Occurrence and distribution of organochlorinated pesticides and flame retardants in Spain and China

Pablo Zapata Corella

2022 · PhD Thesis · Pablo Zapata Corella

Occurrence and distribution of organochlorine pesticides and flame retardants in Spain and China

Pablo Zapata Corella, 2022.

UNIVERSITAT DE BARCELONA

CSIC
Consejo Superior de Investigaciones Científicas

Occurrence and distribution of organochlorine pesticides and flame retardants in Spain and China

Pablo Zapata Corella

Tesis Doctoral. Química Analítica y Medio Ambiente

Occurrence and distribution of organochlorinated pesticides and flame retardants in Spain and China

A Theis submitted for the doctoral degree in Analytical Chemictry by:

Pablo Zapata Corella

Directores:

Silvia Lacorte Bruguera (IDAEA-CSIC)

Anna Rigol Parera (UB)

Xiaojun Luo (GIG-CAS)

Tutora:

Anna Rigol Parera (UB)

Dr. Silvia Lacorte Bruguera, Research Scientis in the Department of Environmental Chemistry of the Institute of Environmental Diagnosis and Water Research, Dr. Anna Rigol Parera, Professor in the Department of Chemical Engirneering and Analytical Chemistry of the University of Barcelona and Dr. Xiaojun Luo, Research Scientist in the State Key Laboratoy of Organic Geochemistry in the Guangzhou Institute of Geochemistry,

STATE THAT:

The current PhD report entitled "**Occurrence and distribution of organochlorinated pesticides and flame retardants in Spain and China**" has been elaborated under our supervision by **Pablo Zapata Corella** in the Department of Environmental Chemistry of the Institute of Environmental Diagnosis and Water Research and the State Key Laboratoy of Organic Geochemistry in the Guangzhou Institute of Geochemistry, and all the resultspresented in this manuscript are consequence of the research work of the hereby mentioned doctoral student.

And in order to make it certain, we sign the current certificate.

Barcelona, July of 2022

Dr. Silvia Lacorte Bruguera **Dr. Anna Rigol Parera** **Dr. Xiaojun Luo**

“When DDT arrived, within two
weeks, my results and safety data
supplied by Geigy proved that we
had found the panacea “

Kenneth Mellanby

Acknowledgements

First acknowledge needs to be for my directors, Silvia, Anna and prof. Luo. This Thesis would not be possible without them. I am so thankful for all their support, the knowledge they have share with me and for making possible the Chinese interchange, which were the two longest and most adventurous years of my life so far.

Un agradecimiento especial a Silvia por las conversaciones que empiezan con un problema de laboratorio y terminan con todo tipo de curiosidades y anécdotas sobre el mundo de la investigación en química ambiental. Posiblemente es lo que mas he disfrutado de este camino que ahora termina. Muchas gracias.

Mi segundo agradecimiento debe se para mi hermana, Celia Zapata, que lleva conmigo desde el primer momento de mi vida y me ha demostrado en numerosas ocasiones que siempre puedo contar con ella. Esta etapa final no ha sido una excepción.

Gran agradecimiento también a toda la gente con la que he compartido laboratorio en Barcelona, desde estudiantes de prácticas a doctorandos que “ya estaban ahí cuando llegue”. Pero sin duda, entre estas personas hay dos que merecen una mención especial por el cariño que les tengo: Miguel Velazquez y Rubén Ballesteros. Gracias por todo, por la buena relación desde el primer momento, por hacer de válvula de escape para estrés de los momentos en que nada sale y por compartir las alegrías y satIsfaciones que producen los aciertos, por todas las risas y buenos momentos que hemos tenido tato dento como fuera del laboratorio. No puedo mas que desearos lo mejor en todos los aspectos de vuestra vida.

Gracias a Joel Fabregat y a Pere Colomer por compartir la experiencia en China y el apoyo mutuo que allí desarrollamos. Gracias Pere por abrir camino en la jungla tropical y monzónica del sur de China, y gracias Joel por todo; Ha sido un gran placer caminar en paralelo todos estos años.

I also need to acknowledge all the good people I found in GIG. Thanks to Mahadi for the good conversations in the balcony of the sixth floor even with your always pessimistic point o view. Thanks for Dilanka and Kaimook for extra spicy and exotic food they cook. I built some spicy tolerance with Chinese food, but yours were often too much for me. Thanks to my research group partners in China. Thanks to Yin-e Liu for her help providing everything I need in the laboratory, to Zihe Ren for the sampling trips, this Thesis would not be possible without all your help. Special thanks to the “language interchange” group we made, mostly spurred by Chengyou Zhu. I consider you all my Chinese friends. Thanks to Tao Wang, Lin Tao, Zongrui Li and Yu Liu. Thanks to all the basketball people in GIG. Special thanks to Xueyin Niu aka Yan for being my Chinese protector and showing me a different point of view nearly about everything. You have been my inspiration and I will always keep a good memory from you.

Gracias también a toda la comunidad española en Guangzhou. Al “alcalde” Fausto, a Unai, Ignasi, Irene y “Evani”. A todos los becarios del ICEX y a los demás participantes españoles del SEW-REAP, Olga, Bet, Clara y Eva, pero en especial a Purificación Melgarejo, la Puri, simplemente por ser como eres.

Gracias también a todos los viejos amigos de Madrid, no os puedo nombrar a todos, no terminaría, y a toda la nueva gente buena que he conocido en Barcelona. Soys parte de mi y por tanto parte de esta tesis.

Por último, gracias a mi padre y a mi madre por todo el apoyo incondicional que siempre he tenido por vuestra parte. Por los consejos, advertencias y discrepancias que me han hecho crecer y ser como soy. Os lo debo todo.

Abstract

The present Thesis is focused on the determination of the distribution and impact of legacy persistent organic pollutants (POPs) and organophosphate esters (OPEs) in environmentally protected areas from Spain and China and in an e-waste site form China.

Environmental pollution have received increased interest from the scientific community since the second half of the 20th century and several legal frameworks and governmental administrations have been developed to regulate the production and use of hazardous substances that can have an impact for the environment and human health. In 2004, the Stockholm Convention on POPs came into force, urging signing countries to legislate and control the so called “dirty dozen”. Under the Stockholm Convention, monitoring programs started to be implemented and some matrices such as soils, air or biota were proposed to determine the geographical distribution and time-evolution of POPs. In this Thesis, the families of POPs analyzed include organochlorine pesticides (OCPs) used as pesticides and as industrial products, polychlorinated biphenyl (PCBs) used as industrial fluids for heat exchange or as dielectric fluids in bobbins or capacitors, and polybrominated diphenyl ethers (PBDEs) used as flame retardant. Despite the ban on the production and use of these substances, they are still detected in different environmental compartments, mostly the biotic compartment, soils and sediments and are a cause of concern due their bioaccumulation, biomagnifications and overall persistence properties. In addition, in this Thesis organophosphate esters (OPEs) have been also studied. These compounds are used as flame retardants, as plasticizers and polymer additives and have been introduced into the market after PBDEs phase out, as less environmentally harmful substitutes. In this Thesis, the presence of these four families of pollutants has been evaluated in soil, sediments, and water from 2 contrasted areas in China with very different anthropogenic pressures: (i) in 4 National Parks and (ii) in an e-waste site affected by electronic dismanting activities. POPs have also been monitored in gull eggs of protected areas in Spain, which are used as bioindicators of chemical contamination. The work has been divided in three chapters briefly described down below.

In the first chapter, an instrumental method based on gas chromatography coupled with tandem mass spectrometry has been developed and validated for the detection and quantification of OCPs and PCBs in bird eggs. PBDEs were analyzed by gas chromatography coupled with mass spectrometry with negative chemical ionization. The presence of Stockholm Convention POPs were determined in protected areas from the Iberian peninsula through the use of eggs from two seagull species, namely yellow legged gull (*Larus michahelis*) and audouin gull (*Larus audouinii*), as bioindicators. Results showed high pollution burdens of PCBs ranging from 60 to more than 1000 ng/g ww with higher contribution of the higher chlorination degree congeners. The level of 4,4´-DDT ranged from 0.04 to 1.35 ng/g ww and was lower than its main metabolite, 4,4´-DDE, which ranged from 31.4 to 760 ng/g ww, which suggests a non-recent use of the technical formulation. Higher levels of DDTs were observed in audouin gulls than in yellow legged gulls from the same colony in the Ebro Delta and attributed to the strict fish based diet of the former contrarily to the opportunistic and scavenging behavior of the later. PBDEs were also frequently detected but at lower levels (3 to 20 ng/g ww) with predominance of BDE 47 and BDE 209. Other OCPs like chlordane, heptachlor epoxides, penta- and hexachlorobenzene and hexachlorobutadiene were widespread and quantified at levels lower than 10 ng/g ww. In the 9 colonies studied, a different contamination pattern was observed according to the different activities carried out in each study area, and it was observed that Mediterranean colonies showed a higher concentration of POPs than Atlantic areas.

The second chapter is centered in the occurrence of OPEs, PBDEs, PCBs and OCPs in soils and sediments from 4 environmentally protected areas of South China with different protection degrees, namely, Pudacuo and Danxia Shan National Parks, Gaoqiao Mangrove National Nature Reserve and Heshan National Field Research Station of Forest Ecosystem. Found pollution levels were in general high for OPEs, of which TPhP was often the main contributor, followed by PBDEs, with dominance of BDE 209, PCBs and finally OCPs. α-HCH, however, was widespread and frequently detected at low levels (mean of 0.5 ng/g dw). Among study National Parks, Heshan holds the lowest protection category and showed the highest pollution levels, probably related with garbage burning as a point source and the presence of furniture production industry in the county. Pudacuo is a high mountain

National park located at the beginning of the Tibetan plateau and the most remote and isolated area among studied ones. However it contained the highest levels of OPEs in sediments (median of 340 ng/g dw). A forest soil sample from Pudacuo was the only sample free of pollutants except for the ubiquitous α-HCH. Gaoqiao mangrove is an ecosystem influenced by the Daba river runoff and the ocean tides of Beibu gulf. The mangrove sediments have accumulated legacy pollutants and showed the highest levels of PCBs (median of 0.35 ng/g dw) and OCPs (median of 3.3 ng/g dw). PBDEs were also detected at median levels of 48 and 54 ng/g dw in soils and sediments respectively. In Danxia Shan National Park had the lowest pollution impact considering median levels of Σpollutants, sediments contained PBDEs (median concentration of 58 ng/g dw), attributed most probably to the different recreational and touristic activities taking place in the area.

The third chapter reflects one of the main modern environmental problems taking place in developing countries: the e-waste disassembling and recycling in non-formal workshops and associated uncontrolled residues discharged to the environment. Baihe village is located in the Qingyuan county (South China) which has been identified as one of the three biggest e-waste areas in China. Noneconomically profitable plastic residues have been dumped during years in a small pond emerging as a plastic lump in the opposite side. Soils, sediments and free ranged hen eggs samples were collected and analyzed for OPEs, PBDEs, PCBs and OCPs and water samples were analyzed for the more polar pollutants, OPEs. Results showed that the pollutants contained in the plastic lump migrate to the pond water and sediment proportionally to their hydrophilic/lipophilic characteristics (evaluated as K_{ow}) but they are also present in the surroundings. The highest levels were found for OPEs, with more than 100 µg/g dw in sediment from the pond and up to 76 µg/g dw in soils followed by PBDEs (39 µg/g dw in the pond sediment and from 0.08 to 4.4 µg/g dw in soils) and PCBs (20 µg/g dw in sediments from the polluted pond and up to 75 µg/g dw in soils). OCPs were detected al much lower levels. Total OPEs levels in water samples from the pond and nearby freshwater bodies ranged from 0.36 to 1.6 µg/L. Hen eggs were also polluted and estimated daily intakes of PCBs due to their consumption represented health risks for adults and children while PBDEs showed risks just for children.

Overall, this Thesis demonstrates that POPs, despite are not used, are a main cause of environmental pollution and human health associated problems. These compounds are present at concentrations at the μg/g level in soils and sediments of an e-waste site in China and concentrations 1000 times lower are still found in National Parks, which are the areas of the highest protection status. This means that POPs are widespread and that past production and use has affected remote areas theoretically not impacted by humans. For the emerging OPEs, the high concentrations detected in e-waste sites and also in National Parks can lead to future serious environmental and health impacts. Therefore, monitoring strategies to determine the levels of POPs and OPEs are important to define contamination status of a given area and contribute to the implementation of conservation measures against chemical pollution.

Index

List of figures

List of tables

List of acronyms and abbreviations

ABS- Acrylonitrile-butadiene-styrene
Ace- Acetone
ACN- Acetonitrile
AhR- Aryl hydrocarbon receptor
BAF- Bioaccumulation factor
BFR- Brominated flame retardants
BMF- Biomagnification factor
DBP- p,p´-dichlorodibenzophenone
DCM- Dichloromethane
ECD- Electron capture detector
EDI- Estimated daily intake
EEE- Electrical or electronic equipments
EI- Electron ionization
EIC- Extracted ion chromatogram
EtOH- Ethanol
FAO- Food and Agriculture Organization of the United Nations
GABA- γ-aminobutyric acid
GC- Gas chromatography
HCB- Hexachlorobenzene
HCBu- Hexachlorobutadiene
HCH- Hexachlorocyclohexane
Hex- Hexane
HIPS- High impact polystyrene
HLB- Hidrophilic lipophilic balance
IUCN- International Union for the Conservation of Nature
MDL-Method detection limit
MeOH- Methanol
MOE- Margin of exposure
MQL- Method quantification limit
MRM- Multiple reaction monitoring

MS- Mass spectrometry

MSMS- Mass spectrometry coupled to mass spectrometry (tandem MS)

NCI- Negative chemical ionization

OCP- Organochlorine pesticide

OPFR- Organophophosphourus flame retardant

OPE- Organophosphoshate ester

PA- Polyamide

PAH- Polycyclic aromatic hydrocarbon

PBDE- Polybromodiphenyl ether

PCB- Polychlorobiphenyl

PCDD- Polychlorinated dibenzo-*p*-dioxins

PCDF- Polychlorinated dibenzofurans

PE- Polyethylene

PFAS- Perfluoroalkyl substances

PLE- Pressurized liquid extraction

POP- Persistent Organic Pollutant

PP- Polypropylene

PPCP- Pharmaceutical and personal care products

PS- Polystyrene

PVC- Polyvinyl chloride

SIM- Single ion monitoring

SPA- Special Protection Area for Birds

SPE- Solid phase extraction

TCDD- 2,3,7,8-Tetrachlorodibenzo-p-dioxin

TIC- Total ion chromatogram

TMF- Trophic magnification factor

TOC-Total organic carbon

TOF- Time of flight

UAE- Ultrasound assisted extraction

UNEP- United Nations Environment Programme

WHO- World Health Organization

1. Introduction

Anthropogenic chemical pollution is related to the discharge of synthetic compounds from different industrial, urban, agricultural and domestic activities to the environment. In 1962, Rachel Carson published the book entitled “Silent Spring” that has been considered by many as the beginning of the scientific discussion on environmental pollution (Carson, 1962). In this book, Carson describes the undesired effects of pesticides and biocides in the ecosystems from a multidisciplinary point of view, with special focus on 4,4´-dichlorodiphenyl trichloroethane, more commonly known as DDT, and its impact on birds. Since the second half of the 20th century, the hazards that synthetic chemicals can produce to the environmental and human health have been a cause of concern. Environmental incidents with huge impacts on human health, the development of analytical techniques able to achieve very low detection limits and, in general, new knowledge on the identification of new contaminants and associated toxicity have led to regulation and legal policies for many potentially hazardous substances. Figure 1.1 shows a timeline with some historical events and milestones regarding environmental pollution including some relevant environmental incidents, the creation of governmental environmental protection commission in China, which was the embryo of the Ministry of Ecology and Environment, and the Environmental Protection Agency in USA as well as some local and international legal policies on different pollutants.

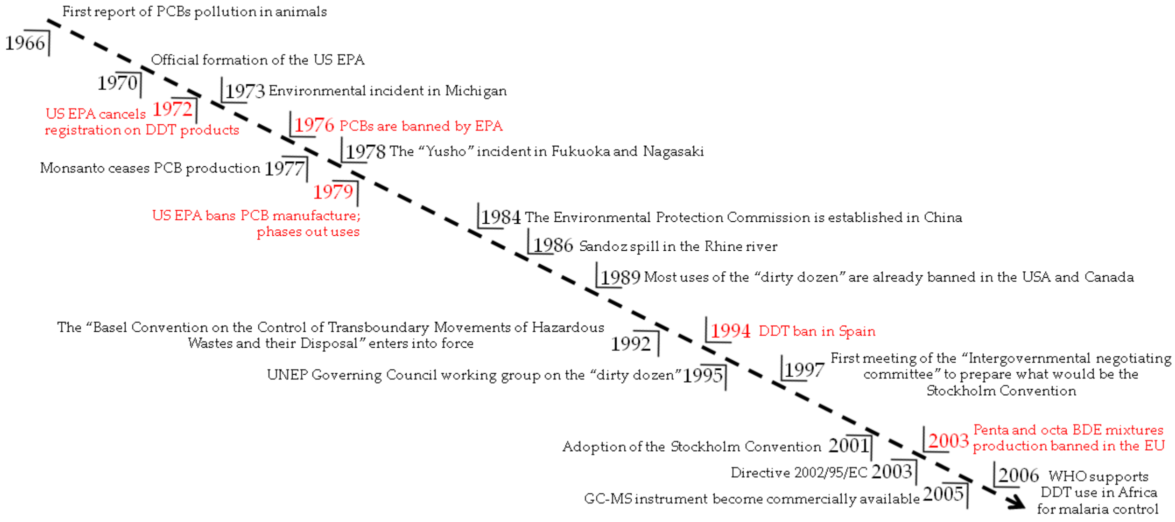


Figure 1.1. Historical events and milestones in environmental pollution.

Organic pollutants include legacy contaminants and emerging contaminants. Legacy contaminants include long lasting pollutants with supposedly no new environmental inputs mostly due to production and/or use banning policies. They are commonly known as persistent organic pollutants (POPs). The fate and impact of these pollutants has been widely studied as they are still detected in the environment due to their low degradation rates and accumulation properties. Emerging contaminants, on the other hand, are compounds which are nowadays in use for different purposes and, therefore, primary sources to the environment are still active. They are generally not regulated or regulatory standards are still being developed and are less studied than legacy ones. They include pharmaceuticals, drugs of abuse, personal-care compounds, in-use pesticides, perfluoroalkyl substances, plasticizers, flame retardants or chloroparaffins, among others (Richardson and Kimura, 2017). Knowledge gaps are usually related with uncertainties on their toxic properties, mostly in the chronic or long-term exposure both for humans or wildlife, their sources and environmental fate, including transport, accumulation or degradation (Gondi et al., 2022, Ahmad et al., 2022).

Depending on their properties, organic contaminants distribute among the different environmental compartments: air, water, soils and sediments and biota. Complex physical, chemical and biological processes determine the transport and fate of pollutants in the environment, and the most persistent compounds are accumulated in biota, while many emerging contaminants can be degraded (Jain et al., 2022). The more volatile ones can be transported through air, reaching cold and remote areas (Li et al., 2017). Their effects depend on the intrinsic toxic properties of each compound or their mixtures, but in general organic contaminants affect the endocrine system, are neurotoxic, affect the reproduction of organisms and moreover there is evidence that some compounds are bioactive and have chronic effects (Dishaw et al., 2014, Marlatt et al., 2021).

1.1. Target compounds

Compounds studied in the present PhD Thesis include legacy POPs and organophosphate esters (OPEs). The former have been selected based on their inclusion in the Stockholm Convention whereas OPEs have been studied as they are in use flame retardants used in replacement of polybrominated diphenyl ethers (PBDEs) but also because of their widespread use as plasticizers.

Despite POPs were used in the past, they are still widespread in the environment and their presence is associated to toxic impairment in many wildlife species. The Stockholm Convention is a legal binding instrument internationally developed to face the problems derived from the extensive use of POPs. As described in the main text of the Convention, “the objective of this Convention is to protect human health and the environment from persistent organic pollutants” (UNEP, 2019). It was firstly raised in 1995 by the Governing Council of the United Nations Environment Programme (UNEP), but it was not until 2001 when it was firstly signed in Stockholm and came into force in 2004. To date, there are 152 signatories of the Convention, including Spain and China, and 185 parties. Other countries such as Israel, Italy, Malaysia, USA, Haiti or Brunei Darussalam have not ratified nor implemented the Convention.

Initial steps were focused on the so-called dirty dozen, twelve compounds identified as POPs, which include pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, HCB, mirex and toxaphene), industrial chemicals (HCB and PCBs) and by-products (HCB, PCDD/PCDF and PCBs), but the list is permanently being revised and new substances have been added to the Convention or proposed by the parties to be listed as intended in article 8.

POPs are defined by the Stockholm Convention as “… organic chemical substances which possess a particular combination of physical and chemical properties such that, once released into the environment they fulfill 4 characteristics” (Stockholm Convention, 2004):

- Remain intact for exceptionally long periods of time (many years);
- Become widely distributed throughout the environment as a result of natural processes involving soil, water and, most notably, air;
- Accumulate in the living organisms including humans, and are found at higher concentrations in the food chain;
- Are toxic to both humans and wildlife.

Substances proposed with those characteristics are introduced in the list and ratified by the parties. Listed POPs are classified in three Annexes (table 1.1):

Table 1.1. Compounds listed in each annex of the Stockholm Convention (UNEP, 2019).

	Listed compounds
Annex A (Elimination)	Aldrin, Chlordane, Chlordecone, Decabromodiphenyl ether (commercial mixture, c-decaBDE), Dicofol, Dieldrin, Endrin, Heptachlor, Hexabromobiphenyl, Hexabromocyclododecane, Hexabromodiphenyl ether and Heptabromodiphenyl ether, Hexachlorobenzene (HCB), Hexachlorobutadiene, α-hexachlorocyclohexane, β-hexachlorocyclohexane, γ- hexachlorocyclohexane (Lindane), Mirex, Pentachlorobenzene, Pentachlorophenol and its salts and esters, Polychlorinated biphenyls (PCBs), Polychlorinated naphthalenes, Perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds, Short-chain chlorinated paraffins (SCCPs), Technical Endosulfan and its related isomers, Tetrabromodiphenyl ether and Pentabromodiphenyl ether, Toxaphene
Annex B (Restriction)	DDT, Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride
Annex C (Unintentional production)	Hexachlorobenzene (HCB), Hexachlorobutadiene (HCBu), Pentachlorobenzene, Polychlorinated biphenyls (PCBs), Polychlorinated dibenzo-p-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs), Polychlorinated naphthalenes

Annex A includes most of the listed chemicals used as pesticides or in industrial activities. Signing countries should avoid their use and end the production of these chemicals. However, some specific exceptions allow production and use of certain compounds as the use of aldrin and chlordane as “local ectoparasiticide” and insecticide, hexachlorobenzene as intermediate product in chemical synthesis or in closed systems and deca-BDE for polyurethane foams used in buildings insulation or plastic and textile additive for products which require anti-flammable or fire retardancy properties.

Production and use of substances listed in Annex B should be restricted to acceptable purposes with specific exceptions such as disease vector control or intermediate for dicofol production in the case of DDT. Use exceptions of perfluoroalkyl substances (PFAS) and related compounds are metal plating in closed-loop systems, certain fire-fighting foams or as intermediate in the production of the pesticide sulfuramid.

Chemicals listed in Annex C are unintentionally produced during combustion processes of organic matter containing chlorine or thermal processes due to incomplete chemical reactions. Some examples of industrial activities that may release substances listed in this annex are waste incinerators, cement kilns, metallurgical production of copper, iron aluminum or zinc, textile dyeing or non-industrial combustion processes (residential) or motor vehicles powered by leaded gasoline. Best available techniques and best environmental practices guidelines are available or being developed by the conference of the parties in order to reduce, minimize or eliminate the release of these pollutants to the environment.

On the other side, OPEs are not regulated but they account for an emerging family of compounds with high production volumes which are used as flame retardants in substitution of brominated flame retardants and also as additives in the production of polymeric materials. They are detected in both biotic and abiotic matrices, despite their effects are still under study.

In the following sections the compounds studied are listed, and information on their physico-chemical properties, their toxic effects and production volumes are depicted. More detailed information is provided for Spain and China as in this Thesis study areas comprise these two countries.

1.1.1. Organochlorine pesticides (OCPs)

The chemical structure of OCPs is characterized by a C chain, normally including benzene rings or other C cycles and Cl atoms in the outer part of the structure. Molecular structures and main physicochemical properties of OCPs are summarized in Table 1.2. Studied OCPs have low polarity and their log K_{OW} values range from 3.72 to 6.91. They are classified as semi-volatile pollutants (World Health Organization, 1989) with Henry´s law constants ranging between 10^{-2} and 10^{-8} atm/m^3 mol.

Table 1.2. Physicochemical properties of OCPs. Values obtained from the Estimation Program Interface (EPI) suite (US EPA, 2012).

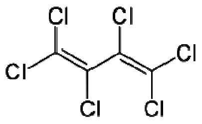
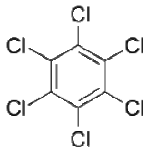
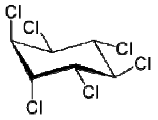
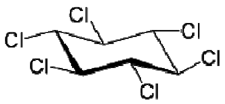
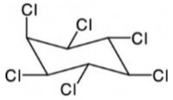
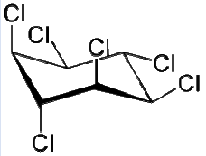
Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (^{o}C)
Hexachlorobutadiene (HCBu)		87-68-3	260.76	3.2	4.8	2.9	1.03E-2	216
Hexachlorobenzene (HCB)		118-74-1	284.78	0.0047	5.9	3.8	2.28E-3	291
α-HCH		319-84-6	290.83	8.0	3.7	3.0	5.14E-6	304
β-HCH		319-85-7	290.83	8.0	3.7	3.4	5.14E-6	304
δ-HCH		319-86-8	290.83	8.0	3.7	3.4	5.14E-6	304
γBHC (lindane)		58-89-9	290.83	8.0	3.7	3.4	5.14E-6	304

Table 1.2. Continued

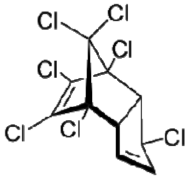
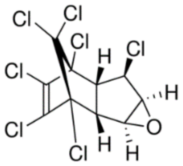
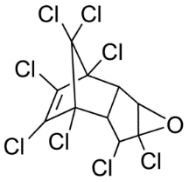
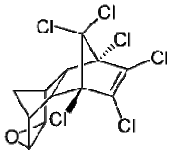
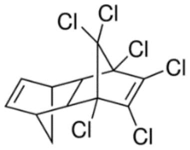
Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (^{o}C)
Heptachlor		76-44-8	373.32	0.18	6.1	4.6	2.94E-3	330
Heptachlor epoxides		1024-57-3	389.32	0.2	5.0	4.0	2.10E-5	340
Oxychlordane		27304-13-8	423.76	0.045	5.48	4.2	8.64E-8	353
Endrin		72-20-8	380.91	0.25	6.5	4.3	1.00E-5	363
Aldrin		309-00-2	364.91	0.017	6.5	4.9	4.4E-5	330

Table 1.2. Continued

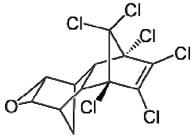
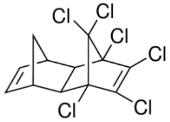
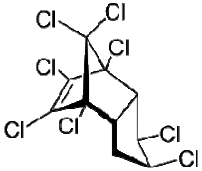
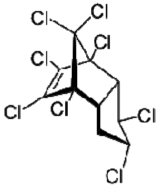
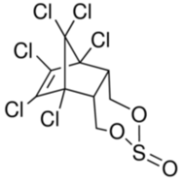
Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (^{o}C)
Dieldrin		60-57-1	380.91	0.25	5.2	4.3	9.44E-6	340
Isodrin		465-73-6	364.91	0.017	6.1	4.9	3.87E-4	330
Cischlordane		5103-71-9	409.78	0.056	6.2	4.8	8.30E-4	351
Transchlordane		5103-74-2	409.78	0.056	6.2	4.8	8.30E-4	351
α endosulfan		959-98-8	406.93	0.45	3.8	3.8	4.50E-007	401

Table 1.2. Continued

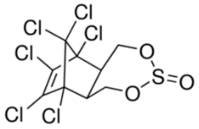
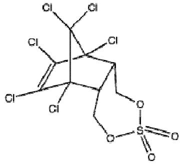
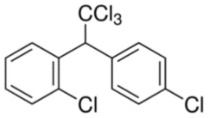
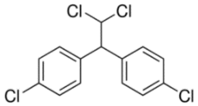
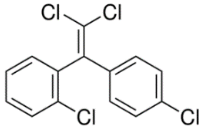
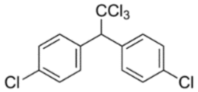
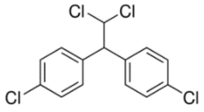
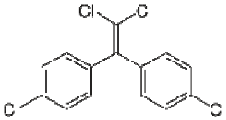
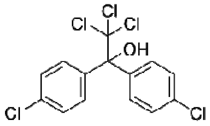
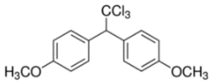
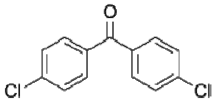
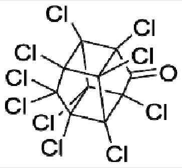
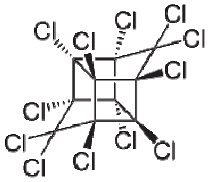
Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (ºC)
β endosulfan		33213-65-9	406.93	0.45	3.8	3.8	4.50E-007	401
Endosulfan sulfate		1031-07-8	422.92	0.48	3.7	4.0	3.91E-008	409
2,4´DDT		789-02-6	354.49	0.085	6.8	5.2	2.65E-004	368
2,4´DDD		53-19-0	320.04	0.1	6.0	5.1	1.21E-004	367
2,4´DDE		3424-82-6	318.03	0.14	6.0	5.1	5.09E-005	363
4,4´DDT		50-29-3	354.49	0.0055	6.9	5.2	4.77E-004	368

Table 1.2. Continued

Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (ºC)
4,4´DDD		72-54-8	320.04	0.09	6.0	5.1	1.21E-004	367
4,4´DDE		72-55-9	318.03	0.04	6.5	5.1	1.08E-003	363
Dicofol		115-32-2	370.49	0.8	5.0	4.1	4.32E-008	398
Metoxychlor		72-43-5	345.65	0.1	5.1	4.4	6.28E-005	378
DBP		90-98-2	251.11	3.8	4.4	3.5	1.07E-6	342
Chlordecone		143-50-0	490.6	2.7	5.4	4.2	5.38E-08	371
Mirex		2385-85-5	545.54	0.085	6.9	5.5	8.11E-04	366

Attending the carbon skeleton, OCPs can be classified in 5 types or groups: norbornane or norbornene, benzene, cyclohexane, “DDT-like” and others.

The carbon structure of norbornane/norbornene type is characterized by a cyclohexane or cyclohexene ring with a methylene bridge between non-consecutive carbon atoms (C_1-C_4) (Figure 1.2). Different subfamilies can be defined based on substitutions in the basic structure. Three C atoms forming a pentane ring together with C_5 and C_6 lead to chlordanes, containing 8 Cl atoms, and heptachlors, with 7 Cl atoms, respectively. A sulfur oxide group in the third ring lead to endosulfans type structure; sulfite (SO_3) group forms alpha and beta isomers and sulfate (SO_4) group forms endosulfan sulfate. In “endrin-like” compounds, the carbon structure can be characterized as double norbornene with common C_1 and C_4 and they contain 6 Cl atoms and an epoxy group in the case of endrin and dieldrin.

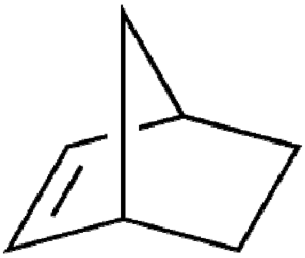


Figure 1.2. norbornene structure.

The benzene type OCPs are constituted by a benzene ring totally or partially substituted with Cl atoms; pentacholorobenzene (PCBe) with 5 Cl atoms and hexacholorobenzene (HCB) with 6 Cl atoms.

The cyclohexane type OCPs (Figure 1.3) comprise the α-, β-, γ- and δ- isomers of hexachlorocyclohexane. The γ-isomer, also called lindane, was intentionally produced as pesticide in large quantities while the rest of isomers are generated during the production process.

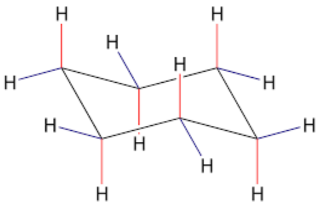


Figure 1.3. Cyclohexane structure.

The “DDT-like” type OCPs have a common structure with a central C atom bonded with 2 benzene rings (Figure 1.4). DDTs, methoxychlor and dicofol contain a $-CCl_3$ group bonded to the central C atom. $–CCl_2$ group, single or double bonded to the central atom characterize DDE and DDD isomers, respectively. DBP (p,p´-dichlorobenzophenone) can also be classified in this group even though it is a degradation product of dicofol, either metabolic, photolytic or formed during instrumental analysis (Zamariola et al., 2017).

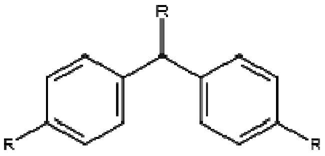


Figure 1.4. "DDT-like" structure.

Finally, other OCPs such as hexachlorobutadiene, chlordecone and mirex cannot be classified in the rest of the groups. The former is a perchlorinated C_4 alkene and the other two are C_{10} alkane structures with 10 Cl atoms and a cetone group in the case of chlordecone and 12 Cl atoms in the case of mirex.

OCPs were extensively used since late 30s decade, mostly for agricultural applications, although during World War Two or during the Vietnam War, European and North American soldiers were usually directly treated with technical DDT when they were destined to tropical countries. Technical endosulfan has been widely used in coffee cultures in South America (UNEP, 2004), aldrin and its degradation product, dieldrin, were used to kill termites and other organisms like worms or grasshoppers. Dicofol has been used as fungicide and acaricide and was permitted with a maximum content of DDT impurities of 0.1% (Council Directive 79/117/EEC, 1978). Heptachlor and its epoxides were used to kill cotton insects and grasshoppers and have been responsible for the decline of several wild bird populations, including Canadian Geese and American Kestrels (Stockholm Convention, 2001).

OCPs were banned during 1950-1980, depending on the country. Exceptions to the worldwide ban are health related applications to fight vector transmitted diseases. Malaria is the classical example. It is mainly transmitted through the direct ingestion of non-treated infected stagnant waters or through the *Anopheles* genus mosquito bite and huge efforts has been made to avoid its spread in non-developed countries, mostly in the African continent (van den Berg, 2017). The use of DDT was approved for these purposes years after the global agreement about the need to stop its production and use because its effectiveness was demonstrated and health benefits are a priority despite the environmental problem that it represents in the long term. Therefore it has been reintroduced in areas with increasing infections of typhus or malaria (Van den Berg et al., 2009).

Reliable information about production volumes and OCPs inventories at a global scale is scarce and emission and fate models are used to estimate those quantities (Wania and Mackay, 1999). The highest worldwide production of OCPs has been estimated for DDT with 4,500 kt and for technical HCH (mixture of isomers with 67-70% of α-HCH (Zhang et al., 2018)) with 10,000 kt (Lohmann et al., 2007). Other OCPs have been used and produced at smaller quantities and related information is scarce and usually not reliable due to confidentiality or the lack of trustable records (Breivik et al., 2006, Voldner and Li, 1995).

Spain produced several OCPs during the 1960s to 1980s decades. DDT was produced by a variety of companies such as Fábrica Española de Productos Químicos y Farmacéuticos SA (FAES), Zeltia, Fabricación Nacional de Colorantes y Explosivos, Sociedad Electroquímica de Flix or Cruz Verde. Additionally, dicofol production in Spain was among the highest in Europe, with approximately 0.0125 kt produced each year (Van der Gon et al., 2007). DDT production and use restrictions started in 1977 (BOE, 1975), however, recent environmental inputs of DDT and related compounds such as DDE and DDD have been described in Spain (Muñoz-Arnanz and Jiménez, 2011) and China (Qiu et al., 2005) associated to the production and use of technical dicofol, which contains traces of DDT as impurities and degradation leads to DDE and DDD. In Spain, HCHs was produced in four factories, two in the Basque country, one in Galicia and another one in Aragón. These companies together have discharged more than 200 kt of HCHs contaminated subproducts to the surrounding environment, which have led to concerning environmental issues (Fernández et al., 2013). Figure 1.5 was extracted from Qui et al. (2005) from the Sino-Italy Joint Project (UNDP Project CPR/01/R51/A/CC/31) and shows the amount of DDT, dicofol and DDT used for dicofol synthesis.

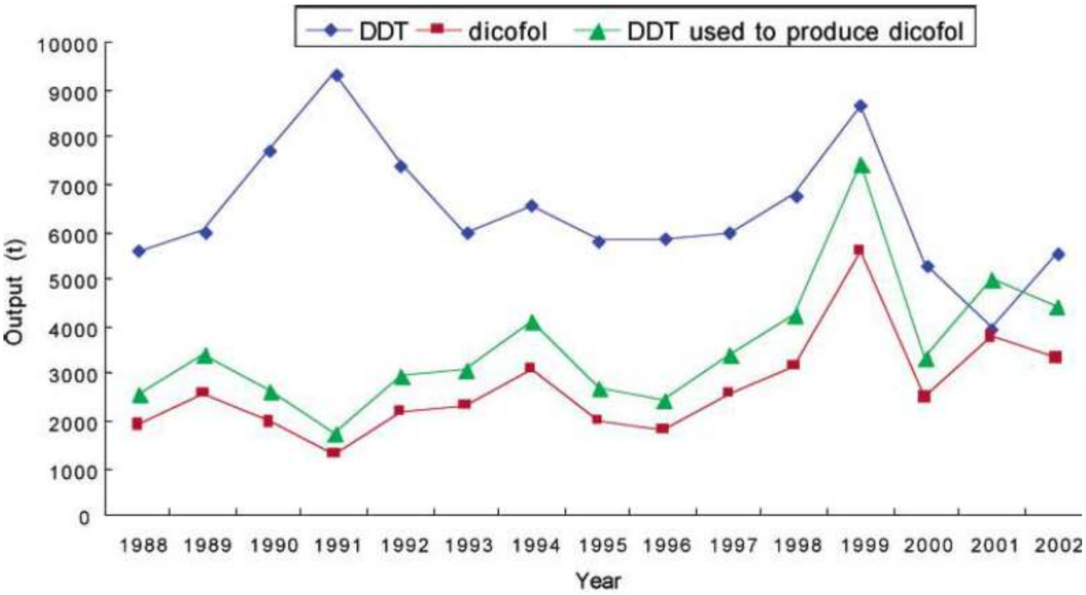


Figure 1.5. Evolution of technical DDT and DDT as an intermediate for dicofol production.

In China, 270 kt of DDT were produced since 1950 until its use in the country was banned for agricultural purposes in 1983, but the production for exportation continued, with approximately 97 kt of technical DDT produced from 1988 to 2002, because it was used for malaria control in African countries.

Technical HCH was also produced in large quantities in China. More than 4,000 kt (33% of global production) were produced until late 1980s and then decreased because it was substitued by the production of technical lindane, containing more than 90% content of the γ-isomer. Its production implies large quantities of other isomers as wastes and byproducts (Ma et al., 2020). From 8 to 12 tonnes of byproducts are generated for each ton of produced lindane (Bodenstein 1972).

Once released to the environment, their fate is conditioned by the physicochemical properties of each OCP, being accumulated in soils and sediments, especially when the organic matter content is high (Ali et al., 2018), or bioaccumulated and biomagnificated in the trophic webs. They are seldom detected in water or at very low concentrations (Bhutto et al., 2021).

The high persistence and bioaccumulation and biomagnification properties lead to undesirable effects both for human health and the environment. Major toxic effects are related with neurotoxicity, which includes neurodevelopmental, neurodegenerative, and neurobehavioral disorders (Saeedi Saravi et al., 2016). This same review describes neurodevelopmental effects related with the capacity of OCPs to cross the placenta and also to be released by breast milk, reaching the individual in an early developmental stage, causing autism, impairment on fine motor skills or cognitive deficits. Neurodegenerative effects include abnormalities in psychomotor development and dementia, both of them being studied at epidemiological level in OCPs occupational exposed populations. Among neurobehavioral disorders, anxiogenic effects as well as attention deficit hyperactivity disorder, hyperexcitability or tremors were associated with different OCPs.

Other toxic properties in vertebrates are related with their action as endocrine disruptors that can impair the metabolism homeostasis. Toxicity mechanisms of OCPs were reviewed by Mrema et al. (2013). Endocrine disruption properties of OCPs are related with their interaction with nuclear and intracellular receptors, interfering with hormone signaling pathways. These interactions are due to structural similarities with natural hormone such as steroids and thyroxine. OCPs exposure at high concentrations (50 to 100 mg/kg of body weight for 10 consecutive days) in mammals has been reported to affect the levels of thyroid hormones, decreasing T4 activity and producing hypothyroidism. Aromatic rings containing OCPs can bind with the aryl hydrocarbon receptor (AhR), which is a transcription factor activated with planar ligands (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)) and involved in xenobiotic detoxification mechanisms through activation of cytochromes such as CYP450, but also in immune response, stem cell maintenance and cell differentiation (Kawajiri and Fujii-Kuriyama, 2017). The interaction of OCPs with the AhR induces the transcription of cytochromes P450 such as CYP1A1 or CYP1B1 but with low efficiency due to the non-planar structure. OCPs toxicity is also related with their ability to trigger apoptosis which may provoke immunodeficiency, autoimmunity diseases and reproductive anomalies. Their relation with oxidative stress, by affecting the redox homeostasis, decrease the antioxidant defenses which produce an accumulation of reactive oxygen species which in turn may lead to oxidative DNA damage, endoplasmic reticulum stress and alterations in the mitochondrial function. Finally, epigenetic effects of OCPs have been suggested and may be related with uncontrolled cell proliferation and potential carcinogenicity. In fish species, interactions with the central nervous system led to neurodevelopment and neuroendocrine system disruption, as OCPs behave in a similar way than sexual hormones, with anti-androgen and weak estrogen activity in juvenile and adult individuals, interfere with the immune system and alter metabolic function in adults or act as teratogenic xenobiotics in developing stages (Martyniuk et al., 2020). In birds, the presence of OCPs has been linked to shell thickness diminution which ultimately derives in unsuccessful hatching and reproduction impairment (Miljeteig et al., 2012). In humans, their activity as xenoestrogens has been linked to breast and other cancers; concretely for DDT and related compounds anti-androgenic and estrogenic activities have been linked to their interaction with the estrogen receptor.

1.1.2. Polychlorinated biphenyls (PCBs)

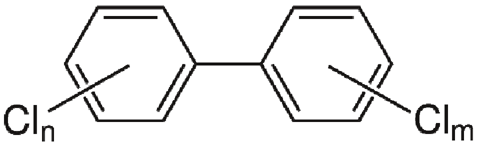
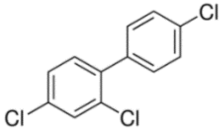
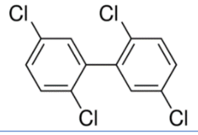
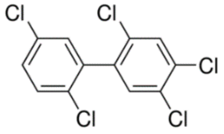
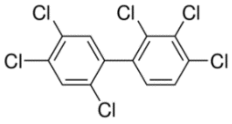
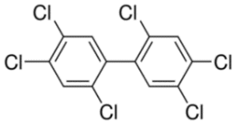
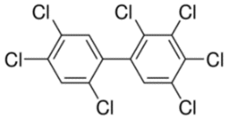


Figure 1.6. PCBs structure.

PCBs are a series of 209 congeners with a common hydrocarbon chain consisting in 2 C-C bonded benzene rings substituted with 1 to 10 Cl atoms (Figure 1.6). Each possible Cl substitution in the benzene rings defines each of the 209 possible congeners and therefore is used to appoint them. Their physicochemical properties are similar to OCPs and are indicated in Table 1.3. They are non-polar ($5.62< \log K_{OW} <8.27$), semi-volatile compounds, with high chemical, thermal and photo stability. In this Thesis, 6 marker PCBs have been selected based on their widespread environmental distribution. The number of Cl atoms determines the properties of the congener’s series. The higher the Cl content in the molecule, the higher molecular mass, molecular volume and boiling point, but lower polarity and volatility. Isolated individual congeners are white crystalline solids but mixtures are liquids or resins with increasing viscosity along the number of Cl substitutes.

PCBs were first synthesized in 1881 and their commercial manufacture began in 1929 by Swann Chemical Company (USA) with the purpose of providing a fluid with flame retardation properties to avoid risks associated with fires in oil containing transformers, but their uses were expanded during 1930s decade as heat exchangers or hydraulic fluids or even as plasticizers (Erickson and Kaley, 2011). Their industrial uses and applications are commonly related with fire hazardous processes because of their physicochemical properties, such as thermal and chemical stability, electrical insulation properties, low flammability and flame retardation. When combustion reactions occur, PCBs produce HCl, which reduce the spread of fire because it can inhibit or slow down the combustion reactions. The uses of PCBs can be classified attending to uses: i) completely closed systems, such as capacitors and transformers, in which they act as dielectric fluids and comprise 60% of their total use, ii) nominally closed systems, like pumps or heat transfers, with 15% of the total use, and iii) open-ended applications, as additives and plasticizers in PVC and other Cl containing rubbers or as additives in paints, inks, adhesives, or lubricating oils, which covers the remaining 25% (Antolín Rodríguez, 2011; WHO, 1993).

Table 1.3. Physicochemical properties of PCBs. Values obtained from the Estimation Program Interface (EPI) suite (US EPA, 2012).

Compound	Molecular structure	CAS number	Mm (g/mol)	Water solubility (mg/L, 25 ºC)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	Teb (ºC)
PCB 28		7012-37-5	257.54	0.42	5.6	4.7	2E-4	341
PCB 52		35693-99-3	291.99	0.015	6.3	4.9	2.25E-4	359
PCB 101		37680-73-2	326.43	0.015	6.8	5.1	7.132E-5	378
PCB 138		35065-28-2	360.88	0.0015	7.44	5.3	1.170E-4	397
PCB 153		35065-27-1	360.88	0.0035	7.44	5.3	2.154E-4	397
PCB 180		35065-29-3	395.32	0.0038	8.27	5.5	2.379E-4	416

Industrial synthesis of PCBs is done by direct ferric chloride catalyzed chlorination of biphenyls and subsequent treatments to neutralize pH and removal of impurities (Hubbart, 1964). Obtained product consists in a mixture of PCB isomers and congeners whose chlorination degree depends on the reaction time and conditions. These mixtures were commercially named Aroclors® and are followed by a 4 digit number. The first 2 digits refer to the commercial series, while the last 2 digits refer to the weight percentage of Cl. Despite Aroclor is the most famous name for PCB containing commercial mixtures, some other commercial names have been used by Monsanto (e.g. Therminol for heat exchange fluids or Pydraul for hydraulic fluids) or other producers (Phenoclor, Clophen, Sovol or Pyralene among others).

Production of PCBs increased from 1930 until 1970s when Monsanto voluntarily stopped the production 2 years before the ban in USA. An estimation of around 300 kt of 6 marker PCBs were globally produced and are distributed in waste or in-use stocks, emitted to the environment or dissipated (Li and Wania, 2016). However, previous studies based on literature review and reports from the major production countries, such as USA, Germany, France and URSS (nowadays Russia), estimates a global production of PCBs higher than 1,300 kt (Breivik et al., 2002).

In Spain, the only PCB production plant was set in Flix, north-east Spain, by Cross S.A. and Rhône-Poulenc under French license. From 1955 to 1984 it produced a total amount of nearly 30 kt of technical mixtures under the commercial name of Fenochlor and/or Pyralene (de Voogt and Brinkman, 1989). In China, approximately 8 kt of PCBs were produced during the 1960’s and 1970’s (Jiang et al., 1997).

PCBs toxicity derives from their interaction with the AhR. This interaction depends on the position of the Cl atoms which defines the relative position of the benzene rings. Congeners with both benzene rings in the same geometrical plane have much higher interactions with the AhR than those ones where benzene rings form an angle. PCBs with at least 4 Cl atoms in other positions than *ortho* (congeners 77, 81, 126 and 169) can acquire the necessary planar conformation and have a high interaction with the AhR and therefore are called coplanar or dioxin like PCBs. PCBs with at least 4 Cl atoms in *non-ortho* positions and another one in an ortho position can also interact with the receptor but in a weaker way and are called *mono-ortho* PCBs. Toxicity of both groups can be referred to 2,3,7,8-TCDD through toxic equivalency defined by the World Health Organization (WHO) (Van den Berg et al., 2006). Remaining congeners are called *ortho* PCBs and their toxic properties are less related with the AhR. As rewieved by Buha Djorjevic et al. (2020), PCBs have been related with miss functions in the immune system, cardiovascular diseases or developmental impairments in epidemiological studies. They have been widely identified as endocrine disruptors, including estrogenicity, insulin enhancing effects and arachidonic acid secretion, but the most important endocrine disruption takes place in the reproductive system and the hypothalamo–pituitary–thyroid axis. Even though no effects in the levels of thyroid-stimulating hormone have been found in the thyroid gland, PCBs and OH-PCBs (main metabolites) have been related with both increases and decreases in the thyroid hormones due to structural similarities. Higher chlorinated congeners act as agonist while low chlorinated ones act as antagonists. PCBs can also bind to the T4 thyroxine carrier and the transthyretin receptor and are capable of crossing the blood-brain barrier, causing neurotoxicity.

Toxic effects have been also identified to be related with the interactions in the Ca^{2+} cellular functions, which impair gene transcription, protein translation, cellular signaling and the motor system. They enhance the activity of ryanodine receptors, which are a family of Ca^{2+} channels, affecting the cytoplasmatic Ca^{2+} concentrations. These interactions may be related with toxicity problems in the central and peripheral nervous system such as decrements in neonatal reflexes, cognitive function, motor activity, tremors or hearing impairments (Pessah et al., 2009).

1.1.3. Polybromodiphenyl ethers (PBDEs)

PBDEs are a series of 209 congeners with a common hydrocarbon chain consisting in 2 benzene rings as substituent groups for the central ether atom. Each possible Br substitution in the benzene rings defines each of the 209 congeners (Figure 1.7). Variation in their physicochemical properties relies on the number and position of Br atoms in a similar way than PCBs. They are semivolatile non-polar compounds with Henry´s constants values around 10^{-4} atm/m^3 mol and log Kow between 5.88 and 12.11 (Table 1.4). Higher bromination congeners are heavier, less volatile, less polar

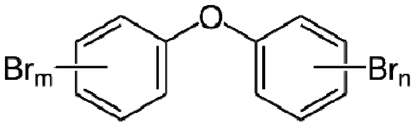
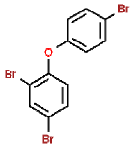
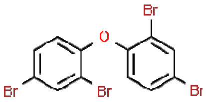
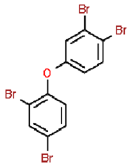
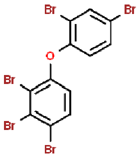
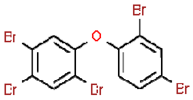
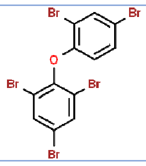
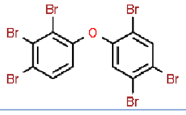
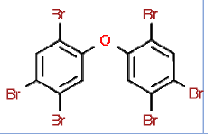
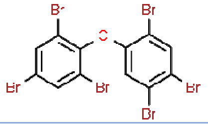
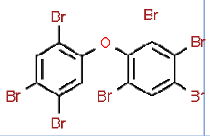
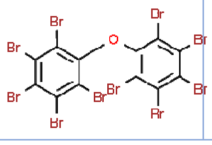


Figure 1.7. PBDEs structure.

and less water soluble. In this Thesis, 11 PBDE congeners of environmental relevance are studied.

Table 1.4. Physicochemical properties of PBDEs (Estimation Program Interface (EPI) suite (US EPA, 2012).

Compound	Molecular structure	CAS number	M_m (amu)	Water solub (mg/L, 25^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (ºC)
BDE 28		41318-75-6	406.9	0.026	5.9	3.9	4.7E-5	375
BDE 47		5436-43-1	485.8	0.0015	6.8	4.1	1.05E-4	405
BDE 66		189084-61-5	485.8	0.0015	6.8	4.1	1.05E-4	405
BDE 85		182346-21-0	564.7	7.9E-5	7.7	4.3	2.3E-4	436
BDE 99		60348-60-9	564.7	0.0024	7.7	4.3	2.3E-4	436
BDE 100		189084-64-8	564.7	7.9E-5	7.7	4.3	2.3E-4	436
BDE 138		182677-30-1	643.6	4.1E-6	8.6	4.6	5.9E-4	467
BDE 153		68631-49-2	643.6	4.1E-6	8.6	4.6	5.9E-4	467
BDE 154		207122-15-4	643.6	4.1E-6	8.6	4.6	5.9E-4	467
BDE 183		207122-16-5	722.5	2.2E-7	9.4	4.8	1.5E-3	498
BDE 209		1163-19-5	959.2	1E-4	12.1	5.4	2.3E-3	590

PBDEs are mostly used as flame retardants due to their Br content, which inhibit the spread of fire through the caption of free radicals and subsequently reducing the combustion reaction intermediates and therefore slowing down the whole process. They are added in a wide variety of polymers such as epoxy and phenolic resins, polyamides (PA), polyethylene (PE), polypropylene (PP), polystyrene (PS) or polyvinyl chloride (PVC) among others, but also in rubbers and textile fibers. Those materials are later used in different applications, mostly related with electrical and electronic equipment (EEE) like printed circuit boards and panels, coating, connectors, moisture barriers, wires or cables but also in transportation packages and coatings. As a result, they are present in many end products like computers, televisions, cell phones, coils, bobbins, construction material, furniture, clothes, household appliances, etc. (Rahman et al., 2001). They are not chemically bonded to the material so they can be easily released during the lifetime of the commercial item but also during disposal and recycling processes.

PBDEs are synthesized by direct bromination of diphenyl ether catalysed by a metal such as Fe or Al. The bromination occurs preferably in positions 2 and 4 of both rings and different congeners are produced by subsequent bromination of reaction products (Ryden, 2013).

PBDEs have been largely produced and consumed since the 1970s decade until early 2000s, when awareness of associated environmental problems started to be raised. Penta and octa BDE mixtures were included in the Stockholm Convention in 2009 while decaBDE was not listed until 2017. The global demand was largely increased due to their widespread aplicability; for example, global production of PBDEs increased from 40 kt in 1992 to 63.3 kt (54.8 kt of decaBDE) in 1999 and to 67 kt (56 kt of decaBDE) in 2001 (Ma et al., 2012, Rahman et al., 2001). Historical PBDEs production, consumption and distribution between in-use and waste stocks since 1970s decade have been recently estimated; total productions were estimated at 1600 kt for decaBDE, followed by 175 kt of pentaBDE mixtures and 130 kt of octaBDE. Global production peaked at approximately 85 kt in 2003 (Figure 1.8) from which around 75 kt were decaBDE and 10 kt were pentaBDE mixture. OctaBDE mixtures global production peaked 6 years earlier, in 1997, with 8.5 kt (Abassi et al. 2019).

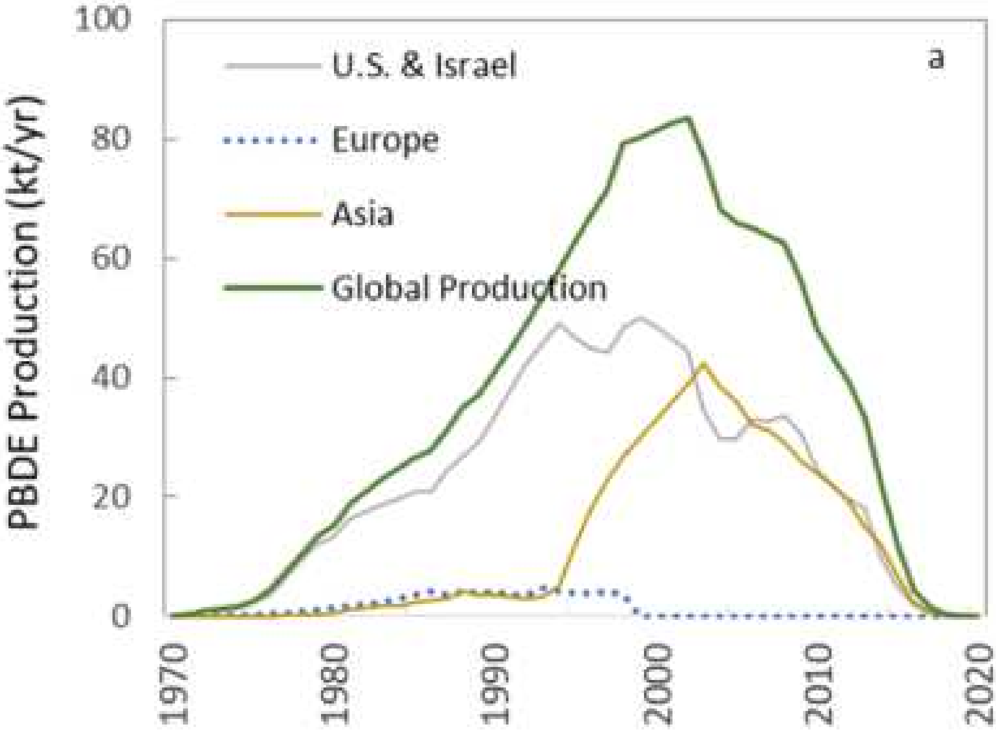


Figure 1.8. Evolution of global PBDEs production. Extracted from Abassi et al. (2019).

PBDEs production in China started in the early 1980s. Historical pentaBDE production was less than 10 kt while octaBDE was never produced. However, decaBDE showed the highest production and use rates among PBDEs in China by far and historical production was over 500 kt, with the peak in 2005 with more than 40 kt produced. However, consumption is expected to be higher than production because before 2000 more than half of used decaBDE was imported.

PBDEs have low acute toxicity levels, with oral LD_{50}>5 g/kg, and therefore chronic toxicity is the major cause of concern regarding hazardous effects related with these compounds. They can be considered as endocrine disruptors as they interact with the estrogen, progesterone and androgen receptors (both as agonist and antagonists) and because they can cross the placenta, they have been linked to neurodevelopmental effects during brain development, which may affect motor activity, memory or learning. They are present at high concentrations in human breast milk resulting in high human prenatal and early life stages exposures in humans, especially in North America (Costa and Giordano, 2007). Both PBDEs and OH-PBDEs, their main metabolites, can interact with thyroid systems, concretely with thyroid transporter proteins and the thyroid nuclear receptor due to their structural similarity with the endogenous thyroid hormones. For example, a reduction in serum thyroxine (T4) or triiodothyronine (T3) have been described in rodents, while in humans there are reports about T4 increase (Dishaw et al., 2014). All PBDEs seem to act by the same mechanisms, but dose-effects differ among congeners. PBDEs can also bind to the AhR like PCBs, and subsequently toxic effects have been described, but in a much lower extent. OH-PBDEs and MeO-PBDEs, however, may play a more important role than PCB metabolites regarding health effects derived from their interaction with the above mentioned receptor (Zhang et al., 2022a).

1.1.4. Organophosphate esters (OPEs)

OPEs are a group of emerging pollutants whose common structure is derived from the phosphoric acid. A central P atom is double bonded to an O atom and single bonded to 3 organic side chains as triple ester (Figure 1.9).

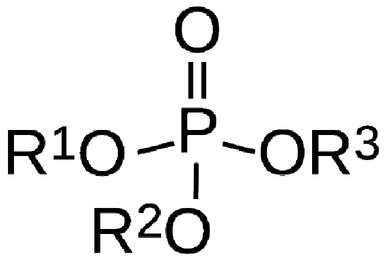


Figure 1.9. OPEs structure.

Their physicochemical properties (Table 1.5) are determined by the nature of the side chains and can be classified as alifatic or alkyl-OPEs, such as TEP or TBP, aromatic or aryl-OPEs, which include TPhP or TCPs and chlorinated OPEs containing chlorine chains like TCPP or TCEP. The different nature of the side chains implies a wide range in physicochemical properties. Aryl- and alkyl-OPEs are generally more hydrophobic, and this hydrophobity is increased with the length of the side chains. For example, the logK_{ow} values are increased from TEP (0.87) to TBP (3.82), TPhP (4.70) and TCPs (6.34). Since the assignation of acronyms for OPEs is not unified among literature, full name and acronyms used by van der Veen and de Boer (2012) and Bergman et al. (2012) are included in the first column when differing to avoid unambiguitis.

Table 1.5. Physicochemical properties of OPEs. Values obtained from the Estimation Program Interface (EPI) suite (US EPA, 2012).

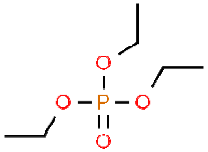
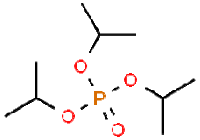
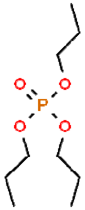
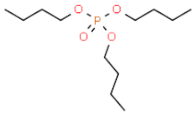
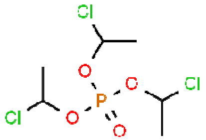
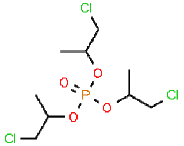
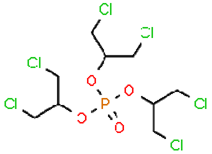
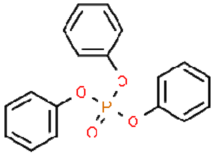
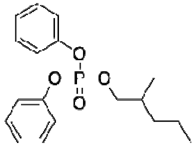
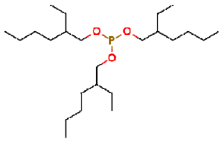
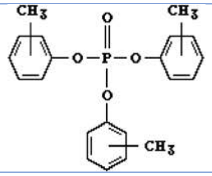
Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (ºC)
Triethyl phosphate (TEP)		78-40-0	182.15	1.1E4	0.9	1.8	3.6E-8*	233
Triisopropyl phosphate (TiPP, TIPP)		513-02-0	224.24	501	2.1	2.4	8.1E-5	254
Tripropyl phosphate (TnPP, TPP)		513-08-6	224.24	827	2.3	2.6	6.8E-7*	284
Tributyl phosphate (TnBP, TNBP)		126-73-8	266.32	7.3	3.8	3.4	1.4E-6*	327
Tris(2-chloroethyl) phosphate (TCEP)		115-96-8	285.49	7940	1.6	2.6	7.7E-10*	352

Table 1.5. Continued

Compound	Molecular structure	CAS number	M_m (g/mol)	Water solubility (mg/L, 25 ^{0}C)	Log K_{OW}	Log K_{OC}	K_H (atm/m^3 mol)	T_{eb} (^{o}C)
Tris(1-chloro2-propyl) phosphate (TCPP,TCIPP)		13674-84-5	327.57	1080	2.9	3.2	4.2E-9*	365
Tris(1,3-dichloroisopropyl) phosphate (TDCPP,TDCIPP)		13674-87-8	430.91	7	3.6	4.0	1.1E-7	459
Triphenyl phosphate (TPhP,TPHP)		115-86-6	326.07	1.9	4.7	4.0	3.3E-6*	441
2-ethylhexyl diphenyl phosphate (EHDPP)		1241-94-7	362.41	1.9	6.3	4.5	5.4E-5*	443
Tris(2-ethylhexyl) phosphate (TEHP)		78-42-2	434.6	0.6	9.5	6.4	7.9E-8*	446
Tricresyl phosphate /tris(methylphenyl) phosphate (TCP,TMPP)		1330-78-5	368.37	0.36	6.3	4.7	8.1E-7*	476

OPEs can be used as flame retardants by two different mechanisms which affect the propagation of the combustion reaction in the gas or in the solid phase. The solid phase mechanism is common to all OPEs and is based on the formation of a passivation layer which inhibits the diffusion of Oxygen species (atoms, molecules or radicals) in a similar way than stainless steel or other rustproof metals. Once the burning process has started, OPEs are decomposed and a solid phosphoric acid layer is formed on the surface of the burning material. This is the main action mechanisms for non-halogenated OPEs. Chlorinated OPEs, however, can also act in the gas phase, slowing or even inhibiting the spread of combustion chain reactions by deactivation of free radicals during fire propagation. This gas phase flame retardation mechanism is analog to PCBs and PBDEs. Therefore, halogenated OPEs are mostly used as flame retardants and were considered as feasible substitution for brominated flame retardants due to their less persistency in the environment.

Non-halogenated OPEs are most commonly used as plasticizers or other uses such as additives, lubricants, hydraulic fluids or pesticides. They are present in different kinds of polyurethane (PU) like rigid PU foam, used as a building material for insulation and refrigeration, or flexible PU foam used in furniture or EEE. Other polymers potentially containing OPEs are PE, PP, PA, acrylonitrile-butadiene-styrene (ABS) or high impact polystyrene (HIPS). PVC is a typical OPE containing polymer which may contain TPhP, TEP, TCEP or TEHP. They are also applied to different textile fibers such as cellulose or cotton, in glues, in epoxy and phenolic resins or to easily recover copper from printed circuit boards (Andrae, 2007, Van der Veen and de Boer, 2012, Yang et al., 2019).

OPEs industrial synthesis uses phosphorous oxychloride ($POCl_3$) as the initial raw material, which is combined with an organic alcohol (R-OH). The triple dechlorination and ester bond formation lead to the corresponding organophosphorous triester and the obtained product depends on the carbon chain of the used organic alcohol. The main reaction can be expressed as follows:

$$POCl_3 + 3\ R\text{-}OH \rightarrow PO(O\text{-}R)_3 + 3\ HCl$$

OPEs production has been increased year by year during last three decades, especially after ban policies on legacy halogenated flame retardants like PBDEs took place. Global production and consumption estimations slightly vary among studies. Olivero-Verbel et al. (2021) reported an increment from 300 to 1,000 kt over the last decade which can be in agreement with data compiled by Yang et al. (2019), which reports that global consumption of OPEs of 100 kt in 1992 has been increased to 108 kt in 1995, 186 kt in 2001, 500 kt in 2011 and to 680 kt in 2015.

Nowadays, organophosphorus flame retardants (OPFRs), mostly OPEs but also organophosphines, organophosphonates or organophosphate salts are produced at least in 367 factories around the world, of which 201 of them are located in China, followed by USA with 69. In China nearly 600 kt of OPFRs are produced annually and two thirds of them are OPEs. Concretely, Guangdong province hosts 39 OPFRs production facilities, making it the third Chinese manufacturing province after Shandong and Jiangsu. The highest OPEs production volumes were estimated for TEP (65 kt/year), TCPP (45 kt/year) and TCEP (more than 30 kt/year) (Huang et al., 2021).

Toxic properties have been described for this family of pollutants. However, the wide range in physicochemical properties and differences in the side chains of OPEs leads to a variety of toxicity mechanisms and adverse outcomes. Information about toxicity mechanisms is still limited and studies are generally focused on model organisms. Neurotoxicity properties of OPEs are probably related with their similarities with organophosphate pesticides as they can cross the blood-brain barrier, reaching the target organ (brain) and interacting with neurotransmitters like γ-aminobutyric acid (GABA), acetylcholinesterase, acetylcholine or histamine (Bekele et al., 2021). Endocrine disruption and affection on steroid hormones modulation have also been reported; chlorinated OPEs have been reported to be carcinogenic, inducing tumors in tissues where they are accumulated such as liver or testis (Chokwe et al., 2020). Endocrine disruption effects are expected to occur through interactions of TPhP, TEHP or TDCPP with specific receptors such as estrogen, androgen, thyroid hormone or glucocorticoid receptors, either by agonistic or antagonistic mode of action (Yan et al., 2021).

OPEs may affect gonadal development and gametogenesis through interactions with the hypothalamus-pituitary-gonad axis, induce malformations and growth inhibition, infertility and even lethality effects (Yan et al., 2021). In fact, reproductive and developmental toxicity is of special concern because it can affect not only at the individual organisms level but also at population level.

Hepatotoxcity and cardiotoxicity are related with the transport of OPEs through blood and tissue fluids. In the liver, OPEs increase the formation of reactive oxygen species which may induce oxidative stress, DNA damage and apoptosis (Chen et al., 2018). Environmentally relevant concentrations of OPEs have been linked with cardiotoxicity effects including pericardium edema, arrhythmia or heart malformations among others in zebra fish (Du et al., 2015). Some OPEs impair developmental toxicity, e.g. TPhP has been linked to visual function related gene transcription inhibition in zebra fish and TDCPP have been proven to be transferred from parent to larvae (Shi et al., 2019).

1.2 POPs and OPEs in the environment

Once released to the environment, POPs and OPEs are distributed in the different environmental compartments. The partitioning depends on the physicochemical properties of each pollutant but also on each environmental matrix.

1.2.1. Air

The air compartment is a gaseous phase which covers the whole surface of the planet. Due to the semivolatility of POPs, they can be transported by air and therefore air fluxes and dynamics are important in the global scale. The phenomena of long and short range transport or the so called grasshopper effect are responsible of the presence of POPs in remote areas, far away from any possible sources (Mackay and Wania, 1995). Air transport is also responsible of their presence in cold areas and high mountains, like the Arctic or the Tibetan plateau through cold deposition. In those remote areas, the pollution profiles of POPs depends on the volatilization/deposition behavior; more volatile compounds with higher fugacity are able to travel further and be easier revolatilized from soil which becomes a secondary source of lighter and more volatile POPs to the atmosphere (Zhan et al., 2017).

OPEs have been detected in air generally associated to air particulate matter of different particle sizes (Cao et al., 2022, Wang et al, 2020). However, atmospheric transport and global fate of OPES is still under study and information about influencing factors such as degradation or deposition-volatilization processes is scarce. However, OPEs have been detected in air and particulate matter (mostly PM2.5) in areas far away from their sources (Zeng et al., 2020, Zhang et al., 2022b).

1.2.2. Water

Water bodies can be divided in two main groups, namely marine water, which includes oceans and seas and are characterized by high ionic strength due to the presence of dissolved salts and the rest of water bodies or freshwater, which includes rivers, lakes, groundwater or glaciers among others. Because of the hydrophobic characteristics, POPs are not usually present in water or their levels are quite low. Nevertheless, some studies have reported the presence of small concentrations of dissolved hydrophobic POPs in water bodies when using high sample volumes to decrease detection limits or alternatively passive samplers to accumulate studied chemicals over time (Habibullah-Al-Mamun et al., 2019, Martinez et al., 2019). However, water bodies can play an important role in the transportation of hydrophobic POPs adsorbed to suspended particles and marine environments are considered as sinks and reservoirs for these compounds (Avellan et al., 2022).

OPEs, on the other hand, have a wide range of water solubility (0.36 to 11,000 mg/L at 25 ^{0}C; see Table 1.5), generally higher than POPs and therefore, they are widespread in water bodies. In freshwater, Bekele et al. (2021) reviewed the occurrence of OPEs and reported individual OPEs levels from ng/L to tens of μg/L in rivers and lakes from Europe, North America and Asia with TCPP, TCEP or TnBP as the main compounds detected. Water treatment plants have been identified as sources of OPEs to receiving waters (Lian et al., 2022a). Wang et al. (2020) reported OPEs levels from 1 to 10 μg/L in influents of water treatment plants from different cities in Australia, China and Europe and higher elimination rates were found for alkyl-OPEs due to easier degradation than chlorinated OPEs, which are better removed by physical treatment than biological. TCPP is usually the dominating compound in effluents and, in some cases, higher levels have been found in effluents than in influents. Both alkyl and chlorinated OPEs have been detected in influent and effluents of water treatment plants from Spain (Rodriguez et al., 2006). OPEs degradation in water (photolytic, biologic or abiotic) plays an important role to understand the presence or absence of more labile OPEs like TPhP or TCPs, while chlorinated ones such as TCEP or TCPP are more resistant to degradation (Cristale et al., 2017). Because some OPEs are stable, they have also been detected in drinking water (bottled, filtered or tap water) from Asia or USA with total sums of a least 6 OPEs ranging from <MDL to 1,660 ng/L (Wang et al., 2020a).

1.2.3. Sediments

Sediments encompass particulate matter previously transported and deposited at the bottom of a water body. Similarly to water bodies, sediments can be divided in marine and freshwater sediments. The close contact between the sediments and the corresponding water creates a partitioning of the compounds present in both compartments with a distribution equilibrium. The equilibrium is affected by the polarity, hydrophobicity or solubility of the compounds, which can be used to estimate their tendency to remain in water or to adsorb in sediments. Some factors like sediment particle size or water flow may influence the distribution, but the most influencing factor is the sediment composition, especially the presence of organic matter where pollutants can be sorbed (Bayen, 2012). Sediments, thus, incorporate and accumulate pollutants through time deposition of suspended organic matter containing sorbed pollutants and, therefore, reflect the pollution status of their corresponding water body; the first layers of the sediment reflect relatively recent pollution inputs while deeper ones reflect historical ones. Moreover, pollution of sediments is related with upstream inputs and can be an indirect measure of the river basin pollution. In coastal marine areas, sediments reflect the pollution status not just from the marine environment but also from continental waters transporting inland pollutants (Sanchez-Avila et al., 2012). Therefore, sediments become a sink for pollutants, and can also act as secondary source to the water body and biota. Table 1.6 compiles some studies about the occurrence of OPEs, PBDEs, PCBs and OCPs in sediments from different ecosystems around the world, including marine and freshwater.

Table 1.6. Pollution levels in sediments, expressed in ng/g dw.

Area (Country). *indicates protected area	Analyzed compounds	Matrix	PBDEs	PCBs	OCPs	OPEs	Refference
ISIKLI (Turkey)	7 PCBs	marine sediment	0.2	0.3	2.1		Kocagöz et al., 2014
SÖKE plateau (Turkey)		marine sediment	1.3	0.3	3.2		
SÖKE estuary (Turkey)		river sediment	1.2	0.8	2.2		Levels are expressed as mean values
Yes¸ilırmak River (Turkey)	11 OCPs, 15 PCBs	river sediment		0.9 (0.7-1.15)	7.6 (4.25 -27.75)		Dinç et al., 2021
Samsun bay, Black sea (Turkey)		marine sediment		1.88 (1.03-23.72)	5.485 (1.36 - 14.81)		
Coastal areas (Taiwan)	PCBs	marine sediment	(nd- 64)	(nd- 649)	0.02-2.48		Several studies Cheng et al., 2021
Coastal areas (Korea)	23 PCBs, 3 HCHs, 6DDXs	marine sediment		2.26 (nd-15.03)	1.54 (0.02-7.79)		Choi et al., 2011
Todos os Santos Bay (Brazil)	51 PCBs, 6DDXs	marine sediment		0.28 (nd-4.66)	0.33 (0.07-0.89		Sot˜ao Neto et al. 2020
Albufera de Valencia Natural park* (Spain)	TCEP	lagoon sediment				5.8	Lorenzo et al., 2019
	TCPP	lagoon sediment				53.8	
	TDCPP	lagoon sediment				2.5	
	TPhP	lagoon sediment				14.1	
	TCP	lagoon sediment				3.9	
	TEHP	lagoon sediment				15.8	
	6 OPEs	lagoon sediment				95.9	
Gulf of Lyon* (France)	8 OPEs	marine sediment				2.4	Alkan et al., 2021
Gulf of Lyon* (France)		marine sediment				3.3	
Rhone river (France)		river sediment				96.6	
Marseille (France)		marine sediment				304.4	

Table 1.6. Continued

Area (Country). *indicates protected area	Analyzed compounds	Matrix	PBDEs	PCBs	OCPs	OPEs	Refference
Sungei Buloh mangroove (Singapore)	50 PCBs, 5 PBDEs (no BDE 209), 17 OCPs	Mangrove sediment	1	0.88 (0.59-1.14)	4.2		Bayen et al., 2005
Yifengxi mnagrove (China)	10 PBDEs	Mangrove sediment	23.8				Liu et al., 2017
Shuanghan mangrove (China)		Mangrove sediment	37.1				
Su´ai wan mangrove (China)		Mangrove sediment	56.6				
Pearl river mangrove, Guangzhou (China)	11 OPEs	Mangrove sediment				55.5	Hu et al., 2017
Pearl river mangrove, Zhuhai (China)	12 OPEs	Mangrove sediment				16.4	
Pearl river mangrove, Shenzhen (China)	13 OPEs	Mangrove sediment				50.5	
Industrial and e-waste mangrove in Nansha (China)	18 PBDEs	Mangrove sediment	135.6				Wu et al., 2017
Albania	8 PBDEs, 6 OPEs	Aquaculture sediment	<LOD			1.99	Aznar-Alemany et al., 2018
Greece		Aquaculture sediment	<LOD			24.4	
Italy		Aquaculture sediment	9.68			12.5	
Norway		Aquaculture sediment	1.08			25.8	
Portugal		Aquaculture sediment	1.06			58.2	
Spain		Aquaculture sediment	1.79			9.55	
United kingdom		Aquaculture sediment	37.9			15.2	
Sava river (Italy)	14 OPEs	River sediment				29.54	Köck-Schulmeye et al., 2021
Adige river (Croatia)		River sediment				55.88	
Ebrotas riv (Greece)		River sediment				8.95	

Table 1.6. Continued

Area (Country). *indicates protected area	Analyzed compounds	Matrix	PBDEs	PCBs	OCPs	OPEs	Refference
Yangzonghai lake (China)	19 OCPs	Lake sediment			0.3-4.5		Yuan et al., 2017
Nalón river (Spain)	10 OPEs	River sediment				15 (4.3-32)	Cristale et al., 2013
Arga river (Spain)	10 OPEs	River sediment				86 (3.8-292)	
Besos River (Spain)	10 OPEs, 8 PBDEs	River sediment	293 (<LOD-812)			261 (153-824)	
Western Scheldt estuary (Netherlands)	9 OPEs, 6 PBDEs	River sediment	9.86			19.2	Brandsma et al., 2015
Hainan Island (China)	10 OPEs, 17 PBDEs	Coastal riverine sediment	3.53			19.1	Mo et al., 2019
		Near-shore marine sediments	1.46			16.4	
		Off-shore marine sediments	2.31			15.1	
Miramane* (Itay)	40 PCBs	Marine sediment		84			Pozo et al., 2009
Punta campanella* (Italy)		Marine sediment		4			
Sant Stefano island* (Italy)		Marine sediment		0.3			
Liaohe River* (China)	12 PCBs	River sediment		0.12	30.3		Ke et al., 2018
Imam Khomeini Port, Shadegan* Persian Gulf (Iran)	7PCBs	Marine sediment		4.3			Zahed et al., 2009
Majidieh Khoor, Shadegan* Persian Gulf (Iran)		Marine sediment		45.8			
Mahshahr Port, Shadegan* Persian Gulf (Iran)		Marine sediment		16.35			
Musa Khoor Estuary, Shadegan* Persian Gulf (Iran)		Marine sediment		6.4			

According to the data from Table 1.6., it is observed that POPs and OPEs are ubiquitous in both in marine and freshwater sediments around the globe. In Asia, Chinese rivers have been reported to contain mean values of Σ_{10}OPEs from 31 ng/g dw in Xijiang river to 236 ng/g dw in the Pearl river (Zhujiang) (Wang et al., 2020a). Coastal sediments from Hainan island contained Σ_{10}OPEs ranging from 1 to 60 ng/g dw and PBDEs ranging from 0.39 to 12.9 (Mo et al., 2019). Riverine sediments from the Liao River showed levels of Σ_{13}OPEs ranging from 19.7 to 234 ng/g dw and riverine OPEs inputs to Bohai and Yellow sea was estimated at 16 tons/year (Hu et al., 2021). Marine sediments from the Korean coast were reported to contain PCBs (nd to 15 ng/g dw), DDTs (0.02 to 4.03 ng/g dw) and HCHs (nd to 3.76 ng/g dw) in 73, 54 and 25 % of the samples, respectively (Choi et al., 2011). In European river sediments from Spain reported levels were between 3.8 and 824 ng/g dw for 10 OPEs and from nd to 812 ng/g dw for 8 PBDEs (Cristale et al., 2013). Σ_8PBDEs levels were between nd and 16.7 ng/g dw in three river basin sediments from south Europe while Σ_{13}OPEs ranging between 0.31 and 549 ng/g dw were reported (Giulivo et al., 2017). Reported median levels of Σ_{15}PBDEs in freshwater sediments from North America were of 40, 320 and 12 ng/g dw in lakes Eire, Michigan and Superior, respectively (Zhu and Hites, 2004). Regarding the African continent, high POPs pollution levels have been also detected in sediments from Nigeria, with mean ΣPCBs in river sediments ranging from 688 to 2964 ng/g dw and ΣOCPs up to 5000 ng/g dw (Adebusuyi et al., 2022).

1.2.4. Soil

Soils cover a huge area of the earth´s surface and are a valuable resource. Their pollution status largely depends on the uses and anthropogenic pressures. For example, agricultural and rural soils are commonly exposed to pesticides or fertilizers but also from non-point sources (Sun et al., 2018). Urban and industrial soils, on the other hand, are more influenced by punctual sources and suffer rapid modifications in their uses (De Kimpe and Morel, 2000). Hydrophobic POPs tend to get adsorbed to the organic matter of the soil and, given a common source, their levels can be correlated with the amount of organic matter (Zhang et al., 2011). Other soil characteristics (e.g. particle size, porosity and black carbon content) can also affect the sorption of POPs but in a lesser extent (Ali et al., 2015). This sorption behavior has led to identify soils as primary sinks for non-polar POPs. Potential hazards are related with the mobility and bioavailability of different POPs and therefore soils also can act as secondary source when conditions change and remobilization occurs. Mobilization potential is decreased during time in a process called soil ageing until an equilibrium value (Vlková and Hofman, 2012). On the other hand, bioavailability is related with the accumulation potential in soil organisms and transfer to the trophic webs. Another important mechanism to consider soils as pollution sources is the volatilization in the air-soil interface, which is related to volatility and fugacity properties (Tao et al., 2008). There are evidences of the presence of POPs and OPEs in soils from different parts of the world (Table 1.7).

Most studies refer to a single chemical family and therefore comparisons are sometimes difficult to make. In the Czech Republic higher levels of DDT were detected in mountain soils (median of 46 ng/g dw) than in agricultural soils (median of 20.4 ng/g dw), which was attributed to atmospheric cold deposition (Holoubek et al., 2009). German forest soils showed similar distribution of DDTs and PCBs associated with historical local sources, with higher levels in more industrialized areas and PCB profiles related with Aroclor technical mixtures used in the past (Aichner et al., 2013). Few European studies have reported levels of OPEs in soils. Levels Lower than 10 ng/g dw were reported in grassland and forest soils from Poland and Germany, attributed to air deposition (Wang et al., 2020b). In China, higher OPEs levels (410 to 1,370 ng/g dw) have been found in urban soils near e-waste or plastic recycling sites in Tianjin and Guangzhou cities than those found in nearby agricultural and farmland areas (Cui et al., 2017, Wang et al., 2018a). Among the rest of continents, high levels of PCBs (up to 24 ng/g in soils near Okobaba in Nigeria), HCHs (up to 50 ng/g in soils from Ghana) or DDTs (up to 118 ng/g in soils also from Nigeria) have been reported in West Africa (Adebusuyi et al., 2022). 20 OPEs analyzed in American soils near an airport in New York ranged from 1.2 to 73 ng/g dw (Li et al 2019). Some monitoring studies have been also done in soils from different parts of Kuwait as a part of the National Implementation plan of the Stockholm Convention on POPs and levels between 10 and 200 ng/g were reported for PBDEs with BDE 209 as the major contributor, while OCPs were detected at lower concentrations, with just 2 out of 16 sites over the 1 ng/g dw level (Alshemmari, 2021).

Table 1.7. Pollution levels in soils expressed in ng/g dw.

Area (Country). * indicates protected area	Analyzed compounds	PBDEs	PCBs	OCPs	OPFRs	refference
Veneto* (Italy)	TCPP				0.11-11	Vecchiato et al., 2021
Albufera de Valencia* (Spain)	TCPP				14.1–89.7	Lorenzo et al., 2018
Wooded area (France)	8 PBDEs	1.2 (0.225- 5.11)				Muresan et al., 2010
Agricultural (France)	8 PBDEs	1.93 (0.242-43.9)				
Urban (France)	8 PBDEs	2.24 (0.324 -18.0				
Rural and background 2007 (Scotland)	7 PBDEs	0.856 (0.09-4.52)				Rhind et al., 2013
Rural and background 2008 (Scotland)	7 PBDEs	1.22 (0.107 -15.4)				
Rural and background 2009 (Scotland)	7 PBDEs	1.42 (0.09-10.5)				
Forest soils (Germany)	6 PCBs, 6 DDXs, Dieldin, HCB		18	153		Aichner et al., 2013
Alpine forest (Austria)	6 PCBs, 6 DDXs and HCB		3.3	8.3		Weiss et al., 2000
Mountain forest (Czech Republic)	7 PCBs, 3 DDXs, 4 HCHs, HCB		22.7	71.26		Holoubek et al., 2009
Grassland (Czech Republic)			6.31	11.74		
Agricultural (Czech Republic)			3.6	41.6		
E-waste site (China)	32 PCBs, 8 PBDEs	225 (13-1080)	38 (23-310)			Jiang et al., 2017
E-waste site (Pakistan)		699 (49-1010)	6630 (473-8780)			
Agricultural soil Shanghai (China)				26.5		Different studies compiled by Sun et al., 2018
Agricultural soil Guangdong (China)			18.4			
Agricultural soil Guangdong (China)			66			
Agricultural soil Jiangxi (China)			6.75	17.81		
Agricultural soil Sichuan (China)			1.005	4.91		
Agricultural soil Shaanxi (China)			0.67	6.2		
Agricultural soil Whole China (China)			0.424			
Indus river basin (Pakistan)	8 PBDEs (no BDE 209)	0.272 (0.047-2.377)				Ali et al., 2015

Table 1.7. Continued

Area (Country). * indicates protected area	Analyzed compounds	PBDEs	PCBs	OCPs	OPFRs	refference
Urban soils shanghai (China)	DDT, 4 HCHs				0.022 (0.002-0.117)	Liu and Meng, 2011
Haihe plain (China)	4 HCHs, 3 4,4´DDXs				67.5 (0.42-2700)	Tao et al., 2008
Tibetan plateau (China)	4 DDXs, 4 HCHs				2.2 (ND-4.74)	Fu et al., 2001
Fengjiang e-waste area (China)	7 m-PCBs		2310			Liu et al., 2020b
Binhai e-waste area (China)			27.8			
Farmland			69.9			
Urban park in Guangzhou (China)	11 OPEs				75	Cui et al., 2017
paddy field in Guangzhou (China)					110	
Urban residential area in Guangzhou (China)					180	
Open e-waste in Tianjin (China)	12 OPEs				696 (122-2100)	Wang et al., 2018a
Semi-closed e-waste in Tianjin (China)					116 (58.5-316)	
Farmland near e-waste in Tianjin (China)					56.3 (37.7-156)	
e-waste dismantling sites (India)	26 PCBs		6.5 (1.2-13.9)			Chakraborty et al., 2018
e-waste shredding sites (India)			8.2 (0.4-15)			
e-waste metal recovery sites (India)			148 (8.8-488)			
Open dumpsite (India)			3.4 (0.3-21)			
Municipal waste dumpsite (Cambodia)	14 PBDEs	32 (0.54-91)				Eguchi et al., 2013
River Basin (Pakistan)	8 PBDEs	0.272 (0.047-2.380)				Different studies compiled by McGrath et al., 2017
River Basin (Pakistan)	8 PBDEs	40 (0.6-500)				
Urban (Pakistan)	10 PBDEs	0.484 (0.120- 1.36)				
Agricultural (Pakistan)	10 PBDEs	0.11 (0.046- 0.219)				
Industrial (Pakistan)	10 PBDEs	0.681 (0.148- 1.91)				
Dumping site and surrounding areas (Pakistan)	8 PBDEs	1.55 (0.12- 8.93)				
Agriculture (Pakistan)	8 PBDEs	21.1 (6.88-37.7)				
E-waste site (Ghana)	16 PBDEs	54.8				
Background levels (Tanzania)	13 PBDEs	0.386				
Industrial (Mexico)	5 PBDEs	14.2 (1.8-127)				

Table 1.7. Continued

Area (Country). * indicates protected area	Analyzed compounds	PBDEs	PCBs	OCPs	OPFRs	refference
Agricultural (USA)	8 PBDEs	2.24 (<MQL-11)				
Rural background (Brasil)	15 PBDEs	0.99				
Polyurethane manufacturing (USA)	5 PBDEs	29.9				
Rice paddy (e-waste processing region) (Vietnam)	14 PBDEs, 7 OPEs	2.2			12.3	Matsukami et al., 2015
E-waste open burning site (Vietnam)		24			221.6	
E-waste recycling workshop (Vietnam)		1900			199.7	
Rural (Kenya)	7 PBDEs	3.15 (0.93-6.60)				Sun et al., 2016
Suburban (Kenya)	7 PBDEs	2.19 (1.12-4.20)				
Wildlife conservancy (Kenya)	7 PBDEs	1.03 (0.19-3.13)				
Rural (Kenya)	7 PBDEs	2.54 (0.25-14.1)				
Brick kiln industry (Mexico)	5 PBDEs	25 (1-122)				Perez-Vazquez et al., 2015a)
Urban (Mexico)	5 PBDEs	34.5 (2.25-98)				
Industrial (Mexico)	5 PBDEs	8 (1.6-17.8)				
Agricultural (Mexico)	5 PBDEs	16.6 (2.7-41.8)				
Flood plain (USA)	10 PBDEs	13.8 (0.94-51.1)				Yun et al., 2008
Flood plain (USA)	10 PBDEs	3.03 (0.09-19.4)				
Flood plain (USA)	10 PBDEs	0.88 (0.2-2.98)				
Airport in New York (USA)	17 OPEs				14.3 (1.16-73.1)	Li et al., 2019

1.2.5. Biota

This compartment covers the living organisms in the aquatic, terrestrial or aerial ecosystems. Contaminants are uptaken through diet, adsorbed through skin or through respiration (air breathing or through gills). Once uptaken, hydrophobic pollutants can be stored in fatty tissues, which may lead to biomagnification and bioacummulation properties while others can be metabolized.

Bioaccumulation refers to the process of gradual concentration of a given pollutant in the body of a living organism during time, which means that intakes are higher than elimination, which biologically occurs through metabolization and/or excretion of the pollutant. It has been defined by the Food and Agriculture Organization of the United Nations as “the net result of uptake, transformation and elimination of a substance in an organism due to all routes of exposure” (FAO, 2022). It is usually quantified by bioaccumulation or bioconcentration factors (BAF and BCF, respectively) as ratios between the concentration of the pollutant in the uptaking organism and the exposure source; while BAFs are defined for any exposure route, especially dietary, BCFs refers just to abiotic sources (Borgå, 2013).

Biomagnification refers to the process of concentration of a given pollutant along a trophic web, which implies higher pollution levels and therefore higher ecotoxicity potential in predatory species. It has been defined as “Sequence of processes in an ecosystem by which higher concentrations are attained in organisms at higher trophic levels” (FAO, 2022). It can be quantified in a single prey-predator relationship as the ratio of the concentrations in the predator and the pray as a biomagnification factor (BMF). However, trophic magnification factors (TMF) provide information on the magnification potential of a pollutant along more prey-predator steps and take into account the trophic level of studied organisms, evaluated as the proportion $^{15}N/^{14}N$ expressed as $\delta^{15}N$. TMFs are calculated as the antilogarithm of the slope of the linear regression resulting from the representation of the log-transformed concentration of the pollutant in different species versus the trophic level of the species ($\text{Log } C_i = (m \times TL) + n$; $TMF=10^m$) (Weijs et al., 2015). TMFs over 1 implies higher pollution burdens in higher trophic levels and therefore biomagnification processes are taking place, while TMFs lower than 1 implies biodilution.

POPs accumulate and transfer differently according to their physicochemical properties. In a congener's series like PCBs or PBDEs, accumulation and magnification potential can be related with the characteristics of the homologues through polarity, which is in turn related with other physicochemical properties such as K_{ow}, number of halogen substituents or molecular size. Generally, less polar compounds have higher TMFs, which have been positively correlated with log K_{ow} for OCPs, PCBs and PBDEs (Fremlin et al, 2020). However, polynomial regression was used to obtain a relation between TMFs and log K_{ow} for PBDEs in a marine food web in north China; TMFs increased with log K_{ow} until log K_{ow}=6.87 and then started to decrease (Shao et al., 2016). Chen et al. (2010a) found similar BMFs for PBDEs in a terrestrial food web in Virginia (USA) than those for PCBs and DDE. On the other hand, Kelly et al. (2008) found that recalcitrant PCBs were biomagnified in an Arctic marine food web while BDE 47 was the only PBDEs with TMFs significantly higher than one. Discrepancies among studies are related with differences in the biological system such as the nature of the food web (e.g. terrestrial or aquatic, benthic or pelagic etc), the trophic chain length (higher TMFs are found in longer chains), the migration behavior of different species or even variability among individual organisms (Kobayashi et al., 2015, Wang et al., 2021).

OPEs, on the other hand, can be transported by the plasma and distributed through the body. They are usually detected at higher levels in non-metabolic tissues like fatty ones where they are accumulated. Chlorinated OPEs with lower K_{OW} values than non-chlorinated ones have less affinity with lipidic tissues and have a weak or non-correlation with the lipid content while that correlation has been reported to be significant for aryl-OPEs such as TCPs or TPhP (Liu et al., 2019a). TEP and TnBP showed higher BCFs from water and sediment to different tissues in a riverine ecosystem in South Korea than the rest of studied OPEs (Choo et al., 2018). The bioaccumulation potential of OPEs show discrepancies among authors and a wide range of BCFs, encompassing 2 orders of magnitude, have been reported (Hou et al., 2016). These variations have been attributed to species and ecosystem differences but also to differences on physicochemical characteristics of OPEs; chlorinated OPEs generally showed lower BCFs while some aryl-OPEs can be classified as bioaccumulative under the REACH criterion (BCF>500) (European Union, 2008). Biomagnification potential of OPEs is also not clear. Differences in

TMFs among trophic webs, species, sex and individual OPEs have been described. Concretely two chlorinated OPEs, namely TCEP and TCPP, were found to bioaccumulate, with TMFs of 2.6 and 2.2 respectively, in an estuary benthic food web in the Netherlands, but all studied OPEs underwent trophic dilution in a pelagic food web from the same estuary (Brandsma et al., 2015). Total and individual TMFs of OPEs were found to be lower than 1 in a coral reef ecosystem from south China, indicating trophic dilution and attributed to the much lower metabolization of OPEs in plankton and coral than in fish (Ding et al., 2020). The same study suggested a latitudinal gradient of TMFs for different marine food webs where higher TMFs were found at higher latitudes. OPEs are rapidly metabolized to the corresponding di- and mono-esters, which are often used for exposure assessment. The metabolization process takes hours to few days depending on the species and the congener, and are commonly faster in chlorinated OPEs because phase II metabolic reactions can occur directly by dechlorination followed by glutathione conjugation even though they can also be metabolized previously by phase I O-dealkylation like aryl and alkyl OPEs (Hou et al., 2016).

1.2.6. Bioindicators of chemical pollution

Many organisms have been used as biomonitors of chemical pollution as they reflect the bioavailable fraction of pollutants. Mussels as well as other mollusks and bivalves have been used to study metals and organic pollutants in marine and freshwater ecosystems since late 70s because they accumulate pollutants in their tissues through filtration, but they have been also used as control organisms for environmental health issues such as population dynamics disturbance (Reguera et al., 2018, Strehse and Maser, 2020). Snails are also suitable as biomonitoring organisms mostly for terrestrial habitats but also for marine ecosystems, depending on the snail species; they are used to study soil, plants and air pollution in urban, rural and wild environments (Baroudi et al., 2020). For metals present in waters, a variety of organisms including algae, macrophites, zooplankton (including protozoa, crustacean, amphipod or copepod species), insects, bivalves, gastropods, fish or amphibians have been proposed to monitor Hg, Cd, Cr, Cu, Fe, Ni or Pb and potential associated effects (Zhou et al., 2008). Larger fish species or marine mammals are also useful to study the concentration and impact of pollutants in apex consumers. Some examples of these bioindicators are skin biopsies samples from whale sharks (*Rhincodon typus*) of Mexico to study POPs like PBDEs, PCBs or OCPs (Fossi et al., 2017), stranded striped dolphins (*Stenella coeruleoalba*) blubbers or fat tissues from the Mediterranean sea to study PBDEs, PCBs and PCDD/Fs (Capanni et al., 2020) or different elasmobranchs species such as sharks or rays to study the presence of metals, POPs and plastics and their potential effects through biomarkers in oceanic habitats, as reviewed by Alves et al. (2022). In marine mammals the levels of all POPs are much higher than in fish and denote that these compounds are bioacumulated and biomagnified along marine food webs, making these species very vulnerable.

Bioindicators are living organisms or biological responses able to reflect properties and/or changes in their surroundings. In the case of environmental pollution, bioindicators provide information about the pollution status of a given habitat, usually through accumulation of the studied pollutants or their degradation products. Desirable properties of bioindicators include easy sampling, wide distribution to enable comparisons among different ecosystems, long live span, have high tolerance to chemical pollution or a well characterized dose-effect relationship, occupy a high trophic level and capacity to reflect the pollution status of a sufficiently defined area (Michalak and Chojnacka, 2014).

Birds have been widely used as environmental bioindicators for chemical pollution, climate change, ecosystems composition and biodiversity or ecological strain factors because they comply with many desirable characteristics such as i) easy detection and observation in their natural environment, ii) well treated classification and easy identification, iii) wide distribution among habitats and ecological niches, iv) their distribution, abundance, habitat preference, biology, ecology and life history are well known and v) presence of different species in the top levels of the food web. But birds may also present some disadvantages with respect to other organisms because their mobility may affect the studied property if their habits and behaviors are not well defined and some species may be affected by environmental stressors in a different way than the studied ecosystem (Egwumah et al., 2017). Among birds, seagulls (Laridae genre) have been used as bioindicators for POPs because they have a long lifespan and are placed in high positions of the trophic webs so they accumulate pollutants through life and biomagnificate them through the trophic chain. Additionally, different gull species are distributed around the globe, making them a suitable species for biomonitoring and for comparison purposes. Lipophilic pollutants are uptaken through diet and annually transferred to the eggs, so eggs are excellent bioindicators of environmental pollution and represent a non-invasive biotic sample (Pastor et al., 1995). The first laid is the one with higher pollution levels and reflect recent inputs from the marine area where they get fed, while the second and third ones are influenced by endogenous reserves and therefore reflect the long term exposure (Vicente et al., 2015). Gulls breed in large colonies and they come back to the colony they were born to lay the eggs (a natural behavior called philopatry) so pollution found in eggs reflect the pollution status of the breeding area.

Several gull eggs biomonitoring studies from Europe have been published, including herring gulls (*Larus argentatus*), yellow legged gulls (*Larus michahellis*) and also the more vulnerable Audouin gull (*Larus audouinii*), among others, for monitoring of different POPs and evaluation of the temporal evolution and potential effects (Tongue et al., 2021). In North America, eggs of 4 laridae species have been used to monitor legacy and emerging pollutants in Atlantic and Pacific coasts of Canada and the Great Lakes (Chen et al., 2012, Su et al., 2015). In studies from South America, gull nests from the Argentinean Patagonia have been used to study the human influence through the presence of anthropogenic debris (Yorio et al., 2022). Black tailed gull (*Larus crassirostris*) eggs have been used to monitor chlorinated paraffins and OPEs in South Korea (Choo et al., 2022) or chlorinated POPs in Hokkaido, Japan (Choi et al., 2001). There are also few examples in Africa about metal pollution monitoring using Kelp gull (*Larus dominicanus*) eggs (van Aswegen et al., 2022). Studies reporting POPs and/or OPEs in bird eggs from different parts of the world have been compiled in Table 1.8. Most of the studies report the data in ng/g wet weight, but some of them are provided on lipid based, which makes comparisons difficult especially when the water or lipid content is not available from the original paper. In Table 1.8 data is provided as indicated in the bibliography.

Table 1.8. Occurrence of POPS and OPEs in bird eggs.

Analyzed compounds	Bird species	Concentration (median)	Concentration (range)	Units	Area (Country). * indicates protected area	Reference
7 PBDEs	Greateer flamingo	8.95	nd-25.9	ng/g lw	Doñana National Park * (Spain)	Baron et al., 2015
	Black kite	19.5	1.8-192	ng/g lw		
	White stork	41.4	2.05-251	ng/g lw		
9 PBDEs	Peregrine falcon	4400	1500-7300	ng/g lw	Great Lakes (aquatic) (Canada)	Guerra et al., 2012
		4300	530-38.000	ng/g lw	New Brunswik (terrestrial) (Canada)	
		1200	710-5300	ng/g lw	Bilbao (aquatic), (Spain)	
		227	160-3600	ng/g lw	Guadalajara (terrestrial) (Spain)	
32 PCBs	Common Eider	76.9	41.6-168	ng/gww	Røst (Norway)	Huber et al., 2015
	European Shag	161	145-167	ng/g ww	Røst (Norway)	
	Herring Gull	10012	9289-13379	ng/g ww	Røst (Norway)	
15 OCPs	Common Eider	54.8	33-104	ng/g ww	Røst (Norway)	
	European Shag	80.5	75-89	ng/g ww	Røst (Norway)	
	Herring Gull	535	455-709	ng/g ww	Røst (Norway)	
17 PBDEs	Common Eider	0.84	0.55-1.4	ng/g ww	Røst (Norway)	
	European Shag	3.29	2.46-3.87	ng/g ww	Røst (Norway)	
	Herring Gull	13.9	nd-16.8	ng/g ww	Røst (Norway)	
9 OPEs	Common hen (yolk)	3.6	2.2-6.8	ng/g ww	(Australia)	Li et al., 2020
28 PBDEs	White stork	0.83	0.214-9.5	ng/g ww	Doñana national park * (Spain)	Muñoz-Arnanz et al., 2011
		6.59	2.79-20-5	ng/g ww	Madrid (Spain)	
13 OPEs	Black tailed gull	499		ng/g lw	Baekryeong-do (Korea)	Choo et al., 2022
		391		ng/g lw	Hong-do (Korea)	
62 PCBs	Kelp gull	241		ng/g ww	Quiriquina Island (Chile)	Muñoz Cifuentes et al., 2003
		149		ng/g ww	Maiquillahue Bay (Chile)	
11 OCPs	Kelp gull	296		ng/g ww	Quiriquina Island (Chile)	
		307		ng/g ww	Maiquillahue Bay (Chile)	
7 m-PCBs	Neotropic cormorant	47.9		ng/g ww	Cruces River * (Chile)	

Table 1.8. Continued

Analyzed compounds	Bird species	Concentration (median)	Concentration (range)	Units	Area (Country). * indicates protected area	Reference
11 OCPs	Neotropic cormorant	198		ng/g ww	Quiriquina Island (Chile)	
		122		ng/g ww	Cruces River * (Chile)	
		111		ng/g ww	Quiriquina Island (Chile)	
62 PCBs	Brown-hooded gull	181		ng/g ww	Teja island (Chile)	
11 OCPs	Brown-hooded gull	187		ng/g ww	Angachilla River (Chile)	
		142		ng/g ww	Puerto Varas (Chile)	
		963		ng/g ww	Teja island (Chile)	
		1067		ng/g ww	Angachilla River (Chile)	
		508		ng/g ww	Puerto Varas (Chile)	
62 PCBs	Pink-footed Shearwater	163		ng/g ww	Mocha island (Chile)	
		102		ng/g ww	Juan Fernandez islands (Chile)	
11 OCPs	Pink-footed Shearwater	267		ng/g ww	Mocha island (Chile)	
		271		ng/g ww	Juan Fernandez islands (Chile)	
26 PBDEs	Glaucus gull	76.8	59.5–470	ng/g lw	Chuckchi Sea (Alaska)	Vander Pol et al., 2009
		92.8	51.1–142	ng/g lw	Bering Sea (Alaska)	
		348	243–4130	ng/g lw	Gulf of Alaska	
		61.4	47.2–99.2	ng/g lw	Bering Sea (Alaska)	
36 PCBs	Glaucus gull	1720	1620–2500	ng/g lw	Chuckchi Sea (Alaska)	
		1690	1580–2280	ng/g lw	Bering Sea (Alaska)	
		1710	700–2920	ng/g lw	Gulf of Alaska	
		1400	1130–3870	ng/g lw	Bering Sea (Alaska)	
4 HCHs	Glaucus gull	278	194–313	ng/g lw	Chuckchi Sea (Alaska)	
		215	98.3–274	ng/g lw	Bering Sea (Alaska)	
		116	33.0–221	ng/g lw	Gulf of Alaska	
		184	85.6–243	ng/g lw	Bering Sea (Alaska)	
DDXs (4,4´-isomers)	Glaucus gull	1470	1280–1910	ng/g lw	Chuckchi Sea (Alaska)	
		1590	1040–2020	ng/g lw	Bering Sea (Alaska)	

Table 1.8. Continued

Analyzed compounds	Bird species	Concentration (median)	Concentration (range)	Units	Area (Country). * indicates protected area	Reference
		1960	594–3700	ng/g lw	Gulf of Alaska	
		1060	893–3780	ng/g lw	Bering Sea (Alaska)	
12 PCBs	Ivory gull	246	244–248	ng/g lw	Seymour Island (Canada)	Lucia et al., 2015
		1284	399–1996	ng/g lw	Station Nord (Greenland)	
		1363	881–2557	ng/g lw	Svenskøya, Svalvard (Norway)	
		2658	1276–3389	ng/g lw	Nagurskoe (Russia)	
		1359	825–1772	ng/g lw	Cape Klyuv (Russia)	
		823	420–3318	ng/g lw	Domashny (Russia)	
4,4´-DDT	Ivory gull	11.4	8.8–14.0	ng/g lw	Seymour Island (Canada)	
		6.3	1.6–41.2	ng/g lw	Station Nord (Greenland)	
		22.7	10.1–47.0	ng/g lw	Svenskøya, Svalvard (Norway)	
		40.9	32.4–45.7	ng/g lw	Nagurskoe (Russia)	
		32.5	20.9–69.5	ng/g lw	Cape Klyuv (Russia)	
		21.2	18.3–45.1	ng/g lw	Domashny (Russia)	
4,4´-DDE	Ivory gull	1098	1034–1161	ng/g lw	Seymour Island (Canada)	
		1450	483–3621	ng/g lw	Station Nord (Greenland)	
		1328	933–3239	ng/g lw	Svenskøya, Svalvard (Norway)	
		3177	1532–3864	ng/g lw	Nagurskoe (Russia)	
		1366	813–1915	ng/g lw	Cape Klyuv (Russia)	
		984	341–7407	ng/g lw	Domashny (Russia)	
5 PBDEs	Ivory gull	391	3.67–4.14	ng/g lw	Seymour Island (Canada)	
		15.6	4.01–26.5	ng/g lw	Station Nord (Greenland)	
		11.8	8.21–20.6	ng/g lw	Svenskøya, Svalvard (Norway)	
		26.6	14.5–55.3	ng/g lw	Nagurskoe (Russia)	
		23.5	6.57–32.9	ng/g lw	Cape Klyuv (Russia)	
		3.97	2.10–6.08	ng/g lw	Domashny (Russia)	
8 PBDEs	Great black-backed gull	640	46–2100	ng/g lw	west Scotland landfill (United Kingdom)	Tongue et al., 2021
	Herring gull	780	18–2900	ng/g lw	west Scotland landfill (United Kingdom)	

Table 1.8. Continued

Analyzed compounds	Bird species	Concentration (median)	Concentration (range)	Units	Area (Country). * indicates protected area	Reference
	Lesser black-backed gull	1100	52–6800	ng/g lw	west Scotland landfill (United Kingdom)	
5 OPEs	Herring gull	0.93	0.6-4.91	ng/g ww	Laurential great lakes (Canada)	Chen et al., 2012
7PCBs	Yellow-legged gull	191	124-565	ng/g ww	Cies islands * (Spain)	Viñas et al., 2020 (SI)
		723	580-858	ng/g ww	Vigo (Spain)	
4,4´-DDXs		30.3	22.5-69.4	ng/g ww	Cies islands * (Spain)	
		202	183-273	ng/g ww	Vigo (Spain)	
HCB		4.56	0.43-7.13	ng/g ww	Cies islands * (Spain)	
		5.33	1.14-9.08	ng/g ww	Vigo (Spain)	
9 PBDEs (no BI 209)		11.42	3.54-29.7	ng/g ww	Cies islands * (Spain)	
		20.66	19.59-27.7	ng/g ww	Vigo (Spain)	

Table 1.8 shows that bird eggs are excellent biomonitors of POP as they have contamination patterns characteristic of a given habitat. This pattern reflects the anthropogenic pollution of each study area and indicates that other species sharing habitat can be equally affected by POP pollution. Thus, the use of gull eggs as bioindicators is very adequate to determine the geographical and temporal trends of the pollution caused by POPs (Colomer-Vidal et al., 2022). Finally, this information can be used to define conservation actions to be implemented in areas of high ecological interest.

1.3. Chemical analysis

1.3.1. Sampling and sample pretreatment

Sampling is an important step of the analytical process which aims to obtain the most representative possible sample for a measured property. To extract robust conclusions, the sample has to be representative of the study area. The techniques and sampling protocols depend on the matrix and the property to be measured. For soil pollution assessment, simple sampling techniques such as soil collection with a scoop, a shovel o a screw are commonly used. Surface or top soils (horizon A) are commonly sampled because they reflect recent pollution inputs. The very first layer, containing grass, small vegetation and other kind of residues which are considered as impurities is usually discarded (Orgiazzi et al., 2018, Wu et al., 2022). Surface sediments are harder to obtain and Van Veen or other drags are needed (Lian et al., 2022b, Lorenzo et al., 2019). Sampling with stainless steel shovels have also been reported to simplify the sampling procedure (An et al., 2022). Often pooled samples are used to increase the representativeness of the sample. Other techniques for soil and sediment sampling include core sampling, which encompass a range of depths. This sampling is useful to study temporal trends or punctual discharges in a wider time lapse. The dating of each depth is related with the sedimentation or soil deposition rate (Liu et al., 2016). Samples are stored in precleaned containers, usually glass ones to ensure inertness, transported in coolers or portable fridges and stored at low temperatures until analysis.

Water samples are collected with grab samplers and generally from the middle course of the water body and dosed in preferably glass bottles to avoid external contamination from the plastic material. Special caution is needed to store samples in cold conditions to avoid degradability of target contaminants.

For biotic samples, a variety of techniques are available depending on the organism to be studied and the sample type. When the whole organism is sampled (e.g. small fishes or birds, mollusks or small amphibians) handmade traps or nets are commonly used (Fu et al., 2020). Many studies rely on local markets to obtain edible samples (Andersen et al., 2015, Castro-Jiménez et al., 2021, Govaerts et al., 2018). Naturally dead or heavily injured individuals are also an adequate alternative because of the reduction on the disturbance (Luo et al., 2009, Ranjbar Jafarabadi et al., 2021). When possible, less invasive techniques like capture, sample collection (e.g. feathers or blood) and further release of the individual organism are used (Fernie et al., 2017, Moreno et al., 2011). Bird eggs are grabbed directly from nests and usually the first laid one is selected when possible (Van Asvegen et al., 2019).

Samples are transported to the laboratory and stored at low temperature or frozen. Previously to sample extraction, a preparation step is performed. The sample pretreatment is usually conducted to assure the matrix suitability for further extraction and clean-up. Common pretreatments include water elimination by freeze drying techniques (liophylization), homogenization of the sample to ensure the reproducibility of the results, sieving in the case of soils and sediments or filtration in the case of water samples.

1.3.2. Extraction and clean-up processes

Sample treatment is an important step aiming to obtaining an extract where pollutants are preconcentrated and quantitatively transferred, while impurities and potential interfering substances are discarded. It is usually performed in two steps, namely extraction and clean-up or purification. The extraction is a process which transfers the analytes from the original matrix to a less complex one, usually an organic solvent. Several techniques such as Soxhlet extraction, ultrasound assisted extraction (UAE), pressurized liquid extractions (PLE) and Quechers are used.

Soxhlet extraction is a widely used technique due to its suitability for a wide range of pollutants and matrixes, however it has some disadvantages; it is high time (12 to 36 h) and solvent consuming (100 to 300 mL) and is not suitable for thermo labile analytes (analytes should not be degraded at the boiling point of the solvent mixture or lower temperature). The sample is placed in a cellulose or glass thimble together with a desiccant agent (typically Na_2SO_4) and placed in the extraction chamber. Solvents such as acetone, dichloromethane (DCM) or hexane or solvents mixtures are selected based on the physicochemical characteristics of the analytes. The extraction solvent enters the extraction chamber as a gas which is then condensed over the thimble containing the sample. Then, solvent refluxes to the boiling flask through a siphon arm after being accumulated in the extraction chamber, finishing a full cycle. Several cycles ensure the quantitative extraction from the sample to the solvent. Soxhlet extraction has been used as a standard technique for POPs extraction but is nowadays being substituted by less time and solvent consuming techniques such as PLE (Xu et al., 2013). It has also been successfully applied for OPEs determination in different abiotic matrixes such as soil, beach sand and sediments (Jimenez-Skrzypek et al., 2021, Lee et al., 2018).

Ultrasound assisted extraction (UAE) is a simple, fast, cheap and low solvent consuming method. It is based on the distribution equilibrium of a given pollutant between the solid matrix and a solvent. It is based on the improvement of the extraction efficiency due to the acoustic cavitation phenomenon, which consist in the formation of micro bubbles caused by the expansion and compression of the molecules of a liquid exposed to sonic waves over 20 kHz and under 10 MHz frequency. Those micro bubbles rapidly grow and collapse, generating turbulences at a microscopic level, inter-particle collisions and agitation in the micro porous of the extracted matrix, which accelerates and improves the diffusion processes (Tiwari, 2015). The sample needs to be previously dried and homogenized, decreasing particle size and increasing the specific surface and therefore, the solid-liquid contact area. Extraction solvents are added and extraction is performed by vortexing and placing the sample in an ultrasound bath typically for 10 min at room temperature (time and temperature can be modified to fulfill method requirements). This process can be repeated twice to 3 times with fresh solvent to assure the quantitative transfer of analytes to the liquid phase. Most important parameters improving efficiency of

UAE are the sonication time, the liquid/solid ratio and the temperature but high temperatures may produce thermal degradations for more labile compounds so room temperature is usually chosen to ensure a quantitative extraction of the analytes (Albero et al., 2019). Used solvents or solvent mixtures would also affect the method performance and a similar polarity than analytes should be selected. Commonly used solvents include hexane, DCM, acetone, acetonitrile (ACN), ethanol (EtOH), methanol (MeOH) or water depending on the chromatographic technique to be used. This extraction method offers some advantages such as low solvent and time consumption and suitability for thermo labile compounds with respect to Soxhlet extraction or easier operation and lower apparatus costs with respect to PLE.

PLE is a technique where the contact between the extractant and the matrix is enhanced, increasing the diffusion and desorption rates, allowing low solvent volumes and extraction times. For POPs, extraction temperatures are commonly over 100 ^{0}C and extraction times are short, usually ranging from 5 to 25 minutes. In PLE techniques, high pressure above 1000 psi is commonly used (Xu et al., 2013). PLE has also been applied for OPEs analysis in solid matrixes such as sediment or sludge (Pantelaki and Vousta, 2019). The extraction process is normally repeated twice or three times with fresh solvents to ensure the quantitative extraction of analytes. This technique is characterized for being reproducible, time-effective and efficient. However, many interferents are coextracted and an efficient clean-up step is needed.

Quechers stands for Quick, Easy, Cheap, Effective, Rugged, and Safe. This method aims to simplify to the maximum the sample treatment process, with minimal sample manipulation and minimize economic and time costs. It comprises two main steps, namely extraction or partitioning and clean-up by dispersive SPE, with centrifugation and separation of the liquid and solid phases after each step. Quechers method was originally developed for pesticide analysis in agricultural products, but they have evolved and can be applied for several analytes such as POPs, polycyclic aromatic hydrocarbons (PAHs) or pharmaceutical and personal care products (PPCPs) in different environmental matrixes (Kim et al., 2019).

Once the analytes have been extracted from the matrix, a clean-up or purification process is needed in order to eliminate all the potentially interfering substances in the analysis, which commonly include lipids and pigments. Lipids removal can be enhanced with acid treatment (usually sulfuric acid, H_2SO_4) through acid wash or using an acid pretreated sorbent during the clean-up. However it should be applied with caution because side reactions with analytes may occur, degrading them and impeding their subsequent instrumental determination or reducing the recovery rates (Muir and Sverko, 2006). OPEs are not suitable for acid treatment and lipid removal can be problematic for biotic samples. An additional step between the extraction and the clean-up can be performed to remove lipids and improve the clean-up results. This key step consists in a freeze lipid precipitation and removal, which reduce the amount of potential interfering substances. The extracted solvent is evaporated and a polar solvent (MeOH) is added previous to the freezing step which allows to discard most of the lipid content in the solid phase (Liu et al., 2018).

In soil and sediment extraction, coextracted sulfur is also problematic because it can affect the method performance but mostly because it may damage MS analyzers, reducing their lifetime. Sulfur removal is commonly done with acid activated copper either during extraction or as a pre clean-up step (Mechlińska et al., 2012).

The clean-up process is usually made by using sorbents where analytes and interferents are retained followed by an elution step to quantitatively desorb pollutants while potential interferents are retained in the sorbent. For this process, solid phase extraction (SPE) is commonly used. Sorbents such as florisil, alumina, silica or hydrophilic lipophilic balance (HLB) have been successfully applied for the analysis of a wide variety of pollutants in different matrixes. SPE commercial serially produced cartridges containing different amounts (from milligrams to grams) are available and used according to the nature of analytes and the lipid content of the original matrix (Reddy et al., 2019). They provide high reproducibility, are easy to use and reduce the sample treatment time. On the other hand, laboratory made chromatographic columns with a variety of sorbents have been successfully applied both for POPs and OPEs (Alkan et al., 2021). They permit the use of multiple layers containing different sorbents such as silica (often acid pretreated), C18 phases or alumina depending on the nature of the extract as well as to select used amounts of each of them (Chung and Chen, 2011).

After the clean-up step, the extract volume is reduced by evaporation under N_2 flow to typically 100-1000 μL prior to instrumental analysis. To reduce potential evaporation of volatile analytes during this process, a high boiling point organic solvent (e.g. isooctane or toluene), compatible for posterior chromatographic analysis is added (Dąbrowski, 2015).

1.3.3. GC-MS analysis

Gas chromatography (GC) is the separation technique based on the analytes partition between a stationary and a mobile phase. POPs and OPEs are GC-amenable compounds but liquid chromatography methods have also been proposed for OPEs, especially when their metabolites (organophosphorous diesters) are analyzed (Chung and Chen, 2011, Pantelaki and Voutsa, 2020). For the analysis of POPs, electron capture detectors (ECD) were widely used during 1970s-90s decades because they contain electronegative atoms which produce high signals at low concentrations. ECD were progressively substituted by mass spectrometry analyzers because of the structural information they provide, which allows quantifying coeluted compounds with different mass spectrums and enables their identification with high certainty.

The extract containing the analytes is injected in the GC-MS system and several injection modes such as direct injection, on-column injection, split injection or splitless-split mode are available. Splitless-split is the most commonly used one. In this mode the injector vents are closed during a certain period of time (splitless) and are opened afterwards (split) allowing reproducible results for compounds present at low concentration in samples. Main factors to control are the injection temperature and the injected volume, but the splitess time has been demonstrated to also affect high molecular weight PBDEs (Björklund et al., 2004). Most studies use injection volumes of 1-2 µL using splitless injection mode for the analysis of organic micropollutants which are usually found at trace levels in environmental samples. Higher injection volumes produce higher signal and therefore higher sensitivity and lower detection and/or quantification limits, which can be achieved using programmed temperature vaporization injection. The temperature of the liner which connects the needle with the head of the column also needs to be set according to the analyzed compounds physicochemical characteristics. Higher temperatures produce more effective vaporization of the analytes but thermal degradation processes can occur. Typically, temperatures from 225 to 300 ^{0}C are chosen as a compromise between quantitative vaporization and minimization of thermal degradation (Badea et al., 2020).

After injection, the analytes flow through the chromatographic column at different speed depending on their vapor pressure, being separated from each other. To achieve a good separation, the temperature gradient and a proper column should be selected. Temperature gradient should comprise all the analytes boiling points to assure their volatilization but without exceeding the column limits to avoid excessive bleeding and column deterioration. Lower slopes (^{0}C/min) provide better separations but also longer running times and wider peaks so an overall compromise must be considered (Lepper et al., 2020).

GC columns have evolved rapidly since the last century and nowadays fused silica capillary columns have demonstrated good performance in terms of separation efficiency and reproducibility, stability at high temperatures, and suitability for different stationary phases. The stationary phase influences the analytes retention times depending on the interactions between them and therefore a stationary phase with chemical similarities with target compounds increases the interactions, retention and ultimately the separation between analytes. Dimethyl polysiloxane stationary phase columns modified with a 5% of phenyl groups to enhance the π interactions are commonly used for POPs and OPEs (Pantelaki and Vousta, 2019, Xu et al., 2013). Physical characteristics of the column such as length, internal diameter and stationary film thickness are critical to achieve a good chromatographic resolution. 0.25 mm internal diameter columns with film thickness of 0.25 μm permit good retention capacity and high temperature resistance, typically over 300 ^{0}C, and therefore low bleeding of the column and longer durability. 15 m length columns enable detection of big molecular mass PBDEs while efficient separation of other congeners is not affected (Mametov et al., 2019). Standard columns of 30 m are usually needed for multiresidue methods for POPs and OPEs to obtain a good separation of analytes, often resulting in longer running times. Even 60 m long columns have been used to avoid coelutions when analyzing a large congener series like PCBs (Muir and Sverko, 2006).

For POPs and OPEs analysis, GC is coupled to several MS analyzers. In the MS detection the intensity of the signal is proportional to the amount of ions produced and the instrument is able to discriminate those ions based on the mass to charge ratio (m/z). Most widely used MS instruments consist in quadrupoles mass analyzers mostly because they have reasonable prices while keeping a good resolution power, enough for the discrimination of ions with m/z differences as small as 1 amu. However, high resolution mass spectrometry analyzers such as Orbitrap or Time of Fight (TOF) permits lower m/z discrimination which together with spectral libraries comparison enable non-target screening and distinguish congeners such as PCDD/Fs, PBDD/Fs or dioxin like-PCBs in multiresidue methods (Badea et al., 2020, Bichon et al., 2015).

As mass spectrometry analyzers need a previous ionization step, electron ionization (EI) and chemical ionization (CI) are commonly used as ionization sources depending on the analytes to be monitored. EI is a strong ion source, and molecules coming from the GC separation are exposed to a potential difference of 70 eV, which usually does not produce molecular ions in the spectrum and rather produces fragment ions. Those fragments are characteristic of each molecule and provide structural information for unequivocal identification. Chromatographic coelution problems can be avoided due to the high specificity that it provides and it also enables isotopic dilution (because can discriminate isotopes such as D (deuterium, H^2) or C^{13} for a better quantification of target compounds. However, isotopic dilution determination for all studied compounds is not economically viable due to the high costs of commercially synthesized isotopically labeled compounds and most of them are quantified using similar isotopically labeled surrogates (e.g. $13C^{13}$ 4,4´-DDE for the quantification of DDT isomers and degradation products) or non-environmentally relevant compounds as surrogate standards such as PCB 65. This ionization source is commonly used for the determination of OCPs, PCBs and OPEs. On the other hand, NCI has been used for PBDEs determination because it allows lower detection limits than EI with the disadvantage that it provides nearly non-structural information because the common ion $[Br]^-$ (m/z 79 and 81) is used to quantify all congeners except for BDE 209 and just chromatographic retention time is used for identification purposes (Petrovic et al., 2007).

Once the ions have been formed, they are analyzed in the mass spectrometer. Quadrupole type ion analyzers are constituted by four metallic bars which create specific electromagnetic fields in order to stabilize certain ions of interest for further detection. Single quadrupole instruments can be operated in full scan mode in ranges typically from 70 to 500 amu to determine all formed ions or in selected ion monitoring (SIM) mode for identification and quantification of analytes by selecting the specific fragment ions of each compound. Single quadrupole provides high noise/baseline chromatograms, which produce higher detection and quantification limits, and the signal is less specific and can be affected by matrix interferences. Triple quadrupole instruments (QqQ or tandem mass spectrometry (MS/MS)) enable a first ion selection in Q1 followed by a second fragmentation in Q2 which acts as a collision cell prior to a second ion selection in the third quadrupole (Q3). MS/MS enables the monitoring of fragmentation reactions (single reactions, SRM, or multiple, MRM) which are determined by the chemical structure of the analyte, providing chromatograms with nearly no noise and allowing very low detection and quantification limits. However, method optimization requires some additional steps:

- The ion selection in Q1 (precursor/parent ion selection) is a common optimization step for triple quadrupole instruments. In a tentative first analysis, all produced ions for each target compound are scanned and the ones with higher produced signals are selected. A compromise between signal intensity and fragments ions should be adopted and specific ions with high m/z are selected as they are more selective and specific and are not affected by the matrix or by other ions which may interfere with the analyte signal and therefore lead to wrong quantification or even identification of the target compounds.
- Precursor ion fragmentation in Q2 is tested with different collision energies to select the most specific and intense ion. Collision energies in Q2 are optimized in the range from 5 to 50 eV. Generally, increasing collision energies increase the fragmentation and thus the signal intensity of smaller product ions.
- Ion selection in the Q3 (product/daughter ion) can be done following two strategies (which should provide similar results) to find the optimum product ion and collision energy: i) set the third quadruple in scan mode (product ion

scan), so all product ions are detected, or ii) select potential MRM to monitor and test different collision energies.

- Dwell, scan cycles and peak definition and intensity optimization. Ions behavior inside the quadrupole depends on their m/z ratio and therefore different ions cannot be selected at the exact same time. Dwell refers to the time (measured in ms) spent in the detection of a concrete ion. Higher times provide higher signal but increase the scan cycle time. The chromatogram is actually not a continuous line but rather one point is drawn every scan cycle. The scan cycle time is the sum of dwell times for each monitored reaction in a given chromatographic window. In order to obtain well defined peaks, at least 10-15 points should be drawn so total scan time should be 10-15 times lower than the thinnest peak width at the base. Dwell can be adjusted consequently.

During recent years, improvements in GC such as tandem GC (GCxGC), also called bidimensional GC, has been introduced to fully characterize complex mixtures of organic contaminants in environmental matrices. GCxGC is based on a double chromatographic separation; analytes are separated by two columns with different stationary phases to distinguish potential coelutions in the single peaks obtained by the first separation. A two dimension surface is obtained as the chromatogram. It is usually coupled with TOF analyzers and allows the determination of coeluted compounds and non-target screening methods (Stefanuto et al., 2021). Finally, the advent of GC-OrbitrapMS has increased the scope of POPs and OPEs analysis by its hability to perform full scan at high resolution. It allows the non-target analysis so that contaminants in a sample are fully characterized (Pico et al., 2020).

1.4. Study areas

1.4.1. Protected areas

Protected areas have been defined by the International Union for the Conservation of Nature (IUCN) as “the places where we aim to retain the extraordinary beauty and richness of the Earth and all its benefits to humanity, the evolutionary heritage of more than four billion years and for all we know unique in the vastness of the universe” (Worboys, 2015). However a more recent, less-poetical and more practical definition is also available: “a protected area is a geographical clearly defined space, recognized, dedicated and managed, through legal or other efficient kinds of resources/tools to achieve the long term conservation of nature and its ecosystems services and associated cultural values” (Dudley, 2008).

Protected areas legislations were developed during final decades of the last century. However, some natural landscapes have been protected and conserved traditionally during history for cultural or religious reasons. Good examples are sacred Tibetan forests from Yunnan province (China), which have act as *de facto* protected areas becoming biodiversity hotspots with very low anthropogenic influence (Brandt et al., 2015). Nowadays, the most recognizable legal figure of environmental protection is National Park (NP). The first NP was declared in Yellowstone (USA) more than 100 years ago, in 1872, and it was followed by Abysko and Garphyttan national parks in Europe. Since then, the earth´s surface covered by protected areas has been increased gradually, and each country has developed its legislation individually. Nowadays standard IUCN categories can be used as a reference to establish comparisons and equivalences with national legal denominations. Table 1.9 summarizes the above mentioned categories:

Table 1.9. IUCN categorization of protected areas.

IUCN category	Denomination
Ia	Strict nature reserve
Ib	Wilderness area
II	National park
III	Natural monument or feature
IV	Habitat or species management area
V	Protected landscape or seascape
VI	Protected areas with sustainable use of natural resources

Even though the protection objectives are not the same in each category, all protected areas must fulfill the given definitions, but they are also meant to:

- Conserve the composition, structure, function and evolutionary potential of biodiversity.
- Contribute to regional conservation strategies (as core reserves, buffer zones, corridors, stepping-stones for migratory species etc.).
- Maintain diversity of landscape or habitat and of associated species and ecosystems.

- Be of sufficient size to ensure the integrity and long-term maintenance of the specified conservation targets or be capable of being increased to achieve this end.
- Maintain the values for which it was assigned in perpetuity.
- Be operating under the guidance of a management plan, and a monitoring and evaluation programme that supports adaptive management.
- Possess a clear and equitable governance system.

In Spain, the first national park´s law is from 1916, two years before the establishment of “Montaña de Covadonga” and “Ordesa y Monte Perdido” NPs (Real Decreto de 16 de agosto de 1918). Figure 1.10 shows the location of differently categorized protected areas in Spain.

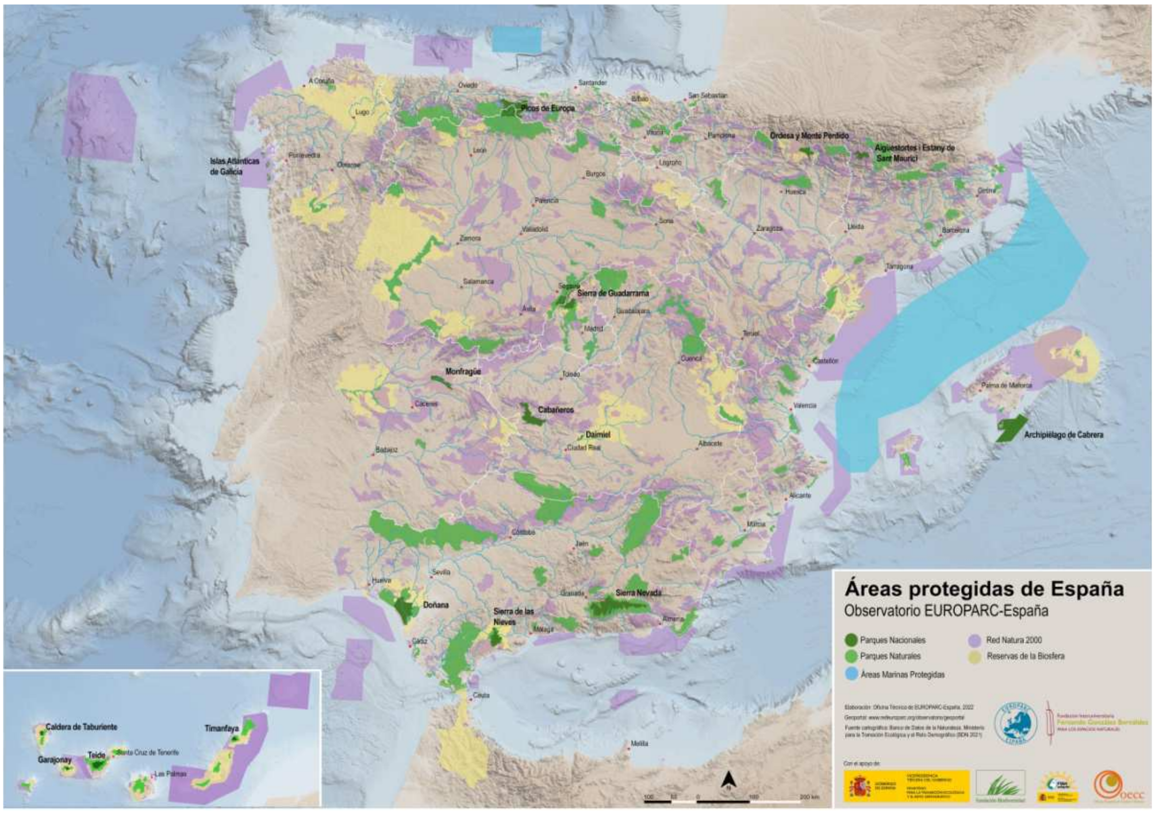


Figure 1.10. Protected areas in Spain. Extracted from Europarc, 2022.

There are 1.958 protected areas in Spain, including 15 NPs (IUCN category II), 151 natural parks and 290 natural reserves. All together (including the “Natura 2000” net for which Spain is the major European contributor), they cover more than 25% of the terrestrial area and nearly 10% of the marine one; NPs cover an area of 385.000 ha and had a governmental budget of 79 million € in 2014 (Múgica de la Guerra et al., 2017).

The development of China´s protected areas framework was previous to the IUCN categorization and the designation of Chinese protected areas differs considerably. In a homogeneization attempt Zhang et al. (2017) proposed equivalences between both systems. Table 1.10 compiles the Chinese categorization, the governmental agencies responsible of the management and the proposed equivalence with IUCN categories.

Table 1.10. Equivalences of protected areas categories between Chinese and IUCN systems, adapted from Zhang et al (2017). Used acronyms: State Forestry Administration (SFA), Ministry of Environmental Protection (MEP), State Oceanic Administration (SOA), Ministry of Housing and Urban-Rural Development (MOHURD), Ministry of Land and Resources (MLR), Ministry of Water Resources (MWR), Ministry of Agriculture (MOA), Chinese Academy of Sciences (CAS) State Oceanic Administration (SOA).

Protected areas of China	IUCN categories	Chinese governmental agency
Nature Reserve (the core zone and buffer zone)	Ia, Ib or III	SFA, MEP, SOA, MOHURD, MLR, MWR, MOA, CAS
Nature Reserve (the experimental zone)	V or VI	SFA, MEP, SOA, MOHURD, MLR, MWR, MOA, CAS
National Park Pilot	II	The Government of Yunnan Province
Scenic Spot	II, III, V	MOHURD
National Forest Park of China	V	SFA
National Wetland Park of China	II or V	SFA
National Geopark of China	III	MLR
Special Marine Reserve	III, IV, V or VI	SOA
Community-based Conservation Area	IV	Coordinated by SFA
No-logging natural forest area	V	SFA
No-grazing grassland area	V	MOA
National Aquatic Germplasm Resource Conservation Zone	VI	MOA

In China, the first protected area was established in Dinghu Shan (Guangdong province) as a national nature reserve in 1956 (Miller-Rushing, 2017), but the first national park meeting the IUCN standards was Pudacuo in 2007, which was based on an already existing natural reserve, and categorized in type II under IUCN classification (Ma, 2013). Nowadays there are 2740 Nature Reserves in China (Figure 1.11) which covers nearly 15% of the territory (142 million ha) and 4.56 million ha of Marine Protected Areas. The number of Protected Areas as well as the covered territory has increased during last decades, especially during late 1990s and early 2000s, but it was followed by a slight decrease in protected surface area after

2005. The decrease in the covered area was related with individual management and in many cases was due to legal redefinition of the protected area borders in an attempt to better exploit the associated natural resources (Huang et al., 2019).

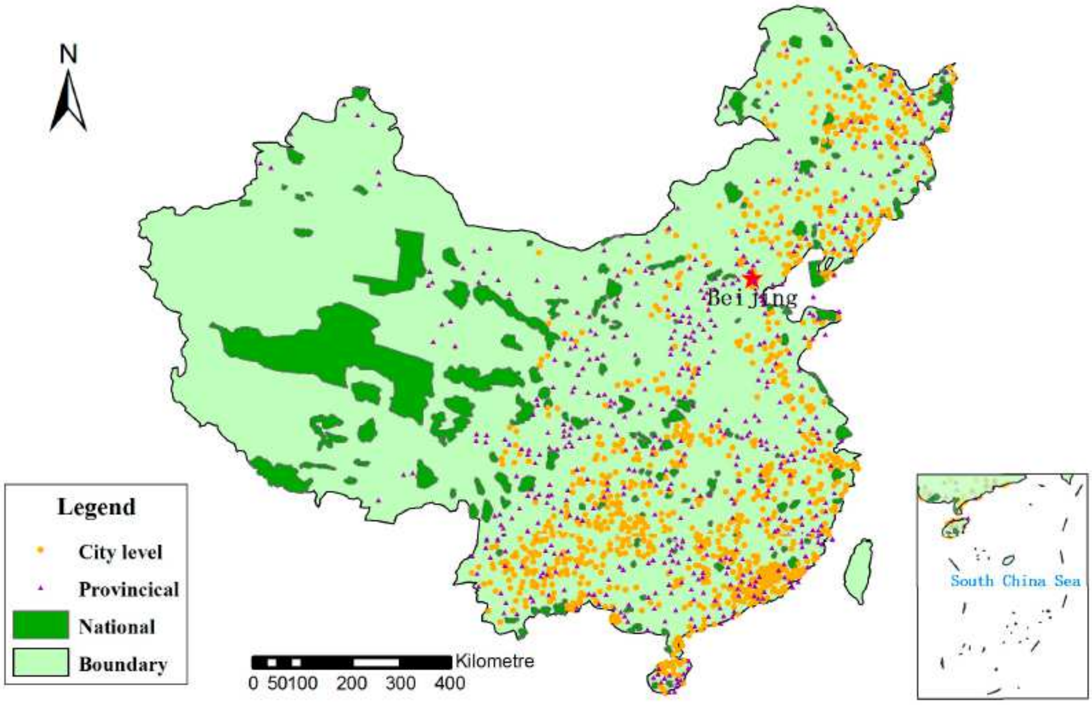


Figure 1.11. Protected areas in Peoples Republic of China. Extracted from Cao et al., 2015.

Despite the legal environmental protection and restrictions, several anthropogenic activities are performed inside or in the surroundings and may affect the quality and temporal evolution of the ecosystems (Xu et al., 2016).

Studies about organic pollution in protected areas around the world are scarce mostly due to the assumption that they are pristine areas because no industrial activities are allowed inside them and therefore no ecotoxicological impacts are expected to occur. However, some studies have pointed to the necessity to increase the available knowledge on the pollution status of protected areas to improve their management and conservation strategies (Abessa et al., 2018). In this Thesis, the pollution status of protected areas in Spain and South China has been evaluated and each area is briefly descripted down below.

The Montgrí, Medes Islands and Baix Ter Natural Park was created with the main objective of unifying previous protection regulations. It is an area with a unique landscape, made up of a coastline with cliffs, coves and beaches that penetrate into seabeds of unquestionable ecological value, a mountain massif that rises between the alluvial plains of El Baix and L'Alt Empordà and the wetlands formed by the river Ter. In this Natural Park, birds form a group of special interest, both for the protected species that find refuge and for their diversity. Between the Montgrí and the Medes Islands, 189 taxa have been counted, among which seabirds and some birds of prey stand out. Rupicolous birds are also well represented. On the coastal strips of the massif and on the islands, various species of birds of prey such as the peregrine falcon (*Falco peregrinus*), the common kestrel (*Falco tinnunculus*), the eagle owl (*Bubo bubo*) and the jackdaw (*Corvus monedula*) find refuge. The yellow-legged gull (*Larus michaellis*) is undoubtedly the most abundant bird on the islands, with a population of 7200 pairs and forms one of the most numerous colonies in the Mediterranean.

The Ebro Delta Natural Park is a wetland area, a highly productive environment on which countless species of plants and animals depend for their survival. The marshes perform many vital functions, such as water storage, protection against storms, stabilization of the coast, recycling of nutrients, etc. The confluence of the marine and continental environments gives rise to a high diversity of fish species (around 50). As far as birds are concerned, the number of specimens varies between 50,000 and 100,000 distributed in some 330 species, representing 60% of the species in Europe, and with a local nomenclature that includes some 250 names, making it one of the richest in the world. It is home to some of the most important seabird breeding colonies in the Mediterranean. It has been declared a Special Protection Area for Birds (SPA) and was included in the List of Wetlands of International Importance (Ramsar) on 26 March 1993. The Delta's avifauna is the most characteristic and its quantitative and qualitative importance goes beyond local interest and is of great international importance. For this reason, the Ebro Delta is profiled in various conventions as an area of maximum interest for breeding colonies, anatidae, waders and seabirds, and for the passage of migratory wintering birds, always included in category A, of urgent protection priority. The number of pairs *of L. michahellis* is of 9744 and for *L. audouinii* of 14177.

The Columbretes Islands Natural Park is formed by four little groups of volcanic islands, located 49 km in front of Castellón. This archipelago, due to its isolation and excellent state of conservation, is home to several animal and plant species rare in the rest of the Mediterranean, such as the Audouin's gull, the Eleonora's falcon or the very rare Reseda hookeri, and a considerable number of species unique in the world, such as the Columbretes lizard or the tree alfalfa. They hosted around 500 yellow-legged gull pairs in 2008 (Molina and Bermejo, 2009).

Mar Menor is located in Murcia autonomous community (south-east Spain). It hosts several colonies of yellow-legged gull located in some islands and saltpans reserves, a salty lagoon just separated from the sea by a sandy strip where many touristic activities take place. In this reserve breed >8000 yellow-legged gull pairs (Molina and Bermejo, 2009).

The Chafarinas Islands have been declared a "National Hunting Refuge" since 1979, and subsequently declared a SPA. Due to their isolation and rocky configuration, the Chafarinas Islands are home to important colonies of birds, considered until recently to be in danger of extinction. The yellow-legged gull is an abundant and generalist species that shares a breeding colony with the scarcer and more specialized Audouin's gull on the Chafarinas Islands. Since the Chafarinas Islands were declared a National Hunting Reserve, the traditional collection of gull eggs, practiced by the islanders and people from Melilla who came by boat, was prohibited. This led to an immediate increase in seabird populations. Today more than 7000 pairs of yellow-legged gulls have been identified.

The Berlengas is a small archipelago of 3 granite islands, which lie to the west of Cabo Carvoeiro in Peniche, 10–15 km off the coast of Portugal. Considered a Nature Reserve, the natural beauty of this small archipelago is incredible due to the minimal human intervention. It has been considered a protected area since 1465, when King Afonso V forbade any kind of actions against nature there, and since 2011, it has been declared a UNESCO World Biosphere Reserve. The Berlengas are home to colonies of herring gulls, crested cormorants, peregrine falcons, among others. Berlengas host around 8500 breeding pairs of yellow-legged gulls (Ceia et al., 2014).

National Park of the Atlantic Islands of Galicia is located off the Galician Rías Baixas and are an area of extraordinary ecological wealth. Due to the high natural value of this area and the rapid deterioration it was suffering from human activities, it was declared a National Park in 2002. The archipelagos of Ons, Sálvora and Cortegada represent examples of Atlantic marine ecosystems of great biological richness. The scarce human presence and the availability of good breeding sites and, above all, the abundance of marine resources make this National Park an ideal shelter for a wide variety of species, especially marine species. In this sense, the archipelago of Ons and Cies are classified as ZEPA (Special Protection Areas for Birds), in accordance with Directive 79/409/EEC. In the Atlantic Islands, this gull is the most abundant and emblematic species, with a population of 30,000 breeding pairs, one of the largest in the world.

Heshan National Field Research Station of Forest Ecosystem is located in the Heshan county (Guangdong province) and was established in 1984 by the Heshan Institute of Forestry and the South China Institute of Botany, nowadays Guangzhou Botanical Garden from the Chinese Academy of Science. It is part of the National Ecological and Environmental Field Research Station Network. It shows a subtropical monsoon climate with mean annual precipitations of 1700 mm and mean temperatures of 21.7 0C (Fu et al., 2011). Its evergreen broad-leaved forest is dominated by the Schima superba and Michelia macclurei tree species (Hu et al., 2018). Research here is mainly focused on ecological restoration and ecosystem management, hosting successful reforestation projects during the last decades (Wang et al., 2010). It is the area of this study with the lowest environmental protection degree. 17.1% of the territory is used for agricultural activities, and furniture production facilities are present in its surroundings. Its main problems nowadays are soil erosion and vegetation degradation.

Pudacuo National Park was set in 2007 as the first national park in China meeting the International Union for Conservation of Nature (IUCN) standards and was based on an already-existing nature reserve. It covers a mountainous area of 2000 km2 at 3400 m above sea level and is part of the UNESCO´s world natural heritage site. It holds a huge plants and mammals biodiversity in the high mountain ecosystems. Conservation actions are aimed at achieving a sustainable and balanced management between tourism, economic development and the ecosystems and biodiversity preservation (Xinhua News Agency, 2007, Zhou and Chen, 2005).

Gaoqiao Mangrove National Natural Reserve is one of the multiple mangrove forests in Zhangjian, Suixi County, located in the west south of Guangdong province, close to the neighbor province of Guangxi. Its climate is subtropical monsoon marine, with mean temperatures of 23.2 0C and mean annual precipitations of 1168 mm (Gao et al., 2019). It has a huge influence in the coastal dynamics, linking terrestrial and marine environments, reducing the erosion and impact of ocean tides in the shore and receiving nutrients not just from the river upstream but from the regular seawater floodings.

Danxia Shan National Park is located in the Renhua County, north of Guangdong province. It is a UNESCO world heritage site since 2010 and holds the national park and natural reserve titles as well as 5A touristic attraction. Located in the Renhua county, north part of Guangdong province, it covers an area of 290 Km2 and its climate can be classified as subtropical with monsoon influence, with average temperature of 19.7 0C and average daily precipitations of 1715 mm. Geologically, it was formed 140 to 65 million years ago by fluvial deposition which in turn generated today´s eroded sedimentary red sandstone landscape. Several studies have been conducted about it is geological formation and evolution. It has been termed as “a red terrestrial clasolite characterized by the red walls and cliffs” which turns into an isolation and differential evolution of plants and animals in the hilltops (Wu et al., 2008).

1.4.2. E-waste sites

E-waste refers to discarded, obsolete, broken or out of use electrical or electronic equipment (EEE) which includes any device with a battery or a power cord. EEE was legally defined by the European Parlament (2003) as “equipment which is dependent on electric currents or electromagnetic fields in order to work properly, and equipment for the generation, transfer and measurement of such currents” and e-waste includes “all components, subassemblies and consumables which are part of the product at the time of discarding”. E-waste can be classified in 6 groups which comprise different amount of the global generated e-waste (Balde et al., 2017): temperature exchange equipment (17%), screen and monitors (14.8%), lamps (8.7%), large equipment (1.6%), which include washing machines, photovoltaic panels or electric stoves among others, small equipment (36.7%) like microwaves, video cameras, toasters etc. and small IT (20.4%) such as cell phones or tablets.

Globally from 20,000 to 50,000 kt of e-waste is generate every year of which USA is the major producer. For instance, in 2016, nearly 45,000 kt of e-waste, more than

6 kg/year per inhabitant were generated in the USA and it was expected to be increased to more than 50 million tons in 2021, representing 6.8 kg/year per inhabitant. In Europe, 8,700 kt of e-waste are generated each year while in China, more than 11,000 kt of e-waste were generated in 2012. The increase in e-waste generation worldwide is related with economic growth in developing countries but also with the short electronic innovation cycles, which lead to faster obsolescence of EEE and a diminution in their acceptable life span, which decreased from 4 to 2 years in the case of personal computers (Perkins et al., 2014). Most of the e-waste generated in developed countries is exported to developing ones, mostly in Asia and Africa and the most important receiving countries are China, India, Pakistan and Nigeria. Even though formal international policies like the Basel Convention aim to ban and control e-waste exportations, legal loopholes permit those shipments under figures such as donations, second hand markets and recycling purposes (UNEP, 1992). Concretely in China, local and central governments have developed laws and enforcements for banning and controlling e-waste imports since 2002, but it still occurs in illegal ways (Shinkuma et al., 2009). In developed countries a small percentage of the e-waste is processed in a formal and controlled way but the remaining (75% approximately in Europe and 80% in USA) is not registered and ends dumped into landfills, incinerated or unofficially exported. The waste export is economically profitable for both parts. On one hand, the big labor costs and strict environmental policies and regulations for waste disposal in developed countries increase the costs of proper e-waste management. On the other hand, developing countries receive short-term economic benefits such as employment opportunities and contribution to the raw materials market. Even more, countries receiving e-waste, even in formal, semi-formal or non-formal ways may impose fees on weight basis, contributing to the administration budget. As an example, China used to impose a 50$ fee per imported ton of e-waste in the early 1990s (Sthiannopkao and Wong, 2013). Chinese imports of e-wastes rose from 1 million tons in the early 1990s to 17.5 million tons in 2000. In e-waste, 30% by weight are valuable metals Ag, Pd, Pt, Ge, Ru, Rh, Al, Sn or Cu which can be recovered and used as new raw materials or shelled (Islam et al., 2020). Some precious metals are present in e-waste at higher proportions than natural ores; for gold, natural ores contain 0.5 to

13.5 g/ton which is lower than the proportion in e-waste, which ranges from 10 to 1000 g/ton (Tipre et al., 2021).

Commonly used techniques for the non-formal e-waste recycling in developing countries are mechanical dismantling, like hammer smashing or cable peeling to recover copper, open burning to calcine plastics for later recover metals or even acid washes and baths (Wu et al., 2019). These recycling techniques lead to high pollution inputs for the surrounding environment. Common pollutants associated with those activities are flame retardants such as PBDEs, OPEs, alternative flame retardants, other halogenated flame retardants, combustion byproducts like PCDD/Fs, PAHs, Pb, Zn, Cu or Cd, chlorinated paraffins or PCBs metals that could not be recovered (Guan et al., 2020, Luo et al., 2020, Zhao et al., 2009).

China´s fast economic growth during last decades have led to an increase on EEE consumption and disposal, which, together with above mentioned imports of e-waste have generated a considerable environmental problem, especially in areas where non-formal e-waste recycling are among the major economical activity, usually after agriculture and livestock. In order to deal with non-formal e-waste recycling and dismantling associated problems, 3 major legal enforcements have been set in China, namely Circular Economy Promotion Law, Solid Waste Pollution Control Law and Clean Production Promotion Law. They provide a framework with guidelines and recommendations about pollution prevention during the whole lifecycle of EEE in a similar way than European directives and involve various governmental agencies and/or ministries, which develop more specific laws. National agencies involved include the National Development and Reform Commission, the Ministry of Environmental Protection, the Ministry of Industry and Information Technology or the Ministry of Finance, and developed laws include corrective actions regarding increases on resources efficiency, restrictions in hazardous substances used during production or producers responsibility on the recovery of obsolete EEE. However, some loopholes have been also identified; territorial restrictions are just applied to mainland China, which makes Hong Kong one of the main entrance ports for illegal e-waste imports and effective regulation is lacking or poorly defined (Lu et al., 2014).

The number of formal e-waste recycling facilities in China is growing during recent years due to above mentioned legal improvements, which also include license expeditions and subsidies for companies which comply with the legal framework. In 2015 there were 109 companies legally qualified for the disassembling and disposal of EEE. However, 3 big non-formal e-waste recycling areas remain and are known to be associated with environmental problems (Figure 1.12).

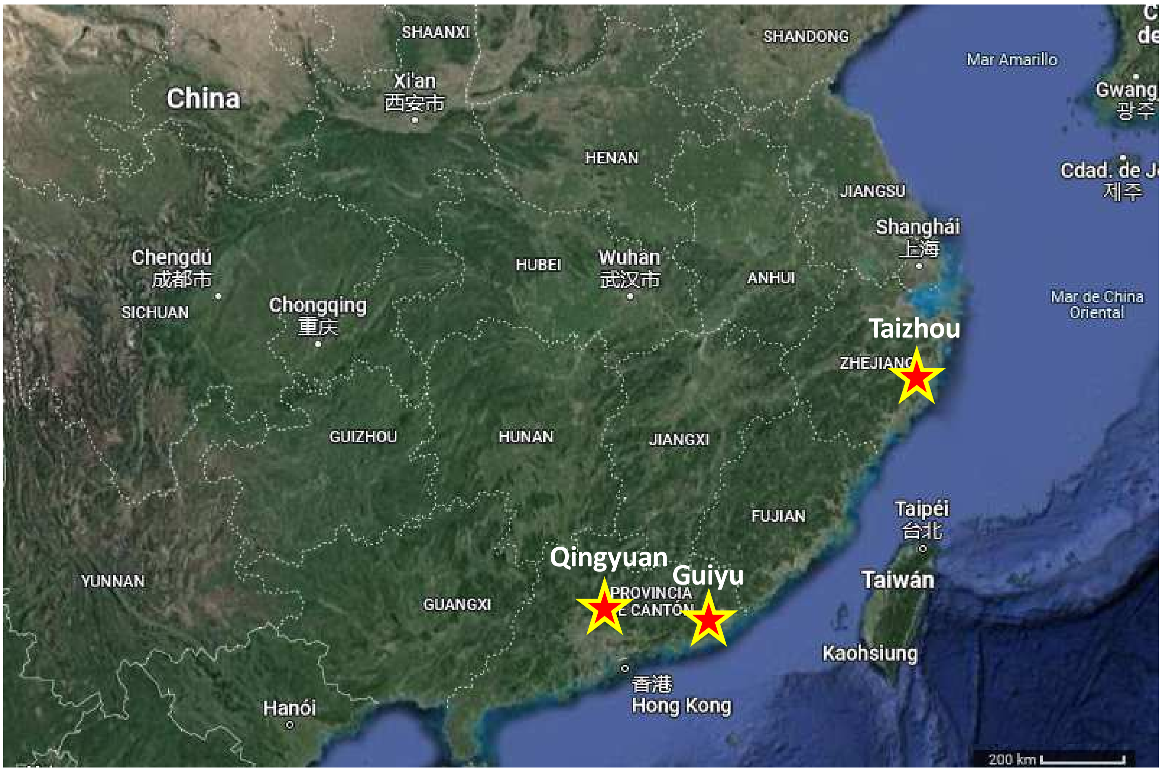


Figure 1.12. The 3 biggest and more environmentally problematic e-waste areas in China.

In Guiyu, located in the east of Guangdong province, these activities started in the 1980s decade and grew in the following years. More than 6,000 workshops were established, attracting workers from other sectors due to the high volumes of e-waste treated there (Zeng et al., 2016). It has been considered as the paradigmatic example of an e-waste area, the biggest in China, with more than 500 kt of e-waste processed each year which represent an industry valued at 1,000 million Chinese yuans (more than 130 million US dollars), and a major receiver of e-waste from North America (Chan et al., 2013). The area also hosts reparation and renovation of EEE workshops, which, together with the dismantling, form what local government has called an “industrial chain extension”. Some of these activities have been ceased since 2013 due to the installation of a new and modern industrial park. However, non-treated EEE as well as scraps and other residues remains there after workshops or even little villages are abandoned (Wu et al., 2019).

Taizhou is a small town of 400,000 inhabitants located in the Zhejiang province, east China. E-waste recycling activities started in Taizhou and surrounding villages in the late 1970s mostly with PCB-containing capacitors coming from inside the country. In the early 1990s decade, the dismantling of imported transformers and capacitors started, occupying more than 10 % of the local population and a total of more than 2,200 kt of e-waste have been treated in the area (Chan et al., 2007, Li and Achal, 2020).

Qingyuan is another big e-waste area in south China, center of Guangdong province. More than 1,000 kt of e-waste have been treated there since 1990 and more than 12 kt of non-recyclable waste were produced annually in around 1,300 workshops which employ almost 80,000 people (Han et al., 2019). EEE treated there is varied and include cell phones, printed circuit boards, transformers, capacitors or even heavy machinery (Zeng et al., 2016).

Released pollutants associated to e-waste sites are spread in the surrounding environment, being detected in soils, sediments, water, indoor and outdoor dust, wild and domestic animals and even humans. For human health, main exposure pathways include direct exposure through inhalation of air pollutants or polluted dust particles, through skin contact or through ingestion of polluted food. These exposures are of special concern firstly for workers which are in close contact with the e-waste and polluted environments in the workshops; secondly for children living in e-waste cities or villages, whith potential health risks over 8 times higher than adults and thirdly for the general population inhabiting those cities or villages. In fact, human exposures in e-waste hotspots are often above reference levels established by national or international organisms such as WHO or Food and Agriculture Organization of the United Nations (FAO) (Li and Achal, 2020). Associated health issues and illnesses depends on the toxicity of each pollutant and include endocrine disruption, especially affecting the thyroid system, reproductive toxicity and birth defects, affections on the immune system, neurotoxic effects, developmental issues etc. The impact of e-waste released pollutants depends mostly on the types of recycled e-waste, recycling techniques, the nature of released substances and their environmental fate but also from environmental factors such as the habitats and characteristic fauna and flora or climatologic conditions.

Baihe Tang, a little village located in the Qingyuan County, has been studied in this Thesis and represents an example of environmental and human health impact of e-waste non-formal recycling activities and the distribution and fate of associated pollutants. The presence of flame retardants in water and sediment samples, in soils from agricultural crops and in domestic and wild fauna reveals that the whole ecosystem is being affected. E-waste recycling areas like Baihe village are so called hotspots due to the high pollution rates derived from the methods used to recover high market value materials from all kinds of EEE. The environmental impact is in fact a problem and a cause of concern to scientists and policy makers but they also create a field laboratory to better understand the behavior of pollutants associated to e-waste in real environmental conditions. Several studies have been performed in this area during the last two decades. Main results are summarized in Table 1.11.

Table 1.11. Compilation of various studies performed in Baihe village and found pollution levels.

Year	Reference	Matrix	PCBs	OCPs	PBDEs	OPEs	Other pollutants
2015	Zheng et al.	Indoor dust	7 PCBs (median 74-2900 ng/g)		8 PBDEs (median 685-23600 ng/g)	8 OPFRs (median 2180- 6750 ng/g)	
2016	Zeng et al.	Surface particles	159 PCBs (660–180,000 ng/g dw)		17 PBDEs (130–1,100,000)		Chloroparaffins (30000-890000 ng/g dw), OH-FRs (62,000–140,000 ng/g dw)
2009	Luo et al.	Bird muscle (5 species)	54 PCBs (median 10000-120000 ng/g lw)	OCPs; DDTs + HCHs (medians 500-4500 ng/g lw)	13 PBDEs (median 37-2200 ng/g lw)		
2014	Sun. et al.	Light vented bulbuls muscle	median 34 PCBs (7300 ng/g lw)	median DDTs (170 ng/g lw)			
2019	Peng et al.	Kingfisher muscle	60 PCBs (median 2100- 1500000 ng/g lw)		14 PBDEs (2100 - 130000 ng/g lw)		
2008	Wu et al.	Water	44 PCBs (196–206 ng/L)		18 PBDEs (23.8–25.0 ng/L)		
		Aquatic organisms	44 PCBs (20.2–25900 ng/g ww)		18 PBDEs (46.6–1072 ng/g ww)		
2019b	Liu et al.	Water snake				9 OPFRs (mean 1.9 ng/g ww)	
		Water snake eggs				9 OPFRs (mean 12 ng/g ww)	
		Common carp				9 OPFRs (mean 14 ng/g ww)	
2020a	Liu et al.	Frog muscle				15 OPFRs (0.62-15 ng/g ww)	
		Frog eggs and gonads				15 OPFRs (2.2-59 ng/g ww)	
2018	Liu et al.	Bird eggs	30 PCBs (2.8e5-3.5e5 ng/g lw)	3 DDTs (220-230 ng/g lw)	17 PBDEs (2.4e3-2.8e3 ng/g lw)		
		Watersnake eggs	30 PCBs (1.4e5-2.3e5 ng/g lw)	3 DDTs (640-1200 ng/g lw)	17 PBDEs (4e3-8.2e3 ng/g lw)		
		Aquatic organisms	30 PCBs (5.9e4-1.8e5 ng/g lw)	3 DDTs (260-490 ng/g lw)	17 PBDEs (4.8e3-1.6e4 ng/glw)		
		Amphibious organisms	30 PCBs (3.1e3-1.1e4 ng/g lw)	3 DDTs (24-3200 ng/g lw)	17 PBDEs (210-1400 ng/g lw)		
		Terrrestrial organisms (no lizards)	31 PCBs (2.8e4-2.8e5 ng/g lw)	3 DDTs (2500-8500 ng/g lw)	17 PBDEs (2.7e3-1.3e4 ng/g lw)		

Table 1.11. Continued

Year	Reference	Matrix	PCBs	OCPs	PBDEs	OPEs	Other pollutants
2012	Zheng et al.	Hen eggs			19 PBDEs (2640–14100 ng/g lw)		PBBs (700–1620 ng/g lw) HBCDs (44–350 ng/g lw), AHFRs (720–3920 ng/g lw)
2018	Zeng et al.	Hen eggs	159 PCBs (326-8870 ng/g lw) (median 1130-1360 ng/g lw)				
2018	Huang et al.	Hen eggs 2010			18 PBDEs (mean 14,100±3220 ng/g lw)		
		Hen eggs 2013			18 PBDEs (mean 4736±959 ng/g lw)		
		Hen eggs 2016			18 PBDEs (mean 4741±619ng/g lw)		
2014	Wang et al.	Roots			55 PBDEs (3.3-94.3 ng/g dw)		
		Leaves			55 PBDEs (4.7-45.1 ng/g dw)		
		Soil			55 PBDEs (13.9-13250 ng/g dw)		
2019a	Liu et al.	Water				10 OPFRs (sum 255 ng/L)	
		Sediments				10 OPFRs (sum 135 ng/g dw)	
		Aquatic organisms				10 OPFRs (sum 4.3-34 ng/g ww)	

The main pollution reservoir in the Bahie village is a natural pond, where non profitable subproducts have been dumped during years, covering the whole bottom part and even raising the opposite shore of the pond creating a “deposition plastic sedimentary delta”. Pollutants contained in those subproducts are distributed by chemical equilibriums in the system formed by the original matrix (mostly plastic debris), the organic sediments and the water as reported by Liu et al. (2019a) for OPEs. This equilibrium is mostly affected by the pollutant polarity (normally expressed as K_{ow}) and the organic carbon content of the solid matrix. Then pollutants are introduced in the food webs from the sediments to small aquatic filter-feeders organisms or from the water to other kind of aquatic organisms through skin respiration or gills. Much higher levels of PBDEs and PCBs were found in aquatic organisms than water from the polluted pond due to their hydrophobicity (Wu et al., 2008). Bioaccumulation factors were always >1 which means that these pollutants tend to accumulate preferently in biota rather than in water. BAFs linearly increased with K_{ow}, both expressed as logarithms, for PBDEs, but followed a parabolic curve for PCBs. Once introduced to the food webs, POPs are transported along the food web, suffering a biomagnification process in most cases although trophic dilution has also been described and linked to either metabolization or excretion; TMFs were lower for PBDEs (3 out of 18 higher than 1) than for same substitution degree PCBs (45 out of 53 higher than 1) and followed a parabolic dependence with K_{ow} for PCBs in a freshwater food web (Wu et al., 2009). On the other hand, OPEs underwent trophic dilution between carps and their predator, watersnakes, in the polluted pond and metabolic processes played an important role (Liu et al., 2019b). In that same study, the maternal transfer to the watersnake eggs was reported to be positively correlated with K_{ow} for compounds with $\log K_{ow}<6$ and some differences between chlorinated and non-chlorinated OPEs were described. The tissue distribution, BCFs between water and biota, biota-sediment accumulation factors (BSAFs) and TMFs for OPEs were also investigated in this freshwater food web (Liu et al., 2019a). Studied OPEs generally showed higher concentrations in livers, gills and kidneys and were positively correlated with lipid content. BCFs ranged from 3.6 to 1,109, while logarithmic transformed BSAF ranged from -2 to 0.41, both following significant correlation with K_{ow} of studied OPEs. TMF for total OPEs were of 0.72, suggesting biodilution effects when all studied OPEs were considered together.

Pollutants are also affecting terrestrial food webs through amphibians and predation. High levels of PCBs and PBDEs have been detected in various species of frogs and toads, lizards and birds (Table 1.11) (Liu et al., 2018, Luo et al., 2009, Peng et al., 2019, Sun et al., 2014). Parental transfer and sex differences have been also described for OPFRs in frogs and negatively correlated with K_{ow} in the case of maternal transfer ratio (Liu et al., 2020a).

Other ways for the pollutants to enter the food web can be through the plant-soil system in polluted crops or through free ranged hens picking plastics and other residues while they get fed, also affecting humans (Wang et al., 2014, Zeng et al., 2018). PBDEs were 10 to 100 times higher in hen eggs from the e-waste sites than those from the control site, both collected in 2010, and estimated daily intakes (EDI) in human beings ranged from 4,200 to 20,000 ng/day (Zheng et al., 2012). In eggs collected in 2013 and 2016, PCBs daily intake was above the minimal risk levels for adults in 70% of the samples and for children in all the samples (Zeng et al., 2018). Same samples were also evaluated for PBDEs and EDIs were not over the reference those established by EPA, but BDE47 and BDE99 showed a margin of exposure (MOE) lower than 2.5, which implies potential health risks for humans and wildlife (Huang et al., 2018). Finally, another way for these pollutants to enter human bodies is the involuntary breathing and/or ingestion of fine particles or polluted air, which effects on human health are of special concern for workers and habitants of Baihe village (Zeng et al., 2016, Zheng et al., 2015).

Even though Baihe village is a well documented e-waste hotspot several research projects are still ongoing in order to better understand the behavior of e-waste associated organic pollutants, which mainly include flame retardants and plastisizers, the potential impacts for environmental and human health and their time evolution. In this Thesis, this site has been studied to identify the pollution source and determine the partitioning of OPEs in the sediment-water system and the accumulation of POPs and OPEs in soils and hen eggs.

References

Abbasi, G., Li, L., Breivik, K., 2019. Global Historical Stocks and Emissions of PBDEs. Environ Sci Technol 53, 6330-6340, 10.1021/acs.est.8b07032

Abessa, D.M.S., Albuquerque, H.C., Morais, L.G., Araujo, G.S., Fonseca, T.G., Cruz, A.C.F., Campos, B.G., Camargo, J., Gusso-Choueri, P.K., Perina, F.C., Choueri, R.B., Buruaem, L.M., 2018. Pollution status of marine protected areas worldwide and the consequent toxic effects are unknown. Environ Pollut 243, 1450-1459, 10.1016/j.envpol.2018.09.129

Adebusuyi, A.T., Sojinu, S.O., Aleshinloye, A.O., 2022. The prevalence of persistent organic pollutants (POPs) in West Africa – A review. Environ Challen 7, 100486, 10.1016/j.envc.2022.100486

Ahmad, H.A., Ahmad, S., Cui, Q., Wang, Z., Wei, H., Chen, X., Ni, S.Q., Ismail, S., Awad, H.M., Tawfik, A., 2022. The environmental distribution and removal of emerging pollutants, highlighting the importance of using microbes as a potential degrader: A review. Sci Total Environ 809, 151926, 10.1016/j.scitotenv.2021.151926

Aichner, B., Bussian, B., Lehnik-Habrink, P., Hein, S., 2013. Levels and spatial distribution of persistent organic pollutants in the environment: a case study of German forest soils. Environ Sci Technol 47, 12703-12714, 10.1021/es4019833

Albero, B., Tadeo, J.L., Pérez, R.A., 2019. Ultrasound-assisted extraction of organic contaminants. TrAC, Trends Anal Chem 118, 739-750, 10.1016/j.trac.2019.07.007

Ali, U., Mahmood, A., Syed, J.H., Li, J., Zhang, G., Katsoyiannis, A., Jones, K.C., Malik, R.N., 2015. Assessing the combined influence of TOC and black carbon in soil-air partitioning of PBDEs and DPs from the Indus River Basin, Pakistan. Environ Pollut 201, 131-140, 10.1016/j.envpol.2015.02.035

Ali, U., Sweetman, A.J., Riaz, R., Li, J., Zhang, G., Jones, K.C., Malik, R.N., 2018. Sedimentary black carbon and organochlorines in Lesser Himalayan Region of Pakistan: Relationship along the altitude. Sci Total Environ 621, 1568-1580, 10.1016/j.scitotenv.2017.10.071

Alkan, N., Alkan, A., Castro-Jimenez, J., Royer, F., Papillon, L., Ourgaud, M., Sempere, R., 2021. Environmental occurrence of phthalate and organophosphate esters in sediments across the Gulf of Lion (NW Mediterranean Sea). Sci Total Environ 760, 143412, 10.1016/j.scitotenv.2020.143412

Alshemmari, H., 2021. Inventories and assessment of POPs in the State of Kuwait as a basis for Stockholm Convention implementation. Emerg Contam 7, 88-98, 10.1016/j.emcon.2021.02.003

Alves, L.M.F., Lemos, M.F.L., Cabral, H., Novais, S.C., 2022. Elasmobranchs as bioindicators of pollution in the marine environment. Mar Pollut Bull 176, 113418, 10.1016/j.marpolbul.2022.113418

An, N., Yu, L., Yan, L., Yang, D., 2022. Assessment of some trace elements accumulation in Karst lake sediment and Procambarus clarkii, in Guizhou province, China. Ecotox Environ Safe 237, 113536, 10.1016/j.ecoenv.2022.113536

Andersen, M.S., Fuglei, E., Konig, M., Lipasti, I., Pedersen, A.O., Polder, A., Yoccoz, N.G., Routti, H., 2015. Levels and temporal trends of persistent organic pollutants (POPs) in arctic foxes (Vulpes lagopus) from Svalbard in relation to dietary habits and food availability. Sci Total Environ 511, 112-122, 10.1016/j.scitotenv.2014.12.039

Andrae, N.J., 2007. Durable and Environmentally Friendly Flame Retardants for Synthetics. M.Sc. Thesis, North Carolina State University, Raleigh, North Carolina.

Antolín Rodriguez, Juan Manuel, 2011. Influencia en la acumulación de PCBs por fertilización con bio-residuos en suelos agrícolas (doctoral tesis). Universidad de Valladolid, dto. Ciencias agroforestales (in spanish). https://www.educacion.gob.es/teseo/mostrarRef.do?ref=915018 (accesed february 2022)

Avellan, A., Duarte, A., Rocha-Santos, T., 2022. Organic contaminants in marine sediments and seawater: A review for drawing environmental diagnostics and searching for informative predictors. Sci Total Environ 808, 152012, 10.1016/j.scitotenv.2021.152012

Aznar-Alemany, O., Aminot, Y., Vila-Cano, J., Kock-Schulmeyer, M., Readman, J.W., Marques, A., Godinho, L., Botteon, E., Ferrari, F., Boti, V., Albanis, T., Eljarrat, E., Barcelo, D., 2018. Halogenated and organophosphorus flame retardants in European aquaculture samples. Sci Total Environ 612, 492-500, 10.1016/j.scitotenv.2017.08.199

Badea, S.L., Geana, E.I., Niculescu, V.C., Ionete, R.E., 2020. Recent progresses in analytical GC and LC mass spectrometric based-methods for the detection of emerging chlorinated and brominated contaminants and their transformation products in aquatic environment. Sci Total Environ 722, 137914, 10.1016/j.scitotenv.2020.137914

Balde C, Forti V, Gray V, Kuehr R, Stegmann P (2017) The Global E-waste Monitor-2017. United Nations University (UNU), International Telecommunication Union (ITU) and International SolidWaste Association (ISWA), Bonn/Geneva/Vienna

Baroudi, F., Al Alam, J., Fajloun, Z., Millet, M., 2020. Snail as sentinel organism for monitoring the environmental pollution; a review. Ecol Indic 113, 106240, 10.1016/j.ecolind.2020.106240

Bayen, S., Wurl, O., Karuppiah, S., Sivasothi, N., Lee, H.K., Obbard, J.P., 2005. Persistent organic pollutants in mangrove food webs in Singapore. Chemosphere 61, 303-313, 10.1016/j.chemosphere.2005.02.097

Bayen S., 2012. Occurrence, bioavailability and toxic effects of tracemetals and organic contaminants in mangrove ecosystems: a review. Environ Int 101, 48:84

Bekele, T.G., Zhao, H., Yang, J., Chegen, R.G., Chen, J., Mekonen, S., Qadeer, A., 2021. A review of environmental occurrence, analysis, bioaccumulation, and toxicity of organophosphate esters. Environ Sci Pollut Res Int 28, 49507-49528, 10.1007/s11356-021-15861-8

Bergman, A., Ryden, A., Law, R.J., de Boer, J., Covaci, A., Alaee, M., Birnbaum, L., Petreas, M., Rose, M., Sakai, S., Van den Eede, N., van der Veen, I., 2012. A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environ Int 49, 57-82, 10.1016/j.envint.2012.08.003

Bhutto, S.U.A., Xing, X., Shi, M., Mao, Y., Hu, T., Tian, Q., Cheng, C., Liu, W., Chen, Z., Qi, S., 2021. Occurrence and distribution of OCPs and PAHs in water, soil and sediment of Daye lake. J Geochem Explor 226, 106769, 10.1016/j.gexplo.2021.106769

Bichon, E., Guiffard, I., Venisseau, A., Marchand, P., Antignac, J.P., Le Bizec, B., 2015. Ultra-trace quantification method for chlordecone in human fluids and tissues. J Chromatogr A 1408, 169-177, 10.1016/j.chroma.2015.07.013

Bjorklund, J., Tollback, P., Hiarne, C., Dyremark, E., Ostman, C., 2004. Influence of the injection technique and the column system on gas chromatographic determination of polybrominated diphenyl ethers. J Chromatogr A 1041, 201-210, 10.1016/j.chroma.2004.04.025

Bodenstein, G., 1972. Disposal of wastes from Lindane manufacture. In: Uhlmann E, Verlag K (eds) Lindane monograph of an insecticide. Schillinger, Freiburg im Breisgau, pp 23–77

Borgå, K., 2013. Ecotoxicology: Bioaccumulation. Reference Module in Earth Systems and Environmental Sciences, Elsevier. 6-8. 10.1016/B978-0-12-409548-9.00765-X

Brandt, J.S., Butsic, V., Schwab, B., Kuemmerle, T., Radeloff, V.C., 2015. The relative effectiveness of protected areas, a logging ban, and sacred areas for old-growth forest protection in southwest China. Biol Conserv 181, 1-8, 10.1016/j.biocon.2014.09.043

Brandsma, S.H.; Leonards, P.E.G.; Leslie, H.A.; De Boer, J., 2015. Tracing organophosphate and brominated flame retardants and plasticizers in an estuarine food web. Sci. Total Environ 505, 22–31

Breivik, K., Vestreng, V., Rozovskaya, O., Pacyna, J.M., 2006. Atmospheric emissions of some POPs in Europe: a discussion of existing inventories and data needs. Environ Sci Policy 9, 663-674, 10.1016/j.envsci.2006.09.001

Brinkman UATh, De Kok A (1980) Production, properties and usage. In: Kimbrough RD (ed) Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, New York, 1–40

BOE, 1975 Boletín Oficial del Estado Núm 308. Ministerio de Agricultura, Orden del 4 de diciembre por la que se restringe el uso de ciertos plaguicidas (December 24th, 1975)(in Spanish)

Buha Djordjevic, A., Antonijevic, E., Curcic, M., Milovanovic, V., Antonijevic, B., 2020. Endocrine-disrupting mechanisms of polychlorinated biphenyls. Curr Opin Toxicol 19, 42-49, 10.1016/j.cotox.2019.10.006

Busvine, J., 1992. The DDT story. By Kenneth Mellanby. Bull Entomol Res 82, 554-556, 10.1017/S0007485300042693

Cao, M., Peng, L., Liu, S., 2015. Analysis of the Network of Protected Areas in China Based on a Geographic Perspective: Current Status, Issues and Integration. Sustainability 7, 15617-15631, 10.3390/su71115617

Cao, Z., Xu, X., Zhao, Y., Du, R., Fan, Y., Wei, P., Ma, K., Zhu, Y., Huang, X., Hu, F., Hu, P., Liu, X., 2022. Gas-particle partition and size-segregated distribution of flame retardants in indoor and outdoor air: Reevaluation on the role of fine particles in human exposure. Chemosphere 292, 133414, 10.1016/j.chemosphere.2021.133414

Capanni, F., Munoz-Arnanz, J., Marsili, L., Fossi, M.C., Jimenez, B., 2020. Assessment of PCDD/Fs, dioxin-like PCBs and PBDEs in Mediterranean striped dolphins. Mar Pollut Bull 156, 111207, 10.1016/j.marpolbul.2020.111207

Carson, R., 1962. Silent Spring. Bluejay

Castro-Jimenez, J., Banaru, D., Chen, C.T., Jimenez, B., Munoz-Arnanz, J., Deviller, G., Sempere, R., 2021. Persistent organic pollutants burden, trophic magnification and risk in a pelagic food web from coastal NW Mediterranean Sea. Environ Sci Technol 55, 9557-9568, 10.1021/acs.est.1c00904

Ceia, F.R., Paiva, V.H., Fidalgo,V.,Morais, L., Baeta, A., Crisóstomo, P.,Mourato, E.,Garthe, S.,Marques, J.C., Ramos, J.A., 2014. Annual and seasonal consistency in the feeding ecology of an opportunistic species, the yellow-legged gull Larus michahellis. Mar Ecol Prog Ser 497, 273–284

Chan, J. K., Xing, G.H., Xu, Y., Liang, Y., Chen, L.X., Wu, S.C., Wong, C.K.C., Leung, C.K.M., Wong, M.H., 2007. Body loadings and health risk assessment of polychlorinated dibenzo-p-dioxins and dibenzofurans at an intensive electronic waste recycling site in China. Environ Sci Technol 41, 22, 7668-7674, 10.1021/es071492j

Chan, J. K., Man, Y. B., Wu, S. C., Wong, M. H., 2013. Dietary intake of PBDEs of residents at two major electronic waste recycling ites in China. Sci Total Environ 463-464, 1138-1146, 10.1016/j.scitotenv.2012.06.093

Chakraborty, P., Selvaraj, S., Nakamura, M., Prithiviraj, B., Cincinelli, A., Bang, J.J., 2018. PCBs and PCDD/Fs in soil from informal e-waste recycling sites and open dumpsites in India: Levels, congener profiles and health risk assessment. Sci Total Environ 621, 930-938, 10.1016/j.scitotenv.2017.11.083

Chen D, Watts B, La Guardia MJ, Harvey E, Mojica EK, Hale RC., 2010a. Species-specific accumulation of polybrominated diphenyl ether flame retardants in birds of prey from the Chesapeake Bay. Environ Pollut 158:1883–9

Chen, D., Letcher, R.J., Burgess, N.M., Champoux, L., Elliott, J.E., Hebert, C.E., Martin, P., Wayland, M., Chip Weseloh, D.V., Wilson, L., 2012. Flame retardants in eggs of four gull species (Laridae) from breeding sites spanning Atlantic to Pacific Canada. Environ Pollut 168, 1-9, 10.1016/j.envpol.2012.03.040

Chen, H.,Wang, P., Du, Z.,Wang, G., Gao, S., 2018. Oxidative stress, cell cycle arrest,DNA damage and apoptosis in adult zebrafish (Danio rerio) induced by tris(1,3-dichloro-2-propyl) phosphate. Aquat Toxicol 194, 37-45, 10.1016/j.aquatox.2017.11.001

Cheng, J.O., Tseng, P.H., Chou, P.H., Hsieh, C.Y., Ko, F.C., 2021. Revisiting of persistent organic pollution occurrence and distribution in the surface sediment along western Taiwan coast. Mar Pollut Bull 173, 113118, 10.1016/j.marpolbul.2021.113118

Choi, J.W., Matsuda, M., Kawano, M., Wakimoto, T., Iseki, N., Masunaga, S., Hayama, S., Watanuki, Y., 2001. Chlorinated persistent organic pollutants in black-tailed gulls (Larus crassirostris) from Hokkaido, Japan. Chemosphere 44, 1375–1382, 10.1016/S0045-6535(00)00358-1

Choi, H.G., Moon, H.B., Choi, M., Yu, J., 2011. Monitoring of organic contaminants in sediments from the Korean coast: Spatial distribution and temporal trends (2001-2007). Mar Pollut Bull 62, 1352-1361, 10.1016/j.marpolbul.2011.03.029

Choo, G.; Cho, H.S.; Park, K.; Lee, J.W.; Kim, P.; Oh, J.E., 2018. Tissue-specific distribution and bioaccumulation potential of organophosphate flame retardants in crucian carp. Environ Pollut 239, 161–168

Choo, G., Ekpe, O.D., Park, K.W., Chung, D., Lee, J., Oh, J.E., 2022. Temporal and spatial trends of chlorinated paraffins and organophosphate flame retardants in black-tailed gull (Larus crassirostris) eggs. Sci Total Environ 803, 150137, 10.1016/j.scitotenv.2021.150137

Chung, S.W., Chen, B.L., 2011. Determination of organochlorine pesticide residues in fatty foods: a critical review on the analytical methods and their testing capabilities. J Chromatogr A 1218, 5555-5567, 10.1016/j.chroma.2011.06.066

Colomer-Vidal, P., Bertolero, A., Alcaraz, C., Garreta-Lara, E., Santos, F.J., Lacorte, S., 2022. Distribution and ten-year temporal trends (2009-2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies. Environ Pollut 293, 118555, 10.1016/j.envpol.2021.118555

Costa, L.G., Giordano, G., 2007. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. Neurotoxicology 28, 1047-1067, 10.1016/j.neuro.2007.08.007

Council Directive 79/117/EEC of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances. http://eur-lex.europa.eu/LexUriServ/site/en/consleg/1979/L/01979L0117-20040520-en.pdf

Cristale, J., Garcia Vazquez, A., Barata, C., Lacorte, S., 2013. Priority and emerging flame retardants in rivers: occurrence in water and sediment, Daphnia magna toxicity and risk assessment. Environ Int 59, 232-243, 10.1016/j.envint.2013.06.011

Cristale, J., Dantas, R.F., De Luca, A., Sans, C., Esplugas, S., Lacorte, S., 2017. Role of oxygen and DOM in sunlight induced photodegradation of organophosphorous flame retardants in river water. J Hazard Mater 323, 242-249, 10.1016/j.jhazmat.2016.05.019

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, 10.1016/j.chemosphere.2017.02.070

Dąbrowski, Ł., 2015. Review of use of keepers in solvent evaporation procedure during the environmental sample analysis of some organic pollutants. TrAC, Trends Anal Chem 80, 507-516, 10.1016/j.trac.2015.10.014

Dandeneau, R. D.; Zerenner, E. H. 1979. An investigation of glasses for capillary chromatography. J High Resol Chromatogr 2, 351–356. 10.1002/jhrc.1240020617

De Kimpe, C.R., Morel, J.-L., 2000. Urban soil management: A growing concern. Soil Sci 165, 31-40

de Voogt P, Brinkman UATh, 1989. Production, properties and usage of polychlorinated biphenyls. In: Kimbrough RD, Jensen AA, editors. Halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins and related products. Top Environ Health. Elesevier, 3–45. ISBN 0-444-81029-3

Dinc, B., Celebi, A., Avaz, G., Canli, O., Guzel, B., Eren, B., Yetis, U., 2021. Spatial distribution and source identification of persistent organic pollutants in the sediments of the Yesilirmak River and coastal area in the Black Sea. Mar Pollut Bull 172, 112884, 10.1016/j.marpolbul.2021.112884

Dishaw, L.V., Macaulay, L.J., Roberts, S.C., Stapleton, H.M., 2014. Exposures, mechanisms, and impacts of endocrine-active flame retardants. Curr Opin Pharmacol 19, 125-133, 10.1016/j.coph.2014.09.018

Du Z., Wang G., Gao S., Wang Z., 2015. Aryl organophosphate flame retardants induced cardiotoxicity during zebrafish embryogenesis: by disturbing expression of the transcriptional regulators. Aquat Toxicol 161:25–32. 10.1016/j.aquatox.2015.01.027

Dudley, N. (Editor) 2008. Guidelines for Applying Protected Area Management Categories. Gland, Switzerland: IUCN. x + 86pp. WITH Stolton, S., P. Shadie and N. Dudley (2013). IUCN WCPA Best Practice Guidance on Recognising Protected Areas and Assigning Management Categories and Governance Types, Best Practice Protected Area Guidelines Series No. 21, Gland, Switzerland: IUCN

Egwumah FA, Egwumah PO, Edet DI., 2017. Paramount roles of wild birds as bioindicators of contamination. Int J Avian & Wildlife Biol.2(1):194–200. 10.15406/ijawb.2017.02.00041

Eguchi, A., Isobe, T., Ramu, K., Tue, N.M., Sudaryanto, A., Devanathan, G., Viet, P.H., Tana, R.S., Takahashi, S., Subramanian, A., Tanabe, S., 2013. Soil contamination by brominated flame retardants in open waste dumping sites in Asian developing countries. Chemosphere 90, 2365-2371

Europarc España, 2022. https://redeuroparc.org. Figure available at https://redeuroparc.org/descargas-2/ (Accesed May 2022)

European Parlament, 2003. Directive 2002/96/EC of the European Parliament and of the council of 27 January 2003 on waste electrical and electronic equipment (WEEE). Available at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002L0096&from=EN (accesed February 2022)

European Union, 2008. European Union Risk Assessment Report: Tris (1-chloro-2-propyl) Phosphate (TCPP). EINECS No: 237-158-7. Office for Official Publications of the European Communities, Luxembourg

Erickson, M.D., Kaley, R.G., 2nd, 2011. Applications of polychlorinated biphenyls. Environ Sci Pollut Res Int 18, 135-151, 10.1007/s11356-010-0392-1

Fernandez, J., Arjol, M.A., Cacho, C., 2013. POP-contaminated sites from HCH production in Sabinanigo, Spain. Environ Sci Pollut Res Int 20, 1937-1950, 10.1007/s11356-012-1433-8

Food and Agriculture Organization of the United Nations (FAO), 2022. https://www.fao.org/pesticide-registration-toolkit/information-sources/terms-and-definitions/terms-and-definitions-b/en/. Accesed June 2022

Fremlin, K.M., Elliott, J.E., Green, D.J., Drouillard, K.G., Harner, T., Eng, A., Gobas, F., 2020. Trophic magnification of legacy persistent organic pollutants in an urban terrestrial food web. Sci Total Environ 714, 136746, 10.1016/j.scitotenv.2020.136746

Fu, S., Chu, S., Xu, X., 2001. Organochlorine Pesticide Residue in Soils from Tibet, China. Bull Environ Contam Toxicol 66, 171-177, 10.1007/s001280000221

Fu, Z., Chen, G., Wang, W., Wang, J., 2020. Microplastic pollution research methodologies, abundance, characteristics and risk assessments for aquatic biota in China. Environ Pollut 266, 115098, 10.1016/j.envpol.2020.115098

Giulivo, M., Capri, E., Kalogianni, E., Milacic, R., Majone, B., Ferrari, F., Eljarrat, E., Barcelo, D., 2017. Occurrence of halogenated and organophosphate flame retardants in sediment and fish samples from three European river basins. Sci Total Environ 586, 782-791, 10.1016/j.scitotenv.2017.02.056

Gondi, R., Kavitha, S., Yukesh Kannah, R., Parthiba Karthikeyan, O., Kumar, G., Kumar Tyagi, V., Rajesh Banu, J., 2022. Algal-based system for removal of emerging pollutants from wastewater: A review. Biores Technol 344, 126245, 10.1016/j.biortech.2021.126245

Govaerts, A., Verhaert, V., Covaci, A., Jaspers, V.L.B., Berg, O.K., Addo-Bediako, A., Jooste, A., Bervoets, L., 2018. Distribution and bioaccumulation of POPs and mercury in the Ga-Selati River (South Africa) and the rivers Gudbrandsdalslagen and Rena (Norway). Environ Int 121, 1319-1330, 10.1016/j.envint.2018.10.058

Guan K.L., Liu Y., Luo X.J., Zeng Y.H., Mai B.X., 2020. Short- and Medium-Chain Chlorinated Paraffins in Aquatic Organisms from an E-Waste Site: Biomagnification and Maternal Transfer. Sci Total Environ 708, 134840, 10.1016/j.scitotenv.2019.134840

Habibullah-Al-Mamun, M., Kawser Ahmed, M., Saiful Islam, M., Tokumura, M., Masunaga, S., 2019. Occurrence, distribution and possible sources of polychlorinated biphenyls (PCBs) in the surface water from the Bay of Bengal coast of Bangladesh. Ecotox Environ Safe 167, 450-458, 10.1016/j.ecoenv.2018.10.052

Han Y., Tang Z., Sun J., Xing X., Zhang M., Cheng J., 2019. Heavy Metals in Soil Contaminated through E-Waste Processing Activities in a Recycling Area: Implications for Risk Management. Proc Safe Environ Prot 125, 189-196, 10.1016/j.psep.2019.03.020

Holoubek, I., Dusek, L., Sanka, M., Hofman, J., Cupr, P., Jarkovsky, J., Zbiral, J., Klanova, J., 2009. Soil burdens of persistent organic pollutants--their levels, fate and risk. Part I. Variation of concentration ranges according to different soil uses and locations. Environ Pollut 157, 3207-3217, 10.1016/j.envpol.2009.05.031

Hou, R., Xu, Y., Wang, Z., 2016. Review of OPFRs in animals and humans: Absorption, bioaccumulation, metabolism, and internal exposure research. Chemosphere 153, 78-90, 10.1016/j.chemosphere.2016.03.003

Hu, Y.X., Sun, Y.X., Li, X., Xu, W.H., Zhang, Y., Luo, X.J., Dai, S.H., Xu, X.R., Mai, B.X., 2017. Organophosphorus flame retardants in mangrove sediments from the Pearl River Estuary, South China. Chemosphere 181, 433-439, 10.1016/j.chemosphere.2017.04.117

Huang, C.C., Zeng, Y.H., Luo, X.J., Tang, B., Liu, Y.E., Ren, Z.H., Mai, B.X., 2018. Level changes and human dietary exposure assessment of halogenated flame retardant levels in free-range chicken eggs: A case study of a former e-waste recycling site, South China. Sci Total Environ 634, 509-515, 10.1016/j.scitotenv.2018.03.386

Huang Y., Fu J., Wang W., Li J., 2019. Development of China’s Nature Reserves over the Past 60 Years: An Overview. Land Use Policy 80, 224-232, 10.1016/j.landusepol.2018.10.020

Huang, J., Ye, L., Fang, M., Su, G., 2022. Industrial Production of Organophosphate Flame Retardants (OPFRs): Big Knowledge Gaps Need to Be Filled? Bull Environ Contam Toxicol, 10.1007/s00128-021-03454-7

Hubbard, H.L. 1964 Chlorinated biphenyl and related compounds. In: Kirk-Othmer Encycl Chem Tech 5:289–297

Islam, A., Ahmed, T., Awual, M.R., Rahman, A., Sultana, M., Aziz, A.A., Monir, M.U., Teo, S.H., Hasan, M., 2020. Advances in sustainable approaches to recover metals from e-waste-A review. J Cleaner Prod 244, 118815, 10.1016/j.jclepro.2019.118815

Jain, M., Khan, S.A., Sharma, K., Jadhao, P.R., Pant, K.K., Ziora, Z.M., Blaskovich, M.A.T., 2022. Current perspective of innovative strategies for bioremediation of organic pollutants from wastewater. Bioresour Technol 344, 126305, 10.1016/j.biortech.2021.126305

Jensen S. Report of a new chemical hazard. New Scientist.1966;32:312

Jiang, L., Cheng, Z., Zhang, D., Song, M., Wang, Y., Luo, C., Yin, H., Li, J., Zhang, G., 2017. The influence of e-waste recycling on the molecular ecological network of soil microbial communities in Pakistan and China. Environ Pollut 231, 173-181, 10.1016/j.envpol.2017.08.003

Jiménez-Skrzypek, G., Hernández-Sánchez, C., Ortega-Zamora, C., González-Sálamo, J., González-Curbelo, M.Á., Hernández-Borges, J., 2021. Microplastic-adsorbed organic contaminants: Analytical methods and occurrence. TrAC, Trends Anal Chem 136, 116186, 10.1016/j.trac.2021.116186

Kawajiri, K., Fujii-Kuriyama, Y., 2017. The aryl hydrocarbon receptor: a multifunctional chemical sensor for host defense and homeostatic maintenance. Exp Animals 66, 75-89, 10.1538/expanim.16-0092

Ke, X., Bao, Q., Qi, Y., Huang, X., Zhang, H., 2018. Toxicity assessment of sediments from the Liaohe River Protected Area (China) under the influence of ammonia nitrogen, heavy metals and organic contaminants. Environ Toxicol Pharmacol 59, 34-42, 10.1016/j.etap.2018.02.008

Kelly BC, Ikonomou MG, Blair JD, Gobas FAPC., 2008. Bioaccumulation behaviour of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. Sci Total Environ 401:60–72

Kierkegaard, A., Sellstrom, U., McLachlan, M.S., 2009. Environmental analysis of higher brominated diphenyl ethers and decabromodiphenyl ethane. J Chromatogr A 1216, 364-375, 10.1016/j.chroma.2008.05.058

Kim, L., Lee, D., Cho, H.-K., Choi, S.-D., 2019. Review of the QuEChERS method for the analysis of organic pollutants: Persistent organic pollutants, polycyclic aromatic hydrocarbons, and pharmaceuticals. Trends Environ Anal Chem 22, e00063, 10.1016/j.teac.2019.e00063

Kobayashi, J., Imuta, Y., Komorita, T., Yamada, K., Ishibashi, H., Ishihara, F., Nakashima, N., Sakai, J., Arizono, K., Koga, M., 2015. Trophic magnification of polychlorinated biphenyls and polybrominated diphenyl ethers in an estuarine food web of the Ariake Sea, Japan. Chemosphere 118, 201-206, 10.1016/j.chemosphere.2014.08.066

Kocagoz, R., Onmus, O., Onat, I., Cagdas, B., Siki, M., Orhan, H., 2014. Environmental and biological monitoring of persistent organic pollutants in waterbirds by non-invasive versus invasive sampling. Toxicol lett 230, 208-217, 10.1016/j.toxlet.2014.01.044

Kock-Schulmeyer, M., Ginebreda, A., Petrovic, M., Giulivo, M., Aznar-Alemany, O., Eljarrat, E., Valle-Sistac, J., Molins-Delgado, D., Diaz-Cruz, M.S., Monllor-Alcaraz, L.S., Guillem-Argiles, N., Martinez, E., Miren, L.A., Llorca, M., Farre, M., Pena, J.M., Mandaric, L., Perez, S., Majone, B., Bellin, A., Kalogianni, E., Skoulikidis, N.T., Milacic, R., Barcelo, D., 2021. Priority and emerging organic microcontaminants in three Mediterranean river basins: Occurrence, spatial distribution, and identification of river basin specific pollutants. Sci Total Environ 754, 142344, 10.1016/j.scitotenv.2020.142344

Lee, S., Cho, H.J., Choi, W., Moon, H.B., 2018. Organophosphate flame retardants (OPFRs) in water and sediment: Occurrence, distribution, and hotspots of contamination of Lake Shihwa, Korea. Mar Pollut Bull 130, 105-112, 10.1016/j.marpolbul.2018.03.009

Leppert, J., Muller, P.J., Chopra, M.D., Blumberg, L.M., Boeker, P., 2020. Simulation of spatial thermal gradient gas chromatography. J Chromatogr A 1620, 460985, 10.1016/j.chroma.2020.460985

Li, J., Xie, Z., Mi, W., Lai, S., Tian, C., Emeis, K.C., Ebinghaus, R., 2017. Organophosphate Esters in Air, Snow, and Seawater in the North Atlantic and the Arctic. Environ Sci Technol 51, 6887-6896, 10.1021/acs.est.7b01289

Li, W., Wang, Y., Kannan, K., 2019. Occurrence, distribution and human exposure to 20 organophosphate esters in air, soil, pine needles, river water, and dust samples collected around an airport in New York state, United States. Environ Int 131, 105054, 10.1016/j.envint.2019.105054

Li W., Achal V., 2020. Environmental and Health Impacts Due to E-Waste Disposal in China - a Review. Sci Total Environ 737, 139745, 10.1016/j.scitotenv.2020.139745

Lian, M., Lin, C., Li, Y., Hao, X., Wang, A., He, M., Liu, X., Ouyang, W., 2022a. Distribution, partitioning, and health risk assessment of organophosphate esters in a major tributary of middle Yangtze River using Monte Carlo simulation. Water Res 219, 118559, 10.1016/j.watres.2022.118559

Lian, M., Lin, C., Xin, M., Gu, X., Lu, S., Wang, B., Ouyang, W., Liu, X., He, M., 2022b. Organophosphate esters in surface waters of Shandong Peninsula in eastern China: Levels, profile, source, spatial distribution, and partitioning. Environ Pollut 297, 118792, 10.1016/j.envpol.2022.118792

Lipsky, S. R.; Mcmurray, W. J. Role of Surface Groups in Affecting the Chromatographic Performance of Certain Types of Fused-Silica Glass Capillary Column. J. Chromatogr. 1984, 289, 129–142. doi:10.1016/S0021-9673(00)95082-9

Liu, Y., Meng, F., 2011. Distribution of HCHs and DDTs in Agricultural Soil of Pudong New Area, Shanghai. Proc Environ Sci 11, 1296-1301, 10.1016/j.proenv.2011.12.194

Liu, L., Zhang, K., Zhang, Z., 2016. An improved core sampling technique for soil magnetic susceptibility determination. Geoderma 277, 35-40, 10.1016/j.geoderma.2016.04.030

Liu, X.C., Wu, W.C., Zhang, Y.B., Wang, T., Zhao, J.G., Chen, Z.H., 2017. Occurrence, profiles, and ecological risks of polybrominated diphenyl ethers in mangrove sediments of Shantou, China. Environ Sci Pollut Res Int 24, 3608-3617, 10.1007/s11356-016-8112-0

Liu, Y.E., Huang, L.Q., Luo, X.J., Tan, X.X., Huang, C.C., Corella, P.Z., Mai, B.X., 2018. Determination of organophosphorus flame retardants in fish by freezing-lipid precipitation, solid-phase extraction and gas chromatography-mass spectrometry. J Chromatogr A 1532, 68-73, 10.1016/j.chroma.2017.12.001

Liu, Y.E., Luo, X.J., Zapata Corella, P., Zeng, Y.H., Mai, B.X., 2019a. Organophosphorus flame retardants in a typical freshwater food web: Bioaccumulation factors, tissue distribution, and trophic transfer. Environ Pollut 255, 113286, 10.1016/j.envpol.2019.113286

Liu, Y.E., Tang, B., Liu, Y., Luo, X.J., Mai, B.X., Covaci, A., Poma, G., 2019b. Occurrence, biomagnification and maternal transfer of legacy and emerging organophosphorus flame retardants and plasticizers in water snake from an e-waste site. Environ Int 133, 105240, 10.1016/j.envint.2019.105240

Liu, Y.E., Luo, X.J., Guan, K.L., Huang, C.C., Zhu, C.Y., Qi, X.M., Zeng, Y.H., Mai, B.X., 2020a. Legacy and emerging organophosphorus flame retardants and plasticizers in frogs: Sex difference and parental transfer. Environ Pollut 266, 115336, 10.1016/j.envpol.2020.115336

Liu, C., Wei, B.K., Bao, J.S., Wang, Y., Hu, J.C., Tang, Y.E., Chen, T., Jin, J., 2020b. Polychlorinated biphenyls in the soil-crop-atmosphere system in e-waste dismantling areas in Taizhou: Concentrations, congener profiles, uptake, and translocation. Environ Pollut 257, 113622, 10.1016/j.envpol.2019.113622

Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Global fate of POPs: current and future research directions. Environ Pollut 150, 150-165, 10.1016/j.envpol.2007.06.051

Lorenzo, M., Campo, J., Morales Suarez-Varela, M., Pico, Y., 2019. Occurrence, distribution and behavior of emerging persistent organic pollutants (POPs) in a Mediterranean wetland protected area. Sci Total Environ 646, 1009-1020, 10.1016/j.scitotenv.2018.07.304

Lu C., Zhang L., Zhong Y., Ren W., Tobias M., Mu Z., Ma Z., Geng Y., Xue B., 2014. An overview of e-waste management in China. J Mater Cycles Waste Manage 17, 1-12, 10.1007/s10163-014-0256-8

Luo, X.J., Zhang, X.L., Liu, J., Wu, J.P., Luo, Y., Chen, E.J., Mai, B.X., Yang, Z.Y., 2009. Persistent halogenated compounds in waterbirds from an e-waste recycling region in South China. Environ. Sci. Technol. 43, 306–311, 10.1021/es8018644

Luo C., Hu B., Wang S., Wang Y., Zhao Z., Wang Y., Li J., Zhang G., 2020. Distribution and chiral signatures of polychlorinated biphenyls (PCBs) in soils and vegetables around an e-waste recycling site. J Agric Food Chem 68, 10542-10549, 10.1021/acs.jafc.0c00479

Ma, J., Qiu, X., Zhang, J., Duan, X., Zhu, T., 2012. State of polybrominated diphenyl ethers in China: an overview. Chemosphere 88, 769-778, 10.1016/j.chemosphere.2012.03.093

Ma, Y., 2017. Environmental Reviews and Case Studies: Contextualization of National Parks in the Nature Conservation Scheme in China: A Case Study of Pudacuo National Park in Yunnan Province. Environ Pract 15, 293-312, 10.1017/s1466046613000203

Ma, Y., Yun, X., Ruan, Z., Lu, C., Shi, Y., Qin, Q., Men, Z., Zou, D., Du, X., Xing, B., Xie, Y., 2020. Review of hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT) contamination in Chinese soils. Sci Total Environ 749, 141212, 10.1016/j.scitotenv.2020.141212

Mametov, R., Ratiu, I.A., Monedeiro, F., Ligor, T., Buszewski, B., 2021. Evolution and Evaluation of GC Columns. Crit Rev Anal Chem 51, 150-173, 10.1080/10408347.2019.1699013

Marlatt, V.L., Bayen, S., Castaneda-Cortes, D., Delbes, G., Grigorova, P., Langlois, V.S., Martyniuk, C.J., Metcalfe, C.D., Parent, L., Rwigemera, A., Thomson, P., Van Der Kraak, G., 2021. Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans. Environ Res 208, 112584, 10.1016/j.envres.2021.112584

Martinez, A., Awad, A.M., Herkert, N.J., Hornbuckle, K.C., 2019. Determination of PCB fluxes from Indiana Harbor and Ship Canal using dual-deployed air and water passive samplers. Environ Pollut 244, 469-476, 10.1016/j.envpol.2018.10.048

Martyniuk, C.J., Mehinto, A.C., Denslow, N.D., 2020. Organochlorine pesticides: Agrochemicals with potent endocrine-disrupting properties in fish. Mol Cell Endocrinol 507, 110764, 10.1016/j.mce.2020.110764

Matsukami, H., Tue, N.M., Suzuki, G., Someya, M., Tuyen le, H., Viet, P.H., Takahashi, S., Tanabe, S., Takigami, H., 2015. Flame retardant emission from e-waste recycling operation in northern Vietnam: environmental occurrence of emerging organophosphorus esters used as alternatives for PBDEs. Sci Total Environ 514, 492-499, 10.1016/j.scitotenv.2015.02.008

McGrath, T.J., Ball, A.S., Clarke, B.O., 2017. Critical review of soil contamination by polybrominated diphenyl ethers (PBDEs) and novel brominated flame retardants (NBFRs); concentrations, sources and congener profiles. Environ Pollut 230, 741-757, 10.1016/j.envpol.2017.07.009

Mechlińska, A., Wolska, L., Namieśnik, J., Wolska, L., 2012. Removal of sulfur from a solvent extract. TrAC, Trends Anal Chem 31, 129-133, 10.1016/j.trac.2011.06.022

Michalak, I., Chojnacka, K., 2014. Effl Biomonitor. 312-315, 10.1016/b978-0-12-386454-3.01008-3

Miljeteig, C., Gabrielsen, G.W., Strom, H., Gavrilo, M.V., Lie, E., Jenssen, B.M., 2012. Eggshell thinning and decreased concentrations of vitamin E are associated with contaminants in eggs of ivory gulls. Sci Total Environ 431, 92-99, 10.1016/j.scitotenv.2012.05.018

Miller-Rushing, A.J., Primack, R.B., Ma, K., Zhou, Z.-Q., 2017. A Chinese approach to protected areas: A case study comparison with the United States. Biol Conserv 210, 101-112, 10.1016/j.biocon.2016.05.022

Mo, L., Zheng, J., Wang, T., Shi, Y.G., Chen, B.J., Liu, B., Ma, Y.H., Li, M., Zhuo, L., Chen, S.J., 2019. Legacy and emerging contaminants in coastal surface sediments around Hainan Island in South China. Chemosphere 215, 133-141, 10.1016/j.chemosphere.2018.10.022

Molina, B., Bermejo, A., 2009. Gaviota patiamarilla (Larusmichahellis). In:Molina, B. (Ed.),Gaviota reidora, sombría y patiamarilla en España. Población en 2007–2009 ymétodode censo. SEO/BirdLife,Madrid, pp. 50–111. (In spanish)

Moreno, R., Jover, L., Diez, C., Sanpera, C., 2011. Seabird feathers as monitors of the levels and persistence of heavy metal pollution after the Prestige oil spill. Environ Pollut 159, 2454-2460, 10.1016/j.envpol.2011.06.033

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74-88, 10.1016/j.tox.2012.11.015

Múgica de la Guerra, M., Martínez Alandi, C., Atauri Mezquida, J.A., Gómez-Limón García, J., Puertas Blázquez, J., García Ventura, D., 2017. Anuario 2016 del estado de las áreas protegidas en España. ISBN: 978-84-940457-4-5 (In spanish)

Muir, D., Sverko, E., 2006. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal. Anal BioanalChem 386, 769-789, 10.1007/s00216-006-0765-y

Muñoz-Arnanz, J., Jimenez, B., 2011. New DDT inputs after 30 years of prohibition in Spain. A case study in agricultural soils from south-western Spain. Environ Pollut 159, 3640-3646, 10.1016/j.envpol.2011.07.027

Muresan, B., Lorgeoux, C., Gasperi, J., Moilleron, R., 2010. Fate and spatial variations of polybrominated diphenyl ethers in the deposition within a heavily urbanized area: case of Paris (France). Water Sci. Technol. 62, 822-828

Olivero-Verbel, R., Moreno, T., Fernandez-Arribas, J., Reche, C., Minguillon, M.C., Martins, V., Querol, X., Johnson-Restrepo, B., Eljarrat, E., 2021. Organophosphate esters in airborne particles from subway stations. Sci Total Environ 769, 145105, 10.1016/j.scitotenv.2021.145105

Orgiazzi, A., Ballabio, C., Panagos, P., Jones, A., Fernández-Ugalde, O., 2017. LUCAS Soil, the largest expandable soil dataset for Europe: a review. European J Soil Sci 69, 140-153, 10.1111/ejss.12499

Pantelaki, I., Voutsa, D., 2019. Organophosphate flame retardants (OPFRs): A review on analytical methods and occurrence in wastewater and aquatic environment. Sci Total Environ 649, 247-263, 10.1016/j.scitotenv.2018.08.286

Pantelaki, I., Voutsa, D., 2020. Occurrence, analysis and risk assessment of organophosphate esters (OPEs) in biota: A review. Mar Pollut Bull 160, 111547, 10.1016/j.marpolbul.2020.111547

Pastor, D., Jover, L., Ruiz, X., Albaiges, J., 1995. Monitoring organochlorine pollution in Audouin’s Gull eggs: the relevance of sampling procedures. Sci. Total Environ. 165, 215–223

Peng, Y., Wu, J., Luo, X., Zhang, X., Giesy, J.P., Mai, B., 2019. Spatial distribution and hazard of halogenated flame retardants and polychlorinated biphenyls to common kingfisher (Alcedo atthis) from a region of South China affected by electronic waste recycling. Environ Int 130, 104952, 10.1016/j.envint.2019.104952

Perez-Vazquez, F.J., Orta-García, S.T., Ochoa-Martinez, A.C., Pruneda-Alvarez, L.G., Ruiz-Vera, T., Jimenez-Avalos, J.A., Gonzalez Palomo, A.K., Perez-Maldonado, I.N., 2015. Polybrominated diphenyl ethers (PBDEs) concentration in soil from San Luis Potosi, Mexico: levels and ecological and human health risk characterization. Int J Environ Health Res 26, 239-253

Pessah, I.N., Cherednichenko, G., Lein, P.J., 2010. Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. Pharmacol Ther 125, 260-285, 10.1016/j.pharmthera.2009.10.009

Petrovic, M., Eljarrat, E., Diez, S., Kowalewska, G., Barcelo, D., 2007. Chemical Analysis of Contaminants in Sediments. 1, 61-129, 10.1016/s1872-1990(07)80075-4

Pico, Y., Alfarhan, A.H., Barcelo, D., 2020. How recent innovations in gas chromatography-mass spectrometry have improved pesticide residue determination: An alternative technique to be in your radar. TrAC, Trends Anal Chem 122, 115720, 10.1016/j.trac.2019.115720

Pozo, K., Lazzerini, D., Perra, G., Volpi, V., Corsolini, S., Focardi, S., 2009. Levels and spatial distribution of polychlorinated biphenyls (PCBs) in superficial sediment from 15 Italian Marine Protected Areas (MPA). Mar Pollut Bull 58, 773-776, 10.1016/j.marpolbul.2009.03.003

Qiu, X., Zhu, T., Yao, B., Hu, J., Hu, S. 2005. Contribution of dicofol to the current DDT pollution in China. Environ Sci Technol, 39, 4385-4390

Rahman, F., Langford, K.H., Scrimshaw, M.D., Lester, J.N., 2001. Polybrominated diphenyl ether PBDE flame retardants. Sci Total Environ 275, 1-17, 10.1016/S0048-9697(01)00852-X

Ranjbar Jafarabadi, A., Mashjoor, S., Mohamadjafari Dehkordi, S., Riyahi Bakhtiari, A., Cappello, T., 2021. Emerging POPs-type cocktail signatures in Pusa caspica in quantitative structure-activity relationship of Caspian Sea. J Hazard Mater 406, 124334, 10.1016/j.jhazmat.2020.124334

Real Decreto de 16 de agosto de 1918 creando el Parque Nacional del Valle de Ordesa o del río Ara, en el Pirineo del Alto Aragón, fijando sus límites y extensión y los del Parque Nacional de Covadonga. (in spanish). Available online at: https://www.boe.es/eli/es/rd/1918/08/16/(1) (accesed march 2022)

Reddy, A.V.B., Moniruzzaman, M., Aminabhavi, T.M., 2019. Polychlorinated biphenyls (PCBs) in the environment: Recent updates on sampling, pretreatment, cleanup technologies and their analysis. Chem Eng J 358, 1186-1207, 10.1016/j.cej.2018.09.205

Reguera, P., Couceiro, L., Fernandez, N., 2018. A review of the empirical literature on the use of limpets Patella spp. (Mollusca: Gastropoda) as bioindicators of environmental quality. Ecotox Environ Safe 148, 593-600, 10.1016/j.ecoenv.2017.11.004

Richardson, S.D., Kimura, S.Y., 2017. Emerging environmental contaminants: Challenges facing our next generation and potential engineering solutions. Environ Technol Innovation 8, 40-56, 10.1016/j.eti.2017.04.002

Rhind, S.M., Kyle, C.E., Kerr, C., Osprey, M., Zhang, Z.L., Duff, E.I., Lilly, A., Nolan, A., Hudson, G., Towers,W., Bell, J., Coull, M., McKenzie, C., 2013. Concentrations and geographic distribution of selected organic pollutants in Scottish surface soils. Environ Pollut 182, 15-27

Rodriguez, I., Calvo, F., Quintana, J.B., Rubi, E., Rodil, R., Cela, R., 2006. Suitability of solid-phase microextraction for the determination of organophosphate flame retardants and plasticizers in water samples. J Chromatogr A 1108, 158–165

Ryden, Andreas, 2013. Synthesis of organobromines as a tool for their characterisation and environmental occurrence assessment. Stockholm University, Department of Materials and Environmental Chemistry (doctoral thesis) ISBN 978-91-7447-800-6

Saeedi Saravi, S.S., Dehpour, A.R., 2016. Potential role of organochlorine pesticides in the pathogenesis of neurodevelopmental, neurodegenerative, and neurobehavioral disorders: A review. Life sciences 145, 255-264, 10.1016/j.lfs.2015.11.006

Sanchez-Avila, J., Tauler, R., Lacorte, S., 2012. Organic micropollutants in coastal waters from NW Mediterranean Sea: sources distribution and potential risk. Environ Int 46, 50-62, 10.1016/j.envint.2012.04.013

Shao, M., Tao, P., Wang, M., Jia, H., Li, Y.F., 2016. Trophic magnification of polybrominated diphenyl ethers in the marine food web from coastal area of Bohai Bay, North China. Environ Pollut 213, 379-385, 10.1016/j.envpol.2016.02.034

Shi, Q., Wang, Z., Chen, L., Fu, J., Han, J., Hu, B., Zhou, B., 2019. Optical toxicity of triphenyl phosphate in zebrafish larvae. Aquat Toxicol 210, 139-147

Shinkuma TH, Minh Nguyen Thi, 2009. The flow of e-waste material in the Asian region and a reconsideration of international trade policies on e-waste. Environ Impact Assess Rev 29(1):25–31

Sotao Neto, B.M.T., Combi, T., Taniguchi, S., Albergaria-Barbosa, A.C.R., Ramos, R.B., Figueira, R.C.L., Montone, R.C., 2020. Persistent organic pollutants (POPs) and personal care products (PCPs) in the surface sediments of a large tropical bay (Todos os Santos Bay, Brazil). Mar Pollut Bull 161, 111818, 10.1016/j.marpolbul.2020.111818

Stefanuto, P.-H., Smolinska, A., Focant, J.-F., 2021. Advanced chemometric and data handling tools for GC×GC-TOF-MS. TrAC, Trends Anal Chem 139, 116251, 10.1016/j.trac.2021.116251

Sthiannopkao S., Wong M. H., 2013. Handling E-Waste in Developed and Developing Countries: Initiatives, Practices, and Consequences. Sci Total Environ 463-464, 1147-1153, 10.1016/j.scitotenv.2012.06.088

Stockholm Convention, 2004. What are POPs? Available at: http://chm.pops.int/TheConvention/ThePOPs/tabid/673/Default.aspx (accessed February 2022)

Stockholm Convention, 2001. Available at: http://chm.pops.int/default.aspx (Accesed January 2022)

Strehse, J.S., Maser, E., 2020. Marine bivalves as bioindicators for environmental pollutants with focus on dumped munitions in the sea: A review. Mar Environ Res 158, 105006, 10.1016/j.marenvres.2020.105006

Soxhlet, F., 1879. "Die gewichtsanalytische Bestimmung des Milchfettes". Dingler's Polytechnisches Journal (in German). 232: 461–465

Su, G., Letcher, R.J., Moore, J.N., Williams, L.L., Martin, P.A., de Solla, S.R., Bowerman, W.W., 2015. Spatial and temporal comparisons of legacy and emerging flame retardants in herring gull eggs from colonies spanning the Laurentian Great Lakes of Canada and United States. Environ Res 142, 720-730, 10.1016/j.envres.2015.08.018

Sun, Y.X., Hao, Q., Zheng, X.B., Luo, X.J., Zhang, Z.W., Zhang, Q., Xu, X.R., Zou, F.S., Mai, B.X., 2014. PCBs and DDTs in light-vented bulbuls from Guangdong Province, South China: levels, geographical pattern and risk assessment. Sci Total Environ 490, 815-821, 10.1016/j.scitotenv.2014.05.066

Sun, H., Qi, Y., Zhang, D., Li, Q.X., Wang, J., 2016. Concentrations, distribution, sources and risk assessment of organohalogenated contaminants in soils from Kenya, Eastern Africa. Environ Pollut 209, 177-185

Sun, J., Pan, L., Tsang, D.C.W., Zhan, Y., Zhu, L., Li, X., 2018. Organic contamination and remediation in the agricultural soils of China: A critical review. Sci Total Environ 615, 724-740, 10.1016/j.scitotenv.2017.09.271

Tao, S., Liu, W., Li, Y., Yang, Y., Zuo, Q., Li, B., Cao, J., 2008. Organochlorine pesticides contaminated surface soil as reemission source in the Haihe Plain, China. Environ. Sci. Technol 42, 8395–8400

Tipre D. R., Khatri B. R., Thacker S. C., Dave S. R., 2021. The brighter side of e-waste-a rich secondary source of metal. Environ Sci Pollut Res Int 28, 10503-10518, 10.1007/s11356-020-12022-1

Tiwari, B.K., 2015. Ultrasound: A clean, green extraction technology. TrAC, Trends Anal Chem 71, 100-109, 10.1016/j.trac.2015.04.013

Tongue, A.D.W., Fernie, K.J., Harrad, S., Drage, D.S., McGill, R.A.R., Reynolds, S.J., 2021. Interspecies comparisons of brominated flame retardants in relation to foraging ecology and behaviour of gulls frequenting a UK landfill. Sci Total Environ 764, 142890, 10.1016/j.scitotenv.2020.142890

UNEP, 2019. Stockholm Convention on Persistent Organic Pollutants. Text and annexes. Available at: http://chm.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP-CONVTEXT-2021.English.pdf. Accessed January 2020

UNEP, 1992. Basel Convention on the control of transboundary movements of hazardous wastes and their disposal. Available at: http://www.basel.int/Portals/4/Basel%20Convention/docs/text/BaselConventionText-e.pdf

UNEP, 2004. http://chm.pops.int/Home/tabid/2121/Default.aspx

US EPA, 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.1. United States Environmental Protection Agency, Washington, DC, USA

Van Aswegen, J.D., Nel, L., Strydom, N.A., Minnaar, K., Kylin, H., Bouwman, H., 2019. Comparing the metallic elemental compositions of Kelp Gull Larus dominicanus eggs and eggshells from the Swartkops Estuary, Port Elizabeth, South Africa. Chemosphere 221, 533-542, 10.1016/j.chemosphere.2019.01.013

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci: an official journal of the Society of Toxicology 93, 223-241, 10.1093/toxsci/kfl055

Van den Berg, H., 2009. Global status of DDT and its alternatives for use in vector control to prevent disease. Environ Health Perspect, 117, 1656-1663

Van den Berg, H., Manuweera, G., Konradsen, F., 2017. Global trends in the production and use of DDT for control of malaria and other vector-borne diseases. Malaria J 16, 401, 10.1186/s12936-017-2050-2

Van der Gon, H.D., Van het Bolscher, M., Visschedijk, A., Zandveld, P., 2007. Emissions of persistent organic pollutants and eight candidate POPs from UNECE-Europe in 2000, 2010 and 2020 and the emission reduction resulting from the implementation of the UNECE POP protocol. Atmos Environ 41,9245e9261

Van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119-1153, 10.1016/j.chemosphere.2012.03.067

Vecchiato, M., Bonato, T., Barbante, C., Gambaro, A., Piazza, R., 2021. Organic pollutants in protected plain areas: The occurrence of PAHs, musks, UV-filters, flame retardants and hydrocarbons in woodland soils. Sci Total Environ 796, 149003, 10.1016/j.scitotenv.2021.149003

Vicente, J., Sanpera, C., Garcia-Tarrason, M., Perez, A., Lacorte, S., 2015. Perfluoroalkyl and polyfluoroalkyl substances in entire clutches of Audouin's gulls from the Ebro Delta. Chemosphere 119 Suppl, S62-68, 10.1016/j.chemosphere.2014.04.041

Vlckova, K., Hofman, J., 2012. A comparison of POPs bioaccumulation in Eisenia fetida in natural and artificial soils and the effects of aging. Environ Pollut 160, 49-56, 10.1016/j.envpol.2011.08.049

Voldner, E.C., Li, Y.-F., 1995. Global usage of selected persistent organochlorines. Sci Total Environ 160/161, 201-210

Wang, S., Zhang, S., Huang, H., Niu, Z., Han, W., 2014. Characterization of polybrominated diphenyl ethers (PBDEs) and hydroxylated and methoxylated PBDEs in soils and plants from an e-waste area, China. Environ Pollut 184, 405-413, 10.1016/j.envpol.2013.09.021

Wang, Y., Sun, H., Zhu, H., Yao, Y., Chen, H., Ren, C.,Wu, F., Kannan, K., 2018a. Occurrenceand distribution of organophosphate flame retardants (OPFRs) in soil and outdoor settled dust from a multi-waste recycling area in China. Sci. Total Environ. 625, 1056–1064, 10.1016/j.scitotenv.2018.01.013

Wang, X., Zhu, L., Zhong, W., Yang, L., 2018b. Partition and source identification of organophosphate esters in the water and sediment of Taihu Lake, China. J Hazard Mater 360, 43-50, 10.1016/j.jhazmat.2018.07.082

Wang, X., Zhu, Q., Yan, X., Wang, Y., Liao, C., Jiang, G., 2020a. A review of organophosphate flame retardants and plasticizers in the environment: Analysis, occurrence and risk assessment. Sci Total Environ 731, 139071, 10.1016/j.scitotenv.2020.139071

Wang, Y., Li, Z., Tan, F., Xu, Y., Zhao, H., Chen, J., 2020b. Occurrence and air-soil exchange of organophosphate flame retardants in the air and soil of Dalian, China. Environ Pollut 265, 114850, 10.1016/j.envpol.2020.114850

Wang, Z., Li, Y., Kong, F., Li, M., Xi, M., Yu, Z., 2021. How do trophic magnification factors (TMFs) and biomagnification factors (BMFs) perform on toxic pollutant bioaccumulation estimation in coastal and marine food webs. Regional Studies in Marine Science 44, 101797, 10.1016/j.rsma.2021.101797

Wania, F., Mackay, D., 1999. Global chemical fate of alpha -hexachlorocyclohexane. 2. Use of a global distribution model for mass balancing, source apportionment, and trend prediction. Environ Toxicol Chem 18 (7), 1400-1407

Weijs, L., Dirtu, A.C., Malarvannan, G., Covaci, A., 2015. Bioaccumulation and Biotransformation of Brominated Flame Retardants. 67, 433-491, 10.1016/b978-0-444-63299-9.00014-4

Weiss, P.; Lorbeer, G.; Scharf, S., 2000. Regional aspects and statistical characterisation of the load with semivolatile organic compounds at remote Austrian forest sites. Chemosphere 40, 1159−1171

Worboys, G. L., 2015. ‘Concept, purpose and challenges’, in G. L.Worboys, M. Lockwood, A. Kothari, S. Feary and I. Pulsford (eds)Protected Area Governance and Management, pp. 9–42, ANU Press, Canberra

World Health Organization, 1989. "Indoor air quality: organic pollutants." Report on a WHO Meeting, Berlin, 23-27 August 1987. EURO Reports and Studies 111. Copenhagen, World Health Organization Regional Office for Europe

WHO, 1993. Polychlorinated biphenyls and terphenyls. In: Dobson S, van Esch GJ (eds) Environ Heal Criteria 140, 2nd edn. World Health Organization (WHO), Geneva

Wu, J.P., Luo, X.J., Zhang, Y., Luo, Y., Chen, S.J., Mai, B.X., Yang, Z.Y., 2008. Bioaccumulation of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in wild aquatic species from an electronic waste (e-waste) recycling site in South China. Environ Int 34, 1109-1113, 10.1016/j.envint.2008.04.001

Wu, J.P., Luo, X.J., Zhang, Y., Yu, M., Chen, S.J., Mai, B.X., Yang, Z.Y., 2009. Biomagnification of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls in a highly contaminated freshwater food web from South China. Environ Pollut 157, 904-909, 10.1016/j.envpol.2008.11.001

Wu, Q., Liu, X., Liang, C., Leung, J.Y.S., Li, H., Chen, S., Mai, B., Miao, S., Chen, Y., Wu, Z., Chen, Z., 2017. Historical trends and ecological risks of polybrominated diphenyl ethers (PBDEs) and alternative halogenated flame retardants (AHFRs) in a mangrove in South China. Sci Total Environ 599-600, 181-187, 10.1016/j.scitotenv.2017.05.002

Wu Q., Du Y., Huang Z., Gu J., Leung J. Y. S., Mai B., Xiao T., Liu W., Fu J., 2019. Vertical profile of soil/sediment pollution and microbial community change by e-waste recycling operation. Sci Total Environ 669, 1001-1010, 10.1016/j.scitotenv.2019.03.178

Wu, J., Jia, R., Xuan, H., Zhang, D., Zhang, G., Xiao, Y., 2022. Priority Soil Pollution Management of Contaminated Site Based on Human Health Risk Assessment: A Case Study in Southwest China. Sustainability 14, 3663, 10.3390/su14063663

Xu, W., Wang, X., Cai, Z., 2013. Analytical chemistry of the persistent organic pollutants identified in the Stockholm Convention: A review. Analytica Chimica Acta 790, 1-13, 10.1016/j.aca.2013.04.026

Xu, W., Li, X., Pimm, S.L., Hull, V., Zhang, J., Zhang, L., Xiao, Y., Zheng, H., Ouyang, Z., 2016. The effectiveness of the zoning of China's protected areas. Biol Conserv 204, 231-236, 10.1016/j.biocon.2016.10.028

Yuan, H., Liu, E., Zhang, E., Luo, W., Chen, L., Wang, C., Lin, Q., 2017. Historical records and sources of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in sediment from a representative plateau lake, China. Chemosphere 173, 78-88, 10.1016/j.chemosphere.2017.01.047

Zhang,Y., Qi, S., Xing,X., Yang,D., Devi,N. L., Qu,C., Liu,H.X., Zhang,J.Q., Zeng, F.M., 2018. Chapter 21 - Legacies of Organochlorine Pesticides (OCPs) in Soil of China—A Review, and Cases in Southwest and Southeast China, Editor(s): Benedetto De Vivo, Harvey E. Belkin, Annamaria Lima, Environmental Geochemistry (Second Edition), Elsevier, 543-565,ISBN 9780444637635,10.1016/B978-0-444-63763-5.00022-7

Yun, S.H., Addink, R., McCabe, J.M., Ostaszewski, A., Mackenzie-taylor, D., Taylor, A.B., Kannan, K., 2008. Polybrominated diphenyl ethers and polybrominated biphenyls in sediment and floodplain soils of the Saginaw River Watershed, Michigan, USA. Archives Environ Contam Toxicol 55, 1-10

Zahed, M.A., Nabi Bidhendi, G., Pardakhti, A., Esmaili-Sari, A., Mohajeri, S., 2009. Determination of polychlorinated biphenyl congeners in water and sediment in North West Persian Gulf, Iran. Bull Environ Contam Toxicol 83, 899-902, 10.1007/s00128-009-9874-6

Zamariola, N., Toledo Netto, P., da Silva Franchi, C.A., de Camargo, J.L.V., de Marchi, M.R.R., 2017. QuEChERS-Based Method for Pesticides Analysis in Adipose Tissue Associated with Rat Ovaries. J Anal Toxicol 41, 399-406, 10.1093/jat/bkx023

Zeng Y. H., Tang B., Luo X. J., Zheng X. B., Peng P. A., Mai B. X, 2016. Organohalogen pollutants in surface particulates from workshop floors of four major e-waste recycling sites in China and implications for emission lists. Sci Total Environ 569-570, 982-989, 10.1016/j.scitotenv.2016.06.053

Zeng, Y., Huang, C., Luo, X., Liu, Y., Ren, Z., Mai, B., 2018. Polychlorinated biphenyls and chlorinated paraffins in home-produced eggs from an e-waste polluted area in South China: Occurrence and human dietary exposure. Environ Int 116, 52-59, 10.1016/j.envint.2018.04.006

Zeng, Y., Ding, N., Wang, T., Tian, M., Fan, Y., Wang, T., Chen, S.J., Mai, B.X., 2020. Organophosphate esters (OPEs) in fine particulate matter (PM2.5) in urban, e-waste, and background regions of South China. J Hazard Mater 385, 121583, 10.1016/j.jhazmat.2019.121583

Zhan, L., Lin, T., Wang, Z., Cheng, Z., Zhang, G., Lyu, X., Cheng, H., 2017. Occurrence and air-soil exchange of organochlorine pesticides and polychlorinated biphenyls at a CAWNET background site in central China: Implications for influencing factors and fate. Chemosphere 186, 475-487, 10.1016/j.chemosphere.2017.08.003

Zhang, N., Yang, Y., Tao, S., Liu, Y., Shi, K.L., 2011. Sequestration of organochlorine pesticides in soils of distinct organic carbon content. Environ Pollut 159, 700-705, 10.1016/j.envpol.2010.12.011

Zhang, L., Luo, Z., Mallon, D., Li, C., Jiang, Z., 2017. Biodiversity conservation status in China's growing protected areas. Biol Conserv 210, 89-100, 10.1016/j.biocon.2016.05.005

Zhang, W., Xie, H.Q., Li, Y., Zhou, M., Zhou, Z., Wang, R., Hahn, M.E., Zhao, B., 2022a. The aryl hydrocarbon receptor: A predominant mediator for the toxicity of emerging dioxin-like compounds. J Hazard Mater 426, 128084, 10.1016/j.jhazmat.2021.128084

Zhang, L., Xu, W., Mi, W., Yan, W., Guo, T., Zhou, F., Miao, L., Xie, Z., 2022b. Atmospheric deposition, seasonal variation, and long-range transport of organophosphate esters on Yongxing Island, South China Sea. Sci Total Environ 806, 150673, 10.1016/j.scitotenv.2021.150673

Zhao G., Zhou H., Wang D., Zha J., Xu Y., Rao K., Ma M., Huang S., Wang Z., 2009. PBBs, PBDEs, and PCBs in foods collected from e-waste disassembly sites and daily intake by local residents. Sci Total Environ 407, 2565-2575, 10.1016/j.scitotenv.2008.11.062

Zheng, X.B., Wu, J.P., Luo, X.J., Zeng, Y.H., She, Y.Z., Mai, B.X., 2012. Halogenated flame retardants in home-produced eggs from an electronic waste recycling region in South China: levels, composition profiles, and human dietary exposure assessment. Environ Int 45, 122-128, 10.1016/j.envint.2012.04.006

Zheng, X., Xu, F., Chen, K., Zeng, Y., Luo, X., Chen, S., Mai, B., Covaci, A., 2015. Flame retardants and organochlorines in indoor dust from several e-waste recycling sites in South China: Composition variations and implications for human exposure. Environ Int 78, 1-7, 10.1016/j.envint.2015.02.006

Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta 606, 135-150, 10.1016/j.aca.2007.11.018

Zhu, L.Y., Hites, R.A., 2004. Temporal Trends and Spatial Distributions of Brominated Flame Retardants in Archived Fishes from the Great Lakes. Environ Sci Technol 38, 2779-2784, 10.1021/es035288h

2. Objectives

Based on the bibliography and on the importance to determine the presence of legacy POPs and OPEs in the environment, and given that few studies determine the different families of POPs that are included in the Stockholm Convention, the general objective of this Thesis is to develop a multiresidue analysis capable to determine 4 different chemical families and to design monitoring studies that permit to evaluate the contamination status of different areas. On a first instance, National Parks and other areas with lower categories of environmental protection such as natural reserves have been monitoring as they constitute ecosystems with high biodiversity and natural interest and the legal figures are meant to preserve their natural evolution from anthropogenic pressure. The objective was to obtain new knowledge on the chemical pollution status of environmentally protected areas from Spain and China in order to better understand the potential sources and impacts to improve the management and conservation actions. On a second instance, this Thesis has evaluated the occurrence of POPs and OPEs in an e-waste recycling and disposal area in Bahie village, China, to determine the impact on the environment and human health.

Therefore, the following specific objectives were set:

- To develop suitable analytical methods for the multiresidue analysis of OCPs, PCBs, PBDEs and OPEs in environmental matrixes, including biotic and abiotic.
- To evaluate the pollution status of areas with different degrees of environmental protection in Spain by using gull eggs as bioindicators of environmental pollution.
- To asses the occurrence and sources of pollution in protected areas of South China through soils and sediments monitoring studies.
- To determine the pollution levels of soils, sediments, water and hen eggs from an e-waste hotspot in Baihe village and identify the source, partitioning and the potential impact for the environment and human health.

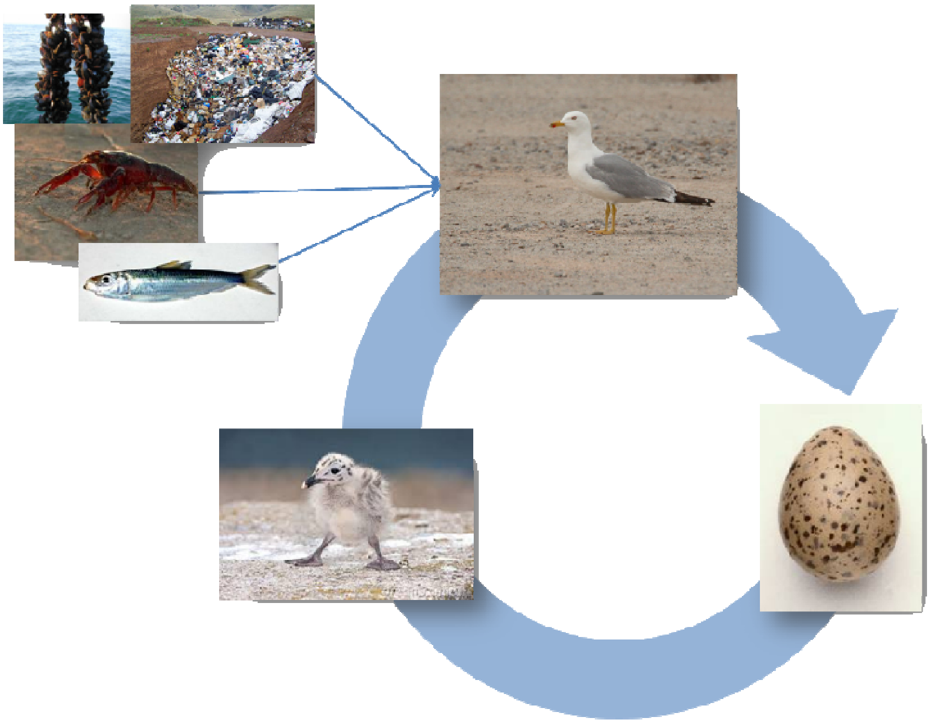
This Thesis has been structured in 3 different chapters.

In the first chapter, a multiresidue analytical method based on GC-MS/MS for the detection and quantification of chlorinated POPs such as PCBs and OCPs has been developed, validated and applied to evaluate the pollution status of National and Natural Parks of the Iberian Peninsula through the use of gull eggs as bioindicators.

The second chapter investigates the presence of OPEs, PBDEs, PCBs and OCPs in four environmentally protected areas in South China through the analysis of abiotic matrixes like soils and sediments and identifies the potential sources of pollution considering the anthropogenic activities carried out in each area.

In the third chapter, the sources of pollutants and environmental and human health risks of non-formal e-waste recycling activities have been studied in an already identified e-waste hotspot in Guangdong province, South China. Finally, this Thesis includes a general discussion where the levels detected in Dpain and China are compared to the bibliography and draws general conclusions on the analytical development and on the monitoring studies, and the implication it has for the environment.

3. Presence and impact of Stockholm Convention POPs in natural and national parks of the Iberian Peninsula



3.1 Introduction

Nowadays many habitats are strongly affected by anthropogenic and environmental pressures which can affect the wellbeing of many species. National and natural parks are protected spaces meant to maintain the functioning of the natural ecosystems and to act as refuge for many biota species. These areas have a high degree of protection in order, among others, to avoid and minimize any anthropogenic contamination that can alter the landscape, the integrity and the evolution of their natural systems, as well as to prevent the impact of pollution. Protected spaces serve as a migratory refuge for many bird species as well as breeding areas. However, these habitats are strongly affected by environmental pollution caused by many families of persistent organic pollutants (POPs) (Morales et al., 2016).

Seabirds are commonly used as sentinel species for monitoring POPs levels in the marine environment because they are very widespread and are sensitive to environmental changes. Among birds, gull eggs have been reported as excellent bioindicators of POPs (OSPAR, 2000; Fliedner at al., 2012) as they reflect the contamination pattern of an area. Gulls have the following characteristics: (i) they have a long lifespan (up to 20 years) and accumulate the contaminants through food and transfer them annually to the eggs; (ii) gulls are secondary consumers and are apex organisms of the seashore food chain and therefore biomagnify the POPs present in the marine foodweb; (iii) they are colonial breeders and exhibit high levels of breeding philopatry, so they reflect the contamination of a given area; (iv) gulls are monogamous that display a relatively high degree of mate fidelity life-long and therefore, the transfer of POPs from parents to chicks can be assessed; (v) gulls are abundant around the world and are able to reflect geographical distribution of POPs; (vi) many gull species, especially *Larus michahellis*, are non-protected species whose eggs represent a non-invasive sampling with high simplicity protocol, due to the large colonies settled in many coastal areas.

The Iberian Peninsula hosts several maritime-terrestrial natural and national parks along the Atlantic and Mediterranean shores, where birds have an important ecological role. These locations are considered by the EU Birds Directive (Council

Directive 2009/147/EC on the conservation of wild birds) as Special Protection Areas (SPAs), Sites of Community Importance (SCIs) and/or special Areas of Conservation (SACs), which host a large number of bird species and a huge biodiversity. Despite the high degree of protection of natural and national parks, they are often affected by human activities related to tourism, harbor and maritime activities, agriculture and hunting, and even industrial activities in the surroundings. As a result, waters around these areas are affected by the input of contaminants via discharges of wastewater treatment plant effluents, marine emissaries or run-off. The different degrees of anthropogenic pressures might pose these rich ecosystems at risk and may affect the bird populations. Specifically, in Spain birds are exposed to chemicals used in the past in industry (e.g., PCBs) (Gonzalez et al., 1991) and agriculture (e.g., organochlorine pesticides) (Jiménez et al., 2007; Merino et al., 2005; Muñoz-Arnanz and Jimenez, 2011) and relatively high concentrations of these environmental contaminants may impair neurodevelopment, endocrine, reproductive and survival effects (Gonzalez, 1988; Iwaniuk et al., 2006; Ortiz Martinez and Martinez Conde, 1995), and in extreme cases, mortality.

These findings reinforce the necessity of a better knowledge of the presence of POPs on sensitive areas which are refuges for numerous wildlife bird species. Thus, the aim of this study was to evaluate the occurrence of several families of POPs using gull eggs as bioindicators in seven *L. michahellis* colonies and three *L. audouinii* colonies located in the Iberian Peninsula and in the North of Africa (Figure 3.1). The POPs considered in this study include marker PCBs, PBDEs, OC pesticides and PFOS, which are listed in the Stockholm Convention (2009). The potential risks of POPs on eggshell thickness and egg size have also been evaluated.

3.2. Materials and Methods

3.2.1. Chemicals and Reagents

Standard Pesticide Mix 1 containing aldrin, α-, β-, γ- and δ-hexachlorocyclohexane (HCH), cis- and trans-chlordane, 2,4´-DDD, 2,4´-DDE, 2,4´-DDT, 4,4´-DDD, 4,4´-DDE, 4,4´-DDT, dieldrin, α- and β-endosulfan, endrin, heptachlor, heptachlor exoand endo-epoxides, hexachlorobenzene (HCB), isodrin, methoxychlor, mirex, and oxychlordane, at a concentration of 10 µg/mL, and the PCB congeners 28, 52, 101, 138, 153 and 180, at 1 µg/mL in toluene was purchased from AccuStandard (New Haven, USA). Dicofol was also obtained from AccuStandard (New Haven, USA) at a concentration of 100 µg/mL in methanol. Endosulfan sulphate (20 µg/mL in methanol) was supplied by Supelco (Bellefonte, PA, USA), while hexachlorobutadiene (5000 µg/mL in methanol) and pentachlorobenzene (solid, 98% purity) were acquired from Sigma-Aldrich (St. Louis, MO, USA). The surrogate internal standards used for quantification were: $^{13}C_6$-hexachlorobenzene (100 µg/mL in acetone) and PCB 209 (10 µg/mL in cyclohexane), both acquired from Dr. Ehrenstorfer (Augsburg, Germany), $^{13}C_{12}$-4,4´-DDE at a concentration of 100 µg/mL in nonane and laballed MBDE77, supplied from Cambridge Isotope Laboratories (Andover, MA, USA), and PCB 65 (solid, 98% purity), purchased from AccuStandar.

PBDE standard mixture containing the following congeners: BDE 28, 47, 99, 100, 153, 154, 183 (1 µg/mL), and BDE 209 (10 µg/mL) in nonane/toluene were from Wellington Laboratories (Guelph, Canada). Sodium perfluoro-1-octane sulfonate (PFOS) at a concentration of 2 µg/mL in methanol as well as its labeled surrogate internal standard used for quantification, sodium perfluoro-1-(1,2,3,4-$^{13}C_4$) octane sulfonate (m-PFOS) at 50 µg/mL in methanol, were supplied by Wellington Laboratories (Ontario, Canada).

For the clean-up of extracts, Strata FL-PR Florisil cartridges 10 g/60 mL gigatubes, of 170 µm particle diameter and 80 Å were supplied by Phenomenex (Torrance, California, USA). Active carbon 120/400 was from Supelco (Bellefonte, USA). Dichloromethane, n-hexane and isooctane, for gas chromatography analysis, and water and acetonitrile of HPLC grade were purchased from Merck (Darmstadt, Germany). Glacial acetic acid was provided by Panreac (Barcelona, Spain). Nitrogen for the evaporation of the sample extracts (purity> 99.995%), helium (purity> 99.9995%), used as carrier gas for GC separation, and ammonia (purity> 99.995%) as moderate gas for for chemical ionisation were supplied by Air Liquid (Barcelona, Spain).

3.2.2. Study areas

The Natural and National Parks studied host the main gull colonies of the Iberian Peninsula.

The National Park of Atlantic Islands of Galicia (thereafter Atlantic Islands) is located in the north-west of Spain and is composed of three archipelagos (Ons, Cíes and Sálvora). Its environmental value lies on its geographical position, because it protects the Galician coast and its estuaries from the ocean´s tides, creating a unique ecosystem. Many industrial and transport activities are carried out near this area, including a chloroalkaly industry that produced organochlorine pesticides until their ban around 1977 (Lackmann et al., 2004). The yellow-legged gull is the most abundant specie with more than 15,000 pairs in Cíes Islands in 2004 (Molina and Bermejo, 2009).

Berlengas is a group of three islets placed between 10 and 15 km away of Portugal coast, in front of Peniche town. They were declared as Biosphere Reserve by UNESCO in 2011 and host around 8,500 breeding pairs of yellow-legged gulls (Ceia et al., 2014). The Chafarinas Islands (Spain) are a group of volcanic outcrops located in the north coast of Morocco, about 50 km east of Melilla.

The Chafarinas Islands (thereafter Chafarinas) are designated as a ‘National Hunting Refuge’ and comprises three small islands: Congreso and Rey Islands hosted 3,244 pairs and 4,200 pairs of yellow-legged gulls in 2011 and 2005, respectively (Martín et al., 2015), while Isabel Island is inhabited by human and has had negligible seabird influence since 1848 (García et al., 2002).

Illa Grossa is located in Mar Menor, in Murcia autonomous community (south-east Spain) host several colonies of yellow-legged gull located in islands and saltpans reserves in Illa Grossa close to the Mar Menor (thereafter Mar Menor), a salty lagoon just separated from the sea by a sandy strip where many touristic activities take place. In these reserves jointly breed more than 8,000 yellow-legged gull pairs (Molina and Bermejo, 2009).

The Columbretes Islands Natural Park (thereafter Columbretes) is composed by four little groups of volcanic islands, located 49 km in front of Castellón. They hosted 500 yellow-legged gull pairs in 2008 (Molina and Bermejo, 2009).

The Ebro Delta Natural Park (thereafter Ebro Delta) is one of the biggest wetlands in the Mediterranean region. It is a special protection area (SPA) and it was included on the international Ramsar Convention for the wetlands in 1993 (http://www.ramsar.org/). Ebro Delta supports great bird diversity (> 350 species), with large breeding colonies of gulls, among other water birds, and also serves as a migratory refuge and a wintering area for more than 250,000 birds. Among the gull species that breed, the yellow-legged gull hosted more than 9,000 pairs in 2007 (Molina and Bermejo, 2009) and Audouin’s gull hosted a mean of 11,560 pairs in the period 1995-2009, where until mid-2000s hosted 70% of the world population (Fernández-Chacón et al., 2013). Many industrial activities take place upstream in the riverside of the Ebro River. Among them, a chloroalkali factory was operating in Flix for decades, discharging tons of highly polluted residues to the river. This has led to high pollution levels in all the downstream river basin.

The Medes Islands (thereafter Medes) is a small archipelago composed by seven little islands and some more islets located near the Mediterranean coast of Girona, and it is a part of the Montgrí, les Illes Medes i el Baix Ter Natural Park. In 2001 it was designated as a Specially Protected Area of the Mediterranean Importance (SPAMI) and host 8,000 breeding pairs of yellow-legged gull (Jácome Sanz, 2014).

Finally, Tarragona´s and Castellón´s ports are not national or natural parks, but hold part of the Ebro Delta Audouin´s gulls population, which has migrated to nearby areas to form new colonies (Payo-Payo et al., 2017). These ports are located in big cities of the western Mediterranean coast

3.2.3. Sample collection

We sampled yellow-legged gull (*Larus michahellis*) and Audouin´s gull (*Larus audouinii*) eggs in nine colonies from the Iberian Peninsula and one located in North Africa (Figure 3.1). Yellow-legged gull is a common, large and opportunistic gull, with an omnivorous diet that often scavenges on refuse tips. On the other hand, Audouin’s gull is a medium size gull, endemic of the Mediterranean area. It is a piscivorous species, considered a specialist in the capture of clupeiforms (pelagic fishes), but they can also feed on fishery discards and terrestrial preys (Navarro et al., 2010). Audouin’s gull was classified globally as threatened species in 1988, but due an increasing number of breeding pairs (mainly in the Ebro Delta colony) it is actually classified as Least Concern (BirdLife International, 2017). However, in the Spanish Red Data Book it is classified as Vulnerable (Martínez-Vilalta and Oro, 2004).

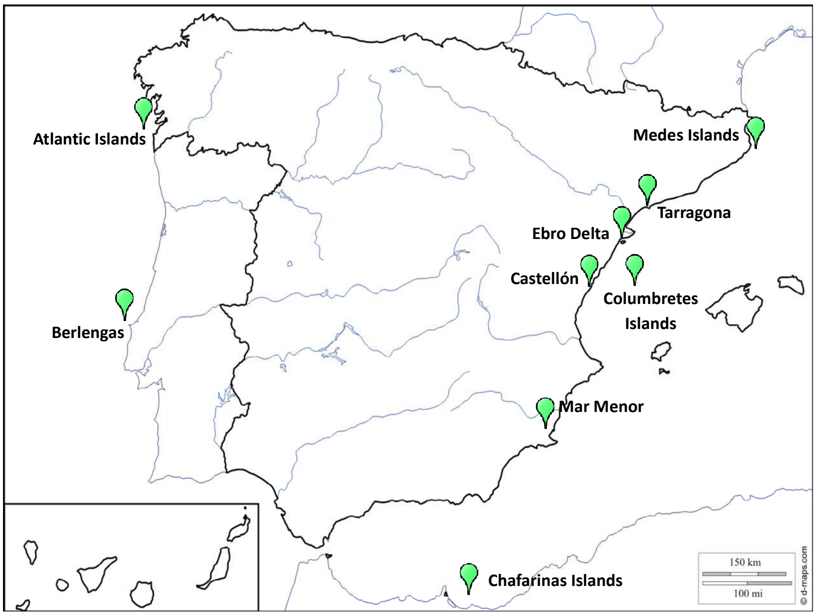


Figure 3.1. Map of the location of the National and Natural parks studied in this work.

Fresh eggs of yellow-legged gull or Audouin's gull were collected from each colony during the breeding season of 2016. For the yellow-legged gulls, 12 eggs were randomly collected from 3 subcolonies (36 eggs in total in each locality), while for the Audouin's gulls, only six eggs from each subcolony (18 eggs in total) were collected since it is a protected species. The eggs were transported to the laboratory in a cool box. For comparing the results between different colonies, only the first egg of each nest was sampled since it represents the maximum pollutant transference level from female to eggs, as demonstrated for PCBs, dioxins and furans (Pastor et al., 1995) and perfluorinated compounds (Vicente et al., 2015). Embryonated eggs were discarded of analytical analysis.

3.2.4. Analytical method

3.2.4.1. OC pesticides and PBDEs

One gram of lyophilized eggs was spiked with 100 ng (100 µL of a 1 µg/mL) of a surrogate mixture of all internal standards. Then, 25 mL of a solvent mixture hexane:dichloromethane (1:1, v/v) was added and the samples were vortexed (1 min) and ultrasonic extracted at room temperature for 10 min. This extraction procedure was repeated 3 times without changing the solvent. Samples were then centrifuged at 3,000 rpm for 10 minutes at room temperature and the supernatant was transferred into a 40 mL amber glass vial and placed in a Turbo Vap LV (Caliper, Hopkinton, Massachusetts), where the extract was evaporated until approximately 1 mL under a gentle stream of N_2 at 30ºC. To purify the extracts, Florisil SPE cartridges of 10 g were conditioned with 35 mL of a solvent mixture of hexane:dichloromethane (1:1, v/v) and the extract was then added and eluted by gravity with 35 mL hexane:dicloromethane (1:1, v/v). The eluate was evaporated to about 1 mL under a gentle stream of N_2 after adding 500 µL of isooctane as a keeper (Dąbrowski, 2016). The final extract (*c.a*. 500 µL) in isooctane was transferred into a 1.5 mL chromatographic vial and analysed by GC-MS/MS.

OCs were determined on an Agilent 7890 GC system coupled with a 7000A GC/MS triple quad mass spectrometer (Agilent, Santa Clara, CA, USA). The chromatographic separation was accomplished on a HP-5MS (5%-phenyl, 95% dimethylpolysiloxane) fused-capillary column (Agilent, Santa Clara, CA, USA) of 30 m of length, 0.25 mm of internal diameter (I.D.) and 0.25 µm film thickness. Two µL were injected (inlet temperature 225ºC) on splitless mode during 1 min. The oven temperature was programmed from 70 ºC to 175 ºC at 6 ºC/min (held for 4 min) then raised to 235 ºC at 3 ºC/min, and finally to 315 ºC at 7 ºC/min (held for 10 min). Helium was used as carrier gas at a flow of 1.2 ml/min (75.9 kPa). Three inlet (200 ºC, 250 ºC and 300 ºC) and ionization source temperatures (200 ºC, 225 ºC and 250 ºC) were tested to obtain maximum signal without the degradation of target compounds (e.g. dicofol). Acquisition was performed in multiple reactions monitoring mode (MRM). To optimize the fragmentation conditions, the most abundant ion of each compound was selected as precursor ion and the resulting product ions were monitored at increasing collision energies from 5 to 50 eV using product ion scan mode. The two most abundant product ions that produced the highest signal to noise ratio were chosen. Two MRM transitions were set for each target compound for quantification and confirmation purposes (Table 3.1). In this way, the unambiguous identification of each compound can be assured by the two MRM transitions and the chromatographic retention time. Quantification was performed by internal standard method using $^{13}C_6$-hexachlorobenzene, $^{13}C_{12}$-4,4´-DDE, CB-65 and CB-209 as internal standards. Agilent´s Mass Hunter (Version B.03.01/Build 3.1.170.0) software (Agilent, Santa Clara, CA, USA) was used in this study to process acquisition data.

PBDEs were analyzed by GC-NCI-MS on an Agilent 6890 gas chromatograph coupled to an Agilent 5973 Network mass spectrometer (Santa Clara, CA, USA). Chromatographic separation was carried out on an Agilent HP-5MS (15 m x 0.25 mm I.D., 0.25 µm film thickness) fused-capillary column using helium as the carrier gas at a flow rate of 1.1 mL/min (15 psi). The temperature program was from 120 ºC (held for 1 min) to 205 ºC at 8 ºC/min and then to 310 ºC at 6ºC/min (held for 10 min). Two µL of sample were injected using pulsed splitless injection mode (15 psi) during 2 min at a temperature of 300 ºC. The GC-NCI-MS operating conditions were as follows: ion source and transfer line temperature of 255 and 310 ºC, electron energy of 190 eV, emission current 50 µA, and ammonia was used as the chemical ionization moderating gas (ion source pressure of 1.9 10^{-4} torr). MS acquisition was performed in selected ion monitoring mode (SIM) using for quantification the bromine ion (*m/z* 79), and *m/z* 486.5 for BDE 209, and for identification purposes the ions at *m/z* 81 and 161 $[HBr_2]^-$ (Table 3A2) (Eljarrat et al., 2002). MSD ChemStation software version 5.0.0.0 from (Agilent, Santa Clara, CA; USA) was used for data acquisition and processing of the results. PBDEs quantification was performed by internal standard method using PCB 209 as surrogate standard and MBDE77 as internal standard.

3.2.4.2. PFOS

Sample preparation for PFCs determination was based on the method described by Meyer et al. (2009) using solid-liquid extraction from wet samples with acetonitrile. Briefly, about one gram of sample was weighted in a polypropylene tube and internal standard (m-PFOS) was added at a concentration of 100 ng/g. After an incubation period of 18 hours at 4ºC, 9 mL of acetonitrile were added to the samples and they were thoroughly mixed using a vortex. Samples were then extracted in an ultrasonic bath for 10 minutes at room temperature (3 times). Clean-up was performed using 25 mg of activated carbon and 50 µL of glacial acetic acid. After evaporation under a gentle N_2 stream, the extract was reconstituted with 350 µL of acetonitrile and 150 µL of HPLC-water, and the final extract was analyzed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) using an Acquity LC system (Waters Corp., Milford, MA, USA) coupled to a Triple Quadruple tandem mass spectrometer (Water Corp.) using the instrumental conditions reported by Vicente et al. (2012). Chromatographic separation was performed on an Acquity UPLC BEH C18 column (1.7 µm particle size, 50 mm×2.1 mm, Waters, USA). Quantification of PFOS was performed by isotopic dilution.

3.2.5. Quality assurance/quality control (QA/QC)

For each analytical procedure quality control analysis were performed to ensure the quality of the results. Procedural blanks were analyzed using hen eggs spiked with the surrogate standard (100 ng) and any of the target compounds were detected at levels above method limits of detection. During the chromatographic sequences, isooctane was injected every 5 samples to ensure no carryover between samples. Extraction efficiency was evaluated using commercial lyophilized hen eggs (n=3). One gram of sample was spiked at 100 ng/g of target organochlorine compounds (10 ng/g for PCBs), 50 ng/g of PBDEs (500 ng/g of BDE 209) and PFOS (50 ng/g) and it was extracted and cleaned up following the above described protocols. Method and instrumental detection and quantification limits were also determined, using a signal to noise ratio (S/N) of 3 and 10, respectively. Intra-day precision (n=5) and inter-day precision (n=5 in 5 non-consecutive days) were performed with a standard mixture of all analytes at 0.5 µg/mL for OCs and PBDEs (0.05 µg/mL for PCBs) (5 µg/mL for BDE 209) and 0.1 µg/mL for PFOS. The lipid content was gravimetrically determined and was of 8.2±0.9 and 6.9±05% for yellow-legged gull and Audouin's gull, respectively, and the average water content was of 76.5±1.5%.

3.2.6. Egg and eggshell parameters

Maximum length (L) and width (Wd) were measured with a caliper (DigitalPro Ratio 880063) with a precision of ±0.01 mm and the weight (W) of the whole egg with a precision balance (±0.1 g; Pesola MS1000, Pesola AG, Schindellegi, Switzerland). Afterwards, eggshells were dried at room temperature for one month and then the following was measured: the weight of the dried shell (WS) and the eggshell thickness. The eggshell thickness was measured at six points around the equator (three at each half eggs), using a caliper (±0.01 mm). The eggshell thickness index (I) values were calculated for all the eggs according to the method given by Ratcliffe (1967).

The egg volume was calculated using the formula $V=Kv^{*} L^{*}Wd^{2}$ (Hoyt, 1979), where Kv is a species-specific constant whose value is 0.000477 for the yellow-legged gull (Oro, 2008) and 0.000476 for the Audouin's gull (Oro et al. 1995). Desiccation index (Di) was calculated for each egg as a measure of functional quality using the formula $Di = (W - WS)/V$ (Helander et al., 2002). Desiccation index expresses the mean density of the entire egg content at the time of laying because the egg immediately begins to lose weight by diffusion of water vapor. A low Di value indicates desiccated eggs. Egg parameters within each species were compared using ANOVA and Tukey post-hoc test for paired comparisons. Egg parameters are always shown as mean ± standard deviation (SD). Analyses were implemented using the R 3.3.2 software (R Development Core Team, 2016) with the package multcomp (Hothorn et al., 2008). In addition, egg parameters were correlated with the load of POPs detected, according to each chemical family.

3.3. Results and discussion

3.3.1. Performance of the analytical method

The GC-MS/MS method developed in the present work was applied to the determination of a wide range of organochlorine pesticides in gull eggs and is based on a previous GC-MS method optimised for the analysis of a lower number of pesticides (Morales et al., 2012). As an example, Figure 3.2A shows, the GC-MS/MS chromatogram of a standard solution with the resolved 36 OCPs and 6 marker-PCBs. Nearly all compounds showed a good chromatographic resolution. However, heptachlor epoxide isomers could not be separated due to their chemical nature, either chromatographically nor with specific MRM transitions, so results are expressed as the sum of the endo- and exo- heptachlor epoxide isomers. In addition, these compounds coeluted with oxychlordane, but this coelution can be solved by selecting specific MRM transitions as can be seen in Figure 3.A1A on the annex, where the MS spectra obtained along the chromatographic peak as well as the specific MRM transition chromatograms for heptachlor epoxides (353→191) and oxychlordane (185→121) (Figure 3.A1B) are given. Table 3.1 shows the selected transition and collision energies, along with chromatographic retention times for each target compound. Two MRM transitions were selected, the most abundant one for quantification and the other for confirmation. The transitions were specific for each analyte and no other coelutions were observed.

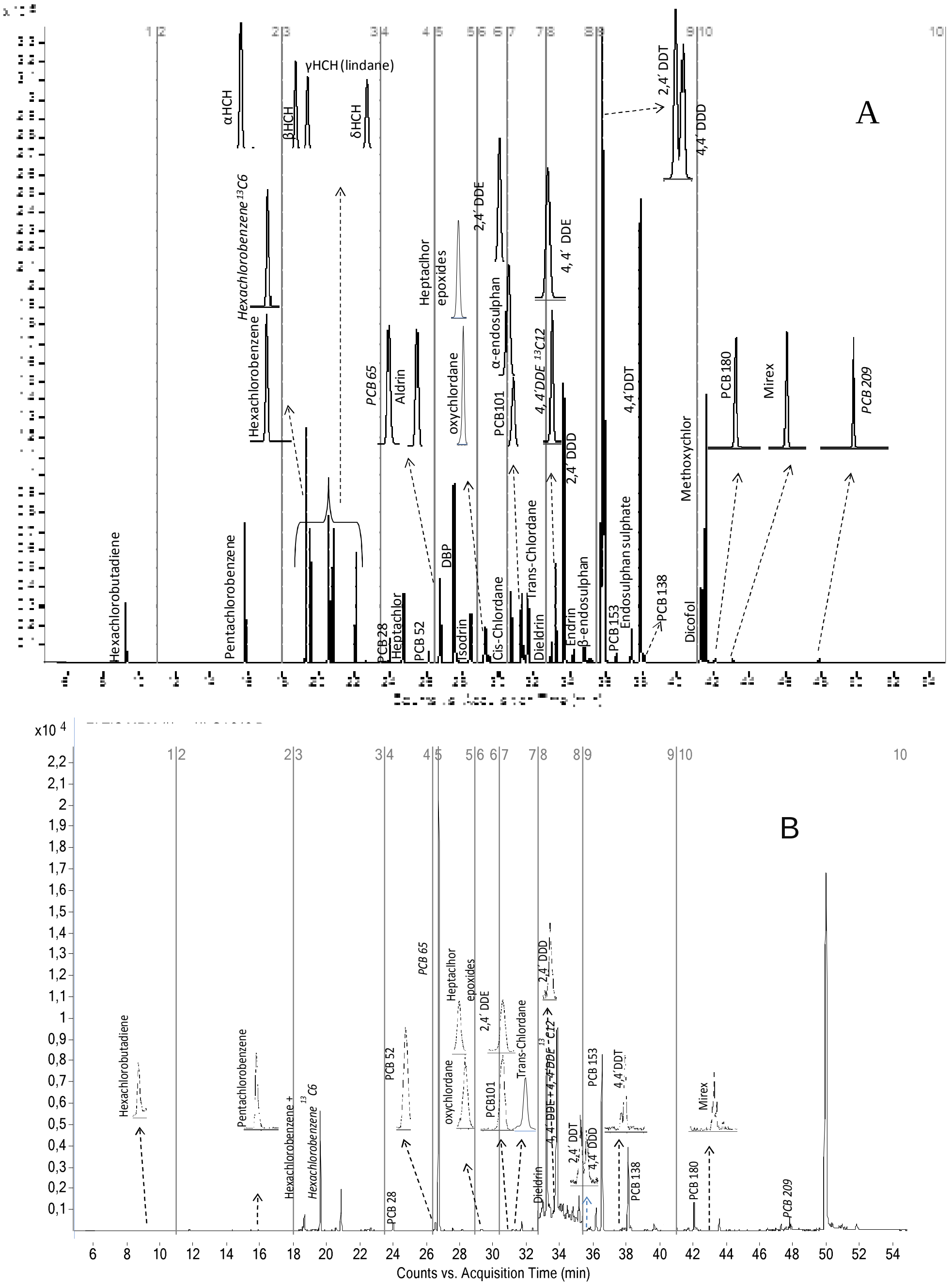


Figure 3.2. A) GC-MS/MS (MRM) total ion chromatogram of 1 ng/mL standard mixture (0.1 ng/ mL for PCBs) of all the compounds at optimal conditions; B) GC-MS/MS (MRM) total ion chromatogram of a Chafarinas´ gull egg sample.

Table 3.1. Retention time, MRM quantifier and qualifier transitions and collision energies (CE) of the target compounds and surrogates internal standards used for GC-MS/MS determination.

Compound	Retention time (min)	Quantifier transition	CE (eV)	Qualifier transition	CE (eV)
Hexachlorobutadiene	9.40	260→225	10	260→190	45
Pentachlorobenzene	15.90	250→215	30	250→180	30
α-HCH	19.36	219→147	20	219→183	15
Hexachlorobenzene	20.55	284→249	20	284→114	20
β-HCH	20.80	219→147	20	219→183	15
γ-HCH (lindane)	22.10	219→183	15	219→147	20
δ-HCH	23.98	219→147	20	219→183	15
PCB 28	24.02	258→186	30	258→188	40
Heptachlor	24.80	272→237	20	272→235	20
PCB 52	26.10	292→222	45	292→220	40
Aldrin	26.80	263→193	40	263→191	40
Isodrin	28.50	263→193	30	263→228	20
Heptachlor epoxides	29.30	353→191	45	353→193	45
Oxychlordane	29.35	185→121	20	185→85	20
Cis-Chlordane	30.70	375→266	30	375→339	20
2,4´-DDE	31.30	318→246	20	318→248	20
α-endosulfan	31.40	195→159	10	195→125	20
PCB 101	31.43	326→256	50	326→219	50
trans-Chlordane	31.65	373→266	30	373→264	30
Dieldrin	32.98	241→206	30	241→170	40
4, 4´-DDE	33.25	318→248	20	318→246	20
2,4´-DDD	33.70	235→165	30	235→200	20
Endrin	34.20	263→193	20	263→228	20
β-Endosulfan	34.80	241→206	15	241→170	25
2,4´-DDT	35.73	235→165	40	235→199	10
4,4´-DDD	35.86	235→165	40	235→199	10
PCB 153	36.53	360→290	30	360→288	30
Endosulfan sulphate	37.40	272→237	10	272→143	40
4,4´-DDT	37.90	235→165	40	237→167	40
PCB 138	38.10	360→290	40	360→288	40
Dicofol	41.30	139→111	30	139→75	30
Methoxychlor	41.53	227→169	30	227→115	50
PCB 180	42.10	394→324	30	394→359	20
Mirex	43.06	332→262	40	332→260	50
Surrogates					
$^{13}C_{12}$-4, 4´-DDE	33.25	330→258	30	330→260	30
PCB 65	26.74	292→222	30	292→220	30
$^{13}C_6$-Hexachlorobenzene	19.64	290→218	20	290→253	20
PCB 209	47.80	498→428	50	498→391	50

During the optimization of the GC-MS/MS method, it was observed the degradation of dicofol at a temperature of 300ºC on the injection port and 250ºC on the ionization source. This behavior is attributed to low thermal estability and has been described before (Zamariola et al., 2017). To minimize this effect, the thermal stability of dicofol was evaluated at different injection port (200, 250 and 300 ºC) and ionization source temperatures (200, 225 and 250 ºC). Generally, all the target compounds, except dicofol, showed the highest response at high temperatures, due to the better evaporation process and a good thermal stability. In contrary, the dicofol degration was significantly reduced at low temperatures. Figure 3.A2 and 3.A3 (Annex) shows the different response for dicofol and its main degradation product, p,p´dichlorodibenzophenone (DBP), at different inlet and source temperatures. As a compromise and to maintain the sensitivity for the other target compounds, a temperature of 250 ºC for the injection port and 225 ºC for ionization source were selected as optimum values.

Quality parameters for PCBs and OCs method are shown in Table 3.2. All calibration curves fitted well with a linear model ($R^2 \geq 0.99$), with response factors ranging from 0.04 to 6.90. Good intra-day precision was achieved when injecting a standard five times consecutively, with values of the relative standard deviation (RSD%) always below 5.5%, except for dicofol, which was 12.1%. Inter-day precision, expressed as %RSD, was in all cases, lower than 19.1%. Instrumental detection limits, calculated as the amount (in ng) that produce a signal to noise ratio (S/N) of 3 ranged from 0.1 pg for pentachlorobenzene to 1.82 pg for endrin. Recoveries of whole method ranged between 71±11% for trans-chlordane and 119±3% for pentachlorobenzene. These recoveries are acceptable showing the adequate efficiency of the extraction procedure. In addition, no matrix effects or interferences related to the high lipid content were observed that could affect quantification. As an example, Figure 3.2B shows the total ion MRM chromatogram of a gull egg sample from Chafarinas. The developed method provided good sensitivity with method detection limits in the low ng/g ww level.

Table 3.2. Quality parameters of the GC-MS/MS method. a: Compounds quantified at the MDL.

Compound	Response factor	R^2	IDL (pg)	MDL (ng/g ww)	MQL (ng/g ww)	% R (±SD)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
Hexachlorobutadiene	0.40	0.9955	0.02	0.003	0.01	114 ± 9	3.0	1.6
Pentachlorobenzene	1.08	0.9951	0.01	0.002	0.01	119 ± 3	1.8	1.2
α-HCH	1.87	0.9975	0.08	0.28	0.92	95 ± 15	1.0	0.9
Hexachlorobenzene	1.02	0.9968	0.02	0.002	0.01	107 ± 5	0.6	0.4
β-HCH[a]	1.41	0.9987	0.11	0.23	0.77	116 ± 12	4.3	3.7
γ-HCH (lindane)	0. 43	0.9994	0.12	0.03	0.10	90 ± 9	1.7	9.7
δ-HCH	1.16	0.9989	0.30	0.31	1.04	76 ± 15	3.5	4.8
PCB 28	0.53	0.9995	0.19	0.09	0.31	118 ± 0.2	0.8	0.8
Heptachlor	0.91	0.9998	0.04	0.003	0.01	103 ± 20	3.2	2.8
PCB 52[a]	0.25	0.9995	0.17	0.06	0.21	100 ± 0.5	2.2	1.6
Aldrin	0.16	0.9997	0.15	0.05	0.17	93 ±3	0.3	0.6
Isodrin	0.11	0.9998	0.23	0.08	0.27	86 ± 7	0.7	1.8
Heptachlor epoxides	0.01	0.9995	0.52	0.09	0.30	81 ± 12	1.4	2.8
Oxychlordane	0.10	0.9993	0.03	0.02	0.07	83 ± 10	1.5	4.4
Cis-Chlordane	0.14	0.9996	0.08	0.03	0.09	74 ± 13	2.6	5.4
2.4´-DDE[a]	0.46	0.9999	0.06	0.03	0.11	114 ± 2	1.5	3.8
α-Endosulfan	0.08	0.9998	0.20	0.28	0.94	110 ± 7	1.6	3.4
PCB 101	0.15	0.9996	0.37	0.11	0.38	77 ± 2	3.2	6.4
trans-Chlordane	0.07	0.9997	0.08	0.03	0.10	71 ± 11	2.8	8.3
Dieldrin	0.04	0.9999	0.47	0.55	1.84	105 ± 1	1.2	2.6
4. 4´-DDE	0.58	0.9998	0.05	0.03	0.11	102± 4	0.6	1.3
2.4´-DDD[a]	2.52	0.9998	0.07	0.04	0.14	99 ± 7	1.3	16.5
Endrin	0.09	0.9934	1.82	0.25	0.82	99 ± 12	1.1	4.5
β-Endosulfan	0.10	0.9996	0.26	0.32	1.05	74 ± 6	2.0	4.5
2.4´-DDT[a]	6.90	0.9992	0.04	0.02	0.08	95± 11	2.2	19.1
4.4´-DDD[a]	5.26	0.9986	0.14	0.05	0.16	92 ± 18	4.2	0.9
PCB 153	0.21	0.9992	0.29	0.12	0.41	107±3	5.5	16.1
Endosulfan sulph.	0.32	0.9978	0.12	0.04	0.15	73 ± 1	3.1	10.7
4.4´-DDT	5.00	0.9958	0.12	0.03	0.09	90 ± 11	3.5	7.6
PCB 138	0.18	0.9988	0.27	0.14	0.46	102 ± 3	5.4	16.3
Dicofol	0.66	0.9939	1.54	0.16	0.53	112 ± 6	12.1	12.9
Methoxychlor	4.88	0.9916	0.11	0.04	0.13	77 ± 15	2.8	17.3
PCB 180	0.12	0.9985	0.49	0.19	0.63	97 ± 2	2.7	12.5
Mirex	0.20	0.9998	0.41	0.11	0.37	98 ± 3	3.9	9.3
BDE 28	0.96	0.9936	0.02	0.01	0.03	86 ± 3	0.8	7.5
BDE 47	0.84	0.9922	0.03	0.01	0.04	88 ± 2	0.6	6.0
BDE 100	1.04	0.9917	0.03	0.06	0.20	76 ± 2	0.3	4.1
BDE 99	1.08	0.9922	0.09	0.05	0.18	81 ± 2	0.6	4.4
BDE 154	0.96	0.9951	0.09	0.04	0.12	89 ± 2	0.6	5.7
BDE 153	0.97	0.9969	0.08	0.09	0.29	90 ± 3	0.5	4.3
BDE 183	0.78	0.9982	0.06	0.02	0.07	71 ± 1	1.0	5.7
BDE 209[a]	0.16	0.9928	0.36	0.46	1.52	83 ± 3	4.0	7.2
PFOS	0.15	0.9962	0.09	0.50	0.16	92± 3	11.3	8.9

For PBDEs determination, extraction procedure was effective with recovery rates from 71±1% for BDE 183 to 90±3 % for BDE 153. In addition, the calibration curves showed good linearity for all compounds at concentrations between 0.01 and 0.5 µg/mL (0.1 and 5 µg/mL for BDE 209). The method detection and quantification limits ranged from 0.01 (BDE 28 and BDE 47) to 0.46 ng/g ww for BDE 209 and from 0.04 for BDE 47 to 1.52 ng/g ww for BDE 209, respectively. Finally, consecutive injections gave variation coefficients below 5% and below 10% for injections of a standard during different days for all PBDEs (table 3.2). Because of lack of selectivity of the NCI mode using *m/z* 79/81 for PBDEs, which are common ions for all brominated compounds, *m/z* 161 was used as confirmation ion and no interferences were observed in any of analyzed samples. Figure 3.A4 shows, as an example, the resulted total ion GC-NCI-MS chromatogram of a standard mixture of PBDEs. Finally, PFOS was detected with good analytical parameters, following the results of previous studies that report the method used for its analysis and the performance obtained (table 3.2) (Meyer et al., 2009).

3.3.2. Levels on gull eggs

Gull eggs have been proposed as bioindicators of environmental pollution. POP levels in eggs reflect the contaminant burden of the female at the time of egg laying, especially the uptake of contaminants from food recently ingested around the colony, although some contaminants may derive from the previously accumulated levels in the adipose tissue (Vicente et al., 2015). The concentrations of individual POPs in the different colonies are indicated in table 3.3 and the main families detected were PCBs and DDTs. This is in accordance to a previous study in glaucous gull from the Arctic where PCB and p,p′-DDE accounted for 90% of the contaminants detected (Herzke et al., 2003).

Table 3.3. Levels (ng/g ww) of POPs detected in gull eggs of Natural and National parks of the Iberian Peninsula. Nd: not detected.

	Larus michahellis					*Larus audouinii*				
Compounds	Columbretes (n=1)	Ebro delta (n=3)	Chafarinas (n=1)	Mar Menor (n=1)	Atlantic Is. (n=3)	Medes (n=3)	Berlengas (n=1)	Castellón (n=1)	Ebro delta (n=3)	Tarragona (n=1)
PFOS	40.5	52.7±11	19.3	10.9	13.5±3.0	82.73±9.1	10.1	80.0	91.7±21.3	65
PCB 28	1.38	1.91±0.8	0.13	0.70	1.02±0.4	0.79±0.1	0.43	1.71	1.23±0.2	1.14
PCB 52	0.16	0.11±0.1	0.31	0.07	0.14±0.1	0.07±0.1	0.03	0.39	0.20±0.1	0.25
PCB 101	4.80	3.26±0.3	0.65	0.93	0.59±0.2	0.39±0.1	0.42	6.23	3.54±0.4	4.60
PCB 138	244	134±20	73.5	64.2	77.3±26	23.3±3.0	18.3	340	175±22.9	168
PCB 153	388	253±35	104	110	128±44	39.1±5.6	29.6	597	304±29	295
PCB 180	219	132±24	68.1	52.2	47.2±16	23.9±3.4	10.6	333	163±16.4	174
BDE 28	nd	nd	nd	nd	nd	nd	nd	nd	0.26±0.1	0.47
BDE 47	2.60	4.43±5.3	1.05	5.76	2.11±0.8	2.15±0.7	2.15	1.29	1.99±0.4	1.48
BDE 99	2.11	1.87±1.2	1.03	8.13	3.34±1.5	4.44±1.9	1.09	0.26	0.55±0.2	0.74
BDE 100	0.79	0.74±0.3	0.43	2.24	1.13±0.4	1.15±0.4	0.73	0.45	0.65±0.1	0.56
BDE 153	1.87	0.84±0.5	0.80	3.33	1.15±0.8	1.87±0.7	0.80	0.36	1.17±0.1	0.96
BDE 154	0.83	0.48±0.1	0.36	0.94	0.44±0.1	0.63±0.1	0.28	0.63	0.77±0.1	0.55
BDE 183	0.31	0.27±0.2	0.33	0.55	0.65±0.4	0.80±0.1	0.22	0.04	nd	0.20
BDE 209	0.54	5.21±1.4	1.50	1.28	1.52±1.0	2.35±0.4	0.54	0.00	1.03±1.2	1.28
Dieldrin	nd	6.76±0.2	nd	nd	4.94±1.0	4.08±0.2	4.08	5.42	7.86±3.9	nd
Endrin	nd	0.41±0.7	nd	nd	nd	1.94±1.8	nd	nd	nd	nd
β –HCH	nd	0.72±0.2	0.46	nd	nd	0.64±0.2	0.33	nd	0.70±0.3	nd
γ-HCH	nd	0.60±1.0	1.85	nd	nd	nd	nd	1.09	nd	nd
Hexachlorobutadiene	nd	0.04±0.1	0.01	0.03	0.05±0.1	0.04±0.1	0.04	0.04	0.05±0.0	0.11
Hexachlorobencene	4.29	4.95±0.8	0.82	2.82	3.61±1.1	1.76±0.5	3.55	3.06	4.86±0.7	4.02
Pentachlorobenzene	nd	0.33±0.1	0.01	0.24	0.27±0.1	0.20±0.1	0.30	0.22	0.30±0.1	nd
Heptachlor epoxides	4.85	4.74±2.8	2.38	2.05	4.31±0.4	3.92±1.4	2.31	4.68	2.49±0.2	nd
4,4'-DDT	0.09	0.68±0.5	0.19	0.56	0.13±0.1	0.50±0.1	0.12	0.16	1.35±0.7	0.04
4,4'-DDE	238	218±50	61.7	197	31.4±20	42.6±9.4	32.3	252	760±412	170
4,4'-DDD	nd	0.09±0.1	0.04	0.37	0.07±0.1	nd	nd	0.14	0.16±0.1	nd
2,4-DDT	nd	0.31±0.1	0.10	0.11	0.07±0.1	0.02±0.1	0.05	nd	0.45±0.2	nd
2,4-DDE	nd	nd	nd	0.05	0.04±0.1	nd	nd	0.04	0.02±0.1	nd
2,4-DDD	nd	0.05±0.1	0.02	nd	0.04±0.1	nd	nd	nd	0.08±0.0	nd
a-chlordane	0.28	nd	nd	nd	nd	nd	nd	1.40	nd	0.32
g-chlordane	nd	1.45±0.1	nd	1.33	1.47±0.1	nd	1.40	1.50	1.42±0.1	nd
oxyclordane	0.01	5.17±1.3	0.65	3.29	2.92±0.3	3.18±0.5	3.81	1.88	3.44±0.3	nd
Mirex	nd	nd	nd	1.80	2.03±0.1	nd	1.60	nd	2.08±0.4	nd

Figure 3.3A and 3.3B show the ∑POP concentrations found in gull eggs and the percentage of mean concentrations of each family of POPs to the total ∑POP levels in each studied colony, respectively. Audouin´s gull eggs showed, as reported in previous studies (Morales et al., 2012), higher levels of pollutans due to their pelagic fish based diet. The colonies of Castellón and Tarragona derive from the colony of the Ebro Delta (Payo-Payo, 2017). Whereas Castellón and Ebro Delta have similar ∑POP levels (1.6 and 1.5 µg/g ww, respectively), the colony of Tarragona has lower levels probably because it is a younger colony. On the other hand, the POP distribution was different in each of this 3 colonies, with higher contribution of PCBs in Castellón and higher contribution of OCs in Tarragona, compared to Ebro Delta. These colonies were installed in 2011 and 2013, respectively and we presume that the POP contribution reflexes the environment of each area. In fact, Castellón and Columbretes gulls, despite being different species, have similar ∑POPs and similar profiles with a high contribution of PCBs, so probably this reflects a fish based diet.

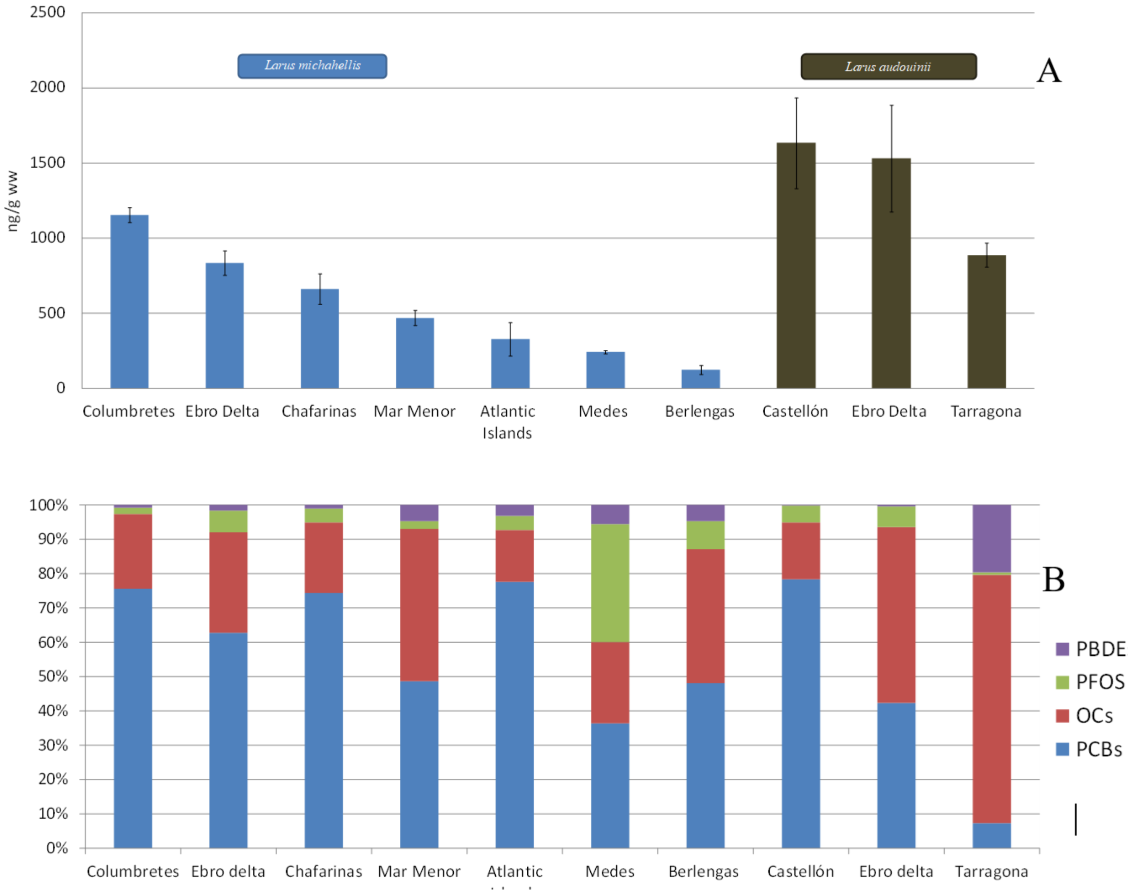


Figure 3.3. A) ∑POP concentration in the different studied colonies and (B) contribution (%) of PBDEs, PFOS, OCs and PCBs to the total ∑POPs in each gull colony.

In yellow-legged gulls, the impact of ∑POPs in eggs was as follows: Berlengas < Medes < Atlantic Islands < Mar Menor < Chafarinas < Ebro Delta < Columbretes. Eggs from Berlengas archipelago, similar to Atlantic Islands, have the lowest ∑POPs, which is highly influenced by the Atlantic ocean tides and dilution capacity of the ocean. Contrarily, Columbretes shows the highest levels and this might be explained by the long distance to the shore which affect gull's feeding habits, based mainly in fish and not so much in rubish tips. Again, this is in accordance to the levels detected in Audouin's gull eggs, which feeds mostly in fish.

In all colonies, PCBs showed the highest levels, dominating pollution profiles and usually followed by OCs except for Medes which has a pollution pattern dominated by PFOS. PBDEs were present at similar levels, with slight variations within colonies (Figure 3.3B). The highest levels were detected in Audouin's gull from Tarragona port, and this might be linked to the high ship traffic in this area. Among PCBs, the most abundant congener was always the PCB 153, followed by PCB 180 and PCB 138. Contribution of low chlorination degree marker-PCBs like 28, 52 and 101 congeners was much lower due to their lower bioaccumulation capacity (Drouillard et al., 2001). Same profiles have been previously reported in apex organisms of marine habitats like whale sharks (Fossi et al., 2017). ∑PCBs in Castellón colony were found at concentrations higher than 1 µg/g ww, followed by Columbretes (850 ng/g ww), and Atlantic Islands and Mar Menor, but at lower levels, whereas Berlengas and Medes colonies were the less affected by PCBs, with ∑PCB of 60 and 85 ng/g ww, respectively.

∑OCs ranged from 48.3 ng/g ww in Berlengas to 783.4 ng/g ww in Audouin´s gull eggs from the Ebro Delta. Among them, aldrin, isodrin, α- and β- endosulfan, α- and δ-HCH, heptachlor, methoxychlor, dicofol and endosulfan sulfate were never detected. This indicates that these compounds were either not used or used historically so that environmental degradation has already occurred. Hexachlorobenzene, 4,4´-DDT and 4,4´-DDE were ubiquitous and detected in all colonies. Hexachlorobenzene is listed in annex C of the Stockholm Convention because of its unintentional production, so the levels detected arise from emissions as a byproduct from several industrial processes. The highest levels of 4,4´-DDE and 4,4´-DDT were detected in Audouin´s eggs from Ebro Delta with 700 ng/g ww and 1.4 ng/g ww respectively. In Spain, mean values of 9,000 and 200 ng/g ww have been reported for 4,4´-DDE and 4,4´-DDT in red kite eggs from Doñana (Gómara et al., 2007) and 721 ng/g ww for the former in flamingo´s eggs from the same site (Guitart et al., 2005), while levels of 700 ng/g ww and 3.7 ng/g ww were respectively detected in peregrine falcon eggs from the central part of the peninsula (Merino et al., 2005). Other studies report levels of 936 and 154 ng/g ww of 4,4´-DDE in booted eagle and goshawk eggs from Murcia (Martínez-Lopez et al., 2007), although 4,4´-DDT has not been detected. Lower levels of 4,4'-DDE have been found for goshawk and buzzard eggs from north-east Spain (292.5 and 113 ng/g ww respectively) (Mañosa et al., 2003). In all cases, DDE levels were much higher than those found for DDT, suggesting a non-recent use of the technical pesticide. However, new environmental inputs of DDT due to dicofol production and use have been suggested in Spain. Previous studies have reported about the $\frac{2,4'DDT}{4,4'DDT}$ ratio being used to distinguish between a "dicofol type" contamination and the historical use of technical DDT when values are over 0.2 (Muñoz-Arranz and Jiménez, 2011; Qiu et al., 2005). Except for Medes which showed a ratio lower than 0.1, all samples presented high values, ranging from 0.19 in Mar Menor to 0.9 in subcolonies of Ebro Delta. Spain produced high quantities of dicofol until 2006, when 90 tons of this acaricide were synthesized (Li et al., 2015) so this kind of pollution is suggested in this study. However, dicofol has not been detected in any sample.

Heptachlor epoxides were detected in all colonies except in Tarragona at 2-8 ng/g ww, which is in the same range than results reported for birds of prey like goshawk (1.6 to 5.2 ng/g ww; Mañosa et al., 2003). Dieldrin, oxychlordane, trans-chlordane, hexachlorobutadiene and pentachlorobenzene were also frequently detected at the ng/g ww level. While the three former compounds have been used in agriculture as pesticides for termite or other insects' control, and therefore its presence is related to its historical use, hexachlorobutadiene and pentachlorobenzene, listed in annex C of the Stockholm Convention, are linked to its unintentional production during industrial processes and were detected at low levels. β and γ HCHs were detected at 0.4 ng/g ww in Medes and from 0.3 to 3.7 ng/g ww in Berlengas and Chafarinas, respectively. Mirex was detected in Chafarinas, Ebro Delta, Mar Menor and Berlengas with around 2 ng/g ww in all of the samples. Endrin was just found in 2 samples, being Medes, one of the less polluted places. However, its fast metabolization by birds was described long ago (Heinz and Johnson, 1979), being its persistence in soils the main environmental problem related to this compound, so this can be the main reason for this chemical not being detected. Cis-chlordane was just detected in Tarragona, Castellón and Columbretes, which can be indicating a local source of the compound, found concentrations were lower than 1.5 ng/g ww in all cases.

∑PBDEs levels ranged from 3 ng/g ww in Castellón to 22 ng/g ww in Mar Menor´s colony. Very different profiles were found among colonies probably related to the different use of PBDE technical formulations. BDE 28 was only detected in Audouin´s eggs from Ebro Delta and Tarragona´s port. BDE 47 was the most abundant congener in Ebro Delta colonies, Chafarinas, Castellón, Tarragona, Columbretes and Berlengas, although BDE 153 was the most abundant in Medes. Profiles of Atlantic Islands and Mar Menor were dominated by BDE 99 followed by BDE 47. The deca-brominated congener (BDE 209) showed levels between 0.54 ng/g ww in Columbretes and Berlengas and 5.2±1.4 ng/g ww in yellow-legged gull´s eggs from the Ebro Delta. In Spain PBDEs are widespread. ∑PBDEs in white stork´s eggs from Doñana and Madrid of 1.64 and 9.08 ng/g ww where reported in 2005, with profiles highly dominated by the decabrominated congener (Muñoz-Arnanz et al., 2005), probably due to the different diet. Higher results were reported by Barón et al. (2011) based on lipid weight, for 14 different bird eggs from the same place, ranging from 1.4 to 90.7 ng/g lw. The levels are in general low if compared with levels detected in different gull species eggs in Canada, which ranged from 37 to 610 ng/g ww (Chen et al., 2012) but are in the same order of magnitude as previously reported from northern Europe herring gull eggs with ∑PBDEs of 40 ng/g ww (Carlsson et al., 2011) and ∑PBDEs of 8.3 ng/g ww (Helgason et al., 2009).

PFOS has been detected in Audouin’s gull eggs at levels from 10.1 to 91.7 ng/g ww and in yellow-legged gull eggs at levels from 13.46 to 82.73 ng/g ww. These levels are very similar to those detected in a 20 year survey of PFOS in gull eggs from Germany (Rüdel et al., 2010). Audouin’s gull eggs have the highest concentration of PFOS in the colony of Ebro Delta (91.7 ng/g ww). Values from Chafarinas and Atlantic Islands (27 and 43 ng/g ww respectively) are similar to those reported by Vicente et al. (2012) for PFOS in Southern Mediterranean colonies (Dragonera, Grosa and Chafarinas) and western Iberian colonies (Berlengas and Atlantic Islands) in 2009. In the other hand, values from Medes (82 ng/g ww) and Ebro Delta (52 ng/g ww) are higher than values form Vicente et al. (2012) for PFOS in northen Mediterranean colonies (Medes Islands, Ebro Delta and Columbretes) in 2009. Reason for this might be that southern Iberian colonies do not receive direct industrial and urban discharges as northern colonies, but rather agricultural run-off (Vicente et al., 2012). Comparing with our results, Vicente et al. (2015) dectected a higher concentration (122-281 ng/g yolk ww) of PFOS in eggs of Audouin's gull from Ebro Delta. In the Comacchio lagoon (Adriatic Sea) concentration of PFOS in the yolk sac of embryos of yellow-legged gull's eggs ranged between 30.6 and 686 ng/g ww (mean 165.9±36.9 ng/g ww) (Parolini et al., 2016). Higher concentrations can be found in eggs of gull species between 2009 and 2014 from across Canada and the United States, with maximums of mean 921±431 ng/g ww (Toronto Harbour in Lake Ontario), 611±176 ng/g ww (Middle Island in Lake Erie), 479±91 ng/g ww (Weseloh Rocks in the Niagara River) and 472±160 ng/g ww (Hamilton Harbour in Lake Ontario; Gewurtz et al., 2016). Laboratory studies have shown female birds to have lower levels of PFOS in their livers than males, due to the deposition of PFOS into the eggs (Newsted et al., 2007).

3.3.3. Eggs shell parameters and relation to POPs

Eggshell parameters were used for characterizing the eggshell indexes in each colony and to evaluate if POPs had an effect on these parameters. We used egg volume as a biometric parameter to compare eggs among colonies, since egg volume is a good predictor of chick size at hatching and further survival (e.g. Blomqvist et al., 1997). On the other hand, eggshell thickness and desiccation index are measures of egg viability and thus, reproduction success. For the Audouin's gull no differences among colonies were found for the volume ($F_{2,73}$ = 2.36, p = 0.102) and the eggshell thickness ($F_{2,73}$ = 2.60, p = 0.081). But the desiccation index was lower at Tarragona than at Ebro Delta and Castellón ($F_{2,73}$ = 4.30, p = 0.017; Tukey test: Tarragona - Ebro Delta p = 0.016, Tarragona – Castellón p = 0.029, Castellón - Ebro Delta p = 0.978; Table 3.A1, Figure 3.4). For Audouin's gull, no relationship between eggshell parameters and POPs could be made because there were too few samples.

For the yellow-legged gull differences among colonies were significant for all egg parameters (volume: F6,185 = 4.41, p < 0.001; eggshell thickness: F6,178 = 8.52, p < 0.001; desiccation index: F6,178 = 11.71, p < 0.001; Table 3.A1). Post-hoc Tukey test showed that the volume of eggs was lower at Atlantic Islands than Ebro Delta and Medes (p = 0.035 and p <0.001, respectively), eggshell thickness was greater at Ebro Delta than at Berlengas, Chafarinas, Atlantic Islands and Medes (p <0.001 for all these pair-wise comparisons), and desiccation index was lower at Chafarinas than at Berlengas, Ebro Delta, Atlantic Islands and Medes (p <0.001 for all these pair-wise comparisons; Table 3.A1, Figure 3.4). None of the compounds are associated with this low DI in Chafarinas. However, in the yellow-legged gull there was no significant correlation between the egg volume, the eggshell thickness index or the desiccation index and the ∑PCBs or ∑DDT concentration (Spearman rank correlation, all test p>0.05; Figure 3.A5). This indicates that the compounds detected and the concentration levels are not associated to any impairment on the eggshell parameters.

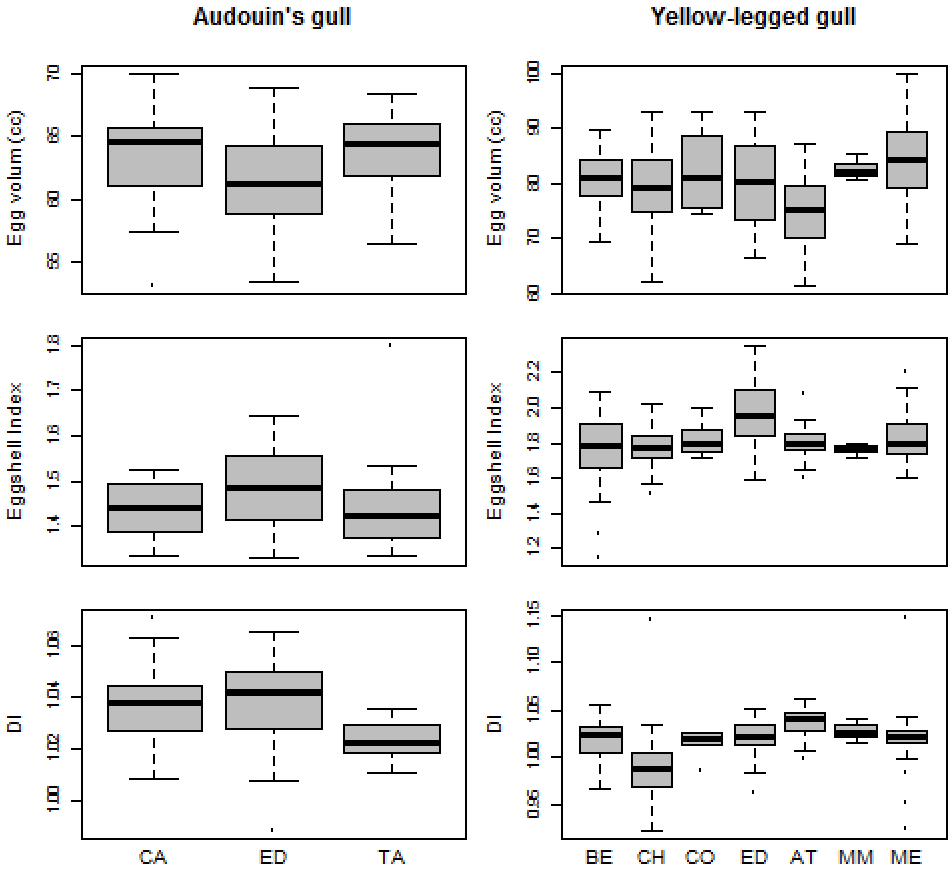


Figure 3.4. Found differences between colonies in egg volumes, eggshell thickness and desiccation index.

3.4. Conclusions

This study has shown that National and Natural Parks are areas with a high chemical impact by POPs, as measured using gull eggs as bioindicators of environmental pollution. In this study, we have shown that gull eggs collected from all 10 colonies of the Iberian Peninsula contain POPs at levels between 125 and 1932 ng/g ww, with the highest levels in areas close to industries or agricultural activities (Ebro Delta) or in gulls feeding basically on fish (Columbretes). This hypothesis is corroborated by the higher levels of POPs detected in Audouin´s gull who feeds exclusively on fish. Among studied compounds, PCBs and OCs where the main contributors to ∑POP, and PBDEs and PFOS were detected at much lower levels, ranging from 3.03 to 91.3 ng/g ww. However, in the Medes Island, the most northern colony, the profile was dominated by PFOS, probably due to a specific source of contamination. The overall presence of POPs in gull eggs dwelling in areas with a high level of conservation points to the relevance of this type of pollution as other birds sharing habitat could be equally affected. In this study, we have optimized a methodology to determine a large number of POPs included in the Stockholm Convention, with emphasis to organochlorine compounds, which will serve to carry out systematic and long term surveys to determine whether conservation actions have an effect on the levels detected in each area, using gull eggs as bioindicators.

References

Barón, E., Máñez, M., Andreu A.C., Sergio, F., Hiraldo, F., Eljarrat, E., Barceló, D., 2014. Bioaccumulation and biomagnification of emerging and classical flame retardants in bird eggs of 14 species from Doñana Natural Space and surrounding areas (South-western Spain). Environ Int 68, 118-126

Blomqvist, D., Johansson, O.C., Götmark, F., 1997. Parental quality and egg size affect chick survival in a precocial bird, the lapwing *Vanellus vanellus*. Oecologia 110, 18-24

BirdLife International, 2017. Species factsheet: *Larus audouinii*. Downloaded from http://www.birdlife.org on 26/11/2017

Carlsson, P., Herzke, D., Wedborg, M., Gabrielsen, G.W., 2011. Environmental pollutants in the Swedish marine ecosystem, with special emphasis on polybrominated diphenyl ethers (PBDE). Chemosphere 82, 1286-1292

Ceia, F.R., Paiva, V.H., Fidalgo, V., Morais, L., Baeta, A., Crisóstomo, P., Mourato, E., Garthe, S., Marques, J.C., Ramos, J.A., 2014. Annual and seasonal consistency in the feeding ecology of an opportunistic species, the yellow-legged gull *Larus michahellis*. Mar Ecol Prog Ser 497, 273-284

Chen D., Letcher R.J., Burgess N.M., Champoux L., Elliott J.E., Hebert C.E., Martin P., Wayland M., Chip Weseloh D.V., Wilson L., 2012. Flame retardants in eggs of four gull species (Laridae) from breeding sites spanning Atlantic to Pacific Canada. Environ Pollut 168, 1-9

Dąbrowski, Ł. 2016. Review of use of keepers in solvent evaporation procedure during the environmental sample analysis of some organic pollutants. Trends Anal Chem 80, 507-516

Drouillard, K.G., Fernie, K.J., Smits, J.E., Bortolloti, G.R., Bird, D.M., Norstrom, R.J. 2001. Bioaccumulation and toxicokinetics of 42 polychlorinated biphenyl congeners in American kestrels (Falco sparverius). Environ Tox Chem 20, 2514-2522

Eljarrat , E., Lacorte, S., Barceló, D., 2002. Optimization of congener-specific analysis of 40 polybrominated diphenyl ethers by gas chromatography/mass spectrometry. J Mass Spectrom 37, 76-84

Fernández-Chacón, A., Genovart, M., Pradel, R., Tavecchia, G., Bertolero, A., Piccardo, J., Forero, M., Afán, I., Muntaner, J., Oro, D., 2013. When to stay, when to disperse and where to go: survival and dispersal patterns in a spatially structured seabird population. Ecography 36, 1117-1126

Fliedner, A., Rüdel, H., Jürling H., Müller, J., Neugebauer, F., Schröter-Kermani, C. 2012. Levels and trends of industrial chemicals (PCBs, PFCs, PBDEs) in archived herring gull eggs from German coastal regions, Env Sci Europe, 24, 7-15

Fossi, M.C., Baini, M., Panti, C., Galli, M., Jiménez, B., Muñoz-Arnanz, J., Marsili, L., Finoia, M.G., Ramírez-Macías, D., 2017. Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies. Comp. Biochem. Physiol. C: Toxicol Pharmacol doi: 10.1016/j.cbpc.2017.03.002

García, L.V., Marañon, T., Clemente, L., 2002. Animal influences on soil properties and plant cover in the Chafarinas Islands (NW Africa), in: Rubio, J.L., Morgan, R.P.C., Asins, S. (Eds.), Man and Soil at the Third Millenium, 1, Geoforma, Logroño, Spain, pp. 705-712

Gewurtz, S.B., Martin, P.A., Letcher, R.J., Burgess, N.M., Champouz, L., Elliott, J.E., Weseloh, D.V.C., 2016. Spatio-temporal trends and monitoring design of perfluoroalkyl acids in the eggs of gull (Larid) species from across Canada and parts of the United States. Sci Total Environ 565, 440-450

Gómara, B., González, M.J., Baos, R., Hiraldo, F., Abad, E., Rivera, J., Jiménez, B., 2008. Unexpected high PCB and total DDT levels in the breeding population of red kite (Milvus milvus) from Doñana National Park, south-western Spain. Environ Int 34, 73-78

Gonzalez, M.J., Fernandez, M.A., Hernandez, L.M., 1991. Levels of chlorinated insecticides, total PCBs and PCB congeners in Spanish Gull eggs. Arch Environ Contam and Toxicol 20, 343-348

Gonzalez, L.M., 1988. Organochlorine and heavy metal contamination in the eggs of the Spanish imperial eagle (Aquila (heliaca) adalberti) and accompanying changes in eggshell morphology and chemistry. Environ Pollut 51, 241-258

Guitart, R., Clavero, R., Mateo, R., Mañez, M., 2005. Levels of persistent organochlorine residues in eggs of greater flamingos from the Guadalquivir marshes (Doñana), Spain. J Environ. Sci Health, Part B 40, 753-760

Heinz, G.H., Johnson, R.W., 1979. Elimination of endrin by mallard ducks. Toxicology 12, 189-196

Helander, B., Olsson, A., Bignert, A., Asplund, L., Litzén, K., 2002. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (Haliaeetus albicilla) in Sweden. Ambio. 31, 386–403

Helgason, L.B., Polder, A., Føreid, S., Boek, K., Lie, E., Gabrielsen, G.W., Barrett, R.T., Skaare, J.U., 2009. Levels and temporal trends (1983-2003) of polybrominated diphenyl ethers and hexabromocyclododecanes in seabird eggs from north Norway. Environ Toxicol Chem 28, 1096-1103

Herzk, D., Wing, G., Gabrielsen, Evens, A., Burkowa, I.C., 2003. Polychlorinated camphenes (toxaphenes), polybrominated diphenylethers and other halogenated organic pollutants in glaucous gull (Larus hyperboreus) from Svalbard and Bjørnøya (Bear Island) Environ Pollut, 121, 293-300

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical J. 50, 346-363

Hoyt, D.F., 1979. Practical methods of estimating volume and fresh weight of bird eggs. Auk 96, 73–77

Iwaniuk, A.N., Koperski, D.T., Cheng, K. M., Elliott, J. E., Smith, L. K., Wilson, L. K., Wylie, D.R.W., 2006. The effects of environmental exposure to DDT on the brain of a songbird: Changes in structures associated with mating and song. Behav Brain Res 173, 1-10

Jácome-Sanz, D., 2014. Las Islas Medas: Pasado, presente y futuro. Biol. on-line, 3, 1 ISSN: 2339-5745 (In Spanish)

Jiménez, B., Merino, R., Abad, E., Rivera, J., Olie, K., 2007. Evaluation of organochlorine compounds (PCDDs, PCDFs, PCBs and DDTs) in two raptor species inhabiting a Mediterranean Island in Spain. Env Sci Pollut Res 14, Special Issue 1, 61–68

Lackmann, G. M., Schaller, K. H., Angerer, J., 2004. Organochlorine compounds in breast-fed vs. bottle-fed infants: preliminary results at six weeks of age. Sci Total Environ 329, 289-293

Li, L., Liu, J., Hu, J. 2015. Global inventory, long range transport and environmental distribution of dicofol. Environ Sci Technol 49, 212-222

Mañosa, S., Mateo, R., Freixa, C., Guitart, R., 2003. Persistent organochlorine contaminants in eggs of northern goshawk and Eurasian buzzard from northeastern Spain: temporal trends related to changes in the diet. Environ Pollut 122, 351-359

Martín, J., López, P., Gutierrez, E. García, L.V., 2015. Natural and anthropogenic alterations of the soil affect body condition of the fossorial amphisbaeian Trogonophis wiegamnni in North Africa. J Arid Environ 122, 30-36

Martinez-Lopez, E., María-Mojica, P., Martinez, J.E., Calvo, J.F., Wright, J., Shore, R.F., Romero, D., Garcia-Fernandez, A.J., 2007. Organochlorine residues in booted eagle (Hieraaetus pennatus) and goshawk (Accipiter gentilis) eggs from southeastern Spain. Environ Toxicol Chem 26, 2373-2378

Martínez-Vilalta, A., Oro, D., 2004. Gaviota de Audouin Larus audouinii, in: Madroño, A., González, C, Atienza, J.C. (Eds.), Libro Rojo de las aves de España. Dirección General para la Biodiversidad-SEO/BirLife, Madrid, Spain, pp. 242-245

Merino, R., Bordajandi, R., Abad, E., Rivera, J., Jiménez, B., 2005. Evaluation of organochlorine compounds in peregrine falcon (Falco peregrinus) and their main prey (Columba livia) inhabiting central Spain. Environ Toxicol Chem 24, 2088-2093

Meyer, J., Jaspers, V.L.B., Eens, M., de Coen, W., 2009. The relationship between perfluorinated chemical levels in the feathers and livers of birds from different trophic levels. Sci Total Environ 407, 4894-4900

Molina, B., Bermejo, A. 2009. Gaviota patiamarilla (Larus michahellis), in: Molina, B. (Ed.), Gaviota reidora, sombría y patiamarilla en España. Población en 2007-2009 y método de censo. SEO/BirdLife, Madrid, pp. 50-111

Morales, L., Martrat, M.G., Olmos, J., Parera, J., Vicente, J., Bertolero, A., Abalos, M., Lacorte, S., Santos, F.J., Abad, E., 2012. Persistent organic pollutants in gull eggs of two species from the Ebro Delta Natural Park. Chemosphere 88, 1306-1316

Morales, L., Martrat, M.G., Parera, J., Bertolero, A., Ábalos, M., Santos, F.J., Lacorte, S., Abad, E., 2016. Dioxins and dl-PCBs in gull eggs from Spanish Natural Parks (2010–2013). Sci Total Eviron 550, 114-122

Muñoz-Arnanz, J., Jiménez, B., 2011. New DDT inputs after 30 years of prohibition in Spain. A case study in agricultural soils from south-western Spain. Environ Pollut 159, 3640-3646

Muñoz-Arnanz, J., Sáez, M., Aguirre, J.I., Hiraldo, F., Baos, R., Pacepavicius, G., Alaee, M., Jiménez, B., 2005. Predominance of BDE 209 and other higher brominated diphenyl ethers in eggs of white stork (Ciconia ciconia) colonies from Spain. Environ Int 37, 572-576

Navarro, J., Oro, D., Bertolero, A., Genovart, M., Delgado, A., Forero, M., 2010. Age and sexual differences in the exploitation of two anthropogenic food resources for an opportunistic seabird. Mar Biol 157, 2453-2459

Newsted, J.L., Coady, K.K., Beach, S.A., Butenhoff, J.L., Gallagher, S., Giesy, J.P., 2007. Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. Environ Toxicol Pharmacol 23, 1-9

Oro, D., Bosch, M., Ruiz, X., 1995. Effects of a trawling moratorium on the breeding success of the Yellow-legged Gull Larus cachinnans. Ibis 137, 547–549

Oro, D., 2008. Living in a ghetto within a local population: an empirical example of an ideal despotic distribution. Ecology 89, 838–846

Ortiz Martinez, A., Martinez-Conde, E., 1995. The neurotoxic effects of lindane at acute and subchronic dosages. Ecotox Environ Safe 30, 101-105

OSPAR, 2000. Comission. JAMP Guidelines for monitoring contaminants in biota. Ref. No: 1992–2

Parolini, M., Colombo, G., Valsecchi, S., Mazzoni, M., Possenti, C. D., Caprioli, M., Dalle-Done, I., Milzani, A., Saino, N., Rubolini, D., 2016. Potencial toxicity of environmentally relevant perfluorooctane sultonate (PFOS) concentrations to yellow-legged gull Larus michahellis embryos. Environ Sci Pollut Res 23, 426-437

Pastor, D., Jover, L., Ruiz, X., Albaiges, J., 1995. Monitoring organochlorine pollution in Audouin's gull eggs: The relevance of sampling procedures. Sci Total Environ 162, 215-223

Payo-Payo, A., Genovart, M., Sanz-Aguilar, A., Greño, J., García-Tarason, M, Bertolero, A., Piccardo, J., Oro, D., 2017. Colonisation in social species: the importance of breeding experience for dispersal in overcoming information barriers. Sci. Rep. 7, 42866 (DOI: 10.1038/srep42866).Qiu, X., Zhu, T., Yao, B., Hu, J., Hu, S., 2005. Contribution of dicofol to the current DDT pollution in China. Environ Sci Technol 39, 4385-4390

Ratcliffe, D.A., 1967. Decrease in eggshell weight in certain birds of prey. Nature, 215, 208-210

Rüdel, H., Müller, J., Jürling, H., Schröter-kermani, C. 2010. Retrospective monitoring of perfluorinated compounds in archived herring gull eggs. Interdisciplinary Studies on Environmental Chemistry — Environmental Specimen Bank, Eds., T. Isobe, K. Nomiyama, A. Subramanian and S. Tanabe, pp. 81–86. © by TERRAPUB, 2010

Stockholm Convention, COP4 Final, Earth Negotiation Bulletin 15, 174, 11th May 2009

Vicente, J., Bertolero, A., Meyer, J., Viana, P., Lacorte, S., 2012. Distribution of perfluorinated compounds in Yellow-legged gull eggs (Larus michahellis) from the Iberian Peninsula. Sci Total Environ 416, 468-475

Vicente, J., Sanpera, C., García-Tarascón, M, Pérez, A., Lacorte, S., 2015. Perfluoroalkyl and polyfluoroalkyl substances in entire clutches of Audouin's gulls from the Ebro Delta. Chemosphere 119, S62-S68.

Zamariola, N.,Toledo Netto, P., da Silva Franchi, C.A., Viana de Camargo, J.L., Rodrigues de Marchi, M.R., 2017. QuEChERS-Based method for pesticides analysis in adipose tissue associated with rat ovaries. J Anal Toxicol 41, 399-406

Annex

Table 3.A1: Mean and standard deviation of the eggs parameters of Audouin's gull (La) and yellow-legged gull (Lm) from each colony (n: sample size).

Species	Colony	Volume (cc)	Eggshell thickness (mm)	Desiccation index	n
La	Castellón	63.63 ± 3.80	1.44 ± 0.06	1.037 ± 0.016	29
	Ebre Delta	61.79 ± 3.61	1.49 ± 0.09	1.038 ± 0.018	35
	Tarragona	63.61 ± 3.39	1.45 ± 0.13	1.023 ± 0.008	12
Lm	Berlengas	80.02 ± 5.49	1.76 ± 0.23	1.020 ± 0.022	26
	Chafarinas	79.53 ± 7.05	1.77 ± 0.11	0.988 ± 0.036	48[a]
	Columbrets	82.32 ± 7.43	1.82 ± 0.10	1.016 ± 0.016	6
	Ebro Delta	79.97 ± 7.76	1.97 ± 0.19	1.022 ± 0.017	46
	Atlantic Islands	74.82 ± 6.30	1.80 ± 0.10	1.037 ± 0.016	27[b]
	Mar Menor	82.71 ± 2.33	1.76 ± 0.04	1.028 ± 0.012	3
	Medes	83.70 ± 7.32	1.82 ± 0.13	1.020 ± 0.031	36

[a] n=42 for eggshell thickness and desiccation index.

[b] n=26 for eggshell thickness and desiccation index.

Table 3.A2: CG-NCI-MS retention times and monitored ions for PBDEs congeners.

Compound	Retention time (min)	m/z 1	m/z 2	m/z 3
PBDE 28	11.67	79	81	161
PBDE 47	14.02	79	81	161
PBDE 100	11.75	79	81	161
PBDE 99	16.34	79	81	161
PBDE 154	17.68	79	81	161
PBDE 153	18.45	79	81	161
PBDE 183	20.37	79	81	161
PBDE 209	27.34	486.5	408.5	
Surrogates				
PCB-209	16.99	498	500	496

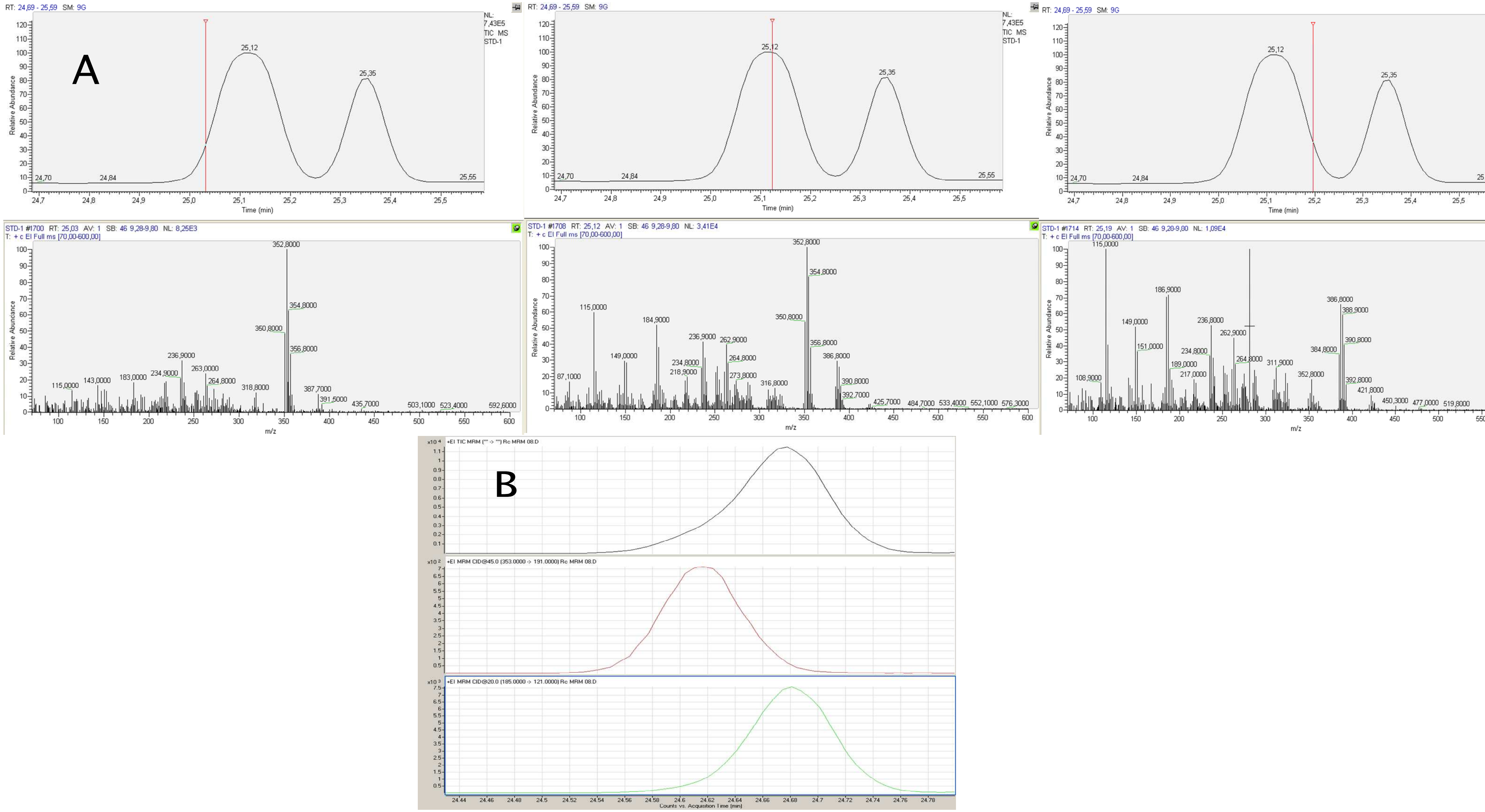


Figure 3.A1. A) Mass spectra along the coelution peak. B) MRM TIC and specific MRM EIC of the specific transitions of oxychlordane and heptachlor epoxides.

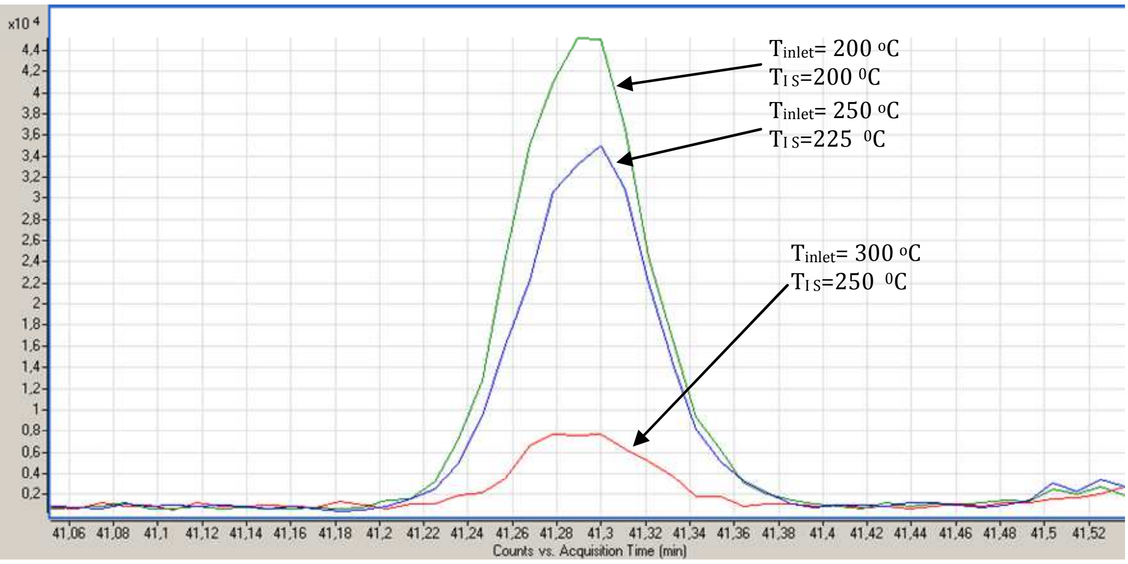


Figure 3.A2. Dicofol signal at three different inlet and ionization source temperatures.

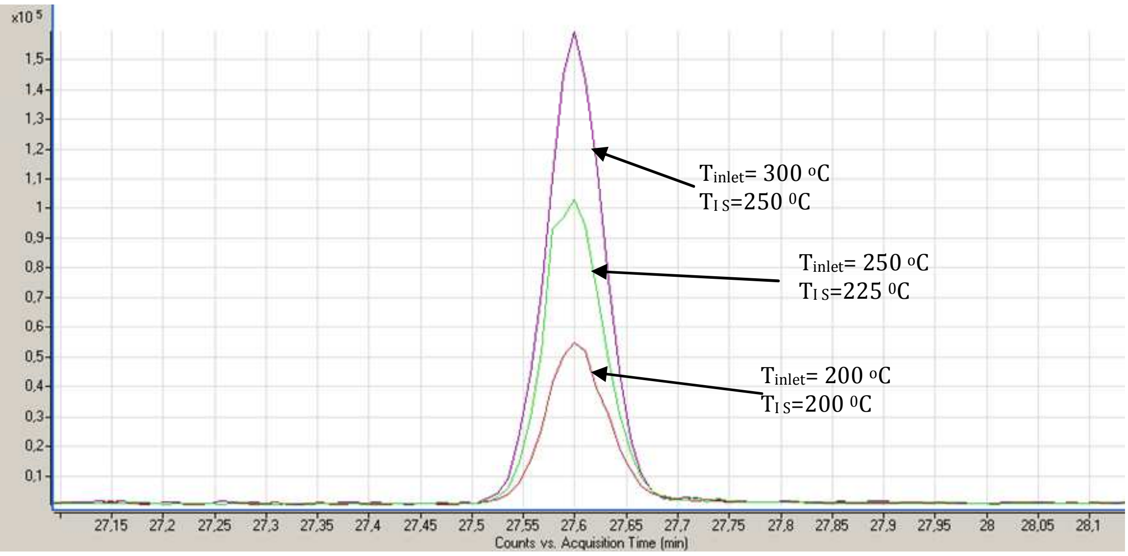


Figure 3.A3. DBP signal at three different inlet and ionization source temperatures.

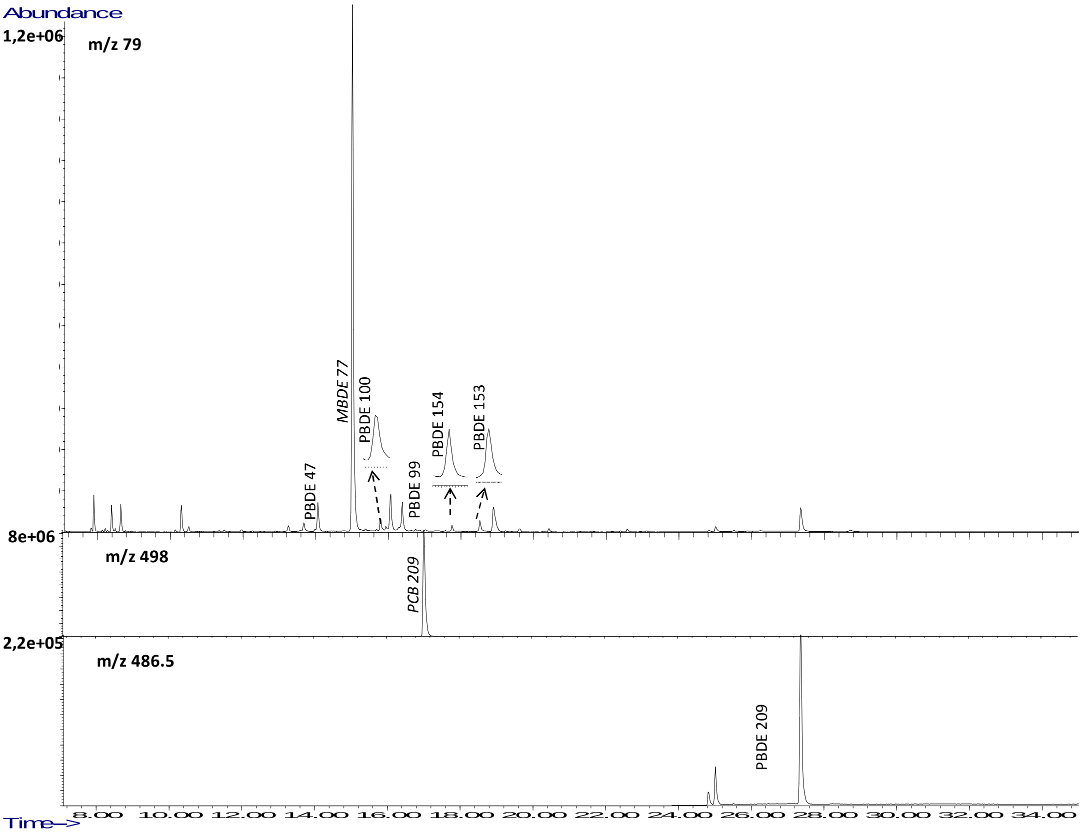


Figure 3.A4. GC-NCI-MS ion chromatogram of a gull egg from Cies Island indicating PBDEs detected.

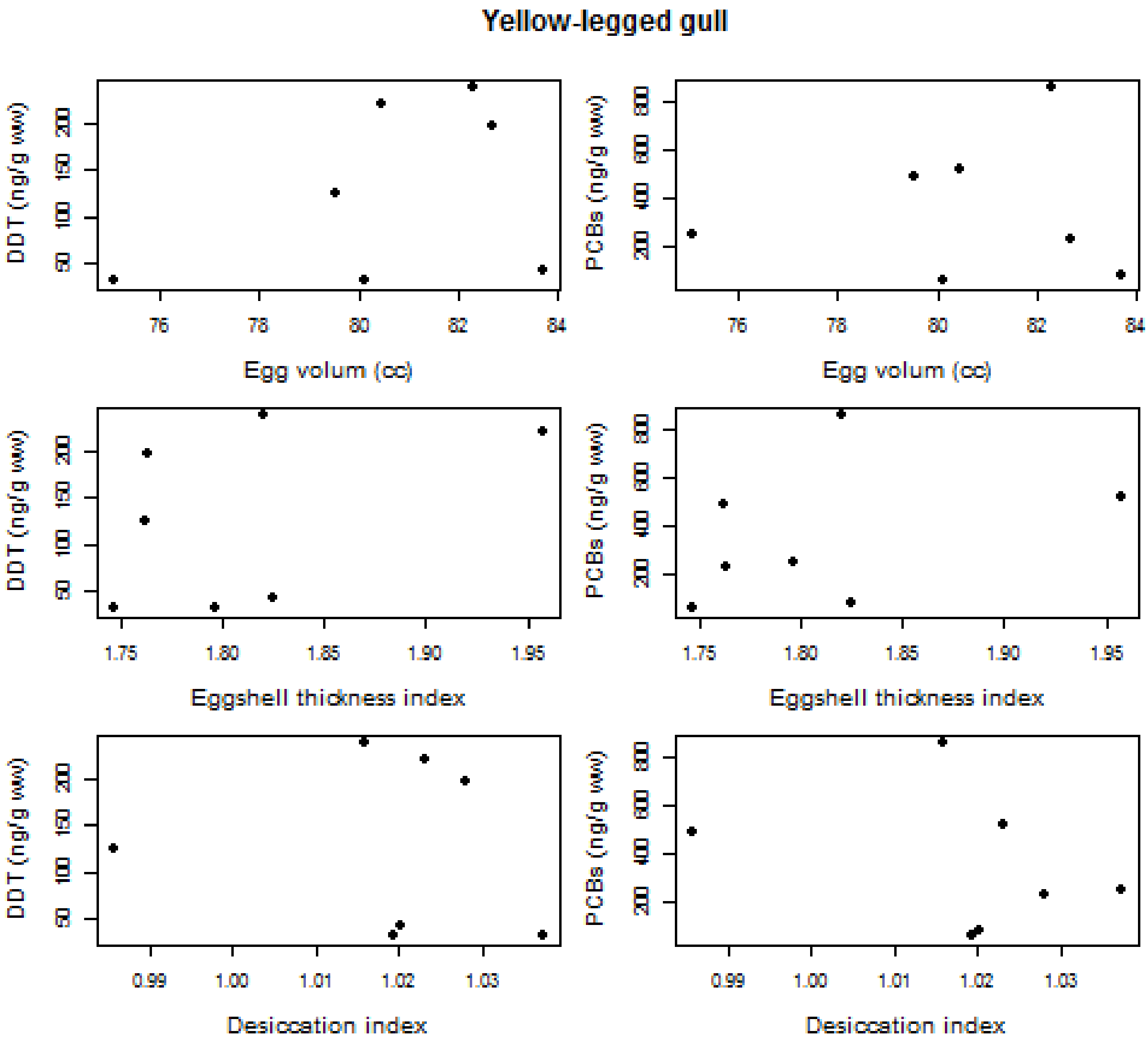
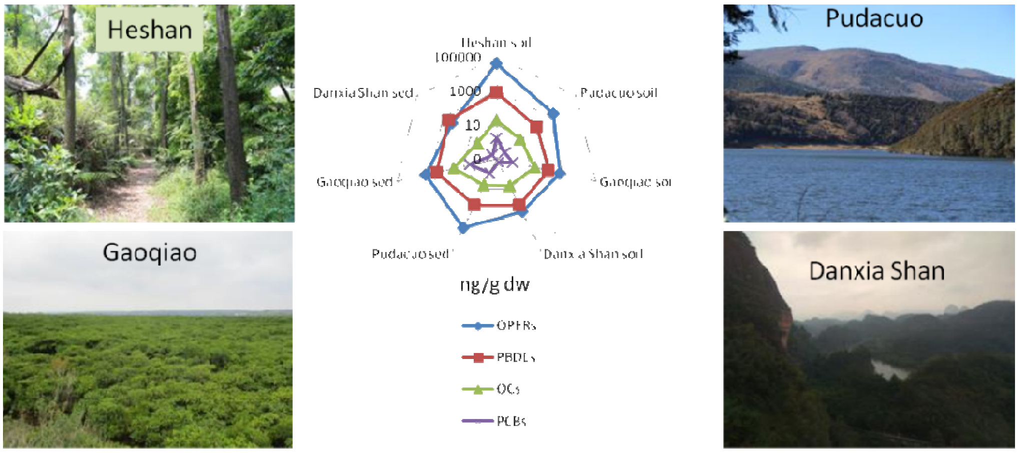


Figure 3.A5. Correlations between ΣDDTs and ΣPCBs with eggshell parameters.

4. Occurrence of legacy POPs and OPEs in soils and sediments in environmentally protected areas in south China



4.1. Introduction

Pollution by persistent organic pollutants (POPs) remains an important and ongoing problem worldwide because they persist in the environment and affect human health and the ecosystems (Stockholm Convention, 2019). Among different chemicals that comprise POPs, polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) deserve attention because of the high production volumes. PBDEs used as flame retardants in textile, plastic, and rubber manufacturing, had a global in-use and waste stock of 500 kt for BDE 209 and ~38 kt for BDEs 28, 47, 99, 153, and 183 in 2018 (Abassi et al., 2019). After being banned, production and use of organophosphate esters (OPEs) increased due to their use as PBDE substitutes, but also as plastic additives in furniture, textile materials, electronic devices, or polyurethane foams, among others (van der Veen and De Boer, 2012). Global OPE consumption has increased during recent decades, from 100 kt in 1992 to 500 kt in 2011 and 680 kt in 2015 (Yang et al., 2019a). In 2007, 70 kt of OPEs were produced in China, with an estimated annual increase of 15% (Wei et al., 2015). PCBs were used as additives in industrial fluids for heat exchange, or as plastic and paint additives (Erickson and Kaley, 2011). In 2015, it was estimated that > 60% of the 300 kt of PCBs were in waste stocks, whereas > 10% (approximately 35 kt) remained in use stocks (Li and Wania, 2016). Finally, OCPs have been used for agricultural pests control and to avoid expansion of insect transmitted diseases such as typhus or malaria (van den Berg et al., 2017). Among pesticides, approximately 4000 kt of technical HCH were produced in China until 1984, and a further 11.4 kt were produced between 1991 and 2000 with α isomer as the major compound (Li et al., 2001). More than 90 kt of technical DDT was produced in China from 1988 to 2002 (Ma et al., 2020). Even after prohibition and use restrictions, POPs continue to be detected in industrialized and urbanized areas (Kim et al., 2017; Wong et al., 2009) as well as in natural environments and remote regions (Wang et al., 2016; Wu et al., 2012). These contaminants can cause harmful effects and disturb the ecological equilibrium of ecosystems (Abessa et al., 2018) and assessment of the contamination status is extremely relevant under global change scenarios.

Soils and sediments, especially those with high organic matter content, act as sinks and reservoirs of legacy POPs such as PCBs, OCPs, or PBDEs, according to soil uses and locations (Holoubek et al., 2009; McGrath et al., 2017) while in-use compounds such as OPEs are deposited from air (Yadav et al., 2018) or directly discharged to the environment (van der Veen and de Boer 2012; Zhang et al., 2022). These matrices are useful to monitor contaminants as they reflect both historical and recent pollution inputs (Choi et al., 2011). Soil and sediment pollution are of concern because they are considered secondary sources of pollutants to wildlife, especially when desorption processes occur and POPs become more bioavailable (Tao et al., 2008). Mobilization of contaminants is of particular relevance in biodiversity hotspots, such as natural and national parks.

Natural and national parks are areas of natural beauty and ecological importance. These areas are intended to maintain the natural evolution of the ecosystem using legal figures to ensure fauna and flora protection and preservation, and ecosystem and biodiversity conservation (Chen, 2020). Currently, there are 2234 protected areas in China listed in the world database on protected areas (Yang et al., 2019) which are promoted to sustain recreational activities and maintain the productive development of the surrounding villages (Zhou and Grumbine, 2011). Different strategies and policies are being implemented to achieve a balance between China's rapid economic growth and ecosystem and biodiversity preservation towards an ecological civilization (Shi et al., 2019; Zhang et al., 2017a). Some of these strategies include: (i) zoning the protected areas into a core zone, an experimental or transition zone, and a buffer zone between them; (ii) prohibitions or restrictions of some human activities or (iii) promotion of educative and "eco-friendly" touristic activities (Brandt et al., 2015; Miller-Rushing et al., 2017; Xu et al., 2016). China also encourages scientific research within the protected environments to achieve an equilibrium between nature and human livelihood (Huang et al., 2019a; Wang et al., 2020). Although not many studies are focused in protected areas, there is some evidence regarding chemical pollution in national parks or natural reserves around the world (Barletta et al. 2019; Pelamatti et al., 2021; Vecchiato et al., 2021).

This study aims to assess the occurrence and distribution of 11 PBDEs, 6 PCBs, 29 OCs including isomers and 14 current-use OPEs with different structures, namely alkyl, aryl and chlorinated, in soils and sediments from four protected areas in south

China. The study areas have different protection status and several activities take place inside the premises, such as agriculture, forestry and tourism. Studied compounds were selected for their environmental relevance, production and use volumes in China. Because information on the pollution status of protected areas is still scarce in China, and generally around the world, the novelty of this study is to provide a first assessment of POPs and OPEs occurrence in areas which have a small anthropogenic footprint.

4.2. Materials and methods

4.2.1. Sampling sites and sample collection

Sampling campaigns took place during late spring and early summer of 2018 in four protected areas in south China (Figure 4.1). Permissions were obtained from each area sampled and the number of samples collected was subjected to these permissions. Detailed information on samples (name, GPS location, altitude, and field observations) is provided in the annex, Table 4.A1.

Heshan National Field Research Station of Forest Ecosystem is located in the Heshan County (Guangdong Province). It holds the lowest environmental protection degree among the study areas, with agricultural activities and furniture production facilities in its surroundings. The area has experienced soil erosion and vegetation degradation problems and has hosted forest restoration projects since 1984 (Wang et al., 2010). Three soil samples were taken in this area (Figure 4.1A): an active crop soil where magnolia trees are grown (HS-S1); an old rice paddy field (HS-S2); and an inside forest soil from a reforested area (HS-S3). Sediments were not available.

Pudacuo National Park was established in 2007 as the first national park in China meeting the IUCN standards (Ma, 2017). Pudacuo is the most remote and isolated studied area. The sampling site was divided in two areas (Figure 4.1B). Two soils were collected from the transition and buffer zones of the park, i.e., one crop soil (PDC-S1) and a forest soil from the riverside near the walking pathway (PDC-S2), and a third sample was collected from the forest soil in the mountain hillside (PDC-S3). Another forest soil was collected close to Shudu Lake (PDC-S4) at an altitude > 3600 m. Sediment samples were taken from a small stream (PDC-sed1) and from the Shudugang River (PDC-sed2), and three additional sediment samples were taken from Shudu Lake (PDC-sed 3–5).

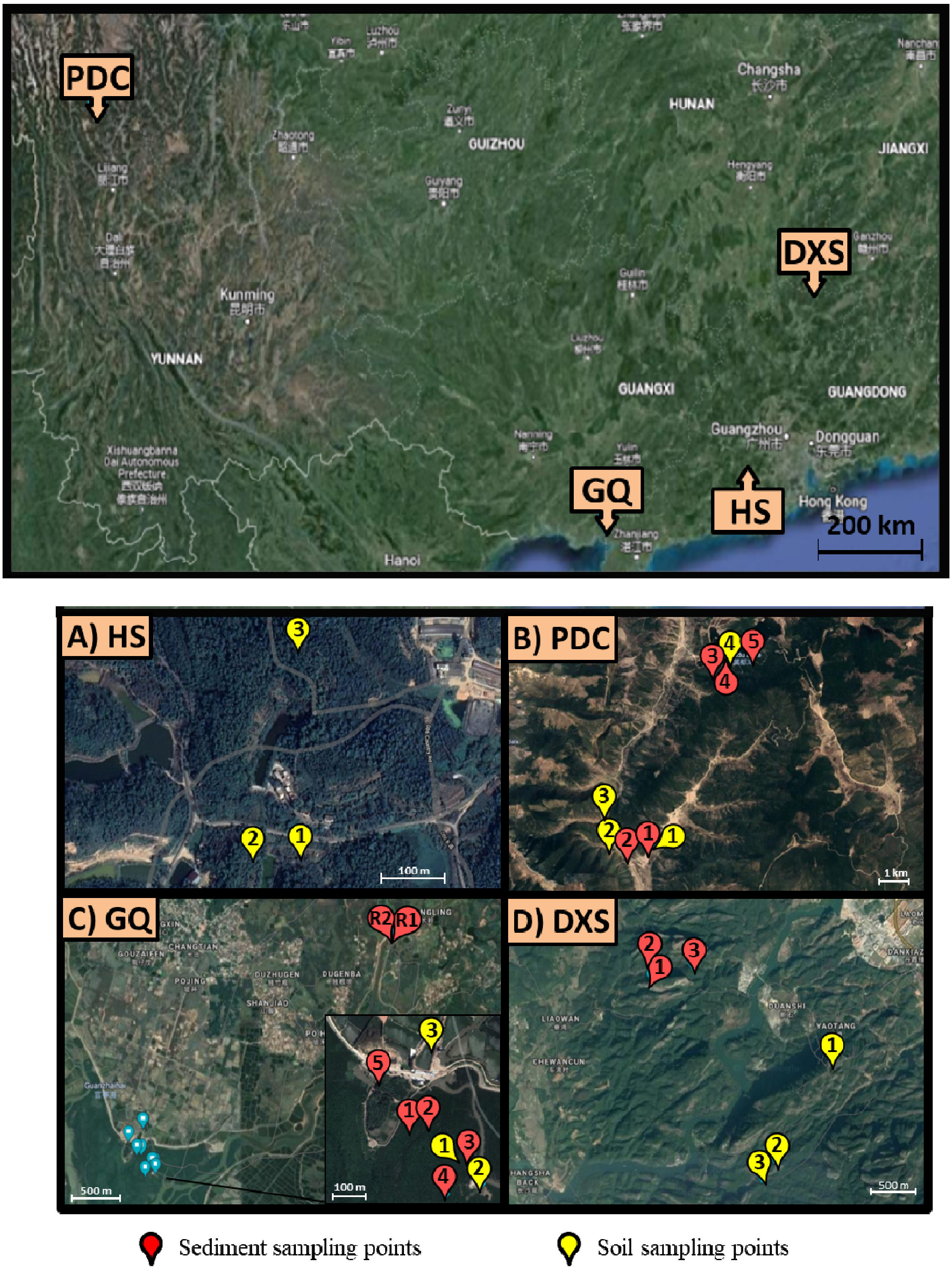


Figure 4.1. General map of studied areas in South China and detailed maps of sampling points (sediment samples marked in red, soil samples marked in yellow). A) Heshan Forest Research Station (HS), B) Pudacuo National Park (PDC), C) Gaoqiao Magrove Natural Reserve (GQ) and D) Danxia Shan National Park (DXS).

Gaoqiao Mangrove National Natural Reserve is one of the multiple mangrove forests in Zhangjian, Suixi County, southwest of Guangdong Province. It has a considerable influence on the coastal dynamics, linking terrestrial and marine environments, reducing the erosion and impact of ocean tides on the shore, and receiving nutrients from the upstream river and regular seawater flooding. Soil samples were collected from an area with low developed vegetation (GQM-S1), in an area affected by visitors (GQM-S2), and in a small agricultural crop (GQM-S3). Five sediment samples were taken from inside the mangrove during the low tide period (Figure 4.1C). These samples were taken from inner (GQM-sed1-4) and outer (GQM-sed5) parts of the forest. GQM-sed2 was collected from a small stream riverside/bank, and GQM-sed3 from a large river branch. Additionally, Daba River upstream sediment samples were taken before (GQR-sed1A and GQR-sed1B) and after (GQR-sed2) a dam fed with that river, to evaluate pollution inputs from the river basin to the mangrove area.

Danxia Shan National Park is located in the Renhua County, north of Guangdong Province. It is a UNESCO world heritage site since 2010 and is categorized as a 5A tourist attraction. Geologically, it has been described as “a red terrestrial clasolite characterized by the red walls and cliffs” formed by water erosion (Wu et al. 2008). One soil sample was taken from a hilltop close to the cliff at 323 m asl (DXS-S1) (Figure 4.1D) and two samples were collected in the well-developed forests in the bottom part of the park (DXS-S2 and DXS-S3). Two river sediments (DXS-sed1 and DXS-sed2) and one lake sediment (DXS-sed3) were also sampled. Soil samples (n = 13) were collected using an ethanol pre-cleaned stainless-steel shovel. After removing the first soil layer (1–2 cm), three to five shovelfuls were taken at a 2–5-cm depth from an approximately 1 m2 area and mixed in a glass pot to prepare a pooled sample. Surface sediment samples (n = 16) were collected with the same stainless-steel shovel from the river or lake shore and placed in glass pots. 10 min after sampling, the supernatant water was discarded. In all cases, pots were covered with aluminum foil, closed, kept in dark, and transported refrigerated to the laboratory where they were kept frozen until analysis.

4.2.2. Chemicals and reagents

For OPEs analysis, triethyl phosphate (TEP), tri-iso-propyl phosphate (TiPP), tri-n-propylphosphate (TnPP), tri-n-butyl phosphate (TnBP), tri(2-chloroethyl) phosphate (TCEP), tri(2-chloro-isopropyl) phosphate (TCPP), tri(2-chloro,1-chloromethy-ethyl) phosphate (TDCPP), tri(2-ethylhexyl) phosphate (TEHP), 2-ethylhexyl diphenyl phosphate (EHDPP), tri-phenyl phosphate (TPhP), and tri-cresyl phosphates (TCP) were purchased from AccuStandard (New Haven, CT, USA). Individual standards were prepared at 100 µg/mL and a working solution containing all OPEs at 1 µg/mL was also prepared, all in toluene. TnPP-D21, TCPP-D18 and TPhP-D15 were used as surrogate standards and were also purchased from AccuStandard.

For PBDE analysis, solutions containing congeners 28, 47, 66, 85, 99, 100, 138, 153 and 154 at 10 µg/mL in isooctane, BDE 183 at 50 µg/mL in isooctane and BDE 209 at 50 µg/mL in isooctane:toluene (1:1) were acquired from AccuStandard. A working solution with all congeners at 1 µg/mL was prepared in isooctane.

For OCP and PCB analysis, Pesticide Mix 1 containing aldrin, α-, β-, γ- and δ-HCHs, cis- and trans- chlordane, 2,4′-DDD, 2,4′-DDE, 2,4′-DDT, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, α- and β-endosulfan, endrin, heptachlor, heptachlor exo- and endo-epoxides, hexachlorobenzene (HCB), isodrin, methoxychlor, mirex, and oxychlordane, at a concentration of 10 µg/mL and the PCB congeners 28, 52, 101, 138, 153 and 180, at 1 µg/mL in toluene, was purchased from AccuStandard. Dicofol at a concentration of 100 µg/mL in methanol, endosulfan sulphate (100 µg/mL in methanol), hexachlorobutadiene (HCBu) (2 mg/mL in methanol) and pentachlorobenzene (solid, 98% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were after combined with the pesticide mix to make two OCPs working solutions at 1 and 0.1 µg/mL in toluene (0.1 and 0.01 µg/mL for PCBs). $^{13}C_6$-hexachlorobenzene (100 µg/mL in acetone) and $^{13}C_{12}$–4,4′-DDE at a concentration of 100 µg/mL in nonane obtained from Cambridge Isotope Laboratories (Andover, MA, USA), solid PCB 209, and PCB 65 (100 µg/mL in isooctane), from Sigma-Aldrich (St.Louis, MO, USA), where used as surrogate standards for PBDEs, OCP and PCBs.

Chromatography grade n-hexane, dichloromethane (DCM) and acetone were purchased from Oceanpk (Sweden), while ethyl acetate was from Honeywell (USA).

4.2.3. Sample treatment

Prior to extraction, samples were freeze dried, grounded with a mortar and pestle, and sieved at 100 µm. The extraction of soils and sediments followed the same procedure. One gram of soil or sediment was weighed with a precision of 0.001 g, placed in a cellulose thimble containing 5 g of anhydrous sodium sulfate, spiked with 100 ng of surrogate standards and Soxhlet extracted (48 h, 60^{o}C) using 200 mL of hexane:acetone (1:1). Copper pieces (soaked with HCl, washed with water until neutral pH and then washed with acetone, DCM and hexane) were added to the flask in order to remove elemental sulfur as copper sulfate. The Soxhlet extract was evaporated at a low pressure in a rotary evaporator R-215 (Buchi, Switzerland) until approximately 2 mL and this extract was cleaned up with ENVI florisil SPE cartridges (Sigma-Aldrich, USA). Cartridges were conditioned with 8 mL of ethyl acetate and 6 mL of n-hexane. After loading the sample, a first fraction was eluted with 5 mL of n-hexane followed by 5 mL of a n-hexane:DCM (1:1) mixture to collect PBDEs, PCBs and OCPs. A second fraction was eluted with 8 mL of ethyl acetate to collect OPEs. Each fraction was evaporated by a gentle N_2 flow and reconstituted in 1 mL of n-hexane for later instrumental determination.

4.2.4. Instrumental analysis

Instrumental analysis was performed by GC-EI-MS/MS using an Agilent 7890A gas chromatograph coupled to an Agilent 7000A MS Triple Quad detector system with two different methods for (i) OPEs and (ii) OCPs and PCBs. A DB-5MS column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) was used.

For OPEs, the temperature gradient started at 90^{o}C (1 min hold time), increased to 220^{o}C at 10^{o}C/min, to 240^{o}C at 20^{o}C/min, to 280^{o}C at 5^{o}C/min (5 min), and finally to 325^{o}C at 30^{o}C/min (5 min hold time), with a total runtime of 35 min. A multiple reaction monitoring (MRM) method was adapted and optimized in terms of collision energy and dwell for all compounds (Poma et al., 2018) and quantification and confirmation transitions were set for TEP, TiPP, TnPP-D21 and TnBP-D27. For OCPs and PCBs analysis, the temperature gradient was set at 70oC (1 min hold time), increased to 175oC at 6oC/min (4 min hold time), to 235oC at 3oC/min, to 315oC at 7oC/min (10 min hold time), with a total run time of 64 min. 1 µL was injected in pulsed splitless mode in both cases and acquisition was performed in Multiple Reaction Monitoring (MRM). Transitions, collision energies, and retention times are indicated in Table 4.A2 and Table 4.A3 of the Annex.

GC-MS with negative chemical ionization was used for the analysis of PBDEs in an Agilent 7890A GC system coupled with an Agilent 5975C mass spectrometer. A DB-5MS column (15 m length, 0.25 mm internal diameter, 0.25 µm film thickness) was used with He as carrier gas at a flow of 1 mL/min. The oven temperature was set at 100^{o}C, increased to 250^{o}C at 10^{o}C/min, to 310^{o}C at 15^{o}C/min (15 min hold time) and the total run time was of 36 min. 1 µL of sample was injected in pulsed splitless mode. Acquisition was performed in Selected Ion Monitoring (SIM). Monitoring ions with mass charge ratio (m/z) of 79 were used for all PBDEs except BDE 209; 486.5 for BDE 209, 290 for PCB 65 and 498 for PCB 209 were set as quantification ions. For confirmation purposes, m/z 81 and 161 were used for PBDEs, while m/z 256 and m/z 464 were used for PCB 65 and PCB 209 respectively, both used as surrogate internal standards.

. In all cases, the chromatographic sequence was programmed to inject a solvent blank at the beginning and end of each working day and every 10 samples to prevent carryover and maintain good cleanliness conditions inside the instruments. The response of the surrogate standards was controlled to determine changes in the MS response during acquisition.

4.2.5. Quality assurance and quality control

Calibration was performed over a concentration range of 1–1000 µg/L for each target compound except PCBs (0.1–100 µg/L). Intra-day precision was calculated as the standard deviation of a 500 µg/L standard solution injected five consecutive times, and inter-day precision was calculated with the same concentration injected during five consecutive days.

Samples were extracted in batches of 12 and included 9 samples, one spiked soil sample at 100 ng/g for each target compound, one matrix blank (same blank soil without spike), one procedural blank (cellulose thimble with sodium sulfate). Both samples and blanks were spiked with 100 ng of surrogate standards. The extraction efficiency, expressed as percentage recovery, was determined using spiked soils (n = 4).

Instrumental limits of detection (LOD) and quantification (LOQ) were calculated with the 1 µg/L standard solution as the concentration which produced a signal to noise ratio of 3 and 10, respectively. In the case that there was an external blank contribution, as for some OPEs, LODs and LOQs were calculated as the mean blank contribution (n = 4) plus 3 and 10 times its standard deviation, respectively. This average blank contribution was subtracted from the spiked and real samples.

Details regarding instrumental detection and quantification limits, recoveries, intra and inter-day precision, and blank contribution are provided in the annex (Tables 4.A5 and 4.A6); whereas for PBDEs they were reported in previous papers from the same research group (Hu et al., 2019; She et al., 2013). No blank contribution was observed for PBDEs, PCBs or OCPs whereas for 8 OPEs, the blank contribution ranged from 1.2 to 126 ng/g. Detection limits were between 0.1–163 ng/g, with higher levels for those compounds that were present in blanks. Recoveries ranged from 69% to 122% except for TnPP and TnBP, with recoveries of approximately 150%. Standard deviations for non-consecutive injections were < 30% for all compounds, except dicofol due to ion-source degradation to the corresponding dibenzophenone (Zapata et al., 2018).

4.2.6. Total organic carbon determination

Around 100 mg of the 100 µm sieved sample was accurately weighed in porous ceramic capsules, treated with HCl (aq) (35%) for 15 h at room temperature and heated at 80 ^{o}C for 1 h to dissolve carbonates, washed with H_2O until neutral pH and dried at 80 ^{o}C. Around 0.7 g of Fe (s) and 1.5 g of W (s) were added to each sample and both calibration standards before being analyzed in an ELTRA CS-800 double dual range C/S determinator (Haan, Germany) working at 2000^{0}C and 100 nm wavelength. Calibration was performed in 2 levels with the certificate reference material for TOC determination Medium organic content soil standard AEB2178 (alpha resources LLC, Stevensville, MI Michigan, USA), with 3.07% C, and the steel chips calibration standard 92400-4020 C215 (Eltra, Haan, Germany) with a content of 0.462% of C for low content samples.

4.3. Results and discussion

4.3.1. General overview of contamination patterns

Our results indicate a widespread pollution of POPs and OPEs in soil and in a lesser extent in sediments from protected areas in China. Table 4.1 indicates the detection frequency (expressed as a percentage of positive results over the total number of analyses in each matrix and site; compounds not-detected were considered as zeros in order not to overestimate the mean concentrations), and the minimum and maximum quantified values, and median concentrations of each family of pollutants in each soil and sediment. ∑pollutants ranged from 1.04–39,900 ng/g dry weight (dw) in soils and 2.54–2,340 ng/g dw in sediments.

Heshan is the area with lowest protection degree, whereas the other study areas hold the distinction of national park and/or natural reserve, and thus, are granted much higher levels of protection. As shown in Figure 4.2A, Heshan was the most polluted area, followed by Pudacuo, Gaoqiao mangrove, and Danxia Shan. In Heshan, the median pollutant concentration in soil was 698 ng/g dw, followed by Gaoqiao, Pudacuo, and Danxia Shan with median concentrations of 223, 111, and 31 ng/g dw, respectively. In sediment, the median concentrations decreased in the order of Pudacuo (349 ng/g dw) > Gaoqiao (191 ng/g dw) > Danxia Shan (59 ng/g dw). In both soil and sediment, OPEs were the most abundant family, followed by PBDEs, OCPs and PCBs. In Heshan and Pudacuo, OPEs in soils accounted for 98 and 94% of ∑pollutants in soil and sediment, respectively, whereas in Gaoqiao mangrove and Danxia Shan, OPEs represented 80 and 71% of ∑pollutants, with 17 and 27% contributions of PBDEs and 3 and 2% contributions of OCPs, respectively (Figure 4.2B).

Table 4.1. Detection frequency, minimum, maximum and median concentrations of total sum (expressed as ng/g dw) for each studied family of pollutants. Minimum values refer to the level of the lowest positive result. Values < MDL were given a 0 to calculate median levels considering detected compounds.

Pollutants		Soils				Sediments		
		HS (n=3)	PDC (n=4)	GQ (n=3)	DXS (n=3)	PDC (n=5)	GQ (n=8)	DXS (n=3)
ΣOPEs	det freq (%)*	48	23	19	14	39	23	5
	min-max	328-39500	132-1667	157-479	3.5-283	47-2327	2.1-1090	nd-236
	median	420	66	160	98	340	105	-
ΣPBDEs	det freq (%)*	24	18	27	18	16	24	18
	min-max	95-397	10-76	40-48	16.9-73.2	1.7-89	12-104	50-330
	median	360	13	48	22	11	54	58
ΣPCBs	detn freq (%)*	17	4	28	6	7	27	11
	min-max	0.19-1.5	nd-0.39	0.19-0.76	nd-0.18	0.16-0.71	0.22-1.4	nd-0.21
	median	0.19	-	0.19	-	0.18	0.35	-
ΣOCPs	det freq (%)*	14	5	14	9	5	15	8
	min-max	1.9-13	0.65-2.5	0.23-18	1.4-4.9	0.1-3.1	0.5-13	0.48-1.6
	median	3.5	1.5	1.6	1.4	0.81	3.3	0.81
ΣPollutant	min-max	520-39900	0.65-1690	40-529	25-357	2.5-2340	15-1120	52-568
	median	698	111	223	31	349	191	59

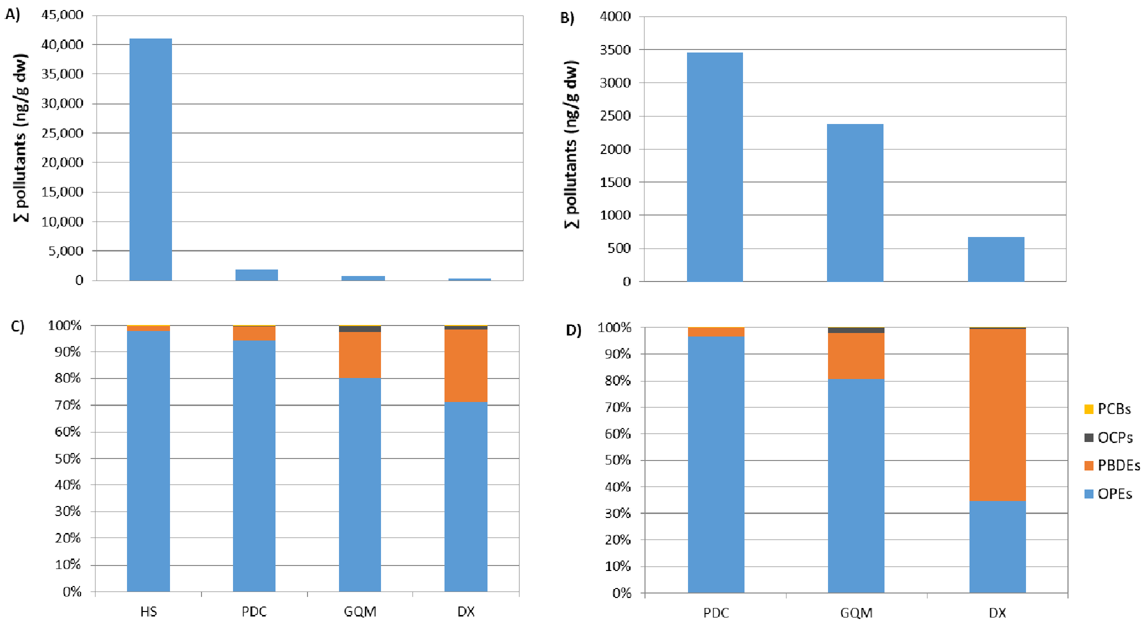


Figure 4.2. Sum of concentration of pollutants (ng/g dw) in soils (A) and sediments (B) and pollution profiles by families in soils (C) and sediments (D)

Considering the sum of individual pollutants in each area, there was a strong relationship among soil and sediment contamination in Pudacuo ($R^2 = 0.79$, $p < 0.01$), and Gaoqiao mangrove ($R^2 = 0.72$, $p < 0.01$), but not in Danxia. This means that in the two former areas there was a high interaction between both matrices due to similar diffusive sources.

Tables 4.A7–4.A10 indicate the individual concentrations of OPEs, PBDEs, OCPs and PCBs in soils and sediments of the 4 studied areas. Of the 60 analyzed compounds, 21 were not detected in any soil or sediment sample and α-endosulfan was not detected in any sediment sample. The absence of these 21 compounds may be related with low uses and/or effectiveness of the ban or restriction policies, and degradation processes may also have played an important role for less persistent pollutants such as OPEs. OPEs were the most frequently detected pollutants with total detection frequencies from 14 to 48% in spoil and 5 to 39% in sediments, followed by PBDEs with 18-27% in soil and 16-24% in sediments. OCPs and PCBs showed lower detection frequencies (Table 4.1) except for PCBs in Gaoqiao, which were over 25% in both soils and sediments. TOC ranged from 0.36% to > 30% (Table 4.A4). Even though some samples with high TOC content were also among the most polluted samples, no significant correlation was found between ΣPOPs, ΣOPEs, or individual compounds as a function of TOC in soils or sediments.

4.3.2. OPEs

OPEs were present in 10 out of the 13 soils and 12 out of the 16 sediments and the number of compounds detected ranged from 1 to 7 in soils and from 2 to 9 in sediments. Figure 4.3 shows the OPE concentrations and profiles in soils and sediments and Table 4.A7 lists the individual concentrations. Individual OPEs ranged from non- detected (nd) to 25,430 ng/g dw in soils and from nd to 1320 ng/g dw in sediments. TPhP, TCPs, and TCPP accounted for the main detected OPEs, which was attributed to recent inputs as they remain in use. TPhP may also be released from plastic garbage/residues such as polyesters or polyvinyl chloride (Wei et al. 2015).

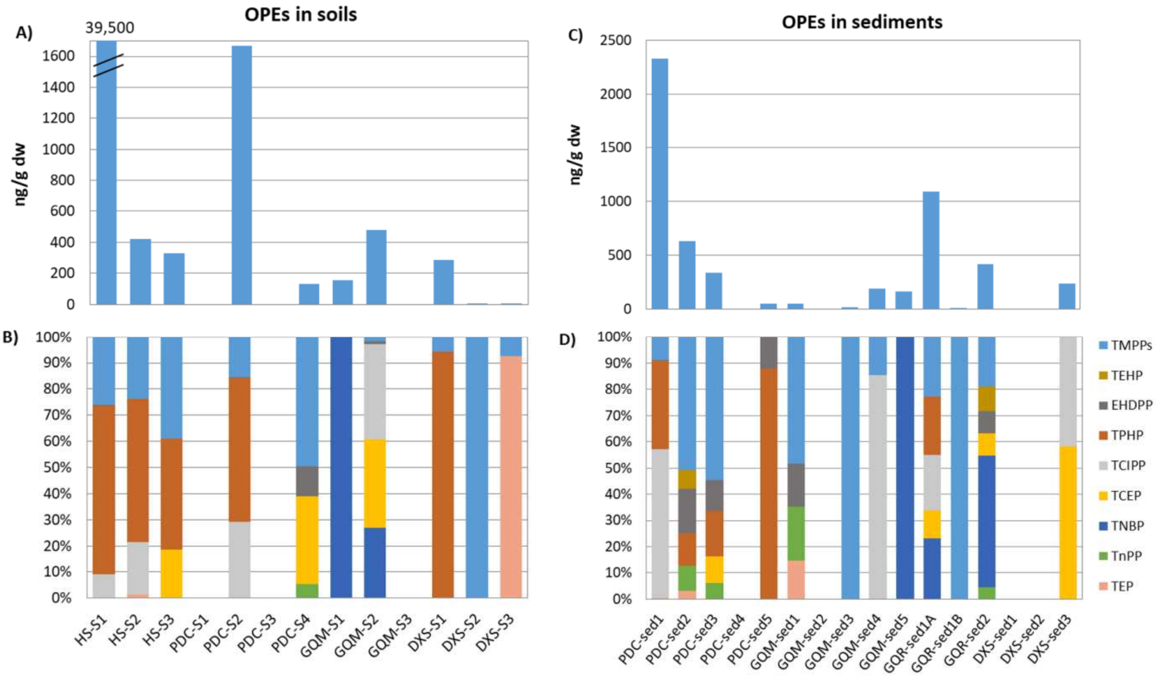


Figure 4.3. Total concentration (ng/g dw) and profiles of OPEs in soils (A and B, respectively) and sediments (C and D, respectively) in the different study areas.

The most polluted area was Heshan (Figure 4.3A), where ∑OPEs in soils ranged from 328 to 39,500 ng/g dw. Soil pollution was dominated by aromatic OPEs (specifically TPhP) accounting for up to 64% of ∑OPEs, followed by TCP isomers (Figure 4.3B). TCPP accounted for 10% and 20% in HS-S1 and HS-S2, respectively, and TCEP accounted for 20% in HS-S3. TEHP, EHDPP, and TEP were detected at much lower concentrations and TnBP and TnPP were never detected. High levels of OPEs, like the ones found in HS-S1, may be associated with illegally dumped garbage and open burning as a point source and visual evidence of these practices were found during the sampling campaign.

Despite Pudacuo being an isolated area at 3600 m altitude, individual OPEs were detected in two of the four analyzed soils at concentrations ranging from 5.10–917 ng/g dw. No OPEs were detected in PDC-S1 or PDC-S3, as the former is a crop soil and the latter is located inside the forest, far from potential visitor influence; both samples had lower TOC than other soils (Table 4.A4). PDC-S2 had the highest concentration with ∑OPEs of 1667 ng/g dw, with high prevalence of TPhP (917 ng/g dw; 54%) and TCPP (488 ng/g dw; 30%). This sample was collected from the side terrace of the river, adjacent to the walking visitor’s pathway, which may have contributed to the detected levels. In PDC-S4, the levels were much lower, with TCPs and TCEP the main contributors (Figure 4.3B). Sediments had lower concentrations than soil (Figure 4.3C). River sediment PDC-sed1 was the most polluted site (∑OPEs 2327 ng/g dw) with TPhP (32%) and TCPP (58%) the main contributors, followed by PDC-sed2 and PDC-sed3 with 50% contribution of TCPs. In contrast, OPEs in lake sediments were low (PDC-sed5) or non-detected (PDC-sed4).

Soils from Gaoqiao mangrove had very low TOC (Table 4.A4) and the ∑OPE concentration was of 157 and 479 ng/g dw in GQM-S1 and GQM-S2. Distinct profile was observed, where GQM-S1 contained only TnBP, a compound used as a reagent for rare metals extraction, as an industrial defoamer and as a chlorine-containing rubber plasticizer (Zhang et al. 2021), GQM-S2 contained TCPP, TCEP and TnBP at concentrations from 129 to 147 ng/g dw and traces of other OPEs. In contrast, no OPEs were detected in GQM-S3 crop soil, which was located outside of the mangrove forest (Figure 4.3B). Sediments from the mangrove were also characterized by low TOC and low concentrations of all OPEs (Figure 4.3C), ranging from nd to 188 ng/g dw; TCEP, TPhP and TEHP were not detected. This contrasted with the high OPE levels in river sediments (except GQR-sed1B) where TnBP, TCEP, TCPP, TPhP and TCP3 were the main compounds detected (Figure 4.3D). The same order of magnitude, with $\sum_{14}$OPEs ranging from 13.2–377 ng/g dw, was reported in mangroves in the Pearl River Delta (Hu et al., 2017).

Danxia Shan is the area with the highest protection status and OPEs were generally lower than in the other three sampled areas. ∑OPEs ranged from 3.5 ng/g dw in DXS-S2 to 283 ng/g dw in DXS-S1. For DXS-S1, the most polluted area, the sample was collected from a view stop point at 323 m altitude, where tourists and visitors spend time resting, eating, drinking and enjoying the views, and had a high prevalence of TPhP (94%). This high contribution of TPhP was also observed in the touristic area of PDC-S2. TPhP is used as plasticizer, flame retardant and as an additive in varnishes, paints or glues, and its presence in soil may be linked with plastic residues from consumable goods (Ali et al. 2017). In contrast, DXS-S2 and DXS-S3 were collected from the bottom part of the geological formation with less touristic influence and showed low OPE concentrations. Among sediments, only lake sediment DXS-sed3 contained OPEs, i.e., TCEP and TCPP at concentrations of 137 and 98.2 ng/g dw, respectively (Figure 4.3D), which were probably released by recreational activities in the lake and its surroundings, such as boating, walking and restaurants.

4.3.3. PBDEs

PBDEs were present in all studied areas and were the second most abundant pollutants after OPEs. PBDE levels and profiles are indicated in Figure 4.4 and individual concentrations are given in Table 4.A8. Despite the deca-formulation being banned in 2008 in Europe for electrical and electronic equipment (European Parliament 2008), soils and sediments from the four protected areas in China were dominated by BDE 209, which accounted for 75–100% of ∑PBDEs in soils and 61–100% in sediments for all studied areas, except for PDC-S2 and PDC-S4 which were dominated by penta-BDEs and PDC-sed5 which contained BDE 47, 99, 154 and 209 congeners. The predominance of BDE 209 agrees with its usage in China (Ji et al., 2017) and has also been described in sediments from different regions such as Korea, Taiwan, Italy, Australia and Japan (Klinčić et al., 2020; Iqbal et al., 2017). Other PBDEs were mainly detected in soil, but at low levels. This can be attributed to the prohibition of penta and octa-BDE formulations during early 2000s in several countries (European Commission, 2003; Stockholm Convention, 2019).

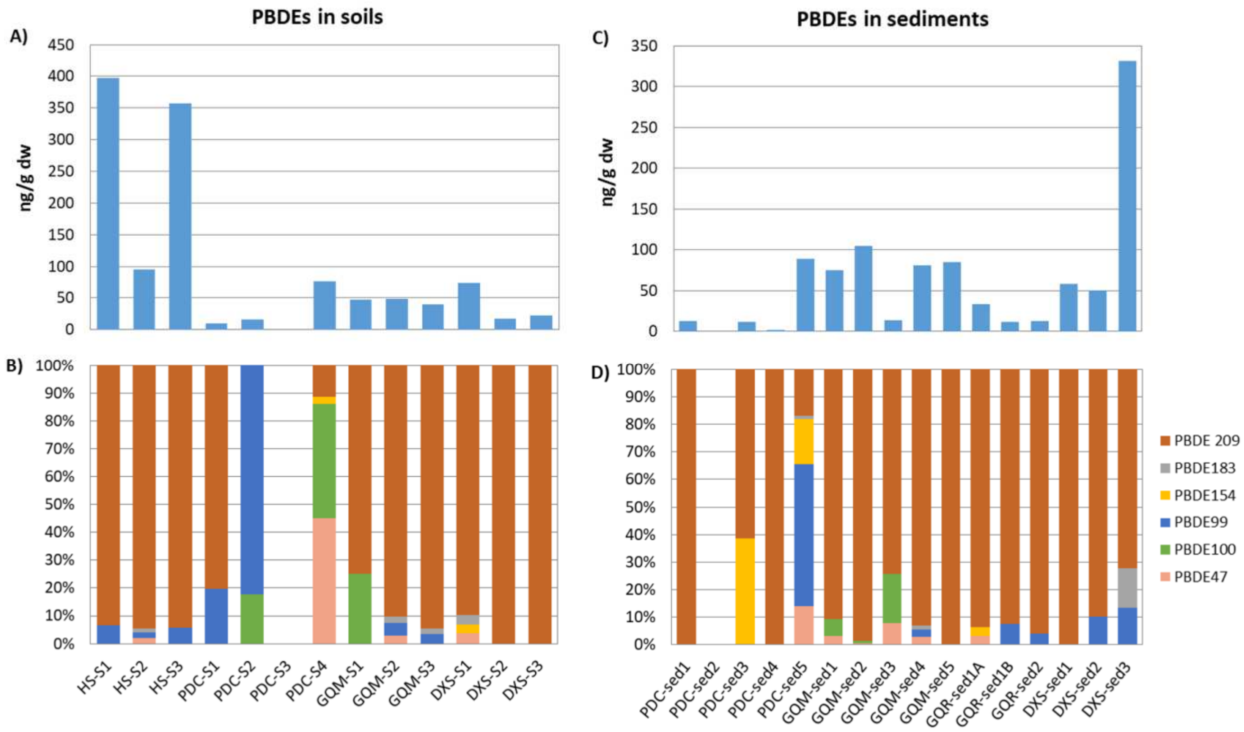


Figure 4.4. Total concentration (ng/g dw) and profiles of PBDEs in soils (A and B, respectively) and sediments (C and D, respectively) in the different study areas.

Consistent with OPEs, soils from Heshan had the highest PBDEs levels (Figure 4.4A). HS-S1 and HS-S3 showed the same PBDE profiles, which were dominated by BDE 209 and BDE 99 to a lesser extent (Figure 4.4B). On the other hand, HS-S2 showed lower levels (∑PBDEs = 94.6 ng/g dw), which may correspond to regenerated soils as local aged soils were removed during the construction of the artificial lake years after the ban of these legacy compounds.

In Pudacuo, PBDEs were detected in three out of the four soils. The concentrations were low and profiles varied among soils, being BDE 209 the dominant compound in PDC-S1 but not in other soils (Figure 4.4B). The concentrations in river and lake sediments were also low (Figure 4.4C) except for PDC-sed5 with ∑PBDEs of 88.6 ng/g dw and had contributions from all PBDEs except BDE 99 (Figure 4.4D).

All three soil samples from Gaoqiao mangrove had PBDEs concentrations ranging from nd to 43.4 ng/g dw and profiles dominated by BDE 209 (Figures 4.4A and 4.4B), with other congeners detected at trace levels. Sediments had higher PBDE levels than soils, which may have been due to their higher TOC. PBDE concentrations in mangrove sediments ranged from 13–104 ng/g dw and in river sediments ranged from 11.6–33 ng/g dw dominate also by BDE 209. Worldwide presence, bioavailability, and toxicity data for various pollutants in mangroves from 1974–2012 were reviewed by Bayen (2012) and indicate that these areas are vulnerable with regards to chemical pollution. PBDEs ranged from 0.08–23.09 ng/g dw in mangrove sediments from Singapore, India and Senegal. Similar findings have been reported for sediments from three mangroves in the Pearl River Delta, south China, where BDE 209 accounted for > 93% of ΣPBDEs (Hu et al. 2019). The same author, in 2020, reported ∑PBDEs from 46.4–219 ng/g dw in sediments from Futian National Nature Reserve, a mangrove forest in Shenzhen City located at approximately 250 km from Gaoqiao (Hu et al., 2020). Another study from south China reported levels from 1.53–75.9 ng/g dw for BDE 209 and from 0.57–14.4 ng/g dw for $\sum_7$PBDEs (excluding BDE 209) in mangroves from Hong Kong (Zhu et al., 2014), which are lower than those found in Gaoqiao in the present study. All these results point again to the widespread presence of BDE 209 in mangroves.

In Danxia Shan, soils and sediments had concentrations from nd to 65.7 ng/g dw and nd to 240 ng/g dw, respectively (Figure 4.4A and 4.4C) and PBDE profiles (Figure 4.4B and 4.4D), except for DXS-sed3. DXS-sed3 which was the most polluted sediment among analyzed areas, with ∑PBDEs of 332 ng/g dw and BDE 209 accounted for 72% of ∑PBDEs concentration, followed by BDE 183 and BDE 99. This sample was a lake sediment which most likely had accumulated PBDEs over time compared to the river sediments collected in this area.

4.3.4. OCPs

Traces of OCPs were detected in all soils and sediments with ∑OCPs < 20 ng/g dw. Concentrations and profiles are represented in Figure 4.5 and individual concentrations are given in Table 4.A9. In soils, from 1 to 5 out of 29 analyzed compounds were present per sample, with α-HCH and HCBu being the most ubiquitous compounds and cis-chlorane and 4,4’-DDT having the highest maximum concentrations (10.4 and 14.8 ng/g dw, respectively). α-HCH was the most frequently detected OCP, being present in 12 out of 13 soil samples at a mean concentration of 0.52 ± 0.3 ng/g, and in 13 out of 16 sediments at a mean concentration of 0.39 ± 0.3 ng/g (Figures 4.5B and 4.5D). HCHs, as for the other analyzed POPs, have high persistence and low degradation rates and are widespread in both marine and terrestrial environments around the world (Alshemmari, 2021; Grung et al., 2015; Taiwo, 2019). HCBu was detected in six soils and five sediments. Only three samples (DXS-S1, PDC-sed5, and GQR-sed1A) did not contain OCPs (Figures 4.5A and 4.5C).

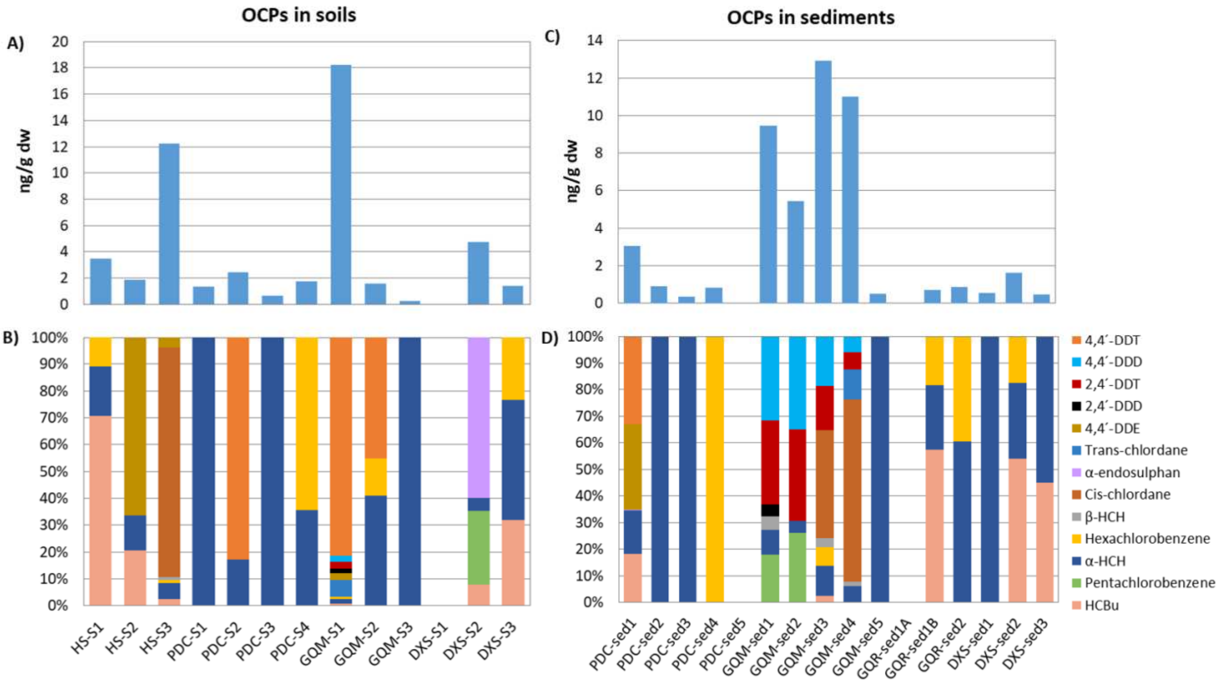


Figure 4.5. Total concentration (ng/g dw) and profiles of OCPs in soils (A and B, respectively) and sediments (C and D, respectively) in the different study areas.

In Heshan, the mean OCP concentration was 6.1 ng/g dw and α-HCH and HCBu were present in all three soil samples, being HS-S3 the one with the highest concentration and with a high contribution from cis-chlordane (10.4 ng/g dw). The presence of 4,4´-DDE may have been related to historical use of technical DDT.

In Pudacuo, α-HCH was present at levels < 1.5 ng/g dw in all soil samples and in three out of the five sediment samples. Traces of 4,4´-DDT, 4,4´-DDE, HCBu and HCB were detected in at least one sample. OCPs have been reported as main environmental pollutants in South Asia (Ali et al., 2014) and China (Huang et al., 2019b) due to their long half-life and atmospheric transport. However, OCP pollution levels in Pudacuo were quite low and were comparable to those in other studies performed in high altitude areas such as the Tibetan Plateau with concentrations < 6 ng/g for DDT and HCHs (Fu et al., 2001), or alpine mountains of Switzerland with concentrations from tens to hundreds pg/g of HCHs or HCB (Shunthirasingham et al., 2013). Melting glaciers can also be a potential source for POPs pollution and have previously been identified as a major source of these compounds in subalpine lakes (Blais et al., 2001; Gouin et al., 2004; Meijer et al., 2002).

Gaoqiao was the area with the highest OCP pollution level, especially in sediments from the mangrove (GQM-sed) which receives agricultural runoff, and α-HCH, DDTs and cis-chlordane were the main compounds detected; contrarily, river sediments (GQR-sed) had trace level concentrations. Similar levels were found by Wu et al. (2015) for α-HCH (0.06–0.17 ng/g) but lower for DDT in sediments from Nansha mangrove, south China. GQM-sed4 contained cis-chlordane at 6 ng/g dw and trans-chlordane at 1.1 ng/g dw, which were within the range of those found in mangroves from Singapore, India and Hong Kong (0.01–11.1 ng/g dw) as reviewed by Bayen et al. (2012).

In Danxia Shan ΣOCPs were between n.d and 4.9 ng/g dw in soils and 0.5–1.6 ng/g dw in sediments, with HCBu detected in two soils and two sediments. Overall, OCPs were detected at very low concentrations and reflect historical diffusive sources.

4.3.5. PCBs

PCBs were detected in 6 soils out of 13 and in 9 sediments out of 16. They showed the lowest concentrations among the analyzed pollutants, with ∑PCBs ranging from nd to 1.5 ng/g dw in soils and from nd to 1.4 ng/g dw in sediments. PCBs pollution levels and profiles are represented in Figure 4.6 and detailed results for each sample are provided in Table 4.A10. PCB 28 was dominant in soils, except for HS-S3 where only PCB 153 was detected. Danxia Shan was the least affected area by PCB contamination.

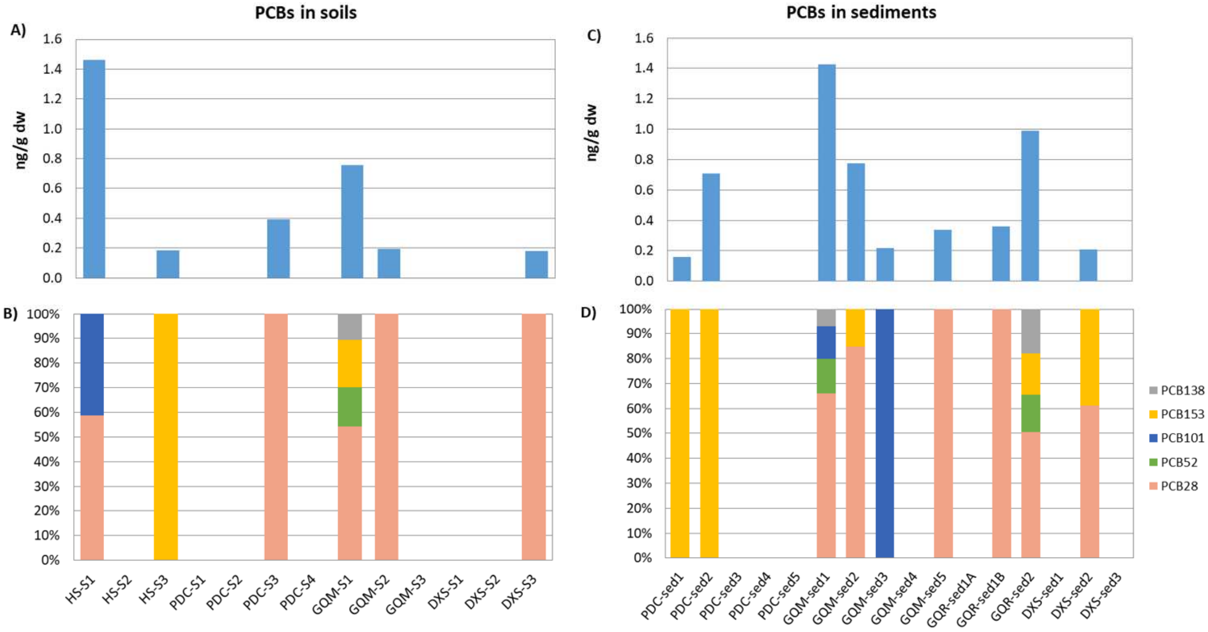


Figure 4.6. Total concentration (ng/g dw) and profiles of PCBs in soils (A and B, respectively) and sediments (C and D, respectively) in the different study areas.

PCBs have been prohibited for more than 30 years, which may explain the low levels detected in the studied protected areas which are related to background pollution rather than point sources (Zhan et al., 2017). The low levels detected in soils agree with the study of Zheng et al. (2014), who reported an average concentration of 0.51 ng/g in 30 Chinese mountain soils. Levels ranging from nd to 0.088 ng/g dw were reported in soils from Pamirs mountains, central Asia, associated with long-range transport (Ding et al., 2020). On the other hand, sediments from four mangrove forests from Shantou, south China, had mean Σ_{41}PCBs ranging from 0.11 to 0.37 ng/g (Ren et al., 2019), in coast mangroves of India (mean Σ_{182}PCBs of 4.4 ± 4.8 ng/g dw) (Zanardi-Lamardo et al., 2019) and in Jobos Bay in Puerto Rico (Σ_{50}PCBs between 0.42–1200 ng/g dw) (Alegria et al., 2016). Traces of PCBs detected in remote areas are most likely associated with accumulation in soils and/or sediments, air transport or cold deposition (Meijer et al., 2002; Olatunji, 2020).

4.4. Conclusions and perspectives

This study has demonstrated the presence of POPs and OPEs in some protected areas in south China which, hypothetically, should be pristine as anthropogenic activities are, in principle, not permitted within their boundaries. Legacy POPs were detected at low levels which reflected the background pollution. However, OPEs were ubiquitous and associated to tourism influence and recreational activities, as observed in Danxia Shan, where higher pollution levels were found near recreational activities. Industrial activities carried out within the premises of the protected areas or nearby as in the case of furniture production industry or garbage burning in Heshan, and agricultural activities upstream of Daba river in Gaoqiao mangrove were also associated with a high level of pollutants. Even Pudaco, a remote area located far away from any direct pollution source, was affected by environmental pollution, likely from diffuse sources.

Soil and sediment monitoring programs provide information on the pollution status and its time-evolution, which can guide decision-making regarding legal policies and evaluate the efficiency of conservation actions. In the short and mid-term, periodic surveillance, effective zoning and risk assessment for organic pollutants in protected areas will contribute to achieve main environmental protection objectives, along with economic development.

References

Abbasi, G., Li, L., Breivik, K., 2019. Global Historical Stocks and Emissions of PBDEs. Environ Sci Technol 53, 6330-6340, 10.1021/acs.est.8b07032

Abessa, D.M.S., Albuquerque, H.C., Morais, L.G., Araujo, G.S., Fonseca, T.G., Cruz, A.C.F., Campos, B.G., Camargo, J., Gusso-Choueri, P.K., Perina, F.C., Choueri, R.B., Buruaem, L.M., 2018. Pollution status of marine protected areas worldwide and the consequent toxic effects are unknown. Environ Pollut 243, 1450-1459, 10.1016/j.envpol.2018.09.129

Alegria, H., Martinez-Colon, M., Birgul, A., Brooks, G., Hanson, L., Kurt-Karakus, P., 2016. Historical sediment record and levels of PCBs in sediments and mangroves of Jobos Bay, Puerto Rico. Sci Total Environ 573, 1003-1009, 10.1016/j.scitotenv.2016.08.165

Ali, U., Syed, J.H., Malik, R.N., Katsoyiannis, A., Li, J., Zhang, G., Jones, K.C., 2014. Organochlorine pesticides (OCPs) in South Asian region: a review. Sci Total Environ 476-477, 705-717, 10.1016/j.scitotenv.2013.12.107

Ali, N., Shahzad, K., Rashid, M.I., Shen, H., Ismail, I.M.I., Eqani, S., 2017. Currently used organophosphate and brominated flame retardants in the environment of China and other developing countries (2000-2016). Environ Sci Pollut Res Int 24, 18721-18741, 10.1007/s11356-017-9336-3

Alshemmari, H., 2021. Inventories and assessment of POPs in the State of Kuwait as a basis for Stockholm Convention implementation. Emerging Contam 7, 88-98, 10.1016/j.emcon.2021.02.003

Barletta, M., Lima, A.R.A., Costa, M.F., 2019. Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. Sci Total Environ 651, 1199-1218, 10.1016/j.scitotenv.2018.09.276

Bayen, S., 2012. Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: a review. Environ Int 48, 84-101, 10.1016/j.envint.2012.07.008

Blais, J.M., Schindler, D.W., Muir, D.C.G., Sharp, M., Donald, D., Lafrenière, M., Braekevelt, E., Strachan, W.M.J., 2001. Melting Glaciers: A Major Source of Persistent Organochlorines to Subalpine Bow Lake in Banff National Park, Canada. AMBIO: J Hum Environ 30, 410, 10.1639/0044-7447(2001)030[0410:mgamso]2.0.co;2

Brandt, J.S., Butsic, V., Schwab, B., Kuemmerle, T., Radeloff, V.C., 2015. The relative effectiveness of protected areas, a logging ban, and sacred areas for old-growth forest protection in southwest China. Biol Conserv 181, 1-8, 10.1016/j.biocon.2014.09.043

Chen, H., 2020. Land use trade-offs associated with protected areas in China: Current state, existing evaluation methods, and future application of ecosystem service valuation. Sci Total Environ 711, 134688, 10.1016/j.scitotenv.2019.134688

Choi, H.G., Moon, H.B., Choi, M., Yu, J., 2011. Monitoring of organic contaminants in sediments from the Korean coast: Spatial distribution and temporal trends (2001-2007). Mar Pollut Bull 62, 1352-1361, 10.1016/j.marpolbul.2011.03.029

Ding, Y., Huang, H., Chen, W., Zhang, Y., Chen, W., Xing, X., Qi, S., 2020. Background levels of OCPs, PCBs, and PAHs in soils from the eastern Pamirs, China, an alpine region influenced by westerly atmospheric transport. J Environ Sci, 10.1016/j.jes.2020.11.022

Erickson, M.D., Kaley, R.G., 2nd, 2011. Applications of polychlorinated biphenyls. Environ Sci Pollut Res Int 18, 135-151, 10.1007/s11356-010-0392-1

European Commission, 2003. Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003 amending for the 24th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). Oficial Journal of the European Union. 45-46. Available at https://op.europa.eu/en/publication-detail/-/publication/0a566c4b-75ab-459b-8546-dbae21493f1b

European Parliament, 2008. Joined Cases C-14/06 and C-295/06. Directive 2002/95/EC– Electrical and electronic equipment – Restriction of use of certain hazardous substances – Decabromodiphenyl ether (‘DecaBDE’) – Commission Decision 2005/717/EC – Exemption of DecaBDE from the prohibition on use – Actions for annulment – Commission’s implementing powers – Infringement of the enabling provision. Available at https://op.europa.eu/en/publication-detail/-/publication/db746402-649a-4561-98e8-1b783be5700e

Fu, S., Chu, S., Xu, X., 2001. Organochlorine Pesticide Residue in Soils from Tibet, China. Bull Environ Contam Toxicol 66, 171-177, 10.1007/s001280000221

Gouin, T., Mackay, D., Jones, K.C., Harner, T., Meijer, S.N., 2004. Evidence for the "grasshopper" effect and fractionation during long-range atmospheric transport of organic contaminants. Environ Pollut 128, 139-148, 10.1016/j.envpol.2003.08.025

Grung, M., Lin, Y., Zhang, H., Steen, A.O., Huang, J., Zhang, G., Larssen, T., 2015. Pesticide levels and environmental risk in aquatic environments in China- A review. Environ Int 81, 87-97, 10.1016/j.envint.2015.04.013

Holoubek, I., Dusek, L., Sanka, M., Hofman, J., Cupr, P., Jarkovsky, J., Zbiral, J., Klanova, J., 2009. Soil burdens of persistent organic pollutants--their levels, fate and risk. Part I. Variation of concentration ranges according to different soil uses and locations. Environ Pollut 157, 3207-3217, 10.1016/j.envpol.2009.05.031

Hu, Y.X., Sun, Y.X., Li, X., Xu, W.H., Zhang, Y., Luo, X.J., Dai, S.H., Xu, X.R., Mai, B.X., 2017. Organophosphorus flame retardants in mangrove sediments from the Pearl River Estuary, South China. Chemosphere 181, 433-439, 10.1016/j.chemosphere.2017.04.117

Hu, Y., Pei, N., Sun, Y., Xu, X., Zhang, Z., Li, H., Wang, W., Zuo, L., Xiong, Y., Zeng, Y., He, K., Mai, B., 2019. Halogenated flame retardants in mangrove sediments from the Pearl River Estuary, South China: Comparison with historical data and correlation with microbial community. Chemosphere 227, 315-322, 10.1016/j.chemosphere.2019.04.075

Hu, Y., Sun, Y., Pei, N., Zhang, Z., Li, H., Wang, W., Xie, J., Xu, X., Luo, X., Mai, B., 2020. Polybrominated diphenyl ethers and alternative halogenated flame retardants in mangrove plants from Futian National Nature Reserve of Shenzhen City, South China. Environ Pollut 260, 114087, 10.1016/j.envpol.2020.114087

Huang, Y., Fu, J., Wang, W., Li, J., 2019a. Development of China’s nature reserves over the past 60 years: An overview. Land Use Policy 80, 224-232, 10.1016/j.landusepol.2018.10.020

Huang, H., Ding, Y., Chen, W., Zhang, Y., Chen, W., Chen, Y., Mao, Y., Qi, S., 2019b. Two-way long-range atmospheric transport of organochlorine pesticides (OCPs) between the Yellow River source and the Sichuan Basin, Western China. Sci Total Environ 651, 3230-3240, 10.1016/j.scitotenv.2018.10.133

Iqbal, M., Syed, J.H., Katsoyiannis, A., Malik, R.N., Farooqi, A., Butt, A., Li, J., Zhang, G., Cincinelli, A., Jones, K.C., 2017. Legacy and emerging flame retardants (FRs) in the freshwater ecosystem: A review. Environ Res 152, 26-42, 10.1016/j.envres.2016.09.024

Ji, X., Ding, J., Xie, X., Cheng, Y., Huang, Y., Qin, L., Han, C., 2017. Pollution Status and Human Exposure of Decabromodiphenyl Ether (BDE-209) in China. ACS omega 2, 3333-3348, 10.1021/acsomega.7b00559

Kim, L., Jeon, J.W., Son, J.Y., Park, M.K., Kim, C.S., Jeon, H.J., Nam, T.H., Kim, K., Park, B.J., Choi, S.D., Lee, S.E., 2017. Monitoring and risk assessment of polychlorinated biphenyls (PCBs) in agricultural soil from two industrialized areas. Environ Geochem Health 39, 279-291, 10.1007/s10653-017-9920-y

Klincic, D., Dvorscak, M., Jagic, K., Mendas, G., Herceg Romanic, S., 2020. Levels and distribution of polybrominated diphenyl ethers in humans and environmental compartments: a comprehensive review of the last five years of research. Environ Sci Pollut Res Int 27, 5744-5758, 10.1007/s11356-020-07598-7

Li, L., Wania, F., 2016. Tracking chemicals in products around the world: introduction of a dynamic substance flow analysis model and application to PCBs. Environ Int 94, 674-686, 10.1016/j.envint.2016.07.005

Li, Y.F., Cai, D.J., Shan, Z.J., Zhu, Z.L., 2001. Gridded usage inventories of technical hexachlorocyclohexane and lindane for China with 1/6 degrees latitude by 1/4 degrees longitude resolution. Archives of Environmental Contamination and Toxicology 41, 261-266, 10.1007/s002440010247

Ma, Y., 2017. Environmental Reviews and Case Studies: Contextualization of National Parks in the Nature Conservation Scheme in China: A Case Study of Pudacuo National Park in Yunnan Province. Environ Pract 15, 293-312, 10.1017/s1466046613000203

Ma, Y., Yun, X., Ruan, Z., Lu, C., Shi, Y., Qin, Q., Men, Z., Zou, D., Du, X., Xing, B., Xie, Y., 2020. Review of hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT) contamination in Chinese soils. Sci Total Environ 749, 141212, 10.1016/j.scitotenv.2020.141212

McGrath, T.J., Ball, A.S., Clarke, B.O., 2017. Critical review of soil contamination by polybrominated diphenyl ethers (PBDEs) and novel brominated flame retardants (NBFRs); concentrations, sources and congener profiles. Environ Pollut 230, 741-757, 10.1016/j.envpol.2017.07.009

Meijer, S.N., Steinnes, E., Ockenden, W.A., Jones, K.C., 2002. Influence of Environmental Variables on the Spatial Distribution of PCBs in Norwegian and U.K. Soils: Implications for Global Cycling. Environ Sci Technol 36, 2146-2153, 10.1021/es010322i

Miller-Rushing, A.J., Primack, R.B., Ma, K., Zhou, Z.-Q., 2017. A Chinese approach to protected areas: A case study comparison with the United States. Biol Conserv 210, 101-112, 10.1016/j.biocon.2016.05.022

Olatunji, O.S., 2020. Partitioning, Translocation Pathways and Environmental Risk Evaluation of Selected Polychlorinated Biphenyls and Pesticides. Water, Air, & Soil Pollut 231, 10.1007/s11270-020-04771-z

Pelamatti, T., Rios-Mendoza, L.M., Hoyos-Padilla, E.M., Galvan-Magana, F., De Camillis, R., Marmolejo-Rodriguez, A.J., Gonzalez-Armas, R., 2021. Contamination knows no borders: Toxic organic compounds pollute plastics in the biodiversity hotspot of Revillagigedo Archipelago National Park, Mexico. Mar Pollut Bull 170, 112623, 10.1016/j.marpolbul.2021.112623

Poma, G., Sales, C., Bruyland, B., Christia, C., Goscinny, S., Van Loco, J., Covaci, A., 2018. Occurrence of Organophosphorus Flame Retardants and Plasticizers (PFRs) in Belgian Foodstuffs and Estimation of the Dietary Exposure of the Adult Population. Environ Sci Technol 52, 2331-2338, 10.1021/acs.est.7b06395

Ren, G., Yan, X., Chu, X., Cai, Y., Shi, Y., Zheng, K., Yu, Z., 2019. Polybrominated diphenyl ethers and polychlorinated biphenyls in mangrove sediments of Shantou, China: Occurrence, profiles, depth-distribution, and risk assessment. Ecotox Environ Safe 183, 109564, 10.1016/j.ecoenv.2019.109564

She, Y.Z., Wu, J.P., Zhang, Y., Peng, Y., Mo, L., Luo, X.J., Mai, B.X., 2013. Bioaccumulation of polybrominated diphenyl ethers and several alternative halogenated flame retardants in a small herbivorous food chain. Environ Pollut 174, 164-170, 10.1016/j.envpol.2012.11.024

Shi, F., Weaver, D., Zhao, Y., Huang, M.-F., Tang, C., Liu, Y., 2019. Toward an ecological civilization: Mass comprehensive ecotourism indications among domestic visitors to a Chinese wetland protected area. Tourism Manag 70, 59-68, 10.1016/j.tourman.2018.07.011

Shunthirasingham, C., Wania, F., MacLeod, M., Lei, Y.D., Quinn, C.L., Zhang, X., Scheringer, M., Wegmann, F., Hungerbuhler, K., Ivemeyer, S., Heil, F., Klocke, P., Pacepavicius, G., Alaee, M., 2013. Mountain cold-trapping increases transfer of persistent organic pollutants from atmosphere to cows' milk. Environ Sci Technol 47, 9175-9181, 10.1021/es400851d

Stockholm Convention on Persistent Organic Pollutants, 2019. http://chm.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP-CONVTEXT-2021.English.pdf. (Accessed August 2021)

Taiwo, A.M., 2019. A review of environmental and health effects of organochlorine pesticide residues in Africa. Chemosphere 220, 1126-1140, 10.1016/j.chemosphere.2019.01.001

Tao, S., Liu, W., Li, Y., Yang, Y., Zuo, Q., Li, B., Cao, J., 2008. Organochlorine pesticides contaminated surface soil as reemission source in the Haihe Plain, China. Environ Sci Technol 42, 8395–8400, 10.1021/es8019676 C

van den Berg, H., Manuweera, G., Konradsen, F., 2017. Global trends in the production and use of DDT for control of malaria and other vector-borne diseases. Malaria Journal 16, 401, 10.1186/s12936-017-2050-2

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119-1153, 10.1016/j.chemosphere.2012.03.067

Vecchiato, M., Bonato, T., Barbante, C., Gambaro, A., Piazza, R., 2021. Organic pollutants in protected plain areas: The occurrence of PAHs, musks, UV-filters, flame retardants and hydrocarbons in woodland soils. Sci Total Environ 796, 149003, 10.1016/j.scitotenv.2021.149003

Wang, F., Li, Z., Xia, H., Zou, B., Li, N., Liu, J., Zhu, W., 2010. Effects of nitrogen-fixing and non-nitrogen-fixing tree species on soil properties and nitrogen transformation during forest restoration in southern China. Soil Sci Plant Nutrit 56, 297-306, 10.1111/j.1747-0765.2010.00454.x

Wang, X., Gong, P., Wang, C., Ren, J., Yao, T., 2016. A review of current knowledge and future prospects regarding persistent organic pollutants over the Tibetan Plateau. Sci Total Environ 573, 139-154, 10.1016/j.scitotenv.2016.08.107

Wang, W., Feng, C., Liu, F., Li, J., 2020. Biodiversity conservation in China: A review of recent studies and practices. Environ Sci Ecotechnol 2, 100025, 10.1016/j.ese.2020.100025

Wei, G.L., Li, D.Q., Zhuo, M.N., Liao, Y.S., Xie, Z.Y., Guo, T.L., Li, J.J., Zhang, S.Y., Liang, Z.Q., 2015. Organophosphorus flame retardants and plasticizers: sources, occurrence, toxicity and human exposure. Environ Pollut 196, 29-46, 10.1016/j.envpol.2014.09.012

Wong, F., Robson, M., Diamond, M.L., Harrad, S., Truong, J., 2009. Concentrations and chiral signatures of POPs in soils and sediments: a comparative urban versus rural study in Canada and UK. Chemosphere 74, 404-411, 10.1016/j.chemosphere.2008.09.051

Wu, J., Zhang, Y., Luo, X., She, Y., Yu, L., Chen, S., Mai, B., 2012. A review of polybrominated diphenyl ethers and alternative brominated flame retardants in wildlife from China: Levels, trends, and bioaccumulation characteristics. J Environ Sci 24, 183-194, 10.1016/s1001-0742(11)60758-4

Wu, Q., Leung, J.Y., Yuan, X., Huang, X., Li, H., Huang, Z., Li, Y., 2015. Biological risk, source and pollution history of organochlorine pesticides (OCPs) in the sediment in Nansha mangrove, South China. Mar Pollut Bull 96, 57-64, 10.1016/j.marpolbul.2015.05.047

Xu, W., Li, X., Pimm, S.L., Hull, V., Zhang, J., Zhang, L., Xiao, Y., Zheng, H., Ouyang, Z., 2016. The effectiveness of the zoning of China's protected areas. Biol Conserv 204, 231-236, 10.1016/j.biocon.2016.10.028

Yadav, I.C., Devi, N.L., Li, J., Zhang, G., 2018. Organophosphate ester flame retardants in Nepalese soil: Spatial distribution, source apportionment and air-soil exchange assessment. Chemosphere 190, 114-123, 10.1016/j.chemosphere.2017.09.112

Yang, J., Zhao, Y., Li, M., Du, M., Li, X., Li, Y., 2019a. A Review of a Class of Emerging Contaminants: The Classification, Distribution, Intensity of Consumption, Synthesis Routes, Environmental Effects and Expectation of Pollution Abatement to Organophosphate Flame Retardants (OPEs). Int J Mol Sci 20, 10.3390/ijms20122874

Yang, H., Vina, A., Winkler, J.A., Chung, M.G., Dou, Y., Wang, F., Zhang, J., Tang, Y., Connor, T., Zhao, Z., Liu, J., 2019. Effectiveness of China's protected areas in reducing deforestation. Environ Sci Pollut Res Int 26, 18651-18661, 10.1007/s11356-019-05232-9

Zanardi-Lamardo, E., Mitra, S., Vieira-Campos, A.A., Cabral, C.B., Yogui, G.T., Sarkar, S.K., Biswas, J.K., Godhantaraman, N., 2019. Distribution and sources of organic contaminants in surface sediments of Hooghly river estuary and Sundarban mangrove, eastern coast of India. Mar Pollut Bull 146, 39-49, 10.1016/j.marpolbul.2019.05.043

Zapata, P., Ballesteros-Cano, R., Colomer, P., Bertolero, A., Viana, P., Lacorte, S., Santos, F.J., 2018. Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks. Sci Total Environ 633, 704-715, 10.1016/j.scitotenv.2018.03.081

Zhan, L., Lin, T., Wang, Z., Cheng, Z., Zhang, G., Lyu, X., Cheng, H., 2017. Occurrence and air-soil exchange of organochlorine pesticides and polychlorinated biphenyls at a CAWNET background site in central China: Implications for influencing factors and fate. Chemosphere 186, 475-487, 10.1016/j.chemosphere.2017.08.003

Zhang, L., Luo, Z., Mallon, D., Li, C., Jiang, Z., 2017. Biodiversity conservation status in China's growing protected areas. Biol Conserv 210, 89-100, 10.1016/j.biocon.2016.05.005

Zhang, H., Liu, T., Song, X., Zhou, Q., Tang, J., Sun, Q., Pu, Y., Yin, L., Zhang, J., 2021. Study on the reproductive toxicity and mechanism of tri-n-butyl phosphate (TnBP) in Caenorhabditis elegans. Ecotox Environ Safe 227, 112896, 10.1016/j.ecoenv.2021.112896

Zheng, Q., Nizzetto, L., Mulder, M.D., Sanka, O., Lammel, G., Li, J., Bing, H., Liu, X., Jiang, Y., Luo, C., Zhang, G., 2014. Does an analysis of polychlorinated biphenyl (PCB) distribution in mountain soils across China reveal a latitudinal fractionation paradox? Environ Pollut 195, 115-122, 10.1016/j.envpol.2014.08.021

Zhou, D.Q., Edward Grumbine, R., 2011. National parks in China: Experiments with protecting nature and human livelihoods in Yunnan province, Peoples’ Republic of China (PRC). Biol Conserv 144, 1314-1321, 10.1016/j.biocon.2011.01.002

Zhu, H., Wang, Y., Wang, X., Luan, T., Tam, N.F., 2014. Distribution and accumulation of polybrominated diphenyl ethers (PBDEs) in Hong Kong mangrove sediments. Sci Total Environ 468-469, 130-139, 10.1016/j.scitotenv.2013.08.0

Annex

Table 4.A1. Sample name, GPS coordinates, height and observations for each studied area.

Area	Sample	GPS coordinates		Height/m	*In situ* observations
Heshan National Field Research Station of Forest Ecosystem	HS-S1	N 22º 40´ 32.04´´	E 112º 54´00.46´´	42	Soil from ornamental trees crop field
	HS-S2	N 22º 40´ 32.07´´	E 112º 53´57.63´´	42	Soil besides an artificial lake which was used to grow rice 4-5 years ago and now was used for ornamental and reforestation trees
	HS-S3	N 22º 40´ 44.49´´	E 112º 54´00.35´´	81	Soil from the forest where *michelia macclurei* trees were planted for reforestation
Pudacuo National Park	PDC-S1	N 27º50´48.61"	E 099º55´12.09"	3474	Crop soil near the entrance of the " visitor´s long trail"; Some vegetables cultivated there
	PDC-S2	N 27º50´56.20"	E 099º54´30.22"	3516	Soil from the riverside, but higher than the river can reach during its biggest flow, near the walking pathway.
	PDC-S3	N 27º51´37.41"	E 099º54´18.01"	3549	Forest soil from the mountain side, far away from the visitor’s pathway.
	PDC-S4	N 27º54´12.84"	E 099º56´40.21"	3620	Soil from the forest surrounding the lake, road less

Table 4.A1. Continued

Area	Sample	GPS coordinates		Height/m	*In situ* observations
					than 150 m far.
	PDC-sed1	N 27º50´48.61"	E 099º55´12.09"	3474	Sediment from a stream near PDC-S1
	PDC-sed2	N 27º50´44.90"	E 099º54´38.36"	3491	Sediment from a side stream of the river Shudugang He
	PDC-sed3	N 27º54´13.76"	E 099º56´28.64"	3615	Sediment near but outside the lake
	PDC-sed4	N 27º54´15.29"	E 099º56´33.58"	3611	Sediment from the lake (in the shore)
	PDC-sed5	N 27º54´29.56"	E 099º57´15.12"	3614	Sediment lakeside full of roots and with dark colour; the forest of PDC-S4 does not cover the lakeside here
Gao Qiao mangrove National Natural Reserve	GQM-S1	N 21º 34´04.78´´	E 109º 45´ 30.42´´	2	Grass covered space, surrounded with young trees
	GQM-S2	N 21º 34´02.80´´	E 109º 45´ 31.65´´	3	No trees area. Near the main river and a little sand hill where visitors can stop to see the mangrove from a high position
	GQM-S3	N 21º 34´18.40´´	E 109º 45´ 26.90´´	8	Small corn crop besides the river

Table 4.A1. Continued

Area	Sample	GPS coordinates		Height/m	*In situ* observations
	GQM-sed1	N 21º 34´09.28´´	E 109º 45´ 24.57´´	7	Inside the mangrove, well developed area
	GQM-sed2	N 21º 34´09.30´´	E 109º 45´ 26.29´´	-3	Little stream inside the mangrove
	GQM-sed5	N 21º 34´13.91´´	E 109º 45´ 21.39´´	15	Outer part; Black colour in the second layer of the sediments
	GQR-sed1A GQR-sed1B	N 21º 35´26.06´´	E 109º 47´ 02.69´´	10	Pond besides the river filled with river water through a gate which was closed while sampling
	GQR-sed2	N 21º 35´25.02´´	E 109º 47´ 02.05´´	-4	Main river outside the mangrove after a dam.
Danxia Shan National Park	DXS-S1	N 25º 2´4.99"	E 113º44´57.04"	323	Soils from Rudder stone viewpoint 4-5 m away from the path
	DXS-S2	N 25º1´23.94"	E 113º44´23.94"	143	Bottom part of the Rudder stone viewpoint
	DXS-S3	N 25º1´16.38"	E 113º44´26.31"	114	Soil from the forest, 20 m far from the river
	DXS-sed1	N 25º2´30.94"	E 113º43´41.89"	179	Sediment from the river
	DXS-sed2	N 25º2´42.76"	E 113º43´36.03"	119	Sediment from the river just before a big lake
	DXS-sed3	N 25º2´40.77"	E 113º43´54.69"	156	Sediment from the lake

Table 4.A2.Studied OPEs and their CAS number to avoid unambiguity and mass spectrometry conditions used.

Compound	CAS no	Ret time (min)	Quantifier transition			Qualifier transition		
			Precursor ion	Product ion	Collision energy (eV)	Precursor ion	Product ion	Collision energy (eV)
TEP	78-40-0	4.3	155	99	30	127	81	30
TPP (TiPP)	513-02-0	5.2	141	81	30	183	99	30
TPP (TnPP)	513-08-6	7.7	141	81	30	183	99	30
TnBP	126-73-8	11.0	155	81	40	211	99	40
TCEP	115-96-8	12.3	249	63	30	205	63	30
TCPP	13674-84-5	12.6	277	125	10	201	125	30
TDCPP	13674-87-8	17.4	381	159	10	209	75	10
TPhP	115-86-6	18.1	326	169	30	215	169	30
EHDPP	1241-94-7	18.4	251	77	30	251	153	30
TEHP	78-42-2	18.6	113	82	20	211	99	20
TCP1	1330-78-5	20.6	368	197	20	368	181	20
TCP2	1330-78-5	21.0	368	197	20	368	181	20
TCP3	1330-78-5	21.3	368	197	20	368	181	20
TCP4	1330-78-5	21.7	368	197	20	368	181	20

Table 4.A3. Mass spectrometry conditions for each studied OCs.

Compound	Ret time (min)	Quantifier transition			Qualifier transition		
		Precursor ion	Product ion	Collision energy (eV)	Precursor ion	Product ion	Collision energy (eV)
HCBu	9.40	260	225	10	260	190	45
PeCB	15.90	250	215	30	250	180	30
α-HCH	19.40	219	147	20	219	183	15
HCB	20.55	284	249	20	284	114	20
β-HCH	20.80	219	147	20	219	183	15
γ-HCH (lindane)	22.10	219	183	15	219	147	20
δ-HCH	23.98	219	147	20	219	183	15
PCB 28	24.02	258	186	30	258	188	40
Heptachlor	24.80	272	237	20	272	235	20
PCB 52	26.10	292	222	45	292	220	40
Aldrin	26.80	263	193	40	263	191	40
DBP	27.60	139	111	20	139	75	30
Isodrin	28.50	263	193	30	263	228	20
Heptachlor epox	29.30	353	191	45	353	193	45
Oxychlordane	29.35	185	121	20	185	85	20
Cis-Chlordane	30.70	375	266	30	375	339	20
2,4´DDE	31.30	318	246	20	318	248	20
α-endosulfan	31.40	195	159	10	195	125	20
PCB 101	31.43	326	256	50	326	219	50
Trans-Chlordane	31.65	373	266	30	373	264	30
Dieldrin	32.98	241	206	30	241	170	40
4, 4´DDE	33.25	318	248	20	318	246	20
2,4´DDD	33.70	235	165	30	235	200	20
Endrin	34.20	263	193	20	263	228	20
β-endosulfan	34.80	241	206	15	241	170	25
2,4´DDT	35.73	235	165	40	235	199	10
4,4´DDD	35.86	235	165	40	235	199	10
PCB 153	36.53	360	290	30	360	288	30

Table 4.A3. Continued

Compound	Ret time (min)	Quantifier transition			Qualifier transition		
		Precursor ion	Product ion	Collision energy (eV)	Precursor ion	Product ion	Collision energy (eV)
Endosulfan sulphate	37.40	272	237	10	272	143	40
4,4´DDT	37.90	235	165	40	237	167	40
PCB 138	38.10	360	290	40	360	288	40
Dicofol	41.30	139	111	30	139	75	30
Methoxychlor	41.53	227	169	30	227	115	50
PCB 180	42.10	394	324	30	394	359	20
Mirex	43.06	332	262	40	332	260	50

Table 4.A4. Total organic carbon percentage in each sample.

Sample	% Organic C	Sample	% Organic C
HS-S1	0.39	GQM-sed1	2.24
HS-S2	0.83	GQM-sed2	1.69
HS-S3	5.50	GQM-sed3	0.87
PDC-S1	4.23	GQM-sed4	3.45
PDC-S2	17.0	GQM-sed5	0.41
PDC-S3	9.46	GQR-sed1A	1.69
PDC-S4	21.6	GQR-sed1B	0.50
PDC-sed1	5.89	GQR-sed2	0.87
PDC-sed2	5.53	DXS-S1	5.34
PDC-sed3	23.7	DXS-S2	0.88
PDC-sed4	0.36	DXS-S3	1.40
PDC-sed5	>30	DXS-sed1	4.41
GQM-S1	1.08	DXS-sed2	0.37
GQM-S2	0.37	DXS-sed3	3.47
GQM-S3	0.78		

Table 4.A5. Analytical parameters for studied OPEs. Nd: not detected

Compound	Intraday precision (%RSD)	Inter-day precision (%RSD)	%Recovery (n=4)	LOQ (ng/g)	LOD (ng/g)	Blank contribution (ng/g) (n=8)
TEP	8.8	11.2	74 ±10	22.7	7.7	1.2 ± 2.2
TiPP	5.5	7.3	91 ±2.5	145	49.9	8.9 ±13.6
TnPP	5.4	2.5	148 ±9	0.4	0.1	nd
TnBP	3.5	5.5	151 ±5	501	238	126 ± 220
TCEP	4.7	3.5	91 ±11	167	91	58.7 ± 10.8
TCPP	3.6	4.6	97 ±12	395	163	63.1 ± 33.2
TDCPP	2.2	4.9	79 ±1	1.1	0.3	nd
TPhP	1.8	2.5	88 ±18	157	57	14.3 ± 14.3
EHDPP	1.3	8.8	109 ±15	20.5	7.5	2.0 ± 1.9
TEHP	0.8	34	82 ±11	82.3	32.4	11.0 ± 7.1
TCP1	2.1	14	69 ±34	3.3	1.0	nd
TCP2	1.3	13	78 ±32	1.3	0.4	nd
TCP3	2.1	13	96±28	1.7	0.5	nd
TCP4	2.2	13	105 ±21	5.7	1.7	nd

Table 4.A6. Analytical parameters for studied OCs.

Compound	Intraday precision (% RSD)	Inter-day precision (% RSD)	% Recovery	LOD (ng/g)	LOQ (ng/g)
HCBu	6.6	13	76 ± 0.1	0.13	0.44
PeCB	8.5	13	80 ± 18	0.19	0.63
α-HCH	6.7	4.1	97 ± 16	0.03	0.08
HCB	6.7	8.7	83 ±17	0.08	0.29
β-HCH	6.9	3.5	115 ± 11	1.40	4.70
γ-HCH (lindane)	6.9	3.5	110 ± 14	0.12	0.40
δ-HCH	6.5	3.7	122 ± 9	0.13	0.45
PCB 28	7.2	4.1	77 ± 0.2	0.13	0.43
Heptachlor	2.7	3.6	78 ±19	0.11	0.36
PCB 52	1.6	1.7	82 ± 2	0.27	0.89
Aldrin	2.8	3.7	88 ± 6	0.95	3.20
Isodrin	3.8	11	97 ± 4	0.55	1.80
Heptachlor epox	4.6	16	105 ± 1	2.50	8.20
Oxychlordane	4.0	18	97 ± 16	0.30	0.99
Cischlordane	2.5	13	85 ± 9	0.17	0.56
2,4´DDE	8.9	7.9	82 ± 1.5	0.37	1.20
PCB 101	6.8	3.9	79 ± 1	0.02	0.05
α-endosulfan	6.0	15	120 ± 18	2.70	9.10
Transchlordane	7.9	16	107 ± 17	0.43	1.40
Dieldrin	5.7	8.9	88 ± 0.5	2.60	8.70
4,4´DDE	5.5	6.3	85 ± 10	0.36	1.20
2,4´DDD	6.7	5.7	97 ± 11	0.14	0.46
Endrin	11	10	100 ± 10	2.90	9.90
β-endosulfan	9.4	16	102 ± 13	1.70	5.70
2,4´DDT	7.2	8.3	112 ± 5	0.11	0.36
4,4´DDD	7.2	8.3	89 ± 6	0.11	0.36
PCB 153	11	13	71 ± 1	0.07	0.22
Endosulfan sulphate	12	26	100 ± 1	2.90	9.70
PCB 138	9.2	14	109 ± 0.1	0.38	1.30
4,4´DDT	6.7	24	98 ± 13	0.20	0.68
Dicofol	8.9	49	125 ± 7	1.00	3.40
Metoxychlor	5.7	28	96 ± 26	0.14	0.45
PCB 180	12	25	112 ± 0.3	0.04	0.14
Mirex	10	22	92 ± 2	0.21	0.69

Table 4.A7. Concentration of OPEs in each sample (ng/g dw). Nd: not detected

Sample	TEP	TnPP	TnBP	TCEP	TCPP	TPhP	EHDPP	TEHP	TCP1	TCP2	TCP3	TCP4
HS-S1	nd	nd	nd	36.1	3600	25400	125	19.8	5130	2540	1635	994
HS-S2	6.4	nd	nd	nd	84.0	232	nd	nd	44.1	26.5	29.8	nd
HS-S3	nd	nd	nd	60.9	nd	139	nd	nd	40.5	nd	87.6	nd
PDC-S1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-S2	nd	nd	nd	nd	488	917	5.1	nd	120	56.7	43.2	37.1
PDC-S3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-S4	nd	7.3	nd	44.1	nd	nd	15.2	nd	nd	16.5	24.8	23.9
PDC-sed1	8.4	nd	nd	nd	1320	787	5.1	nd	86.9	55.8	33.4	29.4
PDC-sed2	19.8	60.7	nd	nd	nd	77.8	108	43.6	68.3	78.8	76.2	98.7
PDC-sed3	nd	20.4	nd	34.3	nd	59.0	39.1	nd	56.3	43.8	42.0	42.6
PDC-sed4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-sed5	nd	nd	nd	nd	nd	41.3	5.7	nd	nd	nd	nd	nd
GQM-S1	nd	nd	157	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQM-S2	nd	nd	130	161	176	nd	5.2	nd	4.0	2.1	2.0	nd
GQM-S3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQM-sed1	6.9	9.8	nd	nd	nd	nd	7.9	nd	7.7	4.9	4.7	5.7
GQM-sed2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQM-sed3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.7	nd
GQM-sed4	nd	nd	nd	nd	16	nd	nd	nd	11.9	nd	15.6	nd
GQM-sed5	nd	nd	163	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQR-sed1A	nd	nd	252	116	231	242	nd	nd	nd	nd	248	nd
GQR-sed1B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.1	nd
GQR-sed2	nd	19.2	210	34.6	nd	nd	35.9	39.5	nd	26.2	31.1	21.9
DXS-S1	nd	nd	nd	nd	nd	268	nd	nd	nd	16.0	nd	nd
DXS-S2	nd	nd	nd	nd	nd	nd	nd	nd	2.5	1.0	nd	nd
DXS-S3	7.0	nd	nd	nd	nd	nd	nd	nd	0.6	nd	nd	nd
DXS-sed1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DXS-sed2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DXS-sed3	nd	nd	nd	137	98.2	nd	nd	nd	nd	nd	nd	nd

Table 4.A8. Concentration of PBDEs in each sample (ng/g dw). Congeners 28, 66, 85, 153 and 138 were not detected in any sample. Nd: not detected

Sample	BDE 47	BDE 100	BDE 99	BDE 154	BDE 183	BDE 209
HS-S1	nd	nd	26.5	nd	nd	370
HS-S2	1.9	nd	1.9	nd	1.2	89.5
HS-S3	nd	nd	20.7	nd	nd	336
PDC-S1	nd	nd	2.0	nd	nd	8.1
PDC-S2	nd	2.9	13.7	nd	nd	nd
PDC-S3	nd	nd	nd	nd	nd	nd
PDC-S4	34.4	31.6	nd	1.8	nd	8.7
PDC-sed1	nd	nd	nd	nd	nd	12.2
PDC-sed2	nd	nd	nd	nd	nd	nd
PDC-sed3	nd	nd	nd	4.3	nd	6.8
PDC-sed4	nd	nd	nd	nd	nd	1.7
PDC-sed5	12.5	nd	45.6	14.7	0.9	15.0
GQM-S1	nd	12.0	nd	nd	nd	35.9
GQM-S2	1.4	nd	2.2	nd	1.1	43.5
GQM-S3	nd	nd	1.4	nd	0.9	37.9
GQM-sed1	2.3	4.7	nd	nd	nd	68.2
GQM-sed2	0.6	1.0	nd	nd	nd	103
GQM-sed3	1.0	2.3	nd	nd	nd	9.6
GQM-sed4	2.3	nd	2.1	nd	1.2	75.1
GQM-sed5	nd	nd	nd	nd	nd	85.1
GQR-sed1A	1.0	nd	nd	1.1	nd	30.7
GQR-sed1B	nd	nd	0.9	nd	nd	10.7
GQR-sed2	nd	nd	0.5	nd	nd	11.5
DXS-S1	2.8	nd	nd	2.2	2.5	65.7
DXS-S2	nd	nd	nd	nd	nd	16.9
DXS-S3	nd	nd	nd	nd	nd	21.8
DXS-sed1	nd	nd	nd	nd	nd	58.2
DXS-sed2	nd	nd	5.1	nd	nd	45.2
DXS-sed3	nd	nd	44.6	nd	47.0	240

Table 4.A9. Concentration of OCPs in each sample (ng/g dw). δ- and γ- HCH, heptachlor, aldrin, isodrin, heptachlor epoxides, oxychlordane, 2,4´-DDE, dieldrin, endrin, β-endosulfan, endosulfan sulfate, dicofol, methoxychlor and mirex were not detected in any sample. Nd: not detected

Sample	HCBu	PCBe	α-HCH	HCB	β-HCH	Cis-chlordane	α-endosulfan	Trans-chlordane	4,4´-DDE	2,4´-DDD	2,4´-DDT	4,4´-DDD	4,4´-DDT
HS-S1	2.4	nd	0.6	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd
HS-S2	0.4	nd	0.2	nd	nd	nd	nd	nd	1.3	nd	nd	nd	nd
HS-S3	0.3	nd	0.7	0.1	0.2	10.4	nd	nd	0.5	0.6	nd	nd	nd
PDC-S1	nd	nd	1.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-S2	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.0
PDC-S3	nd	nd	0.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-S4	nd	nd	0.6	1.1	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-sed1	0.6	nd	0.5	nd	nd	nd	nd	nd	1.0	nd	nd	nd	1.0
PDC-sed2	nd	nd	0.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-sed3	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-sed4	nd	nd	nd	0.8	nd	nd	nd	nd	nd	nd	nd	nd	nd
PDC-sed5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQM-S1	0.1	nd	0.3	0.1	nd	nd	nd	1.1	0.5	0.3	0.4	0.4	14.8
GQM-S2	nd	nd	0.6	0.2	nd	nd	nd	nd	nd	nd	nd	nd	0.7
GQM-S3	nd	nd	0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQM-sed1	nd	0.8	0.4	nd	0.2	nd	nd	nd	nd	0.2	1.4	1.4	5.0
GQM-sed2	nd	0.7	0.1	nd	nd	nd	nd	nd	nd	nd	1.0	1.0	2.6
GQM-sed3	0.2	nd	0.8	0.5	0.2	3.0	nd	nd	nd	nd	1.2	1.4	5.6
GQM-sed4	nd	nd	0.6	nd	0.2	6.8	nd	1.1	nd	nd	0.7	0.6	1.1
GQM-sed5	nd	nd	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQR-sed1A	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GQR-sed1B	0.4	nd	0.2	0.1	nd	nd	nd	nd	nd	0.2	nd	nd	nd
GQR-sed2	nd	nd	0.5	0.3	nd	nd	nd	nd	nd	0.3	nd	nd	nd
DXS-S1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DXS-S2	0.4	1.3	0.2	nd	nd	nd	2.8	nd	nd	nd	0.2	nd	nd
DXS-S3	0.5	nd	0.6	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd
DXS-sed1	nd	nd	0.6	nd	nd	nd	nd	nd	nd	nd	0.2	nd	nd
DXS-sed2	0.9	nd	0.5	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd
DXS-sed3	0.2	nd	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 4.A10. Concentration of PCBs in each sample (ng/g dw). PCB 180 was not detected in any sample.

Soil Sample	PCB 28	PCB 52	PCB 101	PCB 153	PCB 138
HS-S1	0.9	nd	0.6	nd	nd
HS-S2	nd	nd	nd	nd	nd
HS-S3	nd	nd	nd	0.2	nd
PDC-S1	nd	nd	nd	nd	nd
PDC-S2	nd	nd	nd	nd	nd
PDC-S3	0.4	nd	nd	nd	nd
PDC-S4	nd	nd	nd	nd	nd
PDC-sed1	nd	nd	nd	0.2	nd
PDC-sed2	nd	nd	nd	0.7	nd
PDC-sed3	nd	nd	nd	nd	nd
PDC-sed4	nd	nd	nd	nd	nd
PDC-sed5	nd	nd	nd	nd	nd
GQM-S1	0.4	0.1	nd	0.1	0.1
GQM-S2	0.2	nd	nd	nd	nd
GQM-S3	nd	nd	nd	nd	nd
GQM-sed1	0.9	0.2	0.2	nd	0.1
GQM-sed2	0.7	nd	nd	0.1	nd
GQM-sed3	nd	nd	0.2	nd	nd
GQM-sed4	nd	nd	nd	nd	nd
GQM-sed5	0.3	nd	nd	nd	nd
GQR-sed1A	nd	nd	nd	nd	nd
GQR-sed1B	0.4	nd	nd	nd	nd
GQR-sed2	0.5	0.1	nd	0.2	0.2

5. Presence of novel and legacy flame retardants and other pollutants in an e-waste site in China and associated risks

5.1. Introduction

Recycling activities are considered as an important way to minimize waste and residue generation from all kind of manufacturing products in the way towards circular economy and, ideally, aiming at zero waste so that all recycled matter can be used as a new raw material (Nandy et al., 2022). Nevertheless, residues are actually produced during the recycling processes and they need to be considered as a pollution source and therefore managed and treated in a suitable way. Recycling activities are especially important for Electrical and Electronic Equipment (EEE).

EEE is defined as devices with either a battery or a power cord and can be classified in three major groups including (i) large household appliances (e.g. microwaves ovens, freezers or washing machines), (ii) information and telecommunication technology like computers and laptops and (iii) consumer equipment such as cell phones, TVs or video players among others (Perkins et al., 2014). After their operational lifetime they become electrical and electronic waste (e-waste). Recycling of e-waste has economical potential due to the high market value of metals and rare earths contained in them. However, low market value materials are also generated, mostly plastics, which are usually dumped in little land fills or even directly to the environment, especially when processed in non-formal facilities, with the subsequent pollution input to surrounding ecosystems (Li et al., 2020).

During last years, worldwide production of e-waste has increased at an annual rate of 3- 4%, with estimated 52.2 million tons produced in 2021 (Shaikh et al., 2020). Many e-waste recycling facilities have been set in third world countries from Asia, Africa and Latin America, who imports EEEs from more developed countries from Europe or North America (Sthiannopkao and Wong, 2013, Li et al., 2019a). On average, each American generates 29.5 kg of e-waste per year while less than 5 kg per person and year is generated in China (Perkins et al., 2014). Globally, the amount of e-waste produced is enormous but yet recycling is often performed in non-formal facilities which use basic handling and product recovery techniques, producing a high environmental impact (Awasthi et al., 2016) with serious human health implications (Cai et al., 2020). This is basically caused by the release of chemical pollutants linked to the plastics and electronic components, such as chlorinated biphenyls (PCBs) or polybrominated diphenyl ethers (PBDEs), recently replaced by organophosphate esters (OPEs) both used as plasticizers and flame retardants, or polybrominated biphenyls (PBBs), short and medium chain chlorinated paraffins (S/MCCP), polychlorinated dibenzodioxins and dibenzofurans (PCDD/F) or polycyclic aromatic hydrocarbons (PAHs) released from combustion processes, and lower market value metals such as Pb, Cr, Cd, Hg, Zn, Ni or Li, among others (Shen et al., 2017).

This study is focused in Baihe Tang, an e-waste recycling village, located near Longtang town, Qingyuan County (Guangdong province, south China). Qingyuan is the third biggest e-waste recycling county in China after Guiyu and Taizhou, with more than 1300 informal and familiar small workshops in where around 1 million tons of e-waste have been recycled since the early 1990s (Han et al., 2019). Non-formal practices like open burning, acid washing, or manual mechanical dismantling of EEE have been undertaken during years and non-valuable residues, mostly plastics, have been dumped in a non-controlled way in the area (Zeng et al., 2016a). Particularly, a small pond of approximately 5000 m^2 and 2 m depth has received the biggest part of long-lasting dumped residues from several workshops bordering the pond (Liu et al., 2019a). Material accumulated over the years has caused a big sedimentary deposition made of plastic debris and organic matter, which has emerged as a sedimentary delta in the opposite side of the pond from where they were disposed. More recently, new laws and enforcements about e-waste recycling and disposal have been developed and applied by central and local governments in China (Lu et al., 2014). As a result, several workshops surroundings and inside the village have been abandoned and non-treated e-waste is scattered, such as CD lectors, phone batteries (100-240 V, which is a working voltage not used in China but in Europe or USA), capacitors, coils, halogen and LED lamps and bulbs, circuit boards, chips or phone chargers among others. Since it is already a highly polluted area, several studies have been carried out to investigate the accumulation of organohalogen pollutants in aquatic, amphibious and terrestrial organisms (Liu et al., 2018a), their biomagnification along the aquatic food webs (Wu et al., 2009), and human exposure (Zheng et al., 2015). However, little is known on the risk of POPs and OPEs considering the water-sediment-soil system and impacts both for the environment and humans considering the toxicityof each contaminant and the ingestion of hen eggs.

Given the large amount of plastic and electronic debris abandoned in the e-waste site in Baihe village, this study aims to evaluate the distribution and impact of 14 OPEs, 11 PBDEs, 6 marker PCBs and 29 organochlorine pesticides (OCPs) in soils, sediments and free-ranged hen eggs. Additionally, we evaluated the presence of the more soluble compounds, namely OPEs, in the artificial ponds, streams and water channels potentially affected by the e-waste pollution. We provide information on the environmental risks caused by these compounds and human exposure through the consumption of hen eggs.

5.2. Materials and methods

5.2.1. Sample collection

All samples were collected around the Baihe Tang village (50 m asl) which covers an area of approximately 73.000 m^2. The main economic activity is the dismantling of EEE. However, other economic activities including duck and goose farming, tree growing for wood or small agricultural crops mostly dedicated to self-consumption or rural markets selling are also present in the area. Figure 5.1 indicates the study area which comprises 4 artificial ponds surrounded by agricultural areas, and the samples collected in two sampling campaigns during 2018-19.

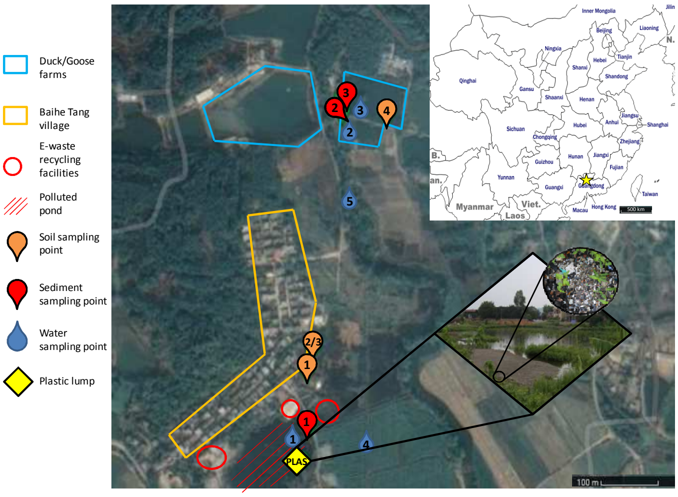


Figure 5.1: Sampling points in Baihe Tang village (Qingyuan County, Guangdong provice) and detail of the plastic lump.

Pond 1 was directly affected by e-waste debris. One sample consisting in a mixture of plastic waste and sediment was collected directly at the “polluted pond delta” (PLAS sample), a sediment sample (SED 1) and a water sample (WAT 1). In Pond 2, located at 578 m from pond 1, sediment (SED 2) and water (WAT 2) were collected. In pond, 3 at 613 m from pond 1, a sediment was collected (SED 3) but at the time of sampling the pond was dry and no water was available. In pond 4, close to pond 3, only water (WAT 3) was collected as there was a fence and sediment could not be reached. Four soil samples were taken around the ponds. SOIL 1 and 2 were natural soils, and SOIL 3 was a dredge, and were collected 174 m and 194 m away from the polluted pond and SOIL 4 was collected from a small corn crop 613 m away of the polluted pond. Additional water samples were taken from an irrigation canal (WAT 4) and a nearby river (WAT 5). Table 5.A1, in annex, shows GPS coordinates of collected samples and water content of soil and sediment samples. For sediment, 4 subsamples were collected with a stainless steel shovel and placed in a glass pot to make a pooled sample. The supernatant water was discarded *in situ*. For soils, 4 subsamples were collected from an approximately 1 m^2 with the hand shovel after removing the first layer (around 2 cm), pooled and placed in a glass pot. In between samples, the shovel was cleaned 3 times with water and ethanol. Soils and sediments were frozen at -70^0C and freeze-dried before analysis. Waters were grab sampled using a glass bottles tied to a long rope and thrown as close as possible to the center of the water body and water was dosed in amber glass bottles. Samples were covered with aluminum foil, capped and kept refrigerated in a portable fridge and transported to the laboratory. Waters were kept at 2^0C until analysis which was performed in less than 15 days to avoid degradation of contaminants.

Also, hen eggs were provided by local peasants who grow free range hens. In 2018, three families provided 3 eggs each from their own hens (samples HEN 1-3, 3 eggs/pool) and in 2019, 3 other families provided 5 eggs each (samples HEN 4-6, 5 eggs/pool). Eggs were wrapped with a bubble plastic to prevent ruptures and transported to the laboratory in a portable fridge. Once in the laboratory, biometric assays were done before opening the eggs (Table 5.A2). Eggs were crack-opened and homogenized in a total of 6 pooled samples, frozen at -70 ^{0}C and freeze-dried.

5.2.2. Chemicals and reagents

Standard solutions of triethyl phosphate (TEP), tri-iso-propyl phosphate (TiPP), tri-n-propylphosphate (TnPP), tri-n-butyl phosphate (TNBP), tri(2-chloroethyl) phosphate (TCEP), tri(2-chloro-isopropyl) phosphate (TCPP), tri(2-chloro,1-chloromethy-ethyl) phosphate (TDCPP), tri(2-ethylhexyl) phosphate (TEHP), 2-ethylhexyl diphenyl phosphate (EHDPP), tri-phenyl phosphate (TPhP), and tri-cresyl phosphates (TCP) were purchased from AccuStandard (New Haven, CT, USA).

PBDEs solutions containing congeners 28, 47, 66, 85, 99, 100, 138, 153 and 154 at 10 µg/mL in isooctane, BDE 183 at 50 µg/mL in isooctane and BDE 209 at 50 µg/mL in isooctane:toluene (1:1) were also provided by AccuStandard.

For OCP and PCB analysis, Pesticide Mix 1 was purchased from AccuStandard. It contains the following compounds: aldrin, dieldrin, endrin, isodrin, α-, β-, γ- and δ-HCHs, cis- and trans- chlordane, 2,4′-DDD, 2,4′-DDE, 2,4′-DDT, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, α- and β-endosulfan, heptachlor, heptachlor exo- and endo-epoxides, hexachlorobenzene (HCB), methoxychlor, mirex, and oxychlordane, at a concentration of 10 µg/mL and the PCB congeners 28, 52, 101, 138, 153 and 180, at 1 µg/mL in toluene. Dicofol at a concentration of 100 µg/mL in methanol, endosulfan sulphate (100 µg/mL in methanol), hexachlorobutadiene (HCBu) (2 mg/mL in methanol) and pentachlorobenzene (solid, 98% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Three types of SPE cartridges were used for sample treatment: ENVI Florisil (500 mg, 3 mL, Supelco Bellefonte, PA, USA) for soils and sediments; Oasis HLB (200 mg, 6 cc, Waters, Milford, Massachusetts, USA) for OPEs extraction from water and eggs samples, and Hyper Sep Florisil cartridges (10 g, 750 mL, Thermo Scientific, Rockwood, TN, USA) for POPs in egg samples. Gas chromatography grade methanol, dichloromethane, acetonitrile and hexane were provided by Oceanpk (Sweden) and ethyl acetate by Honeywell (USA).

5.2.3. Sample extraction

5.2.3.1. Soils and sediments

Samples were fine grounded with a mortar and pestle and sieved to obtain the 100 µm fraction. One gram of sample was spiked with 100 ng of each surrogate and then Soxhlet extracted (48 h, 60 ^{0}C) with 200 mL of n-hexane:acetone (1:1). Copper pieces were added to the flask in order to remove elemental sulfur. Solvent was collected and evaporated in a rotary evaporator R-215 working at 450 bar (Buchi, Switzerland) at room temperature until 1 mL approximately before the clean-up step. ENVI florisil SPE cartridges were conditioned with 8 mL of ethyl acetate and 6 mL of n-hexane, then samples were loaded and eluted by gravity with 5 mL of n-hexane followed by 5 mL of a n-hexane:dichloromethane (DCM) (1:1) to obtain a first fraction containing non-polar compounds like PBDEs, PCBs and OCPs. A second fraction was obtained by elution with 8 mL of ethyl acetate and used for OPEs determination. Both fractions were solvent exchanged to 1 mL of n-hexane and stored at -20ºC until analysis. Pollutant concentrations in sediment and soil are expressed in µg/g dry weight (dw).

Total Organic Carbon (TOC) was analyzed in an ELTRA CS-800 double dual range C/S detector (Haan, Germany) working at 2,000^{0}C and 100 nm wavelength. Around 100 mg of sample were accurately weighed in porous ceramic capsules, then treated with HCl (aq) (35%) for 15 h at room temperature and heated at 80^{o}C for 1 h to dissolve carbonates, washed with double deionized H_2O until neutral pH and dried at 80^{0}C. Around 0.7 g of Fe (s) and 1.5 g of W (s) were added to each sample and each calibration standard. Calibration was performed in two levels with the certificate reference material for TOC determination medium organic content soil standard AEB2178 (alpha resources LLC, Stevensville, MI Michigan, USA), with 3.07 % C, and the steel chips calibration standard 92400-4020 C215 (Eltra, Haan, Germany) with a content of 0.462% of C for low content samples.

5.2.3.2. Waters

OPEs were the only compounds analyzed in water as the rest of compounds are not expected to be detected in this compartment due to their high hydrophobicity and adsorption to the organic matter of soils and sediments or accumulation in biota

(Zhang et al., 2011). Water was filtered through cellulose paper (pore size 10 µm) to remove particulate matter and suspended solids like leaves or roots. 500 mL of water was spiked with 100 ng of OPEs surrogate standards. Extraction was performed with Oasis HLB cartridges (200 mg, 6 cc) which were conditioned with 4 mL ethyl acetate, 4 mL of methanol and 4 mL water. Cartridges were then dried and eluted with 8 mL of ethyl acetate. Approximately 1 g of Na_2SO_4 was then added to the extract to eliminate remaining water and then the extract was filtered through 0.45 µm PTFE filters. Solvent was evaporated under a gentle N_2 stream to approx. 1 mL, transferred to a chromatographic vial, evaporated to dryness and reconstituted with 500 µL of n-hexane. Samples were then stored in the freezer (-20^{0}C) until instrumental analysis. Pollutant concentrations in are expressed as µg/L.

5.2.3.3. Eggs

Due to the high lipid content of eggs, the sample extraction for PBDEs, PCBs and OCPs detection was not compatible with the analysis of OPEs, and thus, two analytical protocols were undertaken. For OPEs, a previously optimized method for fish muscle based on freeze lipid precipitation as a key step for the clean-up process was used (Liu et al., 2018b). One gram of homogenized and lyophilized sample was spiked with 100 ng surrogate standards and Soxhlet extracted with 200 mL of DCM at 50^{0}C during 24 h. The solvent was then evaporated to approximately 5 mL in a Buchi R-215 rotavapor, dosed in a test tube and filled with fresh solvent until 10 mL. One mL of the extract was used for lipid determination, which was performed by gravimetrical measurements after evaporating organic solvent at 60^{0}C for 24 h. The remaining extract was evaporated to dryness under N_2 stream, reconstituted with 8 mL of MeOH and frozen at -20^{0}C overnight. Then, the MeOH supernatant was decanted to separate it from the frozen lipids. The freeze lipid precipitation process was repeated with another portion of 8 mL MeOH. Both MeOH extracts were transferred to a 500 mL flask previously filled with 300 mL water. For the clean-up process, Oasis HLB cartridges (200 mg, 6 cc) were conditioned with 4 mL ethyl acetate followed by 4 mL MeOH and 4 mL of H_2O. Sample was preconcentrated at a flow of 15 mL/min, dried for 30 min under N_2 stream and eluted with 8 mL ethyl acetate. If some lipids remained (turbid liquid), Z-sep C18 sorbent (Supel QuE Z-sep, Supelco) was used to remove them before filtering through 0.45 µm PTFE syringe filters. If some water was also present, same procedure was done with Na_2SO_4

instead of Z-sep C18 sorbent. The extract was evaporated until dryness and reconstituted in 500 µL of n-hexane.

PBDEs, PCBs and OCPs were extracted based on previously published studies (Zapata et al., 2018). Briefly, one gram of homogenized and lyophilized sample was vortexed with 25 mL n-hexane:DCM (1:1) and ultrasound extracted for 10 min and this procedure was repeated 3 times. The extract was further purified with florisil SPE cartridges (10 g, 6 mL) (Supelco, Bellefonte, USA) eluted by gravity with 35 mL of n-hexane:DCM (1:1), evaporated until near dryness and reconstituted in 500 µL n-hexane. Pollutant concentrations in hen eggs are expressed as µg/g wet weight (ww). Mean water and lipid contents were 72.7 ± 1% and 3.3 ± 0.6%, respectively (Table 5.A2).

5.2.4. Instrumental analysis

OPEs, PCBs and OCPs were analyzed in an Agilent 7890A GC system coupled with an Agilent 7000A GC/MS Triple Quad detector. An Agilent J&W DB-5MS column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) was used. Two instrumental methods, one for the analysis of OPEs adapted from Poma et al. (2018) and one for the analysis of PCBs and OCPs (Zapata et al., 2018) were used. For OPEs, 1 µL was injected in pulsed splitless mode at 250 ^{o}C The temperature gradient at the oven started at 90^{o}C (1 min hold time), increased to 220^{o}C at 10^{o}C/min, to 240^{o}C at 20^{o}C/min, to 280^{o}C at 5^{o}C/min (5 min), and finally to 325^{o}C at 30^{o}C/min (5 min hold time), with a total runtime of 35 min. Ion source temperature was 280ºC. For OCPs and PCBs analysis, 1 µL was injected in pulsed splitless mode at 250ºC. The temperature gradient was set at 70^{o}C (1 min hold time), increased to 175^{o}C at 6^{o}C/min (4 min hold time), to 235^{o}C at 3^{o}C/min, to 315^{o}C at 7^{o}C/min (10 min hold time), with a total run time of 64 min.. The ion source temperature was set at 225^{o}C. Monitorred reactions and analytical parameters can be found in the annex, table 5.A3 for OPEs and table 5.A4 for OCPs and PCBs

PBDEs were analyzed in an Agilent 7890A GC system coupled to an Agilent 5975C mass spectrometer working in negative chemical ionization. A short DB-5MS column (15 m length, 0.25 mm internal diameter, 0.25 µm film thickness, Agilent J&W) was used to enable BDE 209 determination. The oven temperature was set at

100^{o}C, increased to 250^{o}C at 10^{o}C/min, to 310^{o}C at 15^{o}C/min (15 min hold time) and the total run time was of 36 min. 1 µL of sample was injected in pulsed splitless mode with the injector temperature set at 300ºC. The MS ion source worked at 250ºC. Details about monitored ions for PBDEs are also indicated in Table 5.A4.

5.2.5. Quality assurance and quality control

Samples were processed in 12 position batches. Each batch included one matrix blank (precleaned soil, commercial hen egg or double deionized water), one procedural blank (no matrix), one spiked Quality Control (QC) and 9 samples. For QC, a blank soil sample was spiked with native compounds at a concentration of 100 ng/g (n=5), commercial hen eggs were spiked at 100 ng/g (n=5) and distilled water was spiked 0.2 µg/L (n=5).

All glass material was baked at 450^{0}C to avoid external contamination. However, OPEs were detected in blank samples due to their presence in filters, plastics or laboratory equipment used for their analysis. Mean contribution for each compound detected in blanks, namely TEP, TiPP, TnBP, TCEP, TCPP, TPhP, EHDPP and TEHP, was subtracted from samples results. Recovery and blank contribution for each sample treatment are compiled in the annex (Table 5.A3).

5.2.6. Solid-liquid partitioning ratios

In order to assess the migration of OPEs from the plastic lump and accumulation to sediment, a partitioning ratio (K) was calculated between PLAS and WAT1 and between SED1 and WAT1, following equations 1 and 2:

$$K_{PLAS/WAT1} = \frac{C_{i,PLAS}\ (\frac{\mu g}{kg})}{C_{i,WAT1}\ (\frac{\mu g}{L})} \qquad (1)$$

$$K_{SED1/WAT1} = \frac{C_{i,SED1}\ (\frac{\mu g}{kg})}{C_{i,WAT1}\ (\frac{\mu g}{L})} \qquad (2)$$

Where $\mathrm{C_{i,PLAS}}$ is the measured concentration of pollutant i in sample PLAS, $\mathrm{C_{i,WAT1}}$is the measured concentration of pollutant i in sample WAT1 from and $C_{i,SED1}$ is the measured concentration of pollutant i in sample SED1.

5.2.7. Risk assessment and estimated daily intake (EDI)

To evaluate the impact of pollutants in soil/sediments and water, the risk quotients (RQ) were calculated. RQ are the ratio between the measured environmental concentration (MEC) and a toxicity reference value. We used the lowest predicted no effect concentration (PNEC) from the NORMAN ecotoxicology database (2021) for each pollutant to represent the worst-case scenario (Table 5.A5). RQ values lower than 1 represents no environmental risk, while RQs over 1 represent that environmental effects are likely to occur; the higher the RQs, the higher the risk. In order to assess the risk associated to chemical mixtures, the concentration addition (CA) model was used to calculate a total RQ for each family of pollutants, assuming the same toxicological action mechanisms for substances of the same family (Backhaus and Faust, 2012).

To estimate the exposure of humans from the ingestion of polluted eggs, estimated daily intakes (EDIs) were calculated using equation 3:

$$EDI = \frac{C_i \times CR}{BW} \tag{3}$$

where C_i is the amount of each pollutant in the eggs expressed in μg/g ww, CR (consumption rate) is the amount of egg consumed daily per person, obtained from a consumption survey in Guangdong province (14 g per person and day in rural areas) (Ma et al., 2005) and BW is the average body weight (63 kg for adults (Zhou et al., 2012) and 14.65 kg for children (Huang et al., 2018). EDIs have been calculated as mean values of the 6 pooled egg samples. Calculated EDI values were compared with reference doses (RfD) or acceptable daily intakes (ADIs), depending on the available data, to obtain the margin of exposure (MOE) as shown in equations 4 and 5:

$$MOE = \frac{RfD}{EDI} \tag{4}$$

$$MOE = \frac{ADI}{EDI} \tag{5}$$

MOE provide information about the risks associated with pollution exposure due to ingestion. MOE values higher than 2.5 represent no health concern; the lower MOE value, the higher risk for human health.

5.3. Results and discussion

5.3.1. POPs and OPEs in sediments from the Baihe e-waste site

Table 5.1 shows the concentration of each studied OPE, PBDE and PCB in the plastic lump, and sediments. The plastic lump (PLAS) had the highest levels among samples, with a ∑pollutants of 8,400 µg/g dw, where OPEs accounted for 95% of ∑pollutants, followed by PBDEs (5%), and PCBs and OCPs accounted for less than 0.1%. ∑OPEs were detected at 8,000 µg/g dw, with a profile dominated by TPhP and TCPP with concentrations of 4,100 µg/g dw and 3,400 µg/g dw, respectively, and represented 51% and 43% of ∑OPEs, followed by TCP isomers (420 µg/g dw, 5% of ∑OPEs). TPhP is a plastic additive but also used as flame retardant for the manufacture of electrical, electronic and optical equipment, machineries and vehicles. TCPP is mainly used as plastic additive for rubber products and polymers, adhesives and sealants, coating products, laboratory chemicals and leather treatment products, and in formulation of mixtures and/or re-packaging (ECHA, 2022). TCPs are used as additive in lubricants, hydraulic fluids, or engine oils, and as plasticizers and flame retardant (de Ree et al., 2014). TCEP, EHDPP, TnBP and TDCP were detected at concentrations from 5.7 to 10.9 µg/g dw and are associated to their use as flame retardants in PVC, polyurethane foams and smartphones and other EEE. ∑PBDEs were detected at 423 µg/g dw, and BDE209 was the main congener detected (45% of ∑PBDEs), followed by BDE 47, 99 and 100. The high levels of OPEs compared to PBDEs may point to their more recent use related with newer EEE devices dumped and/or stored there. PLAS contained 6.37 µg/g dw of ∑PCBs, with a profile dominated by PCB 28 and 180. Finally, ∑OCPs was of 0.97 µg/g dw, with dieldrin at the highest concentration (0.92 µg/g dw), and mirex, transchlordane and DDTs detected at 0.005-0.008 µg/g dw.

Table 5.1. Concentration of pollutants (µg/g dw) in the plastic lump, sediment and sois samples from the e-waste site, total organic carbon (TOC) content in percentage and distance to the main source. Nd: Not detected

	PLAS	SED 1	SED 2	SED 3	SOIL 1	SOIL 2	SOIL 3	SOIL 4
Distance to source (m)	source	0	578	613	174	197	197	614
%TOC		8.7	1.9	1.1	6.9	0.2	5.8	0.9
TEP	0.89	0.14	nd	nd	nd	nd	0.08	0.08
TiPP	0.87	0.15	nd	0.01	nd	0.11	0.01	0.094
TnPP	0.06	0.10	0.003	nd	0.008	0.005	nd	0.01
TnBP	7.17	0.48	0.43	0.06	0.12	0.11	0.47	0.18
TCEP	10.9	2.03	0.17	0.18	1.31	0.06	1.4	0.34
TCPP	3450	10.1	0.24	0.45	1.18	0.08	6.24	22.7
TDCP	5.8	0.30	0.02	0.02	0.22	0.004	0.82	0.14
TPhP	4100	40.8	0.19	1.30	2.55	0.01	8.52	42.6
EHDPP	9.11	0.08	0.01	0.01	0.02	0.005	0.02	0.25
TEHP	1.15	0.27	0.008	0.008	nd	0.007	0.05	0.03
TCP1	184	33.2	0.05	0.85	0.41	nd	0.48	4.52
TCP2	108	14.2	0.03	0.36	0.27	0.015	0.30	2.58
TCP3	70.3	6.54	0.030	0.15	0.17	0.06	0.21	1.66
TCP4	56.7	3.23	0.024	0.065	nd	nd	0.15	1.3
∑OPEs	*8005*	*112*	*1*	*3*	*6*	*0*	*19*	*76*
BDE 28	17.6	0.60	0.003	nd	0.04	0.009	0.05	nd
BDE 47	63.7	2.36	0.03	nd	0.22	0.01	0.21	nd
BDE 66	25.3	0.78	0.005	nd	0.09	0.009	0.08	nd
BDE 100	37.7	1.82	0.005	nd	0.03	0.005	0.06	nd
BDE 99	52.5	2.44	0.03	nd	0.32	0.013	0.59	nd
BDE 85	4.86	0.12	nd	nd	0.011	0.008	0.02	nd
BDE154	3.50	0.13	0.005	nd	0.03	0.0008	0.06	nd
BDE153	15.6	0.37	0.013	nd	0.08	nd	0.12	nd
BDE 138	1.76	0.08	0.003	nd	0.01	nd	0.014	nd
BDE183	10.1	0.63	0.017	nd	0.10	nd	0.07	nd
BDE 209	190	29.8	18.0	0.04	2.72	0.03	3.10	0.16
∑PBDEs	*423*	*39.1*	*18.1*	*0.04*	*3.65*	*0.08*	*4.37*	*0.16*
PCB 28	3.1	3.34	0.33	0.10	2.26	0.018	5.82	0.025
PCB 52	0.97	1.26	0.15	0.008	0.75	0.003	0.78	nd
PCB 101	0.32	0.95	0.22	0.03	0.68	0.006	2.29	nd
PCB 153	0.37	5.63	0.12	0.02	0.46	nd	24.2	0.006
PCB 138	0.27	5.56	0.14	0.02	0.59	nd	22.4	0.007
PCB 180	1.33	3.37	0.08	nd	0.17	nd	19.8	0.005
∑PCBs	*6.36*	*20.1*	*1.04*	*0.18*	*4.91*	*0.03*	*75.3*	*0.04*
HCBu	nd	nd	nd	nd	nd	0.015	nd	nd
PeCB	nd	0.60	nd	0.05	0.031	nd	0.14	nd

Table 5.1. Continued

	PLAS	SED 1	SED 2	SED 3	SOIL 1	SOIL 2	SOIL 3	SOIL 4
α-HCH	0.0003	nd	0.0006	0.001	0.0006	0.0009	nd	0.0007
HCB	0.017	0.24	0.006	0.021	0.009	0.0005	0.12	0.0009
β-HCH	0.0003	nd	0.0006	0.001	0.0006	0.0009	0.0001	0.0007
δ-HCH	nd	nd	0.0003	nd	nd	nd	nd	0.0004
γ-HCH	nd	nd	nd	nd	nd	nd	nd	nd
α-endosulfan	nd	nd	nd	nd	nd	nd	0.007	nd
Transchlordane	0.008	nd	0.004	nd	nd	0.0005	nd	nd
4,4DDE	0.005	0.005	nd	nd	0.006	nd	nd	nd
β-endosulfan	nd	0.06	nd	nd	nd	nd	0.16	nd
4,4DDD	0.005	nd	0.0008	nd	0.002	nd	nd	nd
Chlordecone	nd	nd	nd	nd	nd	nd	nd	nd
4,4DDT	nd	nd	nd	nd	0.01	nd	nd	nd
Metoxychlor	nd	nd	nd	nd	nd	nd	nd	nd
Mirex	0.01	0.06	nd	nd	nd	nd	nd	nd
∑OCPs	*0.05*	*0.97*	*0.01*	*0.07*	*0.06*	*0.02*	*0.43*	*0.00*

SED 1 close to the plastic lump had ∑pollutants of 172 µg/g dw, 49 times lower than PLAS. OPEs (65% of ∑pollutants) were detected at the highest concentrations (0.07 – 40.8 µg/g dw) and were 72 times lower than PLAS. A decrease of 10 times was observed for PBDEs (23% of ∑pollutants, 0.08 – 29.8 µg/g dw). The higher decrease for OPEs was attributed to their higher degradability compared to PBDEs (Cristale et al., 2017). PCBs (12% of ∑pollutants, 0.95 – 3.36 µg/g dw) were higher in the SED 1 than in PLAS, and OCPs (1% of ∑pollutants, nd – 0.24 µg/g dw) were at similar concentrations. Although concentrations differed, a similar profile was observed among pollutants in PLAS and SED 1 (Table 5.1), dominated by TPhP, TCPs and TDCPP, BDEs 209 and 47 and all PCB congeners. Considering logarithmic transformed individual concentrations of OPEs and PBDEs, a good correlation was observed among PLAS and SED 1 ($R^2 = 0.76$, $p<0.001$) (Figure 5.2A), and this indicates that the plastic lump is most probably the source of pollution of the whole pond. On the contrary, PCBs and OCPs did not correlate among PLAS and SED 1, and this indicates that these legacy pesticides originated from other past activities. Specifically, SED 1 contained 0.60 µg/g dw of PeCB, which was not detected in PLAS, and 0.24 µg/g dw hexachlorobenzene (HCB). Both these compounds are classified as unintentionally produced by the Stockholm Convention

(2009) and used as additives in PCB mixtures, rubbers, electronic equipment or as flame retardants.

SED 2 and 3 were located in ponds 2 and 3, at 600 m approximately from the polluted pond and had ∑pollutants of 20.4 and 3.7 µg/g dw, respectively. SED 2 was dominated by PBDEs, which accounted for 89% of ∑pollutants with BDE 209 as the main congener detected, and SED 3 was dominated by OPEs, which accounted by for 92% of ∑pollutants with TPhP, TCP, TCPP and TCEP as the main OPEs. In these 2 sediments, PCBs ranged from 0.009 to 0.33 µg/g dw, dominated by PCB 28, and OCPs ranged from nd to 0.05 µg/g dw, with PeCB, HCB again as the main compounds detected.

5.3.2. Water-sediment partitioning of OPEs

The concentration of OPEs in water samples are compiled in Table 5.2. WAT 1, from the polluted pond, had higher concentration (∑OPEs 1.62 µg/L) than the rest of waters analyzed (∑OPEs 0.36-0.76 µg/L), due to the influence of the plastic lump. TCPP was the main compound detected in all water samples, followed by TEP, TiPP, TnBP, TCEP and TPhP and the rest of compounds (EHDPP, TEHP, TDCP and TCPs) were detected at much lower concentration. TnPP was never detected.

Table 5.2. Concentration of OPEs (µg/L) in water samples from the e-waste site and distance to the main source in m. Nd: not detected.

Compounds	WAT 1	WAT 2	WAT 3	WAT 4	WAT 5
Distance to the source (m)	0.0	569	613	165	430
TEP	0.22	0.10	0.06	0.04	0.08
TiPP	0.39	0.01	0.01	0.02	0.04
TnPP	nd	nd	nd	nd	nd
TnBP	0.03	0.09	0.04	0.04	0.02
TCEP	0.02	0.04	0.03	0.05	0.03
TCPP	0.34	0.48	0.15	0.15	0.11
TDCP	0.00	0.01	nd	0.02	0.00
TPhP	0.52	0.00	0.01	0.01	0.17
EHDPP	nd	nd	nd	nd	nd
TEHP	nd	0.02	0.01	0.02	0.02
TCP1	0.04	nd	0.02	0.01	nd
TCP2	0.02	nd	0.02	nd	nd
TCP3	0.01	nd	0.02	nd	nd
TCP4	0.01	0.02	0.02	nd	0.02
∑*OPEs*	1.62	0.76	0.39	0.36	0.50

OPEs contained in the plastic debris produced during the non-formal recycling processes leached to the surrounding environment, and a water-sediment partitioning was observed in Pond 1 according to the polarity and degradability of contaminants. TPhP was the main OPE in the plastic lump, and also in sediment and in water, and this indicates that leaching from the plastic lump produces water and sediment contamination. Other OPEs detected in WAT 1 were TiPP, TCPP and TEP (0.22 to 0.38 µg/L) and correspond to OPEs with higher solubility and lower Kow than the rest of compounds, indicating that they can be easily solubilized in water. Correlations between log K and log K_{ow} were found to be statistically significant in both PLAS/WAT1 (R^2 = 0.54, p = 0.004) and SED1/WAT1 (R^2= 0.61, p= 0.002, respectively) (Figures 5.2B and 5.2C), which indicate that migration capacity from the plastic lump to the water and from the water to the sediment depends on the polarity of the pollutants. However, waters collected from Pond 2 (WAT2) had a similar OPE profile than the sediment from this pond but no significant correlation was found between them, indicating a differential distribution and sources.

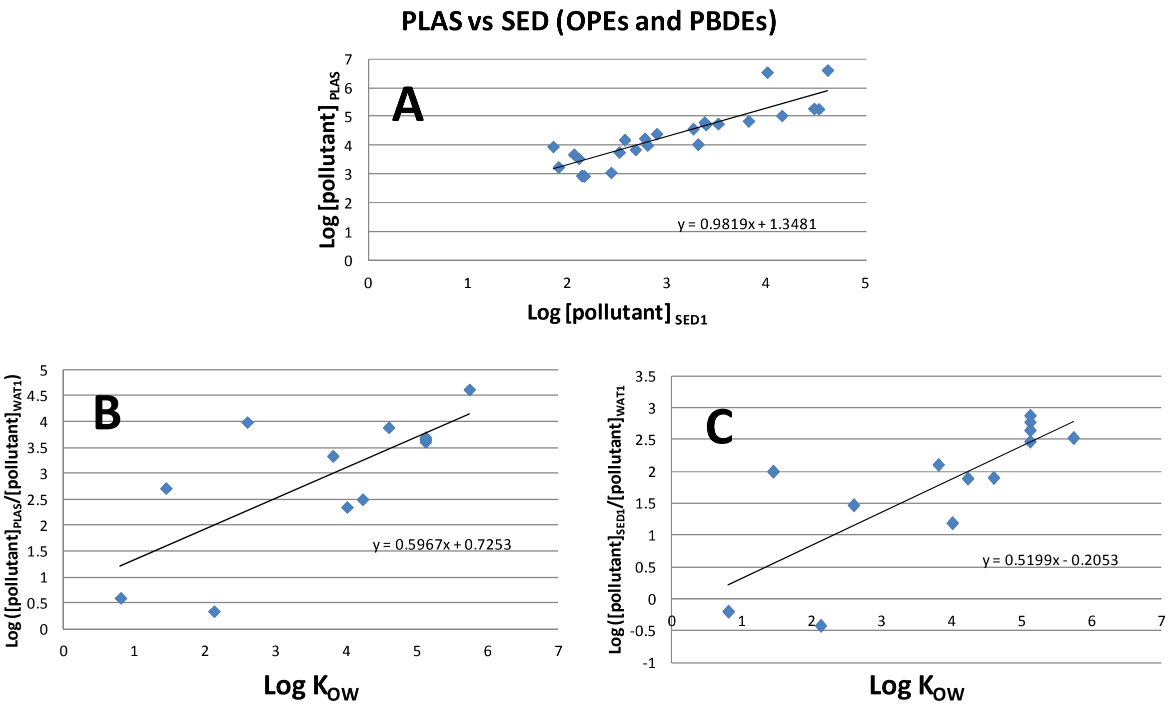


Figure 5.2. Correlations for individual compounds. Log KOW values were obtained from the Estimation Program Interface (EPI) suite (US EPA, 2012). A) Relantionship between individual OPEs and PBDEs logarithmic transformed concentrations in samples PLAS and SED1 (R2=0.76, p=2E-8). B) Correlation between the logarithmic transformed ratio between concentrations of individual OPEs in samples PLAS and WAT 1 and log KOW (R2= 0.54, p=0.004). C) Correlation between the logarithmic transformed ratio between concentrations of individual OPEs in samples SED 1 and WAT 1 and log KOW (R2= 0.61, p= 0.002).

5.3.3. Presence of POPs and OPEs in soils

∑pollutants in soils ranged from 0.61 to 98.8 µg/g dw, with SOIL 3 > SOIL 4 >> SOIL 1 >> SOIL 2 (Table 5.1). Although there was no correlation among pollutants with TOC, SOIL 2 presented a red/brown color characteristic from the iron oxides present in high clay content soils and showed a low carbon content (0.2% TOC) and SOIL 3, the dredge, with a brown-dark color, contained 5.8% of organic carbon. Pollution profiles in these 4 soil samples were very different. SOIL 1 contained OPEs > PCBs > PBDEs, SOIL 2 contained OPEs > PBDEs > PCBs, SOIL 3 was dominated by PCBs (76% of ∑pollutants) especially highly chlorinated ones and SOIL 4 was dominated by OPEs. In all cases OCs were detected at low concentrations.

∑OPEs ranged from 0.46 to 76.6 µg/g dw. Except for SOIL 2, OPEs were dominated by TPhP and TCPP, ranging from 2.55 to 42.6 µg/g dw (from 27 to 56% of ∑OPEs), followed by TCEP, and TDCP.

∑PBDEs in soil 1 and 3 were of 3.66 and 4.39 µg/g dw respectively, and were always dominated by BDE209, which accounted for 74 and 71% of ∑PBDEs, followed by BDE 99 and 47. Much lower concentrations were detected in SOIL 2 (low TOC), that contained all studied PBDE congeners, and SOIL 4 (agricultural soil) where BDE 209 was the only congener detected.

∑PCBs ranged from 0.03 µg/g dw in SOIL 2 to 75.4 µg/g dw in SOIL 3, which showed a profile dominated by PCBs 153, 138 and 180, and 28 in a minor level. The high levels in SOIL 3 may reflect historica PCB contamination. Previous studies in the area suggest that the high levels of PCBs in this site are associated to PCB containing devices dismantled in the past (Zheng et al., 2015). OCPs were sporadically detected in soils with ∑OCPs between 0.03 and 0.43 µg/g dw, with PeCB, HCB, and b-endosulfan the main compounds detected. Some OCPs like heptachlor and its epoxides, oxychlordane, cischlordane, aldrin, isodrin, dieldrin, endrin, 2,4´-DDE, 2,4´-DDD, chlordecone, endosulfan sulphate, dicofol, metoxychlor and mirex were never detected in soils.

5.3.4. Presence of POPS and OPEs in hen eggs

In e-waste sites, contamination of soils affect hens picking plastics and other residues while they get fed (Wang et al., 2014, Zeng et al., 2018) and may raise concerns on human health (Li et al., 2020). In this study we used free ranged hen eggs as an indicator of human exposure through ingestion. ∑pollutants ranged from 0.097 to 2.06 µg/g ww. PCBs were the most abundant compounds, with mean $\Sigma_{6marker}$PCB of 0.37 µg/g ww, followed by PBDEs (mean Σ_{11}PBDE of 0.17 µg/g ww), OPEs (mean ΣOPEs$_{14}$ of 0.06 µg/g ww) and OCPs (mean ΣOCPs of 0.02 µg/g ww) (Table 5.3, Table 5.A6 for individual concentrations). The PCB profile was dominated by PCB 138 and PCB 153, followed by PCB 28; BDE 183 and 209 were the main PBDEs detected. TnBP and TCPP were the most ubiquitous OPEs, and the rest were detected at the low µg/g ww level as these compounds can be metabolized (Wang et al., 2021). Despite OCPs were detected at low concentrations (table 5.3), some compounds such as chlordecone, mirex or DDTs were frequently detected mostly due to past agricultural activities and HCHs, HCBu, PeCB and HCB due to their use as flame retardants, additives and/or plasticizers associated to the EEE dismantling. The rest of the compounds (γ-HCH, heptachlor, aldrin, isodrin, dieldrin, endrin, heptachlor epoxides, oxychlordane, cis or trans isomers of chlordane, 2,4´-DDE, 2,4´-DDD, α- or β- isomers of endosulfan, metoxychlor, endosulfan sulfate, TnPP, TCEP and PBDE 138) were never detected in studied hen eggs.

Table 5.3. Mean levels of pollutants found in hen eggs in ng/g ww (n=6), associated estimated daily intakes (EDI) and reference doses (RfD) and acceptable daily intakes (ADI) used to calculate margin of exposure (MOE) due to eggs consumption for adults and children. Na: not available. Σ TCPs includes 4 TCP isomers. Σ PBDEs (without BDE 209) includes congeners 28, 47, 66, 85, 99, 100, 138, 153, 154 and 183. Σ6m-PCBs includes congeners 28, 52, 101, 138, 153 and 180. Σ HCHs includes α, β, γ and δ isomers. Σ DDTs includes 2,4´-DDT, 2,4´-DDE, 2,4´-DDD, 4,4´-DDT, 4,4´-DDE and 4,4´-DDD. Nd: not detected. Na: not available.

	Mean (µg/g ww) (n=6)	RfD (µg/(kg bw*day))	ADI (µg/(kg bw*day))	Refference	EDI adults (µg/(day*kg body weight))	MOEs adults	EDI children (µg/(day*kg body weight))	MOEs children
TEP	0.035	125	-	Li et al., 2019b	0.002	77000	7.0	18000
TiPP	0.002	na	-		0.0005	na	2.0	na
TnPP	nd	na	-		na	na	na	na
TnBP	0.031	24	-	Li et al., 2019b	0.007	3400	30.3	790
TCEP	nd	22	-	Li et al., 2019b	0.0	-	0.0	-
TCPP	0.016	80	-	Li et al., 2019b	0.004	22000	0.016	5000
TDCP	<0.001	15	-	Li et al., 2019b	0.00002	620000	0.0001	140000
TPhP	0.001	70	-	Li et al., 2019b	0.0002	430000	0.0007	99000
EHDPP	0.001	15	-	Li et al., 2019b	0.0002	79000	0.0008	18000
TEHP	0.005	35	-	Li et al., 2019b	0.001	28000	0.0055	6400
Σ TCPs	0.0019	20	-	Zhang et al., 2021	0.0004	47000	0.002	11000
Σ PBDEs (without BDE 209)	0.11	0.1	-	Huang et al., 2018	0.021	5	0.37	0.3
BDE 209	0.078	7	-	Huang et al., 2018	0.017	400	0.076	92
Σ6m-PCBs	0.377	0.1	-	Arnich et al., 2009	0.084	1	0.36	0.3
Hexachlorobutadiene	0.001	-	0.2	Zhang et al., 2019b	0.0002	930	0.0009	220
Pentachlorobenzene	0.002	-	na		0.0004	na	0.002	na
Hexachlorobenzene	0.001	-	0.6	Mahmoud et al., 2016	0.0003	2200	0.001	500
Chlordecone	<0.001	-	na		0.0001	na	0.0004	na
Mirex	0.006	-	na		0.001	na	0.006	na
Σ HCHs	0.001	-	5	Mahmoud et al., 2016	0.3	19000	1.1	4400
Σ DDTs	0.005	-	10	Arisekar et al., 2022 (SI)	0.001	9200	0.005	2100

Flame retardants or other pollutants in eggs produced near or in the surroundings of the non-formal e-waste recycling facilities or workshops in China have been previously reported. Hen eggs close from e-waste disassembling site and close to a municipal waste incinerator area from Zhejiang province had median $\Sigma_{6marker}$PCBs of 0.001 and 0.0002 µg/g ww), respectively (Shen et al., 2017). In an e-waste site in Taizhou, Zhejiang province, $\Sigma_{indicator}$PCBs in home produced hen eggs were up to 0.004 µg/g ww (Xing et al., 2010). Σ_{27}PCBs of 0.035 µg/g ww and Σ_{12}PBDEs of 0.0004 µg/g ww (BDE 209 not analyzed) were found in eggs from Taizhou e-waste site (Zhao et al., 2009), and in Wenling Σ_7PBDEs mean concentration were of 0.038 ± 42 µg/g ww (Qin et al., 2011), both in Zhejiang province. Chicken and goose home-produced eggs from Guiyu, one of the biggest e-waste areas in China, contained median Σ_{17}PBDEs values between 0.011 and 0.214 µg/g ww (0.4 and 7.7 µg/g lw respectively)(Zeng et al., 2016b). In chicken eggs grown near a municipal dumpsite in Nabuja city, Nigeria, mean Σ_7PBDEs ranged from 0.026 to 0.029 µg/g ww but the study did not include BDE 209 (Oloruntoba et al., 2019).

Considering the median concentration in eggs and daily egg ingestion, individual EDIs ranged from 0.00002 to 0.084 µg/$kg_{body\ weight}$×day in adults and from 0.0001 to 0.36 µg/$kg_{body\ weight}$×day in children (Table 5.3). Regarding MOEs, values for OPEs and OCPs (individually or grouped by families) were always higher than 2.5, which implies no human health concern. On the other hand, the ingestion of marker PCBs due to free ranged hen eggs consumption can be hazardous both for children and for adults and the ingestion of PBDEs (except BDE 209) can be hazardous only for children. Compared with other studies, hen eggs from Qingyuan e-waste sites collected in 2010 contained PCBs 10 to 100 times higher than those from the control site, and estimated daily intake ranged from 0.07 to 0.32 µg/day × $kg_{body\ weight}$ (Zheng et al., 2012). In eggs collected in 2013 and 2016 also in Qingyuan, PCBs daily intake was above the minimal risk levels for adults in 70% of the samples and for children in all the samples (Zeng et al., 2018). Same samples were also evaluated for PBDEs, and EDIs were lower than the EPA reference values but BDE47 and BDE99 showed a MOE lower than 2.5, which implies potential health risks for humans and wildlife (Huang et al., 2018).

5.3.5. Risk assessment and environmental impacts of the Baihe e-waste site

Pollutants detected in water, soil and sediments were present at toxicologically relevant concentrations which can have implications for the environment and human health. Table 5.4 indicates RQs for these 3 matrices to determine the risk they can produce. Values within the 1-10 represent a moderate risk.

Table 5.4. Total risk quotients (RQs) by families of pollutants for each soil and sediment sample and for OPEs in water samples.

SOILS	ΣRQ OPEs (CA)	ΣRQ PBDEs (CA)	ΣRQ PCBs (CA)	ΣRQ OCPs (CA)
SOIL 1	342	8	36	30
SOIL 2	28	0.27	0.18	4.8
SOIL 3	544	9.2	839	51,061
SOIL 4	2,452	0.04	0.31	1.2
SEDIMENTS				
SED 1	8,884	65	197	19,896
SED 2	46	6.0	8.9	29
SED 3	245	0.01	1.2	51
WATERS				
WAT 1	4.8			
WAT 2	1.4			
WAT 3	2.8			
WAT 4	1.1			
WAT 5	2.0			

In waters, RQs, ΣOPEs ranged between 1.12 in WAT4 and 4.83 in WAT1. The highest risk was found for the polluted pond (WAT1), followed by WAT3 around 600 m away from the pollution source. Individual OPEs RQs ranged from 0 to 1.9 and compounds that mostly contributed to the total risk were TCP1 and TCP2 with RQs > 1 in the polluted pond.

RQs in soils ranged from 0.18 for PCBs in SOIL 2 to 51,000 for OCPs in SOIL 3. RQs in sediments were between 0.013 for PBDEs in SED 3 to 20,000 for OCPs in SED 1. High risks associated with OCPs in SOIL3 and SED1 might be due to the high toxicity of endosulfans detected in these samples. Higher RQs were found for OPEs followed by PCBs in the rest of soils and sediments.

5.3.6. Comparison with other e-waste sites

E-waste recycling areas like Baihe village are so called hotspots of environmental pollution derived from the non-formal methods used to recover high market value materials from all types of EEE. OPEs and PBDEs were the main pollutants detected in the Baihe e-waste, and similar findings are reported in other e-waste sites.

Σ_{13}OPFRs were between 0.22 and 14 µg/g dw in sediments from the Liangjiang River (Guiyu), near a non-formal and uncontrolled e-waste dismantling area and ranged from 0.42 to 0.032 µg/g dw in sediments from the Xiaoqing River, close to a flame retardants production site in (Chen et al., 2021), indicating that production can be less harmful than recycling activities. Another study in Guiyu reported Σ_8OPEs from 6 to 2000 µg/g dw in e-waste influenced sediments (Li et al., 2019a). Much lower levels than those reported in the present study were found for OPEs in an e-waste site in northern Vietnam, where Σ_{10}OPEs ranged from 0.006 to 0.125 µg/g dw in river sediments and from 0.018 to 3.43 µg/g dw in soils (Matsukami et al., 2015).

In water, our results are comparable with those ones reported for waters from Taihu lake (Σ_{11}OPEs between 0.16 and 1.53 µg/L) highly influenced by an OPEs manufacturing area (Wang et al., 2018a). Urban waters near Shanghai, showed $\sum_8$OPEs ranging from 0.34 to 1.70 µg/L with TCEP and TCPP as the major contributors (Zhang et al., 2019a).

Soils from a multi-waste recycling area in Tianjin (north-east China) showed levels between 0.12 and 2.1 µg/g, (Wang et al., 2018b), lower than the present study. Lower levels were also found in an e-waste area in Pakistan, near Karachi city, where Σ_7OPEs between 0.046 and 1.69 µg/g dw were reported (Iqbal et al., 2017).

Similar PBDEs levels than the present study, were detected in sediments from other recycling sites in China. Σ_7PBDEs (without BDE 209) ranged from 0.005 to 0.030 µg/g dw, and BDE209 was the main contributor and ranged from 0.19 to 43 µg/g dw in an e-waste site near Shenzhen city, Guangdong province, with no concentration gradient along the depth of the sediment (Zhou et al., 2019). In an abandoned e-waste recycling area in Guiyu (Guangdong), Σ_{16}PBDEs ranged from 5 to 115 µg/g dw in river sediments and from 0.04 to 7.2 µg/g dw in soils (Wu et al., 2019). In a plastic recycling area in Hebei province, Σ_{21}PBDEs ranging from 0.018 to 9.8 µg/g dw were detected in sediments and between 0.001 and 5.5 µg/g dw in soils (Tang et al., 2014). Crops soils affected by e-waste in Guangdong province showed Σ_9PBDEs ranging from 0.002 to 0.020 µg/g dw and BDE 209 from 0.025 to 0.10 µg/g dw as the dominating congener (Zou et al., 2007). Higher levels were detected in soils from 4 e-waste recycling villages in the Qingyuan County, where Σ_{41}PBDEs were up to 13 µg/g dw and BDE 209 was again the dominant congener with levels ranging from 0.005 to 12 µg/g dw (Wang et al., 2014).

Legacy pollutants like PCBs have been also detected in different e-waste sites. Soils from Guiyu contained Σ_{35}PCBs between 0.0006 and 0.004 µg/g dw, which are much lower than in the present study (Luo et al., 2020). Σ_{26}PCBs in 4 e-waste sites in India ranged from 0.0004 to 0.48 µg/g dw (Chakraborty et al., 2018). Lower levels at the pg/g dw were found for Σ_{22}PCBs in soils from a plastic recycling area in east China (Qin et al., 2022). In Zhejiang province, Σ_{19}PCBs ranged from 11.7 to 2.61 µg/g dw in an abandoned e-waste recycling and from 0.0035 to 0.051 µg/g dw in a newly built e-waste facility, suggesting that proper management of residues can reduce environmental emissions (Liu et al., 2020).

Human residing in Baihe village or other e-waste sites are exposed to pollutants through consumption of contaminated eggs but also from breathing and/or ingestion of fine particles or polluted air, and are of special concern for workers (Zeng et al., 2016a, Zheng et al., 2015).

5.4. Conclusions

Baihe village is a well-documented e-waste hotspot with numerous studies reporting the levels and impact of non-formal e-waste recycling activities to the environment and human health. Even with the policies and laws implemented since 2010 to ban e-waste disposal in small family run workshops and the efforts made by the Qingyuan county government to relocate those potentially hazardous activities to modern industrial facilities, high amounts of pollutants associated with e-waste are still present and distributed among environmental compartments.

In this paper the presence of legacy POPs such as PBDEs, PCBs and, in a less extent OCPs, and new pollutants like OPEs at high levels in water, soils and sediments from the most polluted pond in the village but also in the surroundings, where some duck and goose farms are located and in free-ranged hen eggs locally produced have been detected. Pollution patterns of analyzed samples demonstrate a common source: the plastic lump which has been accumulated during years of illegal dumping of plastic residues from the e-waste recycling activities. Moderate to high risks have been associated to the levels detected in the area together with human intakes through consumption of food goods produced here.

References

Arisekar, U., Shakila, R.J., Shalini, R., Jeyasekaran, G., Padmavathy, P., 2022. Effect of household culinary processes on organochlorine pesticide residues (OCPs) in the seafood (Penaeus vannamei) and its associated human health risk assessment: Our vision and future scope. Chemosphere 297, 134075, 10.1016/j.chemosphere.2022.134075

Arnich, N., Tard, A., Leblanc, J.C., Le Bizec, B., Narbonne, J.F., Maximilien, R., 2009. Dietary intake of non-dioxin-like PCBs (NDL-PCBs) in France, impact of maximum levels in some foodstuffs. Regul Toxicol Pharm : RTP 54, 287-293, 10.1016/j.yrtph.2009.05.010

Awasthi, A.K., Zeng, X., Li, J., 2016. Environmental pollution of electronic waste recycling in India: A critical review. Environ Pollut 211, 259-270, 10.1016/j.envpol.2015.11.027

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ Sci Technol 46, 2564-2573, 10.1021/es2034125

Cai, K., Song, Q., Yuan, W., Ruan, J., Duan, H., Li, Y., Li, J., 2020. Human exposure to PBDEs in e-waste areas: A review. Environ Pollut 267, 115634, 10.1016/j.envpol.2020.115634

Chakraborty, P., Selvaraj, S., Nakamura, M., Prithiviraj, B., Cincinelli, A., Bang, J.J., 2018. PCBs and PCDD/Fs in soil from informal e-waste recycling sites and open dumpsites in India: Levels, congener profiles and health risk assessment. Sci Total Environ 621, 930-938, 10.1016/j.scitotenv.2017.11.083

Chen, P., Ma, S., Yang, Y., Qi, Z., Wang, Y., Li, G., Tang, J., Yu, Y., 2021. Organophosphate flame retardants, tetrabromobisphenol A, and their transformation products in sediment of e-waste dismantling areas and the flame-retardant production base. Ecotox Environ Safe 225, 112717, 10.1016/j.ecoenv.2021.112717

Cristale, J., Dantas, R.F., De Luca, A., Sans, C., Esplugas, S., Lacorte, S., 2017. Role of oxygen and DOM in sunlight induced photodegradation of organophosphorous flame retardants in river water. J Hazard Mater 323, 242-249, 10.1016/j.jhazmat.2016.05.019

de Ree,H., van den Berg,M., Brand, T.,Mulder,G.J., Simons, R., van Zanten, B.V.,Westerink, R.H.S., 2014.Health risk assessment of exposure to TriCresyl Phosphates (TCPs) in air-craft: a commentary. Neurotoxicol 45, 209–215. 10.1016/j.neuro.2014.08.011

ECHA, 2022. European Chemicals Agency. https://echa.europa.eu/substance-information/-/substanceinfo/100.033.766 (accesed april 2022)

Huang, C.C., Zeng, Y.H., Luo, X.J., Tang, B., Liu, Y.E., Ren, Z.H., Mai, B.X., 2018. Level changes and human dietary exposure assessment of halogenated flame retardant levels in free-range chicken eggs: A case study of a former e-waste recycling site, South China. Sci Total Environ 634, 509-515, 10.1016/j.scitotenv.2018.03.386

Iqbal, M., Syed, J.H., Breivik, K., Chaudhry, M.J.I., Li, J., Zhang, G., Malik, R.N., 2017. E-Waste Driven Pollution in Pakistan: The First Evidence of Environmental and Human Exposure to Flame Retardants (FRs) in Karachi City. Environ Sci Technol 51, 13895-13905, 10.1021/acs.est.7b03159

Li, H., La Guardia, M.J., Liu, H., Hale, R.C., Mainor, T.M., Harvey, E., Sheng, G., Fu, J., Peng, P., 2019a. Brominated and organophosphate flame retardants along a sediment transect encompassing the Guiyu, China e-waste recycling zone. Sci Total Environ 646, 58-67, 10.1016/j.scitotenv.2018.07.276

Li, J., Zhao, L., Letcher, R.J., Zhang, Y., Jian, K., Zhang, J., Su, G., 2019b. A review on organophosphate Ester (OPE) flame retardants and plasticizers in foodstuffs: Levels, distribution, human dietary exposure, and future directions. Environ Int 127, 35-51, 10.1016/j.envint.2019.03.009

Li, W., Achal, V., 2020. Environmental and health impacts due to e-waste disposal in China - A review. Sci Total Environ 737, 139745, 10.1016/j.scitotenv.2020.139745

Liu, Y., Luo, X.J., Huang, L.Q., Tao, L., Zeng, Y.H., Mai, B.X., 2018a. Halogenated organic pollutants in aquatic, amphibious, and terrestrial organisms from an e-waste site: Habitat-dependent accumulation and maternal transfer in watersnake. Environ Pollut 241, 1063-1070, 10.1016/j.envpol.2018.06.038

Liu, Y.E., Huang, L.Q., Luo, X.J., Tan, X.X., Huang, C.C., Corella, P.Z., Mai, B.X., 2018b. Determination of organophosphorus flame retardants in fish by freezing-lipid precipitation, solid-phase extraction and gas chromatography-mass spectrometry. J Chromatogr A 1532, 68-73, 10.1016/j.chroma.2017.12.001

Liu, Y.E., Luo, X.J., Zapata Corella, P., Zeng, Y.H., Mai, B.X., 2019. Organophosphorus flame retardants in a typical freshwater food web: Bioaccumulation factors, tissue distribution, and trophic transfer. Environ Pollut 255, 113286, 10.1016/j.envpol.2019.113286

Liu, C., Wei, B.K., Bao, J.S., Wang, Y., Hu, J.C., Tang, Y.E., Chen, T., Jin, J., 2020. Polychlorinated biphenyls in the soil-crop-atmosphere system in e-waste dismantling areas in Taizhou: Concentrations, congener profiles, uptake, and translocation. Environ Pollut 257, 113622, 10.1016/j.envpol.2019.113622

Lu, C., Zhang, L., Zhong, Y., Ren, W., Tobias, M., Mu, Z., Ma, Z., Geng, Y., Xue, B., 2014. An overview of e-waste management in China. J Mater Cycles Waste Manage 17, 1-12, 10.1007/s10163-014-0256-8

Luo, C., Hu, B., Wang, S., Wang, Y., Zhao, Z., Wang, Y., Li, J., Zhang, G., 2020. Distribution and Chiral Signatures of Polychlorinated Biphenyls (PCBs) in Soils and Vegetables around an e-Waste Recycling Site. J Agricult Food Chem 68, 10542-10549, 10.1021/acs.jafc.0c00479

Mahmoud, A.F., Ikenaka, Y., Yohannes, Y.B., Darwish, W.S., Eldaly, E.A., Morshdy, A.E., Nakayama, S.M., Mizukawa, H., Ishizuka, M., 2016. Distribution and health risk assessment of organochlorine pesticides (OCPs) residue in edible cattle tissues from northeastern part of Egypt: High accumulation level of OCPs in tongue. Chemosphere 144, 1365-1371, 10.1016/j.chemosphere.2015.10.016

Ma,W.J., Deng, F., Xu, Y.J., Xu,H.F., 2005. The study on dietary intake and nutritional status of residents in Guangdong, 2002. Guangdong J. Health Epidem. Prevent. 01, 1–5

Matsukami, H., Tue, N.M., Suzuki, G., Someya, M., Tuyen, L.H., Viet, P.H., Takahashi, S.,Tanabe, S., Takigami, H., 2015. Flame retardant emission from e-waste recycling operation in northern Vietnam: environmental occurrence of emerging organophosphorus esters used as alternatives for PBDEs. Sci Total Environ. 514, 492–499. 10.1016/j.scitotenv.2015.02.008

Nandy, S., Fortunato, E., Martins, R., 2022. Green economy and waste management: An inevitable plan for materials science. Progress in Natural Science: Materials International 32, 1-9, 10.1016/j.pnsc.2022.01.001

NORMAN ecotoxicology database, 2021. Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances.https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php (accessed november 2021)

Oloruntoba, K., Sindiku, O., Osibanjo, O., Balan, S., Weber, R., 2019. Polybrominated diphenyl ethers (PBDEs) in chicken eggs and cow milk around municipal dumpsites in Abuja, Nigeria. Ecotox Environ Safe 179, 282-289, 10.1016/j.ecoenv.2019.04.045

Perkins, D.N., Brune Drisse, M.N., Nxele, T., Sly, P.D., 2014. E-waste: a global hazard. An Global Health 80, 286-295, 10.1016/j.aogh.2014.10.001

Poma, G., Sales, C., Bruyland, B., Christia, C., Goscinny, S., Van Loco, J., Covaci, A., 2018. Occurrence of Organophosphorus Flame Retardants and Plasticizers (PFRs) in Belgian Foodstuffs and Estimation of the Dietary Exposure of the Adult Population. Environ Sci Technol 52, 2331-2338, 10.1021/acs.est.7b06395

Qin, X., Qin, Z., Li, Y., Zhao, Y., Xia, X., Yan, S., Tian, M., Zhao, X., Xu, X., Yang, Y., 2011. Polybrominated diphenyl ethers in chicken tissues and eggs from an electronic waste recycling area in southeast China. J Environ Sci 23, 133-138, 10.1016/s1001-0742(10)60384-1

Qin, Y., Liu, Y., Wang, J., Lu, Y., Xu, Z., 2022. Emission of PAHs, PCBs, PBDEs and heavy metals in air, water and soil around a waste plastic recycling factory in an industrial park, Eastern China. Chemosphere 294, 133734, 10.1016/j.chemosphere.2022.133734

Shaikh, S., Thomas, K., Zuhair, S., 2020. An exploratory study of e-waste creation and disposal: Upstream considerations. Resour Conserv Recycl 155, 104662, 10.1016/j.resconrec.2019.104662

Shen, H., Guan, R., Ding, G., Chen, Q., Lou, X., Chen, Z., Zhang, L., Xing, M., Han, J., Wu, Y., 2017. Polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in Zhejiang foods (2006-2015): Market basket and polluted areas. Sci Total Environ 574, 120-127, 10.1016/j.scitotenv.2016.09.038

Sthiannopkao, S., Wong, M.H., 2013. Handling e-waste in developed and developing countries: initiatives, practices, and consequences. Sci Total Environ 463-464, 1147-1153, 10.1016/j.scitotenv.2012.06.088

Stockholm Convention, 2009. COP4 final. Earth Negotiation Bulletin 15, 174 11th May.

Tang, Z., Huang, Q., Cheng, J., Yang, Y., Yang, J., Guo, W., Nie, Z., Zeng, N., Jin, L., 2014. Polybrominated diphenyl ethers in soils, sediments, and human hair in a plastic waste recycling area: a neglected heavily polluted area. Environ Sci Technol 48, 1508-1516, 10.1021/es404905u

US EPA, 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.1. United States Environmental Protection Agency, Washington, DC, USA

Wang, S., Zhang, S., Huang, H., Niu, Z., Han, W., 2014. Characterization of polybrominated diphenyl ethers (PBDEs) and hydroxylated and methoxylated PBDEs in soils and plants from an e-waste area, China. Environ Pollut 184, 405-413, 10.1016/j.envpol.2013.09.021

Wang X., Zhu L., Zhong W., Yang L., 2018a. Partition and source identification of organophosphate esters in the water and sediment of Taihu Lake, China. J Hazard Mater 360, 43-50

Wang, Y., Sun, H., Zhu, H., Yao, Y., Chen, H., Ren, C.,Wu, F., Kannan, K., 2018b. Occurrence and distribution of organophosphate flame retardants (OPFRs) in soil and outdoor settled dust from a multi-waste recycling area in China. Sci. Total Environ. 625, 1056–1064, 10.1016/j.scitotenv.2018.01.013

Wang, X., Zhu, Q., Liao, C., Jiang, G., 2021. Human internal exposure to organophosphate esters: A short review of urinary monitoring on the basis of biological metabolism research. J Hazard Mater 418, 126279, 10.1016/j.jhazmat.2021.126279

WHO, 1998. EHC 209: Flame Retardants: Tris-(Chloropropyl)Phosphate and Tris-(2-Chloroethyl)phosphate, Geneva, Switzerland

Wu, J.P., Luo, X.J., Zhang, Y., Yu, M., Chen, S.J., Mai, B.X., Yang, Z.Y., 2009. Biomagnification of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls in a highly contaminated freshwater food web from South China. Environ Pollut 157, 904-909, 10.1016/j.envpol.2008.11.001

Wu, Q., Du, Y., Huang, Z., Gu, J., Leung, J.Y.S., Mai, B., Xiao, T., Liu, W., Fu, J., 2019. Vertical profile of soil/sediment pollution and microbial community change by e-waste recycling operation. Sci Total Environ 669, 1001-1010, 10.1016/j.scitotenv.2019.03.178

Xing, G.H., Wu, S.C., Wong, M.H., 2010. Dietary exposure to PCBs based on food consumption survey and food basket analysis at Taizhou, China - the world's major site for recycling transformers. Chemosphere 81, 1239-1244, 10.1016/j.chemosphere.2010.09.032

Zapata, P., Ballesteros-Cano, R., Colomer, P., Bertolero, A., Viana, P., Lacorte, S., Santos, F.J., 2018. Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks. Sci Total Environ 633, 704-715, 10.1016/j.scitotenv.2018.03.081

Zeng, Y.H., Tang, B., Luo, X.J., Zheng, X.B., Peng, P.A., Mai, B.X., 2016a. Organohalogen pollutants in surface particulates from workshop floors of four major e-waste recycling sites in China and implications for emission lists. Sci Total Environ 569-570, 982-989, 10.1016/j.scitotenv.2016.06.053

Zeng, Y.H., Luo, X.J., Tang, B., Mai, B.X., 2016b. Habitat- and species-dependent accumulation of organohalogen pollutants in home-produced eggs from an electronic waste recycling site in South China: Levels, profiles, and human dietary exposure. Environ Pollut 216, 64-70, 10.1016/j.envpol.2016.05.039

Zeng, Y., Huang, C., Luo, X., Liu, Y., Ren, Z., Mai, B., 2018. Polychlorinated biphenyls and chlorinated paraffins in home-produced eggs from an e-waste polluted area in South China: Occurrence and human dietary exposure. Environ Int 116, 52-59, 10.1016/j.envint.2018.04.006

Zhang, N., Yang, Y., Tao, S., Liu, Y., Shi, K.L., 2011. Sequestration of organochlorine pesticides in soils of distinct organic carbon content. Environ Pollut 159, 700-705, 10.1016/j.envpol.2010.12.011

Zhang, Z., Shao, H., Wu, M., Zhang, J., Li, D., Li, J., Wang, H., Shi, W., Xu, G., 2019a. Occurrence, Distribution, and Potential Sources of Organophosphate Esters in Urban and Rural Surface Water in Shanghai, China. Arch Environ Contam Toxicol 77, 115-126, 10.1007/s00244-019-00633-w

Zhang, H., Shen, Y., Liu, W., He, Z., Fu, J., Cai, Z., Jiang, G., 2019b. A review of sources, environmental occurrences and human exposure risks of hexachlorobutadiene and its association with some other chlorinated organics. Environ Pollut 253, 831-840, 10.1016/j.envpol.2019.07.090

Zhang, Q., Wang, Y., Jiang, X., Xu, H., Luo, Y., Long, T., Li, J., Xing, L., 2021. Spatial occurrence and composition profile of organophosphate esters (OPEs) in farmland soils from different regions of China: Implications for human exposure. Environ Pollut 276, 116729, 10.1016/j.envpol.2021.116729

Zhao, G., Zhou, H., Wang, D., Zha, J., Xu, Y., Rao, K., Ma, M., Huang, S., Wang, Z., 2009. PBBs, PBDEs, and PCBs in foods collected from e-waste disassembly sites and daily intake by local residents. Sci Total Environ 407, 2565-2575, 10.1016/j.scitotenv.2008.11.062

Zheng, X.B., Wu, J.P., Luo, X.J., Zeng, Y.H., She, Y.Z., Mai, B.X., 2012. Halogenated flame retardants in home-produced eggs from an electronic waste recycling region in South China: levels, composition profiles, and human dietary exposure assessment. Environ Int 45, 122-128, 10.1016/j.envint.2012.04.006

Zheng, X., Xu, F., Chen, K., Zeng, Y., Luo, X., Chen, S., Mai, B., Covaci, A., 2015. Flame retardants and organochlorines in indoor dust from several e-waste recycling sites in South China: Composition variations and implications for human exposure. Environ Int 78, 1-7, 10.1016/j.envint.2015.02.006

Zhou, P., Zhao, Y., Li, J., Wu, G., Zhang, L., Liu, Q., Fan, S., Yang, X., Li, X., Wu, Y., 2012. Dietary exposure to persistent organochlorine pesticides in 2007 Chinese total diet study. Environ Int 42, 152-159, 10.1016/j.envint.2011.05.018

Zhou, H., Tam, N.F.Y., Cheung, S.G., Wei, P., Li, S., Wu, Q., 2019. Contamination of polybrominated diphenyl ethers (PBDEs) in watershed sediments and plants adjacent to e-waste sites. J Hazard Mater 379, 120788, 10.1016/j.jhazmat.2019.120788

Zou, M.Y., Ran, Y., Gong, J., Mai, B.X., Zeng, E.Y., 2007. Polybrominated Diphenyl Ethers in watershed soils of the Pearl River Delta, China: Occurrence, inventory, and fate. Environ Sci Technol 41, 8262–8267, 10.1021/es071956d

Annex

Table 5.A1. Sediment, water and soil sampling sites and water content.

SAMPLES	CODE	GPS location		Water content (%)
POLLUTED POND (POND 1)	PLAS	N23º35´28.18"	E113º01´31.88"	17.2
	SED1	N23º35´28.18"	E113º01´31.88"	51.9
	WAT1	N23º35´28.18"	E113º01´31.88"	
	SOIL1	N23º35´34.264"	E113º01´32.45"	31.8
	SOIL2	N23º35´34.47"	E113º01´32.65"	14.1
	SOIL3	N23º35´34.47"	E113º01´32.65"	12.6
POND 2	SED2	N23º35´47.641"	E113º01´34.23"	31.6
	WAT2	N23º35´47.641"	E113º01´34.23"	
	SOIL4	N23º35´47.48"	E113º01´37.27"	19.6
POND 3 (duck farm)	SED3	N23º35´48.695"	E113º01´35.15"	35.1
	WAT3	N23º35' 48.3"	E113º01'35.96"	
Irrigation canal	WAT4	N23º35' 28.88"	E113º01'36.01"	
River	WAT5	N23º 35' 35.38"	E113º01'40.34"	

Table 5.A2. Biometric data of individual eggs and water and lipid content, in %. First number of the sample name indicates the pooled sample; second number indicates the individual egg.

Sample	Length (mm)	Width (mm)	Weight (g)	Eggshell thickness (mm)	% Water content	% Lipid content
HEN1-1	52.72	42.44	50.99	0.28	74.17	2.28
HEN1-2	48.28	37.25	36.56	0.27		
HEN1-3	53.52	43.07	52.96	0.29		
HEN2-1	54.58	40.55	47.45	0.29	73.40	3.50
HEN2-2	56.87	42.95	56.40	0.31		
HEN2-3	54.81	42.29	53.29	0.28		
HEN3-1	51.50	37.68	40.27	0.23	71.78	4.16
HEN3-2	53.74	40.52	46.91	0.20		
HEN3-3	54.53	43.61	55.46	0.25		
HEN4-1	57.85	42.61	56.30	0.33	72.76	3.50
HEN4-2	52.90	40.51	49.13	0.31		
HEN4-3	51.86	40.95	47.22	0.28		
HEN4-4	56.71	47.84	68.72	0.30		
HEN4-5	53.47	40.65	47.98	0.27		
HEN5-1	53.62	40.51	48.56	0.28	71.46	3.16
HEN5-2	54.63	42.50	53.74	0.28		
HEN5-3	50.47	41.63	48.18	0.27		
HEN5-4	52.20	41.06	48.90	0.26		
HEN5-5	51.26	41.30	47.95	0.29		
HEN6-1	53.05	38.14	42.42	0.27	73.04	3.10
HEN6-2	52.53	39.37	42.49	0.24		
HEN6-3	54.45	40.42	48.37	0.27		
HEN6-4	52.95	39.34	43.63	0.26		
HEN6-5	56.60	39.52	46.01	0.23		
Mean	*53.5±1*	*41.1±2.2*	*49.2±6.5*	*0.27±0.03*	*72.7±1*	*3.3±0.6*

Table 5.A3. OPEs studied, CAS number for unambiguous identification and GC-MS/MS chromatographic retention times and monitored transitions and collision energies for studied OPEs. Recoveries (% ± standard deviation) and blank contribution indicated.

Compound	CAS no	Rt (min)	Q1 (CE, eV)	Q2 (CE, eV)	% R±SD Soil	% R±SD Water	% R±SD Eggs	Bk ±SD (ng/g) Soil	Bk ±SD (ng/L) Water	Bk ±SD (ng/g) Eggs
TEP	78-40-0	4.3	155>99 (30)	127>81 (30)	74 ±10	93 ± 9.5	96 ± 9	1.2 ± 2.2	nd	8 ± 7
TPP (TiPP)	513-02-0	5.2	141>81 (30)	183>99 (30)	91 ±2.5	57 ± 1	75 ± 8	8.9 ±13.6	nd	nd
TPP (TnPP)	513-08-6	7.7	141>81 (30)	183>99 (30)	148 ±9	102 ± 26	96 ± 5	nd	nd	nd
TnBP	126-73-8	11.0	155>81 (40)	211>99 (40)	151 ±5	107 ± 6.5	86 ± 20	126 ± 220	4.1 ± 0.6	27 ± 10
TCEP	115-96-8	12.3	249>63 (30)	205>63 (30)	91 ±11	98 ± 1	76 ± 25	58.7 ± 10.8	4.3 ± 0.6	22 ± 25
TCPP	13674-84-5	12.6	277>125 (10)	201>125 (30)	97 ±12	93 ±4.5	118 ± 31	63.1 ± 33.2	4.9 ± 0.8	36 ± 12
TDCPP	13674-87-8	17.4	381>159 (10)	209>75 (10)	79 ±1	78 ± 20	81 ± 4	nd	0.5 ± 0.1	4 ± 3
TPhP	115-86-6	18.1	326>169 (30)	215>169 (30)	88 ±18	101 ± 6	88 ± 2	14.3 ± 14.3	10 ± 0.5	13 ± 7
EHDPP	1241-94-7	18.4	251>77 (30)	251>153 (30)	109 ±15	72 ± 38	72 ± 14	2.0 ± 1.9	0.01 ± 0.02	9 ± 7
TEHP	78-42-2	18.6	113>82 (20)	211>99 (20)	82 ±11	111 ± 5	64 ± 11	11.0 ± 7.1	1.3 ± 1	6 ± 11
TCP 1	1330-78-5	20.6	368>197 (20)	368>181 (20)	69 ±34	72 ± 29	99 ± 7	nd	0.07 ± 0.02	11 ± 2
TCP 2	1330-78-5	21.0	368>197 (20)	368>181 (20)	78 ±32	73 ± 20	86 ± 3	nd	0.17 ± 0.06	4 ± 2
TCP 3	1330-78-5	21.3	368>197 (20)	368>181 (20)	96±28	71 ± 22	90 ± 1	nd	nd	3 ± 2
TCP 4	1330-78-5	21.7	368>197 (20)	368>181 (20)	105 ±21	79 ± 29	75 ± 4	nd	nd	2 ± 3

Table 5.A4. Compounds studied, CAS number, GC-MS/MS retention time and monitored transitions and collision energies for OCPs and PCBs and by GC-NCI-MS for PBDEs, ordered by retention time.

COMPOUND	CAS	Rt (min)	Q1 (CE, eV)	Q2 (CE, eV)	MDL (ng/g dw) Soil	% R±SD Soil	MDL (ng/g ww) Eggs	% R±SD Eggs
HCBu	87-68-3	9.40	260>225 (10)	260>190 (45)	0.13	76 ± 0.1	0.04	81.4 ± 24
PeCB	608-93-5	15.90	250>215 (30)	250>180 (30)	0.19	80 ± 18	0.07	76 ± 1
α-HCH	319-84-6	19.40	219>147 (20)	219>183 (15)	0.03	97 ± 16	0.11	102 ± 10
HCB	118-74-1	20.55	284>249 (20)	284>114 (20)	0.08	83 ±17	0.04	75 ± 10
β-HCH	319-85-7	20.80	219>147 (20)	219>183 (15)	1.40	115 ± 11	0.18	87 ± 16
γ-HCH	58-89-9	22.10	219>183 (15)	219>147 (20)	0.12	122 ± 9	0.20	92 ± 10
δ-HCH	319-86-8	23.98	219>147 (20)	219>183 (15)	0.13	110 ± 14	0.46	108 ± 4
PCB 28	7012-37-5	24.02	258>186 (30)	258>188 (40)	0.13	77 ± 0.2	1.98	92 ± 11
Heptachlor	76-44-8	24.80	272>237 (20)	272>235 (20)	0.11	78 ±19	0.65	100 ± 6
PCB 52	35693-99-3	26.10	292>222 (45)	292>220 (40)	0.27	82 ± 2	1.04	86 ± 1
Aldrin	309-00-2	26.80	263>193 (40)	263>191 (40)	0.95	88 ± 2	0.44	88 ± 6
DBP	90-98-2	27.60	139>111 (20)	139>75 (30)				
Isodrin	465-73-6	28.50	263>193 (40)	263>191 (40)	0.55	97 ± 4	1.21	77 ± 5
Heptachlor-epox.	1024-57-3	29.30	353>191 (45)	353>193 (45)	2.50	105 ± 1	1.07	109 ± 2
Oxychlordane	27304-13-8	29.35	185>121 (20)	185>85 (20)	0.30	97 ± 16	0.14	92 ± 9
Cis-Chlordane	5103-71-9	30.70	375>266 (30)	375>339 (20)	0.17	85 ± 9	0.30	86 ± 11
2,4´-DDE	72-55-9	31.30	318>246 (20)	318>248 (20)	0.37	82 ± 1.5	0.62	80 ± 2
α-endosulfan	959-98-8	31.40	195>159 (10)	195>125 (20)	0.02	120 ± 18	0.55	86 ± 3
PCB 101	37680-73-2	31.43	326>256 (50)	326>219 (50)	2.70	79 ± 1	1.70	90 ± 7
Trans-Chlordane	5103-74-2	31.65	373>266 (30)	373>264 (30)	0.43	107 ± 17	0.99	87 ± 4
Dieldrin	60-57-1	32.98	241>206 (30)	241>170 (40)	2.60	88 ± 0.5	5.21	85 ± 6
4, 4´-DDE	72-55-9	33.25	318>248 (20)	318>246 (20)	0.36	85 ± 10	0.09	76 ± 5

Table5.A4. Continued

COMPOUND	CAS	Rt (min)	Q1 (CE, eV)	Q2 (CE, eV)	MDL (ng/g dw) Soil	% R±SD Soil	MDL (ng/g ww) Eggs	% R±SD Eggs
2,4´-DDD	53-19-0	33.70	235>165 (30)	235>200 (20)	0.14	97 ± 11	0.71	115 ± 3
Endrin	72-20-8	34.20	263>193 (20)	263>228 (20)	2.90	100 ± 10	7.71	99 ± 1
β-endosulfan	33213-65-9	34.80	241>206 (15)	241>170 (25)	1.70	102 ± 13	2.04	74 ± 8
2,4´-DDT	789-02-6	35.73	235>165 (40)	235>199 (10)	0.11	112 ± 5	0.74	125 ± 1
4,4´-DDD	72-54-8	35.86	235>165 (40)	235>199 (10)	0.11	89 ± 6	0.74	126 ± 1
PCB 153	35065-27-1	36.53	360>290 (30)	360>288 (30)	0.07	71 ± 1	0.16	108 ± 6
Endosulfan-sulf	1031-07-8	37.40	272>237 (10)	272>143 (40)	2.90	100 ± 1	5.39	115 ± 10
4,4´-DDT	50-29-3	37.90	235>165 (40)	237>167 (40)	0.38	98 ± 13	1.12	96 ± 15
PCB 138	35065-28-2	38.10	360>290 (40)	360>288 (40)	0.20	109 ± 0.1	3.82	89 ± 13
Dicofol	115-32-2	41.30	139>111 (30)	139>75 (30)	1.00	125 ± 7	1.18	98 ± 9
Methoxychlor	72-43-5	41.53	227>169 (30)	227>115 (50)	0.14	96 ± 26	4.54	110 ± 5
PCB 180	35065-29-3	42.10	394>324 (30)	394>359 (20)	0.04	112 ± 0.3	1.90	106 ± 3
Mirex	2385-85-5	43.06	332>262 (40)	332>260 (50)	0.21	92 ± 2	6.40	106 ± 4
BDE 28	41318-75-6	9.81	79	161/81	0.43	78 ± 5	2.06	87 ± 1
BDE 47	5436-43-1	12.34	79	161/81	0.28	71 ± 7	2.06	96 ± 2
BDE 66	189084-61-5	14.14	79	161/81	0.30	82 ± 4	1.97	101 ± 3
BDE 100	189084-64-8	14.53	79	161/81	0.05	79 ± 3	1.81	97.2 ± 0.1
BDE 99	60348-60-9	14.75	79	161/81	0.09	85 ± 6	1.67	103 ± 5
BDE 85	182346-21-0	15.17	79	161/81	0.45	68 ± 9	1.99	93 ± 1
BDE154	207122-15-4	17.95	79	161/81	0.90	89 ± 8	1.53	99 ± 2
BDE153	68631-49-2	18.50	79	161/81	0.45	86 ± 7	2.16	86 ± 3
BDE 138	182677-30-1	20.01	79	161/81	0.22	74 ± 8	1.44	106 ± 3
BDE 183	207122-16-5	21.18	79	161/81	0.19	95 ± 4	1.43	95 ± 7
BDE 209	1163-19-5	28.34	486.5	79/81/161	20	105 ± 5	6.36	72 ± 21

Table 5.A5. Predicted no effects concentrations (PNEC) used for risk assessment obtained from NORMAN ecotoxycology database (https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php).Na: not available.

COMPOUNDS	Lowest PNEC seds freshwater (µg/kg dw)	Lowest PNEC freshwater (µg/L)
TEP	2903	632
TiPP	11.8	0.89
TnPP	22.8	2.32
TnBP	967	76
TCEP	9.27	4
TCPP	120	111
TDCP	20.5	1.1
TPhP	64.4	0.74
EHDPP	2.24	0.018
TEHP	24.3	0.039
TCP1	11.7	0.023
TCP2	3.4	0.023
TCP3	16.3	0.11
TCP4	7	0.023
BDE 28	646	0.049
BDE 47	129	0.049
BDE 66	614	0.019
BDE 100	1406	0.049
BDE 99	141	0.049
BDE 85	259	0.009
BDE154	609	0.049
BDE153	618	0.049
BDE 138	68.9	0.0054
BDE183	42.8	0.0029
BDE 209	3553	0.2
PCB 28	239	0.11
PCB 52	139	0.021
PCB 101	82.7	0.01
PCB 153	86	0.0046
PCB 138	145	0.0035
PCB 180	57.4	0.0014
HCBu	50.9	0.6
PeCB	1.1	0.007
α-HCH	1.49	0.02
HCB	13.7	0.05
β-HCH	1.49	0.02
δ-HCH	1.49	0.02
γ-HCH	1.49	0.02

Table 5.A5. Continued

COMPOUNDS	Lowest PNEC seds freshwater (µg/kg dw)	Lowest PNEC freshwater (µg/L)
Heptachlor	na	na
Aldrin	24.2	0.01
Isodrin	24.2	0.01
Heptachlor epoxides	0.0001	2.00E-07
Oxychlordane	na	0.013
Cis-chlordane	0.15	0.00005
2.4DDE	11.5	0.00076
α-endosulfan	0.0032	0.000005
Trans-chlordane	0.15	0.00005
Dieldrin	24.2	0.01
4.4´-DDE	81.6	0.025
2.4´-DDD	14.5	0.0039
Endrin	22.1	0.0051
β-endosulfan	0.0032	0.000005
2.4´-DDT	156	0.025
4.4´-DDD	20.1	0.025
Chlordecone	0.78	0.001
Endosulfan sulphate	62.1	0.95
4.4´-DDT	101	0.01
Dicofol	0.32	0.0013
Metoxychlor	3.06	0.00078
Mirex	2100	0.043

Table 5.A6. Concentration of pollutants in hen eggs (µg/g ww) samples from the e-waste site. Nd: not detected.

COMPOUNDS	HEN 1	HEN 2	HEN 3	HEN 4	HEN 5	HEN 6
TEP	0.0016	0.010	0.175	nd	0.007	0.014
TiPP	nd	0.0004	nd	0.0012	nd	nd
TnPP	nd	nd	nd	nd	nd	nd
TnBP	nd	nd	0.095	0.093	nd	nd
TCEP	nd	nd	nd	nd	nd	nd
TCPP	nd	nd	0.099	0.0001	nd	nd
TDCP	nd	nd	0.0005	nd	nd	nd
TPhP	nd	nd	0.004	nd	nd	nd
EHDPP	nd	nd	0.005	nd	nd	nd
TEHP	nd	nd	0.004	0.028	0.002	0.0005
TCP1	0.001	nd	0.006	0.0001	nd	nd
TCP2	0.001	nd	nd	0.0004	nd	nd
TCP3	0.001	nd	nd	nd	nd	nd
TCP4	0.001	nd	nd	nd	nd	nd
∑OPEs	*0.01*	*0.01*	*0.39*	*0.12*	*0.01*	*0.01*
BDE 28	nd	nd	nd	1.48	2.38	nd
BDE 47	0.004	0.0006	0.0004	0.001	0.002	0.005
BDE 66	nd	nd	nd	0.006	0.014	0.004
BDE 100	nd	0.0002	0.0001	0.002	0.003	0.002
BDE 99	1.96	0.001	0.0008	0.009	0.022	0.008
BDE 85	nd	0.0002	nd	0.006	0.017	0.0006
BDE 154	2.88	0.0015	0.002	0.003	0.014	0.014
BDE 153	0.08	0.003	0.03	nd	nd	0.08
BDE 138	nd	nd	nd	nd	nd	nd
BDE 183	0.001	0.004	0.005	0.009	0.024	0.27
BDE 209	0.05	0.04	0.014	0.033	0.111	0.22
∑PBDEs	*4.98*	*0.05*	*0.05*	*1.55*	*2.59*	*0.60*
PCB 28	0.006	0.017	0.018	0.008	0.008	0.34
PCB 52	nd	nd	0.002	0.002	0.34	0.007
PCB 101	0.002	0.03	0.014	0.0004	0.002	0.087
PCB 153	0.004	0.099	0.047	0.004	0.013	0.38
PCB 138	0.008	0.125	0.06	nd	0.018	0.51
PCB 180	0.002	0.022	0.012	nd	0.002	0.071
∑PCBs	*0.02*	*0.29*	*0.15*	*0.01*	*0.38*	*1.40*
HCBu	0.002	0.001	0.001	0.001	0.001	0.001
PeCB	0.002	0.002	0.002	0.002	0.002	0.002
α-HCH	0.001	0.001	0.001	0.0003	0.0005	0.0005
HCB	0.0006	0.001	0.002	0.001	0.001	0.002
β-HCH	nd	0.001	0.001	nd	0.0002	0.0001
δ-HCH	0.0002	0.0003	0.400	0.0003	0.0001	0.0004

Table 5.A6. Continued

COMPOUNDS	HEN 1	HEN 2	HEN 3	HEN 4	HEN 5	HEN 6
γ-HCH	nd	nd	nd	nd	nd	nd
α-endosulfan	nd	nd	nd	nd	nd	nd
transchlordane	0.0001	0.010	0.005	nd	0.0004	0.001
4,4DDE	nd	nd	nd	nd	nd	nd
β-endosulfan	nd	nd	nd	nd	nd	nd
4,4DDD	nd	0.001	0.0004	nd	nd	0.0001
Chlordecone	0.0004	0.0001	0.0004	0.0006	0.0007	0.0002
4,4DDT	nd	0.007	0.002	nd	nd	0.0004
Metoxychlor	nd	nd	nd	nd	nd	nd
Mirex	nd	nd	0.003	nd	nd	0.035
∑OCPs	*0.01*	*0.02*	*0.42*	*0.01*	*0.01*	*0.04*

6. General discussion

6.1. Mass spectrometry fragments assignation.

Few studies develop one single multiresidue method for the detection of POPs and rather analyze a specific family. This section discusses the development of the gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) methods for the different families of contaminants.

For PBDEs, GC-NCI-MS was used. GC-NCI-MS is a common technique for PBDEs analysis, widely used and with already validated results (Kierkegaard et al., 2009). PBDEs congeners were identified by their elution order and $[Br]^-$ (m/z=79,81) was used as the quatification ion for all of them except for BDE 209, which was quantified through $[C_6Br_5O]^-$ (m/z=486.5). For confirmation purposes, $[HBr_2]^-$ (m/z= 161) was used. Short GC column (15 m) was used to minimize BDE 209 degradation inside the column and shorten elution times (Bjorklund et al., 2004). These monitored ions are not suitable for isotopic dilution quantification methods so non-environmentally relevant PCBs such as PCB 65 or PCB 209 were used as surrogate standards due to their chemical similarities with PBDEs.

For OCPs and PCBs, a multiresidue tandem GC-MS/MS method was developed and optimized (See chapter 3). The identification and subsequently quantification of target analytes was based on retention times and two MRM transitions which were specific of each compound and determined by the chemical structure. Tables 6.1 and 6.2 compiles proposed ions or looses monitored in the MS/MS methods applied in this Thesis.

For OCPs, chlorine looses including Cl, Cl_2, HCl etc are commonly monitored. Natural isotopic distribution of Cl can be approximated to 75% of Cl^{35} and 25% of Cl^{37} and the rest of isotopes whose contribution is much lower can be used for identification, but more rarely for quantification. An advantage of this isotopic distribution is that it permits to set multiple specific MRM transitions because it creates fragments containing Cl clusters in the mass spectra and allows setting different MRM based on the different Cl isotope abundance, as in the case of 4,4´-DDE (m/z 246 and 248 belonging to the same chlorinated cluster), 4,4´DDT (m/z 235, 237 and 165,167) or trans-chlordane (m/z 266, 264), among others.

Table 6.1. Proposed fragment asignation for MS/MS transitions of studied OCPs and PCBs. M:molecular ion, NA: not assigned.

Compound	MW (gr/mol)	Quantifier precursor ion	Quantifier product ion	Qualifier precursor ion	Qualifier product ion
HCBu	260.76	260 $[M]^{+}$	225$[M-Cl^{35}]^{+}$	260 $[M]^{\prime +}$	190 $[M-Cl_2^{35}]^{+}$
PeCB	250.34	250 $[M]^{+}$	215 $[M-Cl^{35}]^{+}$	250 $[M]^{\prime +}$	180 $[M-Cl_2^{35}]^{+}$
α-HCH	290.83	219$[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	147 $[219 - Cl^{35}\ Cl^{37}]^{+}$/ $[219 - Cl_2^{35}H_2]^{+}$	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	183$[219-HCl^{35}]^{+}$
HCB	284.78	284 $[M]^{\prime +}$	249 $[M-Cl^{35}]^{+}$	284 $[M]^{\prime +}$	114 NA
B-HCH	290.83	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	147 $[219 - Cl^{35}\ Cl^{37}]^{+}$/ $[219 - Cl_2^{35}H_2]^{+}$	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	183 $[219-HCl^{35}]^{+}$
γ-HCH (lindane)	290.83	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	183 $[219-HCl^{35}]^{+}$	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	147 $[219 - Cl^{35}\ Cl^{37}]^{+}$/ $[219 - Cl_2^{35}H_2]^{+}$
δ-HCH	290.83	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	147 $[219-Cl^{35}\ Cl^{37}]^{+}$/ $[219-Cl_2^{35}H_2]^{+}$	219 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M-Cl_2^{35}H_2]^{+}$	183 $[219-HCl^{35}]^{+}$
PCB 28	257.54	258 $[M]^{\prime +}$	186 $[M-Cl^{35}\ Cl^{37}]^{+}$/ $[M - Cl_2^{35}H_2]^{+}$	258 $[M]^{\prime +}$	188 $[M-Cl_2^{35}]^{+}$
Heptachlor	373.32	272 $[M-H_5C_5Cl^{37}]^{+}$ / $[MI-H_3C_5Cl^{35}]^{+}$	237 $[272–Cl^{35}]^{+}$	272 $[M-H_5C_5Cl^{37}]^{+}$ / $[MI-H_3C_5Cl^{35}]^{+}$	235 $[272-Cl^{37}]^{+}$
PCB 52	291.99	292 $[M]^{\prime +}$	222 $[M-2Cl^{35}]^{+}$	292 $[M]^{\prime +}$	220 $[M-2Cl^{37}]^{+}$
Aldrin	364.91	263 $[M-H_4C_2Cl_2^{37}]^{+}$	193 $[263-Cl_2^{35}]^{+}$	263 $[M-H_4C_2Cl_2^{37}]^{+}$	191 $[263-Cl^{35}Cl^{37}]^{+}$
Isodrin	364.91	263 $[M-H_4C_2Cl_2^{37}]^{+}$	193 $[263-Cl_2^{35}]^{+}$	263 $[M-H_4C_2Cl_2^{37}]^{+}$	228 $[263-Cl^{35}]^{+}$
Heptachlor epox	389.32	353 $[M-HCl^{35}]^{+}$	191 $[353-H_3C_3Cl_2^{35}Cl^{37}O]^{+}$	353 $[M-HCl^{35}]^{+}$	193 $[353-H_3C_3Cl_3^{35}O]^{+}$
oxychlordane	423.76	185 $[H_4C_5Cl_3O]^{+}$	121 $[185-HCCl^{35}O]^{+}$	185 $[H_4C_5Cl_3O]^{+}$	85 $[185-H_2CCl_2^{35}O]^{+}$
Cis-Chlordane	409.78	375 $[M-Cl^{35}]^{+}$	266 $[375-H_3C_3Cl_2^{35}]^{+}$/ $[375-HC_3Cl^{35}Cl^{37}]^{+}$	375$[M-Cl^{35}]^{+}$	339 $[375-HCl^{35}]^{+}$
2,4´ DDE	318.03	318 $[M]^{\prime +}$	246 $[M-Cl^{35}Cl^{37}]^{+}$/$[M-H_2Cl_2^{35}]^{+}$	318 $[M]^{\prime +}$	248 $[M-Cl_2^{35}]^{+}$
α-endosulfan	406.93	195 NA	159 $[195-HCl^{35}]^{+}$	195 NA	125 $[195- Cl_2^{35}]^{+}$
PCB 101	326.43	326 $[M]^{\prime +}$	256 $[M-Cl_2^{35}]^{+}$	326 $[M]^{\prime +}$	219 $[M- Cl^{37}Cl_2^{35}]^{+}$
Trans-Chlordane	409.78	373 $[M-Cl^{37}]^{+}$	266 $[373-HC_3Cl_2^{35}]^{+}$	373 $[M-Cl^{37}]^{+}$	264 $[373-HC_3Cl^{35}Cl^{37}]^{+}$
Dieldrin	380.91	241 $[M-Cl_4^{35}]^{+}$	206 $[241-Cl^{35}]^{+}$	241 $[M-Cl_4^{35}]^{+}$	170 $[241-Cl_2^{35}]^{+}$
4, 4´ DDE	318.03	318 $[M]^{\prime +}$	248 $[M-Cl_2^{35}]^{+}$	318 $[M]^{\prime +}$	246 $[M-Cl^{35}Cl^{37}]^{+}$/$[M-H_2Cl_2^{35}]^{+}$
2,4´ DDD	320.04	235 $[M-HCCl^{35}Cl^{37}]^{+}$ / $[M-H_3CCl_2^{35}]^{+}$	165 $[235-Cl_2^{35}]^{+}$	235 $[M-HCCl^{35}Cl^{37}]^{+}$ / $[M-H_3CCl_2^{35}]^{+}$	200 $[235-Cl^{35}]^{+}$
Endrin	380.91	263 $[M-H_4C_2Cl_2^{37}O]^{+}$	193 $[263-Cl_2^{35}]^{+}$	263 $[M-H_4C_2Cl_2^{37}O]^{+}$	228 $[263-Cl^{35}]^{+}$
β-endosulfan	406.93	241 $[M-H_4CCl_2^{35}SO_3]^{+}$ / $[M-H_2CCl^{35}Cl^{37}SO_3]^{+}$	206 $[241-Cl^{35}]^{+}$	241 $[M-H_4CCl_2^{35}SO_3]^{+}$ / $[M-H_2CCl^{35}Cl^{37}SO_3]^{+}$	170 $[241-HCl_2^{35}]^{+}$

Table 6.1. Continued

Compound	MW (gr/mol)	Quantifier precursor ion	Quantifier product ion	Qualifier precursor ion	Qualifier product ion
2,4´ DDT	354.49	235 $[\mathrm{M\text{-}HCCl_2^{35}Cl^{37}}]^+$ /$[\mathrm{M\text{-}H_3CCl_3^{35}}]^+$	165 $[\mathrm{235\text{-}Cl_2^{35}}]^+$	235 $[\mathrm{M\text{-}H_3CCl_3^{35}}]^+$	199 $[\mathrm{235\text{-}HCl^{35}}]^+$
4,4´ DDD	320.04	235 $[\mathrm{M\text{-}HCCl^{35}Cl^{37}}]^+$ / $[\mathrm{M\text{-}H_3CCl_2^{35}}]^+$	165 $[\mathrm{235\text{-}Cl_2^{35}}]^+$	235 $[\mathrm{M\text{-}HCCl^{35}Cl^{37}}]^+$ / $[\mathrm{M\text{-}H_3CCl_2^{35}}]^+$	199 $[\mathrm{235\text{-}HCl^{35}}]^+$
PCB 153	360.88	360 $[\mathrm{M\text{-}H}]^+$	290 $[\mathrm{360\text{-}Cl_2^{35}}]^+$	360 $[\mathrm{M\text{-}H}]^+$	288 $[\mathrm{360\text{-}Cl^{35}Cl^{37}}]^+$
Endosulfan sulphate	422.92	272 $[\mathrm{M\text{-}H_6C_4SO_4}]^+$	237 $[\mathrm{272\text{-}Cl^{35}}]^+$	272 $[\mathrm{M\text{-}H_6C_4SO_4}]^+$	143 $[\mathrm{272\text{-}C_2Cl_3^{35}}]^+$
4,4´DDT	354.49	235 $[\mathrm{M\text{-}HCCl_2^{35}Cl^{37}}]^+$ /$[\mathrm{M\text{-}H_3CCl_3^{35}}]^+$	165 $[\mathrm{235\text{-}Cl_2^{35}}]^+$	237$[\mathrm{M\text{-}HCCl_3^{35}}]^+$	167 $[\mathrm{237\text{-}Cl_2^{35}}]^+$
PCB 138	360.88	360 $[\mathrm{M\text{-}H}]^+$	290 $[\mathrm{360\text{-}Cl_2^{35}}]^+$	360 $[\mathrm{M\text{-}H}]^+$	288 $[\mathrm{360\text{-}Cl^{35}Cl^{37}}]^+$
Dicofol	370.49	139 $[\mathrm{M\text{-}H_5C_7Cl_3^{35}Cl^{37}}]^+$	111 $[\mathrm{139\text{-}CO}]^+$	139 $[\mathrm{M\text{-}H_5C_7Cl_3^{35}Cl^{37}}]^+$	75 $[\mathrm{139\text{-}HCCl^{35}O}]^+$
Methoxychlor	345.65	227 $[\mathrm{M\text{-}H_2CCl_3^{35}}]^+$	169 $[\mathrm{227\text{-}H_2C_2O_2}]^+$	227 $[\mathrm{M\text{-}H_7C_8O}]^+$ / $[\mathrm{M\text{-}H_2CCl_3^{35}}]^+$	115 $[\mathrm{227\text{-}C_8H_{16}}]^+$
PCB 180	395.32	394 $[\mathrm{M\text{-}H}]^+$	324 $[\mathrm{394\text{-}Cl_2^{35}}]^+$	394 $[\mathrm{M\text{-}H}]^+$	359 $[\mathrm{394\text{-}Cl^{35}}]^+$
Mirex	545.54	332 $[\mathrm{M\text{-}H_3Cl_6^{35}}]^+$/ $[\mathrm{M\text{-}HCl_5^{35}Cl^{37}}]^+$	262 $[\mathrm{332\text{-}\ Cl_2^{35}}]^+$	332 $[\mathrm{M\text{-}H_3Cl_6^{35}}]^+$/ $[\mathrm{M\text{-}HCl_5^{35}Cl^{37}}]^+$	260 $[\mathrm{332\text{-}Cl^{35}Cl^{37}}]^+$

For OPEs, characteristic ions and reactions are related with the central phosphate group and the carbonated side chains. Base peaks are sometimes not large enough to be neither specific of the target compound nor suitable for subsequent fragmentation and therefore cannot be used to distinguish from potential interfering compounds containing phosphate or for quantification purposes. For this reason, precursor ions just containing P, O and H and no carbonated side chains were always discarded. However, small ions with m/z of 99 or 81 among others have been selected as product ions because structural specificity is already assured by the precursor ion, which are always selected to contain carbonated side chains that are specific from the target compound. Unambiguous identification with two MRM transitions and the retention time guarantee the correct identification of the analyte. Table 6.2 compiles monitored fragments and their proposed identification for studied OPEs.

Table 6.2. Proposed fragment asignation for MS/MS transitions of studied OCPs and PCBs. M: molecular ion, NA: not assigned.

Compound	MW (g/mol)	Quantifier precursor ion	Quantifier product ion	Qualifier precursor ion	Qualifier product ion
TEP	182.15	155 $[\mathrm{M-C_2H_3}]^+$	99 $[\mathrm{H_4PO_4}]^+$	127 $[\mathrm{M-C_4H_7}]^+$	81 $[\mathrm{H_2PO_3}]^+$
TPP (TiPP)	224.24	141 $[\mathrm{M-C_6H_{11}}]^+$	81 $[\mathrm{H_2PO_3}]^+$	183 $[\mathrm{M-C_3H_5}]^+$	99 $[\mathrm{H_4PO_4}]^+$
TPP (TnPP)	224.24	141 $[\mathrm{M-C_6H_{11}}]^+$	81 $[\mathrm{H_2PO_3}]^+$	183 $[\mathrm{M-C_3H_5}]^+$	99 $[\mathrm{H_4PO_4}]^+$
TnBP	266.32	155 $[\mathrm{M-C_8H_{15}}]^+$	81 $[\mathrm{H_2PO_3}]^+$	211 $[\mathrm{M-C_4H_7}]^+$	99 $[\mathrm{H_4PO_4}]^+$
TCEP	285.49	249 $[\mathrm{M-Cl}]^+$	63 $[\mathrm{PO_2}]^+$	205 $[\mathrm{M-OC_2H_5Cl}]^+$	63 $[\mathrm{PO_2}]^+$
TCIPP	327.57	277 $[\mathrm{M-H_3CCl}]^+$	125 $[\mathrm{H_6C_2PO_4}]^+$	201 $[\mathrm{M-C_4H_8Cl_2}]^+$	125 $[\mathrm{H_6C_2PO_4}]^+$
TDCIPP	430.91	381 $[\mathrm{M-H_3CCl}]^+$	159 $[\mathrm{H_5ClC_2PO_4}]^+$	209 $[\mathrm{M-H_{10}C_6Cl_4}]^+$	75 $[\mathrm{H_4C_3Cl}]^+$
TPhP	326.07	326 $[\mathrm{M}]^+$	169 $[\mathrm{M-C_{12}H_{13}}]^+$	215 $[\mathrm{M-C_9H_7}]^+$	169 $[\mathrm{M-C_{12}H_{13}}]^+$
EHDPP	362.41	251 $[\mathrm{M-C_8H_{15}}]^+$	77 $[\mathrm{C_6H_5}]^+$	251 $[\mathrm{M-C_8H_{15}}]^+$	153 NA
TEHP	434.6	113 $[\mathrm{C_8H_{17}}]^+$	82 $[\mathrm{C_6H_{10}}]^+$	211 $[\mathrm{M-R_2}]^+$	99 $[\mathrm{H_4PO_4}]^+$
TCPs	368.37	368 $[\mathrm{M}]^+$	197 NA	368 $[\mathrm{M}]^+$	181 NA

6.2. Chromatographic coelutions

Coelution is a common effect in chromatography where more than one peak belonging to different analytes are overlapped and therefore their areas cannot be integrated separately so individual quantification is not possible. To solve these problems, 2 different approaches can be applied: improvement of the chromatographic resolution by temperature gradient or adequate column choice or definition of specific MRM transitions.

The chromatographic approach is based on a separation improvement, which will increase the differences in the retention times of different compounds, eventually avoiding the overlapping. This can be made by using longer columns, which increases the differences in the interaction with the stationary phase of coeluting compounds, or decreasinig the temperature gradient, which increase the interaction time with the stationary phase and the difference between retention times of coeluting analytes. Figure 6.1 illustrate the increased retention time difference between TDCPP and the deuterated surrogate homologe TDCPP-D15 in a common DB-5MS column (30 m length, 0.25mm id, 0.25μm fim thickness) with a faster (A) and slower (B) temperature gradient. Chromatographic displament due to deuterium presence has been previously described (Turowski et al., 2003). This strategy, however, has two main inconveniences: it requires higher running times and it produce wider peaks for all the analytes.

The spectrometric strategy is based on structural differences of the coeluted compounds and is suitable for non isomeric compounds with non-common selected transitions. In these cases, an overlapped peak found in the Total Ion Chromatogram (TIC) can be separated in the Extracted Ion Chromatogram (EIC) or extracted reaction chromatogram for MS/MS methods, because the signal of each coeluted compound is specific thus, enables the identification and quantification of each compound in the coeluted peak. Three coelution problems faced during method optimization are described down below.

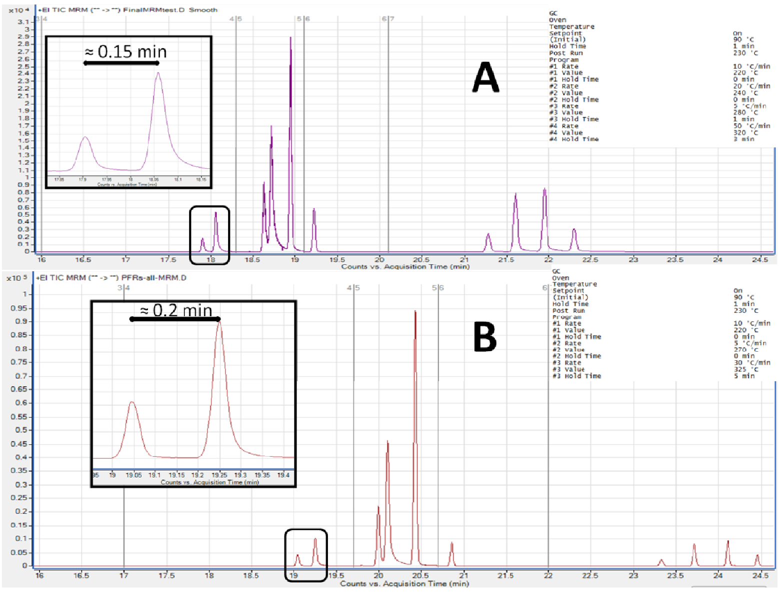


Figure 6.1. Effects of temperature gradient on the chromatographic separation

- Oxychlordane/heptachlor epoxide: Figure 6.2 shows the TIC (A) (obtained in a HP-5MS column (30 m length, 0.25 mm id, 0.25 μm fim thickness), which present a single peak, and EIC of selected reaction for heptachlor epoxides (353 →191) (B) and oxychlordane (185→121) (C) and showing no interferences between them which allows individual determination by specific MRM transitions as monitored ions are selective of each contaminant.

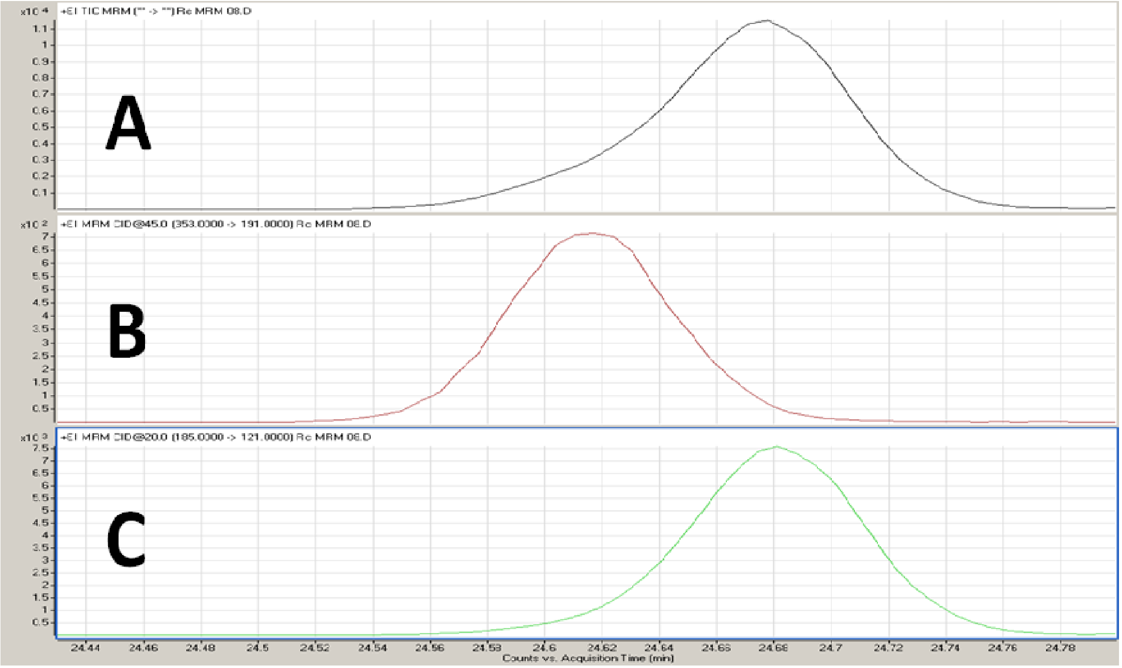


Figure 6.2. Coelution of oxychlordane and heptachlor epoxide. From top to bottom, A) total ion chromatogram, B) extracted reaction chromatogram for heptachlor epoxide and C) extracted reaction chromatogram for oxychlordane.

- 2,4´-DDT/ 4,4´-DDD: In the case of DDT related compounds, the EI fragmentation forms the same fragment ions and thus MRM transitions are the same. Selected precursor ion (m/z 235) is common for both compounds and consists in the double aromatic ring with the central C atom but without the polychlorinated methyl radical (CCl_3 in the case of 2,4´-DDT and CCl_2 for 4,4´-DDD). Therefore, potentially monitored product ions are common for both analytes and efficient chromatographic separation is needed to chromatographically resolve both of them. Figure 6.3 illustrates the separation efficiency with the HP-5MS column (30 m length, 0.25 mm id, 0.25 μm fim thickness) before (A) and after (B) multiple injections. Column bleeding and deterioration produce lower separation rates. Good conservation and maintenance of GC columns as well as good clean-up procedures may delay this problem.

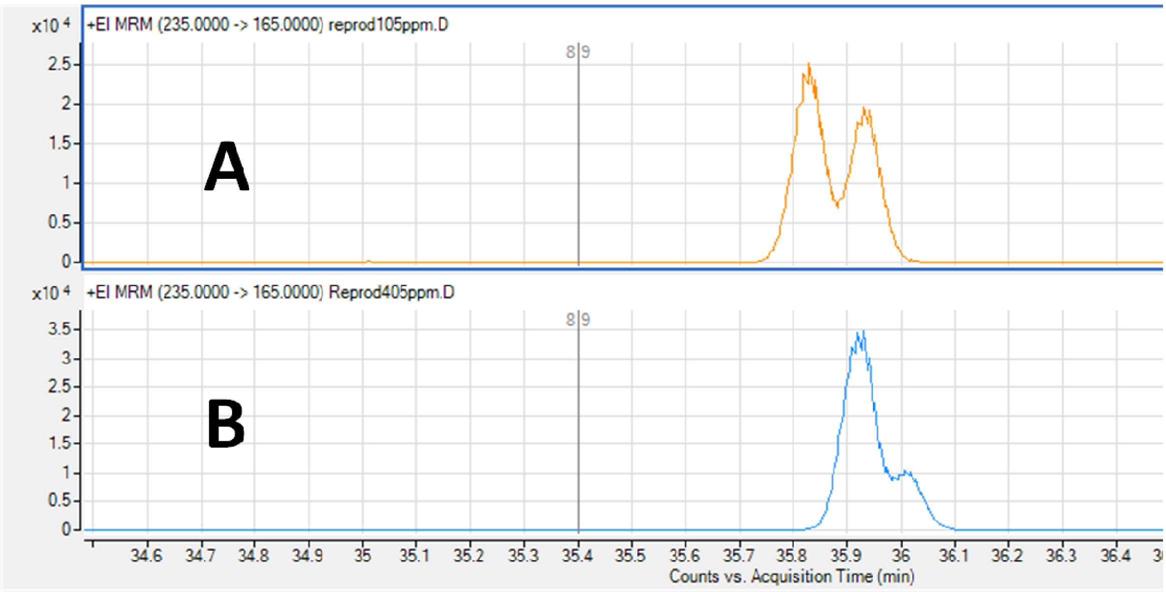


Figure 6.3. Chromatographic separation of 2,4´-DDT and 4,4´-DDD before (A) and after (B) column deterioration.

- TBOEP/TPhP: The coelution of these compounds was detected during method optimization for OPEs. Standard solutions TICs (figure 6.4A) showed a number of peaks lower than the number of target compounds. Injection of individual compounds permitted to identify the retention time of each and select specific transitions for both coeluted compounds (299→55 for tris-butoxyethyl phosphate (TBOEP) and 326→169 for TPhP) and both of them could de effectively separated by their specific MRM transition (figure 6.4B). However, the ion signal for TBOEP was rather small, leading to high detection limits which together with the high variability in quality control samples results and the high blank contribution of blank samples made impossible the detection and quantification of TBOEP at low environmental levels such as the ones expected in protected areas and the compound was dismissed from the multiresidue method.

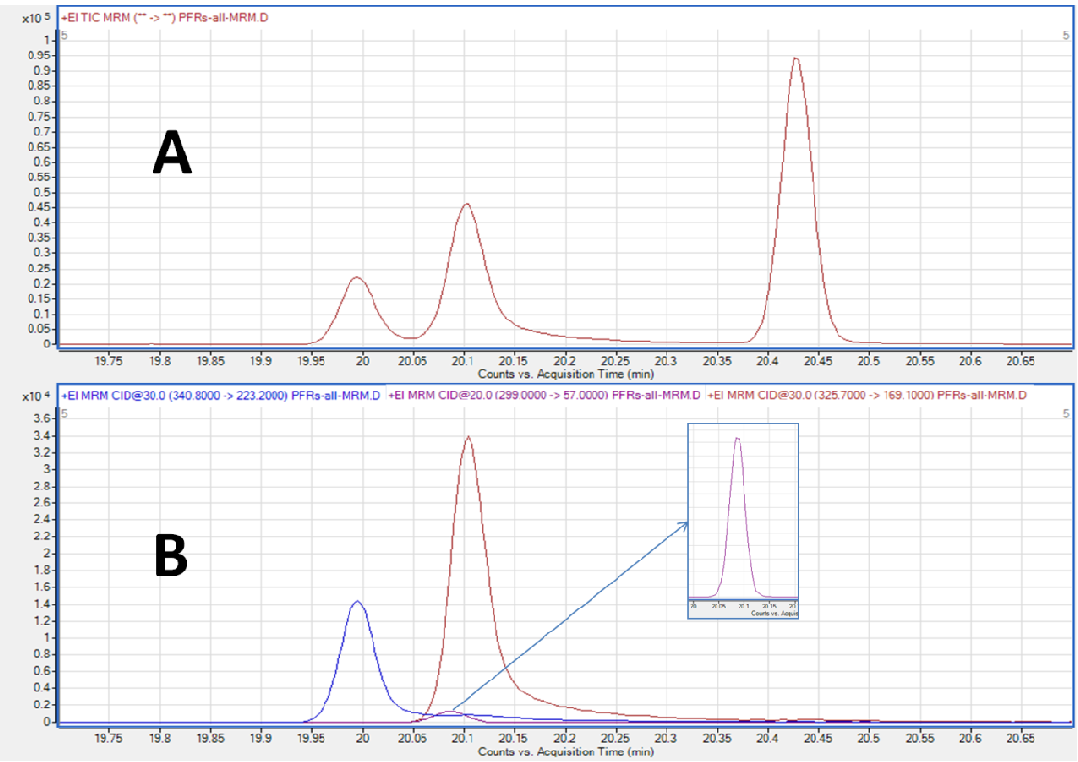


Figure 6.4. Total ion chromatogram (A) and overlapped selected reaction chromatograms (B) for TBOEP and TPhP.

Therefore, in this Thesis a single extraction and a dual analysis by GC-MS/MS and GC-NCI-MS has been developed to allow the trace concentration analysis of OCPs, PCBs, PBDEs and OCPs in biotic and abiotic samples. The methodology developed was validated in terms of recoveries in soil, sediment, water and egg eggs and the method detection limits were calculated. Because the analytical strategy was robust, it was applied in several monitoring studies to evaluate the presence of POPs and OPEs in areas on environmental importance.

6.3. Levels in bird eggs

Bird eggs have been used as bioindicators for environmental pollution in different types of ecosystems. Figure 6.5 shows reported levels in several studies around the world of PBDEs (A), PCBs (B) and OCPs (C) in eggs of laridae and other bird species. Levels are expressed in ng/g ww in a logarithmic scale. Green bars represent obtained results in the present study (see chapter 2).

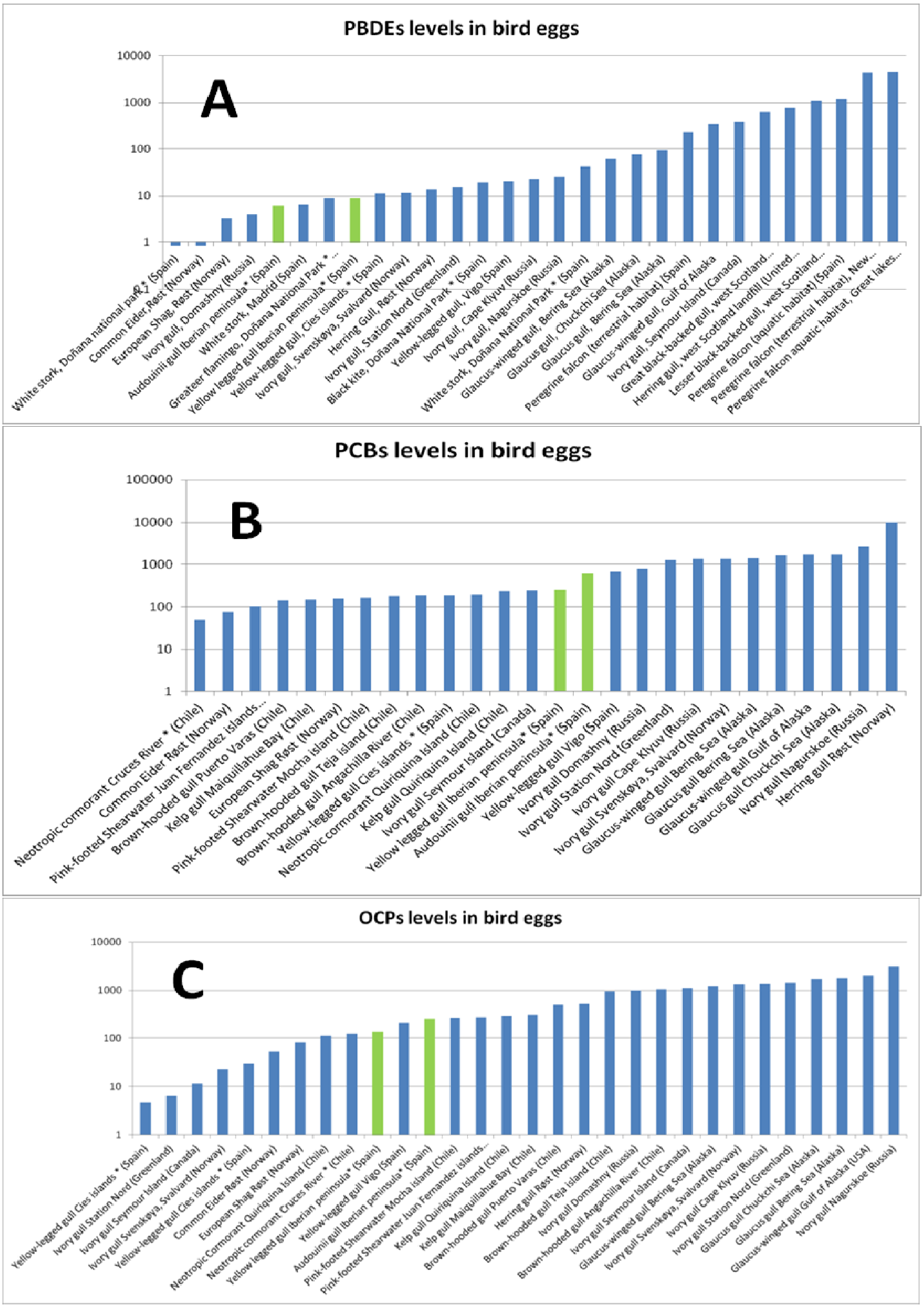


Figure 6.5. Pollution levels in bird eggs from different studies in logarithmic transformed ng/g ww.

The highest PBDEs levels were reported for peregrine falcon eggs due to biomagnification because it is an apex predator (Guerra et al., 2012). On the other hand, the lowest PBDEs levels were found for white storks eggs from Doñana National Park (Spain) and common eider eggs from Norway (Muñoz-Arnanz et al., 2011, Huber et al., 2015). Levels found in the present study are similar to those in the low concentration range found in white storks eggs from central Spain, probably influenced by urban environments and greater flamingo eggs which share a fish based diet (Baron et al., 2015). Differences in reported PCBs levels are lower, encompassing 2 to 3 orders of magnitude, than those ones for PBDEs (variability up to 4 orders of magnitude). Concentrations in audouin and yellow-legged gulls are similar to those found in yellow-legged gull eggs from Vigo and Cies Islands respectively and are in the same order of magnitude than the ones in neotropic cormorant eggs from protected and non-protected areas of Chile but lower than other gull species eggs from cold climates areas as Canada, Norway or Alaska (Lucia et al., 2015, Muñoz-Cifuentes et al., 2005, Vander Pol et al., 2009, Viñas et al., 2020).

OCPs levels in gull eggs from the Iberian Peninsula were above Chilean neotropic cormorant eggs and below pink-footed shearwater, which share feeding habits with audouinii gulls, mostly based on pelagic fish (Muñoz Cifuentes et al., 2005). The highest OCPs levels were reported in eggs from different gull species from high latitudes of the north hemisphere (Alaska, Svalvard island or Nagurskoe in Russia) (Lucia et al., 2015, Vander Pol et al., 2009).

6.4. Levels in soils

Studied compounds have been detected in soils from protected and non-protected areas as well as e-waste sites from different parts of the world. Figure 6.6 compiles reported levels of OPEs (A), PBDEs (B), PCBs (C) and OCPs (D) in soils in order to illustrate the comparison with the results obtained in the present Thesis. Green columns represent soils from studied protected areas in South China (Chapter 4); red columns belong to Baihe village soils; names labeled with an asterisk belong to protected areas. Reported values are expressed in ng/g dw in a logarithmic scale.

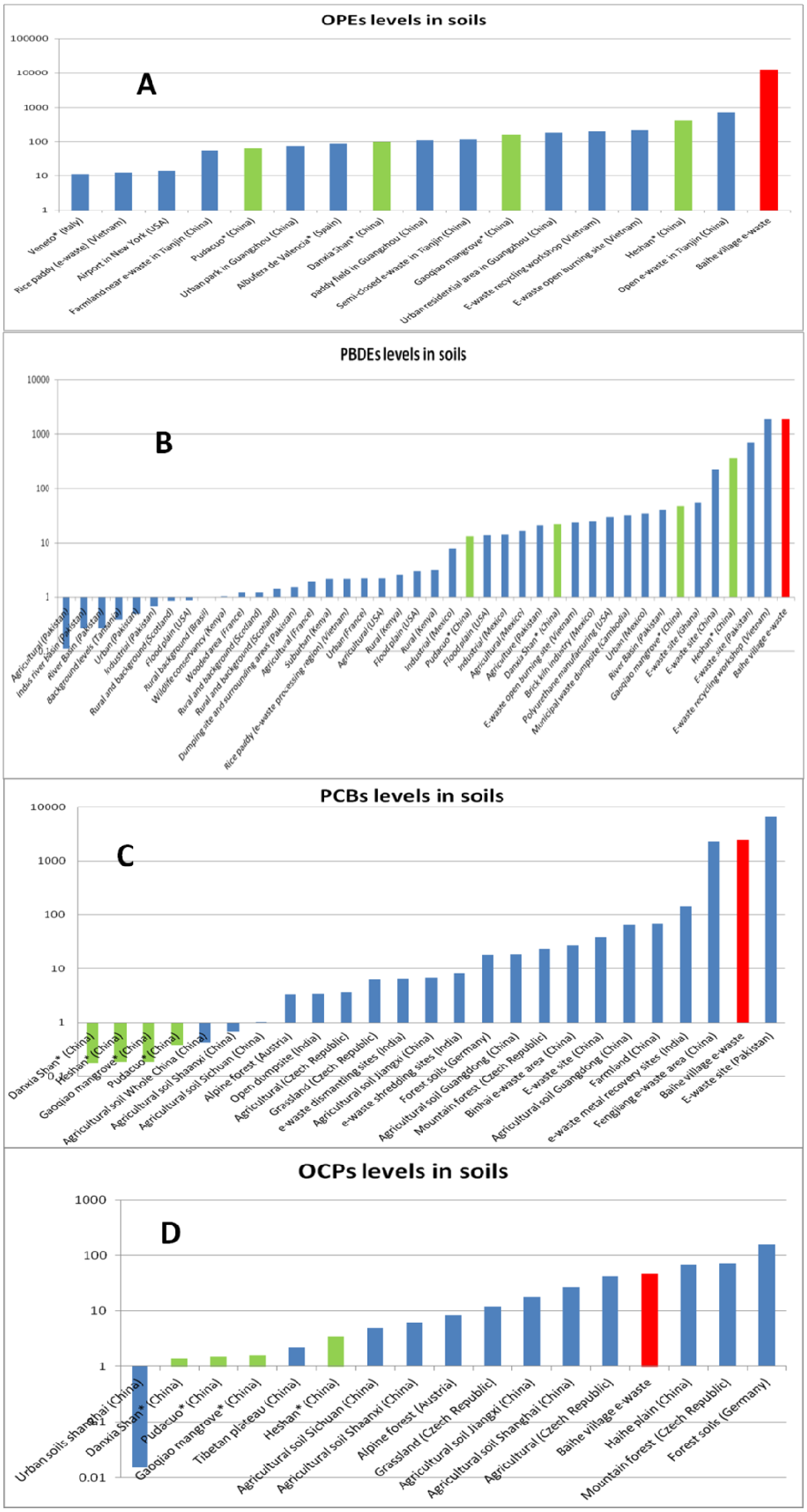


Figure 6.6. Pollution levels in soils compiled from different studies around the world.

OPEs levels in protected areas studied in this Thesis (chapter 4) encompass a range in which urban, agricultural and e-waste areas of Asia are also comprised and are higher than those ones found in a protected area in Veneto (Italy) (Cui et al., 2017, Vechiatto et al., 2021, Wang et al., 2018). Similarly to OPEs, PBDEs levels in chinese protected areas (without considering Heshan research station) are in the same order of magnitude than in industrial and/or agricultural soils from different countries in America and Asia and are higher, from 1 to 2 orders of magnitude, than background levels from Brazil or Scotland (McGrath et al., 2017, Rhind et al., 2013) or rural or agricultural soils levels from Pakistan, Kenya or France, probably related with the high production and consumption volume of BDE 209 in China (Muresan et al., 2010, Sun et al., 2016). E-waste affected soils from Vietnam, Ghana or China were in the 0.01-0.001 ng/g dw range, similar than the ones found in Heshan and slightly lower than Baihe e-waste village (Matsukami et al., 2015, Wang et al., 2018). PCBs concentrations in studied protected areas of South China were, contrarily to PBDEs and OPEs among the lowest ones when compared with natural ecosystems such as grasslands or forests or agricultural soils from Asia and Europe (Holoubek et al., 2009, Sun et al., 2016). E-waste sites are hardly affected by PCBs pollution and levels found in soils from Baihe village are close to those ones found in other e-waste areas in China and Pakistan and higher than e-waste sites in India (Chakraborty et al., 2018, Jiang et al., 2017). OCPs showed a similar situation than PCBs regarding protected areas. Soils from Baihe village e-waste showed similar levels than agricultural or forest soils from Asia and Europe (Aichner et al., 2013, Sun et al., 2018, Weiss et al., 2000).

6.5. Levels in sediments

Sediment pollution has been studied in different ecosystems with varied sources, either local or diffusive, and athropogenic pressures. Reported results of sediment pollution from several studies are represented in Figure 6.7. Levels for OPEs (A), PBDEs (B), PCBs (C) and OCPs (D) are expressed as ng/g dw in a logarithmic scale. Green and red bars represent results from the present Thesis in protected areas and Baihe e-waste respectively.

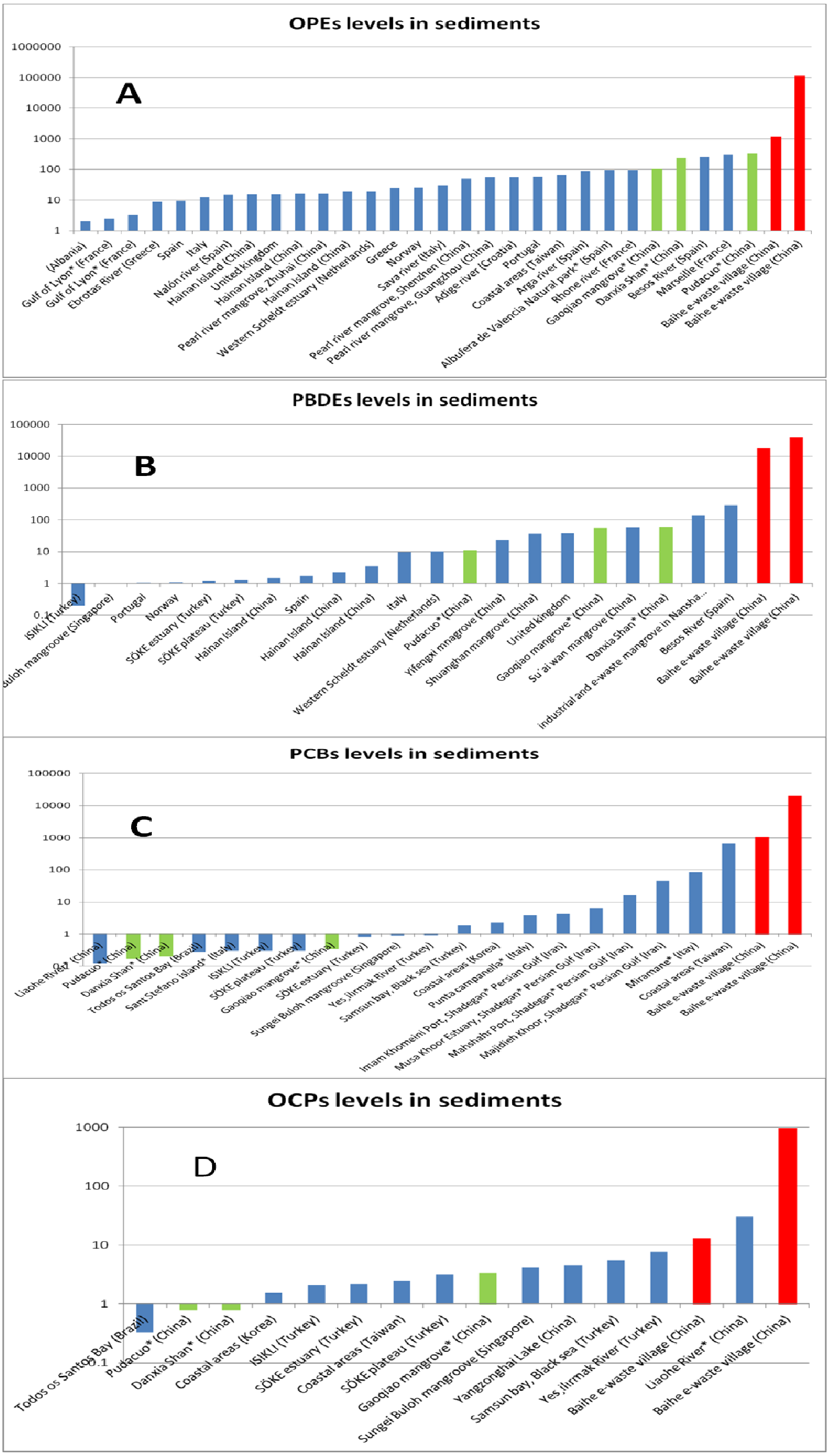


Figure 6.7. Pollution levels in soils compiled from different studies around the world.

OPEs levels in sediments from studied protected areas were within the high range of concentration when compared to the compiled studies around the world, but lower than sediments from the polluted ponds surroundings the e-waste site of Baihe village. Similar OPEs concentrations were reported levels from the Besos river in Spain and marine sediments of Marseille, France (Alkan et al., 2021, Cristale et al., 2013). OPEs levels in studied protected areas from South China were two orders of magnitude higher than protected marine areas of the Gulf of Lyon, France, or Greek rivers and one order of magnitude higher than other mangrove sediments from China or riverine sediments from different countries of Europe such as Spain, Italy or the Netherlands (Aznar-Alemany et al., 2018, Brandsma et al., 2015, Mo et al., 2019, Hu et al., 2017). PBDEs levels from protected areas reported in this Thesis were lower than in the Besos river or in industrially influenced mangrove sediments from Nansha, China (Wu et al., 2017), but one to two order of magnitude higher than marine sediments from Hainan Island, China, or river sediments from Turkey (Kocagöz et al., 2014, Mo et al., 2019) and in the same order of magnitude than other mangrove sediments from China or river sediments from the United Kingdom (Aznar-Alemany et al., 2018, Liu et al., 2017). PCBs, however, showed a different situation; levels from studied protected areas from South China were in the same order of magnitude than sediments from other protected areas such as Sant Stefano Island in Italy or the Liaohe river in China but lower than marine sediments from protected areas in the Iranian Persian Gulf or Miramane and Punta Campanella in Italy (Ke et al., 2018, Pozo et al., 2009, Zahed et al., 2009). Regarding OCPs, levels in sediments from Danxia Shan and Pudacuo were within those reported for Todos os Santos Bay in Brazil and median levels of marine sediments from Korea (Choi et al., 2011, Sotão Neto et al. 2020). Sediments from Gaoqiao showed similar levels than Sungei Buloh mangrove in Singapore, marine sediments from Taiwan or River sediments from Turkey (Bayen et al., 2005, Cheng et al., 2021, Kocagöz et al., 2014). Sediments from the polluted pond in Baihe village were the highest among evaluated studies, while the rest of sedimentfs from the ponds close to the Bahie village were within the range of other sites from China as the Liaohe river sediments (Ke et al., 2018).

References

Aichner, B., Bussian, B., Lehnik-Habrink, P., Hein, S., 2013. Levels and spatial distribution of persistent organic pollutants in the environment: a case study of German forest soils. Environ Sci Technol 47, 12703-12714, 10.1021/es4019833

Ali U, Mahmood A, Syed J H, Li J, Zhang G, Katsoyiannis A, Jones K C, Malik R N, 2015. Assessing the combined influence of TOC and black carbon in soil-air partitioning of PBDEs and DPs from the Indus River Basin, Pakistan. Environ Pollut 201, 131-140, 10.1016/j.envpol.2015.02.035

Alkan, N., Alkan, A., Castro-Jimenez, J., Royer, F., Papillon, L., Ourgaud, M., Sempere, R., 2021. Environmental occurrence of phthalate and organophosphate esters in sediments across the Gulf of Lion (NW Mediterranean Sea). Sci Total Environ 760, 143412, 10.1016/j.scitotenv.2020.143412

Aznar-Alemany, O., Aminot, Y., Vila-Cano, J., Kock-Schulmeyer, M., Readman, J.W., Marques, A., Godinho, L., Botteon, E., Ferrari, F., Boti, V., Albanis, T., Eljarrat, E., Barcelo, D., 2018. Halogenated and organophosphorus flame retardants in European aquaculture samples. Sci Total Environ 612, 492-500, 10.1016/j.scitotenv.2017.08.199

Baron, E., Bosch, C., Manez, M., Andreu, A., Sergio, F., Hiraldo, F., Eljarrat, E., Barcelo, D., 2015. Temporal trends in classical and alternative flame retardants in bird eggs from Donana Natural Space and surrounding areas (south-western Spain) between 1999 and 2013. Chemosphere 138, 316-323, 10.1016/j.chemosphere.2015.06.013

Bayen, S., Wurl, O., Karuppiah, S., Sivasothi, N., Lee, H.K., Obbard, J.P., 2005. Persistent organic pollutants in mangrove food webs in Singapore. Chemosphere 61, 303-313, 10.1016/j.chemosphere.2005.02.097

Brandsma, S.H., Leonards, P.E., Leslie, H.A., de Boer, J., 2015. Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web. Sci Total Environ 505, 22-31, 10.1016/j.scitotenv.2014.08.072

Chakraborty, P., Selvaraj, S., Nakamura, M., Prithiviraj, B., Cincinelli, A., Bang, J.J., 2018. PCBs and PCDD/Fs in soil from informal e-waste recycling sites and open dumpsites in India: Levels, congener profiles and health risk assessment. Sci Total Environ 621, 930-938, 10.1016/j.scitotenv.2017.11.083

Chen, D., Letcher, R.J., Chu, S., 2012. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography-tandem quadrupole mass spectrometry. J Chromatogr A 1220, 169-174, 10.1016/j.chroma.2011.11.046

Cheng, J.O., Tseng, P.H., Chou, P.H., Hsieh, C.Y., Ko, F.C., 2021. Revisiting of persistent organic pollution occurrence and distribution in the surface sediment along western Taiwan coast. Mar Pollut Bull 173, 113118, 10.1016/j.marpolbul.2021.113118

Choi H. G., Moon H. B., Choi M., Yu J., 2011. Monitoring of organic contaminants in sediments from the Korean coast: spatial distribution and temporal trends (2001-2007). Mar Pollut Bull 62, 1352-1361, 10.1016/j.marpolbul.2011.03.029

Choo, G., Ekpe, O.D., Park, K.W., Chung, D., Lee, J., Oh, J.E., 2022. Temporal and spatial trends of chlorinated paraffins and organophosphate flame retardants in black-tailed gull (Larus crassirostris) eggs. Sci Total Environ 803, 150137, 10.1016/j.scitotenv.2021.150137

Cristale, J., Garcia Vazquez, A., Barata, C., Lacorte, S., 2013. Priority and emerging flame retardants in rivers: occurrence in water and sediment, Daphnia magna toxicity and risk assessment. Environ Int 59, 232-243, 10.1016/j.envint.2013.06.011

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, 10.1016/j.chemosphere.2017.02.070

Dinc, B., Celebi, A., Avaz, G., Canli, O., Guzel, B., Eren, B., Yetis, U., 2021. Spatial distribution and source identification of persistent organic pollutants in the sediments of the Yesilirmak River and coastal area in the Black Sea. Mar Pollut Bull 172, 112884, 10.1016/j.marpolbul.2021.112884

Fu, S., Chu, S., Xu, X., 2001. Organochlorine pesticide residue in soils from Tibet, China. Bull Environ Contam Toxicol 66, 171-177, 10.1007/s001280000221

Guerra, P., Alaee, M., Jimenez, B., Pacepavicius, G., Marvin, C., MacInnis, G., Eljarrat, E., Barcelo, D., Champoux, L., Fernie, K., 2012. Emerging and historical brominated flame retardants in peregrine falcon (Falco peregrinus) eggs from Canada and Spain. Environ Int 40, 179-186, 10.1016/j.envint.2011.07.014

Holoubek, I., Dusek, L., Sanka, M., Hofman, J., Cupr, P., Jarkovsky, J., Zbiral, J., Klanova, J., 2009. Soil burdens of persistent organic pollutants--their levels, fate and risk. Part I. Variation of concentration ranges according to different soil uses and locations. Environ Pollut 157, 3207-3217, 10.1016/j.envpol.2009.05.031

Hu, Y.X., Sun, Y.X., Li, X., Xu, W.H., Zhang, Y., Luo, X.J., Dai, S.H., Xu, X.R., Mai, B.X., 2017. Organophosphorus flame retardants in mangrove sediments from the Pearl River Estuary, South China. Chemosphere 181, 433-439, 10.1016/j.chemosphere.2017.04.117

Huber, S., Warner, N.A., Nygard, T., Remberger, M., Harju, M., Uggerud, H.T., Kaj, L., Hanssen, L., 2015. A broad cocktail of environmental pollutants found in eggs of three seabird species from remote colonies in Norway. Environ Toxicol Chem 34, 1296-1308, 10.1002/etc.2956

Jiang, L., Cheng, Z., Zhang, D., Song, M., Wang, Y., Luo, C., Yin, H., Li, J., Zhang, G., 2017. The influence of e-waste recycling on the molecular ecological network of soil microbial communities in Pakistan and China. Environ Pollut 231, 173-181, 10.1016/j.envpol.2017.08.003

Ke, X., Bao, Q., Qi, Y., Huang, X., Zhang, H., 2018. Toxicity assessment of sediments from the Liaohe River Protected Area (China) under the influence of ammonia nitrogen, heavy metals and organic contaminants. Environ Toxicol Pharmacol 59, 34-42, 10.1016/j.etap.2018.02.008

Kocagoz, R., Onmus, O., Onat, I., Cagdas, B., Siki, M., Orhan, H., 2014. Environmental and biological monitoring of persistent organic pollutants in waterbirds by non-invasive versus invasive sampling. Toxicol Lett 230, 208-217, 10.1016/j.toxlet.2014.01.044

Kock-Schulmeyer, M., Ginebreda, A., Petrovic, M., Giulivo, M., Aznar-Alemany, O., Eljarrat, E., Valle-Sistac, J., Molins-Delgado, D., Diaz-Cruz, M.S., Monllor-Alcaraz, L.S., Guillem-Argiles, N., Martinez, E., Miren, L.A., Llorca, M., Farre, M., Pena, J.M., Mandaric, L., Perez, S., Majone, B., Bellin, A., Kalogianni, E., Skoulikidis, N.T., Milacic, R., Barcelo, D., 2021. Priority and emerging organic microcontaminants in three Mediterranean river basins: Occurrence, spatial distribution, and identification of river basin specific pollutants. Sci Total Environ 754, 142344, 10.1016/j.scitotenv.2020.142344

Li, W., Wang, Y., Kannan, K., 2019. Occurrence, distribution and human exposure to 20 organophosphate esters in air, soil, pine needles, river water, and dust samples collected around an airport in New York state, United States. Environ Int 131, 105054, 10.1016/j.envint.2019.105054

Li, Z., He, C., Thai, P., Wang, X., Braunig, J., Yu, Y., Luo, X., Mai, B., Mueller, J.F., 2020. Organophosphate esters and their specific metabolites in chicken eggs from across Australia: Occurrence, profile, and distribution between yolk and albumin fractions. Environ Pollut 262, 114260, 10.1016/j.envpol.2020.114260

Liu Y., Meng F,. 2011. Distribution of HCHs and DDTs in agricultural soil of Pudong new area, Shanghai. Proc Environ Sci 11, 1296-1301, 10.1016/j.proenv.2011.12.194

Liu, X.C., Wu, W.C., Zhang, Y.B., Wang, T., Zhao, J.G., Chen, Z.H., 2017. Occurrence, profiles, and ecological risks of polybrominated diphenyl ethers in mangrove sediments of Shantou, China. Environ Sci Pollut Res Int 24, 3608-3617, 10.1007/s11356-016-8112-0

Liu C., Wei B. K., Bao J. S., Wang Y., Hu J. C., Tang Y. E., Chen T., Jin J., 2020. Polychlorinated Biphenyls in the soil-crop-atmosphere system in e-waste dismantling areas in Taizhou: concentrations, congener profiles, uptake, and translocation. Environ Pollut 257, 113622, 10.1016/j.envpol.2019.113622

Lorenzo, M., Campo, J., Picó, Y., 2018. Determination of organophosphate flame retardants in soil and fish using ultrasound-assisted extraction, solid-phase clean-up, and chromatography with tandem mass spectrometry. J Sep Sci 41, 2595–2603. 10.1002/jssc.201701461

Lorenzo M., Campo J., Morales Suarez-Varela M., Pico Y., 2019. Occurrence, distribution and behavior of emerging Persistent Organic Pollutants (Pops) in a

Mediterranean wetland protected area. Sci Total Environ 646, 1009-1020, 10.1016/j.scitotenv.2018.07.304

Lucia, M., Verboven, N., Strom, H., Miljeteig, C., Gavrilo, M.V., Braune, B.M., Boertmann, D., Gabrielsen, G.W., 2015. Circumpolar contamination in eggs of the high-Arctic ivory gull Pagophila eburnea. Environ Toxicol Chem 34, 1552-1561, 10.1002/etc.2935

Matsukami, H., Tue, N.M., Suzuki, G., Someya, M., Tuyen le, H., Viet, P.H., Takahashi, S., Tanabe, S., Takigami, H., 2015. Flame retardant emission from e-waste recycling operation in northern Vietnam: environmental occurrence of emerging organophosphorus esters used as alternatives for PBDEs. Sci Total Environ 514, 492-499, 10.1016/j.scitotenv.2015.02.008

McGrath, T.J., Ball, A.S., Clarke, B.O., 2017. Critical review of soil contamination by polybrominated diphenyl ethers (PBDEs) and novel brominated flame retardants (NBFRs); concentrations, sources and congener profiles. Environ Pollut 230, 741-757, 10.1016/j.envpol.2017.07.009

Mo, L., Zheng, J., Wang, T., Shi, Y.G., Chen, B.J., Liu, B., Ma, Y.H., Li, M., Zhuo, L., Chen, S.J., 2019. Legacy and emerging contaminants in coastal surface sediments around Hainan Island in South China. Chemosphere 215, 133-141, 10.1016/j.chemosphere.2018.10.022

Muñoz-Arnanz, J., Saez, M., Aguirre, J.I., Hiraldo, F., Baos, R., Pacepavicius, G., Alaee, M., Jimenez, B., 2011. Predominance of BDE-209 and other higher brominated diphenyl ethers in eggs of white stork (Ciconia ciconia) colonies from Spain. Environ Int 37, 572-576, 10.1016/j.envint.2010.11.013

Muñoz-Cifuentes, J., Becker, P.H., Sommer, U., Pacheco, P., Schlatter, R., 2003. Seabird eggs as bioindicators of chemical contamination in Chile. Environ Pollut 126, 123-137, 10.1016/s0269-7491(02)00400-1

Pozo, K., Lazzerini, D., Perra, G., Volpi, V., Corsolini, S., Focardi, S., 2009. Levels and spatial distribution of polychlorinated biphenyls (PCBs) in superficial sediment from 15 Italian Marine Protected Areas (MPA). Mar Pollut Bull 58, 773-776, 10.1016/j.marpolbul.2009.03.003

Sotao Neto B. M. T., Combi T., Taniguchi S., Albergaria-Barbosa A. C. R., Ramos R. B., Figueira R. C. L., Montone R. C., 2020. Persistent Organic Pollutants (POPs) and Personal Care Products (PCPs) in the surface sediments of a large tropical bay (Todos Os Santos Bay, Brazil). Mar Pollut Bull 161, 111818, 10.1016/j.marpolbul.2020.111818

Sun, J., Pan, L., Tsang, D.C.W., Zhan, Y., Zhu, L., Li, X., 2018. Organic contamination and remediation in the agricultural soils of China: A critical review. Sci Total Environ 615, 724-740, 10.1016/j.scitotenv.2017.09.271

Tao S, Liu W, Li Y, Yang Y, Zuo Q, Li B, Cao J, 2008. Organochlorine Pesticides contaminated surface soil as reemission source in the Haihe Plain, China. Environ. Sci. Technol 42, 8395–8400

Tongue, A.D.W., Fernie, K.J., Harrad, S., Drage, D.S., McGill, R.A.R., Reynolds, S.J., 2021. Interspecies comparisons of brominated flame retardants in relation to foraging ecology and behaviour of gulls frequenting a UK landfill. Sci Total Environ 764, 142890, 10.1016/j.scitotenv.2020.142890

Turowski, M., Yamakawa, N., Meller, J., Kimata, K., Ikegami, T., Hosoya, K., Tanaka, N., Thornton, E.R., 2003. Deuterium isotope effects on hydrophobic interactions: the importance of dispersion interactions in the hydrophobic phase. J Am Chem Soc 125, 13836-13849, 10.1021/ja036006g

Vander Pol, S.S., Becker, P.R., Ellisor, M.B., Moors, A.J., Pugh, R.S., Roseneau, D.G., 2009. Monitoring organic contaminants in eggs of glaucous and glaucous-winged gulls (Larus hyperboreus and Larus glaucescens) from Alaska. Environ Pollut 157, 755-762, 10.1016/j.envpol.2008.11.026

Vecchiato, M., Bonato, T., Barbante, C., Gambaro, A., Piazza, R., 2021. Organic pollutants in protected plain areas: The occurrence of PAHs, musks, UV-filters, flame retardants and hydrocarbons in woodland soils. Sci Total Environ 796, 149003, 10.1016/j.scitotenv.2021.149003

Vinas, L., Besada, V., Perez-Fernandez, B., Bode, A., 2020. Yellow-legged gull eggs (Larus michahellis) as persistent organic pollutants and trace metal bioindicator for two nearby areas with different human impact. Environ Res 190, 110026, 10.1016/j.envres.2020.110026

Wang, Y., Sun, H., Zhu, H., Yao, Y., Chen, H., Ren, C., Wu, F., Kannan, K., 2018. Occurrence and distribution of organophosphate flame retardants (OPFRs) in soil and outdoor settled dust from a multi-waste recycling area in China. Sci Total Environ 625, 1056-1064, 10.1016/j.scitotenv.2018.01.013

Weiss, P.; Lorbeer, G.; Scharf, S., 2000. Regional aspects and statistical characterisation of the load with semivolatile organic compounds at remote Austrian forest sites. Chemosphere 40, 1159−1171

Wu, Q., Liu, X., Liang, C., Leung, J.Y.S., Li, H., Chen, S., Mai, B., Miao, S., Chen, Y., Wu, Z., Chen, Z., 2017. Historical trends and ecological risks of polybrominated diphenyl ethers (PBDEs) and alternative halogenated flame retardants (AHFRs) in a mangrove in South China. Sci Total Environ 599-600, 181-187

Yuan, H., Liu, E., Zhang, E., Luo, W., Chen, L., Wang, C., Lin, Q., 2017. Historical records and sources of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in sediment from a representative plateau lake, China. Chemosphere 173, 78-88, 10.1016/j.chemosphere.2017.01.047

Zahed, M.A., Nabi Bidhendi, G., Pardakhti, A., Esmaili-Sari, A., Mohajeri, S., 2009. Determination of polychlorinated biphenyl congeners in water and sediment in North West Persian Gulf, Iran. Bull Environ Contam Toxicol 83, 899-902, 10.1007/s00128-009-9874-6

7. Conclusions

The results and discussion of the present Thesis permit to extract the following conclusions:

- The analytical strategy developed based on GC-MS/MS for the determination of OCPs, PCBs and OPEs, and GC-NCI-MS for the analysis of PBDEs has been validated and successfully applied in a variety of environmental matrixes such as soil, sediment, bird eggs, combined with suitable sample treatments for each matrix.
- An extraction method to determine OPEs in water has been developed and the blank contribution has been carefully evaluated to accurately quantify the concentrations of OPEs without external contamination. Quality parameters are reported to ensure the performance of the method.
- Gull eggs are suitable bioindicators of PBDEs, PCBs and OCPs pollution and different contamination patterns were observed in 9 breeding areas of the Iberian Peninsula. The use of gull eggs as bioindicators provide information on potential pollution levels of other species sharing habitats and with similar feeding habits.
- Pollution burdens in gull eggs are related with the diet and may vary among species; eggs from *Larus audouinii* contained higher concentrations than eggs from *Larus michahellis* due to the strict fish based diet of the former and the opportunistic and scavenging habits of the later.
- The proposed strategy to monitor POPs in gull eggs enable comparison of the pollution status of laridae species in different areas around the world and contribute to the POP biomonitoring using bird species according to the Stockholm Convention.
- Soils and sediments accumulate organic pollutants, becoming sinks and reservoirs of pollutants which reflect the anthropogenic activities carried out in the surrounding environment.
- Protected areas from both Spain and China are not pristine as would be expected and are, in fact, affected by chemical anthropogenic pollution coming from different sources such as industrial activities outside of the protected areas borders, touristic activities taking place inside (usually in the buffer zone) or even background diffusive sources.

- Studied protected areas in Spain are mostly affected by legacy pollutants like OCPs and PCBs which may produce toxic effects in fauna in the long term, disturbing the ecological equilibriums and interfering with the legal environmental protection objectives.
- Studied protected areas in South China showed different pollution levels and profiles, probably related with differential anthropogenic activities, inside or in the surroundings, and differential transport and accumulation of the pollutants in the 4 different studied ecosystems.
- Pollution burden in studied protected areas from South China was higher for OPEs than for legacy POPs due to their recent use and discharge to the environment. Among POPs, BDE 209 was the dominant pollutant in most samples and α-HCH was ubiquitous, in both cases due to the extensive production and use during last decades.
- E-waste disassembling and recycling in non-formal facilities continues to be a concerning environmental problem in China despite the development of new laws and regulations.
- Mismanagement of plastic subproducts and residues in the e-waste recycling facility in Baihe village (China) has lead to their accumulation in a pond, which act as the pollution source for the whole ecosystem. EEE recycling activities was the main economical activity in this area but produced a profound environmental impact.
- Emerging pollutants like OPEs were dominant pollutants in soils and sediments from Baihe village due to their use as flame retardants and plasticizers followed by legacy POPs such as PBDEs and PCBs. OCPs were present at much lower levels.
- Free ranged hens were also affected by studied pollutants and eggs consumption represent a route for human dietary exposure with low to moderate risks for PCBs and PBDEs.
- Overall this Thesis concludes that despite POPs are legacy compounds not used at present, they are widespread in hotspots of contamination, like an e-wast site, but are also present in remote areas with high level of protection and thus, meaning that years after prohibition and despite regulatory issues and controls, they are widespread and ubiquitous in the environment.

Emerging OPEs were also detected at very high concentrations, indicating a future environmental problem. Therefore, monitoring studies as the ones proposed in this Thesis are a good way to determine the occurrence, partitioning and fate of contaminants that have high toxicological implications for the environment and human health.