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Licenciatura em Engenharia do Ambiente

Influence of Abiotic Stress Factors on VOCs Emission from Portuguese Rice Paddy Fields

Relation with increased Climate Change

Dissertação para obtenção do Grau de Mestre em
Engenharia do Ambiente
Perfil de Gestão de Sistemas Ambientais

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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Outubro de 2013

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Acknowledgements

Big opportunities always happen when we less expect – I called Serendipity – and we should never waste them! At least, it was what I have done and never regretted. The challenge of coming up with new tasks and reproduce them on an ended dissertation should be considered important help and motivation from who made it possible and I have a pleasure to thank all of them now.

To start, I want to underline here all my highest honor and admiration and thank my supervisor Professor Dr. Alexandra Ribeiro by orientation, support, friendship and important opportunity of leave me work and explore my field and laboratory skills with maximum vote of confidence.

I also want to recognize the enormous support offered by my Co-supervisor Post-Doc researcher Dr. Maria Nazaré Couto, which was truly tireless in all support, friendship, availability and dedication among the whole dissertation.

I had the experience of working as a researcher with a team from FCT-UNL and partners from INIAV and UTAD, on a project named PTDC/AGR-AAM/102529/2008 "Trace gas emission from Portuguese irrigated rice fields in contrasting soils, by the influence of crop management, climate and increased concentration of CO_2 and temperature in the atmosphere". I get totally involved with the aim of doing my best day by day. It was big step further on my professional enrolment and gained skills. My thesis appears as one of the result marks of the project. And it was only possible thanks to the four months of financial support allowed by FFCT (Portuguese Foundation for Science and Technology) for reach the objectives and make possible the accomplishment of field and laboratory investigation.

I would also like to thank CENSE-DCEA (Center for Environmental and Sustainability Research-Department of Environmental Engineering Sciences) - FCT-UNL for the facilities. As well as the rest of team project from FCT-UNL, the important help from all other researchers and laboratory colleges in technical support and features, and specially Dr. Eduardo Mateus for offered complete availability and support during the analysis, identification and discussion process of results and sharing knowledge about laboratory work technical performance. From INIAV team, I want to thanks to project coordinator Dr. Corina Carranca for conferences invitation and sharing information about data from rice paddy fields, and Ms. Nuno Figueiredo for support in field and informative/technical material. Other partners of the project from CotArroz, Paula Marques, I also want to mention the great reception and permission for collecting plant material for analysis.

By last but not the least, I attribute highest gratifications for having with me my family, my partner and all my friends that always make me believe that everything, with effort and dedication, is possible. A sincerely thank for still walking with me in this important journey of my life.

Thank you all.

Resumo

A resposta ao stress pelas plantas leva à emissão de sinais químicos para a atmosfera - compostos orgânicos voláteis (COVs). Os COVs participam na química da atmosfera: sofrem fotólise ou reagem com oxidantes atmosféricos originando ozono troposférico. Neste trabalho foi estudada a emissão de COVs no ciclo da planta do arroz (*O. sativa L.* cv. Aríete), em campos alagados, em dois ensaios, em blocos aleatórios com três repetições, instalados em dois solos de texturas diferentes (argilo-limoso e areno-franco), avaliando o efeito das emissões ao ar livre e em câmaras abertas sob efeito de tratamentos com indução de stress abiótico (aumento da temperatura e aumento simultâneo da temperatura e da concentração de CO_2). Os COVs foram extraídos da planta utilizando micro extração em fase sólida e destilação por arrastamento de vapor com extracção sumultânea, seguido de deteção por cromatografia gasosa acoplada a espectrometria de massa, utilizando duas colunas capilares, uma apolar (DB-5) e outra polar (DB-WAX). Foram identificados 33 COVs com a coluna apolar e 22 com a polar e identificadas três principais classes: voláteis verdes das folhas, monoterpenos e sesquiterpenos. Tendo em conta o ciclo de vida da planta, observou-se uma mais elevada emissão na fase vegetativa, seguida da reprodutiva e a menor na maturação. No solo argilo-limoso ocorreu uma maior emissão em comparação a de textura areno-franco. Entre as câmaras abertas, verificou-se uma maior emissão quando do aumento apenas da temperatura, em relação ao aumento simultâneo com CO_2. Pelos cenários do Painel Intergovernamental das Alterações Climáticas com aumento de temperatura e concentração de CO_2, há dois efeitos inerentes na emissão de COVs pelo arroz, um negativo com maior emissão devido à temperatura e outro positivo referente à diminuição de COVs na presença de CO_2. A inclusão de dados de campo nos modelos de qualidade do ar ajudará a previsão e entendimento do impacte das alterações climáticas na qualidade do ar à escala global.

Palavras-chave: cultura do arroz; factores de stress abiótico; emissão de COVs; alterações climáticas.

Abstract

Plants are emitting chemical-signals to the atmosphere in response to stress factors - Volatile Organic Compounds (VOCs). VOCs have higher influence on atmosphere chemistry: they are acting as photochemical precursors in tropospheric ozone formation. Present work studies VOCs emission released by rice (*Oryza sativa* L *cv.* Aríete) cycle in paddy fields, in aleatory schemes with three replicates, in two separate soil plots with different textures (silty clay and loamy sand), studying open field conditions and open top chambers (OTCs) under influence of treatments with induced abiotic stress (increase temperature and simultaneously temperature and CO_2 atmospheric concentration enhancement). VOCs were extracted from plant by solid phase micro extraction (SPME) and stem distillation extraction (SDE), and analyzed by gas chromatography coupled to mass spectrometry (GC/MS) using two GC capillary columns with different polarities, one non-polar (DB-5) and other polar (DB-WAX). A total of 33 VOCs using a non-polar column and 22 VOCs using a polar column, in both set of results were identified the three main classes of compounds: green leaf volatiles (GLV), monoterpenes and sesquiterpenes. Between rice cycle VOCs vary their trend and on vegetative stage were observed more VOCs, followed by ripening and lesser on reproductive. Silty clay soil demonstrated higher amount of VOCs released if compared with loamy sand texture. Between OTCs, more compounds were released by increasing temperature than simultaneously temperature and CO_2. In Intergovernmental Panel for Climate Change (IPCC) scenarios with emergent trend of increasing temperature and CO_2 atmospheric concentration, two effects are inherent to rice VOCs emission, one negative with higher emission related with temperature and other positive with less emission associated CO_2. Field data measurements addictions in air quality models will help achievements of realistic previsions and better understand the effect of climate change in air quality on a global scale.

Key-works: rice culture; abiotic stress factors; VOCs emission; climate change.

Table of Contents

ACKNOWLEDGEMENTS III

RESUMO V

ABSTRACT VII

TABLE OF CONTENTS IX

LIST OF FIGURES XIII

LIST OF TABLES XVI

ACRONYMS AND ABBREVIATIONS XIX

1. INTRODUCTION 1

1.1 RESEARCH SCOPE AND OBJECTIVES 2

1.2 DISSERTATION STRUCTURE 2

2. LITERATURE REVIEW 3

2.1 IMPORTANCE OF RICE CULTURE 3

2.1.1 Global status *3*

2.1.2 Economics *6*

2.1.3 Ecological advantage *7*

2.1.4 Rice culture in Portugal *7*

2.2 RICE PLANT 8

2.2.1 Cultivation systems *10*

2.2.2 Influent stress factors *11*

2.2.2.1 Soil characteristics 11

2.2.2.2 Temperature and atmospheric carbon dioxide concentration 12

2.2.2.3 Wind 13

2.2.2.4 Sun Radiation 13

2.2.2.5 Water 13

2.2.3 Chemical responses to stress factors *14*

2.3 VOLATILE ORGANIC COMPOUNDS 14

2.3.1 VOCs main classes *15*

2.3.1.1 Terpenes 15

2.3.1.2 Green Leaf Volatiles 16

2.4 VOCS STUDY 17

2.4.1 Extraction techniques *17*

2.4.2 Analytical techniques *18*

2.4.3 Rice volatile profile studies *20*

2.4.4 VOCs specific meanings and aroma correspondence *21*

2.5 VOCS, RICE AND CLIMATE CHANGE SCENARIOS 23

3. MATERIALS AND METHODS 33

3.1 SITE DESCRIPTION 33

3.1.1 CULTURE PRACTICES 34

3.2 EXPERIMENTAL DESIGN 35

3.2.1 Sampling *35*

3.2.2 VOCs extraction methods *38*

3.2.2.1 Solid phase microextraction 39

3.2.2.2 Steam distillation extraction 40

3.2.3 VOCs analysis *41*

3.2.3.1 Gas chromatography/mass spectrometry 41

3.2.4 VOCs Identification *43*

3.2.4.1 Statistical results treatment 44

4. RESULTS 45

4.1 VOCS PROFILE BY GC/MS USING DB-5 NON-POLAR COLUMN 45

4.2 VOCs PROFILE BY GC/MS USING DB-WAX POLAR COLUMN 49

4.3 DATA TREATMENT 52

4.3.1 Students t-test 52

4.3.1.1 One sample t-test mean (confidence intervals) 52

4.3.1.2 Independent two sample t-test (mean differences) 53

5. DISCUSSION 55

6. CONCLUSIONS 59

7. REFERENCES 60

ANNEXES

List of Figures

FIG. 2.1 – WORLD POPULATION IN 2004 BASED ON STAPLE FOOD CROPS CALCULATED FROM DATA AVAILABLE IN FAOSTAT (SOURCE: FAO, 2004) 4

FIG. 2.2 – WORLD MAP OVERVIEW OF COUNTRIES WITH HIGHER (BLUE COLOUR) AND LOWER (ORANGE COLOUR) RICE AREA HARVESTED/TOTAL CROP AREA HARVESTED (SOURCE: MOHANTY, 2010) 4

FIG. 2.3 - TREND OF THE THREE MAIN CEREAL PRICES (USD PER CEREAL TONNE) SINCE 2011 TO FIRST TWO MONTHS OF 2013. PRICES REFERRED MONTHLY AVERAGE (SOURCE: FAO, 2013) 6

FIG. 2.4 – RICE ECOSYSTEM LANDSCAPE A) FLOCK OF STROKS (SOURCE: APARROZ, 2009) AND B) CRAYFISH IN RICE SOIL SUBSTRATE IN COTARROZ PADDY FIELDS, SALVATERRA DE MAGOS 7

FIG. 2.5 - RICE PLANT CONSTITUENTS (SOURCE: IRRI, 2013A) 8

FIG. 2.6 – EXAMPLE OF GROWTH PHASES OF RICE PLANT (SOURCE: IRRI, 2013B) 9

FIG. 2.7 - IRRIGATE *VS.* RAINFED RICE IN ASIAN CONTINENT (SOURCE: HUKE (1997) IN WASSMANN *ET AL.*, 2009) 10

FIG. 2.8 – METEOROLOGICAL CHARACTERISTICS IN *SALVATERRA DE MAGOS* DURING THE RICE CULTURAL CYCLE OF 2012 (P = ACCUMULATE MONTHLY PRECIPITATION, MM; T MED/GREEN CIRCLES = MONTHLY AVERAGE AIR TEMPERATURE, ^{o}C; RED TRIANGLES = MAXIMUM TEMPERATURE, ^{o}C, AND BLUE SQUARES = MINIMUM TEMPERATURE, ^{o}C) (ADAPTED FROM: INIAV, 2013A) 11

FIG. 2.9 – CHARACTERISTICS OF SAND AND CLAY SOILS TOWARDS TENSION AND WATER CONTENT VARIATION (SOURCE: IRRI, 2013B) 12

FIG. 2.10 – EXAMPLES OF MONO – AND SESQUITERPES POLYCYCLIC STRUCTURES (SOURCE: NIST 08, 2013) 16

FIG. 2.11 – CONSTITUENT PARTS OF A STANDARD SPME HOLDER (SOURCE: CHROMEDIA, 2013) 17

FIG. 2.12 – GC/MS EQUIPMENT LAYOUT (SOURCE: GINSBACH *ET AL.*, 2010) 19

FIG. 2.13 – VOC PROFILES COLLECTED FROM UN- AND INFESTED RICE PLANTS SAMPLES (SOURCE: ZHOU AND WANG, 2011) 20

FIG. 2.14 - RELATION BETWEEN VOC SOURCES EMISSION, MOLECULAR SIZE AND CLIMATE REACTION (SOURCE: RIIPNEN *ET AL.*, 2012) 23

FIG. 2.15 – LOW MOLECULAR COMPOUNDS AND THEIR REACTIONS (ADDAPTED FROM: SHENG *ET AL.*, 2013) 24

FIG. 2.16 – MONTHLY ESTIMATION OF ISOPRENE, MONOTERPENES, SESQUITERPENES, AND OVOCS EMISSIONS FROM EUROPEAN VEGETATION. OTHER LAND AREA USES INCLUDE ALL CATEGORIES EXCEPTED FOREST (SOURCE: STEINBERG *ET AL.,* 2009) 25

FIG. 2.17 – VOCS EMISSION REDUCTION IN EUROPE BETWEEN 1990 AND 2010 (SOURCE: EEA, 2004) 27

FIG. 2.18 – COMPLEMENTATION METHODS FOR INCLUDING MEASUREMENT STUDIES AND MODEL STUDY TO UNDERSTAND REAL PHENOMENA AND PREDICT FUTURE SITUATION (CHO AND OKI, 2012) 28

FIG. 3.1 – LOCATION OF FIELD SITE; (RIGHT SIDE) COTARROZ PLOTS (SOURCE: FIGUEIREDO, 2011) 33

FIG. 3.2 – COTARROZ PARTIAL LANDSCAPE 33

FIG. 3.3 – CULTURE PRATICES DATES AND FLOODING PERIOD (SOURCE: INIAV, 2013C) 34

FIG. 3.4 – COTARROZ FIELD ASPECT AFTER SEEDING (A); WEEDING (B); BEFORE HARVESTED (C) 34

FIG. 3.5 – EXPERIMENTAL DESIGN LAYOUT 35

FIG. 3.6 – DATA COLLECTION LAYOUT IN COTARROZ, SALVATERRA DE MAGOS, 2012, IN OF SOIL PLOT A (A); SOIL PLOT B OTC (B); PLOTS UNDER CONTROLED CONDITIONS (C) 36

FIG. 3.7 – SAMPLING SCHEME AT SILTY CLAY SOIL 36

FIG. 3.8 – DAILY TEMPERATURE AVERAGE DURING THE WHOLE RICE CYCLE 37

FIG. 3.9 – DAILY CO_2 CONCENTRATION AVERAGE AMONG WHOLE RICE CYCLE 38

FIG. 3.10 - MATERIAL FOR SPME EXTRACTION SPME HOLDER AND FIBRE DVB/CAR/PDMS AND (A), ABOUT 0.3 G OF RICE SAMPLES INTO 15 ML VIALS (B) 39

FIG. 3.11 – SDE EXTRACTION; SDE MATERIAL (A) AND FRESH RICE SAMPLES (B) 40

FIG. 3.12 – EXAMPLE OF TIC RESULTING FROM SPME EXTRACTION AND ANALYSED BY GC/MS USING DB-5 CAPILARY COLUMN 41

FIG. 4.1 – RETENTION TIME IN RELATION WITH AMOUNT OF CARBON UNITS OF VOC CONSTITUENTS FROM THE THREE MAIN CLASSES (TIC BY GC/MS USING DB-5 COLUMN) 47

FIG. 4.2 – PREDOMINANCE OF MONO- AND SESQUITERPENES ON VEGETATIVE STAGE 47

FIG 4.3 – VOICES EMISSION DIFFERENCES BETWEEN SOIL TEXTURES 48

FIG. 4.4 – HIGHER NUMBER OF VOCS ON TEMPERATURE INDUCED OTC (TE_C) 48

FIG 4.5 – GLV BETWEEN THREE PHASES (TE SAMPLE COMPARATION) WITH GC DB-WAX POLAR COLUMN 50

List of Tables

TABLE 2.1 – COUNTRY RANGE OF RICE PRODUCTION (TONES AND PERCENTAGE), OCCUPIED AREA (HECTARES AND PERCENTAGE) AND PRODUCTIVITY IN THE YEAR 2009 5

TABLE 2.2 - RANGE OF FIFTH MAIN EU RICE PRODUCERS IN THE YEAR 2011 8

TABLE 2.3 – CLASSIFICATION OF COMMERCIAL RICE BY GRAIN SIZE 9

TABLE 2.4 - OPTIMAL, MINIMAL AND MAXIMAL TEMPERATURES (°C) DURING RICE GROWING PHASES 12

TABLE 2.5 – ISOPRENE UNITS, CARBON AND HYDROGEN ATOMS AND CHEMICAL FORMULA OF DIFFERENT TERPENES 15

TABLE 2.6 – STRUCTURE OF MAIN GREEN LEAF VOLATILES 16

TABLE 2.7 – ASSUMED MEANING AND AROMA DESCRIPTOR OF MAIN VOCS 22

TABLE 2.8 – MAIN VOC EMITTER COUNTRIES IN EU ACCORDING WITH THE UNECE PROTOCOL 27

FIG. 2.18 – COMPLEMENTATION METHODS FOR INCLUDING MEASUREMENT STUDIES AND MODEL STUDY TO UNDERSTAND REAL PHENOMENA AND PREDICT FUTURE SITUATION (CHO AND OKI, 2012) 28

TABLE 2.9 – MAIN CONCLUSIONS OF THE IPCC SCENARIOS PREDICTION 29

TABLE 2.10 – SCENARIO RESULTS FOR RICE PRODUCTION OVER THE THREE ECONOMICAL CATEGORIES GROUP COUNTRIES 30

TABLE 3.1 – FIELD SAMPLING DATA RESUME 37

TABLE 3.2 – SAMPLING DATES AND CORRESPONDING PHASE 38

TABLE 3.3 – SPME EXTRACTION 39

TABLE 3.4 – MASS OF RICE SAMPLES FOR SPME EXTRACTION 39

TABLE 3.5 – SDE EXTRACTION 40

TABLE 3.6 – MASS OF COMPOSITE RICE SAMPLES FOR SDE EXTRACTION 41

TABLE 3.7 – GC PROGRAMMED METHOD 42

TABLE 3.8 – THE VAN DEN DOOL/KRATZ RI FORMULA CALCULATION 43

TABLE 3.9 – MOLECULAR IONS FROM HYDROCARBONS C_8 TO C_{15} 43

TABLE 3.10 – EMISSIONS MEAN $\bar{X}_x$ AND STANDARD DEVIATION S_x OF POPULATION FROM EACH TREATMENT AND RESPECTIVE RICE CYCLE PHASE 44

TABLE 4.1 – RICE CYCLE VOCS EMISSION PERFORMED BY SPME ANALYZED BY GC/MS (DB-5 COLUMN) 46

TABLE 4.2 - VOCS EMISSION ANALYZED GC/MS (DB-WAX POLAR COLUMN) 49

FIG 4.5 - GLV BETWEEN THREE PHASES (TE SAMPLE COMPARATION) WITH GC DB-WAX POLAR COLUMN 50

TABLE 4.3 – GC COLUMNS RESULTS COMPARISON 51

TABLE 4.4 – T-STUDENT TEST $CI_{95\%}$ RESULTS FOR ONE SAMPLE TEST (MEAN μ) WITH 95% OF CONFIDENCE DEGREE 52

TABLE 4.5 - T-STUDENT TEST $CI_{99\%}$ RESULTS FOR ONE SAMPLE TEST (MEAN μ_x) WITH 99% OF CONFIDENCE DEGREE 53

TABLE 4.6 – INDEPENDENT SAMPLES T-TEST BETWEEN SAMPLE TREATMENTS 53

Acronyms and abbreviations

°C	degree Celsius
3-PGA	3-phospoglycerate
AVOC	Anthropogenic Volatile Organic Compound
BVOC	Biogenic Volatile Organic Compound
C_5	isoprene
COTArroz	Centro Operativo e Tecnológico do Arroz
DB-5	non polar capillary column with 5% phenyl-95% methylpolysiloxane
DB-WAX	polar capillary column with polyethylene glycol
DMAPP	dimethylsiphospate
E-nose	Electronic nose
EU	European Union
eV	electric volt
FAO	Food and Agricultural Organization
FCT – UNL	Faculdade de Ciência e Tecnologia da Universidade Nova de Lisboa
Fe	iron
FFCT	Fundação para a Ciência e Tecnologia
g/mol	gram per mole
GC	gas chromatography
GC/MS	GC coupled with Mass Spectrometry
GC-FID	GC with Flame Ionization Detector
GDP	Gross Domestic Product
GLV	green leaf volatiles
ID	Inner diameter
INIAV	Instituto Nacional de Investigação Agrária e Veterinária
IPCC	Intergovernmental Panel on Climate Change
IPP	isopentenyldiposphate
IRGSP	Rice Genome Sequencing Project
Kcal/cap/day	kilocalories per capita per day
Kt	kilo tons
LMC	low molecular compounds
LOX	lipoxygenase
m/z	mass to charge
MEP	methyllerythritol phosphate
MVA	mevalonic acid
MW	molecular weight
NERICA	New Rice for Africa
NO_3	Nitrate
O_3	ozone
OF	open field
OH	hydroxyl radical
OTC	open tot chamber
P	phosphorus
Pa	Pascal
PA	polyacrilate
PAN	peroxyacetylnitrate
PDMS	polydimethylsyloxane

pF	water tension
PI	panicle initiation
Ppb	part per billion
Ppbv	part per billion of volume
Ppm	part per million
PTR-MS	proton transfer reaction mass spectrometry
RI	retention indices
RuBPCO	Ribulose bisphosphate carboxylase oxygenase
SDE	steam distillation extraction
SOA	Secondary Organic Aerosol
SPME	solid phase micro extraction
SRES	Special Report Emission Scenarios
T/ha	ton per hectare
TE	OF silty clay soil texture
TE_c	OTC with induced temperature
TE_{CC}	OTC with simultaneous temperature and CO_2 enhanced
TIC	total ion current
TN	OF loamy sand soil texture
TWB	the World Bank
UNECE	United Nations Economic Commission for Europe
UTAD	Universidade de Trás-os-Montes e Alto Douro
VOC	Volatile Organic Compound
WDI	World Development Indicators
WDI	World Development Indicators
WHO	World Health Organization
µm	micro meter

1. Introduction

The combination of the importance of rice culture as a main staple food crop in the world, with the raise of global population and increasing climate change effects due to higher temperature and atmospheric CO_2 concentration, will set a new valid topic of study – the interaction between rice cycle gas emission and atmospheric chemistry. Rice plant response to abiotic stress factors is expressed by releasing volatile organic compounds (VOCs) to atmosphere; these compounds promote the secondary aerosol oxidation as well as are considered a precursor of tropospheric ozone formation. The major goal of this thesis is to understand the effect of VOCs emitted from rice paddy fields in Portugal, in open field under different treatments as different soil characteristics, and open top chambers (OTCs) where abiotic factors are induced as temperature enhancement and temperature together with atmospheric CO_2 concentration.

The motivation and data collected for the present thesis are part of the research project PTDC/AGR-AAM/102529/2008 entitled "Trace gas emission from Portuguese irrigated rice fields in contrasting soils, by the influence of crop management, climate and increased concentration of CO_2 and temperature in the atmosphere" – carry out by teams from three Portuguese research institutions named: *Instituto Nacional de Investigação Agrária e Veterinária* (INIAV); *Universidade de Trás-os-Montes e Alto Douro* (UTAD) and *Faculdade de Ciência e Tecnologia – Universidade Nova de Lisboa* (FCT-UNL), in cooperation with *Centro Operativo e Tecnológico do Arroz* (COTArroz).

The data collection covered the whole rice cycle, between May and October 2012, in COTArroz paddy fields, *Salvaterra de Magos*, Portugal. In this scope a field scale data measurement has never been done before. This work represents the first step in this domain. Laboratory measurements from different rice plant parts, as roots, leaves and grain, studying abiotic and biotic stresses were already performed. The identification process was difficult due to vestigial emissions released by rice plant, when compared with other plants, as *Pinnus* spp. or *Eucalyptus* spp. For that reason it was only possible to accomplish qualitative analysis from rice VOCs profile.

1.1 Research scope and objectives

This thesis aims to take further steps towards analyzing the impacts of gas emissions from Portuguese irrigated rice fields with relation with climate change predictions, and it involves:

- ✓ Understand the importance of main staple food crop – rice (*Oryza sativa* L.) – on global economic, environment and cultural spheres;
- ✓ Identify VOCs distribution among whole rice field cycle growing phases under different treatments including different soil textures (silty clay and loamy sand) and different abiotic conditions with increasing temperature and simultaneous temperature and CO_2 atmospheric concentration enhanced. Analyse statistical data set significance;
- ✓ Understand the effects of VOCs emission from rice field and their behaviour in climate change forecast scenarios with increasing temperature and CO_2 concentration.

1.2 Dissertation structure

The present dissertation is divided in the following chapters:

1. Introduction: details of research scope, main objectives and dissertation structure
2. Literature review: previous work developed in the scope
3. Materials and methods: description of materials used in sampling, extraction, analysis, identification and data treatments methods
4. Results: presentation of results
5. Discussion: hypothesis formulation and their discussion
6. Conclusions: main outcomes and future research
7. References

Annexes

2. Literature review

2.1 Importance of rice culture

The nutritional properties assimilated by humans to their subsistence and welfare are crucial. Good nutrition status is essential way to have an optimal physical and psychical life. The intake of a right proportion of a big variety of food is necessary, varying from place to place and depending on a geographical and cultural base. Rice is in charge of keeping nutritional safety conditions to humans and is a main source of carbohydrates but has lower percentage of proteins, fiber and lipids. However, it contains vitamin B1, B2 and B6 as well minerals and natural antioxidants (AParroz, 2009).

Historically, China and India were the precursors of the rice production practices and varieties, followed by the African continent. In Portuguese age of discovery (between 1415 – 1543) rice expanded its production to Portuguese colonies, as Guiné – Bissau and Brazil, and also in Spanish colonies of Central America (Kush (1997) in Figueiredo, 2011).

Nowadays, almost 120 000 different species of rice exists and the culture has extended to all continents aside from cold polar zones. More common specie to grow is *Oryza sativa* L., divided on two subspecies: *indica* and *japonica*, depended on geographic position and morphologic characteristics (AParroz, 2009). The *indica* type is usually associated with tropical and subtropical zones (representing 80% of global production) (Mohanty, 2010) and *japonica* is mainly found in temperate climate, Mediterranean, Norwest Asia, United States and some European countries (Glaszmann (1987) in Figueiredo, 2011).

2.1.1 Global status

As a consequence of grow population in last years it is predictable that we will need twice more food than expected, in the period between 2000 – 2025, when compared with 1960's (UNDP, 2010). Modification of land use to create more space and resources for agriculture cannot expand more, and rice fields will increase not in occupied area or different geographical location, but in consequence of intensive agricultural practices. Sustainable rice agriculture management can be achieved by developing adequate water techniques to each location with appropriate technology and applying efficient fertilization methods.

Rice, wheat and maize are the three biggest widely grown crops around the world. Fig. 2.1 shows that in the year 2004 rice was the staple food crop (crop that constitutes predominant calories intake of a standard diet, 700 Kcal/cap/day or more) of about 3.23 billion people, wheat of almost 1.55 billion and lastly maize was responsible for about 288 million (FAO, 2004). United States emphasized this fact by making 2004 as international year of rice.

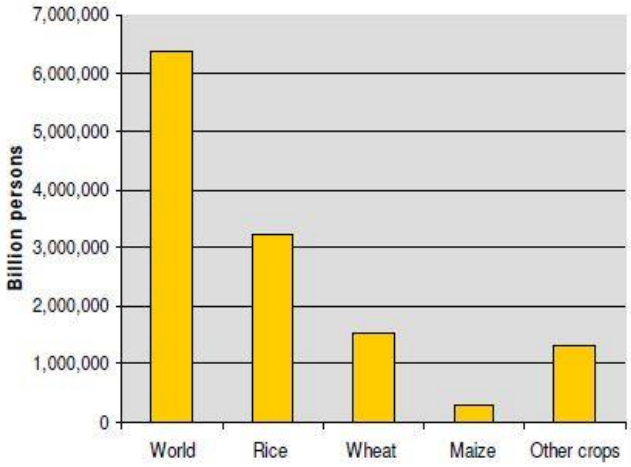


Fig. 2.1 – World population in 2004 based on staple food crops calculated from data available in FAOSTAT (Source: FAO, 2004)

In the 21st century rice harvested area was approximately 152 million ha (Mohanty, 2010), including multiple cropping and their frequency, i.e. how much times crop can be produced in the same land. Literature analysis (AParroz, 2009; Figueiredo, 2011; FAO 2012) and world map in Fig. 2.2 are matching with the blue and violet colour meaning half or more of country area occupied by rice. Fig. 2.2 shows higher rates of yield improvements in Southeast Asian countries and some Latin America ones.

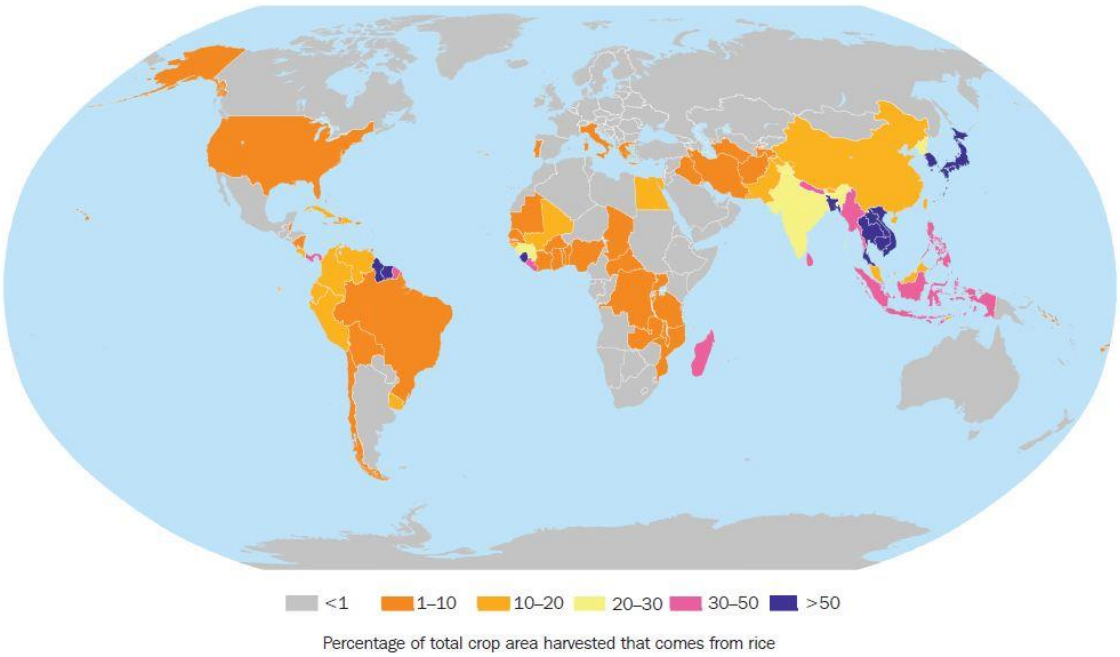


Fig. 2.2 – World map overview of countries with higher (blue colour) and lower (orange colour) rice area harvested/total crop area harvested (Source: Mohanty, 2010)

Global rice production more than tripled between 1961 and 2008 at average annual growth rate of 2.49% (Mohanty, 2010).

Table 2.1 presents the major production of rice, more than 90% (FAO (2010) in Figueiredo, 2011), is associated to the Asian continent, where China and India are the key producers and the smallest production is in Oceania due to agricultural land available space. Second continent is America, with Brazil as a main producer and in Africa the most important producer country is Egypt.

Table 2.1 – Country range of rice production (tones and percentage), occupied area (hectares and percentage) and productivity in the year 2009

Continent /Country	Production (10^6 t)	Area (10^6 ha)	Harvested area (%)	Global production (%)	Productivity (t/ha)
Asia	611	143	88.8	90.1	4.36
America	38.2	7.30	4.50	5.60	5.25
Africa	24.4	10.0	6.20	3.60	2.44
Europe	4.10	0.70	0.40	0.60	6.14
Oceania	0.30	0.00	0.00	0.00	8.03
China	197	29.9	18.5	29.1	6.59
India	131	44.1	27.3	19.3	2.98
Indonesia	64.4	12.9	8.00	9.50	5.00
Bangladesh	45.1	11.5	7.10	6.60	3.92
Vietnam	38.9	7.40	4.60	5.70	5.23
Taiyuan	31.5	11.0	6.80	4.60	2.87
Brazil	12.6	2.90	1.80	1.90	4.37
Japan	10.6	1.60	1.00	1.60	6.52
USA	10.0	1.30	0.80	1.50	7.94
Egypt	7.50	0.80	0.50	1.10	10.0
Global	678	161	100	100	4.20

(Source: FAO (2010) in Figueiredo, 2011)

Rice is grown on both small and large farms. The extent are generally smallest in Asia and Africa (both continents with higher production), less than 1 ha size. European continent vary from 3.90 ha in Greece to 40 ha in Italy. In Latin America farms have a tendency to be larger

but less than 5 ha, except Uruguay (276 ha) and United States (160 ha) (IRRI, 2013b). The heterogeneity within regions and between countries results from a range of production factors, such as: technology used, water regime, amount of rainfall, soil characteristics, climate, cultural practices and others. However, genetic manipulation creates good advantages for plant agrosystem adaptation. Rice genome sequences were completed in 2005 by International Rice Genome Sequencing Project (IRGSP, 2008).

2.1.2 Economics

No country has been able to sustain a rapid transition out of poverty without raising productivity in its agricultural sector. This process involves a successful structural transformation providing food, labour and savings (Mohanty, 2010). The World Development Indicators (WDI) published, by the World Bank (TWB), that agriculture contributed more than 10% of global Gross Domestic Product (GDP) in 1961, but nowadays agriculture is responsible of about 3% of economic output (FAO, 2012).

The rice consumption, in many cases/countries, is done on the original country that is produced. It represents a low importance on trade international market, with only 7 - 8% of total production is transactional (FAO, 2012). Fig. 2.3 shows that rice is the cereal with the highest price per tonne (600 USD/t) in the beginning of 2013 (FAO, 2013), almost the double than maize (300 USD/t) and wheat (350 USD/t) (FAO, 2013).

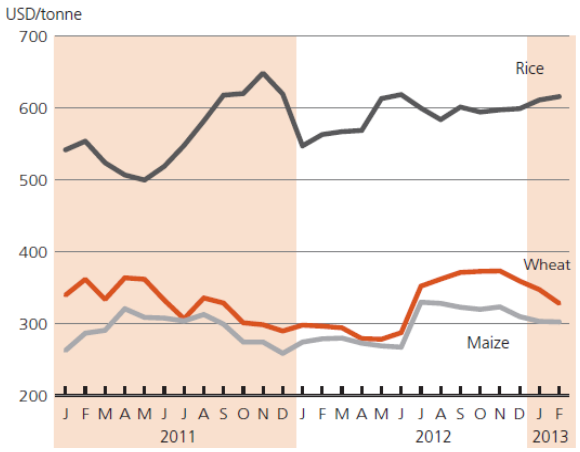


Fig. 2.3 - Trend of the three main cereal prices (USD per cereal tonne) since 2011 to first two months of 2013. Prices referred monthly average (Source: FAO, 2013)

Economics of rice global situation will be one of the big discussion topics on the First International Conference of Food Security to be held this September and October of 2013, in Noordwijkerhout, the Netherlands. It aims better understand the drivers of current and future food security system and the trade-offs between competing environmental, economic and social objectives and outcome (International Conference on Food Security, 2013).

2.1.3 Ecological advantage

Rice wetlands create habitats containing an appreciate number of fauna and flora species, contributing for biodiversity of the region. About 86% of rice flooded lands for at least part of the year has the big ecological function, such as surrogate seasonal migrating, wintering and nesting spot for water birds species, including waterfowl, shorebirds, wading birds (Fig. 2.4 a), and land birds (Taft and Elphick, 2007).

The vegetation roots growing in rice fields are important foraging resources, the presence of a large number of aquatic in- and vertebrates organisms (fishes, reptiles and amphibians) (Fig. 2.4 b), plays an important role on regulation and biologic control of pest and infestations (Taft and Elphick, 2007).

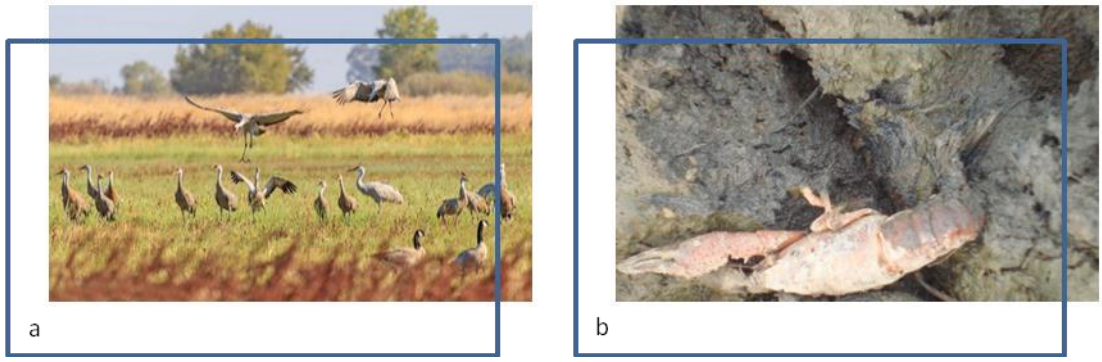


Fig. 2.4 – Rice ecosystem landscape a) Flock of stroks (Source: AParroz, 2009) and b) Crayfish in rice soil substrate in COTArroz paddy fields, Salvaterra de Magos

Around rice ecosystems areas, not only rice crop grows, but also maize, wheat, ryes are cultivated as well as vegetables and fruits are produced (INIAV, 2013b).

2.1.4 Rice culture in Portugal

More than 60% of rice produced in EU is from *japonica* subspecies and *indica* subspecies just takes place in Spain and at a smaller scale in Greece (INIAV, 2013b). In Portugal *japonica* represents about 70% of total national production, which is explained by climate characteristics of growing rice in paddy (INIAV, 2013b).

Portugal is the fourth biggest rice producer (18.2×10^6 t) in the 27 European Union countries (Table 2.2). Italy has the first place (150×10^6 t), followed by Spain (93×10^6 t) (FAO, 2011). Rice is the sixth main crop produced in Iberia Peninsula (Portugal and Spain).

Portugal is the first *per capita* consumer with 18 kg/year in Europe (INIAV, 2013b), being consumed 60% in the north and 40% in the South. Main rice farms are located in *Sado*, *Tejo* and *Mondego* river valleys, covering *Alentejo*, *Ribatejo* and *Beira Litoral* regions, respectively.

Table 2.2 - Range of fifth main EU rice producers in the year 2011

Country	Production (10^6 t)	Area (10^6 ha)
Italy	150	24.6
Spain	92.9	12.1
Greece	24.4	3.23
Portugal	18.2	3.12
France	13.2	2.50

(Adapted from: FAO, 2012)

2.2 Rice plant

Rice is an angiosperm plant, belonging to *Ponocea* family (Wells, 2003). Morphologic constituents are divided in roots, stem, leafs and panicle (Fig. 2.5). The panicles keep the spikelet and this will form rice grain.

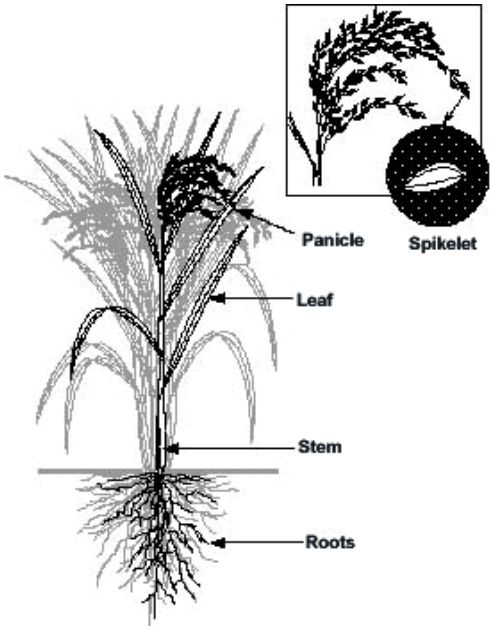


Fig. 2.5 - Rice plant constituents (Source: IRRI, 2013a)

Life cycle of rice cultivars under irrigated systems ranges from 110 to 165 days from germination to maturity, depending on the variety and environment conditions (Wells, 2003). On Mediterranean land this period occurs between May and mid of October.

Fig. 2.6 presents the three main agronomic rice development phases: vegetative (germination to panicle initiation (PI)); reproductive (PI to heading), and ripening (heading to maturity).

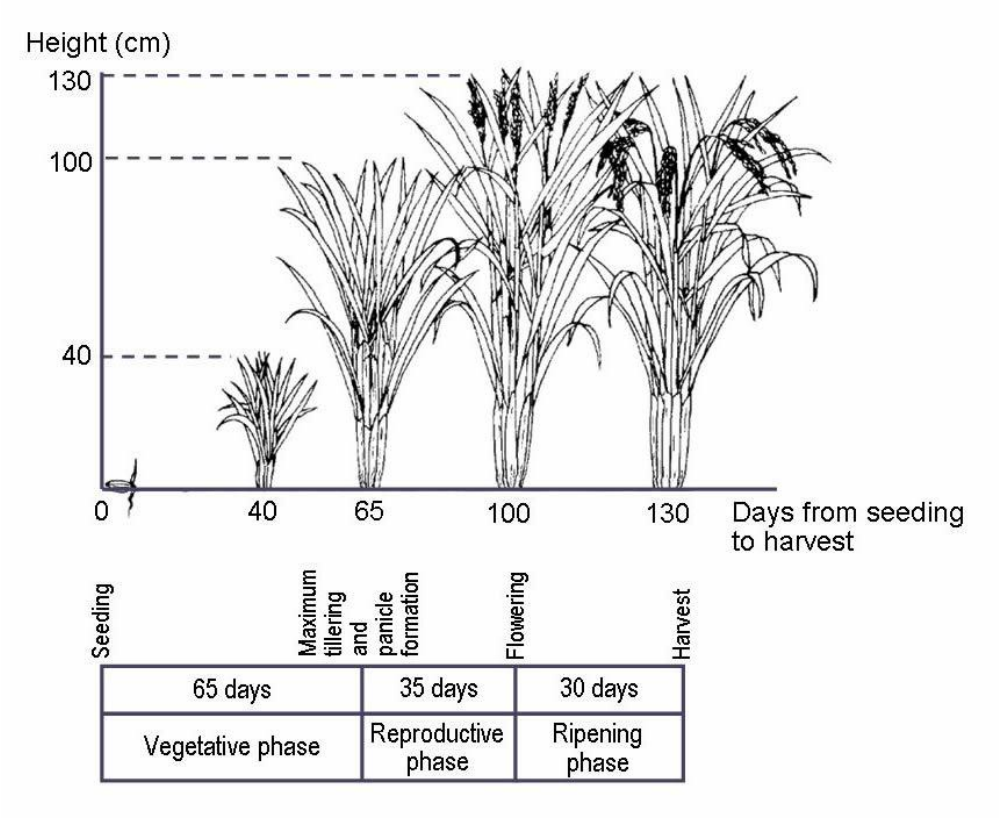


Fig. 2.6 – Example of growth phases of rice plant (Source: IRRI, 2013b)

The duration of vegetative phase differs on rice variety, but reproductive and ripening phases are common between variety types (IRRI, 2013b).

Rice is consumed mainly in the grain form. Table 2.3 presents the classification of rice by size (length) and the relation between length and width of the grain. Short, medium and long categories are following Portuguese legislation (INIAV, 2013b).

Table 2.3 – Classification of commercial rice by grain size

Commercial Classification	Size (length mm)	Relation (length/width)	Type/ Varieties
Short (Round)	≤ 5.2	≤ 2.0	Bomba; Paella rice.
Medium	5.2 – 6.0	≤ 3.0	Sushi rice; Risotto rice.
Long	> 6.0	> 3.0	Carolino; Agulha; Basmati.

(Adapted from: Figueiredo, 2011)

In Portugal, long rice is consumed in larger quantities in different varieties proportion, 72% is *Carolino*, 27% *Agulha* and 1% belonging to other types like sushi rice (INIAV, 2013b).

Rice can be distinguished from other cereals that are just consumed after transformation processes. The only physical process is whitening the rice grain and this can cause losses of mineral salts, vitamins and fiber (Figueiredo, 2011). "Whiteness" can be divided in three types: white (polished rice obtained after process of bleaching); brown (richer in vitamins and fiber) and middle rice (partially removed the shell) (Mahanty, 2010).

The number of complete rice cycle days and respective rice form are depended on the location and the rice cultivation systems (Wells, 2003).

2.2.1 Cultivation systems

Rice systems can be characterized by water source in irrigated and rainfed rice. Fig. 2.7 shows the area occupied on Asian continent by both systems. Rainfeed rice system can be distinguished between lowland, upland/dryland and deepwater rice (Mahanty, 2010).

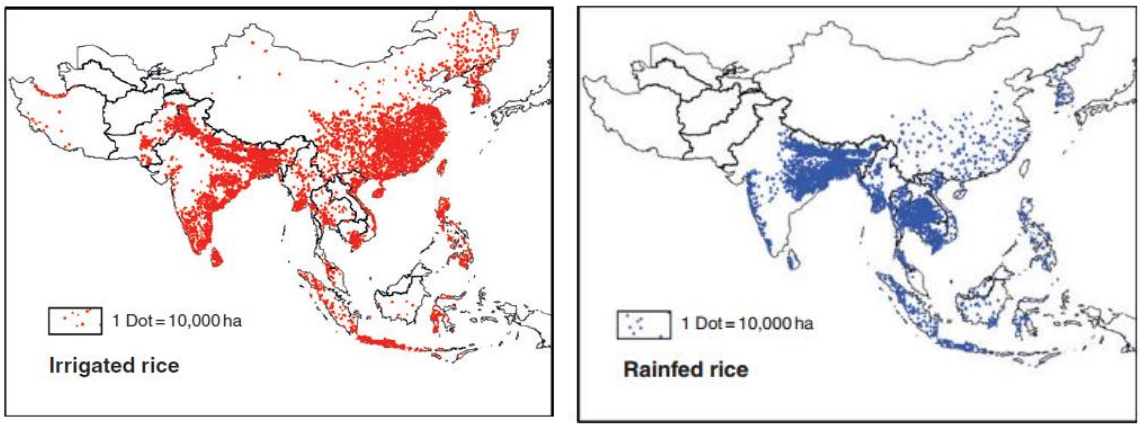


Fig. 2.7 - Irrigate *vs.* rainfed rice in Asian continent (Source: Huke (1997) in Wassmann *et al*., 2009)

Irrigate rice grows using water supplies to reinforce rainfall and natural runoff *via* human-made surface irrigation systems. There exists a greater control for reducing risks associate with drought. More than 75% of the world's rice (FAO, 2012) are produced under irrigated systems, and about 48% of irrigate systems grow more than one crop (multiple cropping) combining rice – wheat, rice – oilseed and rice – ryegrass (Mahanty, 2010).

Rice plant is submerged around 80% of the time (Mahanty, 2010) during vegetative phase. Rainfed rice uses only rainfall and natural runoff. In general, farmers applied less fertilizer in rainfed than in irrigated rice (Figueiredo, 2011).

Fig. 2.8 presents temperature and precipitation data in Portuguese rice paddy fields during the standard rice cycle course. Monthly average temperature oscillated around 13 and 21 ^{o}C. The precipitation values are higher in autumn.

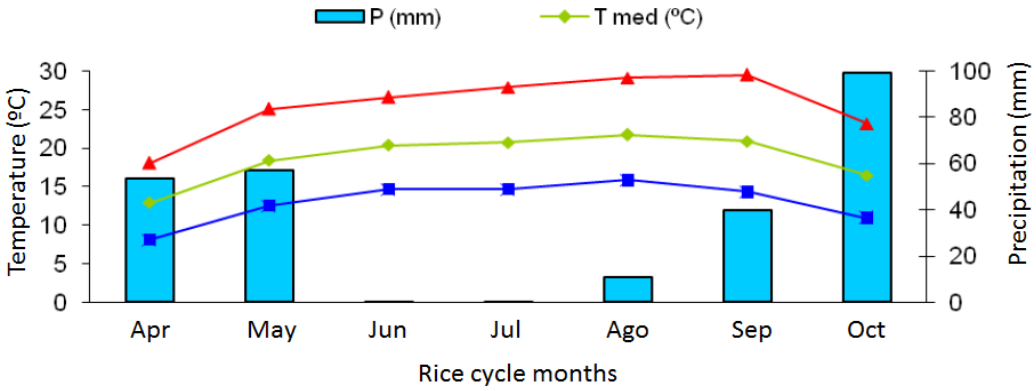


Fig. 2.8 – Meteorological characteristics in *Salvaterra de Magos* during the rice cultural cycle of 2012 (P = Accumulate monthly precipitation, mm; T med/green circles = Monthly average air temperature, ^{o}C; red triangles = Maximum temperature, ^{o}C, and blue squares = Minimum temperature, ^{o}C) (Adapted from: INIAV, 2013a)

Between April and October, normally at temperate Mediterranean climate, any extreme event is observed. Rice systems are chosen according with environmental climate characteristics of each region (Mohanty, 2010).

2.2.2 Influent stress factors

Rice is produced on a big variety of climates, within higher precipitation zones, more than 5 100 mm, and in lower ones with less than 100 mm. Altitude is also a determinant factor but rice can be found from 3 m to high altitudes over 3 000 m (Krishnan *et al.* (2011) in Figueiredo, 2011).

Rice culture depends on external abiotic and biotic factors. The biotic factors cover all intra- and interspecific interactions exchanging actions of attraction, defence, attack and mutual gain. The abiotic ones described below are: soil characteristics; wind intensity and direction; sun radiation; water and temperature and CO_2 concentration in atmosphere.

2.2.2.1 Soil characteristics

Soil profile influences water infiltration. Rice plant has good adaptation to different kinds of soils. Fig 2.9 presents the relation of water content in clay and sandy soils, where water moves from low tension (pF) for high tension. Clay soil particles are colloidal (smaller than 0.05 µm) electrically charged. Those characteristics permit water and nutrient storage (line on right side chart in Fig. 2.9), but above pF=2 water tension, it is difficult for roots to extract the needed contents. A pure sandy soil, are less common for crop cultivation due to holding a little portion of water (large dimension of particles, from 0.02 – 2 mm) (IRRI, 2013b).

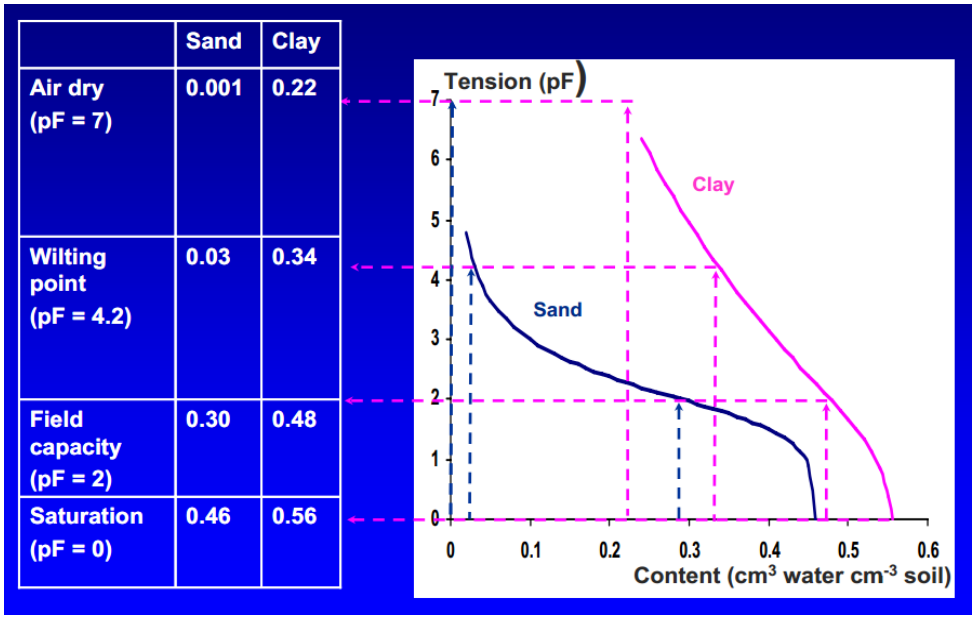


Fig. 2.9 – Characteristics of sand and clay soils towards tension and water content variation (Source: IRRI, 2013b)

In Europe, particularly in Portugal, the rice cropping practices are mainly by flooding/irrigation systems (Maes and Derbergh, 2001). Sandy soils are well drained and aerated, making easier for submersible control than clay ones. Sandy soils become productive for rice cultivation if water and nutrients are correctly supplied. To gain perform rice yields, loamy soils are suitable (see soil textures proportions in annexes T1) (IRRI, 2013b).

2.2.2.2 Temperature and atmospheric carbon dioxide concentration

Rice response to temperature is dependent on its stage (Cho and Oki, 2012). The vegetative period is normally more sensitive to extreme temperatures variation when compared to other growing phases (Table 2.4).

Table 2.4 - Optimal, minimal and maximal temperatures (°C) during rice growing phases

Growing phases	Optimal temp. (°C)	Minimal temp. (°C)	Maximal temp. (°C)
Vegetative	25-28	15	35
Reproductive	27-33	20	38
Ripening	20-25	12	30

(Adapted from: Figueiredo, 2011)

Rice is consider a C3 plant, that uses metabolic pathway for carbon fixation only in Kalvin Cycle, this process transforms CO_2 and enzyme ribulose bisphosphate carboxylase oxygenase (RuBPCO) into two units of 3-phosphoglycerate (3-PGA), essential sugar during the photosynthesis process. C3 plants, as rice, prefer moderate temperatures and optimum photosynthesis rate is gain under CO_2 concentration around 300 ppm or higher (Dudareva *et al.,* 2013). Rice has low photosynthetic efficiency at hot dry conditions, caused by photorespiration – instead of fixing carbon the plant start to fix oxygen (Nomura et al., 2000). Biochemically, higher CO_2 concentration stimulates increase on RuBPCO and photorespiration is reduced (Nomura et al., 2000).

Temperature and CO_2 atmospheric concentration conditions, separately, have higher influence on rice grain yield. Generally, with increasing temperature, yield declined 10% for every degree ^{o}C above the optimal one. The *japonica* type during the vegetative stage (Nomura et al., 2000), at mean temperatures around 36 ^{o}C, will have zero grained yield, explained by pollen sterility effect. According with Boote *et al*. (2005), doubling CO_2 atmospheric concentration, rice grain size and biomass increased by 25 to 32% due to a greater number of reproductive sites (panicles).

2.2.2.3 Wind

Wind direction and velocity does not play a main role on rice growth, but can influence crop production as physiological and mechanical impact, e.g. if the plant does not yet have a deep root or if in earliest stages extreme wind storm events happen, it can damage and break the steam or, in worst cases, cause the death of plant. Beneficial impact can also result like a good balance of hormones. The moderate wind turbulence in surrounding environment creates a good supply of CO_2 levels to the plants resulting in larger photosynthesis rates (TNAU, 2013).

2.2.2.4 Sun Radiation

Light energy is an important factor in rice plant during all its stages (e.g. for seed germination, leaf expansion, stem growth and also influences nutrient assimilation) (FAO, 2004). If rice passes through extensive shade periods in maturation/ripening stage the grains will stop to be filled. On irrigates fields sun reflection helps to develop an optimum plant population and increase its yield production (IRRI, 2013c).

2.2.2.5 Water

Water creates the environment for rice cultivation, and it is responsible for many functions as regulation of temperature in soil and water surface, pest defence repelling pathogenic attacks and in flooded areas water increases heat capacity of soil (heat capacity of water is 23 times higher than the air) (IRRI, 2013c). The tallest of rice plant has direct relation with the water slide and it can make a big impact on plant hardness and mainly on panicle resistance.

Reduced availability of water during vegetative stage will delay the period of panicle initiation by 10 to 20 days (Lansigan *et al.*, 2000), consecutively increased the proportion of unfilled grains along the maturation process and can reduce 1000 times seed weight (Lansigan *et al.*, 2000). Water stress change morphological and physiological measurements like tiller number, photosynthetic rate, leaf area index, leaf nitrogen, roots and shoot biomass and density.

Positive multifunctionality from water management of flooding rice farming is possible. Next to rivers deltas, water released from the flooded rice area can be used in the downstream delta areas and it contributes largely for water quality and salinization control at the beginning of dry season (TNAU, 2013).

2.2.3 Chemical responses to stress factors

Plants produce a large variety of chemicals (Mateus, 2008). They can be divided in two metabolisms, primary – essential and direct involved on plant grow – and secondary ones – not related with primary functions and restrict to certain plant taxa involved on competition and reproduction functions (Dudareva *et al.,* 2013).

Stress in plants can be defined as any change in growth condition that disrupts metabolic homeostasis (equilibrium of plant functions) and requires process of acclimation (Spinelli *et al*., 2011). Stresses are rarely happen singularly, they often occur in combination. Responses to stress factors are made by secondary metabolism – emitting chemical odour signals to the atmosphere – usually called Volatile Organic Compounds (VOCs).

2.3 Volatile Organic Compounds

The VOCs are organic compounds that are gaseous in atmospheric conditions. They have a molecular weight, usually lower than 300 g/mol, and high vapour pressure at ambient temperature (upper than 130 Pa at 20 °C). The physical parameters of these compounds allow them to freely cross low atmosphere/troposphere (Dudareva *et al.,* 2013).

VOCs can be divided in anthropogenic VOCs (AVOCs) and biogenic VOCs (BVOCs), depending on the emission source. AVOCs can be released from two types of fonts, stationary and diffuse. The stationary ones, as fuel industries, domestic uses, sludge treatments on wastewater treatment plants and waste disposal sites, have almost the same environmental impact than diffuse ones, based on fossil fuel burned out motors. From biologic sources, BVOCs are released from diffuse sources as forests and agricultural fires (burning biomass), biochemical soil reaction, but the main source is focusses on the emission from vegetation (Schirmer and Quadros, 2010).

Dudareva *et al.* (2004) found that the formation of VOC can be regulated at the gene expression – VOCs biosynthesis normally occurs in the epidermal cells of plant tissues, or in glandular trichomes in secretory structures like found on plant genera *Artemisia annua* (peppermint) and *Ocimum basilicum* (Dudareva *et al.,* 2004). There are two types of VOCs in

plants: stored VOCs – volatized into the atmosphere by healthy unwounded plants; and induced VOCs – emitted hours or days after a stimuli or stress from both, stressed and undamaged plant leaves, having defenced and attraction functions (Spinelli *et al.,* 2011).

The major difference on emission of VOCs between plants depends on the species, developing plant stage, released plant organs (Rinne *et al.,* 2009) and quantities of a particular VOC mainly depends on the rate of its biosynthesis – availability of substrate, level of stress induced and energy provided by primary metabolism (Dudareva *et al.,* 2013).

2.3.1 VOCs main classes

VOCs are a very heterogeneous group of compounds including alkenes, alkanes, carboxilic acids, nitrogen-containing compounds and alcohols, and there are three main dominating group compounds named terpenes (including mono- and sesquiterpenes) and green leaf volatiles (GLV) (Spinelli *et al.,* 2011).

2.3.1.1 Terpenes

Terpenes are a class of unsaturated hydrocarbons from biological origin and can be named essential oils of plants (Khor and Uzir, 2010).

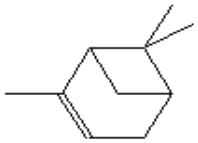
Terpenes constitute the biggest and varied biochemical class of secondary metabolites derived from two common C_5 five-carbon constituents, singular C_5 is named isoprene. Two separated pathways, mevalonic acid (MVA) and methyllerythritol phosphate (MEP) are involved in their synthesis (Dudareva *et al.,* 2013). MEP pathway links two C_5 units producing C_{10} – monoterpenes. MVA pathway originates three units of C_5 creating volatiles C_{15} – sesquiterpenes and four C_5 units forming C_{20} – diterpenes (Rinne *et al.,* 2009). Table 2.5 presents the combination of carbon and hydrogen atoms of each terpene and their chemical formula.

Table 2.5 – Isoprene units, carbon and hydrogen atoms and chemical formula of different terpenes

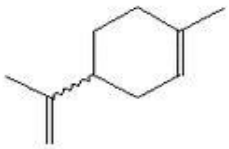
Terpenes	Isoprene units	Carbon atoms	Hydrogen atoms	Chemical formula
Monoterpene	2	10	16	$C_{10}H_{16}$
Sesquiterpene	3	15	24	$C_{15}H_{24}$
Diterpene	4	20	32	$C_{20}H_{32}$
Sesterpene	5	25	40	$C_{25}H_{40}$
Triterpene	6	30	48	$C_{30}H_{48}$
Carotenoid	8	40	64	$C_{40}H_{64}$

(Source: Coutinho *et al*., 2009)

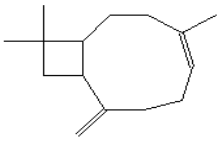
Mono- and sesquiterpenes are more commonly present in the oils, these two groups can be considered a separate classes, where sesquiterpenes are typically fragrances emitted from flowers, and other ones as iso- and diterpenes are usually associated with balsams, resins, waxes and rubbers (Khor and Uzir, 2010; Spinelli *et al.,* 2011). Essential oils normally present polycyclic structures (Fig. 2.10) with C=C double bounds. To date, 55 000 terpenes have been isolated (Khor and Uzir, 2010) and the trend is doubling each decade.



α pinene
$C_{10}H_{16}$



limonene
$C_{10}H_{16}$



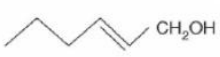
caryophyllene
$C_{15}H_{24}$

Fig. 2.10 – Examples of mono – and sesquiterpes polycyclic structures (source: NIST 08, 2013)

2.3.1.2 Green Leaf Volatiles

Most of the plants have a similar response to artificial damage, such as wounding, drying or freezing (Harren and Cristescu, 2013). Physiological relation was established with the rapid formation of C_6 (six carbon compounds), so-called green leaf volatiles (GLVs). These compounds are produced *via* the lipoxygenase (LOX) pathway and are responsible for 50% of the emissions, within a few seconds, from plant parts after damage (Harren and Cristescu, 2013). Chemically, GLVs are mostly saturated or monosaturated aldehydes, alcohols and esters (Legendre *et al.,* 1978). Table 2.6) shows the name and structure of main GLV.

Table 2.6 – Structure of main Green Leaf Volatiles

Name	Structure
n-hexanal	
n-hexanol	
trans-2-hexenal (leaf aldehyde)	
trans-2-hexenol	
cis-3-hexenal	
cis-3-hexenol (leaf alcohol)	
trans-3 hexanal	
trans-3 hexanol	
cis-3-hexenyl acetate	

(Source: Mateus, 2008)

2.4 VOCs study

The characterization of volatile fraction emitted by plants is a "dynamic process" sequenced by VOCs extraction, analysis and identification. Presents section explains the main techniques used.

2.4.1 Extraction techniques

Solid phase microextraction (SPME) is an efficient extraction technique for plant VOCs collection introduced by Janusz Pawliszyn, in the early 1990's (Zini *et al*, 2002). Several studies have been reported that applied the SPME method due to its high sensitivity, solvent free sample preparation, non-invasive, rapid operation and small amount of sample needed (Maes *et al*., 2001; Zini *et al*, 2002; Mateus, 2008; Santana, 2009; Zeng *et al*., 2011).

The heart of the SPME technique is a fibre coated by an adsorbent/absorbent polymer which extracts the analytes, due physical interactions. The extraction of analytes can be performed on liquid or gaseous phases. The fibre is attached to a stainless steel pluger and is assembled on a syringe-like holder (Mateus, 2008). Fig. 2.11 illustrates constituent parts of a standard SPME system.

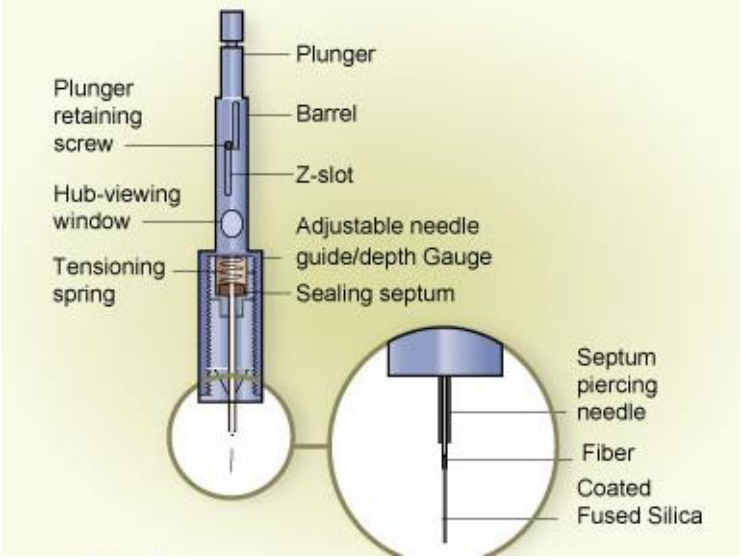


Fig. 2.11 – Constituent parts and detail of a standard SPME system (Source: Chromedia, 2013)

For efficient extraction the analytes must have an affinity to SPME fibre. The principle of SPME technique is based in the equilibrium establishment between the analytes and fibre (Zeng *et al*., 2012). Optimization and reproducibility of the extraction can be achieved by controlling the polarity and thickness of the coating of the fibre and parameters such as operational temperatures, stirring, and extraction time (e.g. constant sampling time for all extractions) (Chromedia, 2013).

The selection of a fibre coating depends on factors that promote the best efficiency of adsorption/absorption process, such as molecular weight and analyte size, analyte's and fibre polarity (Chromedia, 2013). The most used fibres, considered as having a good efficiency for headspace VOCs extraction, are polydimethylsyloxane (PDMS) and polyacrilate (PA) (Parreira and Cardeal, 2005). In order to improve the efficiency of SPME, some mixed coatings, with complementary characteristics from PDMS and PA, have been developed to produce more selectivity of certain compounds and stronger retained analytes (Parreira and Cardeal, 2005) (see recommended applications of usual polymer coating fibres in annexes T2).

Steam distillation extraction (SDE) technique is also a good method for achieving the extraction of volatile compounds from solid matrices (Parreira and Cardeal, 2005). It involves the steam distillation of plant materials, with sequential trapping of the volatiles in the solvent. The solvent used must be immiscible with water in order to achieved two phases (Godefroot *et al.*, 1981).

2.4.2 Analytical techniques

Gas chromatography (GC) is an effective separation method for the qualitative and quantitative VOCs analyses. The inherent principle behind GC techniques is based on the partition of the analytes between an inert carrier gas, the mobile phase, and a solid or liquid stationary phase, that is placed inside the column (Mateus, 2008).

GC mass spectrometry (GC/MS) technique is used for separation, analysis and identification of volatile and semi volatile compounds. The sample molecules after eluting from analytical columns enter into a vacuum chamber where they are ionized in the ion source. The resulting ions are then separated depending of their *m/z* (mass to charge) ratio in the mass analyzer and processed into detector. Detector produces a total ion chromatogram and mass spectrum for each peak with intensity of ions *vs.* their *m/z* (Mateus, 2008).

There are four injection techniques in GC: split, splitless, direct and on-column injection. The first two techniques (see results comparison in annexes F1 and F2) are the most used. Split mode is used for high sample concentrations where a portion of sample is discarded during the injection process through to split vent (RESTEK, 2013). The splitless mode, where all the sample is introduced to the column, is used for trace level analysis (Mateus, 2008). Sample polarity and temperature limits of compounds elution are important concerns to take into account for correct choice of the injection mode (RESTEK, 2013).

In the GC technique, the column plays the most important role. The principle is that analytes like to interact with stationary phase of a similar chemical nature (Agilent Technologies, 2013) (main stationary phases are presented in annexes T3). The capillary GC columns, made by inert fused silica material, provide very good separation efficiency (narrowed and separated peaks) with lower sample amounts, short column length and brief runs (Mateus, 2008). Around 90% of separation problems can be resolved by doing the separation using columns with different

phase polarities (e.g. polar and non-polar) (Mateus, 2008).

Film thickness also influences retention and maximum operation temperature of the column. Thick films (1 to 5 µm), promoting higher residential time in the stationary phase, are used for the separation of compounds with higher volatilities, due to better peak resolution (Agilent Technologies, 2013). Fig. 2.12 illustrates whole GC/MS system process.

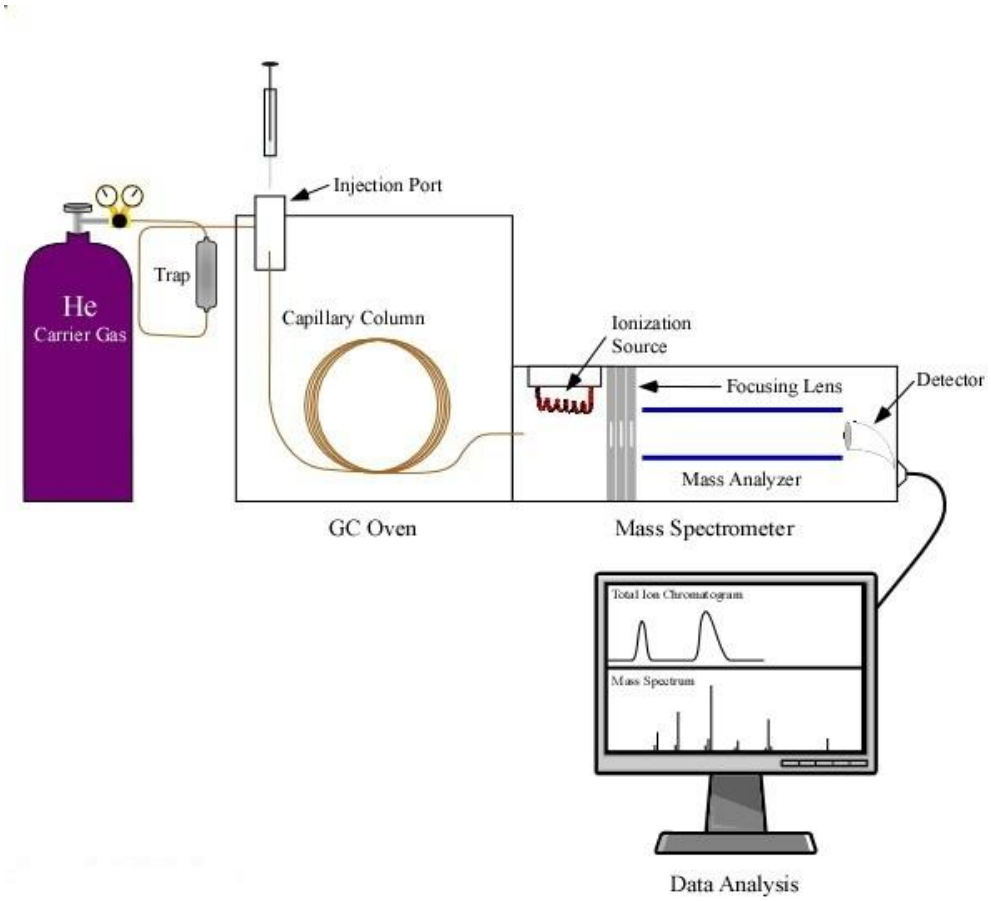


Fig. 2.12 – GC/MS equipment layout (Source: Ginsbach *et al*., 2010)

Different methods have been used for trace gas detection and analysis of rice plants. Instead of GC/MS analyses, proton transfer reaction – mass spectrometry (PTR-MS) has enable monitoring VOC emissions in a more comprehensive way, detecting stable isotopes from various chemical groups, with online real-time capability to distinguish, in the order of seconds, at (sub)part per billion levels, isoprene molecules (Rinne et al., 2009; Harren and Cristesu, 2013).

Gas chromatography – olfactometry (GC-O) uses human nose as a detector and can be employed to determine whether a particular volatile, eluting from GC, has aroma activity (Mahattanatawee *et al.*, 2005). Technique sensitivity depends on the experience from the professional and on the different concentration levels of VOC (Mahattanatawee *et al.*, 2005).

Recent studies also applied the electric-nose (E-nose) technique for detection of VOC profiles. This technique is more used for studying different degrees of insect damage and storage time. The E-nose system do not detect individual compound, although, it is used to identify a wide range group of volatiles, with rapid, sensitive, non-destructive and easy management (Zhou and Wang, 2011). The big advantage of this technique is the efficient discrimination and prediction power without requiring any specific tuning or refinements for optimizing performance (Zhou and Wang, 2011).

2.4.3 Rice volatile profile studies

Zhou and Wang (2011) used the Electronic-nose multisensory technology (E-nose) and GC/MS to discriminate between volatile profiles, emitted from infested and uninfested rice plants by *Nilaparvata lugens*, an herbivorous insect common named brown planthopper, and for VOCs identification. In total they identified 23 VOCs emitted by infested plants, and that amount was 3.37, 3.36 and 6.57 times larger than in uninfected control rice. Fig. 2.13 shows the growing trend of VOCs intensification with ascendant number of insects in rice samples, during first two hours after infestation.

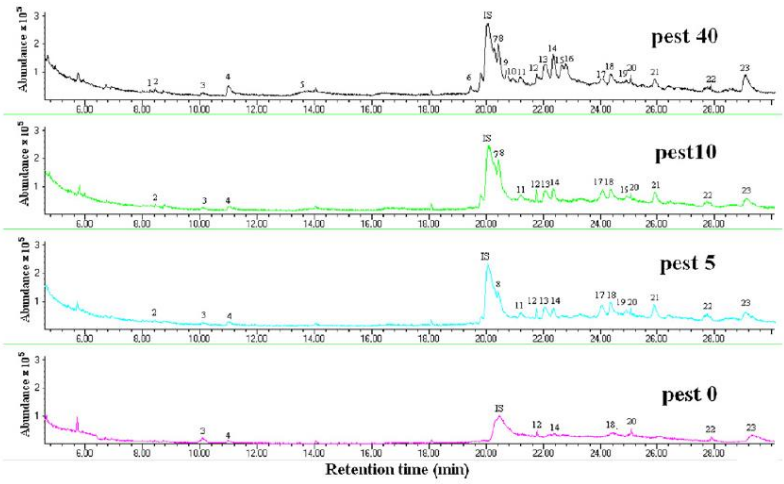


Fig. 2.13 – VOC profiles collected from un- and infested rice plants samples; pest 0 (without infestation); pest 5 (five insects); pest 10 (ten insects) and pest 40 (forty insects) (Source: Zhou and Wang, 2011)

Yuang *et al.* (2008) studied the rice plant defence against *Cotesia marginiventris*, a rice parasitoid, using SPME followed by GC/MS analysis technique, found a total of 28 volatile compounds, where 11 were terpenes. The most abundant compound was linalool and a few sesquiterpenes as zizimbrene and sesquipellandrene. After stress stimuli, plant released just few unclear peaks and breakthrough the time a large number of mono- and sesquiterpenes are came out.

VOCs emission from unprocessed rice grain was study to prove interaction/attraction between *Oryza sativa L.* and rice field rats (*Rattus rattus mindanesis) (*Bullard and Holguim, 1977). A total of 73 compounds were identify, mostly alcohols, aldehydes, ketones and terpenes.

Yatsumatsu *et al.* (1966) in Maga (1984) studied VOCs associated with cooked rice and found out a range of monoterpenes, including limonene and cymene.

A large range of GLV was found by Legendre *et al.* (1978) in commercial rice grain.

Buttery *et al.* (1983) in Maga (1984) identified aldehydes and alcohols in scented rice without any application of induced stress factors.

Zeng *et al.* (2011) investigated flavour components from rice bran (a by-product of rice milling) and a total of 43 out of 76 compounds were identified belonging to esters, alkanes, alcohols, ketones, aldehydes and fatty acids.

Studies were performed to measuring other forms of rice emissions, i.e. parcels of rice constitution in other products. As an example, volatile flavour of commercial rice cakes and also from backed in laboratory (differing in proportion of brown rice grain and wheat flour pastas added) were studied by Butery *et al.* (1999). In total they found 60 compounds, the major compositions were aldehydes and ketones.

VOCs emissions from soil textures were also studied. Wheatley et al. (1996) used GC/MS and identified a total of 35 VOCs in the headspace of a silty clay soil (without testing any plant growth). They concluded that VOCs are depending from nutrient addition into soil. Bastos and Magan (2007) used E-nose system to identify VOCs composition under different temperatures and water potential, between three soil textures loamy sand, calcareous clay and volcanic ash. Most of VOCs were alcohols, ketones and aldehydes. Their conclusions point that identification of volatile profiles responses can help to understand some cause – effect dealings between agricultural crop areas (with different soils textures) and environmental stresses.

2.4.4 VOCs specific meanings and aroma correspondence

VOC profiles collected from agricultural field system possess all necessary condition to be translated into cause-effect relations of plant defence against external abiotic or biotic stress (Dudareva *et al.*, 2013).

Table 2.7 describes the assumed meanings of main representative VOCs found in natural systems (Michael *et al.* (1978) in Maga, 1984; Rinne *et al.,* 2009; Harren and Cristescu, 2013; and Dudareva *et al.*, 2013). All aroma descriptions are based in assumptions from the study performed by Mahattanatawee *et al*. (2005).

Table 2.7 – Assumed meaning and aroma descriptor of main VOCs

VOCs	Assumed meaning	Reference	Aroma descriptor
3-hexenol	After wounding or physical damage has antibiotic properties and inhibit the invasion of bacteria and other microorganisms into damage tissues – Defence	Harren and Cristescu, 2013	Green fruity
2–hexenal	Precursor in the degradation process of plant – storage condition	Michael *et al.*, 1978 in Maga, 1984	Fruity
β–caryophylene	Improve plant defences below-ground attracting entomopatogenic nematodes that kill root pests – Defence	Dudareva *et al.*, 2013	Fruity warm floral
α-copaene	Attract male Mediterranean fruit flies – Intra – specie interaction	Maes and Debergh, 2003	-
limonene	Produced by storage pools in leafs is related with photosynthetic efficiency/yield – environmental condition	Rinne *et al.,* 2009	Fruity floral lemonate
linalool	Attract predatory mites that kill other upper leafs herbivores – Defence	Dudareva *et al.*, 2013	Sweet floral
n-octanal, *n*-nonanal and *n*-decanal	Contribute for pollinators attraction – Intra – specie interaction	Dudareva *et al.*, 2013	Meaty oily and metallic green herbal

2.5 VOCs, rice and climate change scenarios

This section describes a correlation between VOCs influence on air chemistry and behaviour of rice VOCs emission in climate change scenarios prediction.

2.5.1 VOCs influence on air chemistry

VOCs are considered one of the major precursors of photochemical oxidants in atmosphere, e.g. ozone (O_3) and peroxyacetyl nitrate, in presence of sunlight (equation 1.1).

Oxygen + VOCs + sunlight = tropospheric ozone (equation 1.1)

Warmer and sunny locations tend to net formation of tropospheric O_3 (Hobbs *et al.*, 2004). In human population the main complains have been recognized as eye, nose, throat irritation and potential mood or memory effects. Increasing ozone concentrations in troposphere, besides being the big component of smog, can also cause stress to vegetation, in case of agriculture is visible in crops yield welfare (Hobbs *et al.*, 2004).

The average concentration of tropospheric O_3 level had doubled in the past 100 years (NASA, 2013), and there is a tendency of increasing peak concentration events. The World Health Organization (WHO) established the quality standard limit of O_3 concentration in 100 µg/m^3 (measured in 8 hours average). But this legislation limits were frequently exceed, at least in 2003 and again in 2006, for more than 50% of European urban population (Steinbrecher *et al.*, 2009).

Upper-left quadrant in Fig. 2.14 shows VOCs participation in aerosol growth and formation process, named secondary organic aerosol (SOA) with Dp < 100 nm (Matsumoto, 2013).

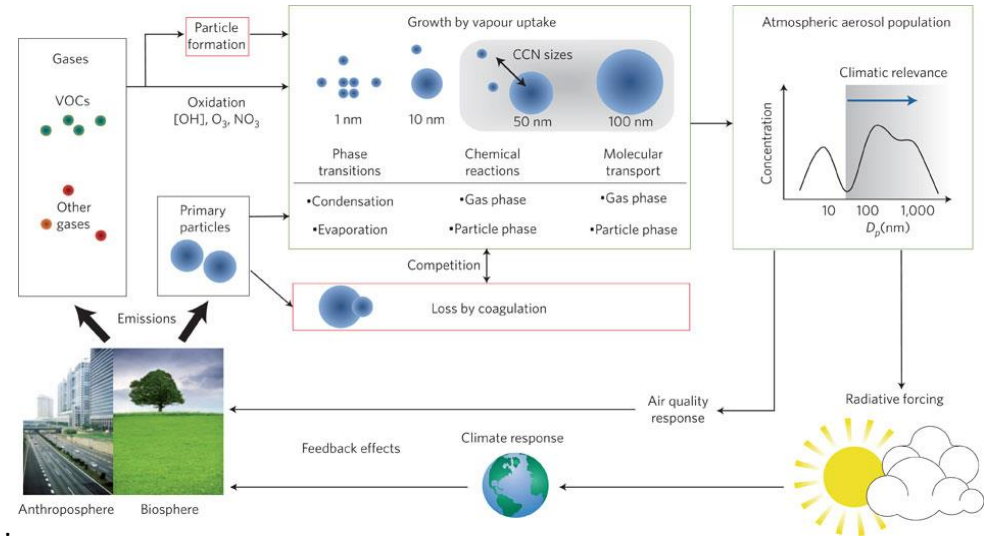
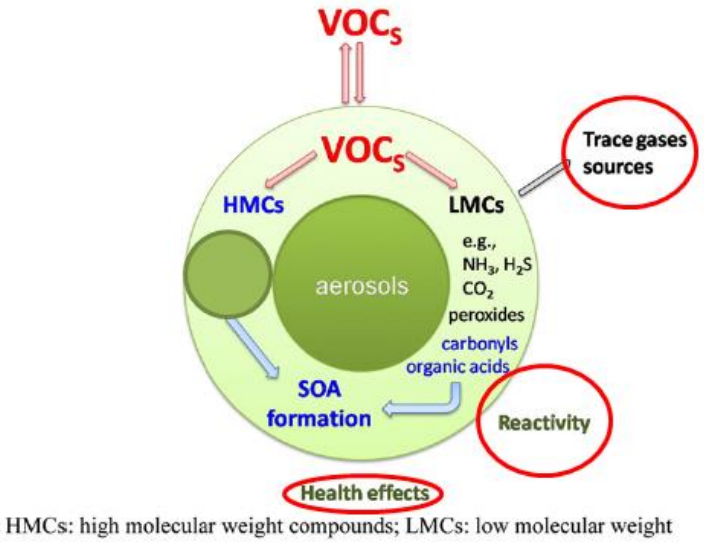


Fig. 2.14 - Relation between VOC sources emission, molecular size and climate reaction (source: Riipnen *et al.*, 2012)

VOCs react with O_3, hydroxyl (OH_x) and nitrate (NO_x) radicals, as the reaction products have lower volatility and thus condense into aerosol particles. O_3 can be abundant during both day and night (Riipnen *et al.*, 2012). VOCs also compete with methane (CH_4) for OH_x. Have influence on CH_4 lifetime and concentration due to the high reaction coefficients (Matsumoto, 2013).

The BVOCs are more reactive in atmosphere than AVOCs (Rinne *et al.,* 2009). Little research has been performed to define mass or type of chemical species originating from agriculture and livestock emission. Heterogeneity of low molecular weight (LMW) propriety (Fig. 2.15) has a direct impact on transportation and transformation of VOCs, e.g. they contribute to 40 to 60% of OH_x loss associated with VOCs in the clear air and more 20% in polluted air (Chen *et al.*, 2012). The VOCs and their products can enter in human body by human breathing and skin exposure. Depending on concentration level, human exposure can cause health problems, and in the worst case, provoke cytotoxicity in lung cells, encouraging lung cancer (Sheng *et al.*, 2013).



HMCs: high molecular weight compounds; LMCs: low molecular weight compounds

Fig. 2.15 – Low molecular compounds and their reactions (Addapted from: Sheng *et al.*, 2013)

2.5.2 VOCs behaviour in atmosphere

In high pressure weather systems, VOCs are more reactive in the atmosphere, with a pronounced emission during the summer months, which have higher probability of low wind speed, high insolation and higher NO_x levels (Steinberg *et al.,* 2009).

Air quality studies have been developed thought the years. Steinberg *et al.* (2009) used modeling techniques to estimate VOCs emission from European vegetation (10 km by 10 km) for an extended area of $22{,}56\times10^6$ m^2 for the years 1997, 2000, 2001 and 2003 (Fig. 2.15). They

divided VOCs by class and concluded that emission are dominated by forest areas, with isoprene being the most abundant category, followed by almost similar distribution of monoterpenes, oxygenate VOCs (OVOCs) where GLV category is included, and sesquiterpnes only contributed with 1-2% of the total VOC emitted.

Fig. 2.16 shows a strong dependence on the annual cycle of temperature and light in North hemisphere, with main emitting months coinciding with the period from May to September. In addition, the most of vegetation also change the emission-active surface throughout the year, with higher area surface during summer, and in consequence, most of the emissions are observed in the leaf-on phase of the year (Steinberg *et al,* 2009).

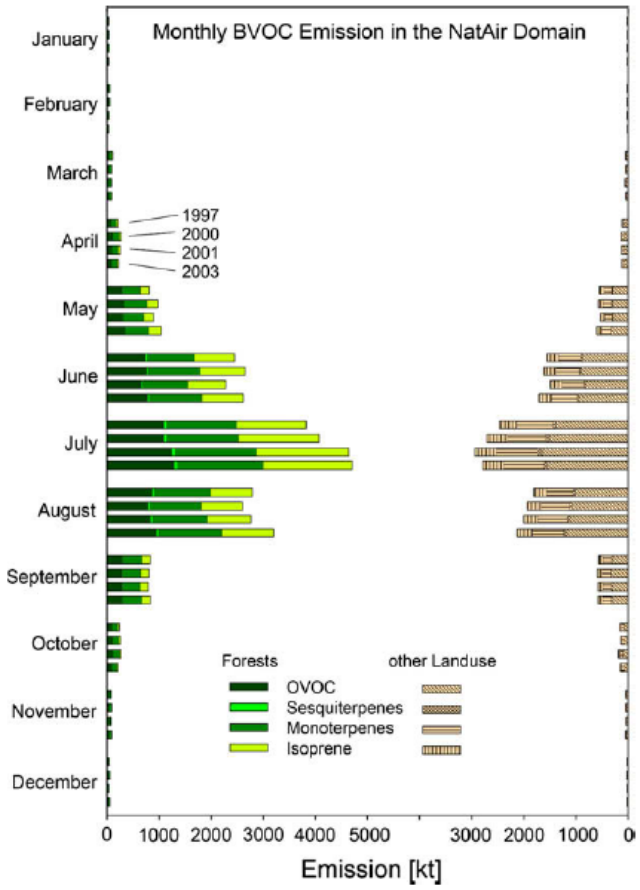


Fig. 2.16 – Monthly estimation of isoprene, monoterpenes, sesquiterpenes, and OVOCs emissions from European vegetation. Other land area uses include all categories excepted forest (Source: Steinberg *et al.,* 2009)

Steinberg *et al.* (2009) emission modelling study also concluded distinct diurnal variations of isoprene and monoterpenes emissions. Isoprene reveals higher peaks emission during mid-day, as monoterpenes, but stops during the night and monoterpenes continue in a smaller rate. Monoterpene emissions occur from stored pools that continue their activity in darkness night environments (see F3 in annexes).

Only about one third of angiosperm species tested, including rice, emit isoprene at vestigial rates (Spinelli *et al.,* 2011). To date incomplete or very limited knowledge are well-known about the release of VOCs from plant tissues, due to not only physical properties – their volatility and polarity – but also to cytological organized process (Hobbs *et al*., 2004).

According to Spinelli *et al.* (2011), some general aspects about isoprene behaviour emission in response to abiotic stress, should be retained:

- ✓ Isoprene synthesis is dependent on photosynthesis, and on the other hand photosynthesis has high dependence of CO_2. It is proved that isoprene emission can be 50% less under elevated CO_2 concentration. But if O_2 is removed, the isoprene emissions stops.
- ✓ Isoprene emission is affected by N nutrition. It means that plants with low N nutrition have lower rates of isoprene. However, plants with higher N availability emitted more isoprene when grown under sun radiation than grown in the shade;
- ✓ Isoprene emission is very sensitive to temperature. It plays an important role on thermotolerance of plants (protection against short high temperature episodes);
- ✓ Low influence on isoprene emission by water – stress, but if several drought affects plant for substantial period of time, isoprene emission is increased;
- ✓ Same as in drought effect, the rate of emission depends on the level of stress caused.

2.5.3 Air quality, climate change scenarios projections and rice VOC emissions

Atmospheric chemists are interested in the origin of air pollution and its consequences (Maes and Derbergh, 2003). There is big pressure on EU member states to reduce emissions to atmosphere from agricultural sources (see T4 in annexes), as defined by UNECE Gothenburg Protocol (UNECE, 2012), the EU National Emission Ceilings Directive (see annexes T5 about Directive 2008/50/EC, 2008) and the Kyoto Protocol.

European Environmental Agency (2004) explored targets of Kyoto Protocol (Fig. 2.17) and revealed positive evolution of VOCs emission (including BVOCs and AVOCs). The analysis was concentrated in the uses of Kyoto mechanisms (air pollution policies control) under three different scenarios using RAINS model – Regional Air pollution Information and Simulations, which included emission reduction from Kyoto strategies, costs and environmental effects of emission control policy actions, taking into account the role of atmospheric dispersion proprieties. Fig. 2.17 shows a progressive change in emissions in 2010 when compared with 1990: the reduction of 44% in all Europe, including Western (-54%) blue colour, Central (-22%) orange and East Europe (-26%) green line (EEA, 2004).

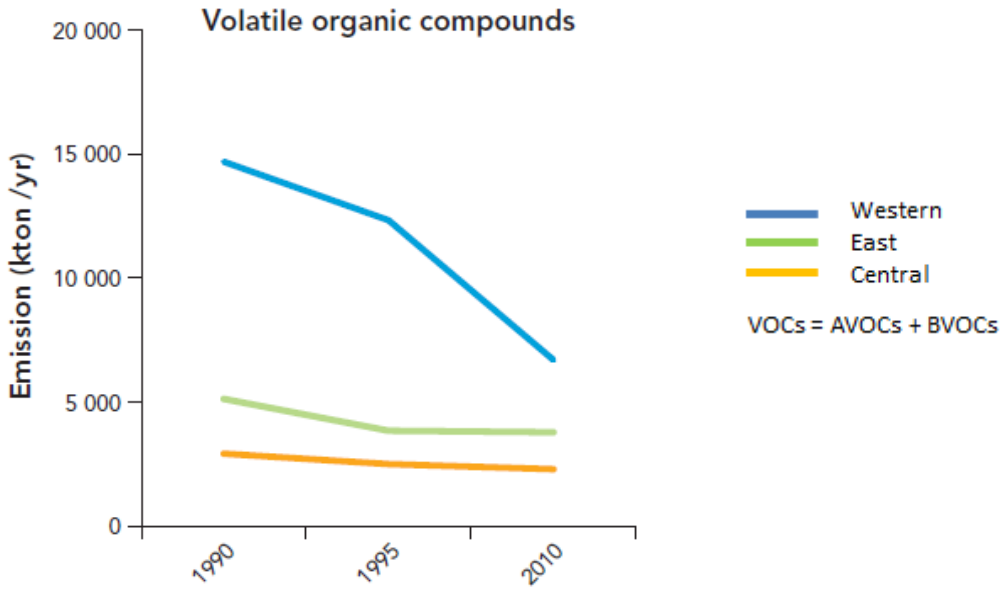


Fig. 2.17 – VOCs emission reduction in Europe between 1990 and 2010 (Source: EEA, 2004)

Table 2.8 indicates the ranking of four main EU VOCs (BVOCs and AVOCs) emitter countries (for entire ranking see T5 in annexes).

Table 2.8 – Main VOC emitter countries in EU according with the UNECE Protocol

Ranking	County	VOCs emission in 2005 (10^3 tons/year)
1st	Italy	1286
2nd	France	1232
3rd	Germany	1143
4rd	United Kingdom	1088

(Adapted from: UNECE, 2012)

From the beginning of 21st century, new issues are pumping out from research scientific community: "If we do not understand what is going on with biogenic (plant – produced) VOCs, we are not going to be able to weigh different air-quality strategies properly" (Purves in Schultz, 2004).

Steinberg *et al.* (2009) carried out air quality studies, at on the European scale, and concluded that countries with highest emission of BVOCs per ground area (including not only isoprene measurements, but also monoterpenes, sesquiterpenes and OVOCs) were Portugal, Spain and Greece. They found a correlation with the Mediterranean highest vegetation emitter species,

such as oaks, eucalyptus, aromatic plants, like rosemary and thyme and interpreted that higher amount of compounds in river valleys (F4 in annexes). Other investigations found also a high BVOCs peak emission in European boreal zone/taiga, due to dominant vegetation of coniferous forests consisting in pines, spruces and larches (Rinne *et al*., 2009). Rinne *et al.* (2009) conclude that isoprene emission from open wetland ecosystems per land area can be of the same order of magnitude than emission from coniferous forests. These authors assume that the modelling results did not include feedback interactions from steadily increasing CO_2 concentrations on the atmosphere neither abiotic nor biotic stresses.

Fig. 2.18 shows the importance of the need of field species VOCs measurements in order to better predict results from air quality modelling efforts about emission influence factors. These approaches of analysis, expression, validation and prediction are a present trend for scientific community research.

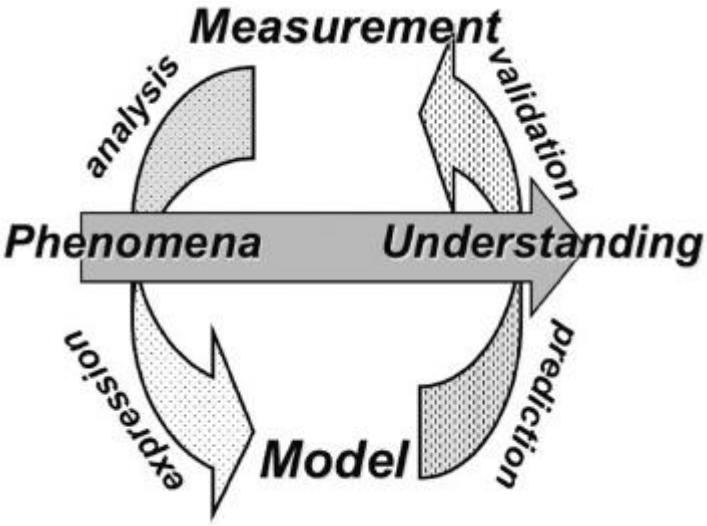


Fig. 2.18 – Complementation methods for including measurement studies and model study to understand real phenomena and predict future situation (Cho and Oki, 2012)

There is a higher temperature trend accepted for the future (see annexes F5 to understand temperature trend until 2012). The projection based climate modelling scenarios from Intergovernmental Panel on Climate Change (IPCC, 2013), set the warming average temperature for the near future from 2014 to 2030, compared to 1980 – 1999 period, in the worst scenario case, at + 0.85 °C. Between 2031 and 2070 projection shows + 3.10 °C and, by the end of 21st century, among 2071 to 2099, temperature will be + 4.49 °C. Table 2.9 shows the impact projections.

Table 2.9 – Main conclusions of the IPCC scenarios prediction

Impact of climate change and direction of trend	Probability of trend[a]	
	Recent decades	Future
Warmer and fewer cold days and nights over most land areas	Very likely	Virtually certain
Warmer and more frequent hot days and nights over most land areas	Very likely	Virtually certain
Frequency of warm spells/heat waves increases over most land areas	Likely	Very likely
Frequency of heavy precipitation events increases over most land areas	Likely	Very likely
Areas affected by drought increase in many regions	Likely	Likely
Intense tropical cyclone activity increases in some regions	Likely	Likely

[a]Probability classes: Likely > 66% probability of occurrence
Very likely > 90% probability of occurrence
Virtually certain > 99% probability of occurrence.

(Source: IPCC, 2013)

Climate change has many facets, including changes in long-term trends in temperature, rainfall regimes and atmospheric gases concentration. Higher air temperature will increase sea level due to the thermal expansion of sea water and rapid melting of glaciers and ice caps. Effects on greater rice productive deltaic cultivation areas will be more unprotected to inundation and salinity intrusion (IPCC, 2013).

Table 2.10 shows the projections for rice production outcomes during the next decades for three category groups of TWB classification scheme (TWB – Country and Leading Groups, 2013), where authors (Nelson *et al.*, 2010) calculated their percentage based on two scenarios: perfect mitigation of climate change effects and under influence of climate change, between 2010 and 2050. The baseline scenario (red circles) assumed standard economic, social and environmental conditions like we are globally having today. Nelson *et al.* (2010) assumed the highest trend of increasing rice production under climate change mean scenario and pessimistic case (low economic grow and high population growth tendency, with continue GHGs increasing concentration due to tropospheric ozone formation). On the other hand, under optimistic conditions (with good and equal economic evolution situation and low population grow), the rice production slowed down and decreased over the years.

Table 2.10 – Scenario results for rice production over the three economical categories group countries

Crop & category	2010 (mmt)	2050 (mmt)	2010-50 increase (%)	2010 (mmt)	2050 (mmt)	2010-50 increase (%)	2010 (mmt)	2050 (mmt)	2010-50 increase (%)
	Pessimistic			Baseline			Optimistic		
Rice									
Developed									
Perfect mitigation	18.8	20.7	9.9	18.8	19.9	5.6	18.9	19.1	1.2
Climate change mean	18.1	18.2	0.6	18.1	17.6	-3.2	18.1	16.8	-7.6
Developing									
Perfect mitigation	388.0	453.4	16.8	388.4	433.4	11.6	388.7	418.1	7.6
Climate change mean	382.1	418.1	9.4	382.3	398.1	4.1	382.8	385.6	0.7
Low-income developing									
Perfect mitigation	81.5	108.2	32.8	81.6	103.5	26.8	81.7	98.6	20.7
Climate change mean	81.0	104.8	29.3	81.1	100.2	23.5	81.2	95.1	17.1
Middle-income developing									
Perfect mitigation	306.5	345.1	12.6	306.8	329.9	7.5	307.0	319.5	4.1
Climate change mean	301.0	313.3	4.1	301.1	297.9	-1.1	301.7	290.5	-3.7

(Adapted from: Nelson *et al.*, 2010)

Rice production is increasing only on single digits (red narrow pointing up), more emphasis are going to low-income developing countries, with more than 6% of growth (Nelson *et al.*, 2010), where some countries with high rice production are included (as Bangladesh, Vietnam and Southeast Asia countries. However, for rising these percentages there will be concern about water scarcity related to irrigate systems for rice crop production (Nelson *et al.*, 2010).

Having in mind abiotic stress conditions, progressive population growth and rice demand, it should expect the negative impacts between these interactions, resulting on decreased of rice yields and increased susceptibility of plant sterility. A good point among the negative projections is the opportunity for rice genome improvement mechanism (Nelson *et al.*, 2010). It will help to reduce losses related with abiotic stresses temperature tolerance, as well as drought, submergence and over salty environmental concentration (Mahonty, 2010).

In 2002, FAO, the West African Rice Development Association, National Agricultural Research Systems in sub-Saharan Africa, and many other partners got involved on the creation of African Rice Initiative promoting the use of New Rice for Africa (NERICA) in upland rice production systems. NERICA varieties have greater tolerance to water scarcity (FAO, 2004).

The growth trend of rice culture will cause impact on the environment, like changing water regime, soil characteristic, N cycle, and other effects. We would also like to identify variation on rice VOCs composition, variation trends and the air quality effects. As referred in previous section *2.1.1 Global status,* more than 166 million ha of global earth terrestrial surface are belonging to rice field cultivation, which should represent concern.

3. Materials and Methods

3.1 Site description

A study of VOCs emission from Portuguese rice paddy fields was carried out during the period between May and October 2012, comprehending the whole life cycle of rice plant (*Oryza sativa* L. *cv.* Aríete). Site study were located in COTArroz fields in *Paul de Magos (Lat. 39º 02' 21, 40''N*, Long. 8º 44' 25, 98'' W) *Salvaterra de Magos*, *Santarém* district part of Tejo river valley, denominated region *Lisboa e Vale do Tejo* (Fig. 3.1).

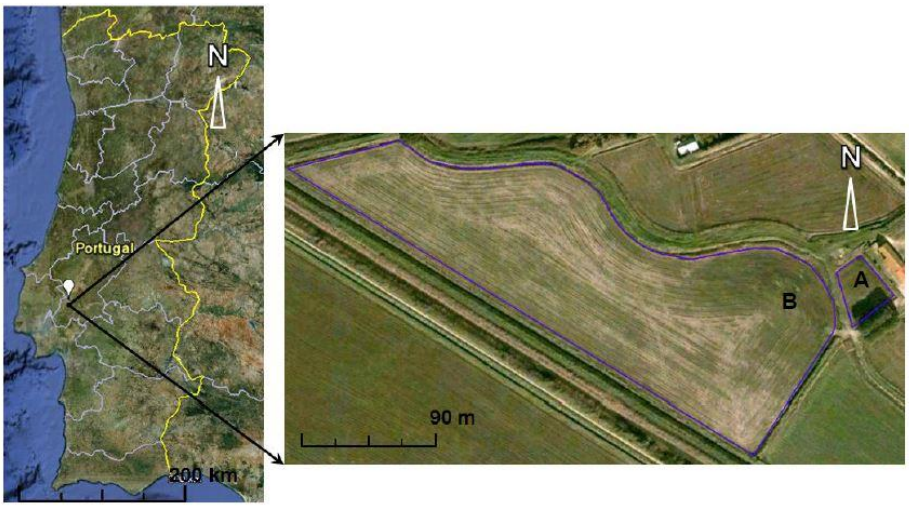


Fig. 3.1 – Location of field site; (right side) COTArroz plots (Source: Figueiredo, 2011)

The sectioned area is characterized in terms of soil characteristics as Fluvisols, from semi-bodied tertiary soil deposits (FAO, 2007). Fig. 3.2 shows COTArroz partial landscape.

Fig. 3.2 – COTArroz partial landscape

3.1.1 Culture practices

Fig. 3.3 presents rice culture practices (since May to October 2012) conducted by COTArroz and detailed growing phases. Seeding was made at 23rd May and harvested at 13rd October.

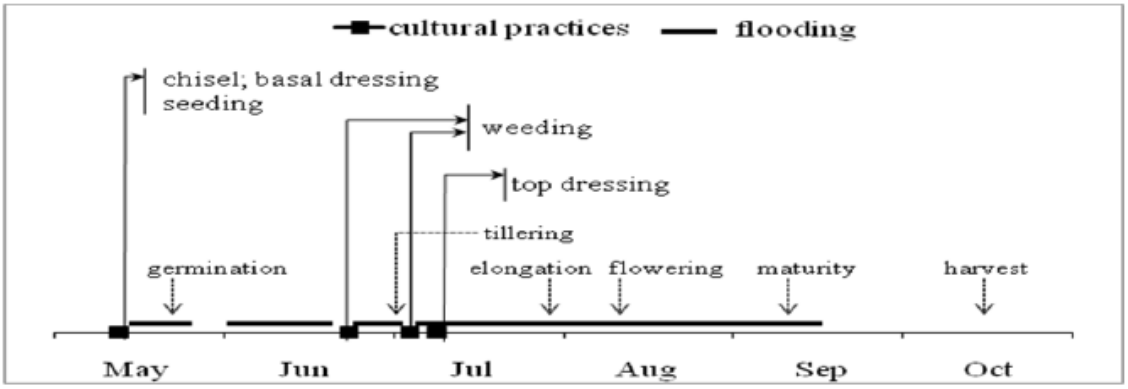


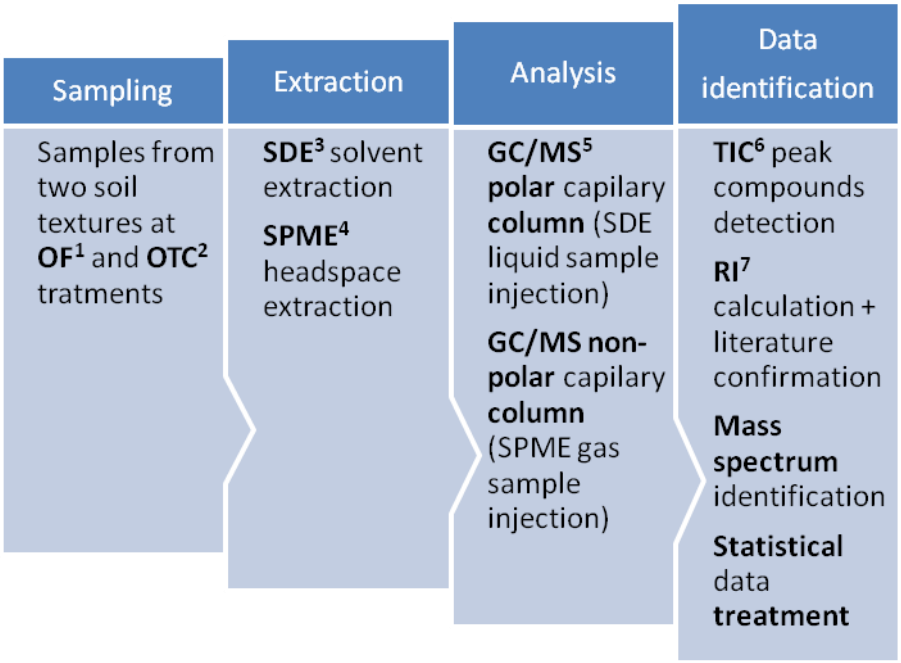
Fig. 3.3 – Culture pratices dates and flooding period (Source: INIAV, 2013c)

Fig. 3.4 shows rice field aspect at after seeding, weeding and before harvested moments.

Fig. 3.4 – COTArroz field aspect after seeding (a); weeding (b); before harvested (c)

3.2 Experimental design

Fig 3.5 presents the layout of whole experiment (from rice sample collection to VOCs qualitative identification).



[1]open field; [2]open top chamber; [3]steam distillation extraction; [4]solid phase micro extraction; [5]gas chromatography coupled to mass spectrometry; [6]total ion chromatogram; [7]retention index

Fig. 3.5 – Experimental design layout

3.2.1 Sampling

Samples were collected from two distinct soil plots A – Loamy sand and B – Silty clay (right side Fig 3.1). In loamy sand soil, three OF arbitrary zones were installed with area of 3.0 m^2 (1.5 m x 1.2 m) and 0,5 m between each sampling zone (Fig 3.6 a). In silty clay soil were installed also three OF areas, and six OTCs (Fig 3.6 b) covered by polyethylene film material with approximate area of 12 m^2 (4 m wide x 3 m high) and 2 m open top hole diameter, three with induced temperature and other three with simultaneous temperature and CO_2 concentration enhancement. In addition, a controlled plant experiment with both types of soil textures was conduct in separated vases (Fig 3.6 c).

Fig. 3.6 – Data collection layout in COTArroz, Salvaterra de Magos, 2012, in OF soil plot A (a); soil plot B OTC (b); plots under controled conditions (c)

Fig. 3.7 outlines the method scheme for sampling at silty clay. Three OTCs with induced temperature symbolized with numbers 1, 3 and 6, three others OTCs with temperature and CO_2 enhancement are numbered 2, 5 and 7 and three OF areas with 4, 8 and 9.

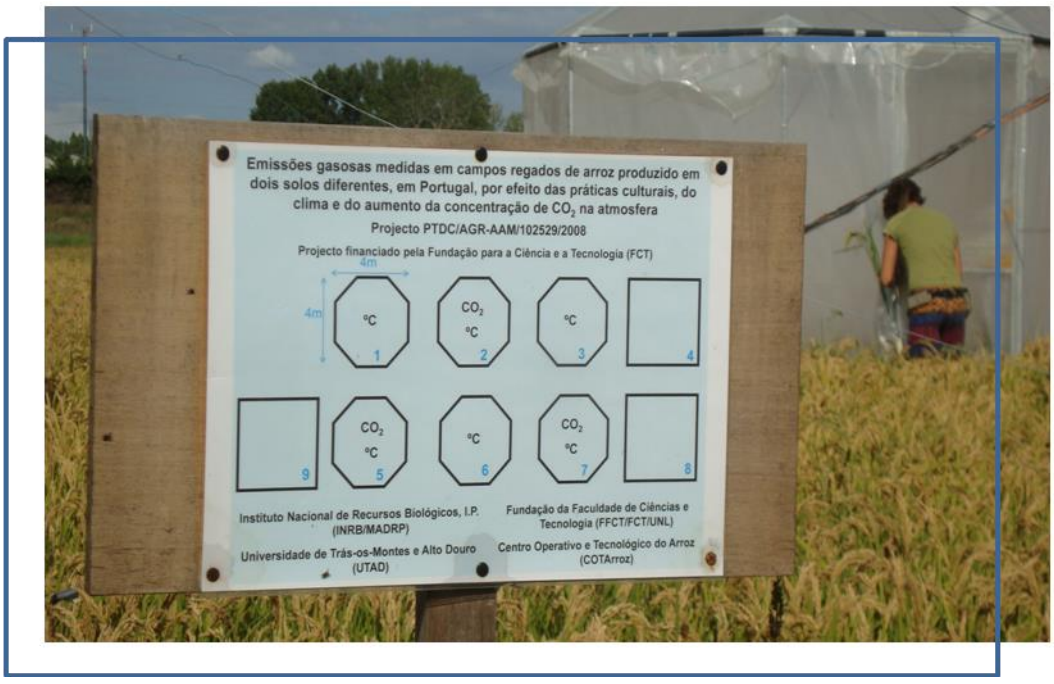


Fig. 3.7 – Sampling scheme at silty clay soil

The plant samples were preferably collected during the early p.m. daily period. The sampling units from plots A and B were made selecting 4 to 5 whole plants. After plant material collection the samples were transported to FCT laboratory in a short period of time.

Table 3.1 presents the field data sampling resume, considering TN – OF loamy sand; TE – OF silty clay; TE_c – OTC with increased temperature, and TE_{cc} – OTC with temperature and CO_2 enhancement. Rice plant completes its cycle within the mean duration days of irrigate rice paddy fields, between 150 – 180 days (Aparroz, 2009). At the beginning of the field sample collection (4th June, 2012), in TE_c and TE_{cc}, some unexpected occurrence did not allow healthy plant development. Rice plants from TE were relocated into chambers at 9th July.

Table 3.1 – Field sampling data resume

Treatments	Rice cycle (days)	Sampling (days)	Nº of samples each day	Average and standard deviation temperature* (ºC)	Average and standard deviation [CO_2]* (ppm)
TN and TE	158	6	6	20,1±2,1	375,4±38,5
TE_C		5	3	22,8±2,3	398,1±33,4
TE_{CC}		5	3	22,0±2,2	547,3±65,7

*Data provide by COTArroz

The average temperature at TE_c and TE_{cc} did not vary much between each other (red and green lines on Fig. 3.8), however comparing with the environmental average temperature (blue line), the maximal differences stayed about + 3 ºC inside TE_C (Table 3.1).The TE_{CC} registered almost +175 ppm atmospheric CO_2 concentration (Table 3.1) and had always the higher concentration level among the cycle (green line on Fig. 3.9).

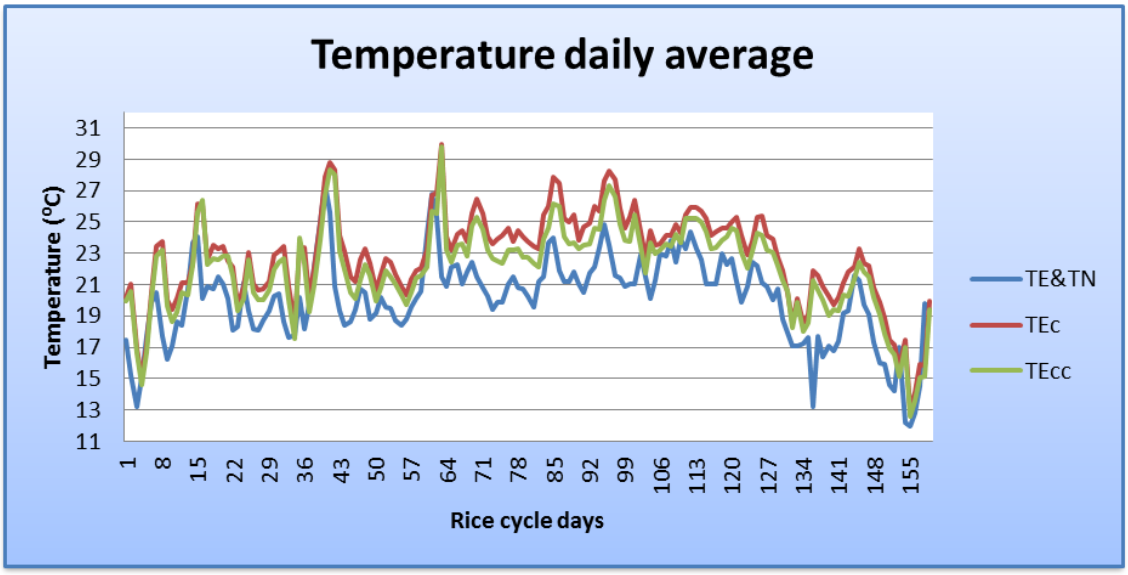


Fig. 3.8 – Daily temperature average during the whole rice cycle

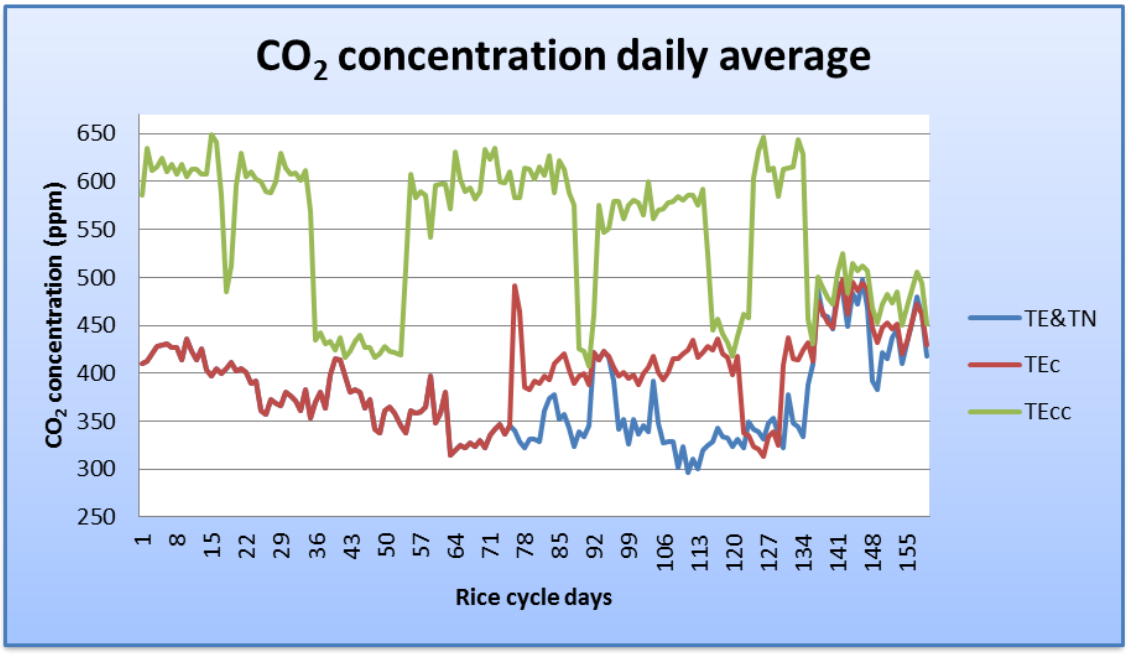


Fig. 3.9 – Daily CO_2 concentration average among whole rice cycle

In total, six field sampling days were made to cover the three main phases of the rice cycle (Table 3.2).

Table 3.2 – Sampling dates and corresponding phase

Sampling date	Rice cycle phase	Samples chosen for analyses
4th July	Vegetative	-
24th July	Vegetative	✓
16th August	Reproductive	✓
4th September	Ripening	-
19th September	Ripening	✓
26th September	Ripening	-

Representative sampling date choice for analyses always symbolizes approximately the mid date of each developing phase.

3.2.2 VOCs extraction methods

Field campaign since 4th of July until 19th of September (Table 3.1) was used for performing SPME and SDE extraction methods.

3.2.2.1 Solid phase microextraction

Table 3.3 describes SPME extraction technique.

Table 3.3 – SPME extraction

	Technique description
SPME	✓ Manual SPME holder (Supelco, Belledort) (Fig. 3.10 a) ✓ Fibre coating: DVB/CAR/PDMS (Polydimethylsyloxane – Divinylbenzene) (1 cm length and 100 µm film thickness) ✓ 15 mL vial
Samples	✓ About 0,3 g (Table 3.4) of fresh cut rice leaves (Fig.3.10 b)
Extraction time	✓ 45 min at room temperature
Fibre desorption	✓ GC injection port at 250 ^{o} for 2 min

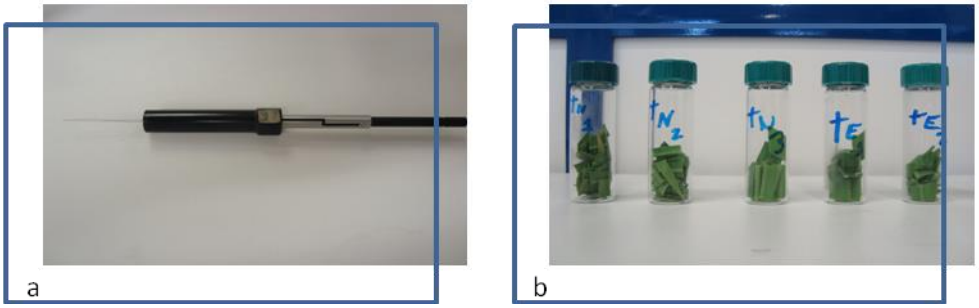


Fig. 3.10 - Material for SPME extraction SPME holder and fibre DVB/CAR/PDMS and (a), about 0.3 g of rice samples into 15 mL vials (b)

Table 3.4 presents the average mean and standard deviations mass of fresh rice samples (three replicates per treatment) extracted by SPME from field campaign dates covering three rice growing phases.

Table 3.4 – Mass of rice samples for SPME extraction

Growing phases	TN (g)	TE (g)	TE_c (g)	TE_{cc} (g)
Vegetative (24th July)	0.28±0.01	0.24±0.06	0.27±0.04	0.27±0.04
Reproductive (16th August)	0.25±0.04	0.25±0.04	0.26±0.04	0.26±0.06
Ripening (19th September)	0.31±0.06	0.31±0.03	0.27±0.08	0.24±0.04

3.2.2.2 Steam distillation extraction

Table 3.5 describes SDE extraction technique.

Table 3.5 – SDE extraction

	Technique description
SDE	✓ Veith and Kiwus exhaustive steam - distillation connected to solvent-extraction distillation apparatus (Fig. 3.11 a) ✓ 250 mL round bottomed flask ✓ Solvent: diethyl ether (Riedel-de Haen; 32203) – pentane (Sigma-Aidrich; 34956) 2:1 (v/v)
Samples	✓ About 7 g (Table 3.6) of fresh cut rice leaves from composite samples (Fig. 3.11 b)
Extraction time	✓ 2 h at boiling point water temperature
Concentration volume	✓ 1 mL under a gentle steam nitrogen
Storage	✓ -20 ^{o}C until analysis

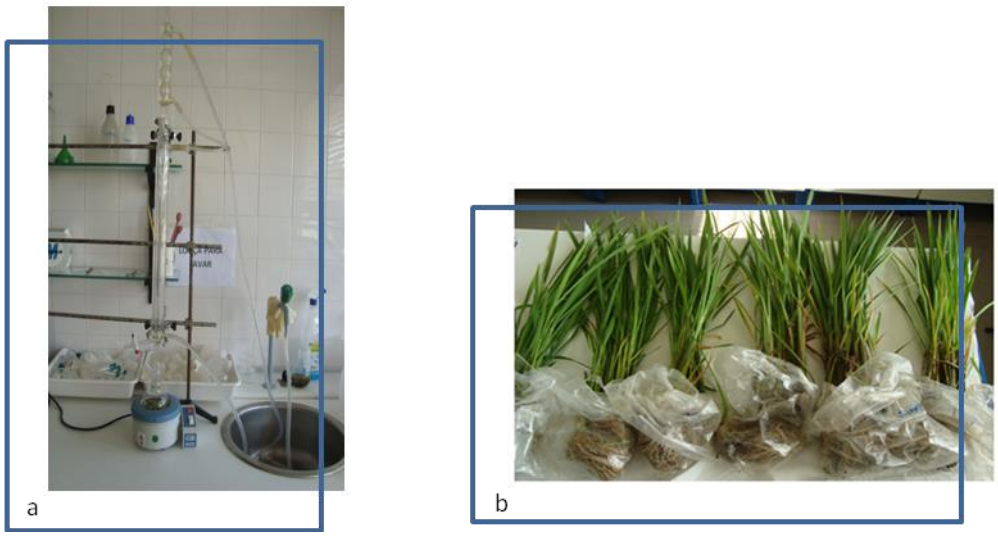


Fig. 3.11 – SDE extraction; SDE material (a) and fresh rice samples (b)

Table 3.6 presents the mass of composite samples extracted by SDE from field campaign dates covering the three rice growing phases.

Table 3.6 – Mass of composite rice samples for SDE extraction

Growing phases	TN (g)	TE (g)	TE_c (g)	TE_{cc} (g)
Vegetative (24th July)	7.01	7.08	7.15	6.98
Reproductive (16th August)	7.10	6.98	7.02	7.11
Ripening (19th September)	7.07	7.03	7.07	6.99

3.2.3 VOCs analysis

The qualitative analysis of VOCs compounds emitted from rice *Oryza sativa* L. *cv.* Ariete were performed by two different equipment of gas chromatography coupled to mass spectrometry (GC/MS) with different column polarities.

3.2.3.1 Gas chromatography/mass spectrometry

SPME samples were analysed by GC/MS system (HP Hewlett Packard, Series II, model 5890/ MSD 5972 mass selective detector). The VOCs separation was achieved on a capillary column coated with a non – polar phase 5% phenyl 95% dimethylpolysiloxane (ZB-5MS equivalent to DB-5) with 30 m length x 320 µm ID and 0.25 µm film thickness (Phenomenex Inc., USA). Data were generated in full screen spectra between mass ranges of 40 – 300 *m/z* and results was recorded in total ion chromatograms (e.g. Fig. 3.12).

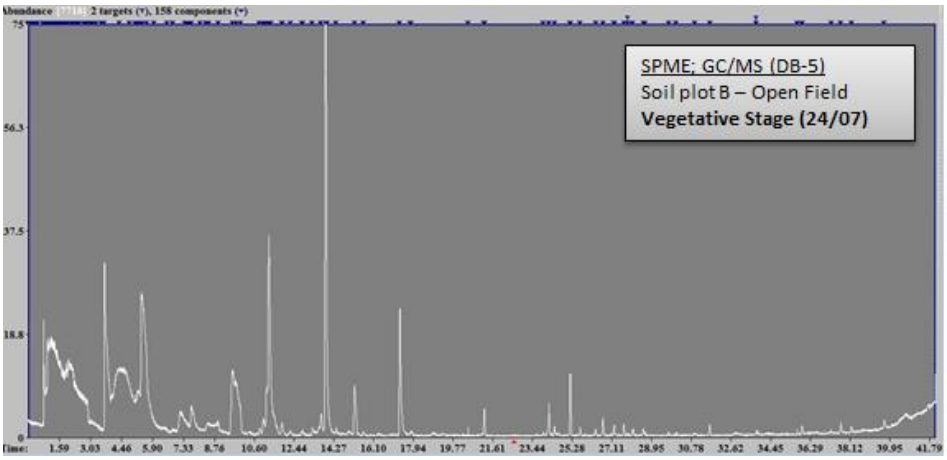


Fig. 3.12 – Example of TIC resulting from SPME extraction and analysed by GC/MS using DB-5 capilary column

SDE samples were analyzed by GC/MS system (SCINSQ – BUNKER model GC – 456) equipped with MS quadrupole mass spectrometer. The VOCs separation was achieved on a capillary column coated with a polar phase polyethylene glycol (BR-SWAX equivalent to DB-WAX) with 30 m length x 320 µm ID and 0.25 µm film thickness (Bruker Daltonics Inc., USA). Total volume injection of 1 µL with a microliter syringe (Hamilton Microliter™ Syringes, Reno, Nevada, USA).

Data were generated in full screen spectra between mass ranges of 40 – 400 *m/z* and results were recorded in total ion chromatogram (e.g. Fig. 3.13).

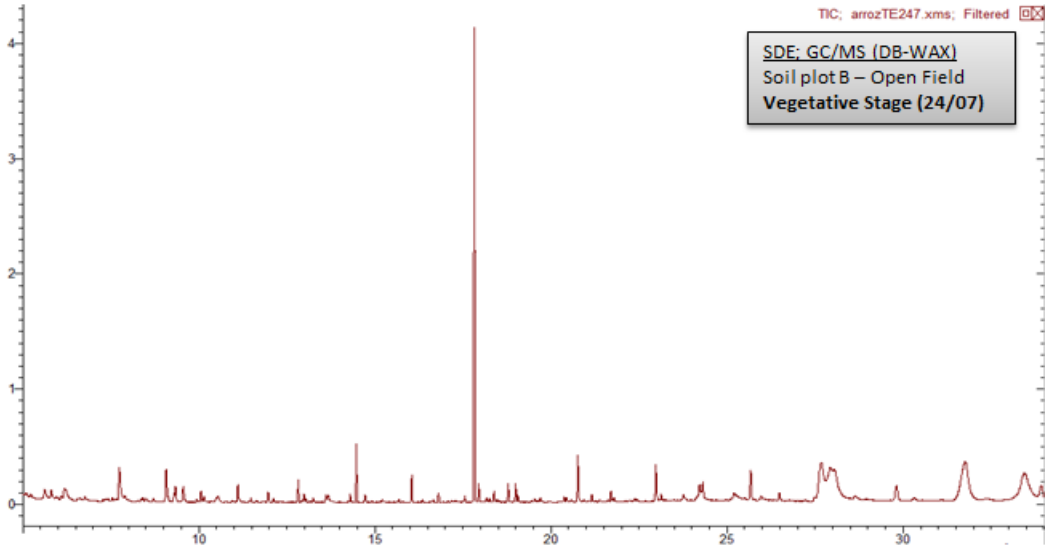


Fig 3.13 – Example of TIC resulting from SDE extracts analysed by GC/MS using DB-WAX capillary column

In both GC equipment was used the same method and Table 3.7 presents respective method parameters.

Table 3.7 – GC programmed method

Method parameters	
Injection type	Manual
Injection mode	Splitless for 2 min
Carrier gas (constant flow)	Helium 1.2 mL/min
Temperature injection	250 ^{o}C
Temperature ramp	4 ^{o}C/min
Temperature initial	40 ^{o}C for 2 min
Temperature final	200 ^{o}C
Ionization mode	EI (70 eV)

3.2.4 VOCs Identification

After extraction and analysis, the fingerprints/fragments/chromatograms of studied compounds were qualitatively analysed. Quantitative analyses were no possible to perform due of the small peak area on the chromatograms. Rice volatile emissions are not as high as those reported for *Pinnus* spp. or *Eucalyptus* spp. leaves that allow high peak areas.

The resulting chromatographic peaks from GC/MS were analysed using the softwares AMDIS from NIST and MS ChemStation from Agilent. Identification process started with peak detection (each compound has the respective retention time), followed by their RI (retention index) calculation (Table 3.8), and comparison with reported literature RI (e.g. Adams, 2001). The maximal absolute index difference admitted comparing with available literature was assumed to fall between a deviation of 25 (Zeng *et al*., 2012) to 30 (Cardeal *et al*, 2006) from the literature reported values. The identification process is confirmed after mass spectra comparison with NIST 08 database libraries (F6 in annexes shows mass spectra examples from the three main VOCs classes).

Table 3.8 – The van den Dool/Kratz RI formula calculation

Common RI	Temperature conditions	Formula	Reference
van den Doll/Kratz	Linear temperature program ramp	$RI_x = 100_n + 100(t_{x\text{-}tn}) / (t_{n+1} - t_n)$	van den Dool and Kratz, 1963
Legend	✓ t_{n+1} and t_n retention times of the reference n-alkane hydrocarbons eluting immediately before and after compound "X" ✓ t_x retention time of compound "X"		

Hydrocarbons RIs were obtained from injection of C_8-C_{20} standard solution using the same GC/MS programmed method parameters. The molecular mass of hydrocarbons was found using the equation $\mathbf{12 \times n + (2n + 2)}$ (with n representing the number of carbon atoms), derived from general formula of *n-alkanes* $\mathbf{C_nH_{2n+2}}$ (Chang and Goldsby, 2012). The equation gives the highest molecular mass (*m/z*) of the peak, so-called molecular ion (MI), and it is represented by the last ion in spectra. Table 3.9 shows molecular ions from C_8-C_{15} hydrocarbons of interest.

Table 3.9 – Molecular ions from hydrocarbons C_8 to C_{15}

$\mathbf{C_nH_{2n+2}}$	C_8H_{18}	C_9H_{20}	$C_{10}H_{22}$	$C_{11}H_{24}$	$C_{12}H_{26}$	$C_{13}H_{28}$	$C_{14}H_{30}$	$C_{15}H_{32}$
MI (*m/z*) = 12×n + (2n+2)	114	128	142	156	170	184	198	212

3.2.4.1 Statistical results treatment

Student's-t test was used to find the statistical truth for treatment means in each rice growing phase (one sample mean test), as well as to comparing the means between two treatments (independent sample test). Only SPME results were statistically treated. A total of 36 samples were analysed by GC/MS using DB-5 column, data were grouped in triplicates from each sample treatment. Population size of for each t-student test was equal for all treatments (n =3). Table 3.10 shows the population VOCs emission mean $\overline{X}_x$ and respective standard deviation S_x (numbers $_x$ from 1 to 12 present the sequential order of treatments).

Table 3.10 – Emissions mean $\overline{X}_x$ and standard deviation S_x of population from each treatment and respective rice cycle phase

Rice cycle phases	TN	TE	TE_C	TE_{CC}
Vegetative	$\overline{X}_1$ = 11,3 S_1 = 5,80	$\overline{X}_4$ = 12,7 S_4 = 3,85	$\overline{X}_7$ = 5,67 S_7 = 1,69	$\overline{X}_{10}$ = 3,00 S_{10} = 0,82
Reproductive	$\overline{X}_2$ = 2,67 S_2 = 0,94	$\overline{X}_5$ = 3,67 S_5 = 1,24	$\overline{X}_8$ = 5,00 S_8 = 1,63	$\overline{X}_{11}$ = 4,03 S_{11} = 0,94
Ripening	$\overline{X}_3$ = 6,67 S_3 = 0,47	$\overline{X}_6$ = 5,00 S_6 = 2,45	$\overline{X}_9$ = 8,67 S_9 = 2,62	$\overline{X}_{12}$ = 6,00 S_{12} = 2,94

The assumptions to measure confidence interval (CI) (one sample mean test) were observed: normal population distribution and standard deviation of treatments is unknown. We were in the case to assume null hypothesis: real mean of treatments are belonging to calculated CI at different confidence levels (99% and 95%), i.e. Can we use this values as representative of field rice plant?; Assuming equal variance between treatments, we are in the case of proving the differences between two treatments (independent unpaired test), where null hypothesis is: there are no differences between treatments, i.e. Do abiotic stress factors influence VOCs emission?

4. Results

This chapter presents all qualitative analysis of VOC fraction emitted by the rice (*Oryza sativa* L. *cv.* Aríete) samples among the whole growing cycle (referred in Table 3.1). Identification of volatile components extracted from SPME was performed by GC/MS using a non-polar (DB-5) column and from SDE extraction VOCs were analysed by GC/MS using a polar (DB-WAX) column. Data treatment was based on t-Student analyses in order to interpret statistical mean results.

4.1 VOCs profile by GC/MS using DB-5 non-polar column

Table 4.1 presents the results obtained from the 36 field rice samples (each sample collection day counted with six OTC plus three open field soil plot B and open field soil plot A). Table legend are divided in compound name and their chemical formulas, ascending order of RI_{calc} calculated according with quasi-linear equation of Van den Dol/Kratz (referred on Table 3.9) and RI_{lit} from literature (Adams, 2001). Right columns represented by gray colour expressing all treatments sampled: TN means plot A OF (loamy sand soil); TE represents plot B OF (silty clay soil), TE_C means plot B OTC with temperature enhanced and TE_{CC} plot B OTC with induced simultaneously temperature and CO_2.

A total of 33 VOCs were found and the composition results points out to a 15,15% of GLV (five compounds counting some aldehydes and alcohols) including 4-pentanal 2-methyl, 3-hexenal, 3-hexenol and 2,4-hexadienal; within the class of terpenes the division is followed by monoterpenes, with 33.3% (11 compounds) like *α*-pinene, myrcene, *α*-phellandrene, cymene, limonene, 1,8-cineol, ocymene, *α*-terpinolene, linalool, *n*-decanal *β*-cyclocitral; and sesquiterpenes with 33.3% (11 compounds) such as coparene, elemenne, *β*-caryophyllene, *α*-farnesene, bergamotene, humulene, aromadendrene, *α*-curcumene, ziginberene, bisabolene and *β*-sesquipheladrene. Other compounds outside of main classes represent 18.2%, such as 1-hexenol 2-ethyl (alcohol), benzoic acid (acid), *n*-nonanal and 2,6-nonadienal (aldehydes), methyl salicylate (ester) and *β*-ionene (ketone).

The GLV trend did not vary as much among the treatments as mono- and sesquiterpenes. Mono- and sesquiterpenes fluctuation occurs essentially between OF (TN and TE) and OTC (TE_C and TE_{CC}) treatments. Sesquiterpenes compounds were less observed and the difference trend among the treatments is mainly present in OTC with zero sesquiterpenes found at vegetative and reproductive stage and only four compounds appeared at ripening stage.

According with Spinelli *et al*. (2011) the VOCs division in classes (GLV, mono- and sesquiterpenes) is based on their C units. With DB-5 capillary column was possible to establish a linear relation between retention time and volatility of the compounds.

Table 4.1 – Rice cycle VOCs emission performed by SPME analyzed by GC/MS (DB-5 column)

				Vegetative					Reproductive					Ripening			
Peaks	Chemical formula	RI Lit	RI Calc	T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}
4-pentanal 2-methyl	$C_6H_{10}O$	776	784		X									X	X		
3-hexenal	$C_6H_{10}O$	796	807	X	X		X		X	X		X					
2-hexenal	$C_6H_{10}O$	850	854	X	X	X	X		X	X		X		X		X	X
3-hexenol	$C_6H_{12}O$	853	868	X	X	X	X		X	X	X	X			X		X
2,4-hexadienal	C_6H_8O	925	928	X	X	X			X	X		X		X	X		
α-pinene	$C_{10}H_{16}$	939	943	X	X		X								X	X	X
myrcene	$C_{10}H_{16}$	989	1001														X
phellandrene	$C_{10}H_{16}$	1004	1014				X				X						
cymene	$C_{10}H_{16}$	1026	1032	X	X	X	X							X	X		X
1-hexenol 2-ethyl	$C_8H_{18}O$	1028	1035		X		X			X		X		X	X		X
limonene	$C_{10}H_{16}$	1035	1038	X	X	X	X				X	X		X	X		X
1,8-cineol	$C_{10}H_{18}O$	1039	1047	X	X	X	X				X	X		X	X	X	X
ocymene	$C_{10}H_{16}$	1050	1056		X												X
α-terpinolene	$C_{10}H_{16}$	1089	1100	X			X										X
benzoic acid	$C_8H_8O_2$	1102	1110	X	X	X					X					X	
n-nonanal	$C_9H_{14}O$	1104	1111												X	X	
linalool	$C_{10}H_{18}O$	1106	1117	X	X												
2,6 nonadienal	$C_9H_{14}O$	1180	1191									X				X	
methyl salicylate	$C_8H_8O_3$	1201	1207	X	X												
n-decanal	$C_{10}H_{20}O$	1212	1221						X					X	X	X	
β-cyclocitral	$C_{10}H_{16}O$	1219	1229													X	
coparene	$C_{15}H_{24}$	1386	1400	X													
elemene	$C_{15}H_{24}$	1406	1418	X	X									X			X
β-caryophyllene	$C_{15}H_{24}$	1425	1440	X	X												
α-farnesene	$C_{15}H_{24}$	1430	1442	X	X					X							
bergamotene	$C_{15}H_{24}$	1435	1450	X	X												
humulene	$C_{15}H_{24}$	1455	1470		X												
aromadendrene	$C_{15}H_{24}$	1462	1479	X					X								
α-curcumene	$C_{15}H_{24}$	1481	1501		X												
β-ionene	$C_{13}H_{18}O$	1493	1504											X	X	X	X
ziginberene	$C_{15}H_{24}$	1500	1514	X	X												
bisabolene	$C_{15}H_{24}$	1511	1527														X
β-sesquipheladrene	$C_{15}H_{24}$	1523	1537	X	X									X	X		
Total				**20**	**22**	**7**	**10**		**6**	**6**	**5**	**8**		**11**	**12**	**9**	**13**

Fig 4.1 shows the linearity between compounds class and retention time, where GLV (C_6 units) appear in earliest retention time, followed by monoterpenes (10 C units) and by more 'heavy' sesquiterpenes (15 C units).

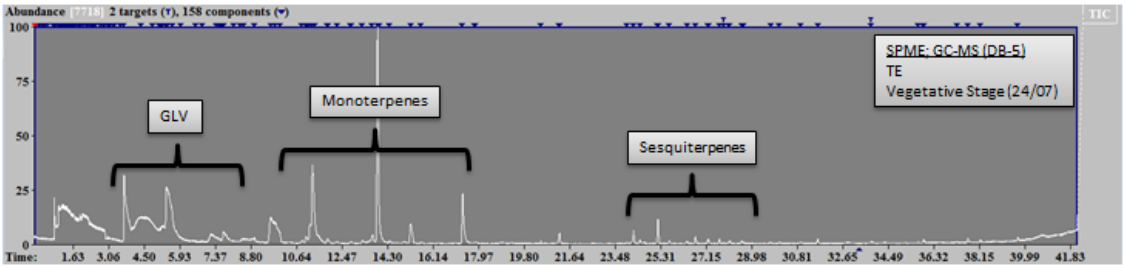


Fig. 4.1 – Retention time in relation with amount of carbon units of VOC constituents from the three main classes (TIC by GC/MS using DB-5 column)

The choice of TE samples in vegetative stage was purposely made to facilitate an easy observation of the relation. In all other sample treatments the same VOC class sequence were observed too. Next result examples reinforced this option following same orientation line for TE chosen samples.

The comparison of all sample treatments among the three rice growing phases point out for a higher number of VOCs during vegetative stage. Analysing TE total results more three times VOCs were identified on vegetative phase (22 compounds) than reproductive (eight compounds) and almost two times of VOCs than ripening (13 compounds). Main VOC classes causing that difference were sesquiterpenes followed by monoterpenes. Fig. 4.2 presents the TICs from TE samples at three growing phases. In every VOC classes, the reproductive stage was responsible for the least number of compounds.

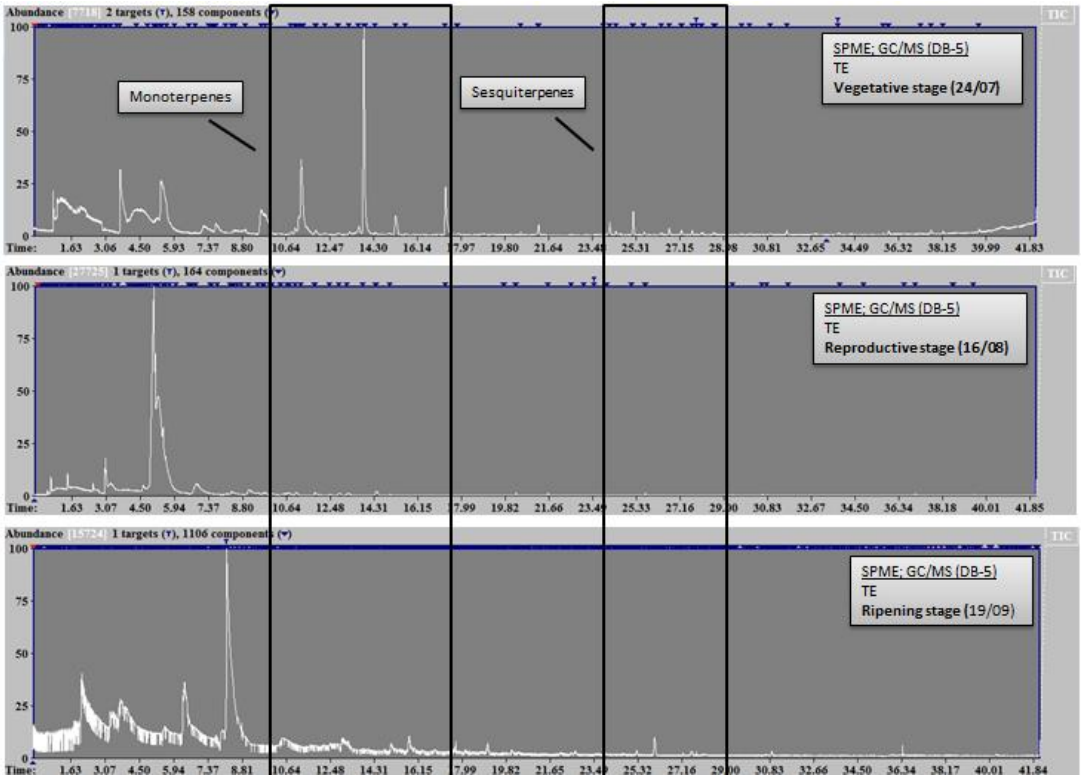


Fig. 4.2 – Predominance of mono- and sesquiterpenes on vegetative stage

Taking into account the two different soil textures used (see section *2.2.1.1 Soil characteristics*), silty clay (TE) and loamy sand (TN), there were differences in VOCs emission trend. Fig. 4.3 presents these results and TE had always more VOCs than soil TN, at least one or two compounds.

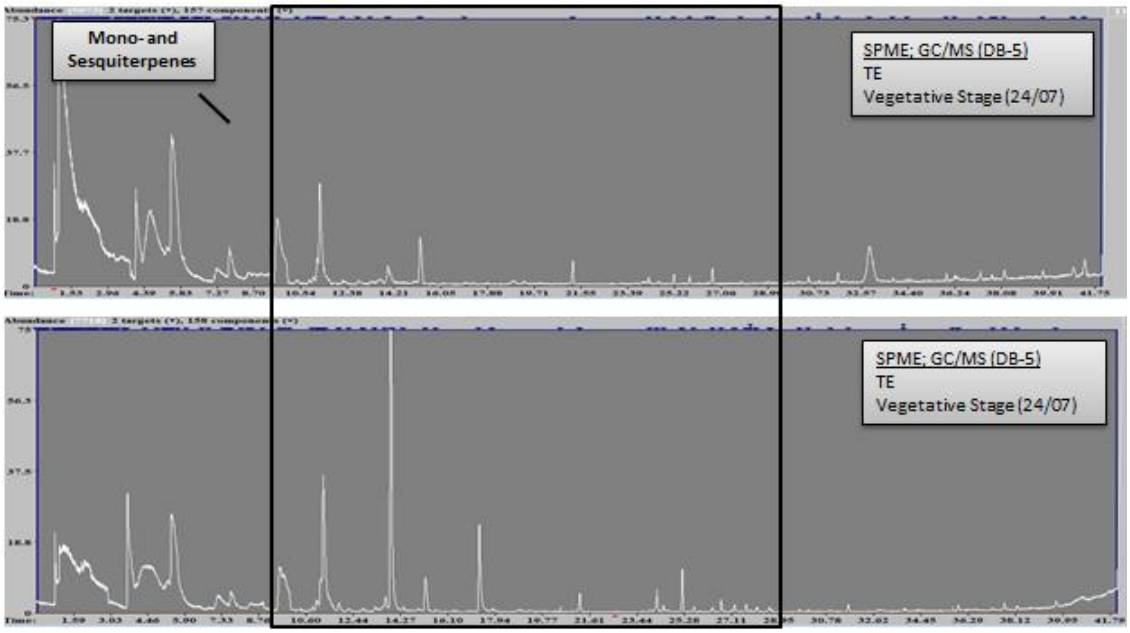


Fig 4.3 – Voices emission differences between soil textures

Silty clay soil increased volatile emission. Every replicate field sample indicates more VOC compounds in OTC under the influence only of temperature (TE_C), when compared with simultaneously increase of temperature and CO_2 (TE_{cc}). Fig. 4.4 shows fewer compounds emitted by rice under influence of simultaneous higher temperature and CO_2 concentration. It should be pointed out that for better peak comparison Fig. 4.4 presents differences on chromatogram scales.

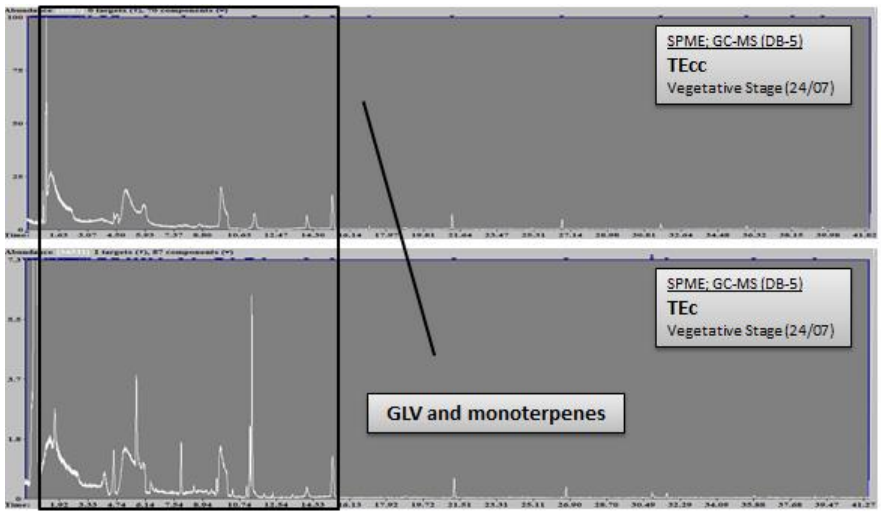


Fig. 4.4 – Higher number of VOCs on temperature induced OTC (TE_C)

4.2 VOCs profile by GC/MS using DB-WAX polar column

Mateus (2008) referred that for better comprehension and complete VOCs profile identification, the samples should be analysed using different GC column polarities. Stored SDE samples were analysed using GC polar column DB-WAX. But fewer samples were analysed when compared with SPME samples because of loss (evaporation) during the storage period. Only seven remaining samples were representative of the rice cycle, and just TE treatment samples could cover all rice phases.

Table 4.2 systematizes the VOCs profile with the same legend description as Table 4.1 but without any RI calculation or comparison. In total, 22 flavour compounds were counted. Predominant categories were alcohols, aldehydes and ketones.

Table 4.2 - VOCs emission analyzed GC/MS (DB-WAX polar column)

		Vegetative			Reproductive		Ripening	
Peaks	**Chemical Formula**	**TE**	**TE_{CC}**	**TE_C**	**TE**	**TN**	**TE**	**TE_{CC}**
2-hexanone	$C_6H_{12}O$		x				x	
n-hexanal	$C_6H_{12}O$	x		x	x			x
2-pentanol	$C_5H_{12}O$	x	x			x		
heptanal	$C_7H_{14}O$				x	x	x	
2-hexenal	$C_6H_{12}O$	x	x	x	x		x	x
3-hexenol	$C_6H_{12}O$	x	x	x	x	x	x	x
n-octanal	$C_8H_{16}O$					x	x	x
cymene	$C_{10}H_{16}$				x			
2-heptenol	$C_7H_{14}O$	x						
1,6 hexanediol	$C_6H_{14}O_2$	x	x	x	x	x	x	x
n-nonanal	$C_9H_{14}O$	x	x	x		x	x	x
1,3 nonadienol	$C_9H_{14}O$				x	x	x	
2,6 nonadienal	$C_9H_{14}O$		x			x	x	x
n-decanal	$C_{10}H_{20}O$	x			x	x	x	x
decanol	$C_{10}H_{20}O$				x			
β-cyclocitral	$C_{10}H_{16}O$	x	x	x	x	x	x	x
β-caryophyllene	$C_{15}H_{24}$	x						
aromadendrene	$C_{15}H_{24}$	x						
cetene	$C_{15}H_{24}$	x	x					
α-curcumene	$C_{15}H_{24}$	x						
β-ionene	$C_{13}H_{20}O$	x					x	
2-pentadecanone	$C_{18}H_{36}O$	x	x		x	x	x	
Total		**15**	**10**	**6**	**12**	**11**	**13**	**9**

The composition of the three main VOC categories was divided in 22.72,% of GLV, 9.09% monoterpenes and 18.18% sesquiterpenes. The GLV comprised five compounds including ketones, aldehydes and alcohols, such as 2-hexanone, hexanal, 2-hexenal, 3-hexenol and 1,6-hexanediol. The division of monoterpenes comprised two compounds including cymene and *β*-cyclocitral, and the sesquiterpenes four compounds: *β*-caryophyllene, cetene, aromadendrene

and *α*-curcumene. Other compounds also included on the Table 4.2 represent 50% of total VOC composition: (four aldehydes) *n*-octanal, *n*-nonanal, 2,6 nonadienal and *n*-decanal; (three alcohols) 2-heptenol, 1,3 nonadienol and decanol; (one ketone) 2-pentaecanone; and (one ester) *β*-ionene.

Comparing the three columns of TE (left column from each phase) between all growing phases in Table 4.2, the highest number of VOCs is present on vegetative (15 compounds) phase, followed by ripening (13 compounds) and reproductive (12 compounds). Fig. 4.5 presents an example of this analysis with a higher number of GLV on vegetative stage. The differences on chromatogram scale are used for simplifying peaks observation.

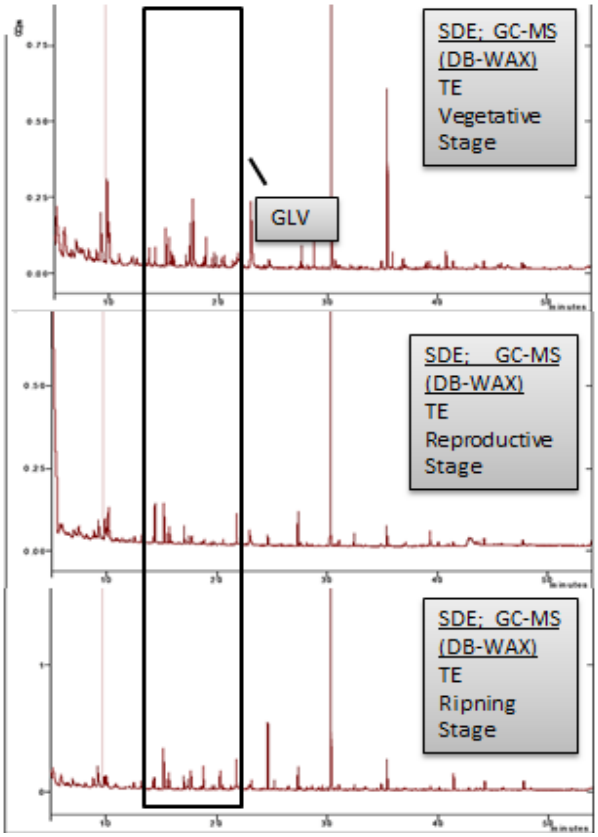


Fig 4.5 - GLV between three phases (TE sample comparation) with GC DB-WAX polar column

Table 4.3 shows, simultaneously, the differences of VOCs distribution from DB-WAX and DB-5 columns between all treatments among the rice cycle. In total DB-WAX identified 22 compounds and DB-5 column 33. Similarities between columns results exist manly on the sesquiterpenes class.

Table 4.3 – GC columns results comparison

		Columns		
Peaks	**Chemical formula**	**DB-5**		**DB-WAX**
2-pentanol	$C_5H_{12}O$		.	X
4-pentanal 2-methyl	$C_6H_{10}O$	X	.	
2-hexanone	$C_6H_{12}O$		.	X
n-hexanal	$C_6H_{12}O$		.	X
3-hexenal	$C_6H_{10}O$	X	.	
2-hexenal	$C_6H_{10}O$	X	.	X
3-hexenol	$C_6H_{12}O$	X	.	X
2,4-hexadienal	C_6H_8O	X	.	
n-heptanal	$C_7H_{14}O$		.	X
n-heptenol	$C_7H_{14}O$		.	X
1,6 hexanediol	$C_6H_{14}O_2$		.	X
n-octanal	$C_8H_{16}O$		.	X
α-pinene	$C_{10}H_{16}$	X	.	
myrcene	$C_{10}H_{16}$	X	.	
phellandrene	$C_{10}H_{16}$	X	.	
cymene	$C_{10}H_{16}$	X	.	X
1-hexenol 2-ethyl	$C_8H_{18}O$	X	.	
limonene	$C_{10}H_{16}$	X	.	
1,8-cineol	$C_{10}H_{18}O$	X	.	
ocymene	$C_{10}H_{16}$	X	.	
α-terpinolene	$C_{10}H_{16}$	X	.	
benzoic acid	$C_8H_8O_2$	X	.	
n-nonanal	$C_9H_{14}O$	X	.	X
linalool	$C_{10}H_{18}O$	X	.	
1,3 nonadienol	$C_9H_{14}O$		.	X
2,6 nonadienal	$C_9H_{14}O$	X	.	X
methyl salicylate	$C_8H_8O_3$	X	.	
n-decanal	$C_{10}H_{20}O$	X	.	X
decanol	$C_{10}H_{20}O$		.	X
β-cyclocitral	$C_{10}H_{16}O$	X	.	X
coparene	$C_{15}H_{24}$	X	.	
elemene	$C_{15}H_{24}$	X	.	
β-caryophyllene	$C_{15}H_{24}$	X	.	X
α-farnesene	$C_{15}H_{24}$	X	.	
bergamotene	$C_{15}H_{24}$	X	.	
cetene	$C_{15}H_{24}$		.	X
humulene	$C_{15}H_{24}$	X	.	
aromadendrene	$C_{15}H_{24}$	X	.	X
α-curcumene	$C_{15}H_{24}$	X	.	X
β-ionene	$C_{13}H_{18}O$	X	.	X
ziginberene	$C_{15}H_{24}$	X	.	
bisabolene	$C_{15}H_{24}$	X	.	
β-sesquipheladrene	$C_{15}H_{24}$	X	.	
2-pentadecanone	$C_{18}H_{36}O$		.	X
Total		**33**	**.**	**22**

4.3 Data treatment

In this section were carried out the statistical analyses of the *Oryza sativa* L. *cv.* Aríete chemical volatile leaves composition, extracted by SPME and analysed by GC/MS (DB-5), proving the significance of the influence of stress factors in VOCs emission among the whole rice cycle.

4.3.1 Students t-test

The rice data were in accordance with statistical t-student hypothesis test conditions: small data sets of treatments samples, data follow approximately standard normal distribution model, standard deviation is unknown and is replaced by an estimate scaling parameter using t-distribution, and the real mean value and standard deviation population are independent.

4.3.1.1 One sample t-test mean (confidence intervals)

Two degrees of freedom were chosen and statistical significance were set at 0,05 and 0,01 levels (95% and 99% of confidence degree, respectively). Null hypothesis defend that real mean values are within the respective CI_x (confidence interval).

Table 4.4 presents the CI with 95% of confidence, where the real mean value (Table 4.1) are within the limits for the respective treatment on correspondent rice cycle phase.

Table 4.4 – T-student test $CI_{95\%}$ results for one sample test (mean μ) with 95% of confidence degree

Rice cycle phases	TN	TE	TE_C	TE_{CC}
Vegetative	$CI_{\mu1}$ = 11,3±14,4	$CI_{\mu4}$ = 12,7±9,56	$CI_{\mu7}$ = 5,67±4,19	$CI_{\mu10}$ = 3,00±2,03
Reproductive	$CI_{\mu2}$ = 2,67±3,67	$CI_{\mu5}$ = 3,67±3,08	$CI_{\mu8}$ = 5,00±2,33	$CI_{\mu11}$ = 4,03±2,36
Ripening	$CI_{\mu3}$ = 6,67±1,17	$CI_{\mu6}$ = 5,00±6,08	$CI_{\mu9}$ = 8,67±6,51	$CI_{\mu12}$ = 6,00±7,30

The real mean values (μ_x) (see last row on Table 4.1), μ_1=20, μ_2=6, μ_4=22, μ_5=6, μ_7=10, μ_9=9, μ_{11}=5, μ_{12}=9 are located between the associated confidence interval limits, and the null hypothesis was not excluded. Other mean values μ_3=11, μ_6=12, μ_8=8 and μ_{10}=10 rejected the null hypothesis, i.e. the real mean values are not within the correspondent $CI_{95\%}$.

Table 4.5 presents the CI where we are having 99% of confidence that the real mean value (Table 4.1) are within the respective limits.

Table 4.5 - T-student test $CI_{99\%}$ results for one sample test (mean μ_x) with 99% of confidence degree

Rice cycle phases	TN	TE	TE_C	TE_{CC}
Vegetative	$CI_{\mu1}$ = 11,3±33,2	$CI_{\mu4}$ = 12,7±19,3	$CI_{\mu7}$ = 5,67±9,68	$CI_{\mu10}$ = 3,00±4,70
Reproductive	$CI_{\mu2}$ = 2,67±5,38	$CI_{\mu5}$ = 3,67±10,1	$CI_{\mu8}$ = 5,00±9,36	$CI_{\mu11}$ = 4,03±5,38
Ripening	$CI_{\mu3}$ = 6,67±2,69	$CI_{\mu6}$ = 5,00±14,0	$CI_{\mu9}$ = 8,67±15,0	$CI_{\mu12}$ = 6,00±16.8

Having 99% of confidence all mean real values μ_x from Table 4.1 are within the $IC_{99\%}$ limits. Null hypothesis are not reject in any test, i.e. we can use this results as representative from field rice culture. Highest CI values were observed in TN and TE samples at vegetative phase.

4.3.1.2 Independent two sample t-test (mean differences)

Student's independent two sample t-test was used to study the difference between two independent treatments. Both samples size are equal (n =3) and the two distributions having the same variance (in concordance with independent two sample t-test assumptions).

Hypotheses were tested between two treatments each time. Triplicate combinations were studied combining all treatments TE, TN, TE_{CC} and TE_C. Four degrees of freedom were used with 1%, 5% and 10% of significance (99%, 95% and 90 confidence degree, respectively), where $T_{tab99\%}$=4,60 $T_{tab95\%}$=2,78 and $T_{tab90\%}$=2,13. Values of T_{calcx} present the t-test calculation. Null hypothesis points out that real means are equal between treatments and hypothesis one is the reverse (exists differences between means). If $T_{calcx} > T_{tabx}$ is proved that means are significantly different at determinate level of probability. Table 4.6 present the T_{calcx} values from each treatment comparison.

Table 4.6 – Independent samples t-test between sample treatments

Rice cycle phases	TN and TE	TE_C and TE_{CC}	TN and TE_{CC}	TN and TE_C	TE and TE_{CC}	TE and TE_C
Vegetative	T_{calc1}=0,28	T_{calc4}=2,01	T_{calc7}=2,00	T_{calc10}=1,32	T_{calc13}=2,51	T_{calc16}=2,35
Reproductive	T_{calc2}=0,90	T_{calc5}=0,52	T_{calc8}=1,73	T_{calc11}=1,20	T_{calc14}=0,57	T_{calc17}=1,75
Ripening	T_{calc3}=0,94	T_{calc6}=2,00	T_{calc9}=0,32	T_{calc12}=1,07	T_{calc15}=0,37	T_{calc18}=1,07

Having 99%, 95% of confidence all t-tests demonstrated $T_{calcx} < T_{tabx}$, i.e. null hypothesis is not rejected and is not statistically proved that exist differences between treatment means. But with 90% ($T_{tab90\%}$=2,13) of confidence T_{calc13} = 2,51 and T_{calc16} = 2,35 proved $T_{calcx} > T_{tabx}$, i.e. results rejected null hypothesis and exists differences between T_E absolute mean at vegetative phase ($TE_{\mu 4}$=22) and TE_{CC} ($TE_{CC\mu 10}$=7) and TE_C ($T_{Ec\mu 7}$=10) real mean values.

Between phases, higher T_{calcx} values show the respective treatments with major absolute mean differences. Vegetative phase had the major values which mean more differences in total released compounds. At vegetative phase, comparing pairs or treatments, TE and TE_{CC} at presents higher value.

5. Discussion

Rice is the most important staple food crop for a large part of the world's population. As the population growth trend increasing, the demand for rice will grow. Portugal is the fourth biggest rice producer in EU and the first *per capita* consumer. Agrosystems occupy a large land areas and rice extends their territory every year. Air quality issues are now focused on the emission rates from biogenic sources, one of the present pollutants is the volatile fraction emitted by plants, so-called VOC. VOCs are released from plants by secondary metabolism in response to biotic and abiotic stress factors, such as: herbivore damage, pathogenic attack or infestation, intra- and interspecies odour signals, temperature, light – time-explosion, plant species physiology and phenology, O_3 and CO_2 exposure, soil physical and chemical characteristics (availability of N and water soil). VOCs are very heterogeneity group of compounds with a higher influence on atmosphere chemistry, acting as photochemical precursors in tropospheric ozone formation.

Portuguese rice paddy fields were a subject study of chemical volatile fraction emission from fresh rice leaves, among 2012 rice (*Oryza sativa* L. *cv.* Ariete) cycle. Field measurements from whole rice cycle allowed a greater interpretation of a complete growth process, being the first study in the scope, followed the one previously carried out by FCT research team of the project.

When we are comparing other studies, referred in this chapter, about VOCs emission from other parts of the rice plant or even though different plant species, a range of different factors should be taking into account: the metabolic type of the plant; morphology and phenology of developing plant phases, environmental plant material conditions during the studies (in- or outdoor), GC equipment features (spectrometers, programmed methods and GC columns) and VOCs methodology identification. These differences are not always representing unfailing results if compared with field sample collection and analysis, but on the other hand, it should be considered as a good methodology to matching environmental responses.

The small peak size represented vestigial VOCs emission from rice leaves, allowing only performing qualitative analysis. From SPME technique, 36 headspace samples were analysed by quadrupole analyzer and DB-5 capillary column, where 33 VOCs have been identified. From SDE extraction, 22 VOCs were identified from 7 liquid samples by quadrupole analyzer and DB-WAX capillary column. In total were identified three main VOC classes GLV, mono- and sesquiterpenes, with different composition between columns. The DB-5 capillary column showed to be a main choice for separation of non-polar compounds and DB-WAX capillary column looked to be greater for polar VOCs, such as aldehydes, ketones and alcohols (see column coating on T3 in annex). Table 4.4 shows a large difference in monoterpenes emission (DB-5 columns had 11 and DB-WAX only two).

The SPME, as equilibrium process is dependent of the volatile concentration in the headspace and it not allows injection repetition, the SDE promote enrichment of the volatiles on the solvent layer and the final solvent extract allows multiple injections (Mahattanatawee *et al*,

2005). The amount of sample used influences the concentration of SDE final extract. Kpoviessi *et al*, (2011) used 500 g of fresh leaves from *Sclerocarya birriea* (deciduous three) samples (in this study we just could steam distilled 7 g of fresh cut rice leaves each time). Zeng *et al.* (2011) observed from same rice bran samples, 65 compounds detected from SDE and 76 from SPME.

VOCs emitted by plants can be direct related as isoprene derivatives (mono- and sesquiterpenes) and, as Spinelli *et al.* (2011) affirmed, the emission is closely related with younger leafs in earlier stages of plant development. Rinne *et al.* (2009) biochemically assumed that the higher amount of isoprene emission only starts few weeks after first leaves come out of plant. Highest VOCs abundance on vegetative phase was observed in our study and this result can be explained by the greater rice plant activity on early stage (Dudareva *et al.*, 2004) to grow a solid steam extension and to fully expend their young leafs.

In 2011 rice cycle, samples were submitted to SPME extraction and GC-FID separation/detection using DB-5 capillary column and also vegetative phase presented maximum VOCs emission from mono- and sesquiterpenes classes (Couto *et al*., 2012). Other example from different plant cycle VOCs profile identification, Maes and Debergh (2009) studied VOCs emission from tomato shoots and the higher amount of volatile compounds was observed in the vegetative phase too. They identified compounds by GC/MS using non-polar capillary column and found out a predominance of mono-and sesquiterpenes.

Common mechanism to reduce leaves temperature is transpiration and it is regulated by stomata opening and air relative humidity. But, rice paddy ecosystem are characterized by elevate humidity caused by flooding environment, where the transpiration is reduced and other mechanism should act as thermoregulation function to protect leaves (Cho and Oki, 2012). Increasing temperature induces stress to rice plant growth. Our study may prove that VOCs are higher under higher temperatures and it is can be related with thermotolerance protection.

According to literature studies involving VOCs emission from other rice varieties, such as wild (Zizania aquatic) (Frank et al. (1976) in Maga, 1984), unpolished California brown pearl rice (Bullard and Holguium (1977) in Maga, 1984) and scented or so-called aromatic rice (Buttery *et al*., 1983), all authors concluded the same phenomena of temperature influencing the increased number of VOCs. From cooked rice (Aisaka, 1977) were identified 3 to 5 times more VOCs then fresh raw rice. And also Tsugita *et al.* (1983) compared the flavour compounds from rice stored at 4 ^{o}C to that stored at 40 ^{o}C for 60 days, where in rice stored at 4 ^{o}C had less 50% of total amount of VOCs than at 40 ^{o}C.

Temperature tolerance has a relevant effect on phosyntethesis rate. This relation has been connected to down-regulation at biochemical and enzymatic level of rice plant (Dudareva *et al*., 2004). A change in emission rates is predictable, resulting on reduced VOCs emission. In our treatments TEcc shows always lesser compounds emission. Also in 2011 rice cycle, plants in OTC under influence of temperature plus CO_2 enhancing showed less volatile compounds than under only temperature treatment (Couto *et al*., 2012). There is a concern about construction material of OTCs, polyethylene film, may or may not have influence on VOCs

composition. Other authors (Rinne *et al*., 2009 and Wassman, *et al*., 2010) referred that enhanced CO_2 concentration on ambient reduce VOCs emission, due to closer relation between photosynthesis and VOCs stomatal biosynthesis on C3 plants.

More VOCs emission was identified in silty clay soil texture when compared with loamy sand soil. Fewer studies focused on VOCs released from plants under different soil textures (Bastos and Magan, 2007). Wheatley et al. (1996) used GC/MS technique to study VOCs from headspace of a silty clay soil (without testing any plant growth). They concluded that VOCs are depending from nutrient addition into soil. Bastos and Magan (2007) used e-nose system to identify VOCs differences between three soils: loamy sand, calcareous clay and volcanic ash under different temperatures and water potentials. They conclude that loamy sand soil had the lowest compound variability and even lower different VOC classes were observed under drier soil conditions.

Other external factor referred by several authors (Coutinho *et al*., 2009; Rinne et al., 2009; Kpoviessi *et al*., 2011; Dudareva *et al*., 2012) that influenced VOC emission peaks remains to photoperiod. According to Rinne *et al.* (2009), monoterpene emissions from Mediterranean specie *Quercus ilex* are almost double in high summer peak. Coutinho *et al.* (2009) and Hobbs *et al.* (2004) also referred that soil moisture can cause emission variations. Spinelli *et al*. (2011) points out the O_3 exposure variation and N availability as other stress factors that can induce fluctuation on VOCs profiles. Some singular compounds released during rice cycle present a particular trend between stages and also between sample treatments. This can be related not only with our studied factors, but also with presence of pest infestation (Aisaka (1977) in Maga, 1984; Michael *et al.* (1978) in Maga, 1984; Zhou and Wang, 2011).

Data analyses allow us to estimate the statistical significance of the results. The results of one sample t-test mean conclude that with 99% of confidence level, all mean values from treatment samples were within the calculated correspondent interval. The results of independent t-test studying the difference between pairs of treatments proved that just two pairs of mean values were statistically different (TE from TE_C and TE_{CC} in absolute mean values at vegetative phase). These treatments had the highest difference (more than ten compounds) between them (see last raw on Table 4.1). The explanation for other values do not statistically concluding the truth are in the compound premises identification, i.e. compound only need to be found in one triplicate to be considered for whole composite treatment.

At this point, hypothesis according to analysed data allowed us to say that increasing rice production will probably influence VOCs emission in air chemistry under climate change scenarios predictions (simultaneous higher temperature and higher CO_2 concentration). Plants produce more vegetative matter under elevated CO_2 concentration, and rice as well (plus 30% rice production) (INIAV, 2013b), encouraging a higher tillers number and grain yield depending of photosyntetical process used (C3 plants use CO_2 less efficiency than C_4 plants) (Wassmann, *et al.*, 2010). Rice (C3 plant) revealed a big sensitivity to higher CO_2 concentration (less emission of VOCs). This fact has been seen as a positive response to compensate effects of future climate change conditions. Based on previous studies, we may associate rice performance under elevated CO_2 on three main parameters: a decreased of stomatal aperture,

increased photosynthetic activity and enhances total biomass (Cho and Oki, 2012). The impact of higher air temperature on rice VOCs emission may cause more volatile compounds emission among the rice growing season (Cho and Oki, 2012).

In general, other plants such as forests of oak and pine, citrus, cottonwood, vegetables, and others agroforestry species also emit higher number of VOCs under warmer climate. It means that when temperatures are higher, associated with a greater AVOCs demand of NO_x and CO_2 in the summer could affect agricultural areas through medium and long distance transportation factors (NASA, 2013). This fact will accelerate photochemical reaction rates in atmosphere, and just showering if in the presence of a cloud cover.

In the past, some of the government's anti-smog efforts have been reducing AVOCs released by motor vehicles, power plants and factories. Since the 90's, this issue starts to change and great attention was given to BVOCs from vegetation. In 1998, United States Environmental Protection Agency reports (USEPA, 1998), concluded that 131 million people lived in countries with higher and unhealthy level of smog. VOCs emission from agriculture is a complex sphere between interaction from all BVOCs and AVOCs sources, climate conditions, location, air chemistry and atmospheric sinks (Cho and Oki, 2012). There is also a linear relation between VOCs concentration and distance of emitter sources. Air quality models are dealing with many uncertainties. To obtain a desired result we need critically understand which factors are making a definitive contribution to achieve the main physiological and phenological responses of rice to abiotic stress. Rice cycle field measurements are crucial tools for the projection of future rice VOCs behaviour under climate change effects, offering important information that is of great use to society.

6. Conclusions

Rice volatile emissions extracted by SDE and SPME techniques and identified by GC/MS using two capillary columns with different phases, present qualitative differences in their volatile fraction composition. Some of the differences maybe explained by the use of different columns phases, DB-5 resolves non-polar compounds and DB-WAX polar compounds. Other factor with possible influence on the results is the extraction method (SPME *vs.* SDE) that, in spite of allowing complementary characterization, produced different "extracts" from the same matrix. Among rice cycle growing phases, vegetative may stimulated a higher VOCs emission, possible to explain due to greater plant activity on early stage of development. Temperature caused more VOCs emission from rice plant when compared with simultaneous temperature and CO_2 concentration, probably revealed by thermotolerance capacity of rice plant under higher temperatures. VOCs emission was lower in presence of elevated CO_2 concentration, possible to explain by photosynthesis plant rate. Other external stress factors should be studied to have a complete interpretation of results, and point out the influence of each factor separately, such as: photoperiod, soil water, O_3 exposure and N availability. Study biotic factors in the field also can help to identify cause-effect relations.

Looking into prediction for climate change scenarios, we may formulate that the hypothesis higher temperature will be a fact, appearing simultaneously with other climacteric parameters. Atmospheric CO_2 concentration it is also expect to increase. Taking into account that VOCs are released when plant experiment stress, as we observed on rice cycle under our experimental conditions, temperature may affect VOCs emission expressed in higher number of compounds, although CO_2 may contribute for lowering this emission. Rice shows to have a vestigial emission per plant, but considering the world rice farms extension, rice can play a role on global air quality. Complete inventory of VOCs emission for main vegetation categories would improve correct estimations and projection of future VOCs behaviour under climate change effects, and help new correct implementation of air quality issues.

Further research:

- ✓ Rice biotic (intra- and interspecific) odour-signals interaction under field conditions;
- ✓ Influence of other abiotic factors on VOCs emission during the rice cycle (photoperiod, soil water, O_3 exposure and N availability);
- ✓ VOCs profile among the other rice varieties cycle;
- ✓ VOCs profile from other crop species (maize and wheat).

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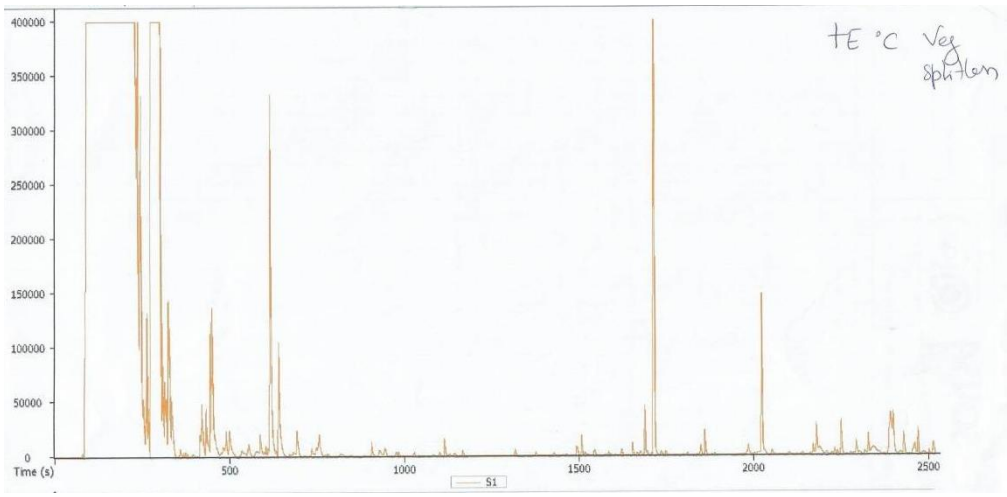
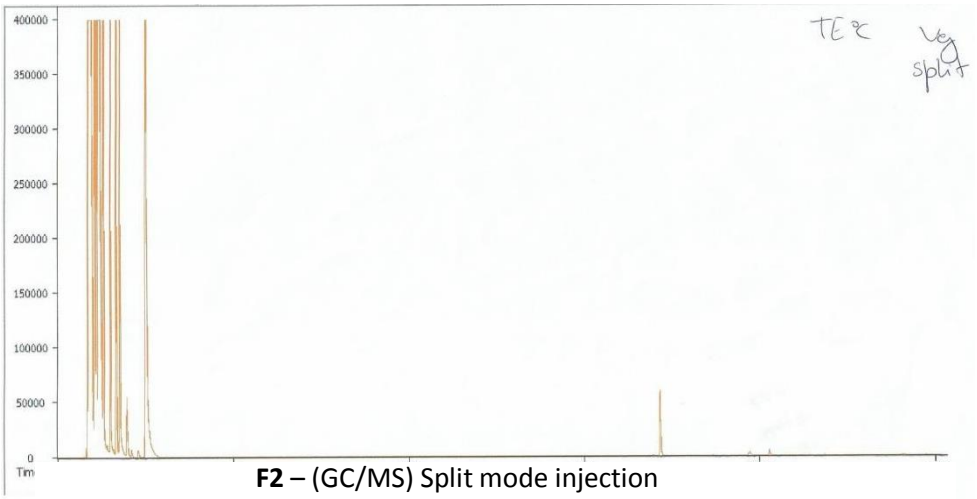
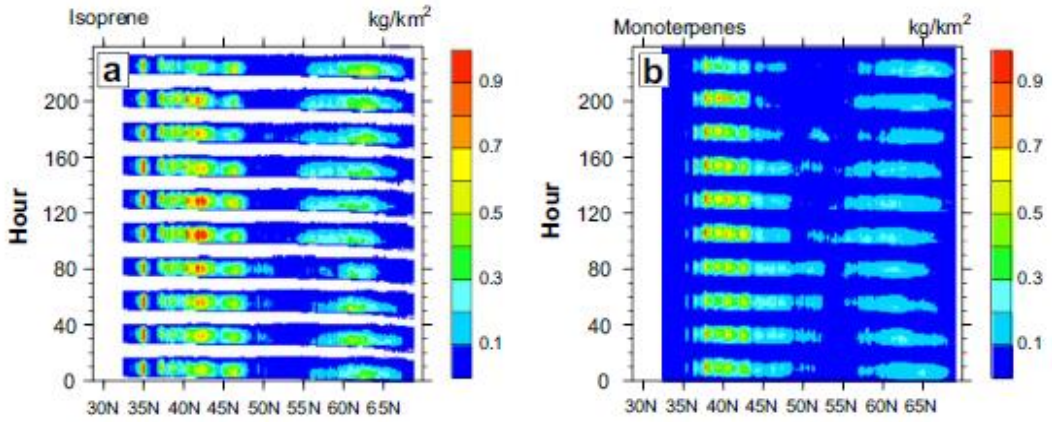
Annexes



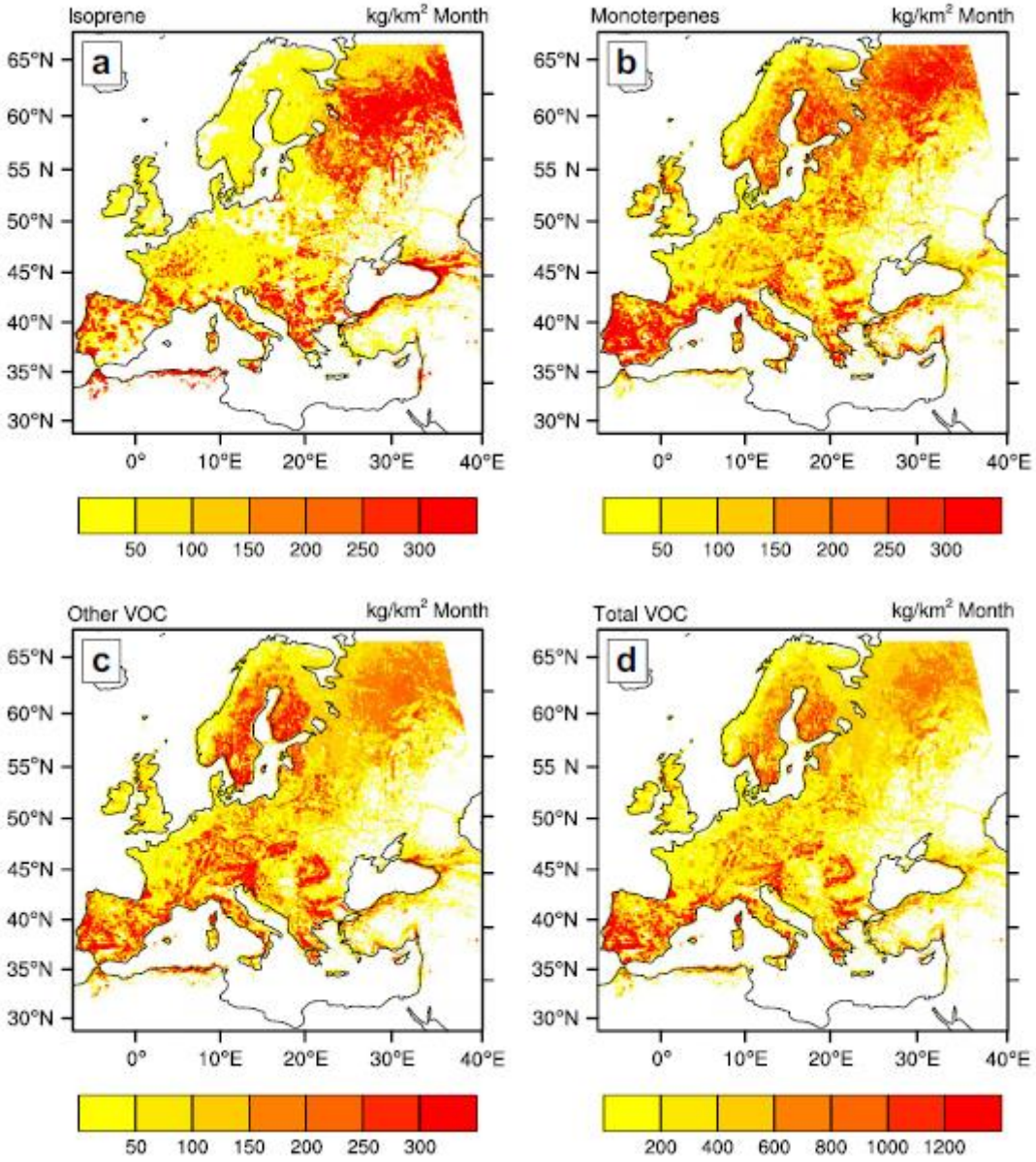
F1 – (GC/MS) Splitless mode injection



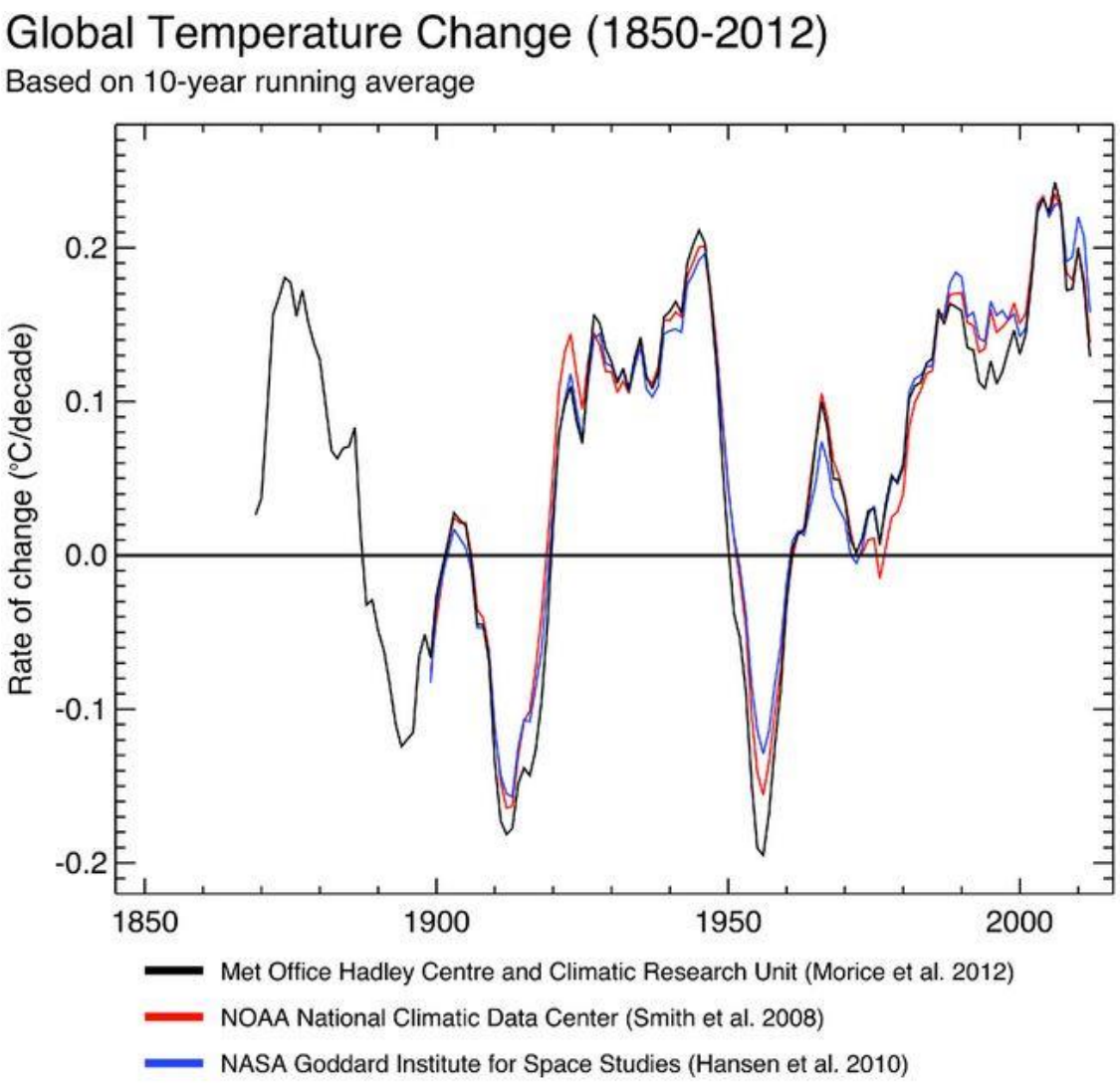
F2 – (GC/MS) Split mode injection



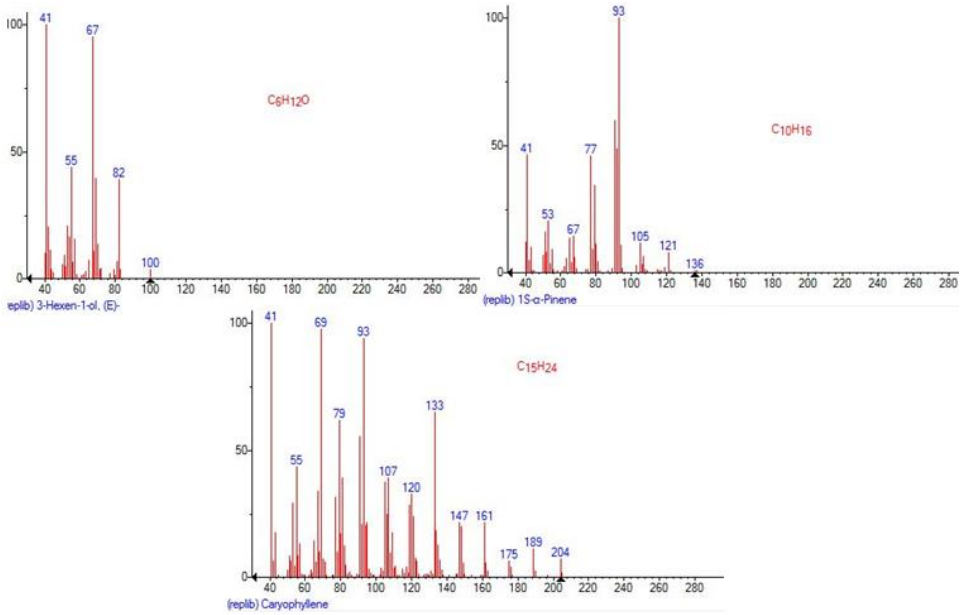
F 3 – Dayly variation of isoprene and monoterpene emissions in Europe from 1st to 10th July (Source: Steinberg *et al.,* 2009)



F 4 – Emissions of BVOCs from terrestrial vegetation in Europe in July 2000 (Source: Steinbrecher *et al.,* 2009)



F5 - Chart of global temperature change since 1850 to 2012, based on three different studies presented by black, red and blue lines (Source: EEA, 2013)



F6 – Mass spectra examples of three mains VOCs categories (GLV, mono- and sesquiterpenes)

T1 – Content of sand, silt and clay of main textural grade soils

Textural grade	Sand (%)	Silt (%)	Clay (%)
Loam	65	15	20
Sandy Loam	80	15	15
Loamy Sand	85	10	5
Sandy	95	2	3
Clay Loam	55	15	30
Silty Clay Loam	30	35	35
Clay	40	10	50
Silty Loam	40	50	10

(Adapted from: IRRI, 2013a)

T2– Standard polymer coatings and their applications

Polymer coating	Thickness (µm)	Recommended extraction temperature (T°)	Recommended applications	MW range (g/mol)
PDMS Polydimethylsyloxane	100	200-270	VOCs	80-300
PDMS/DVB Polydimethylsyloxane – Divinylbenzene	70	200-270	polar VOCs; Amines; Nitroaromatic compounds	50-300
CAR/PDMS Carboxen – Polydimethylsyloxane	75	240-300	Gases and low molecular weight compounds	30-225
CW/DVB Carbowax – Divinylbenzene	70	200-260	Polar compounds	40-275
CAR/PDMS/DVB Carboxen – Polydimethylsyloxane – Divinylbenzene	75	220-280	Polar VOCs and low MW compounds	30-300
PA Polyacrilate	85	220-310	Semi polar VOCs	80-300

(Adapted from: Parreira and Cardeal, 2005)

T 3 - GC columns descriptions and their applications

Stationary phase	*Applications*	Compositions	Polarity	Temperature range (ºC) (Isothermal/Programed) *
HP-1ms,DB-1ms,HP-1, DB-1	Amines, hydrocarbons,pesticides, PCBs, phenols, sulfur compounds, flavours and fragrances	100% Dimethylpolysiloxane	Non-polar	From -60 to 325/350
HP-5ms,DB-5, HP-5	Semivolatiles, alkaloids,drugs, FAMEs, halogenated compounds, pesticides, herbicides	5% Phenyl 95% dimethylpolysiloxane	Non-polar	From -60 to 325/350
DB-5ms	Semivolatiles, alkaloids, drugs, FAMEs, halogenated compounds, pesticides, herbicides	5% Phenyl 95% dimethyl arylene siloxane		From -60 to 325/350
DB-1301	Aroclors, alcohols, pesticides, VOCs	6% Cyanopropyl-phenyl 94% dimethyl polysiloxane	Mid-polar	From -20 to 280/300
DB-35, HP-35	CLP-pesticides, aroclors,pharmaceuticals, drugs of abuse	35% Phenyl 65% dimethyl polysiloxane	Mid-polar	From 40 to 300/320
DB-35ms	CLP-pesticides, aroclors, pharmaceuticals, drugs of abuse	35% Phenyl 65% dimethyl arylene siloxane		From 50 to 340/360
DB-1701,DB-1701P	Pesticides, herbicides, TMS sugars, aroclors	14% Cyanopropyl-phenyl 86% dimethyl polysiloxane	Mid-polar	From -20 to 280/300
HP-50+, DB-17	Drugs, glycols, pesticides, steroids	50% Phenyl 50% dimethylpolysiloxane	Mid-polar	From 40 to 280/300
DB-17ms	Drugs, glycols, pesticides, steroids	50% Phenyl 50% dimethyl arylene siloxane		From 40 to 320/340
DB-200	Residual solvents,	35%	Polar	From 30 to 300/320

	pesticides, herbicides	Trifluoropropyl 65% dimethyl polysiloxane		
DB-225ms,DB-225	FAMEs, alditol acetates, neutral sterols	50% Cyanopropyl-phenyl 50% dimethyl polysiloxane	Polar	From 40 to 220/240
HP-INNOWax	Alcohols, free organic acids, solvents, essential oils, flavours and fragrances	Polyethylene glycol	Polar	From 40 to 260/270
DB-WAX	Solvents, glycols, alcohols	Polyethylene glycol	Polar	From 20 to 250/260
CAM	Amines, basic compounds	Polyethylene glycol-base modified	Polar	From 60 to 220/240
HP-FFAP,DB-FFAP	Organic acids, alcohols, aldehydes, ketones, acrylates	Polyethylene glycol-acid modified	Polar	From 40 to 250
DB-23	FAMEs (requiring cis/trans resolution)	50% Cyanopropyl 50% dimethyl polysiloxane	Polar	From 40 to 250/260
CycloSil-B	Chiral compounds (general purpose)	30%-heptakis (2,3-di-O-methyl-6-O-t-butyl dimethylsilyl)-B-cyclodextrin in DB-1701	Mid-polar	From 35 to 260/280
HP-Chiral b Columns	Chiral compounds (using a Nitrogen selective detector, NPD)	beta-Cyclodextrin in phenyl-based stationary phase	Mid-polar	From 30 to 240/250

*Temperatures vary with column dimensions

(Source: Agilent Technologies, 2013)

T4 - Emission reductions commitments for VOC for 2020 and beyond

	Party	*Emission levels 2005 (thousands of tons of VOC)*	*Reduction from 2005 level (%)*
1	Belgium	143	21
2	Bulgaria	158	21
3	Croatia	101	34
4	Cyprus	14	45
5	Czech Republic	182	18
6	Denmark	110	35
7	Finland	131	35
8	France	1232	43
9	Germany	1143	13
10	Greece	222	54
11	Hungary	177	30
12	Italy	1286	35
13	Latvia	73	27
14	Lithuania	84	32
15	Luxembourg	9.8	29
16	Netherlands	182	8
17	Norway	218	40
18	Poland	595	25
19	Portugal	207	18
29	Romania	425	25
30	Slovakia	73	18
31	Slovenia	37	23
32	Spain	809	22
33	Sweden	197	25
34	Switzerland	103	30
35	United Kingdom of Great Britain and Northern Ireland	1088	32
36	European Union	8842	28

(Adapted from: UNECE, 2012)

T5 - Emission ceilings for 2010 up to 2020 for parties that ratified the 1999 Gothenburg Protocol prior to 2010 (expressed in thousands of tons per year)

	Party	*Ratification year*	*SO_2*	*NO_x*	*NH_3*	*VOCs*
1	Belgium	2007	106	181	74	144
2	Bulgaria	2005	856	266	108	185
3	Croatia	2008	70	87	30	90
4	Cyprus	2007	39	23	9	14
5	Czech Republic	2004	283	286	101	220
6	Denmark	2002	55	127	69	85
7	Finland	2003	116	170	31	130
8	France	2007	400	860	780	1100
9	Germany	2004	550	1081	550	995
10	Hungary	2006	550	198	90	137
11	Latvia	2004	107	84	44	136
12	Lithuania	2004	145	110	84	92
13	Luxembourg	2001	4	11	7	9
14	Netherlands	2004	50	266	128	191
15	Norway	2002	22	156	23	195
16	Portugal	2005	170	260	108	202
17	Romania	2003	918	437	210	523
18	Slovakia	2005	110	130	39	140
19	Slovenia	2004	27	45	20	40
20	Spain	2005	774	847	353	669
21	Sweden	2002	67	148	57	241
22	Switzerland	2005	26	79	63	144
23	United Kingdom of Great Britain and Northern Ireland	2005	625	1 181	297	1 200
25	European Union	2003	7 832	8 180	4 294	7585

(Adapted from: UNECE, 2012)