A MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF ORGANIC CONTAMINANTS IN AGRICULTURAL SOIL

by

Feride Öykü Sefiloğlu

B.Sc. in Chemical Engineering, İstanbul Technical University, 2015

Submitted to the Institute of Environmental Sciences
in partial fulfillment of the requirements for the degree of
Master of Science
in
Environmental Technology

Boğaziçi University

2018

A MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF ORGANIC CONTAMINANTS IN AGRICULTURAL SOIL

APPROVED BY:

Prof Dr. Işıl Balcıoğlu Thesis Advisor	…….
Prof. Dr. Ayşegül Tanık	…….
Assist. Prof. Dr. Ulaş Tezel	…….

DATE OF APROVAL: 07/08/2018

ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor Prof. Dr. Işıl Balcıoğlu for her valuable support, comments, persistent help and guidance throughout my whole study for the last two years. Without her enthusiasm and encouragement this dissertation would not have been possible.

I am also thankful to my jury members Prof. Dr. Ayşegül Tanık and Assist. Prof. Dr. Ulaş Tezel for their valuable critics, suggestions and kindness. I am also grateful to be a part of the same research project with Assist. Prof. Dr. Ulaş Tezel, his passion of science was a great motivation to me.

I would like to thank all of my friends in the Institute of Environmental Sciences, especially to Özgücan Eken, Begüm Şepitçi, Göksu Çelik and Merve Ayaz for their friendships and help in the lab. I am grateful to my room friends and colleagues Ece Özön, Defne Şahin and Çağrı Akyol for their worthy friendships and supports. I am also very thankful to Filiz Ayılmaz for her kind assistance in my laboratory studies.

This thesis is supported by Boğaziçi University Research Fund Grant Number 17Y00P3.

Finally, special thanks to my mum, dad, brother, sister, little nieces Naz and Nil, and Kaan Kalaycıoğlu, for standing behind me with their endless support, care and love.

ABSTRACT

A MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF ORGANIC CONTAMINANTS IN AGRICULTURAL SOIL

Agricultural pollution is one of the biggest environmental concerns regarding the contamination of both soil and water resources. Owing to the mobility of pollutants in soil-water system, contamination of soil can be considered as a risk factor for the human health as well as aquatic ecosystem. Therefore, monitoring of soil contamination has a prime importance not only for the evaluation of the risk for the environment but also for the development of treatment and remediation methods for contaminated sites. Hence, development of a multiresidue analytical method was targeted for a wide range of chemicals selected from 39 frequently used pesticides in rice cultivation and 28 industrial pollutants detected in water samples collected from Ergene River in 2017-2018. Simultaneous extraction of the target analytes from soil samples and their quantification were performed with acetate buffered QuEChERS (quick, easy, cheap, effective, rugged, and safe) method and liquid chromatography coupled with tandem mass spectroscopy (LC-MS/MS), respectively. The developed method gave satisfactory recoveries within 70-120% for 78% of the target compounds. The method was applied to 22 soil samples collected from mainly paddy fields located in southwestern Thrace region adjacent to Ergene River in order to determine the agricultural pollution caused by the pesticide application and irrigational activities. The residues of the selected pesticides were found in all soil samples within the concentration range of 0.04-406 μg/kg, whereas the industrial pollutants were dominantly detected in soil samples taken from paddy fields as 0.05-807 μg/kg.

ÖZET

TARIM TOPRAKLARINDAKİ ORGANİK KİRLETİCİLERİN TAYİNİNDE ÇOKLU KALNTI ANALTİK METOD

Tarımsal kirlilik, hem toprak hem de su kaynaklarının kirlenmesine ilişkin en büyük çevresel sorunlardan biridir. Kirleticilerin toprak-su sistemindeki hareketliliği nedeniyle, toprağın kirlenmesi, insan sağlığının yanı sıra sucul ekosistem için de bir risk faktörü olarak düşünülebilir. Bu nedenle, toprak kirliliğinin izlenmesi sadece çevre için riskin değerlendirilmesi için değil, aynı zamanda kirlenmiş sahalar için arıtma ve iyileştirme yöntemlerinin geliştirilmesi için de büyük önem taşımaktadır. Bu nedenle, pirinç yetiştiriciliğinde sık kullanılan 39 pestisit ve 2017-2018 yıllarında Ergene Irmağı'ndan toplanan su numunelerinde sıklıkla tespit edilen 28 endüstriyel kirleticinin analizi hedeflenerek çoklu kalıntı analitik metodu geliştirilmiştir. Toprak örneklerinden hedef analitlerin eşzamanlı ekstraksiyonu ve bunların nicelleştirilmesi, sırasıyla, asetat tamponlu QuEChERS (hızlı, kolay, ucuz, etkili, sağlam ve güvenli) yöntem ve tandem kütle spektroskopisi (LC-MS / MS) ile birleştirilmiş sıvı kromatografisi ile gerçekleştirilmiştir. Geliştirilen yöntem, hedef bileşiklerin %78'i için %70-120 aralığında tatmin edici geri kazanımlar sağlamıştır. Yöntem, pestisit uygulaması ve sulama faaliyetlerinin neden olduğu tarımsal kirliliği belirlemek amacıyla güneybatı Trakya bölgesinde Ergene nehrinin bitişiğinde yer alan ve başlıca çeltik tarlalarından toplanan 22 toprak örneğine uygulanmıştır. Seçilen zirai ilaçların kalıntıları tüm toprak örneklerinde 0.04-406 µg/kg konsantrasyon aralığında bulunurken, endüstriyel kirleticiler özlellikle çeltik tarlalarından alınan toprak örneklerinde 0,05-807 µg/kg aralığında saptanmıştır.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Explanation
2,4-D	2,4-Dichlorophenoxyacetic acid
5-MeBT	5-Methyl-1H-benzotriazole
ACN	Acetonitrile
AF	Attenuation Factor
AF	Ammonium Formate
AHTN	Tonalide
AOAC	Association of Official Agricultural Chemists
ATN	Atenolol
BACs	Benzalkonium Chlorides
BDDA	Benzyldimethyldodecylammonium
BDTA	Benzyldimethyltetradecylammonium
BDHA	Benzyldimethylhexacylammonium
BFR	Brominated Flame Retardants
BPA	Bisphenol A
CBZ	Carbamazepine
CE	Collusion Energy
CEC	Cation Exchange Capacity
CHL	Chlorpyrifos
CLO	Clothianidin
CXP	Cell Exit Potential
DDT	Dichlorodiphenyltrichloroethane
DEA	Deethylatrazine
DEET	N,N-Diethyl-meta-toluamide
DEHP	Di-(2-Ethylhexyl) Phthalate
DEP	Diethyl Phthalate
DFZ	Difenoconazole
DP	Declustering Potential
dSPE	Dispersive Solid Phase Extraction
DT25	Dissipation Factor (25%)
EC	Emerging Contaminant
EDTA	Ethylenediaminetetraacetic Acid

EHMC	Ethylhexylmethoxycinnamate
EPB	Ethyl Paraben
ESI	Electrospray Ionization
FA	Formic Acid
FQ	Fluoroquinolone
GC	Gas Chromatography
GCB	Graphitized Carbon Black
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
HHCB	Galaxolide
HMMM	Hexa(methoxymethyl)melamine
HyA	Hydroxyatrazine
IBF	Ibuprofen
IMI	Imidacloprid
IPU	Isoproturon
Kd	Solid/liquid partition coefficient
K_{oc}	Soil Organic Carbon-Water Partitioning Coefficient
K_{ow}	Octanol/Water Partition Coefficient
LC	Liquid Chromatography
LC_{50}	Median Lethal Concentration
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LOD	Limit of Detection
LogP	Octanol-Water Partition Coefficient
LOQ	Limit of Quantification
MAE	Microwave Assisted Extraction
MCPA	2-Methyl-4-Chlorophenoxyacetic Acid
MCPP	Meta-Chlorophenylpiperazine
ME	Matrix Effect
MeOH	Methanol
MRM	Multi reaction monitoring
MSPE	Magnetic Solid-Phase Extraction
NP	Nonylphenol
NPEOs	Nonylphenol Polyethoxylate
NSAID	Non- Steroidal Anti-Inflammatory Drugs
OC	Organic Carbon
OPE	Organophosphate Ester

OTC	Oxytetracycline
PAH	Polycyclic Aromatic Hhydrocarbon
PCB	Polychlorinated Biphenyl
PCP	Personal Care Product
pKa	Acid Dissociation Constant
PLE	Pressurized Liquid Extraction
PMD	Primidone
PSA	Primary Secondary Amine
PTFE	Polytetrafluoroethylene
Q1	Parent ion
Q3	Fragment ion
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RSD	Relative Standard Deviation
Rt	Retention time
SFE	Supercritical Fluid Extraction
SOM	Soil Organic Matter
STP	Sewage Treatment Plant
TAED	Tetraacetylethylenediamine
TBEP	Tris(2-butoxyethyl) Phosphate
TC	Tetracycline
TCEP	Tris(2-chloroethyl) Phosphate
TCZ	Tebuconazole
THX	Thiamethoxam
TKN	Total Kjeldahl Nitrogen
TP	Transformation Product
TP	Total Phosphorus
TPPO	Triphenylphosphineoxide
UAE	Ultrasound assisted extraction
UV	Ultraviolet
WWTP	Waste Water Treatment Plant

1. INTRODUCTION

Contamination of environmental resources with thousands of anthropogenic chemicals as a consequence of rapid and uncontrolled development in agriculture, industry, urbanization, and transport is one of the most important global environmental issues. According to a 2016 report of EUROSTAT, the annual production of chemicals was about 340 million tonnes in Europe and over 50% of these compounds were classified as hazardous to the environment (EUROSTAT, n.d.). Although not all these chemicals end up in the environment as pollutants, hundreds of chemicals released to environmental compartments through waste streams, agricultural runoffs, accidental spills, etc. The environmental occurrence of these variety of compounds from different origins, called as "emerging contaminants (ECs)", have drawn attention in recent decades due to their harmful impacts on aquatic and terrestrial life, as well as human health.

Emerging contaminants include chemicals such as personal care products, pharmaceuticals, pesticides, illicit drugs, industrial chemicals, flame retardants, algal toxins, surfactants, metals etc. A broad range of contaminants in water are controlled by legislations of European Commission by Water Framework Directive (2000) including 45 industrial and agricultural compounds described as "priority pollutants", which is also extended by Environmental Protection Agency to 129 contaminants under the Clean Water Act (USEPA 2014). On the other hand, a common regulation under Soil Framework Directive is still under negotiation by EU (European Commission, 2012) despite the global concern over soil contamination. In Turkey, several heavy metals and some organic contaminants including PAHs, PCBs and organochlorine compounds are controlled by Soil Pollution Control Regulation (T.C. Çevre ve Orman Bakanlığı, 2005). While, the legislations are only covering a small part of the pollutants entering the environment continuously, 1000 compounds are classified as emerging substances and listed by EU NORMAN Network (2016), where the detection of various contaminants become the focus of many investigations, but still there are insufficient information about the fate and transport mechanisms and toxicological impacts of most of these pollutants to human and environmental health.

To evaluate the occurrence and fate of contaminants, their quantification at low concentrations has a prime importance. Considering the diversity of the contaminants, multiresidue monitoring become a widely used analytical method providing the simultaneous analysis in a short time by virtue of the recent developments in detection techniques. Although multiresidue analysis is applied frequently to investigate the contamination of freshwater sources around the globe (Bai et al., 2018;

Carpenter and Helbling, 2018; Kolpin et al., 2002), there are limited number of studies conducted for the detection of ECs in the terrestrial environment (Chiaia-Hernandez et al., 2017; Feng et al., 2015; Fernandes et al., 2013; Salvia et al., 2012). However, owing to the mobility of pollutants in soil-water system and uptake by plants, it is important to investigate the presence and fate of pollutants in soil to understand the consequences of anthropogenic pollution from a more holistic perspective.

Since soil is a nonrenewable source with life-supporting functions, agricultural contamination is an important environmental concern. The main sources of this pollution are extensive use of pesticides, application of manure, and sewage sludge as fertilizers and irrigation with reclaimed wastewater or other polluted surface waters. Pesticides are widely applied in farming practices for the protection of plants from weeds, insects, fungi etc., causing migration of the pollutants into soil and water. Additionally, veterinary antimicrobials are diffusing into agricultural soil due to the implementation of animal excrement on topsoil as fertilizer to meet the nutrient needs, which carries the manure-derived pollutants into farmlands (Johnson and Jürgens, 2003; Kuldip Kumar et al., 2005; Shore and Shemesh, 2003; Zhang et al., 2014a). Beside pesticides and antibiotics, other industrial and municipal derived ECs such as plasticizers, personal care products, surfactants, flame retardants can enter to the terrestrial environment through irrigation and sewage sludge amendment (Khan et al., 2017; Zheng et al., 2016; Chen et al., 2014). All these contaminants can damage the terrestrial health due to the accumulation of both the parent compound and their transformation products regarding their persistency and toxicity (Boxall, 2012; L. Du and Liu, 2012; Sinclair and Boxall, 2003).

The analysis of target analytes requires a sensitive and selective extraction and analysis due to the complex nature of soil. The sample preparation is the critical step for the isolation and concentration of the analyte from the matrix, which includes interfering compounds for the analysis. Among the traditional and time-consuming extraction techniques, QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction is increasingly employed for a wide range of pollutants in soil and other matrices with respect to its easily implemented, time effective and environmentally friendly procedure. Although this technique was developed for the extraction of pesticides in food samples (Anastassiades et al., 2003), it is also implemented for the extraction of pesticides (Feng et al., 2015; Fernandes et al., 2013; Yu et al., 2016b), antibiotics and hormones (Lee et al., 2017; Meng et al., 2017; Salvia et al., 2012) and pharmaceuticals (Carmona et al., 2017) individually from soil samples. This method includes the multiple separation of analytes by liquid-liquid extraction and subsequent solid liquid extraction to purify the extract. Clean- up of soil extracts is commonly performed by dispersive solid phase extraction (d-SPE) in most cases to reduce the matrix interferences and increase the extraction efficiency. For the identification and quantification of target analytes liquid

chromatography-tandem mass spectrometry (LC-MS/MS) is mostly preferred instrumental method regarding to its suitability for the analysis of analytes in a wider polarity range at low detection limits.

To date, several multiresidue analysis methods have been applied for the identification of organic contaminants in soil using QuEChERS. However, the simultaneous extraction covering a wide range of ECs including both pesticides and other industrial contaminants from soil has not been published yet. Although in Europe the residue levels of pesticides and some other organic contaminants in agricultural soils were reported, there is lack of information about the agricultural pollution in Turkey. Therefore, the aim of this study was to develop a multiresidue analysis for the extraction and quantification of 67 organic contaminants from different pollutant groups in soil and investigate the occurrence of these pollutants in agricultural soil samples collected from the fields of Ergene River bank in southwestern Thrace region of Turkey. The target pesticides were selected from the priority pollutant list and currently applied pesticides on the sampling region; while, the frequently detected contaminants in Ergene River were considered for the selection of other water derived emerging contaminants. The soil samples were collected mainly from the paddy fields in order to establish a relationship between soil and river contamination based on the occurrence data.

2. THEORETICAL BACKGROUND

2.1. Exposure Routes of Emerging Contaminants in the Environment

ECs can enter to the environment in several pathways, but in most cases the identification of the direct source is not possible. Since these compounds are released from industrial, domestic and agricultural activities and detected in the nature in all countries with varying concentrations, ECs become a global issue consequently. The origin of ECs is classified in two groups as point and non-point sources. Industrial discharges, mining activities, wastewater and sewage treatment plants with a spatially definite location are categorized as the point sources, which release commonly concentrated loads of emerging contaminants in the environment (Naidu et al., 2016). On the contrary, non-point sources are defined as diffuse sources, where the location of the pollution source is indistinct. Common examples of these sources are the overflow by rain in industrial and urban fields and runoff derived from the agricultural fields, where manure or sewage sludge are applied as fertilizing agents. Compared with the point sources, non-point sources generate lower concentrations of ECs and more challenging to detect and control the original source of contamination. Figure 2.1. demonstrates the possible pathways, sources and receptors of the pollution (Naidu et al., 2016).

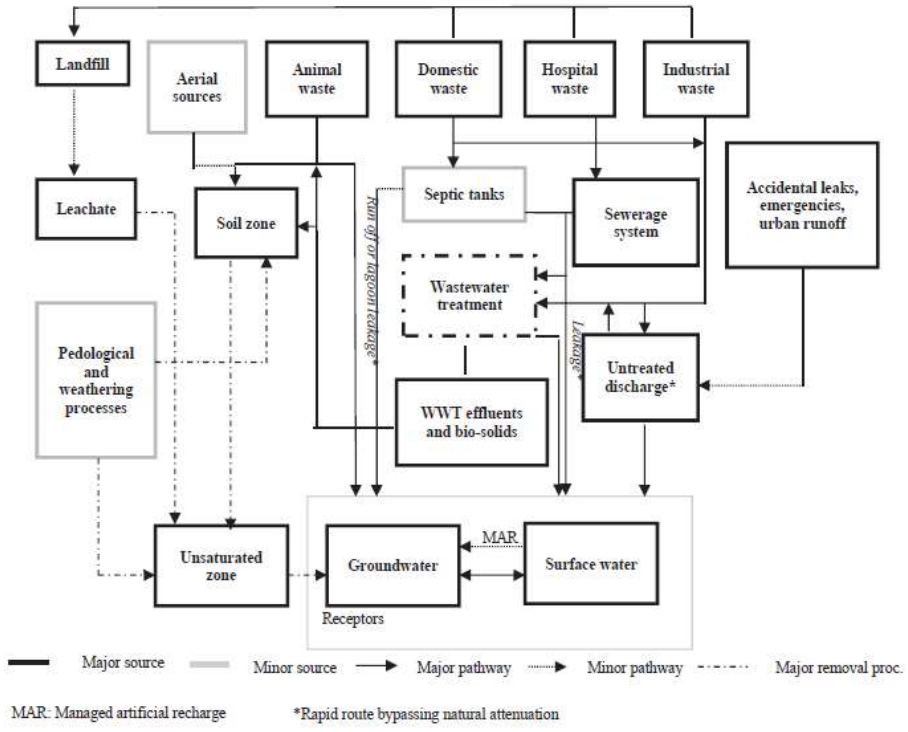


Figure 2.1. Sources, pathways, and receptors of emerging contaminants (Naidu et al., 2016).

2.1.1. Occurrence in the Aquatic Environment

The presence of emerging contaminants in the aquatic environment is mainly related to the discharges of wastewater and sewage treatment plants and industrial plants. WWT plants are the major sources of anthropogenic contaminants as pharmaceuticals, personal care products, plasticizers, endocrine disruptors, surfactants etc., since conventional treatment processes are not designed to remove the ECs. Consequently, these pollutants end up in fresh surface waters as rivers and lakes (Petrie et al., 2015). Although traditional water quality assessments have been focused on the detection of priority pollutants (USEPA 2014), organic contaminants, nutrients, heavy metals, and bacteria; the importance of the monitoring organic contaminants has been recognized over the past decade, regarding the possible adverse effects of these contaminants to the ecosystem. As shown in Figure 2.2, beside the direct discharges of ECs to the receiving water systems, runoff from urban and agricultural fields and leaching from landfills are potential sources of contamination. As wastewater effluents are the major contributor of pharmaceuticals, personal care products and endocrine disrupting compounds; agricultural emissions are the significant sources of pesticides, veterinary medicines and hormones in fresh water systems (Boxall, 2012). Figure 2.2. shows the possible pathways and fate mechanisms of the ECs in the aquatic environment.

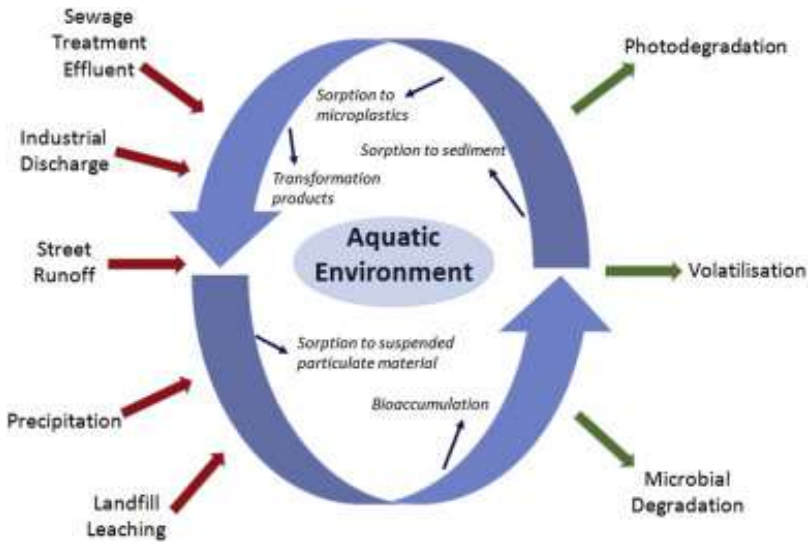


Figure 2.2. Emerging contaminants in the aquatic environment (Wilkinson et al., 2017).

Researches show that a wide variety of ECs are occurring in wastewaters and receiving surface waters (Rimayi et al., 2018; Doummar and Aoun, 2018; Palmiotto et al., 2018). With the new advances in analytical methods and detection techniques, recent studies performed in various countries have been focused on multiresidue monitoring of micropollutants from different classes, instead of investigating a small group of pollutants. Kolpin et al. (2002) investigated the presence of

95 organic contaminants in samples taken from 139 streams US nationwide for two years sampling period and detected 82 of these monitored pollutants including coprostanol (fecal steroid), cholesterol (plant and animal steroid), N,N-diethyltoluamide (pesticide), caffeine (stimulant), triclosan (disinfectant), tri(2-chloroethyl)phosphate (flame retardant), and 4-nonylphenol (nonionic detergent metabolite) were detected frequently. In another study performed in Denmark, Matamoros et al. (2012) reported the occurrence of 17 emerging contaminants in surface waters. Among these diclofenac (anti-inflammatory drug), 2-methyl-4-chlorophenoxyacetic acid MCPA (fungicide), caffeine, and tris(2-chloroethyl) phosphate (TCEP) were contaminants that have concentrations higher than 50 ng/L. It was also revealed that a rain-fall event resulted in higher concentration of pesticides.

Bai et al. (2018) investigated the occurrence of 271 ECs from different classes in South Platte River watershed located in Colorado/ USA within the sampling period of 2014 and 2015 and 109 pharmaceuticals out of 144 were detected in the samples, where metoprolol (beta blocker), gabapentin and lamotrigine (antiepileptic), DEET (insect repellent) were the most frequently found contaminants (>90%) in all samples with high concentrations (>1000 ng/L). Beside the pharmaceuticals, 55 waste indicator compounds were also monitored, and 42 compounds were detected, where the flame retardants namely tri(2-chloroethyl) phosphate, tri (2-butoxyethyl) phosphate, and tri (dichloroisopropyl) phosphate have the highest frequency (>60%) and concentrations (956-6680 ng/L). In this study, 72 pesticides were also analyzed in the samples considering the possible transport of these compounds to fresh waters and 39 of these target contaminants were found at least in one of the samples. Compared to other target micropollutants, pesticides were less abundant, and 2,4-D was the most frequently detected pesticide (98%) with a mean concentration of 114 ng/L, which is followed by MCPP (58.6 ng/L) and diuron (52.4 ng/L).

In a very recent study; 168 compounds out of 200 targeted micropollutants were detected at least in one of 127 sample, collected from Hudson River watershed (USA) (Carpenter and Helbling, 2018). In this study the sources of the contamination were also identified, where 116 of the pollutants were originating from wastewater effluents and 52 were agricultural-derived. Atrazine (herbicide), gabapentin (antiepileptic), metolachlor (herbicide), and sucralose (artificial sweetener) were found in all samples within concentration ranges of 1-204 ng/L, 3-6784 ng/L, 2-298 ng/L and 31-43400 ng/L respectively. Suclarose, atenolol acid (metabolite of atenolol and metoprolol), and metformin (antidiabetic) were measured as the highest concentrations in mg/L range in the samples collected from STP effluents with at least 95% detection frequency.

2.1.2. Occurrence in Agricultural Soils

Emerging contaminants enter to terrestrial environment via multiple pathways as shown in Figure 2.3. While the application of agricultural chemicals for pest control causes direct entry of ECs in soil, the application of manure and biosolids as fertilizers and the use of reclaimed wastewater for irrigation purposes results in indirect input of veterinary antibiotics, personal care products and human pharmaceuticals (Boxall, 2012). Some industrial pollutants may also present in soils, as a consequence of irrigation of the soil with surface waters exposed to severe pollution. Hence, the soil acts as sink of these contaminants, it can act also as a source of ECs to water resources by surface runoff and leaching mechanisms as well. The possible routes of introduction of ECs to the agricultural soils are presented in Figure 2.3.

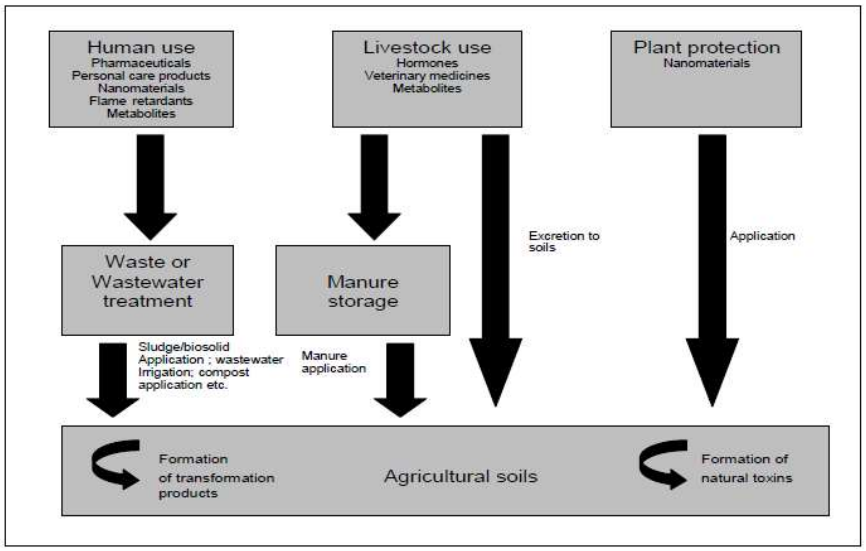


Figure 2.3. Routes of entry of ECs to the agricultural soils (Boxall, 2012).

Although the occurrence of EC in freshwater systems is a major topic in scientific literature, there are only a number of studies investigating the presence of these contaminants in agricultural soils, which are mostly focused on the investigation of pesticides, pharmaceuticals and PAHs in soil. It is well known that synthetic pesticides are applied in modern agricultural practices in a widespread manner to meet the food demand of increasing population. Extensive use of pesticides results in accumulation of persistent contaminants, which are also transferred to humans and other living organisms via food chain. Runoff and leaching of pesticides from soil to surface- and groundwater through rainfalls and irrigation is an important consideration for end users of water (Lu et al., 2015; Feng et al., 2015).

These consequences inspire the interest of researchers to investigate the occurrence of persistent pesticides and their transformation products (TPs) in agricultural fields in a widespread manner. For instance, 80 polar pesticides and their metabolites were analyzed in 29 agricultural soil samples collected between 1995 and 2008 in Switzerland. The results of this study show that in every sampling site at least 10 pesticides are detected at a concentration up to 330 μg/kg (Chiaia-Hernandez et al., 2017) While the detection rate of pesticides were 45%, TPs of the applied pesticides were 47%, which demonstrates the persistency of the agricultural chemicals and their metabolites over the past decade.

Fernandez-Alvarez et al. (2010) studied 36 pesticides in 45 different soil samples. Organochlorine pesticides like aldrin and dieldrin were detected frequently in agricultural soils frequently, although they have been banned years ago. Recent studies on the occurrence of pesticides in agricultural soil samples are listed in Table 2.1.

Table 2.1. Pesticide residues detected in agricultural soils.

Pesticide	Pesticide Type	Substance group	Concentration	Country	Reference
Carbendazim	Fungicide	Benzimidazole	1 - 61	Switzerland	Chiaia-Hernandez et al., 2017
Boscalid	Fungicide	Carboxamide	70-120	Poland	Łozowicka et al., 2017
Imazalil	Fungicide	Imidazole	-	-	-
Prochloraz	Fungicide	Imidazole	2	Switzerland	Chiaia-Hernandez et al., 2017
Quinoxyfen	Fungicide	Quinoline	-	-	-
Azoxystrobin	Fungicide	Strobilurin	2 - 86	Switzerland	Chiaia-Hernandez et al., 2017
Pyraclostrobin	Fungicide	Strobilurin	0.005-0.20	China	Zhang et al., 2012
Trifloxystrobin	Fungicide	Strobilurin	D	Switzerland	Chiaia-Hernandez et al., 2017
Difenoconazole	Fungicide	Triazole	1.1 and 5.1	China	Feng et al., 2015
Epoxiconazole	Fungicide	Triazole	5 - 23	Switzerland	Chiaia-Hernandez et al., 2017
Flutriafol	Fungicide	Triazole	-	-	-
Hexaconazole	Fungicide	Triazole	5.5-36	China	Yu et al., 2016
Myclobutanil	Fungicide	Triazole	D	Switzerland	Chiaia-Hernandez et al., 2017
Propiconazole	Fungicide	Triazole	1 - 5	Switzerland	Chiaia-Hernandez et al., 2017
Tebuconazole	Fungicide	Triazole	1 - 89	Switzerland	Chiaia-Hernandez et al., 2017
Prothioconazole	Fungicide	Triazolinthione	-	-	-
Metolachlor	Herbicide	Chloroacetamide	2 - 25	Switzerland	Chiaia-Hernandez et al., 2017
Pendimethalin	Herbicide	Dinitroaniline	2 - 163	Switzerland	Chiaia-Hernandez et al., 2017
Aclonifen	Herbicide	Diphenyl ether	-	-	-
Imazamox	Herbicide	Imidazolinone	ND	Brazil	Kemmerich et al., 2015
Diuron	Herbicide	Phenylamide	2 - 334	Switzerland	Chiaia-Hernandez et al., 2017
Chloridazon	Herbicide	Pyridazinone	2 - 11	Switzerland	Chiaia-Hernandez et al., 2017
Molinate	Herbicide	Thiocarbamate	10.5	USA	Smalling et al., 2007
Atrazine	Herbicide	Triazine	2 - 249	Switzerland	Chiaia-Hernandez et al., 2017
Simazine	Herbicide	Triazine	1-80	Switzerland	Chiaia-Hernandez et al., 2017

Table 2.1. Continued.

Pesticide	Pesticide Type	Substance group	Concentration	Country	Reference
Lenacil	Herbicide	Uracil	D	Switzerland	Chiaia-Hernandez et al., 2017
Isoproturon	Herbicide	Urea	2 – 4	Switzerland	Chiaia-Hernandez et al., 2017
Methoxyfenozide	Insecticide	Diacylhydrazine	4.61	Spain	Pose-Juan et al., 2015
Acetamiprid	Insecticide	Neonicotinoid	2.20-6.14	China	Zhou et al., 2018
Imidacloprid	Insecticide	Neonicotinoid	4 - 138	Switzerland	Chiaia-Hernandez et al., 2017
Dimethoate	Insecticide	Organophosphate	6.3	China	Liu et al., 2016
Ethoprophos	Insecticide	Organophosphate	-	-	-
Chlorfenvinphos	Insecticide	Organophosphate	31	Canada	Wan et al., 1994
Chlorpyrifos	Insecticide	Organophosphate	37 - 62	Switzerland	Chiaia-Hernandez et al., 2017
Chlorpyrifos	Insecticide	Organophosphate	0.6-19	Spain	Fernandez-Alvarez et al., 2010
Cypermethrin	Insecticide	Pyrethroid	10	Poland	Łozowicka et al., 2017
Mepiquat chloride	Plant growth regulator	Quarternary ammonium	-	-	-

D, detected but not quantified; ND, not detected; "-", no data available.

In addition to pesticides, veterinary antimicrobials and steroids are the pollutants diffused into agricultural soil due to the implementation of animal excrement on topsoil as fertilizer to meet the nutrient needs (Zhang et al., 2014; Kumar et al., 2005; Johnson and Jürgens, 2003; Shore and Shemesh, 2003). Łukaszewicz et al. (2017) detected seven antimicrobial veterinary drugs in 39 agricultural soils of Poland with a detection rate of 54% in the concentration range of 3.6- 57 µg/kg. Among the veterinary antibiotics, enrofloxacin and trimethoprim have the highest concentrations as 57 and 47.8 µg/kg respectively, where these antibiotics were the most frequently used drugs in confined animal feeding operations and they are not easily biodegradable due to strong sorption tendency on soil. Beside the manure implementation, human and animal medicine, personal care products and other untreated contaminants from wastewater treatment plants are spreading in the terrestrial environment due to the application of sewage sludge used as fertilizing agents (Larivière et al., 2017). The sewage sludge use in agriculture is controlled by EU Directive 86/278/EEC (European Commission, 1986) to prohibit the direct application of sludge before any biological, chemical or thermal pretreatment. However, this regulation set limit values only for heavy metals based on the knowledge of that time. Although the legislation was reviewed in 2000 (European Commission, 2000) to extend the analyzed compounds including nonylphenols, PAHs, DEHP, PCBs etc, the new ECs were not taken into account for the revision of the regulation. Dorival-García et al. (2015) studied the occurrence of 17 quinolone antibiotics in sludge samples used for composting purposes. 65 % of the monitored antibiotics were found in sewage sludges, where ciprofloxacin, ofloxacin and enrofloxacin were detected with the highest indicated concentration as 836 µg/kg, 719 µg/kg and 647 µg/kg, respectively.

Another source of emerging contaminants in agricultural soils is the utilization of reclaimed wastewater for crop irrigation. Due to limited available freshwater sources, in semiarid and arid areas, the reuse of treated municipal wastewater is an attractive solution to satisfy high water demand especially for agricultural sector. However, it brings an additional concern, since it is known that the treated wastewater effluents can contain various micropollutants including pharmaceuticals, PCPs, estrogens disinfectants, surfactants etc. (Kolpin et al., 2002). In Turkey, the use of treated wastewater in agriculture is controlled by Water Pollution Control Regulation (T.C. Çevre ve Orman Bakanlığı, 2004), in which the conventional parameters and heavy metals are taken into consideration to determine de suitability of the treated wastewater for irrigation purposes. Calderón-Preciado et al. (2011) investigated 47 organic micropollutants in crops of agricultural soils irrigated with reclaimed wastewater in Spain. In this study 26 of the pollutants including fragrances, pharmaceuticals, flame retardants, disinfection by products and pesticides were detected in the wastewater effluent, where 5 contaminants namely ibuprofen, naproxen, MDHJ, caffeine and tonalide were also detected in the crops grown with these irrigation waters.

2.2. Environmental Effects of Emerging Contaminants

The presence and accumulation of emerging contaminants in the environment have become an important issue due to their adverse effects on the aquatic and terrestrial life and human health. Persistent organic contaminants can be toxic to microbial community of both soil and water systems causing a decrease in biodiversity, sustainability, and quality of the environmental compartments. In addition, the uptake of the contaminants to the edible part of the plants and the bioaccumulation in aquatic animals are another consideration for human health due to the potential transfer of the contaminant residues via food chain.

2.2.1. Ecotoxic Effects

Although most of the studies are focused on the toxic effects of emerging contaminants on aquatic environment (von der Ohe et al., 2011; Farré, Pérez, and Kantiani, 2008; Smital et al. 2013), there are a few studies evaluating the impacts of ECs on soil biota. Even though the contaminants of emerging concern are the same for soil and water systems, the toxic impacts and fate of these pollutants on living-organisms can differ. Soil is a complex ecosystem including organic content, minerals and a wide range of terrestrial organisms. The toxicity of a compound can be reduced by the adsorption mechanisms on humus and minerals, which modulates the availability and mobility of in

soil. Therefore, a toxicity estimation of a contaminant for terrestrial ecosystem from studies conducted with aquatic organisms may not be a factual approach (Uwizeyimana et al. 2017).

Since the agricultural pesticides are the main cause of soil contamination, the ecotoxic effects of these compounds and their transformation products have grown interest. Considering the fact that pesticides are biologically active and toxic compounds; their impact on microorganisms is a serious concern on productivity, quality and sustainability of agricultural soils, which are critical for crop production (Imfeld and Vuilleumier, 2012). Although pesticides can be degraded by microorganisms or by chemical processes as hydrolysis, photolysis oxidation and reduction, the resulting metabolites can be even more toxic in some cases (Gomes et al. 2017). Sinclair and Boxall (2003) analyzed 37 pesticides and their 89 transformation products using the toxicity data on algae, fish and daphnia. The results showed that 70% of the transformation products have similar or less toxicity than their parent compounds. On the other hand, 30% of the TPs are found to be more toxic compared to the corresponding parent compounds.

Although the majority of the studies in the literature have been focused on the effects of pesticides on soil community individually, investigation of the combined effects of different pesticides have gained attention in recent years, since the terrestrial organisms are exposed usually to the mixture of contaminants, which can cause synergistic effects (Panizzi et al., 2017). For the toxicity evaluations, earthworms are commonly used as test species based on the end-points of growth, survival, reproduction and behavioral changes, since they play a crucial role on the nutrient cycle in soil which ensures the sustainability of the soil quality (Uwizeyimana et al. 2017). For instance, Yang et al. (2018) investigated the combined effect of four pesticides as acetochlor, chlorpyrifos, clothianidin, fenobucarb and one heavy metal chromium on the earthworm (Eisenia fetida) using the endpoint of avoidance behavior. The individual evaluation of the target compounds indicated that chlorpyrifos has the highest and fenobucarb has the lowest toxicity on *Eisenia fetida,* whereas the binary mixture of chlorpyrifos and clothianidin and the quaternary combination of chlorpyrifos, clothianidin, acetochlor and chromium showed synergistic effects on the test species. In another study, the acute toxicity of four pesticides including phoxim, chlorpyrifos, and lambda- cyhalothrin were determined using filter paper contact test (OECD 1984) on the same species (Cang et al. 2017). The results showed that imidacloprid is the most toxic compound with a LC_{50} value of 2.82 mg/kg in 14-day soil toxicity test, on the other hand some important synergistic effects were also found from the quaternary combination (LC_{50}=1.27) and ternary mixture of imidacloprid, phoxim and lambda-cyhalothrin (LC_{50}=1.53). These studies show that using individual toxic effects of compounds can be misleading to underestimated predictions by the evaluation of the joint action of contaminants.

Beside the pesticides, municipal and industrial derived emerging contaminants can also present in significant concentrations in agricultural soils due to the manure and biosolids application and irrigation activities. However, the toxicological impacts of these compounds on terrestrial environment are poorly understood considering the lack of information on the occurrence of these compounds in soil to derive researchers to examine their toxicity to exposed organisms (Petrie et al., 2015). Pino-Otín et al. (2017) investigated toxicity of 18 widely used pharmaceutical compounds on the physiological diversity of soil microorganisms, by analyzing the impact of contaminants on the ability of organisms to degrade different carbon sources as carbohydrates, amino acids, carboxylic and ketonic acids, and amines/amides on soils collected from ecological field. This study indicated that antibiotics sulfamethoxazole, trimethoprim, and tetracycline have the highest impact on microbial community at concentrations from 100 mg/L. Beside the antibiotics, ß-blockers as nadolol and blood lipid lowering agents showed intermediate toxicity, where non- steroidal anti-inflammatory drugs (NSAIDs) as ibuprofen, diclofenac and paracetamol have found to be less toxic.

In addition to pharmaceuticals, a large variety of industrial compounds as flame retardants, plasticizers, surfactants etc. end up in the environment resulting to the accumulation and ecotoxic impacts on the terrestrial ecosystem as well. Contamination of soil with industrial compounds in terms of toxicity on soil-dwelling organisms were less studied compared to pesticides and pharmaceutical compounds. Domene et al. (2009) assessed the toxic effects of nonylphenol polyethoxylates (NPEOs), which are used as surfactants in the industry. In this study the toxicity of nonylphenol (NP), which is the final biodegradation product of NPEOs, was evaluated using different soil species including earthworms, enchytraeids, collembolans and plants and reported the lethal endpoints. It is observed that NP have LC_{50} values of 240-523 mg/kg for earthworms, 64-226 mg/kg for soil invertebrate reproduction and >1000 mg/kg for plants, respectively. Results showed the by-product NP is more toxic to terrestrial ecosystem than the parent compounds (NPEOs).

2.2.2. Uptake by Plants

Contamination of soil is a significant concern on behalf of food safety due to the potential transfer of these organic compounds to edible parts of the plants. Accumulation of the contaminants on the root, uptake by the plant and the translocation within plants depend on the soil characteristics and physico-chemical properties of the compounds. The hydrophobicity of a neutral organic compound is used as the major factor for the evaluation of the contaminant accumulation on the root of the plant with respect to the linear relationship between hydrophobicity and root uptake. However,

for ionic compounds different mechanisms as electrical attraction repulsion forces are affecting the uptake of these contaminants by roots (Wu et al., 2015).

The uptake of emerging contaminants on several plants and vegetables is investigated for different types of compounds in the literature. Kumar et al. (2005) focused on the uptake of antibiotic chlortetracycline and tyclosin on corn, green onion and cabbage grown in manure applied soils. In this study it is reported that chlortetracycline was detected in all plant tissues with a concentration range of 2–17 μg/kg fresh weight, where tyclosin was not adsorbed on plants, which is explained by the its low adsorption tendency onto soil resulting less bioavailability for plants. As a consequence of accumulation on plants, antibiotics can also affect the growth of the plants. For instance, Boxall et al. (2006) reported that phenylbutazone, oxytetracycline, and enrofloxacin dramatically drop down the growth rate of plants subjected to these antibiotic substances.

Occurrence and accumulation of pesticide residues in soil brings also an additional concern on the plants grown in that contaminated soils. Ge et al. (2017) assessed the translocation and uptake of commonly used pesticides in paddy cultivation namely imidacloprid (IMI), thiamethoxam (THX) and difenoconazole (DFZ) in rice crops. In this study it is demonstrated that all the pesticides can be accumulated by the rice plants, where imidacloprid, thiamethoxam were detected more in the leaves and difenoconazole rather in the roots. The translocation of IMI and THX from root to leaf were explained by their hydrophilic behavior, whereas lower upward transport of DFZ was attributed to its low water solubility and high $\log K_{ow}$ (4.4). The occurrence of the primary transformation product of THX, clothianidin (CLO), was also investigated in this study, where CLO was found both in soil and plant tissues, suggesting that THX can be degraded in both matrices. However, CLO was detected in plants in much higher concentrations and earlier than soil, which is indicating that THX can be degraded in plants more easily and rapidly.

Wu et al. (2014) studied the effects of wastewater irrigation on plant breeding by investigating uptake of 19 pharmaceuticals and personal care products on 8 vegetables including carrot, cucumber, tomato, lettuce, celery, spinach, cabbage and bell pepper. In this study the crops irrigated with both reclaimed wastewater and fortified-water were assessed. Evaluating the edible parts of the vegetables, in 64% of the wastewater irrigated samples, at least one target contaminant was detected with a concentration range between 0.31 and 3.87 μg/kg. The most frequently detected compounds in edible tissues were found as carbamazepine and meprobamate with a rate of 31%, each, where these rates are increased up to 89% in case of fortified water application. Beside these compounds; caffeine, DEET, dilantin, naproxen, primidone, and triclosan were also found in edible parts of the vegetables

indicating their tendency to move on plant tissues in a soil-plant system. From the results it is concluded that neutral and basic compounds exhibit higher uptake on plants, since naproxen is the only acidic chemical reached to plant tissues, which is explained by the repulsion forces acting on anions by the negatively charged plasma membrane of the plant cells.

Aparicio et al. (2018) examined other 35 industrial and household chemicals including surfactants, disinfectants, UV filters, plasticizers and hormones in root and leafy vegetables. The developed multiresidue method on plants were applied on carrot, potato, turnip, lettuce, spinach, and chard samples supplied from markets. The most frequently detected compounds were perfluorobutanoic (perfluoroalkyl compound), alkylbenzene sulfonates (surfactants) and triclocarban(bioside) in both leafy and root vegetables with concentrations up to 6.8, 45.6 ,6.0 μg/kg, respectively. In this study, dispersion of the contaminants for leafy and root plants showed an alteration. For instance, as the UV-filter benzophene-2 (BP-2) was only found in leaf vegetables with a detection rate of 100%, plasticizer di-(2-ethylhexyl) phthalate (DEHP) and surfactant 4-nonylphenol (NP) were only fund in root plants (92-100%).

2.2.3. Health Effects

The presence and accumulation of emerging contaminants in the environment comportments are considered to be an important issue regarding the human health, since these pollutants enter human body via food chain and drinking water. Although for the detection of the ECs an important progress has been made, the potential adverse effects of these pollutants to human population are poorly known.

Beside the toxic effects on soil and aquatic biota, pesticides and other ECs are associated with adverse effects on human health. Humans are exposed to pesticides via different pathways as inhalation, skin contact or food chain. The negative impacts of pesticides on human body include various carcinogenic, endocrine, neurological etc. problems. Organochlorine pesticides are abandoned in Europe regarding to their persistent behavior in the environment and carcinogen and mutagenic effects on human body (Correia-Sá et al., 2012). As the residues of pesticides can be found in foods and vegetables, which cannot be completely removed by washing, the exposure of the contaminants on human body is inevitable (Nicolopoulou-Stamati et al., 2016). This is also proven by the studies conducted on human tissues, breast milk and urine (Barnett-Itzhaki et al.,2018).

Hartle et al. (2018) investigated the occurrence of 23 persistent organic pollutants including pesticides, plasticizers, flame retardants and polychlorinated biphenyl compounds (PCBs) in 21 human milk samples. 19 contaminant residues were detected in raw milk and whereas 18 of them were still found in pasteurized milk, suggesting the stability of the compounds against pasteurization. Within the monitored contaminants, PCBs and organochlorine pesticides (DDTs) were the most abundant groups with 100% frequency rates and median concentrations of 22.6 μg/kg and 1262 μg/kg, respectively. The only compounds responding to pasteurization were pesticides permethrin and chlorpyrifos, where their mean concentrations were decreased by 62% and 82%. Considering the that milk is the first food for babies ensuring the protection against diseases, the parental transfer of ECs to children is an important concern.

2.3. Fate of Emerging Contaminants in the Environment

After released in the environment the fate and transport of emerging contaminants depends on several mechanisms and parameters. Contaminants may be adsorbed on solid particles, degraded biologically, chemically or physically and transferred to other environmental compartments. ECs undergo these fate processes based on the compound properties, as well as the characteristics of receiving environment and natural conditions. In the following section the behavior of ECs in aquatic and terrestrial ecosystems are reviewed with respect to these factors.

2.3.1. Fate in Water

Natural attenuation of ECs in the aquatic environment depends on various processes involving dilution, hydrolysis, biodegradation, photolysis, and sorption, (Pal et al., 2010). All these processes are affected by the environmental conditions as climate, flow and water hydrology (Wilkinson et al., 2017).

2.3.1.1. Sorption. ECs released in the aquatic environment may attenuate by retaining on sediments and suspended solids with respect to their sorption tendency. However, sorption onto solid particles does not always mean a complete removal from aquatic environment, since it can be remobilize through flooding (Petrovic et al., 2016). Since sorption can affect the mobility it has a profound effect on the other fate processes and impact of pollutants. The sorption of emerging contaminants cannot be evaluated by taking into account only the physicochemical properties (e.g. hydrophobicity, polarity, acid base dissociation constant), as the characteristics of aqueous and solid phase (e.g. pH,

cation exchange capacity, ionic strength, surface area) can influence this fate process(Petrovic et al., 2016). Various sorption mechanism can happen under various environmental conditions.

Hydrophobicity of the compounds and the amount of the organic matter of the natural solids are two important factors determining the sorption behavior especially for neutral organic compounds in water bodies. It is well known that, with increasing K_{ow} and decreasing water solubility of the compound and increasing organic matter of solid particles, the adsorption of organic compounds onto sediments and particulate matter become stronger and faster in return. Gao et al. (1998) investigated the sorption kinetics of 7 pesticides on sediments before and after the removal of organic matter. As expected, the adsorption of more hydrophobic compounds namely bifenox, anilazine and terbutylazine occurred within a short time in a greater extent and as the organic matter is removed by H_2O_2 treatment, the adsorbed amount of all the pesticides were decreased significantly.

For organic contaminants with ionizable functional groups, the partitioning in the aquatic environment is based primarily on the pH of the water and pKa value of the compound. Electrostatic attraction between target contaminant and the solid or dissolved components of aqueous environment can be important on the sorption process. Besides, complexation of ionized contaminant with matrix components of aqueous phase can influence the sorption or desorption Hence, the effect of pH for most of the antibiotics having multiple ionizable functional groups cannot be clear as opposed to some polar pesticides and anti-inflammatory drugs (pKa<7) which retain preferentially in the dissolved fraction of the aqueous system (Petrovic et al., 2016). However, it was shown that basic and hydrophobic contaminants including chloramphenicol, sulfamethazine, famotidine, salbutamol etc. having a pKa value of greater than 7, were prone to bind to solid particles due to the cationic interactions, hydrogen bonding and other complexation mechanisms in river water (Silva et al., 2011)

The presence of exchangeable cations in the solid particles have also a great influence on the sorption of ionizable contaminants. Luo et al. (2011) investigated partitioning of various veterinary antibiotics between sediment and aqueous phase of a river basin located in China considering the effects of chemical composition of river phases. Although both tetracycline (TC; log K_{ow}=0.08-0.09) and sulfonamide (SA; K_{ow}=-0.09-0.91) antibiotics are relatively polar and ionizable compounds, SAs were abundantly detected in river samples, as TCs were rarely found in aqueous phase. The relatively low detected concentrations of tetracyclines in water were explained by their high tendency to adsorb on sediments with respect to the total organic carbon content and cation exchange capacity of the solid particles, which promote the binding of the compounds through exchange and bridging of cations.

Beside the physicochemical properties of compounds and adsorbing material, hydrological conditions like the variation of flow rate of a river system effect the sorption of contaminants on solid phase. As the flow rate of the river increases, limited available contact time of contaminant-sediment system inhibits the sorption of the ECs (Wilkinson et al. 2017). Additionally, high flow rate of river can result in the dilution of contaminants hence the partitioning of them between aqueous and solid phases can be influenced (Luo et al., 2011).

2.3.1.2. Degradation. Contaminants in water can be attenuated through degradation and transformation mechanisms via chemical and biological pathways. The biotransformation of the organic contaminants carried out by microorganisms that are present virtually everywhere in nature can be limited by environmental conditions e.g. depends on many parameters as presence of microbial community, nutrient content, temperature, pH, as well as the river hydrology and salinity, nutrients and oxygen contents. Bioavailability of the contaminants is a fundamental factor for the degradation by aquatic microorganisms, therefore the dissolved contaminants retained in the water fraction are more likely to be degraded (Wilkinson et al., 2017).

The persistency of a contaminant in the aquatic environment may be influenced by the availability of microbial community together with the properties of water as temperature and salinity. Bondarenko et al. (2004) studied the microbial degradation of four widely used pesticides including chlorpyrifos, carbaryl, diazinon, and malathion in surface waters. As the result of experiments degradation of carbaryl and malathion was found to be mainly abiotic, since their half lifes did not affected by the sterilization significantly. However, in case of sterilization half lifes of chlorpyrifos and diazinon is increased up to 4 and 8.5 times, respectively. In this study the effect of temperature and salinity of the water were also investigated. The results of the degradation experiments conducted at 21℃ and 10℃ indicated that persistency of all the pesticides were increased in case of low temperature. With increased salinity, the half lifes of the persistent chemicals diazinon and chlorpyrifos were further increased. These results represent the importance of the spatial conditions for the evaluation of persistency and overall risks of contaminants.

Beside the biological degradation, photolysis of the organic compounds is another important degradation mechanism. Photochemical transformation of contaminants in aquatic environment takes place in two different pathways: as direct and indirect transformation. Direct photolysis is the transformation of a molecule of interest as the consequence of solar light absorption while indirect photolysis involves the reaction of a molecule with reactive and short-lived species formed by photochemical reactions. (Petrovic et al., 2016). The transformation of a contaminant through direct

photolysis depends on the ability of the compound to absorb sunlight based upon its the molecular structure. Here, the π-bond configuration of the compound determines capability of sunlight absorption. Compounds having functional groups with conjugated double bond are more capable to absorb light (Wilkinson et al., 2017). On the other hand, indirect photolysis is mediated by photosensitized chromophoric molecules through solar radiation and the excited energy is emitted which results in the breakdown of organic contaminants. This energy transfer is mainly provided by the natural organic matter or by transient oxidants as singlet oxygen, peroxy radicals and hydroxyl radicals (Petrovic et al., 2016). Beside the physicochemical properties of the contaminant, photodegradation can also be affected by many environmental parameters as the solar fraction, temperature, location, and depth of the water. Since particulate matter in aquatic environment can contribute to the light attenuation due to light absorption and light scattering the probability of direct photolysis decrease with increasing suspended matter concentration of the matrix. Moreover, sorbed target compound on particulate matter can be shielded from the solar light.

Considering the potential persistence of contaminants of emerging concern, the degradation pattern of the pollutants are investigated in many studies. Yamamoto et al. (2009) examined the biodegradation and photochemical transformation of eight pharmaceutical compounds in aquatic environment. All of the targeted compounds were found to be relatively persistent for biological degradation, since about 80 percent of the initial concentrations were remained in the first three days of the experiment. However, some contaminants had degraded in case of direct sunlight like ß-blocker propranolol with a half life of 6 hours, whereas acetaminophen, ifenprodil, and indomethacin found to be moderately photodegradable with about 80% removal rate during 50 hours of exposure. Other four pharmaceutical compounds namely atenolol, carbamazepine, ibuprofen, and mefenamic acid were stable against solar light. In another study, Konstantinou et al. (2001) investigated the photodegradation kinetics of 6 pesticides namely atrazine, propazine, prometryne, propachlor, propanil and molinate in different waters as lake, river, ground marine and distilled water. The degradation rates of all compounds were lower in case of natural waters compared to distilled water according to the light absorbing effect of organic matter which acts as an optical filter for solar light, whereas the particulate matter and sediments scatter the light preventing its penetration beneath the surface layer. As the half lifes of atrazine and molinate were found as 43 and 62.4 days in river, their half lifes in distilled water were calculated as 34.5 and 44.7 days, respectively.

2.3.1.3. Bioaccumulation. Accumulation of the contaminants in living animals is another important concern of freshwater contamination due to the proposed risk for ecological toxicity and biomagnification through food chain. The bioaccumulation of the ECs in aquatic organisms can be

induced by ingestion of particles, direct transfer via skin or gills and biomagnification (Miranda et al., 2008). In Table 2.2 the concentrations of detected ECs in aquatic species were summarized based on the literature.

Table 2.2. The bioaccumulation of emerging contaminants in aquatic biota.

Aquatic Species	Contaminants	Concentrations	Reference
Fish	***Antideprassants***	(in brain tissues)	
	fluoxetine	1.58 ng/g	
	norfluoxetine	8.86 ng/g	Brooks et al., 2005
	sertraline	4.27 ng/g	
	desmethylsertralin	15.6 ng/g	
Fish	***Pharmaceticals***		
	Diphenhydramine	0.2-1.1 ng/g	
	Erythromycin	1.6-4.0 ng/g	
	Acetaminophen,Atenolol, Carbamazepine, Caffeine, Diclofenac Diltiazem, Gemfibrozil, Sucralose Sulfamethoxazole, Trimethoprim	ND	Du et al., 2016
Fish	***Organochlorine Pesticides***		
	Aldrine	44-78 ng/g	
	Dieldrin	26-40 ng/g	
	Endosulfan	91-113 ng/g	
	β + γ HCH	120-193 ng/g	Oliveira Ribeiro et al., 2005
	PAHs		
	Naphtalene	70-224 ng/g	
	Pyrene	104-331 ng/g	
	Benzo[a]pyrene	34-93 ng/g	
Sea urchin	***40 organic pollutants including:***		
	Cypermethrin	0.3-1.95 ng/g	
	Chlorpyriphos	3.8-5.6 ng/g	
	Tonalide	1.4-4 ng/g	Rocha et al., 2018
	Galaxolide	9-42 ng/g	
	TPPO	1.43-4.8 ng/g	
	TBOEP	18 ng/g	
Fish	***Emerging contaminnats***		
	Galaxolide	27000 ng/g (lw)	
	Bisphenol A	490 ng/g (lw)	
	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	470 ng/g (lw)	
	Tris(2-butoxyethyl) phosphate	230 ng/g (lw)	Blum et al., 2018
	Tributyl phosphate	120 ng/g (lw)	
	Triphenyl phosphate	98 ng/g (lw)	
	2-Ethylhexyldiphenyl	92 ng/g (lw)	

ND- not detected; lw- lipid weight

As a result of freshwater contamination, anthropogenic compounds have been increasingly detected in aquatic organisms (Brooks et al., 2005; Du et al., 2016; Letcher et al., 2010). Blum et al. (2018) investigated the occurrence and bioaccumulation of 32 ECs including pesticides, fragrances, organophosphates, plasticizers and surfactants in fish and water samples collected from River Fyris/ Sweden, where the effluents of a sewage treatment plant were discharged. In fish samples, the highest detected concentration of 27000 μg/kg was belonging to galaxolide (fragrance) with the highest bioaccumulation factor, which is explained by the high persistency, low solubility and high hydrophobicity of the compound. In addition, the surfactant 2,4,7,9-tetramethyl-5-decyn-4,7-diol,

organophosphate compounds as tributyl phosphate, triphenyl phosphate, tris(2-butoxyethyl) phosphate and additive bisphenol A were all detected in fish tissues within the concentration ranges of 92-490 µg/kg in lipid weight.

Pesticide residues are also detected frequently in aquatic organisms. Most of the studies have been focused on the detection of organochlorine pesticides in fish tissues with respect to their persistent behavior in the environment (Guo et al. 2008; Singh and Singh, 2008; Oliveira Ribeiro et al., 2005). Miranda et al. (2008) examined both organochlorine and triazine pesticides in the muscles and livers of the fish samples collected from lake Ponta Grossa /Brazil. Among the investigated contaminants, diuron and its metabolite namely dichloroaniline were found in highest concentrations in liver samples as 661 µg/kg in total with a detection rate of 60%. On the other hand, the most abundant pesticides with 100% detection rate in all fish samples were found as hexachlorobenzene, aldrin, DDT and its transformation products with concentrations more than 100 µg/kg. A similar study was also conducted on the edible fish samples from Marmara Sea/Turkey (Coelhan et al., 2006). The organochlorine pesticides namely p,p′-DDE and p,p′-DDD were found as the most abundant contaminants in all 12 fish samples.

Results of the related studies are perturbational considering the fact that a chronic exposure of these contaminants through fish and seafood consumptions can pose a serious risk to human health, since these contaminants were associated with several disorders, carcinogenic and toxic effects on human body (Miranda et al. 2008).

2.3.2. Fate in Soil

Due to the heterogenous and complex structure of soil matrix, the bioavailability, mobility and sorption of the contaminants highly depend on the physico-chemical properties of the compounds and soil characteristics as pH, texture moisture and organic matter content as well as the climatic conditions (Correia-Sá et al., 2012). According to these parameters, ECs released in the terrestrial environment can go through several processes as sorption on soil matter, physical and chemical or biological degradation, plant uptake, runoff to surface waters or leaching to groundwaters. Figure 2.4. demonstrates the fate and transport pathways of the contaminants in soil.

2.3.2.1. Sorption. As mentioned in 2.3.1.1 both properties of target contaminant and solid phase characteristics play role in their interaction during the sorption process. Since sorption can affect the mobility and bioavailability, it has a profound effect on the other fate processes and impact of

pollutants. Various sorption mechanism can happen under various environmental conditions and it is not easy to predict sorption of emerging contaminants by taking into account only the physicochemical properties. However, evaluation of sorption behavior can be performed with sorption coefficient K_d, which indicates the partitioning of contaminant between phases. It is well known that sorption behavior of many ECs is highly related to the organic carbon content of the soil and hydrophobicity of the contaminant (Weber et al., 1983). The sorption of hydrophobic contaminants can be well described with K_{ow} parameter as shown in the study of Papadopoulou et al. (2016) who investigated the sorption of three widespread used pesticides namely, chlorpyrifos (CHL), isoproturon (IPU), and tebuconazole (TCZ) and their metabolites in soil. The comparison of the sorption tendencies of these three pesticides infers the relationship of hydrophobicity and bounding to soil, since CHL has the highest sorption affinity with a $\log K_{ow}$ value of 4.7, whereas IPU was weakly adsorbed to soil ($\log K_{ow}$=2.5).

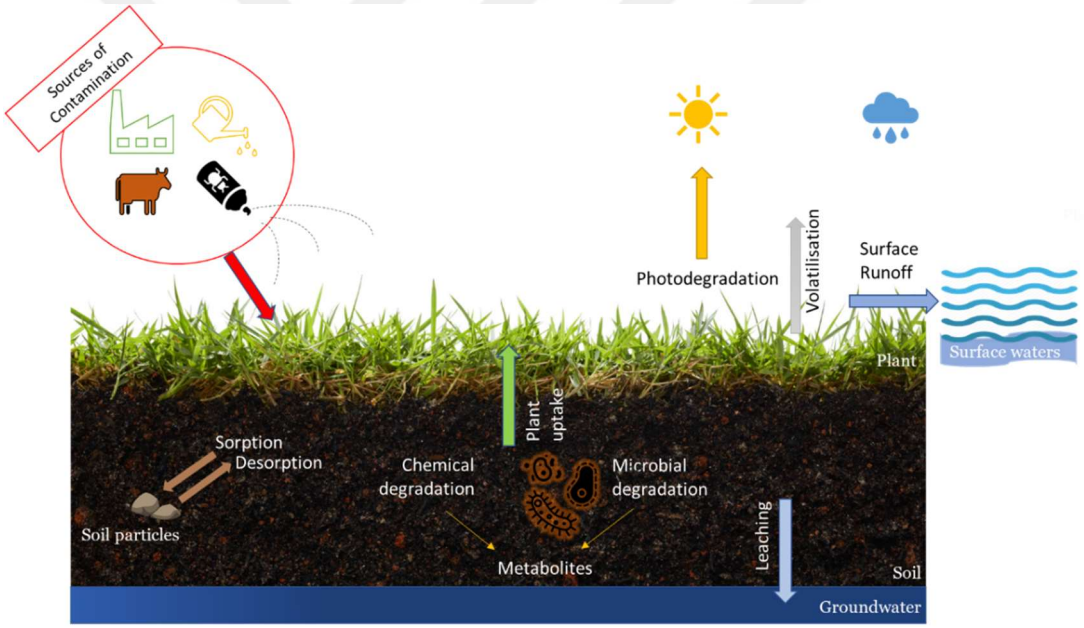


Figure 2.4. Fate and transport pathways of ECs in soil.

The sorption of polar and ionizable contaminants can occur with hydrophobic interactions besides other mechanisms. Especially for charged contaminants, the electrostatic attractions can also cause the partitioning of them between soil and aqueous phase depending upon the pH of soil and the acid-dissociation constant (pKa) of contaminant (Petrie et al., 2015). Accordingly, in the study of Park et al. (2018) who investigated the sorption behavior of three pharmaceuticals ibuprofen (IBF), carbamazepine (CBZ), and atenolol (ATN) to soil organic matter (SOM), it is shown that beside the hydrophobic interactions, electrostatic attractions were responsible on sorption mechanism of charged compounds on organic matter. Results indicated that, as the intensity of the electrical charge

of anionic SOM was increasing, the sorption of charged molecules was enhanced in return. Moreover, at pH 7, the positively charged ATN (pKa=9.5) was absorbed onto SOM with a removal efficiency of 70%, while only 10% of the negatively charged IBF (pKa=4.9) was retained on SOM.

2.3.2.2. Degradation. The persistency of the emerging contaminants in soil depends on the degradation behavior of the compounds for differing conditions. Beside the anaerobic processes as chemical and photochemical degradation, aerobic biodegradation is the main process of depletion for the ECs in soil matrices, contaminants can also degrade via reactions with sunlight or water in soil, which are called as photolysis and hydrolysis, respectively.

The availability of the microbial community is the main factor for the attenuation of organic contaminants in soil by degradation. Hurtado et al. (2017) assessed biotic and abiotic degradation of 8 organic contaminants from different classes with a wide range of physico-chemical characteristics in agricultural soil samples within 40 days period. The results of the study indicated the importance of the microbial community for the degradation of selected contaminants. While the degradation rates of bisphenol A (BPA), carbamezapine (CMZ) and primidone (PMD) were less than 50%, diethyl phthalate (DEP), ethyl paraben (EPB), and 5-Methyl-1H- benzotriazole (5-TTri) exhibited more than 70% degradation. On the other hand, the contribution of sorption and/or hydrolysis to the degradation of DEP, EPB, and tris(2-chloroethyl) phosphate (TCP) was assessed by preventing any possible photolysis reactions.

Beside the presence of microorganisms, the degradation of soil contaminants is highly related to the bioavailability of the compounds, which in return depends upon their water solubility and polarity (Gevao et al., 2000). Depending on the soil properties and the sorption tendency of the organic contaminant, only a part of the concentration become accessible for the terrestrial organisms. Therefore, in most of the studies in the literature, sorption and degradation of contaminants in soil were investigated in tandem. For instance, Yu et al., (2013) investigated the sorption and degradation behavior of 5 personal care products namely carbamazepine (CBZ), bisphenol A (BPA), triclosan (TCS) gemfi- brozil (GFB), triclosan (TCS), and octylphenol (OP) have in 3 different soils with varying texture and organic carbon contents. In this study, it is confirmed that the retention and persistency of the organic compounds in soil are increasing in accordance with the high organic content and higher K_{ow} values of compounds and the absence of the microbial community. For instance, the half life of OP was 9.8 in soil having an organic fraction of 0.41%, which was increased up to 14.3 days in soil with 3.02% organic content. The half lifes of all the target compounds were prolonged up to 3.9 times in case of sterilization, which emphasized the influence of microbial activity

on the persistence of contaminants. In another study, Bending et al. (2006) investigated the the degradation mechanisms of 3 pesticides namely azoxystrobin, diflufenican and isoproturon in two different soils, together with examining the association of biodegradation rates with soil characteristics. As the degradation of diflufenican rate does not change according to the soil type; in case of isoproturon, degradation rates differ largely for the two soil samples, where the 25% dissipation factor (DT25) is increased from 0.56 to 4.4 weeks when soil with higher OC% is tested. Furthermore, the biodegradation of azoxystrobin was found to be strongly dependent on soil pH, where the DT5 factor decreased by 4.25 for one unit increase of pH.

Enhancement of organic matter and contaminants of soil by the amendment of manure and sewage sludge to agricultural fields affect the degradation pattern of the contaminants in soil matrices. Contamination of agricultural fields with veterinary drugs through manure application leads to changes in the microorganism content and decrease in enzymatic activities of the soil (Du and Liu, 2012). It was shown that the co-occurrence of antibiotics and pesticides in soil can inhibit the biological degradation of pesticides (Jiang et al., 2018). Oxytetracycline (OTC) a tetracycline antibiotic adversely affected the bacterial and fungal abundance due to antimicrobial effect hence, decreased the degradation rate of the pesticides namely atrazine, simazine, terbuthylazine, metribuzin, acetochlor and metolachlor. Increasing concentration of antibiotic exerted a dramatic effect on the result. For instance, the half life of metolachlor was increased from 11.7 days to 18.2 days by the presence of OTC at 5 mg/kg, and further increased up to 43.3 days in case of 50 mg/kg OTC application.

It should also be taken into account that the amendment of manure and sewage sludge can enrich the microbial community in the soil increasing the degradation capacity of the medium. This beneficial effect was observed in the study of Sánchez et al. (2004) who investigated the degradation of three different organophosphorus pesticides diazinon, dimethoate and fenitrothion within 90 days incubation period. The results showed that the use of sludge accelerated the degradation of biodegradable pesticides fenitrothion and dimethoate, but it had an adverse effect on the degradation of diazinon, since the use of sludge prevented its bioavailability and chemical dissipation respectively.

2.3.2.3. Transport. Contaminants in the terrestrial environment can be transported to surface- and groundwaters through leaching and runoff. Beside the properties affecting the contaminant-soil interactions, the climatic conditions as amount and heaviness of the rainfall and temperature are significant factors for the transport processes (Boxall, 2012).

Contaminants with less sorption affinity and high persistency are considered to be more mobile in soil suggesting a potential risk to reach to groundwaters. Chefetz et al. (2008) examined the vertical mobility of three pharmaceutical compounds naproxen, diclofenac and carbamazepine in soil profile with laboratory experiments. The retardation of diclofenac and carbamazepine were relatively high contrary to naproxen due to the sorption on SOM in the top layer while the mobility of these contaminants significantly increasing on the deeper SOM-poor layers of soil. The result of the study revealed the leaching probability of these compounds in semi-arid regions where the reclaimed wastewater is used for agricultural irrigation. The contamination risk of groundwater with carbamazepine can be important due to its low degradation rate in soil (Yu et al., 2013).

Besides the organic content, the texture of soil can influence the transport of contaminants. In another study, the sorption and leaching potential of two widely used persistent cationic surfactants benzalkonium chlorides (BACs) are investigated in three different soils with varying textures. The results showed that due to the long carbon chain and positive charge of these compounds and, BACs were retained especially on clay soils surface more likely rather than leaching through the soil layers, where less than 1% of the target contaminants leached through the column in case of sandy loam texture, which does not indicate a possible risk to the aquatic environment (Khan et al., 2017).

The mobility of contaminants can be enhanced under excessive precipitation or flooding conditions even the contaminants exhibit high sorption tendency. In the study of Arias-Estévez et al., 2008) the presence of some pesticides having the K_{oc} value of >1000 in groundwater sources indicated that the transport of these pesticides is possible in case of an excessive precipitation shortly after applied on soil surfaces.

The mobility of the contaminants in the terrestrial environment is also affected by the irrigation water. When wastewater is used for agricultural irrigation, the mobility of strongly sorbed contaminants through soil can be expected due to the complexation of the contaminant with the dissolved organic content of the wastewater. In the study of Salazar-Ledesma et al. (2018), the effect of the wastewater irrigation on the fate and transport on atrazine and its metabolites (deethylatrazine (DEA) and hydroxyatrazine (HyA)) was investigated by collecting the soil samples from different depths, percolating waters and soil solution from the field, periodically. Although atrazine has the highest sorption capacity onto top soil (K_d =5.33), its metabolites have less affinity to retain on soil, especially DEA with a K_d value of 1.49. Irrıgation that was performed right after the application of atrazine with a dose of 0.004 mg/L, resulted in percolation of 65 percent of the applied pesticide through the soil and reached to leachate.

In order to predict the risk of leaching and runoff of the pollutants to freshwater systems, several estimation models and indices are built and developed. For instance, Aravinna et al. (2017) investigated the mobility of 32 pesticides in paddy field evaluating their runoff and leaching potential to aquatic systems. In their study, the leaching potential of the pesticides were estimated using an attenuation factor (AF), which is based on many parameters as the half life and hydrophobicity of the compound, as well as the bulk density and organic content, depth and moisture content of the soil. From the calculations carbofuran, quinclorac and thiamethoxam were found as the most mobile compounds in soil layers having the potential to leach to groundwater systems. A similar estimation was also made for the runoff potential of the pesticides carbofuran and quinclorac were found again in the risk group of a potential runoff to surface waters regarding to their high mobility.

2.4. Multiresidue Analysis of Emerging Contaminants in Soil

Considering the diversity of the contaminants in the terrestrial environment, multiresidue analysis of ECs have become the major focus of researchers, since these methods provide simultaneous analysis of multiple compounds in shorter time and with lower cost (Martins et al., 2014). Compared to water samples, there are limited number of multiresidue analysis conducted for soil samples targeting organic contaminants due to the complex nature of soil, which requires a selective and sensitive extraction and analysis method. Here, sample preparation is the critical step for target contaminant analysis, which contains isolating the analyte from the matrix, cleaning up the matrix, and concentrating the analyte for detectable limits if needed.

For the extraction of target compounds from soil matrix, liquid extraction with an appropriate solvent is performed by an appropriate solvent based on the properties of target compounds and in order to enhance the extraction efficiency several instrumental techniques can be applied. The traditional sample preparation methods based on solvent extraction with mechanical shaker and Soxhlet extraction have been replaced with high pressure and/or high temperature methods; pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE). Although these modern sample preparation techniques increase the extraction efficiency with small amount of solvent and shortened extraction times, all these require expensive equipment and a further clean-up step. Beside these extraction techniques QuEChERS (quick, easy, cheap, effective, rugged, and safe) method have been extensively applied for the extraction of variety of compounds in soil and other matrices, due its low cost and easily implemented procedure.

A further clean- up step is occasionally applied after extraction, in order to eliminate the matrix interferences on the detection and quantification of the analytes. Purification is performed with cartridges like HLB and Florisil or with dispersive solid phase extraction (dSPE) using as primary secondary amine (PSA), C18 and graphitized carbon black (GCB). The separation and quantification of the multiresidue analysis of soil samples are commonly performed by either gas (GC) or liquid (LC) chromatography coupled with mass (MS) or tandem mass spectrometry (MS/MS), since these detectors offer high sensitivity and selectivity, as well as low limit of detection (LOD) values.

2.4.1. Extraction and Purification

2.4.1.1. QuEChERS. This method was first developed by Anastassiades et al. (2003) for the determination of pesticides with a wide range polarity in food samples with high water content like fruits and vegetables. QuEChERS technique provides high quality results in a short time by eliminating complicated steps employed in conventional techniques with low consumption of sample and solvent, low cost, low waste and toxic compounds (Pszczolinska and Michel, 2016). Due to these advantages, this method has become the most conventional extraction technique in recent years with respect to its cost-effective and environmentally friendly aspects.

The procedure involves solvent extraction of target analytes with acidified acetonitrile, followed by a phase separation of organic and aqueous phase by various salts and purification of the extract with dispersive solid phase extraction (d-SPE). The developed sample preparation methodology was further standardized for pesticide analysis in food samples with respect to two buffer type: Acetate buffer is used in the American standard (AOAC) prefers acetate buffer (2007), whereas citrate buffer is utilized in the European standard EN 15662 (2008). The comparison of the original and standardized QuEChERS methods is demonstrated in the following flow charts in Figure 2.5.

The QuEChERS method is used as a template and applied further for multiresidue analysis of different matrices and target compounds with some modifications. This method is widely used for the multiresidue pesticide analysis from different matrices other than fruits and vegetables like milk (Golge et al., 2018), honey (Zheng et al., 2018) and fish (Song et al., 2018). In recent studies, this method is especially preferred for the pesticide analysis in soil (Fernandes et al., 2013; de Gerónimo et al., 2015; Chiaia-Hernandez et al., 2017). Although, QuEChERS method is primarily validated for pesticide analysis, it is further applied for the extraction of antibiotics (Salvia et al., 2012; Lee et al., 2017; Meng et al., 2017), pharmaceuticals (Carmona et al., 2017), hormones (Bergé and Vulliet, 2015) from soil samples.

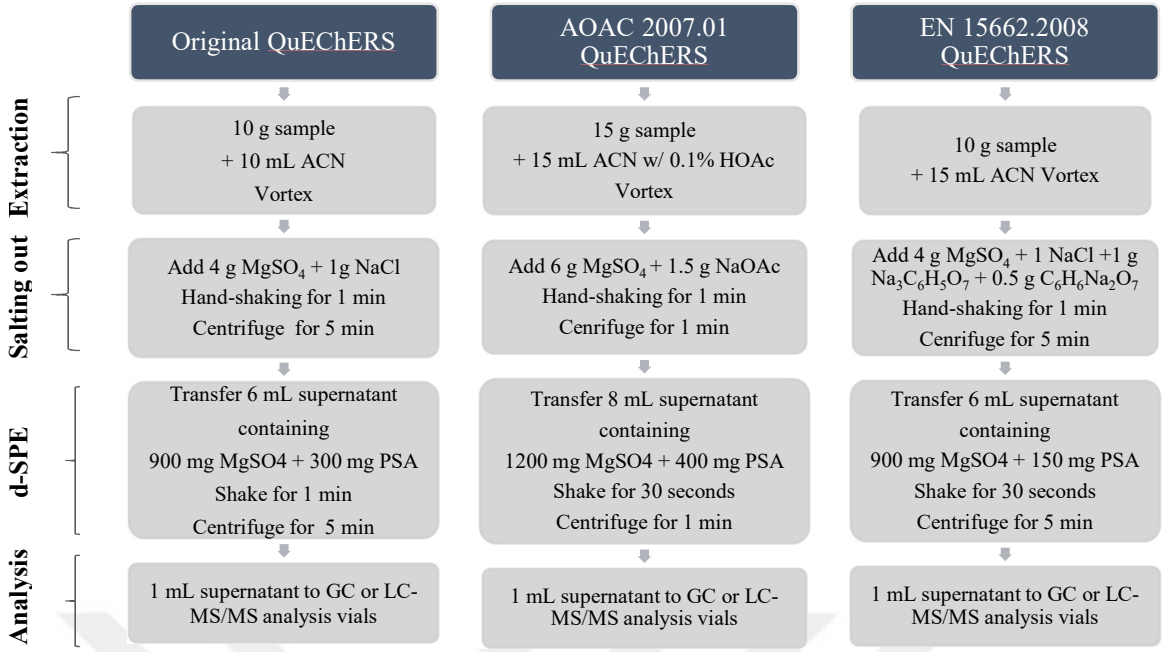


Figure 2.5. Flow chart of original and standardized QuEChERS methods.

For the analysis of pesticides and other pollutants in soil with QuEChERS method, some adjustments are made with respect to the characteristics of target compounds in the literature. Modifications of the procedure are mostly based on the hydration of the soil samples, sample size, extraction solvent, amount and variation of buffers and salts and elimination/revision of d-SPE. These alterations and their impact on the extraction performance on soil analysis are reviewed in the following paragraphs in more detail.

Since the original method is applied to the fruits and vegetables with high water content, hydration of the soil samples as the pretreatment step is necessary to provide the partitioning of water soluble interfering compounds (Lee et al., 2017; Yu et al., 2016; Fernandes et al., 2013). Water is utilized for the homogenization of the sample and accessibility of the soil pores for the extraction solvent (Pszczolinska and Michel, 2016). Fernandes et al. (2013) analyzed 36 pesticides in soil with QuEChERS and compared the extraction efficiencies of hydrated and non-hydrated samples. Results showed that for every target compound, the recovery rates were higher in case water addition at the first step.

Although the original method suggest a sample weight of 10 g, in some of the studies the sample size is reduced to 5 g for soil analysis (Feng et al., 2015; de Gerónimo et al., 2015). Studies showed that reduction of the sample weight provides better homogenization and better segregation of the supernatant (Fernandes et al., 2013).

In most of the studies acetonitrile was used as the extraction solvent as suggested in the original method, since it provides a good compatibility with the target analytes from a wide range of polarity levels and can easily be separated from the aqueous phase. In fact, acidified acetonitrile provides protein denaturation and minimize the lipid coextraction, where the recoveries of polar compounds are improved. In most cases, where LC is used as the separation technique, acetonitrile is acidified with acetic or formic acid to improve recoveries due to the compatibility with mobile phases and chromatographic separation. Alternative to acetonitrile, ethyl acetate, acetone and mixture of other solvents are also used for QuEChERS extraction. Yu et al. (2016) compared the effect of the solvent to the extraction efficiency for the development of a multiresidue analysis targeting 58 pesticides from soil. For this purpose, ethyl acetate, mixture of acetone and n-hexane (1:1 by volume) and acetonitrile with 1% acetic acid were tested and the highest recoveries were obtained for acidified acetonitrile case.

After the initial extraction with acetonitrile, various salts and buffers are used for the phase separation and partition of the target compounds. The choice of the buffer is an important parameter for the control of the pH for the extraction, especially for the pH-sensitive analytes. As the acetate buffer of AOAC method provides a pH of around 4.8, the pH of the citrate buffered EN method is around 5-5.5 (Figure 2.5). The effect of these two buffer types on the extraction recoveries were tested in many studies, and AOAC method is preferred for the analysis of pesticides and antibiotics in soil generally. Salvia et al. (2012) compared the efficiencies of EN and AOAC buffers for the extraction of antibiotics and steroid hormones from soil and indicated that acetate buffered extraction give better recoveries especially for the veterinary antibiotics as macrolides for 1.5-4 times and sulfonamides for 1.5-2 times. Yu et al. (2016) conducted the same comparison experiments for the determination of pesticide recoveries in soil. The results showed that recoveries of citrate buffered extraction was slightly less than acetate buffered method. In this study, extraction without any buffer was also tested and the recoveries were found unsatisfactory for 30% of the analytes with an extraction efficiency of less than 70%.

The amount and type of the salts are important factors to obtain a complete salt induced phase separation in liquid liquid extraction. For salting out, the type of solvent is determinant of the salt type, since the added salts reduce the solubility of the organic phase in water. In case of acetonitrile, $MgSO_4$ is the main salt used for in all versions of QuEChERS method in varying doses based on its capacity of binding a considerable amount of water, which consequently increases the partitioning of the analyte into the organic phase (Pszczolinska and Michel, 2016). The supplement of NaCl have also an additional effect on phase separation (Martins et al., 2014). As less water remains in the

acetonitrile phase, the recovery of polar compounds increases in return (Anastassiades et al., 2003). Caldas et al. (2011) designed the experiments to evaluate of addition of salts with / without NaCl for simultaneous analysis of 5 pesticides from soil and the highest recovery values for all target analytes were found for the joint use of 4 g $MgSO_4$ and 1 g NaCl.

Clean- up step is generally required after the extraction of the target compounds from soil samples with the extraction solvent, in order to eliminate the matrix interferences on the detection and quantification of the analytes. After QuEChERS extraction, for the purification of the extract, dispersive SPE (d-SPE) is preferred in general, where PSA, C18 and GCB sorbents are used in order to minimize the matrix effects and removal of excess water. PSA can ensure the retention of the polar compounds and fatty acids with its high chelating effect due to presence of the primary and secondary amine in its structure (Anastassiades et al., 2003). On the other hand, C18 can effectively bind starch fats and lipids, whereas GCB removes pigments and sterols from the matrix. In the literature, different combinations of d-SPE sorbents are applied to the multiresidue analysis of soil samples using QuEChERS (Feng et al. 2015; Lee et al. 2017; Meng et al. 2017). However, there are also studies for QuEChERS showing that better recoveries can be obtained without any clean up procedure since there is no loss of the analyte due to purification step (Caldas et al., 2011). Although the purification step is used to reduce matrix effects (ME), it can also enhance the MEs of the analytes on the contrary due to the highly increased concentration of soil extract (de Gerónimo et al., 2015).

2.4.1.2. Other extraction techniques and comparison with QuEChERS. Although QuEChERS is the most commonly employed method for the extraction of pesticides and other analytes from soil, some other techniques may also be utilized alternative to QuEChERS as well. The advantages/disadvantages of these alternative techniques are briefly discussed as the following. Additionally, the extraction efficiencies of these methods are compared with QuEChERS with respect to the available data in the literature. In Table 2.3, studies performed for multiresidue analysis of different target compounds in soil are listed and a comparison of the performed sample preparation, extraction, purification, quantification steps and resulting performance of these studies are made.

Ultrasound assisted extraction (UAE) uses the ultrasonic energy for the extraction of target analytes from solid samples. Ultrasonic energy provides an effective contact between solvent and sample with the generated bubbles through cavitation (Tadeo et al., 2010). This technique does not require expensive equipment, since it can easily be implemented on a simple ultrasonic bath. This method is applied for multiresidue analysis of pesticides and pharmaceutical compounds in soil samples (Babić et al.,1998; Chitescu et al., 2012). In the study of Chitescu et al. (2012) a comparison of the extraction

efficiency of UAE and PLE showed that PLE give better results with higher recovery rates and with lower limit of detection. On the other hand, Lesueur et al. (2008) compared the performance of UAE and PLE with QuEChERS for the extraction of 24 pesticides from soil samples. Although UAE as able to extract all the target analytes as QuEChERS, this technique resulted in the lowest recovery values. Moreover, QuEChERS method provide higher recovery rates between 27.3 and 120.9 %.

Pressurized liquid extraction (PLE) utilizes the extraction at elevated pressure and temperature, which results in increased efficiency. At these conditions, the solvent can diffuse in the sample more efficiently, which ensures reduction of the solvent consumption. However, it requires an expensive equipment and an intensive clean up procedure afterwards in connection with the co-extracted matrix components due to the elevated temperatures. Previous studies indicated that PLE method could be used in soil samples for the analysis of antibiotics (García-Galán et al., 2013), pesticides (Homazava et al., 2014) and some other organic contaminants (Hildebrandt et al., 2009) with an extensive clean up procedure. In the literature, several studies are conducted to compare the performances of QuEChERS and PLE for the extraction of pesticides (Homazava et al., 2014; Lesueur et al., 2008; Masiá et al., 2015; Prestes et al., 2012). In all of these studies, QuEChERS was found easier, cheaper and faster option than PLE with higher recovery values. For instance, Masiá et al. (2015) indicated that for the 50 targeted pesticides, the recoveries obtained in soil samples with PLE and QuEChERS were found as 38-85% and 25-92%, respectively. In case of PLE, recoveries of 8 pesticides were found below 50%, whereas by the use of QuEChERS only 3 pesticides were recovered lower than 50% efficiency. Comparing the analysis times, PLE took also 2 times longer than QuEChERS in the same study.

Microwave assisted extraction (MAE) is based on the application of microwave irradiation on the solvent and the sample. This method ensures rapid extraction with low solvent consumption, but has a weak extraction selectivity and requires an additional purification step (Wilkowska and Biziuk, 2011). This technique was applied to soil samples for the extraction of antibiotics (Turiel et al., 2006) and pesticides (Font et al. 1998; Merdassa et al., 2014). Di et al. (2015) inferred that MAE coupled with SPE clean up procedure gave compatible results with QuEChERS for the extraction of 10 organochlorine pesticides. However, Sadílek et al. (2016) found that compared to MAE and PLE, QuEChERS was the best method for the extraction of steroid estrogens from sediment samples providing the highest recovery rates in less time and money consumption.

Supercritical fluid extraction (SFE) utilizes supercritical fluids, mostly CO_2 as the extraction solvent, which is used occasionally together with small amounts of polar organic solvents as alcohols in order

to increase the polarity of the fluid (Raynie, 2006). This technique offers low solvent consumption, low labor intensity and short extraction time. On the other hand, it requires high equipment and maintenance cost, low selectivity and intensive clean-up step afterwards (Wilkowska and Biziuk, 2011). SFE is used for the extraction of pesticide residues from food (Cutillas et al., 2018), honey (Rissato et al. 2004) and soil (Rissato et al. 2005).

Magnetic solid-phase extraction (MSPE) is another extraction method, which is carried out by using magnetic adsorbents under ultrasonic action, followed by the desorption of analytes. This method offers reduced analysis cost and a simplified procedure due to the simultaneous extraction and clean-up opportunity by mixing the soil sample, extraction solvent and magnetic absorbent at room conditions. Based on the magnetic properties, absorbents can be isolated from the extract. However, this method is highly labor intensive, which cannot be operated for a large quantity of samples in a short period (Sun et al., 2010). This technique is performed on the analysis of antibiotics in soil samples (Sun et al., 2010) and pesticide analysis of water samples (Y. Song et al., 2007).

2.4.1.3. Purification of extracts. Clean- up step is generally required after the extraction of the target compounds from soil samples with the extraction solvent, in order to eliminate the matrix interferences on the detection and quantification of the analytes. For the purification of the extract, different methods are applied. Solid phase extraction (SPE) is mainly used technique due to its low solvent requirement and practical use, which can be coupled easily with HPLC. For this purpose, different cartridges are being used in the literature. For antibiotic analysis, HLB cartridges were preferred (Bian et al., 2015; García-Galán et al., 2013) after pressurized liquid extraction, where for pesticide analysis Florisil cartridges were used (Hildebrandt et al., 2009; Homazava et al., 2014).

After QuEChERS extraction, for the purification of the extract, dispersive SPE (d-SPE) is preferred in general, where PSA, C18 and GCB sorbents are used in order to minimize the matrix effects and removal of excess water. PSA can ensure the retention of the polar compounds and fatty acids with its high chelating effect due to presence of the primary and secondary amine in its structure (Anastassiades et al., 2003). On the other hand, C18 can effectively bind starch fats and lipids, whereas GCB removes pigments and sterols from the matrix. In the literature, different combinations of d-SPE sorbents are applied to the multiresidue analysis of soil samples using QuEChERS (Feng et al., 2015; Lee et al., 2017; Meng et al., 2017). However, there are also studies for QuEChERS showing that better recoveries can be obtained without any clean up procedure since there is no loss of the analyte due to purification step (Caldas et al., 2011). Although the purification step is used to

reduce matrix effects (ME), it can also enhance the MEs of the analytes on the contrary due to the highly increased concentration of soil extract (de Gerónimo et al., 2015).

2.4.2. Separation and Quantification

Separation and quantification step of the multiresidue analysis of soil samples are performed by either gas (GC) or liquid (LC) chromatography coupled with mass (MS) or tandem mass spectrometry (MS/MS). Since the selectivity and sensitivity of the method is highly depend on the detector type, instead of a variety of traditional detectors such as electron capture (ECD), diode -array, nitrogen phosphorus, mass spectrometers (MS) are increasingly employed for the detection and quantification of the analytes (Pszczolinska and Michel, 2016). GC analyzers are more preferred in the past, since liquid chromatography was coupled with less selective traditional detectors as UV, fluorescence, and diode array. However, MS/MS detectors with the new ionization techniques like electrospray ionization (ESI) highly increased the sensitivity and performance of LC analyzers by several orders of magnitude in the last decade (Alder et al., 2006). Recent developments in the mass spectrometry provides a great number of analytes in one single run with high resolution and accuracy. The multiple reaction monitoring (MRM) ensures the identification of compounds with MS/MS fragmentation ions and retention time without any need of reference standards (Chiaia-Hernandez et al., 2017).

Compared to GC-MS/MS, LC-MS/MS is becoming as the primary analytical tool for multiresidue analysis of complex matrices, as the developments of analytical instrumentation ensure detection of low concentration of antibiotics with high resolutions and selectivity. As most of the ECs are nonvolatile and in medium to high polarity range compounds, the separation is performed mostly by liquid chromatography for the multiresidue analysis, since GC- MS/MS requires a supplementary derivatization step for the non-volatile and thermally unstable compounds. Correspondingly, LC-MS/MS offers a wider scope detection and increased sensitivity with lower limit of quantifications. Performance comparison of these quantification methods is constructed by several studies, and the results showed that, determination of pesticides by LC-MS is more selective regarding lower LOD and LOQ values (Alder et al. 2006; Pose-Juan et al. 2014). Although LC-MS/MS provides high selectivity, sensitivity and robustness; matrix effects/ interferences are the most common drawback of this detection technique, which can affect the ionization of the compounds (de Gerónimo et al., 2015). Since the matrix of the soil can vary within different soil types, matrix effect analysis and correction are needed in this case to obtain reliable results.

Table 2.3. Selected methods to determine contaminants in soil samples.

Matrix	Target Pollutant	Extraction	Clean-up	Separation	Detection	Recovery (%)	LOD (µg/kg)	Reference
Pesticides & other ECs								
Soil from river basin	22 OCHs products and 16 EPA priority PAHs	PLE hexane, DCM, acetone, MeOH	SPE 5-g Florisil SPE cartridge	HP-5MS (30 m x 0.25 mm , 0.25 µm) Helium	GC-MS	71 - 133	0.19- 7.38**	Hildebrandt et al., 2008
Soil, from drainage sites	38 pesticides	QuEChERS, ACN	-	HP-5MSi (30 m ×0.25 mm, 0.25 µm) Helium	GC-MS	70–120	1.2-9.5	Yang et al., 2010
Soil, rice field	5 pesticides	QuEChERS, ACN w/ 1% HOAc	d-SPE with PSA & C18 compared w/ no clean up	XTerra 3.5 (50 mm x 3mm x 3.5 µm) MeOH : water with 0.1% FA (70:30, v/v),	LC-APCI-MS/MS	70.3 - 120	4-17	Caldas et al., 2011
Soil, organic agricultural areas	10 biopesticides	QuEChERS, ACN w/ 1% HOAc	-	Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 µm) MEOH: AF (aq) 5 mM	LC–MS/MS	68-116	1-5	Prestes et al, 2011
Soil, from from strawberry crops	36 multiclass pesticides	QuEChERS, ACN	d-SPE PSA, MgSO4, and C18	ZB-XLB (30m×0.25mm×0.25 µm) Helium	GC-MS/MS	70–120	1.5-7.6	Fernandes et al., 2012
Soil, CRM & agricultural soil	25 pesticides	PLE CH_2C_{l2}–acetone (1:1, v/v),ACN–H2O QuEChERS, ACN	Silica gel & Florisil d-SPE kit, PSA, C18, MgSO4	BEH C18 (2.1 × 50 mm, 1.7 µm) Water w/ 0.05% FA: MeOH w/ 0.05% FA	LC–MS/MS -ESI	65 - 122	0.1–2.9**	Homazava et al., 2014
Soil	36 pesticides	QuEChERS ACN	d-SPE PSA, MgSO4	C18 column (2.1mm × 100 mm, 2.6 µm) water with 0.01% FA: MeOH	LC-MS/MS EPI	63 - 126	0.04–0.8	Feng et al, 2015
Soil,	18 pesticides	QuEChERS, ACN	-	Brisa C18 (3 mm, 5cm 2.1 mm Water w/ 0.1 mM NH_4Ac): MeOH	LC–MS/MS	70–120	0.1-3.9	Geronimo et al., 2015
Soil, from agricultural areas	58 pesticides	QuEChERS ACN with 1% acetic acid	d-SPE PSA, C18, MgSO4	HP-5 MS UI (30 mm x 0.250 mm 0.25 µm) Helium	GC-MS/MS	69-120	0.1-5**	Yu et al.,2016

Table 2.3. Continued.

Matrix	Target Pollutant	Extraction	Clean-up	Separation	Detection	Recovery (%)	LOD (µg/kg)	Reference
Antibiotics, Pharmaceuticals, Hormones								
Soil, from agricultrual fields	Sulfonamides	MSPE Fe_3O_4/Al_2O_3 nanoparticle, ACN	-	ZORBAX SB-C18 (250mm×4.6mm., 5 µm) Water 0.5% acetic acid: ACN	LC-MS/MS- (ESI)	71-93	0.37 -6.74	Sun et al., 2010
Soil, agricultrual	Pharmaceuticals and fungicides	PLE, AC-citric acid	SPE, Strata X	Acquity C18 (100 mm x 2.1 mm, 1.8 µm) Water w/2 mM AF+FA; MeOH w/ 2 mM AF + FA	LC–MS/MS	>70	≤10	Chitescu et al., 2012
Soil from rural areas & river basin	Sulfonamide antibiotics and metabolites	PLE MeOH–water (90:10, v/v)	SPE Oasis HLB	Atlantis C18 (150 mm × 2.1 mm, 3 µm) Water w/ 10 mM FA: ACN w/ 10 mM FA	LC–MS/MS	60–130	0.01 -4.19	Galán et al, 2013
Soil	14 veterinary products, 11 hormonal steroids and 6 - human contaminants	QuEChERS ACN	SAX Strata-X	Zorbax Eclipse PLUS C18 (50 mm×2.1 mm, 1.8 µm) Water w/ 0.1% FA: MeOH	LC-MSMS	40-110	0.005–0.79 (µg/L)*	Salvia et al., 2013
Soil from livestock farm	Multiclass Antimicrobials	Solvent extraction with ultrasonication ACN:Na_2EDTA–McIlvaine	SPE, Oasis HLB	Zorbax SB-Aq C_{18} (150 mm x 2.1 mm, 3.5 µm) Water w/ 0.1% FA; ACN	LC-MS/MS- (ESI)	>60	0.01 - 2	Bian et al, 2015
Agricultural soil	Antibiotics (10 emerging contminants)	QuEChERS ACN w/ 1% HOAc	-	Eclipse C18 (3.0 mm ×150 mm, 3.5 µm) Water w/ 0.1% FA + 5 mM AF : MeOH w/ 0.1% FA + 5 mM	LC–MS/MS	60.2-120.3	0.15 - 3	Lee et al., 2017

* *_Instrumental Limit of Detection (ILOD)*

** *_ Method LOQ (MLOQ)*

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemical Substances

In this thesis, analytical standards of 75 chemicals that were purchased at their highest purity were used to develop an analytical method for their quantification in soil. The chemical structures and selected physicochemical properties of these target compounds are presented in Table 3.1.

Table 3.1. Structure, molecular weight, solubility LogP and Koc of targeted contaminants.

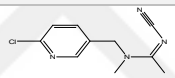
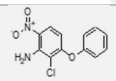
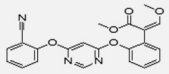
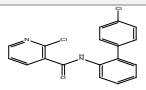
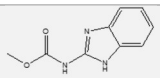
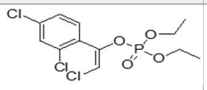
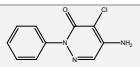
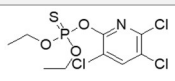
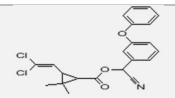
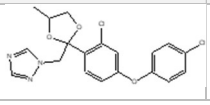
Compound	Molecular Formula	Chemical MW (g/mol)	Structure	Solubility in Water (mg/L)	log P	K_{oc}
			Pesticides			
Acetamiprid	$C_{10}H_{11}ClN_4$	222.06		2.950	0.800	200.0
Aclonifen	$C_{12}H_9ClN_2O_3$	264.66		2.5	4.04	6778.2
Atrazine	$C_8H_{14}ClN_5$	215.68		34.7	2.61	100
Azoxystrobin	$C_{22}H_{17}N_3O_5$	403.39		11.61	2.50	589
Boscalid	$C_{18}H_{12}Cl_2N_2O$	343.2		5	2.960	$4x10^4$
Carbendazim	$C_9H_9N_3O_2$	191.19		8	1.48	176
Chlorfenvinphos	$C_{12}H_{14}Cl_3O_4P$	359.57		124	3.81	380
Chloridazon	$C_{10}H_8ClN_3O$	221.03		14.900	0.91	120
Chlorpyrifos	$C_9H_{11}C_{l3}NO_3PS$	350.59		1.12	4.96	5509
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	416.29		0.004	6.06	307558
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	405.06		15	4.36	$2x10^4$

Table 3.1. Continued.

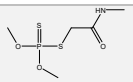
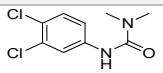
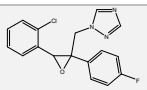
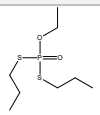
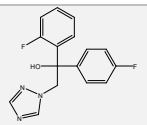
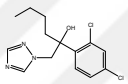
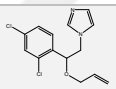
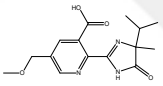
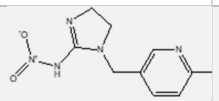
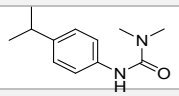
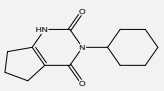
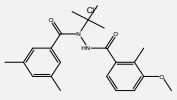
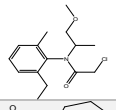
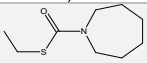
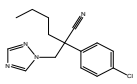
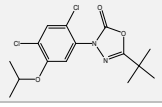
Compound	Molecular Formula	Chemical MW (g/mol)	Structure	Solubility in Water (mg/L)	log P	K_{oc}
Dimethoate	$C_5H_{12}NO_3PS_2$	229.24		6.626	0.78	24
Diuron	$C_9H_{10}Cl_2N_2O$	233.09		42	2.68	813
Epoxiconazole	$C_{17}H_{13}ClFN_3O$	329.07		7.1	3.4	$2x10^5$
Ethoprophos	$C_8H_{19}O_2PS_2$	242.33		1.300	2.99	70
Flutriafol	$C_{16}H_{13}F_2N_3O$	301.1		95	2.3	$5x10^4$
Hexaconazole	$C_{14}H_{17}Cl_2N_3O$	313.07		18	3.9	1040
Imazalil	$C_{14}H_{14}Cl_2N_2O$	296.04		184	2.56	1938
Imazamox	$C_{15}H_{19}N_3O_4$	305.14		626.000	5.36	139
Imidacloprid	$C_9H_{10}ClN_5O_2$	255.05		10	0.6	6719
Isoproturon	$C_{12}H_{18}N_2O$	206.28		65	2.87	251
Lenacil	$C_{13}H_{18}N_2O_2$	234.14		2.9	1.69	165
Mepiquat chloride	$C_7H_{16}ClN$	149.09		500.000	-3.55	n.d.
Methoxyfenozide	$C_{22}H_{28}N_2O_3$	368.21		3.3	3.72	402
Metolachlor	$C_{15}H_{22}ClNO_2$	283.13		530	3.4	120
Molinate	$C_9H_{17}NOS$	187.1		1.100	2.86	190
Myclobutanil	$C_{15}H_{17}ClN_4$	288.11		132	2.89	10^5
Oxadiazon	$C_{15}H_{18}Cl_2N_2O_3$	344.07		0.57	5.33	1915

Table 3.1. Continued.

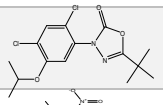
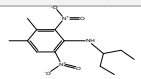
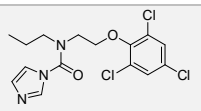
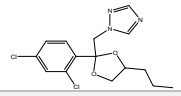
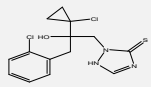
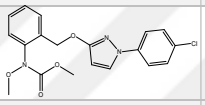
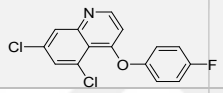
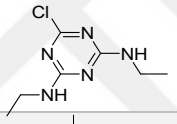
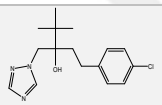
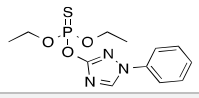
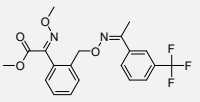
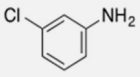
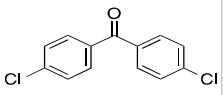
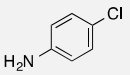
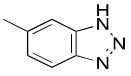
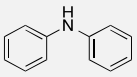
Compound	Molecular Formula	Chemical MW (g/mol)	Structure	Solubility in Water (mg/L)	log P	Koc
Oxadiazon	$C_{15}H_{18}Cl_2N_2O_3$	344.07		0.57	5.33	1915
Pendimethalin	$C_{13}H_{19}N_3O_4$	281.14		0.33	5.2	17491
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	376.67		1.467	4.06	500
Propiconazole	$C_{15}H_{17}Cl_2N_3O_2$	341.06		150	3.7	1086
Prothioconazole	$C_{14}H_{15}Cl_2N_3OS$	343.03		300	3.82	1092
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	387.09		1.9	3.99	9304
Quinoxyfen	$C_{15}H_8Cl_2FNO$	308.13		1.153	4.66	10^5
Simazine	$C_7H_{12}ClN_5$	201.66		6.2	2.18	130
Tebuconazole	$C_{16}H_{22}ClN_3O$	307.15		36	3.7	$2x10^4$
Triazophos	$C_{12}H_{16}N_3O_3PS$	313.31		39	3.34	$3x10^4$
Trifloxystrobin	$C_{20}H_{19}F_3N_2O_4$	408.37		0.00016327	6.62	$4x10^6$
Industrial Emerging Contaminants						
3-Chloroaniline	C_6H_6ClN	127.57		5.400	1.81	72.5
4,4'-Dichlorobenzophenone	$C_{13}H_8Cl_2O$	251.11		3.796	4.62	2826
4-Chloroaniline	C_6H_6ClN	127.57		3.900	1.76	72.5
5-Methyl-1H-benzotriazole	$C_7H_7N_3$	133.15		3.069	1.8	1613
Diphenylamine	$C_{12}H_{11}N$	169.22		53	3.5	1887

Table 3.1. Continued.

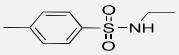
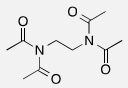
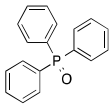
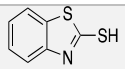
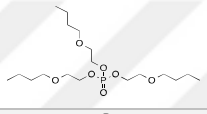
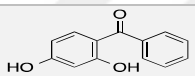
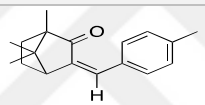
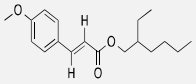
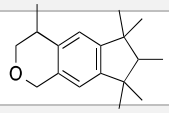
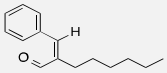
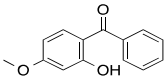
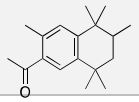
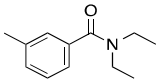
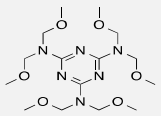
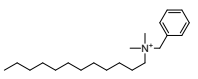
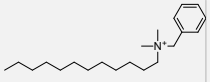
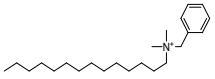
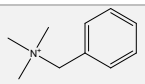
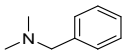
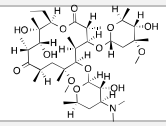
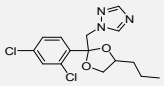
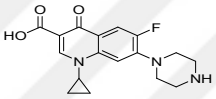
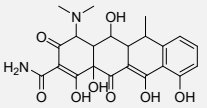
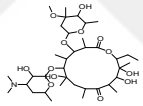
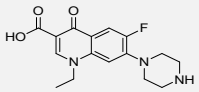
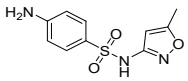
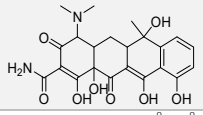
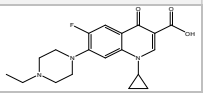
Compound	Molecular Formula	Chemical MW (g/mol)	Structure	Solubility in Water (mg/L)	log P	Koc
N-Ethyl-2-tolysulfonamide	$C_9H_{13}NO_2S$	199.27		1.106	1.96	n.d.
N-methyl-Aniline	C_7H_9N	107.15		5.620	1.66	65
Tetraacetylethylenediamine	$C_{10}H_{16}N_2O_4$	228.25		200	-1.61	415
Triphenylphosphineoxide	$C_{18}H_{15}OP$	278.28		62.76	2.87	$4x10^5$
2-Mercapto-benzothiazole	$C_7H_5NS_2$	167.25		118	2.41	3560
Tris(2-butoxyethyl) phosphate	$C_{18}H_{39}O_7P$	398.47		1.100	4.3	466200
2,4-Dihydroxybenzophenone	$C_{13}H_{10}O_3$	214.22		413.4	3.17	2885
4-Methylbenzylidenecamphor	$C_{18}H_{22}O$	254.37		0.1966	4.95	13720
Ethylhexylmethoxycinnamate	$C_{18}H_{26}O_3$	290.4		0.2	5.66	$1x10^4$
Galaxolide	$C_{18}H_{26}O$	258.4		1.75	6.23	6300
Hexylcinnamaldehyde	$C_{15}H_{20}O$	216.32		2.75	5.33	4025
Oxybenzone	$C_{14}H_{12}O_3$	228.24		69	3.64	1268
Tonalide	$C_{18}H_{26}O$	258.4		1.25	5.7	5195
N,N-Diethyl-m-toluamide	$C_{12}H_{17}NO$	191.13		666	2.18	536
Hexa(methoxymethyl)melamine	$C_{15}H_{30}N_6O_6$	390.44		149.3	3.07	10
Benzyldimethyldodecyl-ammonium	$C_{21}H_{38}ClN$	304.3		1.000000	2.93	10^6
Benzyldimethylhexadecyl-ammonium	$C_{25}H_{46}N$	396.09		-	4.89	10^7
Benzyldimethyltetradecylammonium	$C_{23}H_{42}N$	332.33		1.000	3.91	$4 x10^6$

Table 3.1. Continued.

Compound	Molecular Formula	Chemical MW (g/mol)	Structure	Solubility in Water (mg/L)	log P	Koc
Benzyltrimethylammonium	$C_{10}H_{16}N^+$	150.13		100.000	-2.47	671
N-Benzyldimethylamine	$C_9H_{13}N$	135.21		12.000	1.98	626
Antibiotics						
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.95		1.693	3.16	n.d.
Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	323.13		2.500	1.14	10
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	367.1		11.480	0.28	35.5
Doxycycline	$C_{22}H_{24}N_2O_9$	462.45		630	-0.02	n.d.
Erythromycin	$C_{37}H_{67}NO_{13}$	733.93		2.000	2.7	n.d.
Norfloxacin	$C_{16}H_{18}FN_3O_3$	319.33		177.900	-1.03	92
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.29		610	0.89	1531
Tetracycline	$C_{22}H_{24}N_2O_8$	444.43		231	-1.3	57.6
Enrofloxacin	$C_{19}H_{22}FN_3O_3$	359.16		3397	0.77	83.5

n.d.: No data available

Stock solutions of listed chemicals were individually prepared in acetonitrile or water at 1000 µg/mL according to their solubility's and stored at -20 °C until used for three months at most. A working solution of mixed standard employed for spiking of soil samples was prepared at 1 µg/mL in acetonitrile. Working solutions were used in the week of preparation. Triphenyl phosphate was used as internal standard in all LC-MS/MS analysis.

The chemicals used for extraction of the target analytes from soil samples and for their quantification by LC-MS/MS analysis are listed in Table 3.2.

Table 3.2. Chemical substances used for the extraction and LC-MS/MS analysis.

Chemical	Molecular Formula	Use	Supplier
Extraction			
Acetonitrile	C_2H_3N	Extraction	Merck
Acetic Acid	$C_2H_4O_2$	Extraction	Merck
Deionized water	H_2O	Hydration	-
Magnesium sulfate anhydrous	$MgSO_4$	Salting out	Merck
Sodium Acetate	$C_2H_3NaO_2$	pH adjustment	Merck
Solium Chloride	NaCl	Salting out	Merck
PSA	-	Clean-up	Sigma Aldrich
C18	-	Clean-up	Sigma Aldrich
LC-MS/MS Analysis			
Methanol	CH_3OH	Mobile Phase	Merck
Water	H_2O	Mobile Phase	Merck
Formic acid	HCOOH	Mobile Phase	Sigma Aldrich

The chemicals used for soil characterization studies are given in Table 3.3.

Table 3.3. The chemical substances used in the characterization of soil samples.

Chemical	Molecular Formula	Purpose of Use	Supplier
Potassium chloride	KCl	pH	Sigma Aldrich
Potassium dichromate	$K_2Cr_2O_7$	OC	Tekkim
Concentrated sulfuric acid	H_2SO_4	OC, Digestion of the samples, TP	Sigma Aldrich
Ferrous ammonium sulphate (FAS)	$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$	OC	Merck
1.10-phenanthroline monohydrate	$C_{12}0H_8N_2.H_2O$	OC	Sigma Aldrich
Iron (II) sulfate hepta hydrate	$FeSO_4.7H_2O$	OC	Riedel-de Haën
Hydrogen peroxide	H_2O_2	Digestion of the samples	Sigma Aldrich
TKN indicator	-	TKN	Hach
Potassium hydroxide	KOH	TKN	Merck
Mineral stabilizer	-	TKN	Hach
Polyvinylalcohol	$(CH_2CHOH)n$	TKN	Hach
Nessler reagent	K_2HgI_4	TKN	Hach
Phenolphthalein	$C_{20}H_{14}O_4$	TP	Hach
Potassium hydroxide	KOH	TP	Merck
PhosVer pillow containing ascorbic acid	-	TP	Hach

Table 3.3. Continued.

Chemical	Molecular Formula	Purpose of Use	Supplier
Sodium acetate trihydrate	$NaC_2H_2O_2.3H_2O$	CEC	Sigma Aldrich
Isopropyl alcohol	$(CH_3)_2CHOH$	CEC	Merck
Glacial acetic acid	CH_3COOH	CEC	Merck
Ammonium hydroxide	NH_4OH	CEC	Sigma Aldrich
Sodium hexametaphosphate	$Na_6P_6O_{18}$	Texture	ZAG Kimya
Lanthanium(III) oxide	La_2O_3	Metal analysis	Sigma Aldrich
Nitric acid (65%)	HNO_3	Microwave Digestion	Merck
Hydrochloric acid (36.5-38%)	HCl	Microwave Digestion	Sigma Aldrich

3.1.2. Soil samples

A blank soil sample was obtained from Boğaziçi University's yard to conduct the analytical method optimization studies for the analyses of target emerging contaminants

For the investigation of the target pesticides and other emerging organic contaminants, a total of 22 soil samples were collected from different agricultural fields near to the main river of Ergene watershed and its tributaries in Thrace region of Turkey. Sampling campaign was performed during late January mainly from paddy soils, since rice cultivation is the major activity of the sampling region, where approximately 50% of total rice production of Turkey is supplied (Ocaklı, 2012).

Rice planting in Thrace region is conducted in May to the wet field. After the seeds hold on to the soil, the water is drained from the field. Based on the plant growth the level of the water is increased up to 15 cm and kept until harvesting in September-October at the same level. The fertilization is done with aluminum sulphate, phosphorus, and zinc sulphate. After harvesting, the tilled soil is submerged in water again in winter until the next planting season (Ocaklı, 2012).

Paddy cultivation requires a great amount of water as for 1 kg rice 3000-5000 kg water (Sezer et al., 2012). Therefore, in this thesis, beside the agrochemical contamination, it is also intended to investigate the irrigational pollution on soil based on the usage of contaminated river water (Ergene River). The sampling sites were selected according to the intensity of the fields where irrigational agriculture is applied and closeness to the Ergene river. Since Edirne has the highest share on the rice production of Turkey with a 35.087 ha cultivation area and 341.318 tonnes of product (Ocaklı, 2012) sampling is mainly done from the fields located in Edirne and Kırklareli.

The sampling points selected according to the criterion given above are represented in Figure 3.1., which demonstrates the boarders of Ergene watershed with a claret red outline and the main river (Ergene River) with its tributaries. Sampling locations and belonging ID numbers, number of samples taken, and the coordinates are listed in Table 3.4. For the determination of sampling point coordinates and other mapping activities Google Earth Pro is used.

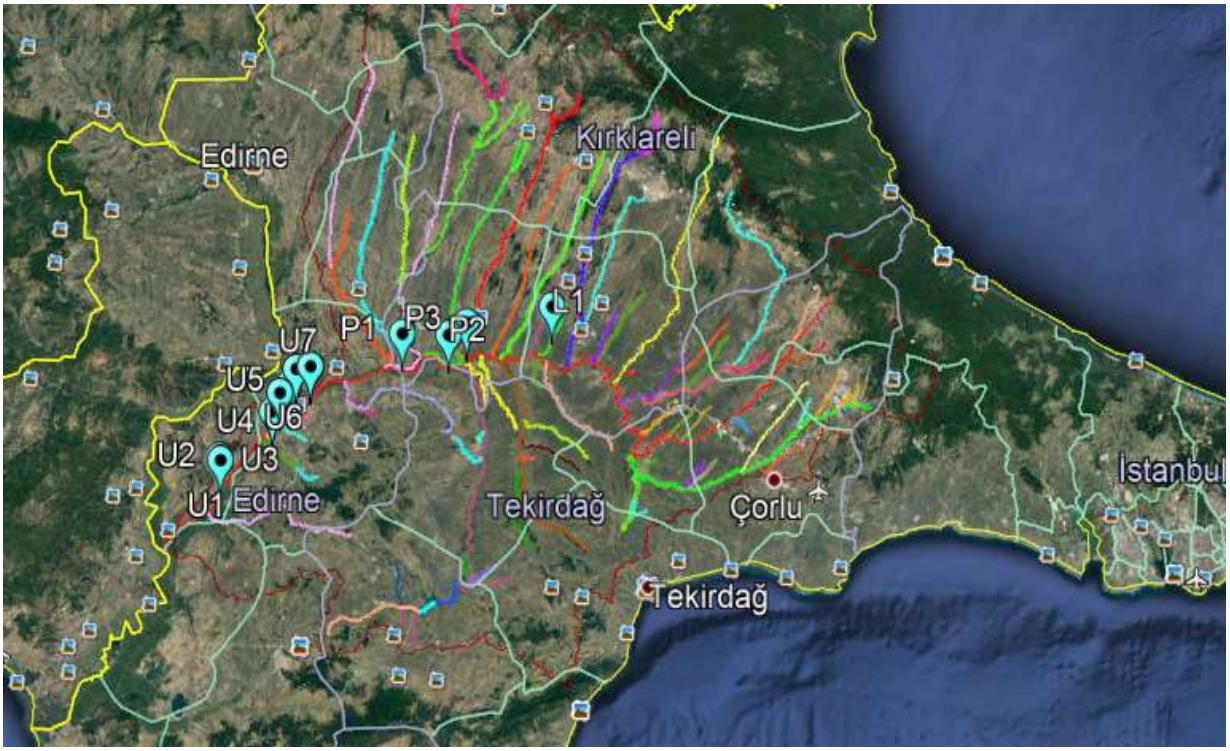


Figure 3.1. Satellite map showing the sampling sites along the river bank of Ergene.

Table 3.4. Description of the sampling sites.

Sample ID	*Sampling Location*	*Number of Samples*	*Crops Grown*	*Latitude*	*Longitude*
U1	Yenicegörece	2	Paddy	41°07'39.8"N	26°28'57.2"E
U2	Yenicegörece	1	Paddy	41°07'21.1"N	26°29'11.3"E
U3	Salarlı	2	Paddy	41°12'18.3"N	26°36'37.6"E
U4	Çiftlikköy	4	Paddy & Unknown	41°14'10.3"N	26°37'42.1"E
U5	Uzunköprü	2	Paddy	41°14'58.9"N	26°39'02.4"E
U6	Uzunköprü	2	Paddy	41°16'34.6"N	26°40'01.5"E
U7	Demirtaş	2	Paddy	41°16'50.7"N	26°42'05.9"E
P1	Pehlivanköy	2	Paddy	41°20'11.3"N	26°55'19.7"E
P2	Katranca	2	Paddy & Unknown	41°20'04.6"N	27°02'00.3"E
P3	Mandıra	2	Paddy & Unknown	41°21'16.1"N	27°04'37.3"E
L1	Düğüncübaşı	1	Unknown	41°22'56.5"N	27°16'54.3"E

3.2. Methods

3.2.1. Soil Sampling

Agricultural soil samples were collected by manual sampling using a trowel approximately 10 cm below the surface layer by mixing random discrete subsamples from every sampling point. Special care was devoted to prevent the possibility of accidental contamination or loss of analytes during sample handling procedures. Each sample was put into a clean, labeled, and sealed plastic bag and delivered to the laboratory immediately after collection.

Samples were air dried at room temperature for two days to prevent any possible errors caused by variable moisture content. After drying, the samples were hammered, since they were molded. Finally, all the samples were sieved with a 2 mm mesh to ensure a homogenous mixture. To minimize the microbial activity, the samples were stored at + 4ºC before analysis.

3.2.2. Extraction Method of the Target Contaminants from Soil Sample

For the extraction of target organic compounds from soil, QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was performed as sample preparation step. The official QuEChERS method was developed for the extraction of pesticides from in fruit and vegetables (Anastassiades et al. 2003). The methodology was standardized with respect to buffer type: the American standard (AOAC) prefers acetate buffer (2007), whereas the European standard EN 15662 utilizes citrate buffer (2008). During the method validation and analysis of agricultural soil, samples were extracted by using acetate-based buffer that was found more effective rates for both antibiotics (Salvia et al., 2012) and pesticides (Yu et al., 2016) than citrate-based buffer regarding to higher recovery.

Since this method is developed for pesticide extraction from food samples, for the extraction of target antibiotics different modifications were tested using different buffers and salts. For the recovery of pesticide and other ECs the original AOAC standard was used with small modifications as demonstrated in Figure 3.2.

Basic steps of sample preparation used in this study were as follows: i) 5 g of soil placed in 50 mL centrifuge tube with target analytes and internal standard. Homogenization with a vortex ii) Extraction of soil that was hydrated with 5 mL with deionized water for 30 minutes by using 10 mL

acetonitrile containing 1% acetic acid. Shaking for 10 min to ensure enough contact time for extraction. iii) Separation of the organic solvent by salting out effect that was induced by the addition of 4 g $MgSO_4$, 1 g NaCl and 1g NaOAc through both vigorous handshaking, and vortexing for 30 seconds and followed by centrifugation at 5000 rpm for 10 min. iv) Filtration through a PTFE filter (0.22 µm) to LC-MS/MS analysis.

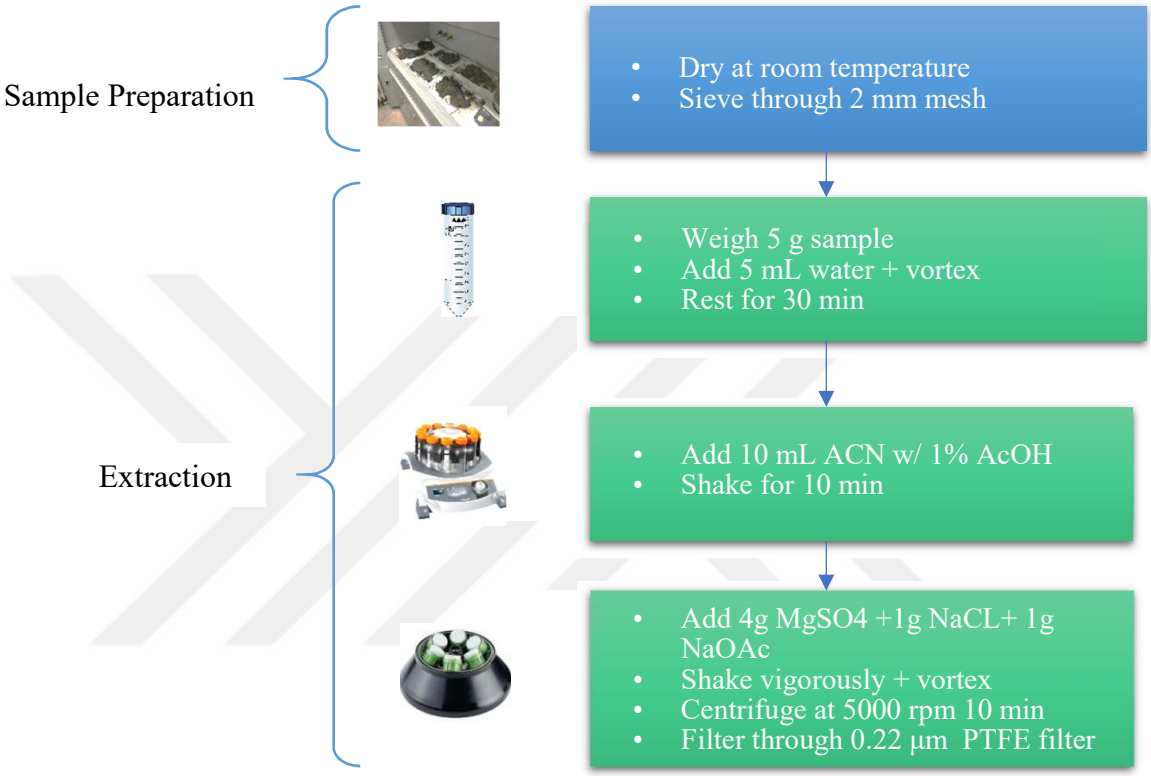


Figure 3.2. Schematic representation of the extraction and analysis.

3.2.3. LC-MS/MS Analysis of Multiclass Organic Pollutants

The analysis of multiclass organic contaminants was performed by using liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). An AB SCIEX QTrap 4500 linear ion trap tandem mass analyzer system coupled with Eksigent Ekspert UltraLC 110 ultra-high-performance liquid chromatography unit was used in developing the analytical technique to quantify the selected pollutants. MS/MS system was operated in multiple reaction monitoring mode (MRM).

The QTrap 4500 MS/MS is a sensitive and selective detector with high resolution capacity. The liquid samples can be pass in to the detector through LC line or syringe pump. The detection system is operated as follows: (i) Ions are formed in the ionization chamber, which are separated from the

matrix via Q0 ion focuser under vacuum. (ii) In ion sorter Q1, the parent ions are sorted and aligned according to their entrance potential (EP). (iii) The parent ions are transferred in the collusion chamber (Q2), where the ions are fragmented to daughter ions due to the applied collusion energy (CE) and the nitrogen gas (CAD). (iv) The fragment ions (Q3) are aligned in the second ion sorter and monitored in the detector. The LC-MS/MS instrumental system and the defined parts of the MS detector is shown in Figure 3.3.

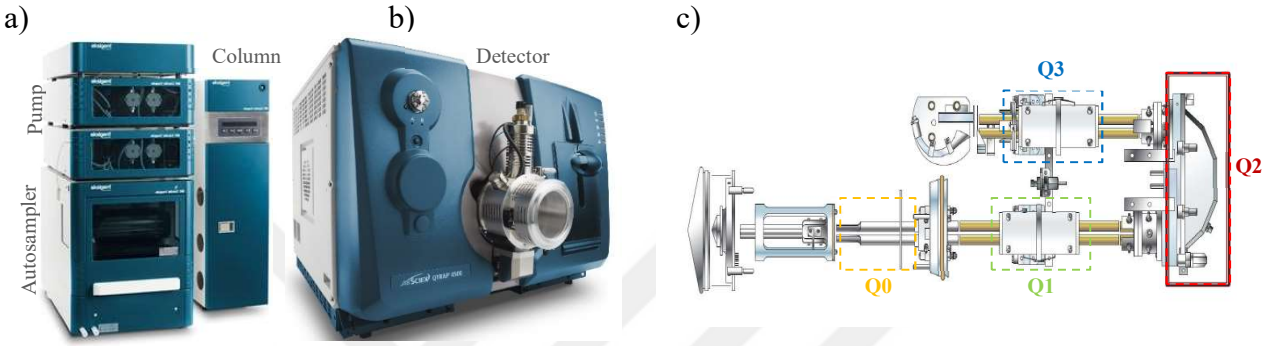


Figure 3.3. a) Liquid chromatography, b) Mass spectrometer, c) Modules of MS/MS detector.

As a preliminary step, MRM transitions and MS/MS conditions for each compound were generated by manual infusion with a syringe pump and the parameters of the ESI source were optimized. After the parent ion (Q1) of a chemical was determined, 2 daughter ions (Q3s), which are the ions with the highest intensities among the other ions generated after the fragmentation of the parent ion in the collision cell, as well as optimum declustering potential (DP), collusion energy (CE) and collision cell exit potential (CXP) to generate those Q3s were determined by "Compound Optimization" mode. After MRM parameters were optimized, the retention time of each analyte was determined with unknown screening method. Following, all the calibration and quantification experiments were done in scheduled MRM (sMRM) mode. The workflow of the optimization method was schematized in Figure 3.4.

A chromatographic separation of the targeted analytes was performed on a Phenomenex Kinetex C18 (50 mm in length and 3 mm in diameter) 2.6µm particle size column. MS grade methanol (Pump A) and MS grade water (Pump B) buffered with 0.1% formic acid were used for gradient elution. A constant flow rate of 0.5 mL/min was applied and the temperature of the column was kept at 40 ºC. A 10 µL aliquot of each sample kept at 4 ºC was used for injection and a solvent flow rate of 500 µL/min was maintained throughout the separation. Total run time was 16 minutes with and initial equilibration time of 2 minutes. The gradient elution program of mobile phase is shown Table 3.5.

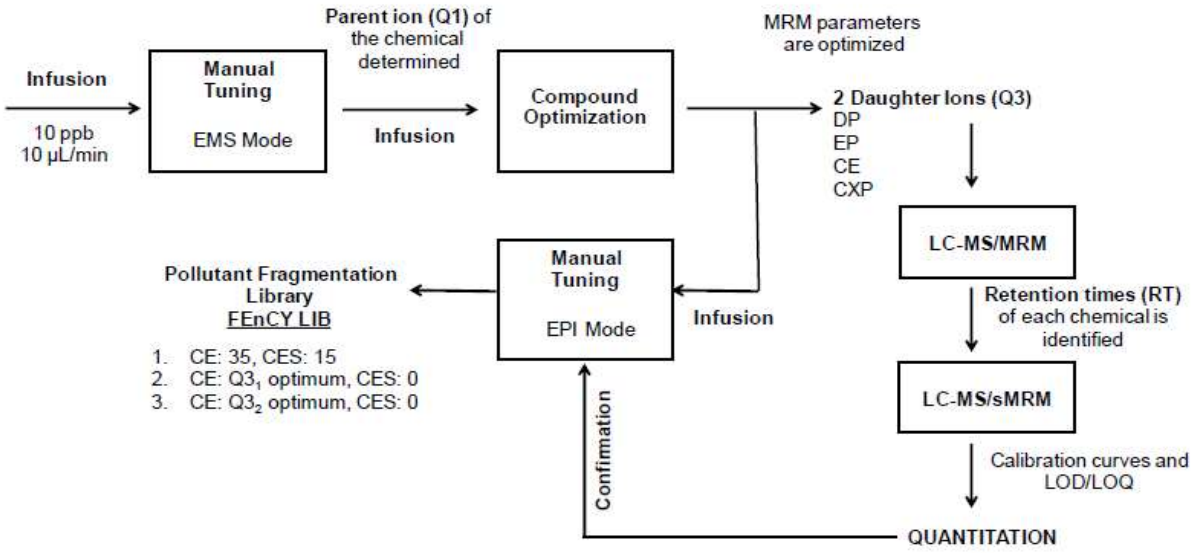


Figure 3.4. Workflow of MRM optimization in LC-MS/MS (Eken et al., 2017).

Table 3.5. Gradient elution program for liquid chromatography separation (A: MeOH 0.1%FA; B: Water 0.1% FA).

Time	*Flow rate (mL/min)*	*A (%)*	*B (%)*
0:00:01	0.50	0	100
0:01:30	0.50	0	100
0:02:00	0.50	50	50
0:04:00	0.50	50	50
0:08:00	0.50	100	0
0:14:00	0.50	100	0

The mass analysis was performed using ESI probe operated at positive ionization mode with MS/MS conditions with respect to the flow rate as follows: Curtain gas (CUR)=30, Ion spray voltage (IS)=5500 V, Temperature (TEM)= 550 ºC, Ion source gas 1 (GS1) = 50; Ion source gas 2 (GS2)= 60

For instrumental control, data acquisition, and processing Analyst Software (AB Sciex) and for the peak control and quantitation Multiquant 3.0.1(AB Sciex) were used respectively.

3.2.4. Evaluation of Analytical Method Performance

The developed analytical method was validated to evaluate its performance with the following parameters in accordance with conventional procedures (SANTE, 2017) specificity, recovery, precision, linearity, limits of detection, limits of quantification, and matrix effect.

In order to evaluate the specificity of developed method, blank and spiked soil samples were analyzed and the absence of interfering species at about the retention time of the target analytes was verified.

3.2.4.1. Recovery studies of the multiclass organic pollutants. Recovery experiments are conducted in order to determine the method performance to extract the targeted compounds from soil simultaneously within performance criteria of 70-120% (SANTE, 2017). These studies were also used to examine the association between recovery rates and soil characteristics.

Recovery experiments were performed with 5 g of blank soil samples spiked with a mixture of the target analytes having the concentration of 1 mg/L at 3 different concentrations as 10, 50 and 100 µg/kg in triplicates. For the sample preparation, the same extraction procedure was applied as demonstrated in Figure 3.2. Recovery and precision were evaluated by analyzing blank soil samples

$$\text{Recovery (\%)} = \frac{Cm\text{-}Cb}{Cs} \text{x}100 \qquad (3.1)$$

C_m=Measured concentration
C_b=Concentration in blank extracts
C_s=Spiked concentration

Recoveries are compared with the response of the corresponding concentrations of the analytes in blank extract. The precision of the method was determined in terms of relative standard deviation (RSD) of the triplicate samples (equation 3.2). Microsoft Excel was used for the mathematical calculations.

$$\text{RSD (\%)} = \frac{(100 \text{ x SD})}{\text{Mean concentration}} \qquad (3.2)$$

RSD= Relative standard deviation
SD= Standard deviation

3.2.4.2. Determination of method LOD and LOQ. The method LOD and LOQ were determined using the calibration curves constructed with lower spiking concentration range applying the same extraction procedure within the range of 1 µg/kg - 5 µg/kg at 3 concentrations in triplicate. The calculations were based on the slope (S) and standard deviation of the response of the calibration curve (Sy) using the following formulas:

$$MLOD = 3.3 \left(\frac{Sy}{S}\right) \quad (3.3)$$

$$MLOQ = 10 \left(\frac{Sy}{S}\right) \quad (3.4)$$

As used in many studies in the literature (Chiaia-Hernandez et al., 2017; Geronimo et al., 2015; Homazava et al., 2014; Fernandes et al., 2012; Prestes et al., 2011) the limit of detection and quantification for each analyte were also calculated by using a matrix matched calibration curve, by spiking the blank extract within the range of 0.05-5 µg/L in 5 concentrations. LOD and LOQ of the analytes were determined by multiplying the signal to noise ratio of the responses of the lowest detectable concentration by 3 and 10 respectively.

$$LOD = S/N \times 3 \quad (3.5)$$

$$LOQ = S/N \times 10 \quad (3.6)$$

S/N= Signal to noise ratio

3.2.4.3. Determination of matrix effects. As soil has a complex matrix, even the purification is applied, residual components can result in either signal enhancement or suppression of the analytes, which can lead inaccurate results and cause errors, when ESI is used as the ionization technique in LC-MS/MS analysis (SANTE, 2017).

In order to evaluate the matrix effects (ME) of soil components on the analysis of target contaminants, the slope of each analytes calibration curve prepared in blank soil extract (Sm) and in pure solvent (Ss) were determined. The linearity of the calibration curves were evaluated within the concentration range of 1 µg/L - 50 µg/L at 5 different concentrations prepared in triplicate. The effects of soil matrix components was estimated as the percentage of signal suppression (-) or enhancement (+). ME value of 0% corresponds to no observed matrix effect. ME values between -20% and 20% demonstrate a mild and negligible effect, while values between -50% and -20% or 20% and 50% indicate a medium signal suppression or enhancement, respectively. ME values below -50% or above 50% represent a strong effect of matrix on signals (Yu et al., 2016).

3.2.5. Characterization of Soil Samples

The characterization of agricultural soil samples collected from 22 different fields was performed by the determination of different parameters including pH, total Kjeldahl nitrogen (TKN), total phosphorus (T-P), organic carbon content (OC), metal content, cation exchange capacity (CEC), and texture. As the results of these experiments demonstrate an extensive data about the soils samples, they are also used to represent the relationship between the soil characteristics and recovery of the targeted analytes.

3.2.5.1. pH. pH values of soil samples were measured with respect to the method described by Forster (1995). Air-dried and sieved soil samples (5 g) were mixed with 12.5 mL 1 M KCl solution and pH measurement was made in the supernatant after 1 h of standing and a second short mixing.

3.2.5.2. Determination of total Kjeldahl nitrogen and total phosphorus. For the determination of total nitrogen and phosphorus, the soil sample (0.5 g) was digested in the presence of hydrogen peroxide (H_2O_2) and sulphuric acid (H_2SO_4) at 440ºC under reflux by using a Hach Digesdahl apparatus according to the procedure of Hach Company (1996)

Total Kjeldahl nitrogen content of the digested soil samples were measured with reference to the Nessler method as described in Hach DR/2010 Spectrophotometer Handbook (1996) and calculated using following formula.

$$\text{TKN (mg/kg)} = \frac{75 \text{ x } A}{B \text{ x } C} \qquad (3.7)$$

A = Concentration in digestate (mg/ L)
B = Weight of sample taken for digestion (g)
C = Volume of digested sample (mL)

Total phosphorus content of the digested soil samples were measured according to the Ascorbic acid method as described in Hach DR/2010 Spectrophotometer Handbook (1996). Reading was performed at 890 nm for the determination of P, P_2O_5 and PO_4^{3-} simultaneously as mg/L. The calculations were made using following formula for each reading:

$$\text{P (mg/kg)} = \frac{A \text{ x } DF \text{ x } 100}{B} \qquad (3.7)$$

A = Concentration in digestate (mg /L)
B = Weight of sample taken for digestion (g)
DF = Dilution factor

3.2.5.3. Determination of metals. For the determination of metals, samples (1 g) were digested in the microwave oven with of nitric and hydrochloric acid according to the Application Report of speed wave MWS-3 of Berghof Products and Instruments (n.d.)

The analysis of Al, As, B, Cr, Cu, Pb, Ni, Zn, Fe and Cd were conducted in digestate using inductively coupled plasma optical emission spectrometry (ICP-OES, Pelkin Elmer Optima 2100 DV). Ca, K, Na and Mg contents of the soil samples were measured with atomic absorption spectrometry (AAS, AAnalyst 300, Pelkin Elmer). The metal concentrations were calculated using following formula:

$$\text{Metal (mg/kg)} = \frac{\text{A x DF x 50}}{\text{B}} \quad (3.8)$$

A = Concentration of digestate (mg/L)
B = Weight of soil sample (g)
DF = Dilution factor

3.2.5.4. Organic carbon content. Organic carbon content of the soil samples were determined according to the method no. TS 8336 of Turkish Standards Institute (TSE) based on the Walkley-Black Method (TSE, 1990). Soil samples (0.1 g) were treated with concentrated H_2SO_4 and 1 N $K_2Cr_2O_7$ and digested in closed COD tubes at 150 C for 2 hours. After the digestion, excess dichromate was back titrated by the addition of ferroin indicator with 0.5 N ferrous ammonium sulphate solution. The OC content of soil samples was calculated using following formula.

$$\text{OC (\%)} = \frac{\text{(B-S) x Nk x 0.389}}{\text{T}} \quad (3.6)$$

B = volume of FAS used for the blank sample (mL)
S = volume of FAS used for the sample (mL)
N_k = Normality of FAS solution (N)
T = weight of soil sample (g)

3.2.5.5. Cation exchange capacity. Determination of cation exchange capacity (CEC) of soil samples was performed as described in Method 9081 of Environmental Protection Agency (EPA 1986). Briefly, this procedure is based on mixing 4 g of soil sample with 1N sodium acetate solution (pH= 8.2) in order to replace the sodium ions with matrix cations, washing with isopropyl alcohol and mixing again with 1N ammonium acetate solution (pH=7) for an exchange of ammonium with adsorbed sodium cation, sequentially by performing each step in several repeats. The resulting washing solutions are combined, and the concentration of displaced Na was determined using atomic absorption spectroscopy and the CEC of each soil sample was calculated by the given formula:

$$\text{CEC (mEq 100 g}^{-1}) = \frac{[\text{Na+}] \text{ x DF x } 10}{23 \text{ m}} \quad (3.7)$$

[Na^+] = Concentration (mg/L)
DF = Dilution factor
m = Weight of soil sample (g)

3.2.5.6. Texture. Texture analysis of the soil samples was performed using sieve analysis and hydrometer method as described in the Method D422 of ASTM (ASTM, 2007). This method covers the determination of particle size distribution of soil quantitively, based on the Stokes' law. With this method, the weight percentages of sand, clay and silt were determined by measuring the density of the soil-water suspension with hydrometer iteratively.

In order to separate sand and silt/clay of the soils, the air-dried samples were sieved through 2 mm mesh (mesh no:10), 0.420 mm (mesh no:40) and 0.074 mm (mesh no: 200) respectively using Endecotts EFL 2000/2 sieve shaker. The weight of each set of particles remained on the sieves were measured. Briefly, soil particles sieved through mesh 200 (50 g) were mixed with 125 mL of sodium hexametaphosphate solution (4%) and resulting mixture allowed to stand for about 16 hours. In the next day, the sample was transferred into 1000 mL graduated cylinder, filled up to the mark with distilled water and the solution was mixed well. First, the hydrometer (ASTM- 152-H) was put in the cylinder filled with only distilled water and the reading was recorded for the zero correction (Fz). Then the hydrometer was inserted to the cylinder containing the soil-water suspension and the readings were performed at cumulative times: t=0 min., 0.5 min., 1 min., 2 min., 4 min., 8min., 15 min., 30 min., 1h, 2h, 4 h and 24 h respectively. From the hydrometer readings, particle diameters and percent finer of each record were calculated using related tables and equations below and plotted respectively.

$$R_{cp} = R + F_T - F_Z \quad (3.8)$$

$$F_T = -4.85 + 0.25\ T \quad (3.9)$$

$$\text{Percent finer} = \frac{\alpha \times Rcp}{Ws} \times 100 \quad (3.10)$$

$$D = K\sqrt{L/t} \quad (3.11)$$

R= Hydrometer reading

Rcp= Corrected hydrometer reading

FT= Temperature correction

Fz= Zero correction

T= Temperature (°C)

α= Correcction factor specific gravity= 0.99

D= Particle diameter (mm)

K= Constant depending on the specific gravity and temperature = 0.0134

L= Effective length (cm)

t= Reading time (min)

The percent of medium and fine sand of the soil were calculated with respect to the sieve analysis and silt/clay content was determined using hydrometer analysis results. Regarding the obtained percentages, the soil type were determined which is demonstrated in Figure 3.5.

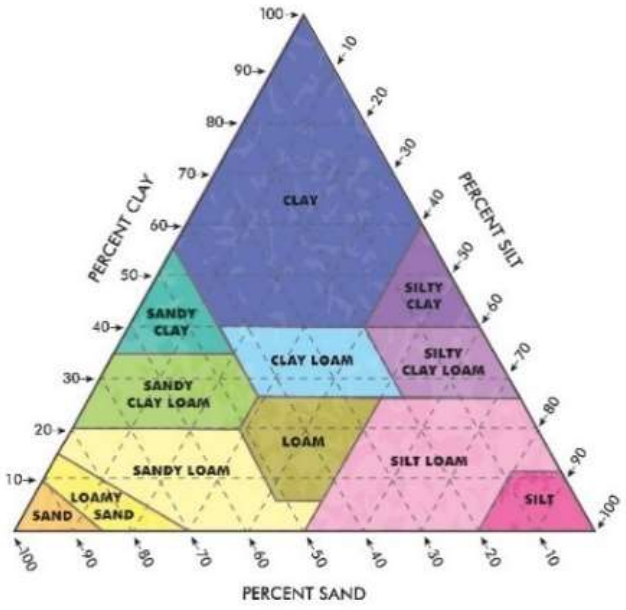


Figure 3.5. Soil texture triangle.

3.2.6. Statistical Analysis

In order to evaluate the relationship of soil properties with each other, with the occurrence of target contaminants and recovery rate of the analytes, Bivariate Correlation Test was used. The significance of the correlations was accepted if $p< 0.01$ and $p<0.05$, where the likelihood to obtain the correlation randomly is at 1% and 5% probability respectively. Statistical analysis was performed using SPSS (Statistical Package for the Social Science) 25 for Windows.

4. RESULTS AND DISCUSSION

4.1. Development of an Analytical Method for Multiclass Organic Pollutants in Soil

4.1.1. Optimization of LC-MS/MS Analysis

Multi reaction monitoring (MRM) experiments were carried out to obtain the maximum sensitivity for the detection of target analytes. For confirmation of the studied pollutants, two MRM transitions and a correct ratio between the abundances of the two optimized MRM transitions were used, along with retention time matching.

MRM transitions and MS/MS conditions for each compound were generated by manual infusion with a syringe pump and the parameters of the ESI source were optimized. After the parent ion (Q1) of a chemical was determined, 2 daughter ions (Q3s), which are the ions with the highest intensities among the other ions generated after the fragmentation of the parent ion in the collision cell, as well as optimum declustering potential (DP), collusion energy (CE) and collision cell exit potential (CXP) to generate those Q3s were determined by "Compound Optimization" mode. The retention time of each analyte was identified by LC/MS-MS system using mixed standards using unscheduled mode. Optimized parameters for each analyte and its fragment ions are summarized in Table 4.1.

Table 4.1. MRM transitions and optimized MS/MS parameters.

	Chemical Name	Q1	Q3	t_R(min)	DP(V)	EP (V)	CE(V)	CXP(V)
1	2,4-Dihydroxybenzophenone	215.0	136.9 / 80.9	4.86	61	10	27 / 51	8 / 6
2	2-Mercapto-benzothiazole	167.9	135 / 65	3.44	71	10	35 / 47	8 / 6
3	3-Chloroaniline	128.0	92.9 / 74.9	2.88	51	10	25 / 43	8 / 6
4	4,4'-Dichlorobenzophenone	251.0	139 / 110.8	6.77	71	10	27 / 55	6 / 8
5	4-Chloroaniline	128.0	93 / 74.8	2.88	56	10	25 / 43	8 / 6
6	4-Methylbenzylidenecamphor	255.1	105 / 114.9	7.23	81	10	45 / 89	6 / 8
7	4-Nonylphenol monoethoxylate	282.2	265 / 69	8.03	61	10	13 / 39	12 / 6
8	5-Methyl-1H-benzotriazole (5-Tolytriazole)	134.0	77 / 79	3.12	56	10	33 / 27	6 / 6
9	Acetamiprid	223.1	126.0 / 99.0	3.01	76	10	31 / 55	11 / 9
10	Aclonifen	265.0	248.0 / 182.1	6.30	61	10	25 / 39	8 / 12
11	Atrazine	216.1	174.1 / 103.9	4.06	21	10	25 / 41	8 / 8
12	Azoxystrobin	404.1	372 / 344.1	5.33	76	10	21 / 35	13 / 25
13	Benzenesulfonamide	158.0	140.9 / 77	2.68	41	10	11 / 31	10 / 6
14	Benzyldimethyldodecylammonium	304.3	90.9 / 212.2	6.03	11	10	53 / 29	8 / 4
15	Benzyldimethylhexadecylammonium	360.4	90.9 / 268.1	7.02	101	10	71 / 33	8 / 12
16	Benzyldimethyltetradecylammonium	332.3	90.7 / 240.1	6.59	96	10	47 / 31	6 / 10
17	Benzyltrimethylammonium	150.1	91 / 64.9	0.49	66	10	31 / 51	6 / 6
18	Boisvelone / Iso-Esuper	235.2	217.2 / 94.9	7.49	71	10	19 / 27	8 / 6
19	Boscalid	343.0	307 / 140	5.46	101	10	27 / 27	18 / 14
20	Carbendazim	192.0	160 / 131.9	2.75	71	10	23 / 39	8 / 10
21	Chloramphenicol	320.8	151.5 / 256.5	3.01	-70	-10	-24 / -	-11 / -

Table 4.1. Continued.

	Chemical Name	Q1	Q3	t_R(min)	DP(V)	EP (V)	CE(V)	CXP(V)
22	Chlorfenvinphos	358.9	155.1 / 98.9	6.46	66	10	19 / 35	8 / 8
23	Chloridazon	222.0	92 / 104	3.02	96	10	35 / 31	14 / 16
24	Chlorpyrifos	349.9	96.8 / 197.9	7.29	56	10	55 / 35	8 / 6
25	Ciprofloxacin	332.2	314 / 230.9	2.72	76	10	29 / 51	12 / 10
26	Clarithromycin	748.4	158 / 82.8	4.22	46	10	37 / 91	10 / 8
27	Cypermethrin	433.0	191 / 415.9	7.62	51	10	21 / 13	8 / 16
28	Difenoconazole	406.0	251 / 188	6.71	106	10	37 / 37	17 / 17
29	Dimethoate	230.1	198.9 / 125	2.97	66	10	15 / 29	19 / 11
30	Diphenylamine	170.0	93 / 92.4	5.66	71	10	37 / 27	8 / 6
31	Diuron	233.0	71.9 / 159.9	4.47	61	10	41 / 37	6 / 8
32	Doxycycline	445.1	428 / 97.9	2.98	86	10	27 / 61	8 / 8
33	Enrofloxacin	360.1	342.2 / 316	2.70	105	10	31 / 27	6 / 12
34	Epoxiconazole	330.1	120.9 / 101.1	5.98	81	10	31 / 69	13 / 11
35	Erythromycin	734.5	158.1 / 83	3.40	21	10	39 / 89	6 / 8
36	Ethoprophos	243.0	131 / 97	5.94	51	10	29 / 41	14 / 12
37	Ethylhexylmethoxycinnamate	291.0	161 / 179	7.89	51	10	27 / 13	8 / 8
38	Flutriafol	302.0	123 / 109	4.21	71	10	39 / 43	14 / 14
39	Galaxolide	257.1	227.1 / 114.9	7.60	91	10	41 / 99	10 / 8
40	Hexa(methoxymethyl)melamine	391.0	177.1 / 283.1	3.94	26	10	39 / 19	6 / 8
41	Hexaconazole	314.0	70 / 159	6.40	66	10	39 / 37	12 / 14
42	Hexylcinnamaldehyde	217.1	129.1 / 127.9	7.28	66	10	25 / 55	6 / 10
43	Imazalil	297.0	159 / 201	3.29	56	10	31 / 23	14 / 15
44	Imazamox	306.2	261.1 / 264.1	3.14	76	10	31 / 27	17 / 19
45	Imidacloprid	256.1	175.1 / 209.1	2.90	71	10	29 / 21	17 / 19
46	Isoproturon	207.1	71.9 / 165.1	4.36	66	10	37 / 21	6 / 8
47	Lenacil	235.1	153.1 / 136.1	4.35	66	10	19 / 43	13 / 11
48	Mepiquat chloride	113.9	58.1 / 98.1	0.50	46	10	29 / 29	6 / 10
49	Methoxyfenozide	369.0	149 / 133	5.71	66	10	23 / 31	14 / 14
50	Metolachlor	284.1	252 / 176.1	6.01	61	10	23 / 37	23 / 15
51	Molinate	188.0	126 / 83	5.56	51	10	19 / 25	14 / 12
52	Myclobutanil	289.0	70 / 125	5.66	66	10	33 / 41	12 / 14
53	N,N-Diethyl-m-toluamide	192.1	118.9 / 90.9	4.36	71	10	25 / 43	6 / 6
54	N-Benzyldimethylamine	136.1	91 / 65	0.49	41	10	25 / 47	6 / 6
55	N-Ethyl-2-tolysulfonamide	200.0	90.9 / 155	3.39	46	10	37 / 15	8 / 8
56	N-methyl-Aniline	108.1	92.9 / 66	0.51	56	10	23 / 39	6 / 6
57	Norfloxacin	320.2	302 / 231	2.71	71	10	29 / 55	12 / 10
58	Ofloxacin	362.2	318 / 261	2.73	46	10	27 / 37	6 / 10
60	Oxadiazon	362.1	303 / 220.1	7.11	56	10	23 / 35	19 / 19
61	Oxybenzone	229.1	151 / 104.8	6.11	61	10	27 / 25	8 / 12
62	Pendimethalin	282.2	211.9 / 193.9	7.31	51	10	17 / 25	17 / 17
63	Prochloraz	376.0	307.8 / 265.8	6.03	26	10	17 / 23	12 / 8
64	Propiconazole	342.1	159 / 69.1	6.40	96	10	43 / 35	15 / 7
65	Prothioconazole	344.1	326 / 328.1	6.42	61	10	17 / 17	7 / 7
66	Pyraclostrobin	388.0	194 / 163	6.57	36	10	19 / 29	15 / 15
67	Quinoxyfen	307.9	197 / 162	7.16	111	10	45 / 65	8 / 8
68	Simazine	202.1	103.9 / 124	3.61	41	10	35 / 25	8 / 8
69	Sulfamethoxazole	254	91.9 / 107.8	2.84	46	10	35 / 35	6 / 8
70	Tebuconazole	308.2	70.1 / 125	6.24	91	10	47 / 53	7 / 13
71	Tetraacetylethylenediamine	229.1	145.1 / 85.9	2.83	36	10	15 / 37	8 / 8
72	Tetracycline	445.1	409.9 / 153.9	2.74	71	10	27 / 35	6 / 8
73	Tonalide	259.1	175.1 / 147.1	7.60	71	10	25 / 37	8 / 6
74	Triazophos	314.0	161.9 / 119	5.83	66	10	27 / 51	8 / 8
75	Trifloxystrobin	409.1	186 / 205.9	6.85	61	10	23 / 21	19 / 19
77	Triphenylphosphineoxide	279.1	201 / 76.9	5.26	96	10	37 / 67	10 / 6
76	Triphenylphosphate	327.0	76.9/ 152.0	6.51	111	10	73 / 59	4 / 8

In LC-MS/MS system chromatographic conditions were also optimized for mixed standard of target pollutants with gradient elution through a reversed phase column to achieve optimum

separation conditions for analytes. Different mobile phase compositions were assayed in the gradient program to increase resolution with high response in short run time. For the chromatographic separation, different organic eluents as methanol and acetonitrile acidified with either formic or acetic acid were tested. When methanol was used as organic eluent, higher sensitivity was obtained, whereas the addition of formic acid provided better ionization compared with acetic acid. Consequently, methanol and water acidified with 0.1% formic acid were found to be the best eluents ensuring high sensitivity and resolution for the separation of analytes.

All the target analytes were analyzed at optimized conditions within 8 minutes. Figure 4.1. shows the chromatogram of the mixed standard at 25 ng/L concentrations. For each analyte, the corresponding peaks extracted from the chromatogram and solvent based calibration curves are demonstrated in Appendix A.

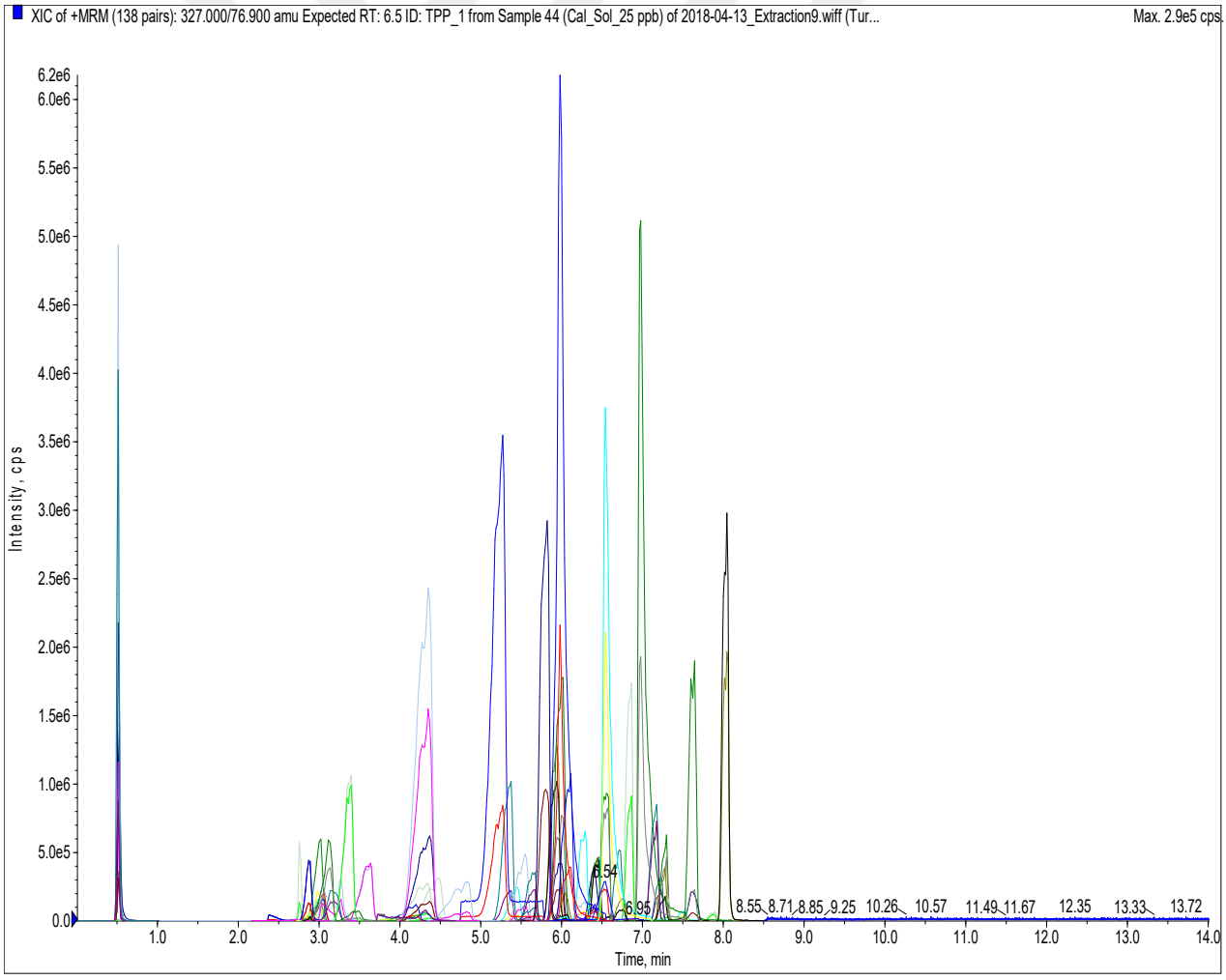


Figure 4.1. LC-MS/MS Chromatogram of analytes.

The separation of the target analytes in the column was mainly achieved with respect to the hydrophobicity of the target compounds as shown in Figure 4.2. Compounds having a logP value less than 2 eluted from the column in the first 3 minutes such as benzyltrimethylammonium, n-benzyldimethylamine, n-methyl-aniline, imidacloprid and chloridazon. Comppounds which have logP values between 2 and 4, detected within 3-6 minutes, including most of the target pesticides, as well as other organic compounds as hexa(methoxymethyl)melamine, 2,4-dihydroxybenzophenone and triphenylphosphineoxide. Compounds having high hydrophobicity (logP>4) as fragrances tonalide and galaxolide, pesticides pendimethalin, cypermethrin, chlorpyrifos and trifloxystrobin retained in the column longer and monitored within 6-8 minutes by the MS detector.

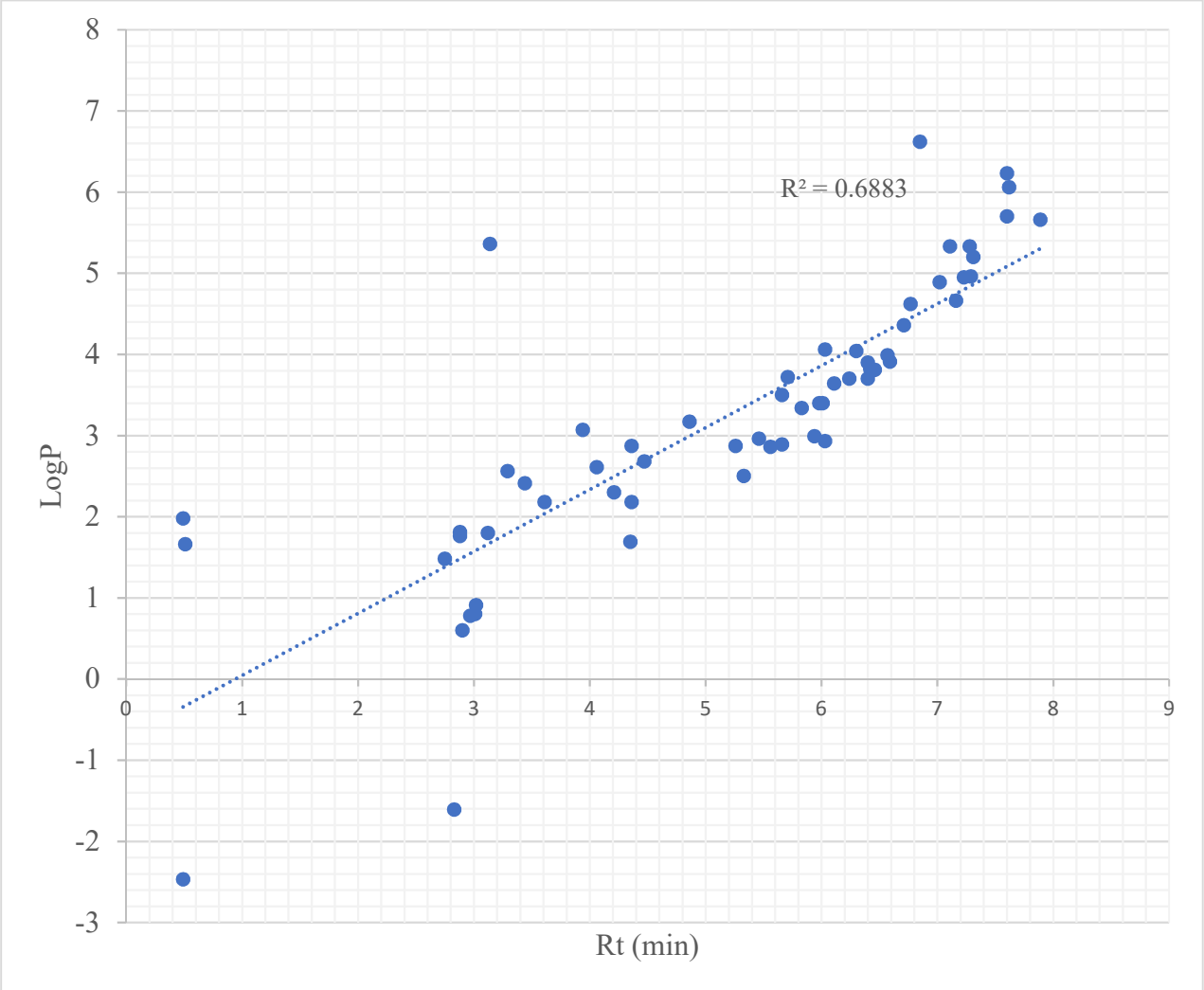


Figure 4.2. The relationship of retention time with LogP of the target analytes.

4.1.2. Optimization of Sample Preparation

4.1.2.1. Simultaneous extraction of antibiotics and pesticides. Considering the complex nature of soils and the different chemical/ physical characteristics of target analytes, the optimization of the sample preparation to obtain a selective and repeatable analysis to achieve high recoveries is the most sensitive and critical step of this study.

For the extraction of organic contaminants from soil, QuEChERS method was performed as sample preparation step. Since official QuEChERS method was developed for the extraction of pesticides from in fruit and vegetables, modifications in the original method was necessary to obtain high recovery rates of target pollutants from soil samples. Samples were extracted using standard AOAC method with some modifications, since the acetate-based buffer was found more effective than citrate-based buffer regarding to higher recovery rates for both antibiotics (Salvia et al., 2012) and pesticides (Yu et al., 2016).

Since there are limited number of studies for the determination of antibiotics in soil using QuEChERS method, modification of the method to extract antibiotics with a number of selected pesticides was the first focus of the study. Keeping in mind the results of previous studies reviewed above and FQs and TCs antibiotics that exhibited strong sorption to soil, the composition of extraction solvent, types of salt and buffer, necessity of cleanup step and concentration of extract by evaporation were all tested to evaluate the recoveries of target analytes. The tested variables in these recovery experiments were listed in Table 4.2 and the recovery values of the target analytes in all experiments were demonstrated in Figure 4.3.

Table 4.2. Experimental parameters tested for the recovery of antibiotics from soil.

Experiment	*Organic solvent*	*Aquous solvent*	*Buffer*	*Salt*	*Clean-up*
1	ACN	Water	NaOAc	$MgSO_4$, NaCl	-
2	ACN	EDTA	NaOAc	$MgSO_4$, NaCl	-
3	ACN	Water	NaOAc	$MgSO_4$, NaCl	PSA, C18
4	ACN	Water	NaOAc	NaCl	-
5	ACN	EDTA	McIlvaine	NaCl	-
6	ACN	EDTA	McIlvaine	Na_2SO_4, NaCl	-
7	MeOH	EDTA	McIlvaine	K_2CO_3	-

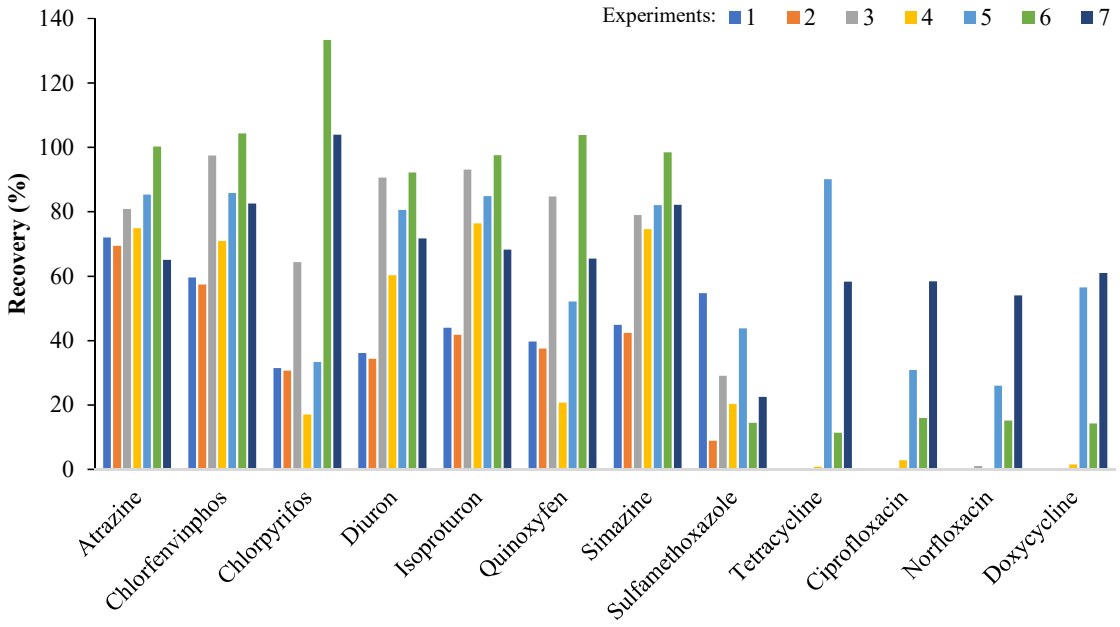


Figure 4.3. Recovery results of antibiotics and pesticides for different parameters.

First, bearing in mind the chelating tendency of the FQ and TC antibiotics with metals found in the composition of soil, the effect of 0.1 M EDTA addition to the aqueous phase of acetate buffered extraction method with the standardized salts was investigated (Experiments 1-2) as preferred in other studies (Salvia et al. 2012). Here, the EDTA solution was used to avoid any complexation of the analytes with cations like Ca^{2+} and Mg^{2+}. However, the recovery of the antibiotics was not achieved under applied extraction conditions (Figure 4.3).

As a next step, the effect of $MgSO_4$ was evaluated on the recovery of antibiotics regarding to its well-known chelating effect to TCs and FQs (Bourdat-Deschamps et al. 2014; Lee et al. 2017). For this purpose, two set of samples were prepared where the salting out was provided with (i) 4 g $MgSO_4$, 1 g NaOAC and 1 g NaCl and (ii) 1 g NaOAC and 1 g NaCl by using 10 mL of 0.1 M EDTA and acetonitrile with 1% acetic acid as extraction solvents. This experiment clearly showed that $MgSO_4$ effects the recovery of TCs and FQs negatively. In the experiment set without $MgSO_4$, responses of antibiotics were detected as sharp peaks in LC-MS/MS for the first time, even the recoveries are in the range of 3%-17%.

In order to increase the recovery of antibiotics, the buffer type was changed in experiment 5. For this purpose, ACN/EDTA/McIlvaine buffer (pH=3) system (2:1:1) was used together with only 3g of NaCl as salting out agent eliminating $MgSO_4$ as applied by the study of Meng et al. (2017), in which

the recoveries of antibiotics were found in a range of 61.4 to 118.9%. Furthermore, the effect of d-SPE on extraction performance was evaluated by the experiments performed with PSA, both PSA and C18, and no clean up. The results did not show a remarkable increase in the recovery values antibiotics in soil samples with the application of any purification step. However, increasing the volume ratio of organic solvent to aqueous buffer (ACN/ EDTA/ Mcllvaine) to 6:1:2 led to 26-100% recovery of antibiotics, whereas the recoveries of selected pesticides deteriorated. To enhance phase separation and hence the concentration of pesticides in organic solvent same procedure was applied with 3 g NaCl+4 g $NaSO_4$ in experiment 6, but simultaneous enhancement in recoveries of antibiotics and pesticides were not achieved (Figure 4.3).

Finally, the effect of organic solvent type was tested on target analytes. K_2CO_3 was used for the phase separation of methanol in experiment 7. Although this extraction trial gave the best results for the antibiotics in all investigated conditions the pesticide recoveries were decreased up to 65% (Figure 4.3). From these results, it is concluded that different extraction conditions are necessary for targeted pesticides and antibiotics.

Method development experiments conducted for the simultaneous extraction of antibiotics and pesticides by testing different parameters showed that it is not possible to extract the target contaminants together based on QuEChERS method within the required recovery values. Further studies were performed with extended target list including both pesticides and industrial micropollutants.

For the extraction of pesticides and other ECs, the standard AOAC method was employed with slight modifications. Sample size was chosen as 5 g, since the decrease in sample weight ensures a better homogenization and partition of the supernatant (Fernandes et al., 2013). After the fortification at required level, sample was vortexed and hydrated with 5 mL deionized water for 30 minutes before the extraction, in order to increase the moisture content for an increased accessibility of the soil pores for the extraction solvent (Pszczolinska and Michel, 2016) and for better recoveries (Fernandes et al. 2013). Extraction was performed by 10 mL acetonitrile acidified with 1% acetic acid, which was chosen as the most efficient solvent for pesticide extraction by several studies (Yu et al., 2016a). Here the ratio of aqueous and organic phase proportion was chosen as 1:2 in order to increase the partition of the target analytes to acetonitrile phase. After the addition of acetonitrile, the tube was shaken for 10 min in order to obtain a sufficient contact time for the extraction solvent and analytes. After extraction, 4 g $MgSO_4$, 1 g NaOAC and 1 g NaCl was added to the centrifuge tubes and hand shaken vigorously. Tubes were centrifuged at 5000 rpm for 5 min afterwards. Here, NaCl was applied

additional to the standard method regarding to its additional contribution on the salting out effect and phase separation, subsequently (Anastassiades et al. 2003; Caldas et al. 2011; Martins et al. 2014). After centrifugation 2 mL of supernatant was filtered through 0.22 µm PTFE filter and analyzed in LC-MS/MS.

4.1.2.2. Effect of d-SPE on the extraction performance. Additional experiments were performed to optimize the sample preparation for the extended list of pollutants detected in agricultural soil samples by AOAC QuEChERS method. In order to examine the effect of purification step on recovery and matrix effect, first 1.5 mL of organic phase of extract was subjected to d-SPE with 30 mg of PSA and 30 mg of C18, then separated by centrifugation at 5000 rpm for 10 min and finally 1 mL of the supernatant was filtered to vials.

For each fortification level, this extraction procedure with d-SPE was applied in triplicates and the recovery rates were compared with no clean up recoveries. Results showed that additional purification step have both positive and negative effects in recoveries for different analytes at different concentration levels. It is observed that as the concentration is increasing the effect of d-SPE on recovery rates is decreasing.

Although d-SPE step have increased the analyte recoveries at 10 µg/kg significantly, the resulting recovery values was found to exceed the limit of 70-120% for 25 compounds compared to no-clean up results. For instance, as the recovery rates of 4,4'-dichlorobenzophenone, aclonifen, boisvelone and hexaconazole without clean up were found as 93%, 101%, 107% and 105 % respectively, in case of d-SPE purification these values were increased up to 138%, 127%, 153% and 134% in return. The comparison of the recoveries with and without the d-SPE step was demonstrated in Figure 4.4.

At 50 µg/kg spiking concentration the differences of recovery rates between d-SPE and no clean up extraction were decreased. At this spiking concentration d-SPE increased the extraction efficiency for some pesticides like diuron, chlorpyrifos, imidacloprid, atrazine more than 10%, for some other compounds purification reduces the extraction efficiency as boscalid, triazophos, carbendazim and methoxyfenozide. As observed for the lowest spiking concentration, for some analytes as epoxiconazole, myclobutanil, prochloraz, propiconazole, tetraacetylethylenediamine and diphenylamine, the desired recovery limits (<120%) were exceeded by clean up.

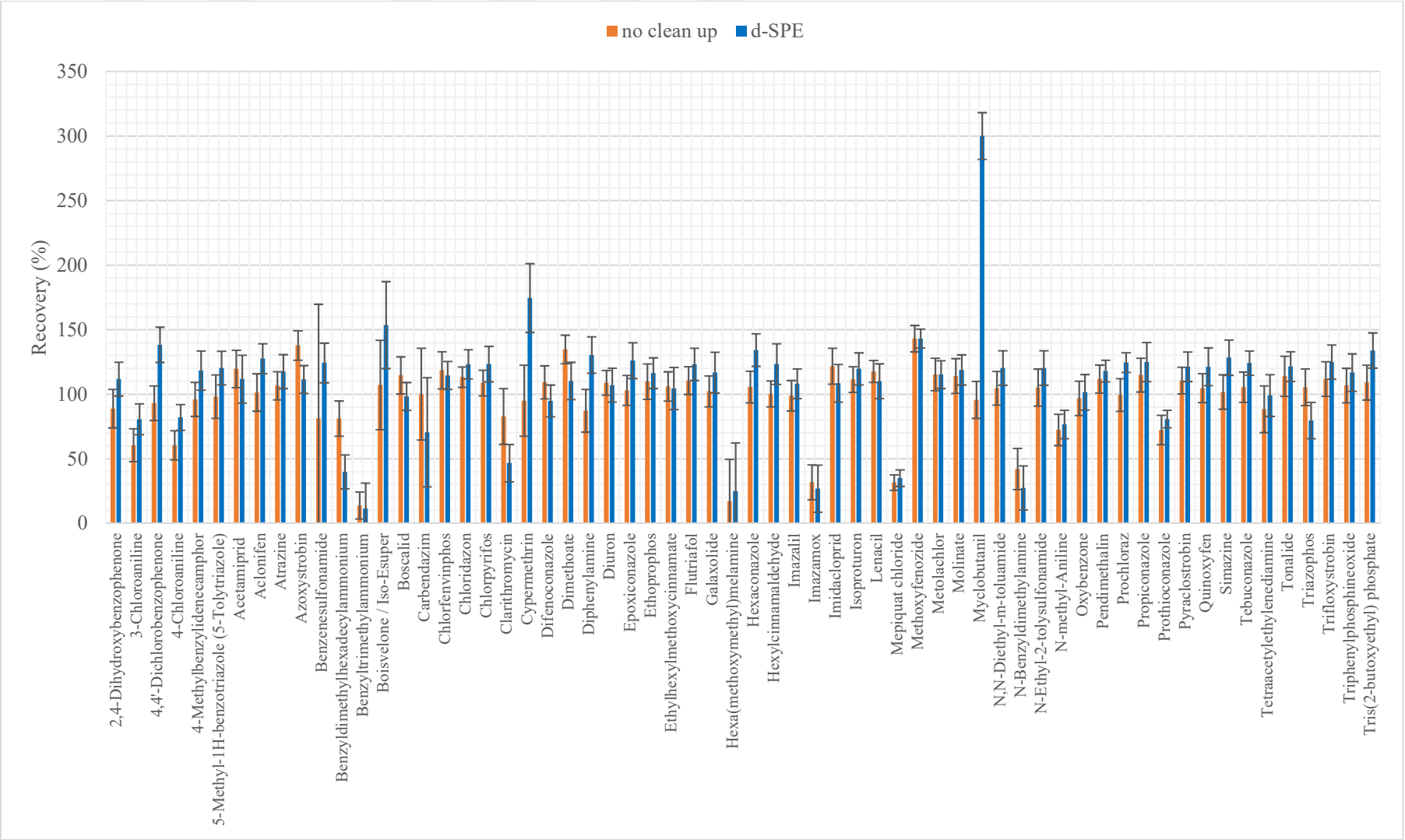


Figure 4.4. Effect of d-SPE on the recovery of ECs at low concentration (10 µg/kg).

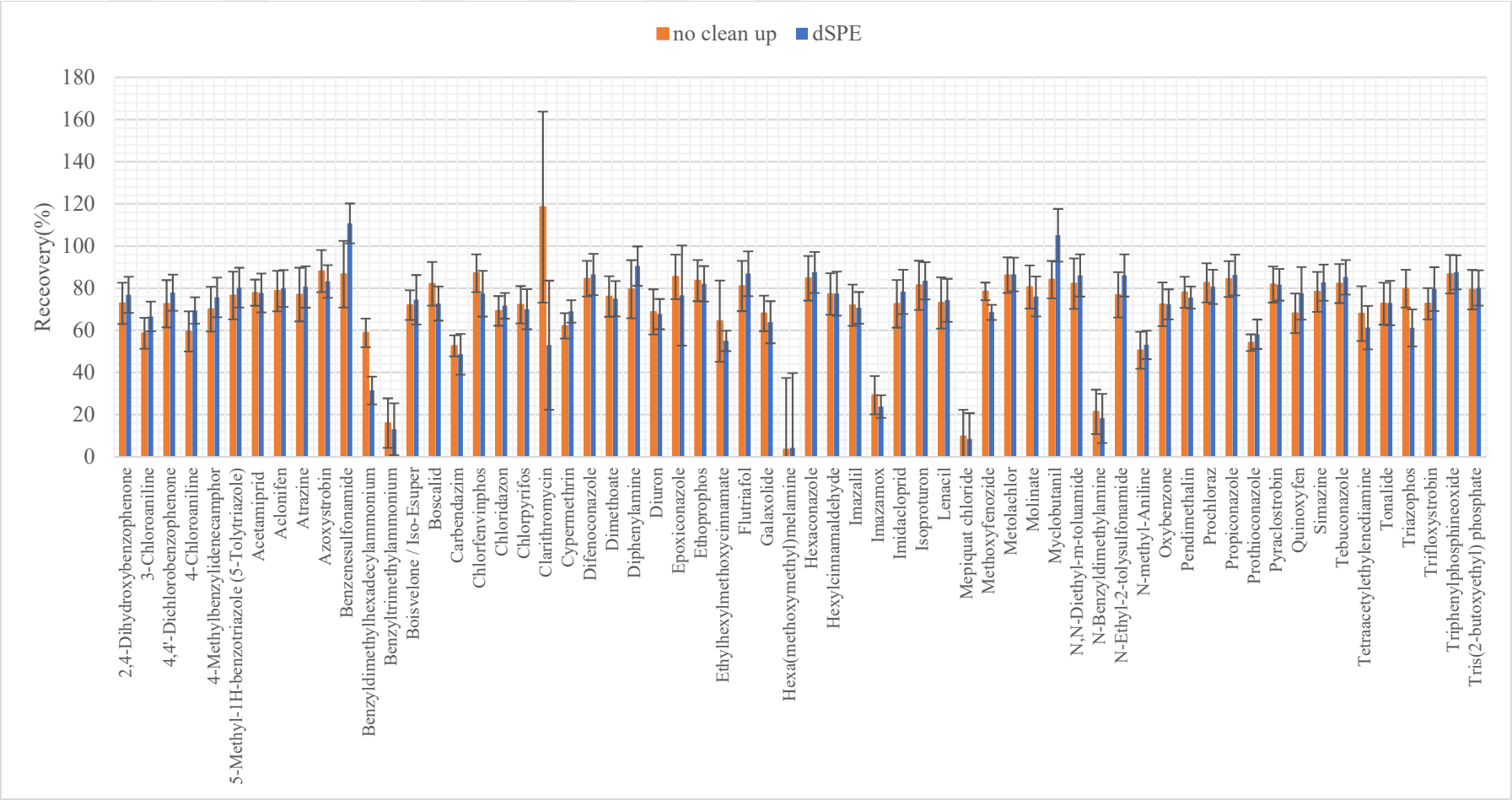


Figure 4.5. Effect of d-SPE on the recovery of ECs at high concentration (100 µg/kg).

As the concentration is increases, the effect of d-SPE on the extraction efficiency was observed to be decreasing. As shown in Figure 4.5. the recovery rates of the extractions with and without cleanup at 100 µg/kg spiking, the recovery rates were found close to each other. In this case, d-SPE step did not show any contribution to raise the recovery rates to the desired range for the compounds which had a recovery rate less than 70%. In fact, for 28 compounds the recovery rates were decreased from 0.2% to 65% by purification.

In order to determine the performance of the d-SPE cleanup, the matrix effects (ME) of both purified and non-purified extracts are evaluated. For this purpose, slopes of solvent based calibration curves and matrix matched calibration curves prepared with blank extracts were compared. As shown in Figure 4.6, the ME for most of the analytes are slightly reduced up to 10% by d-SPE cleanup. The effect of d-SPE was observed on 3 compounds namely 2-mercapto-benzothiazole, clarithromycin and hexa(methoxymethyl)melamine with reduced MEs by 27%, 44% and 34%, respectively. On the other hand, for some compounds (ME >50%) as methoxyfenozide and ethylhexylmethoxycinnamate, cleanup with PSA and C18 did not affect the signal significantly.

The extraction procedure without any purification step gave acceptable recoveries within the range of 70-120% for most of the compounds. The additional cleanup step did not enhance the recovery efficiency to the desired level of the poorly extracted compounds as benzyltrimethylammonium, hexa(methoxymethyl)melamine, n-benzyldimethylamine and mepiquat chloride and resulted in limit excess for a great number of compounds in low concentrations. On the other hand, 87% of the target analytes, the ME rates for the procedure without clean up were in the range of ±50% and d-SPE had only mild effects on ME for most of the other compounds out of this range. Therefore, all the extractions were performed without cleanup, which was found more time and cost effective.

4.1.2.3. Effect of extraction cycle. From the recovery experiments, it is observed that with increasing concentration the extraction efficiency decreases for most of the compounds. This decrease is especially observed by the 100 µg/kg fortification level for each target analyte, which can be attributed to the insufficient extraction solvent in case of high concentrations. Therefore, the effect of an additional extraction cycle was assessed for each fortification level by mixing the sample with the equal volume of acidified acetonitrile twice and combining the resulting supernatants of the two extraction steps.

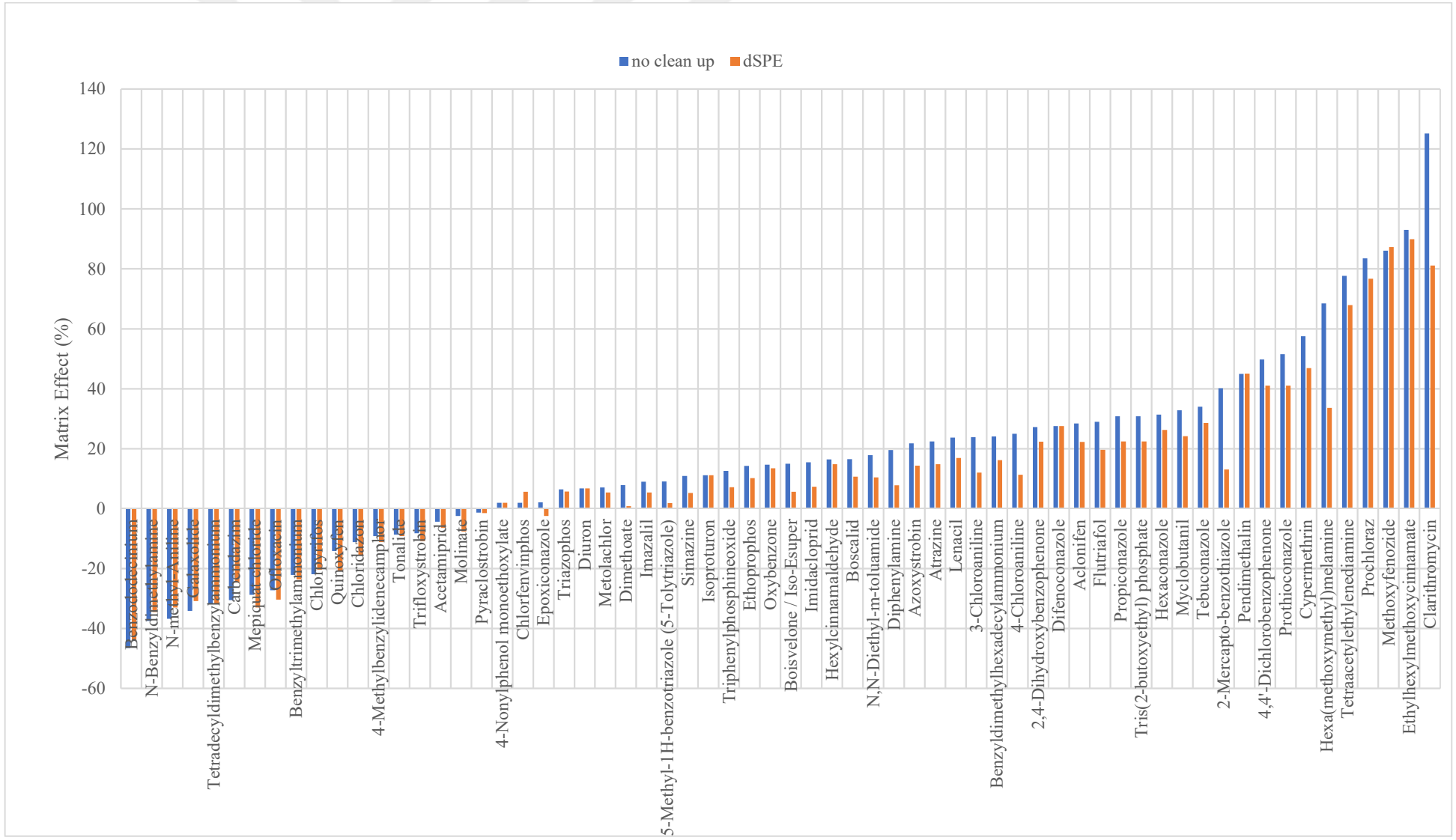


Figure 4.6. Effect of dSPE on matrix effect.

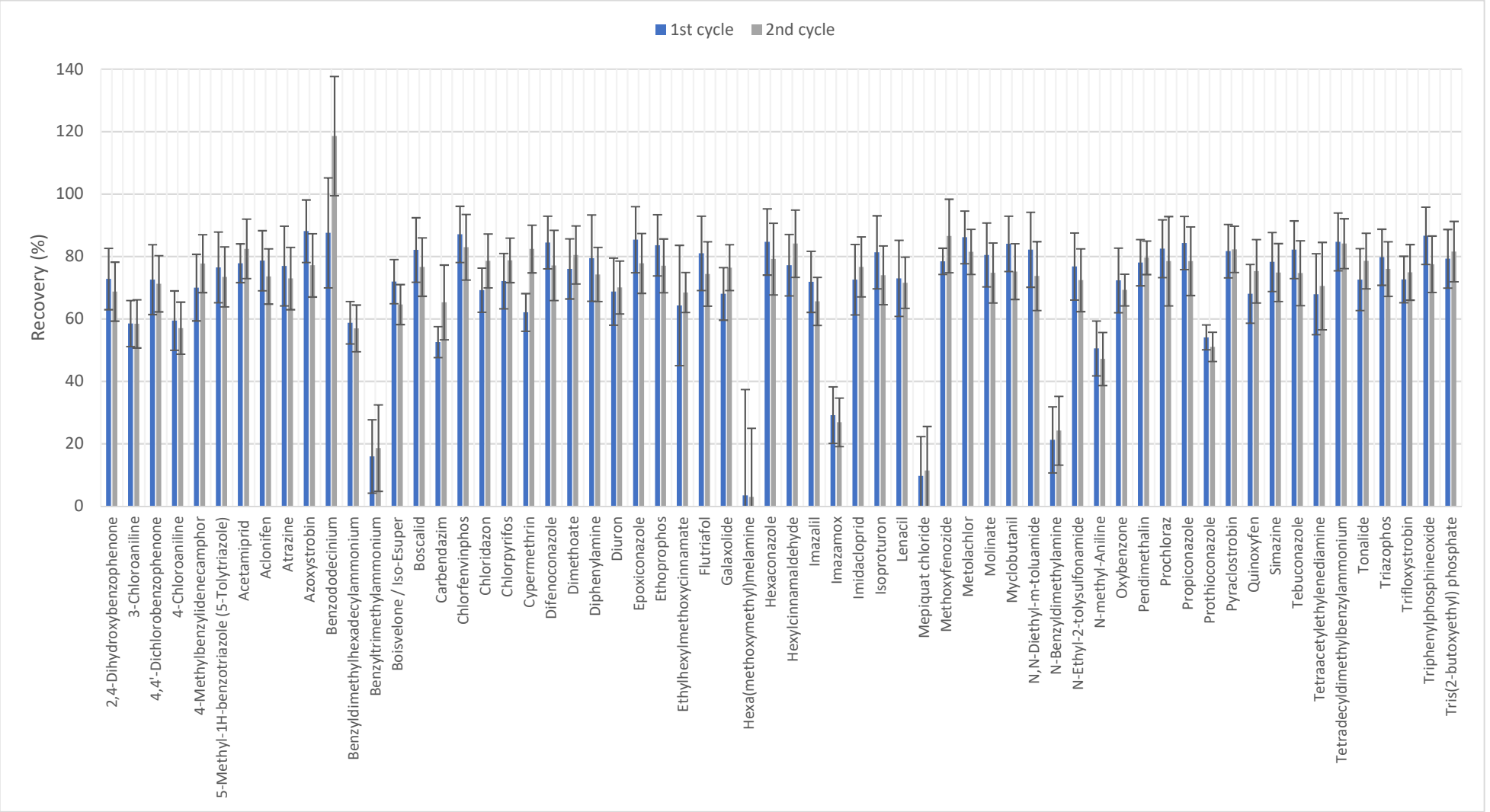


Figure 4.7. Effect of extraction cycle for the recovery target pollutants from soil (100 µg/kg).

The results showed that for 10 μg/kg and 50 μg/kg spiking levels second cycle extraction does not affect the recovery rates of the target analytes significantly. At 10 μg/kg fortification 72% of the target analytes showed higher recoveries with one cycle extraction, whereas at 50 μg/kg 70% of the analytes are more efficiently extracted in one step as well.

In case of 100 μg/kg spiking, second cycle increased the recovery rates of 39% of the analytes in the range of 1.5-20% compared to one cycle extraction, but still for 61% of the target compounds extraction in one step was found to be more efficient. The application of second cycle increased the extraction efficiency of 6 compounds namely chloridazon, cypermethrin, diuron, galaxolide, quinoxyfen and tetraacetylethylenediamine to the desired recovery range of 70-120%. On the other hand, for other compounds this additional step did not reached this recovery limits. The results of two cycle extraction is compared with those of achieved at one time for 100 μg/kg in Figure 4.7. Since the average contribution of the repeated extraction to the recoveries was remained at 6 % in average for less than half of the target analytes, this step was not integrated to the extraction procedure considering the time and solvent consumption.

4.2. Evaluation of the Method Performance

In order to evaluate the specifity of the method, chromatograms of fortified and unfortified blank soil samples were compared to verify the absence of interfering species at about the retention time of the target analytes and interfering peaks were not observed for most of the target pollutants, except 2-mercapto-benzothiazole, 4-nonylphenol monoethoxylate and benzenesulfonamide. Recovery and precision were evaluated by analyzing blank samples spiked at three different concentrations (10, 50, and 100 μg/kg) in triplicates and the results are presented together with other performance parameters in Table 4.3. All the method performance studies were conducted on pseudo blank sample (taken from location U6) to represent the paddy soil.

The recoveries and related RSD values of the target analytes showed changes for each fortification level. In the pie charts (Figure 4.8. and Figure 4.9) the percentages of the analytes meeting and exceeding the desired recovery and RDS values for each fortification level were summarized. Applied method gave mostly satisfactory recoveries for each fortification level out of a few exceptions. The recoveries of 2-mercapto-benzothiazole, 4-nonylphenol monoethoxylate and benzenesulfonamide could not be determined due to the high interfering peaks of those compounds at blank samples. Beside these compounds, the recoveries of benzyltrimethylammonium, imazamox,

Table 4.3. Method validation parameters: Mean recoveries and RSD values at 3 spiking levels, LOD, LOQ, linearity and ME of the target analytes.

	10 ug/kg		50 ug/kg		100 ug/kg		MLOD	MLOQ	R^2	ME
	Mean	%RSD	Mean	%RSD	Mean	%RSD	µg/kg	µg/kg		(%)
2,4-Dihydroxybenzophenone	88.9	14.9	88.6	6.5	72.8	9.8	1.0	3.0	0.9982	27.2
2-Mercapto-benzothiazole	-	-	-	-	-	-	-	-	-	-
3-Chloroaniline	60.5	12.7	70.5	8.7	58.5	7.4	0.7	2.0	0.9906	23.8
4,4'-Dichlorobenzophenone	93.0	13.5	90.6	10.9	72.6	11.2	1.6	5.0	0.9960	49.7
4-Chloroaniline	60.4	11.3	70.6	9.0	59.5	9.5	1.4	4.3	0.9989	24.9
4-Methylbenzylidenecamphor	96.0	13.2	90.1	9.9	70.0	10.7	1.2	3.7	0.9965	-9.2
4-Nonylphenol monoethoxylate	-	-	-	-	-	-	-	-	-	-
5-Methyl-1H-benzotriazole	98.1	16.8	93.7	7.8	76.5	11.3	1.4	4.2	0.9983	9.0
Acetamiprid	119.6	14.5	91.7	6.3	77.8	6.2	2.7	8.1	0.9824	-4.4
Aclonifen	101.3	14.5	97.8	7.6	78.6	9.6	1.2	3.6	0.9993	28.4
Atrazine	106.6	10.9	90.3	8.1	76.9	12.7	0.5	1.6	0.9995	22.3
Azoxystrobin	137.7	11.4	110.3	9.4	88.1	10.0	1.2	3.6	0.9908	21.7
Benzenesulfonamide	-	-	-	-	-	-	-	-	-	-
Benzyldimethyldodecylammonium	718.2	46.9	121.9	6.1	87.6	17.6	29.3	88.8	0.9873	-46.5
Benzyldimethylhexadecylammonium	81.1	13.7	68.6	10.8	58.8	6.8	2.6	7.8	0.9967	24.1
Benzyldimethyltetradecylammonium	171.3	25.6	105.7	5.3	84.7	9.2	14.2	43.1	0.9901	-31.9
Benzyltrimethylammonium	13.8	10.4	16.3	13.3	15.9	11.8	2.3	6.9	0.9950	-22.2
Boisvelone / Iso-Esuper	107.1	34.7	89.5	9.7	71.9	7.0	6.0	18.2	0.9931	15.0
Boscalid	114.6	14.4	102.9	7.3	82.1	10.3	1.0	2.9	0.9956	16.5
Carbendazim	100.0	35.5	69.5	8.2	52.6	5.0	16.7	50.6	0.9855	-30.6
Chlorfenvinphos	118.5	14.3	102.5	8.2	87.1	9.0	1.3	3.9	0.9983	1.9
Chloridazon	113.2	7.9	90.9	5.3	69.2	7.1	2.7	8.2	0.9829	-11.2
Chlorpyrifos	108.6	9.9	88.2	8.0	72.1	8.9	1.3	3.9	0.9988	-21.9
Clarithromycin	82.7	21.6	122.6	16.2	118.5	45.3	0.0	0.2	0.9934	125.1
Cypermethrin	94.9	27.5	94.8	10.6	62.1	6.1	23.0	69.6	0.9735	57.4
Difenoconazole	109.2	12.8	97.7	7.7	84.5	8.5	0.4	1.2	0.9989	27.5
Dimethoate	134.7	11.0	90.8	9.1	76.0	9.6	3.1	9.5	0.9917	7.8
Diphenylamine	87.2	16.6	98.9	9.2	79.5	13.8	0.9	2.7	0.9972	19.5
Diuron	108.9	9.5	87.1	8.2	68.7	10.7	1.3	3.8	0.9921	6.7
Epoxiconazole	102.9	11.6	103.3	0.8	85.4	10.6	0.7	2.0	0.9987	2.0
Ethoprophos	109.7	13.7	99.4	6.8	83.6	9.8	1.0	3.1	0.9984	14.2
Ethylhexylmethoxycinnamate	106.0	11.4	104.1	10.2	64.3	19.3	1.8	5.4	0.9900	93.0
Flutriafol	110.5	10.7	100.7	7.5	81.0	11.9	1.1	3.2	0.9981	28.9
Galaxolide	102.2	11.9	89.5	6.9	68.0	8.4	1.6	4.9	0.9943	-34.2
Hexa(methoxymethyl)melamine	17.2	32.4	4.3	6.0	3.4	34.0	94.1	285.3	0.6861	68.4
Hexaconazole	105.6	12.3	93.0	7.4	84.7	10.6	0.7	2.0	0.9998	31.4
Hexylcinnamaldehyde	100.3	10.0	91.6	9.7	77.2	9.8	1.5	4.6	0.9873	16.4
Imazalil	98.8	11.8	91.4	6.8	71.9	9.8	1.0	3.2	0.9966	8.9
Imazamox	31.8	13.5	33.3	15.9	29.2	9.1	1.5	4.6	0.9909	2.2
Imidacloprid	121.7	13.9	90.0	8.3	72.6	11.3	2.2	6.7	0.9948	15.4
Isoproturon	111.4	9.9	96.0	6.8	81.3	11.7	1.1	3.2	0.9983	11.1
Lenacil	117.6	8.5	93.5	8.8	73.0	12.2	1.2	3.7	0.9947	23.6
Mepiquat chloride	31.6	6.0	13.4	9.3	9.7	12.6	6.9	20.8	0.9825	-28.8
Methoxyfenozide	143.0	10.2	100.8	7.4	78.5	4.2	8.2	24.8	0.9861	86.0
Metolachlor	115.3	12.5	99.3	8.0	86.1	8.4	1.3	4.0	0.9971	7.0
Molinate	114.1	13.5	96.7	5.3	80.5	10.2	0.9	2.6	0.9992	-2.5
Myclobutanil	95.6	14.3	97.6	5.4	84.0	8.8	0.5	1.5	0.9993	32.8
N,N-Diethyl-m-toluamide	104.7	13.0	101.0	7.5	82.2	12.0	0.8	2.6	0.9998	17.8
N-Benzyldimethylamine	42.1	15.9	28.8	9.9	21.3	10.6	0.9	2.7	0.9831	-37.5
N-Ethyl-2-tolysulfonamide	105.1	14.3	95.9	8.9	76.8	10.7	1.8	5.4	0.9997	16.8

Table 4.3. Continued.

	10 ug/kg		50 ug/kg		100 ug/kg		MLOD µg/kg	MLOQ µg/kg	R^2	ME (%)
	Mean	%RSD	Mean	%RSD	Mean	%RSD				
N-methyl-Aniline	72.3	12.1	68.7	8.8	50.5	8.8	2.1	6.3	0.9834	-36.8
Oxadiazon	301.3	21.6	77.3	10.1	94.2	21.3	26.0	78.8	0.9698	45.24
Oxybenzone	96.8	13.3	90.5	6.2	72.3	10.3	0.8	2.5	0.9984	14.6
Pendimethalin	111.7	10.9	90.1	13.5	78.0	7.4	2.0	6.1	0.9952	45.0
Prochloraz	99.4	12.6	95.6	5.1	82.5	9.3	1.3	4.1	0.9959	83.5
Propiconazole	114.8	13.1	97.7	6.2	84.3	8.5	0.7	2.0	0.9982	30.8
Prothioconazole	72.3	11.3	58.8	6.9	54.1	4.0	1.1	3.4	0.9922	51.5
Pyraclostrobin	110.6	10.3	97.8	8.8	81.7	8.5	1.5	4.6	0.9970	-1.4
Quinoxyfen	104.7	11.2	85.3	8.2	68.0	9.4	1.0	3.0	0.9992	-14.2
Simazine	101.7	13.3	93.2	5.9	78.3	9.5	0.4	1.3	0.9993	10.8
Tebuconazole	105.5	11.7	99.9	8.4	82.2	9.2	0.5	1.6	0.9989	34.0
Tetraacetylethylenediamine	88.4	18.1	66.7	12.4	67.9	13.0	2.7	8.1	0.9994	77.6
Tonalide	113.9	15.5	97.0	7.7	72.6	9.9	1.1	3.5	0.9953	-8.7
Triazophos	105.4	14.2	99.0	9.7	79.7	9.0	1.0	3.0	0.9998	6.4
Trifloxystrobin	111.8	13.4	92.3	7.8	72.6	7.4	1.4	4.1	0.9951	-8.2
Triphenylphosphineoxide	106.7	13.4	106.6	7.2	86.6	9.2	1.3	3.9	0.9991	12.5
Tris(2-butoxyethyl) phosphate	109.0	13.5	93.6	10.9	79.3	9.4	2.1	6.2	0.9990	0.0

hexa(methoxymethyl)melamine, n-benzyldimethylamine and mepiquat chloride were found to be less than the acceptable recovery limits for each fortification level. On the other hand, a total of 9 compounds exceeded the acceptable recovery limits (70-120%) at the lowest (10 µg/kg) spiking concentration, 7 compounds at 50 µg/kg and 13 compounds at 100 µg/kg, respectively. Although the average percentages were high in the lowest spiking concentration the RSD values exceeded the limit of 20% for 13% of the target compounds, where this limit excess was dropped at higher spiking levels to 4% at 50 µg/kg and 9% at 100 µg/kg.

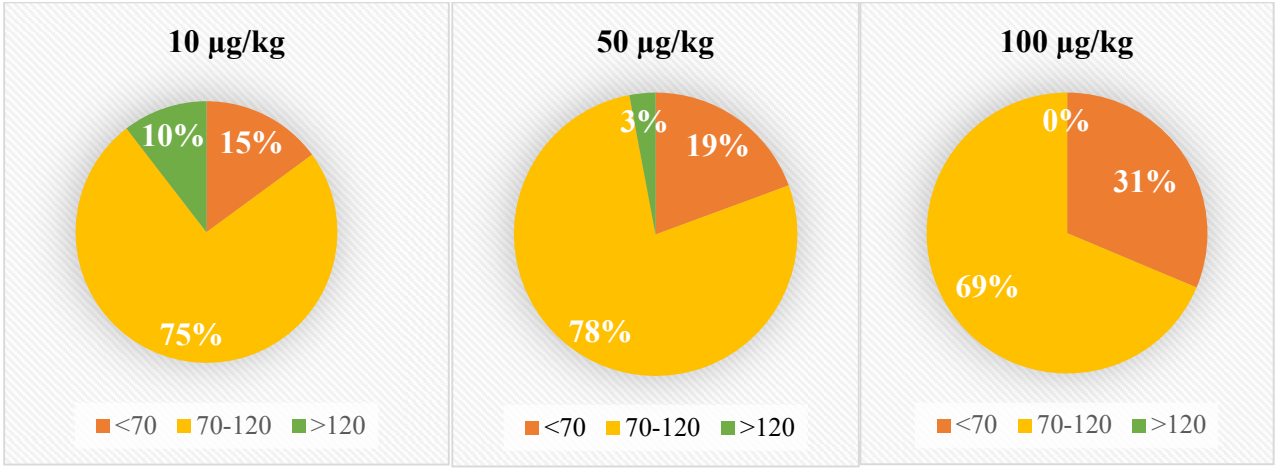


Figure 4.8. The distribution of the analytes meeting and exceeding the desired recovery values.

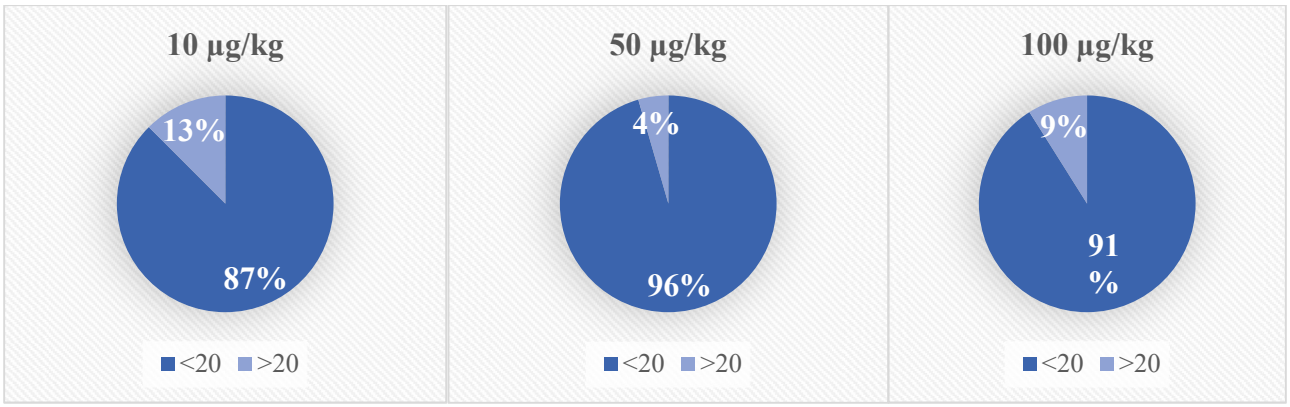


Figure 4.9. The distribution of the analytes meeting and exceeding the desired RSD values.

The linearity of the analytes was determined using the calibration curves constructed with the extract responses of the analytes at the selected range. The linear regression coefficient (R^2) of the extraction matched calibration curves was >0.97 except one target analyte. Although the matrix matched and solvent based calibration curves of hexa(methoxymethyl)melamine showed >0.99 linearity, based upon to its low extraction efficiency, the linearity of the method calibration curve was found as 0.68.

The matrix effects on each analyte was determined comparing the slopes of matrix matched and solvent based calibration curves. As listed in Table 4.3., for most of the analytes (88%), the matrix effect was not dramatic (-50%<ME<50%) and only for 8 compounds out of 67 showed strong matrix effect (ME>50%), which are clarithromycin, cypermethrin, ethylhexylmethoxycinnamate, hexa(methoxymethyl)melamine, methoxyfenozide, prothioconazole, pyraclostrobin and prochloraz and tetraacetylethylenediamine. Even though some strong matrix effect was measured for these compounds, the recoveries were not noticeably affected by this parameter. In Figure 4.10. the distribution of matrix effects of the analytes was summarized in percentages.

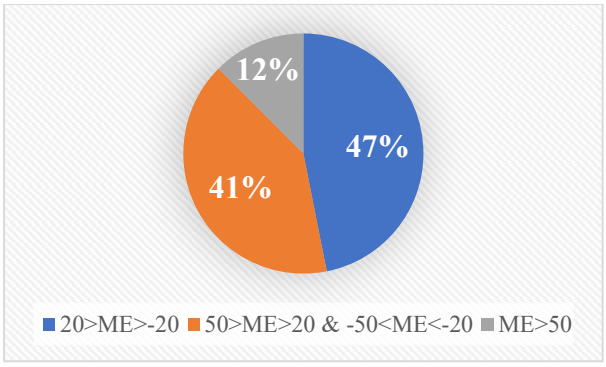


Figure 4.10. The distribution of the analytes with respect to the matrix effect ranges.

Limit of detection (LOD) and quantification (LOQ) are the key elements for the evaluation of the method performance at low concentrations. The determination of LOD and LOQ in soil extraction methods are done in different ways in the literature. One approach on the LOD and LOQ determination is to spike the blank or pseudo blank sample, which represents the characteristics of the analyzed samples, with a lower concentration range close to the expected LOD value. The LOD is calculated using the standard deviation of the response (Sy) and the slope (S) of the calibration curve. On the other hand, in most of the studies, the LOD and LOQ were determined based on the S/N ratio of the lowest detectable point from the matrix matched calibration curve, which provides consequently really low detection limits with respect to the instrumental sensitivity. Although the matrix matched calibration demonstrates the effect of the sample matrix on the analytes responses, this technique does not cover the performance of the whole extraction procedure. As the lowest spiking level meeting the performance criteria was suggested to use by the validation guideline for food samples (SANTE 2017), the determination of the detection limits in soil matrix matched calibration approach is commonly used in the literature.

In order to compare these two LOD estimation approaches, both calibration curves based on spiked pseudo blank sample (U6) at three concentrations (1, 5, 10 µg/kg) and matrix matched calibration curves at 0.05-5 µg/L range were prepared. For the analytes, which did not give a linear response on lower spiking concentrations, the MLOD and MLOQ values were calculated based on the linear response of higher spiking range (10,50, 100 µg/kg). The MLOD and MLOQ values obtained by spiking approach were listed in Table 4.3, while the LOD and LOQ values obtained my matrix matched calibration approach and related linearity values were summarized in Table 4.4.

Table 4.4. LOD and LOQ values based on matrix matched calibration approaches.

	LOD (µg/kg)	***LOQ (µg/kg)***	***R^2***
2,4-Dihydroxybenzophenone	0.04	0.12	0.9995
2-Mercapto-benzothiazole	0.57	1.91	0.9780
3-Chloroaniline	0.05	0.15	0.9994
4,4'-Dichlorobenzophenone	0.67	2.23	0.9988
4-Chloroaniline	0.08	0.27	0.9995
4-Methylbenzylidenecamphor	0.04	0.13	0.9968
4-Nonylphenol monoethoxylate	-	-	-
5-Methyl-1H-benzotriazole (5-Tolytriazole)	0.23	0.78	0.9964
Acetamiprid	0.05	0.16	0.9915
Aclonifen	0.14	0.47	0.9989
Atrazine	0.02	0.06	0.9994
Azoxystrobin	0.01	0.04	0.9979
Benzenesulfonamide	10.00	33.00	0.9880
Benzyldimethyldodecylammonium	2.00	6.67	0.9967
Benzyldimethylhexadecylammonium	0.30	1.00	0.9973
Benzyldimethyltetradecylammonium	2.00	6.67	0.9901
Benzyltrimethylammonium	0.17	0.57	0.9941
Boisvelone / Iso-Esuper	3.60	11.99	0.8813
Boscalid	0.08	0.25	0.9987
Carbendazim	0.08	0.25	0.9936

Table 4.4. Continued.

	***LOD* (µg/kg)**	***LOQ* (µg/kg)**	**R^2**
Chlorfenvinphos	0.52	1.75	0.9995
Chloridazon	0.16	0.52	0.9930
Chlorpyrifos	0.10	0.33	0.9638
Clarithromycin	0.10	0.33	0.9950
Cypermethrin	2.00	6.67	0.9951
Difenoconazole	0.04	0.14	0.9918
Dimethoate	0.05	0.15	0.9993
Diphenylamine	0.30	0.99	0.9858
Diuron	0.07	0.23	0.9979
Epoxiconazole	0.06	0.20	0.9991
Ethoprophos	0.04	0.14	0.9995
Ethylhexylmethoxycinnamate	0.49	1.65	0.9939
Flutriafol	0.11	0.36	0.9972
Galaxolide	0.49	1.62	0.9946
Hexa(methoxymethyl)melamine	0.74	2.45	0.9976
Hexaconazole	0.03	0.10	0.9928
Hexylcinnamaldehyde	0.77	2.57	0.9875
Imazalil	0.03	0.11	0.9983
Imazamox	0.08	0.28	0.9966
Imidacloprid	0.02	0.08	0.9877
Isoproturon	0.05	0.18	0.9995
Lenacil	0.23	0.77	0.9867
Mepiquat chloride	0.11	0.38	0.9958
Methoxyfenozide	8.15	27.17	0.9031
Metolachlor	0.03	0.10	0.9998
Molinate	0.04	0.14	0.9982
Myclobutanil	0.08	0.27	0.9997
N,N-Diethyl-m-toluamide	0.04	0.14	0.9980
N-Benzyldimethylamine	0.05	0.17	0.9822
N-Ethyl-2-tolysulfonamide	0.09	0.30	0.9932
N-methyl-Aniline	0.02	0.06	0.9935
Oxadiazon	10.00	33.00	0.9591
Oxybenzone	0.26	0.86	0.9979
Pendimethalin	0.32	1.06	0.9957
Prochloraz	0.10	0.32	0.9923
Propiconazole	0.08	0.27	0.9913
Prothioconazole	0.27	0.91	0.9965
Pyraclostrobin	0.04	0.13	0.9989
Quinoxyfen	0.02	0.08	0.9996
Simazine	0.03	0.11	0.9999
Tebuconazole	0.01	0.04	0.9952
Tetraacetylethylenediamine	0.85	2.82	0.9769
Tonalide	0.26	0.86	0.9964
Triazophos	0.02	0.08	0.9999
Trifloxystrobin	0.02	0.08	0.9998
Triphenylphosphineoxide	0.15	0.51	0.9930
Tris(2-butoxyethyl) phosphate	0.27	0.89	0.9899

Results of these two methods showed that, lower LOQ values could be obtained when matrix matched calibration curve was used to estimate the limit of detection, as 78% (Figure 4.11) of the analytes showed LOQ values less than 1 µg/kg within the range of 0.04-33 µg/kg. However, the MLOQ values were found in a wider range of 1.18 and 285 µg/kg, in which the method quantification limit of 70% of the target compounds were found less than 5µg/kg (Figure 4.10). These variation of detection limits was also observed in the literature. For instance, although very similar extraction procedure was applied, the LOQ value of chlorpyrifos, triazophos, difeconazole and parathion methyl were found by Feng et al. (2015) with matrix matched calibration approach as 0.1, 0.4, 0.1 and 0.9

µg/kg, though the LOQ values for the same compounds were found as 0.25, 1, 4, 2 µg/kg using Yu et al. (2016a) by spiking approach, respectively.

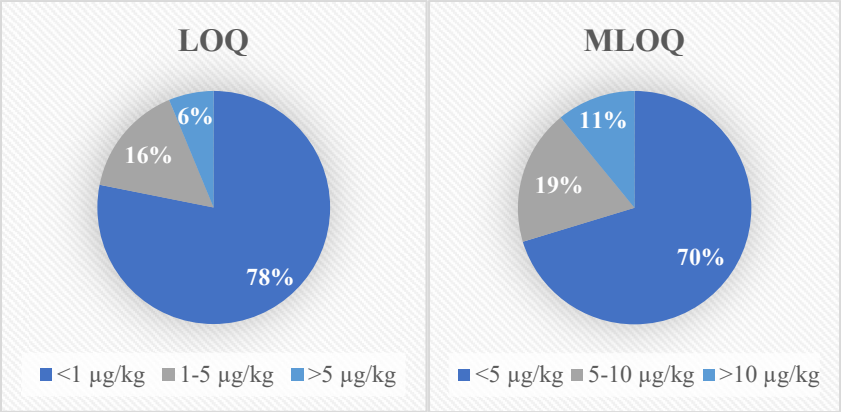


Figure 4.11. The MLOQ and LOQ distribution of analytes in percentages.

4.2.1. Effect of Soil Characteristics on the Method Performance

In order the assess the method performance on different soil types, recovery experiments were both conducted in agricultural soil collected from paddy field (location=U6) and yard soil collected from Boğaziçi University. The recovery rates and related RSD values of both soil samples for 3 different fortification levels as 10, 50 and 100 µg/kg in triplicates were calculated. The recovery results of agricultural soil were summarized in Table 4.3 and the yard soil were listed in Table 4.5.

Table 4.5. Mean recoveries and RSD at 3 spiking levels of yard soil.

	10 ug/kg		50 ug/kg		100 ug/kg		R^2
	Mean	%RSD	Mean	%RSD	Mean	%RSD	
2,4-Dihydroxybenzophenone	90.2	10.8	85.1	9.3	73.5	7.2	0.9812
2-Mercapto-benzothiazole	83.4	3.1	22.8	28.5	10.8	49.6	-
3-Chloroaniline	69.8	8.9	66.3	6.8	58.9	6.1	0.9908
4,4'-Dichlorobenzophenone	95.3	11.8	81.3	7.2	69.5	6.9	0.9819
4-Chloroaniline	67.9	10.5	66.3	7.3	56.9	1.7	0.9908
4-Methylbenzylidenecamphor	96.6	10.4	78.4	7.2	64.9	8.6	0.9801
5-Methyl-1H-benzotriazole	88.6	9.8	82.4	8.0	70.8	7.3	0.9813
Acetamiprid	88.8	6.2	80.5	3.0	75.1	10.3	0.9718
Aclonifen	88.3	6.6	76.7	3.0	73.9	3.2	0.9958
Atrazine	90.1	2.9	81.3	1.8	85.8	7.9	0.9855
Azoxystrobin	94.2	8.1	90.6	2.8	96.7	1.7	0.9980
Benzyldimethyldodecylammonium	73.8	8.5	72.8	8.8	69.9	9.9	0.9422
Benzyldimethylhexadecylammonium	60.6	12.6	59.4	6.6	58.4	8.9	0.9792
Benzyldimethyltetradecylammonium	65.8	9.0	65.7	8.4	62.4	9.4	0.9751
Benzyltrimethylammonium	25.7	9.3	29.2	10.4	33.0	7.5	0.9860
Boisvelone / Iso-Esuper	38.1	45.8	75.9	7.7	72.2	12.0	0.9639
Boscalid	96.5	7.2	91.5	5.1	83.8	1.0	0.9979
Carbendazim	76.8	17.7	76.4	6.4	66.9	5.7	0.9894
Chlorfenvinphos	96.3	3.5	88.9	3.6	90.3	2.1	0.9982
Chloridazon	94.9	6.9	85.6	4.2	76.2	2.5	0.9949
Chlorpyrifos	96.1	5.8	81.7	5.0	71.2	1.7	0.9980
Clarithromycin	40.5	5.1	71.4	25.2	105.0	7.1	0.9668
Cypermethrin	41.5	9.4	55.0	11.8	55.0	2.2	0.9874
Difenoconazole	83.4	3.3	82.4	4.4	80.5	2.8	0.9964

Table 4.5. Continued.

	10 ug/kg		50 ug/kg		100 ug/kg		R^2
	Mean	%RSD	Mean	%RSD	Mean	%RSD	
Dimethoate	90.8	2.3	83.6	3.0	76.4	8.3	0.9844
Diphenylamine	95.4	11.9	88.4	9.4	78.2	8.2	0.9775
Diuron	89.5	4.6	83.0	3.7	84.1	3.2	0.9970
Epoxiconazole	93.0	4.2	88.6	2.9	94.8	2.4	0.9981
Ethoprophos	93.3	5.8	91.3	4.6	96.5	1.1	0.9973
Ethylhexylmethoxycinnamate	81.0	4.2	69.3	12.9	54.7	12.2	0.9488
Flutriafol	98.3	2.1	99.2	7.0	94.1	3.6	0.9958
Galaxolide	97.7	14.5	91.3	6.1	69.7	6.8	0.9877
Hexa(methoxymethyl)melamine	6.6	57.7	3.5	11.9	2.8	18.4	0.8257
Hexaconazole	88.4	3.6	85.8	5.5	90.6	1.4	0.9963
Hexylcinnamaldehyde	88.8	5.4	80.3	9.9	67.8	7.5	0.9724
Imazalil	84.1	4.4	82.0	3.7	81.4	2.9	0.9977
Imazamox	47.4	10.1	48.7	7.2	42.2	8.1	0.9719
Imidacloprid	90.4	6.2	83.9	3.1	75.8	5.0	0.9919
Isoproturon	94.1	3.7	91.4	3.3	97.5	2.4	0.9967
Lenacil	117.2	8.4	97.3	6.0	83.7	4.3	0.9807
Mepiquat chloride	20.8	8.7	13.6	9.5	14.0	4.5	0.9419
Methoxyfenozide	133.3	22.5	88.4	13.4	71.8	8.6	0.9688
Metolachlor	92.2	6.1	91.6	2.7	100.3	0.1	0.9990
Molinate	91.7	3.0	86.7	3.2	85.9	1.3	0.9978
Myclobutanil	59.7	10.5	65.2	5.2	91.0	2.5	0.9931
N,N-Diethyl-m-toluamide	96.9	9.2	93.5	8.8	83.4	9.1	0.9688
N-Benzyldimethylamine	44.7	9.2	37.9	8.4	32.8	6.2	0.9884
N-Ethyl-2-tolysulfonamide	93.9	10.8	87.6	8.6	75.1	7.0	0.9802
N-methyl-Aniline	59.9	14.5	51.5	7.9	41.9	7.4	0.9875
Oxadiazon	75.8	28.2	79.1	17.0	63.1	5.7	0.9810
Oxybenzone	88.6	6.5	83.0	8.4	72.7	6.5	0.9817
Pendimethalin	95.7	0.9	71.8	4.5	71.3	3.7	0.9905
Prochloraz	91.5	5.8	92.0	6.7	105.0	8.9	0.9833
Propiconazole	91.5	3.6	86.8	4.7	89.6	1.9	0.9966
Prothioconazole	70.6	11.1	66.0	17.7	39.3	0.9	0.9825
Pyraclostrobin	98.3	4.1	93.8	4.1	92.7	3.5	0.9973
Quinoxyfen	84.8	3.5	77.7	4.6	71.9	2.9	0.9965
Simazine	94.4	4.4	91.2	4.1	89.1	2.2	0.9973
Tebuconazole	92.1	4.8	84.3	2.7	90.5	1.4	0.9955
Tetraacetylethylenediamine	101.9	12.0	73.3	3.0	57.1	10.0	0.9458
Tonalide	90.9	12.8	76.0	8.0	62.2	7.1	0.9836
Triazophos	95.8	10.1	89.6	8.6	80.0	7.2	0.9746
Trifloxystrobin	93.5	4.5	83.1	2.2	79.2	3.7	0.9970
Triphenylphosphineoxide	96.0	10.0	91.4	9.8	80.9	8.9	0.9622

The comparison of recovery rates on these 2 soil samples at 50 µg/kg spiking concentration was demonstrated on Figure 4.12. As shown in this figure, for 84% of the compounds, the recovery rate of the analytes is higher in the paddy soil. The reason of this difference could be attributed to the higher amount of organic carbon content of yard sample (3.71%) compared to paddy soil having organic carbon as 1.84%. Organic content is the most important parameter enhancing the adsorption of organic compounds onto soil, which is affecting the recovery rates, as the desorption of the compounds from soil having high OC% is more difficult. The soil properties of agricultural and yard soil were listed in Table 4.6.

Table 4.6. Characteristics of paddy and yard soils used for recovery studies.

Sample	***pH***	***TKN (g/kg)***	***T-P (g/kg)***	***P_2O_5 - P (g/kg)***	***PO_4^{3-}-P (g/kg)***	***OC (%)***	***CEC (meq/100 g)***
Paddy soil	7.26	1.07 ± 0.06	1.15 ± 0.04	2.76 ± 0.06	3.68 ± 0.07	1.79 ± 0.07	40.71 ± 0.19
Yard soil	6.89	2.25 ± 0.11	1.57 ±0.07	4.94 ± 0.10	3.70 ± 0.08	3.71 ± 0.33	35.14 ± 0.04

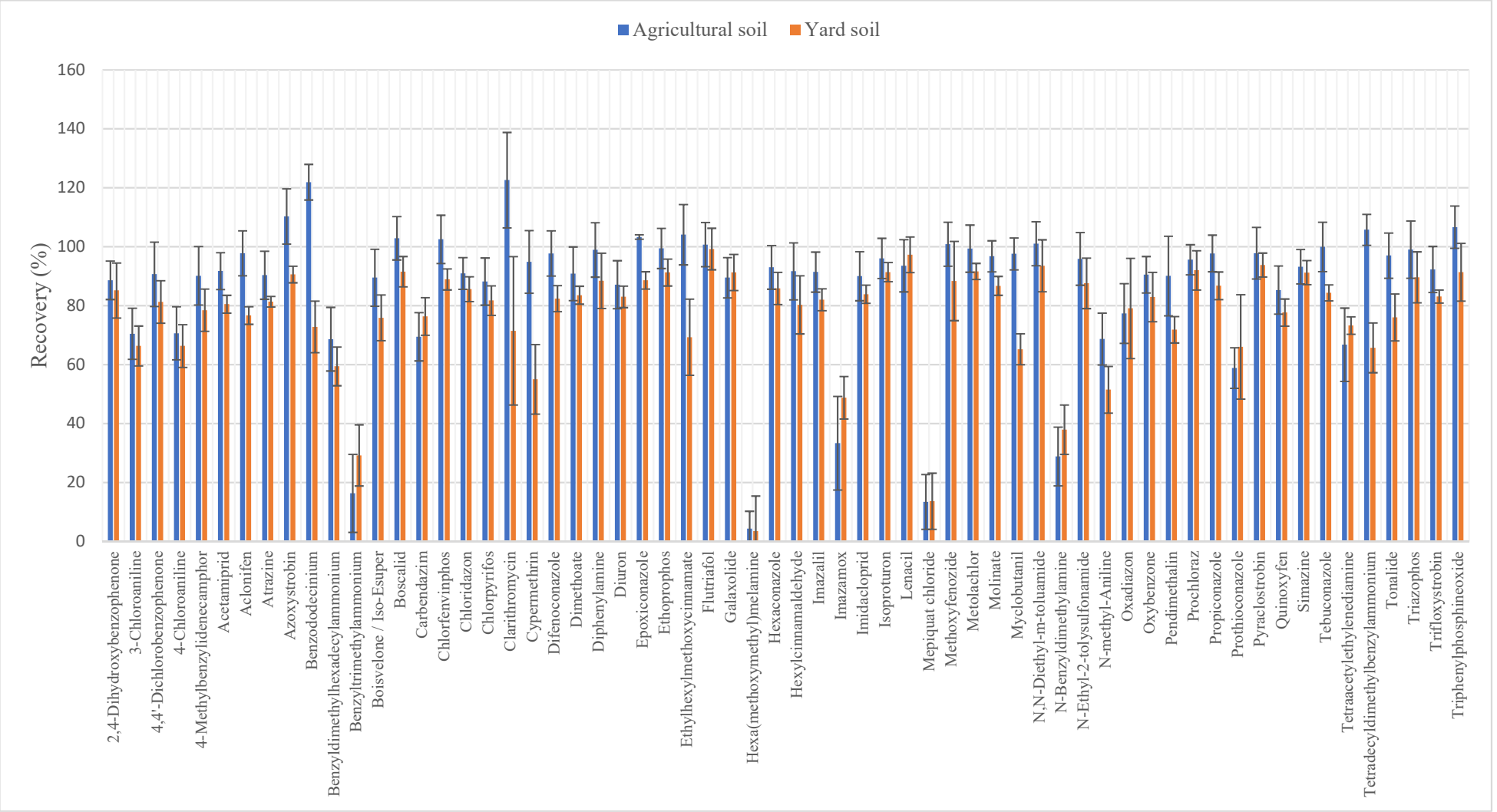


Figure 4.12. Recovery rates of agricultural and yard soil at 50 µg/kg.

For almost all target analytes the recoveries obtained from agricultural soil samples were higher than the yard sample up to 40%. These differences in the recovery were especially remarkable on the compounds having high K_{oc} values. For instance, the recovery of cypermethrin ($LogK_{oc}$=5.5) (PPDB, n.d.) was found as 95% in agricultural soil, which was decreased to 55% in yard soil. Similarly, benzyldimethyltetradecylammonium (BDTA), which has a $LogK_{oc}$ value of 5.5 in sandy soils (Khan, et al., 2017), was extracted from paddy soil with 105 % efficiency, as the recovery was dropped to 66% in case of yard soil recovery was dropped to 66% in case of yard soil. Moreover, the recovery of pendimethalin ($LogK_{oc}$=4.24) was decreased from 90% to 71%.

Although the recovery rates decreased with increasing organic carbon content, the recovery rates of most of the target analytes was found satisfactory in the desired range, which shows the applicability of the method for different soil types. As observed in the paddy soil samples, in case of yard soil the recovery rates were decreased with increasing concentration by a majority of compounds.

4.3. Application of Multiclass Analysis Method to Agricultural Soil Samples

A total of 22 different agricultural soil samples including 18 paddy and 4 unknown soils collected from 11 different locations in Edirne and Kırklareli. Sampling was especially done mainly on paddy fields close to the Ergene River to evaluate the relation of soil and water contamination, since rice is cultivated by submerged irrigation with river water. In order to construct a comprehensive data of the studied samples, the physical and chemical properties as pH, texture, cation exchange capacity, as well as nutrient, organic carbon and metal contents were determined. Samples were extracted and quantified with the developed method and the results were evaluated with the data of the river contamination.

4.3.1. Characterization of Soil Samples

In order to build an extensive qualification data about the studied areas, some chemical and physical characteristics of soils samples from the agricultural fields were evaluated, since soil properties, especially as organic content, pH, cation exchange capacity, texture and metal content have a great influence on the adsorption-desorption mechanisms of the contaminants in soil. The obtained results for pH, TKN, total phosphorus (presented as P, PO_4^{3-}-P, and P_2O_5-P), organic carbon content and cation exchange capacity are tabulated as average concentrations of duplicate samples

and corresponding standard deviations in Table 4.7. The correlations between these parameters were evaluated by Pearson correlations as listed in Table 4.8.

Table 4.7. Physical and chemical properties of soil samples (n=2).

Sample	*pH*	*TKN (g/kg)*	*T-P (g/kg)*	*P_2O_5 - P (g/kg)*	*PO_4^{3-}-P (g/kg)*	*OC (%)*	*CEC (meq/100 g)*
U1_A	6.93	2.71 ± 0.20	1.37 ± 0.00	3.14 ± 0.00	4.20 ± 0.00	3.19 ± 0.22	45.08 ± 0.11
U1_B	6.99	2.27 ± 0.20	1.24 ± 0.01	2.83 ± 0.01	3.80 ± 0.01	3.13 ± 0.04	48.37 ± 0.29
U2	7.12	2.11 ± 0.39	1.28 ± 0.14	3.30 ± 0.07	4.42 ± 0.10	3.15 ± 0.45	63.06 ± 0.17
U3_A	7.12	1.20 ± 0.11	0.95 ± 0.04	2.31 ± 0.22	3.08 ± 0.30	1.74 ± 0.20	49.52 ± 0.49
U3_B	7.21	1.60 ± 0.00	1.08 ± 0.02	2.64 ± 0.17	3.54 ± 0.23	1.51 ± 0.14	44.50 ± 0.01
U4_A	6.84	1.95 ± 0.11	1.27 ± 0.00	3.05 ± 0.14	4.09 ± 0.18	1.60 ± 2.26	34.91 ± 0.00
U4_B	6.85	2.02 ± 0.24	1.17 ± 0.15	2.91 ± 0.13	3.90 ± 0.18	2.39 ± 0.03	48.77 ± 0.01
U4_C	6.92	1.59 ± 0.12	1.18 ± 0.04	2.92 ± 0.13	3.91 ± 0.18	2.50 ± 0.24	58.03 ± 0.24
U4*	5.08	0.79 ± 0.00	1.00± 0.03	2.4 ± 0.03	3.21 ± 0.04	1.23 ± 0.12	20.54 ± 0.03
U5_A	6.96	0.95 ± 0.00	1.09 ± 0.05	2.65 ± 0.01	3.54 ± 0.01	0.97 ± 0.18	20.28 ± 0.01
U5_B	7.03	0.69 ± 0.06	1.08 ± 0.13	2.62 ± 0.14	3.51 ± 0.18	1.05 ± 0.04	16.16 ± 0.04
U6_A	7.15	1.27 ± 0.00	1.19 ± 0.08	2.86 ± 0.06	3.83 ± 0.08	1.84 ± 0.49	33.99 ± 0.08
U6_B	7.26	1.07 ± 0.06	1.15 ± 0.04	2.76 ± 0.06	3.68 ± 0.07	1.79 ± 0.07	40.71 ± 0.19
U7_A	7.08	1.33 ± 0.06	1.10 ± 0.09	2.68 ± 0.05	3.58 ± 0.08	2.45 ± 0.02	38.74 ± 0.09
U7_B	7.11	1.29 ± 0.06	1.07 ± 0.01	2.57 ± 0.09	3.45 ± 0.12	2.08 ± 0.38	41.66 ± 0.05
P1_A	6.88	1.54 ± 0.00	1.25 ± 0.07	2.96 ± 0.05	3.96 ± 0.06	2.50 ± 0.13	40.93 ± 0.63
P1_B	6.95	1.58 ± 0.00	1.23 ± 0.07	2.98 ± 0.01	3.97 ± 0.01	2.23 ± 0.39	50.23 ± 0.09
P2_A	7.22	2.11 ± 0.06	1.44 ± 0.09	3.47 ± 0.01	4.65 ± 0.02	2.50 ± 0.06	47.89 ± 0.08
P2*	5.90	1.13 ± 0.00	1.4 ± 0.07	3.31 ± 0.08	4.42 ± 0.12	2.23 ± 0.06	25.34 ± 0.05
P3_A	7.27	1.2 ± 0.16	1.08 ± 0.03	2.57 ± 0.03	3.45 ± 0.04	1.16 ± 0.18	26.98 ± 0.13
P3*	7.19	1.16 ± 0.11	1.17 ± 0.07	2.76 ± 0.04	3.71 ± 0.06	1.83 ± 0.23	26.80 ± 0.00
L1*	7.04	1.48 ± 0.11	1.04 ± 0.11	2.48 ± 0.14	3.34 ± 0.18	2.30 ± 0.03	46.08 ± 0.09

* = samples from unknown fields.

All the field samples were found as almost neutral with pH values in the range of 6-7, except U4* which has a slightly acidic nature. Neutral pH value is also the favorable acidity for submerged soils of paddy production, since most of the important nutrients as nitrogen, phosphorus potassium, iron, manganese and zinc are available at this pH. However, pH is an important parameter on the mobility of heavy metals and other contaminants in soil-water-plant system. Since all the samples have close pH values no significant correlation was found between pH and other soil characteristics, except the metals Na, Ca and As with a significant factor $p<0.05$.

As it is known that, the cation exchange capacity of soils is mainly related to the organic carbon content and clay content of the soil (Caravaca et al., 1999; Parfitt et al., 1995). In accordance a correlation between OC% and CEC of the field samples was found 0.627 ($p<0.01$). CEC was also highly correlated with the metals in the soil samples.

Table 4.8. Correlation (Pearson) coefficient matrices between soil properties and heavy metals.

	pH	*TKN*	*P*	*PO_4*	*P_2O_5*	*%OC*	*CEC*	*As*	*Zn*	*Pb*	*Ni*	*Fe*	*Cu*	*Cr*	*Cd*	*Al*	*Na*	*K*	*Ca*	*Mg*
pH	1																			
TKN	0.243	1																		
P	0.003	.665**	1																	
PO_4	0.035	.654**	.977**	1																
P_2O_5	0.029	.649**	.977**	1.000**	1															
%OC	0.124	.806**	.693**	.685**	.682**	1														
CEC	0.381	.630**	0.160	0.241	0.239	.624**	1													
As	.505*	.503*	0.112	0.113	0.106	0.397	.501*	1												
Zn	0.198	.868**	.610**	.525*	.522*	.806**	.565**	.535*	1											
Pb	0.301	.795**	0.403	0.389	0.384	.758**	.715**	.633**	.851**	1										
Ni	0.300	.499*	0.421	0.416	0.419	.495*	.559**	.522*	.604**	.571**	1									
Fe	0.284	.807**	.435*	.434*	.433*	.734**	.734**	.662**	.864**	.895**	.776**	1								
Cu	0.264	.856**	.449*	0.404	0.402	.743**	.694**	.698**	.893**	.828**	.662**	.908**	1							
Cr	0.267	.657**	.450*	0.365	0.365	.578**	.444*	.575**	.867**	.688**	.795**	.829**	.805**	1						
Cd	0.018	.601**	0.191	0.160	0.156	.693**	.528*	0.297	.648**	.579**	0.088	.473*	.615**	.425*	1					
Al	0.234	.711**	0.342	0.314	0.309	.708**	.645**	.598**	.724**	.839**	.427*	.749**	.812**	.549**	.649**	1				
Na	.528*	0.246	0.039	0.114	0.117	0.014	0.267	0.360	0.175	0.095	.437*	0.395	0.306	0.406	-0.120	-0.038	1			
K	0.269	.807**	0.304	0.288	0.283	.717**	.719**	.649**	.760**	.744**	.472*	.830**	.865**	.636**	.668**	.787**	0.287	1		
Ca	.509*	0.165	-0.044	-0.011	-0.008	0.185	.477*	.673**	0.199	0.242	.578**	0.381	0.389	0.369	0.038	0.165	0.385	0.344	1	
Mg	0.376	.660**	0.365	0.360	0.363	.651**	.755**	.743**	.743**	.762**	.828**	.891**	.842**	.771**	0.358	.645**	0.354	.748**	.705**	1

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

As the organic content of the soils were found as 0.91%-3.71%; CEC of the soils was found in the range of 16.16-63.06 meq/100 g, respectively.

Total nitrogen content of the soil samples varied in a range of 0.69 to 2.71 g/kg, whereas phosphorus content was measured as 0.95-1.57 g/kg. As well expected results, there was a well correlation between the organic carbon content and nutrient content of the soil samples (Table 4.8). TKN and total P were also well correlated at 0.01 significance level. The positive correlation of OC, N and P in soil is also compatible with other studies (Zhong et al., 2015).

Metal content of the soil samples was also measured to determine the heavy metal pollution in addition to the organic pollutants. The results of metal contents of agricultural soil samples were shown in Table 4.9 as the average concentration of duplicate samples and standard deviations. Each column in the table were formatted with data bars in accordance with the metal concentrations. All monitored metals were found in all samples and all the metals were correlated significantly with each other except Na and Ca. The concentration of metals in paddy samples were found higher than the samples collected from unknown agricultural fields from the same locations, which could be explained by the anthropogenic input of metals through extensive water usage in rice cultivation. The heavy metals were measured in the concentration ranges as 23-102 mg/kg for Zn, 7-20 mg/kg for Pb, 21-118 mg/kg for Ni, 7-40 g/kg for Fe, 9-48 mg/kg for Cu, 25-150 mg/kg for Cr and 0.2-0.40 for Cd. In the study conducted by Wong et al. (2002), heavy metal contamination including Cd, Cr, Cu, Ni, Pb, and Zn in paddy soils in southern China were also investigated and the concentrations were found in the range of 0-0.9, 19-90, 3-44, 4-33, 16-50, 18-107 mg/kg, respectively. This concentration ranges were found to be similar with this study, accept Ni, which have almost 6 times higher upper limit in the present work. The availability of metals in soil strongly depend on the organic content and cation exchange capacity of soil (Micó et al. 2006; Zeng et al. 2011). These positive correlations were also observed in this study, since most of the metals correlated with OC% and CEC at 0.01 significance level as shown in Table 4.8.

In Turkey, some of the heavy metals are controlled under Soil Pollution Control Regulation (T.C. Çevre ve Orman Bakanlığı, 2005) containing Pb, Cd, Cr, Cu, Ni, Zn, and Hg. Upper limit values of these regulated metals with respect to the soil pH and samples exceeding this concentration are listed in Table 4.10. Field samples from the sampling locations U1, P1 and U6 were found higher for both Cr and Ni, whereas U4 exceeded also the limit value set for Ni. Since only chemical fertilizers are used in paddy fields in the sampling region, heavy metal contamination could be attributed to the irrigation water supplied by Ergene River.

Table 4.9. Metal contents of soil samples (n=2).

	As (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Fe (g/kg)	Cu (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	Al (g/kg)	Na (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)
U1_A	1.83 ± 0.06	101.55 ± 8.11	19.94 ± 0.62	88.69 ± 6.89	40.12 ± 2.03	48.47 ± 1.80	149.21 ± 14.82	0.40 ± 0.02	39.49 ± 4.44	1.46 ± 0.02	5.96 ± 0.56	33.09 ± 0.59	11.40 ± 1.03
U1_B	2.03 ± 0.89	96.29 ± 0.38	19.74 ± 0.66	89.96 ± 0.21	37.14 ± 0.42	41.41 ± 1.81	150.12 ± 0.26	0.36 ± 0.00	32.77 ± 3.73	1.54 ± 0.01	4.96 ± 0.45	28.61 ± 1.19	10.47 ± 0.26
U2	2.34 ± 0.32	62.01 ± 1.59	18.61 ± 0.40	74.00 ± 2.29	34.30 ± 2.76	32.32 ± 2.17	68.56 ± 4.13	0.34 ± 0.02	30.73 ± 2.42	1.77 ± 0.13	4.74 ± 0.29	45.67 ± 0.15	10.53 ± 0.40
U3_A	1.38 ± 0.35	34.24 ± 3.07	12.13 ± 0.39	48.94 ± 1.14	19.22 ± 2.95	18.76 ± 0.95	47.64 ± 6.44	0.29 ± 0.04	22.52 ± 5.99	1.41 ± 0.00	4.02 ± 0.88	46.66 ± 0.47	7.70 ± 0.36
U3_B	1.97 ± 0.26	35.78 ± 2.03	12.34 ± 0.15	57.17 ± 0.47	23.72 ± 1.00	31.80 ± 11.98	51.36 ± 3.80	0.26 ± 0.01	24.67 ± 0.69	2.19 ± 0.04	4.48 ± 0.67	39.50 ± 0.05	8.35 ± 0.27
U4_A	1.67 ± 0.28	59.07 ± 2.50	18.10 ± 1.06	98.31 ± 16.10	33.15 ± 1.29	31.12 ± 1.16	96.05 ± 3.15	0.23 ± 0.04	32.72 ± 0.47	1.61 ± 0.08	3.55 ± 0.02	19.04 ± 1.21	7.11 ± 0.29
U4_B	1.05 ± 0.02	53.92 ± 0.52	15.94 ± 0.46	64.53 ± 0.67	28.78 ± 1.41	24.96 ± 0.65	73.08 ± 1.38	0.28 ± 0.01	28.08 ± 0.91	1.68 ± 0.04	3.76 ± 0.35	19.79 ± 0.89	7.18 ± 0.16
U4_C	0.65 ± 0.04	60.36 ± 2.61	17.28 ± 0.63	83.79 ± 3.50	31.07 ± 2.29	27.86 ± 1.25	95.95 ± 5.48	0.35 ± 0.01	31.30 ± 3.26	1.40 ± 0.16	4.19 ± 0.27	15.84 ± 0.07	7.88 ± 0.04
U4*	0.30 ± 0.09	23.40 ± 0.43	8.46 ± 0.25	25.45 ± 0.31	9.05 ± 0.34	9.43 ± 0.06	24.85 ± 0.64	0.26 ± 0.02	8.57 ± 0.85	0.12 ± 0.00	1.77 ± 0.14	8.37 ± 0.00	2.35 ± 0.09
U5_A	0.79 ± 0.00	31.05 ± 1.01	8.25 ± 0.26	26.87 ± 2.34	11.16 ± 2.40	12.04 ± 0.34	43.13 ± 2.79	0.23 ± 0.01	10.47 ± 1.90	2.49 ± 0.12	2.27 ± 0.47	17.80 ± 0.39	2.21 ± 0.10
U5_B	0.72 ± 0.07	26.72 ± 2.53	7.33 ± 0.34	21.01 ± 1.31	7.06 ± 0.99	10.81 ± 1.05	48.64 ± 7.18	0.23 ± 0.00	7.01 ± 1.75	1.56 ± 0.28	0.93 ± 0.80	17.58 ± 0.18	1.93 ± 0.18
U6_A	1.51 ± 0.09	42.18 ± 0.88	9.54 ± 0.10	116.41 ± 0.72	21.01 ± 0.35	20.31 ± 0.09	102.48 ± 0.37	0.24 ± 0.01	17.59 ± 0.07	2.05 ± 0.06	2.98 ± 0.25	37.39 ± 2.60	7.15 ± 0.07
U6_B	1.78 ± 0.05	45.56 ± 0.91	13.73 ± 3.25	118.48 ± 6.08	23.80 ± 0.44	24.26 ± 0.47	103.14 ± 6.05	0.24 ± 0.04	18.33 ± 0.21	1.54 ± 0.15	2.63 ± 0.01	69.58 ± 0.37	11.27 ± 0.24
U7_A	0.82 ± 0.09	41.72 ± 1.35	14.46 ± 0.00	68.07 ± 10.91	23.68 ± 3.44	19.44 ± 0.48	50.95 ± 0.98	0.29 ± 0.05	20.35 ± 3.37	1.60 ± 0.18	2.82 ± 0.13	20.46 ± 0.69	5.08 ± 0.32
U7_B	1.22 ± 0.16	39.23 ± 0.89	13.61 ± 0.13	60.83 ± 3.80	19.07 ± 1.95	18.54 ± 0.63	50.22 ± 1.01	0.21 ± 0.01	15.93 ± 0.68	1.29 ± 0.21	2.69 ± 0.09	26.06 ± 1.69	5.43 ± 0.59
P1_A	1.69 ± 0.11	67.15 ± 2.00	17.34 ± 0.03	101.26 ± 0.86	38.28 ± 1.59	33.00 ± 1.10	96.46 ± 2.51	0.24 ± 0.02	34.23 ± 1.27	1.29 ± 0.11	4.84 ± 0.07	35.63 ± 0.29	12.38 ± 1.73
P1_B	1.59 ± 0.02	63.80 ± 1.23	17.91 ± 0.02	111.66 ± 3.95	32.84 ± 0.21	31.75 ± 1.33	100.59 ± 1.42	0.25 ± 0.02	28.22 ± 0.76	1.63 ± 0.24	2.37 ± 0.11	36.81 ± 0.72	11.34 ± 0.17
P2_A	0.54 ± 0.08	53.17 ± 0.44	12.30 ± 0.21	74.42 ± 0.54	19.72 ± 0.19	22.07 ± 0.17	55.14 ± 0.38	0.24 ± 0.02	17.39 ± 0.34	1.35 ± 0.16	3.01 ± 0.09	27.00 ± 0.11	7.29 ± 0.11
P2*	0.45 ± 0.14	39.51 ± 3.96	9.78 ± 0.39	35.95 ± 0.93	8.98 ± 1.08	14.21 ± 0.60	28.30 ± 2.82	0.27 ± 0.01	18.73 ± 1.95	0.20 ± 0.01	1.94 ± 0.42	9.93 ± 0.35	2.54 ± 0.05
P3_A	1.12 ± 0.07	39.27 ± 0.48	13.12 ± 0.03	32.34 ± 9.16	12.60 ± 0.41	11.73 ± 0.12	32.91 ± 0.23	0.26 ± 0.01	11.57 ± 0.28	0.75 ± 0.05	1.98 ± 0.05	27.85 ± 0.48	4.25 ± 0.09
P3*	1.45 ± 0.10	39.09 ± 0.72	14.79 ± 0.36	23.37 ± 0.92	13.61 ± 1.00	12.60 ± 0.10	48.58 ± 1.28	0.27 ± 0.01	30.35 ± 2.62	0.37 ± 0.03	2.65 ± 0.02	13.30 ± 0.14	4.13 ± 0.11
L1*	1.42 ± 0.20	45.27 ± 0.86	14.52 ± 0.91	42.90 ± 0.73	16.95 ± 0.34	29.13 ± 5.22	36.07 ± 0.63	0.34 ± 0.00	40.50 ± 1.18	0.29 ± 0.02	3.47 ± 0.24	28.55 ± 0.12	5.77 ± 0.18

Table 4.10. Regulative limits of heavy metals in soil and number of samples exceeded these values.

Heavy Metal	Limit Concentration		Samples Exceeded The Limit
	pH 5-6 mg/kg dry weight	pH > 6 mg/kg dry weight	
Pb	50	300	-
Cd	1	3	-
Cr	100	100	U1A, U1B, U6A, U6B, P1A, P1B
Cu	50	140	-
Ni	30	75	U1A, U1B U4A, U4C U6A, U6B, P1A, P1B
Zn	150	300	-
Hg	1	1.5	n.d.

Organic and inorganic contaminants of Ergene River are investigated in the Boğaziçi University in the context of a TUBITAK project (Project No. 115Y064). Among the dataset the results of Novermber sampling campaign was used in the present study to evaluate the association of organic and inorganic contamination between river and soil samples. In the unpublished results of November sampling, the Ni concentrations of Ergene River was found as 113-3807 µg/L with a mean value of 1122.37 µg/L, where the limit of Ni is >200 µg/L in Water Pollution Control Regulation (T.C. Çevre ve Orman Bakanlığı, 2004) for fresh water systems. The same water pollution problem is also observed for Cr, which having a concentration range of 0.20-1222 µg/L. As the Ni and Cr pollution was mainly observed in paddy soils irrigated with the river water, the limit excess could be related to the water pollution, correspondingly.

For the determination of the texture, both sieve and hydrometer analysis were conducted on the field samples and the results were listed in Table 4.11. Figure 4.13. shows an example of grain size distribution graph. From the texture results it can be seen that most of the soils were found to be consist of sand. The low clay content of the samples may be attributed to the submerged irrigation, where the water could drain the small clay particles. It is well known that organic compounds mainly adsorbed on clay content of the soil, these dissolved particles could pose risk to aquatic environment by carrying the contaminants.

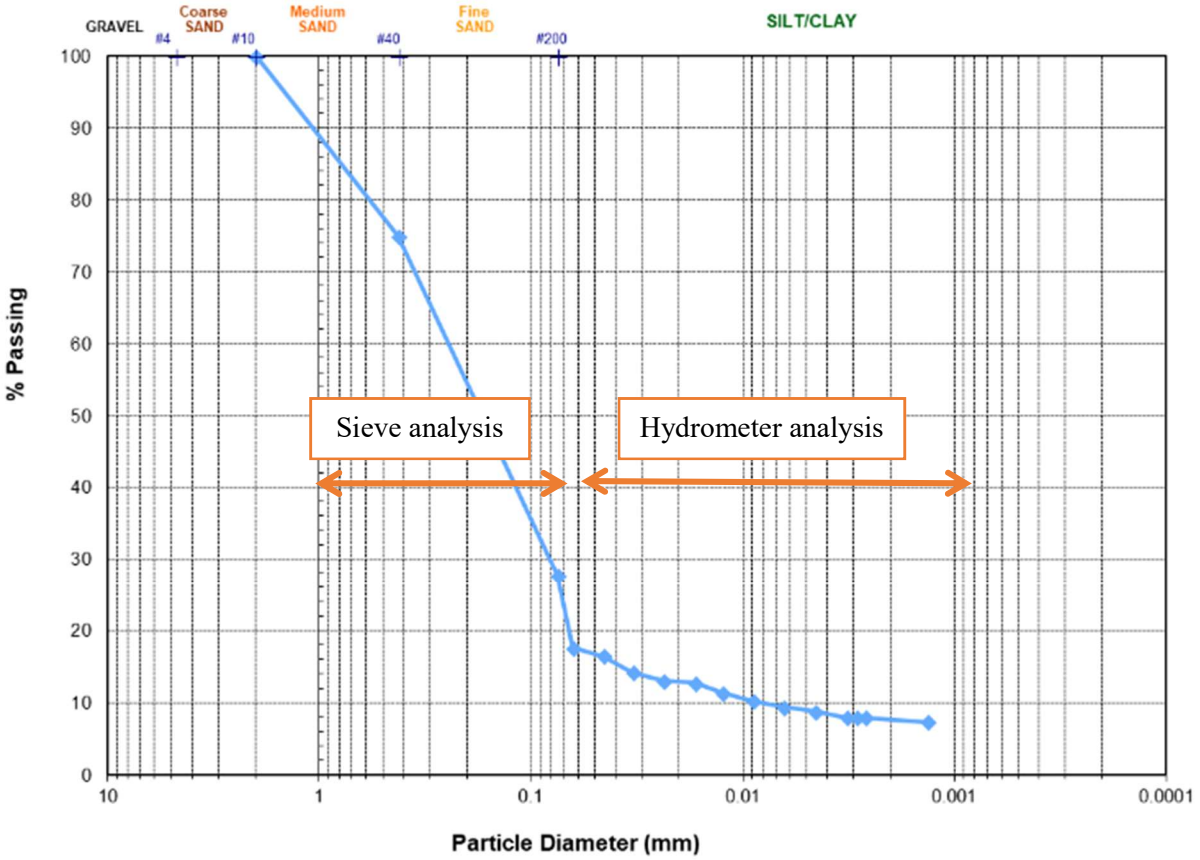


Figure 4.13. Plot of sieve and hydrometer test of sample P3.

Table 4.11. Texture analysis of field samples (n=1).

Sample	Medium sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil Type
U1	80.04	16.31	0.37	3.27	Sand
U3_A	59.03	29.74	5.51	5.72	Sand
U4_A+B	72.74	21.80	1.72	3.74	Sand
U5_A	43.51	44.08	8.16	4.26	Sand
U6_B	54.64	32.38	7.00	5.98	Loamy Sand
U7_A	52.86	30.47	9.42	7.25	Loamy Sand
P1_A	76.61	19.03	4.36		Sand
P1_B	74.16	20.65	5.19		Sand
P2_A	62.66	26.52	4.54	6.28	Sand
*P2**	62.47	26.52	7.38	3.63	Sand
P3_A	25.06	47.16	19.41	8.37	Sandy Loam
*P3**	46.68	30.85	14.91	7.56	Sandy Loam
*L1**	78.23	18.25	3.52		Sand
Blank soil	68.32	28.35	3.33		Sand

4.3.2. Occurrence of Target Organic Pollutants in Agricultural Soil

The developed analytical method was applied on 22 agricultural soil samples collected from Ergene region for the analysis of pesticides and other industrial ECs. The locations of the agricultural fields are particularly selected with respect to the closeness to the Ergene River and its tributaries, in order to assess the association of the soil contamination with the pollutants found in the river. Considering the fact that, the application of the pesticides was performed in June-July in paddy fields at that region, the residuals were remained in the soil for at least 6 months. All the samples were extracted with the developed procedure twice and the mean values of the detected concentrations corrected by mean reoveries and matrix effects were represented in Table 4.12. In order to associate the contamination of soil with river pollutants, the results from the November sampling campaign from river waters were used in this study.

The application rate and possible fate processes are the important factors for the occurrence of the pesticides in the collected samples. Pesticides detected in all the soil samples were found as aclonifen, carbendazim, epoxiconazole, prochloraz, tebuconazole with a concentration range of 0.19-34.12 µg/kg. Furthermore, azoxystrobin, difenoconazole, myclobutanil, propiconazole, pyraclostrobin, oxadiazon pendimethalin, acetamiprid, imidacloprid and molinate were detected at least in 17 samples out of 22. The detection of these pesticides could be expected, since these are the most frequently used compounds for pest control at the sampling region according to data set of the sales rates and field of application of the applied pesticides given by Edirne and Kırklareli Provincial Directorate of Agriculture (Appendix B). As carbendazim, imidacloprid, and prochloraz detected in higher concentration ranges in the boarders of Edirne, the reside levels of azoxystrobin, pyraclostrobin and imazamox were higher in the samples collected from Kırklareli. Some of the targeted pesticides as chlorfenvinphos, dimethoate, simazine, imazalil, isoproturon, lenacil, quinoxyfen and triazophos were not detected in any soil samples.

Triazole pesticides namely epoxiconazole, difenoconazole, myclobutanil and tebuconazole, which are known with their persistent behavior in soil due to their high sorption tendency on soil (Table 3.1), were detected frequently in soil samples as 0.29-34.12 µg/kg, 0.04-0.69 µg/kg, 0.09-1.61 and 0.22-13.27, respectively. Beside the agricultural samples, the residue of tebuconazole, epoxiconazole and myclobutanil were detected in 20 points from Ergene River up to 0.35 µg/L. Similar occurrence values of these pesticides in soil were reported in literature. For instance, Chiaia-Hernandez et al. (2017) and the residual concentrations were reported as 5-23 µg/kg for

Table 4.12. Average micropollutant concentrations in agricultural soils.

Chemical	*U1 A*	*U1 B*	*U2*	*U3 A*	*U3 B*	*U4 A*	*U4 B*	*U4 C*	*U4**	*U5 A*	*U5 B*	*U6 A*	*U6 B*	*U7 A*	*U7 B*	*P1 A*	*P1 B*	*P2 A*	*P2**	*P3 A*	*P3**	*L1**
	Concentration µg/kg																					
2,4-Dihydroxybenzophenone	-	0.17	-	-	-	-	-	-	-	0.06	0.13	0.06	-	-	-	-	-	-	-	-	-	-
3-Chloroaniline	-	-	0.10	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4,4Dichlorobenzophenone	-	-	1.69	-	-	-	-	-	-	1.77	2.34	1.92	-	1.75	1.02	2.53	-	1.12	-	-	-	0.94
4-Chloroaniline	0.28	0.30	0.35	0.30	-	0.26	0.29	0.30	0.26	0.29	0.33	0.29	0.15	0.25	0.20	0.24	0.15	0.21	0.29	0.13	0.29	0.24
4-Methylbenzylidenecamphor	0.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.00
5-Methyl-1H-benzotriazole (5-Tolytriazole)	1.52	2.18	1.54	2.48	-	2.11	1.09	2.75	-	1.69	1.34	1.88	0.64	1.00	0.39	-	-	-	-	-	-	-
Acetamiprid	0.25	0.30	0.82	2.18	0.25	5.78	4.68	3.02	0.08	0.26	0.34	0.31	0.18	6.09	3.56	4.63	0.37	-	-	1.03	-	0.28
Aclonifen	0.61	0.61	0.66	0.67	0.80	0.62	0.62	0.63	0.77	0.71	0.58	0.56	0.68	0.72	0.76	0.90	1.19	1.24	0.78	0.73	0.66	1.30
Atrazine	2.25	3.02	3.22	3.55	4.20	3.61	3.41	3.14	3.13	3.82	3.31	3.21	2.71	3.25	3.48	2.65	3.53	4.01	4.07	4.16	4.41	4.58
Azoxystrobin	0.53	0.46	2.36	0.94	-	0.51	0.40	0.55	0.15	1.60	3.75	0.11	0.10	12.19	10.01	82.40	25.72	59.57	1.61	55.21	1.28	2.69
Benzyldimethyldodecylammonium	792.8	807.7	-	14.28	12.99	72.25	81.55	187.9	-	83.57	442.1	351.9	272.9	16.06	10.05	106.8	107.9	-	-	117.3	104.1	177.3
Benzyldimethylhexadecylammonium	16.82	20.49	-	-	-	1.96	1.75	3.07	-	3.33	3.94	3.46	2.97	-	-	2.59	2.83	-	-	2.74	3.97	23.40
Benzyldimethyltetradecylammonium	398.6	428.1	-	6.41	3.93	21.52	21.99	67.23	-	50.79	192.6	139.3	138.4	4.95	3.21	33.36	34.7	-	-	41.67	36.30	146.8
Benzyltrimethylammonium	-	-	4.69	-	-	-	-	1.93	-	4.27	8.29	-	-	-	-	-	-	-	-	-	-	-
Boscalid	-	-	0.52	0.37	0.30	0.27	-	-	-	0.92	0.29	-	-	-	-	-	-	-	-	-	-	-
Carbendazim	2.18	5.66	9.07	4.24	1.11	5.62	3.74	6.89	0.70	2.20	2.74	3.91	1.13	1.79	1.58	1.77	0.93	1.26	1.63	0.64	0.90	0.49
Chlorfenvinphos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloridazon	-	-	-	0.67	-	-	-	-	-	-	-	-	-	-	-	-	1.99	-	-	0.44	-	0.65
Chlorpyrifos	0.44	0.14	-	-	-	-	-	-	-	-	0.24	-	0.36	-	-	-	-	-	-	-	-	-
Clarithromycin	0.75	0.41	0.06	0.12	-	0.15	0.21	0.30	-	0.08	0.11	0.23	0.14	-	-	0.12	-	-	-	0.14	0.22	-
Cypermethrin	-	-	-	26.62	13.97	6.85	157.9	17.77	-	-	-	-	-	-	-	-	-	-	-	406.51	-	-
Difenoconazole	0.27	0.28	0.06	0.27	0.09	0.17	0.26	0.33	0.05	0.09	0.14	0.44	0.15	0.38	0.07	0.13	0.69	0.54	0.07	0.06	-	0.14
Dimethoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diphenylamine	2.77	0.54	0.95	1.26	1.75	0.55	0.85	1.07	-	0.95	0.58	0.62	0.50	0.40	-	4.06	0.43	0.83	-	0.39	-	1.18

Table 4.12. Continued.

Chemical	*U1 A*	*U1 B*	*U2*	*U3 A*	*U3 B*	*U4 A*	*U4 B*	*U4 C*	*U4**	*U5 A*	*U5 B*	*U6 A*	*U6 B*	*U7 A*	*U7 B*	*P1 A*	*P1 B*	*P2 A*	*P2**	*P3 A*	*P3**	*L1**
	Concentration µg/kg																					
Diuron	0.47	0.55	0.54	0.61	-	0.42	0.22	0.90	-	0.42	1.41	0.41	0.35	0.22	0.11	-	-	-	-	-	-	-
Epoxiconazole	1.31	1.44	0.63	1.74	0.47	5.29	4.82	3.39	1.61	2.10	9.62	1.26	0.68	0.29	0.29	1.13	0.77	34.12	0.37	0.56	0.33	1.81
Ethoprophos	-	0.25	0.39	0.57	1.17	0.76	0.22	0.31	0.13	-	-	-	-	0.55	0.57	0.21	0.13	0.08	0.14	0.13	-	-
Ethylhexylmethoxycinnamate	3.58	3.02	1.62	3.38	1.14	1.07	2.22	1.41	-	2.10	2.20	3.72	1.96	1.42	1.39	2.61	3.37	2.10	-	3.81	-	-
Flutriafol	-	-	0.17	0.17	0.44	0.10	0.12	0.09	-	-	-	-	0.23	-	0.13	0.59	0.26	0.10	0.14	0.10	2.28	-
Galaxolide	6.79	2.54	3.65	1.47	1.65	-	-	-	1.81	2.09	4.33	-	2.40	-	-	-	1.60	-	2.32	-	-	1.39
Hexa(methoxymethyl)melamine	104.85	26.32	8.51	68.51	-	20.45	21.38	13.59	-	-	6.51	13.02	-	28.61	28.97	15.66	5.86	-	-	-	-	-
Hexaconazole	-	0.14	0.16	0.21	-	0.44	0.28	0.33	-	0.15	0.27	-	-	-	-	0.06	-	0.13	-	-	-	-
Hexylcinnamaldehyde	3.31	-	1.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Imazalil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Imazamox	-	-	0.93	-	-	-	0.65	-	-	-	-	0.72	-	1.03	1.34	2.27	-	1.46	-	-	-	1.21
Imidacloprid	0.56	0.63	0.69	5.40	5.96	1.22	1.66	0.98	3.23	-	-	4.65	1.53	0.64	0.68	4.35	0.91	0.27	-	0.71	2.61	1.13
Isoproturon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lenacil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mepiquat chloride	-	-	-	4.10	-	2.00	-	2.52	-	-	1.96	4.59	-	-	-	-	1.29	-	-	-	-	-
Methoxyfenozide	-	-	-	5.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Metolachlor	0.07	-	0.05	0.05	-	0.06	0.05	0.06	-	-	-	-	-	-	-	0.93	0.07	-	-	-	-	-
Molinate	0.61	1.00	14.7	7.45	1.40	4.01	10.19	3.86	0.22	0.92	0.69	0.38	0.25	5.08	3.60	15.20	0.44	0.22	-	1.71	-	-
Myclobutanil	0.25	0.23	-	0.37	0.20	0.22	1.61	0.31	0.11	0.16	0.25	0.20	0.17	0.18	0.11	0.12	0.11	0.14	0.22	0.09	-	-
N,N-Diethyl-m-toluamide	-	-	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N-Benzyldimethylamine	-	-	-	0.68	-	-	-	-	-	-	2.92	-	-	0.47	-	-	-	-	-	-	-	-
N-Ethyl-2-tolysulfonamide	-	-	0.22	0.29	-	0.18	-	0.15	-	0.12	-	0.17	-	-	-	-	-	-	-	-	-	-
N-methyl aniline	2.50	-	0.35	-	-	-	-	0.40	-	-	-	-	-	-	-	0.27	2.28	-	-	-	-	-
Oxadiazon	-	5.35	61.24	128.5	35.7	238.6	151.5	173.3	-	179.1	262.90	69.84	104.9	106.9	25.2	167.2	15.1	6.37	-	228.1	-	-
Oxybenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pendimethalin	1.25	0.45	-	0.64	0.41	0.43	0.40	0.37	-	-	0.50	0.38	0.47	0.44	0.61	0.80	2.84	1.60	0.67	0.39	0.42	0.42

Table 4.12. Continued.

Chemical	U1 A	U1 B	U2	U3 A	U3 B	U4 A	U4 B	U4 C	U4*	U5 A	U5 B	U6 A	U6 B	U7 A	U7 B	P1 A	P1 B	P2 A	P2*	P3 A	P3*	L1*
Prochloraz	2.15	2.29	1.04	1.22	0.39	1.10	3.89	2.19	4.71	0.27	0.54	0.62	0.41	0.40	0.36	0.93	1.11	0.28	0.52	0.46	0.19	0.20
Propiconazole	0.25	0.44	0.57	0.41	0.09	0.42	0.71	0.86	0.06	1.30	4.02	0.40	0.16	0.14	0.09	0.48	0.46	0.63	0.22	11.89	-	1.15
Prothioconazole	6.51	7.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyraclostrobin	0.27	0.12	-	0.28	0.09	1.60	1.60	1.39	0.71	0.71	10.06	0.09	0.06	-	0.05	0.14	0.10	24.79	0.17	0.18	0.41	0.15
Quinoxyfen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tebuconazole	0.59	0.79	1.31	0.75	0.26	1.48	0.51	0.79	5.86	1.03	0.47	0.38	0.30	0.43	0.22	13.27	1.50	1.43	0.28	0.64	1.15	2.58
Tetraacetylethylenediamine	5.52	4.82	-	-	-	7.06	8.05	11.53	-	-	-	2.95	-	-	-	2.86	3.42	-	-	2.09	-	-
Tonalide	4.39	4.27	0.87	0.34	-	-	-	0.85	-	-	1.45	0.75	1.05	-	-	-	0.42	-	-	-	-	-
Triazophos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trifloxystrobin	-	-	0.04	-	-	0.22	0.13	0.11	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-
Triphenylphosphineoxide	0.60	0.68	0.46	0.68	-	0.61	0.48	0.67	-	0.50	0.44	0.60	0.44	0.20	-	-	-	-	-	-	-	-
Tris(2-butoxyethyl) phosphate	9.82	6.96	3.83	10.60	-	15.06	6.46	19.70	-	5.57	5.80	6.44	3.38	5.23	2.94	3.29	1.91	-	-	0.63	0.33	-

epoxiconazole, 1.1-5.1 µg/kg for difenoconazole and 1-89 µg/kg for tebuconazole. A correlation between the occurrence of tebuconazole and azoxystrobin were determined as 0.611 ($p<0.01$), since these pesticides are applied simultaneously to soil in practice, beside their single use.

Pyraclostrobin, a strobilurin fungicide, was detected frequently in soil samples with a residue level of 0.05-24.79 µg/kg. This compound is known with its high sorption behavior with a K_{oc} value of 9120 and half life of 60 days (Fulcher et al., 2014). The residue levels of pyraclostrobin were correlated significantly 98.5% ($p<0.01$) with epoxiconazole, which is an expected association, since these two compounds are used in combination as shown in Table B1. Moreover, oxadiazon is one of the most abundantly found herbicide in 17 soil samples with a concentration range of 5.34-262.9 µg/kg. Although this compound was found as moderately persistant with a half life of 44-45 days in paddy soil (Chakraborty et al., 1999), the high concentrations of oxadiazon can be related to the its high utilization rate and the respective accumulation in the soil.

Imidazole fungicide prochloraz, which is applied individually or in combination with tebuconazole, azoxystrobin and propiconazole in agricultural fields, have a high sorption tendency on soil with an average K_{oc} value of 11829 (Rütters et al., 1999) with a half life ranging from the 11 to 43 days (Hollrigl-Rosta et al., 1999) depending upon field conditions. Pprochloraz was detected in all samples with varying concentrations from 0.19 to 4.71µg/kg most probably due to its high application rate. In addition, a negative correlation was found between the soil pH and the residue levels of prochloraz as -0.623 ($p<0.01$), since the sorption and persistence is increasing of this compound with decreasing pH (Rütters et al., 1999).

Fungicides azoxystrobin and propiconazole, which are applied to the fields both individually and in combination, were detected in 21 soil samples in a concentration range of 0.01- 82.34 µg/kg and 0.06-11.89 µg/kg, respectively. Similarly, high concentrations of these compounds were reported in agricultural soils in Switzerland as 2-86 µg/kg and 1-5 µg/kg, respectively (Chiaia-Hernandez et al., 2017) because of their high persistency in terrestrial environment. DT50 values were found as 58-87 days for azoxystrobin and 99-116 days for propiconazole (Edwards et al., 2016) in field samples. In the same study, it was also shown that these compounds can mobilize horizontally with runoff especially in the first rain event after pesticide application. Correspondingly, propiconazole was also found in 21 river samples from Ergene up to 0.27 µg/L, as azoxystrobin was only detected in 2 points up to 0.11 µg/L.

Neonicotinoid insecticides namely acetamiprid and imidacloprid were found in 19 samples with a concentration range of 0.08-6.09 and 0.27-5.96 µg/kg, respectively. The high detection rates are related to the high consumption rates of these compounds for pest control, as well as their high stability in soil with half lifes of 450-3000 days (Hussain et al. 2016). The occurrence of these compounds were reported in the literature Table 2.1 (Chiaia-Hernandez et al., 2017; Zhou et al., 2018). With respect to the high water solubility of acetamiprid, it was also detected frequently in river samples with a rate of 52% up to 8.63 µg/L.

Despite their low persistency in the terrestrial environment, some pesticide residues as carbendazim, aclonifen and molinate were frequently (>86%) detected in the soil samples, which can be related to the application amount and frequency. Carbendazim is highly applied benzimidazole fungicide, which was detected in all the samples in the present study with a range of 0.49-9.07 µg/kg. It has a strong sorption tendency on soil with a K_{oc} value of 1024 – 2644 (Carbo et al., 2007). However, carbendazim is easily biodegradable with a half life of 9.3 days, which can be decreased with repeated applications (Yu et al., 2009). Aclonifen was found also in all the samples within a low concentration range of 0.56- 1.30 µg/kg and in 18 river samples up to 0.19 µg/L, although it is a modaretely persistant pesticide with a K_{oc} value of 6778.2 and half life in soil as 49 days with a low mobility (Vischetti et al., 2002), Molinate was detected in 19 samples up to 15.2 µg/kg mostly in paddy fields in. Although the DT value of molinate wa reporterd as 5.1 d (Quayle et al., 2006), it was detected in soil samples up to 10.5 µg/kg in USA even it was not apllied at the sampling year (Smalling at al., 2007).. in spite of its high dissipation tendency with a DT50 value of 5.1 d.

Despite their high consumption, some pesticides namely prothioconazole, trifloxystrobin, imazamox and cypermethrin were rarely detected in soil samples, which could be associated to their high dissipation rates. Prothioconazole was only detected in the sample from the location U1 as 6.51-7.07 µg/kg. Although this pesticide is one of the mostly applied active substance of the agrochemicals, the low detection frequency of this compound can be attributed to its rapid degradation with an half life below 5.82 days (Lin et al., 2017). It should be noted that the transformatin reactions can happen in the soil. For instance, it is known that the metabolite of prothioconazole namely prothioconazole-desthio is more toxic and more persistant in soil. Trifloxystrobin, which is another widely used widely fungicide in rice cultivation, was reported as a nonpersistent compound showing half life of 0.7-7.5 d in paddy soil (Cao et al., 2015). Despite its high rates of consumption trifloxystrobin, the residuals were detected only in 5 samples in a low concentration range as 0.04-0.22 µg/kg, which could be attributed to its high degradation rate. In addition, trifloxystrobin residuals were not found in river samples. Similarly, although imazamox is one of the top selling pesticide ingredient in the sampling

region, the residues in paddy soils were found in 8 samples in a low concentration range of 0.65-2.27 µg/kg. This can be related to its high dissipation rate with a half life of 2.2-3.3 days in soil (Milan et al., 2018). Likewise, cypermethrin can be easily degraded in aerobic conditions with a half life of 6-20 days (Jones, 1995). However, it was found in 6 samples with a wide concentration range of 6.84-406.51 µg/kg.

It should be mentioned that, during the sampling campaign some pesticide packages were found around the fields (Figure 4.14). The results of the occurrence study directly matched with these field observations. For instance, prothioconazole was only detected in U1, where we found the empty bottle of the INPUT® EC 460 (Bayer) which consists of prothioconazole and spiroxamine as active compounds. Similarly, at location U4 the package of Mosetam® 20 SP (Safa Tarım) (active ingredient: acetamiprid) was found, where one of the highest concentration of acetamiprid was quantified at this location as 5.78 µg/kg.

Figure 4.14. Pesticide packages found in sampling sites U1 and U4.

Beside the agrochemicals, industrial contaminants were frequently detected in the soil samples. Due to the intensive textile, food, metal and chemical manufacturing activities, industrial ECs are introduced to the environment mainly at the source of the river located in the northeaster Thrace, which are transported to wide distances with the flow of the river. Some persistent compounds end up in the terrestrial environment with irrigational activities and accumulate on the soil regarding to their sorption tendency.

In Figure 4.15 and 4.16, the frequency and detected concentration range of the common contaminants found in both agricultural soils and river samples were demonstrated in two box plot charts. From these studies it can be concluded that the most abundant contaminants in river samples

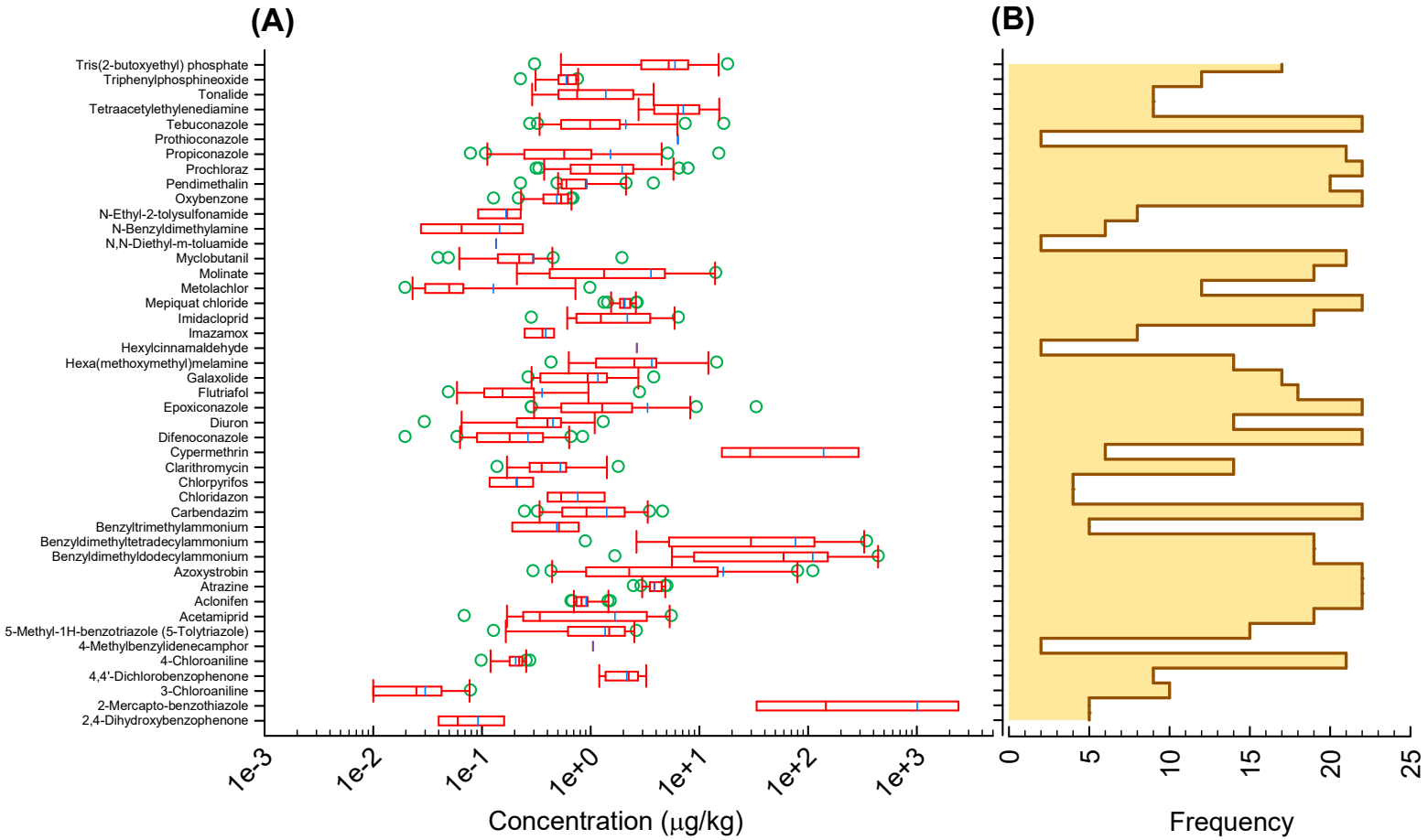


Figure 4.15. Concentration and frequency of common contaminants detected in soil.

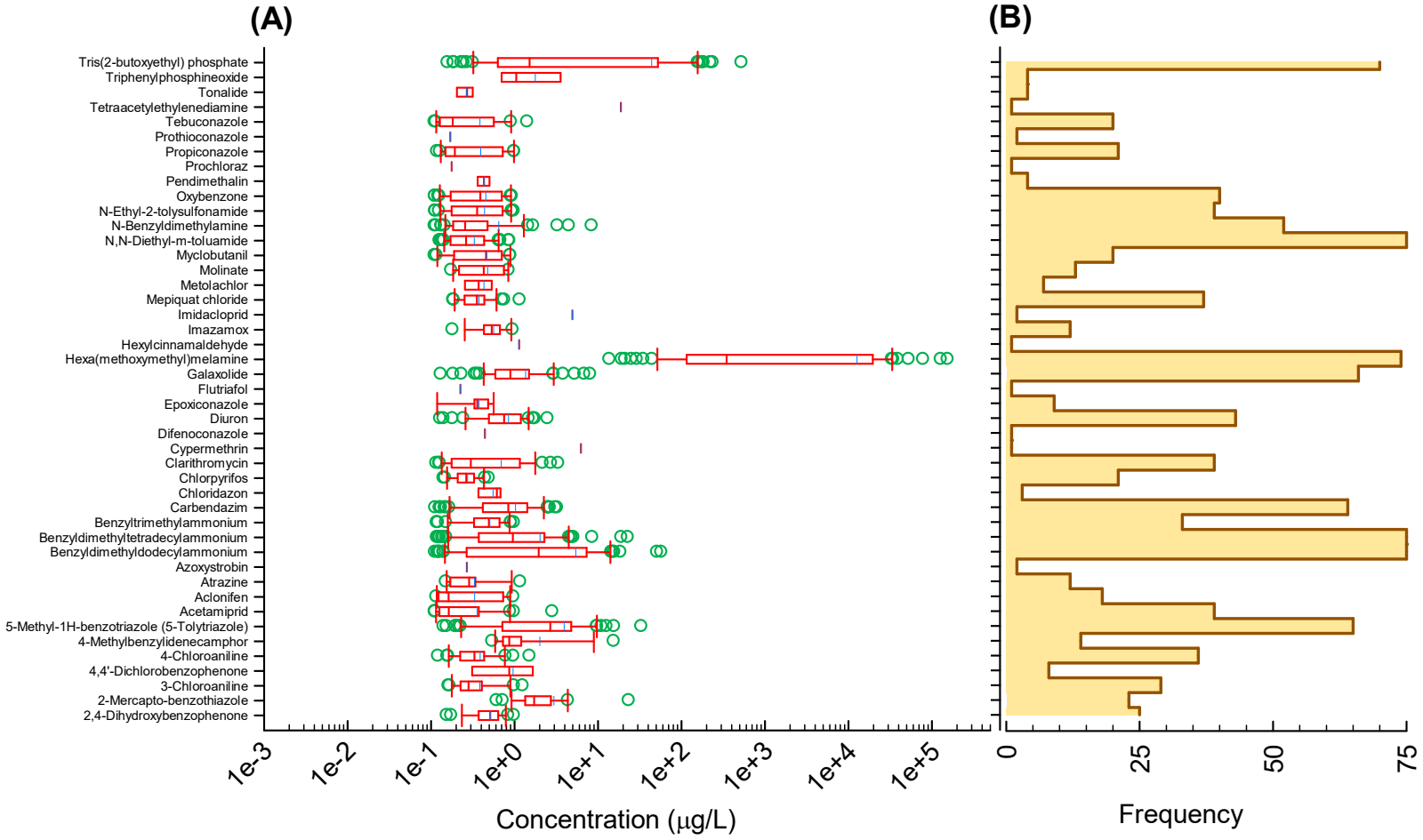


Figure 4.16. Concentration and frequency of common contaminants detected in river.

were also detected frequently in soil samples regarding to their high sorption tendency onto soil, where soil act as a sink of industrial ECs. Most frequently detected chemicals in both soil and water matrices include: benzyldimethyldodecylammonium (BDDA), benzyldimethyltetradecylammonium (BDTA) as surfactant agents, tris(2-butoxyethyl) phosphate (TBEP) as flame retardant, galaxolide (HHCB) and tonalide (AHTN) as synthetic fragrance, 5-Methyl-1H-benzotriazole (5-MeBT) as corrosion inhibitor, hexa(methoxymethyl)melamine (HMMM) as coating agent.

BDDA and BDTA which are two most commonly used cationic surfactants within benzalkonium chlorides (BACs), are the most frequently detected emerging contaminants in the soil samples collected from Ergene watershed. BDDA was found in 19 samples with a wide concentration range of 10.05-807.73 µg/kg, whereas it was found in all of the river samples with a concentration up to 110 µg/L. On the other hand, BDTA have the same detection frequency and quantified as 3.21-428.11 µg/kg and 40.54 µg/L in soil and river samples, respectively. The long hydrophobic carbon chains and the cationic structure of BAC are responsible for their very strongly sorption on soil organic matter and for holding them on the negatively charged soil particles (Khan et al., 2017). Although the biodegradation of these surfactants by the tolerant microorganisms has been suggested it was reported that co-contamination of different BACs can inhibit the biodegradation and consequently this can result in their accumulation in the environment (Khan et al., 2015) .

TBEP which is another anthropogenic compound was found in 17 of the soil samples with a concentration range of 0.32-19.70 µg/kg. The occurrence of TBEP that is used in various manufacturing processes such as polishing agent for floor, plasticizer in plastics and rubber industry, as solvent in resins and conditioner of viscosity in plastisols can be expected mainly in soil and sediments (WHO, 2000), since it has high adsorption coefficient (23988 L/kg) and moderate water solubility (1.1 mg/L). However, this compound was detected in 93% of the samples collected from Ergene River up to 527 µg/L concentration. Moreover, TBEP is categorized as readily biodegradable compound with a half life of 50 days in river waters (WHO, 2000). Similar to the results of this study, TBEP was detected both in river and soil samples in different studies (Wang et al., 2015 ; Fries and Mihajlović, 2011).

Since galaxolide and tonalide are fragrance agents which are widely used in the production of household cleaning products and personal care products as laundry detergents, soaps and in cosmetic industry as an ingredient of perfumes, deodorants and colognes etc. While galaxolide was found in 15 of the soil samples within the concentration range of 0.38-6.79 µg/kg tonalide was detected in 8 soils samples in which the concentrations reached up to 4.39 µg/kg. Although these 2 synthetic musks

have a tendency to sorb onto soil regarding to their high K_{oc} values galaxolide and tonalide were found in 88% and 5% of the river samples up to 8.12 µg/L and 0.32 µg/L, respectively. In literature, the half life of galaxolide and tonalide in soil were reported as 10-17 months and 2−24 years respectively (MacHerius et al. 2012).

High production volume and usage of 5-MeBT that is used as corrosion inhibitor for metals, deicing of airplane fluids, antifreezing agent and ingredient in dishwasher detergents has been reported in surface waters in Germany (Kiss and Fries, 2009) and soil samples in USA (McNeill and Cancilla, 2009). Accordingly, in the investigated area 64% of the soil samples and 89% river water samples have 5-MeBT as contaminant with a concentration range of 0.38-2.74 µg/kg and 0.09-33 µg/L, respectively. The persistency of 5-MeBT was underlined in the study of McNeill and Cancilla (2009) who detected this contaminant in the concentration range of 2.35-424.19 µg/kg in soil samples collected from airports in USA although the deicing activities were not applied over a year.

HMMM is an industrial chemical, which is used as a coating agent for plastics, automobiles and cans. HMMM was the most abundantly detected compound in river samples from Ergene River having concentrations up to 304783 µg/L, which was also detected frequently in German Rivers (Dsikowitzky and Schwarzbauer, 2015). Although the extraction efficiency of HMMM from soil was below 10% in the present study, HMMM showed noticeable sharp peaks in 14 samples despite its low recovery. The resulting peak of HMMM in the chromatograms in blank acetonitrile and sample U1 was shown in Figure 4.17. There is no study about the occurrence and sorption behavior of HMMM in soil matrices.

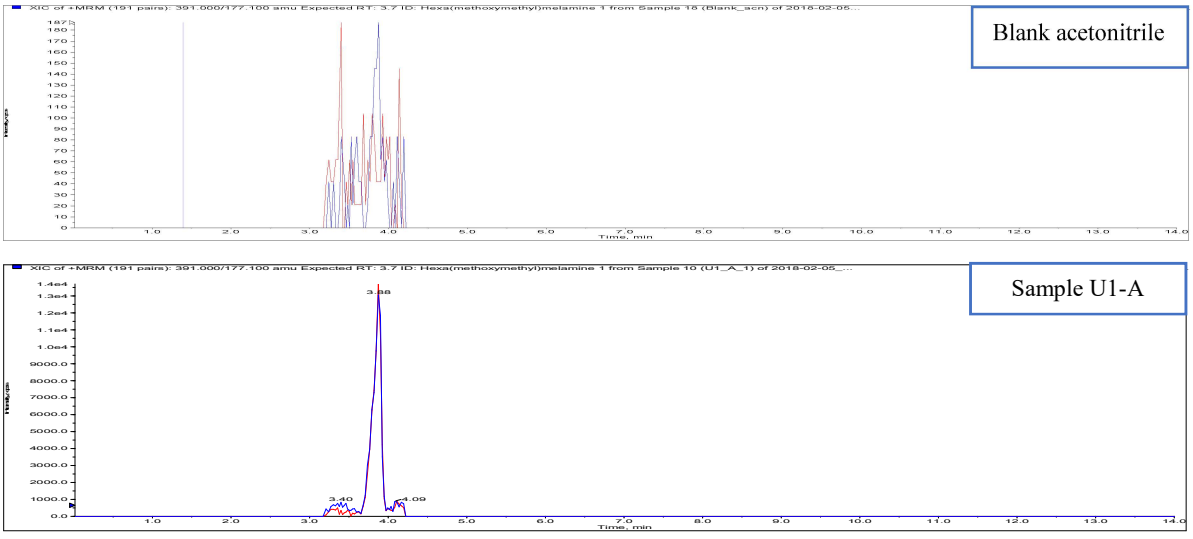


Figure 4.17. Extracted ion chromatogram of HMMM from blank acetonitrile and sample U1-A.

Beside the common contaminants, some industrial contaminants were found abundantly in soil samples, including triphenylphosphineoxide (TPPO) as ingredient of chemical production, tetraacetylethylenediamine (TAED) as bleaching agent, benzyldimethylhexacylammonium (BDHA) as disinfectant, ethylhexylmethoxycinnamate (EHMC) as UV blocker sunscreen, where soil acts as a sink for these contaminants.

TPPO is one of the most widely used organophosphorus compound as the ligand for transitional metals, flame retardant and synthetic additive for the production of pharmaceuticals. The production of organophosphate esters (OPEs) like TPPO is increasingly applied in the industry with the restriction of brominated flame retardants (BFRs), which resulted in an increase occurrence frequency of these compounds in natural environment over the last years (Wang et al., 2015b). In the present study, TPPO was detected in in 54% of the soil samples in a concentration range of 0.20-0.68 µg/kg. Similarly, the occurrence of TPPO was also reported by Cui et al. (2017)in China with a concentration range of 0.5-13 µg/kg, which can be explained by the high soil adsorption potential of TPPO its persistency.

TAED is mainly used as bleaching activator in additives and household detergents for dish- and laundry washing, whereas it is also applied for the production of peracetic acid in sanitizers and for the bleaching of textiles and paper in industry (HERA 2002). It is known that >99% of TAED is converted to the diacetylethylenediamine (DAED) during washing process by perhydrolysis. The 1% of TAED is discharged into sewer. It is investigated that TAED is readily biodegredable with a half life of 9 days in surface waters by aerobic conditions and it has a low sorption behavior with a measured K_{oc} value of 43-80 L/kg (HERA 2002). Although its known low sorption behavior in soil and inherent biodegradation tendency in surface waters, this compound was found in 9 soil samples from Ergene watershed with a high concentration range of 2.09-11.52 µg/kg.

EHMC that is used as UV filter for the manufacturing of cosmetic products and sunscreen agents was one of the most abundantly detected contaminants in agricultural fields and this compound was monitored in 18 soil samples at 1.07-3.81 µg/kg concentration range. However, EHMC was not found in river water samples collected in November while it was detected in 17 samples out of 75 in the concentration range 0.17-13 µg/L in August sampling campaign. This compound was found to be readily biodegradable in surface waters (28 days, 78% removal) although its calculated K_{oc} value was 12280 (EPI-Suite). In various studies, EHMC was detected in surface waters (da Silva et al., 2015), sewage sludge (Gago-Ferrero et al., 2011) and fish samples (Gago-Ferrero et al., 2015) but there are not any study investigated the occurrence of EHMC in soil samples.

Among the sampling points, U1 was found to be the most polluted area, especially with the industrial contaminants. Actually, this result was well expected, since this sampling point was located on the main river, it was a rice cultivation area, which was submerged with the surface water. In overall, the soil samples collected from paddy fields contained industrial pollutants besides the pesticides, while the samples taken from fields other than paddy fields which were not submerged (indicated with "*" at Table 4.12), showed less industrial contamination. This can be especially observed at the location U4, since samples from paddy fields (U4A, U4B and U4C) were contaminated with hexa(methoxymethyl)melamine, tetraacetylethylenediamine, triphenylphosphineoxide, tris(2-butoxyethyl) phosphate and benzyldimethylhexadecylammonium, on the contrary in sample U4* none of these contaminants were detected. These results obviously revealed the the effect of irrigational water on the soil contamination.

In order to assess the source of contamination, the correlations of the spatial contaminant concentrations were examined using bivariate correlation test. The results showed that, the industrial contaminants, which cannot be formed in the terrestrial environment itself, have significant correlations with each other as listed in Table 4.13. These correlations are especially remarkable between the most abundantly detected chemicals in river water samples. For instance, correlations obtained between BDDA and other contaminants as BDTA, BDHA, HHCB, AHTN, TPPO, and HMMM were found as 0.987,0.728, 0.618, 0.938, 0.509 and 0.472, respectively. These high correlation values indicated that Ergene River was the possible source of soil contamination.

As soil can act as a sink of industrial contaminants transported with river water, it can also be the source of contamination for surface waters through runoffs. Beside the industrial chemicals, Ergene River was also polluted with agrochemicals (Figure 4.15), where carbendazim, acetamiprid, mepiquat chloride, propionazole, tebuconazole, myclobutanil and molinate were detected at least 20 samples out of 75. This contamination cannot be directly correlated with the soil contamination determined in this study, since the sampling locations cover only a small area of the fields around the river. However, the similarity of the most frequently detected pesticides both in soil and water samples suggested the relationship, which needs a further investigation for a better understanding of source

Table 4.13. Correlation of industrial ECs in soil samples.

	2,4-Dihydroxybenzophenone	2-Mercapto-benzothiazole	3-Chloroaniline	4-Chloroaniline	4-Methylbenzylidenecamphor	5-Methyl-1H-benzotriazole	Benzyldimethyldodecylammonium	Benzyldimethylhexadecylammonium	Benzyltrimethylammonium	Clarithromycin	Galaxolide	Hexa(methoxymethyl)melamine	N,N-Diethyl-m-toluamide	N-Benzyldimethylamine	N-Ethyl-2-tolysulfonamide	Benzyldimethyltetradecylammonium	Tonalide	Triphenylphosphineoxide	Tris(2-butoxyethyl) phosphate
2,4-Dihydroxybenzophenone	1																		
2-Mercapto-benzothiazole		1																	
3-Chloroaniline			1																
4-Chloroaniline				1															
4-Methylbenzylidenecamphor					1														
5-Methyl-1H-benzotriazole	.439*			.556**		1													
Benzyldimethyldodecylammonium	.658**	.580**					1												
Benzyldimethylhexadecylammonium					.749**		.728**	1											
Benzyltrimethylammonium	.464*								1										
Clarithromycin		.789**				.487*	.820**	.526*		1									
Galaxolide							.618**			.509*	1								
Hexa(methoxymethyl)melamine		.796**				.461*	.472*			.691**	.458*	1							
N,N-Diethyl-m-toluamide												.446*	1						
N-Benzyldimethylamine	.493*								.746**					1					
N-Ethyl-2-tolysulfonamide			.472*	.466*		.746**							.632**		1				
Benzyldimethyltetradecylammonium	.641**	.573**					.987**	.810**		.781**	.630**	.462*				1			
Tonalide	.564**	.652**				.434*	.938**	.644**		.825**	.716**	.588**				.936**	1		
Triphenylphosphineoxide	.449*		.430*	.528*		.951**	.509*			.593**		.482*			.672**	.471*	.538**	1	
Tris(2-butoxyethyl) phosphate						.886**				.514*		.473*			.607**			.813**	1

*. *Correlation is significant at the 0.05 level (2-tailed).*

**. *Correlation is significant at the 0.01 level (2-tailed)*

4. CONCLUSIONS

Although the occurrence of various emerging contaminants in surface waters were frequently investigated in the literature, there are only limited number of studies conducted on soils and the majority of these studies consist of mainly pesticides and pharmaceuticals as target analytes. In the present study, the residue analysis of both pesticides and industrial contaminants was investigated in 22 soil samples collected from Ergene River bank by using a developed multiresidue analytical method. The results were associated with field observations and the available data on river contamination. Based on the results of this study, the following conclusions were drawn:

The analysis of TC an FQ antibiotics was not obtained with the standardized QuEChERS based on AOAC method due to the chelate effect of used salts that deteriorated the extraction performance. Although the recoveries were improved by the use of different solvents, salts, and buffers simultaneous extraction of antibiotics together with pesticides was not achieved at the satisfactory level.

As a result of the application of the optimized extraction method on two different pseudo blank soils for simultaneous analysis of 67 emerging organic contaminants the method performance was found satisfactory for 59 compounds in terms of the recovery, precision, and linearity. The recovery levels were in the satisfactory range for 75%, 78% and 69% of the analytes at 10 µg/kg, 50 µg/kg 100 µg/kg spiking level, respectively. The effect of organic content on the extraction performance was observed, as 84% of the target analytes showed lower recoveries with the blank soil having higher organic carbon content.

A second clean up step of the QuEChERS method by using d-SPE yielded better recoveries for limited target compounds (72% at 10 µg/kg; 14% at 50 µg/kg), the use of d-SPE caused the exceeding of acceptable recovery limits especially at lower concentrations. Since the d-SPE step in the method did not have a positive contribution on the recovery level of benzyltrimethylammonium, hexa(methoxymethyl)melamine, n-benzyldimethylamine and mepiquat chloride as poorly extracted compounds and did not noticeably affect the ME, this step was eliminated in the method for time and chemical saving. To improve the relatively lower recovery achieved at the highest spking level (100 ug/kg) of target analytes, an additional extraction cycle did also not provide considerable enhancement for the recovery.

The limit of detection and quantification of the analytical method was determined with 2 different approaches. LOQ values were found in the range of 0.04-33 µg/kg by matrix matched calibration curve approach, where 78% of the target analytes having a LOQ less than 1 µg/kg. Moreover, the MLOQ values of 70% of the compounds were found below 5 µg/kg, which was determined by the lowest spiking level approach.

The detection of the target pesticides after 6-7 months of application demonstrated the persistency of them in paddy soil. Aclonifen, azoxystrobin, carbendazim, difenoconazole, epoxiconazole, prochloraz, tebuconazole, myclobutanil, propiconazole, pyraclostrobin, oxadiazon, pendimethalin, acetamiprid, imidacloprid, and molinate were detected at least in 17 samples out of 22. The highest concentrations were detected for oxadiazon, azoxystrobin, epoxiconazole, pyraclostrobin, tebuconazole, and propiconazole having concentrations up to 601, 112, 33, 24, 17 and 15 µg/kg, respectively. Moreover, the spatial distribution of the analytes was found in agreement with the sales rates of pesticides in Edirne and Kırklareli.

Beside the agrochemicals, industrial compounds including hexa(methoxymethyl)melamine (coating agent), 5-methyl-1H-benzotriazole (corrosion inhibitor), galaxolide and tonalide (synthetic fragrances), benzyldimethyldodecylammonium and benzyldimethyltetradecylammonium (surfactant agents) and tris(2-butoxyethyl) phosphate (flame retardant) were detected in both soil and water matrices at higher frequency. The residuals of triphenylphosphineoxide (ingredient of chemical production), tetraacetylethylenediamine (bleaching agent), benzyldimethylhexacylammonium, (disinfectant) and ethylhexylmethoxycinnamate (UV blocker sunscreen) were abundantly detected in only soil samples, indicated that soil acted as a sink for these contaminants. In addition to organic contaminants, Cr and Ni concentrations that exceeded the limit values set for the agricultural fields may indicate the metal pollution from river, since these metals were detected at high concentrations in both soil and river samples.

The occurrence of industrial pollutants and heavy metals in paddy soils was associated with the water used for submerged irrigation since 4 soil samples collected from agricultural area other than paddy field did not show the contamination with industrial ECs. A significant correlation was found between the industrial ECs detected in paddy, which were also abundantly detected in river waters. Therefore, river water can be regarded as source of ECs for rice cultivation area.

The findings of this thesis indicated the accumulation potential of both the agrochemicals used for pest control and the contaminants found in irrigation water in agricultural fields. The urgency of a more holistic approach of environmental monitoring of contaminants and the need for further

investigations of ECs in terrestrial systems are obvious to evaluate the possible effects of contaminants on the human and the environmental health. The occurrence data of the monitored pesticides and other industrial ECs in agricultural soil samples are expected to be a significant reference for future works in this field. The obtained data is especially important considering the lack of information related to the agricultural and industrial contamination of the agricultural fields in Turkey.

REFERENCES

Alder, L., Greulich, K., Kempe, G., Vieth, B., 2006. Residue analysis of 500 high priority pesticides: Better by GC-MS or LC-MS/MS?. Mass Spectrometry Reviews, 25, 838-865.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F., 2003. Fast and easy multiresidue method employing acetonitrile. Journal of AOAC International, 86, 412-431.

AOAC, 2007. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. AOAC International, 1-9.

Aparicio, I., Martín, J., Abril, C., Santos, J., Alonso, E., 2018. Determination of household and industrial chemicals, personal care products and hormones in leafy and root vegetables by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, 1533, 49-56.

Aravinna, P., Priyantha N., Pitawala, A., Yatigammana, S.K., 2017. Use pattern of pesticides and their predicted mobility into shallow groundwater and surface water bodies of paddy lands in Mahaweli river basin in Sri Lanka. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 52, 37-47.

Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J., García-Río, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agriculture, Ecosystems & Environment, 123, 247-260.

ASTM D422., 2007. Standard Test Method for Particle-Size Analysis of Soils: ASTM D 422. Annual Book of ASTM Standards, 63, 1-8.

Babić, S., Petrović M., Kaštelan-Macan, M., 1998. Ultrasonic solvent extraction of pesticides from soil. Journal of Chromatography A, 823, 3-9.

Bai, X., Lutz, A., Carroll, R., Keteles, K., Dahlin, K., Murphy, M., Nguyen, D., 2018. Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. Chemosphere, 200, 133-142.

Barnett-Itzhaki, Z., Esteban López, M., Puttaswamy, N., Berman, T., 2018. A review of human biomonitoring in selected southeast asian countries. Environment International, 116, 156-164.

Bending, G. D., Lincoln, S. D., Edmondson, R. N., 2006. Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. Environmental Pollution, 139, 279-287.

Bergé, A., Vulliet, E., 2015. Development of a method for the analysis of hormones and pharmaceuticals in earthworms by quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analytical and Bioanalytical Chemistry, 407, 7995-8008.

Berghof Products and Instruments, n.d. Application Report MWS-3 / Environment Application Report.

Blum, K. M., Andersson, P., Ahrens, L., Wiberg, K., 2018. Persistence, mobility and bioavailability of emerging organic contaminants discharged from sewage treatment plants. Science of The Total Environment, 612, 1532-1542.

Bondarenko, S., Gan, J., Haver, D. L., Kabashima, J. N., 2004. Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. Environmental Toxicology and Chemistry, 23, 2649-2654.

Bourdat-Deschamps, M., Leang, S., Bernet, N., Daudin, J.J., Nélieu, S, 2014. Multi-residue analysis of pharmaceuticals in aqueous environmental samples by online solid-phase extraction-ultra-high-performance liquid chromatography-tandem mass spectrometry: Optimisation and matrix effects reduction by quick, easy, cheap, effective. Journal of Chromatography A, 1349, 11-23.

Boxall, A. B. A., 2012. New and Emerging Water Pollutants Arising from Agriculture. Organisation for Economic Co-Operation and Deveolpment Report, 1-48.

Boxall, A. B. A., Johnson, P., Smith, E., Sinclair, C., Stutt, E., Levy, L., 2006. Uptake of veterinary medicines from soils into plants. Journal of Agricultural and Food Chemistry 54, 2288-2297.

Brooks, B. W., Chambliss, C. K., Stanley, J. K., Ramirez, A., Banks, K. E., Johnson, R. D., Lewis, R. J., 2005. Determination of select antidepressants in fish from an effluent-dominated stream. Environmental Toxicology and Chemistry, 24, 464-469.

Caldas, S. S., Bolzan, C. M., Cerqueira, M. B., Tomasini, D., Furlong, E. B., Fagundes, C., Primel, E. G., 2011. Evaluation of a modified QuEChERS extraction of multiple classes of pesticides from a rice paddy soil by LC-APCI-MS/MS. Journal of Agricultural and Food Chemistry, 59, 11918-11926.

Calderón-Preciado, D., Jiménez-Cartagena, C., Matamoros, V., Bayona, J. M., 2011. Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. Water Research, 45, 221-231.

Cang, T., Dai, D., Yang, G., Yu, Y., Lv, L., Cai, L., Wang, Q., Wang, Y., 2017. Combined toxicity of imidacloprid and three insecticides to the earthworm, eisenia fetida (annelida, oligochaeta). Environmental Science and Pollution Research, 24, 8722-8730.

Cao, M., Li, S., Wang, Q., Wei, P., Liu, Y., Zhu, G., Wang, M., 2015. Track of fate and primary metabolism of trifloxystrobin in rice paddy ecosystem. Science of the Total Environment, 518-519, 417-423.

Caravaca, F., Lax, A., Albaladejo, J., 1999. Organic matter, nutrient contents and cation exchange capacity in fine fractions from semiarid calcareous soils. Geoderma, 93, 161-176.

Carbo, L., Martins, E., Dores, E., Spadotto, C., Weber, O., Ermelinda M., 2007. Acetamiprid, carbendazim, diuron and thiamethoxam sorption in two brazilian tropical soils. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 42, 499-507.

Carmona, E., Andreu, V., Picó, Y., 2017. Multi-residue determination of 47 organic compounds in water, soil, sediment and fish Turia river as case study. Journal of Pharmaceutical and Biomedical Analysis, 146, 117-125.

Carpenter, C., Helbling, D. E., 2018. Widespread micropollutant monitoring in the Hudson river estuary reveals spatiotemporal micropollutant clusters and their sources. Environmental Science and Technology, 52, 6187-6196.

Chakraborty, S. K., Bhattacharya, A., Chowdhury, A., 1999. Degradation of oxadiazon in kalyani alluvial soil. Pesticide Science, 5, 943-948.

Chefetz, B., Mualem, T., Ben-Ari, J., 2008. Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater. Chemosphere, 73,8, 1335-1343.

Chen, F., Ying, G. G., Ma, Y. B., Chen, Z. F., Lai, H. J., 2014. Field dissipation of four personal care products in biosolids-amended soils in North China. Environmental Toxicology and Chemistry, 33, 2413-2421.

Chiaia-Hernandez, A. C., Keller, A., Wächter, D., Steinlin, C., Camenzuli, L., Hollender, J., Krauss, M., 2017. Long-term persistence of pesticides and TPs in archived agricultural soil samples and comparison with pesticide application. Environmental Science and Technology, 51, 10642-10651.

Chitescu, C. L., Oosterink, E., De Jong, J., Stolker, A. A. M., 2012. Ultrasonic or accelerated solvent extraction followed by U-HPLC-high mass accuracy MS for screening of pharmaceuticals and fungicides in soil and plant samples. Talanta, 88, 653-662.

Coelhan, M., Strohmeier, J., Barlas, H., 2006. Organochlorine levels in edible fish from the Marmara Sea, Turkey. Environment International, 32, 775-780.

Correia-Sá, L., Fernandes, V. C., Carvalho, M., Calhau, C., Domingues, V. F., Delerue-Matos, C., 2012. Optimization of QuEChERS method for the analysis of organochlorine pesticides in soils with diverse organic matter. Journal of Separation Science, 35, 1521-1530.

Cui, K., Wen, J., Zeng, F., Li, S., Zhou, X., Zeng, Z., 2017. Occurrence and distribution of organophosphate esters in urban soils of the subtropical city, Guangzhou, China. Chemosphere, 175, 514-520.

Cutillas, V., Galera, M. M., Rajski, Ł., Fernández-Alba, A. R., 2018. Evaluation of supercritical fluid chromatography coupled to tandem mass spectrometry for pesticide residues in food. Journal of Chromatography A, 1545, 67-74.

Di, S., Shi, S., Xu, P., Diao, J., Zhou, Z., 2015. Comparison of different extraction methods for analysis of 10 organochlorine pesticides: Application of MAE-SPE method in soil from Beijing. Bulletin of Environmental Contamination and Toxicology, 95, 67-72.

Domene, X., Chelinho, S., Sousa, J. S., 2009. Soil pollution by nonylphenol and nonylphenol ethoxylates and their effects to plants and invertebrates. Journal of Soils and Sediments, 9, 555-567.

Dorival-García, N., Labajo-Recio, C., Zafra-Gómez, A., Juárez-Jiménez, B., Vílchez, J. L., 2015. Improved sample treatment for the determination of 17 strong sorbed quinolone antibiotics from compost by ultra high performance liquid chromatography tandem mass spectrometry. Talanta, 138, 247-257.

Doummar, J., Aoun, M., 2018. Occurrence of selected domestic and hospital emerging micropollutants on a rural surface water basin linked to a groundwater karst catchment. Environmental Earth Sciences, 77, 1-16.

Dsikowitzky, L., Schwarzbauer, J., 2015. Hexa(Methoxymethyl)melamine: An emerging contaminant in German rivers. Water Environment Research, 87, 461-469.

Du, B., Haddad, S. P., Luek, A., Scott, W. C., Saari, G. N., Burket, S. R., Breed, C. S., Kelly, M., Broach, L., Rasmussen, J., B., Chambliss, C. K., Brooks, B. W, 2016. Bioaccumulation of human pharmaceuticals in fish across habitats of a tidally influenced urban bayou. Environmental Toxicology and Chemistry, 35, 966-974.

Du, L., Liu, W., 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems: A review. Agronomy for Sustainable Development, 32, 309-327.

Eken, Ö., Sefiloğlu, F. Ö., Balcıoğlu, I., Tezel, U., 2017. A Workflow For Targeted and Non-Targeted Quantification of Micropollutants in Surface Water, Proceedings of the 4th International Water Congress, İzmir, 2-4 November 2017, Turkey.

USDA EPA Method 9081, 1986. Cation-Exchange Capacity of Soils (Sodium Acetate).

EU NORMAN Network, 2016. Emerging Substances, https://www.norman-network.net/?q=node/19. Date accessed June 2018.

European Commission, 1986. Protection of the Environment, and in Particular of the Soil, when Sewage Sludge is used in Agriculture. Official Journal of the European Communities, 4, 7, 6-12.

European Commission, 2000. Working Document on Sludge. 3rd Draft. 80(April): 1-19.

European Commission, 2012. Report from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: The Implementation of the Soil Thematic Strategy and Ongoing Activities. Com (2012) 46 final: 15.

European Committee for Stantarization, 2008. Foods of Plant Origin-Determination of Pesticide Residues Using GC-MS and / or LC-MS / MS Following Acetonitrile Extraction / Partitioning and Cleanup by Dispersive SPE - QuEChERS-Method. British Standard: 1-83.

EUROSTAT. Chemicals Production and Consumption Statistics - Statistics Explained, http://ec.europa.eu/eurostat/statisticexplained/index.php?title=Chemicals_production_and_consumption_statistics#Total_production_of_chemicals. Date accessed July 2018.

Farré, M., Pérez, S., Kantiani, L., 2008. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. Trends in Analytical Chemistry, 27, 991-1007.

Feng, X., He, Z., Wang, L., Peng, Y., Luo, M., Liu, X., 2015. Multiresidue analysis of 36 pesticides in soil using a modified quick, easy, cheap, effective, rugged, and safe method by liquid chromatography with tandem quadruple linear ion trap mass spectrometry. Journal of Separation Science 38, 3047-3054.

Fernandes, V. C., Domingues, V. F., Mateus, N., Delerue-Matos, C., 2013. Multiresidue pesticides analysis in soils using modified QuEChERS with disposable pipette extraction and dispersive solid-phase extraction. Journal of Separation Science, 36, 376-382.

Fernandez-Alvarez, M., Lamas, J. P., Garcia-Chao, M., Garcia-Jares, C., Llompart, M., Lores, M., Dagnac, T., 2010. Monitoring of pesticide residues in dairy cattle farms from NW Spain. Journal of Environmental Monitoring, 12, 1864-1875.

Font, N., Hernández, F., Hogendoorn E. A., Baumann, R. A, van Zoonen, P., 1998. Microwave-assisted solvent extraction and reversed-phase liquid chromatography-UV Detection for screening soils for sulfonylurea herbicides. Journal of Chromatography A, 798, 179-186.

Fries, E., Mihajlović, I., 2011. Pollution of soils with organophosphorus flame retardants and plasticizers. Journal of Environmental Monitoring, 13, 2692-2694.

Fulcher, J. M.,. Wayment, D. G., White, P. M., Webber. C. L., 2014. Pyraclostrobin wash-off from sugarcane leaves and aerobic dissipation in agricultural soil. Journal of Agricultural and Food Chemistry 62, 2141-2146.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2011. Occurrence of multiclass UV Filters in treated sewage sludge from wastewater treatment plants. Chemosphere 84, 1158-1165.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2015. UV Filters bioaccumulation in fish from Iberian river basins. Science of The Total Environment, 518-519, 518-525.

Gao, J.P., Maguhn, J., Spitzauer, P., Kettrup, A., 1998. Sorption of pesticides in the sediment of the Teufelsweiher Pond (southern Germany). I: Equilibrium Assessments, effect of organic carbon content and pH. Water Research, 32, 1662-1672.

García-Galán, M. J., Díaz-Cruz, S., Barceló, D., 2013. Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography-electrospray-quadrupole linear ion trap mass spectrometry. Journal of Chromatography A, 1275, 32-40.

Ge, J., Cui, K., Yan, H., Li, Y., Chai, Y., Liu, Xi., Cheng, J., Yu, X., 2017. Uptake and translocation of imidacloprid, thiamethoxam and difenoconazole in rice plants. Environmental Pollution, 226, 47-85.

de Gerónimo, E., Botero-Coy, A. M., Marín, J. M., Aparicio, V. C., Costa, J. L., Sancho, J. V., Hernández, F., 2015. A simple and rapid analytical methodology based on liquid chromatography-tandem mass spectrometry for monitoring pesticide residues in soils from Argentina. Analytical Methods, 7, 9504-9012.

Gevao, B., Semple, K. T., Jones. K. C., 2000. Bound pesticide residues in soils: A review. Environmental Pollution, 108, 3-14.

Golge, O., Koluman A., Kabak. B., 2018. Validation of a modified QuEChERS Method for the determination of 167 pesticides in milk and milk products by LC-MS/MS. Food Analytical Methods, 11, 122-48.

Gomes, A. R., Justino, C., Rocha, S. T., Freitas, A. C., Duarte, A. C., Pereira, R., 2017. Review of the ecotoxicological effects of emerging contaminants to soil biota. Journal of Environmental Science and Health, Part A, 52, 992-1007.

Guo, C., Wang, M., Xiao, H., Huai, B., Wang, F., Pan, G., Liao, X., Liu, Y., 2016. Development of a modified QuEChERS method for the determination of veterinary antibiotics in swine manure by liquid chromatography tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1027, 110-118.

Guo, Y., Meng, X. Z., Tang, H. L., Zeng, E. Y., 2008. Tissue distribution of organochlorine pesticides in fish collected from the Pearl River Delta, China: Implications for fishery input source and bioaccumulation. Environmental Pollution, 155, 150-156.

Hach Company, 1996a. Digestion Procedure for Solids Digestion Procedure for Solids, 43-48.

Hach Company, 1996b. Nitrogen , Total Kjeldahl, 1-8.

Hartle, J. C., Cohen, R. S., Sakamoto, P., Barr, D. B., Carmichael, S. L., 2018. Chemical contaminants in raw and pasteurized human milk. Journal of Human Lactation, 34, 340-349.

HERA, 2002. Human & Environmental Risk Assessment on Ingredients of European Household Cleaning Products- Tetraacetylethylenediamine (TAED).

Hildebrandt, A., Lacorte, S., Barceló. D., 2009. Occurrence and fate of organochlorinated pesticides and PAH in agricultural soils from the Ebro River Basin. Archives of Environmental Contamination and Toxicology, 57, 247-255.

Hollrigl-Rosta, A., Kreuzig, R., Bahadir, M., 1999. Investigations on the metabolic fate of prochloraz in soil under field and laboratory conditions. Pesticide Science, 55, 531-538.

Homazava, N., Aquillon, C. G., Vermeirssen E., Werner, I., 2014. Simultaneous multi-residue pesticide analysis in soil samples with ultra-high-performance liquid chromatography-tandem mass spectrometry using QuEChERS and pressurised liquid extraction methods. International Journal of Environmental Analytical Chemistry, 94, 1085-1099.

Hurtado, C., Montano-Chávez, Y. N., Domínguez, C., Bayona, J. M., 2017. Degradation of emerging organic contaminants in an agricultural soil: Decoupling biotic and abiotic processes. Water, Air, and Soil Pollution, 228, 1-8.

Hussain, S., Hartley C. J., Shettigar M., Pandey, G., 2016. Bacterial biodegradation of neonicotinoid pesticides in soil and water systems. FEMS Microbiology Letters, 363, 1-13.

Imfeld, G., Vuilleumier S., 2012. Measuring the effects of pesticides on bacterial communities in soil: A critical review. European Journal of Soil Biology, 49, 22-30.

Jiang, W., Gao, J., Cheng, Z., Wang, P., Zhou, Z., Liu, D., 2018. The effect of antibiotics on the persistence of herbicides in soil under the combined pollution. Chemosphere, 204, 303-309.

Jin, Y., Zhang, J., Zhao, W., Zhang, W., Wang, L., Zhou, J., Li, Y., 2017. Development and validation of a multiclass method for the quantification of veterinary drug residues in honey and royal jelly by liquid chromatography-tandem mass spectrometry. Food Chemistry, 221, 1298-1307.

Johnson, A., Jürgens, M., 2003. Endocrine active industrial chemicals: Release and occurrence in the environment. Pure and Applied Chemistry, 75, 11-12.

Jones, D., 1995. Environmental fate of chlorpyrifos. Environmental Monitoring and Pest Management, 1-150.

Kang, J., Fan, C. L., Cao, Y. F., Wang, H. J., Peng, X., Wang, Z. B., Chang, Q. Y., Hu, X. Y., Pang, G. F., 2014. Multi-residue screening of 100 multi-class veterinary drugs in milk powder by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Analytical Methods, 6, 8337-8349.

Kemmerich, M., Bernardi, G., Adaime, M. B., Zanella, R., Prestes, O. D., 2015. A simple and efficient method for imidazolinone herbicides determination in soil by ultra-high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, 1412, 82-89.

Khan, A. H., Topp, E., Scott, A., Sumarah, M., Macfie, S. M., Ray, M. B., 2015. Biodegradation of benzalkonium chlorides singly and in mixtures by a pseudomonas sp. isolated from returned activated sludge. Journal of Hazardous Materials, 299, 595-602.

Khan, A. H., Macfie, S. M., Ray, M. R., 2017. Sorption and leaching of benzalkonium chlorides in agricultural soils. Journal of Environmental Management, 196, 26-35.

Kiss, A., Fries, E., 2009. Occurrence of benzotriazoles in the rivers Main, Hengstbach, and Hegbach (Germany). Environmental Science and Pollution Research, 16, 702-710.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D, Barber, L. B., Buxton, H. T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science and Technology, 36, 1202-1211.

Konstantinou, I. K., Zarkadis, A. K., Albanis, T. A., 2001. Photodegradation of selected herbicides in various natural waters and soils under environmental conditions. Journal of Environment Quality, 30, 121-130.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y., Rosen, C. J., 2005. Antibiotic uptake by plants from soil fertilized with animal manure. Journal of Environment Quality, 34, 2082-2085.

Kumar, K., Gupta, S. C., Chander, Y., Singh. A. K., 2005. Antibiotic use in agriculture and its impact on the terrestrial environment. Advances in Agronomy, 87, 1-54.

Larivière, A., Lissalde, S., Soubrand, M., Casellas-Français, M., 2017. Overview of multiresidues analytical methods for the quantitation of pharmaceuticals in environmental solid matrixes: Comparison of analytical development strategy for sewage sludge, manure, soil, and sediment samples. Analytical Chemistry, 89, 453-465.

Lee, Y. J., Choi, J. H., Abd El-Aty, A. M., Chung, H. S., Lee, H. S., Kim, S. W., Rahman, M. M., Park, B. J., Kim, J. E., Shin, H. C., Shim, J. H., 2017. Development of a single-run analytical method for the detection of ten multiclass emerging contaminants in agricultural soil using an acetate-buffered QuEChERSmethod coupled with LC-MS/MS. Journal of Separation Science, 40, 415-423.

Lesueur, C., Gartner, M., Mentler, A., Fuerhacker, M., 2008. Comparison of four extraction methods for the analysis of 24 pesticides in soil samples with gas chromatography-mass spectrometry and liquid chromatography-ion trap-mass spectrometry. Talanta, 75, 284-293.

Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B. M., Jørgensen, E. H., Sonne, C., Verreault, J., Vijayan, M. M., Gabrielsen, G. W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Science of the Total Environment, 408, 2995-3043.

Lin, H., Dong, B., Hu., J., 2017. Residue and intake risk assessment of prothioconazole and its metabolite prothioconazole-desthio in wheat field. Environmental Monitoring and Assessment 189, 1-9.

Liu, Y., Li, S., Ni, Z., Qu, M., Zhong, D., Ye, C., Tang, F., 2016. Pesticides in persimmons, jujubes and soil from China: Residue levels, risk assessment and relationship between fruits and soils. Science of The Total Environment, 542, 620-628.

Lopes, R. P., Reyes, R. C., Romero-González, R., Vidal, J. L. M., Frenich, A. G.. 2012. Multiresidue determination of veterinary drugs in aquaculture fish samples by ultra high performance liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 895-896, 39-47.

Łozowicka, B., Rutkowska, E., Jankowska, M., 2017. Influence of QuEChERS modifications on recovery and matrix effect during the multi-residue pesticide analysis in soil by GC/MS/MS and GC/ECD/NPD. Environmental Science and Pollution Research, 24, 7124-7138.

Lu, Y., Song, S., Wang, R., Liu, Z., Meng, J., Sweetman, A., J., Jenkins, A., Ferrier, R., Li, H., Luo, W., Wang, T., 2015. Impacts of soil and water pollution on food safety and health risks in China. Environment International, 77, 5-15.

Łukaszewicz, P., Kumirska, J., Białk-Bielińska, A., Dołżonek, J., Stepnowski, P., 2017. Assessment of soils contamination with veterinary antibiotic residues in northern Poland using developed MAE-SPE-LC/MS/MS methods. Environmental Science and Pollution Research, 24, 21233-21247.

Luo, Y, Xu, L., Rysz, M., Wang, Y., Zhang, H., Alvarez, P. J. J., 2011. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environmental Science and Technology, 45, 1827-1833.

MacHerius, A., Eggen, T., Lorenz, W. G., Reemtsma, T., Winkler, U., Moeder, M., 2012. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. Journal of Agricultural and Food Chemistry, 60, 7785-7791.

Martins, G. L., Friggi, C. A., Prestes, O. D., Vicari, M.C., Friggi, D. A., Adaime, M. B., Zanella, R., 2014. Simultaneous LC-MS/MS determination of imidazolinone herbicides together with other multiclass pesticide residues in soil. Clean-Soil, Air, Water, 42, 1441-1449.

Masiá, A., Vásquez, K., Campo, J., Picó, Y., 2015. Assessment of two extraction methods to determine pesticides in soils, sediments and sludges: Application to the Túria River Basin. Journal of Chromatography A, 1378, 19-31.

Matamoros, V., Arias, C. A., Xuan, L., Salvadó, V., Brix, H., 2012. Occurrence and behavior of emerging contaminants in surface water and a restored wetland. Chemosphere, 88, 1083-1089.

McNeill, K. S., Cancilla. D. A., 2009. Detection of triazole deicing additives in soil samples from airports with low, mid, and large volume aircraft deicing activities. Bulletin of Environmental Contamination and Toxicology, 82, 265-269.

Meng, M., He, Z., Xu, Y., Wang, L., Peng, Y., Liu, X., 2017. Simultaneous extraction and determination of antibiotics in soils using a method based on quick, easy, cheap, effective, rugged, and safe extraction and liquid chromatography with tandem mass spectrometry. Journal of Separation Science, 40, 3214-3220.

Merdassa, Y., Liu, J., Megersa, N., 2014. Development of a one-step microwave-assisted extraction procedure for highly efficient extraction of multiclass fungicides in soils. Analytical Methods, 6, 3025-3033.

Micó, C., Recatalá, L., Peris, M., Sánchez, J., 2006. Assessing heavy metal sources in agricultural soils of an european mediterranean area by multivariate analysis. Chemosphere, 65, 863-872.

Milan, M., Ferrero, A., Fogliatto, S., De Palo, F., Vidotto, F., 2017. Imazamox dissipation in two rice management systems. Journal of Agricultural Science, 155, 431-443.

Miranda, A. L., Roche, H., Randi, M. A. F., Menezes, M. L., Ribeiro, C. A. O., 2008. Bioaccumulation of chlorinated pesticides and pcbs in the tropical freshwater fish hoplias malabaricus: Histopathological, physiological, and immunological findings. Environment International, 34, 939-949.

Naidu, R., Espana, V. A. A., Liu, Y., Jit., J., 2016. Emerging contaminants in the environment: risk-based analysis for better management. Chemosphere, 154, 350-357.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical pesticides and human health: The urgent need for a new concept in agriculture. Frontiers in Public Health, 4, 1-8.

Ocaklı, I., 2012. Edirne İli Çeltik Sektörü Raporu.

OECD, 1984. 'OECD 207 -Earthworm, Acute Toxicity Tests.' OECD Guideline for Testing of Chemicals.

von der Ohe, P. C., Dulio, V., Slobodnik, J., de Deckere, E., Kühne, R., Ebert, R., Ginebreda, A., de Cooman, W., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Science of The Total Environment, 409, 2064-2077.

Pal, A., Gin, K. Y., Lin, A. Y., Reinhard, M., 2010. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Science of The Total Environment, 408, 6062-6069.

Palmiotto, M., Castiglioni, S., Zuccato, E, Manenti, A., Riva, F., Davoli, E., 2018. Personal care products in surface, ground and wastewater of a complex aquifer system, a potential planning tool for contemporary urban settings. Journal of Environmental Management, 214, 76-85.

Panizzi, S., Nicoleta A. S., Trevisan, M., 2017. Combined ecotoxicological risk assessment in the frame of european authorization of pesticides. Science of The Total Environment, 580, 136-146.

Papadopoulou, E., Karas, P. A., Nikolaki, S., Storck, V., Ferrari, F., Trevisan, M., Tsiamis, G., Martin-Laurent, F., Karpouzas, D. G., 2016. Dissipation and adsorption of isoproturon, tebuconazole, chlorpyrifos and their main transformation products under laboratory and field conditions. Science of The Total Environment, 569-570, 86-96.

Parfitt, R. L., Giltrap, D. J., Whitton J. S., 1995. Contribution of organic matter and clay minerals to the cation exchange capacity of soils. Communications in Soil Science and Plant Analysis, 26, 1343-1355.

Park, J., Cho, K. H., Lee, E., Lee, S., Cho, J., 2018. Sorption of pharmaceuticals to soil organic matter in a constructed wetland by electrostatic interaction. Science of The Total Environment, 635, 1345-1350.

Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Research, 72, 3-27.

Petrovic, M., Sabater, S. Elosegi, A. Barceló, D., 2016. Fate and Behavior in the Aquatic Environment: Biotic And Abiotic Transformations in Rivers. In Emerging Contaminants in River Ecosystems: Occurrence and Effects Under Multiple Stress Conditions. Springer, Switzerland.

Peysson, W., Vulliet., E., 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography-time-of-flight-mass spectrometry. Journal of Chromatography A, 1290, 46-61.

Pino-Otín, M. R., Muñiz, S., Val, J., Navarro, E., 2017. Effects of 18 pharmaceuticals on the physiological diversity of edaphic microorganisms. Science of the Total Environment, 595, 441-450.

Pose-Juan, E., Herrero-Hernández, E., Álvarez-Martín, A., Sánchez-Martín, M. J., Rodríguez-Cruz, M. S., 2014. Development of a procedure for the multiresidue analysis of pesticides in vineyard soils and its application to real samples. Journal of Separation Science, 37, 2215-2224.

Pose-Juan, E., Herrero-Hernández, E., Álvarez-Martín, A., Sánchez-Martín, M. J., Rodríguez-Cruz, M. S., 2015. Pesticide residues in vineyard soils from spain: Spatial and temporal distributions. Science of The Total Environment, 514, 351-358.

PPDB: Pesticide Properties DataBase. University of Herftordshire. https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm#C. Date accessed July 2018

Prestes, O. D., Padilla-Sánchez, J. A., Romero-González, R., Grio, S. L., Frenich, A. G., Martínez-Vidal, J. L., 2012. Comparison of several extraction procedures for the determination of biopesticides in soil samples by ultrahigh pressure LC-MS/MS. Journal of Separation Science, 35, 861-868.

Pszczolinska, K, Michel., M., 2016. The QuEChers approach for the determination of pesticide residues in soil samples: An overview. Journal of AOAC International, 99, 1403-1414.

Quayle, W. C., Oliver, D. P., Zrna, S., 2006. Field dissipation and environmental hazard assessment of clomazone, molinate, and thiobencarb in Australian rice culture. Journal of Agricultural and Food Chemistry, 54, 7213-7220.

Raynie, D. E., 2006. Modern extraction techniques. Analytical Chemistry, 78, 3997-4004.

Ribeiro, C. A. O., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (Anguilla Anguilla) at the Camargue Nature Reserve, France. Aquatic Toxicology, 74, 53-69.

Rimayi, C., Odusanya, D., Weiss, J. M., Boer, J. D. Chimuka, L., 2018. Comparison of several extraction procedures for the determination of biopesticides in soil samples by ultrahigh pressure LC-MS/MS. Science of the Total Environment 627, 1008-1017.

Rissato, S. R., Galhiane, M. S., Apon, B. M., Arruda, M. S. P., 2005. Multiresidue analysis of pesticides in soil by supercritical fluid extraction/gas chromatography with electron-capture detection

and confirmation by gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry, 53, 62-69.

Rissato, S. R., Galhiane, M. S., Knoll, F. R. N., Apon, B. M., 2004. Supercritical fluid extraction for pesticide multiresidue analysis in honey: Determination by gas chromatography with electron-capture and mass spectrometry detection. Journal of Chromatography A, 1048, 153-159.

Rütters, H., Höllrigl-Rosta, A., Kreuzig, R, Bahadir, M., 1999. Sorption behavior of prochloraz in different soils. Journal of Agricultural and Food Chemistry, 47, 1242-1246.

Sadílek, J., Spálovská, P., Vrana, B., Vávrová, M., Maršálek, B., Šimek, Z., 2016. Comparison of extraction techniques for isolation of steroid oestrogens in environmentally relevant concentrations from sediment. International Journal of Environmental Analytical Chemistry, 96, 1022-1037.

Salazar-Ledesma, M., Prado, B., Zamora, O., Siebe, C., 2018. Mobility of atrazine in soils of a wastewater irrigated maize field. Agriculture, Ecosystems & Environment, 255, 73-83.

Salvia, M. V., Vulliet, E., Wiest, L., Baudot, R., Cren-Olivé, C., 2012. Development of a multi-residue method using acetonitrile-based extraction followed by liquid chromatography-tandem mass spectrometry for the analysis of steroids and veterinary and human drugs at trace levels in soil. Journal of Chromatography A, 1245, 122-133.

Sánchez, M. E., Estrada, I. B., Martínez, O., Martín-Villacorta, J., Aller, A., Morán, A., 2004. Influence of the application of sewage sludge on the degradation of pesticides in the soil. Chemosphere, 57, 673-679.

SANTE/11813/2017, 2017. Analytical Quality Control And Method Validation Procedures For Pesticide Residues Analysis In Food And Feed.

Sezer, İ., Akay, H., Öner, F., Şahin, M., 2012. Çeltik üretim sistemleri. Türk Bilimsel Derlemeler Dergisi, 5, 6-11.

Shore, L. S., Shemesh, M., 2003. Naturally produced steroid hormones and their release into the environment. Pure Applied Chemistry, 75, 11-12.

Silva, B. F., Jelic, A., López-Serna, R., Mozeto, A. A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro River Basin, Spain. Chemosphere, 85, 1331-1339.

da Silva, C. P., Emídio, E. S., and Rodrigues de Marchi, M. R., 2015. The occurrence of UV filters in natural and drinking water in São Paulo State (Brazil). Environmental Science and Pollution Research, 22, 19706-19715.

Sinclair, C. J., Boxall, A. B. A., 2003. Assessing the ecotoxicity of pesticide transformation products. Environmental Science and Technology, 37, 4617-4625.

Singh, P.B., Singh, V., 2008. Pesticide bioaccumulation and plasma sex steroids in fishes during breeding phase from North India. Environmental Toxicology and Pharmacology, 25, 342-350.

Smalling, K. L., Orlando, J. L., Kuivila, K. M., 2007. Occurrence of pesticides in water, sediment, and soil from the Yolo California. San Francisco Estuary and Watershed Science, 5.

Smital, T., Terzić, S., Lončar, J., Senta, I., Žaja, R., Popović, M., Mikac, I., Tollefsen, K., Thomas, K. V., Ahel, M., 2013. Prioritisation of organic contaminants in a river basin using chemical analyses and bioassays. Environmental Science and Pollution Research, 20, 1384-1395.

Song, S., Zhu, K, Han, L., Sapozhnikova, Y., Zhang, Z., Yao, W., 2018. Residue analysis of 60 pesticides in red swamp crayfish using quechers with high-performance liquid chromatography-tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 66, 5031-5038.

Song, Y., Zhao, S., Tchounwou, P., Liu, Y., 2007. A nanoparticle-based solid-phase extraction method for liquid chromatography-electrospray ionization-tandem mass spectrometric analysis. Journal of Chromatography A, 1166, 79-84.

Sun, L., Sun, X., Du, X., Yue, Y., Chen, L., Xu, H., Zeng, Q., Wang, H., Ding, L., 2010. Determination of sulfonamides in soil samples based on alumina-coated magnetite nanoparticles as adsorbents. Analytica Chimica Acta, 665, 185-192.

T.C. Çevre ve Orman Bakanlığı., 2004. Su Kirliliği Kontrolü Yönetmeliği. Resmi Gazete.

T.C. Çevre ve Orman Bakanlığı., 2005. Toprak Kirliliginin Kontrolü Yönetmeligi. Resmi Gazete.

Tadeo, J. L., Sánchez-Brunete, C., Albero, B., García-Valcárcel, A. I., 2010. Application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples. Journal of Chromatography A, 1217, 2415-2440.

Turiel, E., Martín-Esteban, A., Tadeo, J. L., 2006. Multiresidue analysis of quinolones and fluoroquinolones in soil by ultrasonic-assisted extraction in small columns and HPLC-UV. Analytica Chimica Acta, 562, 30-35.

USEPA, 2014. Priority Pollutant List.

Uwizeyimana, H., Wang, M., Chen, W., Khan, K., 2017. The eco-toxic effects of pesticide and heavy metal mixtures towards earthworms in soil. Environmental Toxicology and Pharmacology, 55, 20-29.

Voice, T.C, and Weber Jr., W. J., 1983. Sorption of hydrophobic compounds by sediments, soils and suspended solids-i theory and background. Water Resarch, 17.

Wan, M., Szeto, S., Price. P., 1994. Organophosphorus insecticide residues in farm ditches of the lower fraser valley of British Columbia. Journal of Environmental Science and Health, Part B, 29, 917-949.

Wang, R., Tang, J., Xie, Z., Mi, W., Chen, Y., Wolschke, H., Tian, C., Pan, X., Luo, Y., Ebinghaus, R., 2015. Occurrence and spatial distribution of organophosphate ester flame retardants and plasticizers in 40 rivers draining into the Bohai Sea, North China. Environmental Pollution, 198, 172-178.

WHO, 2000. Flame Retardants : TBEP - Tris (2-Butoxyethyl) Phosphate , Tris (2-Ethylhexyl) Phosphate and Tetrakis (Hydroxymethyl) Phosphonium Salts. : 1-154.

Wilkinson, J., Hooda, P. S.,Barker, J., Barton, S., Swinden, J., 2017. Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. Environmental Pollution, 231, 954-970.

Wilkowska, A., Biziuk, M., 2011. Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chemistry, 12, 803-812.

Wong, S. C., Li, X. D., Zhang, G., Qi, S. H., Min, Y. S., 2002. Heavy metals in agricultural soils of the Pearl River Delta, South China. Environmental Pollution 19, 33-44.

Wu, X., Conkle, J. L., Ernst, F., Gan J., 2014. Treated wastewater irrigation: Uptake of pharmaceutical and personal care products by common vegetables under field conditions. Environmental Science and Technology, 48, 11286-11293.

Wu, X., Dodgen, L. K., Conkle, J. L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: A review. Science of the Total Environment, 536, 655-666.

Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y.,Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., Sekizawa, J., 2009. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. Water Research, 43, 351-362.

Yang, G., Chen, C., Yu, Y., Zhao, H., Wang, W., Wang, Y., Cai, L., He, Y., Wang, X., 2018. Combined effects of four pesticides and heavy metal chromium(VI) on the earthworm using avoidance behavior as an endpoint. Ecotoxicology and Environmental Safety, 157, 191-200.

Yu, Y., Liu, X., He, Z., Wang, L., Luo, M., Peng, Y., Zhou, Q., 2016. Development of a multi-residue method for 58 pesticides in soil using QuEChERS and gas chromatography-tandem mass spectrometry. Analytical Methods, 8, 2463-2470.

Yu, Y., Liu, Y., Wu., L., 2013. Sorption and degradation of pharmaceuticals and personal care products (PPCPs) in soils. Environmental Science and Pollution Research, 20, 4261-4267.

Yu, Y., Chu, X., Pang, G., Xiang, Y., Fang, H., 2009. Effects of repeated applications of fungicide carbendazim on its persistence and microbial community in soil. Journal of Environmental Sciences, 21, 179-185.

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., Zhang, G., 2011. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environmental Pollution, 159, 84-91.

Zhang, F., Wang, L., Zhou, L., Wu, D., Pan, H., Pan, C., 2012. Residue dynamics of pyraclostrobin in peanut and field soil by QuEChERS and LC-MS/MS. Ecotoxicology and Environmental Safety, 78, 116-122.

Zhang, X., Li, Y., Liu, B., Wang, J., Feng, C., 2014. The effects of estrone and 17β-estradiol on microbial activity and bacterial diversity in an agricultural soil: Sulfamethoxazole as a co-pollutant. Ecotoxicology and Environmental Safety, 107, 313-320.

Zheng, C., Feng, S., Liu, P., Fries, E., Wang, Q., Shen, Z., Liu, H., Zhang, T., 2016. Sorption of organophosphate flame retardants on pahokee peat soil. Clean-Soil, Air, Water, 44, 1163-1173.

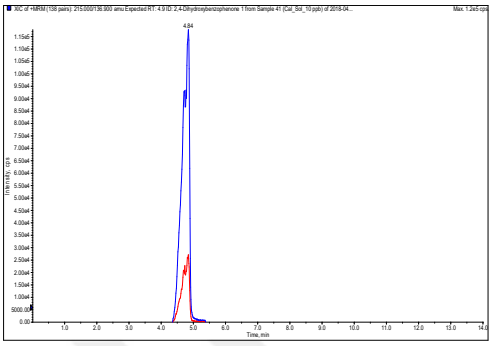
Zheng, W., Park, J. A., Abd El-Aty, A. M., Kim, S. K., Cho, S. H., Choi, J., Yi, H., Cho, S. M., Ramadan, A., Jeong, J. H., Shim, J. H., Shin, H. C., 2018. Development and validation of modified QuEChERS method coupled with lc-ms/ms for simultaneous determination of cymiazole, fipronil, coumaphos, fluvalinate, amitraz, and its metabolite in various types of honey and royal jelly. Journal of Chromatography B, 1072, 60-69.

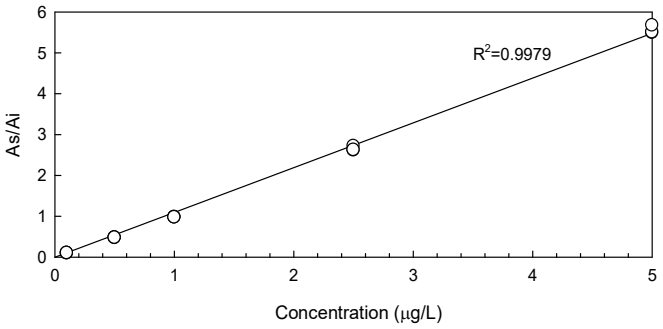
Zhong, Y., Yan, W., Shangguan, Z., 2015. Soil organic carbon, nitrogen, and phosphorus levels and stocks after long-term nitrogen fertilization. Clean-Soil, Air, Water, 43, 1538-1546.

Zhou, Y., Lu, X., Fu, X., Yu, B., Wang, D., Zhao, C., Zhang, Q., Tan, Y., Wang, X., 2018. Development of a fast and sensitive method for measuring multiple neonicotinoid insecticide residues in soil and the application in parks and residential areas. Analytica Chimica Acta, 1016, 19-28.

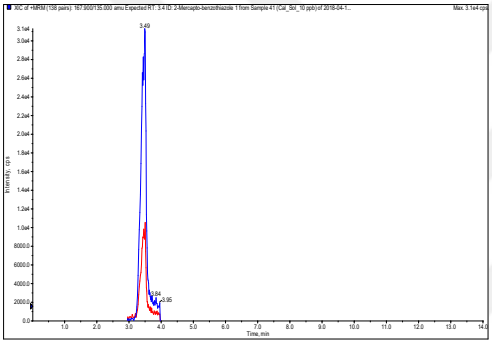
APPENDIX A: CALIBRATION GRAPHS AND CHROMOTOGRAMS OF TARGET CONTAMINANTS

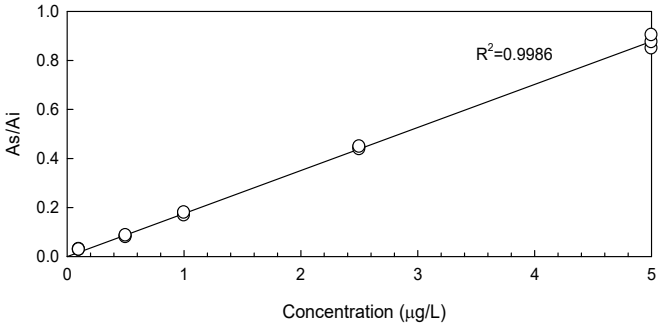
2,4-Dihydroxybenzophenone



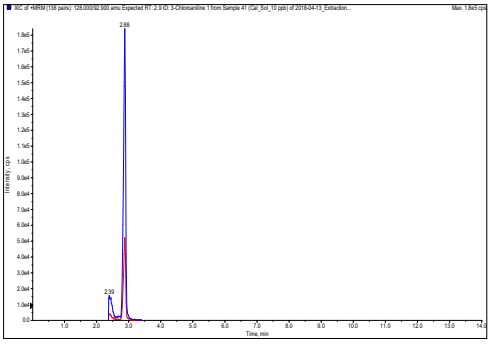


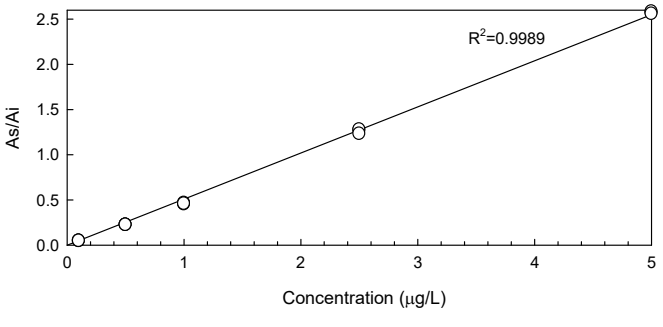
2-Mercapto-benzothiazole



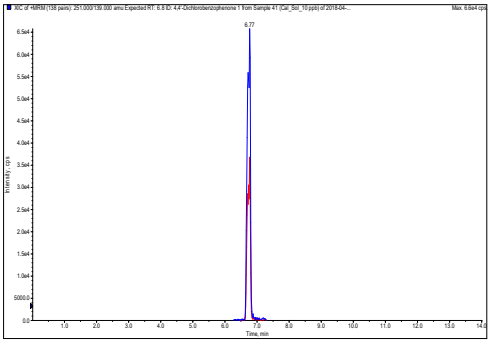


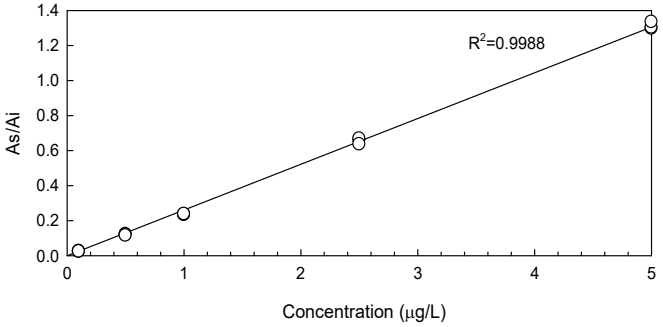
3-Chloroaniline



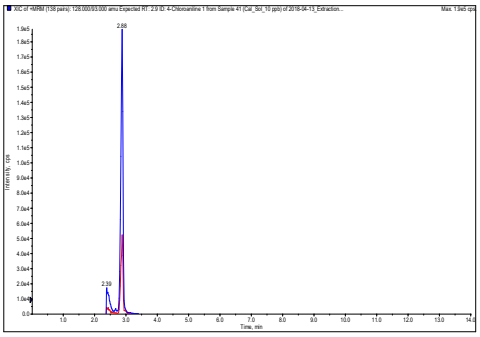


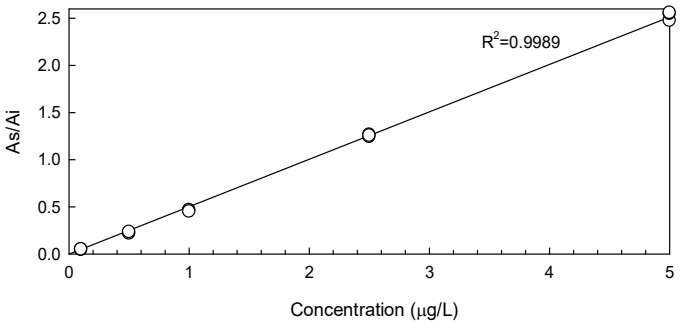
4,4'-Dichlorobenzophenone



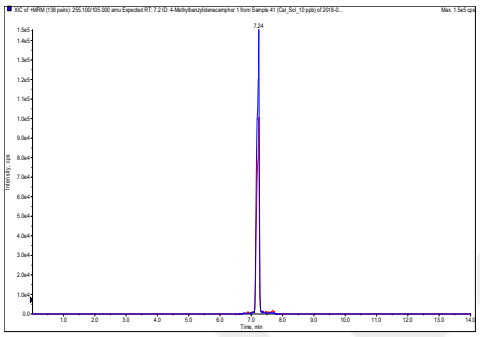


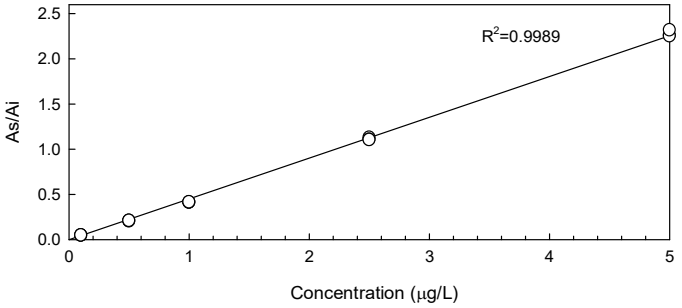
4-Chloroaniline



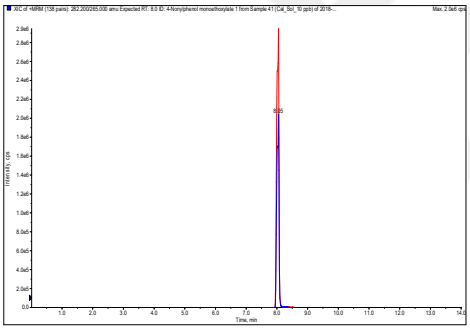


4-Methylbenzylidenecamphor

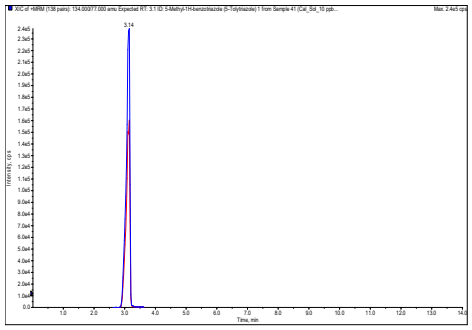


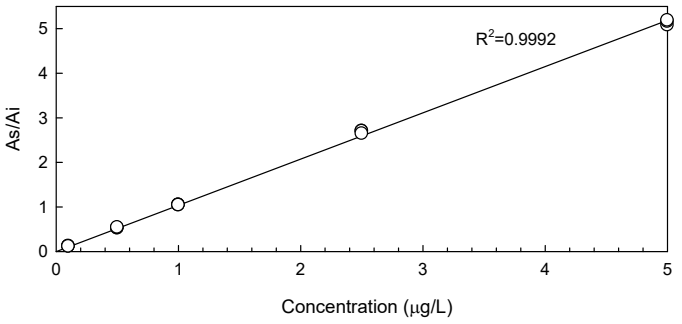


4-Nonylphenol monoethoxylate

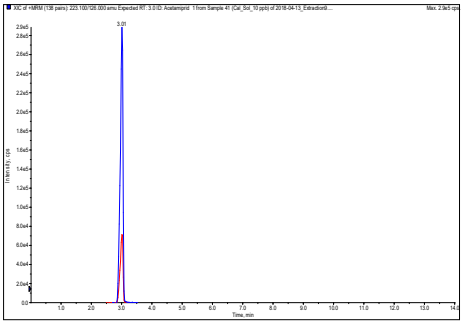


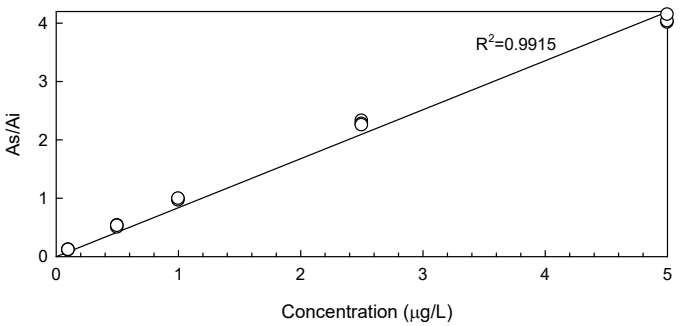
5-Methyl-1H-benzotriazole (5-Tolytriazole)



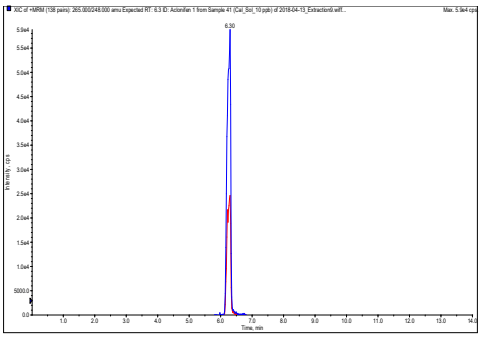


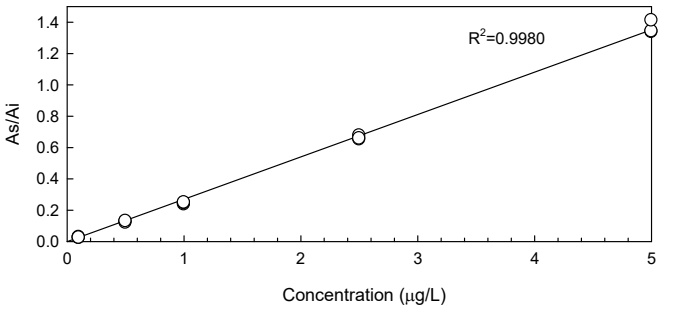
Acetamiprid



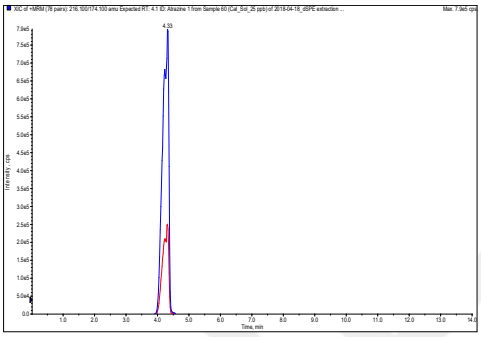


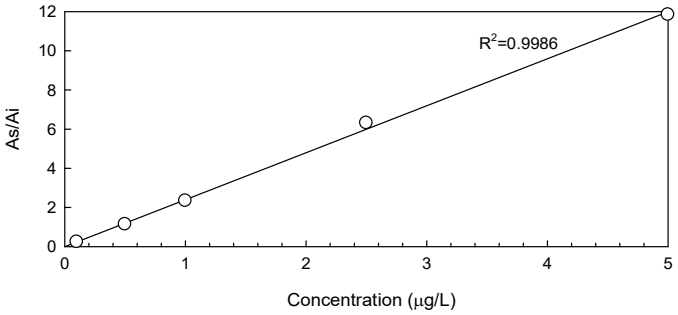
Aclonifen



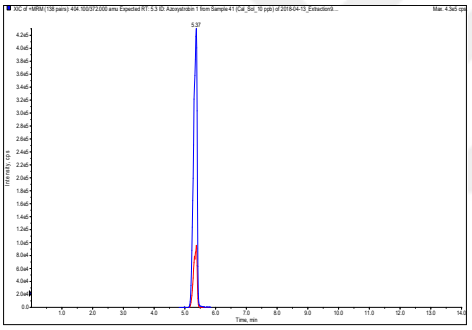


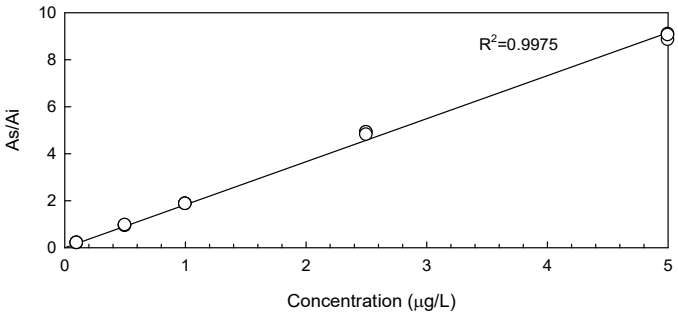
Atrazine



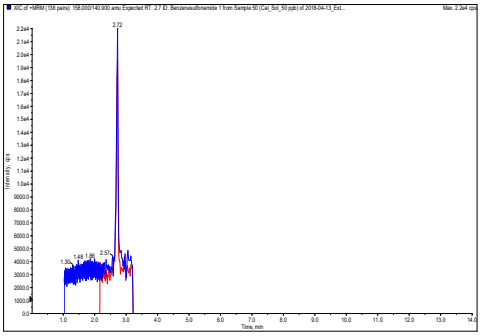


Azoxystrobin

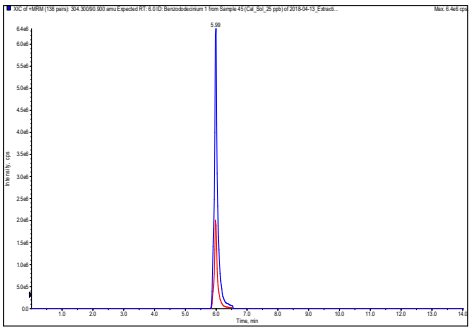


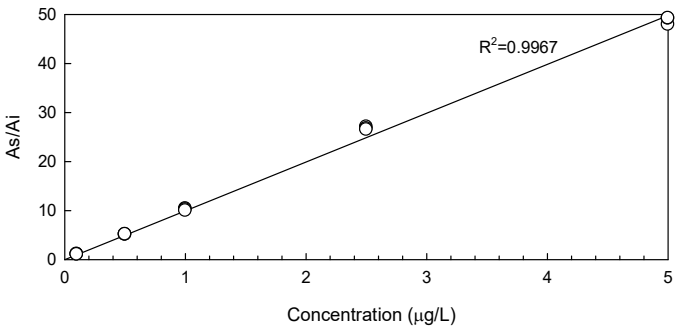


Benzenesulfonamide

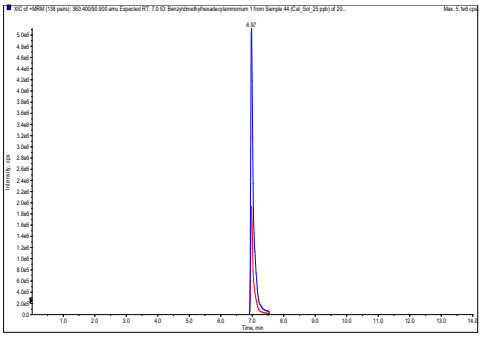


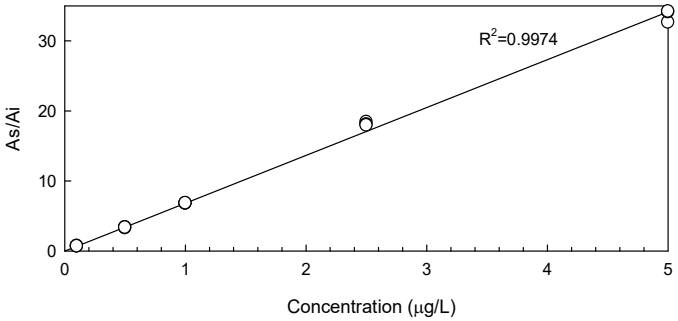
Benzyldimethyldodecylammonium



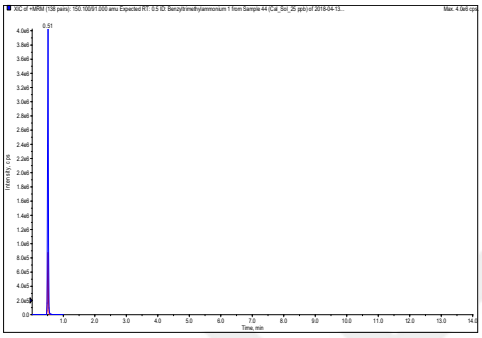


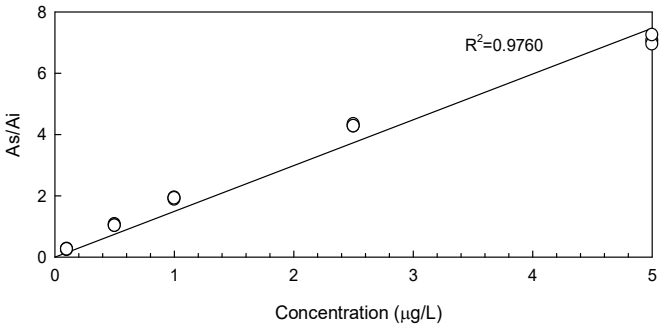
Benzyldimethylhexadecylammonium



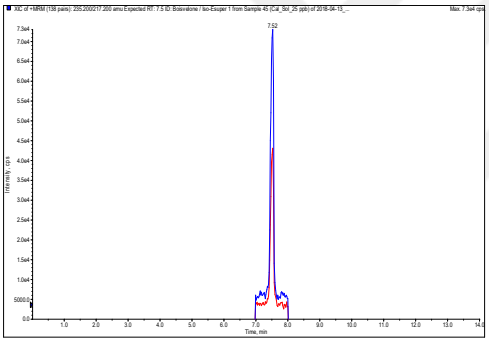


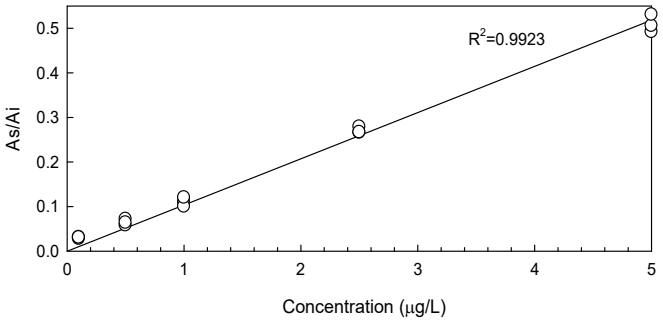
Benzyltrimethylammonium



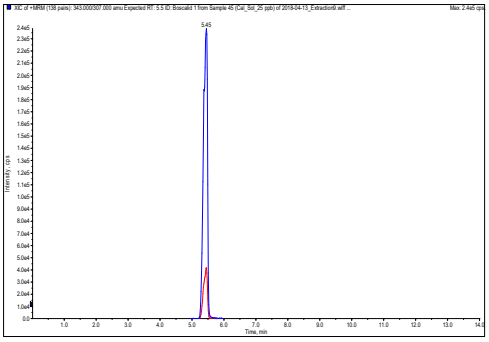


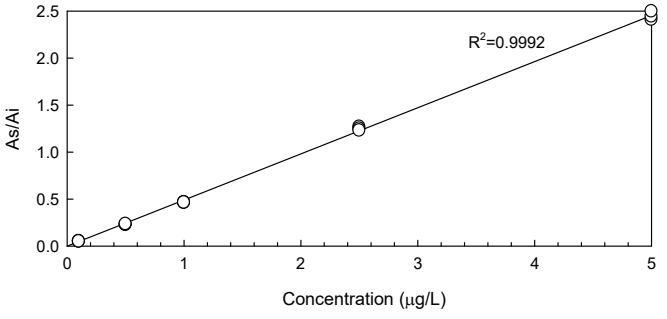
Boisvelone / Iso-Esuper



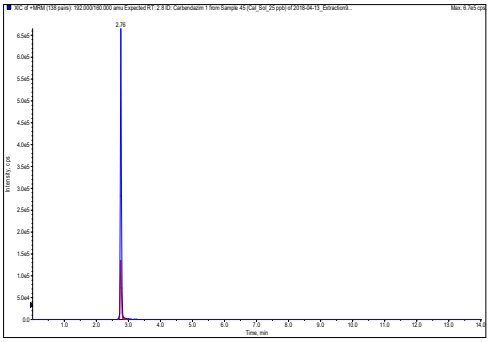


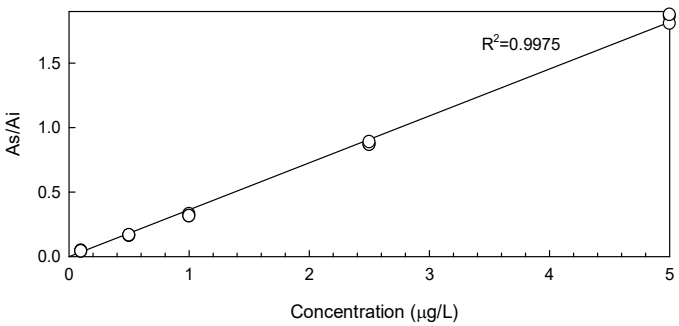
Boscalid



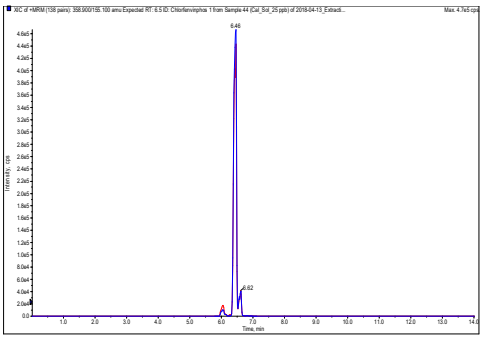


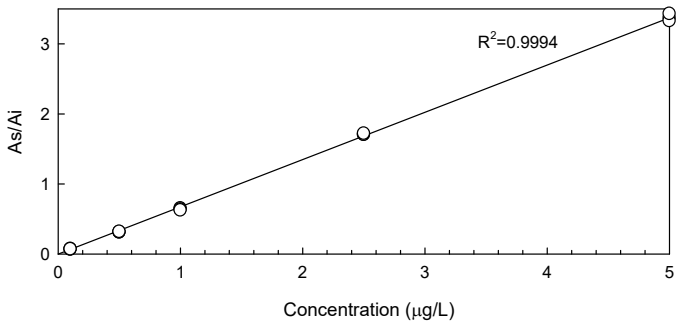
Carbendazim



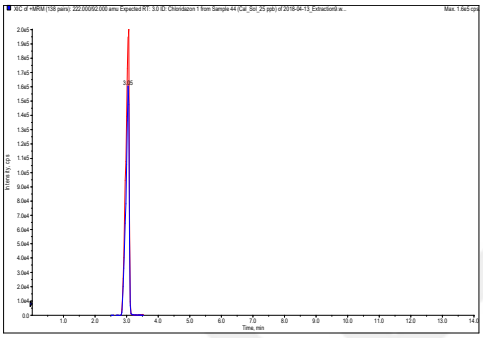


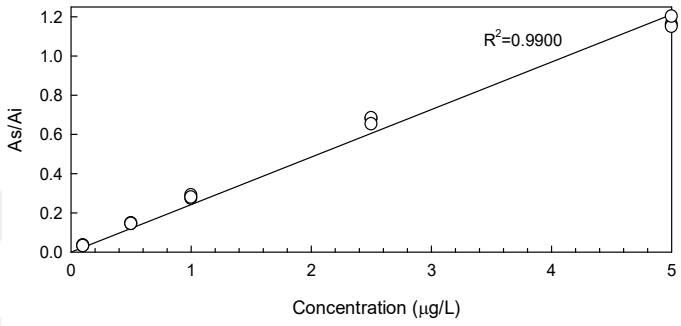
Chlorfenvinphos



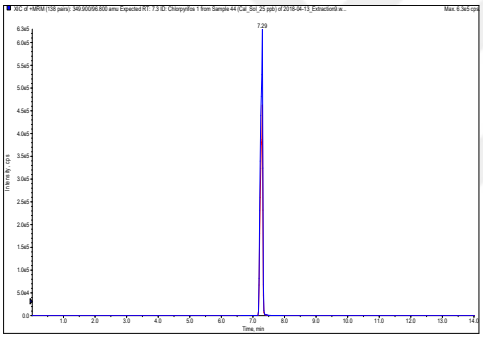


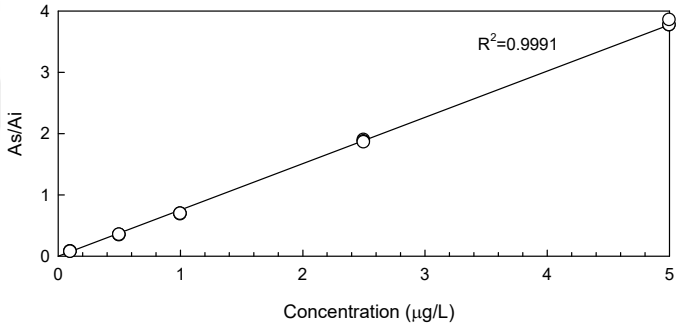
Chloridazon



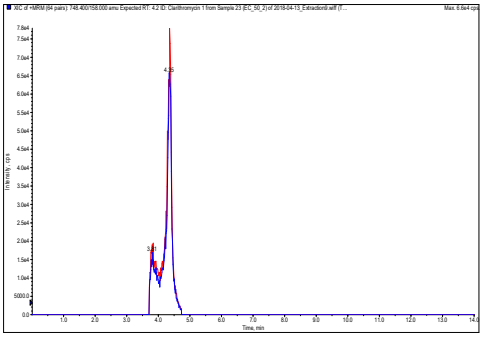


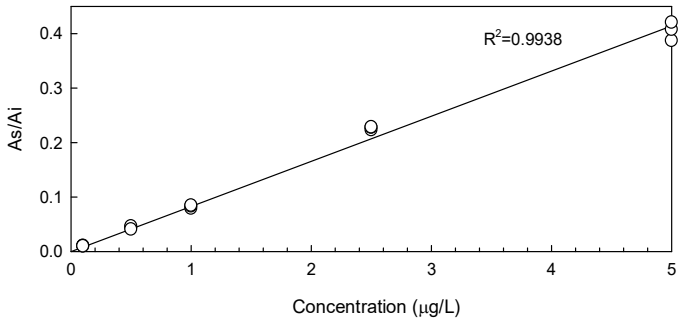
Chlorpyrifos



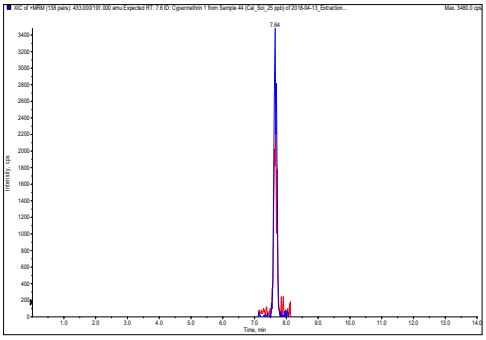


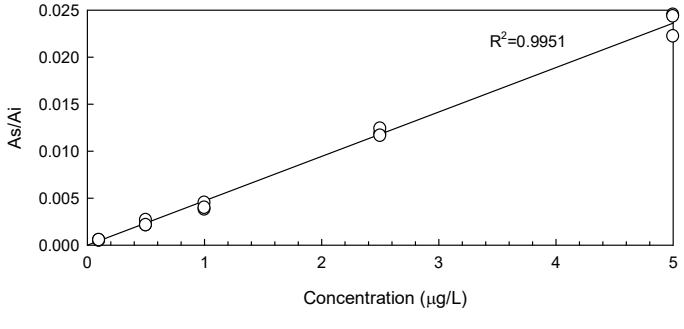
Clarithromycin



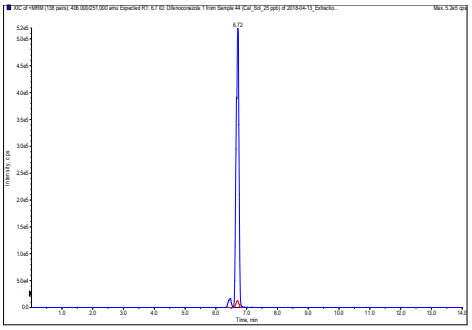


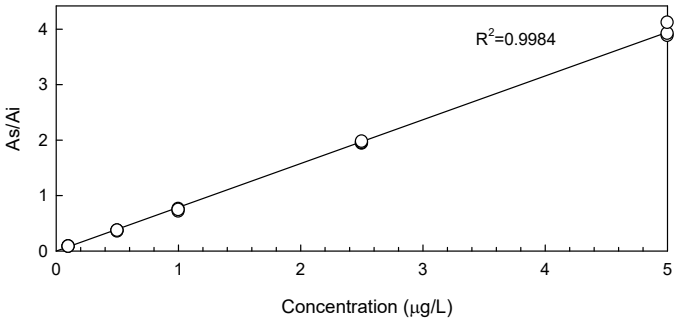
Cypermethrin



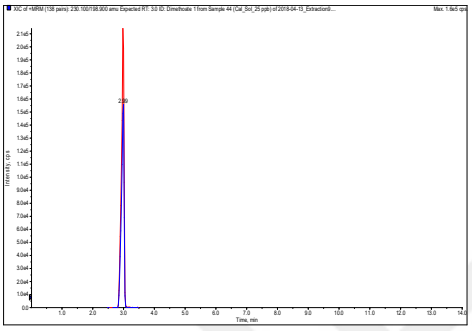


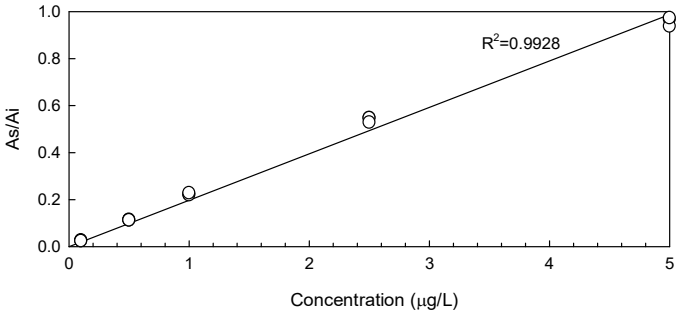
Difenoconazole



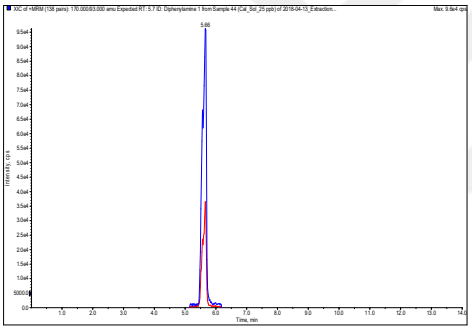


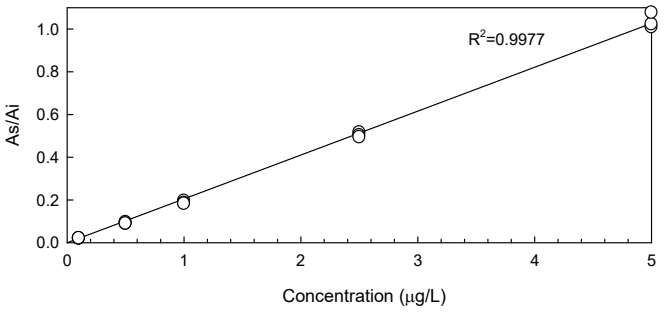
Dimethoate



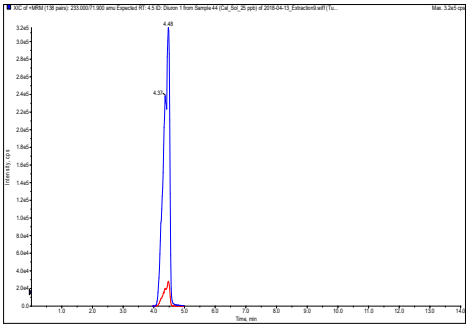


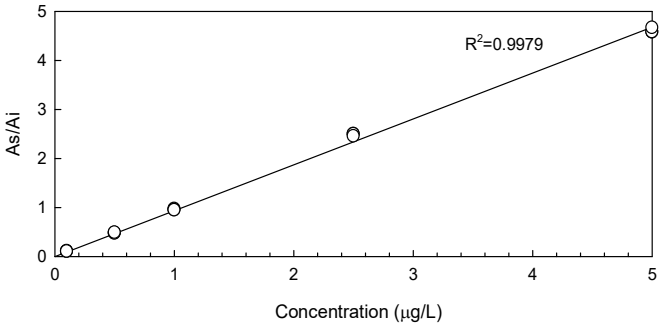
Diphenylamine



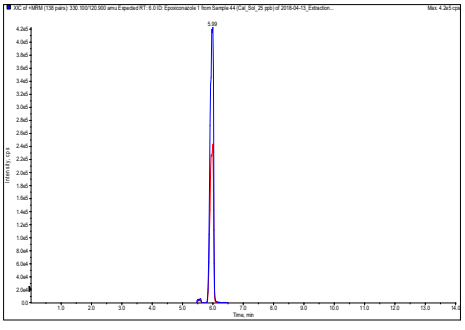


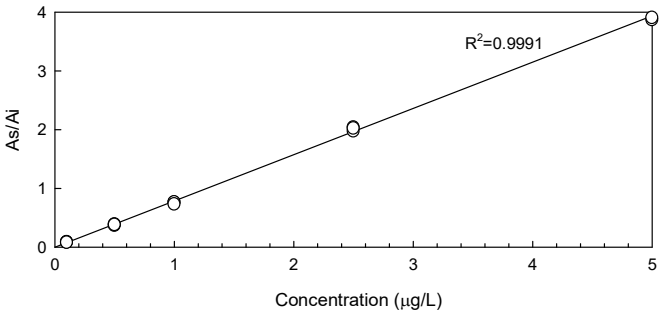
Diuron



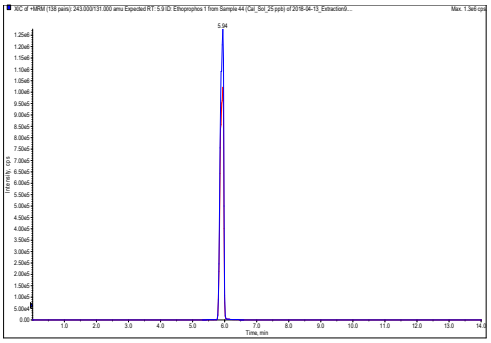


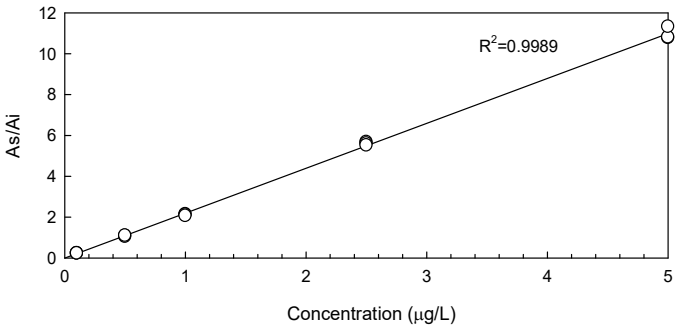
Epoxiconazole



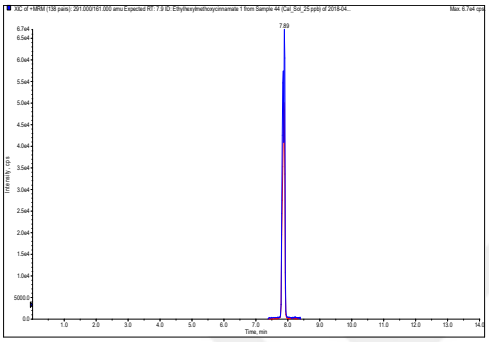


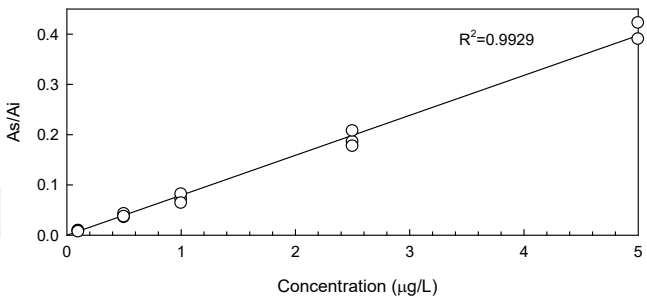
Ethoprophos



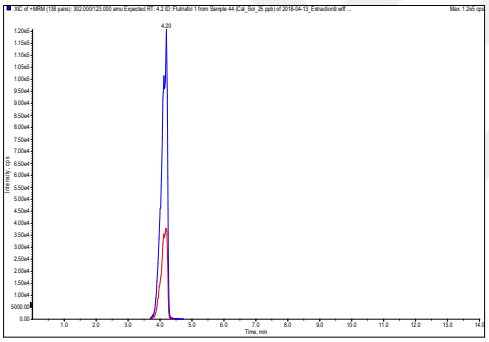


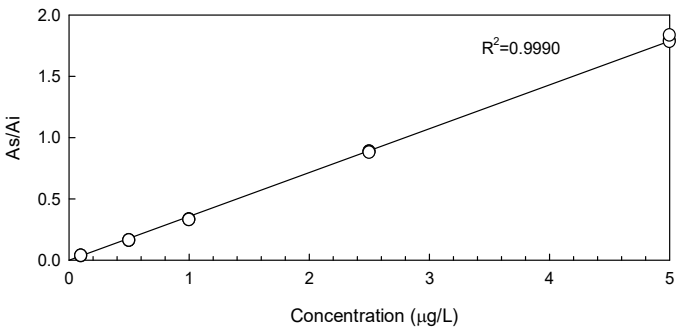
Ethylhexylmethoxycinnamate



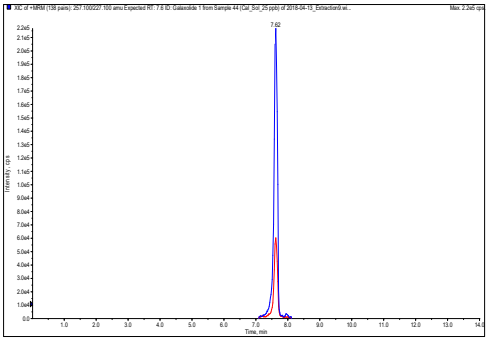


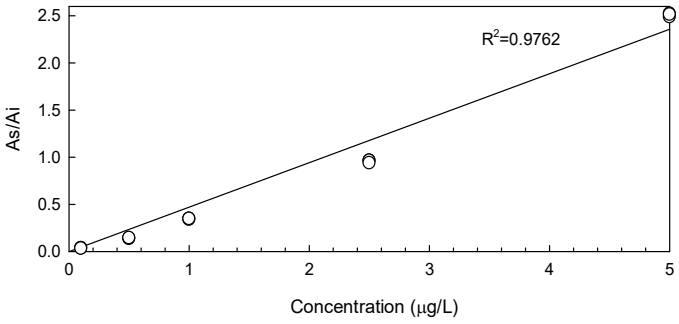
Flutriafol



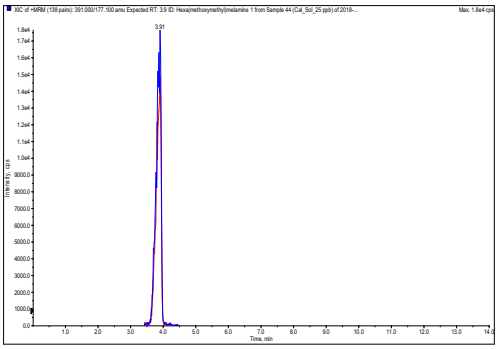


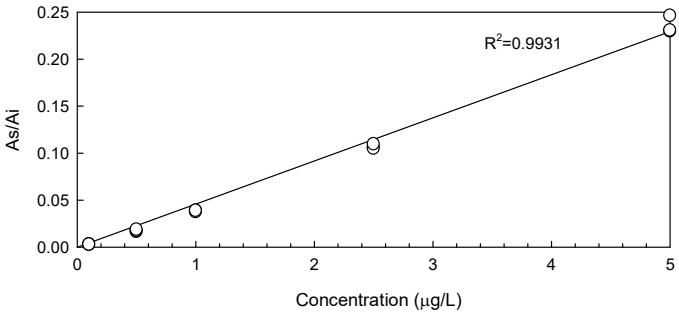
Galaxolide



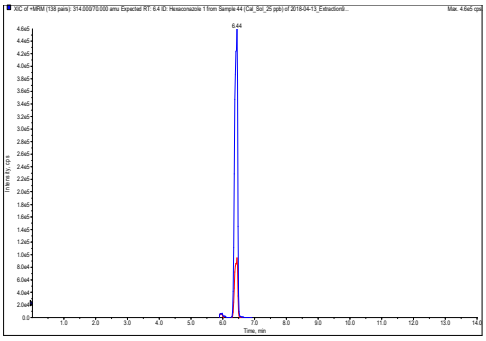


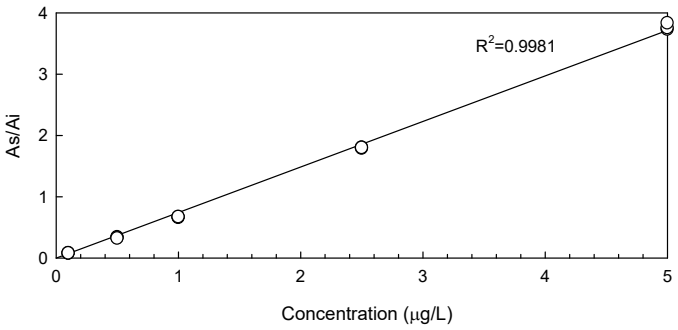
Hexa(methoxymethyl)melamine



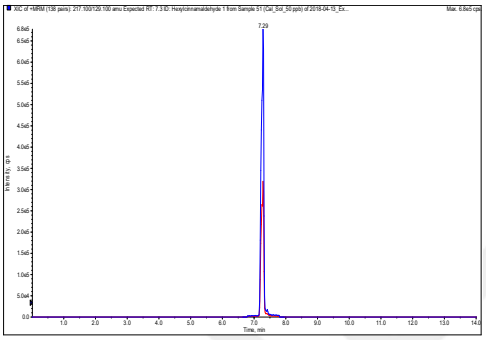


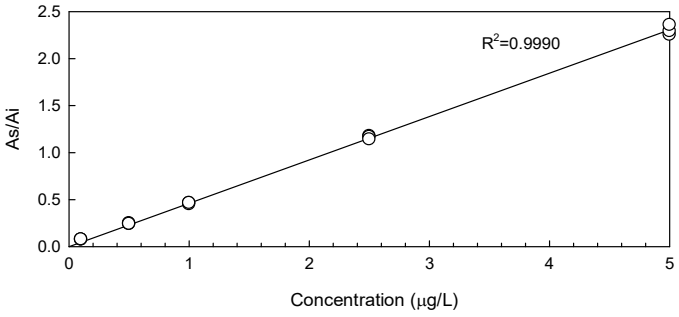
Hexaconazole



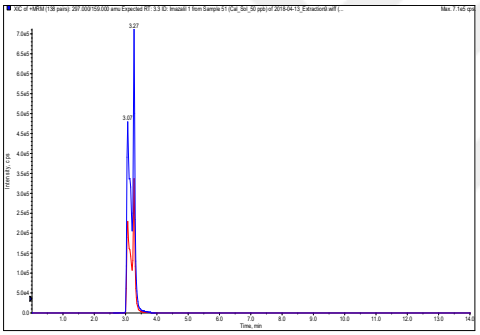


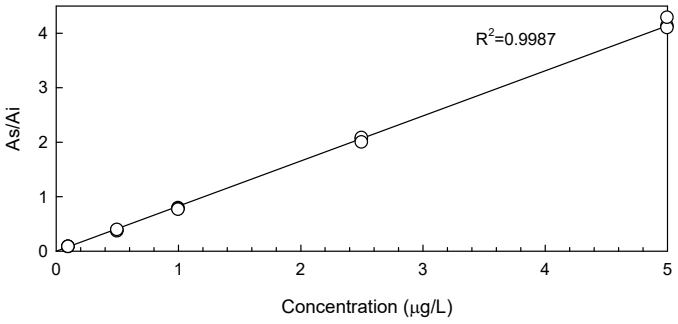
Hexylcinnamaldehyde



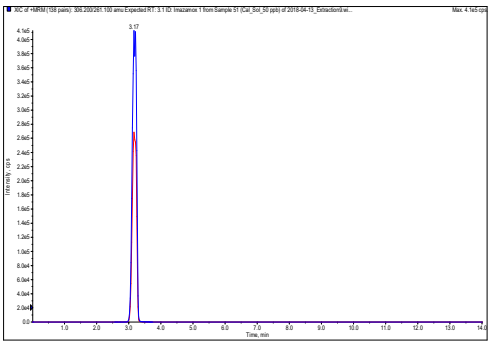


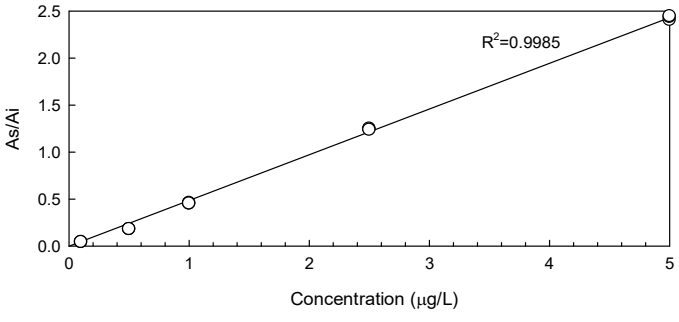
Imazalil



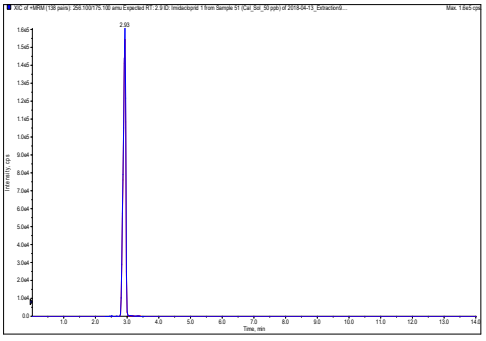


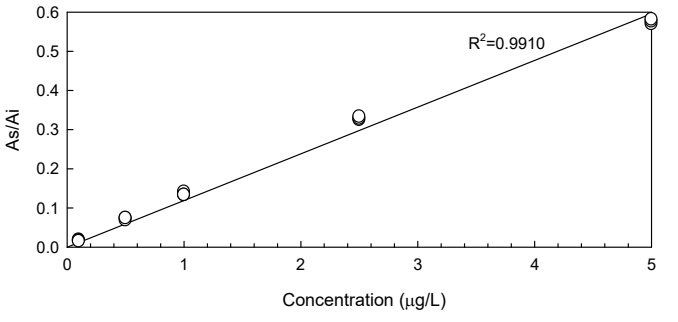
Imazamox



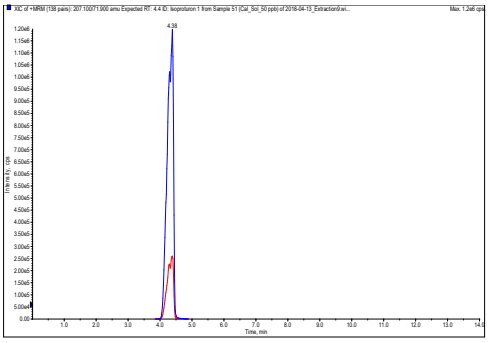


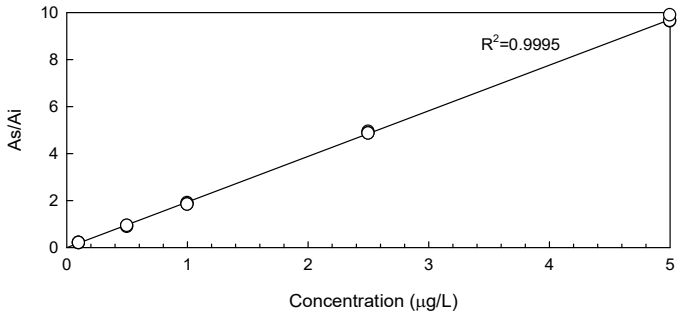
Imidacloprid



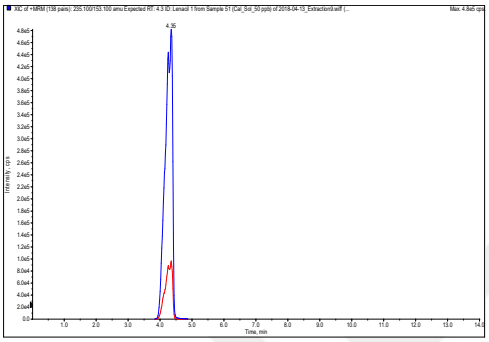


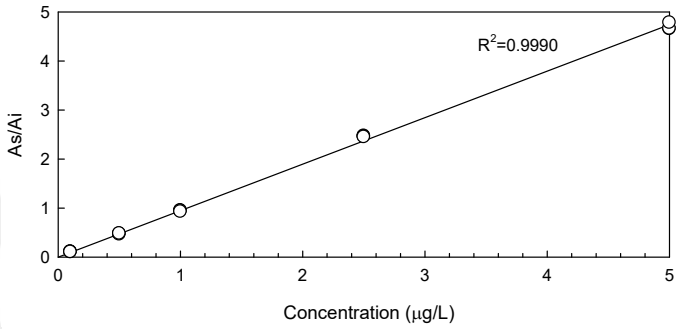
Isoproturon



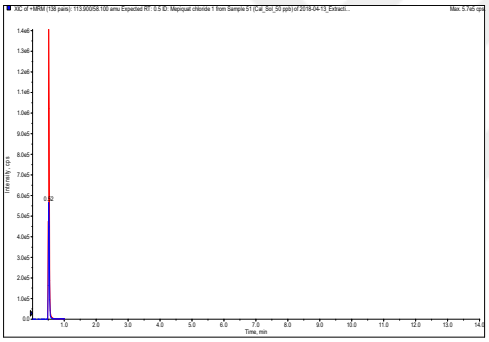


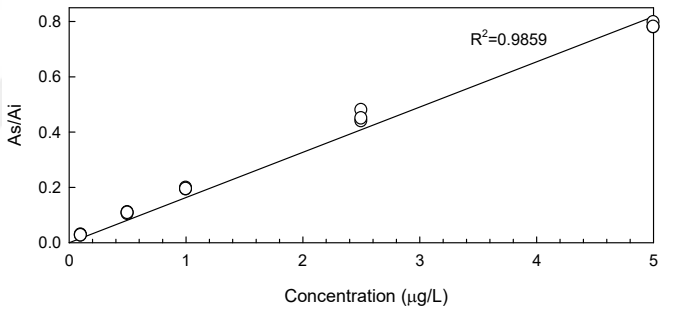
Lenacil



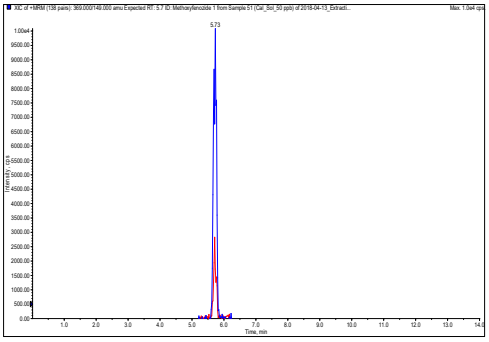


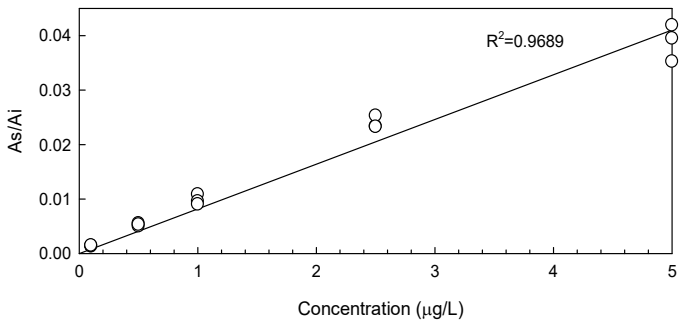
Mepiquat chloride



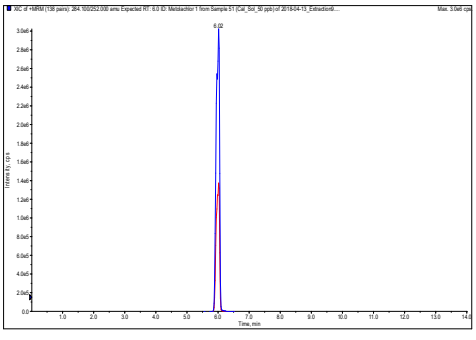


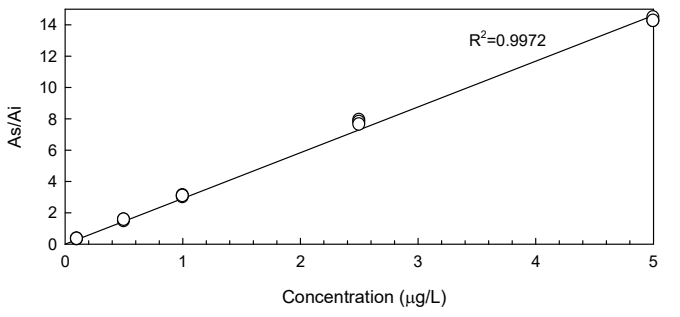
Methoxyfenozide



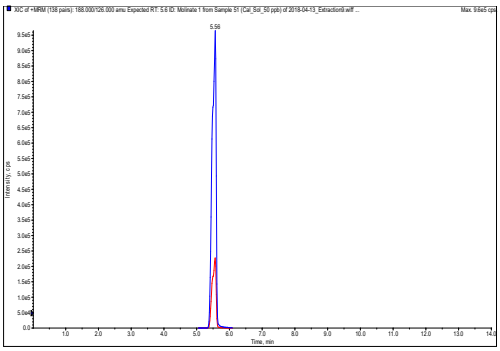


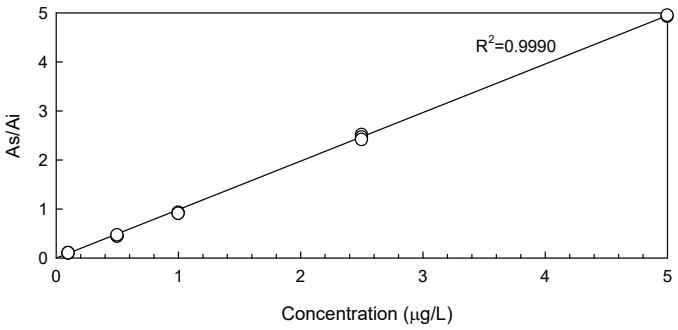
Metolachlor



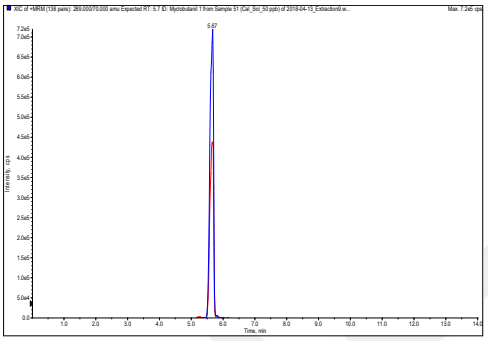


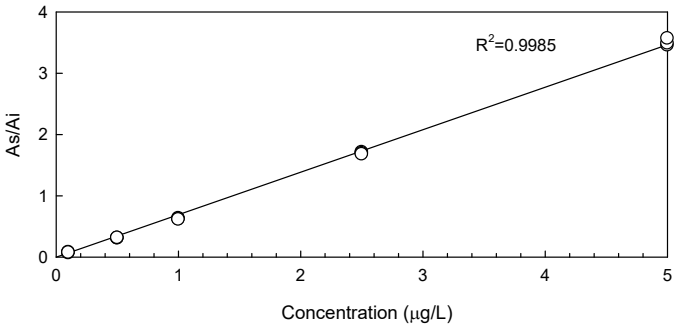
Molinate



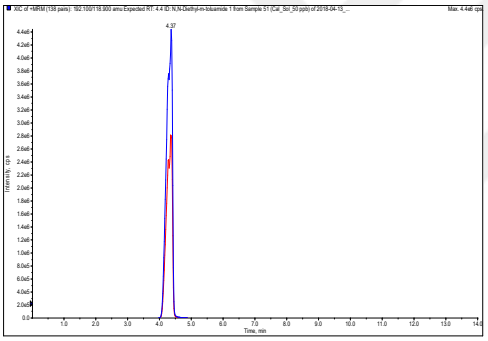


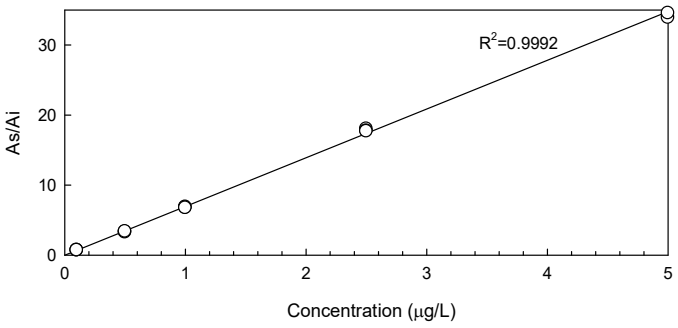
Myclobutanil



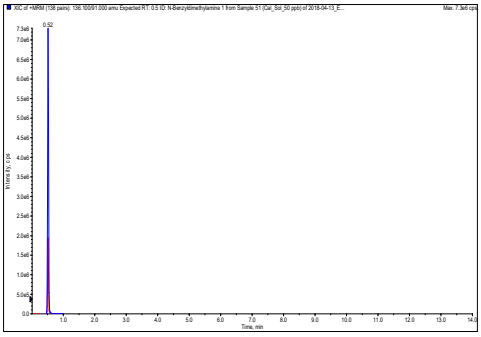


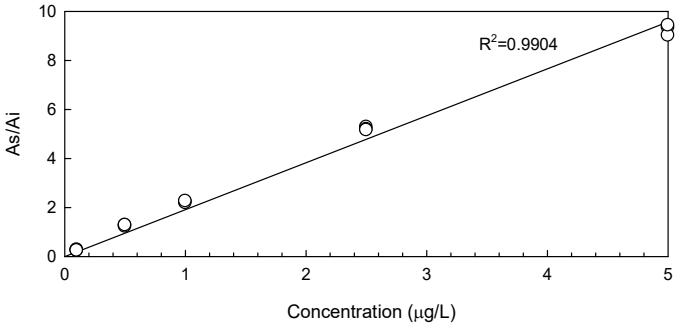
N,N-Diethyl-m-toluamide



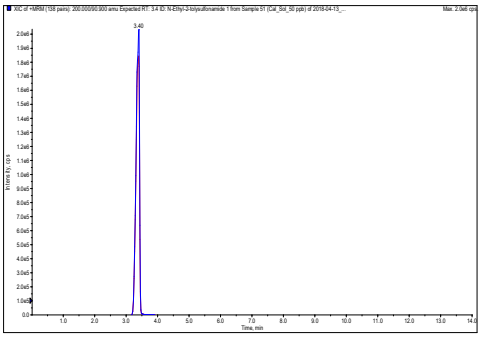


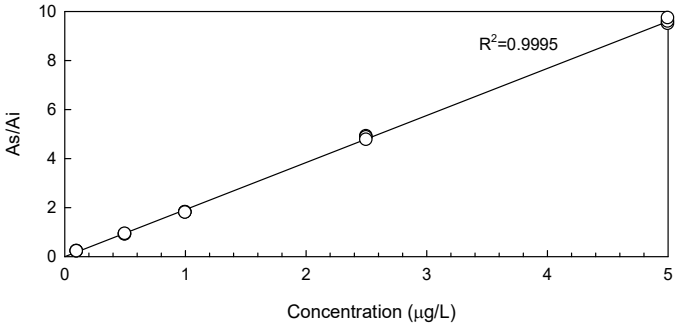
N-Benzyldimethylamine



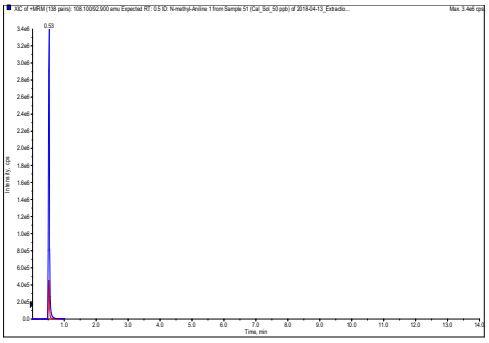


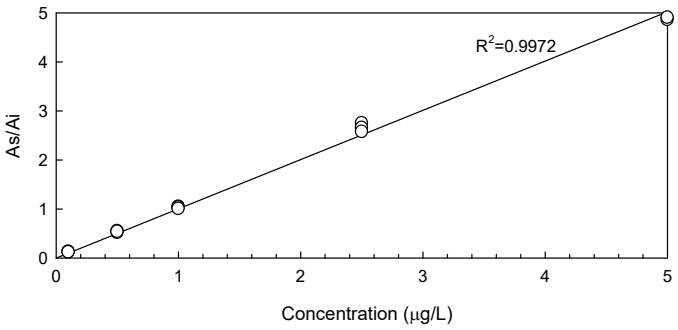
N-Ethyl-2-tolysulfonamide



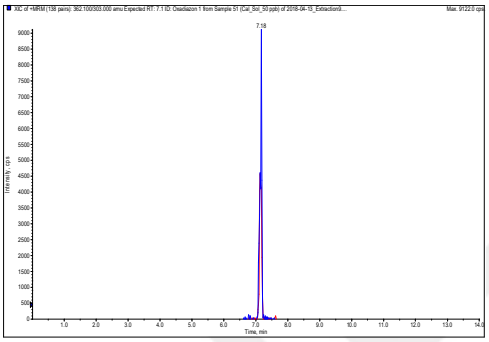


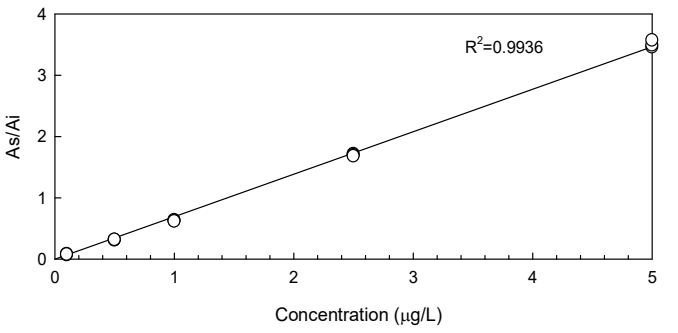
N-methyl-Aniline



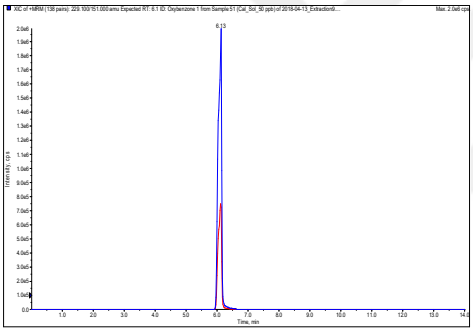


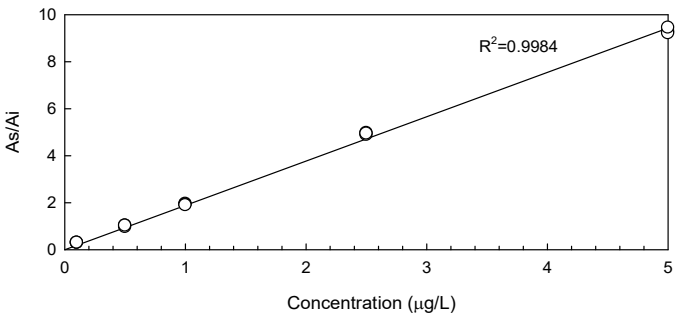
Oxadiazon



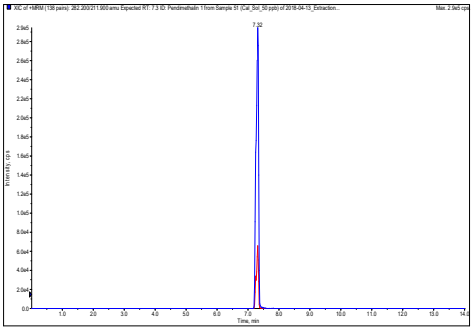


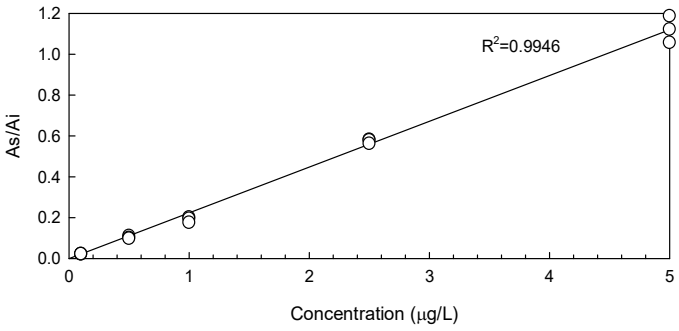
Oxybenzone



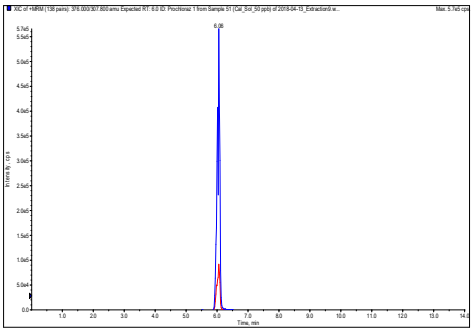


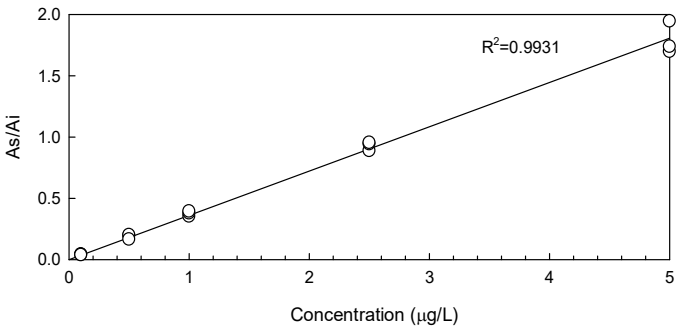
Pendimethalin



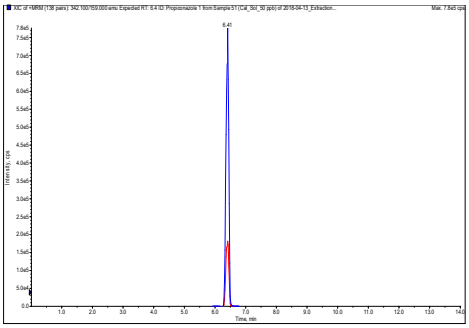


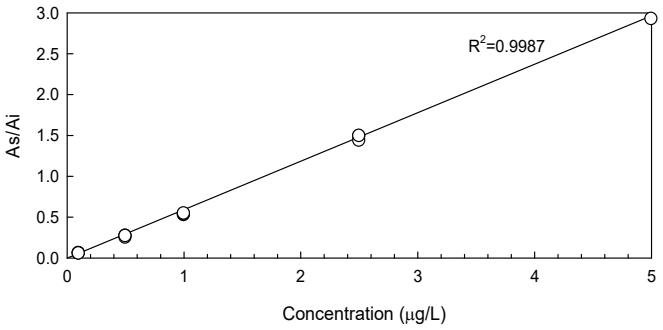
Prochloraz



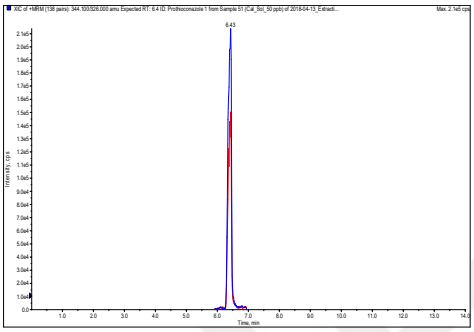


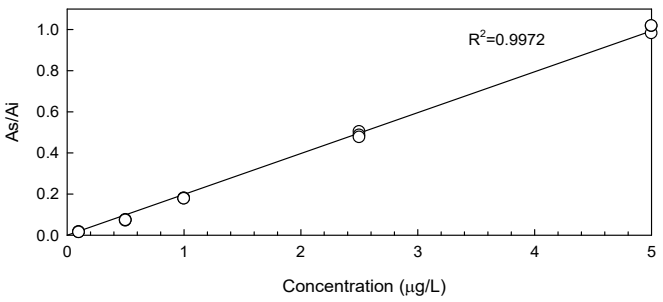
Propiconazole



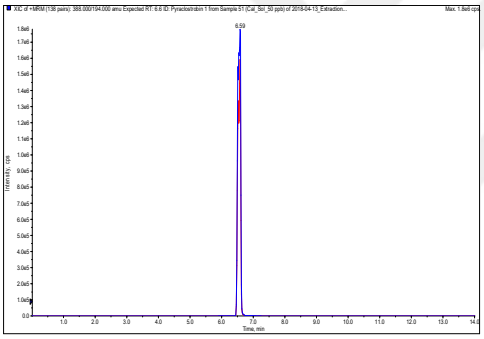


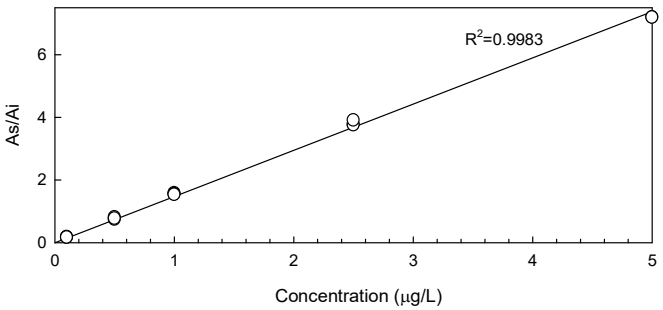
Prothioconazole



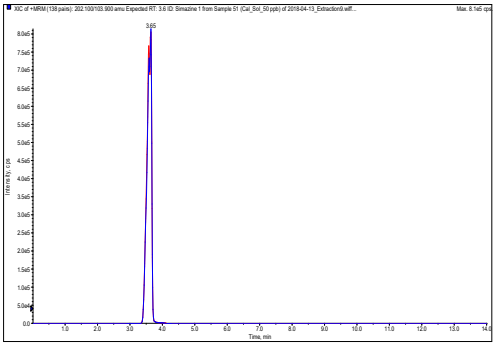


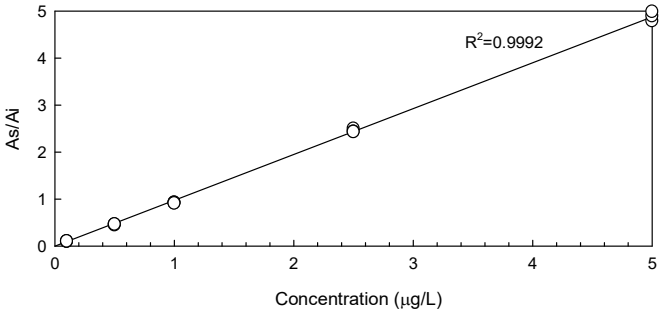
Pyraclostrobin



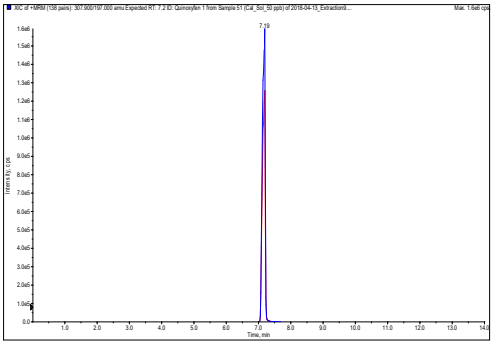


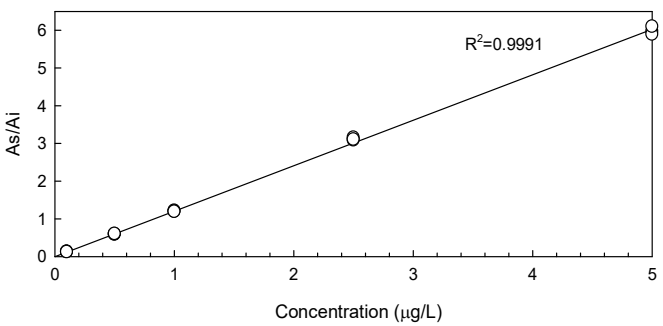
Simazine



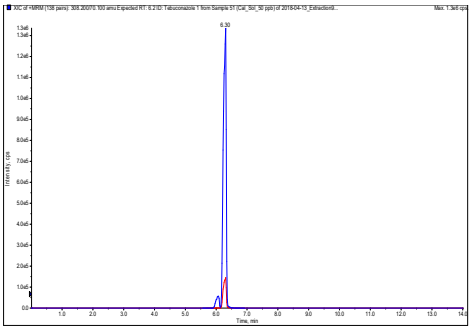


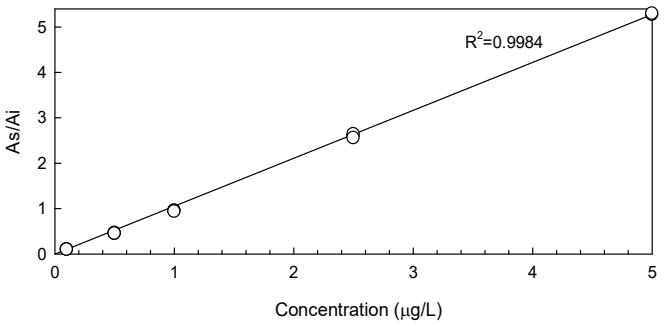
Quinoxyfen



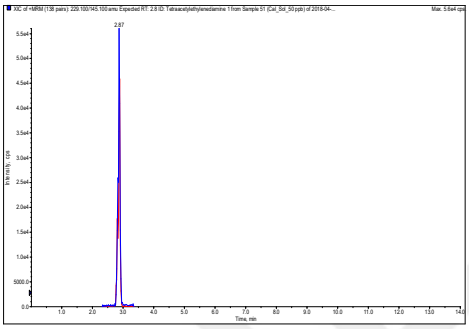


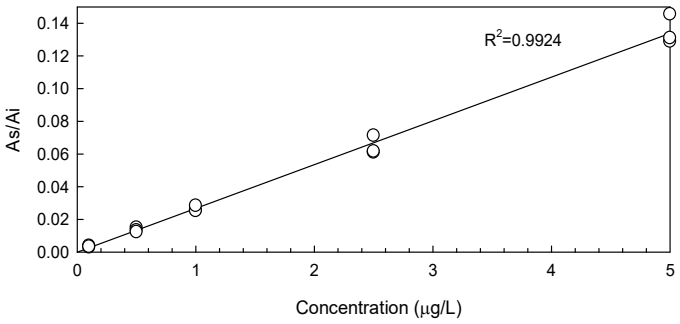
Tebuconazole



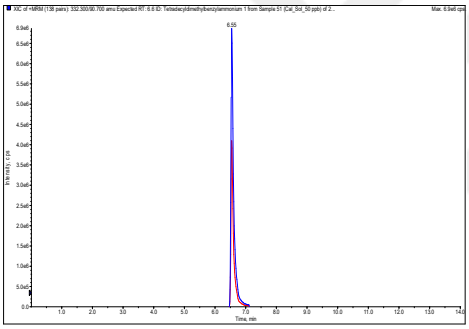


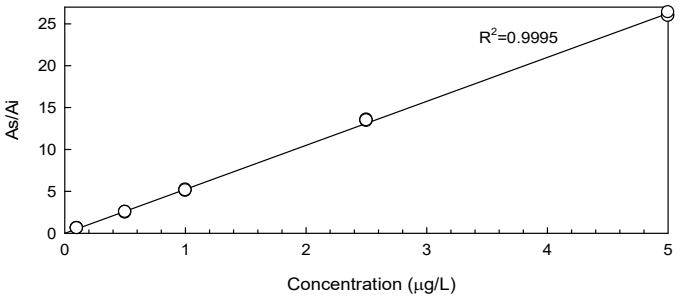
Tetraacetylethylenediamine



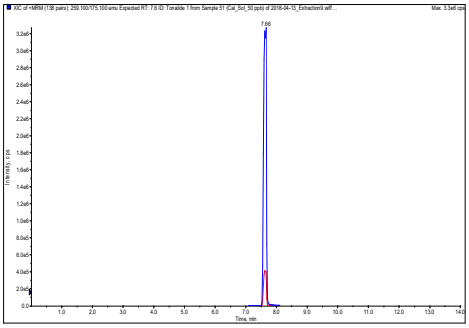


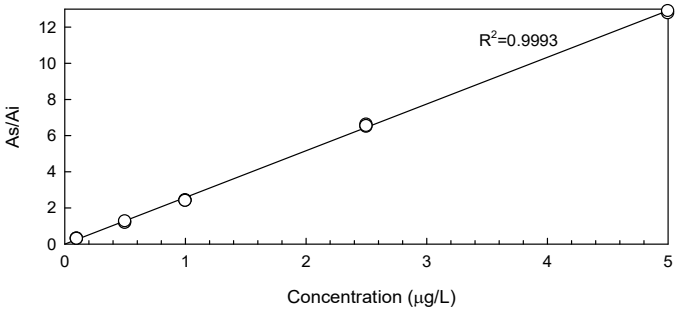
Benzyldimethyltetradecylammonium



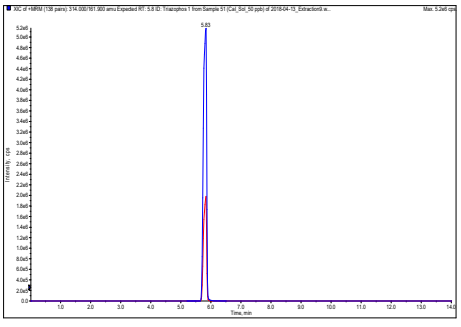


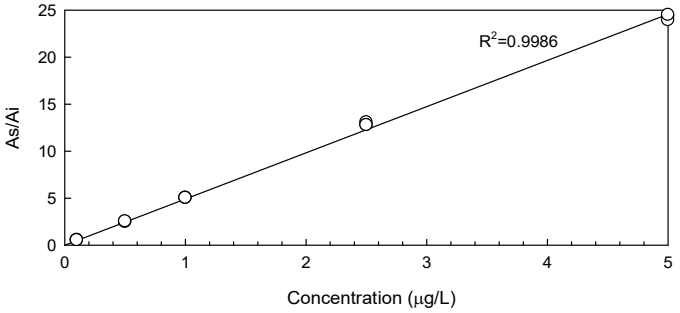
Tonalide



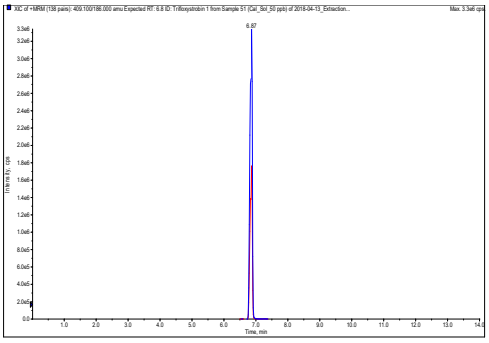


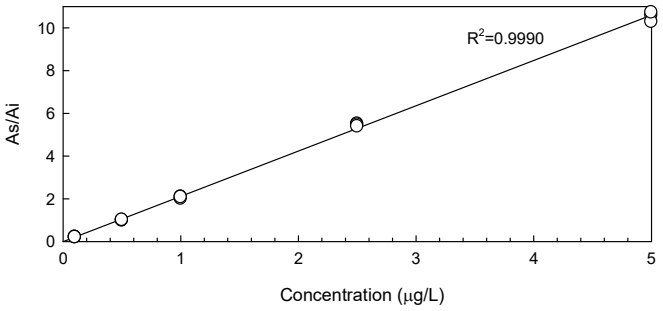
Triazophos



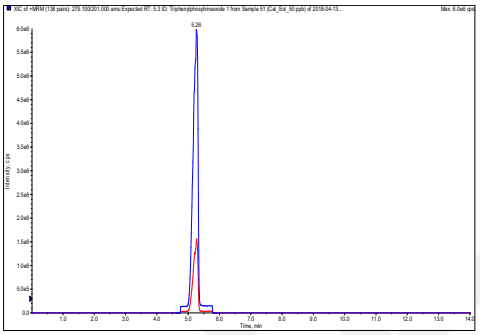


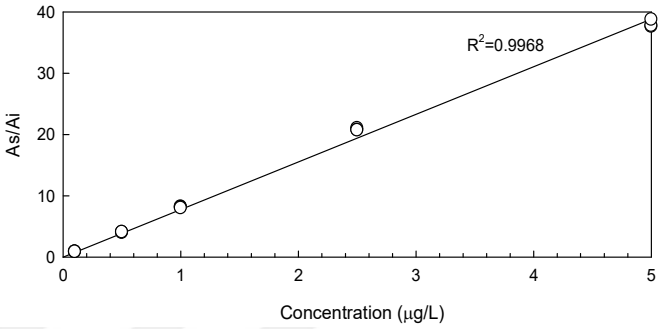
Trifloxystrobin



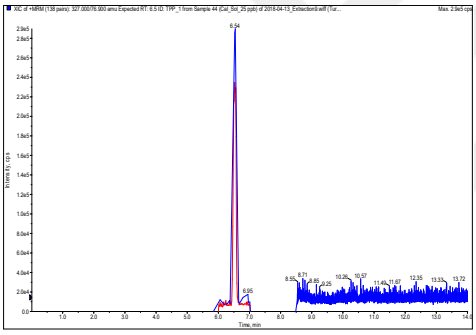


Triphenylphosphineoxide





Triphenylphosphate (Internal Standard)



APPENDIX B: THE SALES RATES AND FIELD OF APPLICATION OF SELECTED PESTICIDES IN EDIRNE AND KIRKLARELI

Table B1. The sales rates and field of application of active compounds for pest control in Edirne and Kırklareli in 2016.

Pesticide	Amount of sale (kg)	Field of application (da)
Edirne		
Imidacloprid	19254	962700
Prochloraz+Fluquinconazole	28761	287610
Pyraclostrobin+Boscalid	11360	284000
Prochloraz	20062	200620
Oxadiazon	28471	189806
Imazamox	18077	144616
Epoxiconazole+Pyraclostrobin	12862	64310
Epoxiconazole+Metrafenone+Fenpropimorph	8892	59280
Pyraclostrobin	3416	56933
Myclobutanil+Quinoxifen	6710	53680
Epoxiconazole+Fenpropimorph	5299	52990
Pyraclostrobin+Metiram	10315	51575
Imidacloprid+Beta Cyfluthrin	1012	50600
Prothioconazole+Spiroxamine	4994	49940
Prochloraz+Tebuconazole	7298	48653
Acetamiprid	1420	47351
Poxiconazole+Prochloraz	8691	43455
Tebuconazole+Azoxystrobin	5420.4	36136
Difenoconazole+Isopyrazam	3457	34570
Azoxystrobin	2349	31320
Epoxiconazole+Carbendazim	2774	27740
Molinate	11737	23474
Boscalid	2625.9	21882
Prochloraz+Triticonazole	3908	19540
Aclonifen	3009	15049
Cypermethrin	434.4	14480
Prothioconazole+Trifloxystrobin	1435	14350
Propiconazole+Difenoconazole	550	13750
Tebuconazole	1950	13000
Carbendazim	909.6	12128
Prochloraz+Propiconazole	1500	12000
Pendimethalin	5483	10966
Trifloxystrobin	203.9	10195
Tebuconazole+Fluopyram	309.8	8851

Table B1. Continued.

Pesticide	Amount of sale (kg)	Field of application (da)
Thiophanate Methyl+Epoxiconazole	448	7466
Propiconazole+Azoxystrobin	835	4175
Epoxiconazole	410.1	4101
Epoxiconazole+Thiophanete-Methyl	140	2333
Propiconazole+Azoxystrobin+Cyproconazole	213	2130
Chloridazon+Triallate	1626	1626
Prothioconazole+Tebuconazole	76	1520
Kırklareli		
Imazamox	71705	573640
Azoxystrobin	16326	217689
Pyraclostrobin	11000	183333
Epoxiconazole+Fenpropimorph	17200	172000
Tebuconazole	22085	147233
Difenoconazole+Isopyrazam	11395	113950
Prothioconazole+Trifloxystrobin	8600	86000
Prothioconazole+Tebuconazole	1491	29820
Imidacloprid	215	10787
Tebuconazole+Fluopyram	168	4805
Chlorpyrifos Ethyl	1288	4294
Prochloraz	200	2000
Carbendazim	110	1468
Acetamiprid	32	1066
Molinate	500	1000
Propiconazole+Azoxystrobin	90	450