al.* (2000) and expressed on the following table (Table 5.7). For tables with the total scores calculated see annex 4. For the following discussion, the pesticides that presented high or very high risks for the different ecosystems were considered.

TABLE 5.7: Final scores for the classification of indexes (Finizio *et al.*, 2000)

	PRHIS_1	PRIHS_2	PRIES_1	PRIES_2	PRIWS_1	PRIWS_2	ERIP
Negligible	≤5	≤5	≤5	≤5	≤5	≤5	≤10
Low	>5 - ≤15	>5 - ≤15	>5 - ≤15	>5 - ≤15	>5 - ≤15	>5 - ≤10	>10 - ≤20
Medium	>15 - ≤40	>15 - ≤30	>15 - ≤50	>15 - ≤40	>15 - ≤40	>10 - ≤30	>20 - ≤40
High	>40 - ≤60	>30 - ≤50	>50 - ≤70	>40 - ≤70	>40 - ≤80	>30 - ≤60	>40 - ≤60
very High	>60	>50	>70	>70	>80	>60	>60

The next figures are related to pesticides who obtained a high or very high class of risk.

The diazinon insecticide shows a very high risk (score = 66) to the short term terrestrial hypogean (Figure 5.8) and this behavior is maintained on the long term index. In the long term index of pesticides that have a high risk to very high, 79% are fungicides (Figure 5.9).

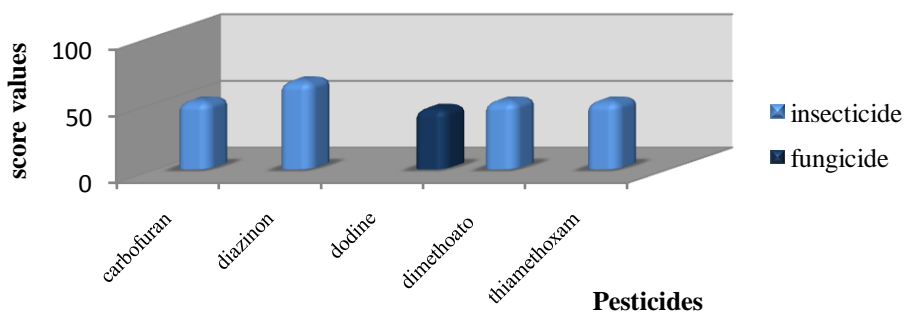


FIGURE 5.8: Pesticides with high (>40 - ≤ 60) and very risk (>60) in short term terrestrial hypogean (PRIHS_1)

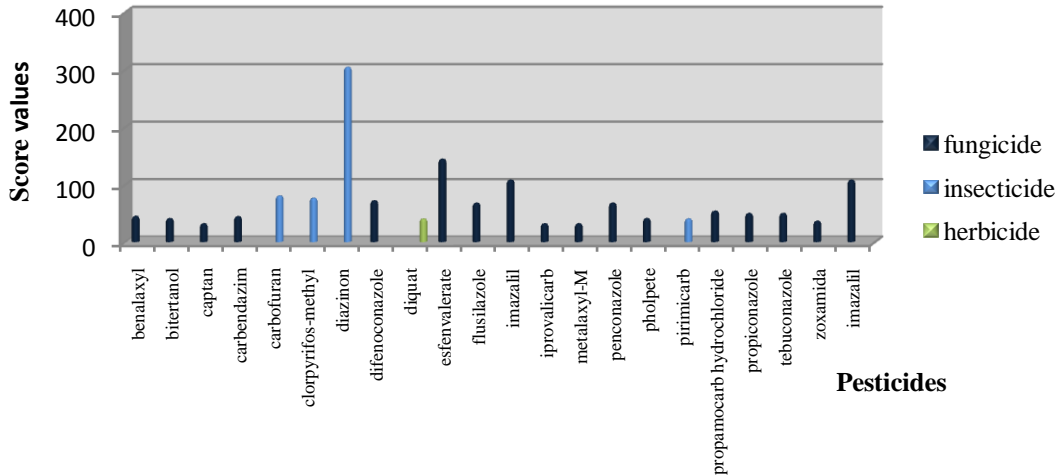


FIGURE 5.9: Pesticides with high (>30 - ≤ 50) and very risk (>50) in long term terrestrial hypogean (PRIHS_2)

In indexes related to the earth epygean system (PRIES_1; PRIES_2) and for pesticides for which it is possible to calculate both index, the score ranged from negligible and medium risk (Annex A4.6 and A4.9), being the insecticides group, the one which shows higher values at short term, and the fungicides in a long term.

Pandey & Singh (1990) mentioned that insecticides were generally shown to a greater direct effect on soil organisms than the herbicides and that fungicide had generally greater effects on soil organisms than the herbicides and insecticides at long term.

In the case of indexes for surface waters, dodine fungicide shows a very high risk (score = 89) to the short term (Figure 5.10) and this behavior is maintained to the long term index (Figure 5.11).

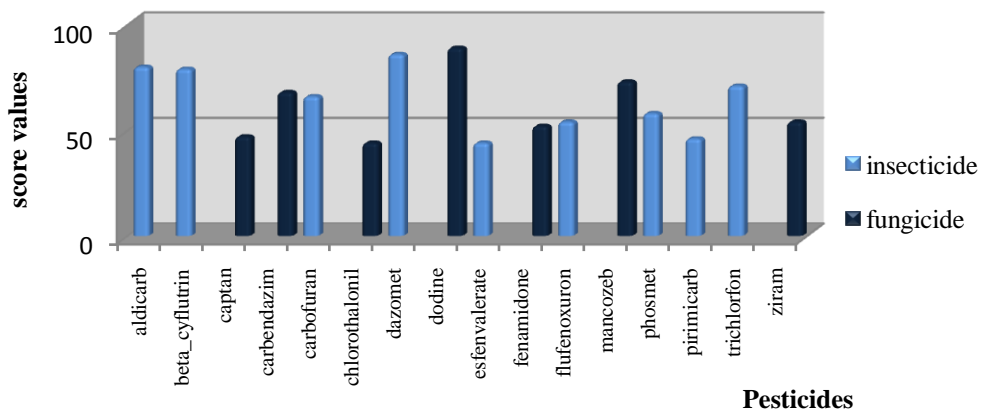


FIGURE 5.10: Pesticides with high (>40 - ≤ 80) and very risk (>80) in short term water system (PRIWS_1)

However, the pesticides that are greater represented with a high or very high score are the insecticides. In the long term index the clorpyrifos (score =77) shows the high score (Figure 5.11). The herbicides that show a high or very high risk on the long term index, on short term index show a low or medium risk.

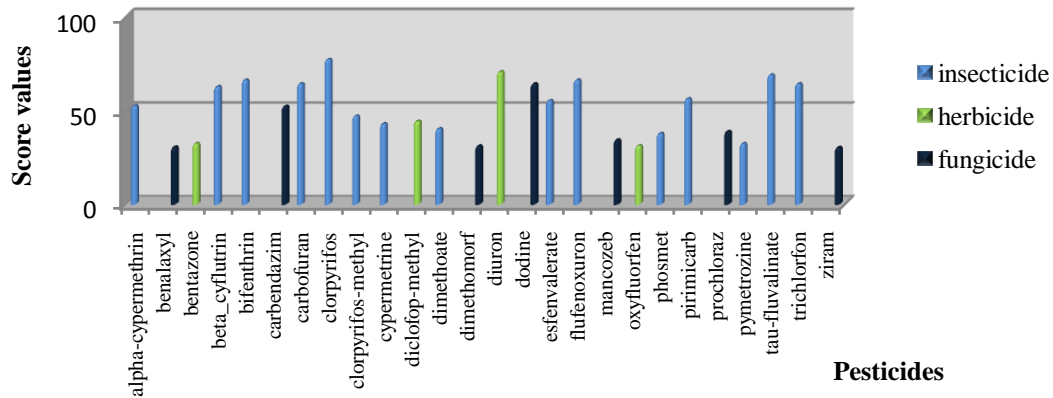


FIGURE 5.11: Pesticides with high (>30 - ≤ 60) and very risk (>60) in long term water system (PRIWS_2)

In the ERIP index the highest score is for clorpyrifos insecticide (Figure 5.12). Pesticides with high risk for the environment represent 9.4% of the total.

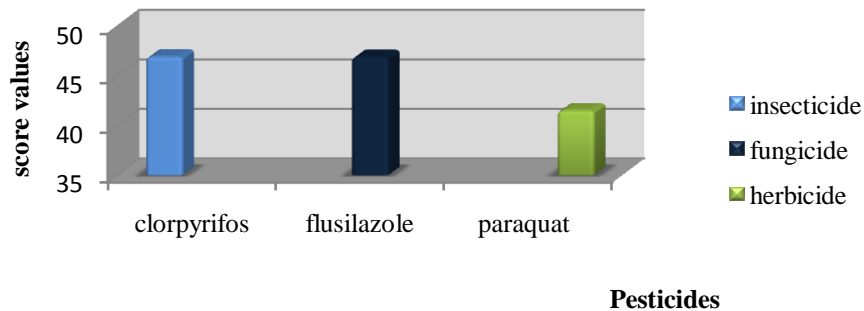


FIGURE 5.12: Pesticides with high (>40 - ≤ 60) and very risk (>60) in ERIP

As a common rule, insecticides tend to be more dangerous than other compound, this tendency is visible in greater or lower extension on all the indexes. With the exception of PRIWS-1, the highest score is always reached by insecticides. Herbicides are possibly overestimated in PRIES-2 and ERIP indexes due to the high score given by default to phytotoxicity. Fungicides are too overestimated in the ERIP due to the high score given by default to microorganisms.

Pyrethroid insecticides show in great part high to very high risk for the aquatic environment in the short term due to their extremely high toxicity for aquatic animals. The risk is identical in the long term that is the opposite of what was verified in Finizio *et al.* 2000, this fact can be related in some cases with the highest affinity for the water compartment and to with a high persistence.

Organophosphates, insecticides are a complex group with variable environmental and toxicological characteristics (Finizio *et al.*, 2000; Konstantinou *et al.*, 2005). For example, the diazinon shows very high risk in the terrestrial hipogean indexes in a long and short term due to high application and high toxicity to some nontarget organisms. As a general rule, for most organophosphates, the risk seems to be higher in the short term, due to high toxicity and relatively low persistence (Finizio *et al.*, 2000; Konstantinou *et al.*, 2005). But in this case diazinon has the same class of risk in the long term and short term, it's possible that it be related to his high application rate. The default used in this index could contribute to this classification.

High variability also applies to carbamates in the different compartments, however the carbamates insecticides have similar behaviors among them, for example pirimicarb (low application rate) with a greater variability, low to very high risk. For the terrestrial epigeal compartment it has a low risk and for the terrestrial hipogean varies between medium to very high in short and long term, respectively, this can be explained by the high toxicity for nontarget organisms, carbofuran (high application rate) shows a very similar behavior.

For the fungicides we must have in consideration that in PRIES_2 and ERIP they are overestimated, this can explain the fact that many fungicides had high risk for the terrestrial hipogean system at long term. Captan, due to its high toxicity for aquatic animals, shows high risk for PRISW-1, but its low persistence highly reduces the risk in PRISW-2, this behavior of risk decrease from the short to long term are observed in Finizio *et al.*(2000). Dodine, due to its high affinity to the water compartment and high toxicity to the nontarget organisms shows very high risk for the aquatic compartment. Tebuconazole presents negligible a low risk for all the indexes in spite of its relatively low toxicity on nontarget organisms, except for the PRIES_2, this can be the result of the default used.

Within the range of herbicides for which it was possible to obtain a rating, its expression was relatively small, the highest scores were observed for the PRISW_2. Most herbicides fall into the "low" to "medium" risk range with some differences among indexes.

The major obstacle to obtain most correct information was the lack of data on the side effects of these substances. So many default values have been used which implies that some

values may be overestimated or underestimated. Beyond the shortcomings also the differences found in different databases vary sometimes several units which can also induce to error. However, several studies show that the synthesis of information on pesticide hazard and exposure into risk indices is found to be useful for providing plausible visions on the *status quo* of pesticide risks and to identify potential trouble spots where risk reduction might be a main concern. The inclusion in the analysis of a set of indicators representing pesticide hazards along a number of ecological dimensions is also found to be important for articulating trade-offs in management objectives across different environmental concerns. Besides, our empirical analysis confirms that multi-criteria techniques constitute a suitable framework to apply risk indices as decision support tools (Travisi, 2006).

5.2.3. Groundwater exposure levels to pesticides

There are at this point, results for the occurrence of pesticides and metabolites in surface and groundwater in agricultural areas of the county's Golegã and Torres Novas.

On a total of 8 sites selected 26 samplings were analyzed by GC-MS, where in 50% of the samples were detected, at least one, of the pesticides and / or metabolites in the analysis (Table 5.8 and Table 5.9).

In a total of 24 samples of groundwater, four (16%) have levels of at least one of the compounds higher than 0.1 µg/L (Table 5.8), considered by Directive on the protection of groundwater, as quality standard for groundwater and is the parametric value for water for human consumption (D.L. No. 243/2001). It is important to note that many times the water of the wells for irrigation is also used to drink for farm workers. This value confirms the vulnerability of the groundwater to contamination in the agriculture areas selected for this study. The two samplings points of surface waters have levels at least one of the compounds greater than 0.1µg/L (Table 5.9).

In the case of ground and surface water and with regard to the 26 active substances considered in the routine of the Ecotoxicology Laboratory DPPF/ISA, in groundwater the following frequencies of detection were observed: alachlor 9%, atrazine 46% and metalachlor 9% (Table 5.8). For the surface water samples was observed the following frequency of detection: 3,4-dicloroaniline 50%, alachlor 50%, atrazine 50%, ethofumesate 50%, metolachlor 50%, propanil 50% and terbuthylazine 100% (Table 5.9).

TABLE 5.8: Level of residues of pesticides and/or metabolites dosed in groundwater samples, in the study area, in 2008

Sampling areas	Wells	Sampling dates	Concentration Levels (µg/L)			Total concentration level (µg/L)
			alachlor	atrazine	metolachlor	
"Campo da Golegã", Golegã	BD	05-Jun	<LOD	<LOD	<LOD
		27-Jun	<LOD	<LOD	<LOD
		22-Jul	<LOD	<LOD	<LOD
		13-Ago	<LOD	<LOD	<LOD
	P	05-Jun	<LOD	<LOD	<LOD
		27-Jun	<LOD	<LOD	<LOD
		22-Jul	<LOD	<LOD	<LOD
		13-Ago	<LOD	<LOD	<LOD
	L	05-Jun	<LOD	<LOD	<LOD
		27-Jun	<LOD	<LOD	<LOD
		22-Jul	<LOD	<LOD	<LOD
		13-Agu	<LOD	<LOD	<LOD
"Quinta dos Pinheiros", Riachos	QP1	05-Jun	<u><0.05</u>	0.11	<LOD	< 0.16
		27-Jun	<LOD	<0.05	<LOD	<0.05
		22-Jul	<LOD	<0.05	<LOD	<0.05
		13-Ago	<LOD	0.08	<LOD	0.08
	QP2	05-Jun	<LOD	0.09	<LOD	0.09
		27-Jun	<LOD	<0.05	<LOD	<0.05
		22-Jul	<LOD	0.07	0.13	0.2
		13-Ag	<LOD	0.07	0.11	0.18
	QP3	05-Jun	<u><0.05</u>	0.10	<LOD	< 0.15
		27-Jun	<LOD	<0.05	<LOD	<0.05
		22-Jul	<LOD	<0.05	<LOD	<0.05
		13-Agu	<LOD	<LOD	<LOD
Frequency of detection (%)			9	46	9	

BD - "Barracão do Duque"; P - "Praias"; L - "Lezíria

QP1 "Quinta dos Pinheiros", well 1; QP2 "Quinta dos Pinheiros", well 2; QP3 "Quinta dos Pinheiros", well 3

<LOD - below the limit of detection

TABLE 5.9: Level of residues of pesticides and/or metabolites dosed in surface water samples, in the study area, in 2008

Sampling areas	Wells	Sampling dates	Concentration Levels (µg/L)							Total concentration level (µg/L)
			alachlor	atrazine	ethofumesate	metolachlor	propanil	3,4 - dicloroaniline	terbuthylazine	
"Alverca da Golegã" (marsh)	DV	13-Aug	<LOD	0.28	<0.05	0.21	<LOD	<LOD	<0.05	<0.59
"Almonda" river	AR	13-Aug	1.73	<LOD	<LOD	<LOD	0.12	3.66	0.21	5.72
Frequency of detection (%)			50	50	50	50	50	50	100	

DV- "Dique dos Vinte"; AR - Almonda river

<LOD - below the limit of detection

In this work was observed mixtures of pesticides in 23% of the samples (of groundwater and surface water), up to four substances in mixture. In 33% of them the total concentration level of pesticides compounds was greater than 0.5 µg/L (parametric value for pesticides total in water for human consumption), registering a maximum total concentration level of approximately 5.72 µg/L in surface water (see Table 5.8 and Table 5.9).

Atrazine, alachlor, metolachlor and terbuthylazine were the pesticides that presented the highest frequencies of detection in this study. The first three occurred in both types of samples (groundwater and surface water) these were the most common detected pesticides in the groundwater at world level (Barbash & Resek, 1997; Funari *et al.*, 1995; Sanchez - Camazano *et al.*, 2005) and in this zone of the study or near the study zone (Cerejeira, 1993; Rei, 2005). While the atrazine highly used herbicide in maize, it has been withdrawn from the market in December 2007, it probably appears in groundwater at a high frequency due to its slow dissipation ($DT_{50} > 200d$, under groundwater conditions). Studies have shown that the dissipation of atrazine is slow, originating the presence of residues in water from leaching in the long term (Ma & Selim, 1994; Rao *et al.*, 1985). The maximum concentration level of atrazine ($0.28 \mu\text{g/L}$) was observed in the surface waters, although according to Gaynor *et al.* (1995) atrazine losses for runoff are much smaller than the quantity capable of leaching.

Alachlor is also present in the two types of samples, this can be related to the fact that alachlor herbicide is very mobile and moderately persistent, these two characteristics are generally observed in chemicals that reach the ground and surface waters (EPA, 1998), in Portugal the final date for his use is 18/12/2008. The same characteristics are also present in metolachlor. Salmeron *et al.* (1991) concluded that metolachlor herbicide is more persistent in soil than alachlor, which could show a higher leaching behavior.

In surface waters terbuthylazine was observed on the both, with a maximum level of $0.21 \mu\text{g/L}$.

Was also observed the presence of propanil and this major metabolite 3,4-dichloroaniline (DCA). Actually, there are no direct uses of 3,4-DCA without chemical transformation. Releases into the environment occur during use of plant protection agents (linuron, diuron, propanil) (European Commission, 2006). Generally propanil is very rapidly degraded in to DCA (Santos, 1998), in this case this can occur because the maximum level found is quite superior for DCA ($3.66 \mu\text{g/L}$) than for propanil ($0.12 \mu\text{g/L}$) (Table 5.9).

In order to broaden the spectrum of analysis of these waters a qualitative analysis of organic compounds by GC-MS was performed by the laboratory of the Portuguese Environment Agency, for each of the different samples of groundwater in data collection June 27. The following compounds were detected:

- for samples collected in the “ Campo da Golegã” designed as QB, P and L: tetrachloroethylene, toluene, trichloroethylene (VOC's) (volatile organic compounds) probably existents in formulations of applied pesticides, and dichloropropene.

- for samples collected in Riachos designed as QP1, QP2 and QP3: xilene, ethylbenzene, tetrachloroethylene, trimethylbenzene (VOC's) probably existents in formulations of applied pesticides, the nematocide dichloropropene and the atrazine herbicide.

The compounds (tetrachloroethylene, trichloroethylene, toluene, ethylbenzene) are used in a wide variety of industrial and commercial applications, including use as metal degreasers, industrial solvents, and ingredients in aerosols, adhesives, and protective coatings herbicide. Probably the occurrence was related with the formulation of some herbicides used in this area. Stackelberg *et al.* (2001) observed in New Jersey, USA, that one or more pesticides were detected with one or more volatile organic compounds (VOCs) in more than 95% of samples for 95 monitoring wells screened in the unconsolidated surficial aquifer system of southern.

Furthermore the VOC's detected on the groundwater were observed in mixture of pesticides in the analyzed samples. This can be a point of future investigation because many studies evidence that the co-occurrence of multiple organic compounds may modify anticipated properties of individual compounds, for example microbial degradation of an individual pesticide can be inhibited by the presence of additional pesticide compounds as a result of the enhanced toxicity of the pesticide mixture to micro-organisms (Stackelberg *et al.*, 2001).

The wells where the groundwater samples were collected, follow the evolution of pesticide residues and metabolites in four dates that are differentiated in terms of application of pesticides on crops that exist (potato and maize), there is in general a variation of the levels determined during the sampling. This dynamic may be related to several factors of agricultural ecosystems, in particular with the edaphical and hydrological features and practices carried out, particularly with the period of application of pesticides and irrigation.

5.3.4. Toxic effects on aquatic organisms in groundwater

In the next points are showed the values of acute toxicity for the *D.magna* and *P. subcapitata* organisms of the sampling of groundwater in the study area (in the zone of Riachos the sampling was in "Quinta dos Pinheiros" (QP) assigned as 1, 2 and 3 for the different wells of collect, and "Barracão do Duque" (BD), "Praias" (P) and "Lezíria" (L) in "Campo da Golegã").

The results of the microbioassay "Daphatoxkit" are expressed as the result of the % of effect on this organism based on the immobilization rate.

In the total of 24 samples of groundwater, 83% showed a % of effect on *D.magna* \geq 50% (Figure 5.13 and Figure 5.14). Concerning QP samples, on 67% of samplings the % of effect was $>$ 50%. Of the 12 samplings on this local 17% had an effect equal to 100%. From the analysis of the results can be assumed that the samples of groundwater from three wells have toxicity for this type of organism (Figure 5.13).

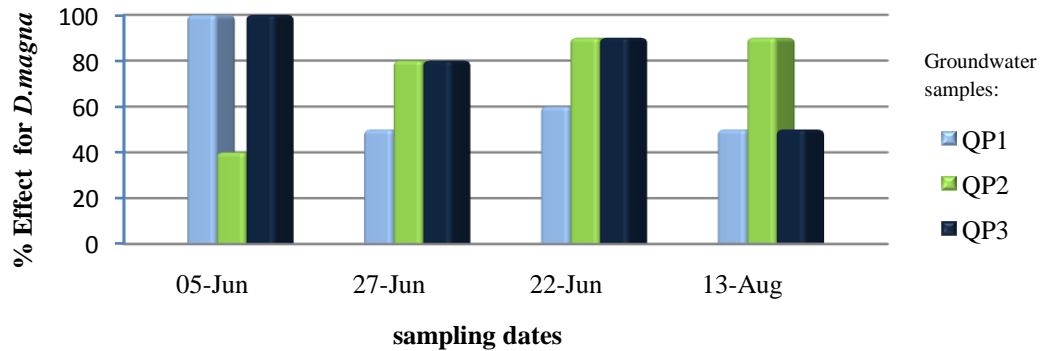


FIGURE 5.13: Immobilization effect % to *D.magna* of the groundwater samples QP1, QP2 and QP3

In “Campo da Golegã “ on 67% of samplings the % of effect was $>$ 50% (Figure 5.14). The percentage of effect was \geq 70% of effect in all the three wells in 13 August samplings. For the 12 samplings for this site 17% have an effect equal to 100%. From the analysis of the results can be assumed that the samples of groundwater from three wells have toxicity for this type of organism (Figure 5.14).

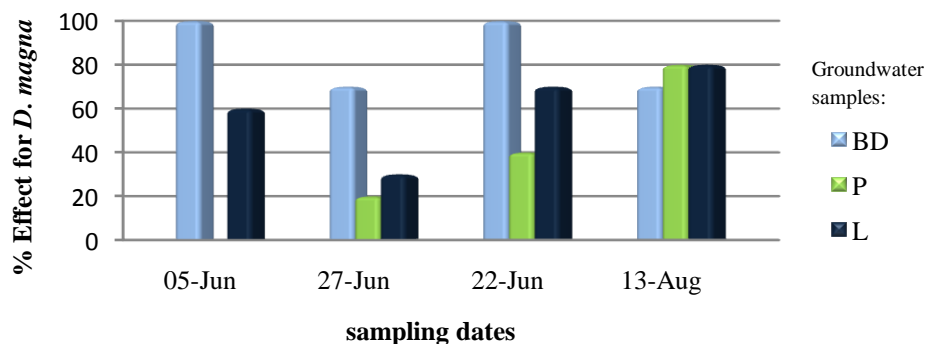


FIGURE 5.14: Immobilization effect % to *D.magna* of the groundwater samples BD, P and L

Yet it is not possible to establish a relationship between the variations in percentage of effect in the six wells based on the different times of water sampling.

The results of the microbioassay “Algatoxkit” are presented in terms of inhibition percentage of the growth registered during the 72 hours of testing.

Regarding the samples QP, of the 12 samples only one of these had a % of inhibition to *P.subcapitata* above 50%, recorded for the sample QP1 on July 22. For samples QP2 and QP1 in the collect of August 13 occurs a stimulation of the *P. subcapitata* growth, respectively of 16% and 22%. Although the levels of atrazine and metolachlor concentrations compared to some of the other dates of sampling have been higher (*vide* Table 5.8), the event may have had origin on various factors. Not just related to the constituents of the samples, which determined that its toxicity resulting from the balance between the inhibitors and stimulating the growth of algae, but also the possibility of any other microalgae in samples collected were able to adapt to the same conditions for the growth of *P. subcapitata* thus the final results (Figure 5.15).

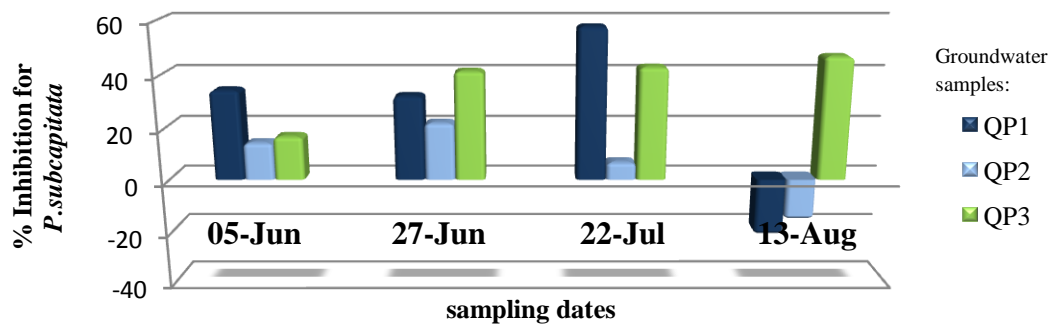


FIGURE 5.15: % of inhibition of *P.subcapitata* growth of the groundwater samples QP1, QP2 and QP3

In “Campo da Golegã” all the samples and for all sampling dates, there was inhibition of *P.subcapitata* growth. In 33% from the 12 samples, inhibition of growth was above 80%, the samples show in this way high toxicity to the organism (Figure 5.16). Yet it is not possible to establish a relationship between the variations in percentage of effect in the six wells based on the different times of water sampling. In the “Campo da Golegã” samples the level of residues was always < LOD (*vide* Table 5.8). So is not possible to establish any relation between toxicity to the organisms and level of residues in samples.

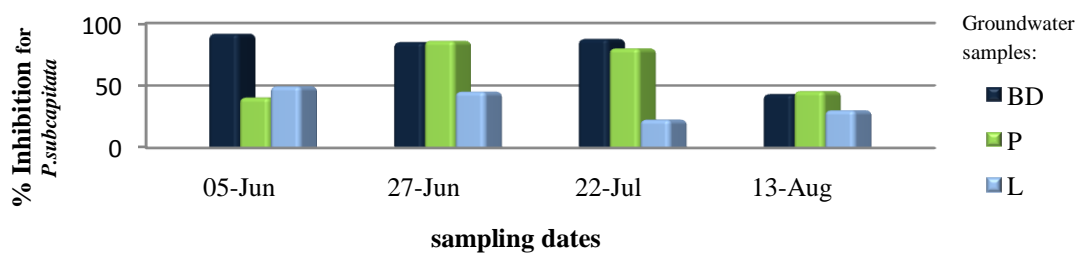


FIGURE 5.16: % of inhibition of *P.subcapitata* growth of the groundwater samples BD, P and L

These results suggest that the groundwater in the area of study may have toxic substances for the algal, being the major concern related to the possibility of bioconcentration of these pollutants up the food chain once the *P. subcapitata* is a primary producer in the ecosystems (Dunnivant & Anders, 2006).

For groundwater samples and for the *D. magna* organism, the largest % of effect were found in the samples were assayed more than one pesticide (*vide* Table 5.8 and Table 5.9), this may be due to one synergistic effect of the mixture.

5.3.5. Toxic effects on aquatic organisms in surface water

All tests fulfilled the validity criteria for control performance required in the respective guidelines.

The *V. fischeri* test didn't reveal toxicity for both samples after 15 min of exposure. For both samples the effect percentage at 100% (v/v) was lower than 10%. NOEC values were considered to be 100% (v/v) of the samples.

For *P. subcapitata*, significant differences in growth between the control and the tested dilutions, were observed for both samples, Almonda river sample (AR) (one-way ANOVA: $F_{5, 15} = 20$, $P < 0.001$) and "Dique dos Vinte" marsh sample (DV) (one-way ANOVA: $F_{5, 15} = 684$, $P < 0.001$). Although Almonda river sample showed a significant inhibition in growth relatively to the control at all tested dilutions, the NOEC and LOEC values were considered to be 50 and 100% (v/v), respectively, because at these dilutions growth was inhibited by merely 10% and by 53%, respectively; the EC_{50} was 99% (v/v). "Dique dos Vinte" marsh sample was shown to be more toxic with NOEC, LOEC and EC_{50} values of 6, 13 and 50 % (v/v), respectively.

When *D. magna* was exposed to the water samples, significant differences in reproduction between the control and the tested dilutions were found both for Almonda river sample (one-way ANOVA: $F_{2, 26} = 11$, $P < 0.001$) and "Dique dos Vinte" marsh sample (one-way ANOVA: $F_{4, 26} = 14$, $P < 0.001$). Although 100% mortality was observed at 50 and 100% (v/v) of Almonda river sample, reproduction was inhibited by merely 5% at the 25% (v/v) dilution (though it is expected to be between 25 and 50% (v/v)). Thus, the NOEC value was 25% (v/v) and it was not possible to estimate the EC_{50} . On the contrary, "Dique dos Vinte" marsh sample showed values of NOEC, LOEC and EC_{50} of < 12.5 , 12.5 and $>100\%$ (v/v), respectively. For this sample, percentages of mortality were of 30, 60, 40 and 50% at the 12.5, 25, 50 and 100% (v/v) concentrations, respectively.

In this study, a battery of tests with organisms bearing different key functions at the ecosystem level was used to evaluate the effectiveness to the toxicity of the water samples.

The *V. fischeri* test didn't revealed toxicity for both samples. According to Ruiz *et al.* (1997), the bacteria is affected by different chemicals in different ways. Sometimes the toxicity was greater when the time of exposure was increased; this purpose was considered and a preliminary assay was performed for a 30 min exposure time. Consequently, two scenarios were considered: no toxicity is present; or the toxicity was not detected, this last aspect is related with the relatively low sensitivity of the test, which could limit its utility to evaluate the toxicity of water (Pintar *et al.*, 2003).

Other limitations are related with the interaction between pesticides and others compounds present in water (including metabolites and other pesticides) (Ruíz *et al.*, 1997).

The results from the algal growth tests showed that *P. subcapitata* revealed slight toxicity (72-h) to the both samples, with an EC₅₀= 50% (v/v) to “Dique dos Vinte” marsh sample and EC₅₀= 99% (v/v) to Almonda river sample. Therefore, a LOEC value was found for Almonda river sample (50% inhibition at the 100% (v/v) dilution) considering that only the 100% (v/v) dilution had significant differences in statistical and ecological terms. The summary results are represented in the table 5.10.

The influence of atrazine on the growth of some cyanobacterias was investigated by Solomon *et al.* (1996). Growth was suppressed even at lowest concentrations of atrazine (0.05 ppm). This concludes that atrazine enhances metabolic activity up to a certain concentration while inhibiting growth.

TABLE 5.10 - Summary of the growth inhibition on the *P. subcapitata* for Almonda river and “Alverca do Campo” (“Dique dos Vinte”).

SAMPLES	NOEC %(v/v)	LOEC % (v/v)	EC ₅₀ (LC at 95%) %
Almonda river (AR)	50	100	99 (94 -104)
“Dique dos Vinte”, marsh (DV)	6.25	12.5	50 (44-56)

NOEC – no-observed-effect-level; LOEC - lowest-observed-effect-level;

EC₅₀ – median effective concentration; LC – level of confidence.

The 21-days *D. magna* reproduction test with “Dique dos Vinte” marsh sample a decrease in the number of neonates released was observed at the lowest dilutions. For the Almonda river sample the same expected monotonicity it was not observed; having an increase in the number of neonates in the 12.5% (v/v) dilution relative to the control. This

increase generally is associated with the hormesis effect - low doses of a substance appear to stimulate an apparently beneficial response in the test organism even though larger concentrations lead to a toxic effect (Bailer & Oris, 1998; Calabrese, 2008; Marwood *et al.*, 2001; Parkhurst *et al.*, 1987).

The results from the *Daphnia* reproductive test showed that *Daphnia magna* $EC_{50} > 100\%$ (v/v) to “Dique dos Vinte” marsh sample and $25 < EC_{50} < 50\%$ to Almonda river sample (Table 5.11). The % of mortality for Almonda river sample was 100% for the 50 and 100% (v/v) dilutions, enabling the determination of EC_{50} . The % of mortality in the subsequent dilution 25, 13, was respectively 10 and 0%. This can evidence the proximity of the lethal and sublethal toxicity as the result of the range factor (2) for this test. It is suggested for future tests, with this sample, the use of a lowest range dilutions between the 25 and 50% (v/v), in order to estimate to accurate the EC_{50} value. For the 25% (v/v) dilution it is evidenced a significant difference to the control, although the % of inhibition was 5%, enabling the estimation of LOEC.

TABLE 5.11 - Summary of the chronic toxicity results on *D.magna* for Almonda river and “Alverca do Campo” (“Dique dos Vinte”).

SAMPLES	NOEC (v/v)	LOEC (v/v)	EC50 (LC at 95%)
Almonda river (AR)	25%	>25% and < 50%	> 25% and < 50%
“Dique dos Vinte”, marsh (DV)	< 12.5%	12.5%	>100%

NOEC – no-observed-effect-level; LOEC - lowest-observed-effect-level;
 EC_{50} – median effective concentration; LC – level of confidence

Considering the NOEC’s and LOEC’s values, the effects on reproduction is more severe for the “Dique dos Vinte” marsh sample. These results suggest that Almonda river sample is more toxic when compared to “Dique dos Vinte” marsh sample, without a meaning EC_{50} value.

In a general view, it doesn’t exist a relation between the % of toxic effects for the organisms and the levels of exposure to the pesticides in the samples. However, *P. subcapitata* is the most sensitive of organisms, what could be expected due to the fact that the pesticides dosed by CG-MS in “Dique dos Vinte” marsh and Almonda river samples were all herbicides. The 3,4-dichloroaniline was observed in Almonda river sample at a maximum level of 3.66µg/L (*vide* Table 5.9).

Yet is not possible to justify the fact of “Dique dos Vinte” marsh sample be most toxic to *P.subcapitata* than Almonda river sample, once the greater levels of herbicides that were observed appears in Almonda river sample. However, this could be related with the mode of action of the different pesticides detected.

Toxicity studies involving pesticides mixtures have resulted in a full spectrum of responses in which the complexity of the interactions depends on differences in the chemical properties and modes of toxic action of pesticides (Bailey *et al.*, 2000).

5.3.6. Toxic effects on aquatic organisms in sediments

For *C. riparius* a significant difference in larval growth between the control and 100% of “Dique dos Vinte” marsh sample was found (nested ANOVA: $F_{1, 5} = 48$, $P = 0.001$). Yet, growth was not inhibited but rather stimulated by 46% relatively to the control and no mortality to 100% (m/m) dilution was observed, not being observed toxic effects in the 7-days *C. riparius* growth for “Dique dos Vinte” marsh sediment sample. Attending the highest values for coefficient of variation (CV), the existence of outliers was considered. Once detected, the statically relevant was tested, concluding that the existence of outliers do not have express in the CV values.

According to these results, two premises were considered:

-The possibility of the increase in food availability resulting from nutrients present in sediment sample that can provide more nutritive value of the food and of its palatability, once when compared with the control, the weight in the 12.5% (m/m) dilution was significantly higher suggesting insufficient available food; consequently it was expected that the midge weight were significantly highest in the others dilutions.

-the toxicity existence once the weight significantly lowers (38%) in the 50 and 100 % (m/m) dilution relatively to the 12.5% (m/m) dilution, considering that the available food was similarly for the three dilutions.

A second test was performed to investigate these possibilities. The results of the second test showed effectively the sediment was not toxic, however the same stimulation effect was verified rejecting the premise that quantity of food provide was limitative. Alternatively the presence of nutrients in the sediment sample as a stimulation factor can be considered as a consequence of the behaviour of *C. Riparius* as opportunistic organisms, tolerant to contamination and a high adaptive capacity.

5.3.7. Potential hazard assessment of pesticides in groundwater of the study area

The actual criteria on the potential hazard assessment is based on the concept of toxicity/exposure ratio (TER).

So the potential hazard assessment of pesticides in groundwater to the nontarget organisms, specifically to the algae *P. subcapitata* and the crustacean *D. magna* was based on the comparison between the maximum levels of residues ($\mu\text{g/L}$) detected on groundwater, and the respective EC_{50} and NOEC values for this aquatic organisms, presented in table 5.12.

In a generally way, doesn't exist a relation between the % of toxic effects for the organisms (*D. magna* and *P. subcapitata*) and the levels of exposure to the pesticides in the samples (Table 5.13). This fact can be related with the maximum levels of residues of atrazine alachlor and metolachlor being lower than the respective NOEC and EC_{50} values in literature for the target organisms (Table 5.12).

TABLE 5.12: Comparison between levels of exposure and NOEC and EC_{50} values

Pesticides dosed in Groundwater	Max. Level of concentration (mg/L)	<i>P. subcapitata</i>		<i>D. magna</i>	
		NOEC (mg/L)	EC_{50} (mg/L)	NOEC (mg/L)	EC_{50} (mg/L)
Alachlor	< 0.00005	0.02	0.96	0.22	10
Atrazine	0.00011	0.1	0.59	0.25	85
Metolachlor	0.00013	----	57.1	0.707	23.5

In this way, the toxicity revealed can be related to the joint presence of pesticides (*vide* Table 5.8) and / or others or possibly other compounds present in water.

TABLE 5.13: Comparison between toxic effects on organisms and levels of exposure

SAMPLING DATE	WELL	% imobilization for <i>D. magna</i>	% inhibition for <i>P. subcapitata</i>	Total concentration levels ($\mu\text{g/L}$)
05-Jun	QP1	100	34	≈ 0.16
	QP2	40	14	<0.05
	QP3	100	17	<0.05
27-Jun	QP1	50	32	0.08
	QP2	80	22	0.09
	QP3	80	41	<0.05
22-Jul	QP1	60	58	0.2
	QP2	90	7	0.18
	QP3	90	43	≈ 0.15
13-Aug	QP1	50	0	<0.05
	QP2	90	0	<0.05
	QP3	50	47

Richards & Baker (1993) concluded that the alachlor, metolachlor and atrazine must be present in high concentrations to be acutely toxic to animal species but however lower concentrations are capable to adversely affect aquatic plants and organisms. The toxicity of the samples for *D. magna* and for *P. subcapitata* may be related to the type of mixtures present, whether of pesticides detected or other types of molecules potentially present. In many studies about this subject the main question is: Can the co-occurrence of pesticide toxicity modify the sample? (Bailey *et al.*, 2000; Lydy & Austin, 2004; Norberg-King *et al.*, 1991). Synergistic effects of pesticides mixtures have been noted in aquatic organisms. Faust *et al.* (2003) found concentration "additivity" (the term is used when the effect of the combination of chemicals can be estimated directly from the sum of the concentrations) for binary mixtures of the S-triazine herbicides atrazine and cyanazine in reproduction tests with the green alga *Chlorella fusca*.

6. MITIGATION STRATEGIES TO REDUCE PESTICIDES INPUTS INTO GROUNDWATER

Known the potential effects of the use of pesticides in agriculture, it is important to consider a policy to prevent contamination of groundwater with pesticides. The contamination of water bodies with agricultural pesticides can pose a significant threat to aquatic ecosystems and drinking water resources. However, the risk for the aquatic community or for human health can often be substantially reduced by appropriate measures.

A number of mitigation measures can be used for leaching: application restrictions for vulnerable soils and/or wet climates, reducing the application rate, and shifting the application to an earlier or later date. Also, creating a fine tilth of the topsoil or other tillage operations (e.g. conventional tillage instead of conservation or zero-tillage) to reduce macropore flow are possible measures to decrease leaching. To reduce pesticide leaching through the bulk soil (“matrix”), a possible mitigation measure is increasing the organic matter content of the soil by agronomic practices like incorporation of crop residues, in order to increase sorption of nonionic pesticides. Another option to reduce leaching by matrix flow would be switching to compounds with higher sorption and/or faster degradation (Reichenberger et al., 2007).

The increase of alternative measures alternative to chemical fight and means of managing the environmental performance of pesticides, namely the adequacy of practices for irrigation and application of pesticides that would allow a reduction of the elements contaminants from agricultural sources, taking into consideration the productive structure socio-economic reality of the region, is important and necessary to protect the groundwater resource.

The integrated protection, as an important component of integrated production and sustainable agriculture, in general, reduces the use of chemical pesticides and encourages the use of alternatives, however when the use of pesticides is necessary promotes properly and carefully use.

Should therefore be increased the need to comply with the recommendations and precautions to achieve a responsible pesticides use. The following measures between others should be followed:

- adopt programs of Integrated Pest Management (IPM) in order to minimize the pesticides use;
- store pesticides safely – away from water resources; minimize pesticide store;
- calibrate and maintain equipment properly; check spray patterns at least daily;

- select pesticides carefully: select pesticides with lower groundwater contamination potential, especially in vulnerable areas;
- consider the location of the pesticide application in relation to groundwater and identify the vulnerability of the area (soil type, geology, and climate);
- follow always label directions and precautions;
- apply the pesticides at the appropriate time: make the fewest applications at the lowest rate that will achieve acceptable pest control; delay an application to avoid periods of heavy rain or irrigation;
- install and maintain antibacksiphoning valves on all pumps and water valves – the valves prevent liquids from moving backwards through water lines;
- triple rinse pesticide containers. Crush, then dispose of containers and unused pesticides legally;
- leave buffer zone around sensitive areas.

Proper disposal of containers is an important factor, namely the delivery of them in reception centers. The change in this system delivery for a system more attractive to farmers, either through an economic return for a percentage of the discount when the purchase of pesticides or an eco-points system that would reduce the distances traveled to surrender and avoid the inconvenience of a fixed time, could bring some advantages at this level. The change in packaging when possible by water soluble plastic will also be an alternative with less environmental impact.

In Portugal there are several entities at different levels to protect the environment and human health from potential contamination with pesticides due to the actual Europe exigencies. In D.L. No 173/2005 of 21 October context, which regulates the activities of distribution, sale, provide services for the implementation of plants protection products and its application by end users, are preparing special training courses for the application of plant protection products with high risk, they will start in 2009, and this training must be pre-marketing of products labeled with words of high risk.

In order to better protect the water resource and its dependent ecosystems, Brock *et al.* (2006) provides a proposal to harmonize the different scientific approaches for Ecotoxicology effect assessment adopted in guidance documents that support different legislative directives in E.U. (Water Framework Directive (2006 / 60/EC) and Uniform Principles (91/414/EEC)).

It is important to promote information campaigns directed to farmers, and also temporary economic compensations for farmers for implementing mitigation measures and personal visits at farms by farm adviser visitors.

7. CONCLUSIONS AND FUTURE DEVELOPMENTS

This study intended to be a contribution to the hazard assessment of groundwater in agricultural area with hydrogeological vulnerability, highlighting cases of contamination. Therefore, the various approaches used contributed to a better management and use of pesticides and provided useful tools to technicians and farmers in the region to assist in decision making.

Moreover the need to manage an integrated manner the groundwater and surface water in the area of study was evidenced, and tried to establish a channel (purely for comparison) between exposure of surface water and groundwater in the same area of influence.

Through the evaluation, of the predicted environmental distribution and the potential for leaching of pesticides registered for selected crops, it was found that there are a considerable number of pesticides with high affinity for water and the potential for leaching (see Chap 5). Calculations of the Mackay Fugacity model and the GUS and Bacci & Gaggi leaching indexes, was proved to be very useful in this work as “screening analysis” to provided initial qualitative assessment of contaminant transport into the environment and specially this facility to reach groundwater. It is also relevant the analysis of the potential for leaching of pesticides on their selection, being increased importance to regions such as study, showing high vulnerability hydrogeology (see Chap 5).

In this work was verified that the models used to assess the environmental behavior of pesticides, were useful to identify pesticides with greater potential for groundwater contamination (see Chap 5).

The risk assessment for three different environmental compartments (surface waters, terrestrial hypogean, and epygean systems) through the use of risk indices is also a powerful tool in conjunction with the previous two, being used to obtain information that constitutes a instrument to the technicians and farmers (see Chap 5), in the area and can assist them in decision making, including a careful selection of pesticides to use, taking into account the characteristics of pesticides and agricultural ecosystems.

The inclusion in the analysis of a set of indicators representing pesticide hazards along a number of ecological dimensions is also found to be important for articulate trade-offs in management objectives across different environmental concerns.

The use of different environmental models in this work allowed the selection of pesticides with higher environmental risk. For example, dicamba insecticide, shows a medium to very high risk to the three environmental compartments and a higher potential to reach

groundwater (PED= 100%; GUS > 2.8 and Bacci & Gaggi >0.1). This type of information must be considered as an elementary tool to assist pesticide users in evaluating their choice of pesticide products on the basis of the potential to impact groundwater quality.

The results obtained by applying the methodology GC-MS, confirmed the high vulnerability of the groundwater contamination by pesticides in the areas selected for study and it was observed in 16% of groundwater samples analyzed, the detection of at least one pesticide and / or metabolites exceeding 0.1µg/L, parametric value in water for human consumption (D.L. n° 243/2001). In the surface water samples were observed in 100% of the samples analyzed levels at least one of the compounds greater than 0.1µg/L.

The pesticides detected in groundwater were: alachlor, atrazine and metolachlor, reaching a maximum total concentration level of 0.2 µg/L (see Table 5.8). In surface water the following pesticides were detected: alachlor, atrazine, ethofumesate, terbutylazine, metolachlor and propanil herbicides and 3,4-DCA metabolite, reaching a maximum total concentration of 5.72 µg/L (see Table 5.9).

Another observation that it is possible to cope with the results is a variation of levels assayed over the collect samples of groundwater, as might expected. This dynamic could result from several factors, such as hydrogeological characteristics and cultural practices in the region, as well as the quantities of water used for irrigation and application of pesticides.

The pesticides alachlor, atrazine and metolachlor found in groundwater were also assayed in surface water (reaching a maximum concentration of 0.28, 1.73 and 0.21µg/L respectively), however it is impossible to establish a relationship between the values found in both cases since it would require a deeper understanding of dynamic groundwater-surface water and should to have made the collection of a larger number of samples and in the same sampling periods, which should be chosen according to the dynamics of local water in order to establish a relationship between both types of samples. However, it is clear the pressure and extreme vulnerability of this area with regard to water resources.

Although both atrazine and alachlor have been withdrawn from the market in Portugal, the levels assayed in the waters possibly demonstrate the continued use of the same over the years prior to its withdrawal, considering that, the modeling and monitoring tools are important for better management of factors and the application of pesticides, namely sustainable use of them, this of course in quantitative and qualitative terms, because enables the establishment of viable alternatives.

In this work mixtures of pesticides in 23% of the samples (of groundwater and surface water), had been detected until four substances in mixture. In 33% of them the total level of

residues was greater than 0.5 µg/L (parametric value for mixtures in water for human consumption), had been dosed a maximum total level of approximately 5.72 µg/L in surface water. In the context of mixtures it is noteworthy that the pesticides and metabolites detected in the water samples in the present study represent different chemical classes and consequently unexpected toxic effects. In future these pesticides should be evaluated as a single “toxic substance” when assessed from the perspective of environmental effects.

Based on the dose-response, acute and chronic toxicity for aquatic organisms were evaluated. The various organisms studied (*P. subcapitata*, *D. magna*, *C. riparius* and *V. fischeri*) played an important role in the maintenance and viability of aquatic ecosystems.

So, with regard to the results of toxicity tests, the groundwater samples revealed toxicity to the aquatic organisms. In the total of 24 samplings of groundwater, 83% shows a % of immobilization to *D. magna* $\geq 50\%$ (v/v) and in 30% is observed a inhibition of *P. subcapitata* growth $>50\%$ (v/v).

Of the two surface water samples analyzed the “Alverca da Golegã” (marsh) sample showed higher toxicity than the sample of the Almonda River for the *P. subcapitata* organism (NOEC, LOEC and EC₅₀, 6, 13 and 50% (v / v)) and also for *D. magna* organism based on effect on reproduction.

It’s difficult to relate the effects detected on this species and the pesticides detected; on one hand, because only the detected pesticides and VOC’s were considered; on the other hand, Junghans *et al.* (2006) suggests that in agricultural areas, pesticides at their application peak, discharged to water bodies from several agricultural areas, have shown to transport a cocktail of diverse pesticides. It’s not possible in any of these cases to establish a relation between the levels of pesticides found in waters and this toxicity to the samples. The results showed that probably is occurring punctual and diffuse contamination.

This study highlights the need for a more conscientious management of the factors which determine the contamination of water resources with pesticides, with special focus for those who determine the contamination of groundwater because of their characteristics previously exposed in chapters 1 and 3. It is therefore essential to promote and disseminate the adoption of protective and integrated production systems. Considering it is also important for such a stimulation of a relationship of confidence by farmers to the technicians who help them. The technicians will be responsible for raising the awareness of farmers to alternative means of struggle and if necessary for a more balanced and judicious use of these chemicals, taking into account the characteristics of pesticides and agricultural ecosystems. In this way is also important the technicians continue formation.

Considering the current state of this area, urgent measures to minimize the continuous impact of the strong agricultural activity must be adopted. It is therefore important to have an appropriate agro-environmental management, considering all its components, in particular the use of pesticides and irrigation in order to safeguard and preserve one of the most important global resources, the groundwater.

Future developments

- ✓ Should be increased the need to comply with the recommendations and precautions to achieve a responsible pesticides use. However the effectiveness of mitigation measures is very variable depending on environmental compartments interaction and climate patterns, therefore, future studies are recommended in order to accomplish the mitigation strategies real consequence in the agricultural area of North Vulnerable area of the Tejo.
 - ✓ Enlarging the pesticides detection spectrum by solid phase microextraction (SPME) and gas chromatography coupled to mass spectrometry (GC-MS) to the pesticides that present higher environmental hazard;
 - ✓ It is elemental the development of decision support systems, still waiting to be implemented in Portugal, in particular in the study area – which represents an area of extreme vulnerability – so that the agricultural activity can contribute to the national economic and social development without neglecting the environmental quality preservation.
 - ✓ Understanding the joint action of toxicant mixtures is a great challenge and its necessary to intensify the studies in this area to implement measures to minimize the impact of the combined action of pesticides in the environment. Lydy *et al.* (2004) have attempted to highlight some of the regulatory issues associated with pesticide mixtures. They conclude with a list of what they consider to be the major challenges in working with pesticide mixtures. This does not represent a comprehensive list of challenges, but will hopefully stimulate additional dialogue among scientists:
- ① If two compounds have an interaction, which chemical is to blame? For instance, in the triazine work, atrazine increases the toxicity of chlorpyrifos. Should this be considered in registration decisions for both compounds?

- 2 Is the assumption of additivity protective for most bodies of water, most of the time? What degree of deviation from additivity is important?
- 3 Guidelines in human health assessments recommend combining similarly acting compounds into a single risk cup. What are the criteria for "similar" modes of toxic action?
- 4 What steps are necessary to choose the correct model? Are any of these models appropriate, or do we need a new model that encompasses both techniques?
- 5 We must not only recognize that a mixture has occurred, but we must also understand the dynamics of the chemicals in the system and how temporal variations influence the toxicity of mixtures.
- 6 The large numbers of chemicals and varying exposure routes in the environment make testing each possible exposure scenario unreasonable. Is it possible to assess the mixture effects of a few high-priority mixtures and develop extrapolation models for the remainder using the available data on the mixture components or similar mixtures?
- 7 What is the priority for choosing the chemical mixtures to test? Should we pick those chemicals with high environmental occurrence, such as atrazine, or choose diverse compounds that will help us understand how to model mixture effects?
- 8 For many mixtures that exhibit greater than additive toxicity, the mechanism is a change in toxicokinetics induced by one compound. Based on this information, could we develop screening assays for compounds to identify their potential role in this type of interaction?

The responses to these challenges could be useful to eliminate some uses and practices that could maximize the impact of the pesticides in the environment by its indiscriminate use.

To achieve these challenges, we need to focus on continuous training of technicians, applicators, farmers, vendors and distributors of pesticides, strengthen supervision and promote planning towards sustainable use of these products.

BIBLIOGRAPHICAL REFERENCES

- Adams, W. J., R. A. Kimerle & Mosher, R. G.** (1985) - Aquatic safety assessment of chemicals sorbed to sediments. In R. D. Cardwell, R. Purdy & R. C. Bahner (eds), *Aquatic toxicology and hazard assessment, 7th Symposium, American Society for Testing and Materials*, Philadelphia, ASTM STP 854: 429–453.
- Adema, D. M. M.** (1978) - *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiology*, vol. 59, pp. 125 -134.
- Agrotejo** (2006) – A força de todos nós. AGROTEJO – União Agrícola do Norte do Vale do Tejo, 19p.
- Aller, L., Bennet, T., Lehr, J.H., Petty, R.J., Hackett, G.** (1987) – DRASTIC: a standardized system for evaluating groundwater pollution potential using hydrogenic settings. U.S. E.P.A, Report 600/2-85/018, Ada, OK, USA, 455 p.
- Alpendurada, M.F.** (2000) - Solid-phase microextraction: a promising technique for sample preparation in environmental analysis. *Journal of Chromatography A*, 889: 153-193.
- Amaro, P.** (2003) - A Protecção Integrada. ISA Press, Lisboa, 446p.
- Amaro, S., Azevedo, J., Ribeiro, L.**(2006) - Avaliação da vulnerabilidade de aquíferos cársicos: DRASTIC versus EPIK. Actas do 8º Congresso da água, 13-17 Março, Figueira da Foz, 13p
- ANIPLA** (2008) – O que é a protecção integrada? (<http://www.anipla.com/ppi.html#4>).
- ASTM** (1981) - Manual on the Use of Thermocouples in Temperature Measurement; ASTM Special Technical Publication 470B (American Society for Testing and Materials, Philadelphia).
- ASTM** (2002a) - Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. American Society for Testing and Materials E729–96. Annual Book of ASTM Standards, vol. 11.05. Philadelphia, PA: ASTM.
- ASTM** (2002b) - Test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. American Society for Testing and Materials E 1706–00e2. Annual Book of ASTM Standards, vol. 11.05. Philadelphia, PA: ASTM.
- Bacci, E.** (1994) – Ecotoxicology of organic contaminants. CRC Press/Lewis Publishers. Inc., Boca Raton, Florida, USA, 165 p.
- Bachmant, Y.** (1994) – Groundwater as part of the water system. In: U. Zoller (Ed) – Groundwater Contamination and Control Management. Dekker, Ink, New York, USA: 5-20.
- Bailer, J. & Oris, J.** (1998) - Incorporating hormesis in the routine testing of hazards. *Human & Experimental Toxicology*, Vol. 17, pp 247-250.
- Bailey, H., Deanovic, L., Reyes, E., Kimball, T., Larson, K., Cortright, K., Connor, V., Hinton, D.** (2000) - Diazinon and chlorpyrifos in urban waterways in northern California, USA. *Environmental Toxicology and Chemistry* 19: 82-87.
- Barbash, J.E.Q., Resek, E.A.** (1997) – Pesticides in Groundwater. Distribution, trends, and governing factors. CRC Press LLC, Boca Raton, USA, 590p.

- Barceló, D. & Hennion, M.C.** (1997) – Trace determination of pesticides and their degradation products in water. *Elsevier*, Amsterdam, The Netherlands, 542 p.
- Barceló, D.** (2000) – Sample handling and analysis of pesticides and their transformation products in water matrices by liquid chromatographic techniques. In: D. Barceló (Ed.) – Sample handling and trace analysis of pollutants. Techniques, applications and quality assurance. Elsevier Science B.V., Amsterdam, The Netherlands: 155-207.
- Barros, M.C.** (1996) – Pesticidas e Ambiente – Uma perspectiva histórica. Actas Simpósio Protecção das Plantas, Agricultura e Ambiente, EAN, Oeiras, 9-10 Maio 1996: 161-174.
- Barros, R.** (2005) – Avaliação da exposição e dos efeitos tóxicos de pesticidas para o biota aquático em águas superficiais e sedimentos da Zona Aluvionar Norte do Tejo. Relatório de fim de curso de Engenharia Agronómica, UTL, ISA, Lisboa, 49 p.
- Bat, L., Akbulut, M.** (1999) – Studies on Sediment Toxicity Bioassays Using *Chironomus Thumm.* K, 1991 Larval Turk. *J. Zoo*, vol 25: 87-93.
- Batista, S.** (2003) – *Exposição da água subterrânea a pesticidas e nitratos em ecossistemas agrícolas do Ribatejo e Oeste e da Beira Litoral.* Diss. Dout., ISA, 464p.
- Batista, S., Cerejeira, M. J., Silva, E., Viana, P., Serôdio, L.** (2000a) – Contaminação de águas subterrâneas no Ribatejo e Oeste e no Centro Litoral com pesticidas e nitratos. Actas 5º Cong. Água, Lisboa, Set. 2000:25-29, 12p.
- Batista, S., Silva, E. & Cerejeira, M.J.** (2006a) – Minimização da contaminação da água subterrânea através do uso sustentável de pesticidas. *Rev. Ciênc. Agr.*, XXIX: 45-57.
- Batista, S., Silva, E. & Cerejeira, M.J.** (2006b) – Exposição da água subterrânea a pesticidas e nitratos em diversos ecossistemas agrícolas do Ribatejo e Oeste e da Beira Litoral (1996-2000). *Rev. Ciênc. Agr.*, XXIX: 102-116.
- Batista, S., Silva, E. & Cerejeira, M.J.** (2007) – Contaminação difusa e pontual da água subterrânea com pesticidas no Ribatejo e Oeste e na Beira Litoral. *Recursos Hídricos*, 28 (1): 53-68.
- Batista, S., Silva, E., Cerejeira, M.J. & Silva-Fernandes, A.** (2001) - Exposure of ground water to alachlor, atrazine and metolachlor in maize areas of Ribatejo and Oeste (Portugal). *Toxicological and Environmental Chemistry*, 79: 223-232.
- Batista, S., Silva, E., Galhardo, S., Viana, P. & Cerejeira, M.J.** (2002) – Evaluation of ground water exposure to herbicides and insecticides in two agricultural areas of Portugal. *International Journal of Environmental Analytical Chemistry*, 82 (8-9): 601-609.
- Batista, S., Viana, P. & Cerejeira, M.J.** (2000b) – Exposição de águas subterrâneas a pesticidas e nitratos (1998-2000). Relatório Final do Projecto DGA/ISA, SAPI, DPPF, ISA, UTL, Lisboa, 93p.
- Bauld, J.** (1994) – Behavior of natural and anthropogenic contaminants in groundwater. In: Zoller, U. (Ed.) – Groundwater Contamination and Control, Marcel Dekker, Inc cap. 3, 37 - 56.

- Beltran, J., López, F. J., Hernández, F.** (2000) – Solid - phase microextraction in pesticide residues analysis. *Journal of Chromatography A*, 885: 389-40.
- BIAC** (2004) – The Sustainable Agriculture Challenge. Comité Consultatif Economique et Industriel Auprés L' OECD, 3p.
- Blaise, C., Vasseur, P.** (2000) - Small-scale Freshwater Toxicity Investigations. *Springer*, Vol I: 137-179.
- Boluda, R., Quintanilla, J.F., Banilla, J.A., Sáez, E., Gamón, M.** (2002) – *Chemosphere* 46:335-369.
- Brock, C.M., Arts, G.H.P., Maltby, L., van den Brink, P.J.** (2006) - Aquatic Risks of Pesticides, Ecological Protection Goals, and Common Aims in European Union Legislation. *Integrated Environmental Assessment and Management*, Volume 2, Issue 4, pp. e20–e46.
- Brown, L.S., Lean, D.R.S.** (1994) - Toxicity of selected pesticides to lake phytoplankton measured using photosynthetic inhibition compared to maximal uptake rates of phosphate and ammonium. *Environmental Toxicology and Chemistry*, Volume 14, Issue 1, pp. 93–98.
- Burton, S.G.A., Leibfritz, S.** (1990) - Effect of sediment test variables on selenium toxicity to *daphnia magna*. *Environmental Toxicology and Chemistry*, Volume 9, Issue 3, pp. 381–389.
- Calabresse, E.J.** (2008) - Hormesis: Why it is Important to Toxicology and Toxicologists. *Environmental Toxicology and Chemistry*, Volume preprint, Issue 2008, pp. 0000–0006.
- Calamari, D. & Vighi, M.** (1987). Quantitative structure activity relationships in ecotoxicology value and limitations. Institute of Agricultural Entomology, University of Milan, Milan, Italy. 106p.
- Calamari, D. & Vighi, M.** (1991) – Scientific bases for the assessment of toxic potential of several chemical substances in combination at low level. Final support. Institute of Agricultural Entomology, University of Milan, Milan, 76p.
- Carvalho, P.M.S.** (2005) – Vulnerabilidade, monitorização e risco na Zona Aluvionar do Tejo. Relatório de Final de Curso, UTL, IST, Lisboa, 91 p.
- Casarett, L.J., Doull, J.** (2007) - Toxicology: the basic science of poisons. Publisher: Macmillan, 1280p.
- CEC** (1992) – Pesticides in ground and drinking water. Water Pollution Research report 27, 135pp.
- Cerejeira, M.J.** (1993) – Estudo da distribuição e destino final dos pesticidas no ambiente numa abordagem integrada. Caso da atrazina na Zona Agrária da Chamusca. Tese de Doutoramento em Engenharia Agronómica, ISA, Lisboa, 222 p.
- Cerejeira, M.J., Silva-Fernandes, A.M., Bacci, E. & Matos, J.** (1995a) – Atrazine and nitrates in the drinking ground water of the Chamusca agricultural area (Portugal). *Toxicological and Environmental Chemistry*, 49:153-160.
- Cerejeira, M.J., Silva-Fernandes, A.M., Viana, P. & Bacci, E.** (1995b) – Atrazine and nitrates levels in the ground water of irrigation wells in the agricultural area of Chamusca (Portugal). *Toxicological and Environmental Chemistry*, 49:123-128.

Cerejeira, M.J., Batista, S., Moura, M. & Silva-Fernandes, A.M. (1997) – Metodologias imunoenzimáticas versus cromatografia gasosa no doseamento de atrazina em águas subterrâneas. *Rev. Ciênc. Agr.*, XX (4): 29-36.

Cerejeira, M.J., Batista, S., Silva, E., Viana, P., Centeno, M. & Silva-Fernandes, A.M. (1998a) – Influência da irrigação na dinâmica de agroquímicos no solo e águas subterrâneas no Ribatejo (Portugal). *Actas VIII SILUBESA*, João Pessoa, Brasil, 26-30 Abril 1998, Vol. II, 648p.

Cerejeira, M.J., Pereira, T., Silva - Fernandes, A. (1998b) - Use of new microbiotests with *Daphnia magna* and *Selenastrum capricornutum* immobilized forms. *Chemosphere*, vol 37,nº 14-15,pp 2949-2955.

Cerejeira, M.J., Batista, S., Silva, E., Viana, P., Centeno, M.S., Silva-Fernandes, A.M. (1999a) – Avaliação do impacte da aplicação de pesticidas na qualidade da água subterrânea de ecossistemas agrícolas do país de 1989 a 1999. *Actas 6ª Conf. Nac. Qual. Ambiente*, Lisboa, Out. 1999, 2: 29-48.

Cerejeira, M.J., Batista, S., Silva, E., Viana, P., Centeno, M.S., Silva-Fernandes, A.M. (1999b) – Ocorrência e dinâmica de pesticidas na água subterrânea de áreas de milho hortícolas, vinha e fruteira do Ribatejo e Oeste. *Actas 5º Enc. Nac. Protec. Integ.*, Bragança, Out., 1999, pp. 448-456.

Cerejeira, M.J., Batista, S., Viana, P. & Silva-Fernandes, A. (2000a) – Dinâmica de agroquímicos no solo e água subterrânea do Ribatejo. *Revista de ciências agrárias*, volume XXIII, 1:30-37.

Cerejeira, M.J., Silva, E., Batista, S., Trancoso, A., Centeno, M.S.L. & Silva-Fernandes, A.M. (2000b) – Simazine, metribuzine and nitrates in ground water of agricultural areas of Portugal. *Toxicological and Environmental Chemistry*, 75: 245-253.

Cerejeira, M.J., Viana, P., Batista, S., Pereira, T., Silva, E., Valério, M.J., Silva, A.M., Ferreira, M. & Silva-Fernandes, A.M. (2003) – Pesticides in Portuguese surface and ground waters. *Water Research*, 37: 1055-1063.

Cerejeira, M.J., Batista, S., Silva, E., Pereira, T. & Caetano, L. (2005) – Acção 19 do Projecto AGRO-DE&D 103 – Estudos ecotoxicológicos. In: A. Lima (Coord.) – *Relatório Final do Projecto AGRO-DE&D 103 “Melhoria das técnicas de produção de arroz com redução de impactes ambientais no Baixo Mondego e Vale do Tejo”*, DPPF, ISA, UTL, Lisboa,50p.

Cisniega M.G., Segovia, N. & López, N. (2002) - Methodology for volatile organic compounds determination in water from wells and springs in Mexico State. *Geofísica Internacional*, Vol. 41, 3: 233-237.

Cohen, S.Z. (1990) – pesticides in groundwater. An overview. In:DH. Hutson & T.R. Roberts (Ed) – *Environmental fate of pesticides*. John Wiley & Sons Ltd, Chichester, UK: 13-25.

Decreto-Lei n.º 47 802 de 19 de Julho (1967) – Estabelece o regime de comercialização dos produtos fitofarmacêuticos destinados à defesa da produção vegetal, com exclusão dos adubos químicos e dos correctivos agrícolas. *Diário da República I Série*, nº 167 de 19/07/1967: 1369-1372.

Decreto-Lei n.º 284/94 de 11 de Novembro (1994) – Transpõe para a ordem jurídica interna a Directiva 91/414/CEE, do Conselho, de 15 de Julho, relativa à colocação dos produtos

fitofarmacêuticos no mercado. *Diário da Republica I Série-A*, nº261 de 11/11/1994: 6809-6810.

Decreto-Lei nº 173/2005 de 21 de Outubro (2005) - regula a distribuição, venda, prestação de serviços de aplicação de produtos fitofarmacêuticos e sua aplicação pelos utilizadores finais. *Diário da república série A*, nº 203, 21/10/2005: 2395 -2396.

Decreto-Lei nº 243/2001 de 5 de Setembro (2001) – Aprova normas relativas à qualidade da água destinada ao consumo humano transpondo para o direito interno a Directiva nº 98/83/CE, do Concelho, de 3 de Novembro de 1998, relativa à qualidade da água destinada ao consumo humano. *Diário da República I Série-A*, nº 206 de 05/09/2001: 3676-3722.

Dietz, C., Sanz, J., Cámara, C. (2006) – Recent developments in solid-phase microextraction. Coatings and related techniques. *Journal of Chromotography A*, 1103: 183-192.

DGADR (2008) – Condições de utilização, DGADR. (<http://www.dgadr.min-agricultura.pt/>)

Dunnivant, F. & Anders, E. (2006) - A Basic Introduction to Pollutant Fate and Transport: An Integrated Approach with Chemistry, Modeling, Risk Assessment, and Environmental Legislation. Wiley-Interscience, 504 p.

Dornefield. C. (2006) - Utilização de *Chironomus* sp (Diptera, Chironomidae) para a avaliação da qualidade de sedimento e contaminação por metais, Tese de Doutoramento em Engenharia Ambiental, EESC, USP, São Carlos – SP, 239: 18 - 56 (<http://www.dominiopublico.gov.br.>)

DPI (2007) – The thinking behind our everyday essentials. Agrochemical risk prediction and assessment: a review of models and tools. 10p. ([http://www.dpi.vic.gov.au/dpi/vro/vrosite.nsf/pages/lwm_farmwater_agrochemical_pdf/\\$FILE/](http://www.dpi.vic.gov.au/dpi/vro/vrosite.nsf/pages/lwm_farmwater_agrochemical_pdf/$FILE/))

EC (1998) – Directive 98/83/CE of Council, of November 3, 1998, relative to the quality of water for human consumption. J. Ofic. nº L 330 de 05/12/1998: 32-54.

EC (2000) – Directive 2000/60/CE of the European Parliament and the Council, of October 23, 2000, which established a framework of action in the field of water policy and includes a list of priority substances. J. Ofic. nº L 327 of 22/12/2000: 1-72.

EC (2001) - Decision No 2455/2001/EC of the European Parliament and of the Council, of November 20, 2001, which establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. J. Ofic. nº L 331 of 15/12/2001: 1-5.

EC (2005) - Regulation (EC) NO 396/2005 of the European Parliament and the Council, of 23 February, 2005, on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. J. Ofic. nº L 70 of 16/03/2005: 1-16.

EC (2006) - Directive 2006/118/CE of the European Parliament and the Council, of December 12, 2006, relative to the protection of groundwater against pollution and deterioration. J. Ofic. nº L 372 of 27/12/2006: 19-31.

EEC (1980) – Directive 80/778/CEE of Council, of July 15, 1980, relative to the quality of water for human consumption. J. Ofic. nº L 229 of 30/08/1980: 11-29.

EEC (1989) - Algal growth inhibition test. EEC Directive 79/831/EEC, Annex V, Part C: Methods for the Determination of Ecotoxicity. Brussels: EEC (European Economic Community).

EEC (1991) – Directive 91/414/CEE of Council, of July 15, 1991, relating to market regulation of the protection plants. J. Ofic. n° L 230 of 19/08/1991: 1-31.

Eisert, R., Levsen, K. (1996)- Solid-phase microextraction coupled to gas chromatography a new method for the analysis of organic in water. *Journal of Chromatography A*, 773: 143-157.

EN ISO 6431 (1996) - Water quality – determination of the inhibition of mobility of *Daphnia magna* straus (Cladocera, Crustacea) – Acute toxicity test. CEN. Brussels.

Environmental Canada (1992a) – Biological test method: toxicity test using luminescent bacteria. Report EPS 1/RM/24. Environmental Canada, catalogue n° EN 49 – 24/24E, 75p.

Environmental Canada (1992b) – Biological test method: growth inhibition test using freshwater Alga *Selenastrum capricornutum*. Environmental Canada, Report EPS 1/RM/25, n° EN49-24/1-25E, 65 p.

Environmental Canada (1997) – Biological test method: test for survival and growth in sediment using the larvae of freshwater midges (*Chironomus tentans* or *chironomus riparius*). Environmental Canada, Report EPS 1/RM/32, n° EN49-24/1-32E, 150 p.

EPA (1993) - Methods for aquatic toxicity identification evaluations: Phase II Toxicity identification procedures for samples exhibiting acute and chronic toxicity.

EPA (1998) – Registration Eligibility Decision (RED). Alachlor. United States Environmental Protection Agency. EPA 738-R-98-020, 338 p.

EPA (2002) – Short term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. United States Environmental Protection Agency.

EPA (2006) – Pesticide field programs. Ensuring Protection through Partnerships. U.S. Environmental Protection Agency, 19 p.

European Commission (2006) – 3, 4 – Dichlozoaniline (3,4 – DCA) Summary Risk Assessment Report. Institute for Health and Consumer Protection. European Chemicals Bureau, 26p ([http:// www.europa.eu.int](http://www.europa.eu.int)).

FAO (2004) - The Ethics of Sustainable Agriculture intensification. FAO Ethic Series, 37 p (www.fao.org/ethics/ser_en.htm)

FAO (2006) - FAO, 2006 livestock report. Food and agriculture organization of the united nations. Rome. (<http://www.fao.org/docrep/009/a0255e/a0255e00.HTM>)

Faust, M., Attenburger, R., Backhaus, T., Blanck, H., Boedecker, W., Gramatica, P., Hamer, V., Scholze, M., Vighi, M., Grimme, L.H. (2003) – Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. *Aquatic toxicology*, 63: 43 – 63.

Feenstra, G. (1997) – What is sustainable agriculture, UC Sustainable Agriculture Research and Education Program, University of California, Davis, CA 95616, (916) 752- 756.

- Finizio, A., Calliera, M., Vighi, M.** (2000) – Rating Systems for Pesticides Risk Classification on different Ecosystems. *Ecotoxicology and Environmental Safety*, vol. 49, pp 262- 274.
- Funari, E., Donati, L., Sandroni, D. & Vighi, M.** (1995) – Pesticide levels in groundwater. Value and limitations of monitoring. In: M.Vighi & E. Funari (Ed.) *Pesticide risk in groundwater*. CRC Press, Boca Raton, USA, p. 3 - 44.
- Focazio, M. J., Reilly, T. E., Rupert, M. G., Helsel, D.R.** (2003) – Assessing Ground-water Vulnerability to contamination: Providing Scientifically Defensible Information for Decision Makers. U.S. Geological Survey, Circular 1224, 33p. (<http://www.pubs.usgs.gov/circ/2002/circ1224/>).
- Gaynor, J.D., Mactarish, D.C. & Findlay, W.I.** (1995) – Atrazine and metolachlor loss in surface and subsurface runoff from three tillage treatments in corn. *J. Environ. Qual.* 24: 246 – 256.
- Gustafson, D.I.** (1989) – Ground water ubiquity score: a simple method for assessing pesticide leachability. *Environ. Toxicol. Chem.*, 8(4):339-357.
- Grote, C., Levsen, K. & Wünsch, G.** (1999) – An automatic analyzer for organic compounds in water based on solid-phase microextraction coupled to gas chromatography. *Anal. Chem.*, 71(20): 4515-4518.
- Hock, W. K. & Brown, C.L.** (1999) – Toxicity and potential health effects of pesticides. Pennsylvania State University, PA, USA. Agrichemical fact shet n° 7, 7p (<http://www.pested.psu.edu/fact7.htm>).
- Hoffman, D. J., Rattner, B. A., Jr. Burton, G. A., Jr. Cairns, J.** (2003) - Handbook of Ecotoxicology. Second Edition. Lewis Publishers, 1290p.
- Hond, F.D., Groenewegen, P., van Straalen, N.M.** (2003) – Pesticides. Problems, Improvements, Alternatives. Blackwell Publishing, 252 p.
- Hornsby, A. G., Wauchope, R.D. & Herner, A. E.** (1996) – Pesticide Proprieties in the environment. Springer-Verlag, New York, USA, 227p.
- INAG** (1999) – Plano de Bacia Hidrográfica do rio Tejo-Análise e diagnóstico da situação de referência, Ministério do Ambiente e Ordenamento, vol.I, Lisboa, 168 p.
- INAG** (2001) – Plano Nacional da Água – Introdução, caracterização e diagnóstico da situação actual dos recursos hídricos. INAG, Lisboa, vol. 1, 542p.
- INE** (2001) – Recenseamento Geral da Agricultura 1999 – Ribatejo e Oeste. Principais resultados. INE, Lisboa, 218p.
- ISO /DIS 8692, 2002** - Water quality – Fresh water algal growth inhibition test with unicelular green algae.
- Junghans, M., Faust, M., Scholze, M., Grimme, L.H.** (2006) - Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures Elsevier. *Aquatic Toxicology*, 76: 93–110.
- Kamwar, R. S** (1996) – Agrochemicals and water management In: L.S.Pereira, R.A. Fedals, J.R. Gilley & B. Lesaffre (Ed.) – Sustainability of irrigated agriculture, Nato Asi series, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 373-393.

- Konstantinou, L.K., Lambropoulou, D.A., Hela, D.G., Albanis, T.A.** (2005) - Environmental monitoring and ecological risk assessment for pesticide contamination and effects in Lake Pamvotis, northwestern Greece. *Environmental Toxicology and Chemistry*, Volume 24, Issue 6, pp. 1548–1556.
- Leitão, T.E.** (1997) – Metodologias para a reabilitação de aquíferos poluídos. Diss. Dout., FCUL, Lisboa, 493 p.
- Lydy, M., Belden, J., Wheelock, C., Hammock, B., Denton, D.** (2004) – Challenges in Regulating Pesticide Mixture. *Ecology and Society* 9: 6p. (<http://www.ecologyandsociety.org/vol9/iss6/art1/>).
- Lydy, M. J. & Austin, K. R.** (2004) – Assessment of pesticide mixtures from the Sacramento – san Joaquin delta using *Chironomus tentans*. *Environmental Contamination and toxicology*, 46: 343 – 349.
- Lobo-Ferreira, J.P.C. & Oliveira. M.M.** (1995) – Síntese da caracterização e do mapeamento das águas subterrâneas de Portugal. In: Lobo-Ferreira, J.P.C. & Ciabatti, P. (eds) – Desenvolvimento de um inventário das águas subterrâneas de Portugal. LNEC, Lisboa, Vol.1:1363-1406.
- Lobo-Ferreira, J.P.C. & Oliveira. M.M.** (2003) - Análise de sensibilidade da aplicação de métodos indexados de avaliação da vulnerabilidade à poluição de águas subterrâneas. Jornadas Luso-Espanholas sobre Águas Subterrâneas no Sul da Península Ibérica Faro, 23 a 27 de Junho de 2003, 10 p.
- Lord, H. & Pawliszyn, J.** (2000) - Evolution of solid-phase microextraction technology. *Journal of Chromatography A*, 885 : 153–193.
- Ma, L. & Selim, H.M.** (1994) - Predicting atrazine transport in soils: second-order and multireaction approaches. *Water Resour. Res.* 30:3489-3498.
- Mackay, D. & Paterson, S.** (1981) - Calculating Fugacity. *Enviro.Sci.Technol.*,15: 1006-1014.
- Mackay, D.** (2001) – Multimedia environmental model: the fugacity approach. 2nd edition. Lewis Publishers, MI.
- Mackay, D. Sni, W. & Ma, K.** (1997) – Illustrated handbook of physical- chemicals proprieties and environmental fate for organic chemicals. Volume V. Pesticide chemicals, CRC Press LLC, Boca Raton, Florida, USA, 812 p.
- MADRP** (Ministério do desenvolvimento rural e pescas). 2008 - A Directiva 2006/118/CE relativa à protecção das águas subterrâneas contra a poluição e a deterioração. Suas implicações na agricultura (<http://portal.min-agricultura.pt/portal/page/portal/MADRP/PT>).
- Mandl, V., Costa, J.S & Tunney, H.** (1994) – Groundwater quality: criteria and standards. In: U. Zoller (Ed) – Groundwater contamination and control, Marcel Dekker, Ink. New York, USA: 87-95.
- Marwood, C.A., Solomon, K.R., Greenberg, B.M.** (2001) - Chlorophyll fluorescence as a bioindicator of effects on growth in aquatic macrophytes from mixtures of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry*, Volume 20, Issue 4, pp. 890–898.
- Mendes, M. P., Ribeiro, L., Paralta, E., Batista, S., Silva, E., Cerejeira, M.J., Leão de Sousa, P.** (2006) – Vulnerabilidade, monitorização e risco na zona vulnerável do Tejo. Actas

8º Congresso da Água “Água, sede de sustentabilidade”, Figueira da Foz, 13-17 de Março de 2006, 15p.

Mendes, M.P., Ribeiro, L., Paralta, E., Batista, S., Silva, E., Cerejeira, M.J. E Leão de Sousa, P. (2008) - Vulnerabilidade, monitorização e risco na Zona Vulnerável do Tejo". *Revista de Ciências Agrárias*, Vol. XXXI (1): 89-91.

Monteiro J. P. Silva J. M, Guerreiro P., Martins J., Reis E. (2007) - modelação de relações entre águas superficiais e superficiais nos aquíferos do Algarve central. Actas do “Seminário sobre águas superficiais 2007”, 8 p.

Moura, M. (1996) – Níveis de Atrazina na água subterrânea numa zona agrícola do Ribatejo. Relatório final de curso Engenharia Agronómica, ISA, UTL, Lisboa, 92 p.

Norberg – King, T., Mount, D., Amato, J., Jensen, D., Thompson, J. (1992) – Toxicity identification evaluation; characterization of chronically toxic effluents, phase I. EPA/600/6 - 91/005. U.S. Environmental Protection Agency. Washington, D.C., USA.

OECD (1984). *Algal growth inhibition test*. OECD Guidelines for Testing of Chemicals vol. 201. Organization for Economic Cooperation and Development Paris: OECD

OECD (1998a) - *Report of the OECD workshop on statistical analysis of aquatic toxicity data*. OECD Series on Testing and Assessment vol. 10. Organization for Economic Cooperation and Development, Paris: OECD. 549 p.

OECD (1998b) - *Daphnia magna reproduction test*. OECD Guidelines for Testing of Chemicals vol. 211. Organization for Economic Cooperation and Development Paris: OECD

OECD (1998d) – OECD series on testing and assessment number 10. Report of the OECD workshop on statistical . Analysis of Aquatic toxicity data. Organization for Economic Cooperation and Development. ENV/MC/CHEM, (98)18, 133p.

OECD (1999) – Environmental Indicators for Agricultura. Vol 2. Issues and design. “The York Workshop”, Organization for Economic Cooperation and Development, 216p. (<http://www.oecd.org/dataoecd/24/36/40680860.pdf>).

OECD (2004) - Sediment-water Chironomid toxicity test using Spiked Water. OECD guidelines for the testing of chemicals. Organization for Economic Cooperation and Development, 219, 21p.

Paralta, E.A., Oliveira, M. M., Batista, S., Francês, A., Ribeiro, L.F. & Cerejeira, M.J. (2001) – Aplicação de SIG na avaliação da vulnerabilidade aquífera e cartografia da contaminação agrícola por pesticidas e nitratos na Região do Ribatejo. Actas Seminário “A hidroinformática em Portugal”, LNEC, Lisboa, 15-16 Novembro 2001, 16 p.

Parkhurst, D.F., SUTER II, G.W., ROSEN, A.E., LINDER, E.(1987)- endpoints for responses of fish to chronic toxic exposures. *Environmental Toxicology and Chemistry*, vol 6, pp.739-809.

Pandey, G. & Singh, K.P. (1990) - Effect of organic amendments on soil microflora and nematode fauna with special reference to Meloidogyne incognita in chick pea. *New Agriculturist.*, 1 : 65-70.

Pereira, T. (2003) – *Impacte da utilização de pesticidas em ecossistemas orizícolas sobre a qualidade de águas superficiais*. Diss. Dout., ISA, UTL, Lisboa, 394p.

- Pereira, T., Brito, F., Viana, P. & Cerejeira, M.J.** (2000) – *Exposição e efeitos de pesticidas em águas superficiais ecossistemas orizícolas (1998-2000)*. Relatório Final do Projecto DGA/ISA, SAPI, DPPF, ISA, UTL, Lisboa, 175p.
- Pereira, T., Cerejeira, M.J., Viana, P. & Serôdio, L.** (2007) – Pesticidas aplicados na cultura de arroz do Baixo Sado e sua influência na qualidade da água do rio Sado. *Recursos Hídricos*, **28** (1): 69-77.
- Pintar, A., Besson, M., Gallezot, P., Gibert, J., Martin, D.** (2003) - Toxicity to *Daphnia magna* and *Vibrio fischeri* of Kraft bleach plant effluents treated by catalytic wet-air oxidation. [Water Research](#), Vol. 38, pp. 289 - 300.
- Portaria nº 563/95** de 12 de Junho (1995) – Relativa à fiscalização de produtos fitomacêuticos. Diário da República I Série, nº 4365 de 12/06/1995.
- Portaria n.º 1100/2004** de 03 de Setembro (2004) – Aprova as listas das zonas vulneráveis e as cartas das zonas vulneráveis do território português. Diário da República I Série B. n.º 208 de 03/09/2004.
- Pretty, J. N.** (2005) – The pesticides DeTox: Towards a More Sustainable Agriculture. Earthscon Ed., 294p, 1 -23.
- Prosen, H. & Kralj, L.** (1999) – Solid-phase microextraction. *Trends Anal. Chem.*, 18 (4): 272-282.
- Rao, P.S.C., Hornsby, A.G. & Jessup, R.E.** (1985) – Indices for ranking the potential for pesticide contamination of groundwater. *Proc. Soil Crop Sci. Soc. Fla*, 44: 1-8. (cit Bacci, 1994).
- Rand, G.M. & Petrocelli, S.R.** (1985)-Introduction. In: Rand, G.M. , Petrocelli, S.R. (Ed.)- *Fundamentals of Aquatic Toxicology*. Hemisphere Publ. Coop., Book Comp., London, 1-28.
- Rei, A.F.** (2005) – Impacto do uso de pesticidas em águas subterrâneas na Zona Aluvionar Norte do Tejo. Relatório Final de Engenharia Agronómica, ISA, Lisboa, 51p.
- Ribeiro, L.** (2000) – IS – um novo índice de susceptibilidade de aquíferos à contaminação agrícola. Nota interna, ERSHA-CVRM, Lisboa, 12 p.
- Ribeiro, L.** (2001) – Vulnerabilidade de aquíferos e medidas de protecção das águas subterrâneas em Portugal Continental. Actas Seminário sobre Geotecnia Ambiental: Contaminação de solos e de águas subterrâneas, Porto, 18 e 19 Outubro 2001: 3-31.
- Ribeiro, L.** (2004) – “ Vulnerabilidade de aquíferos, conceitos e métodos, Lição Síntese”.
- Ribeiro, L.** (2005) – Um novo índice de vulnerabilidade específico de aquíferos – formulação e aplicações. VII SILUSBA, Évora, 30 Maio – 2 Junho de 2005 (in press).
- Richards, R. P., Baker D. B.** (1993) – Pesticide concentrations patterns in agricultural drainage networks in the lake Erie Basin. *Environment toxicology and chemistry*, vol 12: 13-26.
- Reichenberger, S., Bach, M., Skitschak, A., Frede, H-G.** (2007) - Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. *Science of the Total Environment* 384 1–35.

Ruíz, M. J., López-Jaramilho, Redondo, M. J, Font, G. (1997) – Toxicity assessment of pesticides using Microtox test: application to Environmental samples. *Bull, Environment contamination toxicology*, 59:619-625.

Salmeron, V.,Martinez – Toledo, M. V.,Gonzalez – Lopez, J. (1991) – Effects of Alachlor and Metolachlor on the biological activity of *Azospirillum brasilense* grown in chemically defined and dialyzed – soil media. *Environment toxicology and chemistry*. Vol 10: 493 – 499.

Sanches – Camazano, M., Lorenzo, L. F., Sanches – Martin. M. J. (2005) – Atrazine and Alachlor inputs to surface and ground waters in irrigated corn cultivation areas of Castilha – Leon region, *Spain Environmental Monitoring and Assessment*, 105: 11-24.

Santos, T.C.R., Rocha, J.C., Alsonso, R.M., Martinez, E., Ibanez, C., Barceló, D. (1998) - Rapid degradation of propanil in rice crop fields. *Environmental Science and Technology* 32:3479–3484.

Silva, E., Batista, S., Viana, P., Antunes, P., Serôdio, L., Cardoso, A.T. & Cerejeira, M.J. (2006) – Pesticides and nitrates in groundwater from oriziculture areas of the ‘Baixo Sado’ (Portugal). *Intern. J. Environ. Anal. Chem.*, Vol. 86 (13): 955-972.

Silva, E., Batista, S., Viana, P., Antunes, P, Serôdio, L, Cardoso, A.T. & Cerejeira, M.J. (2005) – Avaliação da exposição de amostras de água subterrânea a pesticidas e nitratos. Uso de pesticidas em arrozais numa perspectiva de agricultura sustentada, Projecto AGRO 24, Lisboa, 333:208-272.

Silva, E., Batista, S., Caetano, L. & Cerejeira, M.J. (2008) - Ecotoxicological studies in vineyard freshwaters towards a sustainable use of pesticides. *1.ª Conferência da Tapada, Feira de Ciência e de Tecnologia, Pavilhão de Exposições, no Instituto Superior de Agronomia, 29 e 30 de Outubro de 2008*, poster.

Silva, E., Caetano, L., Batista, S. & Cerejeira, M.J. (2009) – Pesticidas em águas subterrâneas e superficiais no Norte da Zona Aluvionar do Tejo. *In Plano de intervenção e desenvolvimento de um Sistema de Apoio à Decisão para o Norte da Zona Aluvionar do Tejo* (ed. Leão de Sousa, P., Ribeiro, L., Cerejeira, M.J. & Marçal, R.), ISA, UTL, Lisboa.

Silva-Fernandes, A.M. (2002) – Classificação química de produtos fitofarmacêuticos. 10ª actualização. SAPI, DPPF, ISA, UTL, Lisboa, 26p.

Silva-Fernandes, A.M., Cerejeira, M.J., Curto, M.J.M & Centeno, M.S.L. (1999) – *avaliação do efeito poluente dos agroquímicos em águas subterrâneas do ribatejo e oeste*. relatório final do projecto pamaf-ied nº4024, sapi, dppf, isa, utl, lisboa, 123p.

Silva-Fernandes, A.M., Cerejeira, M.J., Pereira, T., Batista, S., Silva, E., Caetano, L. et al. (2005) – *Relatório Final do Projecto AGRO-DE&D 24 “Uso de pesticidas em arrozais numa perspectiva de agricultura sustentada”*, SAPI, DPPF, ISA, UTL, Lisboa, 333p.

Smith, C.J. (1990) – Hydrogeology with respect to underground contamination. In: D.H. Hutson & T.R. Roberts (Ed.) – *Environmental fate of pesticides*. John Wiley & sons, Chichester, UK: 48-99.

Solomon, W.K.R., Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, J., la Point, T.W., Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Giesy, J.P., Hall JR, L.W., Williams, W.M.(1996)- ecological risk assessment of atrazine in north american surface. *Environmental Toxicology and Chemistry*, Vol 15, pp. 31-76.

Somasundaram, L., Coats, J.R., Racke, K. D. & Shanbhag, V.M. (1991). – Mobility of pesticides and their hydrolysis metabolites in the soil. *Environ. Toxicol. Chem.* 10:185 – 194.

SOP (Standard Operational Procedure) (1996a) – Daphtoxkit F magna, Crustacean toxicity screening test for freshwater, Creasel, Deinze, Belgium, 16p.

SOP (Standard Operational Procedure) (1996b) – Algaltoxkit FTM, Freshwater toxicity test with microalgae. Creasel, Deinze, Belgium, 28p.

Sparks, T. C., Crouse, G. D., Durst, G. (2001) - Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. *Pest Management Science* 57:896-905.

Stackelberg, P.E., Kauffman, L.J., Ayers, M.A., Baehr, A.L. (2001) - Frequently co-occurring pesticides and volatile organic compounds in public supply and monitoring wells, southern New Jersey, USA. *Environmental Toxicology and Chemistry*, Volume 20, Issue 4, pp. 853–865.

Stashenko, E.E., Jaramillo, B.E., Martínez, J.R. (2004) - Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity. *Journal of Chromatography A*, Volume 1025, Issue 1, pp 93-103.

Stein, J. R. (1973) - Handbook of phycological methods, culture methods, and growthmeasurements. London, United Kingdom: Cambridge University Press.

Tarkpea, M., Eklund, B., Linde, M., Bengtsson, B.E. (1999) - Toxicity of conventional, elemental chlorine-free, and totally chlorine-free kraft-pulp bleaching effluents assessed by shortterm lethal and sublethal bioassays. *Environmental Toxicology and Chemistry*, Vol. 18, pp. 2487 – 2496.

Tomlin, C.D.S. (2006) – The Pesticide Manual. 14th edition, BCPC, Hampshire, UK, 1349 p.

Trautmann, N.M., Porter, K.S. & Wagenet, R.J. (1998) – Pesticides: Health effects in drinking water. Natural Resources Cornell Cooperative Extension, Cornell University, Ithaca, New York, USA, 5p. (<http://pmep.cce.cornell.edu/facts-slides-self/facts/pes-heefgrw85>)

Travisi, C.M. (2006) - Managing pesticide risks for non-target ecosystems with pesticide risk indicators: a multi- criteria approach. *Int: J. Environment and Management*, 112: 141-162.

U.S.E.P.A (1996) - Daphnid Chronic Toxicity Test. Ecological Effects Test Guidelines. U.S. EPA , 12p.

U.S.E.P.A (2000) – Ecological effects test guidelines. OPPTS 850.1300. Daphnid Chronic toxicity test. United States Environmental Protection Agency. EPA 712 – C -96 – 120,12p.

U.E.S.P.A. (2004) – Drinking water standards and health advisories. Technical Information Pockges. USEPA, Washington, DC, USA, 17p.

U.S.E.P.A (2006) – 2006 Edition of the drinking water standards and health advisories. U.S. EPA, Washington DV, USA, 12p.

Urruty, L. & Montury, M. (1998) – Elaboration D'une method de determination de pesticides por SPME/GL .Actes XXVIII^{ème} Congrès du Groupe Français des Pesticides, Orleans, France, 22-22 Mai 1997: 41-46.

Vrana, B., Allan, I.J., Greenwood, R., Mills, G.A., Dominiak, E., Svenson, K., Knutsson, J., Morrison, G. (2005) - Passive sampling techniques for monitoring pollutants in water. Trends in Analytical Chemistry, Vol. 24, No. 10, 845-866.

Vighi, M. & Funari, E. (1995) – Preface. In: M. Vighi & E. Funari (ed.) – Pesticide risk in groundwater. CRC Press, Boca Raton, Florida, USA: i-iii.

Vighi, M. & Di Guardo, A. (1995) – Predictive Approaches for the evaluation of pesticide exposure. In: M. VIGHI & E. Funari (Ed)- Pesticide Risk in Groundwater. CRC Press, Boca Raton, Florida, USA: 73-100

Waldron, A.C. (1997) – Pesticides and groundwater contamination. Ohio , USA, Extention Bulletin 820, 19p. (<http://ohioline.org.ohio-sate.edu/b820/index.html>)

Webster, E., Cowan – Ellsberry, C., Mccarty, L. (2004) - Putting science into persistence, bioaccumulation, and toxicity evaluations. *Environmental Toxicology and Chemistry*, Volume 23, Issue 10, pp. 2473–2482.

WHO (1987) – Drinking water quality: guidelines for selected herbicides. WHO Regional Office for Europe, Copenhagen, Denmark.

WHO (1993) – Guidelines for Drinking Water Quality. Recommendation 2nd ed, vol 1, World Health Organization, Geneva, Switzerland 181p

WHO (1996) – Guidelines for Drinking Water Quality. Health criteria and other supporting information 2nd ed, vol 2, World Health Organization, Geneva, Switzerland.

WHO (1998) – Guidelines for Drinking Water Quality. Addendum to vol 2. World Health Organization, Geneva, Switzerland (http://www.who.nt/water_sanitation_health/DG-WQ/summarytables)

Winter, T. C, Morvey, J.M., Franke, O.L, Alley, W.M. (1998) – Ground water and surface water a single resource U.S. Geological Survey Circular 1139. Denver Colorado. USA, 79 p.

Wilson, M. F. (2003) - Optimising Pesticide Use. Wiley series in agrochemicals and plant protection. Central science laboratory, Sand Hutton, York, UK, (Ed.) Wiley, 214p

Wills, R. & Yeh, W. (1987) – Groundwater Systems Planning and Management, Prentice – Hall.

Witkowski, A.J., Kowalczyk, A., Vrba, J. (2007) - Groundwater Vulnerability Assessment and Mapping. (Ed.): Taylor & Francis, 260p.

Wong, M.K., Wan, H.B. (1996) - Minimization of solvent consumption in pesticide residue analysis. Journal of Chromatography A, 754: 43-47.

Zar, J.H. (1996) – Biostistical analysis. Prentice – Hall, Upper Saddle River, NJ, USA.