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RESUME

Depuis l'explosion de l'industrie chimique au début du XX^{ème} siècle et l'utilisation de produits de synthèse dans la vie quotidienne, des quantités croissantes de substances chimiques d'origines industrielles, agricoles ou domestiques, n'ont cessé d'être émises dans l'environnement. Toutefois, dans les dernières décennies, il y a eu une préoccupation croissante au sujet du sort de ces produits chimiques qui menacent la ressource en eau. L'accroissement des connaissances sur la performance analytique et les données toxicologiques sur les produits actifs a conduit les autorités dans les pays industrialisés et en développement à imposer diverses restrictions (normes). La surveillance des contaminants dans les milieux aquatiques a été mise en place pour atteindre le bon état écologique des milieux. Cette surveillance a aussi pour but de mieux comprendre les origines, le devenir et les conséquences des contaminants sur les organismes aquatiques. Parmi les polluants organiques les plus dangereux et les plus couramment détectés dans les milieux aquatiques, on trouve les hydrocarbures aromatiques polycycliques et leurs dérives alkylés, les polychlorobyphényles, les phtalates et les pesticides. Ces contaminants présents à l'état de trace ou ultra trace (du pg.L^{-1} au µg.L^{-1} dans l'eau ou du pg.kg^{-1} ps au mg.kg^{-1} ps dans les sédiments) dans les milieux aquatiques. Leurs analyses nécessitent des méthodes analytiques appropriées à la fois spécifiques et sensibles. L'Extraction sur Phase Solide (SPE, pour les matrices liquides) et l'Extraction Accélérée par Solvant (ASE, pour les matrices sédimentaires) suivies d'une étape de purification et d'une étape d'analyse par Chromatographie Gaz couplée à la Spectrométrie de Masse (GC-MS) est la méthode la plus couramment employée.

Les travaux de cette thèse s'orientent ainsi dans une direction visant à développer des nouvelles méthodes analytiques pour l'extraction et l'analyse de plusieurs familles de contaminants organiques (pesticides, phtalates, hydrocarbures, polychlorobiphényles…) présents dans l'eau (eau de surface et souterraine) et dans les sédiments. Un modèle mathématique Umetrics (MODDE) a été employé pour étudier l'influence de chaque paramètre d'intérêt et aussi pour étudier les interactions entre les différents paramètres simultanément. Comparé à des méthodes classiques, ce modèle MODDE permet de réduire le nombre d'expériences, d'économiser des solvants organiques en gardant des rendements très satisfaisants. En GC-MS, les modes de détection SIS et MS/MS ont été optimisés afin d'améliorer la précision d'identification et de quantification des molécules d'intérêts. Les méthodes optimisées ont été ensuite appliquées à étudier des milieux naturels en France et au Liban.

Mots-Clés : Eau, sédiments, contaminants organique, pesticides, HAP, extraction sur phase solide, GC-MS, France, Liban.

ABSTRACT

Since the explosion of the chemical industry in the early twentieth century and the use of synthetic products in daily life, increasing amounts of chemicals in industrial, agricultural and domestic sources, have continued to be issued in the environment. However, in recent decades there has been a growing concern about the fate of these chemicals that leave the ground and threaten water resource. Increased knowledge about the performance and toxicological properties of the actives compounds has led authorities in industrialized and developing countries to impose various restrictions. Constant monitoring of environmental contaminants in aquatic environments has been established to maintain clean environments ecological status. This monitoring also aims to a better understanding of the origins, fate and effects of contaminants in attendance. Among the most dangerous organic pollutants that are detected in aquatic environments, we can site the polycyclic aromatic hydrocarbons (PAHs) and their alkylated counterpart, polychlorobyphenyls, phthalates and pesticides. These contaminants are present at trace or ultra trace levels (from $pg.L^{-1}$ to $\mu g.L^{-1}$ in water or $pg.kg^{-1}$ dw to $mg.kg^{-1}$ dw in sediment) in aquatic environments. Their analysis requires appropriate analytical methods both specific and sensitive. Solid Phase Extraction (SPE) for liquid matrices and accelerated solvent extraction (ASE) for sediment matrices followed by a purification step and analyzed by Gas Chromatography coupled to Mass Spectrometry (GC -MS) is the method most commonly used.

In this thesis we have developed new analytical methods for the extraction and analysis of several families of organic contaminants (pesticides, phthalates, hydrocarbons, polychlorinated biphenyls...) in water (surface and groundwater) and sediments. A mathematical model Umetrics (MODDE) was used to study the influence of each parameter and also to study the interactions between different parameters simultaneously. Compared to conventional methods, this model allows reducing the number of experiments, saving organic solvents and gaining very satisfactory yields. GC-MS detection modes SIS and MS/MS were optimized to improve the accuracy of identification and quantification of molecules of interest. The optimized methods were then applied to study the natural environment in France and in Lebanon.

Keywords: Water, sediment, organic contaminants, pesticides, PAH, solid phase extraction, GC-MS, France, Lebanon.

TABLE OF CONTENTS

Remerciment

Résumé

Abstract

Table of contents

List of figures

List of tables

Acronyms and abbreviations

Introduction ………………………………………………………………………….. 1

Introduction………………………………………………………………………… 5

Chapter 1: Literature review ………………………………………………..…… 10

I. Persistent Organic Pollutants in the environmental matrix ……………...…….…... 11

1. Persistent Organic Pollutants (POPs) …………………………….…………..… 11

1.1. Polycyclic Aromatic Hydrocarbons (PAHs) ……………..………………..… 11

1.2. Origins of PAHs …………………………………………………….……….... 13

1.3. PAHs in aquatic system ………………………………………….……………… 13

1.4. PAHs toxicity ………………………………………………….……………… 14

1.5. Reglementation ………………………………………….…….…….…. 15

References …………………………………………………………………..……. 16

II. Review on pesticides and analytical instruments …………………………………… 19

Abstract ……………………………………………………………………………..… 20

Introduction ………………………………………………..…………………………... 23

I. Pesticides Generalities and water regulations………..………………………..…. 25

I.1 Definitions and history……………………………….……..……..….. 25

I.2 World consumption of pesticides………………………………..……..… 26

I.3 Ecotoxicological effects and health impacts……………….…………….…… 26

I.3.1 Health impacts …………………………………..……………………….…... 26
I.3.2 Environmental impacts ………………………………………….……….. 27
I.4 Water resources……………………………………………………...…...… 28
I.4.1. WHO (International guidelines) ……………………………………….... 29
I.4.2. European directives on drinking water and compliance at the national level..…… 29
I.4.3. USEPA Standards ………………………………………………..……...… 29

II. Extraction technique ………………………….…………………………... 31

II.1 Solid phase-based extraction technique…………………………...……… 31
II.1.1 Solid phase extraction (SPE) …………………………………………… 32
II.1.2 Solid phase Micro extraction (SPME) ……………………………......… 33
II.1.3 Matrix solid phase dispersion (MSPD) …………………………….… 34
II.1.4 Stir-bar sorptive extraction (SBSE) ……………………………..………… 36
II.2 Passive sampling (SPMD, POCIS, Chemcatcher)……………………...… 38
II.3 Immunoassay techniques (ELISA)……………………………………….. 40

III. Analytical instruments for identification and quantification ……………..……... 41

III.1. Chromatographic separation (GC and LC) ……………………………….... 42

III.2. Detection technique ………………………………………………………... 43

III.2.1. Electron capture detector (ECD) …………………………………………... 43
III.2.2. Nitrogen and phosphorus detector (NPD) ……………………………..…... 43
III.2.3. Flame ionization detection (FID) ………………………………………….. 43
III.2.4. Mass spectrometry detector (MSD) ……………………………………….. 44

Conclusion ………………………………………………………………...…… 45

References ……………………………………………………………………. 46

Chapter 2: Sampling process protocol: A state of knowledge ……………….……… 53

Introduction ……………………………………………………….…………..…. 54

I. Materials and Methods …………………………………………….……….. 55
1. Reagents ………………………………………………………………...… 55

2. Analytical instruments …………………………………………………………. 55
3. Sampling and storage …………………………………………………………... 57
4. Extraction Steps ……………………………………………………..…………… 58
4.1. Water samples ………………………………...………………..…………. 58
4.2. Liquid Liquid extraction (LLE) …………………………...………………… 58
4.3. Solid Phase extraction (SPE) …………………………………...……………… 58
4.3.1. The choice of cartridges …………………………….…………….. 59
4.3.2. Choice of factors and Optimization strategy …………………...……. 59
4.3.3. Selection of the factors o the experimental domain and of the responses ……………………………………………………………………………………… 60
4.3.4. Mathematical model postulated: D-optimal design and exchange algorithm ..……………………………………………………… 61
4.2.Sediments …………………………………………………………………… 63
4.2.1. Accelerated solvent extraction (ASE) …………………………….….. 63
5. Analytical Analysis …………………………………………………………….. 66
5.1.Gas Chromatography/ Mass spectrometry (GC/MS/MS) …………………………… 66

II. Study Site …………………………………………………………………….. 66

Chapter 3: Results and discussion ………………………………..………………… 68

I. Experimental design approach for the optimization of organic pollutants extraction from water and sediments

Résumé ………………………………………………………………………………. 69
Summary ………………………………………………………………………………... 70

Article 1: Experimental design approach to the optimization of pesticides extraction from water ……………………………………………………….…………….. 71

Abstract …………………………………………………………………….…………….. 72

1. Introduction ……………………………………………………………...……. 72
2. Materials and Methods ……………………………………..…………….………… 73
2.1. Chemicals …………………………………...………..………….……… 73

2.2. Experimental procedure ……………………………………………..……………73
2.3. Choice of operating variables and their variation levels ……………………..….. 73
2.4. Optimization strategy ………………………...…………………………….. 73

2.5. Mathematical model postulated: D-optimal design and exchange algorithm…… 74

2.6. GC-MS analysis ……………………………………………………………. 74
3. Results and discussion ………………………………………………………… 76
3.1. Analysis of designed experiments: Effect of factors …………………………….. 76
3.2. Analysis of designed experiments: Surface response curve ……………………... 77
3.3. Optimal conditions and method validation ……………………….……….. 77
Conclusion …………………………………………………………………………….. 78

References ……………………………………………………………..……………… 79

Article 2: Experimental design approach to the optimization of hydrocarbons extraction from the sediment: Method development and application …………………………… 80

Abstract ………………………………………………………………………………….. 81

1. Introduction …………………………….…………………………………………81

2. Materials and Methods …………………………………………...…………………. 82
2.1. Reagents ………………………………………………………………………. 82
2.2. Sampling ……………………………………………………………….……. 82
2.3. Extraction procedure ………………………………………………..………… 82
2.4. Choice of operating variables and their variation levels …………………...…….. 82
2.5. Experimental design ……………………………………………………...…… 83
2.6. Purification and pre-separation …………………………………………..……... 83
2.7. Gas Chromatography ……………………………………………………...….. 83

3. Results and discussions …………………………………………………………...… 84

3.1.Analysis of the designed experiments ……………………………………………….. 84
3.1.1. Mathematical model ………………………………………………………… 84
3.2. Determination of optimal conditions …………………………………………… 85
3.3 Method validation ………………………………………………………..………. 85
3.4 Contamination levels of PAHs and Me-PAHs and source apportionment ………….. 86

3.5 Composition profiles of polycyclic aromatic hydrocarbons and source apportionment……………………………………………………………………….. 86
3.5.1 PAHs composition profile……………………………………………….. 86
3.5.2 Me-PAHs composition profile …………………………………….... 86
3.5.3 Source apportionment ………………………………………………. 87
3.6 Relative potency of PAHs recorded concentration ………………………...…….. 87

Conclusion ……………………………………………………………………………….. 88

Appendix A supplementary material ……………………………………………………..89

References ……………………………………………………………………………………89

II. Study case of organic pollution in Water and sediments in France and Lebanon…. 97

Résumé ………………………………………………………………………………… 97
Summary ………………………………………………………………………………. 98

Article 3: Solid phase extraction of organochlorine pesticides residues in ground water samples (Akkar plain, Northern Lebanon) ………………………...…………….. 99

Abstract ……………………………………………………………………………… 100

1. Introduction ……………………………………………………………………….. 101

2. Materials and methods ………………………………………………….…………. 102
   2.1. Location and sampling ……………………………………………….....…… 102
   2.2. Chemicals and instrumentation…………………………………………...…… 104
   2.3. Analytical procedure ……………………………………………………….. 105

3. Results and discussion ………………………..…………………………………… 107
   3.1. α-, β-, γ- and δ-HCH ……………………………………………………….. 111
   3.2. 4,4’-DDT and chlorobenzilate ……………………………………………... 111
   3.3. Heptachlor epoxide (isomers A and B) ………………………………………. 111
   3.4. Aldrin, endrin and endrin ketone ……………………………...……………… 112
   3.5. Trans-Nonachlor and endosulfan sulphate …………………………………… 112
   3.6. Chloroneb, chlorothalonil and DCPA methyl ester…………………………… 113

Conclusion …………..……………………………………………………………… 113

References …………………………………………………………………………… 114

Article 4: Determination of pesticides, nitrates and nitrites level in ground water of Akkar plain ………………………………………………………………………… 118

Abstract ……………………………………………………………………………….. 119

1. Introduction …………………………………………………………………………. 119
2. Materials and Methods ……………………………………………………………. 120
3. Results and discussions …………………………………………………………..... 121
4. Conclusion ……………………………………………………………….………..… 122

References …………………………………………………………………………… 122

Article 5: Overview of persistent organic pollution in sediments from northern France: Study case ………………………………………………………..………………… 124

Abstract ……………………………………………………………………………… 125

1. Introduction ………………………………………………………………………….. 126
2. Materials and methods …………………………………………………...……….. 127
   - 2.1. Reagents …………………………………………………………………………… 127
   - 2.2. Sampling sites ………………………………………………………………… 127
   - 2.3. Sampling ………………………………………………………………………….. 129
   - 2.4. Targeted analytes ……………………………………………………………… 129
   - 2.5. ASE extractions ……………………………………………….……………….. 130
   - 2.6. Purification and pre-separation ………………………………………………… 130
   - 2.7. Gas chromatography analyses ………………………………..………………… 130
3. Results and discussion ……………………………………………………………….. 131
   - 3.1. PCBs distribution and composition profiles ……………………………………….. 131
   - 3.2. PAH and Me-PAHs distribution and composition profiles ……………..………… 133
   - 3.3. Sources apportionment ……………………………………………………………….. 134
   - 3.4. Sediment quality evaluation …………………………………………………….. 138
4. Conclusion ……………………………………..……………………………… 139

References …………………………………………………………………………… 140

Article 6: Case study of PAHs, Me-PAHs, PCBs, Phthalates and pesticides contamination in the Somme river water, France ………………………………………………… 147

Abstract ………………………………………………………………………………… 148

1. Introduction ………………………………………………………………………….. 149

2. Materials and methods ………………………………………………………………….. 150
   2.1. Reagents……………………………………………………………………………. 150
   2.2. Sampling sites…………………………………………..…………………….. 151
   2.3. Targeted analytes ……………………………………………………………….. 152
   2.4. Sampling and extraction procedures ……………………….…………………… 153
   2.5. Sample analysis …………………………………….…………………………… 153

3. Results and discussion ………………………………………………..…………… 154
   3.1. PAHs and Me-PAHs ……………………………………………………………… 154
   3.2. Phtalates……………………………………………………………………………… 156
   3.3. Pesticides…………………………………………………………………………….. 157
   3.4. PCBs……………………………………………………………………………………. 157
   3.5. Water quality evaluation …………………………………………………….. 159

4. Conclusion ………………………………………………………………………….... 161

Acknowledgment ……………………………………………………………………. 162

References…………………………………………………………………………….. 162

Conclusion and perspectives ……………………………………………….……. 169

Conclusion Générale …………………………………………………….…………170

General conclusion ……………………………………………………….…….….172

LIST OF FIGURES

CHAPTER 2

Figure 2.1. ***Sediment*** core Sampler

Figure 2.2. Diagram of the analytical procedure.

Figure 2.3. Accelerated solvent extraction protocol and principle.

CHAPTER 3

ARTICLE 1:

Figure 1. Effects of different factors on the extraction of 32 pesticides. The statistical values are: regression coefficient, $R^2 = 1.00$; adjusted coefficient of regression, R^2 Adj = 1.00; reliability of the mathematical model, $Q^2 = 0.999$ and mean square residuals, RSD = 0.0165.

Figure 2. The surface response curve and the fixed parameters are: pH 6, Flow rate: 2-4 $mL.min^{-1}$; pH 6; elution flow rate, 0.5 $mL.min^{-1}$; NaCl = 100 $g.L^{-1}$ and a mixture of AcOEt/MeOH (1/1 *v/v*) as eluent

ARTICLE 2:

Figure 1. Location of the sampling site (Don station on the deule river)

Figure 2. Effects of different factors on the extraction of PAH, Me-PAH and n-alkanes

Figure 3. Fig. 3. Surface response curve determined from the mathematical model. The total PAHs and n alkanes concentrations are plotted against time and temperature, with pressure and solvent (DCM) levels respectively set at high (14 MPa) and low (0% of DCM) values. Total concentrations of Me-PAH are plotted against time and temperature with pressure and solvent (DCM) levels respectively set at low (10 MPa) and low (0% of DCM) values.

Figure. 4. Total concentration repartition of P16 PAHs and P17 Me-PAHs (mg kg_1 dw).

Figure. 5. Depth profile of the ratio of low molecular weight and high molecular weight (LMW/HMW) of PAHs in the sediment core

ARTICLE 3:

Figure 1. View of Middle East region, Lebanon and AKKAR district with the sampling locations.

Figure 2 Chromatograms standard solution of OCPs in SIS mode. A/ 1- alpha- lindane, 2- Beta BHC, 3- Gamma BHC, 4- Delta BHC, 5- Heptachlor, 6- Aldrin, 7- Heptachlor epoxide, 8- Cis-chlordane, 9- Trans-chlordane, 10- DDE, 11- Dieldrin, 12- Endrin, 13- Endosulfan I, 13- DDD, 14- Endrin aldehyde, 15- Endosulfan sulfate, 16- DDT, 17- Endrine ketone, 18- Metoxychlor.) and B/ 1’- Chloroneb, 2’- Chlorothalonil, 3’- DCPA, 4’- Heptachlor-epoxide, 5’- Trans-nonachlor, 6’- Chlorobenzilate, 7’- Cis-permethrin,8’- Trans-permethrin.

Figure 3. Pollution level in the sampled sites in AKKAR region (sampling of September 2012).

ARTICLE 4:

Figure 1. Location of the sampling sites at Akkar plain

Figure 2. The concentrations of nitrate and nitrite in each studied site

Figure 3. The concentrations of the pesticides detected by G.C. in each studied site (p.p.b.)

Figure 4. The concentrations of pesticides detected by L.C. in each studied site (p.p.b.)

ARTICLE 5:

Figure 1. Location of the three study sites

Figure 2. PCB depth profiles in the three sediment cores of (a) Σ_{28}PCBs, (b) Σ_{12}PCB-DL, (c) Σ_{7}PCBi and (d) the average concentrations of Σ_{28}PCBs, Σ_{12}PCB-DL and Σ_{7}PCBi in each sediment core.

Figure 3. Sedimentary depth profiles in the three sediment core of (a) Σ_{18}Me-PAHs and (b) Σ_{16}PAHs.

Figure 4. Principal component analysis of PCB compositions and comparison with standard mixtures

Figure 5. (a) Depth profiles of low molecular and high molecular weight PAHs ratios (LMW/HMW) of PAHs in the sediment cores. (b) Cross plots of C_0/C_0+C_1 in the Phe/Ant vs the Fl/Pyr series. (c) Cross plots isomeric ratios of: Ant/(Ant+Phe) vs. BaA/(BaA+Ch).

Figure 6. Levels of sediment quality compared to recommended sediment quality guidelines.

ARTICLE 6:

Figure 1. Location of sampling sites in the Somme River in Picardie region, Northern France.

Figure 2. PAHs and Me-PAHs repartition in surface water samples of the Somme River

Figure 3. PAHs and Me-PAHs composition profiles in the Somme River water samples

Figure 4. Concentration of Σ_{28}PCB, dl-PCB and PCBi in Somme River.

LIST OF TABLES

CHAPTER 1

Table A. 1: Structure of 16 PAHs studied in this work and classed as priority compounds in the U.S. EPA.

Table B.1.1. Comparison of standards and guideline values for pesticide residues in drinking water. *Abbreviation*: GV (Guideline Value), MCL (Maximum Contaminant Level), MCLG (Maximum contaminant level goal), MAC (maximum acceptable concentration).

Table B.1.2. Different types of pesticides with their most used pretreatment technique and quantification method.

Table B.1.3. Summary of main types of passive sampling used for pesticides extraction in water matrices

CHAPTER 2

Table 2.1. information for pesticides compounds used in this study.

Table 2.2. MES Results for water samples collected on February 2014.

Table 2.3. Summary of factors and their levels as coded and natural variables.

Table 2.4. Mathematical design acquired from the model with the coded and real values of each variable.

Table 2.5. Sampling sites in Lebanon and their characteristics.

Table 2.6. Sampling sites in the Nord Pas de Calais region and their characteristics.

CHAPTER 3

ARTICLE 1:

Table 1. Targeted pesticides with their classification group, their function, their retention time, their limit of quantification (LOQ) and their qualifier ions.

Table 2. Summary of factors and their levels as coded and natural variables.

Table 3. Mathematical design acquired from the model with the coded and real values of each variable.

Table 4. Average recoveries of the 15 pesticides validated in this work.

ARTICLE 2:

Table 1. Summary of factors and their levels as coded and natural variables

Table 2. Comparison of the mathematical model with experimental extraction performances.

Table 3. Validation of the optimized method comparing to selected NIST SRM 1944 mass fraction values.

ARTICLE 3:

Table 1. Sampling sites details and coordinates

Table 2. General structures of OCPs detected

Table 3. Concentration of Organochlorine pesticide detected in groundwater samples of the Akkar district. (N.D.: Not detected)

Table 4. Comparison of 9 OCPs pesticide concentrations in groundwater of Lebanon with others sites (concentrations in µg L^{-1})

ARTICLE 6:

Table 1. Individual phthalate concentrations in the 13 sampling sites of the Somme River

Table 2. Pesticides repartition in the surface water in Somme River.

Table 3. Threshold values of EQS of individual PAH, phthalate and pesticide (Canadian Environmental Quality Guidelines, 2007; Directive n° 2013/39/UE of 12/08/13 modifying the directives 2000/60/CE and 2008/105/CE), and contamination levels detected in Somme River.

ABBREVIATIONS

A-d10	Acenaphthene-d10
Ant	Anthracene
ASE	Accelerated Solvent Extraction
BaA	Benz[a]anthracene
BIT	1, 2-benzisothiazoline-3-one
CBSQGs	Consensus-based sediment quality guidelines
CE	Capillary Electrophoresis
CPB	Colorado Potato Beetle
CW	Carbowax
DAD	Diode Array Detection
DCM	Dichloromethane
DDD	Dichlorodiphényldichloroéthane
DDT	Dichlorodiphenyltrichloroethane
DET	Desethyl terbuthylazine
DVB	Divinylbenzene
EC	European Directives
ECD	Electron Capture Detection
EQS	Environmental Quality Standards
ESI	Effective Carryover Influence
FAO	Food and Agriculture Organization
FI	Fluoranthene
FID	Flame Ionization Detection
FPD	Flame photometric Detection
FS	Full Scan
GC	Gaz Chromatography
GV	Guideline Value
HLB	Hydrophylic Lipophilic Balanced
HMW	High molecular weight
HPLC	High Performance Liquid Chromatography
HS	Head Space
IFEN	French Institute of the Environment
IPBC	Iodo-2-propynylbutyl-carbamate
IPEP	International Program for Elimination of Pesticides
IT	Ion Trap

LC	Liquid Chromatography
LDPE	Low density polyethylene
LLE	Liquid Liquid Extraction
LMW	Low molecular weight
Kow	Coefficient de partage Octanol / Eau
Log Kow	Logarithm de Kow
MAC	Maximum Acceptable Concentration
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MCPA	2-methyl-4-chlorophenoxyacetic
Me-PAH	Methyl Polycyclic Aromatic Hydrocarbons
MIP	Molecularly Imprinted Polymers
MLLE	Micro Liquid Liquid Extraction
MS	Mass Spectrometry
MSPD	Matrix Solid Phase Dispersion
MWCNT	Multi Walled Carbon Nanotubes
N-d8	Naphtalene-d8
NGO	Non Governmental Organization
NP	Normal Phase
NPD	Nitrogen-Phosphorus detection
NPDWR	National Primary Drinking Water Regulation
OCN	Octachloronaphtalene
OCP	Organochlorine Pesticides
OIT	Octhilinone
OPP	Organophosphorus Pesticides
PA	Poly Acylate
PAH	Polycyclic Aromatic Hydrocarbons
PAH	Polycyclic Aromatic Hydrocarbons
PC	Principal Components
PCB	Polychlorinated biphenyls
PCB-DL	Polychlorobiphenyl- Dioxin like
PCBi	Polychlorobiphenyl indicators
PDMS	PolyDimethylSiloxane
PEC	Probable effect concentration
Per-d12	Perylene-d12
PES	Polyether sulfone

PGC	Porous graphitic carbon
Phe	Phenanthrene
Phe-d10	Phenanthrene-d10
POCIS	Polar Organic Chemical Integrative Samplers
POP	Polar Organic Pollutants
PRESS	Prediction residual sum of squares
PS-DVB	Polystyrene divinyl benzene
PTH	Polythiophene
Pyr	Pyrene
Pyr-d10	Pyrene-d10
Q^2	Fraction of the response variation
R^2	Fraction of the response variation
RP	Reversed Phase
RSD	Relative Standard Deviation
SBSE	Stir Bar Sorptive Extraction
SDME	Single Drop Microextraction
SDWA	Safe Drinking Water Act
SIS	Selected Ions storage
SM	Suspended matters
SPE	Solid Phase Extraction
SPMD	Semi Permeable Membrane Device
SPME	Solid Phase Micro Extraction
SQGs	Sediment quality guidelines
SS	Sum of corrected *y* square for a mean
TCN	Tetrachloronaphtalene
TD	Thermal Desorption
TEC	Threshold effect concentration
TOF	Time of Flight
UHPLC	Ultra High Performance Liquid Chromatography
UNEP	United Nations Environment Program
UNIDO	United Nations Industrial Development Organization
US EPA	United Stated Environmental Protection Agency
WHO	World Health Organization
ZB-XLB	Zebron Fused Silica GC Column

INTRODUCTION

Depuis l'explosion de l'industrie chimique au début du XX^{ème} siècle et l'utilisation de produits de synthèse dans la vie quotidienne, des quantités croissantes de substances chimiques d'origines industrielles, agricoles ou domestiques, n'ont cessé d'être émises dans l'environnement. Ces activités répandent divers polluants d'origines anthropiques dans les milieux aquatiques. Ces polluants ont des natures physiques, chimiques, microbiologiques très variées. Parmi les principaux polluants organiques les plus détectés dans les milieux aquatiques, figurent les hydrocarbures aromatiques polycycliques (HAP), les polychlorobiphényles (PCB), les phtalates et les pesticides. Compte tenu, de leurs caractères toxiques, bioccumulables, leurs capacités à être transportés à des longues distances, les pesticides organochlorés sont parmi les composés les plus critiques, et sont classés dans la liste des polluants organiques persistants (POP). Certains d'entre eux sont inclus dans la liste des polluants prioritaires de la Convention de Stockholm (2006). De nos jours, bien que l'utilisation de la plupart de pesticides organochlorés ont été interdit, certains d'entre eux sont encore présents dans l'environnement en raison de leur forte persistance. Les pesticides organochlorés interdits ont été progressivement remplacés par les pesticides organophosphorés et organoazotés en raison de leur plus court temps de demi-vie dans l'environnement. Ces derniers sont universellement utilisés en raison de leur faible coût, disponibilité, large gamme d'efficacité, capacité à lutter contre un grand nombre d'espèces de ravageurs. Actuellement, les pesticides organophosphorés sont devenus les insecticides les plus utilisés. Les pesticides organophosphorés comprennent tous les composés organiques contenant des atomes de phosphore. Principalement sous la forme d'esters, qu'ils sont dégradés facilement. En outre, ils sont aussi très peu solubles dans l'eau, bien que mieux ainsi dans les solvants organiques que dans les matières grasses. Le terme « pesticides organoazotés » couvre un grand nombre de composés, qui réfèrent généralement aux carbamates et les triazines. Les dérivés de triazine figuraient parmi les herbicides sélectifs les plus utilisés et les plus efficaces. Ils sont connus pour être très toxique et persistant dans le sol, l'eau, les plantes et les animaux. Il est estimé que plus de 98% des insecticides et 95% d'herbicides n'atteint pas leurs destinations ciblées ; ils se dispersent dans différents compartiments de l'environnement, dans l'air, dans l'eau et les sédiments.

La contamination de l'eau par les pesticides et autres contaminants (hydrocarbures, polychlorobiphényles,…) peuvent provenir des sources diffuses (retombés atmosphériques, ruissellement,…) et des sources ponctuelles (effluents industriels, accidents).

Quelles que soient les sources de pollution et le compartiment de l'environnement dans lequel les contaminants sont émis, une grande partie de ces substances rejetées passent dans les milieux aquatiques. Le vent, les cours d'eau, rivière, fleuves sont des vecteurs de transports de ces polluants à des longues distances et les mers et les océans sont les réceptacles finaux. L'enjeu écologique et socio-économique que représente cette ressource, la pollution des eaux est une problématique pesant à la fois pour les pays industrialisés et les pays en développement. Des suivis environnementaux s'avérèrent alors nécessaires afin de mieux comprendre des origines, des devenirs et des conséquences de la présence de ces polluants dans l'environnement aquatique et de trouver des solutions adaptées. Les contaminants organiques sont présents à l'état de traces et ultra trace dans les milieux aquatiques. Leur analyse nécessite, par conséquence, des méthodes analytiques à la fois spécifiques et sensibles. Pour répondre à des besoins de surveillances et de contrôles, des différentes techniques de séparation et de détection sont devenues de plus en plus performantes suite aux nombreux développements scientifiques et technologiques. Cependant, ces outils mis à la disposition des laboratoires de contrôle et de recherche ne permettent pas systématiquement d'identifier et de quantifier les espèces recherchés présents en très faibles teneurs dans des matrices très diluées et très complexes. Une étape de prétraitement d'échantillon s'avère alors nécessaire pour la préconcentration de polluants, et/ou la purification de l'échantillon afin de s'affranchir d'éventuels effets de matrice. Cette étape est souvent banalisée, alors qu'elle représente la deuxième importante source d'erreurs de la méthode analytique après l'échantillonnage. Les méthodes de prétraitement utilisées mettent en jeu des principes différents selon les propriétés physico-chimiques des composés à extraire (polarité, volatilité..) et selon la nature de la matrice de l'échantillon (eaux de surface, eaux souterraines, sédiments...). L'extraction sur phase solide ou Solid-Phase Extraction (SPE) est la méthode la plus couramment employée pour l'analyse des pesticides, HAP et d'autres micropolluants dans l'eau. L'évolution de la SPE a été facilitée par la commercialisation de phases de natures variées et dédiées à des différents composés d'intérêts et à des échantillons différents. Cependant, même si elle est simple et efficace, la technique SPE présente quelques inconvénients au niveau de la consommation des solvants et du nombre d'étapes d'extraction qui peuvent conduire à des pertes de certains composés. Pour les sédiments, l'extraction des micropolluants est classiquement réalisée par extraction Soxhlet. Malheureusement, cette technique nécessite beaucoup de temps et de grands volumes de solvants organiques. D'autres techniques d'extraction ont été mises au point non seulement dans le but de réduire le volume des solvants et des temps d'extraction, mais également pour améliorer la précision des recouvrements analytiques. Ces techniques comprennent l'extraction assistée par micro-ondes, l'extraction par fluide supercritique et extraction par solvant accélérée (ASE). Parmi ces techniques, ASE, connu aussi sous le nom de l'extraction liquide pressurisé (PLE), reste un outil efficace pour extraire des micropolluants organiques dans de différents échantillons solides. En effet, en plus des avantages mentionnés ci-dessus, ASE a reçu une attention croissante en raison de sa facilité de mise en œuvre, un gain de temps, une grande efficacité et aussi il maintient les conditions d'extraction constantes et donne alors une bonne répétabilité par son automatisation. Cependant, l'extractibilité d'un composé peut être conditionnée par la nature des composés ciblés, la nature du solvant et les conditions expérimentales dans lesquelles l'extraction est mise en œuvre. Actuellement, la tendance est plutôt de s’orienter vers des protocoles efficaces, rapides et peu coûteux, mais aussi facilement utilisables pour les analyses de routine. Un point important est également de réduire le volume de solvants utilisés afin de minimiser les risques sanitaires et environnementaux. Mes travaux de thèse s’inscrivent ainsi dans cette optique générale visant à développer des nouvelles méthodes analytiques pour l’extraction et l’analyse des différentes familles de polluants organiques (pesticides, HAP,…) présents dans l’eau et dans les sédiments. Afin de pallier les contraintes de la SPE classique, une nouvelle approche a été employée. Cette approche consiste à utiliser un modèle mathématique (Modde) permettant d’étudier les effets des cinq facteurs influençant sur le rendement d’extraction. Ce modèle permet non seulement d’étudier les influents de chaque paramètre, les interactions entre eux mais aussi de réduire les nombres d’expérience en gardant les rendements satisfaisants. Cette étude a pour l’objectif d’optimiser les conditions optimales d’extraction et, après une étape de validation, d’appliquer les méthodes optimisées pour étudier les milieux naturels. Ainsi, la qualité des eaux souterraines dans la deuxième zone agricole au Liban (Akkar), des eaux de surfaces de la région du Nord Pas de Calais et les sédiments a été évalué.

Ces travaux ont été effectués dans le cadre d’une thèse en cotutelle entre l’Université Libanaise et l’Université Lille 1. Les travaux ont été menés alternativement dans l’établissement d’accueil, le Laboratoire de Géosystéosystèmes à l’université Lille 1 en France et dans l’établissement de l’origine, le Laboratoire de science de l’eau et de l’environnement à la faculté de santé publique, à Tripoli.

Cette thèse est organisée en trois chapitres. Dans le **chapitre 1**, une étude bibliographique sur la généralité des contaminants sera présentée. Il est organisé en deux parties, la première constitue une étude bibliographique sur les HAP et d’autres contaminants et la deuxième partie est une synthèse bibliographique sous forme de review soumis dans le journal

«*International Journal of Environmental Analytical Chemistry*». Les origines, les impacts, les normes de qualité d'eau par rapport au pesticides et autres polluants organiques ; les techniques analytiques les plus couramment utilisés seront présentés.

Le **chapitre 2** constitue le développement analytique, les techniques d'échantillonnage et les techniques analytiques utilisées dans ce travail, ainsi que les sites d'études étudiés. Le **chapitre 3** expose les résultats sous formes de six articles. Il est constitué de deux parties, la première partie présente les études d'optimisation des méthodes d'extraction des pesticides et des hydrocarbures respectivement dans l'eau et dans les sédiments. Deux articles sur l'optimisation des méthodes seront présentés. La deuxième partie est consacrée à des études de cas des différents milieux naturelles en France (Nord pas de Calais) et au Liban (Akkar, Nord de Liban) sous forme de quatre articles.

La conclusion et les perspectives de ce travail seront présentées à la fin de la thèse.

INTRODUCTION

During the twentieth century, the use of synthetic products in daily life, industrial chemicals, agricultural and domestic activities, have contributed in a big part to the environmental pollution. These activities emit various pollutants with anthropogenic origins in natural aquatic environments. Pollutants have different nature, according to their physical, chemical or microbiological properties. Among the main pollutants currently detected in aquatic environments, we can cite polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalates and pesticides. Due to their toxic and bioaccumulative characteristics, they are able to be transported a long distance. Organochlorine pesticides are among the most critical compounds and are classified in the list of persistent organic pollutants (POPs). Some of them are included in the list of priority pollutants in the Stockholm Convention (2006). Nowadays, although the use of organochlorine pesticides was prohibited, some of them are still present in the environment due to their high persistence. Organochlorine pesticides were gradually replaced by organophosphate and organonitrogen pesticides because of their shorter half-life in the environment. Organophosphate and organonitrogen are universally used because of their low cost, availability, wide range of effectiveness and ability to eradicate many pest species. Currently, organophosphate pesticides have become the most widely used insecticides. The oragnophosphosphate pesticides include all organic compounds containing phosphorus atoms. Principally in the form of esters, they are degraded easily. The term "organonitrogen pesticides" covers a large number of compounds, which generally refers to carbamates and triazines. Triazine derivatives are among selective herbicides the most currently used and very effective. They are known to be highly toxic and persistent in soil, water, plants and animals. It is estimated that more than 98 % insecticide and 95 % herbicide do not reach their targeted destination; they disperse in different compartments of the environment; in air, on the ground, in water and sediment.

Contamination of water by pesticides and other pollutants (hydrocarbons, polychlorinated biphenyls …) can occur from diffuse source pollution (atmospheric fallen, runoff ...) and point source pollution (industrial effluents, accidents).

Whatever the sources of pollution and the environmental compartment in which the contaminants are emitted, much of these substances discharged into the aquatic environment. Wind, rivers are vectors of transport of these pollutants at long distances and oceans and seas are the final receiver. The ecological and socio-economic challenge posed by this resource become serious, water pollution is a problem not only for developing countries but also for industrialized countries. Environmental monitoring become necessary to a better understanding of the origin, fate and consequences out of the presence of these pollutants in the aquatic environment and to find appropriate solutions. Organic contaminants are present in trace and ultra trace in aquatic environments. Their analysis requires, consequently, analytical methods both specific and sensitive. To meet the need of monitoring and control, different techniques of separation and detection have become increasingly efficient in response to many scientific and technological developments. However, the tools available do not systematically allows to identify and quantify organic contaminants present in very low level and in very dilute and complex matrices. Therefore a sample pretreatment step is necessary for preconcentration of pollutants and/or purification of the sample in order to overcome possible matrix effects. This step is the second major source of error in the analytical method after sampling. Pretreatment methods used involve different principles according to the physicochemical properties of the compounds to be extracted (polarity, volatility ...) and the nature of the sample matrix (surface water, groundwater, sediment ...). The solid phase extraction (SPE) is the most commonly used method for extracting pesticides and other micropollutants in water. The evolution of the SPE has been facilitated by the availability of various natures of phases and dedicated to different compounds of interest in different samples. However, even if it is simple and effective, the SPE technique has some drawbacks in terms of solvent consumption and the number of extraction steps that can lead to losses of some compounds. For the sediment, the extraction of microcontaminants is conventionally carried out by Soxhlet extraction. Unfortunately, this technique is time consuming and requires large volumes of organic solvents. Other extraction techniques have also been developed not only in order to reduce the volume of solvents and extraction time, but also to improve precision of the analytes recovery. Such techniques include microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE). Among these techniques, ASE, also known as pressurized liquid extraction (PLE), remains an efficient tool for different solid samples extraction. Indeed, in addition to the above mentioned advantages, ASE has received an increasing attention because of its facility to implement, time saving, and efficiency. It maintains constant extraction conditions and gives a good repeatability by its automation. However, extractability of a compound from a matrix such as sediment can be operationally defined by the nature of targeted compounds, the nature of solvents and the experimental conditions under which the extraction is carried out. Currently, the trend is to move towards effective protocols, fast and inexpensive, but also easy to use for routine analysis. An important point is also to reduce the amount of solvents volume to minimize health and environmental risks. My thesis is thus fit into this general approach to develop new analytical methods for the extraction and analysis of different families of organic pollutants (pesticides, PAHs ...) in water and sediments. To overcome the constraints of conventional SPE or ASE, a new approach was used. This approach involves using a mathematical model (Modde) to study the effects of five factors on influences the extraction yield. This model not only studies the influent of each parameter and the interactions between them but also it reduces the numbers of experiment with satisfactory yields. The objective is to optimize the extraction conditions for analyzing organic contaminants from water and sediment. After a validation step, the methods were applied for studying the natural environment. Thus, the quality of groundwater in the second agricultural area in Akkar in Lebanon, surface waters and sediment in Nord Pas de Calais region in France were evaluated.

This work was conducted as part of a joint PhD between the Lebanese University and the University of Lille 1. The work was carried out alternately in the Laboratory Géosystèmes at the University Lille 1 in France and in the Laboratory of water Science and Environment in the Faculty of public Health, Tripoli in Lebanon.

This thesis is organized into three chapters. In Chapter 1, the generality of organic contaminants is presented. It is organized in two parts, the first is a bibliographic study on PAHs and other contaminants and the second part is a summary of literature presented in form of a review submitted to "*International Journal of Environmental Analytical Chemistry*". Origins, impacts, water quality standards and analytical techniques most commonly used are presented.

Chapter 2 provides the analytical development, sampling techniques and analytical techniques used in this work. Chapter 3 presents the results in forms of articles. It is constituted of two parts; the first part is about the optimization methods for pesticides and hydrocarbons respectively in water and in sediments. Two articles on methods optimization will be presented. The second part is about case studies of different natural environments in France in Nord-Pas-de Calais and in Akkar region, North Lebanon. This part is formed of four articles. And finally, a conclusion and prospects of thesis will be presented.

Chapter 1

LITERATURE REVIEW

A. Persistent Organic Pollutants in the environmental matrix

1. Persistent Organic Pollutants (POPs)

Persistent organic pollutants (POPs) are extremely toxic substances for environment and human health. Their physical and chemical properties, particularly their high stability, give them ubiquity and capacity of accumulation in the leaving organisms and nature. Most of POPs are man-made compounds (not naturally found) such as PCB, pesticides, insecticides and polycyclic aromatic hydrocarbons (PAHs). PAHs can be present naturally in the environment but they can also be man-made. PAHs are created during incomplete burning products like coal, oil, gas and garbage. In 1995 the United Nations Environment Program expanded its research and investigation on POPs with an initial focus on what became known as the "Dirty Dozen". These were a group of 12 highly persistent and toxic chemicals: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzen, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and toxaphen. Many of the pesticides in this group are no longer used for agricultural purposes but a few continue to be used in developing countries.

Since then, additional substances such as carcinogenic polycyclic aromatic hydrocarbons (PAHs) and certain brominated flame-retardants, as well as organometallic compounds such as tributyltin (TBT) have been added to the list of Persistent Organic Pollutants. There is several source of pollution, such as industrial, domestic and agricultural. Contaminants can be classed into two groups:

- Organic compounds represented by HAP, polychlorobiphenyl (PCBs), pesticides, insecticides, medicaments, dioxins and many others.
- Inorganic compounds such as metallic compounds, mercury, nitrogen, phosphorus, …

1.1. Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are organic compounds consisting of carbon atoms and hydrogen molecules whose structure comprises at least two fused aromatic rings. The PAH family includes many substances that differ by the number and position of the benzene rings (Neff, 1979), but only a hundred of these molecules have been identified within the environment. Sixteen PAHs were listed by the Agency for Environmental Protection of the United States (U.S. EPA) as priority organic pollutants and therefore a particular interest in monitoring the environment (ATSDR, 1990). The structures of 16 U.S. EPA PAHs studied are shown in Table 1.

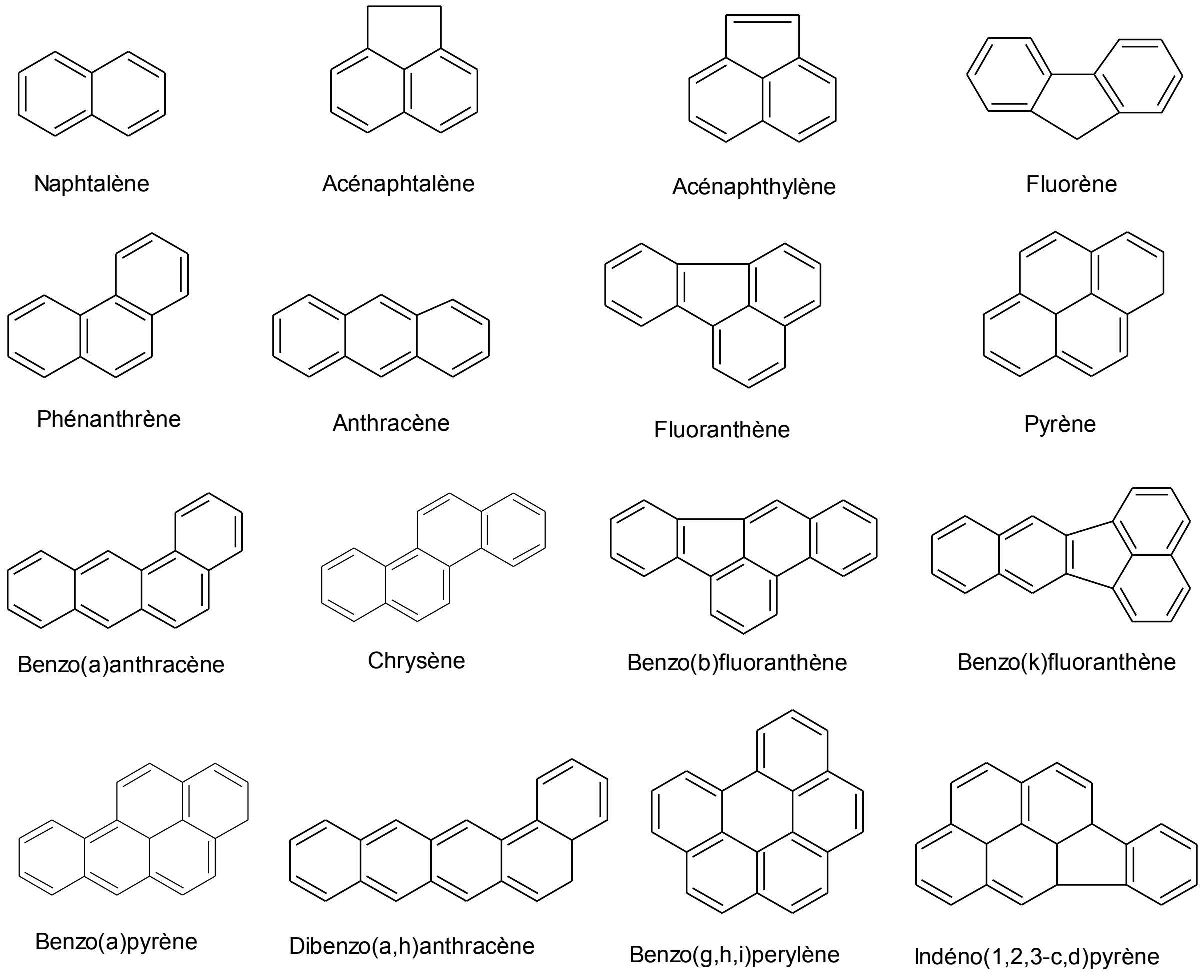
Naphtalène
Acénaphtalène
Acénaphthylène
Fluorène
Phénanthrène
Anthracène
Fluoranthène
Pyrène
Benzo(a)anthracène
Chrysène
Benzo(b)fluoranthène
Benzo(k)fluoranthène
Benzo(a)pyrène
Dibenzo(a,h)anthracène
Benzo(g,h,i)perylène
Indéno(1,2,3-c,d)pyrène

Table 1: Structure of 16 PAHs studied in this work and classed as priority compounds in the U.S. EPA.

The physicochemical properties of PAH vary depending on their structures and molecular masses. These properties determine the fate of PAHs and their distribution in the environment. Generally, PAHs are considered as polar molecules, hydrophobic and poorly soluble in water. Their hydrophobicity increases with the number of aromatic rings, so that their solubility decreases.

1.2. Origins of PAHs

PAH can be originated from natural processes, but human activity is generally considered the major source of introduction of PAHs in the environment. Natural sources include volcanic eruptions, biomass burning during forest fires and leaks of natural reservoirs of oil (Hoyau et al, 1996. Pozzoli et al, 2004). The majority of anthropogenic emissions of PAHs come from the combustion of petroleum, coal, natural gas or garbage (Besombes et al., 2001). In 2004, the estimated global atmospheric emission of 16 PAHs was 520 000 tones, with a percentage of 56.7% from the combustion of biofuels and 17% of forest fires (Zhang and Tao, 2009). Three main sources can be considered for PAH (McElroy, 1989). The largest, called a pyrolytic source comes from the incomplete combustion of organic matter at elevated temperature. PAHs can also be generated by the growth of the organic material subject to natural geothermal gradient therefore in oil and coal. This source is called the petrogenic source. A third source said diagenetic, which is rather minor, corresponds to early diagenesis. In all cases, the PAHs can enter directly in the aquatic environment from point sources such as industrial effluents and urban or through diffuse sources such as runoff in rural areas and in urban areas and atmospheric deposition.

1.3. PAHs in aquatic system

PAHs were detected in all environmental compartments (air, water, soil and sediment) (Menzie et al. 1992). The compounds are formed during the combustion of organic matter and introduced into the atmosphere. They can also have a long residence time in adsorbing on fine particles (<1-3 mm). PAHs are transported in gaseous form or adsorbed particles, to hundreds or even thousands of kilometers from their place of issue (Abrajano et al. 2007). PAHs are then introduced into the aquatic environment *via* dry or wet particles (Neff, 1979). PAHs deposits may also be driven by the runoff to reach marine environment (Latimer et al, 1990). After their introduction in aquatic environments, PAHs are distributed in dissolved form, adsorbed on organic matter or adsorbed on suspended particles. The partition of PAHs between the different phases of the aquatic environment is mainly governed by their hydrophobicity evaluated by K_{ow}. The more soluble PAHs in water with low molecular weights are generally more concentrated in the dissolved phase while PAHs higher molecular weights, more hydrophobic, are more likely to adsorb to suspended solids. Their concentration in the dissolved phase is very low. The dissolved fraction of the less soluble compounds ($K_{ow} > 6$) decreases dramatically in the presence of organic particles suspended in the water column (Cobas and Zhang, 1994). The partition coefficient between the particulate phase and the dissolved phase (Kp) is calculated by the ratio of the concentrations in each phase of the contaminant; the more hydrophobic compounds are characterized by high partition coefficients Kp. In addition to their intrinsic properties, PAHs partition between water and solid phases depends on the characteristics of the medium. Thus, the affinity of the particles for PAH or sediments in suspension can vary depending on various parameters related to the medium (Landrum et al, 1992. Harkey et al, 1994). The nature and concentration of dissolved organic matter and total suspended solids (TSS), the particle size and the time of contact with the pollutant are key parameters (Harkey et al., 1994). The affinity of PAHs for TSS increases with the organic carbon content and for smaller particles (Knezovich et al, 1987, Cobas and Zhang, 1994). The colloidal particles are then subjected to direct or indirect processes sedimentation; sediment mass is the major reservoir of hydrophobic compounds (Neff, 1979). In 1997, a study Lipiatou and Saliot (1991) on the western part of the Mediterranean has estimated that 50% of PAHs introduced in this area eventually incorporated into coastal sediments (0-200 m depth) and nearly 13% sediment between 1000 and 2000 m. PAHs can be released back into the water column by resuspension phenomena due to ocean currents and by bioturbation (Karickhoff and Morris, 1985). Biodegradation phenomena (aerobic or anaerobic) may occur in sediments and particularly affects low molecular weight PAHs (Venosa et al, 1996, Sugiura et al, 1997), while the high molecular weight PAHs seem more resistant to bacterial degradation process and tend to persist longer (Abrajano et al 2007). PAHs may also be subject to photodegradation in the photic zone of the water because of their aromaticity and chemical conjugated bonds (Abrajano et al. 2007), but the bacterial degradation remains the most dominant process to remove contaminated PAH.

1.4. PAHs toxicity

Genotoxic characters (mutagenic) and carcinogenic PAHs are aspects the most studied to date. During the first phase of biotransformation of PAHs, electrophilic intermediates formed may not be supported by the phase conjugation. Some of them , such as epoxy-diols, can bind covalently with the DNA (deoxyribonucleic acid) , RNA (ribonucleic acid) and proteins (Harvey et al, 1999), leading to cellular dysfunction, genetic mutations and the formation of carcinogenic tumors (Nesnow et al, 2002). Specifically, these metabolites may be interposed between the planes of the bases of DNA and form stable adducts with the latter. Upon DNA replication, recognition errors of complementary bases may then take place leading to mutations. B[a] P is one of the most complained on PAH. Indeed, several studies have shown that its carcinogenicity is the highest among its peers. Its toxicity is due in part to a strong carcinogenic metabolite, benzo[a]pyrene -7, 8-dihydrodiol -9, 10- epoxide (BPDE) (Figure 1.5), which binds at the DNA of the cells and causes mutations that ultimately lead to the development of a cancer (Harvey et al, 1999).

1.5. Reglementation

In Europe, the European Parliament and the Council of the European Union have signed on October 23th, 2000 the text of the Framework Directive (WFD), establishing a framework for Community action in the field of water (EU, 2000). The text of the WFD, which applies to all waters (inland surface waters, groundwater and coastal waters), aims including the preservation or improvement of the quality of these in order to protect aquatic ecosystems and sustainable use of water. The objectives are to reduce discharges of priority substances presenting a significant risk to the aquatic environment first and elimination of discharges of priority hazardous substances that are toxic, persistent and bioaccumulative. The list of priority substances in the field of water includes a total of 41 substances of different chemical groups including PAHs. The list of PAH substances is 8: benzo[b]fluoranthene (B[b]FL), benzo[k]fluoranthene (B[k]FL), B[a]P, B[ghi]P and InP, plus Nap, Ant and FL. The last compound is considered as an indicator of the presence of other more dangerous PAHs. It may be noted that the selected compounds are not necessarily the most toxic to aquatic environments, as are the Chr or B[a]A.

The Directive sets an objective to achieve good chemical and ecological status of water bodies in 2015. The chemical status will be assessed against threshold values, the environmental quality standards or Environmental Quality Standards (EQS) set for each priority substance. The EQS are built from the risk assessment of the relevant substance poses to the environment or human health. These values must help ensure good status of surface waters by incorporating the concept of acute and chronic toxicities of contaminants. Two types of EQS are distinguished: The annual average (AA- EQS) and the maximum acceptable concentration (MAC- EQS). For priority substances of the WFD that exists naturally in the aquatic environment, such as PAHs, there is a natural concentration called "noise" or "geochemical background" that is taken into account in the calculation of standard quality

(EU, 2008). It should be noted that the values of EQS are total concentrations in the water and not only those of the dissolved phase.

Considering water intended for human consumption (except for natural mineral waters), the EU states that the sum of the concentrations of B[b]FL , B[k]FL , B[a]P , B[ghi] P and InP do not exceed 0.1 µg.L^{-1}. The concentration of B[a]P does not exceed the value of 0.01 µg.L^{-1} (EU, 1998). The World Health Organization (WHO) defines the limits for drinking water at 5 µg.L^{-1} for FL and 0.7 µg.L^{-1} for B[a]P (WHO, 2011).

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Solid-phase based extraction techniques for the extraction and determination of pesticides in environmental matrix

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Abstract

Pesticides are organic compounds used to protect crop production from harmful species. Regardless of their important role in crop production, they can harm the environment because of their toxicity, their stability and their by-products found in the environmental matrices. The quantification of these compounds requires appropriate extraction/separation method with high efficiency, unique selectivity and high sensitivity. Residual analysis has been performed to find a suitable extraction method to quantify these compounds and their metabolite in different matrices. Since 1990, new and effective sampling techniques based on solid-phase-extraction have been developed. Advanced device formats facilitate processing of problem samples combined with a high level of automation. The selection of an isolation and/or concentration technique depends largely on the class of pesticides to be determined. It is often necessary to determine simultaneously a wide variety of compounds in water samples. Several approaches for application of Solid-phase-based extraction techniques currently used such as solid phase extraction (SPE), solid phase micro-extraction (SPME), matrix-solid phase dispersion (MSPD), stir-bar sorptive extraction (SBSE) and lately polar organic chemical integrative sampling (POCIS) and Immunoassay (ELISA) were developed and will be described in this review.

Keywords: SPE, SPME, MSPD, SBSE, POCIS, ELISA Environmental matrix, pesticides, GC/MS, LC/MS.

Table of Content

Abstract …………………………………………………………………………………..… 2

Introduction ……………………………………………………….………………………... 4

I. Pesticides Generalities and water regulations………….…………………………..….. 6

I.1 Definitions and history……………………………………….…………..…... 6

I.2 World consumption of pesticides…………………………………………..… 7

I.3 Ecotoxicological effects and health impacts……………………………..… 7

I.3.1 Health impacts …………………………..……………………….…... 7

I.3.2 Environmental impacts ……………………………………….……….. 8

I.4 Water resources……………………………………………...……...… 9

I.4.1. WHO (International guidelines) ………………………………………..9

I.4.2. European directives on drinking water and compliance at the national level…… 10

I.4.3. USEPA Standards ……………………………………………..…....… 10

II. Extraction technique ………………………….…………………………... 11

II.1 Solid phase-based extraction technique……………………………...……… 12

II.1.1 Solid phase extraction (SPE) ……………………………………………… 13

II.1.2 Solid phase Micro extraction (SPME) ……………………………....… 14

II.1.3 Matrix solid phase dispersion (MSPD) ………………………………..… 15

II.1.4 Stir-bar sorptive extraction (SBSE) ……………………………...………… 16

II.2 Passive sampling (SPMD, POCIS, Chemcatcher)……………………………..… 19

II.3 Immunoassay techniques (ELISA)…………………………………………..

III. Analytical instruments for identification and quantification ……………..……... 21

III.1. Chromatographic separation (GC and LC) ………………………………….... 22

III.2. Detection technique ………………………………………………………... 23

III.2.1. Electron capture detector (ECD) …………………………………………... 23

III.2.2. Nitrogen and phosphorus detector (NPD) …………………………..…... 23

III.2.3. Flame ionization detection (FID) …………………………………………….. 23

III.2.4. Mass spectrometry detector (MSD) …………………………………….. 24

Conclusion ………………………………………………………………..…… 25

Introduction

Pesticides are organic compounds with various polarity, functional groups and degree of ionization. They belong to the class of persistent organic pollutants (POPs). Due to their toxicity, bioaccumulation, long term transport and persistence characters [1], several pesticides have been listed as priority pollutants by the United States Environmental Protection Agency (US EPA). When sprayed in the environment, these compounds are found in atmosphere than fall as particulate deposition to join shallow surface waters or falling on the soil and leaching to reach and contaminate groundwaters. Actually, global population consists of 7 billion of food consumers [2] and this number tends to increase drastically with the coming years. If the use of pesticides is mandatory for a better recovery regarding agriculture, the downside is the frequent occurrence of these substances in aquatic environments. Within nature, most chemical substances tend to decompose to relatively harmless compounds [3-6]. However, some pesticides are persistent enough to bear the decomposition action of chemical, biological and thermal process. In the reason to protect the ecosystem and to keep water resources clean, it is important to identify the nature of pesticides compounds, their contamination levels as well as their sources. Extraction and analysis of organic pollutants from environmental matrices constitute an essential step in environmental research. However, the extraction for quantitative analysis can turn out to be difficult because these compounds are present in trace levels and can be strongly bound to the sorbent matrix. The analysis of pesticides residues in environmental matrices has received increasing attention in the last few decades, as can be assumed from the large number of papers published dealing with this important subject [7-9].

The accuracy and precision of analysis are depended on both sample preparation and instrumental performance. The analysis is usually carried out using gas chromatography (GC), liquid chromatography (LC) or capillary electrophoresis (CE), depending on their polarity, volatility and thermal stability [10-14]. Regulations were set concerning the acceptable level of pesticides in water [15-18]. This Directive sets concentration limits tolerated in surface water for a number of substances or groups of substances identified as priority because of their significant risk for the aquatic environment. EQS (Environmental Quality Standards) were mainly defined for priority substances and these values are specific for substances detected in surface water. Concerning groundwater, quality standard were set by the European directive on 12 December 2006 (2006/118/EC). Quality standards are not specific for each compound and the majority of active substances have the same limits. As a large number of pesticides are present in water, a regular monitoring is necessary to control the water quality and ensure compliance with established standards [4, 19, 20].

In general, environmental samples cannot be analyzed without preliminary sample preparation due to their present in the environment in trace levels, their large variety of physicochemical property and their behavior regarding the natural surroundings they're present in [21]. Due to low detection level required by regulations and the complex matrices of the environment in which chemical compounds are present, sample preparation would be a challenge for analytical analysis [4]. In order to obtain a reliable method for analyzing pesticides in water, sample preparation, include extraction, concentration and recover of analytes should be optimized [7].

In the last few years, we witness the evolution and progress of analytical methods dealing with environmental samples [22-31]. This has led to new methods by being eco-friendly with less use of solvents, economy of time and consumable matter and reliable results. Liquid-liquid extraction or LLE has always been a reference method for pesticides extraction, but has been replaced by an alternative SPE, owing to its simplicity and economy in term of time and solvent needs [22]. This technique is now the communally used for sample preparation technique in many areas of chemistry, including environmental, pharmaceutical, clinical, food and industrial chemistry [32]. LLE become less used because it cannot allow extracting polar pesticides or their degradation products, it is laborious, time-consuming, large solvent volumes consuming, expensive and subject to problems arising from the formation of emulsion. In addition, recent regulations pertaining to the use of organic solvents have made LLE technique unacceptable. Alternative solid-phase-based extraction techniques can be used for the sample preparation before chromatographic analysis. This include: solid phase extraction (SPE), solid phase micro extraction (SPME), matrix solid phase dispersion (MSDP), stir-bar sorptive extraction (SBSE) and lately polar organic chemical integrative sampling (POCIS) [33-39] and Immunoassay (ELISA) [40].

Three mains objectives will present in this review. Firstly guidelines and regulations for drinking water, persistent pesticides and consequences on the environment will be described. Second objective which focuses on papers appearing the last few years, an overview of the large variety of materials concerning solid phase extraction procedures developed to isolate and pre-concentrate pesticides residues as well as the principles. Thirdly, a section on chromatographic techniques used for identification and quantification in pesticides analysis is presented.

I. Pesticides Generalities and Water Regulations

I.1. Definitions and History

Food and Agriculture Organization of United Nations (FAO) and the World Health Organization (WHO) define "Pesticide" as any substance preventing or controlling unwanted plants or animals. Usually, the term pesticide is widely used, but the agricultural profession speaks of "product" or "plant product" for Crop Protection. These products consist of one or more chemical substances, organic or inorganic, of natural or synthetic origin.

The introduction of pesticides in crops dates back to ancient times where ashes and common salts were used as herbicides. Chinese farmers used arsenicals and nicotine in the form of tobacco extracts as insecticides in the 16^{th} century-while in the early 1900's, sulfuric acid, copper nitrates and potassium salts have been used for crop production. In the same period of time 1900-1950, sodium arsenate become the standard herbicide and was used in large quantities. After that, fungicides treatment based on copper sulfate (which the famous Bordeaux mixture), or based on mercury were introduced.

Subsequently, pesticides benefit greatly from the development of organic chemistry before the Second World War [39-45]. The first group being synthetized were the organochlorine pesticides, but especially after the Second World War, that several organic pesticides were created and commercialized (organophosphates, triazines, carbamates…). In the 1950's, insecticides such as DDD and DDT were introduced and used in large quantities in preventive medicine (to destroy the mosquito responsible for malaria) and in agriculture (elimination of Colorado potato beetle CPB).

Until the early 20th century, these pesticides were mainly derived from mineral compounds, based on substances example copper salt, manganese and arsenic. Since the 1940's the development of synthetic organic chemistry took place and here appeared the first synthetic organic molecules. This period marks the beginning of a rapid expansion of plant protection products in the world. The use of organic pesticides has increased steadily since the 1990s [41] and actually, most of pesticides used are organic substances.

Worldwide consumption structure of pesticides has undergone significant changes since 1960s. The proportion of herbicides in pesticide consumption increased rapidly, from 20% in 1960 to 48% in 2005 [42]. The proportion of consumption of insecticides and fungicides/bactericides declined despite their sales increased. The rapid increase of herbicide consumption enhanced agricultural intensification and productivity.

I.2. World consumption of pesticides

Over 1990s, pesticide sales remained reasonably constant, among 300 billion dollars (of which 47% were herbicides, 79% were insecticides, 19% were fungicides/bactericides, and 5% the others) [42]. The global uses of pesticides have increased considerably during the second part of the 20th century. Some of the problems with diseases and insects have increased with increasing use of nitrogen fertilizers due to a higher susceptibility of the crop to attack at higher nitrogen input [43]. Some countries in Western Europe have seen a reduction in pesticide consumption in recent years, primarily due to policies that promote or enforce management strategies with reduced pesticide use [44]. Future pesticide consumption is likely to grow more rapidly in developing countries than in developed ones [45]. The treatment of pests and diseases, in both plants and livestock, has become more important to safeguard investments in farm output. There are about 25 000 agricultural pesticides with about 700 active substances on the world market. The value of the global market for pesticides in 2010 was around 38 billion$ [46]. France represents the largest pesticides consumer in Europe followed by Germany, Italy, Spain and UK [47].

I.3. Ecotoxicological effects and health impacts

Despite the advantages associated with the use of these substances in agriculture, many questions arise about the fate of these compounds in the environment and toxicity in relation to human and ecosystems.

I.3.1. Health impact

The human contamination by pesticides can be done through different ways, including in the food and water consumption, inhalation or skin contact (case of farmers). The risk of harm from pesticides and their degradation products depends on several factors such as their toxic properties in the short term (acute) or long term (chronic), their persistent nature and ability to form toxic combinations with other chemical compounds [48]. Disorders observed in the case of an acute effect mainly concern the skin and mucous membranes. Many studies on chronic toxicity of pesticides to humans have demonstrated that these compounds can cause neurological disorders, affecting growth [49], cause a malfunction of the immune, reproductive systems [50] and endocrine [51]. Cancer is also listed among the diseases that can be caused by pesticides [52]. For nearly fifty years, pesticides have been detected in all environmental compartments. If pesticides in the environment may have impacts on human health, these compounds may also have a significant effect on the ecosystem.

I.3.2. Environmental Impact

To assess the mechanisms of pesticide contamination on aquatic ecosystems, it is important to understand their mode of dispersal and the dynamics of these compounds in various environmental compartments. After spraying on crops, pesticides can volatilize to the atmosphere, set in the ground, being dragged by the water in the deeper layers, or end up in surface runoff. During the application of pesticides on crops, these products can escape into the atmosphere even before the product reaches the ground. This phenomenon is observed in the spray treatments, and is emphasized by the movement of air masses (drift). After application, pesticides can be transported to the atmosphere by volatilization from soil or through wind erosion. In the atmosphere, pesticides can be found in the gas phase in the dissolved or adsorbed to suspended particulate phase [53-54]. During the transport of these compounds by air masses, pesticides can undergo changes in response to light, oxidizing agents and/or atmospheric radicals, which can lead to the elimination of pesticides, or training by-products of degradation, sometimes more toxic than the parent compounds [55].

Several pesticides have been frequently observed in rainwater, snow and fog [56]. Studies of rainwater showed particular sporadically and irregularly, the presence of a number of herbicides and their metabolites (atrazine, simazine, alachlor, metolachlor, dinoterb, deethylatrazine déisopropylatrazine). Some have also been found in the gas and particulate phases as desethyl terbuthylazine (DET), a degradation product of terbuthylazine [57]. These compounds can be deposited on the earth's surface (land or aquatic) through atmospheric fallout such as wet deposition (rain, snow) and dry deposition (gases and particles). Surface waters can be also contaminated by rain or snow that leads pesticides to a wetland (river, lake, pond, sea, etc.) or on the floor.

The soil is an interface, so that the behavior of pesticides in the soil will determine their persistence and spread to other compartments of the environment. Chemical interactions in soil can be very complex. Indeed, the behavior of pesticides will depend firstly on the soil characteristics (pH, composition of clay, organic matter, porosity, moisture, biological populations, bacterial activity, etc.) and physicochemical properties of the molecules, that can be transformed into the soil by chemical or biological degradation (enzymatic activity of the microbial flora), or be adsorbed by the solid soil particles. But despite the progressive degradation of the molecules and their retention by the soil, a fraction remains potentially mobilized by water (depending on the properties of molecules) [58]. The fraction of mobilized pesticides is driven to streams by runoff or washed into the underground water by infiltration. It is now recognized that the presence of pesticides in the aquatic environment is essentially a problem of diffuse pollution, identified as the major problem of the degradation of water quality [59]. Furthermore, the results of a 2005 study of IFEN (French Institute of the Environment, 2007) on the French water system showed a significant dispersion of pesticides and widespread presence in aquatic environments.

Prevention of risks related to the contamination of water bodies by pesticides requires quality monitoring and pollution of surface water and groundwater. Different regulations are well defined, including the establishment of threshold concentration limits for all water use.

I.4. Water resources

Access to safe drinking water is important as a health and development issue at national, regional and local levels. In some regions, it has been shown that investments in water supply and sanitation can yield a net economic benefit, as the reductions in adverse health effects and health-care costs outweigh the costs of undertaking the interventions. Increase of water demand in all sectors especially in agriculture due to the growth of population lead to a pressure on water resources all over the world. In the European countries, groundwater supplies 65% of all drinking water, groundwater quality and consequently human health are threatened by high concentration of nitrate, pesticides and their by-products, heavy metals and hydrocarbons [60].

Providing a safe and acceptable drinking water is an important issue. In most of the countries, standards and regulations are set. Although the scientific approach for a “clean drinking water” is practically the same, there are differences in both mechanisms producing standards and the way of application.

I.4.1. WHO (International guidelines)

The primary purpose of the guidelines for drinking water quality is the protection of public health. The guidelines provide the recommendations of the World Health Organization (WHO) for managing the risk from hazards that may compromise the safety of drinking water. The recommendations should be considered in the context of managing the risk from other sources of exposure to these hazards, such as waste, air, food and consumer products. WHO guidelines represent the concentration of a component that does not result in any significant risk to the health of consumers. These guidelines have been use in several countries as a basis for the development of their national standards [61-63]. The guideline values for pesticide residues in drinking water set by WHO is presented in table 1.

I.4.2. European directives on drinking water and compliance at the national level

Several directives were developed by the European Union to present water quality standards on drinking water. Council Directive 75/440/EEC of 16 June, 1975 (Amended by Directive 79/869/EEC and Directive 91/692/EEC) concerns the quality required of surface water used or intended for the abstraction of drinking water after appropriate treatment and supplied by public distribution networks.

In order to protect human health, some requirements set by these Directives must be met in drinking water. The European Council adopted (3 November 1998) Directive 98/83/EC on the quality of water intended for human consumption (Official journal L 330 of 05 December 1998) established new requirements for the quality of drinking water in the Member States. It repealed and replaced Directive 80/778/EEC from 25 December 1998, and it applies to all water intended for human consumption apart from mineral and medicinal waters supplies [64].

Member States had to bring into force the laws and regulations necessary to comply with the new Directive by 25 December 2000. Most of the new and tighter standards were met by December 2003. Member States were left the possibility to set values for additional national parameters, in light of local conditions, to protect human health. The guideline values for pesticide residues in drinking water set by European Committee is presented in table 1.

I.4.3. USEPA Standards

The Safe Drinking Water Act (SDWA), passed in 1974 and amended in 1986 and 1996, gives the Environmental Protection Agency (EPA) the authority to set drinking water standards.

The EPA, therefore, sets water regulations (NPDWRs) and maximum contaminant level goals (MCLGs) to control the level of contaminants in the USA drinking water. The Nation Primary Drinking Water Regulation (NPDWR), or primary standard, is a legally enforceable standard that applies to public water systems. It takes the form either of a treatment technique or a maximum contaminant levels (MCLs). MCLs are set as close to the MCLGs as is feasible with the use of best available technology, but also taking cost into account [65, 67].

National Secondary Drinking Water Regulations (NSDWR) or secondary standards set by the EPA are consisting in non-enforceable guidelines. They regard all contaminants that may cause cosmetic effects (such as skin or tooth discoloration) or aesthetic effects (such as taste, odor, or color) in drinking water.

Regulatory limits and guidelines for pesticide residue limits in water are derived from various criteria and are difficult to understand and interpret for experts, administrators, and the general public. Limits and GVs for pesticides residues in drinking water are compared in Table 1 [68].

Pesticides	WHO GV (µg/l)	USA MCL (µg/l)	USA MCLG (µg/l)	USA, Health advisory, lifetime (µg/l)	USA cancer risk (µg/l)	European Committee (µg/l)	Canada (µg/l)
Aldrin					0.2	0.03	
Dieldrin					0.2	0.03	
Aldrin / Dieldrin	0.03						0.7
Chlordane	0.2	2	0		1	0.1	
Cyanazine	0.6			1		0.1	10
Heptachlor		0.4	0		0.8	0.03	
Heptachlor+epoxide	0.03						
Heptachlor epoxide		0.2	0		0.4	0.03	

Table 1: Comparison of standards and guideline values for pesticide residues in drinking water.

Abbreviation: WHO GV (World Health Organization Guideline Value), MCL (Maximum Contaminant Level), MCLG (Maximum contaminant level goal), MAC (maximum acceptable concentration).

II. Extraction technique

Over the past few decades, scientific concern about environmental pollution has increased and environment-friendly methodologies have been developed. These methods include modified and less hazardous sample pre-treatments [66].

Environmental analysis has focused on the extraction and the determination of a wide range of emerging organic contaminants with an apolar or moderately polar character. Indeed, conventional sample-preparation techniques were unable to extract many compounds with such different chemical properties simultaneously. The aim of sample pre-treatments has been to extract more polar contaminants, simplify the procedure, reduce the volumes of sample and organic solvent used, miniaturize the analytical devices, and remove the maximum of interferences from complex matrices. In the recent years, the frequent finding of pesticides in water including surface water, groundwater and drinking water lead to increase the study on pesticide contaminations. During these times, many different extraction techniques have been developed for the sample pre-treatments for pesticides analysis. The objectives were to obtain the cheap screening methods with short time of analysis, low solvent volume needed and sensitive. The methods currently used for pesticide analysis with their advantages and disadvantages will be described in this part.

II.1. Solid-phase-based extraction technique

The determination of organic contaminants in the environment at a very low concentration requires the use of appropriated analytical methods. Extraction and enrichment steps are the important steps before chromatographic separation and detection especially when matrixes are complex [69]. During the extraction and enrichment step, the trace solutes are purified and isolated from the matrix, and concentrated for a better precision of identification and quantification. In the last decades, the development and evolution of the analytical processes for environmental samples become a necessity [70-78]. These methods provide many benefits; it reduces solvent volume and exposure disposal costs and extraction time for sample preparation. Although most of standard methods for the analysis of pesticides use liquid/liquid extraction (LLE), solid-phase extraction (SPE) has been developed as an alternative method, due to its simplicity and economy of time and solvent volume [79]. SPE was initially developed to replace conventional LLE technique. Conventional LLE is labor intensive, frequently met with practical problems, such as emulsion formation. In addition, LLE needs large volumes of high-purity solvents. Contrarily, SPE has numerous advantages including time, money, materials and solvents saving. Actually, SPE is the most commonly used for the extraction/purification for different samples including environmental, pharmaceutical, food and industrial chemistry. These techniques include also solid phase micro-extraction (SPME), matrix solid-phase dispersion (MSPD), stir-bar sorptive extraction (SBSE). The main objective of this section is to describe the current trends of SPE for pesticides analysis with special highlighting on articles published in the last few years. The SPE extraction procedures developed to isolate and pre-concentrate pesticide residues and principles of each procedure are briefed and discussed. Isolation and pre-treatments steps in SPE for pesticides residues analysis in food and in environmental matrices are outlined. An overview of practical application is given for SPE, SPME, MSPD, SBSE and passive sampling.

II.1.1. Solid Phase Extraction (SPE)

SPE was introduced in the 1970's [83], it become commercially available in 1978 as an alternative method to LLE. SPE is currently used as sample preparation technique for the isolation of selected analytes, usually from a mobile phase. The analytes are transferred to the solid phase where they are retained. The solid phase is then isolated from the sample and the analytes recovered by elution requiring an appropriate solvent that depends on the type of analytes (polarity, K_{ow} …). The SPE allows to the enrichment the targeted compounds from the interest matrix, concentration of the sample due to the very low concentration of targeted compounds, matrix simplification, clean up from all undesired substances and medium exchange, transfer of the analytes from solid or liquid phase to a liquid or gas phase for the final analysis.

SPE for liquid samples become widely used in laboratory in the early 1980's with the introduction of commercialized cartridges containing silica based chemically bonded sorbents. Typical cartridges devices consist of short column, conventionally an open syringe barrel containing a sorbent with different particulate size usually between 50-60 µm.

However, a difficulty results in determining polar pesticides when presence of interfering substances such as humic acids and other organic substances in water. The use of specific solid phases such as immunosorbents or molecularly imprinted polymers (MIPs) can solve these problems [84]. MIP is used because of their affordable price compared with immunosorbents [85-87].

Reversed phase LC system is compatible with aqueous samples which allows on-line coupling of SPE with analytical identification/quantification system. This online system is global for water samples typically handle the pre-concentration of analytes starting from 20 ml [88] of aqueous sample on a small cartridges filled with a suitable sorbent. The sorbent is chosen not only for its efficiency in trapping analytes, but also for its compatibility with the stationary phase packed into the chromatographic column to prevent losses in analysis efficiency. For the case of two different sorbent being used, the retention of the analytes in the pre-columns should be lower than in the analytical column to ensure band refocusing at the head of the chromatographic column. On-line systems with several detectors have been reported, such as (UV) detector [89]. Automated SPE on-line sampling can be performed with commercialized or hand-made cartridges as well as 96 well SPE plate [90-92]. On-line procedures allow to analysis the sample without off-line extraction and pre-concentration step. Consequently, it allows minimizing the analytes losses and risk of contamination and thus it provides higher reproducibility [93]. In addition, this technique requires only a small sample volume. However, for complex samples, off-line SPE should be preferred due to its greater flexibility, and the opportunity to analyze the same extract using various techniques.

II.1.2. Solid-phase micro-extraction (SPME)

The excessive use of organic solvent and related pollution problems increase the need to develop a new solvent free method. Moreover, organic solvents used are costly and require time consuming procedure. It is a new approach of sample preparation technique used both in the laboratory and on-site. Developed in the early 1990s at the University of Waterloo by Dr. Pawliszyn's group, it is a simple and inexpensive technique where the use of solvents is not necessary [94-95]. Subsequently, the technique has been currently used and its utilization is in increasing [96-98].

SPME involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes from different kinds of media, that can be in liquid or gas phase. The quantity of analyte extracted by the fiber is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation. After extraction, the SPME fiber is transferred to the injection port of separating instruments, such as a Gas Chromatograph, where desorption of the analyte takes place and analysis is carried out. The extraction involves the equilibrium sorption of analytes onto a microfiber coated with hydrophilic polymer. Nowadays, there are numerous fiber coating based on solid sorbents are available and poly(dimethylsiloxane) PDMS which is relatively non-polar, is the most frequently used. Derivatization in situ with butylchloroformate before on-line SPME extraction using PDMS fiber have been applied successfully for analyzing the acidic pesticides (phenoxy acids mechorprop, dichlorprop, MCPA and 2,4-D and their phenol degradation products 4-chloro-2-methylphenol and 2,4-dichlorophenol) [100]. However, for the determination of triazines, poly(acylate) PA coated fibers is more appropriate since it is more hydrophilic [99], namely, PDMS/divinylbenzene (DVB), and DVB/ Carboxen/PDMS-coated fibers [101-102,105,108-110]. Generally, it is difficult to obtain total extraction with 100% recoveries of targeted compounds. Method validation is an important step which includes a comparison of the recoveries with those obtained from a reference extraction technique on the same analytes in a similar matrix. Surrogates and standard additions are needed to control for the matrix effect [128]. The attraction of SPME is that the extraction is fast and simple and can be operated without solvents, and detection limits can reach parts per trillion (ppt) levels for certain compounds. SPME also has great potential for field applications; on-site sampling can be done even by nonscientists without the need to have gas chromatography-mass spectrometry equipment at each location. When properly stored, samples can be analyzed days later in the laboratory without significant loss of volatiles. Analysis of pesticides and their degradation products proved the validity of SPME using CW-DVB fiber and with GC/MS determination [129]. The results confirmed the robust and reproducible of SPME for the analysis of several pesticides but the use of this technique for extraction of more polar pesticides is limited by the types of fiber available.

Improvements are being made to the SPME technique. The LC-SPME interface has been improved and new mixed phases based on solid/liquid sorption (CW-DVB and PDMS-DVB) have been advanced in recent years for the analysis of compounds by LC [130].

II.1.3. Matrix solid phase dispersion (MSPD)

Matrix solid phase dispersion (MSPD) was firstly introduced by Barker et al. (1989) to isolate drugs from tissues [131]. The main difference between MSPD and SPE is that, in SPE, samples must be in liquid state before application to the column while MSPD can handle solid or viscous liquid samples directly. Interactions of the system components are greater in MSPD and different, in part, from those in SPE [132]. MSPD was an inventive process in 1993 and the applications of MSPD have been widely reviewed [133-135]. Actually, MSPD has found particular application as a somewhat generic analytical process for the preparation, extraction and fractionation of solid, semi-solid and/or highly viscous biological samples. The technique is based on several simple principles of chemistry and physics, involving forces applied to the sample by mechanical blending to produce complete sample disruption and the interactions of the sample matrix with a solid support bonded-phase (SPE) or the surface chemistry of other solid support materials. The process of MSPD requires simple devices and can be readily performed in the laboratory. MSPD is generally applied for food (solid samples) and/or liquid samples (milk, oil…) [136-141]. Semi-solid or solid sample can be placed in a glass mortar containing a bonded phase solid support material and mechanically blended with a glass pestle to attain complete disruption and dispersion of the sample.

The materials employed as dispersant varied from the classic reversed-phase (RP) C18- and C18-bonded silica, to normal phase (NP) [118-120]. In recent years, the greatest innovation in MSPD has been unusual supporting materials (i.e. highly selective molecularly-imprinted polymers (MIPs) [142-148] and the less specific multi-walled carbon nanotubes (MWCNTs) [149-151]. More specifically, MWCNTs, especially after suitable functionalization, are being proposed as emerging sorbents for numerous solid-phase extraction (SPE) applications [152].

MSPD has been also developed for the extraction of pesticides from both vegetables and animals matrices. This application was recently studied [153]. However, new methods, as well as the application to different matrices or compounds, were developed in very recent years.

Gas chromatography (GC) equipped with electron-capture detection (ECD) [154], nitrogen-phosphorus detection (NPD) [155], flame-photometric detection [156], mass spectrometry (MS) [157-159], and tandem MS (MS/MS) [160] are used most frequently for instrumental analysis. However, high-performance liquid chromatography (HPLC) equipped with triple-quadrupole MS/MS [161-162], time-of-flight (TOF) MS [163] detection, and with diode-array detection (DAD) [164-165], is used increasingly. Traditional NP, RP or inert materials are still the most widely used as dispersants and as co-column sorbents for cleanup. Florisil, neutral alumina and silica (with anhydrous Na_2SO_4 as desiccant) are generally recommended before GC analysis of non-polar or slightly-polar compounds. Different solvents could be used for recovery depending on the dispersant and co-sorbent. However, for the extraction of more polar compounds (e.g., some organophosphorus pesticides), RP-silica as sorbent and ethyl acetate as recovery solvent [157,159] have been applied successfully.

II.1.4. Stir-Bar Sorptive extraction (SBSE)

Stir-bar sorptive extraction was first presented by Baltussen, Sandra, David and Cramers in 1999 [166]. SBSE is a solvent less sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. This method has the same basics as SPME. It’s constituted by magnetic stir bar incorporated into a glass jacket basically coated with PDMS (non polar polymeric phase). This polymer allows operating in a wide range of temperature and with association of SBSE and thermal desorption (TD) followed by chromatographic analysis, better recoveries can be attained [167]. Another new coat with polythiophene (PTH) was reported by Cong Hu et al. 2013 [168]. This method is a complex of PDMS/PTH for the detection of OP in water samples, where recoveries range of between 77.7 and 119.8%.

The principle of SBSE consists of two major steps, the extraction of the analytes from the sample and desorption of analytes from the sorbent phase to the chromatographic system [169]. The extraction process should be carried out under stable conditions in order to have better performance i.e. better extraction recoveries. Once the extraction is done, the stir bars are removed, rinsed with tap water than submitted to the break extraction process, though TD or Liquid desorption (LD). TD is limited to thermally stable volatile to semi-volatile solutes, whereas LD is usually more indicated for semi to non-volatile compounds. Some examples of SBSE application are the determination of volatile and semi-volatile organic compounds in air samples [170] and pesticides in water [171]. Table 2 summarizes pesticides families with the most appropriate extraction and quantification techniques.

Pesticides	Sample type	Extraction techniques					Analytical Tool			Ref
		SPE	SPME/HS-SPME	MSPD	SBSE	Passive sampling	GC	LC	Other	
Cl/OCL	Surface water Groundwater Human lung Soil	-C18 -Lichrolut C18	Fbers: -polyacrylate -Polydimethylsiloxane/carboxen/divinilbenzene	Florisil C18 silica	PDMS PDMS	SBSE POCIS Chemcatcher (SDB-XC)	MS/MS ECD MS TOF-MS	HPLC MS/MS	-GCxGC-HRTOF-MS -TD-GC-MS	101, 102, 104, 107, 119, 121, 123, 124, 125, 126, 127, 128
NP	Surface water Wastewater Groundwater									
OP	Surface water Groundwater Fruits peel Fruits Seaweed	C18 -Lichrolut C18	Fibers: -Polyacrylate -Polydimethylsiloxane/carboxen/divinilbenzene -ppy/sol-gel	Dispersant: RP-C8 RP-C18 Florisil Graphitized carbon	Coating: PTH PDMS/PTH	SBSE POCIS Chemcatcher (SDB-XC)	MS/MS NPD MS MS/SIM TOF-MS	DAD MS/MS	LD-LUI-GC-FPD	100, 102, 105, 106, 108, 111, 113-118, 120, 122, 124, 126, 127
ON	Surface water	-Lichrolut C18	Fibers: -polyacrylate -Poly-dimethylsiloxane		PDMS	SBSE POCIS Chemcatcher (SDB-XC)	MS MS/MS TOF-MS	MS/MS		106, 109, 126,127,
Carbamates	Water Seaweed		Fibers: -Polyacrylate -Poly-dimethylsiloxane	Florisil Graphitized carbon		Chemcatcher (SDB-XC)	MS/SIM MS TOF-MS			108, 120, 128
Triazines	Water Groundwater Fruits peel		-Polyacrylate -Poly-dimethylsiloxane -Poly-dimethylsiloxane/divinilbenzene	Dispersant: RP-C8	PDMS	Chemcatcher (SDB-XC)	MS FID MS/SIM MS/MS TOF-MS	HPLC		102, 103, 110, 111, 113, 114, 126, 128
Phenylureas	Vegetables Rice Natural water			Florisil	PDMS	SBSE POCIS		MS/MS	Capillary electrophoresis-electrochemiluminescence detection	112, 124, 127
Pyrethroid	Surface water Seaweed	-Lichrolut C18	Fibers: -Polyacrylate -Poly-dimethylsiloxane	Florisil Graphitized carbon			MS MS/SIM			106, 108, 109, 120

<u>Table 2</u>: Different types of pesticides with their most used pretreatment technique and quantification method.

II.2. Passive sampling (SPMD, POCIS, Chemcatcher)

Passive sampling, called also diffusive sampling, devices have been used for over two decades for the measurement of nonpolar organic contaminants in water [172]. In 2005, passive sampling was developed for monitoring the air quality [173] and only during this last decade that passive sampling technique start to be used for monitoring different categories of pollutants in aquatic environments [174]. Passive sampling is based on the static exposure of devices capable of accumulating by diffusion contaminants present dissolved in water [172, 181]. This diffusive process is driven by a concentration gradient between water and the sampler initially free of the contaminant of interest. Passive sampling systems accumulate micro pollutants and from which reliable exposure concentration can be calculated. These systems are usually designed either as “kinetic samplers” or as “equilibrium samplers”. The equilibrium process is similar to the octanol-water equilibrium partition coefficient (K_{ow}) used since 1970s. K_{ow} have been used to predict the potential for persistent nonpolar contaminants to concentrate in aquatic organisms [175]. Passive sampling is based on the equilibrium of sampler-water partition coefficient (K_{sw}) which defined as the ratio of sampler to water concentration of the interest compound at thermodynamic equilibrium. The prediction of time-average concentrations of pesticides for the period when samplers were exposed depends on the kinetic-based passive samplers for ultra-trace pollutants in water. Kinetic samplers relay on a large sampler capacity, or a large sampler-water partition coefficient, for the contaminants to be sampled. This ensures that under sampling conditions the concentration of the targeted compounds within the sampler does not approach an equilibration state during sampler exposure. The calculation the concentration in the water-phase (Cp) sampled are based on the assumption that uptakes in linearly related to the exposure concentration throughout exposure. Resistance at either the hydrodynamic boundary layer or within the sampler membrane/sequestering phase acts to control contamination flux into the sampler. The mean concentration in the water-phase Cp can then be predicted from $\mathrm{Cp} = \frac{Cs\,Vs}{Rs\,t}$. Where Cs is the concentration of the analyte in the sampler, Vs is the sampler volume, Rs is the specific sampling rate, and t is the exposure time.

These time-integrating passive sampling techniques have become wildly used in the last decade. The performance reference compounds introduced into the sampler has been used. The use of reference compounds enable adjustment of field data from the samplers using kinetic data from laboratory and thus has increased user confidence in these sampling techniques. There is a wide variety of passive sampler currently available, allowing analysis of a wide range of organic pollutants. Among the samplers adapted for the analysis of hydrophobic organic pollutants, SPMD (Semi Permeable Membrane Device) [176-177] allows quantifying organic compounds such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons and pesticides. For nonpolar pesticides, the sampler the most widely used is SPMD. Two other passive samplers type are POCIS (Polar Organic Chemical Sampler) and Chemcatcher (polar version) [178]. To date, these two samplers are under development step, their applications in the environment primarily concern qualitative studies for diagnosis of the presence of pollutants in the environment and identification the nature of pollutant [179]. SPMDs are commercially available and the most currently used type of passive sampler. Indeed, pesticides often occur at ultra-trace levels so that a relatively large surface area of non-polar membranes is required. The advantages for SPMDs are the availability of the largest set of calibration data together with an extensive literature and the use of performance reference compounds for in situ calibration is routine. SPMDs allow to quantitative studied. However, analysis of SPMDs is relatively complex. Some uncertainty factors associated with the use of passive sampling remain, such as their representativeness as mimics of biota uptake and measures of bioavailable fractions, as well as issues relating to biofouling and nonlinear uptake. The utility of these new sampling tools in part reflects the limitations of the other techniques available. Accordingly, these sampling techniques provide an additional set of tools often useful for modern monitoring programs.

Due to their greater ability to concentrate ultra-trace compounds, passive samplers allow to increase the sensitivity of the analysis and thus to achieve low detection limits. Consequently, this technique could not be used for monitoring accidental pollution (industrial effluent, structural damage, severe weather conditions, collision…). These techniques differ from one another, having a point of exposure sampler (depending on experimental setup), but also in terms of exposure (type of water, temperature, turbulence level, etc.) [180]. However, sampling of pesticides available in literatures concerning the use of Chemcatcher and POCIS are limited, and the quantifying conditions are not always well detailed. Table 3 summarizes the three main passive samplers used for pesticides extraction in water.

	SPMD	POCIS	Chemcatcher	References
Compounds	**Organic/Non-polar**	**Organic/polar**	**Organic/inorganic**	
Membrane	Non porous Low density polyethylene (LDPE)	Microporous PES (polyether sulfone)	Teflon Polycarbonate Polytetrafluoroethylene (PTFE) Grafted silica-alkyl group Polystyrene divinyl benzene (PSDVB)	181, 182, 183, 184
Synthetic polymer	Triolein	HLB + N vinyl pyrolidine isolute Env+ PS-DVB modified by hydroxylation	Teflon Polycarbonate	182, 185, 186, 187, 188
Membrane Diameter	L:91.4cm w: 2.5 cm cavity diameter: 1nm	Thickness: 130µm diameter of pores: 100nm	Diameter: 47-50 mm	182, 189
Exchange surface		41 cm^2	17.5 cm^2	182, 190
Duration of deployment of the disk in the environment		15-21 days	15 days	
Inconvenient	Large amount of solvent to elute compounds trapped in the triolein Detects only non-polar compounds (Log $K_{ow} > 3$)			173
Advantages	Allow to quantification analysis	No passage of microorganisms	Organic/ inorganic compounds	172, 180

Table 3: Summary of main types of passive sampling used for pesticides extraction in water matrices.

II.3 Immunochemical techniques

Bioanalytical methods such as immunoassays, immunosensors, and immunoaffinity chromatography are providing information regarding the presence and concentration of contaminants that may impact human health and the environment [205]. Immunochemical methods can be used for both sample preparations (i.e., extraction, cleanup and concentration) and detection. Methods specific for food analysis have been reported, while methods developed for other matrices could be adapted for foods [206-211].

Immunochemical detection was first applied to clinical situations for diagnostic purposes. The sensitivity and selectivity of the interaction between antibodies and their typical large target antigens (i.e., hormones, bacteria, toxins, etc) was aptly used in highly successful diagnostic methods for medical and health-care applications. To stimulate the immune system for generation of specific antibodies the target must be ≥10,000 daltons. The development of specific antibodies for small molecules (<200 daltons) can be obtained through the use of a carrier molecule. Pesticide residue chemists astutely recognized the potential of the technology for small molecule detection in the 1970s and advances in the technology have been reported ever since for a variety of environmental contaminants [205,208-211]. Regardless of the molecular weight of the analyte, immunochemical methods are based on selective antibodies combining with a particular target or members of a closely related analyte group.

The detailed development and applications of many bioanalytical techniques, as well as, quality assurance measures, and how to integrate bioanalytical methods into an instrumental analytical laboratory have been extensively reviewed [205].

Indirect competitive immunoassays were developed on protein microarrays for the sensitive and simultaneous detection of multiple environmental chemicals [212]. In this assay, a DNA/SYTOX Orange conjugate was employed as an antibody label to increase the fluorescence signal and sensitivity of the immunoassays. Epoxy-modified glass slides were selected as the substrate for the production of 4 _ 4 coating antigen microarrays. With this signal-enhancing system, competition curves for 17b-estradiol (E2), benzo[a]pyrene (BaP) and 2,20,4,40-tetrabromodiphenyl ether (BDE-47) were obtained individually on the protein microarray. The results of the microarray immunoassay were within 15% of chromatographic analysis for all three pollutants in spiked river water samples, thus verifying the immunoassay. There was no cross-reaction in the immunoassay between these three environmental chemicals.

III. Analytical instruments for identification and quantification

The identification and quantification of pesticides and their decomposition products in water requires an appropriate extraction step followed by an analysis using different detection techniques. In principle, the targeted compounds are firstly separated in chromatography column and each compound can be detected with different type of detectors. Gaz chromatography (GC) and high-performance liquid chromatography (HPLC) prove their potential efficiency to separate targeted organic compounds in complex mixtures.

The aim is to separate each compound with a good resolution and a short time of analysis. In addition, the chromatographic methods must allow to separate all target compounds but also to separate them from the interfering which present initially in the sample. In recent years, GC equipped with mass spectrometry (GC/MS, GC/MS/MS) had made a progress in the field of monitoring of pesticides in the environment due to their high separation level, selectivity and identification abilities of MS [192]. In order to achieve accurate quantification, compounds must be fully resolved from each other. In a number of cases, this may be possible using selective detection where the compounds are resolved using mass spectrometry (MS) [191]. The variety of detectors coupled with GC improved the detection of pesticides residues, such as electron capture detector (ECD) [194-196], nitrogen phosphorus detector (NPD) [197], flame ionization detector (FID) [198,203]. Beside GC/MS techniques, HPLC equipped with mass spectrometry (LC/MS, LC/MS/MS) [201]. Since the early 2000s, ultra-high performance liquid chromatography (UHPLC) has also been successfully used for many environmental separations [201].

III.1. Chromatographic separation (GC and LC)

GC is an analytical technique for separating compounds based primarily on their volatilities. It was firstly introduced in 1959 by James and Martin [192]. It has shown to be an effective, flexible, and sensitive technique for the analysis of food samples. GC is the technique of choice for several applications aimed to characterizing the volatile food fraction (aroma and flavor), identifying and quantifying several organic contaminants present in trace levels in complex food samples. It is also used for authenticating the origin of a given matrix and verifying compliance with quality standards and safety requirements. To date, GC technique has been promptly applied for analyzing a large variety of organic compounds in complex samples obtained from different matrix [193]. The performance of GC method relay on the choice of capillary columns, pre-column and temperature program under which the analysis is carried out. Another type of chromatographic separation is HPLC. A big different between GC and HPLC is mobile phase; the mobile phase of GC is a gaz (e.i. He) and the mobile phase of LC is present in liquid phase (water, organic solvent, or mixture of solvents). Some compound can be separated either with GC or LC. However, some compounds which have a high level of volatility could not be separated by GC technique and thus LC separation technique must be used.

III.2. Detection technique

For the identification and quantification analysis, the targeted compound in the sample should be firstly separated with chromatographic techniques and follow by the detection step. Numerous detector could be used for the detection of pesticides and the most frequently used is described briefly in this part.

III.2.1. Electron Capture Detector (ECD)

The ECD is used for detecting electron-absorbing components (high electronegativity) such as halogenated compounds. Shen et al. (2013) [194] have optimized the EPA standard operating procedure CH-IN-002.3 [195] for the detection of organochlorine pesticides in water samples. Recoveries obtained for pesticides in different types of water at various levels were higher than 70%, with a RSD ranged between 1.6 and 9.6 %. Concha-Graña et al. (2009) [196], have developed a GC/ECD analytical method based on micro liquid liquid extraction (MLLE) for the extraction of 21 organochlorine pesticides in water. The recovery was between 69% and 107% with a relative standard deviation less than 1.3%.

III.2.2. Nitrogen and phosphorus detector (NPD)

The NPD is a very sensitive and specific detector which is currently used for detecting the nitrogen and phosphorus compounds. It is based on the FID but differs in that it contains a rubidium or cesium silicate (glass) bead situated in a heater coil, a little distance from the hydrogen flame. A method for the determination of three organphosphorus (OPPs) in water and vegetables was developed by using Polypyrrole/sol–gel composite as a solid-phase micro-extraction fiber coating and detected with GC/ECD. The recoveries for the pesticides in tap water, well water, cucumber and lettuce varied from 86 to 109% with a RSD between 3.5 and 9.9 % [197].

III.2.3. Flame ionization detection (FID)

FID is based on the detection of ions formed during combustion of organic compounds on hydrogen flame. The generation of these ions is proportional to the concentration of organic compounds in the sample gas stream. Hydrocarbons are the most detected by the FID [203]. However, GC-FID detector equipped with a single drop micro-extraction (SDMD/GC-FID) allow to analyzing some pesticides with a good efficiency. Pinheiro et al. (2009) have developed a SDME/GC-FID method for analyzing four organophosphate pesticides in water.

Relative recovery obtained is ranging from 73 to 104% with a coefficient of variation between 7.7-18.8% [198].

III.2.4. Mass spectrometry detector (MSD)

MS has been largely used for the identification and quantification analysis of various organic compounds. It is the universal technique and it is generally used coupling with GC or LC. GC/MS is irreplaceable in pesticide analysis. The key characteristics of GC/MS in pesticide analysis are its selectivity and sensitivity. El-Osmani et al. (2014) have used GC-MS for analyzing the organochlorine pesticides residues in ground water samples [204]. The GC-MS is also currently used for other family of organic trace contaminants and other class of pesticides (i.e. organonitrogen pesticides) [199, 204]. Martinez et al. (2013) have used GC-MS method for the determination of 76 micro-pollutants in water samples [199]. The detections limit for these compounds varied from 0.0005 - 0.1 ng.ml^{-1} while the precision of the method was evaluated by spiking three replicates of a sample at 1 µg L^{-1} levels. Repeatability and reproducibility between 5 days were calculated as the percentage of the relative standard deviation ($n = 5$), and were mostly lower than 20%.

For MS detector, a second phase of mass fragmentation can be added. It is called tandem mass spectrometry (MS/MS). MS/MS allow quantifying low levels of target compounds in the presence of a high sample matrix background. Derouiche et al. (2007) have established a GC-IT/MS/MS method for the determination of 15 organochlorine pesticides (OCPs) and 20 polychlorinated biphenyls (PCBs) in aqueous samples. The authors have obtained the recovery ranging from 74.9 to 105.1 % with and RSD that ranges between 5 and 22 %. However, there is a considerable potential advantage with regard to specificity and selectivity for the associations of HS-SPME with GC–tandem ion trap MS in the multi residue analysis. Therefore, applicability of the methodology has been demonstrated without difficulty in the analysis of real water sample in which targeted compound present at low level, below µg.L^{-1} [200].

The limitation in the use of GC-MS depends on GC limits. Thus, very polar and thermally labile pesticides are not suitable for GC separation and cannot be analyzed by GC-MS. In this case, the powerful features of LC-MS should be used. It is very attractive to the field of pesticides residue analysis. LC-MS allow a high efficiency of separation, identification, and quantification of polar analytes. Moreover, it has been shown that, in combination with tandem mass spectrometry (ion trap or triple quadrupole), LC is a very sensitive technique for analysing pesticides residues in water. Singer et al. (2010) have used an online solid phase extraction equipped with LC ESI/MS/MS method for the determination of different biocidal compounds (1,2-benzisothiazoline-3-one (BIT), 3-Iodo-2-propynylbutyl-carbamate (IPBC), irgarol 1051 and 2-N-octyl-4-isothiazolinone (octhilinone, OIT), carbendazim, diazinon, diuron, isoproturon, mecoprop, terbutryn and terbutylazine) and pharmaceuticals (diclofenac and sulfamethoxazole) in wastewater and surface water [201] .

Conclusion

To summarize, in recent years there has been considerable improvement in pesticide residues extraction and analysis techniques. Numerous pretreatment techniques and detection methods have been applied for identifying and quantifying pesticides in environmental matrix such as soil, surface water, ground water, rainwater. But there is also a rising necessity to develop more efficient, rapid and less expensive methods for the analysis of pesticides residues in water in large scale because the increasing of world population, the decreasing the stock of drinking water and especially the decrease in water quality. Each day, various toxic chemicals are discharges into the aquatic system and for some cases; the tendency of water contamination is growing.

Some progresses in the extraction, clean-up steps have been introduced and changes are occurring in the determination steps. Pre-treatment of environmental sample techniques such as SPE and SPME techniques are developing and improving each day. GC-MS, LC-MS and MS/MS have been used for the identification and quantification targeted compounds after the pre-treatment step. The most important issue is that high throughout analysis techniques for hundreds of pesticides in different environmental matrices with a rapid, green and repeatability methods are now available [202].

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Chapter 2

SAMPLING PROCESS PROTOCOL A STATE OF KNOWLEDGE

The principle of sampling process of phytosanitary products can be divided into five elementary steps: sampling onsite, storage and transportation, extraction and concentration, analysis and finally detection and quantification of compounds. The sampling step is the first and one of the most important stages of the analytical protocol because it corresponds to the first phase of analysis. The choice of sampling site is sensitive to have a representative sample in the studied zone. This choice is most likely to be difficult when it's an estuarine environment where various water masses are found. For these reasons, the location and timing should be chosen carefully. The material used for the sampling and the storage of samples should be selected in a certain way to not contaminate the samples. For this, the use of inert or clean containers materials (Teflon, or calcined containers) are needed. For the sample treatment, the procedures depend on the nature of the sample. Some pesticides cannot be detected in all matrices due to their physic-chemical properties. Herbicides such as triazines are also complex to be detected in the particulate phase due to their strong hydrophilic character, while some Organochlorines are not detected in the dissolved phase due to their low solubility in water. Water samples are filtered, extracted, purified, if necessary, and analyzed by gas chromatography (GC).

Sediment and particulate materials are extracted and purified before analysis. These steps will actually extract, purify and concentrate the pollutants contained in original matrix (water, sediment) to remove the maximum amount of compounds that could interfere in the analysis.

This Chapter will be divided in two parts. The first section comprises the materials and methods used for the development of new analytical method for the detection and quantification of pesticides in pure water samples by the mean of mathematical model which we will discuss below, and the second section is about the application of the optimized method to natural water samples in France and Lebanon.

The main objective of the first section is to develop an analytical method based on the Solid Phase Extraction (SPE) for the detection of five different class families of pesticides (carbamates, triazines, thiocarbamate, pyridazinone…). After a wide bibliographic study, five factors were chosen for the optimization of the SPE method. Due to the high number of experiences we had resorted to a mathematical model (MODDE) that allowed us to test all the factors by reducing the number of experiences to 34. The factors and the mathematical model chosen will be discussed in this chapter.

The second part is consecrated to apply this study on natural water samples including North Lebanon, where these are the first study conducted in the region and in France (La Canche, La Somme …).

1. Reagents

All chemicals and solvents used were analytical grade. Ultra pure water with an 18.2 MΩ/cm resistivity was obtained using a Milli-Q system (Millipore, USA). Glassware were washed cautiously by soaking them for almost two hours in a Milli-Q/ decon bin (alkaline solution), then transferred into another bin containing Milli-Q/HCl (30%) for two hours, and the final step would be washing with pure water.

Pesticides and HAP stock solutions (1000mg/L) with solvents and chemicals were supplied from sigma Aldrich (France). pH was adjusted by using suprapur Hydrochloric acid HCl (37%) and potassium Hydroxide (KOH, 0.5 mol.L-1 in methanol) purchased from Panreac Quimica (Barcelona, Spain). SPE HLB Oasis cartridges were supplied from dislab France.

2. Analytical instruments

The pH measurements and adjustment were conducted by Methrom 713 pH meter (HEvi Sau, Switzerland), calibration using two standard buffer solutions pH 4 and pH 7. The flow rate of the samples was adjusted using a pump. SPE manifold was used for the extraction of water samples.

During this study, GC/MS and GC/MSMS were used for the detection and quantification of the organic compounds. The optimized method is shown in the table below.

Compounds	*Chemical class*	*Function*	*LogK_{OW}*	*RT (min)*	*LOD (µg.l^{-1})*	*Qualifier Ions*
EPTC	Thiocarbamate	Herbicide	3.2	11.8	0.05	126;134
Butylate	Thiocarbamate	Herbicide	4.1	13.1	0.05	145;188
Vernolate	Thiocarbamate	Herbicide	3.8	14.0	0.05	127;147;160
Tebuthiuron	Urea	Herbicide	1.8	14.8	0.05	155;170
Etridiazole	Thiazole	Fungicide	2.6	15.8	0.05	183;211
Molinate	Thiocarbamate	Herbicide	3.2	17.8	0.05	126
Propachlor	Thiocarbamate	Herbicide	2.4	19.4	0.05	120;176

Cycloate	Thiocarbamate	Herbicide	4.1	19.7	0.05	83;154; 215
Fluridone	Pyridinone	Herbicide	1.9	58.7	0.05	328
Fenarimol	Pyrimidine	Fungicide	3.7	46.7	0.05	139; 219; 251
Terbacil	Uracil	Herbicide	1.9	24.2	0.05	161
Chlorpropham	Carbamate	Herbicide	3.4	20.2	0.05	127;171;213
Trifluralin	Dinitroaniline	Herbicide	5.3	19.9	0.05	264;306
Atraton	Triazine	Insecticide	2.7	21.8	0.05	169;196;211
Prometon	Triazine	Herbicide	4.3	21.9	0.05	168;210;226
Simazine	Triazine	Herbicide	2.2	22.5	0.05	186;200;203
Atrazine	Triazine	Herbicide	2.6	22.5	0.05	172;200;230
Propazine	Triazine	Herbicide	2.9	22.7	0.05	231
Pronamide	Amide	Herbicide	3.4	23.2	0.05	173;175;254
Simetryn	Triazine	Herbicide	2.6	26.2	0.05	213
Metribuzine	Triazine	Herbicide	1.6	26.6	0.05	198
Alachlor	Chloroacetamide	Herbicide	2.9	25.9	0.05	160;188
Ametryn	Triazine	Herbicide	3	26.3	0.05	213;227
Terbutryn	Triazine	Herbicide	3.6	26.9	0.05	170;185;;242
Napropamide	Amide	Herbicide	3.3	32.6	0.05	128;171;271
Metolachlor	Chloroacetamide	Herbicide	2.9	28.0	0.05	162;238
Triadimefon	Triazole	Fungicide	3.2	28.4	0.05	208;210
Diphenamid	Amide	Herbicide	2.2	29.4	0.05	166
MGK-264	Dicarboximide	Insecticide	3.7	29.0	0.05	164
Butachlor	Chloroacetamide	Herbicide	4.5	31.6	0.05	176;188
Norflurazon	Pyridazinone	Herbicide	2.3	38.8	0.05	102;145;303
Hexazinone	Triazine	Herbicide	1.2	39.4	0.05	171
Alpha-BHC	Organochlorinated	Insecticide	3.8	13.6	0.05	181;183
Endrin	Organochlorinated	Insecticide	5.6	27.0	0.05	281
Chloroneb	Substituted benzene	Fungicide	3.4	17.35	0.05	191
DCPA	Chlorinated benzoic acid	Herbicide	5.3	28.39	0.05	302
Heptachlor epoxide	Organochlorinated	Insecticide	5.44	31.10	0.05	289
Trans nanochlor	Organochlorinated	Insecticide	2.78	33.12	0.05	409

Chlorobenzilate	Organochloriated	Acaricide	4.58	35.45	0.05	252
Gama BHC	Organochlorinated	Insecticide	3.8	24.04	0.05	219
Beta BHC	Organochlorinated	Insecticide	3.8	25.79	0.05	219
Delta BHC	Organochlorinated	Insecticide	3.8	26.99	0.05	219
Aldrin	Chlorinated	Insecticide	6.5	28.37	0.05	293
Endrine Ketone	Organochlorine	Insecticide	5.6	37.97	0.05	345
Endosulfan sulfate	Organochlorine	Insecticide	3.6	39.63	0.05	387

Table1: information for pesticides compounds used in this study.

3. Sampling and storage

Water is collected in pre-cleaned (2L or 5L) amber glass bottles, transported in ice boxes, and stored at 4^0C, filtered with Whatman GF/A circles filter (Ø 110mm, 0.7 µm), extracted and analyzed. Samples are kept for a maximum duration of two weeks before analysis. For groundwater, samples are collected either manually or pumped depending on the water type. If possible, water is pumped for approximately 5 min from the well to purge the pipes in order to obtain a highly representative sample of the groundwater. In some wells, there was no pump and therefore sampling had to be performed manually. On the other hand, surface water samples were collected, when possible, from a bridge at the middle of the water bed. Otherwise, they were collected from the shore. When needed, manual sampling of groundwater and surface waters was carried out at approximately 1 m below the surface with amber glass bottles.

For sediments, there are two types of sampling, either surface sediment (0-2 cm) using a stainless steel grab. For deep sediment, core sampler is used sectioned at 1cm interval, with a 70 cm length. Corers are preferentially used in order to study the fate of these organic contaminants depending on the nature of the soil and their accumulation over time.

Fig. 1 Sediment core Sampler

4. Extraction Steps

4.1.1 water samples

Whether it's surface or groundwater, it is always recommended to filter sample before extraction due to the presence of suspended matters. They can bother during extraction like clogging problems in SPE (J. Chrom. A. 872 200, 309-314). In case of LLE extraction, this is shown by the formation of emulsions (Boussahel et al. 2000).

In another hand, the objective of filtration step is to avoid adsorption/ desorption of pesticides on these suspended matters. Lartiges, 1994 has proved that kinetic degradation of carbamates is easier when sample is filtered.

4.1.2 LLE

In this work, water was filtered using Whatman GF/F (ϕ 47mm, 0.7 μm pores) that was previously calcined at 450^{o}C for 12 hrs. The filtration step was conducted immediately after sampling to prevent adsorption/desorption of pesticides onto particulate matters.

After filtration, MES was calculated for each sample. Results are figured in table 1. The extraction protocol used in this work was inspired from EPA (USEPA, 1996; USEPA 2008), where 1L of filtered water was spiked with internal standards, than extracted four times with 60ml of DCM. The extracts were than collected and dried using Na2SO4 (to prevent any trace of water). Finally extracts were concentrated using a rotary evaporator followed by a light stream of nitrogen.

Sample name	Lagune Fresnois sortie	Lagunae Fresnois entrée	Cojeul	Step Arras entrée	Step Arras sortie	Canche	La Lys
MES (g/L)	0.181	0.207	0.0397	0.387	0.018	0.043	0.159

Table2: MES Results for water samples collected on February 2014.

Results are discussed in article 3 chapter 3.2

4.1.3 SPE

The solid phase extraction consists on percolating the water sample onto a solid support to trap the analytes. The retrieval of the compounds is realized by an elution step with an organic solvent. The usage of solvent is acute depending on the chemometrics of targeted compounds. This step serves as a concentration before analytical analysis.

The extraction consist of seven steps as following: (1) Conditioning step, the cartridge was conditioned with 5 mL of appropriated eluent according to the experiment set (Table 2), followed by 5 mL of methanol, (2) Equilibration, 10 mL of ultra-pure water was added to wet the sorbent surface. (3) Percolation, the sample was added to the cartridge. (4) After the sample was loaded, the cartridge was washed with 5 mL of ultra-pure water followed by 5 mL H2O/MeOH (95/5 v/v). (5) Drying the cartridge with high purity nitrogen flow for 5 min. (6) Eluting with 2 x 5 mL (MeOH/eluent. 1/1 v/v). (7) Evaporating using the rotary evaporator until reaching a volume of 2 mL, then continuing evaporation under gentle nitrogen stream. (8) Fixing the final volume to 500 µL before GC-MS analysis.

4.1.3.1 the cartridges choice

HLB cartridges contain a resin made with co-polymer of divinybenzene and vinyl pyrrolidinone. The pyrrolidinone functionality acts as an imbedded hydrophilic group. The pyrrolidinpne also provides enhanced retention for some polar analytes. HLB is a combination of a Hydrophilic monomer and a lipophilic monomer. The hydrophilic monomer provides wetting properties, reduces contact of analytes with water, enhanced retention for polar compounds while the lipophilic monomer provides reversed-phase property analyte retention. Furthermore, this cartridge has some specific physical properties as surface area 800 m^2/g and pore diameter 80 Å.

4.1.3.2 Choice of factors and Optimization strategy

In this study, hydrophilic-lipophilic balanced (HLB) polymer was selected as sorbent with the aim to cover the range of lipophilicity of the targeted compound (Log K_{OW} ranging from 1.2 to 5.6). Five variables known to affect the extraction efficiency were studied: sample flow rate, sample pH, elution flow rate, ionic strength (NaCl addition) and nature of eluent. The selected variables in this study and their variation levels are summarized in table 3.

Variable	**Factor**	**Level**		
		- 1	**0**	**+1**
X1	Sample flow rate ($mL.min^{-1}$)	2 - 4	5 - 7	8 - 10
X2	pH	3	6	9
X3	Elution flow rate ($mL.min^{-1}$)	0.2	0.5	1

X4	Ionic strength (g.L^{-1} of NaCl)	0	50	100
X5	Eluent (1/1 v/v)	DCM/MeOH	AcOEt//MeOH	ACN/MeOH

Table 3: Summary of factors and their levels as coded and natural variables.

Three levels of variable have been tested in this study, with sample flow rate ranging from 2 to 10 mL.min^{-1}, pH from 3 to 9, elution flow rate from 0.2 to 2 mL.min^{-1} and ionic strength from 0 to 100 g.L^{-1} of NaCl. Eluent is a qualitative variable; the solvent composition was performed with DCM/MeOH, AcOEt/MeOH and ACN/MeOH in a 1/1, *v/v* proportion.

Optimization strategy

In this work, two practical restrictions were found: the stages of sample pre-treatment, extraction of the analytes and clean-up of the extracts are time-consuming. The 34 experiments to be performed were selected according to the D-optimality criterion[49]. D-optimal designs have the property that the estimations derived from the mathematical model postulated are the most precise ones. Thus, the experimental conditions maximizing the accuracy can be obtained from the analysis of the coefficient of the model. Designs based on the D-optimality criterion have already been used in those cases in which either any combination of values in the experimental variables is not possible, or the number of experiments is limited.

The aim of this work is to establish by means of a D-optimal design, which factors influence the extraction of pesticides from water select the best extraction conditions from the point of view of the accuracy. Specifically, we will explore those conditions which ensure high recoveries.

4.1.3.3. Selection of the factors of the experimental domain and of the responses

The five factors mentioned previously together with their variation levels, coded high level (+), low level (−) and the natural values used in the paper are listed in Table 2. The effect of these five factors and interactions between factors on the extraction yields of pesticides specific will be determined.

4.1.3.4. Mathematical model postulated: D-optimal design and exchange algorithm

The selection of the mathematical model constitutes the second step of the experimental design methodology. In this paper, a second order polynomial model was postulated:

$$y = \beta 0 + \sum_{i} \beta iXi + \sum_{i} \beta iiXi^{2} + \sum_{i,j} \beta ijXiXj + \square \qquad (1)$$

Where X is the model matrix or effect matrix with dimensions N × p (N is the number of experiments and p is the number of coefficients of the model), y is the vector of the experimental responses, βi is the vector of the coefficients of model and ε is the vector of the experimental errors.

When the model is adjusted to the experimental data, not only the experimental error but also an error in the selection of the model is transmitted to the coefficients and through them to the analysis of the significance of the factors.

Consequently, the proper selection of the model according to a priori knowledge is important to get satisfactory results. The estimation of the coefficients of model (1), βi, βii and βij allows one to know the effect of a factor and the effect of interaction between factors on the response and is obtained by least squares method:

$$\beta = (Xt . X)-1 . Xt . y \qquad (2)$$

Where (X^{t} X) is called the information matrix and $(X^{t} X)^{-1}$ is the dispersion matrix. The smaller determinant of the dispersion matrix means obtain the more precise estimates of the model and the more reliable conclusions drawn from the analysis of the coefficients. This means that the quality of the coefficients depends on the model matrix: X. By proper selection of X, step previous to experimentation, the determinant of the dispersion matrix $|(X^{t} X)^{-1}|$, and consequently, the variance of the coefficients and hence the influence of the factors on a given response is analyzed. However, the determinant of the information matrix increases when an experiment is added to the design. For this study, the D-optimal design has been built through the exchange algorithm Federow and is shown by natural and coded variables in table 4.

	Coded variables					Natural variables				
Experiment	X1	X2	X3	X4	X5	Sample flow rate	pH	Elution speed	NaCl ($g.L^{-1}$)	Eluent

						(mL.min^{-1})		(mL.min^{-1})		
1	+1	-1	-1	-1	DCM/MeOH	8 - 10	3	0.2	0	DCM/MeOH
2	-1	+1	-1	-1	DCM/MeOH	2 - 4	9	0.2	0	DCM/MeOH
3	+1	+1	+1	-1	DCM/MeOH	8 - 10	9	2	0	DCM/MeOH
4	+1	+1	-1	+1	DCM/MeOH	8 - 10	9	0.2	100	DCM/MeOH
5	+1	-1	+1	+1	DCM/MeOH	8 - 10	3	2	100	DCM/MeOH
6	-1	+1	+1	+1	DCM/MeOH	2 - 4	9	2	100	DCM/MeOH
7	-1	-1	-1	0	DCM/MeOH	2 - 4	3	0.2	50	DCM/MeOH
8	-1	-1	0	+1	DCM/MeOH	2 - 4	3	1.1	100	DCM/MeOH
9	-1	0	-1	+1	DCM/MeOH	2 - 4	6	0.2	100	DCM/MeOH
10	-1	0	+1	-1	DCM/MeOH	2 - 4	6	2	0	DCM/MeOH
11	0	-1	+1	-1	DCM/MeOH	5 - 7	3	2	0	DCM/MeOH
12	-1	-1	-1	-1	AcOEt/MeOH	2 - 4	3	0.2	0	AcOEt/MeOH
13	+1	+1	-1	-1	AcOEt/MeOH	8 - 10	9	0.2	0	AcOEt/MeOH
14	+1	-1	+1	-1	AcOEt/MeOH	8 - 10	3	2	0	AcOEt/MeOH
15	-1	+1	+1	-1	AcOEt/MeOH	2 - 4	9	2	0	AcOEt/MeOH
16	+1	-1	-1	+1	AcOEt/MeOH	8 - 10	3	0.2	100	AcOEt/MeOH
17	-1	+1	-1	+1	AcOEt/MeOH	2 - 4	9	0.2	100	AcOEt/MeOH
18	-1	-1	+1	+1	AcOEt/MeOH	2 - 4	3	2	100	AcOEt/MeOH
19	+1	+1	+1	+1	AcOEt/MeOH	8 - 10	9	2	100	AcOEt/MeOH
20	0	0	0	0	AcOEt/MeOH	5 - 7	6	1.1	50	AcOEt/MeOH
21	-1	-1	-1	-1	ACN/MeOH	2 - 4	3	0.2	0	ACN/MeOH
22	-1	+1	-1	+1	ACN/MeOH	2 - 4	9	0.2	100	ACN/MeOH
23	+1	-1	+1	+1	ACN/MeOH	8 - 10	3	2	100	ACN/MeOH
24	-1	-1	+1	0	ACN/MeOH	2 - 4	3	2	50	ACN/MeOH
25	-1	+1	0	-1	ACN/MeOH	2 - 4	9	1.1	0	ACN/MeOH
26	-1	0	+1	+1	ACN/MeOH	2 - 4	6	2	100	ACN/MeOH
27	+1	-1	0	-1	ACN/MeOH	8 - 10	3	1.1	0	ACN/MeOH
28	+1	+1	-1	0	ACN/MeOH	8 - 10	9	0.2	50	ACN/MeOH
29	+1	+1	0	+1	ACN/MeOH	8 - 10	9	1.1	100	ACN/MeOH

30	+1	0	-1	-1	ACN/MeOH	8 - 10	6	0.2	0	ACN/MeOH
31	0	-1	-1	+1	ACN/MeOH	5 - 7	3	0.2	100	ACN/MeOH
32	0	+1	+1	-1	ACN/MeOH	5 - 7	9	2	0	ACN/MeOH
33	0	0	0	0	ACN/MeOH	5 - 7	6	1.1	50	ACN/MeOH
34	0	0	0	0	ACN/MeOH	5 - 7	6	1.1	50	ACN/MeOH

Table 4: Mathematical design acquired from the model with the coded and real values of each variable.

4.2 Sediments

2.2.1 ASE extraction

Accelerated solvent extraction is an automated extraction technique that uses liquid solvents and solvent mixture to extract solid or semi-solid samples. High Temperature (40-200^{0}C) and pressure (150-200 psi) accelerates the extraction process. ASE uses small quantities of solvent and short periods of time (15ml and 15 mins for 20 g samples).

Upon sampling, and before ASE step, there’s a preparation step of the sample (sediment). The steps are shown in the diagram below:

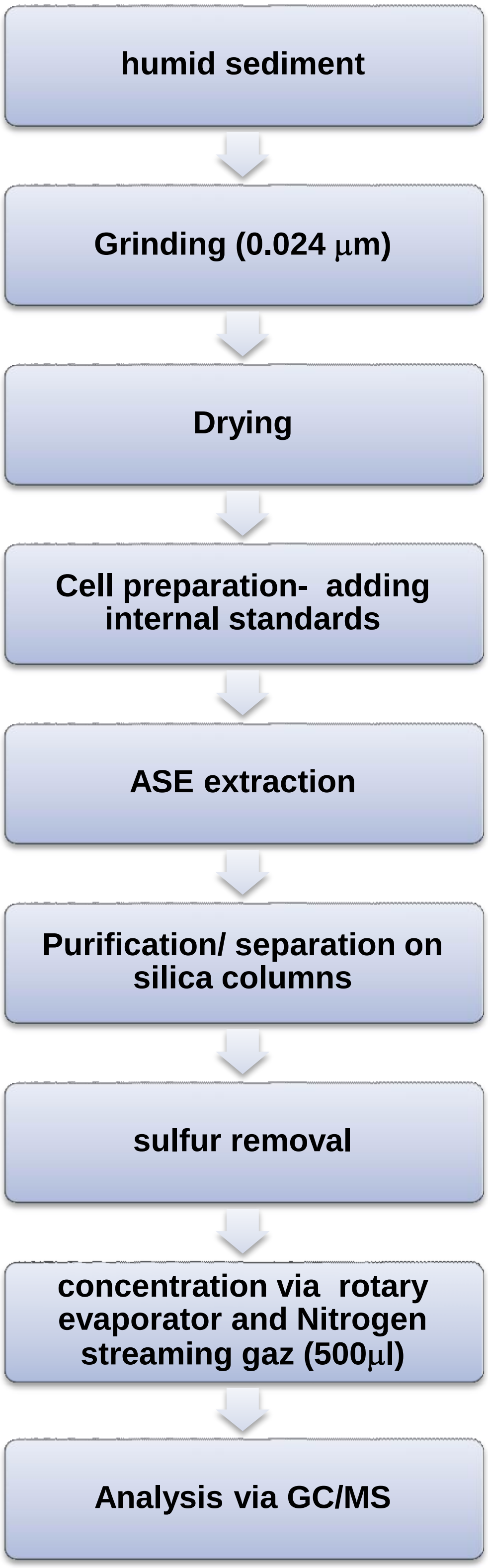

Fig. 2. Diagram of the analytical procedure.

ASE operates by moving the extraction solvent through an extraction cell containing the sample. The sample cell is heated by direct contact with the oven. The extraction is performed by direct contact of the sample with the hot solvent in both static and dynamic modes. When the extraction is complete, compressed nitrogen moves all of the solvent from the cell to the vial for analysis. The filtered extract is collected away from the sample matrix, ready column filtration than analysis. The diagram below shows the ASE extraction protocol.

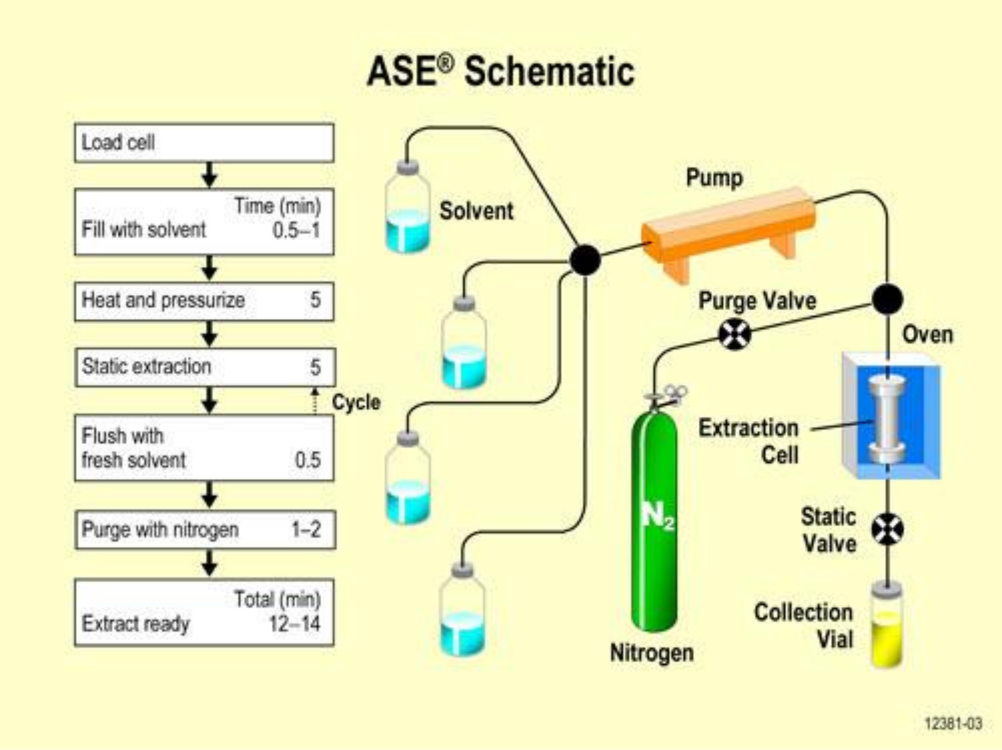

Fig. 3. Accelerated solvent extraction protocol and principle.

After extraction step, the extracts was concentrated to around 1-2 ml in a rotary evaporator and then purified by the mean of column separation.

This step was conducted through a multi-layer column packed orderly from the bottom with: a small amount of fiber glass previously calcined at 450^{0}C. 5g of deactivated silica dissolved in MeOH are added into the column. Add Na_2SO_4, to prevent water particles affecting the extraction.

The extract is pored into column, eluted approximately at 1 drop/second (il faut mettre un debit). To separate the analytes of interest sufficiently from the disturbing co-extracts, it is necessary to select the eluting solvent with appropriate polarity according to targeted compounds. The extraction procedure was as follows: F1: 20 ml of hexane, F2(1), 15 ml of Hexane/DCM (v:v/ 3:1), F2(2), 10 ml of Hexane/DCM (v:v/ 1:1), F3 15ml of DCM and F4 15ml of ACN. The last step before analyzing on GC/MS is concentrating the purified samples separately (each fraction aside) on rotary evaporator followed by a light stream of nitrogen to reach 500µ as a final volume.

5. Analytical analysis

5.1. Gas Chromatography/ Mass spectrometry (GC/MS/MS)

GC MS MS analysis

Pesticides were analyzed using a Varian 3900 gas chromatograph, equipped with a deactivated fused-silica guard column (5 m x 0.25 mm i.d.) and a low polarity si-arylene ZB-XLB capillary column (60 m x 0.25 mm i.d. x 0.25 µm film thickness), and coupled with an Ion Trap Saturn 2000 Mass Spectrometer (Varian Inc.) operating in either selected ion storage (SIS) or full scan (FS) mode. Helium was used as carrier gas at a constant flow rate of 1 $mL.min^{-1}$. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. The temperature of the GC was programmed as follows : initial temperature 80°C, held for 1 min, 10°$C.min^{-1}$ ramp to 170°C then 4°$C.min^{-1}$ ramp to 230°C and finally 3°$C.min^{-1}$ to 280°C and held for 2 min. The total analysis time was 43.67 min. The transfer line and the ion trap mass spectrometer were respectively held at 260°C and 220°C. Identification of each compound was done on the basis of the retention time and the mass spectrum obtained from chromatogram of standard solution acquired in full scan mode. Quantification was then performed in the SIS mode using the most abundant ions. Response factors were determined relative to the internal standards response.

II. Study site

The site chosen for our study in Lebanon is the Akkar zone situated in North Lebanon. Akkar is the second agriculture zone in Lebanon, where the use of pesticide is considerably high. Concerning water consumption, the daily use for the most habitant in this zone uses the groundwater. An epidemiological study was conducted in 2013 by the students of the Faculty of public health in Tripoli; the results show that the residents uses mostly the ground water for drinking, and other usage (average 70%).

The study was focused on Northern Lebanon near Syrian borders (34.5506° N, 36.0781° E). Akkar district is an area of intensive agricultural uses that cover 788 km^2 characterized by the presence of a relatively large coastal plain, with high mountains to the east. As the second agricultural zone of Lebanon, the most widespread crops are cereals (wheat and corn), potatoes, grapes, fruit trees, olives and vegetables. Sampling sites and their characteristics are listed in the table below.

Sampling sites	Site	Depth (m)	pH	GPS coordinates
1	Al Arida	65	6.5	34°37'60" N 35°58'60" E
2	Semmakieh	60	7.2	34°37'60" N 36°0'0" E
3	Al Knayseh	60	6.8	34°37'0" N 36°1'0" E
4	Massoudieh	60	6.5	34°60'55" N 36°04'90" E
5	Marlyat Hawara	65	6.4	34°56'06" N 36°02'39" E
6	Tall Mayan	70	6.8	34°59'80" N 36°03'78" E
7	Tall Abbas El Gharbi	70	6.5	34°34'60" N 36°4'0" E
8	Haret Al Jedideh	65	7.1	34°31'60" N 36°4'0" E
9	Qaabrine	65	6.9	34°57'28"N 36°02'95" E
10	Kobbet Al Chamra	60	7	34°53'90" N 35°99'41" E

Table 5: Sampling sites in Lebanon and their characteristics.

In France samples were conducted on two different campaigns, both rivers where situated in the ‘Nord Pas de Calais’ region; “La Somme” river and “La Canche” river. Water samples were collected from the Somme River from the first station at Béthencourt-sur-Somme (Station 001103) to Gauchy (station 116500). Caracteristics and coordinates of sampling areas are listed below.

	La Canche	La Somme
Coordinates	50^0 19’ 35” N 2^0 24’ 04” E	50^0 13’ 16” N 1^0 34’ 0” E
Length (km)	88	245
Basin (km^2)	1274	6550
Sump	La Canche	La Somme
Flow (m^3/s)	15.1	35

Table 6: Sampling sites in the Nord Pas de Calais region and their characteristics.

Chapter 3

Results and discussions

I. Experimental design approach for the optimization of organic pollutants extraction from water and sediments

Résumé

La reproductibilité, la sélectivité et l'automation ou semi-automation font de la ASE et SPE les méthodes les plus adéquates pour l'analyse des contaminants organiques présents à l'état de trace respectivement dans le sédiment et dans l'eau. SPE est une méthode adéquate pour les extractions des différentes familles de molécules donc les pesticides, dans les milieux aqueux. Et ASE est une technique très efficace pour extraire des contaminants organiques dans les matrices solides tels que les sédiments. Cette partie de manuscrit présente les protocoles d'optimisation des méthodes d'extraction des contaminants organiques en milieu aquatiques. Les extractions de deux familles de molécules sont optimisés : (1) l'extraction des pesticides dans l'eau en utilisant la technique SPE et (2) l'extraction des hydrocarbures (HAP, Me-HAP et les *n*-alcanes) des sédiments avec la technique ASE.

Le modèle mathématique MODDE est utilisé afin de faciliter l'étude des effets de chaque paramètre d'intérêt et leur interaction. Ce modèle mathématique permet non seulement de visualiser facilement les effets de chaque paramètre mais aussi il permet de réduire le nombre d'expérience en préservant les rendements très satisfaisants. Cette partie sera présentée sous forme des articles. L'article 1, présente les différentes étapes et stratégies d'optimisation de la technique d'extraction les pesticides avec la technique SPE. La GC-MS est ensuite utilisée pour analyser des extraits finaux et le modèle mathématique MODDE est employé pour faciliter le traitement de donnés. L'article 2, détaille le protocole et stratégie d'optimisation de la méthode d'extraction des HAP, leurs homologues méthylés (Me-HAP) et les alcanes dans les sédiments. Les extraits sont analysés avec la GC-MS pour les HAP et Me-HAP et avec la GC-FID pour les *n*-alcances. Dans ce deuxième article, la méthode développée est validée avec le matériel de référence et ensuite appliquée pour étudier les niveaux de contamination par les hydrocarbures de la rivière Deûle.

Abstract

Reproducibility, selectivity and automation or semi- automation make the ASE and SPE extraction technique the most appropriate methods for the extraction of organic contaminants at trace respectively in the sediment and water. SPE is a suitable method for the extraction of different families of molecules therefore pesticides in aqueous matrix. And ASE is an effective technique to extract organic contaminants in solid matrices such as sediment. This part of the manuscript presents the protocols of optimization for the extraction of organic contaminants in aquatic environment. Extractions of two families of contaminants are optimized: (1) the extraction of pesticides in water using SPE Technical and (2) the extraction of hydrocarbons (PAHs, Me- PAHs and *n*-alkanes) in sediment with ASE technique.

The mathematical model MODDE is used to facilitate the study of the effects of each parameter of interest and theirs interactions. This mathematical model can not only easily visualize the effects of each parameter on the extraction yield but also it allows reducing the number of experiment with very satisfactory efficiency. This part will be presented in the form of articles. Two articles will be presented Article 1, presents the different steps and strategies of optimization of pesticide extraction using the SPE technique. GC-MS is used to analyze the final extracts and the mathematical model “MODDE” is used to facilitate data treatment. Article 2 details the protocol and optimization strategy for the extraction of PAHs, Me-PAHs and *n*-alkanes in sediments. The extracts were analyzed with GC-MS for PAHs and Me- PAHs and with GC -FID for *n*- alcances analysis. In this second article, the developed method is validated with standard reference material and then the method was applied to study the levels of contamination by hydrocarbons in Deûle River.

- Article 1 -

EXPERIMENTAL DESIGN APPROACH TO THE OPTIMISATION OF PESTICIDES EXTRACTION FROM WATER

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An experimental design approach to the optimisation of pesticide extraction from water

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In the last few decades, pesticides have been used increasingly throughout the world. Nowadays, contamination of aquatic systems by pesticides has become a global problem. Due to their stability, mobility and long-term effects on living organisms, pesticides are among the most dangerous pollutants that can be monitored in the environment, and the determination of accurate contamination levels also constitutes a crucial step in environmental research. However, in the case of quantitative analyses, extraction of targeted analytes can turn out to be difficult since these compounds are often present below the detection limits. Consequently, the accuracy of environmental analyses mainly depends on the efficiency and the robustness of the extraction–preconcentration step. In this work, a solid-phase extraction (SPE) procedure using hydrophilic modified styrene-based polymer (HLB) cartridges was optimized for the extraction of organonitrogen and organochlorine pesticides from water. An experimental design was carried out for modeling SPE optimal extraction conditions of thirty four pesticides. The five parameters studied were the flow rate, pH, elution speed, ionic strength of the sample and the nature of the eluting solvent. Extracts were analyzed using gas chromatography equipped with a mass spectrometer (GC-MS). The optimal extraction conditions selected for the flow rate, pH, elution speed, ionic strength and the nature of the eluting solvent were respectively 2–4 mL min^{-1}, pH = 6, 0.5 mL min^{-1}, 100 g L^{-1} of NaCl and ethyl acetate–methanol (1/1 v/v). The analytical procedure was validated for fifteen pesticides, which include thirteen organonitrogens and two organochlorines.

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1. Introduction

Pesticides play a key role in world food production and have been widely used during the last few decades. They can easily reach aquatic ecosystems by direct application, spray drift, aerial spraying, erosion, runoff from factories and sewage. It is commonly said that more than 95% of pesticides used in agriculture are dispersed in the environment, in air, water and sediments.[1] They can now be detected in surface waters, ground waters and even in glaciers.[2,3] However, problems related to continuous use of pesticides have increased worldwide since contamination became a serious threat to both the aquatic ecosystem and the human health.[4–8] Various regulations have come into force concerning permissible levels of pesticide residues[9,10] and they led either to the development of new analytical techniques or to the improvement of the existing ones.

Extraction of pesticides from water can be carried out by various techniques such as liquid–liquid extraction (LLE),[11] matrix solid-phase dispersion (MSPD),[17] stir-bar sorptive extraction (SBSE),[18] solid-phase extraction (SPE)[12] or solid phase micro-extraction (SPME).[13] Among these techniques, SPE has received increasing attention because of its easy implementation, time saving steps, elimination of emulsions, and the fact that it considerably reduces the amount of solvent required.[19,20] In addition, better enrichment factors are usually obtained by SPE.[21] Nowadays, the solid-phase extraction has been proven to be a powerful method for sample preparation.[10,22–25] Furthermore, SPE presents high potential for automation.[9,26,27] Indeed, even if the application of LLE in water has been widely accepted in standard methods, the LLE procedure is time consuming and requires a large volume of solvent. Micro-LLE has been introduced in US Environmental Protection Agency (EPA) methods, but such a technique does not allow trace detection at low concentration level (0.1 μg L^{-1}) as required for monitoring pesticides in drinking water in European countries.[14–16]

Selection of the sorbent is an important aspect in using the SPE technique.[28] Chemically bonded silica (*e.g.* C_{18} silica) and styrene/divinylbenzene (PS-DVB) copolymers are the most frequently used sorbents for extraction from water samples.[9,24,29–32] However, these sorbents often present low

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recoveries for extraction of polar compounds. Moreover, C_{18} silica has been found to be unstable at extreme pH.[33,34] Porous graphitic carbon (PGC) has been used as a sorbent due to its great adsorption capacity associated with its chemical, thermal and mechanical resistance. However, the use of such a carbon sorbent can lead to excessive or even irreversible retention.[35,36] Recently, new hydrophilic polymeric materials, obtained by copolymerizing monomers containing suitable functional groups or by introducing a functional group to the existing hydrophobic polymers, have been developed as SPE materials. Targeted benefits of these new functionalized polymeric sorbents are improvement of wetting characteristics, mass transfer and retention of polar or ionized compounds. The most commonly used functionalized sorbent for large multi-residue extraction is the Oasis HLB (Waters®), which is a macroporous copolymer made from a balanced ratio of the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone, thus providing reversed-phase capability with a special hook for polar compounds.[37–43]

SPE methods often involve investigation of many variables, which may affect the efficiency of extraction. By considering such a multi-criterion approach, an experimental design can be used to optimize important variables.[10] Optimization through experimental design often assumes factorial designs or non-linear models called response surface models, which require in this case three levels for each parameter.[44]

In this work, Supel-Select HLB SPE (Sigma-Aldrich, USA) cartridges were employed through an experimental design model to optimize the extraction conditions of 34 pesticides in water. Five variables (sample flow rate, pH, ionic strength, nature of the eluent and elution flow rate) were studied. Optimal conditions were then validated using natural water samples.

2. Materials and methods

2.1. Chemicals

Thirty four pesticides were first selected as target compounds (Table 1 and Fig. 1). Pesticide standards were provided by Restek (Bellefonte, USA). Supel-Select HLB SPE cartridges (200 mg/6 mL) were purchased from Sigma-Aldrich (Saint-Louis, USA). The sorbent phase was characterized by the particle size of 55–60 μm offering a pore size of 87 Å and a surface area of 400 m^2 g^{-1}. HPLC grade ethyl acetate (AcOEt), dichloromethane (DCM), methanol (MeOH), acetonitrile (ACN) and hexane were purchased from Dislab (Lens, France). Ultrapure water (Milli-Q) was produced by using a Millipore apparatus (18.2 MΩ cm^{-1} resistivity). Sodium chloride (NaCl, 99.5%) and analytical grade hydrochloric acid (HCl, 37%) were purchased from Merck (Darmstadt, Germany). Potassium hydroxide (KOH, 0.5 mol L^{-1} in methanol) was purchased from Panreac Quimica (Barcelona, Spain). Pentachloronitrobenzene as the internal standard with a purity of 94% was purchased from Sigma-Aldrich (Saint-Louis, USA). All pesticide solution standards were prepared with acetonitrile. GC-MS calibration was performed using eight calibration solutions with concentrations ranging from 5 μg L^{-1} to 5 mg L^{-1} directly prepared from stock solutions.

2.2. Experimental procedure

750 mL of ultra-pure water was spiked with standard solution (at 3 μg L^{-1}) of targeted compounds. The pH was adjusted by addition of 1 M aqueous hydrochloric acid or 0.5 M methanolic solution of potassium hydroxide, and controlled using a Metrohm 713 pH meter (Herisau, Switzerland). Ionic strength was set up by addition of sodium chloride. Extraction was carried out using an SPE vacuum manifold system. The extraction process consists of seven steps as follows: (1) conditioning step, the cartridge was conditioned with 5 mL of appropriate eluent according to the experiment set (Table 2), followed by 5 mL of methanol; (2) equilibration step, 10 mL of ultra-pure water was added to wet the sorbent surface; (3) percolation step, the sample was percolated into the cartridge; (4) after the sample was loaded, the cartridge was washed with 5 mL of ultra-pure water followed by 5 mL H_2O–MeOH (95/5 v/v); (5) the cartridge was dried with high purity nitrogen flow for 5 min; (6) eluting with 2 × 5 mL (MeOH–eluent 1/1 v/v); (7) evaporating using the rotary evaporator until reaching a volume of 2 mL, and then continuing evaporation under a gentle nitrogen stream; (8) fixing the final volume to 500 μL before GC-MS analysis.

2.3. Choice of operating variables and their variation levels

Numerous factors are known to influence significantly the efficiency of SPE extraction of pesticides from water. To increase the recovery of the studied compounds, the pH must also be optimized according to the targeted analytes' chemical properties. Besides, some studies have reported that ionic strength can also influence the recovery of pesticides based on the fact that the water solubility of polar organic compounds is diminished in high ionic strength aqueous media.[46] Likewise, nature of the eluent and flow rate while elution can as well influence the extraction of pesticides. For example, Baugros *et al.* (2008)[45] and Guardia-Rubio *et al.* (2007)[47] reported that a low flow rate increases interaction between the sorbent and targeted compounds thus leading to a better recovery.

In this study, a hydrophilic–lipophilic balanced (HLB) polymer was selected as sorbent with the aim to cover the entire range of lipophilicity of targeted compounds (log K_{OW} ranging from 1.2 to 5.6).[37–43] Five variables that are known to affect the extraction efficiency were studied: sample flow rate, sample pH, elution flow rate, ionic strength (NaCl addition) and the nature of the eluent. The selected variables in this study and their variation levels are summarized in Table 2.

Three levels of variables have been studied, with the sample flow rate ranging from 2 to 10 mL min^{-1}, the pH from 3 to 9, the elution flow rate from 0.2 to 2 mL min^{-1}, and the ionic strength from 0 to 100 g L^{-1} of NaCl. The eluent is a qualitative variable; the solvent composition was made up with DCM–MeOH, AcOEt–MeOH and ACN–MeOH in a 1/1, v/v proportion.

2.4. Optimization strategy

Several models of experimental design such as factorial designs, central composites, Doehlert designs and a simplex method can be applied.[48–52] Factorial designs are appropriate to evaluate

Table 1 Targeted pesticides with their classification group, function, retention time, limit of quantification (LOQ), and qualifier ions[a]

Compounds	Chemical class	Function	log K_{OW}	RT (min)	LOD ($\mu g\ L^{-1}$)	Qualifier ions
EPTC	Thiocarbamate	Herbicide	3.2	11.8	0.05	126; 134
Butylate	Thiocarbamate	Herbicide	4.1	13.1	0.05	145; 188
Vernolate	Thiocarbamate	Herbicide	3.8	14.0	0.05	127; 147; 160
Tebuthiuron	Urea	Herbicide	1.8	14.8	0.05	155; 170
Etridiazole	Thiazole	Fungicide	2.6	15.8	0.05	183; 211
Molinate	Thiocarbamate	Herbicide	3.2	17.8	0.05	126
Propachlor	Chloroacetamide	Herbicide	2.4	19.4	0.05	120; 176
Cycloate	Thiocarbamate	Herbicide	4.1	19.7	0.05	83; 154; 215
Fluridone	Pyridinone	Herbicide	1.9	58.7	0.05	328
Fenarimol	Pyrimidine	Fungicide	3.7	46.7	0.05	139; 219; 251
Terbacil	Uracil	Herbicide	1.9	24.2	0.05	161
Chlorpropham	Carbamate	Herbicide	3.4	20.2	0.05	127; 171; 213
Trifluralin	Dinitroaniline	Herbicide	5.3	19.9	0.05	264; 306
Atraton	Triazine	Insecticide	2.7	21.8	0.05	169; 196; 211
Prometon	Triazine	Herbicide	4.3	21.9	0.05	168; 210; 226
Simazine	Triazine	Herbicide	2.2	22.5	0.05	186; 200; 203
Atrazine	Triazine	Herbicide	2.6	22.5	0.05	172; 200; 230
Propazine	Triazine	Herbicide	2.9	22.7	0.05	231
Pronamide	Amide	Herbicide	3.4	23.2	0.05	173; 175; 254
Simetryn	Triazine	Herbicide	2.6	26.2	0.05	213
Metribuzine	Triazine	Herbicide	1.6	26.6	0.05	198
Alachlor	Chloroacetamide	Herbicide	2.9	25.9	0.05	160; 188
Ametryn	Triazine	Herbicide	3	26.3	0.05	213; 227
Terbutryn	Triazine	Herbicide	3.6	26.9	0.05	170; 185; 242
Napropamide	Amide	Herbicide	3.3	32.6	0.05	128; 171; 271
Metolachlor	Chloroacetamide	Herbicide	2.9	28.0	0.05	162; 238
Triadimefon	Triazole	Fungicide	3.2	28.4	0.05	208; 210
Diphenamid	Amide	Herbicide	2.2	29.4	0.05	166
MGK-264	Dicarboximide	Insecticide	3.7	29.0	0.05	164
Butachlor	Chloroacetamide	Herbicide	4.5	31.6	0.05	176; 188
Norflurazon	Pyridazinone	Herbicide	2.3	38.8	0.05	102; 145; 303
Hexazinone	Triazine	Herbicide	1.2	39.4	0.05	171
Alpha-BHC	Organochlorinated	Insecticide	3.8	13.6	0.05	181; 183
Endrin	Organochlorinated	Insecticide	5.6	27.0	0.05	281

[a] ON and OCl are respectively organonitrogen and organochlorine pesticides.

principal effects as well as interactions between chosen factors. However, any experimental design remains empirical and requires replicate experiments to account for the variation and the uncertainty of measurements. Optimal designs reduce the costs of experimentation by allowing statistical models to be estimated with fewer experimental runs. In this work, 34 experiments were selected according to the D-optimality criterion[49] with the aim to estimate parameters without bias and with minimum variance.

2.5. Mathematical model postulated: D-optimal design and exchange algorithm

The selection of the mathematical model constitutes the second step of the experimental design methodology. In this study, a second-order polynomial model was postulated:

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i,j} \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

X represents the model matrix or effect matrix with an $N \times p$ dimension (where N is the number of experiments and p is the number of coefficients of the model), y is the vector of the experimental responses, β_i is the vector of the coefficients of the model, and ε is the vector of the experimental errors. When the model is adjusted to the experimental data, any experimental error is transmitted to the coefficients and to the significance of the factors. The estimation of the coefficients β_i, β_{ii} and β_{ij} allows determination of the effects of both the factors and the interactions between them:

$$\beta = (X^t \cdot X)^{-1} \cdot X^t \cdot y \qquad (2)$$

(X^tX) is the information matrix and $(X^tX)^{-1}$ is the dispersion matrix. The D-optimality criterion tends to minimize the dispersion matrix corresponding to the variance, and conversely allows maximization of the determinant of the information matrix. For this study, the D-optimal design has been built through the exchange algorithm Fedorov[53,54] that is shown by natural and coded variables in Table 3.

2.6. GC-MS analysis

Analyses of pesticides were carried out using a Varian 3900 gas chromatograph, equipped with a deactivated fused-silica guard column (5 m × 0.25 μm i.d.) and a low polarity Si-arylene ZB-

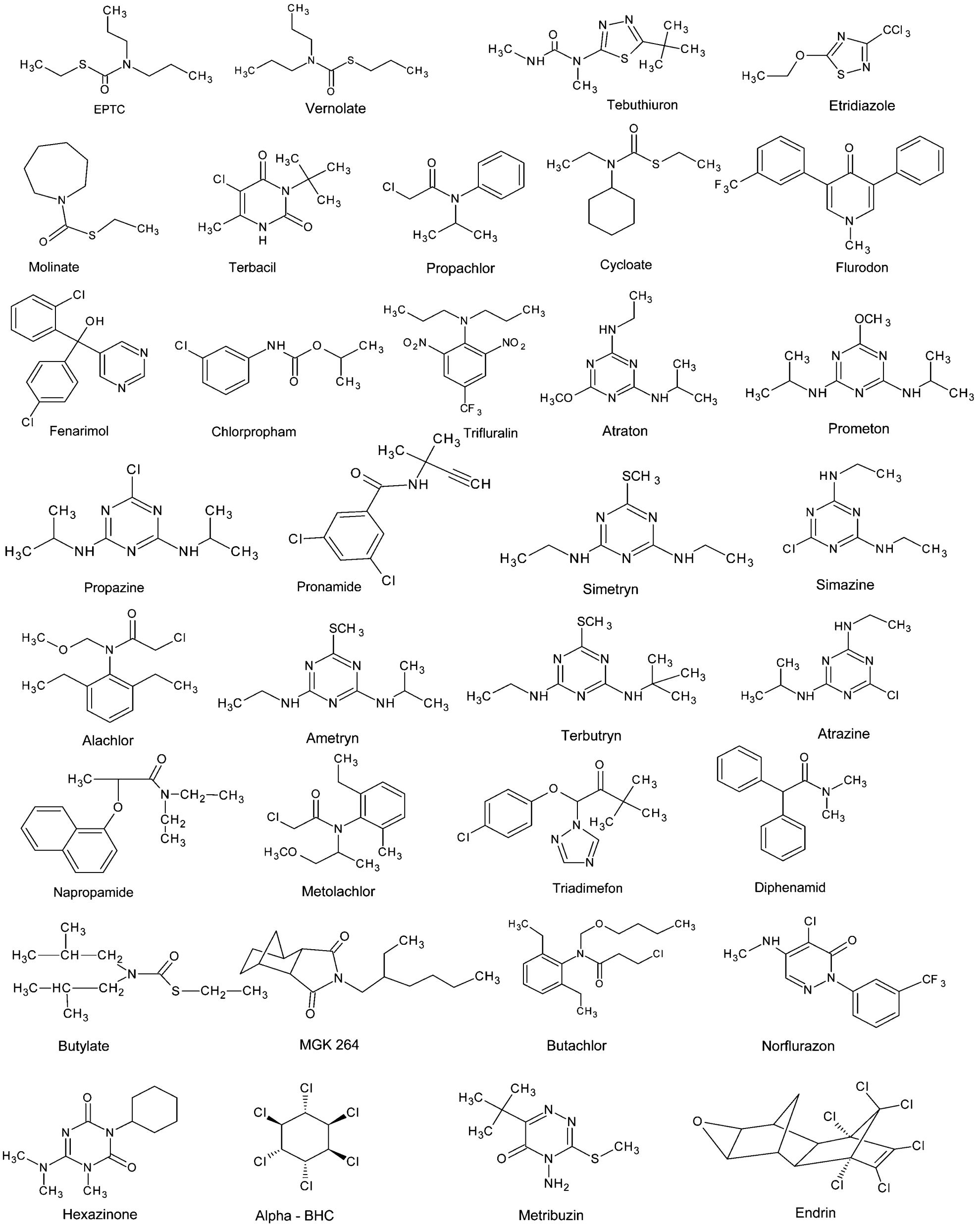


Fig. 1 Structures of the thirty four targeted pesticides.

Table 2 Summary of factors and their levels as coded and natural variables

Variable	Factor	Level		
		−1	0	+1
$X1$	Sample flow rate (mL min^{-1})	2–4	5–7	8–10
$X2$	pH	3	6	9
$X3$	Elution flow rate (mL min^{-1})	0.2	0.5	1
$X4$	Ionic strength (g L^{-1} of NaCl)	0	50	100
$X5$	Eluent (1/1 v/v)	DCM–MeOH	AcOEt–MeOH	ACN–MeOH

XLB capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness), coupled to an Ion Trap Saturn 2000 Mass Spectrometer (Varian Inc.) operating either in selected ion storage (SIS) or in full scan (FS) mode. Helium was used as the carrier gas with a constant flow rate of 1 mL min^{-1}. Samples were injected in splitless mode at 280 °C and the injector was purged with helium after 1 min. The temperature of the GC was programmed as follows: initial temperature 80 °C, held for 1 min,

Table 3 Mathematical design acquired from the model with the coded and real values of each variable

	Coded variables					Natural variables				
Experiment	$X1$	$X2$	$X3$	$X4$	$X5$	Sample flow rate (mL min^{-1})	pH	Elution speed (mL min^{-1})	NaCl (g L^{-1})	Eluent
1	+1	−1	−1	−1	DCM–MeOH	8–10	3	0.2	0	DCM–MeOH
2	−1	+1	−1	−1	DCM–MeOH	2–4	9	0.2	0	DCM–MeOH
3	+1	+1	+1	−1	DCM–MeOH	8–10	9	2	0	DCM–MeOH
4	+1	+1	−1	+1	DCM–MeOH	8–10	9	0.2	100	DCM–MeOH
5	+1	−1	+1	+1	DCM–MeOH	8–10	3	2	100	DCM–MeOH
6	−1	+1	+1	+1	DCM–MeOH	2–4	9	2	100	DCM–MeOH
7	−1	−1	−1	0	DCM–MeOH	2–4	3	0.2	50	DCM–MeOH
8	−1	−1	0	+1	DCM–MeOH	2–4	3	1.1	100	DCM–MeOH
9	−1	0	−1	+1	DCM–MeOH	2–4	6	0.2	100	DCM–MeOH
10	−1	0	+1	−1	DCM–MeOH	2–4	6	2	0	DCM–MeOH
11	0	−1	+1	−1	DCM–MeOH	5–7	3	2	0	DCM–MeOH
12	−1	−1	−1	−1	AcOEt–MeOH	2–4	3	0.2	0	AcOEt–MeOH
13	+1	+1	−1	−1	AcOEt–MeOH	8–10	9	0.2	0	AcOEt–MeOH
14	+1	−1	+1	−1	AcOEt–MeOH	8–10	3	2	0	AcOEt–MeOH
15	−1	+1	+1	−1	AcOEt–MeOH	2–4	9	2	0	AcOEt–MeOH
16	+1	−1	−1	+1	AcOEt–MeOH	8–10	3	0.2	100	AcOEt–MeOH
17	−1	+1	−1	+1	AcOEt–MeOH	2–4	9	0.2	100	AcOEt–MeOH
18	−1	−1	+1	+1	AcOEt–MeOH	2–4	3	2	100	AcOEt–MeOH
19	+1	+1	+1	+1	AcOEt–MeOH	8–10	9	2	100	AcOEt–MeOH
20	0	0	0	0	AcOEt–MeOH	5–7	6	1.1	50	AcOEt–MeOH
21	−1	−1	−1	−1	ACN–MeOH	2–4	3	0.2	0	ACN–MeOH
22	−1	+1	−1	+1	ACN–MeOH	2–4	9	0.2	100	ACN–MeOH
23	+1	−1	+1	+1	ACN–MeOH	8–10	3	2	100	ACN–MeOH
24	−1	−1	+1	0	ACN–MeOH	2–4	3	2	50	ACN–MeOH
25	−1	+1	0	−1	ACN–MeOH	2–4	9	1.1	0	ACN–MeOH
26	−1	0	+1	+1	ACN–MeOH	2–4	6	2	100	ACN–MeOH
27	+1	−1	0	−1	ACN–MeOH	8–10	3	1.1	0	ACN–MeOH
28	+1	+1	−1	0	ACN–MeOH	8–10	9	0.2	50	ACN–MeOH
29	+1	+1	0	+1	ACN–MeOH	8–10	9	1.1	100	ACN–MeOH
30	+1	0	−1	−1	ACN–MeOH	8–10	6	0.2	0	ACN–MeOH
31	0	−1	−1	+1	ACN–MeOH	5–7	3	0.2	100	ACN–MeOH
32	0	+1	+1	−1	ACN–MeOH	5–7	9	2	0	ACN–MeOH
33	0	0	0	0	ACN–MeOH	5–7	6	1.1	50	ACN–MeOH
34	0	0	0	0	ACN–MeOH	5–7	6	1.1	50	ACN–MeOH

10 °C min^{-1} ramped to 170 °C then 4 °C min^{-1} ramped to 230 °C, and finally 3 °C min^{-1} to 280 °C and held for 2 min. The total analysis time was 43.67 min. The transfer line and the ion trap mass spectrometer were respectively held at 280 °C and 220 °C. Identification of each compound was done on the basis of the retention time and the mass spectrum obtained from the chromatogram of the standard solution acquired in full scan mode. Quantification was then performed in SIS mode using the most abundant ions (Table 1).

3. Results and discussion

The D-optimal design was used for the optimization of the five selected parameters that are presumed to impact the solid-phase extraction efficiency of various pesticides in water.

3.1. Analysis of designed experiments: the effect of the factors

The results were explored using statistical and graphical analysis software (Modde 5.0 Umetrics, Sweden). This software was used for regression analysis of the data obtained from the set of 34 experiments and to estimate the coefficients of regression equation. Analysis of variance (ANOVA), squared and interaction terms were applied to test the significance of each term in the equation. The coefficient of correlation R^2 represents the fraction of the response variation explained by the model whereas Q^2 accounts for the fraction of the response variation that can be predicted by the model. The effects of different factors on the extraction yield of 34 pesticides are displayed in Fig. 2.

These results indicate that the sample flow rate ($X1$) has negative influence on the extraction yield of the targeted compounds. This observation is in accordance with literature reviews where the extraction efficiency increases when working at low flow rate.[10,55,56] Consequently, this variable should be fixed at its lower level (2–4 mL min^{-1}). The pH ($X2$) can also be considered to have a negative effect. Likewise, the interaction of pH with ionic force ($X4$) is negative while interactions with $X5$(ACN), $X5$(AcOEt) and the elution flow rate ($X3$) are not significant. Given the neutral nature of the studied analytes, neutral pH values should be prefered.[10,56] The elution speed ($X3$) shows positive influence that can significantly affect the extraction efficiency. Meanwhile, interactions of $X3$ with the sample flow rate ($X1$), pH ($X2$), ionic force ($X4$) and $X5$(ACN)/$X5$(AcOEt) are not significant. These parameters will also be discussed later by analyzing the surface response curves in the next part. The main effect of ionic strength ($X4$) is weakly positive. However, the interactions between $X4$ and the other factors do not allow for evident conclusion, and this part will also be discussed later by analyzing the surface response curves. Concerning the eluent nature, among the three mixtures of the eluent employed, AcOEt–MeOH generally led to better extraction yields.

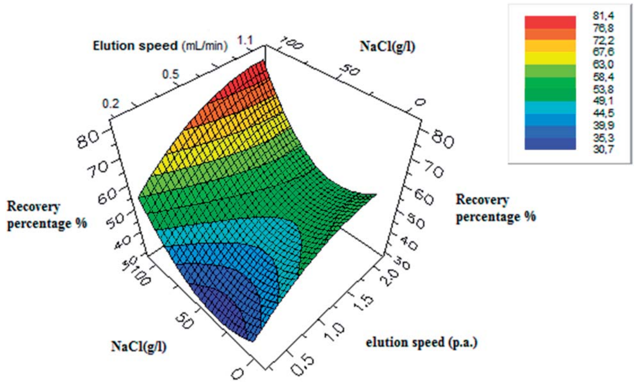


Fig. 3 The surface response curve of targeted pesticides. The fixed parameters are: pH 6, sample flow rate 2–4 mL min^{-1} and a mixture of AcOEt–MeOH (1/1 v/v) as the eluent.

3.2. Analysis of designed experiments: surface response curve

Analysis of factors indicates that a low sample flow rate gives better recovery. In the same manner, working at neutral pH and using AcOEt–MeOH for elution have resulted in better extraction yields. By setting the sample flow rate at its low level (2–4 mL min^{-1}) combined with a neutral pH level (pH = 6) and using AcOEt–MeOH as the eluent, the response surface curve (Fig. 3) shows the influence of the elution speed and the ionic strength (NaCl) on the extraction efficiency.

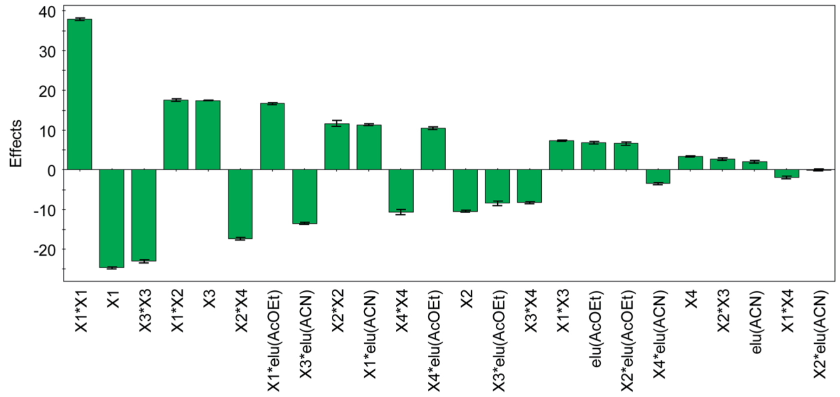
It can be seen that an increase in the ionic strength influences positively the extraction efficiency. NaCl ($X4$) should also be set at its maximum level (100 g L^{-1}) for optimal extraction. This observation is in accordance with other studies focused on the impact of ionic strength on pesticide extraction efficiency. Indeed, Bagheri *et al.* (2000)[10] reported that the highest recovery for the extraction of diazinon was obtained when adding 5% NaCl, while Tolosa *et al.* (1996)[57] used 60 g L^{-1} of NaCl for multiresidue extraction of organophosphorus and organochlorine pesticides. According to Font *et al.* (1993),[9] it can be admitted that an increase in the ionic strength of aqueous samples leads to weakening of the interactions between undissociated molecules and water, thus resulting in an increase of the extraction efficiency. Concerning the elution speed, good recoveries were obtained with a value ranging from 0.4 to 1 mL min^{-1}. Since desorption of targeted analytes should be greater at a moderate flow rate, it was decided to set this factor at its middle level (0.5 mL min^{-1}).

The defined model predicts the optimal solid-phase extraction conditions as: the sample flow rate at low level (2–4 mL min^{-1}), pH = 6, AcOEt–MeOH (1/1 v/v) as the eluent, NaCl = 100 g L^{-1}, and elution speed at 0.5 mL min^{-1}. By setting up these conditions, the concentration of targeted pesticides (34 compounds) can be calculated using the mathematical model given below:

$$\begin{aligned} y = {} & 65.56 - 3.97X1 - 1.93X2 + 4.53X3 + 6.91X4 + 18.94X1^2 \\ & + 5.85X2^2 - 11.49X3^2 - 5.32X4^2 + 8.78X1X2 + 3.66X1X3 \\ & - 0.97X1X4 + 1.37X2X3 - 8.68X2X4 - 4.11X3X4 \end{aligned}$$

3.3. Optimal conditions and method validation

The optimized method was first validated by comparing the average of experimental values with that of predicted values obtained from the mathematical models. The procedure was



* *X1, X2, X3 and X4 are respectively sample flow rate, pH, elution speed and NaCl.*

Fig. 2 Effects of different factors on the extraction of 34 pesticides. Statistical values are: regression coefficient, $R^2 = 1.00$; adjusted coefficient of regression, $R_{\text{Adj}}{}^2 = 1.00$; reliability of the mathematical model, $Q^2 = 0.999$ and mean square residuals, RSD = 0.0165.

Table 4 Average recoveries of the 15 pesticides validated in this study

Pesticide	Extraction yield in ultra-pure water (%)	Extraction yield in river water (%)
Alachlor	105	79
Ametrin	131	101
Atraton	78	94
Butachlor	43	54
Chlorpropham	65	65
Metolachlor	99	76
Molinate	132	74
Napropamide	100	73
Prometon	100	91
Propachlor	103	91
Pronamide	107	89
Terbutryn	82	89
Triadimefon	105	77
alpha-Lindane	82	85
Endrin	119	104
Mean value	97 ± 23	83 ± 13

validated for 15 pesticides using both ultra-pure water and river water (Table 4). The mean of the predicted extraction yield for 32 pesticides initially chosen was 91.0%. Extraction yields of the 15 selected pesticides obtained from spiked ultra-pure water ranged from 43% for butachlor to 132% for molinate with a mean value of 97% (Table 4). The low recovery of butachlor compared to the other chloroacetamide compounds such as alachlor (recovery of 105%, $\log K_{\mathrm{OW}} = 2.9$), propachlor (recovery of 103%, $\log K_{\mathrm{OW}} = 2.4$) or metolachlor (recovery of 99%, $\log K_{\mathrm{OW}} = 2.9$) can be attributed to the higher hydrophobicity of butachlor ($\log K_{\mathrm{OW}} = 4.5$) due to its butyl chain. On the other hand, the modest recovery of carbamate chlorpropham (65%) compared to the one obtained for the thiocarbamate molinate could be associated with the higher hydrogen bonding capacity of chlorpropham (both H-bond donor and acceptor). Our results were comparable to those found in the literature. Kouzayha *et al.* (2012)[56] reported extraction yields of 106%, 106% and 116 for respectively alpha-lindane, alachlor and propizamide, while the developed method gave recoveries of 82%, 105% and 107% respectively for the same compounds. Robustness of the method was also tested by applying the analytical procedure to spiked river water samples originated from the Canche River, in northern France. These additional analyses were performed in triplicate. Non-spiked river water revealed no trace of the targeted analytes. Extraction yields were found to vary from 54% for butachlor to 104% for endrin with a satisfactory mean recovery of 83%.

4. Conclusions

This work presents an optimized SPE strategy using HLB cartridges followed by GC-MS analysis. The method was optimized for determination of fifteen pesticides embedded with various structural characteristics (triazine, carbamate, and thiocarbamate as well as organochlorine compounds). The validated method could be considered as a green method with respect to the volume of eluents used, and by the fact that ethyl acetate can be regarded as an environmentally friendly solvent. The other benefit of the developed procedure is its simplicity, which leads to the possible application for routine analyses. Optimized conditions include percolation of 750 mL of filtered-water samples at natural pH on HLB cartridges at 2–5 mL min^{-1} flow rate. The ionic strength of the sample is controlled by the addition of sodium chloride (100 g L^{-1}), whereas elution is performed at a moderate speed fixed at 0.5 mL min^{-1} with AcOEt–MeOH (1/1 v/v) as the eluent. This method was optimized using a mathematical model, D-optimal designs. The D-optimal matrix designed in this work clearly reveals the effects of different important parameters and their interactions affecting the extraction efficiency in detail. Many aspects of these results confirm the previously reported experimental data. Using the D-optimal method, not only the optimum extraction conditions for different types of pesticides were achieved, but also a great deal of information about the effects of each factor on the recovery could be obtained while performing the minimum number of experiments.

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- Articles 2 –

EXPERIMENTAL DESIGN APPROACH TO THE OPTIMISATION OF HYDROCARBONS EXTRACTION FROM THE SEDIMENT: METHOD DEVELOPMENT AND APPLICATION

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Experimental design approach to the optimisation of hydrocarbons extraction from the sediment: Method development and application

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ABSTRACT

Extraction and analysis of organic pollutants from matrices such as sediment constitute an essential step in environmental research. However, the extraction for quantitative analysis can turn out to be difficult because these compounds are present in trace levels and can be strongly bound to the sorbent matrix. Consequently, accuracy of environmental analyses mainly depends on the efficiency and the robustness of the extraction step. In this work, a sequential ASE extraction procedure was applied to the extraction of polycyclic aromatic and aliphatic hydrocarbons (PAHs, Me-PAHs and *n*-alkanes) in sediment samples. The extraction protocol was developed for 26 PAHs, including the 16 PAHs of the United-States Environmental Protection Agency (EPA) priority list, for 17 alkylated PAHs homologues and for 29 *n*-alkanes (from n-C_{12} to n-C_{40}). A set of 30 experiments was carried out for the determination of the optimal extraction conditions. The four parameters studied were pressure, temperature, extraction time and nature of the solvent. Extracts were analyzed by gas chromatography (GC-MS and GC-FID) after clean-up and concentration. The optimal extraction conditions selected for pressure, temperature, extraction time and nature of solvent were respectively 14 MPa, 160 °C, 24 min and hexane/acetone (1/1 *v*/*v*). The analytical procedure was validated by comparing predicted and experimental values of sediment samples and by analyzing standard reference material. The validated method was then applied to establish a depth profile contamination in the sediment of the Deûle River in Northern France.

1. Introduction

Hydrocarbons are widely disseminated in environment, with sources that can be both natural and anthropogenic. Combustion-derived (pyrogenic) hydrocarbons are formed as a result of incomplete combustion of organic matter, while petroleum-derived (petrogenic) hydrocarbons derived from organic material at relatively low temperatures over geologic time scales (Laflamme and Hites, 1978; Tronczynski et al., 1999). Natural sources of pyrogenic hydrocarbons include biomass fires, volcanic eruptions and diagenesis, while anthropogenic sources include vehicular and industrial emissions (Yunker et al., 2002; Wang et al., 2007). Natural petrogenic hydrocarbon sources include crude oil seeps and coal, while anthropogenic sources include oil spills, chronic discharges and coal burning (Achten and Hoffmann, 2009; Mostert et al., 2010). Several qualitative and quantitative indexes based on polycyclic aromatic hydrocarbons (PAHs and Me-PAHs) and *n*-alkanes (aliphatic hydrocarbons) can be used to determinate the source apportionment (Colombo et al., 1989; Commendatore et al., 2000; Charriau, 2009). Hydrocarbons are highly lipophilic compounds, ubiquitous in coastal, estuarine and river water column, as well as sediments in which they tend to accumulate (Cailleaud et al., 2007; Chiou et al., 1998; Manodori et al., 2006; Gaspare et al., 2009; Ko and Baker, 1995; Yunker et al., 2012). Recent studies have reported that marine organisms are prone to bioaccumulate these substances, particularly in lipid-rich tissues (Neff, 2002; Francioni et al., 2005; Dugan et al., 2005). Due to their toxic, carcinogenic and mutagenic effects (Straif et al., 2005; IARC, 2010; U.S. Department of Health and Human Services, 2011), sixteen PAHs have been recommended as priority pollutants by the United States Environmental Protection Agency (US EPA, 2002).

High level of hydrocarbons represents a serious threat to the ecosystem functioning and human health via food chain and water resources. The analysis of hydrocarbons in sediments generally includes extraction, cleanup, column fractionation and gas chromatographic separation. Extraction of hydrocarbons from sediments is conventionally carried out by Soxhlet extraction (Barnabas et al., 1995). Unfortunately, this technique is time-consuming and requires large volumes of organic solvents. Other extraction techniques have also been developed not only in order to reduce the volume of solvents and extraction times, but also to improve precision of the analytes recoveries. Such techniques include microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) (Camel, 2001; Song et al., 2002; Charriau, 2009; Itoh et al., 2009; Itoh

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et al., 2010). Among these techniques, ASE, also known as pressurized liquid extraction (PLE), remains an efficient tool for different solid samples extraction. Indeed, in addition to the above-mentioned advantages, ASE has received an increasing attention because of its facility to implement, time saving, but also because it allows extracting with high pressure which means that solvents can be heated to high temperatures above their boiling points, which make them much efficient to dissolve target compound from their matrix (Björklund and Nilsson, 2000), it maintains constant extraction conditions and gives a good repeatability by its automation (Hubert et al., 2001; Schantz, 2006). However, extractability of a compound from a matrix such as sediment can be operationally defined by the nature of targeted compounds, the nature of solvents and the experimental conditions under which the extraction is carried out (Ma et al., 2010).

The aim of this study was to define optimal extraction conditions of both polycyclic aromatic hydrocarbons (PAH and Me-PAH) and aliphatic hydrocarbons (*n*-alkanes) from sediment using a sequential ASE extraction technique. An experimental design methodology was employed to facilitate data treatments. The main advantage of design of experiments is to facilitate the data treatment with a limited number of experiments to perform. The optimized extraction method was then applied to determine the concentration of hydrocarbons in different depth of natural sediment, and to determine the hydrocarbon fingerprint and sources of pollution of the Deûle River (North of France).

2. Materials and methods

2.1. Reagents

Sediments were analyzed for 26 non-alkylated and 17 alkylated polycyclic aromatic hydrocarbons as well as for 29 *n*-alkanes (from n-C$_{12}$ to n-C$_{40}$). Studied organic compounds and their limit of quantification are described in Tables S1 and S2 in the Supplementary Information. Deuterated internal standards for PAHs and Me-PAHs (acenaphthene-d_{10} (A-d_{10}), naphtalene-d_8 (N-d_{10}), perylene-d_{12} (Per-d_{12}), phenanthrene-d_{10} (Phe-d_{10}) and pyrene-d_{10} (Pyr-d_{10})) were purchased from Protochem. 1-eicosene, used for *n*-alkanes quantification, was obtained from Dr. Ehrenstorfer Gmbh.

HPLC-grade solvents (hexane, dichloromethane, methanol and acetonitrile) were purchased from Dislab (France). No significant amount of analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70–230 mesh ASTM) activated at 450 °C was heated at 120 °C for 12 h prior to use. Glassware was washed with detergent (Decon), rinsed with ultrapure water and acetone and was dried at 120 °C prior to use.

2.2. Sampling

The Deûle River is located in the Scheldt basin and is one of the main tributaries of the Lys River. The Deûle River was chosen because of the historical metallic and PAH pollution in both sediment and superficial water (Charriau, 2009; Lesven et al., 2010; Agence de l'Eau Artois-Picardie, 2007). Sediments were collected in November 2011 at the Don Station (Fig. 1) known for its large lock and for the presence of two former industrial wastelands. Sediments cores of approximately 30 cm length and 10 cm diameter were collected using 35-cm long Perspex tubes. Sediment cores were sectioned in centimeter-sized slices. Each slice was homogenized before being transferred into pre-calcinated aluminum containers capped with aluminum foils. Sediment samples were transported in the laboratory and were dried at room temperature without storage step.

2.3. Extraction procedure

Sediment samples were air-dried at room-temperature (~20 °C) in a laminar hood, finely ground and sieved at 224 µm. 14 g of sieved sediment samples were spiked with deuterated internal standards (A-d_{10}, N-d_{10}, Per-d_{10}, Phe-d_{10}, Pyr-d_{10}) for PAHs and Me-PAHs analysis and with 1-eicosene for *n*-alkanes analysis. After a delay of equilibration, sediments were then extracted using an accelerated solvent extraction (ASE 200, Dionex Corp., USA). The extraction stages were preheat 0 min, heat 5 min, temperature, static solvent extraction time, pressure and solvent were presented in Table 1, purge 3 min, static cycle ($n = 2$), 60% flush. High purity nitrogen was employed as the purge gas. Extraction procedure affords a total volume of extract of 40 mL.

2.4. Choice of operating variables and their variation levels

Six main factors are known to influence significantly the efficiency of ASE extraction of organic pollutants from sediment matrix. Those are temperature, pressure, nature of the solvent, extraction time (Hubert et al., 2000), number of extraction cycles and volume of solvent used for rinsing. However, the number of

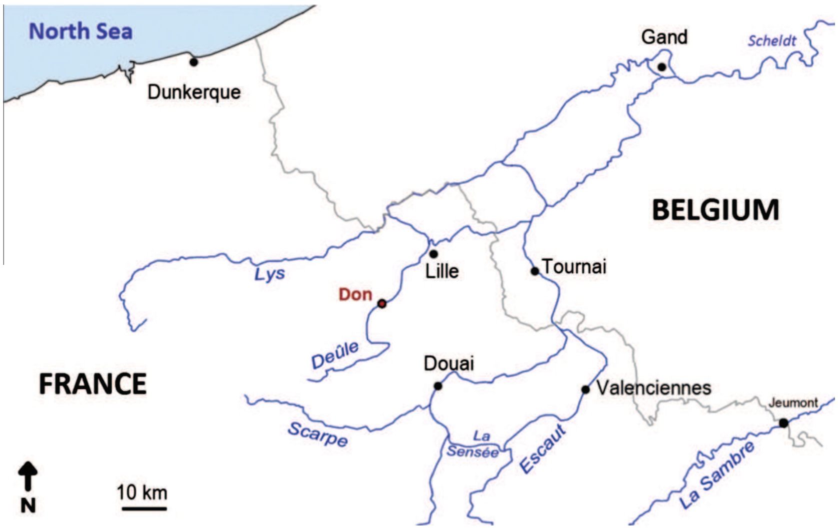

Fig. 1. Location of the sampling site (Don Station on the Deûle River).

Table 1
Summary of factors and their levels as coded and natural variables.

Variable	Factor	Level				
		−α	−1	0	+1	+α
*X*1	Temperature (°C)	40	70	100	130	160
*X*2	Pressure (MPa)	8	10	12	14	16
*X*3	Solvent (DCM/mixture[a]) (%)	0	25	50	75	100
*X*4	Static extraction time (min)	(2 × 3)	(2 × 6)	(2 × 9)	(2 × 12)	(2 × 15)

[a] Mixture corresponds to a mixture of hexane and acetone 1/1 *v*/*v*.

extraction cycles and volume of solvent used for rinsing were not considered as variable parameters in this study and they were fixed at 60% flush combined with two extraction cycles. Indeed, the volume of solvent for rinsing was previously optimized in our laboratory where preliminary tests showed that increasing number of extraction cycles ($n \geqslant 3$) did not provide significant improvement on extraction of targeted analytes comparing to 2 extraction cycles. Moreover, it was observed that clogging of ASE cell occurred more frequently using three extraction cycles. In this study, the temperature, pressure, nature of the solvent and static extraction time were tested for optimum conditions.

Elevated temperature is supposed to improve diffusion of organic compounds through the sediment and should also improve desorption and solubilization of analytes from the sediment during extraction procedures. In our case, ASE allows to perform extraction within a range from room temperature up to 200 °C, and at pressures ranging from 0.3 to 20 MPa. In addition, the nature of the solvent depends on several factors, one of them being the degree of contamination of the matrix (Berset et al., 1999). Relatively polar solvents or binary mixtures containing a polar solvent have been often used for multi-residues extraction. While DCM has frequently been employed (Gaspare et al., 2009; Smith et al., 2009; Mielke et al., 2001; Tronczynski et al., 2005), recent studies recommend the use of mixture such as hexane/acetone (Shu and Lai, 2001; Pena et al., 2007; Ruiz-Fernandez et al., 2012) or hexane/DCM (Boitsov et al., 2011). The selected variables in this study and their variation levels are summarized in Table 1.

With temperature ranging from 40 to 160 °C, pressure from 8 to 16 MPa and time of extraction from 6 to 30 min, five levels have been selected for this study. Variation of the solvent composition was permitted by varying the proportion of dichloromethane (DCM) in a hexane/acetone (1/1 *v*/*v*) mixture.

2.5. Experimental design

In order to obtain the optimal conditions for extraction PAH, Me-PAH and *n*-alkanes, a study was performed by using a face centered composite design (Box and Wilson, 1951; Goupy, 1978; Box, 1978). This experimental design was subjected to a second order multiple regression analysis to explain the behavior of the system using the least square regression methodology. Optimization was performed using superficial sediment from the Don Station (Deûle River).

This experimental design includes:

- A 2^k full factorial design where 2^k experiments are required to cover all possible combinations of factor levels (the low and high levels are coded $X_i = -1$ and $X_i = +1$ respectively).
- Axial points (or star points) that are placed on the axis of each factor in order to encircle the experimental domain where the star distance α between the axial points and the centre of the domain is generally given by: $\alpha = [n_f]^{0.25}$ (n_f is equal to 2^k in this study).
- Central points that are repeated and conducted for estimation of experimental error.

The relation between coded and natural variables is given as follows:

$$u_i = u_i^0 + \Delta u_i \cdot X_i \tag{1}$$

With u_i the real value, u_i^0 the real value at the center point, X_i the coded value and Δu_i the step change value.

The central composite experimental design is represented by a mathematical model obtained by multiple regressions and fitted with a second order polynomial function according to the following form where y is the predicted response:

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j + \varepsilon \tag{2}$$

Coefficients of the model β are determined by matrix algebra according to the relation:

$$\beta = (X^t X)^{-1} X^t \cdot y \tag{3}$$

X is the experiment matrix in coded variables; X^t is the transposed experiment matrix and $(X^t X)^{-1}$ is the reverse of the matrix product of X^t by X, y is the matrix of the answers.

β_0, β_i, β_{ii}, and β_{ij}, are the regression coefficients for intercept, linear, quadratic and interaction terms respectively. ε *represents* the experimental error.

Coded and real values of each variable as well as the response of each experiment are described on Table S3 in the Supplementary Information.

Results given on Table S3 were analyzed using the software Modde 5.0. Once, a Q^2 above 0.8 was reached, the model was assumed to be sufficiently to not rule out additional experiments. The number of experiments was always chosen greater than the number of factors in the mathematical model.

2.6. Purification and pre-separation

After the extraction with ASE, molecular sulfur was removed by addition of activated metallic copper (Blumer, 1997) to the extracts. Extracts were then purified and fractioned by liquid chromatography on a silica column to eliminate organic interferences (Jeanneau, 2007). Aliphatic hydrocarbons were recovered by elution with 20 mL of hexane (Fraction 1), and aromatic hydrocarbons (PAHs and Me-PAHs) were recovered by 15 mL of hexane/dichloromethane mixtures (3/1 *v*/*v*) followed by 10 mL of hexane/dichloromethane mixture (1/1 *v*/*v*) (Fraction 2). Each fraction was concentrated using a rotary evaporator followed by a slight stream of nitrogen before analysis.

2.7. Gas chromatography analyses

PAHs and Me-PAHs were analyzed using a Varian 3900 gas chromatograph equipped with a deactivated fused-silica guard column (5 m, 0.53 mm i.d.) and a fused-silica capillary Optima 5-MS Accent (60 m length, 0.25 mm i.d., 0.25 mm film thickness, Macherey–Nagel) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer. The carrier gas was helium held at a constant flow rate of 1 mL/min. Samples were injected in the splitless mode at 280 °C and the injector was purged with helium after 1 min. Temperature of the GC oven was programmed as follows: from 70 °C (1 min) to 170 °C at 10 °C/min, then to 230 °C at 4 °C/min, and then to 280 °C at 3 °C/min (10 min). The transfer line and the ion trap were respectively held at 260 °C and 220 °C. PAH and Me-PAH identification was done on the basis of the retention time and the mass spectrum from chromatograph of standard solutions acquired in full scan mode. Quantification was then performed in the single ion storage (SIS) mode for better selectivity. An example of chromatogram of PAHs and Me-PAHs in SIS mode was shown in Fig. S1 in the Supplementary Information.

Response factors were determined relative to the deuterated internal standards response and to standard mixtures. Deuterated standards were chosen in order to better fit to the properties of each group of PAHs. Limits of quantification (LOQ) were estimated as ten times the baseline of blank chromatograms and were confirmed by experimental analysis (Table S1).

Alkanes analyses were performed using a Trace GC Ultra (Thermo Fisher Scientific, USA) equipped with a flame ionization detector (FID) at 300 °C. Injection was performed on-column using a deactivated fused-silica guard column (5 m, 0.53 mm i.d.). Separation was permitted using a fused-silica capillary Rtx-5 SIL MS (60 m length, 0.25 mm i.d., 0.25 mm film thickness, Restek) with helium as carrier gas (1 mL/min). Temperature of the GC oven was programmed as follows: from 40 °C (1 min) to 110 °C at 30 °C/min, then to 320 °C at 6 °C/min (32 min). Identification and quantification of *n*-alkanes was done on the basis of the retention times of the calibration mixture containing (DRH hydrocarbon mixture, AccuStandard). An example of chromatogram of *n*-alkanes was shown in Fig. S2 in the Supplementary Information. Response factors were determined relative to 1-eicoseine as internal standard. Concentrations of individual hydrocarbons in each sample were reported in mg kg^{-1} of dry sediment. The individual concentrations of PAHs, Me-PAHs and *n*-alkanes were calculated to give the total concentration of each family for each experience. The later were then treated with the experimental design software to evaluate the influence of each parameter and their interactions.

3. Results and discussions

Analysis of results was performed using statistical and graphical analysis software (Modde 5.0, Umetrics, Sweden). This software was used for regression analysis of the data obtained and to estimate the coefficients of regression equation. ANOVA (analysis of variance), which is a statistical testing of the model in the form of linear term, squared term and interaction term were also used to test the significance of each term in the equation and the best R^2 is the fraction of the response variation explained by the model; Q^2 is the fraction of the response variation that can be predicted by the model. Therefore, R^2 is a coefficient of determination and is equal to (*SS-SSresidus*)/*SS*, and Q^2 is equal to (*SS-PRESS*)/*SS*, where *SS* is the sum of corrected *y* square for a mean and *PRESS* is the prediction residual sum of squares.

3.1. Analysis of the designed experiments

The effects and the interactions between each parameter are summarized on Fig. 2.

Our results indicate (Fig. 2) that high *T* (temperature) has a strong and positive influence on amount hydrocarbons extracted from sediments. This observation is in accordance with most of analytical studies using ASE where extraction efficiencies were observed to generally increase with elevated temperatures (Ramos et al., 2000; Richter et al., 2006). Moreover, interactions between *T* and other factors are generally considered as positive and sometimes as significant (*T* * *P* and *T* * *t*). In contrast, the combination of high temperature with the presence of DCM does have a negative effect, especially for Me-PAHs. Although DCM has been widely used as extraction solvent, it has also been found elsewhere that this solvent resulted in low recoveries for polycyclic aromatic hydrocarbons comparing to hexane/acetone mixtures (Shu and Lai, 2001; Pena et al., 2007). This was confirmed in our case, where main effects and interactions relative to DCM were found to be negative or negligible. Pressure (*P*) lightly influences extraction performances when it changes from its low value (10 MPa) to its high value (14 MPa). However, setting *P* at its high value (14 MPa) is preferable since a positive effect can be observed for the interaction factors associated with *P*, *T* and *t* (time). Even if pressure variations do not significantly affect the extraction efficiency, high pressure allows maintaining the solvent in a liquid state, thus limiting in the same time the possible evaporation of low molecular weight compounds. The last variable is extraction time (*t*), which has an overall positive effect on extraction.

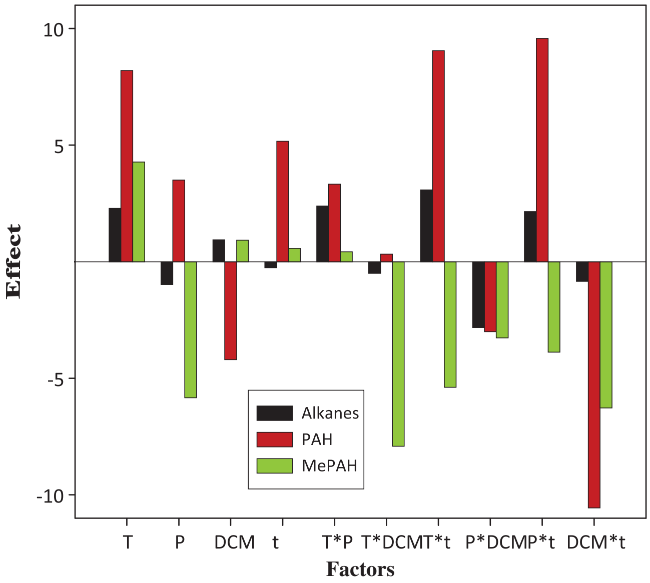

Fig. 2. Effects of different factors on the extraction of PAH, Me-PAH and *n*-alkanes.

Setting up the pressure parameter at high level (14 MPa) combined with a low solvent level (0% of DCM), study of response surface curves for each hydrocarbon family allowed us to define optimal time and temperature for common extraction.

3.1.1. Mathematical model

Mathematical model for each response was written from Eq. (2) part 2.5. The concentration of each family of hydrocarbons could be calculated as below:

$$\begin{aligned}\text{Concentration of PAHs} = {} & 108.04 + 4.1X_1 - 1.75X_2 - 2.10X_3 \\ & + 2.58X_4 - 1.89X_1{}^2 - 0.19X_2{}^2 \\ & + 3.22X_3{}^2 - 0.91X_4{}^2 + 1.66X_1X_2 \\ & + 0.16X_1X_3 + 4.53X_1X_4 - 1.5X_2X_3 \\ & + 4.79X_2X_4 - 5.28X_3X_4\end{aligned}$$

$$\begin{aligned}\text{Concentration of Me-PAHs} = {} & 68.06 + 2.13X_1 - 2.92X_2 + 0.46X_3 \\ & + 0.28X_4 + 0.13{X_1}^2 - 0.03{X_2}^2 \\ & + 9.03{X_3}^2 + 0.09{X_4}^2 + 0.21X_1X_2 \\ & - 3.96X_1X_3 - 2.69X_1X_4 - 1.63X_2X_3 \\ & - 1.94X_2X_4 - 3.14X_3X_4\end{aligned}$$

$$\begin{aligned}\text{Concentration of } n - \text{alkanes} = {} & 10.06 + 1.14X_1 - 0.49X_2 \\ & + 0.47X_3 - 0.13X_4 + 0.61{X_1}^2 \\ & - 0.07{X_2}^2 + 0.63{X_3}^2 - 0.73{X_4}^2 \\ & + 1.19X_1X_2 - 0.25X_1X_3 \\ & + 1.54X_1X_4 - 1.41X_2X_3 \\ & + 1.07X_2X_4 - 0.42X_3X_4\end{aligned}$$

Table S4 in the Supplementary Information is a summary of statistical values of R^2, R^2 Adj, Q^2 and RSD. Fig. 3 show the surface response curve determined from the mathematical model for PAHs, Me-PAHs and *n*-alkanes.

3.2. Determination of optimal conditions

According to the recoveries response surface curves of the three families of hydrocarbons, it can be seen that the temperature and the time of extraction offer better results when they are set up at their high levels (respectively 160 °C and 24 min), and combined with a low level of solvent (hexane/acetone 1/1 *v*/*v* without DCM) and a high pressure (14 MPa). A single experimental condition can also be implemented for a simultaneous optimized extraction of PAHs, Me-PAHs and *n*-alkanes. Factor levels were determined according to their effects and are presented in Table S5 in the Supplementary Information.

The optimal extraction conditions were preheat, 0 min; heat, 5 min; static solvent extraction time, 12 min ($n = 2$); purge 3 min, 60% flush. Extraction procedure affords a total volume of extract of 40 mL in a total time of 31 min.

3.3. Method validation

As presented earlier, the conditions leading to the best performance of extraction for these three families of hydrocarbons were 160 °C, 14 MPa, mixture of hexane/acetone (1/1 *v*/*v*) and for a period of 24 min. This method was validated for each family of hydrocarbons by comparing experimental concentration with values

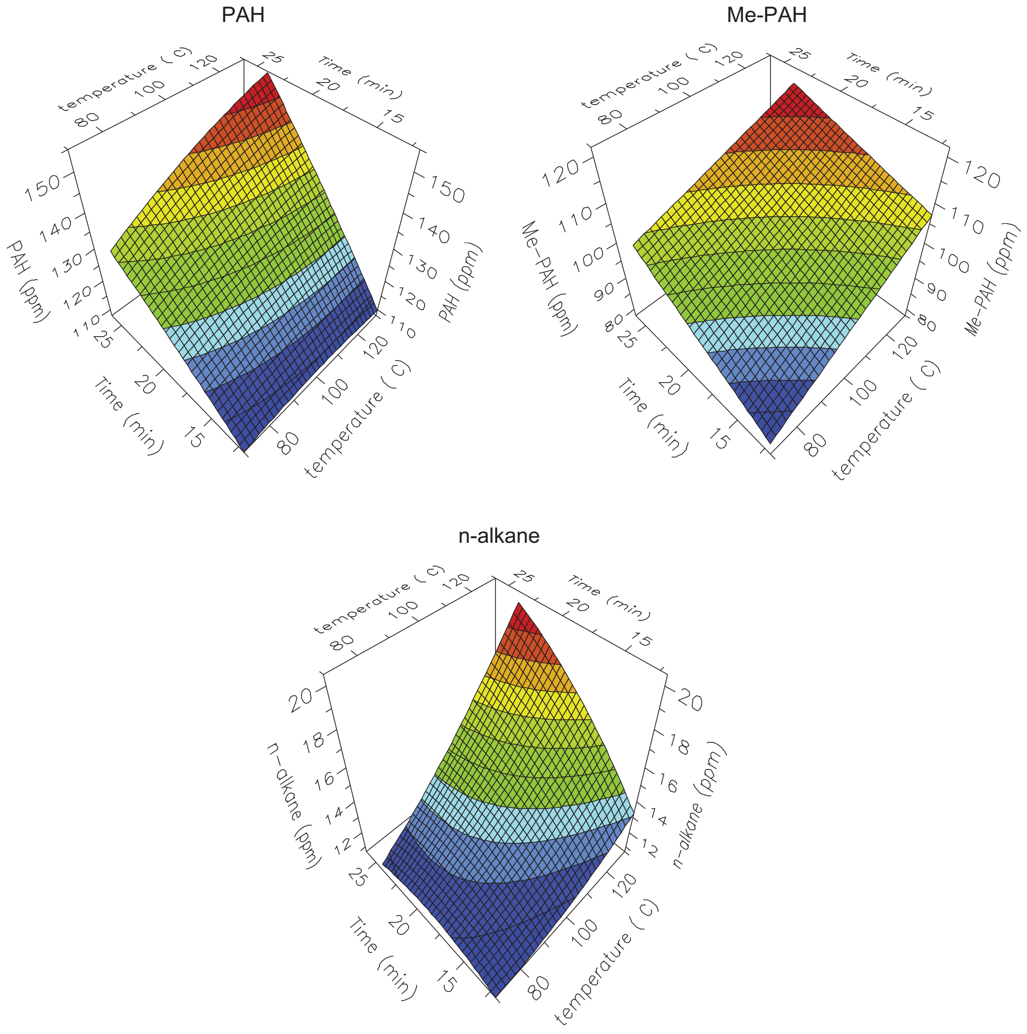

Fig. 3. Surface response curve determined from the mathematical model. The total PAHs and n-alkanes concentrations are plotted against time and temperature, with pressure and solvent (DCM) levels respectively set at high (14 MPa) and low (0% of DCM) values. Total concentrations of Me-PAH are plotted against time and temperature with pressure and solvent (DCM) levels respectively set at low (10 MPa) and low (0% of DCM) values.

Table 2
Comparison of the mathematical model with experimental extraction performances.

Hydrocarbon family	Mathematical predicted values $\sum$ concentrations (mg kg^{-1} dw)	Experimental values $\sum$ concentrations (mg kg^{-1} dw)
PAHs	155.6	151.7 ± 11.9
Me-PAHs	123.8	178.5 ± 8.8
n-Alkanes	26.1	16.5 ± 0.4

predicted by the mathematical models. Results are presented on Table 2.

As depicted on Table 2, predicted and experimental performances for the extraction of PAHs are comparable (151.7 vs. 155.6 mg kg^{-1} dw). Conversely, experimental concentrations for the extraction of Me-PAHs and *n*-alkanes are found to be respectively higher and lower than the predicted values. However, this can be explained by supposing that the extraction of these compounds is total and that the mathematical model can overrate concentrations since no maximum of contamination has been fixed.

The total static extraction time of 24 min was further validated by comparing extraction performances of the selected conditions (160 °C, 14 MPa, mixture of hexane/acetone (1/1 *v*/*v*), 2 × 12 min) with a shorter time of static extraction consisting in 2 cycles of 5 min. The result showed clearly that extraction yield for 2 × 12 min offers the better extraction yields (151.7 ± 11.9 and 178.5 ± 8.8 mg kg^{-1} dw respectively for PAHs and Me-PAHs) compared to 2 × 5 min (93.3 ± 8.3 and 142.7 ± 21.2 mg kg^{-1} dw respectively for PAHs and Me-PAHs). This extraction time is similar to the microwave technique and much faster than ultrasons (~60 min) and soxhlet (~24 h) technique. In addition, by its automation and its facility to implement (programmable for 24/24 h), the extraction condition selected is acceptable and can be a good agreement of extraction time and efficiency.

The accuracy of the analytical procedure for extraction of PAHs and Me-PAHs was verified in triplicate analyses using Certified Reference Material sediment (NIST SRM 1944) purchased from Promochem. For PAHs, average recovery of 119% was achieved, ranging from 79% (fluorene) to 134% (fluoranthene) with the exception for benzo[b]fluoranthene (195 %). Average recovery of 114% was achieved for Me-PAHs, ranging from 73% (1-methylnaphthalene) to 138% (1-methylpyrene) with the exception of 1-methylnaphthalene (180%) (Table 3).

3.4. Contamination levels of PAHs and Me-PAHs and source apportionment

The extraction procedure developed above was applied to assess the contamination levels of polycyclic aromatic hydrocarbons and to determine sources of pollution in the Deûle River. For this study, we focused on the 16 PAHs ($\sum_{16}$ PAHs) listed on the European Union priority pollutants lists as well as 17 methylated homologues ($\sum_{17}$ Me-PAHs) described on Table S1 in the Supplementary Information. Depth contamination levels were evaluated in a sediment core collected at the Don Station. The depth profile is presented on Fig. 4.

3.5. Composition profiles of polycyclic aromatic hydrocarbons and source apportionment

3.5.1. PAHs composition profile

According to Fig. 4, the total concentration of the 16 PAHs (Σ_{16}PAHs) varies significantly with depth and exhibits two subsurface maxima at −8 cm, and −26 cm close to 180 mg kg^{-1} dw Concentrations in PAHs are in the same order of magnitude comparing with values that have already been found in the sediments of the Deûle River. The high level of contamination can be explained by the strong historical inputs due to urban and industrial emissions in the watershed near the Don station. The Deûle River catchment has indeed a great numbers of current and former industrial and polluted sites combined with an extensive urbanization including parts of Lille and its suburbs. In terms of the compositional profiles (Fig. S3A in the Supplementary Information), higher proportions of PAHs species with three rings (19–50%) and four rings (31–70%) were observed.

For superficial sediment, it can be noticed a larger fraction of four-rings PAHs. Besides, no significant traces of six ring-membered PAHs were found in the studied sediment core.

3.5.2. Me-PAHs composition profile

Methylated PAH concentrations were also found to vary significantly with values ranging from 5 to 83 mg kg^{-1} dw with a

Table 3
Validation of the optimized method comparing to selected NIST SRM 1944 mass fraction values.

PAH	Certified Value (mg kg^{-1})	Experimental value (mg kg^{-1})	Recovery (%)	Me-PAH	Certified Value (mg kg^{-1})	Experimental Value (mg kg^{-1})	Recovery (%)
Naphtalene	1.28 ± 0.04	1.35 ± 0.03	105	1-Methyl naphthalene	0.47 ± 0.02	0.85 ± 0.07	180
Acenaphthene	0.39 ± 0.03	0.44 ± 0.01	113	2-Methyl naphthalene	0.74 ± 0.06	0.54 ± 0.06	73
Fluorene	0.48 ± 0.04	0.38 ± 0.01	79	1-Methyl phenanthrene	1.7 ± 0.1	2.11 ± 0.19	124
Phénanthrene	5.27 ± 0.22	5.43 ± 0.72	103	2-Methyl phenanthrene	1.90 ± 0.06	1.79 ± 0.16	94
Anthracene	1.13 ± 0.07	1.48 ± 0.30	131	9-Methyl phenanthrene	1.6 ± 0.2	1.22 ± 0.11	76
Fluoranthene	8.92 ± 0.32	11.96 ± 0.43	134	3-Methyl phenanthrene	2.1 ± 0.1	2.54 ± 0.23	121
Pyrene	9.70 ± 0.42	11.04 ± 0.60	114	2-Methyl anthracene	0.58 ± 0.04	0.65 ± 0.00	112
Chrysen	4.86 ± 0.10	5.26 ± 0.17	108	1,7-Dimethyl phenanthrene	0.62 ± 0.02	0.50 ± 0.03	80
Benz(a)anthracene	4.72 ± 0.11	6.16 ± 0.04	131	1-Methyl fluoranthene	0.39 ± 0.01	0.51 ± 0.05	130
benzo(b)fluoranthene	3.87 ± 0.42	7.54 ± 0.25	195	3-Methyl fluoranthene	0.56 ± 0.02	0.61 ± 0.02	109
Benzo(k)fluoranthene	2.30 ± 0.42	2.41 ± 0.04	105	1-Methyl pyrene	1.29 ± 0.03	1.79 ± 0.07	138
Benzo(a)pyrene	4.30 ± 0.13	4.57 ± 0.03	106	4-Methyl pyrene	1.44 ± 0.03	1.91 ± 0.06	133

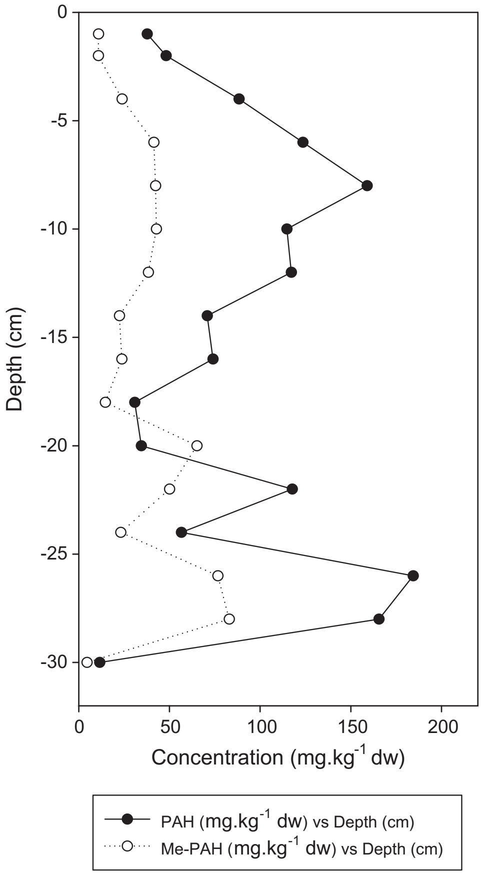

Fig. 4. Total concentration repartition of $\sum_{16}$ PAHs and $\sum_{17}$ Me-PAHs (mg kg^{-1} dw).

maximum occurring at -28 cm depth (Fig. 4). As depicted on Fig. S3B in the Supplementary Information, two-rings Me-PAHs were found to be predominant with proportions ranging from 43% to 66%. Correlations between alkylated and non-alkylated parent PAHs has been found with correlation coefficients (*r*) equal to 0.89, 0.62, 0.55 and 0.59 for naphthalene, phenanthrene, fluoranthene and pyrene respectively.

3.5.3. Source apportionment

PAHs The anthropogenic releases of PAHs can be attributed to petrogenic and pyrogenic origins. PAHs of petrogenic origins are usually characterized by the predominance of 2- and 3-rings PAHs, while a higher proportion of above 4-rings PAHs characterizes the PAHs from pyrogenic origins. The ratio of low molecular weight and high molecular weight (LMW/HMW) is commonly used to distinguish the petrogenic (LMW/HMW > 1) from pyrolytic origins (LMW/HMW < 1) (De Lucas et al., 2005). As depicted on Fig. 5 for the sediment core of the Deûle River, LMW/HMW ratio was found to vary significantly with depth with values ranging from 0.49 (at 2 cm depth) to 1.93 (at 8 cm depth) with a mean value of 1.06. Sources of pyrolytic contamination also seem to dominate surface sediment, whereas petrogenic inputs are suspected for depth below.

3.6. Relative potency of PAHs recorded concentration

The potential toxicological concerns of sedimentary PAHs levels were firstly evaluated by comparing the recorded concentrations in the Deûle River with the Canadian Sediment Quality Guidelines (SQGs) for protection of aquatic life, which range from 5.87 to 111 ng g^{-1} for 13 PAHs (Canadian Council of Ministers of the Environment, 2003). It was found in this studied case that all PAHs guidelines were exceeded whatever the depth of interest. The toxicological assessment of sedimentary PAHs was also performed by calculating dioxin-equivalent toxicity factors (TEQs) using the compilation of fish potency factors (FPFs) provided by Barron et al. (2004) and derived from CYP1A induction and AhR binding. PAH TEQ values of the Deûle sediment core were found to range from 211 to 4279 pg g^{-1} (with a mean value of 1943 pg.g^{-1}), exceeding the no observed effect concentration (NOEC) of 200 pg TEQ g^{-1}.

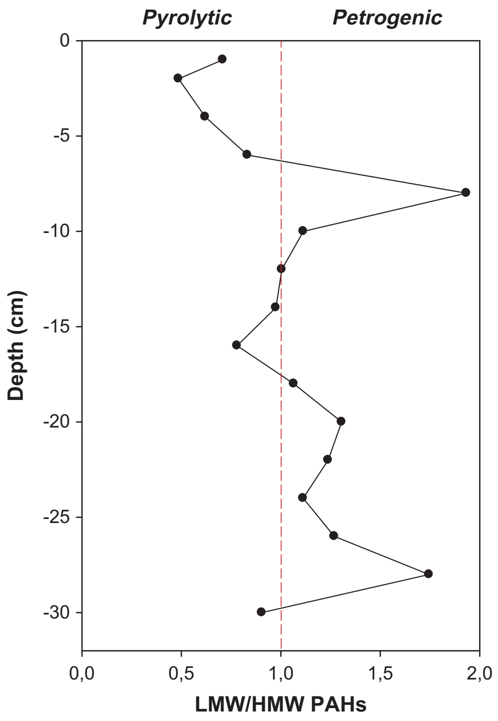

Fig. 5. Depth profile of the ratio of low molecular weight and high molecular weight (LMW/HMW) of PAHs in the sediment core.

4. Conclusion

The extraction of aromatic and aliphatic hydrocarbons from river sediment by ASE was optimized using an experimental design approach. Four parameters (temperature, pressure, solvent composition and time of extraction) were also optimized for a simultaneous extraction of PAHs, Me-PAHs and *n*-alkanes. Influence of each parameter was in accordance with previous related studies. The developed method was then applied to assess the contamination levels in polycyclic aromatic hydrocarbons of the Deûle River in northern France. Recorded concentrations (from 11 to 184 mg kg^{-1} dw for PAHs and from 4 to 82 mg kg^{-1} dw for Me-PAHs) show a high contamination level that is comparable to the ones recorded in the Espierre Canal (Charriau, 2009) and the Moselle River (Jeanneau et al., 2006). The Deûle River is also known to experience serious metallic contamination (Kadlecová et al., 2012; Lourino-Cabana et al., 2011; Boughriet et al., 2007). The high-recorded concentrations in aromatic hydrocarbons may be the result of former industrial and urban wastewater exhausts and of the high population density in this area. According to sediment quality guidelines, such concentrations might cause adverse effects to the aquatic biota. Using molecular ratios, the main source of contamination was combustion while petroleum inputs were suspected for the two recorded maxima of PAH concentration. This study thus provides useful information on the hydrocarbon contamination levels of the Deûle River that allows a quick access to Belgium *via* the Scheldt and the Lys River, and more recently *via* the Roubaix and Espierre Canals, for which navigation has been restored since 2011.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apgeochem.2013.11.009.

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Supplementary Information

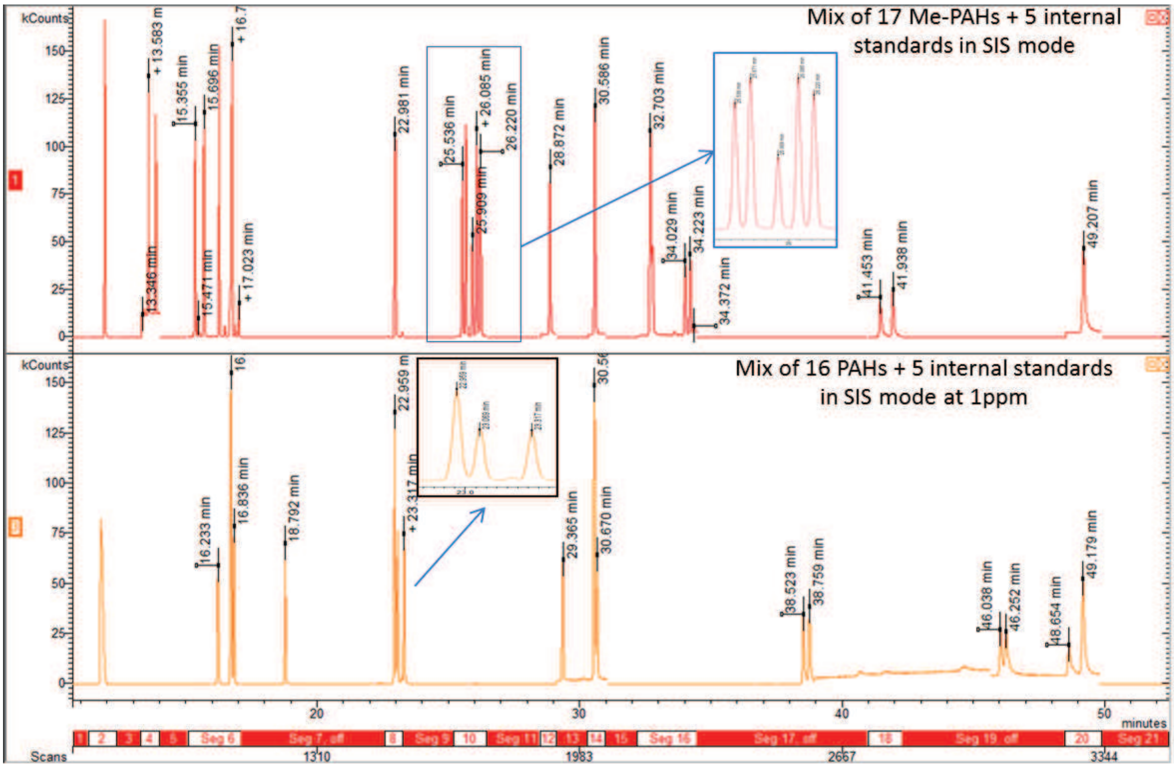

Figure S1: Chromatograms standard solution of Me-PAHs and PAHs in SIS mode

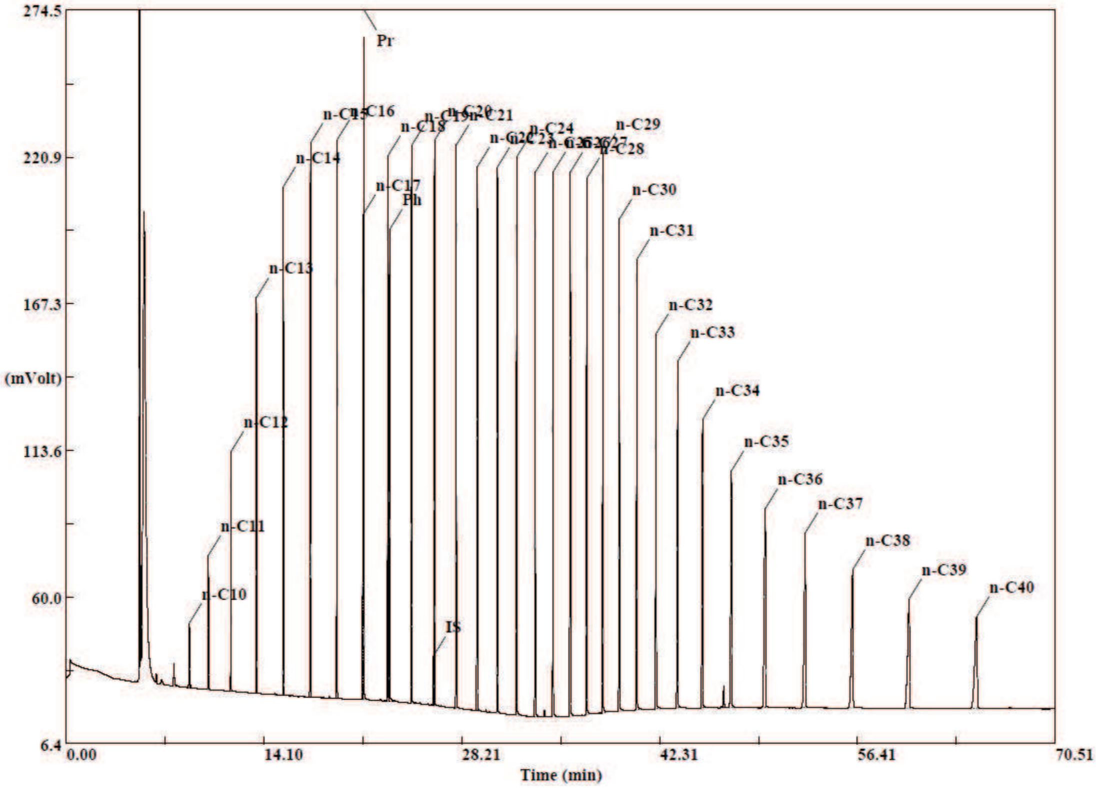

Figure S2: Chromatograms of standard solution of *n*-alkanes C_{10}-C_{40} at 50ppm

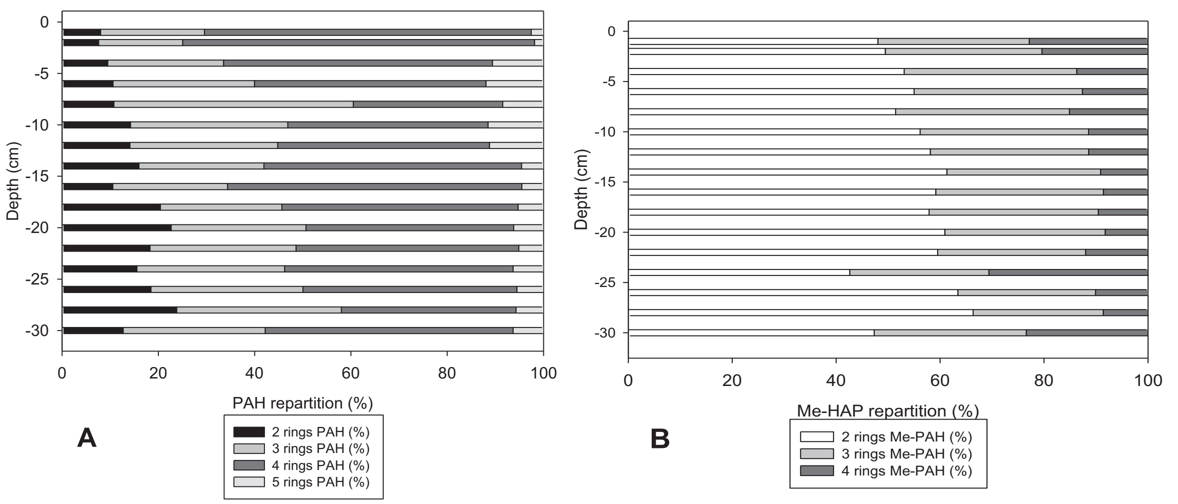

Figure S3: PAHs composition profile in the sediment core (Don Station, Deûle River) for (A) PAHs and (B) Me-PAHs

PAHs	Number of aromatic rings	Retention time (min)	Qualifier ions	Quantifying ions	Deuterated internal standards	LOQ (µg.kg^{-1} dw)
Naphtalene	2	11.93	128	128	N-d_8	0.3
Acenaphthylene	3	16.23	152	152	A-d_{10}	0.3
Acenaphthene		16.82	153	152, 153, 154		0.5
Fluorene		18.77	165	165, 166		0.5
Dibenzothiophene		22.43	184	184	Phe-d_{10}	0.5
Phenanthrene		23.08	178	178		0.3
Anthracene		23.30				0.5
4H-cyclopenta-[*d,e,f*]-phenanthrene		26.02	190	189 190	Pyr-d_{10}	0,3
Pyrene	4	29.32	202	202		0.5
Fluoranthene		30.63				1
Benzo[*c*]phenanthrene		37.11	228	226, 227, 228		0.5
Benzo[*g,h,i*]fluoranthene		37.14	226	227 226		1
Cyclopenta[*c,d*]pyrene		38.44	226			1
Benzo[*a*]anthracene		38.53	228	226 228 229		0.5
Chrysene		38.71	228			1
Triphenylene		38.77	228			0.5
Dibenzo[*a,h*]anthracene	5	41.68	278	278	Per-d_{12}	50
Benzo[*b*]fluoranthene		46.01	252	252 253		5
Benzo[*j*]fluoranthene		46.06				5
Benzo[*k*]fluoranthene		46.21				5
Benzo[*a*]fluoranthene		46.78				5
Benzo[*e*]pyrene		48.16				0.5
Benzo[*a*]pyrene		48.61				5
Perylene		49.34				5
Indeno[*1,2,3-c,d*]- pyrene	6	40.86	276	276		50
Benzo[*g,h,i*]perylene		44.72				5
Me-PAHs						
1-Methylnaphthalene	2	13.58	141	115 141 142	N-d_8	1
2-Methylnaphthalene		13.85				1
1,2-Dimethylnaphthalene		15.36	156	141 155 156		1
1,6-Dimethylnaphthalene		15.68				1
2,6-Dimethylnaphthalene		16.27				0.5
1-Methylphenanthrene	3	25.53	192	191 192	Phe-d_{10}	0.5
2-Methylphenanthrene		25.67				0.5
3-Methylphenanthrene		25.89				1
9-Methylphenanthrene		26.08				0.5
2-Methylanthracene		26.22				0.5
1,7-Dimethylphenanthrene		28.87	206	191, 206	Pyr-d_{10}	0.5
Retene		32.70	219	215, 316, 219, 234		0.5
1-Methylfluoranthene	4	32.78	215	215 216		1
3-Methylfluoranthene		34.02				1
1-Methylpyrene		34.22				1
3-Methylchrysene		41.45	242	241 242	Per-d_{12}	1
6-Methylchrysene		41.94				1

Table S1: Details on the individual PAHs and Me-PAHs studied.

***n*-alkanes**	**Formula**	**Retention time (min)**	**LOQ (µg.kg^{-1} dw)**
C_{12}	$C_{12}H_{26}$	11.73	500
C_{13}	$C_{13}H_{28}$	13.54	
C_{14}	$C_{14}H_{30}$	15.45	200
C_{15}	$C_{15}H_{32}$	17.38	
C_{16}	$C_{16}H_{34}$	19.28	
C_{17}	$C_{17}H_{36}$	21.12	100
C_{18}	$C_{18}H_{38}$	22.89	
C_{19}	$C_{19}H_{40}$	24.59	
C_{20}	$C_{20}H_{42}$	26.22	
C_{21}	$C_{21}H_{44}$	27.78	
C_{22}	$C_{22}H_{46}$	29.26	
C_{23}	$C_{23}H_{48}$	30.69	
C_{24}	$C_{24}H_{50}$	32.07	
C_{25}	$C_{25}H_{52}$	33.39	
C_{26}	$C_{26}H_{54}$	34.66	
C_{27}	$C_{27}H_{56}$	35.89	
C_{28}	$C_{28}H_{58}$	37.07	
C_{29}	$C_{29}H_{60}$	38.21	
C_{30}	$C_{30}H_{62}$	39.38	
C_{31}	$C_{31}H_{64}$	40.62	
C_{32}	$C_{32}H_{66}$	41.99	
C_{33}	$C_{33}H_{68}$	43.53	
C_{34}	$C_{34}H_{70}$	45.28	
C_{35}	$C_{35}H_{72}$	45.31	
C_{36}	$C_{36}H_{74}$	49.70	
C_{37}	$C_{37}H_{76}$	52.52	
C_{38}	$C_{38}H_{78}$	55.88	
C_{39}	$C_{39}H_{80}$	59.86	
C_{40}	$C_{40}H_{82}$	64.66	

Table S2: Details on the individual *n*-alkanes studied in this work

Experiment	**X1**	**X2**	**X3**	**X4**	**T (°C)**	**P (MPa)**	**Solvent (%DCM)**	**Time (min)**	**Y= extraction (mg.kg^{-1} dw)**		
									∑ PAH	**∑ Me-PAH**	**∑ *n*-alkanes**
1	-1	-1	-1	-1	70	10	25	12	104.73	63.44	11.87
2	+1	-1	-1	-1	130	10	25	12	101.66	82.13	8.35
3	-1	+1	-1	-1	70	14	25	12	98.51	63.54	8.29
4	+1	+1	-1	-1	130	14	25	12	102.30	80.99	10.72
5	-1	-1	+1	-1	70	10	75	12	114.75	82.05	9.74
6	+1	-1	+1	-1	130	10	75	12	111.74	83.57	12.60
7	-1	+1	+1	-1	70	14	75	12	103.55	76.45	12.43
8	+1	+1	+1	-1	130	14	75	12	103.81	78.96	9.55
9	-1	-1	-1	+1	70	10	25	24	104.73	78.93	5.91
10	+1	-1	-1	+1	130	10	25	24	116.65	86.37	10.16
11	-1	+1	-1	+1	70	14	25	24	102.66	72.97	8.34
12	+1	+1	-1	+1	130	14	25	24	114.38	79.67	16.25
13	-1	-1	+1	+1	70	10	75	24	113.33	87.44	10.00
14	+1	-1	+1	+1	130	10	75	24	106.37	76.72	12.51
15	-1	+1	+1	+1	70	14	75	24	97.61	70.45	8.90
16	+1	+1	+1	+1	130	14	75	24	120.78	88.09	12.63
17	$-\alpha$	0	0	0	40	12	50	18	93.16	66.18	10.21
18	$+\alpha$	0	0	0	160	12	50	18	108.14	73.16	14.90
19	0	$-\alpha$	0	0	100	8	50	18	103.74	73.14	10.76
20	0	$+\alpha$	0	0	100	16	50	18	111.16	64.91	8.88
21	0	0	$-\alpha$	0	100	12	0	18	125.89	105.42	11.74
22	0	0	$+\alpha$	0	100	12	100	18	116.26	72.34	9.59
23	0	0	0	$-\alpha$	100	12	50	6	101.32	69.17	6.96
24	0	0	0	$+\alpha$	100	12	50	30	107.82	69.81	7.39
25	0	0	0	0	100	12	50	18	93.69	69.06	10.97
26	0	0	0	0	100	12	50	18	105.70	72.98	7.60
27	0	0	0	0	100	12	50	18	107.63	76.97	10.61
28	0	0	0	0	100	12	50	18	96.11	64.73	9.91
29	0	0	0	0	100	12	50	18	105.96	65.46	8.73
30	0	0	0	0	100	12	50	18	112.88	99.43	8.30

Table S3: Coded and real value of each variable and concentration (ppm) of PAH, Me-PAH and *n*-alkanes.

Hydrocarbon family	**R^2**	**R^2 Adj.**	**Q^2**	**RSD**
PAHs	0.957	0.896	0.808	2.4043
Me-PAHs	0.964	0.919	0.828	2.6989
n-alkanes	0.963	0.904	0.802	0.7457

Table S4: Summary of statistical values where R^2 is the regression coefficient. R^2 Adj is the adjusted coefficient of regression and Q^2 represents the reliability of the mathematical model and RSD is the mean square residuals.

	T(°C)	**P (MPa)**	**Solvent (% DCM added)**	**Static extraction time (min)**
Coded levels	+ α	+1	-α	+1
Real values	160	14	0	24 (2 x 12)

Table S5: Summary of levels selected for the various factors studied.

II. Study case of organic pollution in water and sediments in France and Lebanon

Résumé

Les activités urbaines, industrielles et agricoles émettent constamment des micropolluants dans l'environnement. Une fois émis, les contaminants peuvent contaminer les milieux aquatiques à la fois l'eau de surface et l'eau sous terraine. Parmi ces contaminants, on trouve les hydrocarbures (HAP, Me-HAP), les PCB, les phtalates et les pesticides. Ils peuvent avoir des impacts directs et indirects sur la santé humaine, et les écosystèmes aquatiques.

L'évaluation des états écologiques des milieux et les études des impacts des contaminants organiques doivent passer d'abord par l'identification la nature et les niveaux de contaminations des polluants. Cette deuxième partie du chapitre 3 « Résultats et discussion » sont présentés les études cas des différents sites en France et au Liban. La nature de polluant, le niveau de contamination, l'origine et l'évaluation d'état des milieux aquatiques d'intérêt sont présentés sous forme de 4 articles publiés, acceptés ou soumis. L'article 3 concerne l'étude des niveaux de contamination par les pesticides organochlorés des eaux souterraines à Akkar, la deuxième région d'agriculture au Liban. L'article 4 porte sur l'étude de la contamination des eaux sous terraines à Akkar (Liban) par certains autres pesticides, les nitrates et les nitrites. L'article 5 concerne l'étude des niveaux de concentration des polluants organiques persistants (HAP, Me-HAP et les PCB) dans les sédiments de la région Nord-Pas-de Calais en France. Et le 6ème article présente l'étude de cas de la rivière Somme en Picardie en France. Dans cet article, la contamination des eaux de la Somme par les HAPs, Me-HAP, PCB, phtalates et les pesticides sera présentée.

Abstract

Urban, industrial and agricultural activities emit continually micropollutants in the environment. Once present in the environment, these undesired compounds can contaminate aquatic environments both surface and ground water. Among these contaminants, hydrocarbons (PAHs, Me- PAHs), PCBs, phthalates and pesticides are the most currently detected. They may have direct and indirect impacts on human health and aquatic ecosystems.

The assessment of ecological status of the environment and studies of impact related to organic contaminants, identification of the nature and levels of contamination of pollutants must be firstly evaluated. This second part of chapter 3 "Results and discussion" presents case studies of various contaminants obtained from different sites in France and in Lebanon. The nature of the pollutant, the level of contamination, origin and evaluating status of aquatic environments of interest are presented in form of four articles published, accepted or submitted numbered from 3 to 6. Article 3, concerns the study of contamination by organochlorine pesticides in groundwater of Akkar region which is the second agriculture region in Lebanon. Article 4, focuses on the study the contamination of ground water in Akkar (Lebanon) by some other pesticides, nitrates and nitrites. Article 5 concerns the study of the contamination by persistent organic pollutants (PAH, Me- PAHs and PCBs) in sediments obtained from three sites of Nord-Pas- de-Calais region in France. And article 6 presents the case study of the River Somme in Picardy region in France. In this article, the contamination of the Somme River by PAHs, Me-PAHs, PCBs, phthalates and pesticides will be presented.

- Article 3 -

SOLID PHASE EXTRACTION OF ORGANOCHLORINE PESTICIDES RESIDUES IN GROUNDWATER SAMPLES
(AKKAR PLAIN, NORTH LEBANON)

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Abstract

Due to the uncensored use of pesticides in the agricultural regions of Lebanon, the contamination risks of drinking water by organic residues increase periodically in planting seasons. No previous work have been considered in North Lebanon plain concerning pesticide pollution though it is the second agricultural zone in Lebanon with an excessive activity. In this context, the aim of this study was to evaluate the contamination and to map the pollution level of groundwater by organochlorine pesticides in Northern Lebanon. Solid-phase extraction (SPE) cartridge embedded with Hydrophilic-lipophilic-balanced (HLB) copolymer were used for the isolation and trace enrichment of pesticide from water samples followed by gas chromatography coupled with mass spectrometry (GC/MS) to quantify pesticide concentrations. The levels of organochlorine pesticide recorded in groundwater of Akkar district exceeded the limits set by the Stockholm Convention on persistent organic pollutants with total amounts that can reach 14.2 µg L^{-1}. Contamination was also found to be more important inland with the frequent presence of banned pesticide such HCH isomers, 4,4’-DDT, aldrin and endrin. Appropriate remedial measures and systematic investigation of Organochlorine residues in water resources of the AKKAR district are necessary to check further aggravation of the situation.

Keywords**:** SPE, HLB, GC-MS, organochlorine, Groundwater, Lebanon.

1. Introduction

Pesticides are commonly used in agriculture not only to protect crops from being harmed but also to increase crop production. Their usage often constitutes an essential part of massive crop production. Organochlorine pesticides cover a large group of compounds that have the tendency for long range transport and trans-boundary dispersion and may lead to contamination of surface and ground waters. In Lebanon, the groundwater constitutes an important source of freshwater, although it is highly used for agriculture purpose (61% of groundwater in Lebanon is used for irrigation include 26.66% in North Lebanon) (Ministry of energy and water 2010) in water and soil. Contamination by these compounds has spread all over the world and continues to be detected although it usage is prohibited in European countries (Laws, 2000). In general, intensive agriculture combined with factors enhancing leaching and hydrogeogical characteristics of the unsaturated zone may lead to increased levels of nutrients and pesticide in groundwater (Hancok et al., 2008). The effect of topography on the variation of pesticide concentration in groundwater due to focused recharge has also been examined (Dellin and Landon, 2002).

Organochlorine pesticides (OCPs) are chlorinated hydrocarbons that were extensively used from the 1940s through the 1970s for agricultural purposes and mosquito control. Representative compounds in this group include dichlorodiphenyltrichloroethane (DDT), aldrin, endrin and some isomers of hexachlorocyclohexane (HCH) such lindane. Due to their high persistence in the environment, low biodegradability and toxicological effects on human beings (Tanable et al., 1994, Barceló and Hennion 1997, Wania and Mackay 1999, Huen et al., 2012). Many Organochlorine pesticides were banned in developed countries (Fenster et al., 2006). However, even though the use of pesticide has been drastically reduced with settings of more rigorous regulations and innovative application methods. OCPs continue to be detected in natural waters (Mattice et al., 2010, Kaushik et al., 2010, Navarro et al., 2010, IMO, S.T. et al., 2007). In the European Union, water intended for human consumption must meet minimum specified requirements, including a maximum level of 0.1 μgL^{-1} for individual pesticide and a maximum of 0.5 μgL^{-1} for the sum of all pesticide, with an exception for aldrin, dieldrin, heptachlor and heptachlor epoxide, for which the limits have been set at are 0.03 μgL^{-1} (European Union, 1997). While most of the developed countries have already forbidden, the reality is that some developing countries continue to produce and to use OCPs for agricultural activities (Monorith et al., 2003). Remobilization of stock pesticide in soil, unsaturated zones as well as the time needed to renew the water of saturated zones have been identified as determinant factors explaining pesticide persistence in contaminated groundwater several years after the last application (Gutierrez and Baran, 2009). The water's apparent age (residence time) determined through tritium concentrations has been used to explain the spatial and temporal distribution of contaminants in groundwater (Tesoriero et al., 2007, Gourcy et al., 2009). In 2001, the Stockholm convention on persistent organic pollutants (POPs) underlined the necessity to control the global contamination produced by toxic chemicals including OCPs.

The Government of Lebanon signed the Stockholm Convention on May 2001, and ratified it in 2003 in favor of a global Non-Governmental organization (NGO) project called the International POPs Elimination Project (IPEP) in partnership with the United Nations Industrial Development Organization (UNIDO) and the United Nations Environment Program (UNEP) (Abu Jawdeh, 2006).

The aim of this study was to obtain information about the occurrence of organochlorine pesticide in groundwater in Akkar to understand one of the most pressing public health issues.

Facing rural areas in North Lebanon, especially where there are no available data concerning this site of study even though it's the second agricultural zone in Lebanon and that is known to be highly susceptible for water pollution (Halwani et al., 1999).

2. Materials and methods

2.1. Location and sampling

The study was focused on Northern Lebanon near Syrian borders (34.5506° N, 36.0781° E). Akkar district is an area of intensive agricultural uses that cover 788 km^2 characterized by the presence of a relatively large coastal plain, with high mountains to the east. As the second agricultural zone of Lebanon, the most widespread crops are cereals (wheat and corn), potatoes, grapes, fruit trees, olives and vegetables. Organochlorine pesticides are used in this zone without any control particularly at the Syrian borders where farmers are tempted to favor low cost and high efficiency on the back of ecological impacts. It has indeed been recently reported that underground water in this region is strongly contaminated by nitrates, nitrites and pesticide residues (Baroudi et al. 2012). For this study, the sampling network was limited to ten most vulnerable sites in term of frequency of usage according to an inquiry made with the agricultural in North Lebanon 2010. Samples were collected from wells in September 2012 (Table1). Geographical locations of the sampling sites according to the Middle East region and Lebanon are presented on Figure 1.

Sampling sites	Site names	Depth (m)	pH	GPS coordinates
1	Al Arida	65	6.5	34°37'60" N 35°58'60" E
2	Semmakieh	60	7.2	34°37'60" N 36°0'0" E
3	Al Knayseh	60	6.8	34°37'0" N 36°1'0" E
4	Massoudieh	60	6.5	34°60'55" N 36°04'90" E
5	Marlyat Hawara	65	6.4	34°56'06" N 36°02'39" E
6	Tall Mayan	70	6.8	34°59'80" N 36°03'78" E
7	Tall Abbas El Gharbi	70	6.5	34°34'60" N 36°4'0" E
8	Haret Al Jedideh	65	7.1	34°31'60" N 36°4'0" E
9	Qaabrine	65	6.9	34°57'28"N 36°02'95" E
10	Kobbet Al Chamra	60	7	34°53'90" N 35°99'41" E

Table 1: Sampling sites details and coordinates.

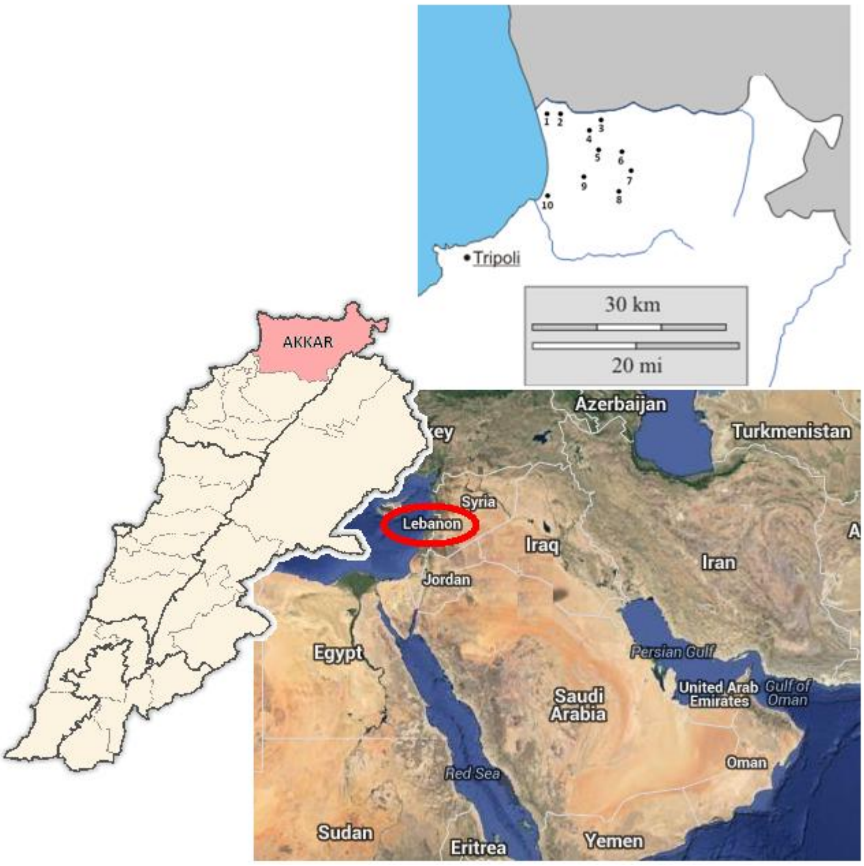


Fig. 1: View of Middle East region, Lebanon and AKKAR district with the sampling locations.

Triplicate samples were collected from every single site using 1.5 L clean amber glass bottles. Prior collection, groundwater was pumped for 10 minutes using the drilling pump installed for each well. Glass bottles were rinsed three times with the well's water before collection. Samples were immediately stored at 4°C in ice boxes for transport and were stored under refrigeration until analyzed (within 48 hours from sampling).

a. Chemicals and instrumentation

All solvents and chemicals were supplied from Dislab (France) and were HPLC or reagent-grade. No significant amount of analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 $M\Omega.cm^{-1}$ resistivity. Whatman GF/A circles filter (ø 110 mm, 0.7µm) were purchased from Whatman (England). Supel-Select HLB SPE cartridges (200 mg / 6 mL) were purchased from Sigma-Aldrich (Saint-Louis, USA). 1-bromo-2-nitrobenzene was purchased from Restek (Bellefonte, USA) and was used as internal standard. Organochlorine pesticide standards (Organochlorine Pesticide Mix AB #3 and Method 525.2 Revised Chlorinated Pesticide Mix #2) were also supplied by Restek (Bellefonte, USA). Detected pesticides and their general structures are detailed in table 2. Eight calibration solutions ranging from 5 $\mu g.L^{-1}$ to 5 $mg.L^{-1}$ were prepared from stock solutions using hexane as solvent and were stored at 4°C. Glassware was washed with detergent (Decon, King of Prussia, USA), rinsed with ultrapure water and acetone and was dried at 120°C prior to use.

Pesticides residues were analyzed by gas chromatography (Varian 3900) equipped with a deactivated fused-silica guard column (5 m x 0.53 mm i.d.) and a RTX-5 SIL MS fused-silica capillary column (60 m x 0.25 mm i.d. x 0.25 µm film thickness), and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS) operating either in selected ion storage (SIS) or in full scan (FS) mode. Helium was used as carrier gas at a constant flow rate of 1 $mL\ min^{-1}$. Injection was performed in the splitless mode at 280°C and the injector was purged with helium after 1 min. The temperature of the GC was programmed as follows: initial temperature 80°C (1 min), 10°$C.min^{-1}$ ramp to 170°C then 4°$C.min^{-1}$ ramp to 230°C and finally 3°$C.min^{-1}$ to 280°C and held for 2 min. The transfer line was help at 260°c. The ion trap mass spectrometer was held at 220°C with a scan time of 1 second/scan and emission current was at 10 uamps. Pesticide identification was completed on the basis of the retention time and the mass spectrum in full scan mode. Quantification was performed in the SIS mode using the most abundant ions; concentrations were determined relative to 1-bromo-2-nitrobenzene used as internal standard.

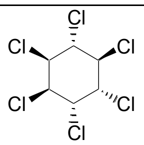
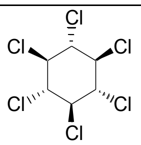
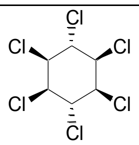
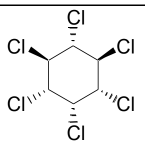
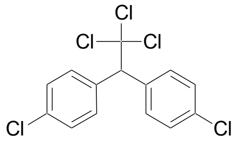
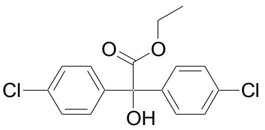
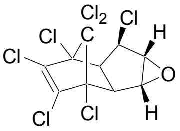
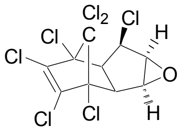
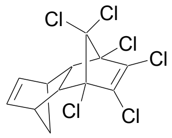
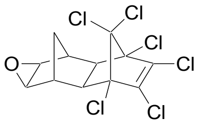
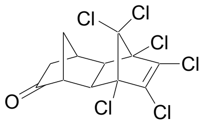
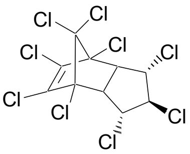
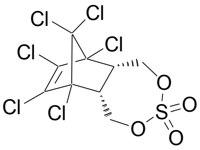
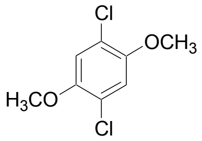
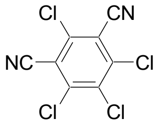
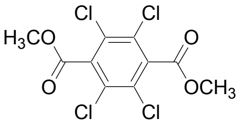
			
α-HCH	β-HCH	γ-HCH (Lindane)	δ-HCH
			
4,4'-DDT	Chlorobenzilate	Heptachlor epoxide (isomer A)	Heptachlor epoxide (isomer B)
			
Aldrin	Endrin	Endrin ketone	Transnonachlor
			
Endosulfan sulfate	Chloroneb	Chlorothalonil	DCPA methyl ester

Table 2: General structures of OCPs detected.

b. Analytical procedure

Water samples were filtered through a 0.7 µm particle retention glass filters, 750 mL of the filtered aqueous solutions were spiked with 25 µL of a 100 µg.L^{-1} solution of 1-bromo-nitrobenzene and were adjusted to pH 6 using hydrochloric acid (37%) and was extracted by solid phase extraction (SPE). Hydrophilic-lipophilic-balanced (HLB) copolymer cartridges were chosen because of their ability to extract a broad range of compound. Each cartridge was firstly conditioned with 5 mL of a MeOH/AcOEt (1/1 v/v) mixture followed by 5 mL of methanol and 10 mL of ultra pure water. Filtered sample was then passed through the cartridge at a flow-rate of (2-5 mL min^{-1}) by mean of a vacuum SPE manifold. Once the retention step had been completed, the cartridge was washed with 5 mL of ultra-pure water followed by 5 mL H_2O/MeOH (95/5 v/v), and then dried in a nitrogen current for 5 min. The SPE cartridges were wrapped in aluminum foils in order to protect them from contamination and were kept frozen until they were sent to France for analysis. Retained components were then eluted with 2 x 5 mL of a MeOH/AcOEt (1/1 v/v) mixture. The organic phase obtained was concentrated under reduced pressure at 45°C followed by a slight stream of nitrogen to fix the final volume to 500 µL before analysis. Prior application onto real water samples, this extraction protocol was validated using pesticide-spiked ultra-pure water. Recoveries of organochlorine pesticide (OCPs) were found to range from 53 to 132% with a mean value of 98 %.

The peaks observed for the standards are showed in Fig.2.

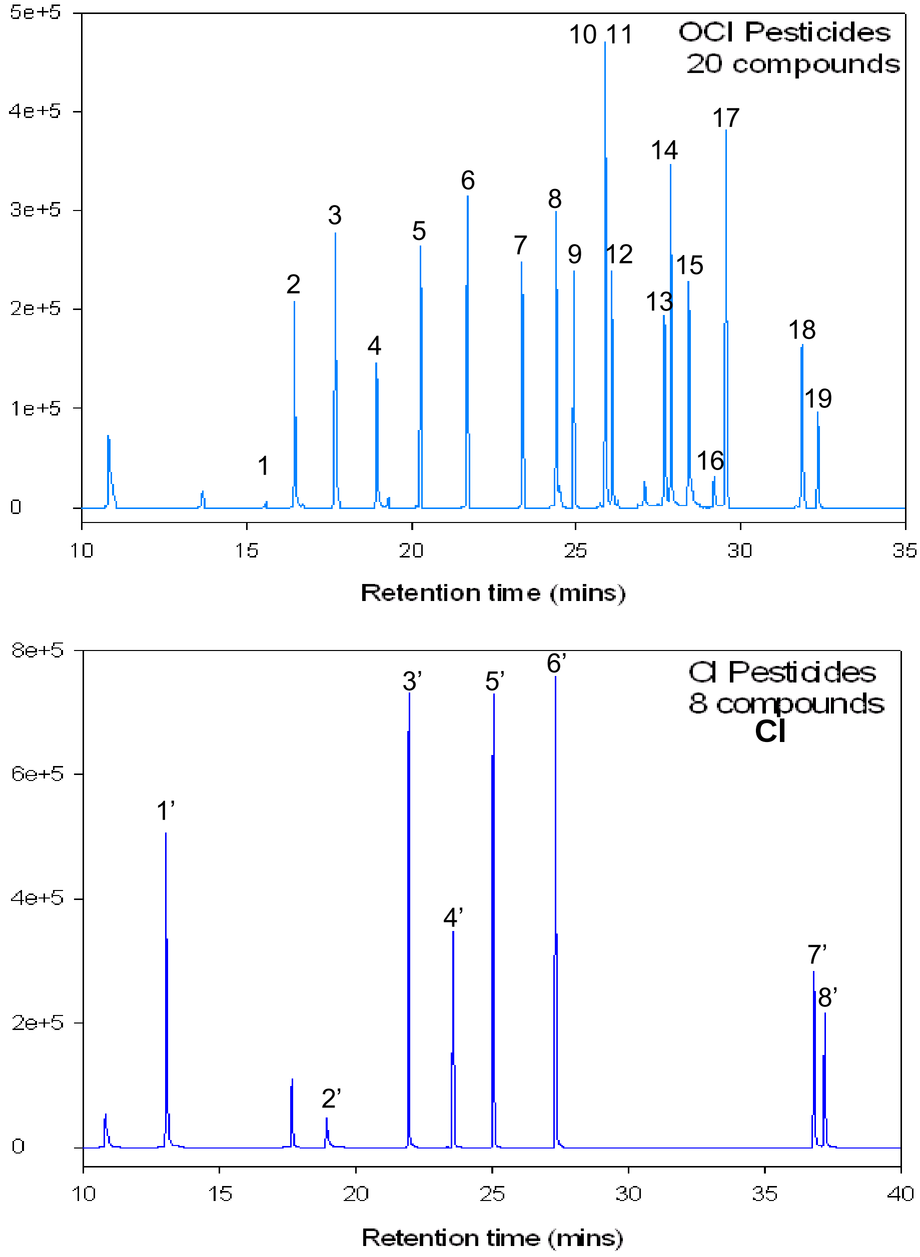


Fig. 2: Chromatograms standard solution of OCPs in SIS mode. A/ 1- alpha- lindane, 2- Beta BHC, 3- Gamma BHC, 4- Delta BHC, 5- Heptachlor, 6- Aldrin, 7- Heptachlor epoxide, 8- Cis-chlordane, 9- Trans-chlordane, 10- DDE, 11- Dieldrin, 12- Endrin, 13- Endosulfan I, 13- DDD, 14- Endrin aldehyde, 15- Endosulfan sulfate, 16- DDT, 17- Endrine ketone, 18- Metoxychlor.) and B/ 1'- Chloroneb, 2'- Chlorothalonil, 3'- DCPA, 4'- Heptachlor-epoxide, 5'- Trans-nonachlor, 6'- Chlorobenzilate, 7'- Cis-permethrin,8'- Trans-permethrin.

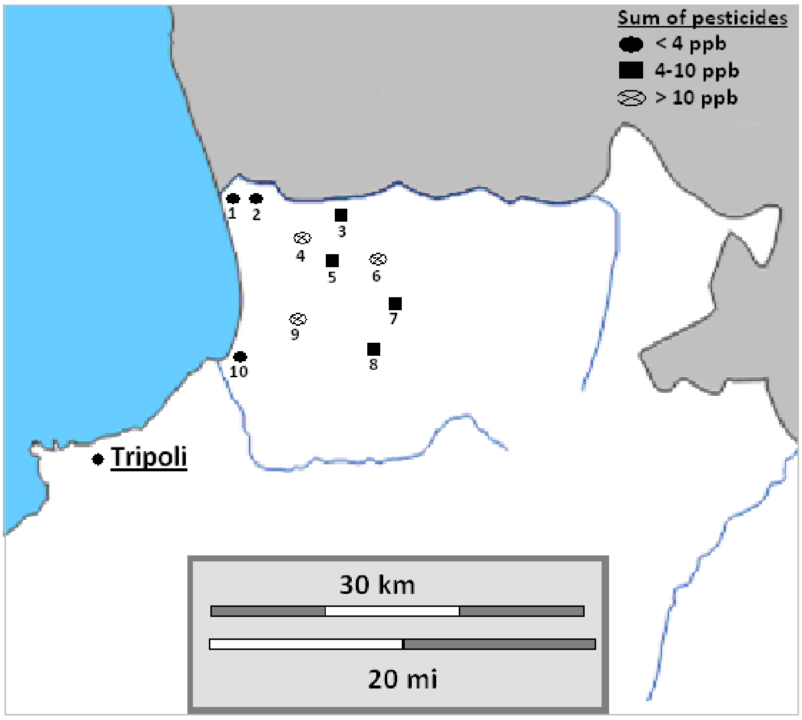


Fig. 3: Pollution level in the sampled sites in AKKAR region (sampling of September 2012).

3. Results and discussion

From a total of 28 OCPs (2 fungicides, 25 insecticides, 1 herbicide), 12 of them were not detected in any sample: *cis*- and *trans*-chlordane, 4, 4'-DDD and 4, 4'-DDE, heptachlor, dieldrin, endrin aldehyde, endosulfan I and II, methoxychlor, *cis*- and *trans*-permethrin. Our results showed the high levels of some organochlorine pesticides in the ten groundwater samples of the Akkar region. Recorded levels were found to be important with the sum exceeding the European Commission legislation in every sampling site (Fig.3). The most frequent pesticide residues were the isomer forms of hexachlorocyclohexane (HCH), chlorobenzilate, aldrin and heptachlor epoxide. The less abundant were DCPA methyl ester and chlorothalonil. Most contaminated sites were found inland (sites 4, 6, 9) whereas groundwater sampled near of the coast showed significant lower concentrations of OCPs (sites 1 and 10). Detailed occurrence of pesticide concentrations for each site is summarized in table 3. The concentrations obtained in this work are compared to other sites in the world (Table 4).

	Abundant ions	**LOD**	**site 1**	**site 2**	**site 3**	**site 4**	**site 5**	**site 6**	**site 7**	**site 8**	**site 9**	**site 10**
Compounds		**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**	**[µg.L^{-1}]**
α-HCH	**181;219**	**0,06**	N.D.	N.D.	N.D.	N.D.	N.D.	6,42	N.D.	2,85	5,33	N.D.
β-HCH	**181;219**	**0,005**	N.D.	N.D.	N.D.	N.D.	N.D.	0,57	N.D.	N.D.	0,45	N.D.
γ-HCH (Lindane)	**181;219**	**0,06**	N.D.	0,21	0,69	0,18	0,77	0,41	0,17	0,54	0,72	N.D.
δ-HCH	**181;219**	**0,06**	N.D.	1,17	0,25	N.D.	1,35	0,46	0,23	0,27	0,61	0,23
4,4'-DDT	**165;235**	**0,06**	N.D.	0,26	0,19	N.D.	0,69	0,23	0,25	0,21	0,35	0,31
Chlorobenzilate	**409;411**	**0,005**	0,18	0,38	0,14	6,09	1,14	0,61	0,17	0,31	1,05	1,04
Heptachlor	**237;272**	**0,005**	0,11	0,05	0,08	0,07	0,09	0,07	N.D.	0,12	0,05	N.D.
Heptachlor	**237;272**	**0,005**	0,87	0,76	0,13	0,93	1,33	0,88	1,61	0,15	0,13	N.D.
Aldrin	**220;263;293**	**0,005**	0,42	0,34	0,15	0,16	0,72	0,28	0,19	0,29	0,29	N.D.
Endrin	**263; 281**	**0,06**	1,11	N.D.	2,46	2,47	1,44	1,37	1,73	2,28	N.D.	N.D.
Endrin ketone	**281;317**	**0,06**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2,15	N.D.	N.D.
Transnonachlor	**289;291**	**0,005**	N.D.	0,04	N.D.	N.D.	0,13	N.D.	N.D.	N.D.	0,15	N.D.
Endosulfan sulfate	**229,272**	**0,06**	1,23	0,61	0,39	1,57	1,51	1,33	1,03	N.D.	0,24	N.D.
Chloroneb	**191;209**	**0,06**	N.D.	0,13	0,11	2,75	0,55	0,17	0,13	0,14	2,63	0,28
Chlorothalonil	**266**	**0,06**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0,33	N.D.	N.D.	0,33
DCPA methyl ester	**302;335**	**0,005**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0,03	0,04	0,04	N.D.

Table 3: Concentration of Organochlorine pesticide detected in groundwater samples of the Akkar district. (N.D.: Not detected)

	Chloroneb	4.4-DDT	Heptachlor (isomer A)	Endrin	α HCHc	β HCH	γ HCH	δ HCH	Aldrin	Heptachlor (isomer B)	References
Syria					10.6 – 13.6		20.2 – 53.7				Hajjar and Mouna (2011)
Gaza		0.3 - 1	0.4	0.1							Shomar et al. (2006)
Saudi Arabia	114										El-Saeid et al. (2011)
Thailand			1.369								Hudak and Thapinta (2005)
China		0.0049			0.0052	0,0025	0.0057	0.0006			Yang et al. (2013)
Guam Island	0.02-3.4		0.01-0.02	0.01-0.30					0.01	0.01-0.05	Denton and Sian-Denton (2010)
Norway		0.05									Haarstad and Ludvigsen (2007)
India			0.0124	0.0046	0.383	0.744	0.247	2.813	1.35	0.291	Singh et al. (2005)
Lebanon (Akkar)	0.06-2.75	0.06-0.69	0.005-0.12	0.06-2.47	0.06-6.42	0.005-0.57	0.06-0.77	0.06-1.35	0.005-0.720	0.005-1.330	This work

Table 4: Comparison of 9 OCPs pesticide concentrations in groundwater of Lebanon with others sites (concentrations in µg L^{-}

3.1. α-, β-, γ- and δ-HCH

From the ten drilled wells that were subject to sampling, only one site (Al Arida, site 1) did not contain any isomers of HCH. Highest concentrations were found in samples of Tall Mayan (site 6), Qaabrine (site 9) and Haret Al Jedideh (site 8) with sums of HCH concentrations respectively equal to 7.86, 7.11 and 3.66 µg.L^{-1}. γ-HCH (lindane) and δ-HCH were the most common isomers with 80% of occurrence in the sampled sites and with concentrations respectively ranging from 0.17 to 0.77 µg.L^{-1} and from 0.23 to 1.35 µg.L^{-1}. The α- and β- isomer forms were less frequently detected (in respectively 3 and 2 sites) but with high concentrations in the range of respectively 2.85-6.42 µg.L^{-1} and 0.45-0.57 µg.L^{-1}. The above levels of HCH isomers are lower than those that have been recorded in 2005 in Syrian groundwater samples with concentrations ranging from 20.2 to 53.7 µg.L^{-1} and from 10.6 to 136 µg.L^{-1} for γ-HCH and δ-HCH respectively (Hajjar and Mouna, 2011). However, such levels of HCHs are cause of concern since these compounds are not only persistent in the environment, but are also highly toxic and can bioaccumulate in food chain (ATSDR, 2005).

3.2. 4,4'-DDT and chlorobenzilate

4,4'-DDT residues were detected in all sampling site excepting at Al Arida (site 1) and Massoudieh (site 4). Other samples showed concentrations in the range from 0.19 to 0.69 µg.L^{-1}, all exceeding the European Commission limit set at 0.1 µg.L^{-1}. No significant traces of degradation products (4,4'-DDE and 4,4'-DDD) was found in studied samples. These levels of contamination are comparable with those recorded in groundwater samples of the Gaza Strip that varied from 0.3 to 1 µg.L^{-1} (Shomar et al., 2006) and in Norway groundwater 0.5 µg.L^{-1} (Haarstad and Ludvigsen, 2007).

Chlorobenzilate was found to be present in all wells with levels ranging from 0.14 µg.L^{-1} at Al Knayseh (site 3) to 6.09 µg.L^{-1} at Massoudieh (site 4). Although structurally similar to DDT, chlorobenzilate is much more subject to biodegradation (Neilson, 1995). Recorded levels of groundwater contamination by chlorobenzilate tend to show that this compound is still used in the Akkar plain.

3.3. ***Heptachlor epoxide (isomers A and B)***

Heptachlor epoxide, which exists in two isomeric forms endo-heptachlor epoxide (isomer A) and exo-heptachlor epoxide (isomer B), is a transformation product of heptachlor used as insecticide. Heptachlor epoxide has been reported to be of greater toxicological significance because it is more stable and persists longer in the environment, especially isomer (ATSDR, 1993). This three compounds are listed in the Stockholm Convention are classified as possible human carcinogens (Stockholm convention, 2004). Herein, no detectable amount of heptachlor was found, but heptachlor epoxide isomers were present in almost all samples except Kobbet Al Chamra (site 10). Concentrations of isomer B (0.13 - 1.61 µg.L^{-1}) were higher than those of isomer A (0.05 - 0.12 µg.L^{-1}). Such levels largely exceed the limits set at 0.03 µg.L^{-1} and are comparable to polluted ground waters in Thailand where concentrations reach 1.369 µg.L^{-1} (Hudak and Thapinta, 2005).

3.4. Aldrin, endrin and endrin ketone

Only well of Kobbet Al Chamra (site 10) did not show detectable traces of aldrin. For the other sites, concentration was found to range from 0.15 to 0.72 µg.L^{-1}. These high residues levels of aldrin are comparable to those recorded in alluvial groundwater aquifers of Gangetic plains in India, where like in Akkar, potato is one of the major crops grown in the area (Singh et al., 2005).

Endrin is known to have a high potential to leach to groundwater (Montgomery 2010). High levels of endrin were indeed recorded for 70% of sampled wells with concentrations ranging from 1.11 to 2.47 µg.L^{-1}. By contrast, endrin ketone was only once detected at a concentration of 2.15 µg.L^{-1} in Haret Al Jedideh (site 8). The high concentrations in aldrin and endrin ketone suggest that these compounds are still used in the Akkar district. Kobbet Al Chamra (site 10) is the only area where no significant trace of such compounds could be detected.

3.5. trans-Nonachlor and endosulfan sulphate

Trans-nonachlor is not only one of the major constituents of the insecticide chlordane, but it is also the most bioaccumulative of this family of compounds and is also considered as potent carcinogen (Luzardo et al., 2014). *trans*-nonachlor residues were detected in three sites (Semmakieh, Marlyat Hawara and Qaabrine) with concentrations respectively equal to 0.04, 0.13 and 0.15 µg.L^{-1}.

Groundwater samples were found to be more contaminated by endosulfan sulfate, which was detected in 70 % of wells at levels in the range 0.24 - 1.57 µg.L^{-1}. No detectable amounts of parent endosulfan could be found that may suggest that recorded level of endosulfan sulfate is the result of former use of endosulfan in the Akkar plain.

3.6. Chloroneb, chlorothalonil and DCPA methyl ester

Chloroneb and chlorothalonil are two fongicides still used since they are more recent and less toxic than the above-mentioned organochlorine pesticide. Al Arida (site 1) is the only site where no residue of such compounds has been detected. Chloroneb was indeed found to be present in 90 % of the sampled sites with concentrations ranging from 0.11 to 2.75 $\mu g.L^{-1}$. This contamination is lower than the one reported recently in Saudi Arabia groundwater where concentrations can reach 114 $\mu g.L^{-1}$ (El-Saeid et al., 2011) Chlorothalonil was only detected in Tall Abbas El Gharbi (site 7) and Kobbet Al Chamra (site 10) with both levels of 0.33 $\mu g.L^{-1}$.

DCPA methyl ester is an herbicide active ingredient mostly used to control crab grasses and a number of fruit and vegetable crops. This compound was found in sites 7, 8 and 9 (Tall Abbas El Gharbi, Haret Al Jedideh, Qaabrine) at more acceptable levels in the range 0.03 – 0.04 $\mu g.L^{-1}$.

4. Conclusion

The present study demonstrates that agricultural practices in the Akkar district have degraded the water quality and significant pesticide pollution was found in groundwater. Sixteen residues of pesticide have frequently been detected in the groundwater with values exceeding the level limits for drinking water according to the European Union. Coastal groundwater samples (from Al Arida, site 1 and Kobbet Al Chamra, site 10) were found to be less contaminated than groundwater sampled inland where pollution can reach certain scary levels especially in sites Massoudieh, Tall Mayan and Qaabrine. Since the sampling was conducted in September 2012, we highly assume that pesticides found in groundwater were not only brought by irrigation but also by rain and rivers of other close areas. Moreover, the frequent high levels recorded for HCH isomers, 4,4’-DDT, aldrin and eldrin may suggest that such compounds are still in use despite the implication of Lebanon in the International POPs Elimination Project (IPEP).

However the ignorance of farmers about the safe use of pesticide is generated from literacy and poverty; in another hand, factors such as soil characteristics, intensive application and the occurrence of point source contamination in the studied area reflects its relative frequent detection in this study.

Finally it must be pointed out that our research was constrained to only few sampling locations in Akkar. More detailed monitoring studies have to be carried out on different seasons to get a clear reference data for the entire location. Besides this work could be the database for future work that must be taken into account regarding this zone especially with the lack of information regarding Akkar plain.

We recommend continuous monitoring in shallow groundwater and wells near agricultural fields on yearly basis that would provide to prevent contamination of groundwater in general and a better understanding of each individual pesticide variability and persistence in groundwater as well as some of the important metabolites.

Research is needed to investigate the type, quantity and concentration of pesticide used by farmers in order to increase awareness.

Epidemiological studies investigating the health effects of pesticide should be undertaken. Furthermore, policies aimed to reduce the potential contaminations of water by pesticide need to be developed and implemented. The pesticide most frequently occurring should be given a second opinion by the authorities whether they should stay sell.

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- Article 4 -

Determination of pesticides, Nitrates and Nitrites level in Groundwater of Akkar plain in Northen Lebanon

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ORIGINAL ARTICLES

Determination of pesticides, Nitrates and Nitrites level in Groundwater of Akkar plain in Northen Lebanon

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ABSTRACT

To estimate the environmental risk posed by the use of pesticides is a major problem, which involves the identification, characterization, quantification and knowledge of the fate of these compounds in the environment. Their impact is directly related to their concentration and toxicity, control of reliable analytical methods is a necessity. The relative risk of contamination of drinking water by organic residues increased from time to time, in this context, the objective of this work is to build a general idea as an initiative for a further study to evaluate the quality of groundwater in Akkar region.

The water quality is evaluated by studying:
- The level of chemical pollution presented by the concentrations of nitrate, nitrite, ammonium and ortho-phosphate ions by colorimetric methods.
- The level of micropollution by measuring the concentration of residues of pesticides G.C./E.C.D. and L.C./U.V.-visible.

The level of disastrous pollution is reflected by the analysis of nitrate, nitrite and pesticide residues in water, used for direct human consumption without prior treatment, to reduce the risk accompanying pollution especially for sites Zennad Sheikh (the amount of pesticides 29.75 p.p.b., the concentrations of nitrate and nitrite are respectively 145.76 mg / L and 1.86 mg / L), Tall Bibi (the amount of pesticides 13.3 p.p.b., the concentrations of nitrate and nitrite are respectively 135.17 mg / L and 0.45 mg / L), and Har*et al* Jdideh (the amount of pesticides 12.36 p.p.b., the concentrations of nitrate and nitrite are respectively 99.88 mg / L and 0.71 mg / L). There are sites in the study area that show amazing results in lack of proper monitoring national and local awareness of the risks associated with the intensive use of fertilizers and pesticides. For all sites the chemical analyzes of water for ammonium meet the standards value and contain a concentration of less than 0.5 mg / L and non worrying values for ortho phosphate. The results of this study increase the need and importance of surveillance and control of pesticide residues and other pollutants to be carried out continuously and more specialized.

Key words: Groundwater, Contamination, Pesticides, Nitrates, Nitrites.

Introduction

The contamination of surface water and groundwater by chemical pollutants and micropollutants is a recurring problem faced by managers of water system. (Bouman *et al*., 2002; Levet *et al*., 2008). However in the absence of alternative control method applicable to large scale quickly, the use of chemicals in agriculture is expected to continue for several more decades.

The agriculture irrigates the environmental compartments by pesticides (insecticides, herbicides and fungicides ...) and chemical nutrients (nitrates, nitrites, ammonium, phosphates ...). All these products are used extensively for the highest yields, without regard to the surrounding environment or considering the impact of this heavy use on human health and ecological effects (Levario-Carillo *et al*., 2004; Meyer *et al*., 2003; Viel *et al*., 1998).

The absence of data on the level of pesticides and nutrients in northern Lebanon groundwater guide us to take in hand the problem of groundwater pollution in the Akkar plain, which is the second agricultural region of the country. This aquifer characterized by high vulnerability to pollution (Halawani *et al*. 1999; Hatoum, 2007) is the only water resource for consumption and irrigation of the most residences in Akkar.

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Materials and Methods

The sampling strategy adopted in the context of this work was based on a spatial coverage of an entire agricultural system during the month of May 2011, Nine sampling sites on two areas (coastal and interior) were selected taking into account some cultures consuming pesticides and the influence of the position of cultivated areas from wells. Their locations are shown on the map (Figure 1) (1:Al Arida, 2:Sheikh Zennab, 3:Kob*et al* Chamra, 4: Har*et al* Jedideh, 5: Qaabrine, 6: Al Knayseh, 7: Massaoudieh, 8: Tall Bibi, 9: Semmakiyeh).

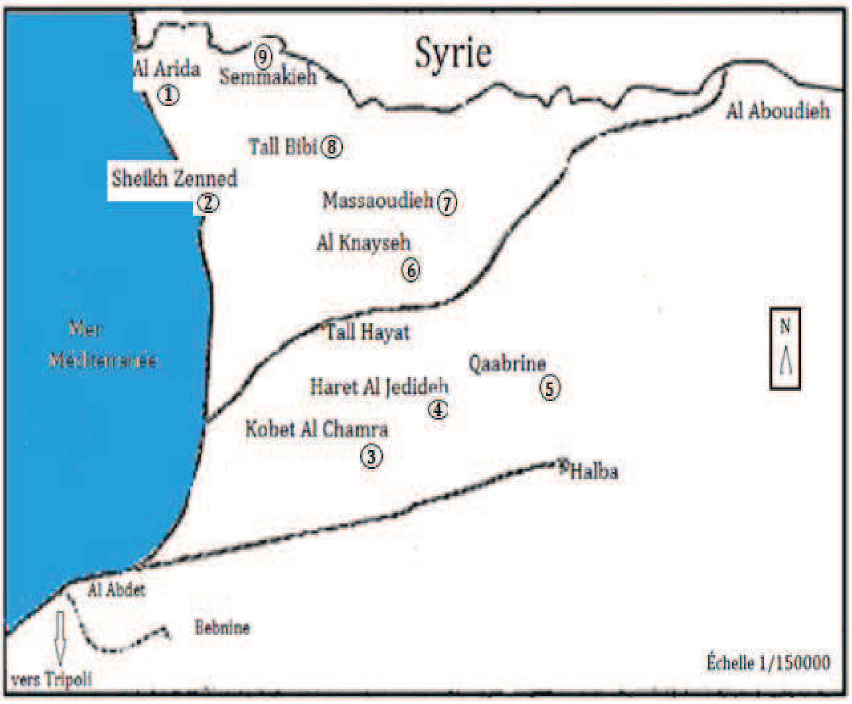


Fig. 1: Location of the sampling sites at Akkar plain

The analysis of nitrate and nitrite were carried out with reference to AFNOR methods 1990.

The analysis of pesticide residues is resumed by the following steps:

1- The Liquid-liquid extraction (Abe *et al*., 2010).

By mixing and stirring 1.0 L of water with 50 ml of dichloromethane for 15 minutes and 5 minutes decantation, obtain the extract residue (organic phase) is obtained. Three successive liquid-liquid extractions are performed; the extracts are combined and evaporated using a rotary evaporator by adding acetonitrile to obtain about 10 ml final volume after evaporation in order to ensure the absence of dichloromethane. The next stage of evaporation is done under nitrogen atmosphere to reach a final volume of 1 ml and use it for injection. The recovery rate is determined using the method of positive and negative control; it is above 70% for all target compounds (Akerbolm, 1995).

2- Identification and Quantification

Depending on the type of pesticide, we used:

- The gas chromatography Agilent technology type (Tranchant *et al*., 1995) equipped with an electron capture detector characterized by capillary chromatographic column with a length of 30 m, a diameter of 0.25 mm and 0.25 micrometer thickness. The carrier gas was high purity nitrogen, flow 1mL/min and the injection is carried out in splitless mode with an injection volume of 1 µl. Its initial temperature is 230 ° C and the detector is 300 °C.

-The liquid chromatography Agilent Technologies coupled to a UV/visible detector and characterized by C18 column 100 mm long, 3 micrometers inner diameter, mobile phase was an acetonitrile / water (ratio v / v respectively 70% / 30%) at a rate of 0.1 ml/mn and an injection volume 5µl, the wavelength is set at 230 nm as a value average (Palma *et al*., 2004; Khim-Heang and Corvi, 1998).

Results and Discussion

Study of chemical contamination by nitrates and nitrites.

Regarding the results of this study, it was find that the nitrate content for all samples are higher than the allowable limit of water for human consumption (50mg/L), two of nine samples only meet the standard value of 0.1 mg / L nitrite. The high concentrations observed, especially for sites 1. 2, 4 and 8, have double exceeded the nitrate and nitrite standards, and this can be explained by the intensive use of fertilizers and the decreased ability of soil degradation (Figure 2).

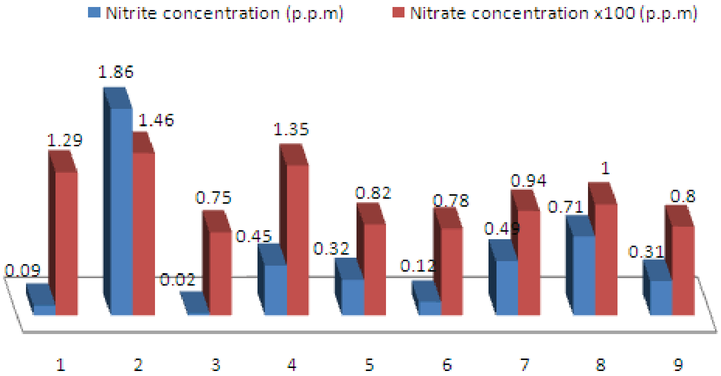


Fig. 2: The concentrations of nitrate and nitrite in each studied site

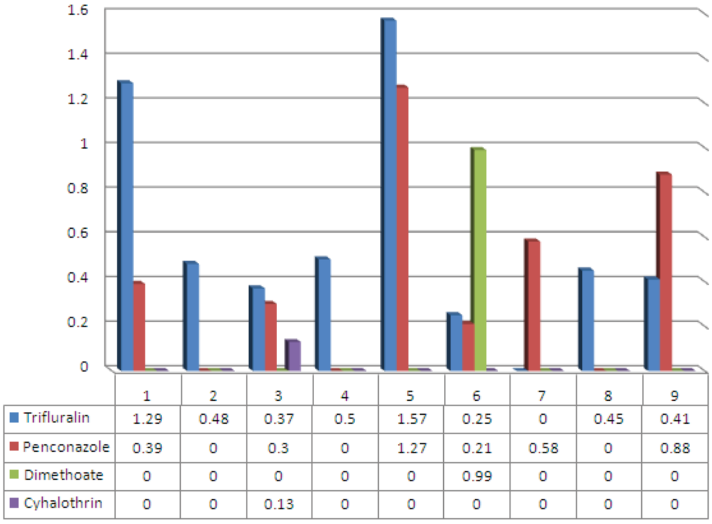
Study in pesticide residue contamination:

For each sample's site, it was tried to quantify the 11 pesticides identified using the two chromatographic methods, however, it must be noted that these 11 pesticides do not summarize all pesticides used in the study area. The analysis of samples shows that approximately all sites are affected by three types of pesticides at least and this confirms that those areas we are faced with the risk of pesticide toxicity.

The Directive 80/778/EEC of 15th July 1980 on the quality of water intended for human consumption set for pesticides, a maximum allowable concentration of 0.1 p.p.b. For each substance and a value of 0.5 p.p.b. for total substances.

For the pesticides studied by G.C./E.C.D. we summarize that:

- The trifluralin is the most popular and its highest concentration is detected for the site number 5 (1.57 p.p.b.).
- The highest concentration of penconazole was observed for site 5 (1.27 p.p.b.).
- The presence of dimethoate for the site 6 with a concentration 0.99 p.p.b.
- The-cyhalothrin comes with an acceptable concentration 0.13 p.p.b. for site 3 (figure 3).



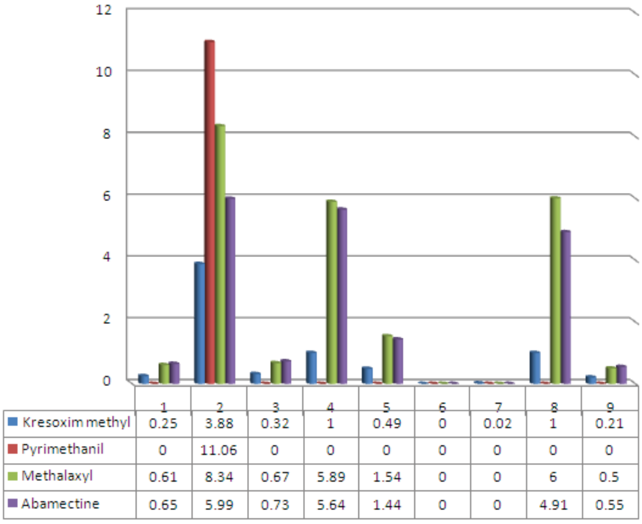
	1	2	3	4	5	6	7	8	9
🟦 Trifluralin	1.29	0.48	0.37	0.5	1.57	0.25	0	0.45	0.41
🟥 Penconazole	0.39	0	0.3	0	1.27	0.21	0.58	0	0.88
🟩 Dimethoate	0	0	0	0	0	0.99	0	0	0
🟪 Cyhalothrin	0	0	0.13	0	0	0	0	0	0

Fig. 3: The concentrations of the pesticides detected by G.C. in each studied site (p.p.b.)

As regards pesticides studied by H.P.L.C.-U.V./visible:

-The presence of kresoxim-methyl is in almost all sites (except site 6) with concentrations between 0.02 and 3.88 p.p.b., the latter is observed for site 2.

- A value alarming pyrimethanil for site 2 (11.06 p.p.b.).
- High levels of abamectin, méthalaxyl are observed especially for sites 2, 4 and 8 (Figure 4).



	1	2	3	4	5	6	7	8	9
🟦 Kresoxim methyl	0.25	3.88	0.32	1	0.49	0	0.02	1	0.21
🟥 Pyrimethanil	0	11.06	0	0	0	0	0	0	0
🟩 Methalaxyl	0.61	8.34	0.67	5.89	1.54	0	0	6	0.5
🟪 Abamectine	0.65	5.99	0.73	5.64	1.44	0	0	4.91	0.55

Fig. 4: The concentrations of pesticides detected by L.C. in each studied site (p.p.b.)

These results are alarming with regard to the recommendations on the quality of water intended for human consumption especially for sites 2, 4 and 8.

By grouping the pesticides detected at each site by type, it was observed that fungicides and insecticides are the most common pesticides detected and this can be explained by the nature of the most widespread crop in the region of Akkar, which is potato.

Conclusion:

By comparing the amount of pesticides detected in each site and the concentrations of nitrate and nitrite, there is some correlations between the two types of chemical pollution (nitrate and nitrite) and micropollutants (in pesticides), especially the sites 2, 4, 8 for which it was observed the highest concentrations in the three pollutants.

In this study, the results show the presence of chemical contamination (mineral and organic) in almost all sites, which may meet certain levels certainly scary especially sites 2, 4 and 8.

During this study, it was found sometimes a situation of widespread contamination with the simultaneous presence of several pesticides on the same site and exceeded the standard for pesticides, nitrate and nitrite in drinking water. This type of observation is one of the main issues raised in the interpretation of analytical results for the cumulative effects of long-term all of these products on health are still poorly understood.

The results of our chemical analysis show that the groundwater is not necessarily good, in contrast to the traditional belief which assumes that the ground water rid of contaminants during its infiltration into the groundwater. The power of retention by the soil micro is linked to the chemical nature of the latter, the soil type and depth of groundwater. The reality is that the ground delays the groundwater contamination but does not eliminate it!

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- Article 5 -

Overview of persistent organic pollution in sediments from Northern France: Study case

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Abstract

PCBs, parent and alkyl-PAHs have been quantified in sediments collected from three canalized rivers (Deûle, Sensée and Scarpe) all located in a highly industrialized zone of the Nord Pas-de-Calais region, Northern France. Quantification using GC-MS allowed to determinate the dispersion trend, the origin as well as the relative potency of the studied sediments. Contamination depth profiles of PCBs, parents and alkyl PAHs have been studied for the three sediment cores. Total concentrations of PCBs (Σ_{28}PCBs) have revealed a higher contamination level for the Scarpe River (ranging from 126.8 to 194.4 µg kg^{-1} dw) by comparison with the Sensée River (from 15.1 to 34.0 µg kg^{-1} dw) and the Deûle River (from *n.d.* to 15.6 µg kg^{-1} dw). Sedimentary depth profiles of total PAHs (Σ_{16}PAHs) and alkyl-PAHs (Σ_{18}Me-PAHs) suggest a significant recent contamination of these three studied sites according to the high concentrations recorded in the surface of sediment cores (up to 33.7 mg kg^{-1} dw for the Scarpe River). The possible sources of PCBs have been identified through a principal component analysis, while the pyrolytic origins of PAHs have been determined using the molecular indexes. The Scarpe River reveals to be the most polluted according to the consensus-based sediment quality guidelines.

Keywords: PAHs, Me-PAHs, PCBs, GC-MS, sediment quality guidelines, source apportionment.

1. Introduction

Organic compounds discharged into aquatic environment can bring negative impacts on aquatic ecosystem by direct and indirect toxic effects on organisms (Fleeger et al., 2003). Actually, organic contaminants are a major environmental cause for concern due to their ubiquitous, their persistence, long-range transportability and their potentially adverse effects on living organisms. Moreover, most of organic contaminants are fat-soluble and can lead to bioaccumulation, thus affecting not only aquatic ecosystems but also human health via drinking water resources and food chain. River water acts as receiving water for various kinds of organic contaminants from municipal and industrial wastewaters (Malve et al., 2003; Singh et al., 2004; Zhang et al., 2004). For the ecosystem protection and to keep water resources clean, it is important to identify the nature of contaminants, their contamination levels as well as their sources.

Organic contaminants are a group of chemicals that have been intentionally or inadvertently produced and introduced into the environment. Polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs and Me-PAHs) belong to the class of persistent organic pollutants (POPs). PCBs have been commonly used as dielectric fluids or transformers and capacitors, and also in paints, inks and pesticides until the hazard posed to both the environment and human health by their use became evident. They are extremely stable compounds under environmental conditions (WHO, 1993). Due to their toxicity, chronic persistence and bioaccumulation, they have been banned or restricted, and some of them have been included in the list of priority pollutants in many countries. However, PCBs remain present in water and sediment (Smith et al., 2009; Dumoulin et al., 2013) and continue to affect aquatic organisms all along the food chain and consequently human health through the diet (Sun et al., 2002). Dissolved PCBs in water only represent a small fraction of total PCBs due to their hydrophobic character, which causes their rapid association to organic entities such as sediments, algae and protozoa (Eganhouse et al., 1991; Hargrave et al., 1992; Brannon et al., 1993; Booij et al., 1994). PAHs and Me-PAHs can be originated from the incomplete combustion of wood, coal, industries and vehicle emissions (Yunker et al., 2002; Wang et al., 2007). They can also come from seepage of crude oil and coal or oil spills. Hydrocarbons are highly lipophilic compounds, ubiquitous in the water column of coastal, estuarine and river, as well as in sediments in which they tend to accumulate (Ko, 1995; Chiou, 1998; Manodori, 2006; Cailleaud, 2007; Gaspare, 2009; Yunker, 2012; Net et al., 2014). Recent studies showed that marine organisms are prone to bioaccumulation of these substances, especially in lipid-rich tissues (Neff, 2002; Francioni, 2005; Dugan, 2005). Because of their toxic, carcinogenic and mutagenic effects (Straif, 2005; IARC, 2010) sixteen PAHs have been listed as priority pollutants by the U.S. EPA.

The aim of the present study was to investigate the concentration levels and the sources of these organic contaminants in order to assess quality of river sediment from three sites in the Nord Pas-de-Calais region, Northern France. Samples were analyzed for 28 PCBs, 16 PAHs and 18 Me-PAHs using gas chromatography/mass spectrometry (GC/MS).

2. Materials et methods

2.1 Reagents

Sediments samples were analyzed for 16 PAHs, 18 alkylated polycyclic aromatic hydrocarbons (Me-PAHs) and 28 PCBs including 12 dioxin-like PCBs (PCB-DL) and the 7 indicators PCB (PCBi). Mixed standard solutions of PAHs and Me-PAHs were purchased from Restek Corp (Bellefonte, PA, USA). PCBs standard solution was obtained from Accustandard Inc. (New Haven, CT, USA). Tetrachloronaphtalene (TCN), 2,3,3’,5,6-tetrachlorobiphenyl (PCB112) and octachloronaphtalene (OCN), used for PCB quantification, were purchased from Dr Ehrenstorfer (Augsburg, Germany) Deuterated internal standards for PAHs and Me-PAHs (acenaphthene-*d*10 (A-*d*10), naphtalene-*d*8 (N-*d*10), perylene-*d*12 (Per-*d*12), phenanthrene-*d*10 (Phe-*d*10) and pyrene-*d*10 (Pyr-*d*10)) were provided by LGC-Promochem (Middlesex, UK). HPLC-grade solvents (hexane, dichloromethane, methanol and acetone) were purchased from Dislab (France). No significant amount of targeted analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70-230 mesh ASTM) activated at 450°C was heated at 120°C for 12h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK), rinsed with ultrapure water and acetone and finally dried at 120°C prior to use.

2.2 Sampling Sites

The three sampling sites selected for this work are located in a 15-km zone in the “Nord Pas-de-Calais region” (France) near Douai city (Figure 1). This heavily industrialized area is already studied by our group due to its severe metallic pollution (Boughriet et al., 2007;

Lesven et al., 2009; Kadlecová, M. et al., 2012; Prygiel et al., 2013; Superville et al., 2014). However, beside data provided by the French Water agencies in connection with the Ministry for Sustainable Development, bibliographic data concerning organic contamination in this area are still lacking.

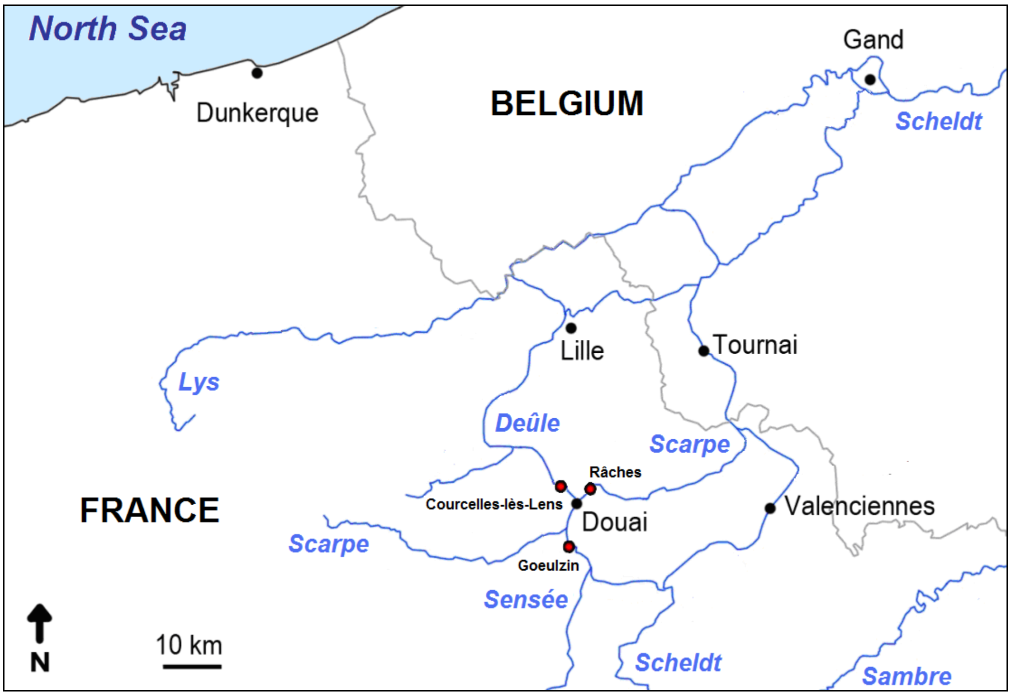


Figure 1: Location of the three study sites

Deûle River at Courcelles-lès-Lens

The Deûle, a tributary of the Lys River, is a 60-km long river beginning at Souchez (Pas-de-Calais, France). As a wide-gauge canal, the Deûle River is highly frequented by commercial barges. The sampling site was chosen at Courcelles-lès-Lens near a former smelter (Metaleurop), which was the third largest nonferrous smelter in the world during the first half of the 20th century. Nowadays, this industrial site has been replaced by a recycling and waste treatment center. Nevertheless, smelting activities are still continuing in this area according to the presence of two metallurgical factories (Nyrstar and Umicore) located at about 4 km downstream. Although contamination of the Deûle River by trace metals is well documented, bibliographic data concerning organic contamination in this area remain scanty (Net et al., 2014).

Scarpe River at Râches

The Scarpe is a 102-km long river flowing from Berles-Monchel (Pas-de-Calais, France) to Mortagne -du-Nord, (Nord, France) where it flows into the Scheldt. Mostly canalized and characterized by a low flow, this river is under the influence of many effluents and various industrial and urban emissions, which widely affect sediment chemistry (Isaure et al., 2002; Alary et al., 2010, 2011). This medium-gauge canal is no longer navigated.

Sensée canal at Gœulzin

Sensée canal has been created to link the canal du Nord to the Deûle and the Scheldt. This area presents similar navigation traffic as the Deûle River according to the data provided by Voies Navigables de France, a public institution in charge of inland waterways. Contrary to the two previous sites, the Sensée canal is less affected by metallurgical activities since this area is dedicated to agriculture (Prygiel et al., 2013).

2.3 Sampling

The sampling campaign was conducted in early spring 2012, on 22/03/2012, 06/04/2012 and 12/04/2012 respectively for the Scarpe, Deûle and Sensée rivers. Sediments cores of approximately 10 cm length and 10 cm diameter were collected using 35-cm long polycarbonate tubes. Sediment cores were sliced in centimeter-sized slices immediately after sampling. Each slice was homogenized before being transferred into pre-calcinated aluminum containers capped with aluminum foils. Sediment samples were transported in the laboratory and were dried at room temperature in a laminar hood without storage step.

2.4. Targeted analytes

In this work, the following PCBs, PAHs and Me-PAHs were analyzed:

PCBs No.(28 PCBs) : 8, 18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209.

PAHs (16 PAHs) : naphthalene (N), acenaphtylene (Ayl), acenaphtene (A), fluorene (F), anthracene (Ant), fluoranthene (Fl), benz*[a]*anthracene (BaA), chrysene (Ch), benz*[a]*pyrene (BaP), phenanthrene (Phe), benzo*[b]*fluoranthene (BbF), benzo*[k]*fluoranthene (BkF), benzo*[ghi]*perylene (Bghi), dibenzo*[a,h]*anthracene (DhA), indeno*[1,2,3-cd]*pyrene (IP), pyrene (Pyr).

Me-PAHs (18 Me-PAHs) : 1-methylnaphthalene (1M-N), 2-methylnaphthalene (2M-N), 1,2-dimethylnaphthalene (1,2DM-N), 1,6-dimethylnaphthalene (1,6DM-N), 2,6-dimethylnaphtalene (2,6DM-N), 1-methylphenanthrene (1M-Phe), 2-methylphenanthrene (2M-Phe), 3-methylphenanthrene (3M-Phe), 9-methylphenanthrene (9M-Phe), 2-methylanthracene (2M-An), 1,7-dimethylphenanthrene (1,7-DMP), retene, 1-methylfluoranthene (1M-Fl), 3-methylfluoranthene (3M-Fl), 1-methylpyrene (1M-Pyr), 4-methylpyrene (4M-Pyr), 3-methylchrysene (3M-Ch), 6-methylchrysene (6M-Ch).

2.5. ASE extraction

Extraction steps were performed on finely ground sediment previously sieved at 224 µm. Sieved sediment samples were spiked with deuterated internal standards A-*d10*, N-*d10*, Per-*d12*, Phe-*d10* and Pyr-*d10* for PAHs and Me-PAHs analysis and with TCN, PCB112 and OCN for PCBs analysis. After a delay of equilibration, sediments were then extracted using an accelerated solvent extraction (ASE 200, Dionex Corp., USA). The extraction conditions were heat 5 min, temperature 100°C, static solvent extraction time 2 min with 5 static cycles, pressure 138 bars, purge 3 min and 35 % flush according to the method developed by Tronczynski et al. (2005). High purity nitrogen was employed as the purge gas.

2.6. Purification and pre-separation

Molecular sulfur was removed by addition of activated metallic copper (Blumer, 1997) to the extracts. The extracts were concentrated, solvent-exchanged to hexane, and were then purified and fractioned by liquid chromatography on a silica column to eliminate organic interferences (Jeanneau, 2007). PCBs were recovered by elution with 20 mL of hexane (Fraction 1), and aromatic hydrocarbons (PAHs and Me-PAHs) were recovered by 15 mL of hexane/dichloromethane mixtures (3/1 v/v) followed by 15mL of hexane/dichloromethane mixture (1/1 v/v) (Fraction 2). Each fraction was concentrated using a rotary evaporator followed by a slight stream of nitrogen before analysis.

2.7. Gas chromatography analyses

Targeted compounds were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.25 mm i.d.) and a fused-silica low polarity si-arylene ZB-XLB capillary column (60 m length, 0.25 mm i.d., 0.25 mm film thickness, Phenomenex) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium held at a constant flow rate of 1 mL/min. Each group of organic compounds was analyzed separately. Temperature of the GC oven was programmed as follows: from 70°C (1 min) to 170°C at 10°C/min, then to 230°C at 4°C/min, and then to 300°C at 3°C/min (13 min) for HAPs and Me-HAPs and from 80°C (1 min) to 170°C at 10°C/min, then to 230°C at 4°C/min, and then to 300°C at 3°C/min (19 min) for PCBs. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. The transfer line and the ion trap were respectively held at 280°C and 220°C. Identification of each compound was done on the basis of the retention time and the mass spectrum from chromatograph of standard solutions acquired in full scan mode. Quantification was then performed in the single ion storage (SIS) mode for better selectivity. Response factors were determined relative to the internal standards previously chosen to better fit to the properties of each compounds.

3. Results and discussions

3.1 PCBs distribution and composition profiles

Among the 28 studied PCBs, nine were detected and quantified at least one time (PCBs 8, 18, 28, 52, 44, 66, 101, 118 and 123). The highest concentration was found in surface sediment of the Scarpe River (Σ_{28}PCBs = 194.4 µg kg^{-1} dw). The Sensée and the Deûle Rivers were found to be less contaminated in PCBs with concentration maxima (Σ_{28}PCBs) reaching 34.0 and 15.6 µg kg^{-1} dw respectively. In this study, special attention was accorded to the seven PCB indicators (Σ_7PCBi _ i.e. PCB 28, 52, 101, 118, 138, 153 and 180), that are among the most frequently detected congeners in the environment, and also to the twelve dioxin-like PCBs (Σ_{12}PCB-DL _ i.e. PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) known to be highly toxic to humans and to persist in the environment (Kimbrough et al., 2010; Tanabe and Minh, 2010). Depth contamination profiles for Σ_{28}PCBs, Σ_7PCBi and Σ_{12}PCB-DL, for the three sediment cores, are presented on Figure 2.

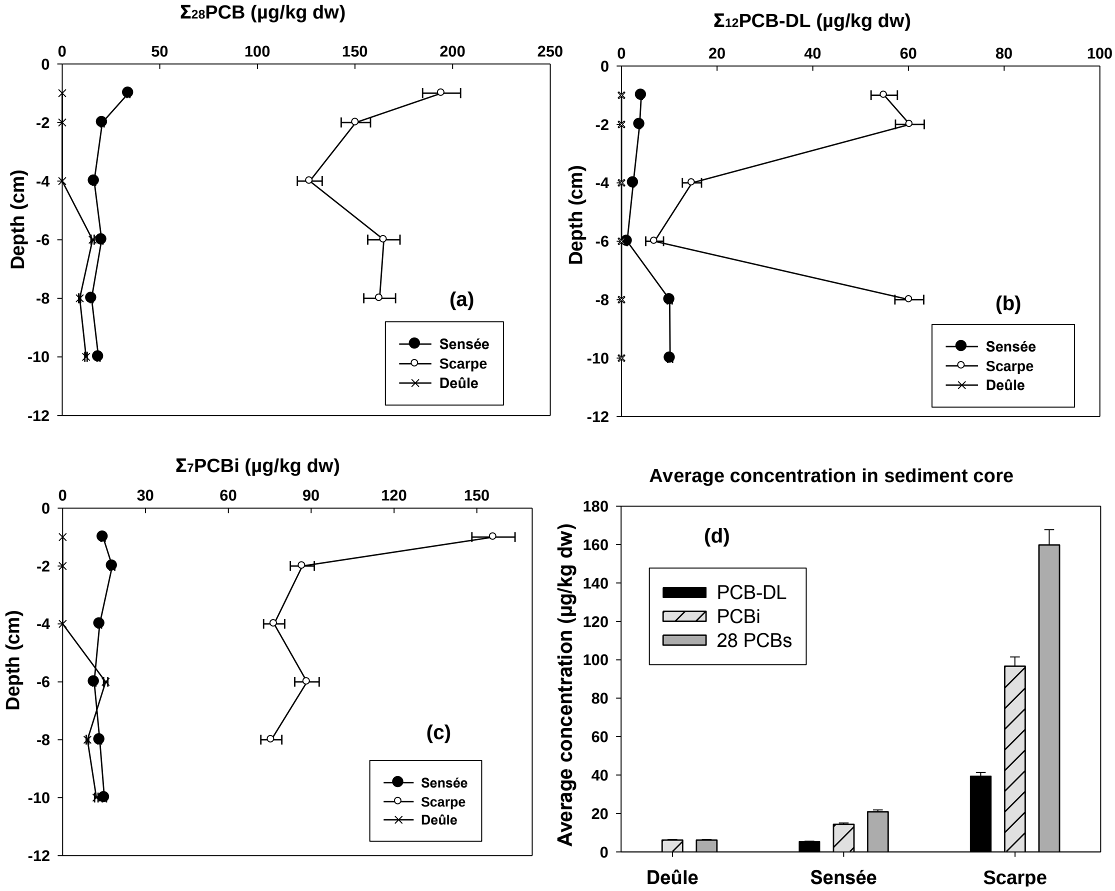


Figure 2: PCB depth profiles in the three sediment cores of (a) Σ_{28}PCBs, (b) Σ_{12}PCB-DL, (c) Σ_7PCBi and (d) the average concentrations of Σ_{28}PCBs, Σ_{12}PCB-DL and Σ_7PCBi in each sediment core.

As above-mentioned, the highest concentrations were measured in the sediment from the Scarpe River with Σ_{28}PCBs varying from 126.8 to 194.4 µg kg^{-1} dw. The maximal concentration was recorded in the surface of the sediment core (0-1 cm depth). Sensée and Deûle sampling sites were found to be less contaminated with Σ_{28}PCBs respectively ranging from 15.1 to 34.0 µg kg^{-1} dw and from *n.d.* to 15.6 µg kg^{-1} dw. In the case of the Sensée River, the highest concentration was also encountered in surface sediment. Indicators PCBs were found to represent 100, 68.9 and 60.5 % of the total detected PCBs for the Deûle, the Sensée and the Scarpe Rivers respectively. As expected, a good correlation could be found between distributions of “total” PCBs (Σ_{28}PCBs) and indicators PCBs (Σ_7PCBi) overall the three sites (R-squared = 0.93, $p < 0.01$). No significant amount of dioxin-like PCB (Σ_{12}PCB-DL) could be found in the Deûle river sediment core. The highest levels of PCB-DL were detected for the Scarpe samples, ranging from 6.9 to 60.3 µg kg^{-1} dw with an average concentration of 39.4 µg kg^{-1} dw. The Σ_{12}PCB-DL depth profile of the Scarpe sediment core was also characterized by a low-contaminated zone at 4-6 cm depth. The Sensée samples showed much lower concentrations with an average value of 5.3 µg kg^{-1} dw. As noticed elsewhere (Babut et al., 2009), Σ_{12}PCB-DL were found to be correlated with Σ_{28}PCBs (R-squared = 0.67, $p < 0.01$) overall the three sites. The homologue composition of PCBs in most samples was characterized by mixtures with a low chlorination level (tri-, tetra- and penta-PCBs). Hexa-, hepta- and octa-PCBs were absent, whereas di-PCB could only be detected in the Sensée canal with a mean proportion of 7.2 % of total PCB concentration.

3.2. PAHs and Me-PAHs distribution and composition profiles

The Σ_{16}PAHs and Σ_{18}Me-PAHs concentrations were also plotted against sediment depth for the three sampling sites (Figure 3). Concerning Σ_{16}PAHs, sediments of the Scarpe river showed the highest contamination with concentrations varying from 13.4 to 33.7 mg kg^{-1} dw, with an average of 20.7 mg kg^{-1} dw. Lower concentrations ranging from 9.1 to 14.4 mg kg^{-1} dw (average value of 9.9 mg kg^{-1} dw) and from 6.1 to 8.2 mg kg^{-1} dw (average value of 6.4 mg kg^{-1} dw) were found respectively for the Deûle and Sensée samples. Surface sediment (0-1 cm) was systematically found to be more contaminated than deeper sediment. This observation was particularly prominent for the Scarpe River, which, in our case, is the only one to be not navigated and thus inclined to a higher sedimentation rate than the Deûle and the Sensée rivers. Such depth profiles tend to indicate a recent contamination and are in agreement with a previous study carried out in the region on sediments under urban influence (Charriau et al., 2009). Concerning methylated homologues (Me-PAHs), all concentrations of individual species were found to be lower than 1 mg kg^{-1} dw, ranging from non-detectable levels to few hundreds µg kg^{-1} dw. The average Σ_{18}Me-PAHs concentrations in sediment cores of Sensée, Deûle and Scarpe were 1.3, 1.3 and 4.6 mg kg^{-1} dw respectively. Overall the three sites, depth profiles of Σ_{18}Me-PAHs were found to be correlated with Σ_{16}PAHs (R-squared = 0.74, $p < 0.01$).

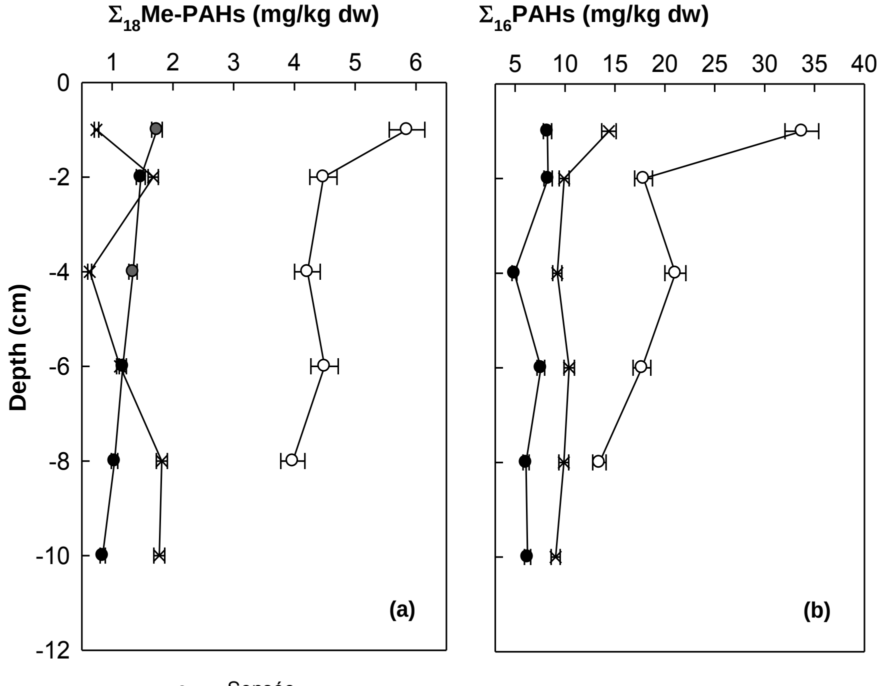


Figure 3: Sedimentary depth profiles in the three sediment core of (a) Σ_{18}Me-PAHs and (b) Σ_{16}PAHs.

In term of composition profiles, PAHS species were dominated by four and five ring structures whatever the depth with average proportions of 20, 36 and 41 % of four rings and 48, 37 and 45 % of five rings respectively for the Scarpe, Sensée and Deûle. Lower molecular weight PAHs (i.e. two ring structures) were predominantly found in the Scarpe River sediment, but did not exceed 12 % of total PAHs (Σ_{16}PAHs) concentration. Such low levels of two ring PAHs can be explained both by their higher water solubility and their lower stability towards (bio)-degradation (Quantin et al., 2005). On the other hand, methylated PAHs were found to be dominated by two- and three-aromatic ring structures with average proportions respectively ranging from 37 to 49 % and from 47 to 53 %.

3.3. Sources apportionment

A relationship could be found between Σ_{28}PCBs and Σ_{16}PAHs distribution profiles (R-squared = 0.65, $p < 0.01$). This observation suggests that their sources are mainly located at similar regions. Atmospheric depositions, runoff from the land, and food chain transport have been regarded as the major sources of PCBs in aquatic environments (Totten et al., 2006). The predominance of lower chlorinated congeners in our samples (tri-, tetra- and penta-PCBs) would favor an atmospheric transport-deposition process reinforced by the strong metallurgic activity nearby (Yang et al., 2009). However, PCBs result from industrial production without any known natural source, and it is also possible to identify the possible sources by evaluating the similarity of the PCB patterns found in the river sediments samples with that of the principal commercial PCB mixtures. A principal component analysis (PCA) was also performed to obtain further information on PCB sources by comparing samples composition and commercial Aroclor mixtures (1221, 1232, 1242, 1248, 1254, 1260 and 1262). (Škribic´ and Durišic´-Mladenovic´, 2007; Zhou et al., 2012). The compositions of Aroclor 1221, 1232, 1242, 1248, 1254 and 1260 (Frame et al., 1996) were normalized with respect to 28 congeners concerned in this study. The first two principal components (PCs) were extracted by PCA, explaining 42.4 % and 22 % of the total variance, respectively. PC1 was basically defined by the contributions of highly chlorinated congeners, whereas PC2 was influenced mainly by di-, tri-, tetra- and penta-PCBs. The results of the PCA are presented Figure 4.

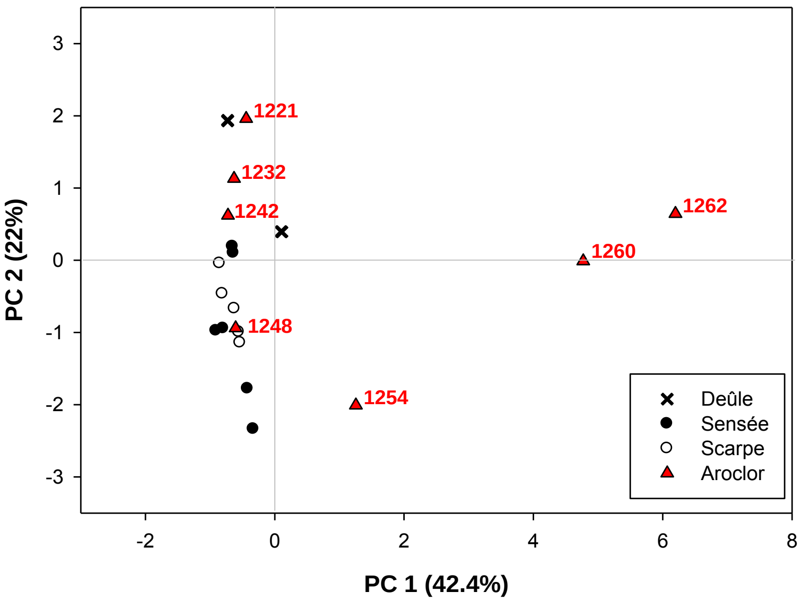
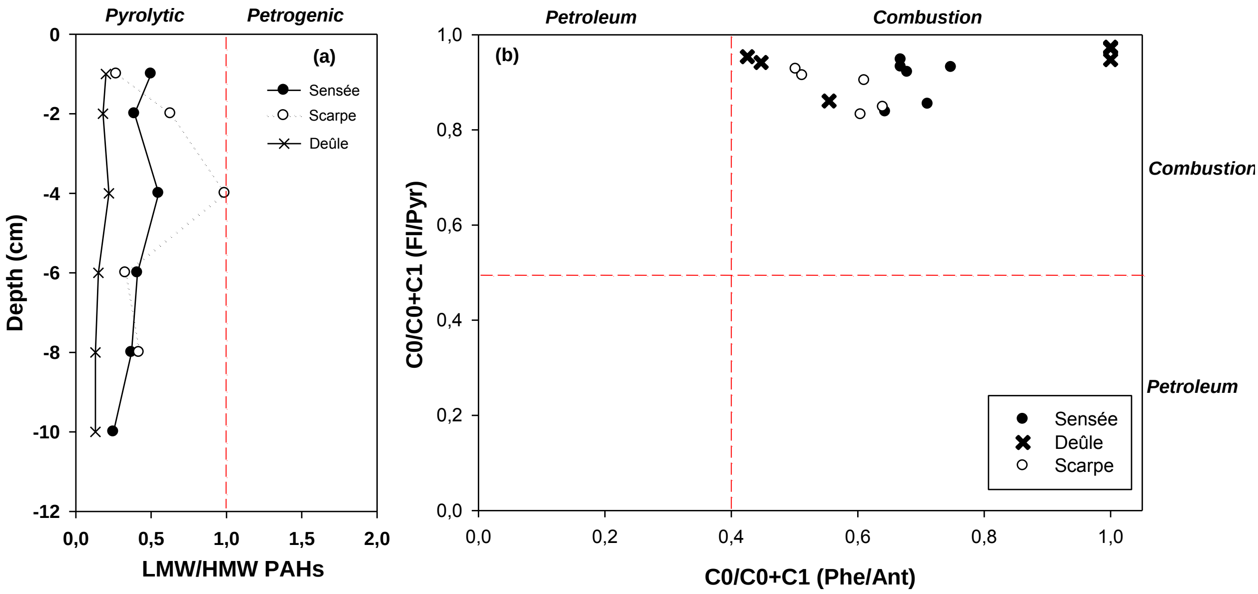
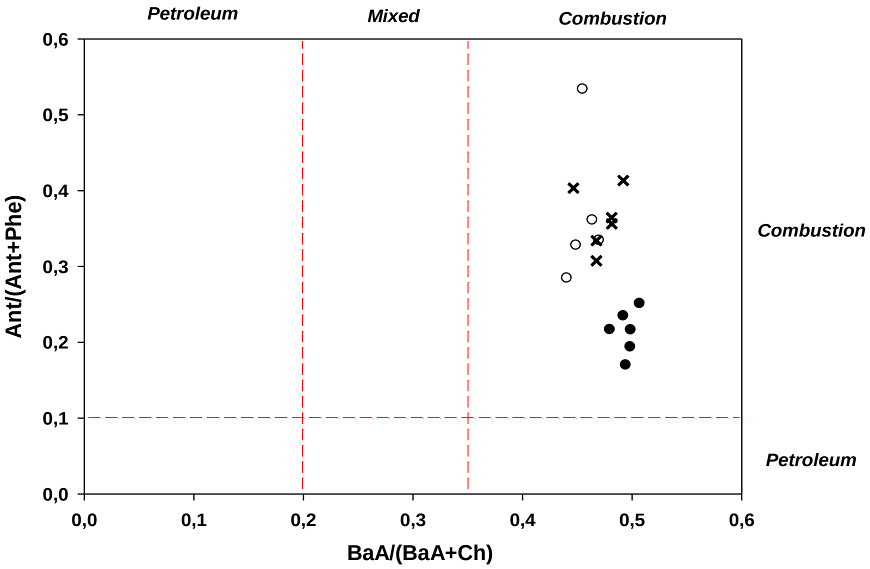


Figure 4: Principal component analysis of PCB compositions and comparison with standard mixtures

The score plot suggests that Aroclor 1248 was the most common mixture used both in the Scarpe and the Sensée rivers areas. However, it cannot be exclude that the contamination profile can also be influenced by other mixtures such as Aroclor 1232, 1242 and, to a lesser extend, 1254. Concerning the Deûle river, the PCBs contamination score plot tends to show a complex influence of Aroclors 1221, 1232 and 1242, all characterized by the prevalence of low molecular weight PCBs. All these Aroclor mixtures were mostly used in electrical capacitors and electrical transformers, and can be released into the environment from landfills containing PCB waste materials and products of municipal refuse and sewage sludge incineration, and improper disposal of PCB materials. Only Aroclor 1260 and 1262 can be dismissed as potential sources in the study areas since their composition is dominated by highly chlorinated PCBs congeners (hexa- to hepta-PCBs).

Concerning polycyclic aromatic hydrocarbons, anthropogenic releases can be attributed to petrogenic and pyrolytic origins. PAHs of petrogenic origins are usually characterized by the predominance of low number of aromatic rings (i.e. 2 and 3 rings), while high proportion of above 4 aromatic rings PAHs characterizes PAHs originated from pyrolytic origins. The ratio of low molecular weight and high molecular weight (LMW/HMW, the sum of (2-3) / (4-6) aromatic rings) is commonly used to distinguish the petrogenic (LMW/HMW > 1) from pyrolytic origins (LMW/HMW < 1) (De Lucas, 2005). As depicted on Figure 5a, LMW/HMW ratios revealed values ranging from 0.09 to 0.99, thus suggesting that combustion should be the dominant source of PAHs in the studied areas even if petrogenic inputs can be suspected for the sediment core of the Scarpe River at 4-6 cm depth. In addition, all the sampling sites showed high proportions of parent PAHs (Σ_{16}PAHs/Σ_{16}PAHs+Σ_{18}Me-PAHs average = 0.84, 0.89 and 0.81 for Sensée, Deûle and Scarpe rivers respectively), which support combustion as the primary PAHs source. Furthermore, discrimination between petrogenic and combustion sources can be ensured using specific ratios involving alkyl PAHs (Gogou et al., 1996; Simo et al., 1997; Yunker et al., 2002). For this purpose, cross plots of C_0/C_0+C_1 ratios in both the phenanthrene/anthracene (Phe/Ant) and fluoranthene/pyrene (Fl/Pyr) series are presented figure 5b. With petroleum/combustion transitions set at 0.4 and 0.5 for the Phe/Ant and the Fl/Pyr series respectively, C_0/C_0+C_1 ratios corroborate the combustion source. Further isomeric molecular ratios can also be used to differentiate potential sources of PAHs in sediment (Yunker and Macdonald, 1995; Budzinski, 1997; Dickhut et al., 2000; Yunker, 2002; Zhang, 2005). Nevertheless, such ratios should be treated with caution as biogeochemical and physical processes may alter PAH signatures due to variations in PAH reactivity, volatility, water solubility and sorption rates (Dickhut et al., 2000; Yunker at al., 2002; Wagener et al., 2010). PAHs of molecular masses 178 and 228 are commonly used to distinguish combustion from petroleum sources according to the values of the anthracene to anthracene plus phenanthrene ratio (Ant/(Ant+Phe)) and the benz*[a]*anthracene to benz*[a]*anthracene plus chrysene ratio (BaA/(BaA+Ch)). In our case, as depicted on figure 5c, cross plots for the ratios Ant/(Ant+Phe) *vs.* BaA/(BaA+Ch) reinforce the combustion process as the major source of contamination in the studied area. Moreover, such high Ant/(Ant+Phe) and BaA/(BaA+Ch) ratios involve high proportions of anthracene and benz*[a]*anthracene that are both among the most photoreactive PAHs (Behymer and Hites, 1988; Gogou et al., 1996). Considering a combustion/transport/deposition process, it can be admitted that no significant photolysis degradation occurred in this case study. These ratios can also imply close contamination sources that can be attributed to the nearby industrial activities.


Pyrolytic
Petrogenic
(a)
Sensée
Scarpe
Deûle
Depth (cm)
0
-2
-4
-6
-8
-10
-12
0,0
0,5
1,0
1,5
2,0
LMW/HMW PAHs
Petroleum
Combustion
(b)
1,0
0,8
0,6
0,4
0,2
0,0
C0/C0+C1 (Fl/Pyr)
Combustion
Petroleum
Sensée
Deûle
Scarpe
0,0
0,2
0,4
0,6
0,8
1,0
C0/C0+C1 (Phe/Ant)


Petroleum
Mixed
Combustion
0,6
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0,1
0,0
Ant/(Ant+Phe)
Combustion
Petroleum
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0,1
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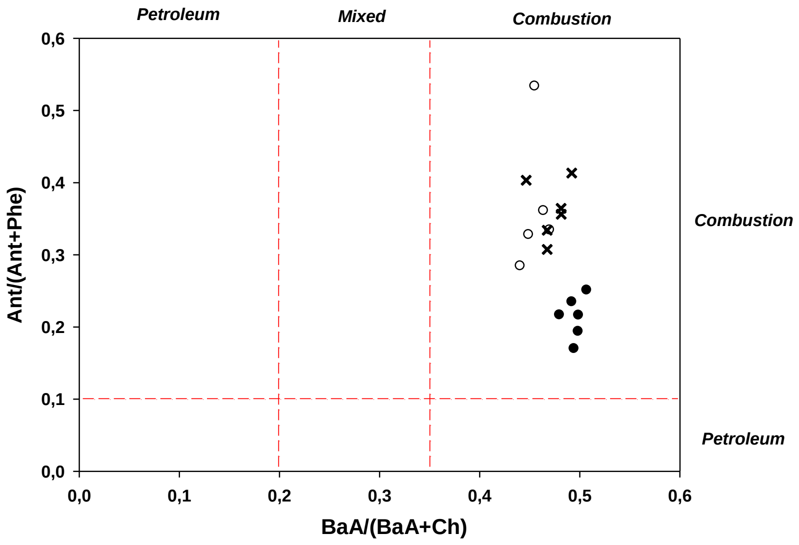
0,4
0,5
0,6
BaA/(BaA+Ch)


Petroleum
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Ant/(Ant+Phe)
Combustion
Petroleum
0,0
0,1
0,2
0,3
0,4
0,5
0,6
BaA/(BaA+Ch)

Figure 5 (a) Depth profiles of low molecular and high molecular weight PAHs ratios (LMW/HMW) of PAHs in the sediment cores. (b) Cross plots of C_0/C_0+C_1 in the Phe/Ant vs the Fl/Pyr series. (c) Cross plots isomeric ratios of: Ant/(Ant+Phe) vs. BaA/(BaA+Ch).

3.4 Sediment quality evaluation

Numerical sediment quality guidelines (SQGs) for river sediment have been developed using a variety of approaches. SQGs include both a threshold effect concentration (TEC), which identifies contaminant concentrations below which adverse effects are not expected to occur, and a probable effect concentration (PEC) which identifies contaminant concentrations above which harmful effects on sediment-dwelling organisms were expected to occur frequently (MacDonald et al., 2000a). SQGs have first been used to identify contaminants of concern in aquatic ecosystems and to rank areas of concern on a regional or national basis (US EPA, 1997). Then, consensus-based SQGs (CBSQGs) have then developed for PCBs and PAHs with the aim to provide a unified synthesis of the existing guidelines, reflecting causal rather than correlative effects, and taking into account the effects of contaminant mixtures in sediment (Swartz et al., 1999; MacDonald et al., 2000b). The certainty in predicting the absence or presence of sediment toxicity occurs at sediment concentration that are < TEC or > PEC values, respectively. For the values between consensus-based TEC (CBTEC) and consensus-based PEC (CBPEC), toxicities and effects to benthic macroinvertebrate species related to reductions in survival, reproduction, and growth, lead to bioaccumulation, and benthic community alterations that correspondingly increase with the increasing of the concentration of contaminants. Consequently, with the aim to better interpret the potential impacts between the TEC and PEC values of the CBSQGs, it has been recommended to consider an additional midpoint effect concentration (MEC = (TEC+PEC/2)). Four possible ranges of concentration can thus be applied for describing the level of sediment quality: Levels 1, 2, 3 and 4 respectively refer to ≤ CBTEC; > CBTEC ≤ CBMEC; > CBMEC ≤ CBPEC; and > CBPEC. Sediment qualities evaluations for the three sampling sites are presented on Figure 6. Sediment quality evaluation based on Σ_{28}PCBs showed a quality level of 1 whatever the depth for sediment obtained from the Sensée and the Deûle Rivers, whereas the Scarpe River sediment quality was found to be at level 2. Based on Σ_{16}PAHs, studied sediments showed poorer quality with average levels reaching 2 and 3. Level 4 was even achieved for top surface sediment (0-1 cm) of the Scarpe River.

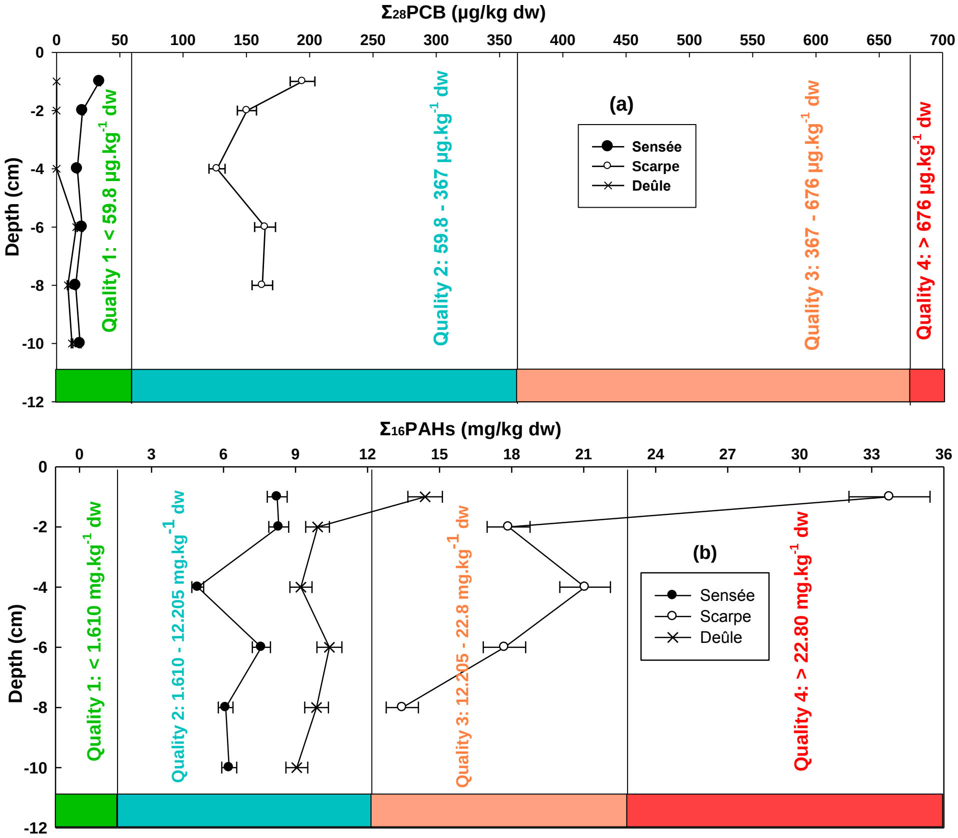

Figure 6: Levels of sediment quality compared to recommended sediment quality guidelines.

Conclusion

Contamination of riverine sediments by persistent organic pollutants (PCBs, PAHs and Me-PAHs) have been studied for the first time for three sites located in the Nord Pas-de-Calais region, Northern France (Scarpe, Deûle and Sensée rivers). The distributions of PCBs and PAHs established to be correlated, thus suggesting that their sources are mainly located at similar regions with highly metallurgical and residential activities. Total concentrations indicate a greater contamination level for the Scarpe River sediment. Recent contamination trend is suspected for all sites according to the higher concentrations recorded in surface sediments. According to the predominance of low chlorinated PCBs congeners, atmospheric deposition can be regarded as the major source of contamination. The possible sources of PCBs have further been studied through a principal component analysis based on PCB composition, while the pyrolytic origin of aromatic hydrocarbons has been determined through the use of molecular indexes. Sediment quality evaluation based on consensus-based SQGs revealed that the Scarpe River is more contaminated with persistent organic pollutants than the Sensée and the Deûle rivers. Complementary studies are already ongoing to go further into the organic contamination in this region heretofore suffering from a severe historical metallic pollution.

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- Article 6 -

Case study of PAHs, Me-PAHs, PCBs, phthalates and pesticides contamination in the Somme River water, France

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Abstract

Surface waters, especially natural rivers always act as receiving waters for various kinds of organic contaminants from municipal and industrial wastewaters, agricultural activities, organic chemicals in use, non-point source pollutions. Due to their toxicity, persistency and wide diffusion, polychlorinated biphenyls (PCBs), pesticides, phthalates, polycyclic aromatic hydrocarbons (PAH) and their alkylated homologues (Me-PAHs) are among the organic contaminants the most often monitored in the environment. Determination of the contamination level is the crucial first step of environmental research. Field investigations have clearly demonstrated their importance on various studies on the contamination levels, the origin, and impact of contaminants in the aquatic ecosystems. The present paper is an effort on the field studies focusing on persistent organic pollutants: PCBs, PAHs and Me-PAHs, phthalates and pesticides in Somme River water located on Northern France. The sampling zone is characterized by fields of agriculture surrounding urbanized and industrialized areas and constitutes a place embedded with strong fishing activities. However, the river section of interest is also known for the high levels of PCB in sediments. The water were analyzed for 28 PCBs, 16 PAHs and 18 Me-PAHs, 6 phthalates and 28 pesticides with the aim to determine the dispersion trend and for some or the water quality of the sampled water.

Keywords: PAHs, Me-PAHs, Phthalates, PCBs, Pesticides, LLE, GC-MS, Somme River

3. Introduction

Actually, organic contaminants are major environmental concern due to their ubiquitous, their persistence, long-range transportability and potentially adverse effects on living organisms. River water acts as receiving water for various kinds of organic contaminants from municipal and industrial wastewaters (Malve et al., 2003; Singh et al., 2004; Zhang et al., 2004). For the ecosystem protection and to keep water resources clean, it is important to identify the nature of contaminants, their contamination levels as well as their sources. Polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues (Me-PAHs), polychlorobiphenyls (PCBs), phthalates and pesticides are the principal classes of organic contaminants in aquatic ecosystem. In this context, twenty-three priority substances and other priority pollutants (PPs) were established by European Commision in the Water Framework Directive 2008/105/EC (European Commission, 2008).

(*i*) Aromatic hydrocarbons including PAHs and Me-PAHs are widely disseminated in the environment with sources that can be both natural and anthropogenic (Yunker, 2002; Wang, 2007; Mostert, 2010). They are highly lipophilic compounds, ubiquitous in coastal, estuarine and river water column, as well as sediments in which they tend to accumulate (Cailleaud, 2007; Chiou, 1998; Manodori, 2006, Gaspare, 2009; Ko, 1995; Yunker, 2012; Net et al., 2014). High level of aromatic hydrocarbons represents a serious threat to the ecosystem functioning and human health via food chain and water resources. Recent studies have indeed reported that marine organisms are prone to bioaccumulate these substances, particularly in lipid-rich tissues (Neff, 2002; Francioni, 2005; Dugan, 2005). Due to their toxic, carcinogenic and mutagenic effects (Straif, 2005; IARC, 2010; U.S. Department of Health and Human Services, 2011), sixteen PAHs have been recommended as priority pollutants by the United States Environmental Protection Agency (US EPA, 2002). (*ii*) Another class of contaminant is represented by the pesticides. It is known that pesticides used for agriculture activities may lead to contamination of surface and ground waters (Kaushik et al., 2010, Navarro et al., 2010; Hancok et al., 2008, El-Osmani et al., 2014). Pesticide contamination of the aquatic environment as well as their ecotoxicological effects for aquatic flora, and human health has also been well documented (Wania and Mackay 1999; Sanborn et al., 2007; Jurewicz and Hanke, 2008; Huen et al., 2012). Runoff from urban areas, return flow waters from agricultural fields, and leaching are considered important inputs to pesticide contamination of surface and groundwater. (*iii*) One other group is characterized by polychlorobiphenyles (PCBs). These compounds have been commonly used as dielectric fluids or transformers and capacitors, in paints, inks and pesticides until the hazard posed to both the environment and human health by their use became evident. They are extremely stable compounds under environmental conditions (WHO, 1993). Due to their toxicity, chronic persistence and bioaccumulation, they have been banned or restricted, and some of them have been included in the list of priority pollutants in many countries. However, PCBs are still present in water and sediment and continue to affect aquatic organisms from the top of food chain (plankton, algae) to predator organisms (fish, birds, marine mammals) and consequently human health through the diet (Sun et al., 2002). (*iv*) Nowadays, phthalates or phthalic acid esters have become also a group of contaminants of environmental concern. Large scales of phthalates have been produced due to the wide variety of uses. They are used in common household products, cosmetics, detergents, flame retardants, plastics, inks, adhesives and medical devices. Several million tons of phthalates have been produced each year. Some phthalates are suspected to act as endocrine disruptors (Gomez-Hens and Aguilar-Caballos, 2003; Ghisari and Bonefeld-Jorgensen, 2009; Lau et al., 2005). Phthalates are currently listed as priority pollutants in many countries due to their link to several human cancer diseases (Kaneco et al., 2006). According to US EPA, DEP, dimethyl phthalate (DMP), DEHP, BBP, DBP and di-*n*-octyl phthalate (DOP) should be considered Priority Toxic Pollutants (US EPA, 1999). Their entry into the surface water occurs directly from the production of plastic materials and indirectly *via* volatile emissions and leaching from their parent polymeric material (Stanley et al., 2003, Petrovic et al., 2001; Gomez-Hens and Aguilar-Caballos, 2003 and Kavlock et al., 2002).

The aim of the present study was to investigate the concentration levels of these four classes of organic contaminants in order to assess quality of river water from fourteen sampling sites of the Somme River in Northern France. Samples were analyzed for 16 PAHs and 18 Me-PAHs, 6 phthalates, 28 pesticides and 28 PCBs, using gas chromatography/mass spectrometry (GC/MS). Results obtained from this multiresidue study aim to fill the lack of data concerning water contamination in this region.

2. Materials and methods

2.1. Reagents

Mixed standard solutions of PAHs and Me-PAHs were purchased from Restek Corp (Bellefonte, PA, USA). PCBs standard solution was obtained from Accustandard, Inc. (New Haven, CT, USA). Phthalates and pesticides standards were purchased from Sigma-Aldrich

(Saint-Louis, USA) and Restek (Bellefonte, USA). Tetrachloronaphtalene (TCN), 2,3,3',5,6-tetrachlorobiphenyl (PCB112) and octachloronaphtalene (OCN), used for PCB quantification, were purchased from Dr Ehrenstorfer (Augsburg, Germany) Deuterated internal standards for PAHs and Me-PAHs (acenaphthene-*d*10 (A-*d*10), naphtalene-*d*8 (N-*d*10), perylene-*d*12 (Per-*d*12), phenanthrene-*d*10 (Phe-*d*10) and pyrene-*d*10 (Pyr-*d*10)) were provided by LGC-Promochem (Middlesex, UK). Benzyl benzoate and pentachloronitrobenzene with a purity of 99% and 94% respectively were used as internal standard for phthalates and pesticides, and they were purchased from Sigma-Aldrich (Saint-Louis, USA). HPLC-grade solvents (hexane, dichloromethane, methanol and acetone) were purchased from Dislab (France). No significant amount of targeted analytes was showed in procedural blanks. Ultrapure water (Milli-Q) was produced by a Millipore apparatus with 18.2 MΩ/cm resistivity. Merck silica gel 60 (70-230 mesh ASTM) activated at 450°C was heated at 120°C for 12h prior to use. Glassware was systematically washed with detergent (Decon, East Sussex, UK), rinsed with ultrapure water and acetone and finally dried at 120°C prior to use.

2.2. Sampling Sites

The sampling campaign was conducted on October 2012 in Somme River (Picardie region in northern France). The sampling was done from downstream at Béthencourt-sur-Somme to Saint-Quentin (Figure 1).

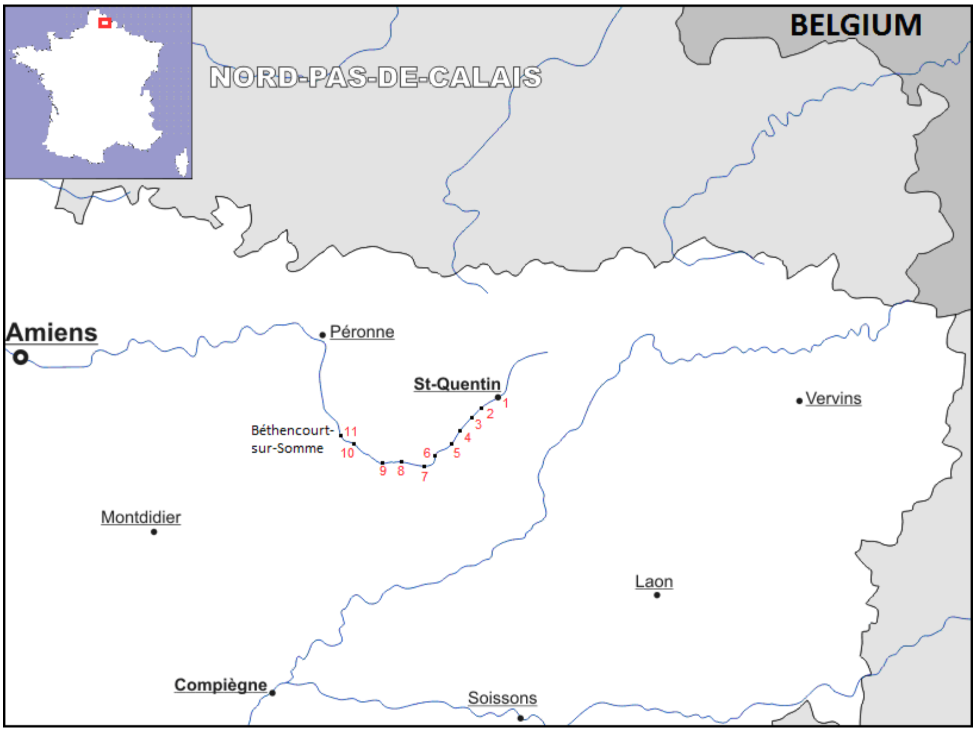

Figure 1*:* Location of sampling sites in the Somme River in Picardie region, Northern France. Water samples were collected from 11 sites of the Somme River from the first station at Béthencourt-sur-Somme (Station 001103) to Gauchy (station 116500). Two additional samplings were performed in two ponds around Béthencourt-sur-Somme with the aim to evaluate a potential accumulation of contaminants. The sampling zone is characterized by fields of agriculture surrounding urbanized and industrialized areas (e.g. Saint-Quentin, Ham) and constitutes a place embedded with strong fishing activities. However, the river section of interest is also known for the high levels of PCB in sediments. (Dumoulin et al., 2013)

2.3. Targeted analytes

In this work, samples were analyzed for 16 PAHs, 18 Me-PAHs, 6 phthalates, 28 pesticides and 28 PCBs including 12 dioxin-like PCBs (dl-PCBs) and 7 PCB indicators (PCBi) as following:

PAHs (16 PAHs) : naphthalene (Na), acenaphtylene (Ayl), acenaphtene (Aen), fluorene (F), anthracene (An), fluoranthene (Fl), benz*[a]*anthracene (BaA), chrysene (Ch), benz*[a]*pyrene (BaP), phenanthrene (Phe), benzo*[b]*fluoranthene (BbF), benzo*[k]*fluoranthene (BkF), benzo*[ghi]*perylene (Bghi), dibenzo*[a,h]*anthracene (DhA), indeno*[1,2,3-cd]*pyrene (IP), pyrene (Py).

Me-PAHs (18 Me-PAHs) : 1-methylnaphthalene (1M-Na), 2-methylnaphthalene (2M-Na), 1,2-dimethylnaphthalene (1,2DM-Na), 1,6-dimethylnaphthalene (1,6DM-Na), 2,6-dimethylnaphtalene (2,6DM-Na), 1-methylphenanthrene (1M-Phe), 2-methylphenanthrene (2M-Phe), 3-methylphenanthrene (3M-Phe), 9-methylphenanthrene (9M-Phe), 2-methylanthracene (2M-An), 1,7-dimethylphenanthrene (1,7DM-Phe), retene, 1-methylfluoranthene (1M-Fl), 3-methylfluoranthene (3M-Fl), 1-methylpyrene (1M-Py), 4-methylpyrene (4M-Py), 3-methylchrysene (3M-Ch), 6-methylchrysene (6M-Ch).

Phthalates (6 PAE): dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate ester (DEHP) and di-*n*-octyl phthalate (DNOP).

Pesticides: alpha-lindane, gamma-lindane, beta-lindane, delta-lindane, heptachlor, aldrin, trans-chlordane, cis-chlordane, 4,4’-DDE, endosulfan I, dieldrin, endrin, 4,4’-DDD, endosulfan II, 4,4’-DDT, endrin aldehyde, methoxychlor, endosulfan sulfate, endrin ketone, chloroneb, chlorothalonil, DCPA methyl ester, heptachlor epoxide (isomer A and B), trans-nonachlor, chlorobenzilate, trans-permethrin and cis-permetrin.

PCBs No.(28 PCBs) : 8, 18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209.

2.4. Sampling and extraction procedures

Water sampling was performed using pre-cleaned amber glass 2.5 L bottles that were immediately capped with Teflon-lined lid. Samples were maintained at 4°C before analysis. Back to the lab, samples were rapidly filtered using 0.7 µm Whatman glass microfiber filters and extracted using liquid–liquid extraction (LLE) technique. The applications of LLE in water and other liquid matrixes have been widely accepted in standard methods for various classes of organic contaminants such as PAHs, pesticides, and PCBs analysis (USEPA, 1996; Japanese Industrial Standard Committee, 2005; Boussahel et al., 2000; Barcéló, 1993; T90-120, AFNOR 1990; USEPA, 2008). Various solvent can be used depending on the nature of interest compounds. Dichloromethane (DCM) has been widely adopted for the extraction of POPs whereas *n*-hexane has often been used for PCB and PAHs extraction (Turrio-Baldassarri et al., 2005; DIN EN ISO 17993). In this study, in order to increase the extraction efficiency, each water sample (1L) was extracted four times with 60 mL of *n*-hexane followed by four times with 60 mL of DCM. The extracts were then pooled and dried using Na_2SO_4. Finally, the extract was concentrated using a rotary evaporator followed by a slight stream of nitrogen before analysis.

2.5. Sample analysis

The extracts were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.53 mm i.d.) and a fused-silica capillary Phenomenex XLB (60 m length, 0.25 mm i.d., 0.25 µm film thickness) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium held at a constant flow rate of 1 mL/min. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. Each group of organic compounds was analyzed separately. The transfer line and the ion trap were respectively held at 280°C and 220°C. Each contaminant was identified based on the retention time and the mass spectrum from chromatogram of standard solutions acquired in full scan mode. Quantification was then performed in the single ion storage (SIS) mode for better selectivity. Response factors were determined relative to the deuterated internal standards response and to standard mixtures. Deuterated standards were chosen in order to better fit to the properties of each group of contaminants.

3. Results and discussions

3.1. PAHs and Me-PAHs

As depicted on figure 2, the total concentration of the 16 PAHs (Σ_{16}PAH) varies significantly along the river section with a mean value of 284 ng.L^{-1}. Two subsurface maxima of concentration were recorded at Fontaine-les-Clercs (station 3 - Σ_{16}PAHs = 513 ng.L^{-1}) and Artemps (station 5 - Σ_{16}PAHs = 831 ng.L^{-1}). On the other hand, the total concentration of 18 Me-PAHs (Σ_{18}Me-PAH) was also found to vary significantly from 75 ng.L^{-1} at Dury (station 7) to 440 ng.L^{-1} at Artemps (station 5) with an average concentration of 185 ng.L^{-1}.

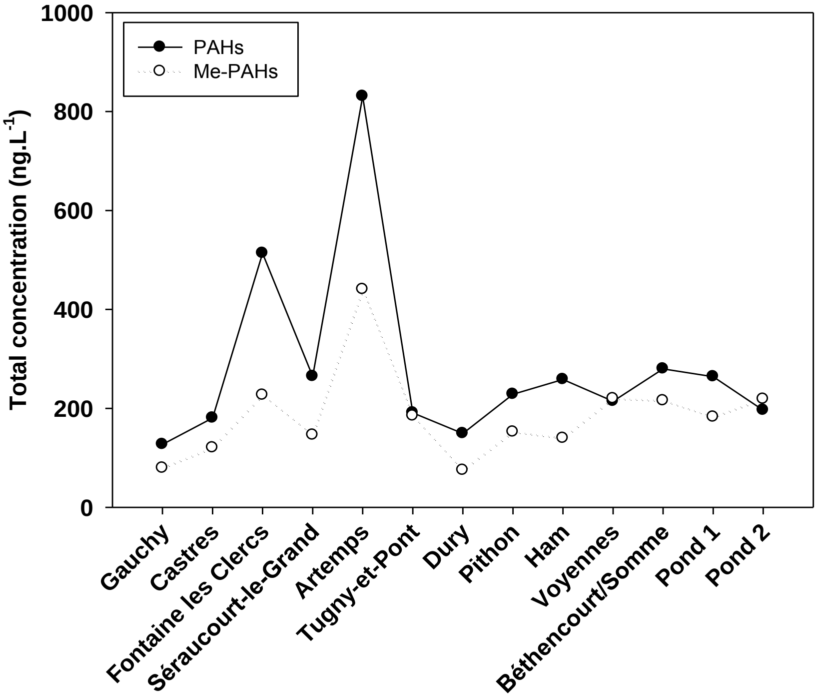

Figure 2: PAHs and Me-PAHs repartition in surface water samples of the Somme River

A strong relationship can be found between the total concentration evolution of Σ_{16}PAHs and Σ_{18}Me-PAHs (R-squared = 0.78, P < 0.01). In terms of the compositional profiles (Figure 3), the concentrations of low molecular weight PAHs (2-3 rings) were significantly higher than high molecular weight PAHs. Three-rings PAHs accounted for the most abundant species with an average of 60 % of the total concentration of PAHs (Σ_{16}PAHs). No significant traces of five and six ring-membered PAHs were detected in the water samples. Concerning Me-

PAHs, two-ring species were predominant with an average proportion of 79 % of Σ_{18}Me-PAHs.

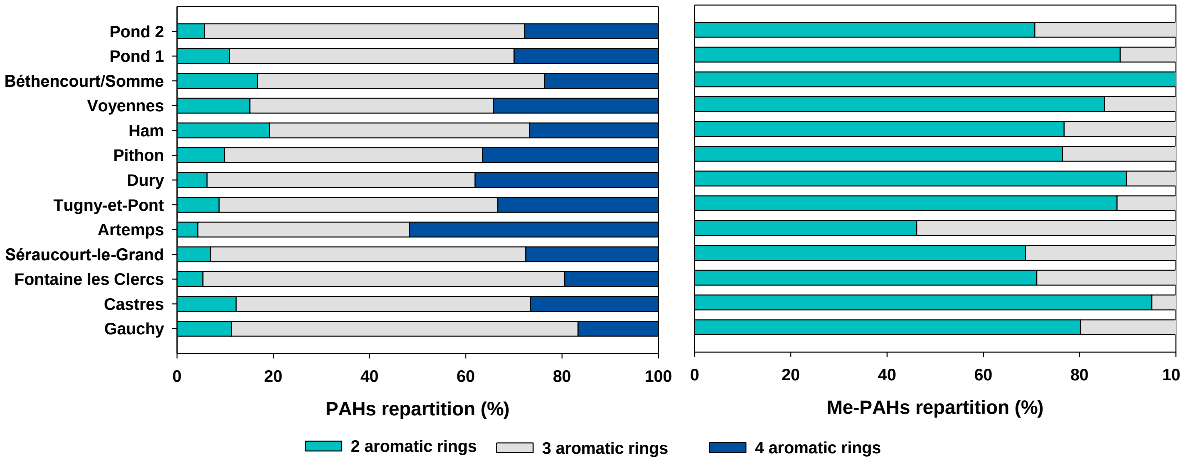

Figure 3: PAHs and Me-PAHs composition profiles in the Somme River water samples

At this stage, some PAHs diagnostic ratios were used as a tool for attempting to identify pollution emission sources (Yunker et al., 2002). For example, the anthracene/(anthracene+phenanthrene) ratio (Ant/(Ant+Phe)) is commonly used to distinguish petrogenic (< 0.1) from pyrogenic (> 0.1) origins, whereas the fluoranthene/(fluoranthene+pyrene) ratio (Fl/(Fl+Py)) allows distinction between petrogenic (< 0.1), fossil fuel combustion (0.4-0.5) and grass/wood/coal combustion (> 0.5). Both these ratio have been widely used to assess contamination sources in water samples (Wang et al., 2009; Opuene et al., 2009; Tobiszewski et al., 2010). In the case of the Somme river water, Ant/(Ant+Phe) ratios support a pyrogenic origin with a mean value of 0.46. The Fl/(Fl+Py) ratios corroborate this hypothesis with a mean value of 0.55 attributable to grass/wood/coal combustion. However, sites 1, 2 as well as the two ponds were not taken into account for the determination of the Fl/(Fl+Py) ratios since no significant trace of fluoranthene could be detected in the corresponding water samples. This might be explained by the fact that PAHs in water samples easily undergo photolysis, which may alter values of diagnostic ratios (Jacobs et al., 2008; Tobiszewski and Namiesnik, 2012). Nevertheless, high proportions of parent PAHs (Σ_{16}PAHs/Σ_{16}PAHs+Σ_{18}Me-PAHs average value = 0.6) support combustion as the primary PAHs source. Moreover, discrimination between petrogenic and combustion sources was ensured using specific ratios involving alkyl PAHs in the phenanthrene/anthracene (Phe/Ant) series. With petroleum/combustion transitions set at 0.4, the C_0/C_0+C_1 (Phe/Ant) ratios determined in our samples corroborate the combustion source with a mean value of 0.77.

3.2. Phthalates

Large variations of concentrations phthalates ($\Sigma_6$phthalates**)** were observed, ranging from 6.93 µg.L^{-1} at Artemps (station 5) to 23.34 µg.L^{-1} at Pond 2 (station 13). DEHP was found to be the most abundant specie with concentration ranging from 5.16 to 20.76 µgL^{-1} for a mean value of 10.23 µg.L^{-1} (accounting for 68 % of mean $\Sigma_6$phthalates). No trace of benzyl butyl phthtalate and di-octyl phthalate could be detected in the samples, whereas low levels of hydrophilic phthalate (DMP) were recorded. No significant correlation could be found between phthalate species distribution. Detailed concentrations are presented in table 1.

Phthalate concentrations (µg.L^{-1})								
N° Station	**Corresponding City**	**DMP**	**DEP**	**DBP**	**BBP**	**DEHP**	**DOP**	**$\Sigma_6$phthalates**
1	Gauchy	0.08	5.35	2.92	-	13.30	-	21.65
2	Castres	-	0.46	0.24	-	20.76	-	21.46
3	Fontaine les Clercs	0.25	2.12	3.28	-	8.88	-	14.53
4	Séraucourt-le-Grand	0.20	-	3.77	-	7.46	-	11.43
5	Artemps	0.02	0.26	0.43	-	6.22	-	6.93
6	Tugny-et-Pont	0.14	1.28	2.01	-	6.57	-	10.00
7	Dury	0.03	0.48	0.45	-	17.93	-	18.89
8	Pithon	0.13	4.92	2.97	-	5.16	-	13.18
9	Ham	0.10	3.01	3.86	-	5.86	-	12.83
10	Voyennes	0.15	3.62	1.98	-	6.84	-	12.59
11	Béthencourt/Somme	0.03	0.52	0.22	-	9.39	-	10.16
12	Pond 1	0.06	6.83	0.77	-	11.23	-	18.89
13	Pond 2	0.22	6.98	2.78	-	13.36	-	23.34

* « - » : not detected « + » : value below the limit of quantification (< 0.01 µg.L^{-1})

Table 1 **:** Individual phthalate concentrations in the 13 sampling sites of the Somme River

The relative high concentrations recorded for phthalates can be explained by the fact that these compounds have now become ubiquitous in water. DEHP and DBP are the most frequently detected and in surface water, wastewater and tap water with concentrations that can easily reach a few dozen µg.L^{-1} (Fromme et al., 2002; Aparicio et al., 2007; Meng et al. 2011). In our case, the low recorded levels of DMP can be linked to the higher degradation rates of short chain phthalates (Staples et al., 1997).

3.3. Pesticides

Among the 28 studied pesticides, only three were detected and quantified at least one time in four samples. The other compounds of interest were below the limit of detection. Gamma-lindane and chloroneb were detected in the four above-mentioned samples corresponding to sites 2, 3, 5 and 11, with concentrations respectively ranging from 177 to 281 ng.L^{-1} and from 90 to 131 ng.L^{-1}. The alpha isomer of lindane was only detected in sites 2 (89 ng.L^{-1}) and 3 (355 ng.L^{-1}) (Table 2). The sources can be attributed to the nearby agricultural activities of sampling stations.

Pesticide concentrations (ng.L^{-1})													
N° Station	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Alpha-lindane	-	89	355	-	-	-	-	-	-	-	-	-	-
Gamma-lindane	-	271	177	-	281	-	-	-	-	-	231	-	-
Chloroneb	-	90	131	-	117	-	-	-	-	-	103	-	-
Σ_{28}pesticides	-	450	663	-	398	-	-	-	-	-	334	-	-

* « - » : not detected

Table 2: Pesticides repartition in the surface water in Somme River.

3.4. PCBs

Somme River sediments are known to be highly contaminated by PCB. The Agence de l'Eau Artois-Picardie (AEAP), which is a public institution of the Ministry for Sustainable Development, has carried out several studies on contamination of sediment of the Somme River by PCBs since 1997. These investigations have led to highlight three sites in the Somme River particularly affected by PCB contamination of sediment: Fontaine-les-Clercs (station 3), Séraucourt-le-Grand (station 4), and Artemps (station 5), with concentrations of PCBi higher than 200 µg.kg^{-1}dw of sediment. No accumulation of PCBs could be noticed downstream watershed (AEAP, 2009; Dumoulin et al., 2013). Besides, no data concerning contamination levels of PCBs in the water column were indicated for these study sites. The present study also aims to provide additional information for these sampling sites with a focus on the water column. Figure 4 showed the total concentrations (Σ_{28}PCBs), dl-PCB and PCBi in surface water of Somme River.

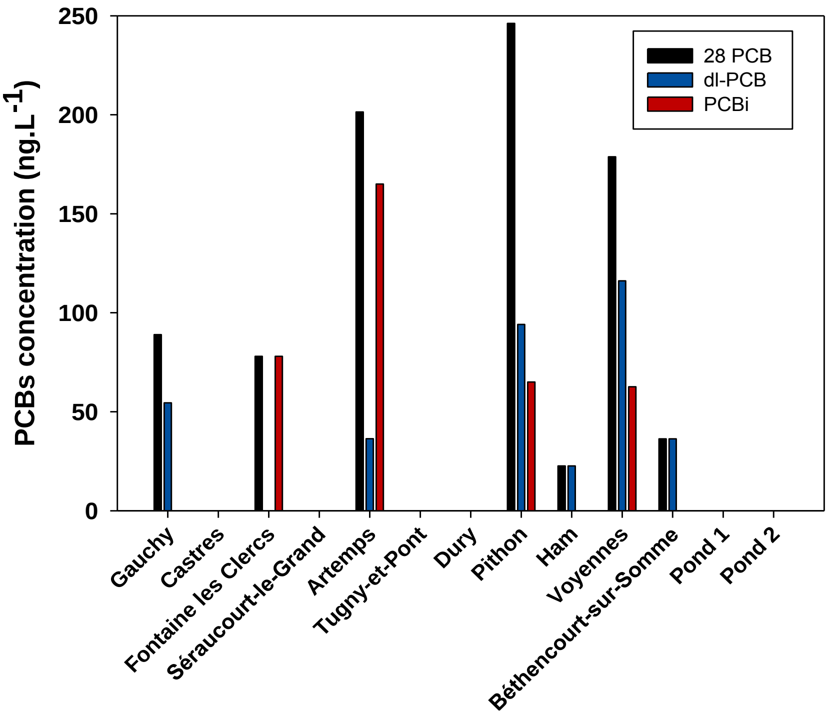

Figure 4: Concentration of Σ_{28}PCB, dl-PCB and PCBi in Somme River.

Large variations of PCBs concentrations were observed for selected sampling sites. High concentrations of Σ_{28}PCB were detected at Artemps (201 ng.L^{-1}), Pithon (246 ng.L^{-1}) and Voyennes (179 ng.L^{-1}) (Figure 4), whereas PCBs were present at non-detectable levels for 6 stations (Castres, Séraucourt-le-Grand, Tugny-et-Pont, Dury, Pond 1 and Pond 2). No specific correlation could be found between Σ_{28}PCB and both indicator and dioxin-like PCBs for the seven concerned stations. PCBi were detected in only four stations and accounted for a mean value of 61 % (ranging from 26 to 100 %). On the other hand, dl-PCB, which are usually measured in biota, were detected at non-negligible levels in six water samples with concentrations ranging from 23 ng.L^{-1} at Ham (station 9) to 116 ng.L^{-1} at Voyennes (station 10) with a mean value of 60 ng.L^{-1}. PCB77 was the dominant dioxin-like congener with an average proportion of 78 %. These concentrations in the water column were not correlated with value previously recorded in sediment for the same sampling sites (Dumoulin et al., 2013). This can be explained by low solubility of PCBs in water by the non-significant remobilization of PCBs from sediment to water column in the studied river section. Moreover, dissolved PCBs in water represent a small fraction of total PCB in the water column due to their rapid association to organic entities such as sediment, algae and protozoa (Brannon, 1993, Hargrave et al., 1992, Eganhouse and Gossett, 1991 and Booij and van den Berg, 1994).

However, it is interesting to note that PCBs are bioaccumulable compounds, which even at low concentration in water, can affect strongly on the aquatic organisms. Moreover, PCBs are extremely stable compounds under environmental conditions (WHO, 1993). Even, their concentrations in surface water were relatively low, it can contaminate underground water which could be source of drinking water and can impact consequently human health.

3.5 Water quality evaluation

Numerous Water Quality Guidelines (i.e. European, Canadian, USA, Australian…) for Marine and Fresh Water Quality have been developed. The specific guidances are corresponding to the type of effluent (surface water, groundwater, freshwater) and to the intended use of the water. Nevertheless, for certain compounds, regulation of hazardous substances (organic micro pollutants) was incomplete or even not available in the literature in the past operation of worldwide water policy (Kallis et al., 2001). Progressively, revisions have been developed to provide a tool for simplifying the reporting quality data. The assessment of the water quality and the contamination level evaluation of each studied site were performed by referring to the environmental quality standards (EQS) indicated through threshold values (European Commission, 2008). For River water quality, the threshold values are currently given for PAHs, phthalates and pesticides. No threshold values available for Me-PAHs. Concerning the PCB contamination, actually, insufficient data allow to set the threshold value. However, according to the circular EU-WFD 2005/12 of 28 July 2005, the interim EQS of PCBs for water inland surface, transitional and territorial marine interior is set to 0.001 $\mu g.L^{-1}$ (INERIS, 2011). Threshold values of EQS of individual PAH, phthalate and pesticides, and contamination levels detected in Somme River are presented in Table 3.

Compounds	EU-WFD	CWQG	Somme River (Average)
PAHs ($\mu g.L^{-1}$)			
Na	130**	1.1*	0.011-0.048 (0.026)
Ayl			0.040-0.081 (0.057)
Aen		5.8*	n.d. – 0.066 (0.017)
F	0.12**	3*	n.d. – 0.060 (0.011)
An	0.1**	0.012*	n.d. – 0.044 (0.027)
Fl	0.12**	0.04*	n.d. – 0.101 (0.032)
Ch		Insufficient data	n.d. – 0.138 (0.011)
BaP	0.27**	0.015*	n.d.
Pn		0.4*	0.008-0.194 (0.047)
BbF	0.017**		n.d.
BkF	0.017**		n.d.
Bghi	0.0082**		n.d.
DhA			

IP	Insufficient data		n.d.
BaA		0.018*	n.d. – 0.068 (0.006)
Py		0.025*	0.021-0.123 (0.041)
Pesticides (µg.L^{-1})			
Alpha-lindane	0.04	0.01	n.d.-0.355 (0.029)
Gamma-lindane	0.04	0.01	n.d. – 0.281 (0.064)
Beta-lindane	0.04	0.01	n.d.
Delta-lindane	0.04	0.01	n.d.
Heptachlor	0.0003	Insufficient data	n.d.
Aldrin	Insufficient data	Insufficient data	n.d.
Trans-chlordane		Insufficient data	n.d.
Cis-chlordane		Insufficient data	n.d.
Endosulfan I	0.01**	0.02	n.d.
Dieldrin	Insufficient data	Insufficient data	n.d.
Endrin	Insufficient data	Insufficient data	n.d.
Endosulfan II	0.01**	0.02	n.d.
4,4’-DDT	Insufficient data	Insufficient data	n.d.
Endrin aldehyde		Insufficient data	n.d.
Endosulfan sulfate		0.02	n.d.
Endrin ketone		Insufficient data	n.d.
Heptachlor epoxide (isomer A and B)	0.0004**	No objet	n.d.
Chloroneb			n.d. – 0.094 (0.046)
Isodrine	Insufficient data		n.d.
P,p-DDT	Insufficient data		n.d.
Phthalates (µg.L^{-1})			
DMP			n.d. – 0.25 (0.110)
DEP		16	n.d. – 6.98 (3.001)
DBP		19	0.22 – 7.58 (2.404)
BBP			n.d.
DNOP		Insufficient data	n.d.
DEHP	Insufficient data		0.34– 20.76 (9.591)

Eu-WFD: European Union Water Framework Directive
CWGQ: Canadian Water Quality Quidelines for the protection of Aquatic life
*Interim Guideline
**Maximum Allowance Concentration

Table 3: Threshold values of EQS of individual PAH, phthalate and pesticide (Canadian Environmental Quality Guidelines, 2007; Directive n° 2013/39/UE of 12/08/13 modifying the directives 2000/60/CE and 2008/105/CE), and contamination levels detected in Somme River.

The results in Table 3 show the contamination level of phthalates and pesticides under the maximum allowance concentration (MAC) of EU-WFD. However, the concentration of alpha-lindane and beta-lindane exceed CWQG value (> 0.01 µg.L^{-1}) for some sampling points (at Fontaine-les-Clercs and Castres for alpha-lindane, and at Fontaine-les-Clercs, Artemps, Castres and Berthencourt-sur-Somme for beta-lindane). For PAH, the concentration detected at Somme River water do not exceed the MAC of EU-WFD. Nevertheless, some measured points showed the value higher than interim guideline set by CWQG for An, Fl, BaA and Py.

Conclusion

The extract water samples was analyzed for 16 PAHs and 18 Me-PAHs, 6 phthalates, 28 pesticides, 28 PCBs, using gas chromatography/mass spectrometry (GC/MS) for the total of 96 targeted compounds. Each contaminant was quantified to assess their contamination levels in 13 sites of Somme River in northern France. Recorded concentrations showed significant contaminations of Somme River. Large variation of concentration was observed from one sampling site to others for the concentration level of hydrocarbons. Hydrocarbons contaminations were dominated by two rings and three rings respectively for Me-PAHs with average of 72% and PAHs with average of 75%. This work reported for the first time the contamination level of phthalates in Somme River. The same order of magnitude of phthalate concentration was observed with fifteen sampling stations in Somme River; the $\Sigma_6$phthalates varies from 7 to 23 µg.L^{-1}. DEHP and DEP are phthalates the most abundance in this River; their average represents respectively around 63% for DEHP and 20% for DEP. Among 28 targeted pesticides, only chloroneb, alpha-lindane and beta-lindane were detected and quantified. Moreover, their concentrations were relatively low close to limited guidelines for drinking water which limited the sum of pesticides at 500 ng.L^{-1}. According to the guideline values set by EU-WFD, Somme River present the good quality respect to these five families selected (PAHs, Me-PAHs, PCBs, phthalates and pesticides). However, these contamination levels could affect the aquatic life because they frequently detected at the values exceeded the guideline values set by CWQG.

It is interesting to note that Somme River is a place where there is a breeding fish and local fishing activities. The generally gap information could be a major source uncertainty in evaluation of water quality and so on the decision that the authorities could take. Nevertheless, there is growing evidence that these kinds of study are potentially important sources of informations which contribute to the quality evaluation of the aquatic ecosystems and some decision of local authority (e.i fishing activities, breeding fish).

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CONCLUSION

Les milieux aquatiques naturels subissent une pression anthropique croissante et reçoivent des quantités non négligeables et inquiétantes des micropolluants organiques. Ces polluants proviennent des diverses activités urbaines, industrielles et agricoles. Des suivis environnementaux permanents se sont alors avérés nécessaires afin de se progresser dans la compréhension des origines et des conséquences de la présence de ces polluants. Du fait de leurs faibles teneurs dans l'eau et les sédiments, leurs analyses nécessitent des méthodes à la fois spécifiques et sensibles. Les méthodes de prétraitement et d'analyses conventionnelles pour les contaminants organiques dans les eaux et les sédiments sont, en générale, consommatrices de temps et de solvants organiques. Les enjeux actuels sont de développer des méthodes appropriées, rapides, consommant moins de solvant, robustes, reproductibles. De plus, ces méthodes doivent permettre l'analyse de composé présent à l'état de trace et ultra-trace dans des matrices environnementales très complexes.

Mes travaux de thèse sont réalisés en collaboration entre le laboratoire de Geosystèmes d'Université Lille 1 et le laboratoire de Science de l'eau de et l'environnement d'Université Libanaise. Ces travaux ont permis de développer des nouvelles méthodes d'extraction rapides, économiques, respectueuses de l'environnement en utilisant moins de solvants toxiques. Le développement de ces méthodes s'est effectué à l'aide d'un modèle mathématique Umetrics (MODDE). Ce modèle couplé avec les expériences en laboratoire permet d'optimiser les méthodes d'extraction des pesticides et des hydrocarbures (HAP, Me-HAP et *n*-alcanes) présents respectivement dans l'eau et dans les sédiments. Comparés aux méthodes conventionnelles (LLE, Ultrason, Soxhlet,…), ces méthodes nécessitent moins de temps, moins de solvants organiques et assez sensibles pour analyser des contaminants organiques à l'état de traces en milieux aquatiques. De plus, les rendements d'extraction sont très satisfaisants avec une bonne reproductibilité grâce à l'automation.

Le deuxième volet de ces travaux de thèse a été consacré à l'application des méthodes optimisées pour étudier les milieux naturels. La nature de polluants, le niveau de contamination et son origine ont été étudiés dans les milieux aquatiques en France et au Liban. Les résultats montrent la présence des pesticides organochlorés dans les eaux souterraines de la région Akkar-Nord du Liban. Cette étude montre des résultats inquiétants en raison des niveaux de contamination élevés malgré l'interdiction d'utilisation des pesticides organochlorés. Compte tenu des résultats obtenus, il est très probable que ces molécules sont encore utilisées au Liban. Les travaux menés sur les sites d'étude en France ont permis de compléter les informations manquantes et d'évaluer les états écologiques des milieux vis-à-vis à ces micropolluants organiques.

Mes travaux de thèse ont conduit à des publications d'articles scientifiques dans des revues de range A et à la participation à des congrès scientifiques nationaux et internationaux.

Ces travaux offrent par ailleurs une première estimation de la qualité des eaux souterraines dans la le Nord de Liban quant au niveau de la contamination par les pesticides. Ils permettent également d'évaluer des états écologiques des sites dans le Nord-Pas-de Calais. Cependant, le nombre d'échantillons analysés durant la période de la thèse et les molécules étudiés sont assez limités. Il serait préférable de mener des études supplémentaires sur des zones plus larges, sur une période plus longue et sur plusieurs saisons pour mieux appréhender la distribution spatio-temporelle des micropolluants organiques en milieu aquatique. D'autres contaminants tels que les produits médicamenteux (à usage humain et vétérinaire) et de soins corporels devraient être également à prendre en considération. En effet, des études récentes ont montrées que ces molécules peuvent présenter des risques importants à la fois pour la santé humaine et pour le fonctionnement des milieux aquatiques. Ces mesures supplémentaires permettraient alors d'avoir des informations globales sur la pollution des eaux et d'évaluer des éventuels impacts liés à ces micropolluants.

CONCLUSION

Natural aquatic environments are under increasing anthropogenic pressure and receive significant and disturbing amounts of organic pollutants. These pollutants come from a variety of urban, industrial and agricultural inputs. Permanent environmental monitoring was then found necessary to make progress in understanding the origins and consequences of the presence of these pollutants. Because of their low concentrations in water and sediment, analyzes methods must be specific and sensitive. Pretreatment methods and conventional analyzes for organic contaminants in water and sediment are, in general, time-consuming and uses large volumes of organic solvents. Current challenges are to develop appropriate methods, fast, consuming less solvent, robust and reproducible methods. In addition, these methods should allow analysis for trace pollutants in complex environmental matrices.

My thesis work is conducted in collaboration between the laboratory of Geosystemes at University Lille 1 and Laboratory Science of water and environment at the Lebanese University. This work led to the development of fast, economical method that respects the environment by using less toxic solvents. The development of these methods is carried out using a mathematical model Umetrics (MODDE). This model coupled with laboratory experiments can optimize the extraction of pesticides and hydrocarbons (PAHs, Me-PAHs and n- alkanes) respectively present in water and sediment. Compared with conventional methods (LLE, Soxhlet ...), these methods require less time, less organic solvents and sensitive enough to analyze trace organic contaminants in aquatic environments. Furthermore, the extraction yields are very satisfactory with good reproducibility through automation.

The second part of the thesis was devoted to the application of optimized methods to study the natural environment. The nature of pollutants, the level of contamination and its origin were studied in aquatic environments in France and Lebanon. The results show the presence of organochlorine pesticides in groundwater in Akkar region of North Lebanon. This study shows worrying results due to high levels of contamination, despite the prohibition on the use of organochlorine pesticides. In view of the obtained results, it is very likely that these molecules are still used in Lebanon. Workin on the sites in France helped to complete the missing and assess the ecological information status of the media towards these organic micropollutants.

The thesis work led to publication of scientific articles in journals ranks A and participation in national and international scientific conferences.

This work provides also a first estimate of the quality of groundwater in the Northern Lebanon on the level of contamination by pesticides. They also assess the ecological status of some sites in the Nord-Pas- de-Calais. However, the number of samples analyzed during the period of the thesis and the molecules studied are quite limited. It would be preferable to conduct additional studies on larger areas, over a longer period and over several seasons to better understand the spatial and temporal distribution of organic micropollutants in the aquatic environment. Other contaminants such as drug products (for human and veterinary use) and body care should also be taken into consideration. Indeed, recent studies have shown that these molecules can pose significant risks to both human health and the functioning of aquatic environments. These additional measures would then have comprehensive information about water pollution and assess potential impacts of such pollutants.