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Transfer and effects of brominated flame retardants (BFRs) on three plant species and one earthworm species in anthroposoils

Cláudia Filipa Coelho Macedo

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Cláudia Filipa Macedo Coelho

**Transfert et effets des retardateurs
de flamme bromés (RFBs) sur trois
espèces végétales et une espèce de
lombric dans des anthroposols**

Devant le jury composé de :

GOURDON, Rémy
BERVOETS, Lieven
SILVA, Eduardo
DE VAUFLEURY, Annette
BAZIN, Christine

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Résumé

La pollution des sols et sédiments par des polluants organiques persistants (POPs) a augmenté ces dernières décennies en raison des processus d'industrialisation et d'urbanisation, affectant la qualité de l'environnement et de la santé humaine. Une étude approfondie a été menée dans deux zones distinctes (Estarreja au Portugal, noté EST, et Casier Peyraud 6 en France, noté PEY) pour évaluer les niveaux de contamination, ainsi que le comportement des contaminants dans des anthroposols et les risques potentiels de ces contaminants pour des organismes vivants du sol. L'objectif principal a été d'évaluer la mobilité environnementale des retardateurs de flamme bromés (RFBs) dans un écosystème terrestre, notamment vers les vers de terre et les plantes, dans une perspective d'évaluation du risque de transferts de ces molécules.

Les niveaux en POPs (RFBs, mais aussi PCBs et PCDD/DFs) dans ces anthroposols ont été quantifiés ainsi que dans les plantes collectées sur les sites étudiés. Une évaluation *in situ* de l'activité biologique a été réalisée par la collecte, le dénombrement et l'identification des organismes vivants visibles (mésosofaune), ainsi que par l'application du test Bait-lamina ©. Une espèce de ver de terre (*Eisenia fetida*) et trois espèces de plants (luzerne (*Medicago sativa*), cresson (*Nasturtium officinale*) et moutarde blanche (*Sinapsis alba*)) ont été choisies pour réaliser des tests en laboratoire de toxicité et de bioaccumulation.

Le facteur de bioaccumulation (BAF) ainsi que les indices Sum of Excess of Transfer (SET) et Evaluation of the Risk of the Transferred Metal Elements (ERITME) ont été calculés. Le BAF permet de déterminer si une substance est accumulée dans un organisme donné et s'il existe un risque d'entrée et de diffusion tout au long de la chaîne alimentaire. Les indices SET et ERITME permettent de classer les sites testés en fonction du transfert efficace des POPs par les anthroposols aux organismes testés, et d'avoir une idée du risque potentiel pour l'écosystème. L'indice SET donne une idée globale de l'excès de transfert pour tous les contaminants dans les matrices étudiées. L'indice ERITME permet d'évaluer le risque environnemental global inhérent associé à l'excès de transfert des contaminants considérés.

Les huit anthroposols étudiés sont assez différents en termes de caractéristiques physico-chimiques ainsi que de contaminations inorganiques et organiques. En général, les anthroposols recueillies sur le site de Peyraud 6 présentent des niveaux plus élevés en POPs. Quelques effets toxiques ont été observés à la fois pour *E. fetida* et pour les espèces de plantes cultivées dans ces huit anthroposols étudiés, notamment en ce qui concerne le taux de reproduction d'*E. fetida*, la masse corporelle d'*E. fetida*, le taux de germination et la hauteur maximale des parties aériennes végétales. Les données obtenues ont clairement révélé la bioaccumulation des POPs quantifiés chez les adultes et les juvéniles d'*E. fetida* ainsi que dans les tissus des espèces végétales cultivées, même à des concentrations très faibles dans le cas des nouveaux

retardateurs de flamme bromées. Différentes familles d'POPs ont montré des comportements différents en ce qui concerne les corrélations avec les paramètres physico-chimiques et l'absorption par *E. fetida*. Les valeurs de BAFs obtenus montrent le potentiel d'accumulation de ces POPs dans les tissus d'*E. fetida*. Les valeurs de BAF obtenus pour les plantes testées indiquent une grande différence dans la disponibilité des contaminants dans les sols des deux sites considérés. Bien que les niveaux d'POPs soient significativement plus élevés dans PEY Ic que dans EST G, il semble que les POPs soient plus disponibles pour être absorbés par les plantes cultivées sur EST G. Cette différence de disponibilité quantifiée peut être due à certains facteurs physico-chimiques du sol, à savoir la nature de la MO, la teneur en COT et le pH légèrement acide de l'anthroposol EST G.

Compte tenu des valeurs ERITME, les matrices étudiées peuvent être classées dans un ordre de toxicité croissant: PEY Ic > PEY Iib > PEY Iva > PEY IIIa > EST G > EST G > EST K > EST C. Cet ordre de toxicité est conforme pour l'ensemble des organismes testés (*E. fetida*, luzerne, cresson et moutarde), et les valeurs d'effet les plus élevées ont été généralement enregistrées dans les échantillons PEY et spécialement dans PEY Ic suivi de PEY Iib. Dans le cas des échantillons d'EST, les valeurs étaient en général toujours inférieures à celles enregistrées dans les échantillons de PEY ; l'anthroposol EST G étant le plus contaminé et les tissus des organismes exposés à cet anthroposol, présentaient les taux les plus élevés dans leurs tissus par rapport aux trois autres sols d'EST.

L'approche traditionnelle suggère l'utilisation du calcul du BAF qui est généralement considérée pour évaluer le risque de contamination. Toutefois, le BAF présente certaines limites, car il ne comporte pas l'effet cumulatif (il considère chaque contaminant individuellement, et donc de manière moins globale). Les approches SET et ERITME ont été développées pour remédier à cette lacune car elles sont basées sur des mesures réelles dans les organismes. Par conséquent, ces index plus réalistes se sont révélés utiles pour l'évaluation des risques et la gestion des sites contaminés, car ils tiennent compte de la somme de tous les contaminants et pas un par un comme dans le calcul du BAF.

Abstract

Soil and sediment pollution with persistent organic pollutants (POPs) has been a rising concern in recent decades mainly due to industrialization and urbanization processes, affecting environmental quality and human health. A comprehensive study was conducted in two distinct areas (Estarreja in Portugal, noted EST, and Casier Peyraud 6 in France, noted PEY) to assess the contamination levels as well as the behavior of contaminants in soils and the potential risks posed by these contaminants to soil organisms. The principal aim was to evaluate the mobility and environmental availability of brominated flame retardants (BFRs) in a terrestrial ecosystem, namely to earthworms and plants, concerning the BFRs transfer risk evaluation. Levels of POPs (BFRs, and also PCBs, PCDD/DFs) in the anthroposoils as well as plants collected in the studied sites were quantified. An *in-situ* evaluation of biological activity was performed through monoliths collection, counting and identification of the visible living organisms (mesofauna) as well as applying the Bait-lamina© test. One earthworm species (*Eisenia fetida*) and three plant species (alfalfa (*Medicago sativa*), watercress (*Nasturtium officinale*) and white mustard (*Sinapis alba*)) were chosen to conduct toxicity and bioaccumulation laboratory tests.

The Bioaccumulation factor (BAF) as well as the Sum of Excess of Transfer (SET) and Evaluation of the Risk of the Transferred Metal Elements (ERITME) indexes were calculated. The BAF allow to determine if a substance is accumulated in a certain organism and if there exists the risk of entry and diffusion along the food chain. SET and ERITME indexes allow to rank the tested sites according to the effective POPs transfer from anthroposoils to the tested organisms and have an idea of the potential risk to the ecosystem. The SET index gives a global idea of the excess of transfer for all the contaminants in the studied matrices. The ERITME index allows to evaluate the possible inherent global environmental risk associated with the excess of transfer on the considered contaminants.

The eight studied anthroposoils are quite different in terms of physico-chemical characteristics as well as in inorganic and organic contamination. In general, anthroposoils collected in Peyraud 6 site presented higher levels of quantified POPs. Some toxic effects were noticed both for *E. fetida* as for the three plant species cultivated in the eight studied anthroposoils, namely concerning the *E. fetida* reproduction rate, *E. fetida* body mass, plants seeds germination rate and maximal height of plants aerial parts. Data obtained clearly revealed the occurrence of bioaccumulation of the quantified POPs in both adults and juveniles *E. fetida* as well as in the cultivated plant species tissues even at very low concentrations in the case of the new BFRs. Different POPs families showed different compartments regarding the correlations with physico-chemical parameters and uptake by *E. fetida*. The obtained BAFs values clearly show

the potential of these POPs to accumulate in *E. fetida* tissues. The obtained BAFs values for plants tested clearly indicates a great difference in the availability of the contaminants in the soils of both considered sites. Despite that the levels of POPs were significantly higher in PEY Ic than in EST G, it seems that the POPs were more available to be taken up by plants cultivated in the anthroposoil EST G. This difference in quantified POPs availability can be due to some physico-chemical parameters of the soil, namely the nature of OM, TOC and the slightly acidic pH of EST G anthroposoil.

Considering the ERITME values, the studied matrices can be classified in an apparent increasing order of toxicity: PEY Ic > PEY Iib > PEY Iva > PEY IIIa > EST G > EST L > EST K > EST C. This order of toxicity is in accordance with the levels of POPs found in the all the tested organisms (*E. fetida*, alfalfa, cress and mustard), where in general the higher values were recorded in PEY samples and specially in PEY Ic followed by PEY Iib. In the case of EST samples, the values were in general always lower than the ones recorded in PEY samples, being the anthroposoil EST G the most contaminated and the tissues of the organisms exposed to this anthroposoil the ones that exhibited higher levels in their tissues when compared with the other three EST samples.

The traditional approach suggests using the BAF calculation which is usually required for evaluating and assessing contamination risk, however BAF present certain limitations, since it do not comprise a cumulative effect, because this index consider each contaminant individually and thus in a less global way. The SET and ERITME approaches were developed to overcome this shortcoming as they are based on real measures in organisms. Therefore, these more realistic indexes have proven useful for risk evaluation and managing contaminated sites as they take into account the sum of all the contaminants, and not one by one as in the BAF.

Résumé long

La pollution des sols et des sédiments par des polluants organiques persistants (POPs) a augmenté ces dernières décennies en raison des processus d'industrialisation et d'urbanisation, affectant la qualité de l'environnement et de la santé humaine. Les POPs englobent plusieurs familles de composés différents. Dans cette thèse l'attention va être portée sur certains POPs tels que : a) les PCBs, les PCDDs et les PCDFs, composés bien connus, largement utilisés et étudiés depuis longtemps et qui persistent dans les réservoirs environnementaux et continuent de poser des problèmes liés à leur persistance et toxicité ; b) les PBDEs et les HBCDs, largement utilisés comme retardateurs de flamme. Une attention particulière est accordée à d'autres composés pas encore déclarés comme des POPs, mais qui présentent des propriétés physicochimiques similaires : les nouveaux retardateurs de flamme bromés (nRFBs) qui ont commencé à être utilisés pour le remplacement de certains composés bromés et chlorés tels que les PCBs, les PBDEs et les HBCDs qui ont vu leur usage interdit au cours des dernières années. Normalement les sols et les sédiments sont considérés comme des matrices différentes, mais les échantillons étudiés dans cette thèse ont été désignés sous le terme d'anthroposols car ce sont des sols et des sédiments qui ont été affectés et contaminés par des activités anthropiques au cours du temps.

Une étude a été menée sur des anthroposols de deux zones distinctes (Estarreja au Portugal, noté EST, et Casier Peyraud 6 en France, noté PEY) pour évaluer leurs niveaux de contamination, ainsi que leurs comportements dans ces anthroposols et les risques potentiels de ces contaminants pour des organismes vivants du sol. L'objectif principal étant d'évaluer la mobilité environnementale des retardateurs de flamme bromés (RFBs) dans un écosystème terrestre, notamment vers les vers de terre et les plantes, dans une prospective d'évaluation du risque de transferts de ces molécules.

Les niveaux en POPs (RFBs, dont les nRFBs, mais aussi les PCBs et PCDD/DFs) dans ces anthroposols ont été quantifiés ainsi que dans des plantes collectées sur les sites étudiés. Une évaluation *in situ* de l'activité biologique a été réalisée par la collecte, le dénombrement et l'identification des organismes vivants visibles (mésafaune), ainsi que par l'application du test Bait-lamina ©. Une espèce de ver de terre (*Eisenia fetida*) et trois espèces de plantes [luzerne (*Medicago sativa*), cresson (*Nasturtium officinale*) et moutarde blanche (*Sinapsis alba*)] ont été choisies pour réaliser des tests en laboratoire de toxicité et de bioaccumulation.

Le facteur de bioaccumulation (BAF) ainsi que les indices SET et ERITME ont été calculés. Le BAF permet de déterminer si une substance est accumulée dans un organisme donné et s'il existe un risque d'entrée et de diffusion tout au long de la chaîne alimentaire. Les indices SET et ERITME permettent de classer les sites testés en fonction du transfert efficace des POPs des

anthroposols aux organismes testés, et d'avoir une idée du risque potentiel pour l'écosystème. L'indice SET donne une idée globale de l'excès de transfert pour tous les contaminants dans les matrices étudiées. L'indice ERITME permet d'évaluer le risque environnemental global inhérent associé à l'excès de transfert des contaminants considérés.

Les huit anthroposols étudiés sont assez différents en termes de caractéristiques physico-chimiques ainsi que de contaminations inorganiques et organiques. Les valeurs de pH sont légèrement acides dans les échantillons d'Estarreja et les teneurs en matière organique (MO) et en carbone organique (COT) sont relativement élevées, reflétant ainsi une activité agricole intensive et l'utilisation du fumier pour la fertilisation du sol dans le cas des anthroposols EST C et EST L. En ce qui concerne les échantillons de Peyraud 6, les valeurs de pH varient de neutres à légèrement basiques. Les teneurs élevées en MO et en COT étaient probablement dues à l'apport de particules fines résultant du dépôt de sédiments provenant de la rivière et du développement de la végétation dans ces casiers. En ce qui concerne la texture, les échantillons d'Estarreja varient de limoneux sableux à sableux, tandis que ceux de Peyraud 6 étaient principalement des limoneux sableux, à l'exception d'un échantillon présentant une texture de sable limoneux. Les paramètres agronomiques mesurés (CaO, K₂O, MgO, P_{Olsen} et rapport C/N) étaient plus élevés dans les échantillons de Peyraud 6, à l'exception du rapport C/N et de la teneur en MO qui était un peu plus élevée dans les anthroposols d'Estarreja. Les niveaux "pseudo totaux" moyens des ETMs analysés sont plus élevés dans les anthroposols de Peyraud 6 que dans ceux d'Estarreja, sauf dans le cas des niveaux en As où les concentrations mesurées dans les échantillons de la zone d'Estarreja considérée dans cette étude ont montré des niveaux relativement élevés par rapport aux valeurs du fond géochimique. Ce fait montre clairement une origine anthropique de ces ETMs dans les anthroposols considérés, principalement liée à la présence et à l'activité du complexe chimique d'Estarreja et/ou à une activité agricole intense. En général, les anthroposols recueillis sur le site de Peyraud 6 présentent des niveaux plus élevés en POPs en comparaison aux anthroposols d'Estarreja. Les niveaux de nRFBs (nHBB, nPBB, nPBT and PBEB) quantifiés dans tous les anthroposols étudiés étaient très faibles par rapport aux autres contaminants organiques quantifiées (comme les PCBs et les PCDD/DFs). De plus, les valeurs en nRFBs obtenues n'étaient pas comparables avec la plupart des rares données trouvées dans la littérature qui correspondent généralement à des sites fortement contaminés dans des pays asiatiques (généralement des sites de production et/ou élimination de déchets électroniques).

Quelques effets toxiques ont été observés à la fois pour *E. fetida* et pour les espèces de plantes cultivées dans ces huit anthroposols étudiés, notamment en ce qui concerne le taux de

reproduction et la masse corporelle d'*E. fetida*, ainsi que le taux de germination et la hauteur maximale des parties aériennes des végétaux testés. Les données obtenues ont clairement révélé la bioaccumulation de POPs quantifiés à la fois chez les adultes et les juvéniles d'*E. fetida* ainsi que dans les tissus des espèces végétales cultivées, même à des concentrations très faibles dans le cas des nouveaux retardateurs de flamme bromés. Différentes familles des POPs ont montré des comportements différents en ce qui concerne les corrélations avec les paramètres physico-chimiques et l'absorption par *E. fetida*. Les valeurs de BAFs obtenues montrent le potentiel d'accumulation de ces POPs dans les tissus d'*E. fetida*. Pour les plantes testées, les valeurs de BAF obtenues indiquent une grande différence dans la disponibilité des contaminants dans les sols des deux sites considérés. Bien que les niveaux des POPs soient significativement plus élevés dans PEY Ic que dans EST G, il semble que les POPs soient plus disponibles pour être absorbés par les plantes cultivées sur EST G. Cette différence de disponibilité quantifiée peut être due à certains facteurs physico-chimiques du sol, à savoir la nature de la MO, la teneur en COT et le pH légèrement acide de l'anthrosol EST G.

Pour l'ensemble des organismes testés (*E. fetida*, luzerne, cresson et moutarde), les valeurs d'effet les plus élevées ont été généralement enregistrées dans les échantillons PEY et spécialement dans PEY Ic suivi de PEY Iib. Dans le cas des échantillons d'EST, les valeurs étaient en général toujours inférieures à celles enregistrées dans les échantillons de PEY ; cependant l'anthrosol EST G, le plus contaminé, présentait les taux les plus élevés dans les tissus des organismes exposés sur cet échantillon par rapport aux trois autres échantillons EST. La plupart des études utilisent le calcul du BAF qui est généralement considérée pour évaluer le risque de contamination. Toutefois, le BAF présente certaines limites, car il considère chaque contaminant individuellement et ne comporte pas l'effet cumulatif.

Les résultats obtenus pour le calcul du BAF ont montré qu'il existe un potentiel de bioaccumulation des POPs dans les tissus de *E. fetida* et que ces organismes peuvent être considérés comme déconcentrateurs de PCDDs, PCDFs, PBDEs et PBBs (BAF<1) et considérés comme macroconcentrateurs de PCBs, nHBB, nPBT et PBEB (BAF>2). Dans le cas des plantes collectées *in situ* et les plantes cultivées sur les anthrosols étudiés, les valeurs de BAF obtenues indiquent l'existence d'une différence aux niveaux de la biodisponibilité des contaminants dans les différentes matrices et aussi une accumulation des certains contaminants dans les tissus des plantes, notamment au niveau des racines. Les valeurs de BAF montrent qu'il existe une possibilité de transfert de ces POPs dans la chaîne trophique (et donc pour des

herbivores via les plantes et des carnivores via les vers de terre) et l'occurrence d'effets toxiques pour les organismes testés.

Les approches SET et ERITME ont été développées car elles sont basées sur des mesures réelles dans les organismes, sans garder l'aspect « mono-molécule » comme c'est le cas pour les BAFs. Par conséquent, ces index se sont révélés utiles pour l'évaluation des risques et la gestion des sites contaminés, car ils tiennent compte de la somme de tous les contaminants et non d'un par un comme dans le BAF.

Les valeurs obtenues pour SET et ERITME sont présentées dans le tableau ci-dessous.

	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
SET	12.96	15.57	12.72	14.60	101.26	96.57	14.25	66.30
ERITME	5220	6095	5392	5860	50886	45695	7784	24907

Compte tenu des valeurs ERITME, les matrices étudiées peuvent être classées dans un ordre de toxicité croissant: PEY Ic > PEY IIb > PEY IVa > PEY IIIa > EST G > EST G > EST K > EST C. Dans cette étude, où les anthroposols étudiés étaient caractérisés par un mélange de différentes familles de POPs et d'ETMs, avec certains contaminants présents à de faibles teneurs, il a été plus approprié d'appliquer les indices SET et ERITME car ils tiennent compte de la somme de tous les contaminants.

Les valeurs de BAFs calculées pour *E. fetida* étaient supérieures à 1 dans le cas des PCBs et des nRFBs, ce qui suggère l'occurrence du transfert de ces contaminants des matrices pour les tissus des vers de terre exposés. Lorsque qu'on considère les valeurs de AQ (le coefficient d'accumulation pris en compte dans les calculs de SET et ERITME), les résultats n'indiquent pas la même tendance que celle indiquée par les BAFs, car les valeurs de AQ indiquent que le transfert excédentaire n'a eu lieu que pour les PCBs-ndl (PCBs non dioxin like), les PCBs dl-noncop (PCBs dioxine like non coplanaires) et les PBDEs.

Ainsi, les approches SET et ERITME ont été développés pour remédier à cette lacune car elles sont basées sur des mesures réelles dans les organismes. Ces indices montrent clairement qu'une bioaccumulation anormale et inattendue de contaminants peut se produire dans des sols même légèrement contaminés et ainsi dépasser les limites tolérées pour la consommation humaine. Ainsi, dans cette étude, où les anthroposols étudiés étaient caractérisés par un mélange

de différentes familles de contaminants, et certaines d'entre elles étaient présentes à de faibles concentrations, il est apparu plus approprié d'appliquer les indices SET et ERITME car ils tiennent en compte de la somme de tous les contaminants, et pas chaque contaminants, un par un comme dans le calcul des BAFs.

Transfer and effects of brominated flame retardants (BFRs) on three plant species and one earthworm species in anthroposoils

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Abbreviations

Transfer and effects of brominated flame retardants (BFRs) on three plant species and one earthworm species in anthroposoils

BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BFRs	Brominated flame retardants
BSAF	Biota-to-soil accumulation factor
Cd	Cadmium
Cr	Chromium
Cu	Copper
dw	Dry weight
<i>E. fetida</i>	<i>Eisenia fetida</i>
ERA	Environmental risk assessment
ERITME	Evaluation of the Risk of the Transferred Metal Elements
FRs	Flame retardants
HBCDs	Hexabromocyclodecanes
HCH	Hexachlorobenzene
K _{oc}	Soil Organic Carbon-Water Partitioning Coefficient
K _{ow}	Octanol-water partition coefficient
LOD	Limite of detection
LOQ	Limit of quantification
lw	Lipid weight
MTEs	Metallic trace elements
nBFR	New brominated flame retardant
nd	Not detected
nHBB	Hexabromobenzene
nPBB	Pentabromobenzene
nPBT	Pentabromotoluene
Ni	Nickel
OECD	Organization for Economic Cooperation and Development
OM	Organic matter
Pb	Lead
PBBs	Polybrominated biphenyls
PBEb	Pentabromoethylbenzene
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls

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PCBs-cop	Coplanar polychlorinated biphenyls
PCBs-noncop	Non-coplanar polychlorinated biphenyls
PCBs-dl	Dioxin-like polychlorinated biphenyls
PCBs-dl-cop	Dioxin-like coplanar polychlorinated biphenyls
PCBs-dl-noncop	Dioxin-like non-coplanar polychlorinated biphenyls
PCBi	Indicator polychlorinated biphenyls
PCBs-ndl	Non-dioxin-like polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
POPs	Persistent organic pollutants
SET	Sum of Excess of Transfer
TOC	Total organic carbon
WHC	Water holding capacity
ww	Wet weight
Zn	Zinc

Introduction and Objectives

Soil contamination has been a rising concern in recent decades due to industrialization and urbanization processes, with considerable waste disposal and the atmospheric deposition of particles by run-off (Ross and Birnbaum, 2003; Liu et al., 2016).

Soils and sediments can be considered as two of the most important compartments in ecosystems, playing key functions for the living community (Blum, 1988). After long periods of continuous emission and considerable deposition, soils and sediments can act as sinks and reservoirs, holding and retaining huge quantities of contaminants, resulting in negative and sometimes irreversible effects (Reddy and DeLaune, 2008). An illustration of the origin and processes associated with contamination dispersion in ecosystems is presented in Figure 1. Due to natural but mainly anthropogenic sources, an appreciable quantity of contaminants can be transported atmospherically, sometimes over long-distances from the source location (Figure 1). Contaminants can thus be deposited directly and/or indirectly on the surfaces of soils and water bodies, with the soils and sediments acting as sinks and reservoirs as well as new sources of contamination (Figure 1).

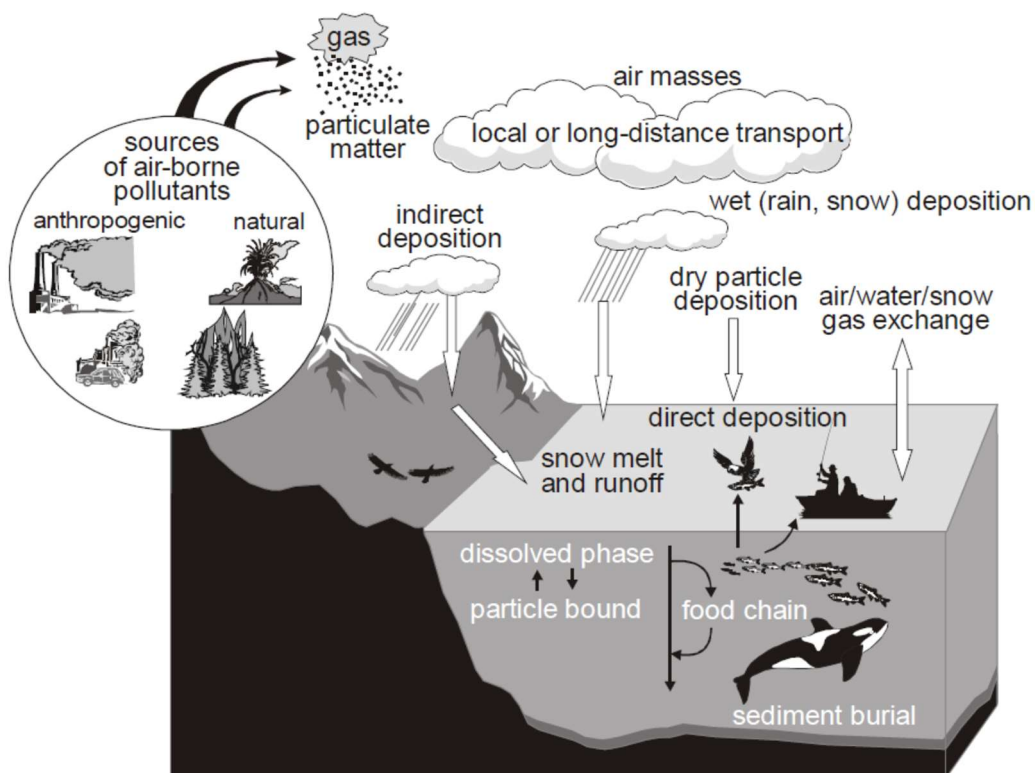


Figure 1 – Illustration of the type of sources and processes associated with contamination dispersion in ecosystems (Ross and Birnbaum, 2003).

Living organisms are in intimate contact with all the ecosystem matrices exposed through contact with and/or the ingestion of the available fraction of these contaminants. Plants as well

as a large share of terrestrial and aquatic organisms occupy the basis of the food chain and are the port of entry through which contaminants reach higher trophic levels.

The contamination of environmental compartments by Metallic Trace Elements (MTEs) has raised worldwide concern over several decades. Due to their non-biodegradable nature and relatively long biological half-lives, MTEs can affect ecological dynamics and ecosystem health. They can easily enter the food chain via different paths such as contaminated soils, water and atmospheric transport and deposition (Fytianos et al., 2001). The main anthropogenic activities responsible for MTEs emissions are urbanization, industrialization, the combustion of fossil fuels, metal smelting processes and the intensive incorporation in soils of pesticides, fertilizers and manures such as sewage sludge (Singh and Kumar, 2006). The accumulation of MTEs such as Cadmium(Cd), Copper (Cu), Chromium (Cr), Nickel (Ni), Lead (Pb) and Zinc (Zn) can be significant in soils in industrialized and urban areas, which may pose environmental risks, and may affect ecosystems and human health due to their potential uptake by plants and animals and leaching by ground- and surface waters (Römken et al., 2009). The risk associated with metals in soils depends on the soil's properties (such as pH and organic carbon content) which affect chemical speciation, solubility and free metal ion activity in the soil solution, which in turn controls metal availability (de Vries et al., 2007). Sediment deposits mainly occur in artificial drainage basins, though they can result from river dredging and natural deposition on river margins. Sediments can also act as a sink or a source of contamination with MTEs, namely for water columns and water bodies, and consequently for the biota (Smith and Pappas, 2007). Large quantities of trace elements can be easily stored in sediments, reaching values that can present risks to biota, and they can even enter the food chain.

Due to the increasing anthropogenic pressures generated by organic compounds in terrestrial ecosystems, it is important to study the presence, fate, and transfer processes of contaminants in organisms, and the potential phenomena of trophic biomagnification. This type of research is essential to correctly evaluate and manage the risks posed by organic pollutants for ecosystems and human health.

Compounds such as polychlorinated biphenyls (PCBs) and the legacy of brominated flame retardants (BFRs), namely polybrominated biphenyls (PBBs), polybrominated biphenyl ethers (PBDEs), hexabromocyclodecanes (HBCDs) and tetrabromobisphenol A (TBBPA), are anthropogenic compounds classified as Persistent Organic Pollutants (POPs). Although their use has already been restricted or prohibited, these historical compounds are ubiquitous, persistent and bioaccumulative (UNEP, 2002).

Since the regulation, restriction and banning of PCBs, PBDEs and HBCDs, new alternative flame retardants have been placed on the market to replace those used previously (Ezechiáš et al., 2014). At present, the new alternative flame retardants (the most common of which are the decabromodiphenyl ethane (DBDPE), the bis(2,4,6-tribromophenoxy) ethane (BTBPE), the bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP), the 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), the hexabromobenzene (nHBB), the pentabromobenzene (nPBB), pentabromotoluene (nPBT) and PBEB (pentabromoethylbenzene)) remain poorly studied and documented (Ezechiáš et al., 2014). These new brominated and chlorinated compounds present physicochemical properties like those of POPs and are potentially hazardous for the environment. The new alternative compounds are expected to occur at lower concentrations than PCBs and PBDEs, whether in the biotic or abiotic compartments.

In the literature review soils and sediments will be dealt with separately, but the samples studied will be designated as anthroposoils throughout this dissertation, due to the fact that these soils and sediments have been formed or heavily modified in consequence of long-term human activity.

The principal aim of the present study is to assess and understand the mobility and environmental bioavailability of POPs in anthroposoils. Mobility criteria and controlling factors will be defined to understand the behavior of the pollutants considered in the environmental matrices examined. The main objectives of the present work correspond to the following questions:

- Do the anthroposoils selected show detectable selected POPs concentrations?
- Which POPs families are present in these anthroposoils?
- Are the POPs available for accumulation by earthworms and plants?
- In the case of bioaccumulation, what are the toxic effects caused by the POPs levels in the animal and plant species investigated?
- What is the degree of bioaccumulation of POPs in the species tested?
- Concerning the evaluation of the real risks, what can be expressed by the indexes calculated such as Bioaccumulation factor (BAF), Sum of Excess of Transfers (SET) and Evaluation of the Risk of the Transferred Metal Elements (ERITME)?
- Are these reference indexes useful in the Environmental Risk assessment approach?

Several methods have been proposed to determine bioavailable contaminant concentrations. Two of the most common ones are the use of earthworms or plants as indicators of soil quality.

The advantage of using earthworms is that they are natural residents in the soil matrix, the present tolerance to different types of soil, and they have a large epidermal surface and ingest more soil than many other soil dwelling organisms (Lanno et al., 2004), resulting in significant pollutant exposure. POPs are taken up by earthworms via passive diffusion from the soil porewater through the cuticle and via internal sorption of the compounds from soil passing through the gut and intestine (Lord et al., 1980; Belfroid et al., 1995). Plant species offer the advantage of being native to the soil environment and the uptake of POPs frequently occurs via a diffusion mechanism from soil particles to soil pore water and subsequent uptake by plant roots as well as via assimilation from the aerial parts following volatilization from the soil (Limmer and Burken, 2016). The use of both earthworms and plants present some disadvantages such as: occurrence of mortality, mobility (in the case of earthworms) that can affect the accumulation kinetics and rates, growth and possible biotransformation of certain POPs into by-products.

In the present study, one species of earthworms (*Eisenia fetida*) and three plant species (*Sinapis alba*, *Medicago sativa*, *Nasturtium officinale*) will be used to estimate the potential toxicological effects of these contaminants regarding parameters such as plant growth and earthworm reproduction. Finally, the potential transfer of the emerging compounds considered to the biocenosis (plants and earthworms) will be evaluated and the bioaccumulation indexes will be calculated to express the magnitude of the bioaccumulation present.

To achieve these objectives and answer the associated questions, this thesis dissertation is divided into six main chapters.

- Chapter 1 comprises an extensive but not exhaustive literature review of the characteristics and environmental presence and significance of the POPs selected (PCBs, PCDDs, PCDFs, PBDEs, HBCDs, PBBs, nHBB, nPBB, nPBT and PBEB). An overview of the relevant legislation and guidelines for selected contaminants in Europe is briefly presented.
- Chapter 2 presents an overview of the mobility of certain POPs as well as their bioavailability and potential bioaccumulation in living organisms, namely earthworms and plants. First, a brief description is given of the assessment of POPs toxicity and bioaccumulation in living organisms on the basis of laboratory tests with earthworms and plants according to ISO guidelines. Secondly, several controlling factors and the

potential mobility of BFRs in the environmental compartments are discussed based on the literature.

- Chapter 3 starts with the description of the methodologies and protocols used for the field sampling campaigns and for the general soil physicochemical characterization. After that, the chemical protocols for the analytical quantification of the POPs and MTEs selected in the anthroposoils studied are described. All the protocols followed for the preparation of earthworms and plants in the reproduction and bioaccumulation tests are described, followed by the chemical procedures used for the quantification of the levels of POPs bioaccumulated in earthworm and plant tissues. The calculation and use of the bioaccumulation factor as well as the SET and ERITME indexes to infer the risks posed by contamination to the ecosystem are described. The approach used for the in-situ evaluation of biological activity and soil quality by monolith sampling and using the Bait-lamina test is briefly described. Finally, the statistical methods that were used to analyse the results obtained are detailed.
- In Chapter 4, all the results are presented and discussed concerning: i) anthroposoil physicochemical characterization; ii) the levels of POPs presents in soils; iii) the laboratory experiments with *Eisenia fetida* (*E. fetida*) to analyze the effects on reproduction and potential contaminant bioaccumulation; iv) the studies of germination and growth of the three plant species used and their potential bioaccumulation of contaminants. Also, several indexes for risk evaluation are calculated and their significance is discussed.
- Finally, Chapter 5 presents the conclusions and final remarks, namely the potential use and possible limitations of using Environmental Risk Assessment (ERA) approaches, considering the presence and effects of the POPs studied.

Chapter 1. Characteristics, environmental presence and significance of several persistent organic pollutants

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This Chapter presents an extensive but not exhaustive literature review concerning the characteristics and environmental presence and significance of the POPs selected (PCBs, PCDDs, PCDFs, PBDEs, HBCDs, PBBs, nHBB, nPBB, nPBT and PBEB). An overview of the relevant legislation and guidelines for selected contaminants in Europe is briefly presented.

Some chemical pollutants are well known for their capacity to persist in the environment, reaching the air, water, soils and/or sediments and for their capacity for accumulation at levels that can be a problem for the living community and human health (El-Shahawi et al., 2010). These pollutants are often designated as persistent organic pollutants and they can be divided into two types according to their sources: (i) chemical compounds intentionally produced and used, such as pesticides and other industrial products; (ii) compounds non-intentionally produced and dispersed during the combustion and/or incineration of wastes, biomass residues and during industrial processes.

POPs comprise several different families of compounds and all of them are characterized by being: a) persistent, thus resisting chemical and/or biological degradation; b) potentially bioaccumulated by living communities and, once inside the food chain, they can be easily biomagnified through the different trophic levels; c) toxic for living beings; and d) mobile over long distances which facilitates their long-range transport even to remote areas (Jones and de Voogt, 1999 ; Li et al., 2006 ; Hertz- Picciotto et al., 2008 ; El-Shahawi et al., 2010).

Due to their typical properties they are widely dispersed for long periods in every environmental compartment and the presence of POPs such as PAHs, PCBs, PCDDs, PCDFs and BFRs has already been reported:

- in aquatic environments and aquatic organisms (Koumanova, 2008; Harman, 2011; Wenning and Martello, 2014);
- in the atmosphere (Castro-Jiménez, 2007; Palm, 2011; Hageman et al., 2011);
- in soils, sediments and terrestrial organisms (Jaspers et al., 2014; Duarte et al., 2018; Ren et al., 2018),
- in the human body (Jaspers et al., 2014; Bruce-Vanderpuije et al., 2019)
- in remote areas such as Arctic regions (Kallenborn et al., 2011; Kallenborn (ed.), 2016).

Regarding soils and sediments, POPs can reach these environmental compartments through the atmospheric deposition of resuspended soil dust, road dust, and air particles of anthropogenic origins (emissions from traffic, industry and incineration processes), both in wet and/or dry conditions (Meuser, 2010; Cachada et al., 2012a); dispersion due to underground leakages of

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wastewater pipes (Meuser, 2010); leaching and dispersion from abandoned industrial sites and illegal residue deposits (Meuser, 2010). POPs can be subjected to several physical, chemical and biological processes that influence their degradation or persistence in the environment (Stokes et al., 2006; Lohmann et al., 2007; Wang et al., 2016). Moreover, atmospheric transport and deposition combined with the phenomena of the “cold condensation effect” explain the presence of the POPs in pristine areas at Arctic and Antarctic latitudes (Wania and Mackay, 1993).

Due to the worldwide impact of these pollutants, in 1998 the Aarhus protocol on POPs was signed as part of the Geneva Convention on Long-distance pollution in European countries (UNECE, 1998). The main goal of this protocol was to promote the elimination of any discharges, emissions and losses of POPs. This protocol also refers to the immediate banning of the production and use of certain products, the later elimination of others such as polychlorinated biphenyls (PCBs) and reducing the emissions of PCDDs, PCDFs and hexachlorobenzene (HCH) below the levels observed in the 1990s. In 2001, a global treaty known as the Stockholm Convention was signed to protect human health and wildlife from POPs (UNEP, 2002). This accord focuses on the elimination and reduction of POPs release into the environment with 5 principal goals: 1) the elimination of dangerous POPs (starting with the 12 worst, usually referred to as the *dirty dozen*); 2) support for transition processes to safer alternative compounds; 3) the targeting of additional POPs for future actions; 4) promoting the cleaning of old stockpiles and equipment that contain POPs; 5) developing strategies and work for an POPs-free future.

In the present thesis work, attention is given to certain POPs such as: a) PCBs, PCDDs and PCDFs, which are well known and widely used compounds that have been studied for a long time and that continue to persist in environmental reservoirs, and still pose environmental problems due to their persistence and toxicity; and b) PBDEs and the HBCDs, widely used as flame retardants. Particular attention is given to other compounds not yet declared as POPs, but which present similar physicochemical properties (Sapozhnikova and Lehotay, 2013): certain BFRs that have begun to replace certain brominated and chlorinated compounds such as PCBs, PBDEs and HBCDs that have been banned. Further details on the characteristics of all these compounds and their toxicity to living organisms will be given in the next subchapters.

1.1. Polychlorinated biphenyls (PCBs)

PCBs are a family of organochloride aromatic compounds expressed by the chemical formula $C_{12}H_{(10-n)}Cl_n$ in which the number of chlorine atoms can vary from 1 to 10. This allows PCBs to be categorized by the degree of chlorination (number of chlorine atoms) in 10 homologous groups from monochlorobiphenyls to decachlorobiphenyls. More than 60% of PCBs are tetra- to hexachlorophenyls. There are a total of 209 PCB congeners from which 12 present a planar structure and properties similar to those of dioxins which are usually referred to as dioxin-like PCBs (PCBs-dl). The remaining 197 congeners – those not conforming to the planar structure – are referred to as non-dioxin like PCBs (PCBs-ndl). PCBs of a given homolog with different chlorine substitution positions are called isomers or congeners. PCBs are named based on a numbering system developed by the IUPAC. This system numbers PCBs from 1 to 209, based on the position of the halogen atoms on the rings (Ballscmitter and Zell, 1980). Seven PCBs can be used to characterize the presence of PCB contamination. Six of these seven are PCBs-ndl (CB-28, -52, -101, -138, -153 and -180), and one is a PCB-dl (CB-118). These seven PCBs are often called “indicator PCBs” (PCBi) (UNEP, 2013). The high-chlorinated PCBs are less volatile than the low chlorinated ones, and the ability to accumulate in lipids increases with increasing chlorine substitution (Ritter et al., 1995a).

The three most important physical properties of PCBs are low vapor pressure, low water solubility (both decreasing with increasing chlorination), and high dielectric constants (IPCS, 1976). The log K_{ow} (octanol – water partition coefficient) of PCBs ranges from 4.3 up to 8.3 due to their high lipophilic nature. Table 1 gives a summary of the main physicochemical properties of each group of PCBs.

Thus, the differences in chemical structure produce varying levels of toxicity. The commercial production of PCBs began in the USA in 1929. Properties such as chemical and physical stability, electrical resistance, low volatility, resistance to degradation at high temperatures and their resistance to degradation by thermal rupture, oxidants and other chemical effects contributed to the intensive production of PCBs and their widespread use (PNUE, 2010).

Table 1 – Principal physicochemical characteristics of each group of PCB isomers (Robertson and Hansen, 2001).

Congener group	Boiling point (°C)	Melting point (°C)	Vapor pressure (Pa) at 25	Water solubility (g/m ³) at 25 °C	log K _{ow}
Biphenyl	71	256	4.9	4.3	0.92
Monochlorobiphenyl	25 – 77.9	285	1.1	4.7	0.25
Dichlorobiphenyl	24.4 – 149	312	0.24	5.1	0.065
Trichlorobiphenyl	28.87	337	0.054	5.5	0.017
Tetrachlorobiphenyl	47 – 180	360	0.012	5.9	4.2 x 10 ⁻³
Pentalorobiphenyl	76.5 – 124	381	2.6 x 10 ⁻³	6.3	1.0 x 10 ⁻³
Hexalorobiphenyl	77 – 150	400	5.8 x 10 ⁻⁴	6.7	2.5 x 10 ⁻⁴
Heptalorobiphenyl	122.4 – 149	417	1.3 x 10 ⁻⁴	7.1	6.2 x 10 ⁻⁵
Octalorobiphenyl	159 – 162	432	2.8 x 10 ⁻⁵	7.5	1.5 x 10 ⁻⁵
Nonachlorobiphenyl	182.8 – 206	445	6.3 x 10 ⁻⁶	7.9	3.5 x 10 ⁻⁶
Decachlorobiphenyl	305.9	456	1.4 x 10 ⁻⁶	8.3	8.5 x 10 ⁻⁷

The commercial products were complex mixtures most commonly used in dielectric fluids in capacitors and transformers. PCBs are now ubiquitous environmental pollutants, occurring in human and animal tissue, and most environmental compartments. Even though PCBs production has been banned in most countries since the 1970s and 1980s, it is estimated that over 1 million tons of PCBs were produced and about one third of this quantity is thought to be still circulating in the environment (Birkett and Lester, 2003). According to Baird & Cann (2005) the major sources of PBCs are mainly urban areas, notably because of their use as coolant fluids in power transformers and capacitors, as plasticizers, as heat transfer fluids in machinery and as waterproofing agents. These compounds are released into the environmental compartments from sources such as poorly maintained waste sites that contain PCBs, improper dumps of PCBs waste like transformer fluids, leaks or releases from electric transformers and the disposal of PCBs-containing products in municipal or other landfills not prepared to stock

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this type of hazardous waste (Faroon et al., 2003). The burning of organic wastes in municipal and industrial incinerators is also a major source of PCBs release (Zhang et al., 2011a).

Most of the historical production of PCBs occurred between 1930 and 1993 and took place in the Northern Hemisphere in the United States, West Germany, the former Soviet Union, France, the United Kingdom, Japan, Czechoslovakia, China, Spain and Italy with the total amount produced around 1.3 million of tons (Breivik et al., 2002, 2007). Most of the intentional production of PCBs has stopped, but considerable amounts are still detected in the environment (Breivik et al., 2002). PCBs are persistent soil contaminants due to their hydrophobicity and resistance to biodegradation (Weber et al., 2008). Soil is very effective in the uptake and retention of PCBs, making PCBs resistant to repeated air-soil exchange. The degradation of PCBs in soils is slow. Soil samples taken from rural sites in western United Kingdom and Norway in 1998 showed that the lighter PCBs are found further north and away from source regions than the heavier PCBs. Heavier molecules struggle to travel long distances such as to Arctic and Antarctic regions (Ockenden et al., 2003). In France, the use of PCBs in certain industrial applications started to be forbidden in 1975. Later, in 1987, the sale, acquisition and use of equipment containing PCBs were forbidden (INERIS, 2011).

In 2001, PCBs were classified as POPs and listed in annexes A and C of the well-known Stockholm Convention that requires the prohibition of PCB usage by 2025 and the correct management of PCBs-containing wastes by 2028 (UNEP, 2009).

Tables 2 and 3 present some of the already recorded median, mean and range values of Σ PCBs in soils and sediments around the world (non-exhaustive lists).

Table 2 – Median, mean concentration and range of Σ PCBs ($\mu\text{g}\cdot\text{kg}^{-1}$ dw) in soils in Europe (non-exhaustive list).

Country	Land-use type	No of PCBs	Σ PCBs		Reference
			Mean / Median	Range (Min – Max)	
Portugal	Urban	Σ_{19} PCBs	- / 8.8	2.3 – 55	Cachada et al., 2012b
Portugal	Urban	Σ_{19} PCBs ; Σ_5 PCBi	- / 7.9 ; - / 2.6	0.62 – 73; 0.15 – 41	Cachada et al., 2009
France	Remote/Urban/Industrial	Σ_7 PCBi	- / -	0.09 - 150	Motelay-Massei et al., 2004
Spain	Urban	Σ_7 PCBi	4.44 / -	0.19 – 10.5	Nadal et al., 2007
	Chemical	Σ_7 PCBi	4.63 / -	0.26 – 15.0	
	Unpolluted	Σ_7 PCBi	0.77 / -	0.29 – 2.11	
Italy	Urban	Σ_{19} PCBs ; Σ_5 PCBi	- / 14 ; - / 6.6	1.8 – 172; 0.72 – 86	Cachada et al., 2009 ; Morillo et al., 2007
Italy	Industrial A	Σ_{24} PCBs	1.14 / 0.55	0.01 – 8.3	Donato et al. (2006)
	Industrial B	Σ_{24} PCBs	0.21 / 0.07	<0.001 – 2.6	
	Industrial C	Σ_{24} PCBs	0.15 / 0.08	0.01 – 0.8	
	Industrial D	Σ_{24} PCBs	0.03 / 0.017	0.0007 – 0.38	
Greece	Landfill	Σ_7 PCBi	- / -	3.96 – 399	Chrysikou et al. (2008)

Table 2 (cont) – Median, mean concentration and range of Σ PCBs ($\mu\text{g}\cdot\text{kg}^{-1}\text{dw}$) in soils in Europe (non-exhaustive list).

Country	Land-use type	No of PCBs	Σ PCBs		Reference
			Mean / Median	Range (Min – Max)	
Germany	Agricultural	Σ_6 PCBs	- / -	0.95 – 3.84	Manz et al. (2001)
Germany	Urban	Σ_{12} PCBs	- / -	1.60 – 21.9	Krauss and Wilcke (2003)
Austria	Forest	Σ_6 PCBs	- / -	6.4 - 95	Weiss et al. (1998)
UK	Rural to urban	Σ_{84} PCBs	4.7 / 1.5	0.39 – 21	Desborough et al. (2016)
UK	Rural	Σ_{33} PCBs	5.07 / 2.5	0.27 – 80.6	Heywood et al. (2006)
UK	Rural to urban	Σ_6 PCBs	31.8 / 6.5	1.7 – 1200	Creaser et al. (1989)
Scotland	Urban	Σ_{19} PCBs ; Σ_5 PCBi	- / 22 ; - / 9.4	4.5 – 78; 1.9 – 43	Cachada et al., 2009 ; Morillo et al., 2007
Slovenia	Urban	Σ_{19} PCBs ; Σ_5 PCBi	- / 6.8 ; - / 2.1	2.8 – 48 ; 0.67 – 29	Cachada et al., 2009 ; Morillo et al., 2007
Sweden	Rural / Urban	Σ_{44} PCBs	- / 7.1	2.3 – 986	Backe et al. (2004)
Sweden	Urban	Σ_{19} PCBs ; Σ_5 PCBi	- / 5.7 ; - / 2.3	2.3 – 77; 0.54 – 47	Cachada et al., 2009
Russia	Agricultural	Σ_{17} PCBs	13.9 / -	5.0 – 31.0	Wilcke et al. (2006)

- = not indicated; nd = not detected.

Table 3 – Median, mean concentration and range of Σ PCBs ($\mu\text{g.kg}^{-1}$ dw) in sediments in Europe (non-exhaustive list).

Country	Origin	No of PCBs	Σ PCBs		Reference
			Mean/Median	Range (Min – Max)	
France	River	Σ_7 PCBsi	- / 41.5	0.13 – 417.1	Mourier et al. (2014)
France	River	Σ_{15} PCBs	- / -	nd – 2310	Lorgeoux et al. (2016)
France	River	Σ_7 PCBi	- / 33.7	0.67 – 234	Liber et al. (2019)
Czech Republic	River	Σ_7 PCBi	- / 38.58	0.33 – 467.08	Stiborova et al. (2017)
England	Marine	Σ_{55} PCBs	- / -	0.082 – 38	Camacho-Ibar and McEvoy (1996)
Russia	Lake	Σ_{34} PCBs	- / -	0.08 – 6.1	Iwata et al. (1995)

- = not indicated; nd = not detected.

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Several earthworm species have been shown to significantly accumulate PCBs. In a study with four different species (*Eisenia andrei*, *Eisenia fetida*, *Eisenia hortensis* and *Lumbricus terrestris*), Ville et al. (1995) observed the accumulation of PCBs in earthworm tissues. Earthworms collected in rice fields in Japan showed levels of 150000 pg.g⁻¹ fresh weight of PCBs-dl in their tissues (Nakamura et al., 2007). Also, Bu et al. (2010) observed the capacity of *E. fetida* to accumulate PCBs-dl. Vermeulen et al. (2010) conducted a study to investigate the accumulation of PCBs in the soil-earthworm-hedgehog food chain, where the accumulation of these compounds was effective in *Lumbricus rubellus* tissues at mean levels of 9.77 up to 21.86 ug.kg⁻¹ dw. *E. fetida* and *Allolobophora caliginosa trapezoides* species were collected from a typical E-waste dismantling area in east China and the accumulation of PCBs in earthworm tissues was observed at levels of 1.17 up to 78.6 ug.kg⁻¹ dw due to ingestion and direct contact with heavily contaminated soils (Shang et al., 2013).

The presence of PCBs in plants has already been reported by some authors. Concentrations of Σ_3 PCBs were reported in *Lolium perenne* (3.5 $\mu\text{g.kg}^{-1}$ dw) and in *Melaleuca leukadendra* (2.64 up to 7.00 $\mu\text{g.kg}^{-1}$ dw) in Australia (Müller et al., 2001). Zhao et al. (2006) detected levels of Σ_{17} PCBs of about 2.80 $\mu\text{g.kg}^{-1}$ dw in grass samples in China. Chrysikou et al. (2008) in a study on the distribution of certain POPs in soils and vegetation near a landfill in Greece found levels of 3.64 up to 25.9 $\mu\text{g.kg}^{-1}$ dw for Σ_7 PCBi in *Solanum eleagnifolium* and *Solanum trifolium* plant species. Also, several studies reported PCBs uptake and translocation by edible vegetables such as: soybeans (Suzuki et al., 1977), carrots (Iwata and Gunther, 1976), beets, turnips and beans (Sawhney and Hankin, 1984). It was also observed that the lower chlorinated congeners (PCB-28, -52 and -101) were found to be more abundant in the shoots than in the roots of these plant species (Iwata and Gunther, 1976; Suzuki et al., 1977; Sawhney and Hankin, 1984).

In France, sediment from cores collected in the Seine river were analyzed and it was found that the concentration gradient over time was similar to the variation of the production and utilization of PCBs over previous decades. Indeed, the significant decrease of these compounds in the sediments deposited during the 80s was directly related to the restriction and/or banning in 1975 of PCBs use in open industrial systems (Mourier et al., 2014; Lorgeoux et al., 2016). Nyberg et al. (2015) concluded that the PCBs levels recorded in the sediments of the Baltic sea and on the west Swedish coast have significantly decreased over the last 40 years, probably as a result of the legislative restriction and/or prohibition implemented in the 1970s. Also, in America, PCBs concentrations in young seals seem to have decreased by about 81% from 1984 to 2009 in Gertrude Island in Washington, probably as a result of the legislation adopted by

USA in 1976 and by Canada in 1977, which led to a reduction in the input of PCBs in the environment (Ross et al., 2013).

THINGS TO KEEP IN MIND

- ✓ PCBs are dispersed worldwide and can be found in every essential ecosystem compartment due to atmospheric transport and subsequent deposition on land surfaces. The sources and the environmental fate and related transfer and dispersion mechanisms have already been thoroughly investigated and extensively reviewed in the literature.
- ✓ Several earthworm and plant species have been shown to significantly accumulate PCBs, especially the lower chlorinated congeners (CB-28,-52 and -101).
- ✓ Although PCBs production has been banned in most countries since the 1970s and 1980s, considerable quantities of PCBs are still circulating in the environment. PCBs are persistent in the environment due to their hydrophobicity and resistance to biodegradation, as they are strongly retained by soil particles.
- ✓ In the particular case of this work, PCBs and their dispersion and mobility mechanisms will be used as an example of a chlorinated family of POPs to try to compare and infer the possible similar fate of BFRs in the environment.

1.2. Polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated dibenzofurans (PCDFs)

PCDDs and PCDFs, usually referred to as “dioxins”, are a group of persistent organic pollutants which comprises a total of 210 compounds, 75 PCDDs and 135 PCDFs congeners (Huwe, 2002; Loganathan and Masunaga, 2015). They are characterized by K_{ow} values ranging from 4.04×10^{-5} to 1.35×10^{-3} and a vapor pressure between 8.3×10^{-13} and 8.1×10^{-7} , depending on the homolog group considered (Srogi, 2007). Dioxins have been referred to as ‘the most toxic man-made compounds’ and have therefore generated much concern regarding their potential health risks. These compounds are chemically very stable, highly hydrophobic, with great affinity to organic matter (OM), and unless transported in association with particulate organic matter, PCDDs and PCDFs usually present limited mobility. Despite this, these organic compounds are highly persistent, and their presence is widespread in the environment, namely in the terrestrial ecosystem. They are easily bioaccumulated in organic matrices, especially in the tissues of organisms, so they are easily introduced into the food chain to reach higher trophic levels (Van den Berg et al., 1998; Baker and Hites, 2000). PCDDs and PCDFs induce toxic responses such as immunotoxicity, carcinogenicity and dermal toxicity, and adverse effects on reproduction development and endocrine functions have already been reported in the literature (Van den Berg, 1998).

PCDDs and PCDFs stem from by-products originating from chlorinated chemical production and combustion processes. They have never been intentionally produced for industrial purposes or even deliberately released into the environment (IPCS, 1989). Since 2001, PCDDs and PCDFs have been included in the Stockholm Convention and subjected to progressive reduction and elimination where feasible, like the other POPs described (UNEP, 2002).

Simonich and Hites (1995) and Barrie et al. (1992) reported that volatilized lower chlorinated PCDDs and PCDFs can be transported over large distances from temperate industrial regions and deposited in cold climate regions (Arctic and Antarctica). Although the distribution of PCDDs and PCDFs occurs mainly via atmospheric transport pathways (Czuczwa and Hites, 1984; Kallenborn et al., 1998; Lohman and Seigneur, 2001), their strong affinity for particulates also leads to significant associated sediment and soil stockage (Eitzer, 1993). PCDDs and PCDFs can be absorbed and accumulated in soils and sediments and are readily redistributed into other ecosystem compartments and therefore bioaccumulated by living organisms and

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easily introduced into the food chain (Connell, 1990; Broman et al., 1992; Berglund et al., 2001).

Tables 4 and 5 present some of the already recorded median, mean and range values of PCDDs and PCDFs in soils and sediments around the world (non-exhaustive lists).

Some earthworm species have been shown to significantly accumulate PCDDs and PCDFs. Reinecke and Nash (1984) observed the accumulation of PCDDs and PCDFs in two earthworm species *Allolobophora catiginosa* and *Lumbricus rubellus* tissues. Earthworms collected in rice fields in Japan showed levels of 900 pg.g⁻¹ dw of PCDDs and PCDFs in their tissues (Nakamura et al., 2007). In another study, earthworms belonging to *E. fetida* and *Allolobophora caliginosa trapezoides* species were collected from a typical E-waste dismantling area in east China. They were found to have an accumulation of PCDDs and PCDFs in their tissues due to ingestion and direct contact with heavily contaminated soils at levels between 0.13 and 0.59 µg.kg⁻¹ dw (Shang et al., 2013). Henriksson et al. (2017) also confirmed the accumulation of PCDDs and PCDFs in *E. fetida* tissues at concentrations of 1500 up to 15000000 pg.g⁻¹ dw as a result of both *in situ* and exposure in laboratory conditions to contaminated soils from Sweden.

Table 4 – Mean concentration and range of PCDDs concentrations in sediments around the world (non-exhaustive list).

Country	Origin	Range (Min – Max)	Reference
France	River sediments	0.013 ^a – 3.77 ^a	Liber et al., 2019
Germany	River sediments	0.30 ^a – 290 ^a	Götz et al., 2007
Germany	Lake sediments	5.27 ^b – 1401.74 ^b	Bruckmeier et al., 1997
Spain	Estuarine sediments	0.08 ^c – 1.76 ^c	Gómez-Lavín et al., 2011
Switzerland	Lake sediments	10 ^b – 1378 ^b	Zennegg et al., 2007

- = not indicated; ^a values expressed in µg.kg⁻¹ dw; ^b values expressed in ng.kg⁻¹ dw; ^c Values expressed in pg.g⁻¹ dw

Table 5 – Mean concentration and range of PCDFs concentrations in sediments in Europe (non-exhaustive list).

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Country	Origin	Range (Min – Max)	Reference
France	River sediments	0.002 ^a – 2.28 ^a	Liber et al., 2019
France	River sediments	0.5 ^a – 478 ^a	Götz et al., 2007
Germany	Lake sediments	8.64 ^b – 2297.78 ^b	Bruckmeier et al., 1997
Spain	Estuarine sediments	0.07 ^c – 2.23 ^c	Gómez-Lavín et al., 2011
Switzerland	Lake sediments	15 ^b – 1069 ^b	Zennegg et al., 2007

- = not indicated; ^a Values expressed in $\mu\text{g.kg}^{-1}$ dw; ^b Values expressed in ng.kg^{-1} dw; ^c Values expressed in pg.g^{-1} dw.

THINGS TO KEEP IN MIND

✓ PCDDs and PCDFs are quite widely dispersed and can be frequently found in essential ecosystem compartments due to atmospheric transport and considerable deposition on land. The sources, the environmental fate and the related transfer and dispersion mechanisms of PCDDs and PCDFs are well-known and have already been reviewed in the literature.

✓ Some earthworm species have been shown to significantly accumulate PCDDs and PCDFs, namely the low molecular weight congeners, in appreciable concentrations in their tissues.

✓ Since PCDDs and PCDFs can easily be bioaccumulated in the tissues of organisms they can then easily enter the food chain and reach higher trophic levels. PCDDs and PCDFs are well-known to induce toxic responses such as immunotoxicity, carcinogenicity and dermal toxicity, as well as adverse effects on reproduction, development and endocrine functions, which have already been reported in the literature.

✓ PCDDs and PCDFs have similar physical-chemical properties but different biological potencies, due to the difference in molecular structure as PCDDs are derivatives of dibenzo-*p*-dioxin and PCDFs are derivatives of dibenzofuran.

✗ These compounds are chemically very stable, highly hydrophobic and have great affinity to OM. Unless transported in association with particulate organic matter, they present limited mobility.

✓ PCDDs and PCDFs are well-known to induce toxic responses such as immunotoxicity, carcinogenicity and dermal toxicity, as well as adverse effects on reproduction, development and endocrine functions, which have already been reported in the literature.

✓ In the particular case of this work, PCDDs and PCDFs and their dispersion and mobility mechanisms will be used as another example of a chlorinated family of POPs in order to compare them and draw inferences regarding the possible similar fate of BFRs in the environment.

1.3. Brominated Flame Retardants (BFRs)

Among all the different and numerous POPs released into the environment, flame retardants are of great concern to the scientific community. Since fires have been one of the major causes of property damage and death throughout history, technological research has been carried out to combat them (Khandual, A., 2014). By incorporating heat resistant chemicals in products, it is possible to reduce the potential of ignition and combustion of a wide range of manufactured products such as textiles, plastics, building materials and electronic equipment widely used both in commerce and residential environments (Alaee et al., 2003).

BFRs are the largest group commonly sold on the market due to their low cost and high performance and efficiency (Birnbaum and Staskal, 2004). BFRs can generate halogen atoms from the thermal degradation of the parent compound to chemically reduce and “retard” the development of fires (de Wit, 2002). At present, there are more than 75 kinds of BFRs (Alaee et al., 2003; Covaci et al., 2011). They are commonly used in polyurethane foam, plastics for electric and electronic equipment, printed circuit boards, expanded and extruded plastic, textile back-coating in furniture, various textiles used in public environments, and rubber for coating wires among other things (de Wit et al., 2010).

The BFRs most widely produced and used are PBBs, PBDEs and HBCDs. The lower brominated congeners (between 1 and 5 bromine-substitutions per molecule) have higher bioavailability due to their high K_{ow} compared to the higher brominated congeners (Darnerud et al., 2001). PBDEs are generally used as mixtures containing multiple PBDEs. HBCDs are composed of three stereoisomers: α -, β -, and γ -HBCD, which have a high capacity for bioaccumulation. BFRs can easily migrate to environmental compartments during the whole lifetime of the products in which they are incorporated. The main release and migration of BFRs occur during the use of these products. Thus, they can be released into the air and dust through volatilization, weathering and absorption processes (Cao et al., 2013). Also, during the end of product life, the BFRs in the products can be released during waste disposal and contaminate soils and groundwater via leaching processes (Chen et al., 2012). Vastag (2008) referred to their environmental persistence, bioaccumulative characteristics and potential toxicity to living organisms. Indeed, the endocrine disrupting potential of BFRs in both animals and man was mentioned in a literature review by Legler and Brouwer (2003).

Regarding their application chronology, BFRs can be classified as “traditional” or “legacy BFRs” and “novel” or “new” BFRs (New BFRs). Legacy BFRs mainly correspond to the

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compounds no longer used or commercialized, namely PBDEs, PBBs and HBCDs. New BFRs correspond to the replacements of legacy BFRs, including DBDPE, nHBB, nPBB, nPBT and PBEB, among others.

Some of these BFRs are similar in structure to PCBs and PBBs. All are persistent, bioaccumulate in living organisms, highly mobile in the food chain, and toxic. BFRs are characterized by low water solubility, high K_{ow} (values > 6) and K_{oa} (values > 8), which means they are lipophilic, and have medium to low vapor pressures (Covaci et al., 2011; Bergman et al., 2012). Their physicochemical and biochemical properties combined with their resistance to chemical and biological degradation lead BFRs to be persistent and easily incorporated in every compartment of the ecosystem, resulting in exposure and negative health effects for animals and humans (Linares et al., 2015; Loganathan, 2012).

1.3.1. Legacy Brominated Flame Retardants

1.3.1.1. Polybrominated diphenyl ethers (PBDEs)

PBDEs are a family of BFRs commonly used to replace chlorinated flame retardants such as PCBs, due to the similarity of their respective properties.

PBDEs are a family of organohalogenated compounds with high molecular weight expressed by the chemical formula $C_{12}H_{(10-n)}Br_n$ in which the number of bromine atoms can vary from 1 to 10. There are a total of 209 PBDEs congeners numbered from 1 to 209, based on the position of the bromine atoms on the rings (Rahman et al., 2001). The industrial production of these compounds is done by mixing diphenyl ethers and bromine and the resulting congeners present different levels of bromination. Therefore, they occur most commonly in the following different forms: tetra-, penta-, octo- and deca-BDEs. Table 6 gives a summary of the main physicochemical properties of the PBDEs congeners usually found in environmental compartments.

According to La Guardia et al. (2006) the commercialized industrial mixtures of PBDEs congeners are c-Penta-BDE, c-Octa-BDE and c-Deca-BDE (where “c-“means “commercial”) which include, respectively, the following BDEs: BDE-47 (tetra-BDE) and BDE-99; BDE-153 (hexa-BDE) and BDE-183 (hepta-BDE); BDE-209 (deca-BDE). Small quantities of BDE-28 (tri-BDE), BDE-100 (penta-BDEs) and BDE-154 can also be found in these mixtures (Lebeuf, 2009).

Table 6 – Physicochemical properties of the most common PBDEs congeners found in the environment (Akortia et al., 2017).

Congeners group	Boiling point (°C)	Melting point (°C)	Vapor pressure (Pa)	Water solubility (µg L ⁻¹)	log K _{ow}
Tetra-BDE	-	79 – 82 (BDE 47)	2.7 – 3.3 x 10 ⁻⁴ (20°C)	10.9 (25°C)	5.9 – 6.2
Penta-BDE	>300°C (decomposition)	92 (BDE 99) 92 – 97 (BDE 100)	2.9 – 7.3 x 10 ⁻⁵ (20°C)	0.0009 (20°C)	6.5 – 7.0
Octa-BDE	-	~ 200	1.2 – 2.7 x 10 ⁻⁷ (20°C)	-	8.4 – 8.9
Deca-BDE	decomposition	290 - 306	<1 x 10 ⁻⁴ (25°C)	-	10

- = not indicated.

The total worldwide production of PBDEs between 1970 and 2005 is estimated to be around 1 300 000 to 1 500 000 tons (UNEP, 2019). These compounds were mainly used in several different types of industrial and manufacturing processes used to produce products such as electrical and electronic equipment, components for vehicles and textiles.

Like certain other POPs, PBDEs are widespread in the environment in appreciable concentrations and can cause harmful toxicity effects. PBDEs can be released into the environment via atmospheric emissions from manufacturing and other industrial processes (Gouteux et al., 2008; Li et al., 2015, Wang et al., 2010a) as well as from waste incineration and recycling processes (Wang et al., 2010b; Hearn et al., 2012).

Emitted contaminants may be transported over large distances in the air and be deposited on land surfaces (Newton et al., 2014; Cetin et al., 2016). Also, the application of sewage sludge to soil as a nutrient amendment can be a significant way of loading BFRs on agricultural-land due to the high levels of contamination usually verified in sewage waste-streams (Kim et al., 2017). The lipophilic properties of PBDEs allow them to easily bind to organic matter and thus persist in soils where half-lives of about 28 years have been reported (Andrade et al., 2010).

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The presence of PBDEs in the environment was reported for the first time in 1966 (Jensen, 1996). Certain regulatory measures were implemented due to the significant worldwide dispersion of PBDEs and their ubiquitous presence in every environmental compartment.

PBDEs are lipophilic substances (Li et al., 2008) and when in the environment they can easily be biomagnified in the food chain (de Wit et al., 2010) and tend to bioaccumulate in living organisms (Kuo et al., 2010). The occurrence of bioaccumulation in wild fauna has already been observed in several studies, even in remote areas with no local point sources or industrial production like the Arctic (Law et al., 2003; Strandberg et al., 2001; ter Schure et al., 2004 a,b). The bioaccumulation of PBDEs in soil dwelling invertebrates, namely earthworms, has already been reported in the literature (Nyholm et al., 2010; Gaylor et al., 2013), since these organisms are one of the main pathways of entry of the contaminants into terrestrial food-chains (Nie et al., 2015). Contaminated soils can be a source of transfer of PBDEs into suspended solids and sediments of aquatic environments due to run-off events (Muresan et al., 2010). Several authors have already reported the bioaccumulation and trophic transfer of PBDEs in the ecosystems of fresh water and marine organisms (Law et al., 2006a, 2006b; Klosterhaus et al., 2012; Poma et al., 2014). Potential human exposure and intake of PBDEs can occur due to the inhalation of soil fragments suspended in outdoor air, the dermal absorption of settled particles, the ingestion of soil particles adhered to vegetables grown on contaminated land and the direct oral intake of soil by toddlers through hand-to-mouth behavior (Abdallah et al., 2015; Akortia et al., 2017). Some plant crops show the ability to translocate PBDEs from soil into vegetative structures and this may also result in consistent low-level exposure through diet (Navarro et al., 2017). Considering the existence of potential risks to human health posed by contaminated soils, reference screening values have already been established by government agencies (NEPC, 2013; USEPA, 2017).

Non-exhaustive lists with the median, mean concentration and range of PBDEs concentrations found in soils and sediments around the world are presented in tables 7 and 8.

Table 7 – Mean concentration and range of PBDEs concentrations in soils in Europe (non-exhaustive list). All values are reported on ng.g⁻¹ dw basis, except if otherwise indicated.

Country	Land-use type	Congeners	Mean / Median	Range (Min – Max)	Reference
France	Urban and rural	Σ ₈ PBDEs	- / -	0.3 – 13	Gaspéri et al., 2016
France	Forest	Σ ₈ PBDEs	1.20 / -	0.225 – 5.11	Muresan et al., 2010
	Agricultural		1.93 / -	0.242 – 43.9	
	Urban		2.24 / -	0.324 – 18.0	
Italy	Pasture (0-1 cm deep)	Σ ₁₃ PBDEs	1.55 ^a / -	0.310 – 3.85 ^a	Parolini et al., 2012
	Pasture (1-4 cm deep)		0.72 ^a / -	0.086 – 1.74 ^a	
	Pasture (4-7 cm deep)		0.43 ^a / -	0.149 – 1.06 ^a	
England	Rural – urban	Σ ₈ PBDEs	15 ^{b,c} / -	2.3 – 49 ^c	Drage et al., 2016
England	Urban	Σ ₆ PBDEs	1.76 ^a / -	0.430 – 4.09 ^a	Harrad and Hunter, 2006
	Suburban		0.321 ^a / -	0.146 – 0.489 ^a	
	Rural		0.22 ^a / -	0.046 – 0.403 ^a	
United Kingdom	Grassland	Σ ₂₂ PBDEs	- / 0.61 ^a	0.065 – 6 ^a	Hassanin et al., 2004
	Forest		- / 2.5 ^a	0.110 – 12 ^a	

Table 7 (cont) – Mean concentration and range of PBDEs concentrations in soils in Europe (non-exhaustive list). All values are reported on ng.g⁻¹ dw basis, except if otherwise indicated.

Country	Land-use type	Congeners	Mean / Median	Range (Min – Max)	Reference
Norway	Forest	Σ_{22} PBDEs	- / 0.97 ^a	0.13 – 3.0 ^a	Hassanin et al., 2004
Sweden	Urban	Σ_{13} PBDEs	12 ^{b,c} / -	0.87 – 46 ^{b,c}	Newton et al., 2015
Slovakia	Urban / industrial	Σ_{15} PBDEs	0.47 ^b / -	0.086 – 1.63	Thorenz et al., 2010
Estonia	Industrial	Σ_{11} PBDEs ^a	- / -	nd – 1.70	Kumar et al., 2009
	Urban / rural		- / -	0.03 – 0.37	
Scotland	Rural and background	Σ_7 PBDEs ^a	0.856 / -	0.09 – 4.52	Rhind et al., 2013
	Rural and background		1.22 / -	0.107 – 15.4	
	Rural and background		1.42 / -	0.09 – 10.5	
Scotland	Background	Σ_7 PBDEs ^a	0.68 ^c / -	0.02 ^c – 1.57 ^c	Zhang et al., 2014
	Background		1.88 ^c / -	0.41 ^c – 10.5 ^c	
	Background		2.55 ^c / -	0.20 ^c – 13.2 ^c	

- = not indicated; nd = not detected; ^a Excluding PBDE-209; ^b value calculated from original data; ^c values expressed in ng.g⁻¹ OM;

Table 8 – Mean concentration and range of PBDEs concentrations in sediments in Europe (non-exhaustive list). All values expressed in $\mu\text{g}\cdot\text{kg}^{-1}$ dw.

Country	Origin	Congeners	Mean / Median	Range	Reference
France	River	Σ_8 PBDEs	- / -	nd – 60	Lorgeoux et al., 2016
France	River	Σ_8 PBDEs	- / 3.81	0.06 – 239	Liber et al., 2019
Portugal	River	Σ_{40} PBDEs	- / -	0.03 – 18	Lacorte et al., 2003
Spain	River	Σ_7 PBDEs	- / -	0.86 – 2.49	de la Cal et al., 2003
Spain	River	Σ_7 PBDEs	- / -	2.4 – 41.7	Eljarrat et al., 2004
Spain	River	Σ_8 PBDEs	- / -	nd – 44.3	Barón et al., 2014
Italy	River	Σ_8 PBDEs	2.14 / -	0.26 – 10.8	Giulivo et al., 2017
Greece	River	Σ_8 PBDEs	1.68 / -	nd – 4.52	
Slovenia					
Croatia	River	Σ_8 PBDEs	5.60 / -	nd – 14.0	
Bosnia and Herzegovina					
Czech Republic	River	Σ_{10} PBDEs	- / 19.34	7.08 – 300.46	Stiborova et al., (2017)
Netherlands	River	Σ_9 PBDEs	- / -	1.4 – 272	Covaci et al., 2002b
United Kingdom	River	Σ_3 PBDEs	- / -	nd – 1170	Allchin et al. (1999)
Norway	Lake	Σ_{11} PBDEs	- / -	0.36 ^a – 26.95	Schlabach et al. (2004)

- = not indicated; nd = not detected.

1.3.1.2. Hexabromocyclodecanes (HBCDs)

HBCDs are nonaromatic brominated alicyclic hydrocarbons expressed by the chemical formula $C_{12}H_{18}Br_6$. The commercial mixture is composed by the three principal diastereomers: alpha (α -HBCD), beta (β -HBCD) and gamma (γ -HBCD) in the following quantities 10-13 %, 1–12 % and 75–89 %, respectively (Covaci et al., 2006). The main physicochemical properties of HBCDs are presented in Table 9.

Table 9 – Physicochemical properties of the three HBCDs isomers (ECHA, 2008).

Isomer	Vapor pressure (Pa) at 21 °C	Water solubility ($\mu\text{g L}^{-1}$) at 25 °C	log K_{ow}	Log K_{oc}
α -HBCD		48.8	5.07	
β -HBCD	6.3×10^{-5}	14.7	5.12	4.66
γ -HBCD		2.1	5.47	

HBCDs is applied in industrial processes and products as a flame-retardant additive. The most common retardant application is the polystyrene foam used in insulation materials and product packaging. HBCDs can also be found in textile materials and electronic/electric equipment. In 2001, the total worldwide production of HBCDs reached 16 700 tons, most of which was consumed in the European region (Bilitewski et al., 2012; Koch et al., 2015). In recent years the production and use of HBCDs has increased and it is now the third most used BFRs worldwide (Bilitewski et al., 2012). Nowadays, around 10 000 to 100 000 tons of HBCDs are produced in or imported to Europe (ECHA, 2008).

HBCDs presents physicochemical characteristics quite similar to those of PBDEs and even other POPs (de Wit, 2002). The presence of HBCDs in remote areas such as the Arctic region has been detected in the blubber of marine animals such as walrus, beluga and narwhal in concentrations of 0.6, 1.4 and 3.4 $\text{ng}\cdot\text{g}^{-1}$ lipid weight (lw), respectively (Tomy et al., 2008).

HBCDs is monitored due to its widespread presence and potential toxicity, and in 2011 this compound was classified as persistent, bioaccumulative and harmful to wildlife and humans. Later, in 2013, HBCDs was included in Annex A of the Stockholm Convention (Lin et al., 2013).

Tables 10 and 11 presented levels of HBCDs found in soils and sediments around the world (non-exhaustive lists).

Table 10 – Mean concentration and range of Σ HBCDs concentrations in soils around the world (non-exhaustive list).

Country	Land-use type	Range	Reference
Sweden	Industrial	<0.1 – 25	Remberger et al. (2004)
China	Industrial to Rural	0.43 – 15.2	Lu et al., 2018
China	Industrial	0.31 – 9.99	Gao et al. (2011b)
	e-waste recycling area	0.22 – 2.34	
China	Residential to agricultural	7.75 – 60.74	Tang et al. (2014)

- = not indicated; nd = not detected; all values expressed in $\mu\text{g.kg}^{-1}$ dw.

Table 11 – Mean concentration and range of Σ HBCDs concentrations in sediments in Europe (non-exhaustive list).

Country	Origin	Range	Reference
France	River	nd – 26.05 ^a	Liber et al., 2019
Spain	River	nd ^b – 513.6 ^b	Eljarrat et al., 2004
Spain	River	nd ^b - 2430 ^b	Guerra et al., 2009
Spain	River	nd ^b - 189 ^b	Herrero et al., 2018
Czech Republic	River	0.21 ^b – 351 ^b	Kukůcka et al., 2015
Czech Republic	River	<0.80 ^b – 126.42 ^b	Stiborova et al., 2017
Netherlands	Estuary	14b – 71b	Verslycke et al., 2005
Belgium	River	<0.2 ^b – 950 ^b	Morris et al., 2004
Netherlands	Estuarine and River	<0.8 ^b – 9.9 ^b	
England	Estuarine and River	<2.4 ^b – 1680 ^b	
Ireland	River	<1.7 ^b – 12 ^b	
England	Lake	0.9 ^b – 4.8 ^b	Harrad et al., 2009
Sweden	River and Lake	<0.1 ^b – 25 ^b	Remberger et al., 2004
Norway	Lake	0.43 ^b – 3.9 ^b	Evenset et al., 2007

- = not indicated; nd = not detected; ^a values expressed in $\mu\text{g}\cdot\text{g}^{-1}$ dw; ^b values expressed in $\mu\text{g}\cdot\text{kg}^{-1}$ dw.

1.3.1.3. Polybrominated biphenyls (PBBs)

PBBs corresponds to the brominated analogs of PCBs and, like the latter, there are 209 possible PBBs congeners. Like other brominated POPs, PBBs were widely used as additives in commercial flame-retardant mixtures. The widely reported accident at the Michigan Chemical Company in St. Louis, Michigan in 1973, where around 250 – 500 kg of the commercial PBBs formulation Firemaster BP-6 was accidentally mixed into cattle feed distributed to several farms, leading to widespread contamination with PBBs. This accident caused the sickness of thousands of animals and the heavy contamination of milk, with severe effects on the health of the resident population (Egginton, 2009). The effects of PBBs were found to be essentially the same as those seen for PCBs. After this accident, PBBs were phased out and their production in Europe ended in 2000.

THINGS TO KEEP IN MIND

- ✓ Legacy BFRs are relatively dispersed and can be found in essential ecosystem compartments due to their atmospheric transport and subsequent deposition on land surfaces, like other POPs.
- ✓ Legacy BFRs were already reported to be present in the tissues of organisms' susceptible to enter the food chain and reach higher trophic levels.
- ✓ These compounds are chemically very stable, highly hydrophobic, with great affinity to OM, and unless transported in association with particulate organic matter, they present limited mobility like PCBs and PCDD and PCDFs, suggesting that their environmental fate and behavior are similar to POPs that have already been thoroughly investigated.
- ✓ Legacy BFRs are classified as persistent, bioaccumulative and harmful to wildlife and humans.
- ✓ PBDEs are already well referenced in the literature on their presence in environmental compartments and organism tissues. Some earthworm species have been shown to significantly accumulate low molecular weight congeners, with appreciable concentrations being found in their tissues.
- ✓ In the case of PBBs and HBCDs, there is a lack of studies and literature data regarding the presence and environmental fate of these compounds in soils, sediments, earthworms and plants.

1.3.2. New or Alternative Brominated Flame Retardants (nBFRs)

As both PBDEs and HBCDs were classified as Persistent Organic Pollutants in the Stockholm Convention, the commercial formulations of PBDEs (c-Penta-BDE, c-Octa-BDE and c-Deca-BDE) and HBCDs were progressively banned and a range of new generation BFRs known as “alternative” or “new” BFRs (nBFRs) emerged and began to be used as replacements for the legacy compounds. Their general physicochemical characteristics are similar to those of PBDEs and similar patterns of environmental contamination and toxicity effects have been reported (Covaci et al., 2011; Ezechiáš et al., 2014). There are more than 75 different compounds that can be used as BFRs but the most common are: DBDPE, which replaces Deca-BDE formulas; BTBPE, used in place of Octa-BDE; BEH-TEBP and EH-TBB that are used to replace Penta-BDE mixtures. nHBB, nPBB and nPBT are also used also to replace PBDEs in a wide range of polymers (Covaci et al., 2011; Ezechiáš et al., 2014).

Different classes of BFRs are characterized by different properties; PBDEs, HBCDs and PBBs are considered as additive BFRs and are more present in the products leached into the environment than the other type of BFRs, i.e. the reactive ones that are more stable and less susceptible to be released into the environment (De Wit, 2002; Eljarrat and Barceló, 2011).

In the present work, we will focus only on four of these nBFRs: nHBB, nPBB, nPBT and PBEB; their main physicochemical properties are presented in Table 12.

Table 12 – Physicochemical properties of the four nBFRs studied in the present work (Bergman et al., 2012; Simonsen et al., 2000).

Compound	Molecular weight (g.mol ⁻¹)	Log K _{ow}	Water solubility (g.L ⁻¹)	Koc	Vapor pressure (Pa)
nHBB	552	6.11	77000	50300	11400
nPBB	473	6.44	-	26886	3510
nPBT	487	6.25	78000	60200	60000
PBEB	501	6.76	35000	114000	15600

The physicochemical properties of the nBFRs presented in Table 8 are similar to those of legacy BFRs. The vapor pressure values of nHBB, nPBB and nPBT are quite low, indicating the low volatility of these compounds and the same range of the values as the PCBs, PBDEs and HBCDs. Log K_{ow} is an important parameter in the estimation of the potential bioaccumulation of contaminants in the tissues of the organisms exposed as it gives an indication of the compounds’ degree of lipophilicity. Lipophilic substances show higher values of log K_{ow}. A

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$\log K_{ow}$ higher than 3 indicates that the substance is hydrophobic and thus potentially bioaccumulative (Amiard, 2011). The $\log K_{ow}$ values observed in nBFRs are similar to those of legacy BFRs and higher than 3, indicating the potential of these compounds to be bioaccumulated in the tissues of living organisms. The K_{oc} parameter is the coefficient of partitioning between the organic carbon fraction and the water present in soils or sediments controlled by the organic carbon levels present (Tissier et al., 2005). Values of K_{oc} higher than 1000 indicate that a given compound is significantly absorbable. The nBFRs considered present K_{oc} values far higher than 1000, thus they are highly absorbable by organism tissues and so more bioaccumulative.

nBFRs are emergent substances and therefore only limited information on them can be found in the literature. The information available usually concerns only the date when commercialization began, the industrial uses and applications of the product, the quantities produced, and only in rare cases certain indications regarding its environmental distribution and fate, toxicity for the biological community and effect on human health. Up to now, nBFRs have not been subject to regulatory control but some of them already feature in Annex III of REACH which describes the potential toxic risks of these substances to the environment. Covaci et al. (2011) already reported the presence of nBFRs in the Arctic region, which shows the occurrence of long-range transportation to remote areas very far from the sites where nBFRs are produced and/or used.

1.3.2.1. Hexabromobenzene (nHBB)

nHBB is an additive nBFRs mainly produced in Eastern Asia and largely used in the industrial production of paper, wood processing, textile materials, plastic products, and electric and electronic components (Covaci et al., 2011; Cruz et al., 2015; Yamaguchi et al., 1988; Watanabe et al., 1986). Although data on producers and production volumes are scarce, the main producers of this compound seem to be the USA, Japan and China (Gauthier et al., 2007; Yamaguchi et al., 1988). Other than by commercial production, nHBB can be released into the environment as a result of the pyrolysis of Octa- and DecaBDE technical products (Buser, 1986).

Levels of nHBB in soils and sediments found worldwide are presented in Tables 13 and 14 (non-exhaustive lists).

Table 13 – Median, mean concentration and range of nHBB concentrations in soils around the world (non-exhaustive list).

Country	Land-use type	Mean/Median	Min - Max	Reference
Sweden	Urban	0.0069 ^c / -	<0.00079 ^c – 6.1 ^c	Newton et al. (2015)
Australia	Polymer industry	0.12 ^a / -	<0.03 ^a – 1.37 ^a	McGrath et al. (2017)
	Waste disposal	17.2 ^a / -	<0.03 ^a – 90.9 ^a	
	Non-industrial	<0.03 ^a / -	<0.03 ^a – <0.03 ^a	
China	E-waste processing region	0.798 ^a / -	0.0983 ^a – 2.93 ^a	Hong et al. (2016)
China	BFR industry	0.89 ^a / -	<0.02 ^a – 17 ^a	Li et al. (2016)
	BFR industry	0.31 ^a / -	<0.02 ^a – 2.0 ^a	
China	Residential, industrial, agricultural, forest	3.4 ^a / -	<0.017 ^a – 720 ^a	Wei et al. (2016)
China	Forest	0.0072 ^b / -	<0.0020 ^b – 0.042 ^b	Zheng et al. (2015)
Vietnam	Rice paddy (e-waste processing region)	- / -	<0.2 ^a – 6.8 ^a	Matsukami et al. (2017)
	E-waste open burning sites	- / -	<0.2 ^a – 0.90 ^a	
	E-waste processing workshops	- / -	<0.2 ^a – 210 ^a	
	Rice paddy (e-waste processing region)	- / -	<0.2 ^a – 0.27 ^a	
Vietnam	Around e-waste processing workshops	- / -	nd – 16 ^a	Someya et al. (2016)
	e-waste open burning sites	- / -	nd – 1.3 ^a	

- = not indicated; nd = not detected; ^a value expressed in ng.g⁻¹dw; ^b value expressed in ng.g⁻¹ww; ^c value expressed in ng.g⁻¹OM.

Table 14 – Median, mean concentration and range of nHBB concentrations in sediments around the world (non-exhaustive list).

Country	Origin	Mean/Median	Range	Reference
Spain	River	- / -	nd – 2.4	Guerra et al. (2010)
China	River	8672 / -	-	Wu et al. (2010)
Japan	River	- / -	6 – 60	Watanabe et al. (1986)
	Estuary	- / -	0.5 – 6	
Japan	River	- / -	nd – 4.3	Watanabe and Sakai (2003)

- = not indicated; nd = not detected; all values expressed in ng.g⁻¹dw.

Over the last decade, nHBB has been also found in air samples collected in the polar regions, showing evidence for its long-range atmospheric transport (Gouteux et al., 2008; Möller et al., 2011). nHBB shows its potential for bioaccumulation once in the environmental compartments. The bioaccumulation of nHBB in soil dwelling invertebrates, namely the earthworm *E. fetida*, has already been reported in the literature by Belfroid et al. (1995). Studies reporting the detection of levels of nHBB in birds' eggs and tissues and in marine organisms can also be found in the literature (Gauthier et al., 2007; Verreault et al., 2007; Wu et al., 2011). On the French Atlantic coast, nHBB was detected at a range from 0.03 up to 4.3 ng.g⁻¹ lw in the tissues of juvenile common sole from three nursery zones (Munsch et al., 2007).

Although not much is known about the processes of transformation and degradation of nHBB in soils, Nyholm et al. (2010) concluded in a test study with soils that the degradation process of this compound is rapid in aerobic conditions and much slower under anoxic conditions, indicating that nHBB is probably persistent in the environment, like the well-known POPs, PCBs, PCDDs and PCDFs.

1.3.2.2. Pentabromobenzene (nPBB)

nPBB is used in the same applications of other polybromobenzenes such as nHBB. Despite this, very little information is available in the literature concerning this compound. Nevertheless, levels of nPBB have already been detected in the soil of an e-waste processing region in China at concentrations of 0.022 up to 0.285 ng.g⁻¹ dw (Hong et al., 2016). Also, Li et al. (2016) reported levels of 0.03 up to 3.4 ng.g⁻¹ dw in soils collected near two BFRs production plants

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in China. Someya et al. (2016) detected levels of nPBB up to $0.56 \text{ ng.g}^{-1} \text{ dw}$ in soils collected around e-waste processing workshops in Vietnam.

1.3.2.3. Pentabromotoluene (nPBT)

nPBT is a halogenated flame retardant expressed by the chemical formula $\text{C}_7\text{H}_3\text{Br}_5$. The commercial designations for this compound are Flammex 5-BT from Berk Ltd[®] (United Kingdom) and FR-105 from Ameribrom (ICL Industrial Products, Israel) and Chemtura US (Covaci et al., 2011; Eljarrat and Barceló, 2011). The nPBT is mostly used in the manufacture of polyesters, polyethylene, polypropylene, polystyrene, textile materials and rubber products. The estimated production of nPBT in Europe is between 1000 and 5000 tons per year. In China, the estimated production is about 600 tons per year (Covaci et al., 2011; Eljarrat and Barceló, 2011). Other than by commercial production, nPBT can be released into the environment as a result of the pyrolysis of Octa- and DecaBDE technical products (Gouteux et al., 2008).

Concentrations of nPBT were detected in soils from urban areas in Sweden at levels up to $0.018 \text{ ug.kg}^{-1} \text{ dw}$ (Newton et al., 2015). Schwarzbauer et al. (2001) recorded levels up to $25 \text{ ug.kg}^{-1} \text{ OM}$ in river sediments in Germany and Lopez et al. (2008) measured concentrations of nPBT in Dutch river sediments ranging from 0.01 to $0.33 \text{ ug.kg}^{-1} \text{ dw}$.

nPBT does not seem to be readily biodegradable (Simonsen et al., 2000). According to de Wit et al. (2006), nPBT has the potential for long-range transportation in the atmosphere like other BFRs such as PBDE. Indeed, levels of nPBT were detected in air samples from the remote area of the Greenland Sea (Möller et al., 2011) and from Egbert station, 70 km north of Toronto, in Canada (Gouteux et al., 2008). The presence of nPBT was also detected in the tissues of small marine organisms (Wu et al., 2011), in the fatty tissues of seals and whales (Montie et al., 2010) and in birds' eggs in Canada (Gauthier et al., 2007).

1.3.2.4. Pentabromoethylbenzene (PBEB)

PBEB is an additive BFR essentially used in polyester resins applied in circuit boards, textile materials, cable coats and polyurethane foams (Hoh et al., 2005). This compound was produced in USA between 1970s and 1980s under the commercial designation FR-105. After 1986, no USA production or import volumes have been reported (Hoh et al., 2005). PBEB appears in the OSPAR list of chemicals, being described as persistent, bioaccumulative and toxic (OSPAR, 2007), but it is not currently produced in any of the OSPAR signatory countries (Covaci et al., 2011; de Wit et al., 2010). Other than by commercial production, PBEB can be released into the environment as a result of the pyrolysis of Octa- and DecaBDE technical products (Gouteux et al., 2008).

Concentrations of PBEB were reported in river sediments in Spain, ranging from non-detected levels to 9.6 ng.g⁻¹ dw (Guerra et al., 2010) and in river sediments in China where the mean concentration measured was 132 ng.g⁻¹ dw (Wu et al., 2010).

Hoh et al. (2005) reported the presence of PBEB in air samples collected in Chicago. The presence of PBEB was already detected in the tissues of small marine organisms (Wu et al., 2011), seals and whales (Montie et al., 2010) and birds in Canada (Gauthier et al., 2007; Venier et al., 2010).

THINGS TO KEEP IN MIND

- ✓ The principal source of nBFRs in the environment are e-waste dismantling areas and the dispersion of nBFRs mainly occurs due to atmospheric transport and its subsequent deposition on land surfaces like other POPs.
- ✓ The log K_{ow} values observed for nBFRs are similar to those of legacy BFRs and higher than 3, indicating the potential of these compounds to be bioaccumulated in the tissues of living organisms, a fact that has already been reported in the literature but mainly for certain aquatic animals and quite often reported, since these compounds are high lipophilic.
- ✓ The risks associated with the presence of legacy BFRs are well-known, but nBFRs are emergent substances and therefore only limited information on them can be found in the literature. The information available usually concerns only the date of beginning of commercialization, industrial uses and applications, the quantities produced and, only in rare cases, indications regarding environmental distribution and fate, toxicity for the biological community and effect on human health. Also, data on the presence of nBFRs in soils, earthworms and plants is scarce. The presence of nBFRs in sediments has already been reported in the literature.
- ✓ Its general, their physicochemical characteristics are similar to those of PBDEs and similar patterns of environmental contamination and toxicity effects have been reported, suggesting that its dispersion mechanisms, availability and bioaccumulation processes might be similar to those of PCBs, PCDDs, PCDFs and PBDEs.

1.4. Regulatory measures and legislation governing BFRs in Europe

The presence of BFRs in environmental matrices gives rise to concerns regarding the fate of POPs and their persistence, their potential for long distance transportation and toxicity to the environment and biota. Also, the potential biomagnification capacity of these contaminants through the different food chain levels represents a danger to ecosystems.

Due to all the concerns posed by POPs, these BFRs compounds have been the subject of several legal proceedings, judgements and even restrictions and bans regarding their usage. Table 15 provides a summary of timelines for decisions on the legislation on BFRs and their banning around the world.

Table 15 – Timelines for decisions around the world regarding legislation and decisions to ban certain BFRs over recent decades (Webster and Stapleton, 2012).

1973	PBBs contamination in Michigan
1975	Technical Bulletin 117 and 133 implemented in California
1977	Ban of TRIS in children's sleepwear
1978	Removal of chlorinated TRIS (TDCPPs) from children's sleepwear
1980s	Early papers on the presence of PBDEs in environmental samples
1998	Discovery of rising trend of PentaBDEs in Swedish breast milk
2004	Ban on use of Penta BDE and Octa BDE in the European Union
2004	Phaseout of the manufacture of PentaBDE and OctaBDE in the United States
	Dust hypothesized to be a major source of exposure to PBDEs
2008	Discovery of the components of Firemaster 550 in dust
2009	PentaBDE and OctaBDE effectively added to the Stockholm Convention
2013	DecaBDE scheduled to be phased out in the United States

Since 2004, the risks associated with PBDEs, HBCDs and PBBs have been assessed through an integrated risk assessment project called the FIRE project (Flame retardants Integrated Risk) that includes the procedures of exposure assessment, laboratory screening studies, QSAR modelling and *in vivo* modelling with the aim of assessing and evaluating the effects of BFRs (FIRE, 2004). In 2006, EU countries adopted legislation to both ban and phase out PBDEs. The Waste of Electrical and Electronic Equipment (WEEE) and Restriction of Hazardous Substances (RoHS) Directives focus on reducing the amount of discarded toxic materials. Another regulation adopted by the EU is the REACH system (Registration, Evaluation,

Chapter 1. Characteristics, environmental presence and significance of several persistent organic pollutants

Authorization, and Restriction of Chemicals) with the aim of identifying and restricting and/or authorizing for use chemical substances of concern due to their potential to be carcinogenic, mutagenic, reproductively toxic, persistent, and bioaccumulative for the biological community. In 2009, several BFRs were included in the list of POPs in the framework of the Stockholm Convention. PentaBDE and OctaBDE were both included in Annex A, which contains substances prohibited from use, production, import and export. Annex A already included other BFRs such as HBB as well as other POPs, namely PCBs, Hexachlorobenzene and toxaphene. More recently, in 2013, HBCDs were also included in this annex with few exceptions and for use in expanded and extruded polystyrene. In the specific case of PBDEs, several regulatory measures were implemented in Europe. Since 2004, the commercial mixtures c-Penta-BDE and c-Octa-BDE can be used or incorporated in industrial products only at concentrations below 0.1% in mass. Also, products or materials that contain more than 0.1% in mass of PBDEs cannot be commercialized as flame retardant materials (INERIS, 2012). After 2006, as defined in the European directive 2002/95/CE, new electric and electronic equipment cannot contain any PBDEs and PBBs. In 2008, the commercial mixture Deca-BDE was also banned from industrial processes (EU official journal). Since 2009, Tetra-BDE and Penta-BDE have appeared in Annex A of the Stockholm Convention (UNEP, 2009). In 2017, Deca-BDE was also included in the annex and some restrictions and phase-out initiatives have been implemented globally. Penta-BDE and Octa-BDE formulations have already been banned from use in many domains (EU, 2003; NICNAS, 2007; Stapleton et al., 2012).

Chapter 2. Persistent organic pollutants mobility in anthroposoils and bioaccumulation in earthworms and plants

Chapter 2. Organic contaminants mobility in anthroposoils and bioaccumulation in earthworms and plants

Chapter 2 presents an overview of the mobility of certain POPs and the occurrence of bioavailability and the potential bioaccumulation in living organisms, namely earthworms and plants. First, a brief description of the assessment of POPs toxicity and bioaccumulation in living organisms based on laboratory tests with earthworms and plants according to ISO guidelines is given. Secondly, several controlling factors and the potential mobility of BFRs in environmental compartments are discussed in the light of the literature.

Soils and sediments are a major part of the terrestrial environment. Their formation is a complex process and they act as sinks and reservoirs, notably for contaminants (Luoma and Ho, 1993). The significant increase of soil contamination with emergent compounds in recent years has led to growing interest from the scientific community and international agencies in developing soil monitoring and assessment strategies. Soil pollution assessment is usually, or conventionally, based on the quantification of the contaminant levels found in the soils studied and their comparison with specific threshold values indicated in the legislation. In addition to not providing any indication of the real effects of contamination on the living community, this classic approach does not consider certain aspects such as: (i) the possible toxicity and effects of other chemicals that were not included in the group analyzed, (ii) the possible interactive effects between the contaminants on the biota, and (iii) their levels of bioavailability or degradation/metabolization.

In the literature, several definitions can be found for the concept of bioavailability. The bioavailability of a contaminant refers to the fraction that can be taken up by an organism from the environmental compartment through both passive and active routes of exposure and which directly influences its toxicity level (Smith et al., 2010). In terrestrial environments, the bioavailability and toxicity of a certain contaminant to terrestrial invertebrates and plants can be influenced and controlled by several important characteristics: the nature of the contaminant (Adriano, 2001); soil-contaminant contact time (Hatzinger and Alexander, 1995; Alexander et al., 2000; Sharer et al., 2003; Ahmad et al., 2004), soil properties such as pH, cation exchange capacity, organic matter and clay contents (Bradham et al., 2006; Spurgeon et al., 2006; Criel et al., 2008); the trophic level and biological characteristics of the organism exposed (Guerin and Boyd, 1992; Katayama et al., 2010; Semple et al., 2007); environmental and climatic conditions, such as temperature and precipitation (Sijm et al., 2000; Pravecek et al., 2005; Ruggiero et al., 2002).

The complexity and wide number of factors governing the fate and bioavailability of the contaminants combined with the site-, chemical- and soil-specific nature of bioavailability leads to the need to consider a more realistic approach for the bioavailability assessment. Bioavailability assessment would surely be more realistic if the organisms exposed were considered rather than only taking into account the total concentrations of contaminants and soil characteristics.

Moreover, the correlation between the efficiency of chemical extractions to mimic natural processes and real bioaccumulation in organisms such as plants and earthworms is a good way to ascertain the real risks (Sheppard and Evenden, 1992).

This chapter is divided in two subchapters. In the first one, the mobility of POPs and their potential bioaccumulation in earthworms and plant tissues is discussed. In the second part, we focus on the mobility of BFRs (the family of POPs that correspond to the main issue of the present study) in the environment and their potential transfer to living organisms.

2.1. Mobility of persistent organic pollutants and potential bioaccumulation in earthworms and plants

2.1.1. Bioaccumulation in earthworms

Soil invertebrates can be considered as good sentinel organisms for soil chemical contamination since they are in direct contact with soil and soil pore water or by food exposure, in contrast to many other invertebrate organisms that are only indirectly exposed through the food chain (Kammenga et al., 2000).

Earthworms tend to migrate over very short distances and represent the largest biomass fraction of most soils (Edwards, 2004). In most terrestrial environments, earthworms are considered as ecosystem engineers owing to their essential role in soil formation, organic matter breakdown processes, soil aeration and nutrient cycling (Edwards, 2004). Thus, earthworms are good indicators of land-use impact and soil fertility, contributing to pedogenesis and the evolution of the soil profile, thereby affecting the physical, chemical and microbiological properties of the soil (Barlett et al., 2010). In addition, earthworms are also responsible for increasing the mineralization and humification of OM by food consumption, respiration and gut passage (Lavelle and Spain, 2001). OM breakdown and the casting activity of earthworms in soils can contribute indirectly to stimulating an increase in microbial mass and activity and the

mobilization of nutrients (Emmerling and Paulsch, 2001). Moreover, earthworm burrowing activities significantly promote the increase of water filtration and soil aeration (Lavelle et al., 2007). Earthworms are primary consumers of OM in soil and thus they can be exposed to both organic and inorganic contaminants that are adsorbed to soil OM particles (Bouche, 1983, 1992; Hopkin, 1989).

The significant role of earthworms in OM breakdown and the cycling of nutrients has contributed to increased interest in their use as indicator organisms in biological impact studies of soil contamination. In laboratory tests for soil ecotoxicological studies, certain organism life-cycle parameters such as growth and reproduction rates and the accumulation and excretion of pollutants can be measured, giving a good idea of what can be observed in the real ecosystem. Due to their intensive interactions with soil, earthworms are very sensitive to, and significantly affected by, contaminants reaching the soil system, so they can be considered as valuable bioindicators (Lanno et al., 2004). Earthworms are exposed to soil contaminants through different routes of exposure. These organisms live in direct contact with the soil pore water and therefore with the dissolved contaminants. Earthworm skin is known to be very permeable to water (Wallwork, 1983) and it represents the main route for contaminant uptake (Jager et al., 2003; Vijver et al., 2005). Earthworms also ingest large amounts of soil, thus they are continuously exposed to contaminants adsorbed to solid particles passing through their digestive tract (Morgan et al., 2004).

Earthworms show a considerable capacity to accumulate organic and inorganic contaminants present in soils (Morrison et al., 2000). Moreover, according to Blouin et al. (2013) earthworms can be responsible for changes in the availability of inorganic and organic pollutants in soils due to the stimulation of the microbial population that accelerates the degradation of the contaminants through metabolization and/or volatilization processes. They can also be responsible for reducing or increasing the level of contaminant sorption onto soil particles through the digestion of organic matter and its subsequent degradation, leading to modifications in soil chemistry (pH and Eh). In a study with the earthworm *Lumbricus rubellus*, Vijver et al. (2005) evaluated the weight of each pathway for metal uptake and concluded that the main route of exposure is dermal absorption whereas pore water uptake via ingestion makes only a small contribution to metal accumulation.

In the case of POPs, earthworms can accumulate them passively by dermal absorption, and actively through ingestion. The relative importance of dermal and dietary exposure depends on the soil contaminant individually and the importance of the dietary pathway increases as a

function of the hydrophobicity of the contaminant (Jager et al., 2003; Ma et al., 1998). Some studies have already demonstrated that earthworms are able to accumulate POPs and therefore represent a factor of exposure and a means for POPs to enter the terrestrial food chain. These POPs are substances such as pharmaceuticals, synthetic fragrances, detergent metabolites, polycyclic aromatic hydrocarbons, biogenic sterols, disinfectants and pesticides (Kinney et al., 2008, 2012; Carter et al., 2014).

Several earthworm species have been shown to significantly accumulate POPs. In a study with four different species (*Eisenia andrei*, *Eisenia fetida*, *Eisenia hortensis* and *Lumbricus terrestris*), Ville et al. (1995) observed the accumulation of PCBs in earthworm tissues. Also, Bu et al. (2010) found the capacity of *E. fetida* to accumulate PCBs-dl. Vermeulen et al. (2010) conducted a study to investigate the accumulation of PCBs and PBDEs in the soil-earthworm-hedgehog food chain, where the accumulation of these compounds was effective in *Lumbricus rubellus* tissues. Reinecke and Nash (1984) observed the accumulation of PCDDs and PCDFs in the tissues of two earthworm species *Allolobophora catiginosa* and *Lumbricus rubellus*. Earthworms belonging to *E. fetida* and *Allolobophora caliginosa trapezoides* species were collected from a typical E-waste dismantling area in east China and showed the accumulation of PCBs, PCDDs, PCDFs and PBDEs in their tissues due to ingestion and direct contact with severely contaminated soils (Shang et al., 2013). Henriksson et al. (2017) also confirmed the occurrence of PCDDs and PCDFs in *E. fetida* tissues resulting from both *in situ* and exposure in laboratory conditions to contaminated soils from Sweden.

Bioaccumulation phenomena can be defined as species-specific (Heikens et al., 2001; Hendrickx et al., 2004; Nahmani et al., 2007). This process is controlled by the various physicochemical properties of the contaminants and the environmental compartment (Vijver et al., 2005), such as contaminant concentration and speciation (Heikens et al., 2001; Hobbelen et al., 2006; Spurgeon et al., 2006; Nahmani et al., 2007), the type and characteristics of the soil (Hendrickx et al., 2004; Kizilkaya, 2005; Hobbelen et al., 2006; Spurgeon et al., 2006), the temperature (Olchawa et al., 2006) and the duration of exposure (Nahmani et al., 2007).

Earthworms are the basis of the terrestrial food chain as they serve as a major food source for amphibians, reptiles, birds and mammals. For this reason, the bioaccumulation of contaminants in earthworm tissues leads to a risk of transfer to higher trophic levels (Marino et al., 1992).

In recent decades, earthworms have received growing recognition as an important test organism for studying the adverse effects of contaminants on soil organisms in view to establishing a set of standard guidelines. At the beginning of the 1980s, the European Union and the Organization

for Economic Cooperation and Development (OECD) defined acute toxicity tests for earthworms based on mortality and growth rate (OECD, 1984). Later, these tests were implemented with chronic toxicity assays based on reproduction rate measurement (OECD, 2004).

In the present work, *E. fetida* was the species chosen to conduct the toxicity and bioaccumulation tests. *E. fetida* is probably the standard test organism used most in terrestrial ecotoxicology studies, due to its rapid life cycle, and easily controlled breeding and reproduction in the laboratory (Reinecke and Reinecke, 2004).

THINGS TO KEEP IN MIND

- ✓ Earthworms can be considered as good test organisms for soil POPs contamination once they are in direct contact with soil and soil pore water or through food exposure. Earthworms are the basis of the food chain and provide privileged pathways for POPs entry, leading to the risk of transfer to higher trophic levels and the occurrence of biomagnification.
- ✓ Several studies have already demonstrated that earthworms can accumulate POPs such as PCBs, PCDDs, PCDFs and PBDEs in their tissues at considerable levels without causing mortality or severe toxic effects.
- ✓ Earthworms accumulate POPs passively through dermal absorption, and actively through ingestion. The importance of the dietary pathway increases as a function of the contaminant's hydrophobicity.
- ✓ The phenomenon of bioaccumulation is species-specific and controlled by the physicochemical properties of the contaminants and the environmental compartment: contaminant concentration and speciation, the type and characteristics of the soil, the temperature and the duration of exposure.

2.1.2. Bioaccumulation in plants

Plants are usually considered as important ecological tools, covering around 47% of the total land surface on Earth. Moreover, plants play an important role in the purification and detoxification of environmental compartments such as air, water and soils (Kvesitadze et al., 2004). The plant root system directly and/or indirectly influences the soil, constituting a zone called rhizosphere, a zone of intense exchange between the soil and the roots (Heller et al., 1998; Lambers et al., 2009; Adhya et al., 2018). Moreover, roots are responsible for modifications in the physico-chemical and biological characteristics of the rhizospheric soil such as: reduction of soil pH due to the emission of protons by roots during the absorption of cations (ionic exchange between root and soil); reduction of redox potential caused by the abundant presence of OM; typology of OM present resulting from the original soil OM modified by the polysaccharides and organic acids typically exuded by roots (Stengel and Gelin, 1998).

The process of root uptake is undoubtedly the principal source of entry and accumulation of POPs present in the soil compartment (Ryan et al., 1988). This process involves a complex combined mechanism of compound-specific active and passive uptake (Carman et al., 1998). Usually, three main pathways through which POPs can enter a plant are considered: a) root uptake and subsequent translocation by the transpiration steam; b) shoot uptake from the air; and c) root uptake and later transport in oil cells which are found in oil-containing plants such as cress and carrot (Topp, 1986; Ryan et al., 1988; Ericksson et al., 1989; Zhang et al., 1999). The uptake of POPs by plants is strongly dependent on the plant species and characteristics (type of root system, tissues and enzymes) as well as on the physico-chemical characteristics of the contaminants (water solubility, vapor pressure, molecular weight, K_{ow}) and the environmental conditions (temperature, pH, OM and soil moisture contents) (Schroll et al., 1994). The plant translocation system is water-based, thus POPs are not drawn into the inner root tissues or xylem fluid (Simonich and Hites, 1995; Kipopoulou et al., 1999). According to Simonich and Hites (1995), organic pollutants are accumulated from the air by the leaf surface. The fact that the migration of POPs from the outer leaf tissues to the inner ones is a slow process and that the phloem system is water-based explains why the higher concentrations of the pollutants are usually found in leaves and not in the tissues of other plant parts (Simonich and Hites, 1995; Boopathy, 2000). Once they have entered the plant structure, POPs can be: a) translocated to other plant tissues associated with the transpiration stream (Schroll et al., 1994),

b) transformed into less toxic compounds by degradation processes (Schnoor et al., 1995; Newman, 1997), or c) incorporated in plant tissues and thus remain stored and unavailable (Field and Thurman, 1996). The translocation of POPs absorbed by roots into different plant organs results from the nutrient transport processes via the: a) transpiration stream, where water and dissolved substances pass from roots to shoots through the xylem, b) assimilate stream, where the transport of assimilates from leaves to the lower plant parts (shoot axis and roots) as well as to apex and fruits occurs through the phloem system.

As mentioned already, plant POPs uptake is species dependent since the degree of uptake is directly related to the plant's lipid content in case of lipophilic POPs (Simonich and Hites, 1995; Meredith and Hites, 1987; Hermanson, 1990). According to Ryan et al. (1988), POPs degradation processes do not occur within the plant tissues and since the plant root uptake and translocation of POPs from the soil is passive, the plant uptake process can be defined as a set of consecutive partition reactions between soil particles and soil water, soil water and plant roots, plant roots and plant transpiration system, and plant transpiration system and plant stems. According to Schnoor (1999), it is possible to predict the uptake of POPs by plants regarding the octane rating of the contaminant once the POPs are absorbed and translocated within the plant tissues only when the POPs present a $\log K_{ow}$ between 0.5 and 4. Moreover, the molecular weight of a compound is one of the main limiting factors during the passage of POPs into plant roots (Söchtig, 1964).

Plant contaminant uptake can pose serious risks from an ecosystem to human health perspective, since plants are at the bottom of the food chain and thus at the beginning of an exposure pathway via food intake to animals (Currado and Harrad, 2001).

The process of bioaccumulation of PCBs in plants can be considered as a complex blend of the physico-chemical nature of the substance and its interaction with the plant biota (Wang et al., 1997). Moreover, the uptake of PCBs with a $\log K_{ow}$ ranging between 4.5 (for the monos) and 8.2 (for the decas) is expected to decrease directly in line with the degree of chlorination (Schnoor, 1999). Liu and Schnoor (2008) concluded that some mono to tetra PCBs are absorbed by plant roots but only the lower chlorinated ones undergo translocation to aerial plant tissues. Members of the Cucurbitaceae family have been shown to accumulate PCBs in their tissues (Hülster et al., 1994). In a study with pumpkin plants, Aslund et al. (2008) also found that the PCBs concentration increased within the stem and leaves after a short period of exposure to contaminated soils, but the concentrations measured in the plant roots remained unchanged. This can be explained mainly by the occurrence of translocation processes (Aken et al., 2010).

Nonetheless, the PCBs levels measured in plant tissues are dependent on PCBs concentrations present in soils as well as on the plant species, the organic components of the soil and temperature (Strek and Weber, 1982). These findings have been also confirmed by other studies by Smith et al. (2007), Mackova et al. (2007) and Aken et al. (2010).

PCDDs and PCDFs are highly lipophilic compounds primarily sorbed by plant roots or soil components, though they are not usually translocated within plant tissues (Reischl, 1989). Despite that, several previous studies showed that bioconcentrations from soil of PCDDs and PCDFs clearly exist in zucchini plants two orders of magnitude higher than those found in vegetables such as pumpkin and cucumber, despite the fact that these three plants belong to the Cucurbita family (Hulster et al., 1994).

Elevated concentrations of PBDEs were also already reported in spinach in Japan (Ohta et al., 2002). An interesting fact is that not only were higher levels of PBDEs quantified in spinach but also the congener composition was different from that of the root vegetables, namely potato and carrot, analyzed in the study, a finding that can be explained by a difference in contamination pathways.

The presence of POPs in plant tissues leads to toxic effects such as the inhibition of plant growth and malformations on newly developed aerial parts (Weber and Mrozek, 1979) as well as a reduction in plant height and fresh weight (Strek and Weber, 1982).

In the present study, alfalfa (*Medicago sativa*), watercress (*Nasturtium officinale*) and white mustard (*Sinapis alba*) were the plant species chosen to conduct the toxicity and bioaccumulation tests. Research on different plant species was carried out and the 3 species selected were chosen according to their growth rates (maximum biomass quantity reached in 30 days), their uses (cultivation, grassland, garden), root type (tracer or pivoting) and family (monocotyledone, dicotyledone).

THINGS TO KEEP IN MIND

- ✓ Plants are usually considered as important ecological tools that play a major role in environmental compartments such as air, water and soils. The plant root system directly and/or indirectly influences the soil properties, constituting a zone of intense exchange between soil and roots, in the so-called rhizosphere.
- ✓ Plants are at the bottom of the food chain and thus at the beginning of an exposure pathway via food intake to animals and humans due to the consumption of aerial parts and root systems in some cases.
- ✓ Several studies have already demonstrated that plants can accumulate POPs such as PCBs, PCDDs, PCDFs and PBDEs in their tissues at considerable levels without causing mortality or severe toxic effects due to root uptake and/or atmospheric deposition.
- ✓ The process of root uptake is undoubtedly the principal source of entry and accumulation of POPs from soil. This process involves a complex combined mechanism of compound specific active and passive uptake that takes place in the rhizosphere zone, comprising three main pathways through which POPs can enter a plant: a) root uptake and subsequent translocation by the transpiration steam, b) shoot uptake from the air, and c) uptake and transport in oil cells in the case of oil containing plants.
- ✓ The bioaccumulation phenomenon is species-specific and strongly dependent on the plant's characteristics (type of root system, tissues and enzymes) as well as on the physico-chemical characteristics of the contaminants (water solubility, vapor pressure, molecular weight, K_{ow}) and the environmental conditions (temperature, pH, OM and soil moisture contents).

2.2 Mobility of BFRs in the environment and potential transfer to living organisms

POPs are well-dispersed in the environment in every ecosystem compartment and can undergo biogeochemical cycles (Figure 2).

It is largely accepted that POPs sorption and bioavailability in soil systems is mainly controlled by the chemical properties of the soil, namely its OM and organic carbon contents (Senesi and Loffredo, 2009). Nevertheless, the structural and functional composition of OM is the main key player in POPs adsorption and behavior in soil, having a greater impact than organic carbon content in these processes (Vlčková and Hofman, 2012). Also, properties of POPs and especially BFRs, such as low solubility, high K_{ow} and high hydrophobicity, are responsible for the prevalence of these compounds to be found mainly in soils, sediments and biota, with these matrices being considered their main environmental reservoirs (Iqbal et al., 2017).

Under specific environmental conditions, POPs and subsequently BFRs can undergo degradation due to several physico-chemical processes such as photodegradation, decomposition, chemical reactions with other compounds present in the compartment and changes in the compound structure due to environmental factors such as temperature and pH (Kot-Wasik et al., 2004; Mamy et al., 2015; Nolte and Ragas, 2017). Biotic processes include the phenomena of bioaccumulation and entry into the food chain, biotransformation and bacterial and microbial biodegradation (Vrkoslavová et al., 2011; Eljarrat et al., 2011; Lal et al., 2010).

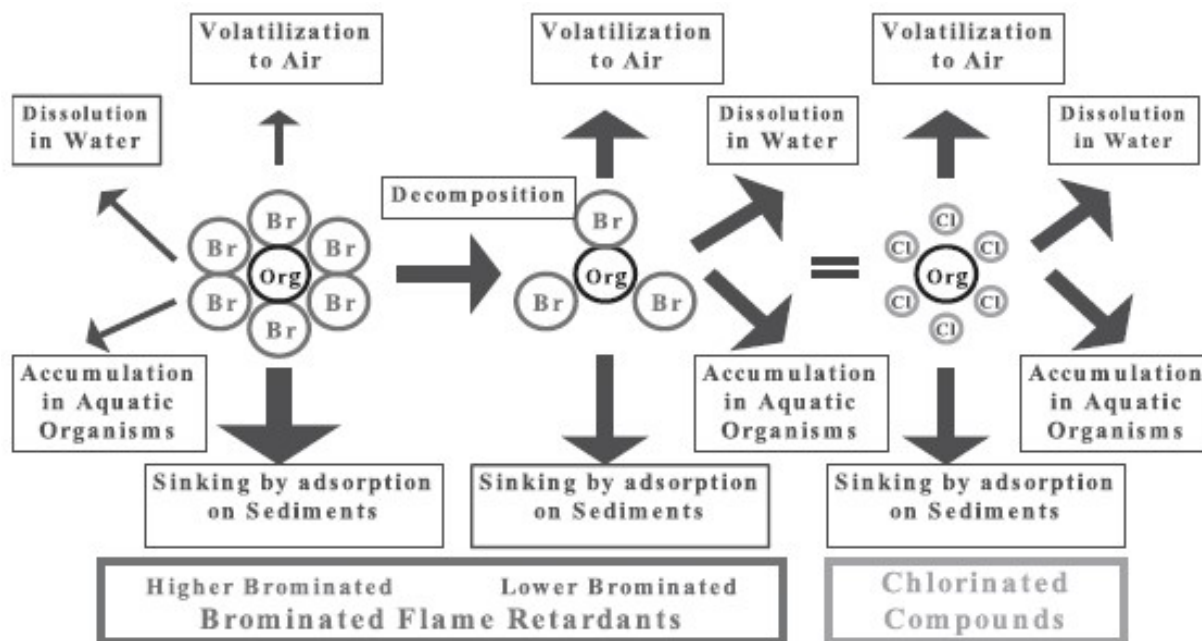


Figure 2 – Schematic representation of the environmental behavior of brominated flame retardants and chlorinated compounds (from Watanabe and Sakai, 2003).

As shown in Figure 2, both BFRs and chlorinated compounds can undergo a large number of chemical (quite significant debromination) and biological processes that control their potential transfer, sinking and accumulation in environmental compartments as well as in the living community. Almost all the BFRs used are highly brominated compounds that are less mobile due to their low volatility, water solubility, bioaccumulation and strong adsorption to soil and sediments. On the contrary, the lower brominated ones that include the decomposition products of BFRs are characterized by high mobility due to their high volatility, water solubility and greater bioaccumulativity than the highly brominated compounds (Watanabe and Sakai, 2003). Both brominated and chlorinated compounds undergo the same processes of volatilization into the atmospheric compartment, dissolution in water with considerable accumulation in aquatic organisms, and sinking by adsorption to soil and sediment particles. These characteristics support the assumption that the environmental behavior of lower brominated compounds is similar to those of chlorinated compounds such as PCBs, PCDDs and PCDFs (Watanabe and Sakai, 2003).

The lipophilicity of POPs and BFRs favor their bioavailability and thus their transfer to organisms, accumulation in the tissues of the latter and their potential entry and transfer through the several trophic levels of the food chain. Three main processes are involved here: bioconcentration, bioaccumulation and biomagnification.

Bioconcentration occurs when an organism absorbs and stores a contaminant at levels higher than those found in the environmental medium. The bioconcentration factor is defined by the ratio between the concentration of a contaminant in an organism and the concentration found in the environmental compartment (Arnot and Gobas, 2006).

Bioaccumulation occurs when the absorption of the contaminant is faster than the excretion process, and the internal concentration increases with time. Absorption can occur through a direct pathway (bioconcentration) and/or through biomagnification along the food chain (Mackay and Fraser, 2000). The potential for bioaccumulation usually tends to be positively correlated with the lipid solubility of the compound, once the chemical crosses the lipid layers of cells to reach the body tissues (Arnot and Gobas, 2006).

Biomagnification is the process that results from the entry of a contaminant via the food supply. This process can be considered as a specific type of bioaccumulation where the contaminant level found in the organism's tissues is greater than the concentration measured in its food and its surrounding environment (Mackay and Fraser, 2000). The level of this input is highly dependent on species-specific biochemical-physiological processes and on the biotransformation (metabolization) and elimination capacities of the compounds.

In recent decades, numerous studies have been conducted in which POPs levels were analyzed in a wide range of environmental matrices with a general trend towards a reduction of the concentrations measured. This decrease is related to the restrictions and even prohibitions of concerning certain POPs.

The levels of POPs and notably BFRs found in the tissues of organisms belonging to different levels of the trophic chain indicate that these compounds are available to be absorbed and bioaccumulated by organisms and readily lead to the occurrence of biomagnification phenomena. In San Francisco Bay, concentrations of PBDEs (330 up to 5500 ng.g⁻¹ lw), HBCDs (330 up to 5500 ng.g⁻¹ lw) and PBEB (< LOD up to 0.2 ng.g⁻¹ lw) were measured in the tissues of white croaker, in double-crested cormorant eggs and in seal tissues (Klosterhaus et al., 2012). These measured concentrations in animals tissues are some orders of magnitude higher than the concentrations of the analysed contaminants in correspondent environmental compartments thus suggesting the occurrence of biomagnification processes. The presence of POPs and legacy and new BFRs has been already observed in remote polar areas. McKinney et al. (2011) detected concentrations of PCBs (1797 up to 10537 ng.g⁻¹ lw), PBDEs (4.6 up to 78.4 ng.g⁻¹ lw) and HBCDs (< 0.3 up to 41.1 ng.g⁻¹ lw) in polar bears from Alaska. Houde et al. (2017) reported range of legacy BFRs (PBDEs (Σ_{13} PBDEs: 0.5 up to 25.8 ng.g⁻¹ lw) and HBCD

(< LOD up to 23.3 ng g⁻¹ lw)) and nBFRs (nHBB (< LOD up to 1.20 ng.g⁻¹ lw) and PBEB (< LOD up to 0.03 ng.g⁻¹ lw)) in seal tissues from the Canadian Arctic zone. In these reference studies, the levels of PCBs were usually higher than those of PBDEs, which in turn were also higher than the levels of HBCDs and BFRs found in animal tissues. The concentrations of PBDEs in these seals showed an increase during the period 1984 – 2003. In 2009 the PBDEs levels measured were significantly lower, an observation probably related to the ban of c-Penta-BDE and c-Octa-BDE formulations since 2004 (Ross et al., 2013).

THINGS TO KEEP IN MIND

- ✓ PCBs levels found in the environment are decreasing but they are still present in available quantities and can be bioaccumulated by living organisms
- ✓ BFRs are present in the environmental compartments at much lower levels than PCBs, PCDDs and PCDFs, but despite this they are available for incorporation and bioaccumulation in the tissues of living organisms.
- ✓ The presence and availability of POPs makes their biomagnification possible through food chain levels.
- ✓ Both brominated and chlorinated compounds undergo the same processes of volatilization to the atmospheric compartment, and dissolution in water with subsequent accumulation in aquatic organisms and sinking by adsorption to soil and sediment particles. This fact supports the assumption that lower brominated compounds presents a similar environmental behavior to chlorinated compounds such as PCBs, PCDDs and PCDFs.

Chapter 3. Material and Methods

This Chapter provides a complete description of the location and context of the sites studied. The methodologies and protocols used for the field sampling campaigns, pre-treatment and storage of soils and plants collected *in situ* are also described. This is followed by a description of the chemical protocols for determining general soil physico-chemical properties and the analytical quantification procedures for the MTEs selected in the anthroposoils and plant samples studied. The analytical procedures for the quantification of POPs in the anthroposoils and plant samples studied are also detailed. All the protocols followed for the preparation of earthworms and plants used in the reproduction, growth and bioaccumulation tests are described, as are the chemical procedures used to quantify the levels of POPs bioaccumulated in the earthworms and plants tissues tested.

The calculation and use of a bioaccumulation factor as well as the SET and ERITME indexes to infer the risks posed by the contamination to the ecosystem are detailed. The approach used for the *in situ* evaluation of biological activity and soil quality by monolith sampling and the use of the Bait-lamina© test is briefly described. Finally, the statistical methods that were used to analyze the results obtained are presented.

3.1. Selection of sampling areas

3.1.1. Origin of anthroposoil samples

The areas selected for this investigation represented different types of land-use (industrial, agricultural and pastoral, forest). The following criteria were considered for the selection of the sampling sites and sample collection:

- Areas with different types of contamination origin and land-use were selected;
- Samples were collected from two distinct areas in two countries that conformed to the study priorities, and data were already available in these specific action zones of the two Human-Habitat Observatories (CNRS Labex DHRIIM Observatoires Homme-Milieux Estarreja and Vallée du Rhône);
- Vegetated areas for soil sampling were selected.

Two different areas were sampled:

- ✓ Site A – Estarreja, close to a chemical complex, a coastal area in the North/Central-West Portugal ($40^{\circ} 46' 37.55''$; $8^{\circ} 34' 44.56''$);
- ✓ Site B - Casier Girardon Peyraud 6 -PK 61.500, in the Rhone river margin, France ($45^{\circ} 18' 04.94''$; $4^{\circ} 47' 54.44''$)

The location of sampling sites A and B is illustrated in Figures 3 and 4.



Figure 3 – Estarreja (Site A), location of sampling points.

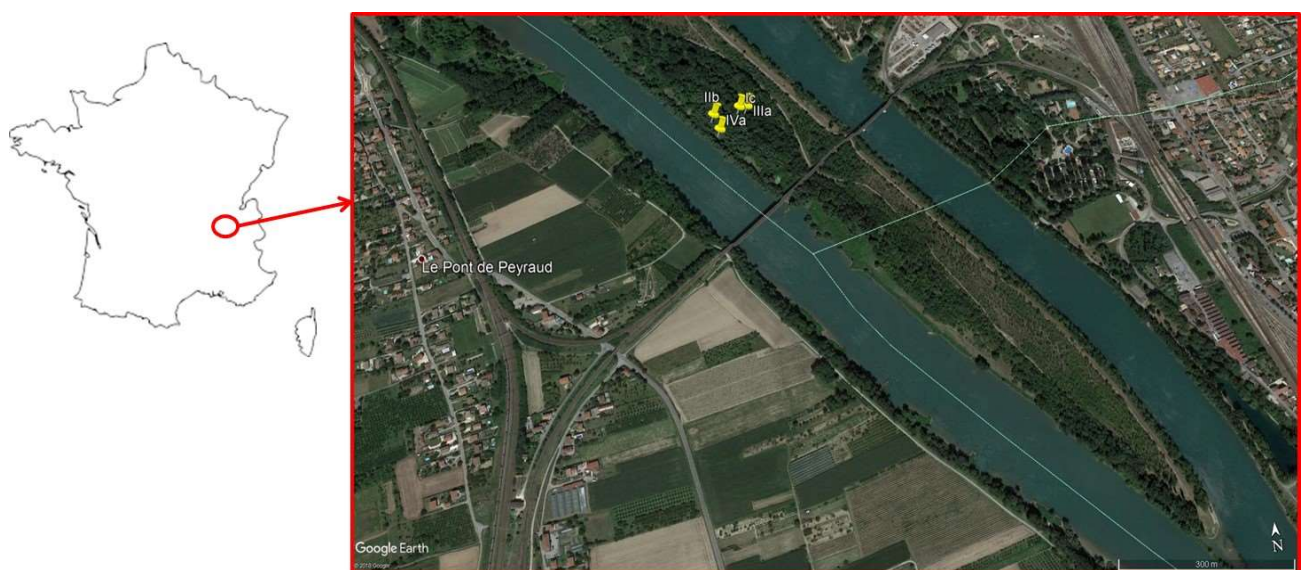


Figure 4 – Casier Girardon Peyraud 6 (Site B), location of sampling points.

Site A: Estarreja, Portugal

The Estarreja sampling site is on the North-Eastern edge of the Aveiro sedimentary basin (Costa and Jesus-Rydin, 2010). In this area, the Precambrian Schistose bedrock is covered by Quaternary deposits (12 m thick), composed of interstratified conglomerates, sandstone, shale and marlstone (Costa and Jesus-Rydin, 2001). The dominant soil types in this area are Podzols and Cambisols (Inácio et al., 2008).

This area is considerably impacted by the industrial activity of the Estarreja Chemical Complex. According to Costa and Jesus-Rydin (2001), the largest industrial companies belonging to this complex are: (i) QUIMIGAL which produced ammonium sulfate from sulfuric acid and ammonia (1952-1990s), nitric acid and ammonium nitrate (1974-1990s), and since 1978 to now it has produced nitric acid, aniline and nitrobenzene in an industrial unit named ANILINA PORTUGAL; (ii) CIRES, which, in 1963, started the production of synthetic resins such as polyvinyl chloride (PVC) from vinyl chloride monomer (VCM, which was also produced there until 1986); (iii) DOW PORTUGAL, which has produced aromatic-based isocyanide polymers since 1978; (iv) UNITECA – a Chlor alkali plant installed in 1956 to produce sodium and chlorine compounds from rock salt. Until 1975, three effluent streams were used to transport the liquid effluents from this Chemical complex: Vala de S.Filipe, Vala de Breja and Vala do Canedo. These man-made streams run through surrounding agricultural fields and were used to transfer industrial liquid effluents containing aniline, benzene, monochlorobenzene, mononitrobenzene and various MTEs into the “Esteiro de Estarreja”, a river branch of the nearby lagoon “Ria de Aveiro” (Costa and Jesus-Rydin, 2001).

Soil contamination by several MTEs and the data describing these soils were reported previously [Inácio et al. (1998), Rodrigues et al. (2006, 2010), Cachada et al. (2009) and Reis et al. (2009)]. Soil contamination with POPs, namely PAHs and PCBs were already reported by Cachada et al. (2012b) in the surroundings of the Estarreja Chemical complex.

Composite soil samples (around 30 kg each) were collected in Estarreja at four sampling points surrounding the Estarreja Industrial Complex. Thus, samples labeled C, G, K and L of soils with different land uses (agricultural, industrial, prairie and forest, respectively) were used (Figure 5).



Figure 5 – Estarreja (Site A), overview of sampling points.

Site B: Casiers Girardon, Lyon, France

At the end of the 19th century, submersible longitudinal and herring-bone dikes were constructed in certain sections of the Rhône river (France), delimiting dike fields called “Casiers Girardon”. These structures were constructed to regulate river flows and form a fixed deep single-bed channel suitable for navigation while at the same time store fine sediments on the banks, leading to the formation of dike fields over time (Besacier-Monbertrand et al., 2014).

In the 20th century, the installation of hydropower plants led to the diversion of the main river channel into a series of parallel secondary channels. The former sections were bypassed and most of the time only filled with a residual minimum water flow. In these bypassed zones, most of the dike fields were progressively filled by sediment and for the most part colonized by trees. Due to the fall in water levels and filling by sediment these fields have become completely disconnected from the main river channel. In very few cases, these fields are intermittently connected to the river with variable degrees of water transfer, mainly due to rainy episodes during the winter which cause the river to overflow.

Considerable sedimentation took place in these zones of the river, leading to the potentially significant accumulation of both organic and metallic pollutants associated with the sediments that have been stored (Thorel et al., 2018).

In the future, dismantling operations will be carried out in the Casiers to improve the ecological and hydraulic functioning of the river. All the sediments stored will be reincorporated in the river bed. The potential existence of organic contaminants such as PCBs in these stored sediments may give rise to concerns about the ecological risk posed by the dismantling operations (Thorel et al., 2018).

Four sediment samples (named PEY Ic, PEY IIb, PEY IIIa and PEY IVa of around 30 kg each) of riverine origin were collected on the Old Rhone from the Casier Girardon Peyraud 6, PK 61.500, Sector Péage-de-Roussillon, located 50 km south of Lyon (France) (Figure 6).



Figure 6 – Casier Girardon Peyraud 6 (Site B), aspect of sampling points.

3.1.2. Origin of plant samples

Several entire plants of Common nettle and Japanese knotweed, belonging to the species *Urtica dioica* and *Fallopia japonica*, respectively (Figure 7), were also collected from the two sampling points of the Casier Girardon Peyraud 6, PK 61.500 (Rhône river margin, France), PEY Ic and PEY IIIa.

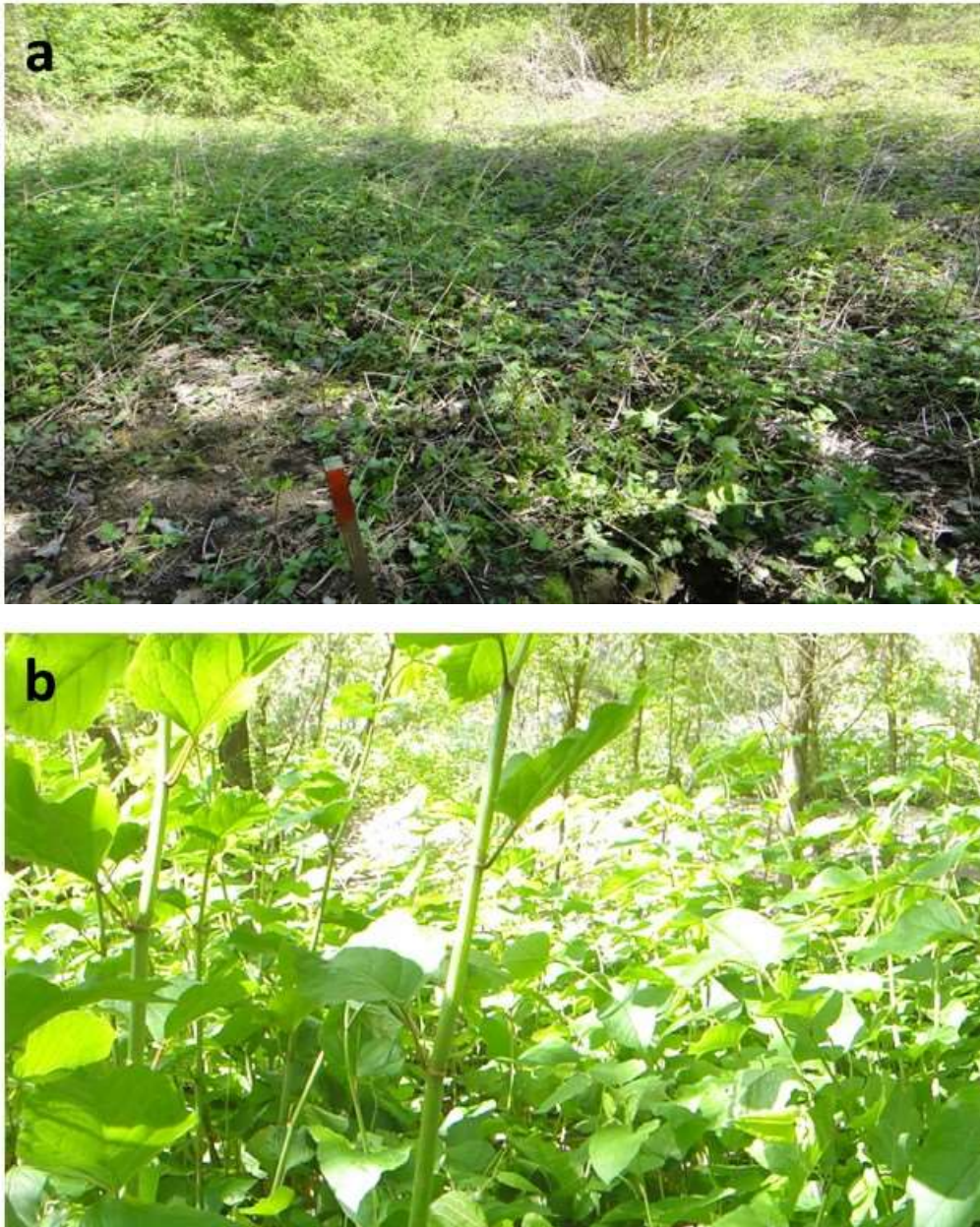


Figure 7 – Plants collected *in situ* from Casier Girardon Peyraud 6 (Site B), from (a) PEY Ic and from (b) PEY IIIa, belonging to the species *Urtica dioica* and *Fallopia japonica*, respectively.

3.1.3. Anthroposoil and plant sampling procedures

Anthroposoil samples were collected from a depth of 0 – 50 cm. At each sampling point, several samples were collected within an area of 1 m² and combined to obtain a single composite sample (approximately 30 kg). After collection, the soil samples were stored in plastic containers lined with aluminium foil in a climatized cold chamber in the laboratory.

Around 10 entire plants specimens (aerial parts + roots) were collected in the same points of the soil sampling and stored in plastic bags until their arrival in the laboratory.

3.1.4. Anthroposoil and plant sample pretreatment and storage

For the quantification of MTEs, a representative portion of each anthroposoil sample collected was dried in an oven at 40°C until reaching constant weight and then sieved at 1 cm. For the organic contaminants, soil and sediment samples were stored in glass jars and/or in aluminium foil in a cold chamber prior to analysis.

After arriving in the laboratory, the plant samples were thoroughly washed with tap water to remove all traces of dust and soil and rinsed with distilled water at least 3 times. In certain cases, an ultrasound bath was used. Roots were separated from aerial parts and both were dried in an oven at 40°C until reaching constant weight. The dried samples were ground and stored in plastic bags at room temperature for further analysis.

3.2. Anthroposoil and plant analysis

3.2.1. Physicochemical characterization

Using standard methods, the following basic parameters were determined for the dried and sieved anthroposoils:

- pH (in KCl and water; NFX 31-117),
- water content (ISO 11465:1993),
- loss on ignition to determine the OM content (Dean, 1974; Heiri et al., 2001),
- organic carbon (OC; ISO 10694:1995),
- cation exchange capacity (CEC; NFX 31-130),
- elemental elements such as Ca, Mg and P analysis (NFX 31-108),
- phosphorus Olsen (NFX 31-160),
- C/N ratio
- particle size distribution (laser diffraction in a Malvern Mastersizer ®2000).

3.2.2. Contents of metallic trace elements in anthroposoil samples

Pseudo-total determination of the MTEs Cd, Cr, Cu, Ni, Pb and Zn were performed on soils and sediments. For the Estarreja soil samples, the analysis was conducted at Acme Labs Canada according to their own protocol AQ200. Briefly, 0.5 g dw of solid sample was leached by a modified aqua regia digestion procedure (1:1:1; HNO₃:HCl:H₂O) and the extracts were analyzed by ICP-ES/MS to determine the “aqua regia fraction” (hereafter referred to as pseudo-total levels). Reference soil materials (DS10 and OREAS45EA) were used to check the efficiency of the mineralization and/or calibration of the metals studied. The limits of quantification (LOQs) of the various trace elements were the following (in mg.kg⁻¹ in between brackets): Cd (0.1), Cr (0.1), Cu (0.1), Ni (0.1), Pb (0.1), and Zn (1.0).

For the Peyraud sediment samples, 1.0 g dw of each sample was dissolved using an acid mixture (6:2; HCl:HNO₃), followed by heating in a CEM Mars 5 type microwave oven. After filtration through ash-free filter paper (Whatman No. 541), the filtrate was adjusted to a final volume of 25 ml with ultrapure water. The trace elements Cd, Cr, Cu, Ni, Pb and Zn were measured by ICP and/or FAAS (Zeeman effect) (Zhao et al., 1994). Certified Reference Material (CRM 281 from BCR) and control samples, such as Surface Water Level 2 (Spectrapure standards), were

used to check the efficiency of the mineralization and/or calibration of the metals studied. LOQs of the various trace elements were the following (in $\mu\text{g}\cdot\text{kg}^{-1}$ for Cd, Cr, Cu, Ni, Pb and in $\text{mg}\cdot\text{kg}^{-1}$ for Zn, in between brackets: Cd (0.1), Cr (0.1), Cu (1.0), Ni (5.0), Pb (0.22) and Zn (0.1). All the measurements were performed in triplicate for each sample. The reagents used were of analytical or ultrapure grade and the glassware was subjected to a cleaning procedure prior to use, with a 5% HNO_3 (v/v) cleaning solution for at least 12 hours, and then rinsed thoroughly with deionized water.

3.2.3. Contents of persistent organic pollutants in anthroposoil samples

POPs include a large number of compounds belonging to different families. In the present study, only certain compounds will be considered due to their presence in the environmental matrices studied.

Despite the existence of 209 PCB congeners, the present study will focus only on 18 PCB congeners distinguished as dioxin-like (PCB congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) and non-dioxin like, such as PCB congeners 28, 52, 101, 138, 153 and 180. The PCB indicator ($\sum 7\text{PCBi}$) comprises the non-dioxin like congeners plus congener 118. The latter are considered the most persistent with a predominant presence in both biotic and abiotic matrices, and characterized by higher toxicity, representing around 80% of the total PCBs (INERIS, 2011d).

The PCDDs were targeted by analyzing seven congeners (2.3.7.8 TCDD, 1.2.3.7.8 PeCDD, 1.2.3.4.7.8 HxCDD, 1.2.3.6.7.8 HxCDD, 1.2.3.7.8.9 HxCDD, 1.2.3.4.6.7.8 HpCDD and OCDD) and the PCDFs by analyzing ten congeners (2.3.7.8 TCDF, 1.2.3.7.8 PeCDF, 2.3.4.7.8 PeCDF, 1.2.3.4.7.8 HxCDF, 1.2.3.6.7.8 HxCDF, 1.2.3.7.8.9 HxCDF, 2.3.4.6.7.8 HxCDF, 1.2.3.4.6.7.8 HpCDF, 1.2.3.4.7.8.9 HpCDF and OCDF). The compounds referred to are usually subjected of regulatory actions (Commission Directive 2006/13/EC).

Regarding the BFR compounds, seven indicator PBDEs were analyzed (congeners 28, 47, 99, 100, 153, 154, 183 and 209) as representative of the principal constituents of Penta and Octa-BDE, widely used commercial formulations. PBDE 209 is the main constituent of the Deca-BDE commercial formulation. Three PBB congeners (52, 101 and 153) and the three HBCD isomers (α , β and γ) were quantified. Fourteen nBFRs were assessed in the samples: PBEB, nPBT, TBCT, nHBB, nPBB, pTBX, OBIND, T23BPIC, ETHBB, TBBPA-bME, BTBPE, TBBPA-bDiBPrE, Tri BRPs, Tetra BRPs and Penta BRPs (see Table of abbreviations).

For the 8 composite anthroposoil samples, an aliquot of each soil sample was placed in an extraction cell for POPs extraction and the Accelerated Solvent Extraction procedure was performed using a SpeedExtractor (Buchi). A mixture of toluene/acetone [70/30, v/v] was injected into the cell at a pressure of 100 bar and a temperature of 120°C. Three injection cycles of 5 min each were carried out to extract all the apolar compounds. The extracted organic phase was dried in a rotary evaporator (Buchi R200), weighed, dissolved in 15 mL hexane and the ¹³C labelled internal standards were added.

To separate PCBs and PBDEs for the analysis, three cleaning steps were carried out using acidic silica, Florisil® and celite/carbon purifying columns. In the first cleaning step, the interfering compounds were removed in the first silica gel column activated with sulfuric acid. PCBs and PBDEs were separated on the second Florisil® column while the separation of coplanar (non-ortho) PCBs from non-planar PCBs and PBDEs was performed on a mixture of Florisil®/Carbopack C/Celite 545 activated overnight at 130°C. Recovery standards were added to the extracts obtained and they were evaporated until dryness under a gentle stream of nitrogen. The dry extracts were diluted to a final volume of 20 and 50 µL toluene to analyze the coplanar PCBs and non-coplanar PCBs with PBDEs, respectively.

To separate nRFBs from the other compounds, the sample extract was loaded twice in 15 ml hexane in the coupled columns. PBBs and several nBFRs (pTBX, TBCT, PBBz, HBBz, PBT, PBEB and OBIND) were eluted twice with 45 mL hexane (Extract 1). The eluted fraction was evaporated to 1ml solvent and purified in a carbon/Florisil® column. The extract obtained was evaporated to dryness under a gentle nitrogen stream and the solvent changed to 20 ml of toluene.

To separate HBCDs and other nBFRs another aliquot of the extract obtained in the chemical extraction step was eluted through three silica columns with 130 ml of dichloromethane. The eluted fraction was evaporated to 150 ml dichloromethane after which sodium hydroxide was added (1 ml, 1 M). HBCDDs, T23BPIC, EHTBB, BEHTEBP, BTBPE, DBDPE, TBBPA-bME and TBBPA-bDiBPrE were extracted from the aqueous phase by 5 ml followed by an additional 2 ml of hexane.

Polychlorinated and BFRs compounds quantification was carried out by gas chromatography coupled with high-resolution mass spectrometry (GC/HRMS) using a 7890A gas chromatograph (Agilent) coupled with a JEOL JMS 700D or a JMS 800D double-sector high resolution mass spectrometer (JEOL, Tokyo, Japan). The system included a HT8PCB capillary column (60 m × 0.25 mm × 0.25 µm) for PCBs, a DB5MS (J&W) capillary column (30 m × 0.25 mm × 0.25 µm) for PCDD/Fs, PBDEs, PBBs and HBCD and an RTX1614 capillary

column (15 m × 0.25 mm × 0.25 μm) for PBDE 209. Injection was carried out in splitless mode and helium was used as a carrier gas (1 ml/min). The injection volume was 2 μl. The GC program for PCBs was: 1 min at 120°C, 20°C/min to 200°C, 3°C/min to 260.5°C and 30°C/min to 330°C and held for 3.5 min. For PBDEs and PBBs, the program was: 2 min at 120°C, 10°C/min to 215°C, 3°C/min to 270°C, 30°C/min to 330°C and held for 3.5 min. For PCDDs, PCDFs and HBCD, the program was: 3 min at 120°C, 20°C/min to 170°C, 3°C/min to 260.5°C and 25°C/min to 300°C and held for 5 min. Finally, the following program was used for PBDE 209: 1 min at 180°C, 20°C/min to 280°C and 3°C/min to 260.5°C. The mass spectrometer resolution was set up at 10 000 (FWHM) and the spectrometric signals were recorded in Single Ion Monitoring (SIM) mode while focusing on two abundant ions selected in the molecular ion isotopic pattern. Ionization was achieved in electron ionization mode (40 eV electron energy). The signals obtained were integrated by JEOL DioK software (v.4). A quality assurance/quality control A procedural blank (consisting of a sodium sulfate and celite matrix treated with the same SOP as the sediment samples) and an internal quality control (QC) standard were included in each batch of ten samples. The associated in-house charts were in accordance with the acceptable limits fixed for these QC runs, *i.e.* set at $\pm 2\sigma$ of the average value. The analytical methods were conducted according to ISO 17025 standards: the QA/QC requirements were fulfilled throughout the whole study. Furthermore, the laboratory continuously participated in proficiency tests on PCDDs, PCDFs, PCBs and PBDEs with acceptable results (Z -score $b \pm 2$). The limit of detection (LOD) was determined by a signal-to-noise ratio equal to 3 ($S/N=3$) and systematically calculated by JEOL Diok software for each congener in each sample. For the PCB_i, the LOD value varied from 0.0049 (PCB 118) to 0.01 (PCB 28) μg.kg⁻¹ dw. For the PBDE_i, the LOD was from 0.032 (BDE 28) to 13.55 (BDE 209) ng.kg⁻¹ dw. For the PCDD/Fs, the LOD values ranged from 0.051 (2, 3, 7, 8 TCDD) to 1.645 (1.2.3.6.7.8 HxCDD) ng.kg⁻¹ dw. The recoveries ranged from 60 to 120%. They were calculated for every analyte quantified under isotopic dilution, based on their internal standard response as well as expressed in terms of the sample mass for each POPs families.

The POPs levels were then normalized according to organic carbon contents, by dividing the POPs concentrations measured in the anthroposol samples by the TOC (total organic carbon) content.

In addition, a DTPA extraction was performed on the 8 samples studied, for which 10 g dw of soils and sediments were shaken for 2 h in 20 ml of the extraction solution (0.005 M DTPA, 0.1 M triethanolamine TEA, 0.01 M CaCl₂ and buffered at pH 7.3) (Lindsay & Norvell, 1978).

After centrifugation (10 min at 4000 rpm), the suspension was filtered (0.45 μm cellulose acetate filter) and acidified with HNO_3 (ultrapure grade reagent) before analysis. The extract analysis for POPs determination was performed as already described above in this subsection. MTEs and POPs concentrations in the DTPA extracts obtained were also measured by GC/HRMS.

3.2.4. Contents of persistent organic pollutants in plant samples

For the POPs extraction at least 1 g of dry material of each plant part tissue was placed in an extraction cell of a SpeedExtractor (Buchi) with a mixture of toluene/acetone (70/30, v/v). Three pressurizing cycles at 120°C for 5 minutes each were carried out to extract all the apolar compounds. The extracted organic phase was evaporated (Buchi R200), weighed, dissolved in 15 ml of hexane and the internal standards ^{13}C were added.

To separate the PCBs and PBDEs for analysis, the purification steps were performed using acid silica, Florisil® and celite/carbon purification columns. The quantification of these compounds was performed by gas chromatography coupled with high resolution mass spectrometry (GC-HRMS, 7890A (Agilent) / JEOL 800D (JEOL, Tokyo, Japan)). A procedure blank (consisting of sodium sulfate and celite treated according to the same procedures used for the samples analyzed) as well as an internal quality standard were included for each sequence of ten samples. Acceptably limits for the quality control were defined as $\pm 2\sigma$ of the mean value. For the PCB_i, the LOD value varied from 0.0049 (PCB 118) to 0.01 (PCB 28) $\mu\text{g.kg}^{-1}$ dw. For the PBDE_i, the LOD ranged from 0.032 (BDE 28) to 13.55 (BDE 209) ng.kg^{-1} dw. For the PCDD/Fs, the LOD values ranged from 0.051 (2,3,7,8 TCDD) to 1.645 (1,2,3,6,7,8 HxCDD) ng.kg^{-1} dw.

3.3. Earthworm reproduction and bioaccumulation assays

Reproduction and bioaccumulation essays with *E. fetida* were conducted according to the standard guidelines OECD 222 (OECD, 2004) and OECD 317 (OECD, 2010), respectively. Earthworms were obtained from Provademse laboratory cultures (Lyon). Laboratorial cultures of earthworms were bred in large containers and under controlled conditions (temperature of $20 \pm 2^\circ\text{C}$ and continuous lightning) and the individuals were maintained in a mixture of peat,

dried manure and distilled water (the substrate was periodically moistened, monitored and renewed).

The assays were performed in plastic boxes (20 per 10 cm height) with approximately 600 g (not dried) of each soil or sediment sample and an artificial ISO soil used as control soil. The water content was adjusted for around 70% of soil or sediment maximum water holding capacity (WHC). Individual sexually matured earthworms with well-developed clitellums (earthworm weight 0.65 ± 0.05 g wet weight) were maintained in the ISO soil at least 24 h before being exposed to the soil and sediment samples. Four replicates per sample studied and for the control soil (ISO soil) were performed. For each replicate, ten adult earthworms were added to each container and remained there for 28 days (Figure 8). Once a week, 5 g of a mixture of baking powder and dry biological horse manure (previous analysed to MTEs and POPs concentrations and presenting negligible levels) were added to each replicate, as well as deionized water if necessary, to maintain constant soil water content.

After 28 days of exposure corresponding to the reproduction period, adults were collected by hand from each test box, counted and weighed. Juveniles continued to be exposed for another period of 28 days and at the end of the test they were removed by water filtration through a fine sieve (the soil quantity was reduced and only the fine fraction and the earthworms remained, thus making it easier to collect them). Juveniles were also counted and weighed. After counting, both adults and juveniles were dried and freezed stored in prior to analysis.



Figure 8 – Experimental system used for *E. fetida* reproduction and bioaccumulation tests.

3.4. Earthworm extraction and analysis

Adults and juveniles collected respectively at the 28th day and 56th day of the reproduction test were used to quantify the bioaccumulated levels of the POPs studied in their tissues.

The lipid content of *E. fetida* tissues was determined using the procedure described by Smedes (1999), consisting in a gravimetric determination after an extraction with 2-propanol and cyclohexane. The extraction ratio used was of 1g tissue (fresh weight, fw) to 1.6 ml of 2-propanol and 2.0 ml cyclohexane.

For the POPs extraction at least 1 g of dry earthworm tissue was placed in an extraction cell of a SpeedExtractor (Buchi) with a mixture of toluene/acetone (70/30, v/v). Three pressurizing cycles at 120°C for 5 minutes each were carried out to extract all the apolar compounds. The extracted organic phase was evaporated (Buchi R200), weighed, dissolved in 15 ml of hexane and the internal standards ¹³C were added.

To separate the PCBs and PBDEs for analysis, three purification steps were performed using acid silica, Florisil® and celite/carbon purification columns. The quantification of these compounds was performed by gas chromatography coupled with high resolution mass

spectrometry (GC-HRMS, 7890A (Agilent) / JEOL 800D (JEOL, Tokyo, Japan)). A procedure blank (consisting of sodium sulfate and celite treated according to the same procedures used for the samples analyzed) as well as an internal quality standard were included for each sequence of ten samples. Acceptability limits for the quality control were defined as $\pm 2\sigma$ of the mean value. For the Σ PCB_i, the LOD value varied from 0.0049 (PCB 118) to 0.01 (PCB 28) $\mu\text{g}\cdot\text{kg}^{-1}$ dw. For the Σ PBDE, the LOD ranged from 0.032 (BDE 28) to 13.55 (BDE 209) $\text{ng}\cdot\text{kg}^{-1}$ dw. For the PCDD/Fs, the LOD values ranged from 0.051 (2,3,7,8 TCDD) to 1.645 (1,2,3,6,7,8 HxCDD) $\text{ng}\cdot\text{kg}^{-1}$ dw. Two extraction and analysis replicates were done for each considered sample.

The POPs levels were then normalized according to lw for *E. fetida*, by dividing the POPs concentrations measured by the lw content.

3.5. Plant germination, growth and bioaccumulation assays

For the plant essays, previous works in the literature on several different plant species, regarding their characteristics, use, germination and growth time needs, helped us to choose several candidates. Finally, according to their growth rate (maximum biomass reached in 30 days), their use (cultivation, grassland, garden), type of roots (tracer or taproot) and family (monocotyledone, dicotyledone), the following three plant species: alfalfa (*Medicago sativa*), watercress (*Nasturtium officinale*) and white mustard (*Sinapis alba*), were selected for cultivation in the anthroposoils considered.

Terracotta pots with a diameter x height (15 x 15 cm) were coated with aluminum foil to avoid contamination from pot material that could interfere with the POPs quantification results.

To limit the output of fine soil particles, river gravel (previously washed and oven dried at 500°C) was placed at the bottom of the pots, obstructing the inlet but leaving space for the water to rise by capillarity. All the pots were then filled with the 8 anthroposoils studied up to 1 cm from the top, 5 pots per soil per plant species.

The filled pots were placed on aluminum trays to facilitate watering with demineralized water, and under artificial lighting (Light type Sylviana).

According to seed size, white mustard was sown about 2-3 cm deep at a rate of 100 seeds per pot. Alfalfa was sown about 1 cm deep at a rate of 200 seeds per pot. The watercress was sown about 1 cm deep at a rate of 200 seeds per pot (Figure 9).

The pots were exposed to a photoperiod of 16h daylight and 8h darkness for about 6 weeks in an acclimatized chamber. They were watered three times a week (Monday, Wednesday and Friday) by pouring water into the aluminum trays. The amount of water added to the trays depended on the quantity remaining inside the trays. The pots were randomly changed on the shelves to avoid any place effect on the plant germination and growth rates (Figure 9).



Figure 9 – Experimental system used for plant growth and bioaccumulation tests.

After 6 weeks exposure, the cultivated plants were removed. The aerial parts (stems and leaves) were separated from the roots and rinsed with demineralized water several times (4 to 5 times), after which an ultrasonic bath was used to wash the root parts (Figure 10). Aerial parts of each plant were counted and measured to determine the germination rate and the height at the end of the growth test. The aerial and root parts are then weighed (to obtain the fresh weight) and then put in an oven at 35°C for one week to slow dry prior to storage for chemical analysis. After drying period, the aerial and roots parts were reweighed to obtain the water content of plant tissues.

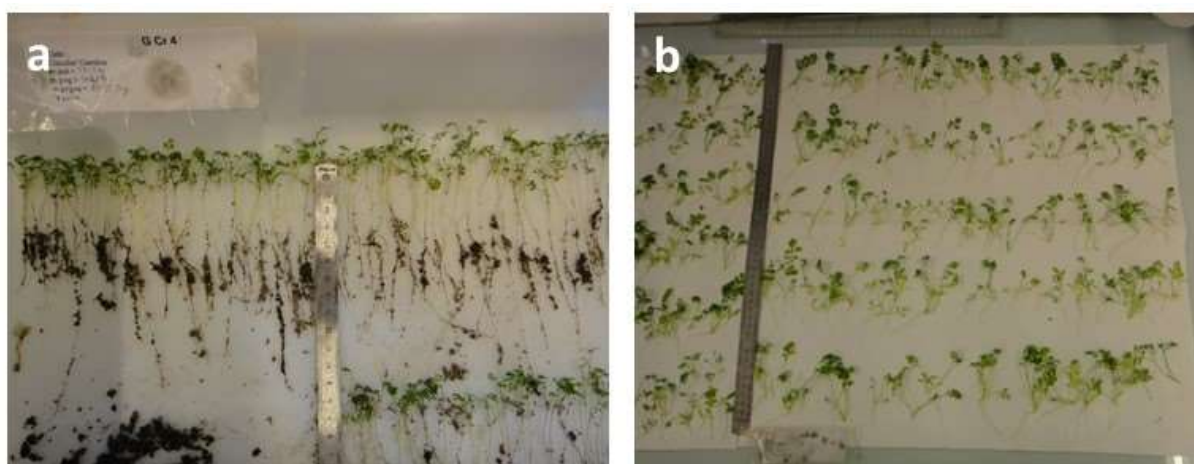


Figure 10 – Cultivated plants after harvesting (a) and after rinsing with distilled water (b) prior to weighing and storage for chemical analysis.

3.6. Plant extraction and analysis

For the POPs extraction at least 1 g of dry plant tissue part (after combining all the plants from the same culture pot) was placed in an extraction cell of a SpeedExtractor (Buchi) with a mixture of toluene/acetone (70/30, v/v). Three replicates were used to all plant tissues, except in case of cress and alfalfa roots that had only 2 replicates for each tested anthroposoil. Three pressurizing cycles at 120°C for 5 minutes each were performed to extract all the apolar compounds. The extracted organic phase was evaporated (Buchi R200), weighed, dissolved in 15 ml of hexane and the internal standards ^{13}C were added.

To separate the PCBs and PBDEs for analysis, there purification steps were performed using acid silica, Florisil® and celite/carbon purification columns. The quantification of these compounds was performed by gas chromatography coupled with high resolution mass spectrometry (GC-HRMS, 7890A (Agilent) / JEOL 800D (JEOL, Tokyo, Japan)). A procedure blank (consisting of sodium sulfate and celite treated according to the same procedures used for the samples analyzed) as well as an internal quality standard were included for each sequence of ten samples. Acceptability limits for the quality control were defined as $\pm 2\sigma$ of the mean value. For the ΣPCBi , the LOD value varied from 0.0049 (PCB 118) to 0,01 (PCB 28) $\mu\text{g.kg}^{-1}$ dw. For the ΣPBDE , the LOD ranged from 0.032 (BDE 28) to 13.55 (BDE 209) ng.kg^{-1} dw. For the PCDD/Fs, the LOD values ranged from 0.051 (2,3,7,8 TCDD) to 1.645 (1.2.3.6.7.8 HxCDD) ng.kg^{-1} dw.

3.7. Bioaccumulation Factor (BAF), SET and ERITME index determination

When a contaminant enters the soil ecosystem, not all the organisms in the soil will be affected in the same way and magnitude. Biological mechanisms such as uptake, sequestration, biotransformation and excretion of specific contaminants are highly variable and determine an organism's vulnerability. This vulnerability also depends on the way that a contaminant reaches the soil system as well as on the contaminant's properties (Van Straalen, 2004). Differences in the organism's body characteristics can also explain the differences in the way that a contaminant can affect a certain organism. Hard-bodied species and those that usually remain

in the soil top layer are preferentially exposed via food, while the soft-bodied and soil-dwelling species that live in the soil pore space are more exposed to the contaminants found in the pore water (O'halloran, 2006). At the end of the food chain, predators can be exposed to contamination through food or prey (Van Straalen et al., 2001).

Bioaccumulation factors (BAFs) are used in risk assessment to estimate the trophic transfer of contaminants from soil to wildlife and can be helpful for predicting risks associated with this transfer. BAFs can be derived from laboratory studies through the determination of concentrations or kinetic estimation methods. In the present study, bioaccumulation tests were performed on the soils and sediments collected to determine the uptake by *E. fetida* of the POPs analyzed. Bioaccumulation factor for earthworms (BAF) was calculated by the expression:

$$\text{BAF} = [\text{POPs}]_{\text{earthworm}} / [\text{POPs}]_{\text{anthroposoil}} \text{ (Egeler, 2009; OECD, 2010).}$$

BAF calculation is usually required for the evaluation and risk assessment of contamination, but questions can arise regarding the quality and correctness of the BAF values obtained. For example, in many of the experiments reported, it is not clear if the equilibrium of contaminant levels in soils and in earthworm tissues was reached if the uptake kinetics was not measured and if only a fixed exposure time was considered (e.g., Kelsey et al., 2005; Gao et al., 2009; Hallgren et al., 2006). Jager et al. (2003) suggested that BAFs can be better expressed dynamically as a ratio of the uptake and elimination rate constants (k_s and k_e). Indeed, only a few studies have reported these ratio values [Amorim et al., 2002; Mäenpää et al., 2008; Sousa et al., 2000]. Most of the studies of POPs uptake kinetics in earthworms showed only BAFs [Ma et al., 1995; Belfroid et al., 1994, 1995; Zhang et al., 2009].

The POPs levels considered to calculate the BAFs were normalized according to lipid weight and organic carbon contents, for *E. fetida* and anthroposoils, respectively.

The SET index (Pauget and De Vaufleury, 2015) quantifies the excess transfer in comparison with an internal concentration of reference (Ciref) which is the concentration of a contaminant in the tissues of the species exposed to a non-contaminated matrix. Figure 11 presents a schematic representation of the SET and ERITME calculation methods.

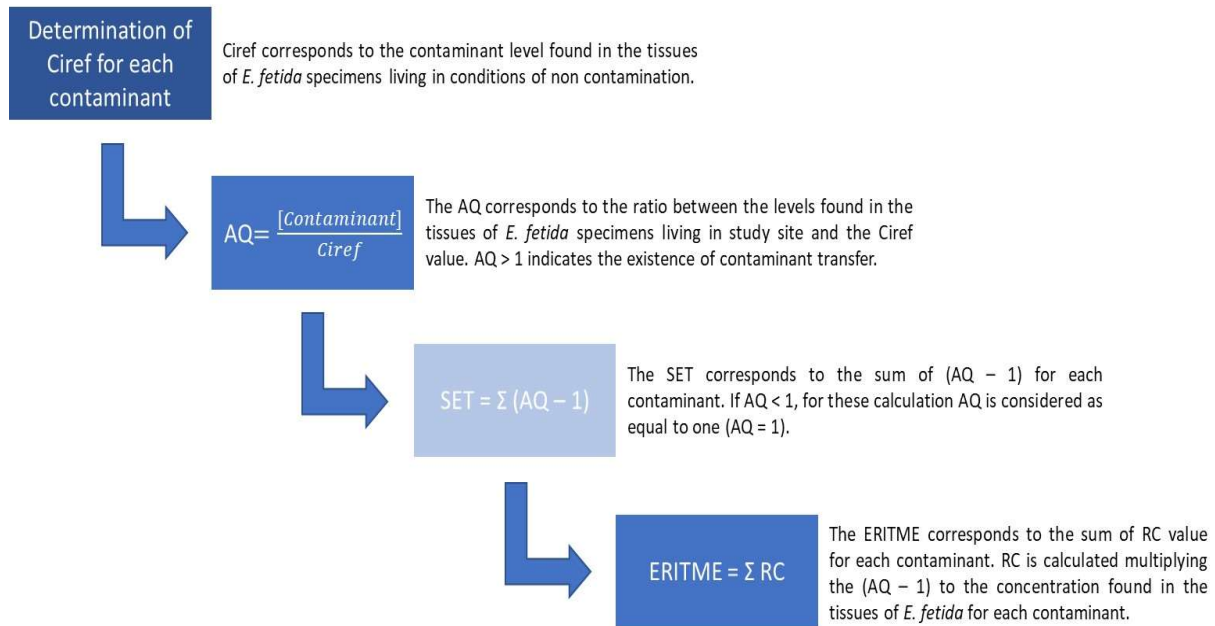


Figure 11 – Schematic representation of the SET and ERITME calculation method (adapted from Baures (2018), based in Pauget and De Vaufleury (2015)).

A coefficient (AQ) is obtained by dividing the concentration level of a certain contaminant in an organism exposed to the soil or sediment tested by the Ciref. If $AQ < 1$ a normal transfer occurs, but if $AQ > 1$ the transfer can be considered as anomalous, thus an excess transfer for the element considered. Finally, the SET value for a soil or sediment is the sum of the individual AQ values obtained for each of the elements considered:

$$SET = \sum (AQ - 1).$$

The ERITME index (Pauget and De Vaufleury, 2015) is useful for evaluating the potential risks associated with the excess transfer of the contaminants quantified. This excess transfer associated with each contaminant is weighted according to a risk coefficient, a toxicity point (TP) defined by ASTDR (ASTDR, 2019). In fact, it is extremely difficult to evaluate the global risk associated with a complex matrix with a cocktail of contaminates that have different toxicity risks. The ERITME index allows assigning a weight to each contaminant toxicity and at the end a sum of the individual weights provides an idea of the global risk associated with the matrix considered. The ERITME value for a soil or sediment can be calculated by the expression:

$$ERITME = \sum RC, \text{ where } RC = (AQ - 1) \times TP.$$

In the present work, the BAFs will be calculated for each POP studied in all the organisms tested, as well as the SET and ERITME which will be adapted to evaluate the *E. fetida* bioaccumulation results and that will since now named ERITME-POP, when calculated for POPs.

3.8. *In situ* evaluation of biological activity - SQBI approach and Bait-lamina test

Soil organisms can be divided into different classes according to their body width, a parameter that can vary by several orders of magnitude within soil communities (Barrios et al., 2007; Parker et al., 2010). Soil diversity comprises microbiota such as bacteria, archaea and fungi, as well highly diverse microfauna, mesofauna, macrofauna and even megafauna (Bardgett, 2002; Wurst et al., 2012). Another part of the soil ecosystem is constituted by several types of photosynthetic organisms such as lichens and plants which play an important role in the ecosystem structure (Orgiazzi et al., 2016). Soil microbiota is responsible for the decomposition processes that allow C and nutrient cycling and is important in the regulation of plant growth regulation and disease defence mechanisms (Wurst et al., 2012). The soil microfauna (organisms <100 μm) is composed mainly by nematodes, protozoa and rotifers that act to improve nutrient availability for other species, as well as facilitating the dispersion of the rhizosphere microbiota through their constant contact with plant roots (Wurst et al., 2012). Soil mesofauna correspond to organisms with a width of 100 μm –2 mm belonging to the following main groups: Acari, Collembola, Tardigrada, Protura, Diplura and Enchytraeidae. Usually, these organisms live in very close contact with the soil porewater and soil pore air and are highly dependent on soil aeration and moisture, thus greatly influenced by soil physico-chemical properties and health (Wurst et al., 2012). Soil macrofauna (width > 2mm), usually known as “ecosystem engineers”, play a key role in litter fractionation and predation on other soil-dwelling organisms. The most significant group of the soil macrofauna are the Macroarthropods which include isopods, spiders and insects as well as soft-bodied organisms (annelids and gastropods) (Jouquet et al., 2006). This group of soil organisms are the main actors of the changes occurring in the physical, chemical and structural characteristics of the soil. They are also important in several soil functions, such as decomposition and nutrient cycling and water infiltration processes. As predators, macroarthropods control other biota and

they also have a positive effect on plant growth and yield as well as on the elimination of certain plant diseases (Wurst et al., 2012).

Due to the entry of contaminants in the soil system, species abundance can be affected and even significantly decline. The most common ecological indexes are sometimes unable to show the real effect of contamination in the soil ecosystem and living community. Species can decline, sometimes until their extinction or, on the other hand, indirect effects and/or compensatory mechanisms can favor certain groups, leading to their higher abundance in contaminated sites (Pedersen et al., 1999; Nahmani and Lavelle, 2002).

In the present study, three monoliths were collected in each of the EST and PEY selected anthroposoils with the dimensions of 20 cm x 20 cm x 20 cm, and the visible living organisms (such as earthworms, spiders, ants, larvae and snails) present in these anthroposoils portions were identified.

The number and diversity of organisms can be related to their feeding activity in the soil and the Soil Quality Biological Index (SQBI) can be calculated (Parisi et al., 2005). To calculate this index, the living organisms found were grouped according to their main function in the ecosystem as Predators (spiders, chilopods), Decomposers (millipedes, gastropods), Ecosystem engineers (ants, earthworms) and Phytophages (larvae, beetles, insects).

Due to the low number of organisms sampled and the difficulty in their correct identification and several inaccuracies in the calculation, it was not possible to calculate the SQBI. However, differences in the quantity and distribution of organisms between the 8 anthroposols studied were observable.

The Bait-lamina© test is an ecological tool commonly used for the refined screening of a contaminated site in view to performing a simple, cheap and relatively fast on-site assessment of the functioning and/or biological activity of soils and sediments (Von Törne, 1990; Helling et al., 1998; Kratz, 1998; Kula and Römbke, 1998; Paulus et al., 1999; Geissen and Brummer, 1999; Larink and Sommer, 2002; Knacker et al. 2003; Van Gestel et al., 2003; Filzek et al., 2004).

Bait-lamina© strips are PVC strips containing small holes filled with a suitable bait substrate used to examine the feeding rates of invertebrates (and microorganisms) in soil or sediment.

The strips with food material are deployed in the field, inserted into the substrate and left for approximately 4 weeks. Perforation rates depend on the activity and density of the soil community, especially the bacterial community, micro-organisms and to a lesser extent

springtails, enchytraeids and earthworms. A lightbox is used to score the bait strips as either 'fully pierced', 'partially pierced' or 'not pierced' by the invertebrate fauna, depending on whether the holes are "empty", "partially empty" or "full". In Figure 12, a Bait-lamina© strip and the holes filling criteria used are presented along with the test apparatus in the soil.

Although many bait strips can be deployed *in situ* (reducing the variability in the results), making it is easy to see the differences in the feeding rates of the several invertebrates present, it is the environmental conditions (namely the water content in the soil or sediments) rather than the degree of contamination that impact the feeding routine, thereby influencing the results (Kula and Römbke, 1998).

In the present study, 60 strips were placed *in situ* during spring time in 2018 at intervals of 15 centimeters into each of the studied soils and sediments (with the exception of soil IVa from the Peyraud-6 sampling site due to the occurrence of a flood episode). Each strip had 16 holes filled with a mixture of cellulose, activated carbon and wheat bran. The exposure time of the strips depends on various parameters of which soil water content is the most important (ISO 18311:2016; Römbke et al. 2006).

The strips were collected twice, with 30 strips collected after 4 weeks of exposure (as it is indicated in the ISO 18311:2016 procedure); the last 30 strips were collected after 6 weeks. After collection, each strip was carefully examined to identify and count the totally empty, the partially empty and the remaining filled holes. This allowed to calculate the global feeding activity of each soil and sediment. The final global feeding activity coirresponds to the average value obtained by considering both the percent of feeding activity per 16 holes of 1 strip and the percent of holes per horizon of the buried bait-lamina strips.

This method is useful for performing rapid, easy and inexpensive evaluations of soil biological activity and quality, giving indications on the potential biological activity and capacity of the soil.

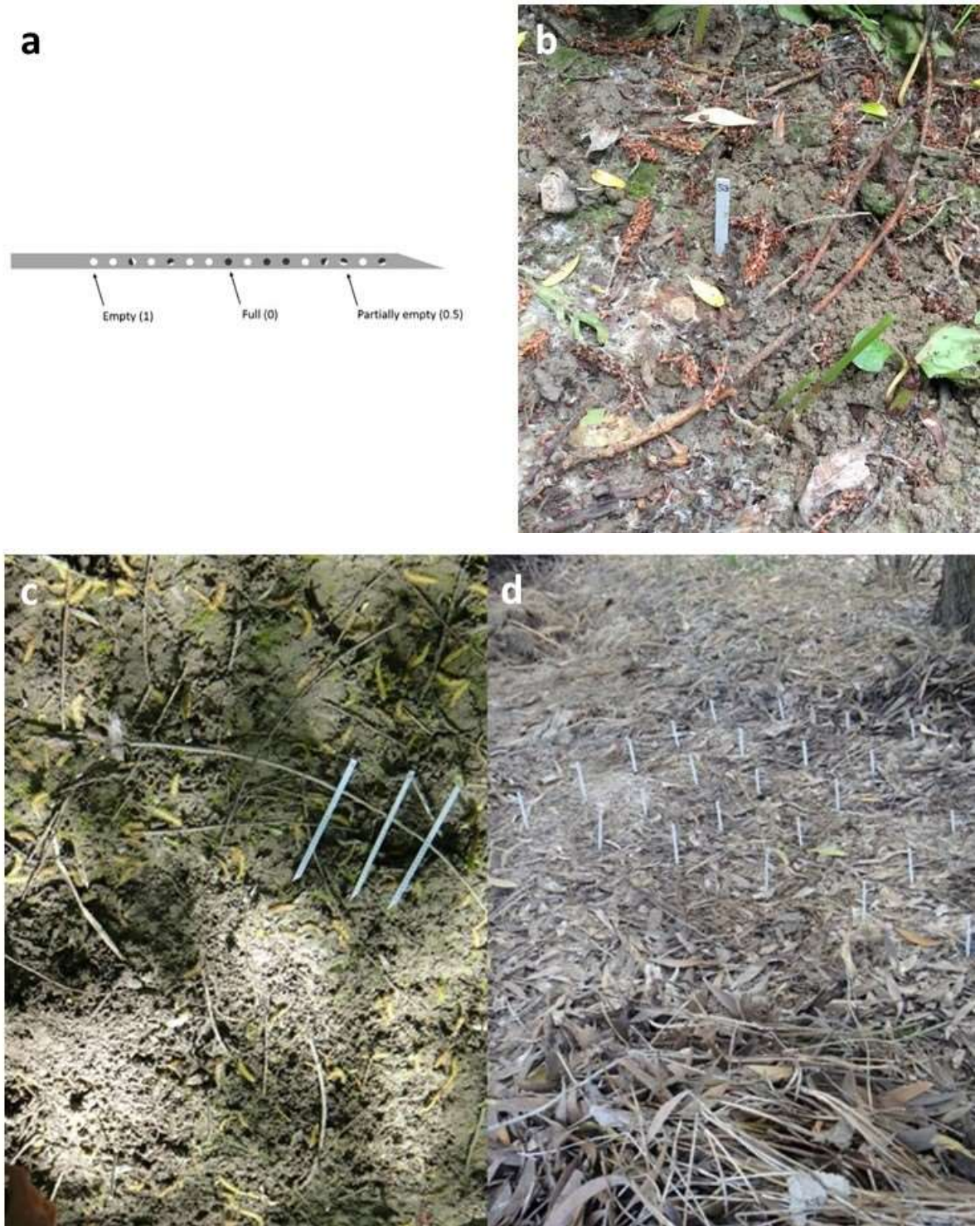


Figure 12 – Bait-lamina© test apparatus: (a) lamina-bait strip; (b) strip in the soil; (c) strips after being collected from the soil; (d) set of 20 bands in the soil.

3.9. Statistical methods

SPSS statistics v.23 software© was used for the calculation of the descriptive statistics and for the statistical analysis of the data (correlation, linear regressions and factor analysis). Factor analysis with the “Principal Component Analysis” (PCA) extraction method is a statistical technique often used to simplify complex data-sets. PCA reduces the dimensionality of the data by transforming it into a set of new uncorrelated reference variables called principal components (PC) (Johnson et al., 2002).

The PCA analysis was performed with the aim of identifying the underlying factors that can explain the pattern of correlations within the set of variables observed.

All the variables were standardized to make the variables comparable and eliminate possible incorrect effects in the statistical analysis due to differences in units and /or scales. The approach used consisted in data transformation by subtracting a reference value (the average of the variable) from each value and dividing it by the standard deviation (Kent, 2011). At the end of this transformation, the data obtained were centric-reduced data. A Standard PCA was applied to these transformed data.

Also, linear regression analysis was performed to check the existence of correlations or not between all the soil parameters and the POPs concentrations found in the soil, earthworms and plant tissues analyzed.

Concerning the results of *E.fetida* reproduction, the homoscedasticity and normality of the results were tested and if not validated, non-parametrical statistic tests were performed.

A Kruskal-Wallis statistical test was performed with the results from the *E. fetida* reproduction test to assess the possible significant differences in the mean mass gain or loss results during the exposure time. The p value was below 5%, indicating the existence of significant differences, so a Wilcoxon pairs comparison test was performed to distinguish the different statistical classes.

A Kruskal-Wallis test was also applied to the mean mortality results to detect possible significance differences between the samples studied. The p value was 0.9995 indicating the absence of differences within the results.

In the case of the number of juveniles produced (reproduction rate), the results showed a normal distribution and good homoscedasticity. Thus, an ANOVA test was performed to determine the existence of significant differences within the results. The p value (=0.0005) was below 5%, indicating the existence of significant differences, thus a Wilcoxon pairs comparison test was performed to distinguish the different statistical classes.

A Kruskal-Wallis statistical test was performed with the results from the plants germination and growth test to assess the possible significant differences in the mean mass gain or loss results during the exposure time. The p value was below 5%, indicating the existence of significant differences, so a Wilcoxon pairs comparison test was performed to distinguish the different statistical classes.

Chapter 4. Results and discussion

In this chapter, the results will be presented and discussed in four parts:

- Part A: The physicochemical, chemical and biological characterization of the anthroposoils studied:
 - ✓ the physicochemical characterization of anthroposoils,
 - ✓ the quantified levels of POPs in the anthroposoils studied,
 - ✓ the quantified levels of POPs in plants collected *in situ*,
 - ✓ the biological characterization of the anthroposoils studied,→ in order to know in particular which detectable POPs concentrations and families are present in these anthroposoils and if such concentrations are limiting/toxic for life; also whether such POPs are transferred to plants *in situ*.

- Part B: Toxicity and bioaccumulation laboratory tests with *E. fetida* and plants:
 - ✓ the results of laboratory tests with *E. fetida* concerning the effects on reproduction, and potential POPs bioaccumulation in their tissues;
 - ✓ the results of germination and growth tests of the three plants species based on the potential bioaccumulation of POPs in their tissues;→ in order to know, in particular, if the POPs available can be accumulated, and up to what degree, by soil organisms and plants. Also, in case of bioaccumulation, what are the toxic effects caused by POPs levels in the animal and plant species tested?

- Part C: Statistical analysis by Principal Component Analysis (PCA): the PCA analysis of the results obtained (anthroposoils and *E. fetida* only):
 - ✓ MTEs and all POPs,
 - ✓ Only POPs,
 - ✓ Only PCBs, PCDDs and PCDFs,
 - ✓ Only BFRs,→ in order to see if relations/correlations exist between the different anthroposoils, notably with the different POPs families, and the effects on and bioaccumulation in *E. fetida*.

- Part D: The calculation of indices to infer potential risk to the ecosystem:
 - ✓ BAF,
 - ✓ SET, ERITME and ERITME-POP index calculations,

→ in order to know, in an Environmental Risk assessment approach, what can be expressed by the indexes calculated such as BAF, SET, ERITME and ERITME-POP. Also, whether these reference indexes will be useful in an Environmental Risk Assessment approach.

In each part, the results obtained for the samples of the two different sites [Estarreja (called EST; Portugal) and Peyraud 6 (called PEY; France)] will be compared with each other and discussed, likewise with the available literature data.

PART A: The physicochemical, chemical and biological characterization of the anthroposoils studied

4.1. Physicochemical characterization of anthroposoils

The selected soils and sediments have different origins and had been subjected to different pollution sources, thus presenting significant differences in their physicochemical parameters. Table 16 shows the results obtained for the general physicochemical and agronomic parameters of anthroposoils collected in Estarreja (Portugal) and Peyraud 6 (France).

Table 16 – Results of the physicochemical and agronomic parameters determined in Estarreja and Peyraud 6 anthroposoils: pH, organic matter content (OM); total organic carbon (TOC); cation exchange capacity (CEC) and particle size distribution (sand, silt and clay) (n= 3).

Parameter	Estarreja				Peyraud 6			
	Mean	Median	Min	Max	Mean	Median	Min	Max
pH (KCl)	4.41	4.50	3.66	4.98	7.57	7.52	7.48	7.74
pH (H ₂ O)	5.25	5.24	4.74	5.77	8.08	8.06	7.86	8.35
OM (g.kg ⁻¹ dw)	20.6	19.9	18.2	24.2	32.6	31.2	26.3	41.5
TOC (g.kg ⁻¹ dw)	11.9	11.6	10.6	14.1	18.9	18.2	15.3	24.1
CEC (méq.kg ⁻¹)	40.9	41.1	19.2	62.4	89.3	93.1	63.3	108
Sand (%)	80.8	82.5	64.6	93.6	68.1	66.9	62.3	76.0
Silt (%)	18.2	16.6	6.10	33.4	27.7	28.7	20.9	32.7
Clay (%)	1.05	0.95	0.30	2.00	4.23	4.40	3.10	5.00
CaO (g.kg ⁻¹ dw)	1.17	1.10	0.95	1.55	6.76	6.48	6.06	8.02
K₂O (g.kg ⁻¹ dw)	0.03	0.04	0.01	0.06	0.09	0.10	0.07	0.12
MgO (g.kg ⁻¹ dw)	0.03	0.03	0.02	0.05	0.19	0.18	0.17	0.20
P_{olsen} (g.kg ⁻¹ dw)	0.04	0.03	0.01	0.11	0.09	0.11	0.01	0.14
C/N ratio	24.0	18.5	15.0	44.0	12.3	12.0	12.0	13.0

The pH values are acid in the samples from Estarreja and both OM and organic carbon (TOC) contents are relatively high, thus reflecting intensive agricultural activity and the use of manure for soil fertilization in the case of EST C and L anthroposoils (see Figure 5, Subchapter 3.1.1). Concerning the Peyraud 6 samples, pH values ranged from neutral to slightly basic and the high contents in OM and TOC were probably due to the input of fine particles resulting from river deposition as well as vegetation development. Regarding texture, the samples from Estarreja varied from sandy loam to sandy while the samples from Peyraud 6 were mainly sandy loam except one sample that presented a loamy sand texture.

The agronomic parameters measured (CaO, K₂O, MgO, P_{Olsen} and C/N ratio) were higher in the Peyraud 6 samples except for the C/N ratio and OM content which was somewhat higher in the Estarreja anthroposoils.

In general, the results of the parameters determined are in accordance with and of the same order as those obtained in other previous studies performed in these two areas (Cachada et al., 2009, 2012a; Rodrigues et al., 2010, 2013; Franquet et al., 2016; Thorel et al., 2018), and thus not limiting for the presence of living organisms and their development.

A summary of the pseudo-total concentrations of quantified MTEs is shown in Table 17. The measured levels showed a considerable degree of variation with many elements exhibiting wide ranges far beyond the mean.

The mean “pseudo total” levels of the MTEs analyzed are higher in Peyraud 6 samples than in Estarreja samples, except in the case of As concentrations. The results obtained in these Estarreja samples studied for the various MTEs are in accordance with the values reported by Cachada et al. (2012b) and Rodrigues et al. (2013) for soils collected in the same area. Inácio et al. (2008) reported that the Portuguese Western region, characterized by the presence of sedimentary detritic rocks and where Arenosols and Podzols are the typical soil types (as verified in Estarreja area), is naturally impoverished in most MTEs. Despite this, the samples from the Estarreja area considered in the present study showed relatively high levels of MTEs when compared with the background values found by Inácio et al. (2008). This fact clearly shows an anthropogenic origin (mainly related to the presence and activity of the Estarreja Chemical Complex and/or intense agricultural activity) of these MTEs in the anthroposoils considered.

The results obtained in the PEY samples for the various MTEs are in accordance with the values reported by Elbaz-Poulichet et al. (1996) and Thorel et al. (2018) in the sediments of the Rhone

river and Chiffolleau et al. (1994) in sediments collected in the Seine Estuary, both of which correspond to drainage basins long impacted by high human activity.

Table 17 – Mean, median, minimum and maximum of pseudo-total concentrations of quantified MTEs (in mg.kg⁻¹ dw) measured in Estarreja (EST) and Peyraud 6 (PEY) anthroposoils (n= 3).

MTE	Local	Mean	Median	Min	Max
Al	EST	2950	2500	200	6600
	PEY	20054	19228	16721	25041
As	EST	38.6	35.3	1.60	82.2
	PEY	10.7	10.5	8.80	13.0
Cd	EST	0.10	0.10	0.10	0.10
	PEY	0.88	0.70	0.52	1.59
Cr	EST	4.00	4.00	1.00	7.00
	PEY	52.4	45.4	43.4	75.5
Cu	EST	13.3	11.0	5.50	25.6
	PEY	31.9	24.7	23.9	54.3
Fe	EST	3825	3450	700	7700
	PEY	14041	13698	11909	16860
Hg	EST	0.14	0.12	0.08	0.26
	PEY	0.43	0.31	0.09	0.99
Mn	EST	39.5	29.5	2.00	97.0
	PEY	453	426	401	558
Ni	EST	3.03	2.60	1.40	5.50
	PEY	24.9	20.9	20.0	37.7
Pb	EST	14.9	15.1	9.90	19.6
	PEY	24.9	20.9	22.5	47.0

Zn	EST	28.5	25.5	13.0	50.0
	PEY	158	131	131	248

The MTEs levels obtained for the 8 anthroposoils considered are also comparable to those found in a large number of industrial and urban areas worldwide. In a study in Tarragona (Spain) Nadal et al. (2007) measured the mean levels of Cd, Cr and Pb in soils from 4 different land uses (petrochemical, chemical, urban/residential area and unpolluted sites) that ranged from 0.11 to 0.21 mg.kg⁻¹ dw, 11.9 to 17.5 mg.kg⁻¹ dw and 18.3 to 42.0 mg.kg⁻¹ dw, respectively. The values are in the same range as the EST samples studied and slightly lower than the concentrations measured in the PEY samples.

The Reference Values of the MTEs considered defined in the legislation in Spain for soils and in France and the Netherlands for soils and sediment quality, are presented in Table 18.

Table 18 - Reference values or total concentrations for some MTEs that figures in some European countries for soils and sediments (in mg.kg⁻¹ dw).

Country	Legislation	Matrice	Specification	Cd	Cu	Fe	Ni	Pb	Zn
Spain	Laws of 06/2009, 26/02/2009	Soils	Urban	4	100	No value	100	100	500
			Industrial	20	200	No value	200	500	1000
France	Law of 08/01/1998	Soils	-	2	100	No value	50	100	300
	Law of 14/06/2000	Sediments	Level 1	1.2	45	No value	37	100	276
			Level 2	2.4	90	No value	74	200	552
Netherlands	New Dutch List, 2000	Soil or sediment	Target value	0.8	36	No value	35	85	140
			Intervention value	12	190	No value	210	530	720

For Fe, no target and/or limit values are indicated in any of the legislations considered.

In the case of the Estarreja samples, most of the “pseudo total” levels measured were all above the regulatory target values in force in Spanish, French and The Netherlands. Some exceptions were noticed for Cu levels quantified in EST C, EST G and EST K that were higher than the

values indicated in the three legislations considered. In EST G and EST L, Pb levels were also above the Dutch target value ($85 \text{ mg.kg}^{-1} \text{ dw}$) and higher than the values defined by Spanish legislation for urban soils and by French guidelines ($100 \text{ mg.kg}^{-1} \text{ dw}$ in both cases). In EST C, EST K and EST L, values were higher than the Dutch target level ($140 \text{ mg.kg}^{-1} \text{ dw}$) and the level indicated by French legislation for soils ($300 \text{ mg.kg}^{-1} \text{ dw}$).

“Pseudo total” MTE concentrations measured in PEY samples for some elements were above the reference values defined in the French and Dutch guidelines. Cadmium levels in the PEY samples were lower than the Dutch target value ($0.8 \text{ mg.kg}^{-1} \text{ dw}$) except for PEY Ic ($1.59 \text{ mg.kg}^{-1} \text{ dw}$). Considering the French legislation, only the PEY Ic sample presented a higher Cd content than contamination Level 1 (L1), value ($1.2 \text{ mg.kg}^{-1} \text{ dw}$). The Cu level in the PEY Ic sample was higher than contamination Level 1 (L1), value ($45 \text{ mg.kg}^{-1} \text{ dw}$) defined by French standards and higher than the target value defined by Dutch legislation ($36 \text{ mg.kg}^{-1} \text{ dw}$). Copper levels in the other PEY samples were lower than the regulatory values. Regarding Ni concentrations, only the PEY Ic presented a value above the Dutch target level ($35 \text{ mg.kg}^{-1} \text{ dw}$). All the PEY samples presented Pb levels below the values defined in the legislations considered. Regarding Zn concentrations, only PEY Ic presented a value above the Dutch target level ($140 \text{ mg.kg}^{-1} \text{ dw}$) while all the values were lower than that stipulated by French legislation for sediments. The anthroposoils studied presented levels of certain of the MTEs analyzed in ranges above the values defined by the legislation considered. In the case of use and/or remobilization of these anthroposoils, decontamination procedures are required to avoid the dispersion of contamination through the ecosystem, especially in the case of the PEY site as it is located on the river margin in direct contact with the Rhone river.

4.2. Levels and sources of persistent organic pollutants in anthroposoils

The concentrations of the POPs obtained in the surface soils of both sampling sites are presented in Tables 19 and 20. The concentrations of PCBs, PCDDs, PCDFs, PBDEs, HBCD, PBBs, nHBB, nPBB, nPBT and PBEB in the 8 anthroposol samples studied are expressed in Table 19 in dw basis and in Table 20 the concentrations are normalized to the TOC contents. The TOC contents in EST anthroposoils ranged from 10.6 to 14.1 gC.kg^{-1} and from 15.3 to 24.1 gC.kg^{-1} in the case of PEY anthroposoils (Table 16 in section 4.1). The TOC normalized concentrations will be used by preference in the results and discussion.

The mean concentrations of Σ PCBs-cop in the soils studied were in the range of 0.49 to 0.80 $\mu\text{g.kg}^{-1}$ TOC and 0.80 to 2.65 $\mu\text{g.kg}^{-1}$ TOC, for EST and PEY samples respectively. Among the 4 congeners considered (CB-77, -81, -126, -169), CB-77 explained 38% to 90% of the total sum of coplanar ones. Regarding the Σ PCBs-noncop, the mean levels found in EST anthroposoils were from 1.92 to 15.4 $\mu\text{g.kg}^{-1}$ TOC, and in the range of 63.9 to 437 $\mu\text{g.kg}^{-1}$ TOC in the PEY samples. Among the 8 congeners considered (CB-105, -114, -118, -123, -156, -157, -167, -189), CB-118 explained about 30 to 50% of the total concentration. The mean levels of Σ PCBs-ndl were in the range of 21.7 to 143 $\mu\text{g.kg}^{-1}$ TOC in the EST samples and from 364 to 2461 $\mu\text{g.kg}^{-1}$ TOC in the PEY samples. Among the 6 congeners considered (CB-28, -52, -101, -138, -153, -180), CB-153, CB-138 and CB-180 explained about 80 to 90% of the total sum in the anthroposoils studied. The concentrations of Σ PCBs-ndl also represented around 90% of the total sum of all the PCB congeners quantified.

The levels of PCBs measured in the Estarreja sample were below those detected in the same area by Cachada et al. (2009; 2012a) and below those detected in soils from industrial, urban and remote areas along the Seine River Basin in France, some of them corresponding to areas severely impacted by industrial pollution linked to processing and recycling or lubricants as well as transformer maintenance and conditioning procedures (Motelay-Massei et al., 2004). Despite that, the levels measured in the Estarreja samples are all in the same range as the values measured in an area close to a chemical factory in Italy that produced PCBs until the 1980s (Donato et al., 2006). Among all the existing PCB congeners (except 209), Buckland et al. (1998) indicated that CB-153 and CB-138 are the most abundant and most frequently found in urban and forest soils due to atmospheric input, a fact also verified in the samples studied.

The French legislation considers a limit of 0.68 mg.kg^{-1} dw for the 7 indicator PCBs in sediments (*Arrêté du 9 août 2006*, NOR: DEVO0650505A). According to the Dutch legislation for soil remediation purposes, the admissible reference value for the sum of the 7 indicator PCBs is 0.02 mg.kg^{-1} dw and the intervention value is defined at 1.0 mg.kg^{-1} dw (Verbruggen and Brand, 2014). The values found in the anthroposoils studied were significantly lower when compared with these legislation values, so the areas studied cannot be considered as contaminated sites based in the regulatory indications considered. The levels of PCBs detected in the PEY samples (old sediments deposited for several years, reaching a historical record of PCB inputs) were somewhat lower than the values found in sediments from the same or nearby areas (Mourier et al., 2014; Lorgeoux et al., 2016; Liber et al., 2019). The levels found in the PEY samples were of the same order of values as those recorded in marine and lake deposited sediments from England and Russia (Camacho-Ibar and McEvoy, 1996; Iwata et al., 1995).

Among the large number of studies, there are contradictory reports on the dominant removal mechanisms for PCBs in soil including volatilization, physical transport processes and biodegradation (Gan & Berthouex, 1994). According to Alcock et al. (1996) the two most important mechanisms of PCB removal are biodegradation and volatilization, with volatilization being dominant. However, this idea contradicts an experiment that compared the vapor loss of PCBs from soil and sand, and which found that there was almost no loss of PCBs by volatilization from either matrix (Haque et al., 1974). In a long-term field experiment on agricultural soils amended with sewage sludge (1968 – 1994), Alcock et al. (1996) obtained values for Σ PCBs half-lives of 8.5 years.

In the EST samples, the mean concentrations of Σ_7 PCDDs ranged from 1.21 to 5.52 $\mu\text{g.kg}^{-1}$ TOC. In the PEY anthroposoils the mean levels measured ranged from 6.23 to 39.9 $\mu\text{g.kg}^{-1}$ TOC. Generally, octachlorodibenzo-p-dioxin (OCDD) and 1,2,3,4,6,7,8-hepta CDD (1,2,3,4,6,7,8-HpCDD) accounted for the largest proportion of PCDD/Fs (>80%) in all the anthroposoils studied. The mean levels of Σ_7 PCDDs found in the samples studied were in the same range as those detected by Liber et al. (2019) in the Rhone river core sediments and by Gómez-Lavín et al. (2011) in estuarine sediments from Spain.

In the EST samples, the mean concentrations of Σ_{10} PCDFs ranged from 0.16 to 1.05 $\mu\text{g.kg}^{-1}$ TOC. In the PEY anthroposoils the mean levels measured ranged from 1.06 to 5.38 $\mu\text{g.kg}^{-1}$ TOC. Among PCDF homologs, octachlorodibenzofuran (OCDF) and 1,2,3,4,6,7,8-HeptaCDF (1,2,3,4,6,7,8-HpCDF) were the predominant congeners and contributed around 60% of the PCDFs in the anthroposoils studied. In the case of Σ_{10} PCDFs, the mean concentrations measured were of the same order as those recorded by Liber et al. (2019) in sediments from the River Rhone, but significantly lower than the values found by Götz et al. (2007) in sediments from the River Elbe in Germany.

Dioxin-like compounds have been detected in most Australian soils, ranging from the limit of detection 0.54 to 3.8 ngWHO₉₈TEQ kg^{-1} dw (Muller et al., 2004). Dioxin-like compounds in soils from urban (with sources such as combustion of fuels, residential wood burning, incineration of domestic waste) and industrial locations (with sources such as chemical manufacturing, metal smelting and refining activities) were substantially higher than in agricultural and remote locations (Muller et al., 2004). The overall loss of organic chemicals from soils is often biphasic, where a short period of rapid dissipation is followed by a longer period of slow chemical release (Beck et al., 1995). The extractability and bioavailability of POPs in soil has been demonstrated to decrease with time (Mueller et al., 2006). It has already been reported that the half-lives of dioxin-like compounds vary from more than four years to

more than twenty years, with greater persistence observed for higher chlorinated homologues (Molina et al., 2000). The elimination of PCDDs/Fs in soil may take a much longer period of time since the principal removal mechanism is thought to be off-site removal (McLachlan et al., 1996).

The mean concentrations of PBDEs (excluding BDE-209) were relatively lower compared with those of PCBs. The mean Σ_7 PBDEs (excluding BDE-209) were from 0.74 to 3.04 $\mu\text{g.kg}^{-1}$ TOC in EST samples and from 3.01 to 25.30 $\mu\text{g.kg}^{-1}$ TOC in PEY anthroposols. The Σ_7 PBDEs (excluding BDE-209) measured are correspond to the sum of the levels of congeners BDE-47 and BDE-99. When including BDE-209, the mean levels of Σ_8 PBDEs are significantly higher, ranging from 13.96 to 343.63 $\mu\text{g.kg}^{-1}$ TOC in the EST samples and from 268.06 to 4073.61 $\mu\text{g.kg}^{-1}$ TOC in the PEY anthroposols. Composition analysis clearly showed that in all the anthroposols studied, BDE-209 is the most predominant congener, explaining more than 90% of the total PBDEs, followed by congeners BDE-47 and BDE-99. Congener BDE-28 was close to the LOD in both the EST and PEY samples.

Concerning the PBDEs, the levels detected in the EST samples were very low when compared with those obtained in the PEY samples and with the values found in other studies on industrial, urban and agricultural soils in Europe (Gaspéri et al., 2016; Muresan et al., 2010; Hassanin et al., 2004). In the PEY samples, the PBDE concentrations measured were slightly lower than the values detected in river sediments in France (Liber et al., 2019) and in terrestrialized river sediments from Italy, Greece, Slovenia, Croatia and Bosnia and Herzegovina (Giulivo et al., 2017). The persistence of PBDEs in soil was demonstrated in measurements from various field experiments, to be more than four years (Eljarrat et al., 2008) and even more than twenty years (Sellström et al., 2005). Soderstrom et al. (2004) stated that PBDEs and BDE209 are more environmentally persistent than currently believed, based on laboratory degradation studies. Atmospheric deposition is thought to be the dominant mechanism of soil PBDE contamination globally, as PBDEs have been detected in remote areas with no possible source of transport other than atmospheric deposition (Schmid et al., 2007). PBDEs were measured in surface soils from remote/rural locations throughout the United Kingdom and Norway with Σ PBDEs concentrations ranging between 0.07 and 12.0 ng.kg^{-1} dw (Hassanin et al., 2004).

The Σ HBCDs mean concentrations measured were from 10.2 up to 2063 $\mu\text{g.kg}^{-1}$ TOC in EST anthroposols and 4.31 to 211 $\mu\text{g.kg}^{-1}$ TOC in PEY samples. Contrary to the trend of the other POPs quantified, Σ HBCDs exhibited higher levels in EST samples. This fact is related to the past and present industrial activities in the Estarreja Chemical Complex and confirmed by the high level of Σ HBCDs measured in the EST G sample (2063 $\mu\text{g.kg}^{-1}$ TOC) collected near the

Chemical Complex area. Regarding the 3 HBCD isomers, α - and γ -HBCD dominated (in equal proportions) followed by β -HBCD.

The levels of HBCDs detected in the PEY samples studied were in the same range as those detected in sedimentary cores also collected in the same zone in the River Rhone (10 km North in a straight line from the PEY site) by Liber et al. (2019).

The Σ_3 PBBs mean levels were somewhat lower in all the samples studied, with values ranging from 0.06 to 0.15 $\mu\text{g.kg}^{-1}$ TOC in the EST samples and from 0.33 to 1.01 $\mu\text{g.kg}^{-1}$ TOC in the PEY samples. Among the 3 congeners quantified, only BB-153 was higher than the LOD but in very low quantities in anthroposoils PEY IIb and PEY IIIa. BB-52 and BB-101 were close to the LOD value for all the EST and PEY samples.

The nHBB mean concentrations found ranged from 0.06 to 0.14 $\mu\text{g.kg}^{-1}$ TOC and from 0.03 to 1.68 $\mu\text{g.kg}^{-1}$ TOC, in the EST and PEY samples, respectively. The nPBB mean levels measured were from 0.02 to 0.06 $\mu\text{g.kg}^{-1}$ TOC in the EST samples and from 0.29 to 1.15 $\mu\text{g.kg}^{-1}$ TOC in the PEY samples. Mean levels of nPBT in the EST anthroposoils ranged from 0.03 to 0.04 $\mu\text{g.kg}^{-1}$ TOC and from 0.04 to 1.37 $\mu\text{g.kg}^{-1}$ TOC in the PEY samples. The PBEB mean concentrations measured were from 0.002 to 0.02 $\mu\text{g.kg}^{-1}$ TOC and from 0.02 to 0.05 $\mu\text{g.kg}^{-1}$ TOC, in the EST and PEY anthroposoils, respectively.

The levels of quantified nRFBs (nHBB, nPBB, nPBT and PBEB) in all the anthroposoils studied were very low in comparison with the other POPs quantified in these samples. In addition, the nBFRs values obtained were not comparable with most of the few data found in the literature which usually correspond to highly contaminated sites in Asian countries (usually e-waste manufacturing and/or disposal sites) (Hong et al., 2016; Someya et al., 2016; Matsukami et al., 2017). Despite this, the values of nHBB and nPBT detected in the samples studied are in the same range as some of those obtained by Newton et al. (2015) in urban soils from Sweden. The concentration of PBEB obtained in the samples studied were lower than those measured in river sediments collected along the Llobregat river basin in Spain as reported by Guerra et al. (2010). This basin is severely impacted by effluents from different industries (tannery, textile, pulp, and paper) and urban wastewaters. PBEBs are present in a wide range of everyday products released into the environment, mainly from household product waste disposal and incineration.

The anthroposoils studied presented appreciable and quantifiable levels of the POPs analyzed, showing historical contamination due to mainly past and/or present industrial activities. In the case of the PEY samples it is well known that PCBs remain present in the ecosystem, despite all the regulations and actions taken to ban their use in recent decades (Liber et al., 2019).

PBDEs were also detected in the studied soils. nBFRs, whose use in industrial manufacturing is recent, have not yet been detected or quantified in the anthroposoils studied. Due to the presence of all these POPs, especially in the PEY site, POPs decontamination or immobilization procedures will be required if these sites are subject to dismantlement or changes in anthroposol storage methods (Thorel et al., 2018).

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Table 19 – Mean concentrations of the POPs (in $\mu\text{g}\cdot\text{kg}^{-1}$ dw) quantified in Estarreja (EST) and Peyraud 6 (PEY) anthroposoils (n=2).

	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
ΣPCBs-cop	0.004	0.01	0.001	0.01	0.50	0.14	0.04	0.11
ΣPCBs-noncop	0.08	0.17	0.03	0.11	8.30	6.71	0.98	2.38
ΣPCBs-ndl	0.74	1.55	0.31	0.10	46.6	28.7	5.57	14.7
Σ_7PCDDs	0.03	0.06	0.02	0.02	0.76	0.34	0.10	0.15
Σ_{10}PCDFs	0.003	0.01	0.002	0.003	0.10	0.03	0.05	0.03
Σ_7PBDEs	0.02	0.03	0.03	0.01	0.41	0.44	0.05	0.41
Σ_8PBDEs	0.44	3.37	0.20	0.20	74.1	70.7	4.10	50.9
ΣHBCDs	0.31	22.4	0.14	0.16	3.99	0.91	0.07	2.39
Σ_3PBBs	0.001	0.002	0.001	0.001	0.02	0.01	0.01	0.01
nHBB	0.001	0.001	0.001	0.002	0.01	0.03	0.002	0.001
nPBB	0.0004	0.001	0.0002	0.0003	0.02	0.02	0.01	0.01
nPBT	0.0004	0.0003	0.0004	0.001	0.03	0.001	0.001	0.002
PBEB	0.0001	0.0001	0.0002	0.0003	0.0004	0.0003	0.001	0.001

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Table 20 – Mean concentrations of the POPs (in $\mu\text{g}\cdot\text{kg}^{-1}$ TOC) quantified in Estarreja (EST) and Peyraud 6 (PEY) anthroposoils (n=2).

	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
Σ PCBs-cop	0.35	0.80	0.10	0.49	26.6	0.80	2.65	4.69
Σ PCBs-noncop	7.41	15.4	1.92	9.08	438	387	63.9	98.5
Σ PCBs-ndl	70.3	143	21.7	80.8	2461	1654	364	609
Σ_7 PCDDs	3.01	5.52	1.21	1.40	39.9	19.3	6.23	6.38
Σ_{10} PCDFs	0.31	1.05	0.16	0.23	5.38	1.98	3.54	1.06
Σ_7 PBDEs	1.88	3.04	1.86	0.74	21.4	25.3	3.01	17.1
Σ_8 PBDEs	41.4	344	14.0	16.5	3911	4074	268	2111
Σ HBCDs	29.1	2063	10.2	12.7	211	52.5	4.31	99.1
Σ_3 PBBs	0.09	0.15	0.06	0.08	1.01	0.64	0.48	0.37
nHBB	0.10	0.06	0.08	0.14	0.29	1.68	0.15	0.03
nPBB	0.04	0.06	0.02	0.02	1.15	1.11	0.29	0.52
nPBT	0.04	0.03	0.03	0.04	1.37	0.04	0.06	0.10
PBEB	0.01	0.01	0.02	0.002	0.02	0.02	0.05	0.03

4.3. Biological characterization of the anthroposoils studied

The living population inhabiting soil includes macrofauna, mesofauna, microfauna and microflora. Soil microorganisms mainly live within the pores between soil particles, free or attached to surfaces such as in thin water films surrounding soil particles.

Figure 13 shows the distribution and the number of organisms found in the monoliths collected from EST and PEY anthroposoils classified as phytophagous, decomposers and predators. The monoliths were collected from 7 of the 8 anthroposoils studied. In soil PEY IVa, sampling was not possible because this site was flooded.

The number of decomposers found in the soils was significantly higher than the other ecological categories (Figure 13). Despite that, the low quantity of decomposers (group represented mainly by earthworm species) verified in anthroposoil EST L can be explained by the high sand fraction (93.6 %). The sandy texture (and coarse sand) can be a negative factor for earthworms, affecting their presence either because of the abrasive action of sand grains that damage earthworm skin or because sandy soil usually retains less water (Laossi et al., 2010).

Figure 14 shows the organisms found in the monoliths of the PEY anthroposoils studied.

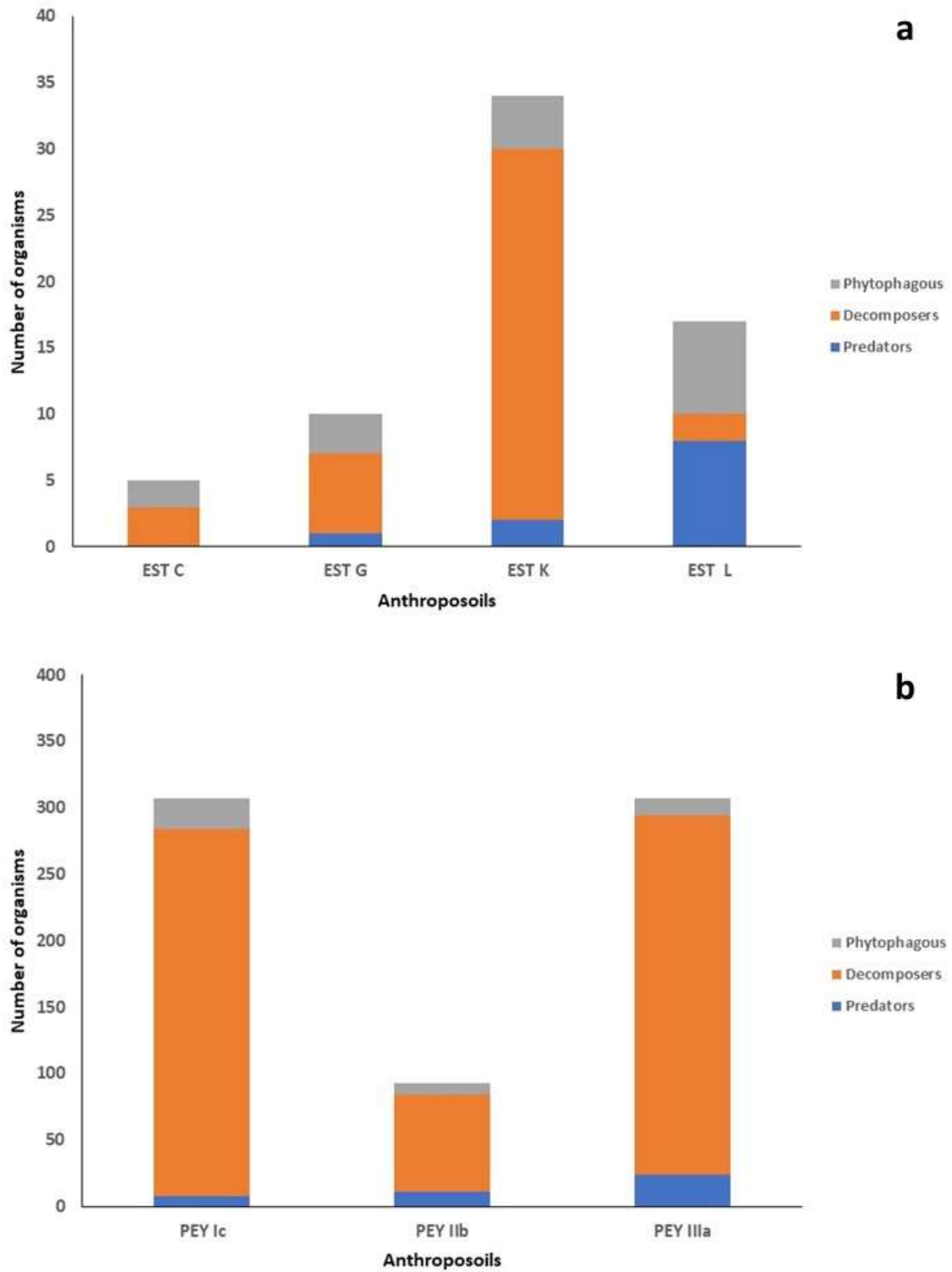


Figure 13 – Distribution of the number of individual organisms found in the monoliths collected from EST (a) and PEY (b) anthroposoils (n = 3).



Figure 14 – Organisms found in the monoliths collected of PEY anthroposoils.

The Bait-lamina© test was performed in 7 of the 8 anthroposoils studied. In soil PEY IVa it was not possible to perform the test once this site was flooded. Figure 15 shows the Bait-lamina© test apparatus.



Figure 15 – Bait-lamina© test apparatus: (a) lamina-bait strips; (b) strips in the soil; (c) strips after collection from the soil.

Table 21 presents the results for the global feed activity after 6 weeks of strip exposure.

Table 21 – Results for the global feed activity (GFA) after 6 weeks of exposure of the Bait lamina© test strips in the anthroposoils tested (n=60 strips per anthroposoil).

Anthroposoils	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa
GFA (%)	58.4	31.4	37.7	7.40	49.0	29.9	34.3

In Figure 16, the results of global feed activity are graphically presented, and certain differences can be noticed between the anthroposoils tested. Soil L presented a much lower value (7.4 %) when compared with the other anthroposoils, which can be explained by its slightly acidic pH (4.74) and high sand content (93.6 %) that do not favor the presence or biological activity of certain soil organisms. Soil EST C presented the higher value for global feed activity (58.4 %), related to the fact that this anthroposoil was still used for farming and presented significant OM content and C/N values (Chapter 3, Figure 5), thus it was rich in macro and micronutrients, favoring the existence of a significant quantity of soil organisms.

The results obtained for GFA are in agreement with the macrofauna present verified in each of the anthroposoils studied.

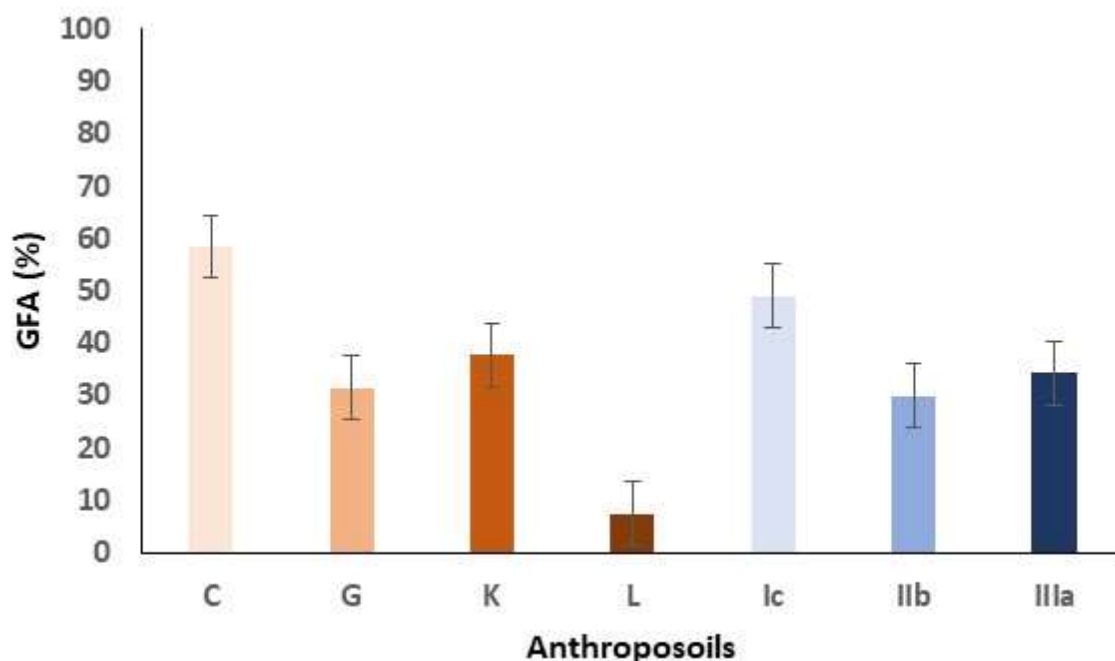


Figure 16 – Global feed activity (GFA) after 6 weeks of exposure of the strips (Bait-lamina© test) in the anthroposoils tested (n=60 strips per anthroposoil).

4.4. Levels and sources of organic contaminants in plants collected *in situ*

Several entire plants of Common nettle and Japanese knotweed, belonging to the species *Urtica dioica* and *Fallopia japonica*, respectively (see Chapter 3, Figure 7), were collected *in situ* from the two sampling points, PEY Ic and PEY IIIa. The POPs analyses were performed on the different parts of the plants: roots and aerial parts (as well as, when possible, the distinction between stem and leaves) (Table 22).

Table 22 – Concentrations of POPs quantified (in $\mu\text{g.kg}^{-1}$ dw) in the plants collected *in situ* in soils PEY Ic and PEY IIIa (mean \pm standard deviation; n = 3 or more).

	Common nettles (PEY Ic)		Japanese knotweed (PEY IIIa)		
	Aerial parts	Roots	Leaves	Stems	Roots
Σ PCBs-cop	0.01 \pm 0.001	0.01 \pm 0.002	0.03 \pm 0.003	0.01 \pm 0.001	0.01 \pm 0.004
Σ PCBs-noncop	0.14 \pm 0.001	0.33 \pm 0.02	0.61 \pm 0.01	0.09 \pm 0.01	0.42 \pm 0.46
Σ PCBs-ndl	1.43 \pm 0.18	3.39 \pm 0.32	3.80 \pm 0.05	0.66 \pm 0.10	2.21 \pm 1.11
Σ 7PCDDs	0.001	0.003	0.003 \pm 0.001	0.001	0.006 \pm 0.001
Σ 10PCDFs	0.000	0.001	0.001	0.001	0.001
Σ 7PBDEs	0.05 \pm 0.02	0.05 \pm 0.01	0.23 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.02
Σ 8PBDEs	0.45 \pm 0.20	0.23 \pm 0.13	2.25 \pm 0.39	0.53 \pm 0.38	2.42 \pm 0.19
Σ HBCDs	0.06 \pm 0.03	0.04 \pm 0.01	0.09 \pm 0.03	0.06 \pm 0.04	0.68 \pm 0.37

Among the POPs quantified in the aerial parts and roots of the two plants analyzed, the PCBs-ndl are those that show higher levels, followed by the sum of the 8 PBDEs. Similar levels of PCBs-noncop and HBCDs were recorded. On the contrary, the PCDDs and PCDFs appear only in very low concentrations (0.01 $\mu\text{g.kg}^{-1}$ dw or less).

The presence of PCBs in plants has already been reported by some authors. The levels obtained in the plants studied were in the same range as the concentrations of Σ 3PCBs reported in *Lolium perenne* (3.50 $\mu\text{g.kg}^{-1}$ dw) and in *Melaleuca leukadendra* (2.64 up to 7.00 $\mu\text{g.kg}^{-1}$ dw) in a suburban residential area in Brisbane in Australia (Müller et al., 2001). Zhao et al. (2006)

detected levels of Σ_{17} PCBs of about $2.80 \mu\text{g}\cdot\text{kg}^{-1}$ dw in grass samples in China. In a study on the distribution of certain POPs in soils and vegetation near a landfill in Greece, Chrysikou et al. (2008) found levels from 3.64 to $25.9 \mu\text{g}\cdot\text{kg}^{-1}$ dw for Σ_7 PCBi in *Solanum eleagnifolium* and *Solanum trifolium* plant species. Also, several studies reported PCBs uptake and translocation by edible vegetables such as: soybeans (Suzuki et al., 1977), carrots (Iwata and Gunther, 1976), beets, turnips and beans (Sawhney and Hankin, 1984). It was also observed that the lower chlorinated congeners (PCB-28, -52 and -101) were found to be more abundant in the shoots than in the roots of these plant species (Iwata and Gunther, 1976; Suzuki et al., 1977; Sawhney and Hankin, 1984). Liu and Schnoor (2008) concluded that some mono to tetra PCBs are absorbed by plant roots but only the lower chlorinated ones undergo translocation to aerial plant tissues. Members of the Cucurbitaceae family have been shown to accumulate PCBs in their tissues (Hulster et al., 1994; White et al., 2005). In a study with pumpkin plants, Aslund et al. (2008) also found that the PCBs concentration increased within the stem and leaves after a short period of exposure to contaminated soils, but the concentrations measured in the plant roots remained unchanged.

PCDDs and PCDFs are highly lipophilic compounds primarily sorbed by plant roots or soil components, though they are not usually translocated within plant tissues (Reischl, 1989). Despite this, several previous studies showed that bioconcentrations of PCDDs and PCDFs clearly exist in zucchini plants two orders of magnitude higher than those found in vegetables such as pumpkin and cucumber, even though these three plants belong to the Cucurbita family (Hulster et al., 1994). Levels measured in the two plant species collected *in situ* in the present study were much lower than those measured by Müller et al. (1994) in carrots, lettuce and peas (values from 0.05 up to $0.48 \mu\text{g}\cdot\text{kg}^{-1}$ dw).

Some plant crops show the ability to translocate PBDEs from soil into vegetative structures and this may also result in consistent low-level exposure through diet (Navarro et al., 2017). Elevated concentrations of PBDEs were also already reported in spinach in Japan (Ohta et al., 2002). An interesting fact is that not only were higher levels of PBDEs quantified in spinach but also the congener composition was different from that of the root vegetables, namely potato and carrot, analyzed in the study, a finding that can be explained by a difference in contamination pathways.

Hassanin et al. (2005) investigated time trends in atmospheric BDEs concentrations in archived grass samples. The general trend observed was of non-detectable levels of BDEs in the early samples, increasing PBDE concentrations in the 1970s, the highest concentrations in the 1980s/1990s (with a peak in 1999) and recent declines. The PBDE concentrations in grass varied

substantially, by a factor of 120 from 0.01 to 1.20 $\mu\text{g.kg}^{-1}$ dw. BDE-47 and BDE-99 generally dominated the profiles, with BDE-28, -35, -100 and -153 contributing less than 25% of PBDE. BDE-28 showed an anomalous behavior as concentrations of BDE-28 have risen whilst concentrations of other congeners have fallen in recent years. Possible explanations include additional recent sources of BDE-28 in brominated products other than the penta-mix PBDE formulation (now banned in the EU) and the possible formation of BDE-28 from other congeners in the environment. The values obtained in the two plant species collected *in situ* were in the same range of those found by the author referred to.

The occurrence of POPs transfer from soil to plant parts is shown by the levels detected in plant parts for most of the POPs analyzed. This fact emphasizes the need to carry out laboratory tests with different plants to try to better understand the potential toxicity associated with the uptake of POPs and to evaluate the degree of potential bioaccumulation of POPs in different plant parts.

MAIN RESULTS

- ✓ The 8 anthroposoils studied are quite different in terms of physico-chemical characteristics as well as regarding inorganic and organic contamination.
- ✓ Despite the levels of contamination found, plants still grow on such sites and the transfer of POPs was well demonstrated.
 - What is the bio-availability or toxicity effect of POPs?
- ✓ Special attention was given to the emergent nBFRs. These compounds have begun to be even more present in environmental compartments but there is very little literature or studies available regarding their distribution, fate, potential uptake and accumulation by terrestrial organisms, namely earthworms and plants.
- ✓ Once nBFRs are present in the anthroposoils studied and in the plant species collected *in situ*, these compounds can easily reach other soil organisms and be incorporated, thus they can migrate along the food chain. The presence of nBFRs raises the issue of toxicity effects, so it is important to conduct toxicity tests with representative terrestrial organisms (Subchapter 4.6).
 - What is the bio-availability or toxicity effect of BFRs?

PART B: Toxicity and bioaccumulation laboratory tests with *E. fetida* and plants

4.5. Toxicological tests

4.5.1. Tests with earthworms - Reproduction test

The results from the reproduction tests are summarized in Table 23, including the effects on body mass, mortality rate and the number of juveniles produced after the exposure period. For each series of samples tested, an ISO soil was used as control sample (ISO EST and ISO PEY).

Table 23 – Results from the reproduction tests at the end of exposure time.

MATRICE	EFFECT ON BODY MASS (%)	MORTALITY (%)	JUVENILES NUMBER
EST ISO	-22.2 ± 7.75	2.50 ± 5.00	307 ± 46
EST C	-5.64 ± 3.86	2.50 ± 5.00	256 ± 32
EST G	10.9 ± 1.70	2.50 ± 5.00	93 ± 23
EST K	7.82 ± 12.5	5.00 ± 10.0	166 ± 104
EST L	-5.43 ± 11.1	2.50 ± 5.00	242 ± 38
PEY ISO	-32.3 ± 4.32	0	120 ± 82
PEY Ic	-42.1 ± 3.07	2.50 ± 5.00	122 ± 58
PEY IIb	-39.7 ± 6.15	27.5 ± 48.6	127 ± 87
PEY IIIa	-38.4 ± 7.23	7.50 ± 5.00	121 ± 61
PEY IVa	-41.4 ± 6.64	2.50 ± 5.00	286 ± 87

Mean values (n=4) and ± =Standard Deviation (SD).

At the end of the 28 days of exposure, the mortality rate was very low in the anthroposoils studied, except in the case of PEY IIb where all 10 earthworms in a replicate were dead after 2 weeks exposure. A Kruskal-Wallis test for the comparison of mean values was performed and no significant differences in mortality rate were found between the *E. fetida* exposed to the 8 anthroposoils studied.

Differences were observed in the weight loss or gain of earthworms considering the mean values of adult body weight at the beginning and at the end of the exposure time. Changes in biomass were measured on adult *E. fetida* (negative values indicate a mass loss). A Kruskal-Wallis test showed significant differences between all the EST samples (p value = 0.01) and all the PEY samples (p value = 0.03 in the lockers). The p value was below 5%, indicating the

existence of significant differences, so a Wilcoxon pairs comparison test was performed to distinguish the different statistical classes. The highest mass losses were found in *E. fetida* exposed to ISO soils, probably due to the low nutrient quantities in this control sample.

Mass gains were observed for *E. fetida* exposed to EST G and EST K anthroposoils and only slight losses of mass were observed for individuals exposed to anthroposoils EST C and EST L (Table 23). This is probably related to the relatively high OM contents of EST anthroposoils. Mass losses in *E. fetida* were observed in all the PEY samples despite these anthroposoils being rich in OM content, while the pH values are within the range of ideal values for the good growth and development of *E. fetida*. It is probable that the sandy character of these anthroposoils and the presence of relatively high concentrations of certain MTEs and POPs inhibited the growth and physiological development of *E. fetida*. Statistical analysis highlighted the existence of significant differences in the loss or gain of mass in adults at the end of the test when compared with the initial *E. fetida* mass (p value = 0.001 in EST samples; p value = 0.01 in PEY samples). Figure 17 presents the boxplot diagrams showing the variation in the mass (loss or gain) of *E. fetida* adults at the end of the bioaccumulation test. Different colors correspond to different statistical groups.

After 8 weeks exposure, the *E. fetida* juveniles were collected and counted (Table 23). Statistical analysis highlighted the existence of significant differences in the number of juveniles by soil (p value = 0.002 in EST samples; p value = 0.01 in PEY samples). Figure 18 presents the boxplot diagrams showing the distribution of *E. fetida* juveniles at the end of the bioaccumulation test. Different colors correspond to different statistical groups.

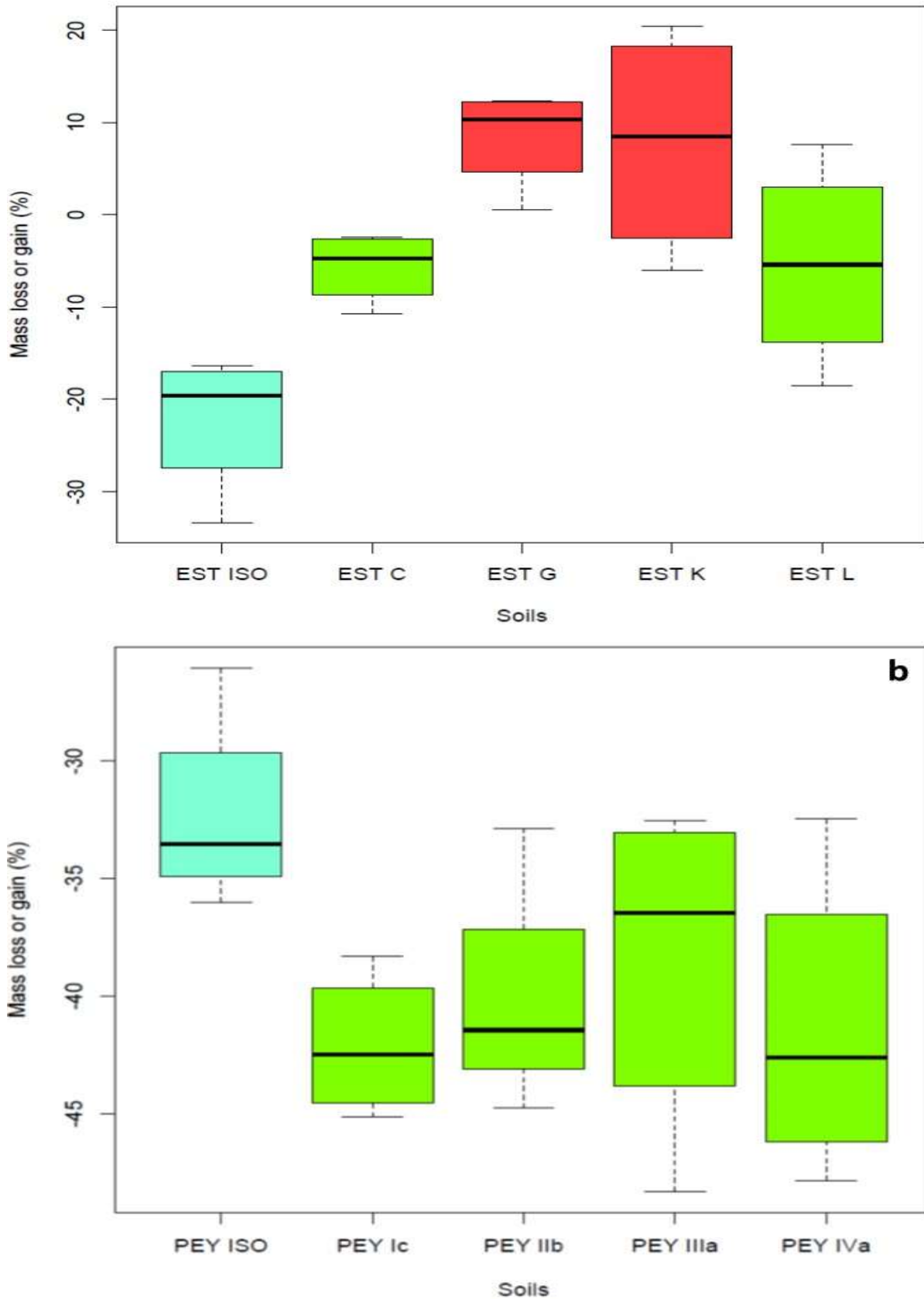


Figure 17 – Boxplots showing the variation in the mass (loss or gain) of *E. fetida* adults at the end of the bioaccumulation tests in EST (a) and PEY (b) anthroposols. Different colors correspond to different statistical groups (p value = 0.001 in EST samples, p value = 0.01 in PEY samples) ($n=4$).

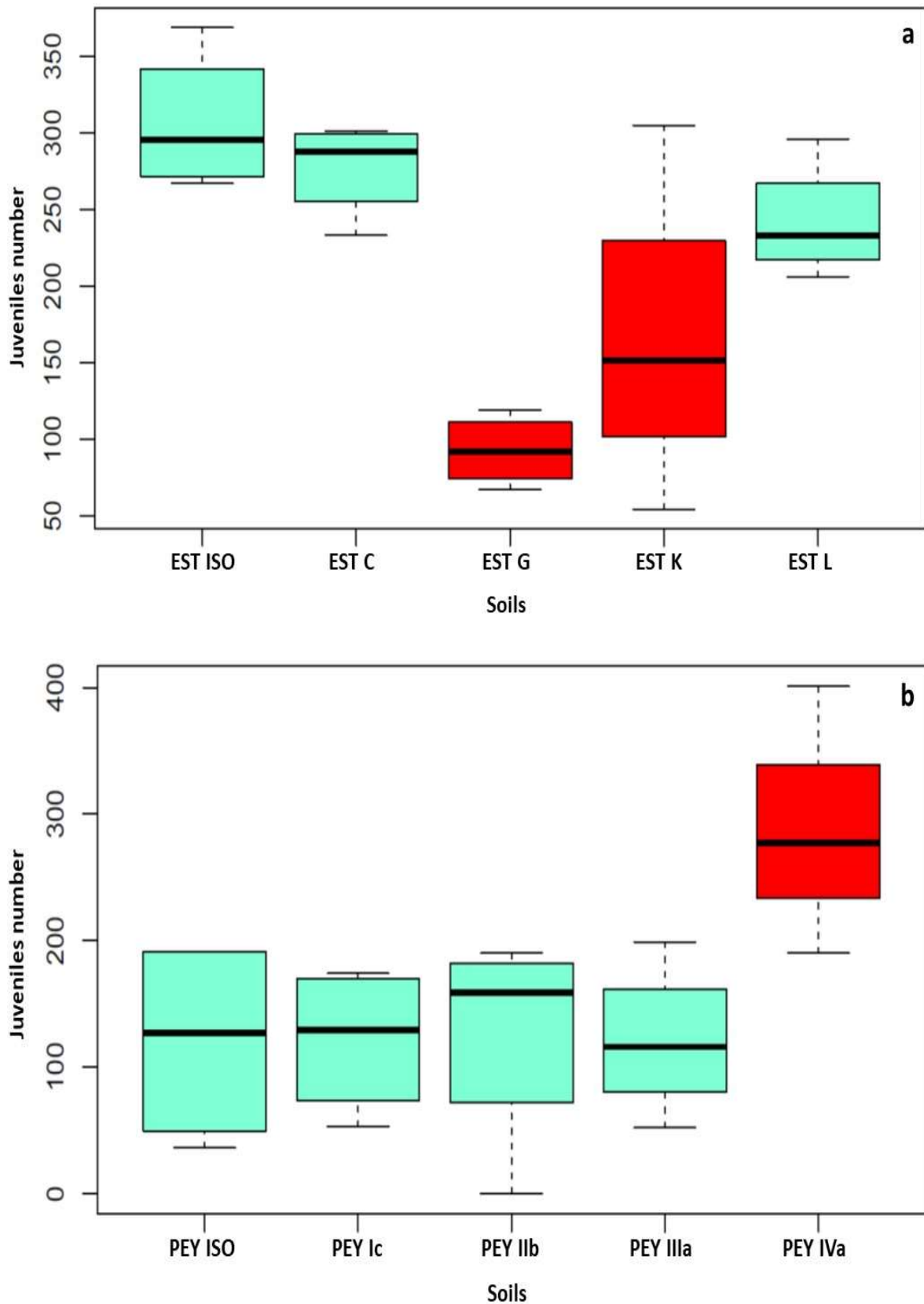


Figure 18 – Boxplots showing the distribution of *E. fetida* juveniles at the end of the bioaccumulation test in EST (a) and PEY (b) anthroposoils. Different colors correspond to different statistical groups (p value = 0.002 in EST samples, p value = 0.01 in PEY samples) (n=4).

Very low mortality was verified in the earthworms exposed to the PEY samples (Table 23) and the numbers of juveniles produced were in general lower than those from EST anthroposoils, probably due to the inhibition effects caused by the sandy texture that can negatively affect the normal biological activities of earthworms in addition to the higher MTE and POPs levels found in the PEY samples. The results obtained in the reproduction assay were in agreement with those reported by Lemtiri et al. (2016) obtained in a study where the role of *E. fetida* on the availability of metals and their effects on metal uptake by plants at different soil concentrations was evaluated. Exposure to high levels of POPs (higher than 100 ng.g⁻¹ dw) is quite often related to an impairment in earthworm reproduction (Datta et al., 2016; Lin et al., 2012; Yasmin and D'Souza, 2010).

Among the EST samples, a large number of juveniles was observed in ISO soil while the lower number corresponds to soil G (Figure 18a). There was a slight difference in the production of juveniles between soils K and G. Soils C and L showed a number of juveniles close to the number observed for ISO soil. Concerning the PEY samples, no significant differences were observed (Figure 18b). It should be noted that a larger number of juveniles occurred in PEY IVa which can be related to the high OM content that served as feed for the earthworms and thus helped their development. For the EST samples, two statistical groups were observed. The first consisted of the ISO, C and L soils with a higher number of juveniles (average between 224 and 307), while the second group consisting of G and K soils, had fewer juveniles (averages of 93 and 166, respectively) (Figure 18a).

Significant levels of Zn found in the PEY anthroposoils can explain the reproduction inhibition observed in the *E. fetida* individuals exposed. Van Gestel et al. (1993) showed that earthworms can regulate their internal Zn concentration for a certain range of concentrations (80 up to 100 mg.kg⁻¹). The Zn values found in the PEY anthroposoils were higher (from 131 up to 248 mg.kg⁻¹), thus the internal concentrations of *E. fetida* exposed to these anthroposoils may probably exceed 100 mg kg⁻¹ and become toxic/inhibitive for earthworm development. In Coelho et al. (2018) (see Annex I), already analyzed the effect of the presence of a mix of contaminants (MTEs) on *E. fetida* reproduction. In the present study, another 3 sediments were considered and verified the same trend of inhibition, notably in the range of MTEs.

The decrease in growth as well as reproduction rate can also be correlated with the reduction of feeding activity as a strategy for the earthworms to avoid exposure to contaminants (Ribeiro et al., 2001; Mosleh et al., 2003).

The results of the reproduction test tend to show that the physico-chemical properties of soils (pH, particle size, sand content) probably play a major role in the effects of anthroposoils on *E.*

fetida. Pearson correlation coefficients higher than 0.7 were obtained for the relation between the physico-chemical properties and the levels of POPs detected in *E. fetida* tissues (Annex II, Table I.1). Despite this, and since there are significant differences between the EST and PEY anthroposoils, the effects on growth, body mass and reproduction rates observed may have been due to the bioaccumulation of certain contaminants. This emphasizes the need to perform bioaccumulation tests with *E. fetida* to assess and understand the bioaccumulation of POPs in *E. fetida* tissues.

4.5.2. Tests with plants - Germination and growth test

The germination and growth tests with plants were carried out with alfalfa (*Medicago sativa*), watercress (*Nasturtium officinale*) and white mustard (*Sinapis alba*), firstly to evaluate the effect on germination (up to 7 days) then on growth (for 60 days) (see subchapter 3.5). Figure 19 shows the boxplots for the distribution of the germination rate of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils. The germination rate was significantly higher for the control (noted T), EST G, PEY Ic and PEY IIb for all the three plants tested compared to the other samples. This good germination can be associated with the relatively high but not toxic levels of some MTEs (such as Zn and Cu) found in these anthroposoils that are oligoelements for plant development. The three plant species tested, cultivated in anthroposoils EST C, EST K and PEY IVa showed very low germination rates, perhaps due to the compactness of these three anthroposoils.

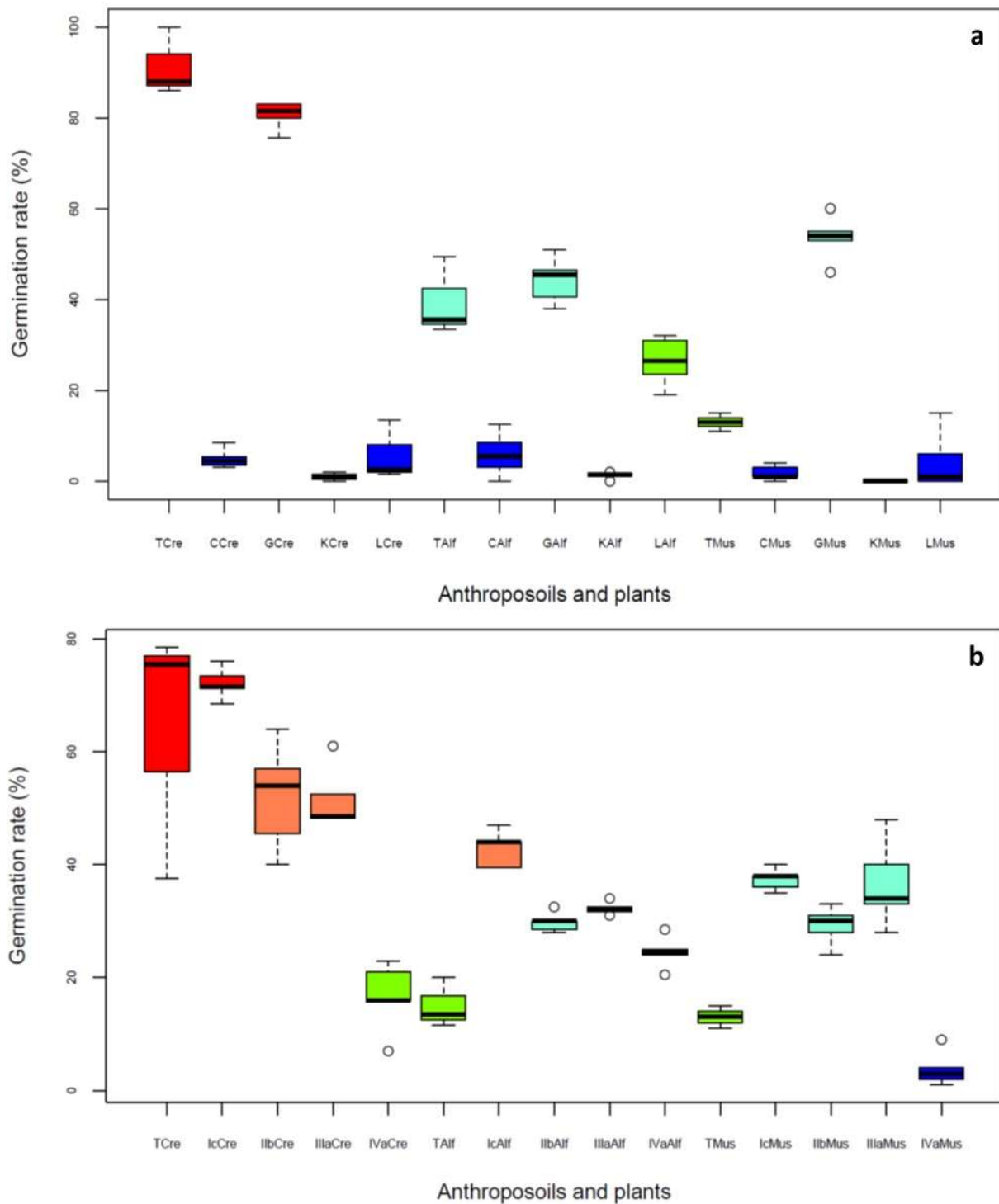


Figure 19 – Boxplots showing the distribution of the germination rate of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils (T = Test control; Cr = Cress; Alf = Alfalfa and Mus = Mustard). (n=3 for T and n=5 for others). Different colors correspond to different statistical groups (p values of 0.0007, 0.0005 and 0.014 in EST samples for Cr, Alf and Mus; p values of 0.003, 0.0005 and 0.0009 in PEY samples for Cr, Alf and Mus).

It has been found that different species do not respond similarly to toxic chemicals (Gong et al., 1999). For Gong et al. (2001), seed germination that depends on the energy reserves in cotyledons is a less sensitive endpoint than early seedling growth.

Nevertheless, two physiological phenomena during germination were established: an increase in oxygen use (when respiration begins) and imbibition (when the seed rehydrates). The dormant seed is in a dehydrated state and has a moisture content of only 10% of its fresh weight (Ernst, 1998). During the germination process, the plant seeds incorporate a great quantity of water that can influence the uptake rate of contaminants, mainly at the initial growth stage (Wolny et al., 2018). Also, soil texture (sand and silt contents) and compacity influence the availability of water in soil, since water is less available to seeds in more compact and sand-rich soils. Our results also suggest that the respiration of the seeds may be affected, particularly since the MTEs (Hg, Ars), or POPs as PCBs contents of the anthroposoils used may be potential inhibitors of respiration.

Figure 20 shows the boxplots for the distribution of aerial part mean heights of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils. The aerial part mean heights were higher for the control, EST G, EST C, PEY Ic and PEY Iib for all the three plants tested. In the case of alfalfa and cress, the anthroposoils played an important role in maximum height, but in the case of mustard a higher aerial part height was expected when compared with the other two species tested due to the plant's characteristics. In the case of anthroposoil EST L, the low plant height may be linked to the slightly acid pH (4.75), lower OM content, the noticeable Pb concentration and the presence of several POPs seen at low levels.

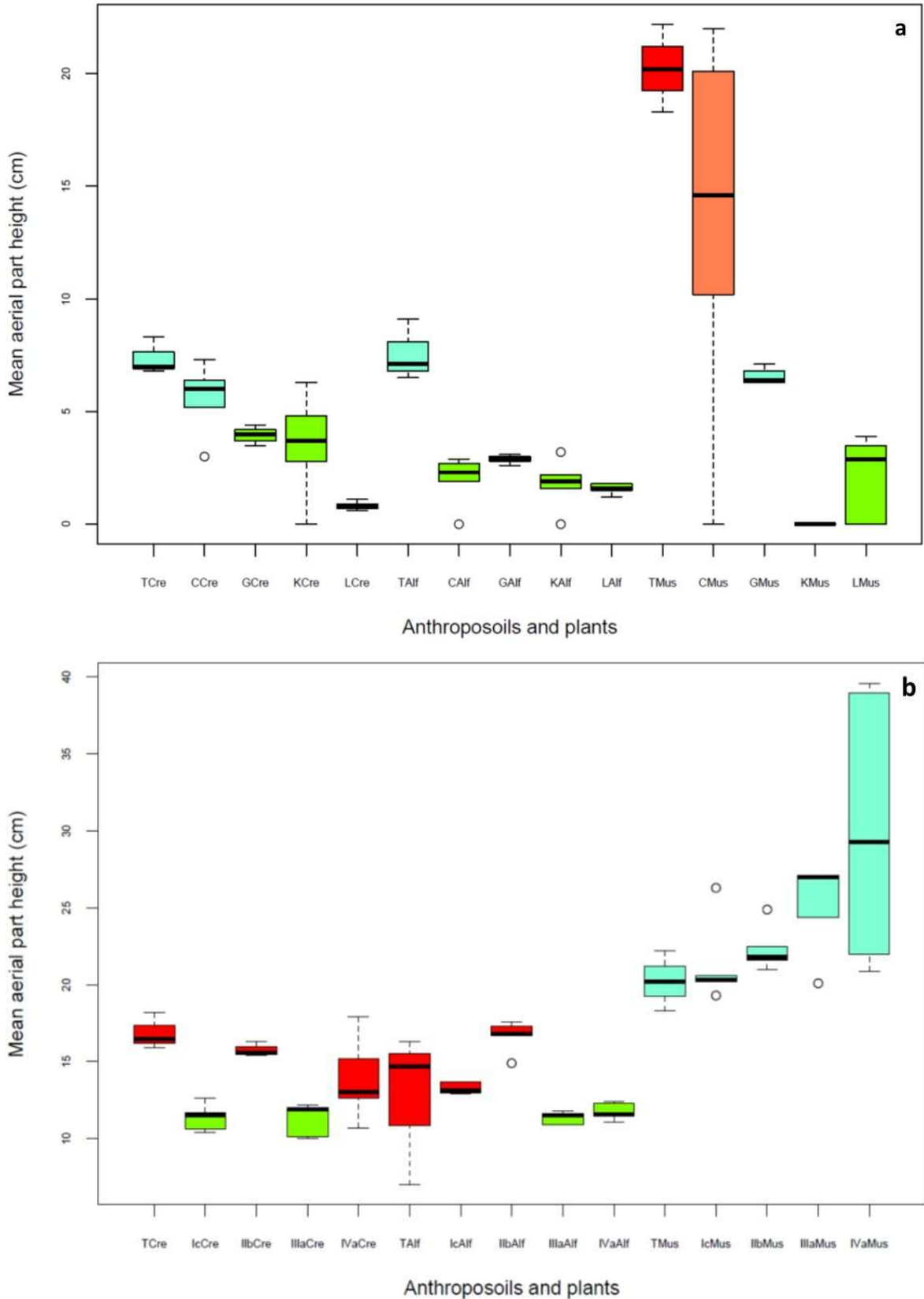


Figure 20 – Boxplots showing the distribution of aerial parts height of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils (T = Test control; Alf = Alfalfa; Cr = Cress; Mus = Mustard). (n=3 for T and n=5 for others). Different colors correspond to different statistical groups (p values of 0.005, 0.007 and 0.003 in EST samples for Cr, Alf and Mus; p values of 0.004, 0.003 and 0.073 in PEY samples for Cr, Alf and Mus).

Figure 21 shows the boxplots for the distribution of the dry weight of aerial parts of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils. As expected, the dry weight of the aerial parts was higher for the control, EST G, EST C, PEY Ic and PEY Iib anthroposoils for all the three plants tested. This was obviously related to the fact that the higher germination rates and the higher weight of the aerial parts were verified in these samples.

The differences in aerial part dry weight between the species tested were certainly related to the differences in germination rates and to the physiological characteristics that determine the capacity of each plant species to cope with contamination.

Also, the presence of certain POPs can be linked to toxicity effects that inhibit the correct development of the aerial tissues; also, the presence of the contaminants can cause damage in the plant tissues already developed as a result of exposure. In a study on the phytotoxicity of contaminated sediments deposited on soils, Bedell et al. (2006) verified that the biomass of aerial parts of maize and ryegrass decreased due to the presence of certain contaminants.

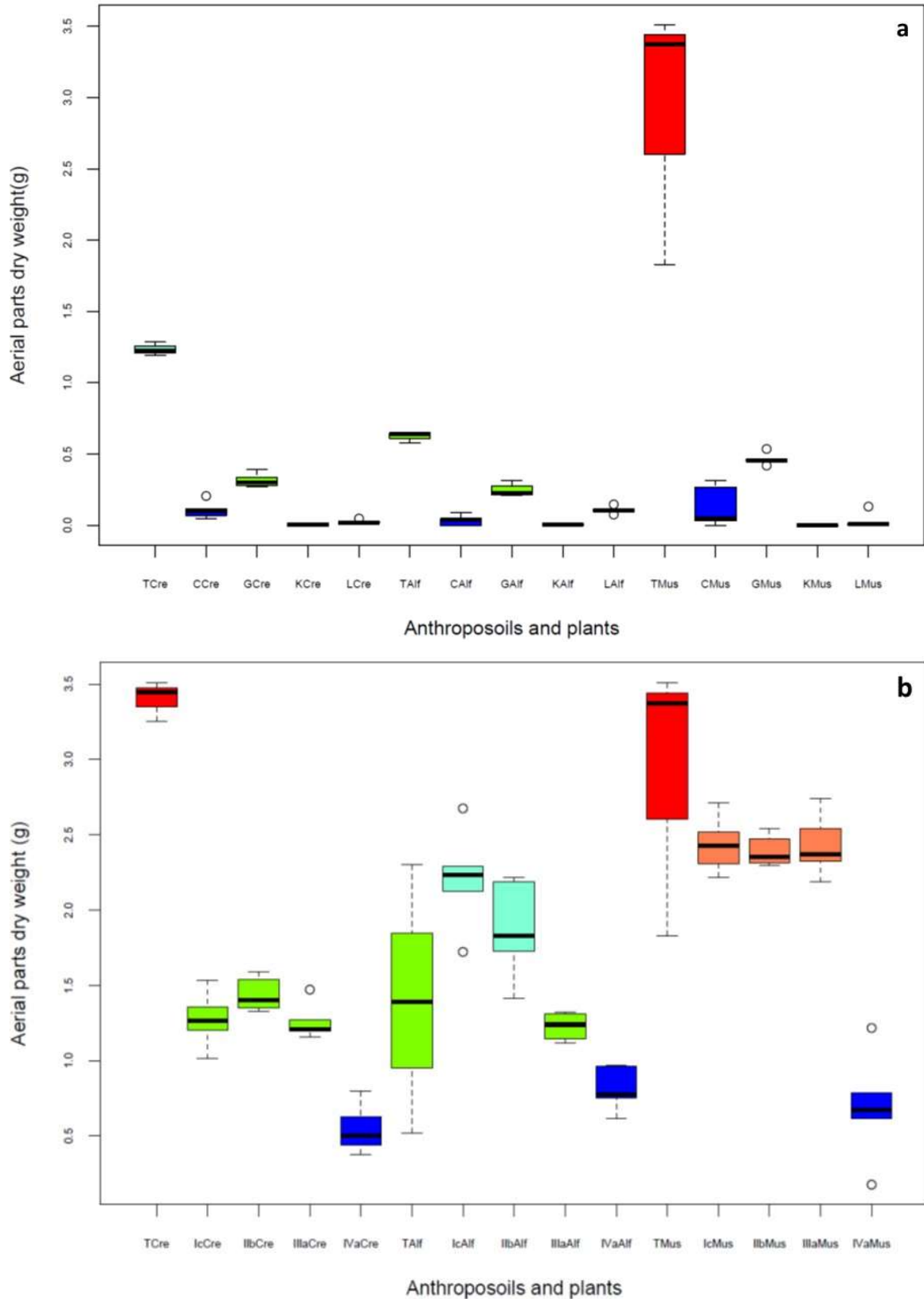


Figure 21 – Boxplots showing the distribution of dry weight of aerial parts of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils (T = Test control; Alf = Alfalfa; Cr = Cress; Mus = Mustard). (n=3 for T and n=5 for others). Different colors correspond to different statistical groups (p values of 0.0003, 0.0006 and 0.008 in EST samples for Cr, Alf and Mus; p values of 0.001, 0.004 and 0.019 in PEY samples for Cr, Alf and Mus).

Figure 22 shows the boxplots for the distribution of the dry weight of aerial parts of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils. As expected, the dry weight of the aerial parts was higher for the control, EST G, PEY Ic and PEY IIb anthroposoils for all the three plants tested. Root development did not seem to be inhibited in these anthroposoils. Low root dry weight was verified for anthroposoils EST C, EST K, EST L and PEY IVa, which may have been due to the physico-chemical characteristics of these anthroposoils, such as pH, OM content, compactness to the presence of certain levels of MTEs and/or POPs that can affect the correct development of plant roots.

Moreover, the inhibition effect of the presence of PCBs on plant growth and development in soils has already been well documented. Streck and Weber (1982) found that PCBs induced an inhibition up to 47% for soybean, beet and pigweed growth and height. Weber and Mrozek (1979) performed a toxicity study of PCBs in soybean plants, where they found that PCBs applied to the soil tested significantly inhibited the height and fresh weight of aerial parts in application rates of 1-100 ppm of PCBs. In the present study, inhibition effects were already observed, particularly in the case of plants cultivated in PEY samples. This could have occurred due to the high levels of PCBs found in these anthroposoils. Also, the biomass loss can be explained by the fact that the products of PCBs and their degradation products become phytotoxic to plants due to the increase in their concentration, bioavailability and solubility (Mehmannavaz et al., 2002).

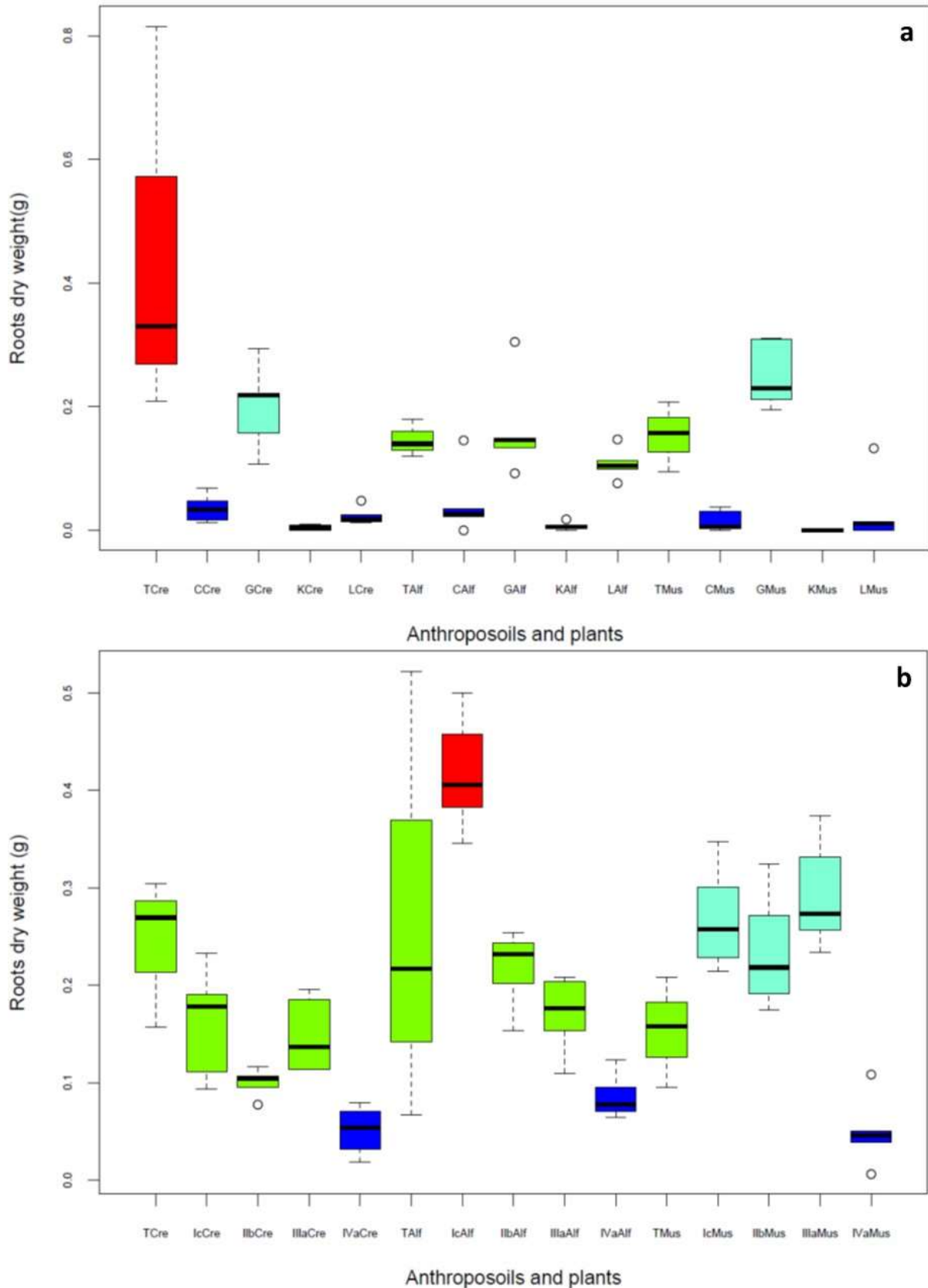


Figure 22 – Boxplots showing the distribution of dry weight of roots (b) of alfalfa, cress and mustard cultivated in the EST (a) and PEY (b) anthroposoils (T = Test control; Alf = Alfalfa; Cr = Cress; Mus = Mustard). (n=3 for T and n=5 for others). Different colors correspond to different statistical groups (p values of 0.0007, 0.005 and 0.0013 in EST samples for Cr, Alf and Mus; p values of 0.003, 0.006 and 0.003 in PEY samples for Cr, Alf and Mus).

MAIN RESULTS

- ✓ Toxic effects were observed for *E. fetida* and the three plant species cultivated in the anthroposoils studied, in particular *E. fetida* reproduction rate, *E. fetida* body mass, plant seed germination rates and the maximal height of plant aerial parts.
 - ✓ Levels of POPs accumulated in organisms (Subchapters 4.7.1 and 4.7.2).
 - ✓ It can be assumed that the toxic effects were caused mainly by the mix of contaminants present in the anthroposoils tested, with in some cases the slight influence of certain physico-chemical parameters.
- A PCA will be performed to identify possible correlations between the physico-chemical soil properties and POPs levels in anthroposoils and *E. fetida* tissues, capable of explaining the effects of contamination in exposed individuals of *E. fetida* (Subchapter 4.8).
- SET and ERITME indexes will be calculated to rank the sites tested according to the effective POPs transfer from anthroposoils to the organisms tested and provide an idea of the potential risk to the real ecosystem (Subchapter 4.9).

4.6. Levels of organic contaminants in *Eisenia fetida* and in the plants tested

4.6.1. *Eisenia fetida*

Since hydrophobic organic contaminants are mainly distributed to the lipids within the organism, the concentrations of POPs measured in earthworms are expressed as lipid-normalized chemical concentrations (lw). The lipid contents were determined according to the chemical procedure described in subchapter 3.4. The lipid contents of *E. fetida* obtained in the present study ranged from 1.73 % in juveniles to 4.07 % in adults based on dw. The results obtained were a little lower than the lipid contents of 9 – 10 % reported by Fadaee (2012) and Gunya et al. (2016) for *E. fetida*.

The concentrations of PCBs, PCDDs, PCDFs, PBDEs, PBBs, nHBB, nPBB, nPBT and PBEB in the tissues of *E. fetida* exposed to the 8 anthroposoils studied are listed in Tables 24 and 25 in dw basis, for adults and juveniles respectively. In Tables 26 and 27 the concentrations are normalized to the lipid contents. The lw normalized concentrations will be used by preference in the results and discussion.

Chapter 4. Results and discussion

Table 24 – Mean concentrations of the quantified POPs measured in adult earthworm tissues (in $\mu\text{g}\cdot\text{kg}^{-1}$ dw) after exposure to Estarreja (EST) and Peyraud 6 (PEY) soils (n=2).

	ADULTS									
	EST ISO	EST C	EST G	EST K	EST L	PEY ISO	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
$\Sigma\text{PCBs-cop}$	0.06	0.05	0.06	0.04	0.05	0.05	0.64	0.23	0.14	0.29
$\Sigma\text{PCBs-noncop}$	8.83	5.38	6.22	5.48	5.73	4.16	43.9	57.0	11.6	21.8
$\Sigma\text{PCBs-ndl}$	63.5	41.7	50.3	40.5	43.8	34.4	348	308	91.9	141
$\Sigma_7\text{PCDDs}$	0.002	0.004	0.005	0.002	0.003	0.003	0.20	0.13	0.05	0.05
$\Sigma_{10}\text{PCDFs}$	0.002	0.002	0.003	0.002	0.002	0.001	0.04	0.02	0.04	0.01
$\Sigma_7\text{PBDEs}$	0.058	0.004	0.003	0.003	0.004	0.19	0.29	0.39	0.33	0.36
$\Sigma_8\text{PBDEs}$	0.43	0.09	0.08	0.08	0.09	0.55	0.89	1.10	1.01	1.03
$\Sigma_3\text{PBBs}$	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.002	0.003	<LOD	<LOD
nHBB	0.04	0.01	0.01	0.01	0.01	0.06	0.06	0.08	0.05	0.03
nPBB	0.004	0.001	0.001	0.001	0.001	0.014	0.016	0.019	0.018	0.018
nPBT	0.010	0.002	0.001	0.001	0.002	0.077	0.085	0.068	0.062	0.051
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD	0.001	0.001	0.002	0.001	0.001

LOD = Limit of detection.

Chapter 4. Results and discussion

Table 25 – Mean concentrations of the quantified POPs measured in juvenile earthworm tissues (in $\mu\text{g.kg}^{-1}$ dw) after exposure to Estarreja (EST) and Peyraud 6 (PEY) soils (n=2).

	JUVENILES									
	EST ISO	EST C	EST G	EST K	EST L	PEY ISO	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
Σ PCBs-cop	0.11	0.11	0.20	0.19	0.08	0.03	0.40	0.15	0.07	0.14
Σ PCBs-noncop	5.77	4.57	7.57	5.33	2.50	1.86	22.7	30.1	4.72	10.2
Σ PCBs-ndl	38.0	32.7	55.7	33.3	15.5	13.6	216	211	43.6	75.8
Σ_7 PCDDs	0.004	0.02	0.03	0.02	0.03	0.01	0.27	0.23	0.07	0.07
Σ_{10} PCDFs	0.01	0.01	0.02	0.02	0.01	0.004	0.04	0.03	0.04	0.01
Σ_7 PBDEs	0.03	0.03	0.19	0.12	0.02	0.16	0.75	0.54	0.29	0.31
Σ_8 PBDEs	0.98	0.96	5.67	3.94	0.49	3.85	5.67	3.73	2.84	2.14
Σ_3 PBBs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
nHBB	0.04	0.03	0.16	0.12	0.02	0.29	0.19	0.10	0.21	0.12
nPBB	0.01	0.01	0.04	0.03	0.003	0.06	0.05	0.03	0.03	0.02
nPBT	0.02	0.02	0.15	0.10	0.01	0.18	0.12	0.07	0.09	0.05
PBEB	0.002	0.003	<LOD	0.01	0.002	0.001	0.001	0.002	0.001	0.001

LOD = Limit of detection.

Chapter 4. Results and discussion

Table 26 – Mean concentrations of the quantified POPs measured in adult earthworm tissues ($\mu\text{g.kg}^{-1}$ lw) after exposure to Estarreja (EST) and Peyraud 6 (PEY) soils (n=2).

	ADULTS									
	EST ISO	EST C	EST G	EST K	EST L	PEY ISO	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
Σ PCBs-cop	3.47	2.86	3.32	2.39	2.96	2.98	36.9	13.4	8.09	16.7
Σ PCBs-noncop	510	311	360	317	331	240	2540	3293	670	1261
Σ PCBs-ndl	3670	2408	2909	2339	2532	1988	20088	17789	5312	8154
Σ 7PCDDs	0.09	0.21	0.32	0.11	0.15	0.16	11.3	7.55	2.81	2.87
Σ 10PCDFs	0.11	0.13	0.20	0.09	0.12	0.06	2.38	1.22	2.14	0.59
Σ 7PBDEs	3.53	0.23	0.17	0.17	0.23	10.9	16.8	22.3	18.9	20.8
Σ 8PBDEs	25.0	5.03	4.62	4.39	5.32	31.7	51.4	63.3	58.1	59.7
Σ 3PBBs	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.12	0.17	<LOD	<LOD
nHBB	2.08	0.35	0.29	0.29	0.35	3.64	3.30	4.39	2.72	1.62
nPBB	0.20	0.06	0.06	0.06	0.06	0.81	0.93	1.10	1.04	1.04
nPBT	0.58	0.12	0.06	0.06	0.17	4.45	4.91	3.93	3.58	2.95
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD	0.06	0.06	0.12	0.06	0.06

LOD = Limit of detection.

Chapter 4. Results and discussion

Table 27 – Mean concentrations of the quantified POPs measured in juveniles earthworm tissues (in $\mu\text{g}\cdot\text{kg}^{-1}$ lw) after exposure to Estarreja (EST) and Peyraud 6 (PEY) soils (n=2).

	JUVENILES									
	EST ISO	EST C	EST G	EST K	EST L	PEY ISO	PEY Ic	PEY IIb	PEY IIIa	PEY Iva
$\Sigma\text{PCBs-cop}$	3.19	3.17	5.60	5.34	2.17	0.96	11.3	4.09	2.02	3.97
$\Sigma\text{PCBs-noncop}$	162	128	213	150	70.2	52.3	637	844	133	288
$\Sigma\text{PCBs-ndl}$	1066	919	1564	934	435	381	6078	5922	1226	2130
$\Sigma_7\text{PCDDs}$	0.19	0.42	0.78	0.44	0.74	0.20	7.65	6.33	1.92	1.93
$\Sigma_{10}\text{PCDFs}$	0.21	0.25	0.59	0.44	0.26	0.11	1.22	0.89	1.08	0.35
$\Sigma_7\text{PBDEs}$	0.96	0.84	5.20	3.43	0.42	4.61	21.2	15.2	8.01	8.74
$\Sigma_8\text{PBDEs}$	27.5	27.1	160	111	13.7	108	159	105	79.7	60.0
$\Sigma_3\text{PBBs}$	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
nHBB	1.18	0.82	4.52	3.37	0.42	8.12	5.34	2.84	5.76	3.48
nPBB	0.23	0.17	1.07	0.70	0.08	1.80	1.26	0.82	0.79	0.48
nPBT	0.59	0.65	4.07	2.70	0.34	4.97	3.34	1.82	2.39	1.38
PBEB	0.06	0.08	<LOD	0.34	0.06	<LOD	<LOD	<LOD	<LOD	<LOD

LOD = Limit of detection.

The levels of all the POPs quantified were higher in *E. fetida* exposed to PEY anthroposoils and higher in juveniles rather than in adults exposed to both the anthroposoils tested.

Mean concentrations of \sum PCBs-cop in the adult *E. fetida* tissues were in the range of 2.39 to 3.32 $\mu\text{g.kg}^{-1}$ lw and 8.09 to 36.9 $\mu\text{g.kg}^{-1}$ lw, for the EST and PEY samples, respectively. Mean concentrations of \sum PCBs-cop in the juveniles *E. fetida* tissues were in the range of 2.17 to 5.60 $\mu\text{g.kg}^{-1}$ lw and 2.02 to 11.3 $\mu\text{g.kg}^{-1}$ lw, for EST and PEY samples respectively. Among the 4 congeners considered (CB-77, -81, -126, -169), CB-77 explains more than 50% of the total sum of coplanar ones. Regarding the \sum PCBs-noncop, the mean levels found in the tissues of *E. fetida* adults exposed to EST anthroposoils were from 311 to 360 $\mu\text{g.kg}^{-1}$ lw, and in the range of 670 to 3293 $\mu\text{g.kg}^{-1}$ lw in those exposed to PEY samples. The mean levels found in the tissues of *E. fetida* juveniles exposed to EST anthroposoils were from 70.2 to 212 $\mu\text{g.kg}^{-1}$ lw, and in the range of 133 to 844 $\mu\text{g.kg}^{-1}$ lw in those exposed to PEY samples. Among the 8 congeners considered (CB-105, -114, -118, -123, -156, -157, -167, -189), CB-118 explains more than 50% of the total concentration. The mean levels of \sum PCBs-ndl measured in *E. fetida* adults were in the range of 2339 to 2909 $\mu\text{g.kg}^{-1}$ lw in the EST samples and 5312 to 20088 $\mu\text{g.kg}^{-1}$ lw in the PEY samples. The mean levels of \sum PCBs-ndl measured in *E. fetida* juveniles were in the range of 435 to 1564 $\mu\text{g.kg}^{-1}$ lw in EST samples and 1226 to 6078 $\mu\text{g.kg}^{-1}$ lw in PEY samples. Among the 6 congeners considered (CB-28, -52, -101, -138, -153, -180), CB-153, CB-138 and CB-180 explain about 80 to 90% of the total sum. The concentrations of \sum PCBs-ndl also represent around 90% of the total sum of all the PCB congeners quantified. The levels of PCBs detected in the *E. fetida* exposed are significantly higher than those indicated by Shang et al. (2013) in a study with contaminated soils from an E-waste dismantling area in China where *E. fetida* and *Allolobophora caliginosa trapezoides* showed PCB levels of 0.31 to 29.3 $\mu\text{g.kg}^{-1}$ dw in their tissues. Despite the difference in the ranges of values, the same trend in the large contribution of PCB-28, -138 and -153 to the total sum (more than 80% of the total sum of PCBs) was also verified in both the study of Shang et al. (2013) and the present study.

In the tissues of *E. fetida* adults exposed to EST samples, the mean concentrations of \sum ₇PCDDs were in the range of 0.11 to 0.32 $\mu\text{g.kg}^{-1}$ lw. In adult *E. fetida* exposed to PEY anthroposoils, the mean levels measured ranged from 2.81 to 11.3 $\mu\text{g.kg}^{-1}$ lw. In the tissues of juveniles, *E. fetida* exposed to EST samples, the mean concentrations of \sum ₇PCDDs were in the range of 0.42 to 0.78 $\mu\text{g.kg}^{-1}$ lw. In *E. fetida* adults exposed to PEY anthroposoils, the mean levels measured were from 1.92 to 7.65 $\mu\text{g.kg}^{-1}$ lw. Generally, octachlorodibenzo-p-dioxin (OCDD) and

1,2,3,4,6,7,8-hepta CDD (1,2,3,4,6,7,8-HpCDD) accounted for the greatest proportion of PCDD/Fs (>80%) in all the *E. fetida* tissues analyzed.

In the tissues of *E. fetida* adults exposed to EST samples, the mean concentrations of Σ_{10} PCDFs were in the range of 0.088 to 0.196 $\mu\text{g.kg}^{-1}$ lw. In the tissues of *E. fetida* adults exposed to PEY anthroposols, the mean levels measured ranged from 2.81 to 11.3 $\mu\text{g.kg}^{-1}$ lw. In the tissues of *E. fetida* juveniles exposed to EST samples, the mean concentrations of Σ_{10} PCDFs were in the range of 0.25 to 0.59 $\mu\text{g.kg}^{-1}$ lw. In the tissues of *E. fetida* juveniles exposed to PEY anthroposols, the mean levels measured were from 0.35 up to 1.22 $\mu\text{g.kg}^{-1}$ lw. Among PCDF homologs, octachlorodibenzofuran (OCDF) and 1,2,3,4,6,7,8-HeptaCDF (1,2,3,4,6,7,8-HpCDF) were the predominant congeners and contributed about 60 % of the PCDFs in the anthroposols studied. The levels of PCDDs and PCDFs detected in exposed *E. fetida* were in the same range as those indicated by Shang et al. (2013) in a study with contaminated soils from an E-waste dismantling area in China, where *E. fetida* and *Allolobophora caliginosa trapezoides* showed levels of PCDDs and PCDFs from 0.19 to 2.18 $\mu\text{g.kg}^{-1}$ lw in their tissues. The same trend of considerable contributions of octachlorodibenzo-p-dioxin (OCDD), 1,2,3,4,6,7,8-HeptaCDD (1,2,3,4,6,7,8-HpCDD), octachlorodibenzofuran (OCDF) and 1,2,3,4,6,7,8-HeptaCDF (1,2,3,4,6,7,8-HpCDF) to the total sum was verified in both the study referred to and the present study.

The mean Σ_7 PBDEs (excluding BDE-209) quantified in adult *E. fetida* tissues ranged from 0.17 to 0.23 $\mu\text{g.kg}^{-1}$ lw in those exposed to EST samples and from 16.8 to 22.3 $\mu\text{g.kg}^{-1}$ lw in those exposed to PEY anthroposols. The mean Σ_7 PBDEs (excluding BDE-209) quantified in juvenile *E. fetida* tissues ranged from 0.42 to 5.20 $\mu\text{g.kg}^{-1}$ lw in those exposed to EST samples and from 8.01 to 21.2 $\mu\text{g.kg}^{-1}$ lw in those exposed to PEY anthroposols. The Σ_7 PBDEs (excluding BDE-209) measured almost completely correspond to the sum of the levels of congeners BDE-47 and BDE-99. When including the BDE-209, the mean levels of Σ_8 PBDEs detected in adult *E. fetida* tissues were significantly higher, ranging from 4.39 to 5.32 $\mu\text{g.kg}^{-1}$ lw in the EST samples and from 51.4 to 63.3 $\mu\text{g.kg}^{-1}$ lw in the PEY anthroposols. In the *E. fetida* juvenile tissues, the mean levels of Σ_8 PBDEs ranged from 13.7 to 159 $\mu\text{g.kg}^{-1}$ lw in the EST samples and from 60.0 to 159 $\mu\text{g.kg}^{-1}$ lw in the PEY anthroposols. Composition analysis clearly showed that in all the *E. fetida* tissues analyzed, BDE-209 was the highly predominant congener, explaining more than 90 % of the total PBDEs, followed by congeners BDE-47 and BDE-99. Congener BDE-28 was close to the LOD in both the EST and PEY samples. The levels of PBDEs detected in exposed *E. fetida* were in the same range as those indicated by Shang et al. (2013) in a study on contaminated soils from an E-waste dismantling area in China where *E. fetida* and

Allobophora caliginosa trapezoides showed levels of PBDEs from 0.15 to 2.44 $\mu\text{g.kg}^{-1}$ dw in their tissues. The same trend of considerable contribution of CB-47 and -99 to the total sum (more than 60 % of the total sum of PBDEs) was also verified in both the study referred to (although BDE-209 was not analyzed in that study) and in the present study.

Σ_3 PBBs were detected only in the tissues of *E. fetida* adults exposed to two PEY anthroposoils, PEY Ic and PEY Iib with values of 0.12 and 0.17 $\mu\text{g.kg}^{-1}$ lw, respectively.

The nHBB mean concentrations found ranged from 0.29 to 0.35 $\mu\text{g.kg}^{-1}$ lw and from 1.62 to 4.39 $\mu\text{g.kg}^{-1}$ lw, in the tissues of *E. fetida* adults exposed to EST and PEY samples, respectively. In the tissues of *E. fetida* juveniles, the levels of nHBB found ranged from 0.42 to 4.52 $\mu\text{g.kg}^{-1}$ lw and from 2.84 to 5.76 $\mu\text{g.kg}^{-1}$ lw, in those exposed to the EST and PEY anthroposoils, respectively. The nPBB mean levels measured were 0.06 $\mu\text{g.kg}^{-1}$ lw in adult *E. fetida* exposed to the EST samples and from 0.93 to 1.10 $\mu\text{g.kg}^{-1}$ lw in adult *E. fetida* exposed to the PEY samples. The nPBB mean levels measured were from 0.08 to 1.07 $\mu\text{g.kg}^{-1}$ lw in juvenile *E. fetida* exposed to the EST samples and 0.48 to 1.26 $\mu\text{g.kg}^{-1}$ lw in the juvenile *E. fetida* exposed to the PEY samples. Mean levels of nPBT in the tissues of adult *E. fetida* exposed to EST anthroposoils ranged from 0.06 to 0.12 $\mu\text{g.kg}^{-1}$ lw and from 2.95 to 4.91 $\mu\text{g.kg}^{-1}$ lw in the tissues of adult *E. fetida* exposed to the PEY anthroposoils. Mean levels of nPBT in the tissues of juvenile *E. fetida* exposed to EST anthroposoils were from 0.34 to 4.07 $\mu\text{g.kg}^{-1}$ lw and from 1.38 to 3.34 $\mu\text{g.kg}^{-1}$ lw in the tissues of adult *E. fetida* exposed to PEY anthroposoils. PBEB was detected only in the tissues of *E. fetida* exposed to PEY soils with measured mean concentrations from 0.06 to 0.12 $\mu\text{g.kg}^{-1}$ lw. On the contrary, in the case of juvenile *E. fetida*, PBEB was detected only in the tissues of those exposed to the EST anthroposoils with measured mean concentrations from 0.06 to 0.34 $\mu\text{g.kg}^{-1}$ lw.

In general, the levels of quantified nBFRs (nHBB, nPBB, nPBT and PBEB) in *E. fetida* tissues were very low in comparison with the other POPs quantified in these samples.

The data obtained clearly revealed the occurrence of bioaccumulation of the POPs quantified in both adult and juvenile *E. fetida* tissues even at very low concentrations in the case of nBFRs. Indeed, in the case of PCBs, PCDDs, PCDFs and PBDEs, strong correlations with r values up to higher than 0.70 (see Annex II, Table I.1) were observed between the levels quantified in earthworm tissues and the levels found in the anthroposoils for these POPs. In the case of the PBBs and the nBFRs considered in the present study (nHBB, nPBB, nPBT and PBEB), no significant correlations (r values lower than 0.7) were found between the levels quantified in earthworm tissues and the levels measured in the anthroposoils.

Indeed, the levels of POPs present in juvenile *E. fetida* tissues may be due to the occurrence of POPs transfer from adults to juveniles or only be the result of a new accumulation of POPs from the anthroposoils. These two hypotheses are not mutually exclusive, and another fact is that the POPs found in higher levels in *E. fetida* tissues correspond to ancient and more persistent ones in the environment, providing a clear indication that juveniles could accumulate POPs from the matrix during the second phase of the reproduction assay (until days 28 and 52). Moreover, the influence and contribution of POPs transfer from adults to juveniles cannot be neglected. The difference in POPs accumulation in adult and juvenile tissues may be related to differences in metabolism and lipid contents between both age stages, and it may also be due to the possible occurrence of changes in the chemical properties tested in the anthroposoils during the first 28 days of the test, caused by adults foraging and subsequent changes in POPs levels and (increasing) bioavailability for the juveniles.

The same trend of different degrees of accumulation in adults and juveniles was observed for the accumulation of MTEs in the same anthroposoils, a phenomenon already reported in Coelho et al. (2018).

The activity of earthworms can decrease soil organic pollutants by stimulating microbial biodegradation (Schaefer and Filser, 2007), by enhancing soil aeration, by their own metabolism (Blouin et al., 2013) and by gut symbionts (Verma et al., 2006). Microorganisms are the foremost agents in the degradation of organic contaminants in soil, as they enhance in an important way the bioavailability of contaminants, facilitating their uptake by other soil organisms, namely earthworms (Chaudhry and Ali, 1988; Masciandaro et al., 2013).

Earthworms live in direct contact with soil porewater and particles and they can accumulate POPs via both alimentary and dermal uptake (Krauss et al., 2000; Vijver et al., 2003). It has been also observed that worm gut uptake becomes the dominant exposure route for strongly hydrophobic contaminants with a log K_{ow} higher than 6 (Jager et al., 2003).

Earthworms living in highly contaminated soils are expected to accumulate high tissue PBDE concentrations since these animals represent the base of the terrestrial food chain for many organisms, making them a pathway for the accumulation of PBDEs in organisms at higher trophic levels. Moreover, PBDEs are lipophilic and bioaccumulate in the fatty tissues of organisms (Voorspoels et al., 2006), thus significant correlations have usually been found in biota between PBDE concentrations and lipid content (Law et al., 2006a; Yu et al., 2009; Xiang et al., 2007). Literature data indicate that levels of BDE-47 are usually higher than those of BDE-99 in adipose tissue and they are much higher than those of BDE-209 (Choi et al., 2003; Covaci et al., 2002a; Meironyte et al., 2001). This occurs partly due to the relative absorption

and persistence of these compounds and it may also be due to the fact that BDE 209 is so structurally large that it has difficulty in moving from environmental matrices to organism tissues.

Some of the HBCDs present are probably not eliminated or depurated by the organisms, so instead this compound can be transformed into another diastereomer via metabolic processes. In earthworms, estimated soil-biota accumulation factors (ww soil / ww worm) ranged between 0.03 and 0.08 based on the total concentration of HBCDs in worm tissue and estimates for the w/w concentration in soil after 28 days of exposure to a range of soil concentrations (Swedish Chemicals Agency European Commission Risk Assessment hexabromocyclododecane, 2008). The diastereomer specific biota-soil accumulation factor for α -HBCDs is more than one order of magnitude higher than the value for γ -HBCD (Swedish Chemicals Agency European Commission Risk Assessment hexabromocyclododecane, 2008). The referred study indicated the existence of a degree of bioisomerization of γ -HBCD to α -HBCD and the preferential biotransformation of γ -HBCD.

Moreover, studies on the accumulation of nBFRs in living organisms are still scarce, a fact that makes it difficult to study potential exposure risks and discuss the environmental fate of these compounds (Iqbal et al., 2017). In subchapter 4.9, the BAF, SET, ERITME and ERITME-POP indexes will be calculated to infer the transfer of contaminants and evaluate the real ecotoxicological risk.

MAIN RESULTS

- ✓ The data obtained clearly revealed the occurrence of bioaccumulation of the POPs quantified in both adult and juvenile *E. fetida* tissues even at very low environmental concentrations in the case of nBFRs.
- ✓ In the case of PCBs, PCDDs, PCDFs and PBDEs clear strong correlations with r values up to higher than 0.70 were observed between the levels quantified in earthworm tissues and the levels found in the anthroposoils for these POPs.
- ✓ In the case of the PBBs and the nBFRs considered in this study (nHBB, nPBB, nPBT and PBEB), no significant correlations were found between the levels quantified in earthworm tissues and the levels measured in the anthroposoils.
- ✓ The levels of POPs quantified in juvenile *E. fetida* tissues may be due to the occurrence of POPs transfer from adults to juveniles or it may only be the result of a new accumulation of POPs from the anthroposoils.
 - A PCA will be conducted to find the existence of possible correlations between the physico-chemical soil properties and POPs levels in anthroposoils and *E. fetida* tissues capable of explaining the effects of the in *E. fetida* individuals exposed to contamination (Subchapter 4.8).
 - BAFs will be calculated to infer the real risk of entry and diffusion along the food chain posed by each of the POPs families quantified (Subchapter 4.9).

4.6.2. Plants

The levels of POPs quantified in the three plant species cultivated in laboratory conditions in the EST G and PEY Ic soils are presented in Table 28. Among the 8 anthroposoils studied, the most contaminated of each sampling site was selected to conduct the analysis of POPs in plant tissues (Figures 5 and 6, Subchapter 3.1.1).

Table 28 – Concentrations of quantified POPs (in ug.kg⁻¹ dw) in the plants cultivated in the EST G and PEY Ic soils. (mean ± standard deviation; n = 3 or more except for roots indicated by * (n = 2)).

	EST G						PEY Ic					
	CRESS		ALFALFA		MUSTARD		CRESS		ALFALFA		MUSTARD	
	AP	R*	AP	R*	AP	R	AP	R*	AP	R*	AP	R
ΣPCBs-cop	0.10 ± 0.004	0.03	0.12 ± 0.03	0.02	0.05 ± 0.002	0.03 ± 0.01	0.11 ± 0.002	0.36	0.09 ± 0.01	0.24	0.07 ± 0.01	0.34 ± 0.14
ΣPCBs-noncop	1.74 ± 0.08	0.88	1.67 ± 0.37	0.55	0.85 ± 0.04	0.85 ± 0.17	2.31 ± 0.07	10.7	1.62 ± 0.24	7.22	1.54 ± 0.16	7.22 ± 3.65
ΣPCBs-ndl	7.97 ± 0.48	9.98	7.86 ± 1.94	6.77	4.00 ± 0.15	10.9 ± 2.17	14.0 ± 0.46	84.4	10.6 ± 1.88	54.3	9.53 ± 0.73	50.2 ± 28.9
Σ₇PCDDs	0.01 ± 0.002	0.09	0.01 ± 0.003	0.04	0.01 ± 0.001	0.06 ± 0.03	0.06 ± 0.002	0.46	0.02 ± 0.01	0.18	0.04 ± 0.01	0.35 ± 0.18
Σ₁₀PCDFs	0.01	0.02	0.004 ± 0.001	0.01	0.002 ± 0	0.02 ± 0.01	0.01 ± 0.001	0.08	0.01 ± 0.002	0.03	0.01 ± 0.002	0.06 ± 0.03
Σ₇PBDEs	0.53 ± 0.03	0.17	0.45 ± 0.17	0.11	0.31 ± 0.03	0.13 ± 0.01	0.40 ± 0.05	0.64	0.24 ± 0.03	0.36	0.20 ± 0.02	0.42 ± 0.04
Σ₈PBDEs	2.29 ± 1.32	2.62	1.52 ± 1.15	1.46	4.81 ± 1.22	7.46 ± 7.56	8.16 ± 4.53	23.5	6.86 ± 4.65	21.0	3.75 ± 0.94	17.2 ± 5.96
ΣHBCDs	1.28 ± 0.60	86.58	1.80 ± 1.30	19.96	2.58 ± 0.72	55.0 ± 27.1	2.41 ± 1.14	3.16	0.22 ± 0.02	0.60	0.25 ± 0.12	3.86 ± 5.21

AP=aerial parts; R=roots

The concentrations of POPs measured in the tissues of the plants cultivated in the EST anthroposoil are lower than the levels measured in the plants cultivated in PEY anthroposoils (Table 28). This was expected since the levels of POPs in the PEY anthroposoils were much higher than those detected in the EST anthroposoils. Despite this general trend, HBCDs are an exception as the levels measured in the different plant parts were higher in the case of those cultivated in EST anthroposoils.

Another expected and verified finding was that the roots accumulated higher quantities of POPs than the aerial parts for all the POPs considered.

Usually POPs in soil are in direct contact with the plant root system. The rhizosphere can be considered as one of the most important regions where plants can interact with POPs, considering the existence of the plant roots together with the root exudates, the rhizosphere soil and the microbe community (Gerhardt et al., 2009; Zeng et al., 2017). Usually, the movement of POPs into the root area is blocked as the POPs are usually bound to the soil before reaching the rhizosphere zone and they are hydrophobic and insoluble in water. Commonly, the bioavailable fractions of POPs include only the water-soluble fractions that can be desorbed by water and acid-soluble fractions that can be desorbed by root exudates such as carbohydrates, organic acids and amino acids (Wu and Zhu, 2016). Certain properties of POPs such as water solubility, vapor pressure, molecular weight and octanol/water partition coefficients affect POPs availability and uptake by plant roots (Zieve and Peterson, 1984). In the case of hydrophobic POPs, root uptake is certainly not a significant pathway of accumulation (Simonich and Hites, 1995). It is expected that the most lipophilic POPs ($\log K_{ow}$ higher than or around 4) enter the epidermis of the root and are transported through the xylem system. However, POPs with a $\log K_{ow}$ higher than 5 will not reach the above ground plant tissues due to their hydrophobicity (Ryan et al., 1988). In this case, volatilization may be the major potential source of leaf contamination through the soil-air pathway (Collins and Finnegan, 2010).

Plants that grow in soils contaminated with POPs contribute to the increase in the density of the microbial population and its diversity in rhizosphere soils, b the higher metabolic capacities for POPs in rhizosphere soils than in bulk soils (Fletcher et al., 1995; Alkorta and Garbisu, 2001). Also, plant root exudates may change the porosity of the soil and physico-chemical conditions, thus facilitating the POPs biodegradation (Oleghe et al., 2017).

Usually the transport of POPs from the roots to the aerial plant parts is insignificant due to the low hydro solubility of these compounds as well as the fact that they are always strongly bound to the organic matter fraction of the soil. Moreover, limited and sometimes contradictory information on PCBs uptake by plants has been given in the literature while most of the studies

have focused only on a single congener or commercial formulation (Iwata and Gunther, 1979; Aken et al., 2010; Anyasi and Atagana, 2011).

The uptake mechanisms of PCBs by plants are currently well-referenced in the literature and well-understood: they appear to occur mainly through two general ways. The first is through the root system and the other is by adsorption in the foliage and stems that can also involve subsequent migration through the epidermal layers (Mackova et al., 2007). The first route considered is probably the most important route of direct entry of PCBs in plant tissues, while the second is related to the uptake of airborne PCBs by terrestrial plants when the compounds absorb to the outer surface of the plants and are dissolved by the lipophilic compounds present in the plant cuticle (Gilbert and Crowley, 1997). Plant roots tend to transfer contaminants from the bulk soil to the rhizosphere. A wide range of plant species has been proven to enhance the dissipations of PCBs in soil, from trees to different forages, grasses and legumes (Dzantor et al., 2000; Chekol et al., 2004; Mackova et al., 2009; Ding et al., 2011). *Medicago sativa L.* is widely known due to its capacity to selectively support the growth of PCBs-degrading bacteria (Li et al., 2013; Teng et al., 2011). The increase in soil microbial and enzymatic activity due the presence of plants is usually correlated with the increase in PCBs-degradation level (Chekol et al., 2004; Aken et al., 2010). Compared to our results, the levels obtained in the parts of the three plant species exposed were in the same range, or in some cases a little above, those of the PCBs concentrations measured in several laboratory and *in situ* studies. An accumulation of Σ_3 PCBs in *Lolium perenne* ($3.5 \mu\text{g.kg}^{-1}$ dw) and in *Melaleuca leukadendra* (2.64 to $7.00 \mu\text{g.kg}^{-1}$ dw) collected in Brisbane in Australia was reported (Müller et al., 2001). Also, Zhao et al. (2006) detected levels of Σ_{17} PCBs of about $2.80 \mu\text{g.kg}^{-1}$ dw in grass samples in China. In a study on the distribution of certain POPs in soils and vegetation near a landfill in Greece, Chrysikou et al. (2008) found levels from 3.64 to $25.9 \mu\text{g.kg}^{-1}$ dw for Σ_7 PCBi in *Solanum eleagnifolium* and *Solanum trifolium* plant species. Also, several studies reported PCB uptake and translocation by edible vegetables such as: soybeans (Suzuki et al., 1977), carrots (Iwata and Gunther, 1976), beets, turnips and beans (Sawhney and Hankin, 1984). It was also observed that the lower chlorinated congeners (PCB-28, -52 and -101) were found to be more abundant in the shoots than in the roots of these plant species (Iwata and Gunther, 1976; Suzuki et al., 1977; Sawhney and Hankin, 1984). Liu and Schnoor (2008) concluded that some mono to tetra PCBs are absorbed by plant roots but only the lower chlorinated ones undergo translocation to aerial plant tissues and this was also verified in the present study. Members of the Cucurbitaceae family have been shown to accumulate PCBs in their tissues (Hulster et al., 1994). In a study with pumpkin plants, Aslund et al. (2008) also found that the PCB concentration increased

within the stem and leaves after a short period of exposure to contaminated soils, but the concentrations measured in the plant roots remained unchanged.

Sandermann (1994) and Coleman et al. (1997) considered a green liver model for the metabolism of xenobiotics by plants that comprises a three-way process starting with the activation phase consisting of the oxidation of PCBs to very soluble and reactive hydroxylated products. The second phase involved the conjugation of activated compounds with plant molecules forming lesser toxic and more soluble compounds. In the third and final phase of this sequestration process, the products formed are adsorbed into plant organs. Although the studies on plant metabolism capacities for PCBs degradation are quite recent (Aken et al., 2010), several studies have already shown the effective transformation, degradation and metabolization of PCBs in plant cells (Mackova et al., 2007; Kucerova et al., 2000; Chroma et al., 2003; Harms et al., 2003; Rezek et al., 2007). These works lead to the conclusion that plant metabolism of PCBs is dependent on plant species and on the strain and degree of chlorination, as it is more effective in the case of lower chlorinated PCBs. The present study verifies this trend and shows that PCBs uptake and accumulation can be selective and different for different plant species.

PCDDs and PCDFs are highly lipophilic compounds primarily sorbed by plant roots and soil components, though they are not usually translocated within plant tissues (Reischl, 1989). Despite that, several previous studies showed that bioconcentrations of PCDDs and PCDFs clearly exist in zucchini plants two orders of magnitude higher than those found in vegetables such as pumpkin and cucumber, even though these three plants belong to the Cucurbita family (Hulster et al., 1994).

As already stated by Hulster et al. (1994) and Kersten et al. (1995), although PCBs, PCDDs and PCDFs show quite similar characteristics, the uptake of these POPs by plant species can be different probably due to the difference in plant physiology and root exudate composition.

The roots and xylem exudates of zucchini (Cucurbitaceae) can solubilize PCDDs and PCDFs (Held and Door, 2000), thus explaining the entry of these POPs in root tissues. In the case of the plants cultivated in the anthroposoils studied, this was verified with some differences in uptake by the different species but in general the levels of PCBs, PCDDs and PCDFs measured were higher in the plant roots than those found in the aerial parts.

The levels measured in the three plant species tested were much lower than those measured by Müller et al. (1994) in carrots, lettuce and peas (values from 0.05 to 0.48 $\mu\text{g}\cdot\text{kg}^{-1}$ dw).

Vegetables are usually expected to contain relatively low BFRs concentrations because of their high-water contents, low lipid contents, and their primary positions in ecosystems. But our

results showed that PBDEs and HBCDs can be accumulated at appreciable levels in plant roots and aerial parts.

The levels of PBDEs obtained in the parts of the three plant species exposed were quite low when compared to those obtained in laboratory tests by Yang et al. (2018) with the cultivation of sweet potato vines where mean levels of $19.4 \mu\text{g.kg}^{-1}$ dw were recorded. Some plant crops show the ability to translocate PBDEs from soil into vegetative structures and this may result in consistent low-level exposure through diet (Navarro et al., 2017). Several laboratory studies have demonstrated the capacity of vegetables such as spinach (*Spinacia oleracea*), potatoes (*Solanum tuberosum*), carrots (*Daucus carota*), lentils (*Lens culinaris*) as well as some cereals (*Triticum aestivum* and *Oryza sativa*) to accumulate PBDEs in their tissues (Ohta et al., 2002; Bocio et al., 2003). Interestingly, Ohta et al. (2002) in a study on PBDEs accumulation in spinach, found that not only the levels of PBDEs quantified in spinach were higher than in the other vegetables studied but also the congener composition was different from that of the root vegetables, namely potato and carrot, analyzed in the study. As in the studies referred to above, in our cultivated plants also some differences in PBDEs accumulation were observed between plant species as well as in the congener composition analyzed. In both the roots and aerial parts of our cultivated plant species, the congeners present in higher quantities were BDE-209, followed by BDE-47 and BDE-99.

The authors suggested that this can be explained by a difference in contamination pathways. Moreover, there are several factors that may greatly affect the uptake of PBDEs by plants. According to Vrkoslavova et al. (2010), plants from different species show different affinities to PBDEs, even when cultivated in the same soil and under the same conditions. In laboratory study with various plant species, Huang et al. (2010) found that uptake levels of PBDEs in ryegrass (*Lolium multiflorum*), alfalfa (*Medicago sativa*), pumpkin (*Cucurbita moschata*), summer squash (*Cucurbita pepo*) and radish (*Raphanus sativus*) were very distinct even when they were cultivated in the same conditions. These authors also found that different tissues within the same plant have different affinities to accumulate PBDEs. For maize (*Zea mays*), pumpkin and ryegrass, it was demonstrated that their roots exhibit a higher capacity to accumulate PBDEs than their stems and leaves (Huang et al., 2010). Also, in the cultivated plants considered in the present study, roots showed a greater affinity to accumulate PBDEs in comparison to aerial parts.

PART C: Statistical analysis by Principal Component Analysis (anthroposoil and *E. fetida* data)

4.7. Principal Component Analysis (PCA) of the entire data set

To investigate the spatial distribution and establish possible existing relations between: (i) the levels of POPs and MTEs found in the anthroposoils studied, (ii) the levels of POPs in *E. fetida* tissues, and (iii) the physicochemical parameters of the anthroposoils, a principal component analysis (PCA) was applied. The results obtained with this PCA will be exploited and explained, from the most global approach to the narrowest one, following 4 different steps, focusing on:

- First step: considering the key soil properties, MTEs levels and POPs concentrations quantified in the soils and in *E. fetida* samples;
- Second step: considering the key soil properties and POPs concentrations quantified in the soils and in *E. fetida* samples;
- Third step: considering the key soil properties and the levels of PCDDs, PCDFs and PCBs quantified in the soils and in *E. fetida* samples;
- Fourth step: considering the key soil properties and the BFRs concentrations quantified in the soils and in *E. fetida* samples.

Data on POPs accumulation in the plant species tested will not be considered in this analysis since at this moment only a few data are available, thus they are not representative enough to be considered. Moreover, for the risk assessment approach using the BAF, ERTIME and SET indexes, the data were focused on earthworm availability and transfer.

Step I: Considering the key soil properties, MTEs levels and POPs concentrations quantified in soils and *Eisenia fetida* samples

The first PCA was run to consider the key soil properties, the MTE pseudo-total levels quantified in the anthroposoils studied and POPs concentrations quantified in the anthroposoils and in the *E. fetida* tissues. Table 29 gives the loadings of the parameters considered for the first five components.

Table 29 – Loadings of PCA including key soil properties, pseudo-total levels of MTEs in anthroposoils and POPs concentrations in anthroposoils and *E. fetida* tissues (noted *Ef*).

	Components				
	PC1	PC2	PC3	PC4	PC5
Sand	-0.69	-0.49	-0.03	0.27	-0.39
Silt	0.62	0.50	0.04	-0.29	0.45
Clay	0.91	0.39	-0.02	-0.09	-0.00
OM	0.74	0.30	0.35	-0.23	-0.02
TOC	0.74	0.30	0.35	-0.23	-0.02
NT _{Dumas}	0.87	0.38	0.27	-0.13	0.03
C/N	-0.64	-0.40	-0.20	-0.00	-0.26
Carbonates tot	0.90	0.26	0.02	0.16	-0.25
CEC _{Metson}	0.87	0.22	0.24	-0.32	0.16
pH _{H2O}	0.90	0.28	0.10	0.13	-0.23
pH _{KCl}	0.90	0.29	0.14	0.13	-0.21
CaO	0.86	0.31	0.01	0.17	-0.27
K ₂ O	0.87	0.13	0.41	-0.06	0.04
MgO	0.94	0.25	0.08	0.07	-0.16
P _{Olsen}	0.60	-0.23	0.56	-0.26	0.01
Cd tot	0.96	-0.15	-0.21	-0.11	-0.06
Cu tot	0.85	-0.10	-0.27	-0.28	-0.02
Cr tot	0.98	0.10	-0.07	-0.09	-0.10
Ni tot	0.98	0.06	-0.11	-0.12	-0.06
Pb tot	0.86	0.12	-0.39	-0.08	-0.02
Zn tot	0.97	0.05	-0.16	-0.17	-0.04
ΣPCBs-dl-cop soil	0.84	-0.32	-0.32	-0.26	0.03
ΣPCBs-dl-cop <i>Ef</i>	0.72	-0.33	-0.15	-0.12	0.35
ΣPCBs-dl-noncop soil	0.88	-0.41	0.05	0.01	-0.09
ΣPCBs-dl-noncop <i>Ef</i>	0.67	-0.48	0.30	0.21	0.20
ΣPCBs-ndl soil	0.90	-0.38	-0.07	-0.09	-0.07
ΣPCBs-ndl <i>Ef</i>	0.75	-0.48	0.14	0.09	0.20
Σ ₇ PCDDs soil	0.86	-0.39	-0.26	-0.14	-0.02
Σ ₁₀ PCDDs <i>Ef</i>	0.91	-0.39	-0.05	0.02	0.02
Σ ₇ PCDFs soil	0.85	-0.01	-0.50	0.05	-0.12
Σ ₁₀ PCDFs <i>Ef</i>	0.79	-0.02	-0.35	0.29	0.07
Σ ₇ PBDEs soil	0.86	-0.25	0.37	-0.04	-0.10
Σ ₇ PBDEs <i>Ef</i>	0.90	0.04	0.12	0.23	-0.03
Σ ₈ PBDEs soil	0.87	-0.35	0.25	-0.03	-0.10
Σ ₈ PBDEs <i>Ef</i>	0.48	0.16	-0.24	0.37	0.13
Σ ₃ PBBs soil	0.95	-0.18	-0.20	0.02	-0.13
Σ ₃ PBBs <i>Ef</i>	0.52	-0.55	0.23	0.28	0.26
PBEB soil	0.49	0.71	-0.13	0.33	-0.15
PBEB <i>Ef</i>	-0.03	0.05	0.12	0.25	0.81
nPBT soil	0.69	-0.29	-0.52	-0.38	0.11
nPBT <i>Ef</i>	0.75	-0.01	-0.21	0.45	0.33
nHBB soil	0.43	-0.42	0.51	0.42	-0.21

Table 29 (cont) – Loadings of PCA including key soil properties, pseudo-total levels of MTEs in anthroposoils and POPs concentrations in anthroposoils and *E. fetida* tissues (noted *Ef*).

	Components				
	PC1	PC2	PC3	PC4	PC5
nHBB <i>Ef</i>	<i>0.67</i>	0.23	-0.22	0.45	0.05
nPBB soil	0.93	-0.29	0.15	0.02	-0.13
nPBB <i>Ef</i>	<i>0.79</i>	0.14	-0.11	0.45	0.14
Total variance explained (%)	64.37	9.89	6.57	5.15	4.39
Cumulative variance explained (%)	64.37	74.26	80.83	85.98	90.37

Extraction Method: Principal Component Analysis

Note: Strong loadings >0.80 are shown in bold style; moderate loadings 0.60 – 0.80 in italics.

With the exception of the PBEB levels measured in *E. fetida* (PBEB *Ef*), the component matrix shows that strong loadings of all the other variables considered are associated with PC1 which explains 64.37 % of the total variance. PC2, which concerns 9.89 % of the total variance, seems to be essentially explained by the PBEB contents measured in the anthroposoils (PBEB soil). The PBEB concentrations in *E. fetida* tissues (PBEB *Ef*) are strongly associated with PC5. This fact clearly indicates the possibility of a difference in the behavior and distribution of PBEB in the anthroposoils as well as its availability and subsequent uptake and accumulation dynamics in *E. fetida* tissues when compared to the other POPs considered. The PC3, PC4 and PC5 components contain a mix of all the variables considered for this factor analysis, explaining 6.57 %, 5.15 % and 4.39 %, respectively, of the total variance.

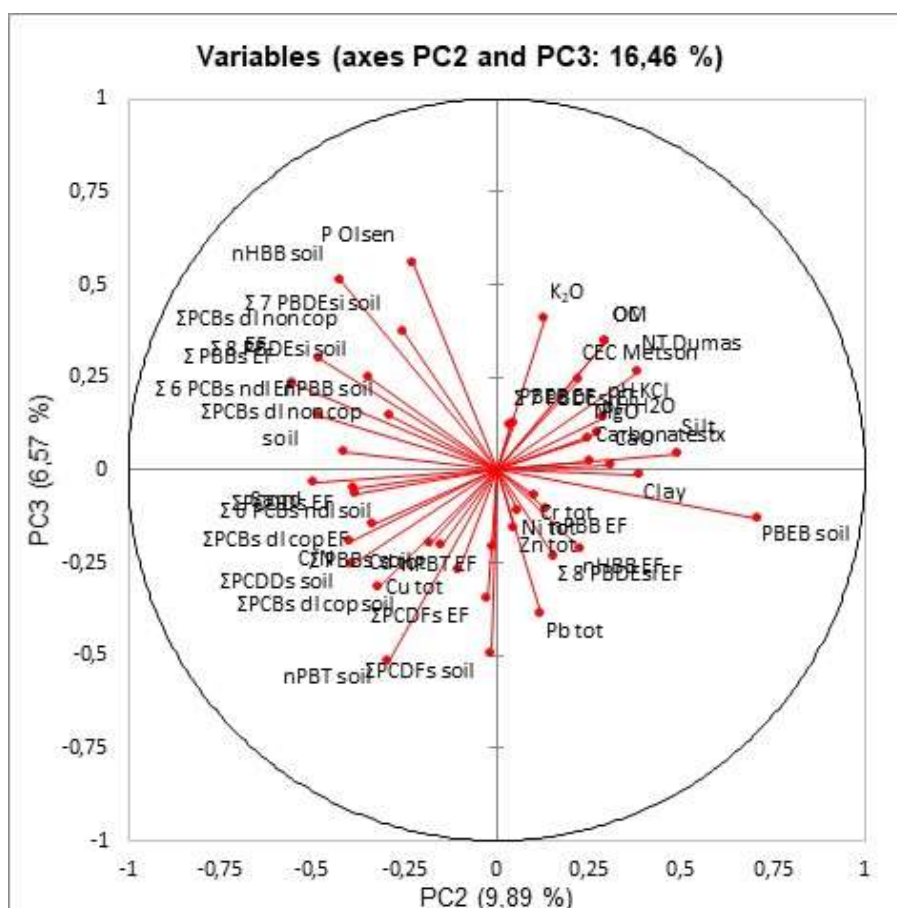


Figure 24 – Loading plot for PC2 and PC3 showing that most part of the variables considered (key anthroposoil properties, pseudo-total levels of MTEs in anthroposoils and POPs concentrations in anthroposoils and *E. fetida* tissues) have identical significance for these two principal component factors.

Regarding the loading plots presented in Figures 23 and 24, it can be seen that the major part of the variables are well represented on the loading plots considered once they are positioned relatively close to the circumference of the correlation circle. In the specific case of the two physicochemical parameters, anthroposoil sand content (Sand) and the C/N ratio (C/N), they appear to be both negatively correlated with PC1 and PC3 but positively correlated with PC2, explained by the PBEb contents measured in the anthroposoils (PBEb soil). The C/N ratio is also positively correlated with PC5, explained by the PBEb concentrations in *E. fetida* tissues (PBEb Ef). The loading map corresponding to axes PC2 and PC3 (Figure 24) shows that, except for the PBEb levels measured in the anthroposoils, all the other variables seem to have similar loads.

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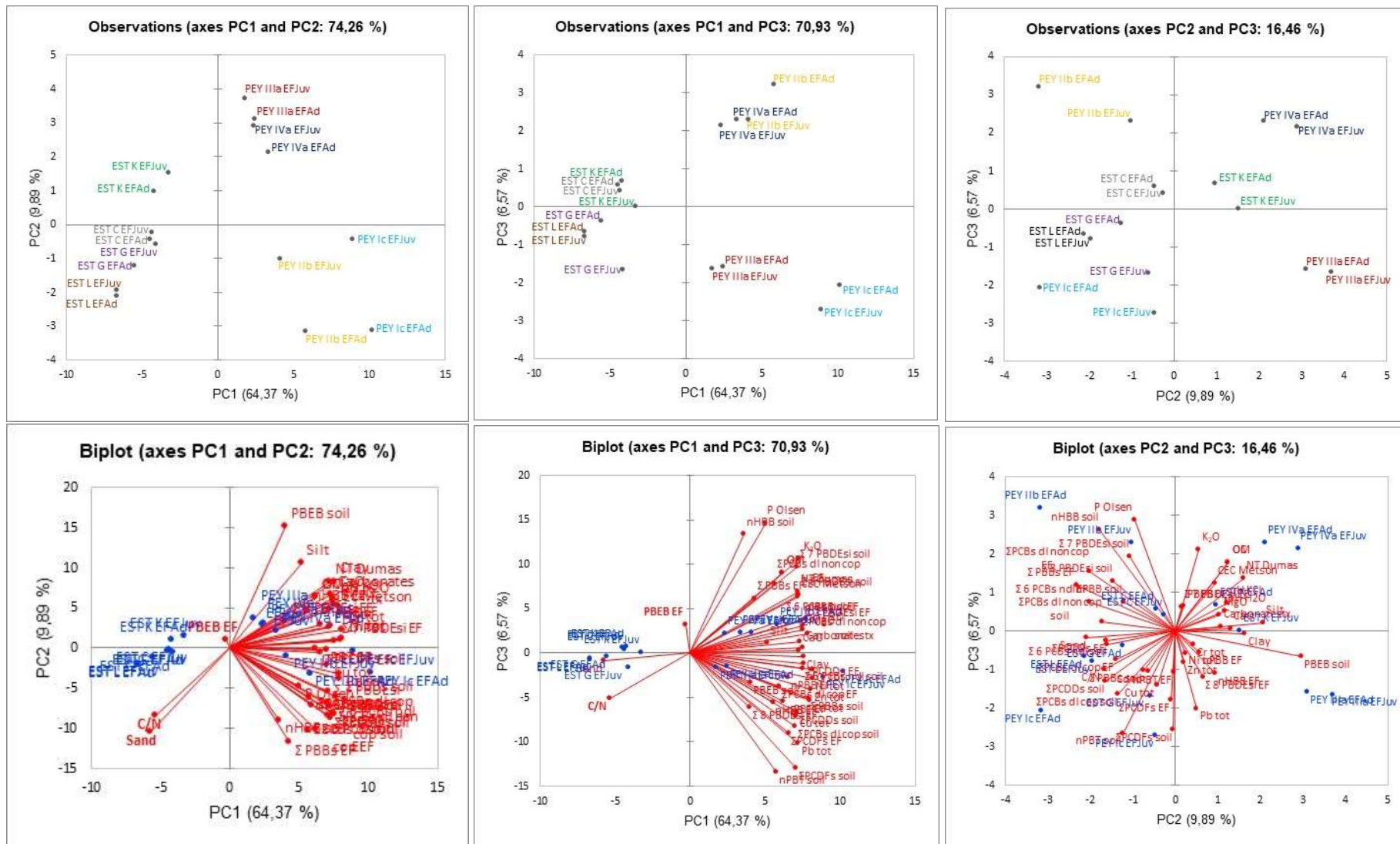


Figure 25 – Observations plots and biplots of PC1, PC2 and PC3 showing the distribution of the variables (key anthroposol properties, pseudo-total levels of MTEs in anthroposols and POPs concentrations in anthroposols and *E. fetida* tissues) and the observations (samples) considered in the present study.

In the observation plots displaying axes PC1 vs PC2 and PC1 vs PC3 (Figure 25), the EST samples are negatively correlated with PC1 and the PEY samples are positively correlated with this axis, which can be explained by the significantly higher POPs contents in the tissues of the *E. fetida* individuals exposed to PEY anthroposoils. Moreover, the distribution of the observations seems to be site dependent since the samples corresponding to adult and juvenile *E. fetida* for each site appears close to each other. The fact that there are physiological differences between the adults and juveniles seems to be a non-conditioning parameter for POPs accumulation.

Step II: Considering the key anthroposoil properties and POPs concentrations quantified in the anthroposoil and *Eisenia fetida* samples

To discard the possible existence of a considerable influence of MTEs contents in the distribution of variables and results, as well as focus on POPs and notably RFBs in the present study, a PCA was run, considering only the most common key anthroposoil properties considered and the POPs concentrations quantified in the anthroposoils and in *E. fetida* tissues. Table 30 gives the loadings of the parameters considered for the first five components. With the exception of the sand quantities, C/N ratio and the levels of PBEB measured in *E. fetida* (PBEB *Ef*), the component matrix shows that strong loadings of all the other variables considered are associated with PC1 which explains 61.12% of the total variance. PC2, which concerns 11.26% of the total variance seems to be essentially explained by the PBEB contents measured in the anthroposoils (PBEB soil). The PBEB concentrations in *E. fetida* tissues (PBEB *Ef*) are strongly associated with PC5. Also, in this case the results clearly indicate the possibility of a difference in the behavior and distribution of PBEB in anthroposoils as well as its availability and consequent uptake and accumulation dynamics in *E. fetida* tissues when compared to the other POPs considered. The PC3, PC4 and PC5 components contain a mix of all the variables considered for this factor analysis, explaining 6.8 %, 5.3 % and 4.95 % of the total variance, respectively.

Table 30 – Loadings of PCA including key anthroposoil properties and POPs concentrations in anthroposoils and *E. fetida* tissues (noted *Ef*).

	Components				
	PC1	PC2	PC3	PC4	PC5
Sand	-0.68	-0.50	-0.09	0.40	-0.25
Silt	<i>0.62</i>	0.50	0.10	-0.44	0.30
Clay	0.90	0.39	-0.01	-0.12	-0.06
OM	<i>0.75</i>	0.30	0.36	-0.14	-0.08
TOC	<i>0.75</i>	0.30	0.36	-0.14	-0.08
NT _{Dumas}	0.88	0.39	0.25	-0.08	0.00
C/N	-0.66	-0.41	-0.14	0.05	-0.25
Carbonates tot	0.90	0.26	-0.05	0.22	-0.19
CEC _{Metson}	0.87	0.23	0.30	-0.30	0.04
pH _{H2O}	0.90	0.28	0.03	0.21	-0.17
pH _{KCl}	0.90	0.29	0.07	0.21	-0.14
CaO	0.87	0.31	-0.06	0.24	-0.20
K ₂ O	0.89	0.14	0.37	0.04	0.06
MgO	0.94	0.25	0.03	0.12	-0.13
P _{Olsen}	<i>0.62</i>	-0.21	0.57	-0.07	-0.01
ΣPCBs-dl-cop soil	0.82	-0.32	-0.23	-0.38	-0.16
ΣPCBs-dl-cop <i>Ef</i>	<i>0.71</i>	-0.33	-0.13	-0.32	0.25
ΣPCBs-dl-noncop soil	0.88	-0.41	0.02	0.02	-0.11
ΣPCBs-dl-noncop <i>Ef</i>	<i>0.70</i>	-0.48	0.18	0.18	0.31
ΣPCBs-ndl soil	0.89	-0.38	-0.06	-0.11	-0.14
ΣPCBs-ndl <i>Ef</i>	<i>0.76</i>	-0.48	0.08	0.03	0.24
Σ ₇ PCDDs soil	0.84	-0.39	-0.22	-0.23	-0.14
Σ ₁₀ PCDDs <i>Ef</i>	0.91	-0.39	-0.08	-0.04	-0.01
Σ ₇ PCDFs soil	0.82	-0.02	-0.50	-0.09	-0.20
Σ ₁₀ PCDFs <i>Ef</i>	<i>0.78</i>	-0.03	-0.44	0.11	0.10
Σ ₇ PBDEs soil	0.88	-0.25	0.33	0.07	-0.09
Σ ₇ PBDEs <i>Ef</i>	0.92	0.04	-0.00	0.21	0.04
Σ ₈ PBDEs soil	0.89	-0.34	0.21	0.04	-0.11
Σ ₈ PBDEs <i>Ef</i>	0.48	0.16	-0.41	0.14	0.13
Σ ₃ PBBs soil	0.94	-0.18	-0.21	-0.03	-0.18
Σ ₃ PBBs <i>Ef</i>	0.54	-0.55	0.11	0.21	0.39
PBEB soil	0.48	<i>0.71</i>	-0.24	0.31	-0.04
PBEB <i>Ef</i>	-0.01	0.05	-0.00	-0.05	0.86
PBT soil	<i>0.65</i>	-0.29	-0.37	-0.56	-0.15
PBT <i>Ef</i>	<i>0.76</i>	-0.00	-0.41	0.15	0.39
nHBB soil	0.47	-0.42	0.33	0.58	0.02
nHBB <i>Ef</i>	<i>0.67</i>	0.23	-0.40	0.26	0.12
nPBB soil	0.93	-0.28	0.10	0.06	-0.13
nPBB <i>Ef</i>	0.80	0.14	-0.31	0.24	0.21
Total variance explained (%)	61.12	11.26	6.88	5.37	4.95
Cumulative variance explained (%)	61.12	72.38	79.26	84.63	89.58

Extraction Method: Principal Component Analysis

Note: Strong loadings >0.80 are shown in bold style; moderate loadings 0.60 – 0.80 in italics.

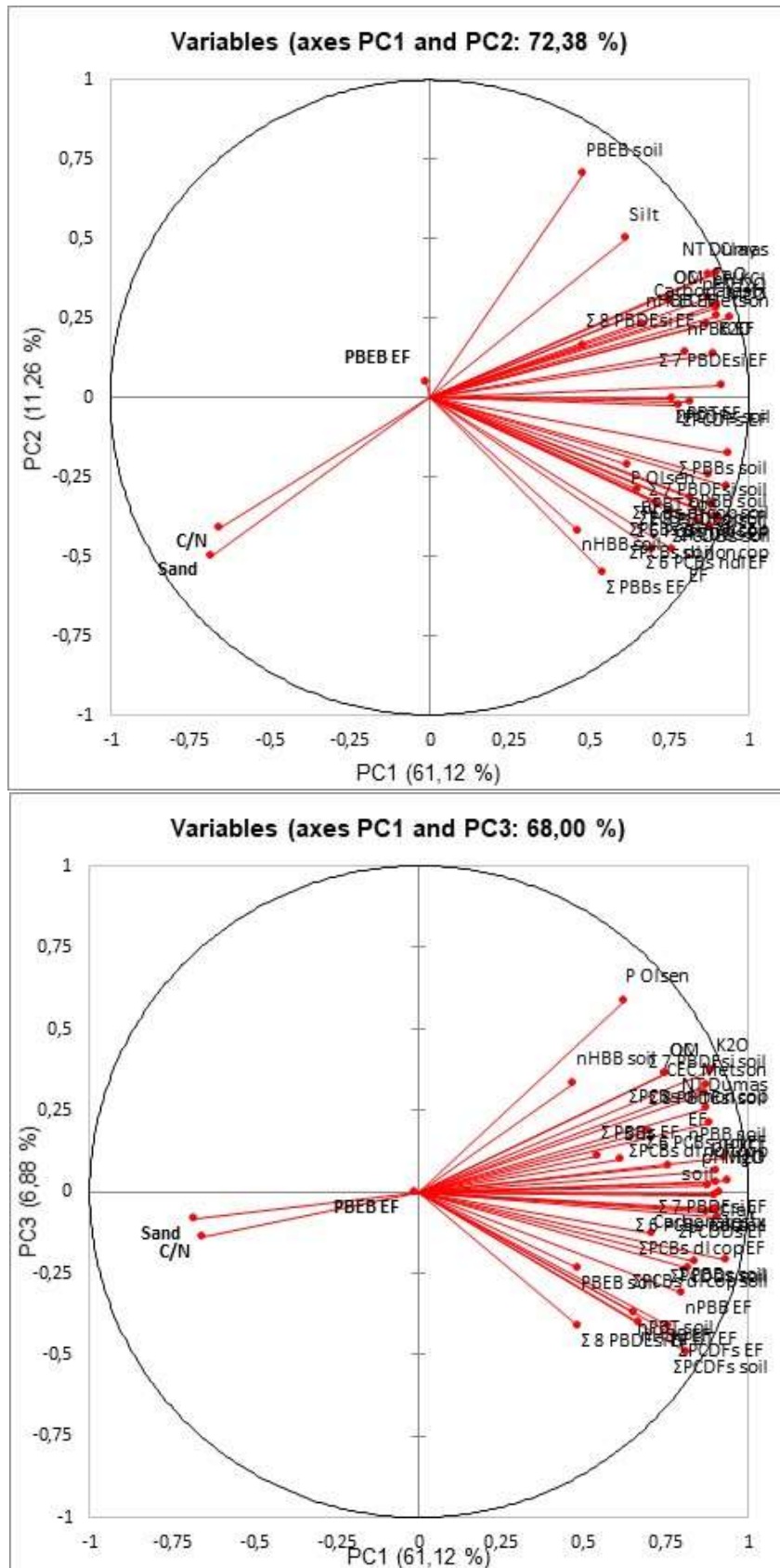


Figure 26 – Loading plots for PC1 and PC2 and PC1 and PC3 showing a clear separation between the two physicochemical anthroposoil properties, anthroposoil sand fraction and C/N ratio and all the other

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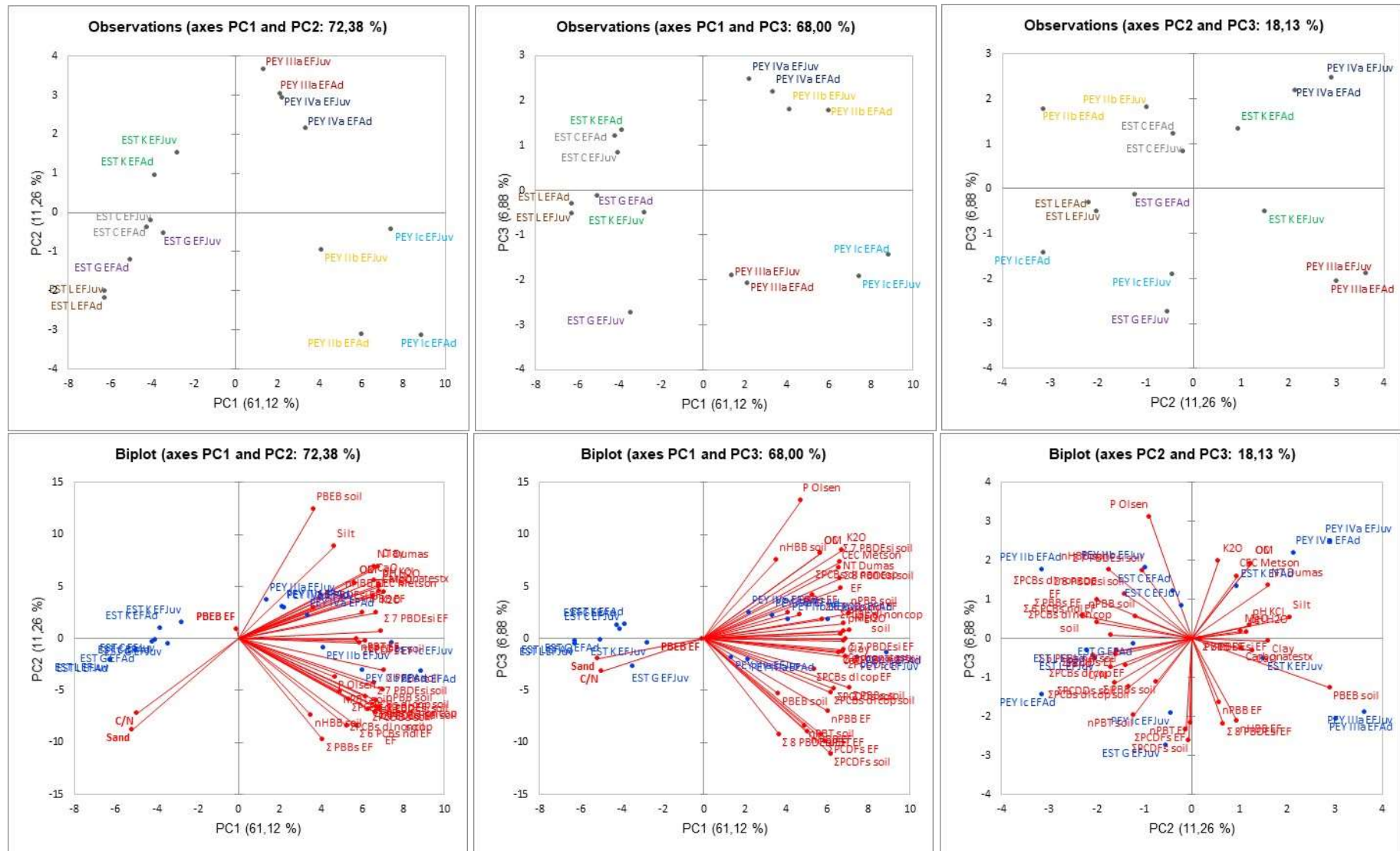


Figure 28 – Observation plots and biplots of PC1, PC2 and PC3, showing the distribution of the variables (key anthroposoil properties and POPs concentrations in anthroposils and *E. fetida* tissues) and the observations (samples) considered in the present study.

In the observation plots displaying axes PC1 vs PC2 and PC1 vs PC3 (Figure 28), the EST samples are negatively correlated with PC1 and the PEY samples are positively correlated with this axis, which can be explained by the significantly higher POPs contents in the tissues of the *E. fetida* individuals exposed to the PEY anthroposoils. Moreover, the distribution of observations seems to be site-dependent once the samples corresponding to adult and juvenile *E. fetida* for each site appear close to each other in most of the cases. Some physiological differences between the adults and juveniles seem to be a non-conditioning parameter for POPs accumulation, although the accumulation of POPs in the *E. fetida* tissues contributes to the mass loss in the adults and the inhibition of reproduction (see subchapter 4.5.1).

Step III: Considering the key anthroposoil properties and the levels of PCDDs, PCDFs and PCBs quantified in anthroposoils and *Eisenia fetida* samples

After performing a PCA considering the key anthroposoil properties and all the POPs concentrations quantified in anthroposoils and in *E. fetida* tissues, in Step III only 3 families of the POPs studied will be considered for the PCA (PCBs, PCDDs and PCDFs). The objective is to see in more detail the existence (or not) of significant differences in the distribution of variables and results when only PCBs, PCDDs and PCDFs are considered. In Table 31, the loadings of the parameters considered for the first five components are displayed.

With the exception of the sand quantities and the C/N ratio, the component matrix shows that strong loadings of all the other variables considered are associated with PC1 which explains 67.5 % of the total variance. PC2, which concerns 12.4 % of the total variance, seems to be partially explained by the PCB, PCDD and PCDF contents measured in the anthroposoils and *E. fetida* samples. In this case the results clearly indicate the similarity in the behavior and distribution of the 3 POPs families considered in anthroposoils as well as their availability and subsequent uptake and accumulation dynamics in *E. fetida* tissues. The PC3, PC4 and PC5 components contain a mix of all the variables considered for this factor analysis, explaining 6.37%, 4.90% and 3.56% of the total variance, respectively.

Table 31 – Loadings of PCA including key anthroposoil properties and the PCDDs, PCDFs and PCBs concentrations in anthroposoils and *E. fetida* tissues (noted *Ef*).

	Components				
	PC1	PC2	PC3	PC4	PC5
Sand	-0.74	0,47	-0,08	-0,46	-0,08
Silt	<i>0.68</i>	-0,48	0,13	0,52	0,09
Clay	0.93	-0,28	-0,17	0,05	0,01
OM	<i>0.78</i>	-0,35	0,18	-0,25	-0,02
TOC	<i>0.78</i>	-0,35	0,18	-0,25	-0,02
NT _{Dumas}	0.91	-0,38	0,08	-0,07	0,02
C/N	-0.69	0,40	-0,11	-0,31	-0.10
Carbonates tot	0.91	-0,12	-0,33	-0,22	0,00
CEC _{Metson}	0.91	-0,27	0,27	0,10	-0,07
pH _{H2O}	0.92	-0,17	-0,24	-0,21	0.00
pH _{KCl}	0.92	-0,20	-0,20	-0,22	0.01
CaO	0.88	-0,16	-0,36	-0,23	0.02
K ₂ O	0.91	-0,20	0,23	-0,10	-0,04
MgO	0.95	-0,15	-0,20	-0,15	-0.02
P _{Olsen}	<i>0.63</i>	0,02	0,62	-0,18	-0,19
ΣPCBs-dl-cop soil	<i>0.80</i>	0,42	0,01	0,25	-0.28
ΣPCBs-dl-cop <i>Ef</i>	<i>0.71</i>	0,43	0,13	0,20	0,35
ΣPCBs-dl-noncop soil	0.84	0,43	0,12	-0,06	-0,23
ΣPCBs-dl-noncop <i>Ef</i>	<i>0.67</i>	0,46	0,27	-0,20	0.43
ΣPCBs-ndl soil	0.86	0,42	0,08	0,03	-0,25
ΣPCBs-ndl <i>Ef</i>	<i>0.74</i>	0,50	0,21	-0,07	0.38
Σ ₇ PCDDs soil	<i>0.80</i>	0,48	0,01	0,19	-0.28
Σ ₁₀ PCDDs <i>Ef</i>	0.88	0,46	0,04	0,01	-0,04
Σ ₇ PCDFs soil	0.80	0,27	-0,46	0,22	-0.13
Σ ₁₀ PCDFs <i>Ef</i>	<i>0.77</i>	0,28	-0,45	0,13	0.27
Total variance explained (%)	67.52	12.42	6.37	4.90	3.56
Cumulative variance explained (%)	67.52	79.95	86.31	91.21	94.77

Extraction Method: Principal Component Analysis Note: Strong loadings >0.80 are shown in bold style; moderate loadings 0.60 – 0.80 in italics.

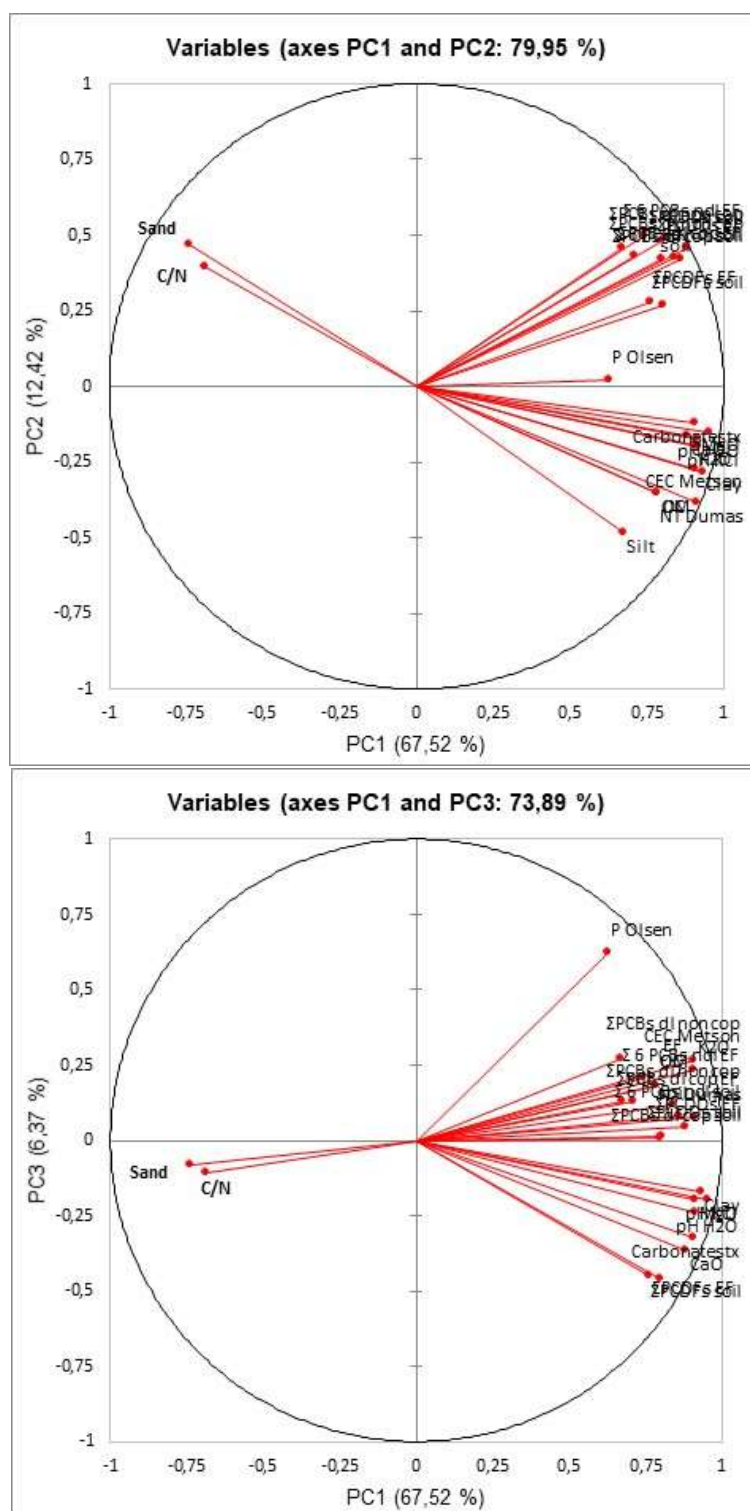


Figure 29 – Loading plots for PC1 and PC2 and PC1 and PC3 showing a clear separation between the two physicochemical anthroposoil properties, anthroposoil sand fraction and C/N ratio and all the other variables considered (other key anthroposoil properties and PCDDs, PCDFs and PCBs concentrations in anthroposoils and *E. fetida* tissues).

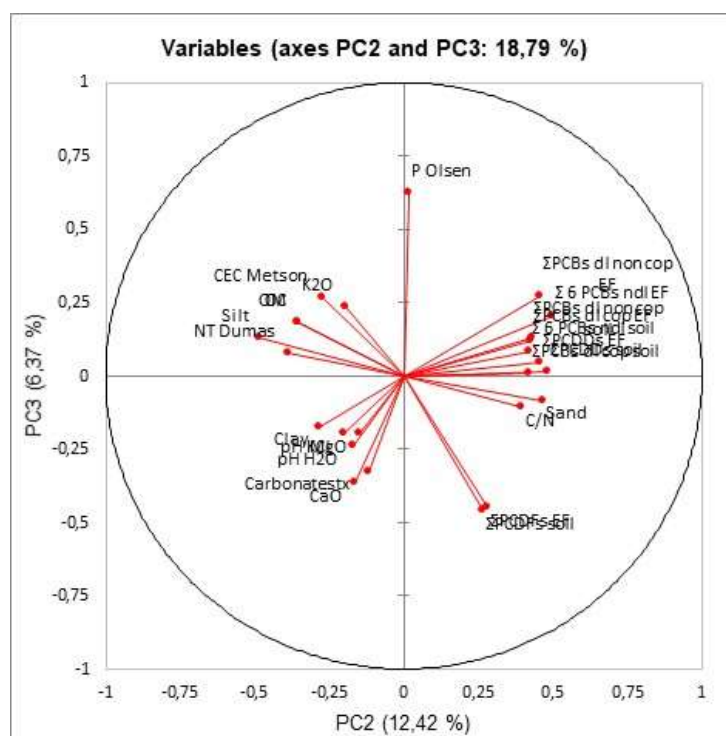


Figure 30 – Loading plot for PC2 and PC3 clearly showing that most of the variables (key anthroposol properties and PCDDs, PCDFs and PCBs concentrations in anthroposols and *E. fetida* tissues) have identical significance for these two PCA factors.

Regarding the loading plots presented in Figures 29 and 30, it can be seen that the major part of the variables are well represented on the loading plots considered as they are positioned relatively close to the circumference of the correlation circle. In the specific case of the two physicochemical parameters, anthroposol sand content (Sand) and the C/N ratio (C/N), they appear to be both negatively correlated with PC1 and PC3 but positively correlated with PC2. The loading map corresponding to axes PC2 and PC3 (Figure 30) shows that all the variables seems to have similar loads, except P_{Olsen} which appears to be strong correlated with PC3.

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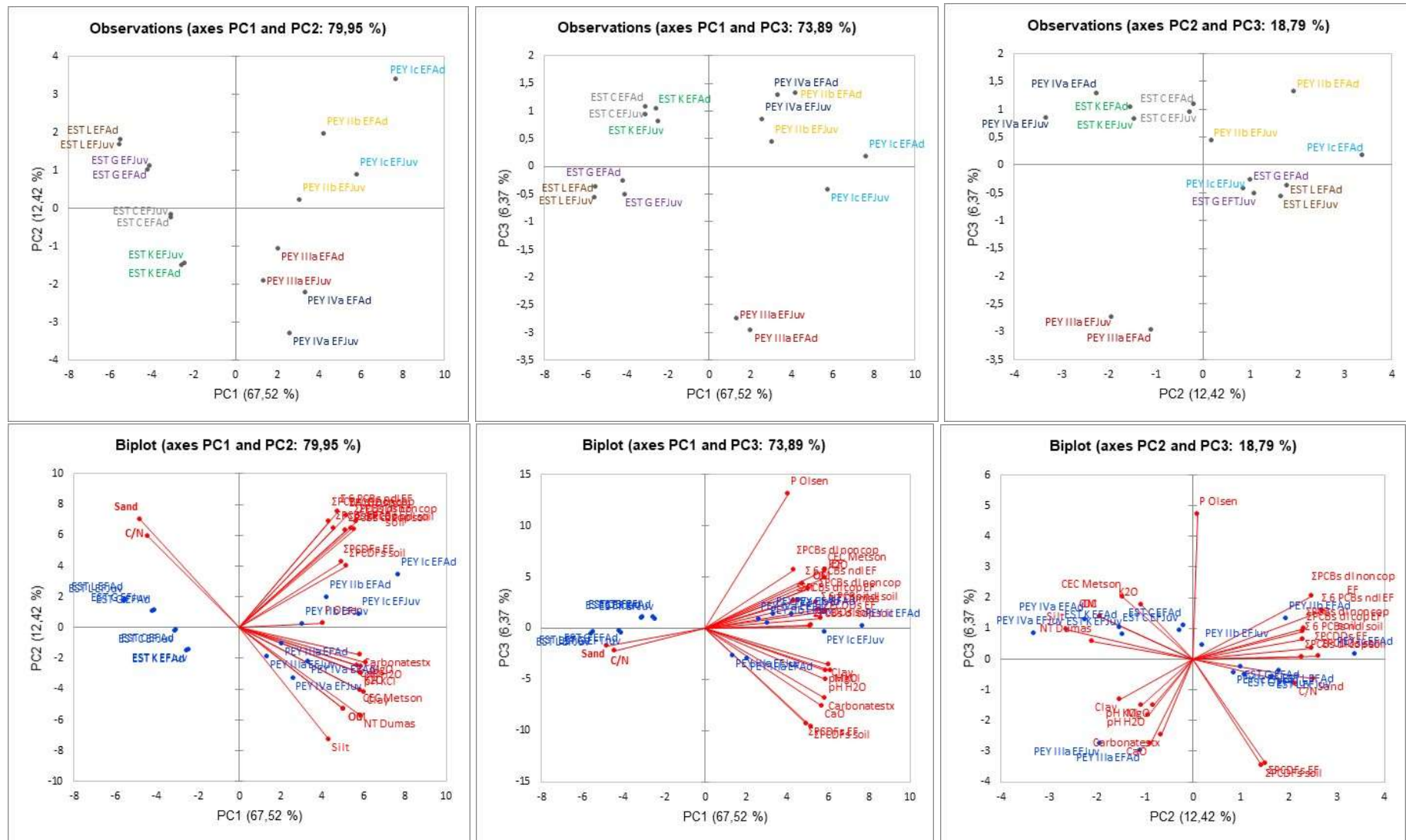


Figure 31 – Observation plots and biplots of PC1, PC2 and PC3 showing the distribution of the variables (key anthroposoil properties and PCB, PCDD and PCDF concentrations in anthroposoils and *E. fetida* tissues) and the observations (samples) considered in the present study.

In the observation plots displaying axes PC1 vs PC2 and PC1 vs PC3 (Figure 31), the EST samples are negatively correlated with PC1 and the PEY samples are positively correlated with this axis, which can be explained by the significantly higher POPs contents in the tissues of the *E. fetida* exposed to PEY anthroposoils. Moreover, the distribution of observations seems to be site-dependent as the samples corresponding to adult and juvenile *E. fetida* for each site appear close to each other in most of the cases. The fact that there are physiological differences between the adults and juveniles seems to be a non-conditioning parameter for PCBs, PCDDs and PCDFs accumulation.

Step IV: Considering the key anthroposoil properties and the BFRs concentrations quantified in anthroposoils and *Eisenia fetida* samples

In this final step, only the key anthroposoil properties and the BFRs concentrations studied in anthroposoils and *E. fetida* tissues will be considered for the PCA. The objective in this step is to see in more detail the existence or not of significant differences in the distribution of variables and results, when considering only the BFRs levels measured. Table 32 gives the loadings of the parameters considered for the first five components.

Except for the sand quantities, the C/N ratio and the concentrations of PBEB measured in *E. fetida* tissues (PBEB *Ef*), the component matrix shows that in general strong loadings of all the other variables considered are associated with PC1, which explains 61.38% of the total variance. PC2, which concerns 10.11% of the total variance, seems to be partially explained by the nHBB contents measured in the anthroposoils. In this case the results indicate similarities in the behavior and distribution of the nBFRs in the anthroposoils as well as its availability and consequent uptake and accumulation dynamics in *E. fetida* tissues. The PC3, PC4 and PC5 components contain a mix of all the variables considered for this factor analysis, explaining 7.67%, 6.27% and 4.63% of the total variance, respectively.

Table 32 – Loadings of PCA including key anthroposoil properties and BFRs concentrations in anthroposoils and *E. fetida* tissues (noted *Ef*).

	Components				
	PC1	PC2	PC3	PC4	PC5
Sand	-0.74	0.48	0.26	-0.36	-0.03
Silt	<i>0.68</i>	-0.50	-0.23	0.42	0.01
Clay	0.94	-0.28	-0.07	-0.07	0.11
OM	0.81	-0.11	-0.34	-0.02	0.10
TOC	0.81	-0.11	-0.34	-0.02	0.10
NT _{Dumas}	0.94	-0.20	-0.24	0.02	-0.03
C/N	-0.72	0.29	0.09	-0.25	0.27
Carbonates tot	0.93	-0.08	0.07	-0.32	-0.03
CEC _{Metson}	0.90	-0.09	-0.34	0.22	0.09
pH _{H2O}	0.94	-0.08	0.01	-0.29	-0.09
pH _{KCl}	0.95	-0.08	-0.01	-0.26	-0.11
CaO	0.91	-0.14	0.07	-0.36	-0.06
K ₂ O	0.92	0.09	-0.24	0.10	-0.21
MgO	0.97	-0.07	-0.01	-0.20	-0.03
P _{Olsen}	<i>0.62</i>	0.45	-0.42	0.22	-0.11
Σ ₇ PBDEs soil	0.85	0.47	-0.14	0.05	0.03
Σ ₇ PBDEs <i>Ef</i>	0.92	0.13	0.15	-0.07	-0.04
Σ ₈ PBDEs soil	0.83	0.52	-0.06	0.06	0.11
Σ ₈ PBDEs <i>Ef</i>	0.51	-0.06	<i>0.63</i>	0.27	0.13
Σ ₃ PBBs soil	0.87	0.24	0.17	-0.08	0.28
Σ ₃ PBBs <i>Ef</i>	0.45	0.56	0.10	0.16	-0.22
PBEB soil	0.58	-0.61	0.20	-0.39	-0.27
PBEB <i>Ef</i>	-0.00	-0.13	0.19	<i>0.74</i>	-0.39
PBT soil	0.55	0.14	0.08	0.21	<i>0.72</i>
PBT <i>Ef</i>	<i>0.73</i>	0.00	0.55	0.21	0.03
nHBB soil	0.44	<i>0.69</i>	0.08	-0.08	-0.48
nHBB <i>Ef</i>	<i>0.70</i>	-0.12	0.58	0.06	-0.00
nPBB soil	0.88	0.45	0.01	-0.01	0.11
nPBB <i>Ef</i>	0.81	-0.03	0.51	0.10	-0.01
Total variance explained (%)	61.38	10.11	7.67	6.27	4.63
Cumulative variance explained (%)	61.38	71.49	79.16	85.43	90.06

Extraction Method: Principal Component Analysis

Note: Strong loadings >0.80 are shown in bold style; moderate loadings 0.60 – 0.80 in italics.

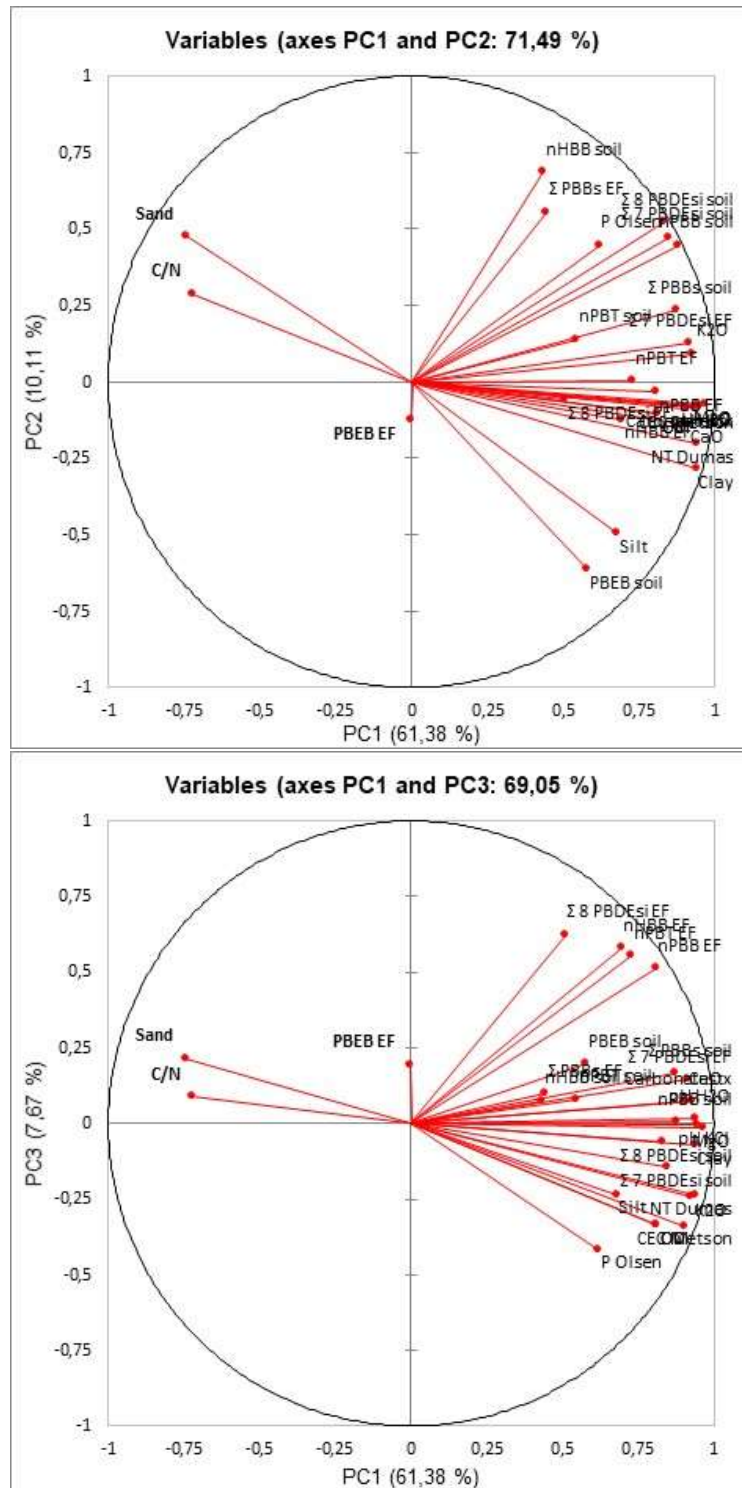


Figure 32 – Loading plots for PC1 and PC2 and PC1 and PC3, showing a clear separation between the two physicochemical anthroposoil properties, anthroposoil sand fraction and C/N ratio and all the other variables considered (other key anthroposoil properties and the BFRs concentrations in anthroposoils and *E. fetida* tissues).

Chapter 4. Results and discussion

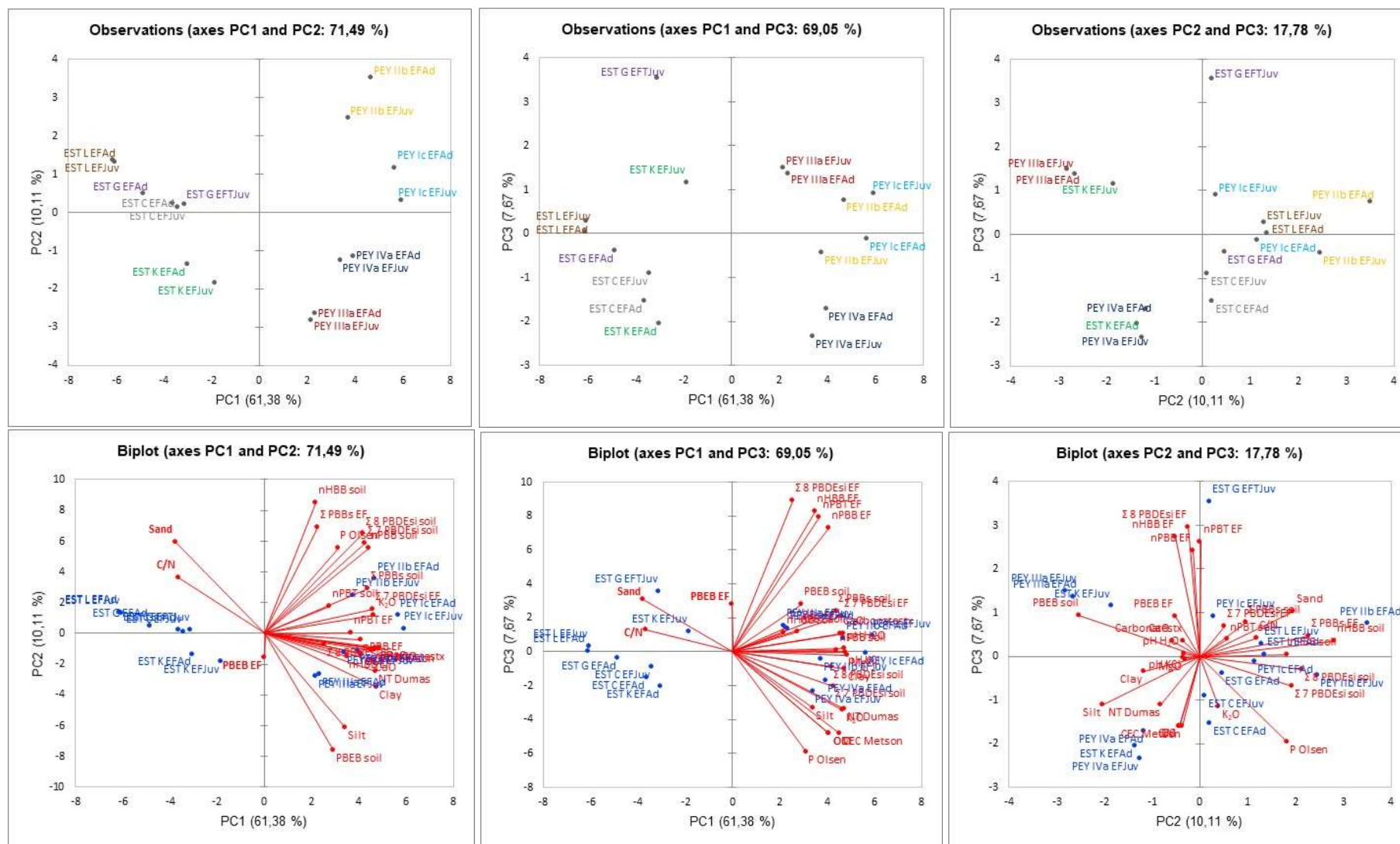


Figure 34 – Observation plots and biplots for PC1, PC2 and PC3 showing the distribution of the variables (key anthroposol properties and BFRs concentrations in anthroposols and *E. fetida* tissues) and the observations (samples) considered in the present study.

In the observation plots displaying axes PC1 vs PC2 and PC1 vs PC3 (Figure 34), the EST samples are negatively correlated with PC1 and the PEY samples are positively correlated with this axis, which can be explained by the significantly higher POPs contents in the tissues of the *E. fetida* exposed to PEY anthroposoils. Moreover, the distribution of observations seems to be site-dependent as the samples corresponding to adult and juvenile *E. fetida* for each site appear close to each other in most cases. The fact that physiological differences exist between the adults and juveniles seems to be a non-conditioning parameter for BFRs accumulation. Despite this, for some of the POPs analyzed a lower accumulation in juvenile tissues was verified perhaps due to differences in metabolism between adults and juveniles. As already stated by Chevillot et al. (2017) in a study on the bioaccumulation of pharmaceuticals and pesticides in *Eisenia andrei*, these differences in accumulation between adults and juveniles could also reflect changes in anthroposoil chemical properties due to adults foraging habits and a decrease in POPs concentrations during the first 28 days. Another fact that must be considered is that the juveniles had a shorter time of exposure to anthroposoil contaminated by POPs than the adults, since the cocoons did not hatch on the first day of the second exposure phase (day 28 to 56).

MAIN RESULTS

- ✓ The EST samples are negatively correlated with PC1 and the PEY samples are positively correlated with this axis. This can be explained by the significantly higher POPs contents in the tissues of the *E. fetida* individuals exposed to PEY anthroposoils.
- ✓ The PCA evidenced a difference in the behavior and distribution of PBEB in soils as well as its availability and subsequent uptake and accumulation dynamics in *E. fetida* tissues when compared to the other POPs considered.
- ✓ In the specific case of the two physicochemical parameters, soil sand content (Sand) and the C/N ratio (C/N) appear to be both negatively correlated with PC1 and PC3 but positively correlated with PC2. This is explained by the PBEB contents measured in the anthroposoils (PBEB soil).
- ✓ The C/N ratio is also positively correlated with PC5, explained by the PBEB concentrations in *E. fetida* tissues (PBEB *Ef*).
- ✓ Despite the correlations found in all the PCA steps between Anthroposoil sand content and C/N ratio, the physico-chemical properties are not the only explanation of the POPs availability considered and the levels found in *E. fetida* tissues.
- ✓ The distribution of observations seems to be site-dependent as the samples corresponding to adult and juvenile *E. fetida* for each site appear close to each other. The fact that physiological differences exist between the adults and juveniles seems to be a non-conditioning parameter for POPs accumulation.
- ✓ PCA results indicate similarities in the behavior and distribution of nBFR in soils as well as its availability and subsequent uptake and accumulation dynamics in *E. fetida* tissues
- ✓ Different POPs families showed different behaviors regarding the correlations with physico-chemical parameters and uptake by *E. fetida*.
 - BAFs will be calculated to infer the real risk of entry and diffusion along the food chain posed by each of the POPs families quantified (Subchapter 4.9).
 - SET and ERITME indexes will be calculated to rank the sites tested according to the effective transfer of POPs from anthroposoils to the organisms tested and obtain an idea of the potential risk to the real ecosystem (Subchapter 4.9).

PART D: Calculation of indexes to infer the potential risk to the ecosystem

4.8. The interest of calculating indexes (Bioaccumulation factor (BAF), SET, ERITME and ERITME-POP)

Regarding the types of organisms tested in the present study, an earthworm and three plant species, toxicological effects were observed relating to growth, reproduction rate and germination. Moreover, significant levels of POPs were detected in the tissues of the organisms tested. Therefore, it can be useful to evaluate the real risk posed by POPs to living organisms by calculating the BAF, SET and ERITME-POP indexes; BAF is widely used but SET and ERITME have only been used on MTEs until now (Mariet et al., 2017). BAF is widely used in risk assessment to determine whether a substance is accumulated in a certain organism and if there is a risk of entry and diffusion along the food chain. Despite its wide use, BAF presents some limitations as it is calculated for a substance and an organism and small quantities or differences of availability between substances can lead to high BAF values that do not signify a risk for the organism and thus induce errors in the assessment of the real risk posed by certain substances.

SET and ERITME-POP indexes will allow ranking the sites tested according to the effective transfer of POPs from anthroposoils to the organisms tested and obtaining an idea of the potential risk to the real ecosystem. SET and ERITME have proven in certain studies with MTEs to be effective in predicting the risk for ecosystems and have been used with terrestrial organisms, such as snails, that together with earthworms and plants constitute the preferential routes of entry of contaminants in the terrestrial food chain (Pauget et al., 2013; Pauget and de Vaufleury, 2015; Mariet et al., 2017).

4.8.1. Determination of the bioaccumulation factor (BAF)

4.8.1.1. BAF for earthworms (*E. fetida*)

Bioaccumulation values are usually reported through the calculation of the bioaccumulation factor (BAF), which is defined as the ratio between the concentrations of a certain contaminant in the organism considered and the levels found in the environmental medium (OECD, 2010).

BAFs are widely used in studies with MTEs and BAFs have already been calculated for some of the anthroposoils studied, the results of which can be found in Coelho et al., 2018 (see Annex I), and the occurrence of transfer of MTEs from soils to *E. fetida* tissues was clearly well observed.

In this section, BAF values will be presented for the POPs considered.

Tables 33 and 34 present the BAFs calculated for the POPs measured in the tissues of *E. fetida* adults and juveniles, respectively.

For the *E. fetida* adults, the BAFs were higher than 1 for the PCBs. In the case of PCDDs and PCDFs, the BAFs were lower than 1 for all the samples (Table 33). For the PBDEs, only the adult *E. fetida* exposed to anthroposoil PEY IIIa showed a BAF higher than 1. The BAFs for PBBs were generally zero or close to zero for all the samples (Table 33). On the contrary, nHBB and nPBT always presented BAFs higher than 1. For nPBB, the BAFs were higher than 1, except for the *E. fetida* exposed to anthroposoils EST G, PEY Ic and PEY Iib. In the case of PBEB, the BAFs for *E. fetida* juveniles were higher than 1 only in the case of those exposed to PEY anthroposoils and were zero for *E. fetida* individuals exposed to EST samples (Table 33).

For the *E. fetida* juveniles, the BAFs for PCBs were generally higher than 1 (Table 34). The BAFs were generally lower than 1 for the PCDDs, PCDFs and PBDEs with a few exceptions for the *E. fetida* exposed to anthroposoils EST K and L (for PCDFs) and anthroposoils EST G, EST K and PEY IIIa (for PBDEs). BAFs for PBBs were zero for all the samples. On the contrary, nHBB and nPBT always presented BAFs higher than 1 (Table 34). For nPBB, BAFs were always higher than 1, except for the *E. fetida* exposed to anthroposoil PEY Iib. In the case of PBEB, the BAFs for *E. fetida* juveniles were higher than 1 only in the case of those exposed to anthroposoils EST C, EST K and EST L (Table 34).

Table 33 –BAFs calculated for the POPs quantified in the adult *E. fetida* tissues exposed to EST and PEY anthroposoils.

	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
Σ PCBs-dl-cop	8.17	4.14	24.2	6.11	1.39	16.8	3.05	3.56
Σ PCBs-dl-noncop	42.0	23.4	165	36.5	5.80	8.51	10.5	12.8
Σ PCBs-ndl	34.3	20.3	108	31.3	8.16	10.8	14.6	13.4
Σ_7 PCDDs	0.07	0.06	0.09	0.11	0.28	0.39	0.45	0.45
Σ_{10} PCDFs	0.42	0.19	0.57	0.53	0.44	0.62	0.61	0.55
Σ_7 PBDEs	0.12	0.06	0.09	0.31	0.78	0.88	6.28	1.22
Σ_8 PBDEs	0.12	0.01	0.31	0.32	0.01	0.02	0.22	0.03
Σ_3 PBBs	0	0	0	0	0.12	0.26	0	0
nHBB	3.53	5.02	3.60	2.50	11.2	2.61	14.9	48.1
nPBB	1.43	0.96	4.00	2.61	0.81	0.99	3.54	2.01
nPBT	2.94	2.28	1.94	3.00	3.57	101	55.7	30.9
PBEB	0	0	0	0	3.11	6.19	1.12	2.30

NB: BAF values >1.00 are shown in bold type.

Table 34 –BAFs calculated for the POPs quantified in the juvenile *E. fetida* tissues exposed to EST and PEY anthroposoils.

	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
Σ PCBs-dl-cop	9.07	6.98	53.9	4.47	0.43	5.11	0.76	0.85
Σ PCBs-dl-noncop	17.3	13.8	78.1	7.74	1.45	2.18	2.07	2.92
Σ PCBs-ndl	13.1	10.9	43.0	5.38	2.47	3.58	3.37	3.50
Σ_7 PCDDs	0.14	0.14	0.37	0.52	0.19	0.33	0.31	0.30
Σ_{10} PCDFs	0.79	0.56	2.83	1.12	0.23	0.45	0.31	0.34
Σ_7 PBDEs	0.45	1.71	1.84	0.57	0.99	0.60	2.66	0.51
Σ_8 PBDEs	0.65	0.46	7.92	0.83	0.04	0.03	0.30	0.03
Σ_3 PBBs	0	0	0	0	0	0	0.00	0.00
nHBB	8.27	78.2	41.9	3.00	18.2	1.69	39.4	103
nPBB	4.05	17.1	46.7	3.48	1.09	0.74	2.69	0.93
nPBT	15.9	154.8	87.1	8.50	2.43	46.8	37.2	14.4
PBEB	8.60	0	22.7	26.1	0	0	0	0.00

Note: BAF values >1.00 are shown in bold style.

The BAFs values calculated clearly show the potential of the POPs analyzed to accumulate in *E. fetida* tissues. Earthworms in general are a major food supply to many animals (birds, reptiles and small mammals), thus the presence of POPs in their tissues can be a significant vector of POPs transfer into the higher trophic levels through biomagnification. According to the classification of Dallinger (1993), and regarding the BAF values presented in Tables 33 and 34, both exposed adult and juvenile *E. fetida* can be considered as deconcentrators of PCDDs, PCDFs, PBDEs and PBBs (BAF<1) but macroconcentrators of PCBs, nHBB, nPBB, nPBT and PBEB (BAF>2), with exceptions in the case of a few samples for each of the reference POPs.

Sellström et al. (2005) determined the levels of PBDEs congeners in earthworm samples collected in three research stations and two farms in Sweden and found that BAFs declined as the degree of bromination increased, a trend that was also verified in the *E. fetida* exposed to the EST and PEY anthroposoils tested.

Navarro et al. (2016) observed that *Eisenia andrei* individuals exposed to contaminated soils were able to metabolize certain PBDEs congeners, namely BDE-99 and BDE-153. This data also corroborated the fact that the BAFs were lower for these congeners than for the others and this classification/graduation of BAFs for BDE congeners was also verified in the present study.

The difference in bioaccumulation rates of the POPs considered in *E. fetida* tissues suggests the existence of differences in the bioavailability of POPs in the soils. The soil characteristics, the physicochemical properties of POPs together with the physiology and habitat of the organisms exposed could play an important role in the availability, bioaccumulation and possible migration of POPs through the soil food chain. This shows that higher brominated BDEs, including BDE-209, are still bioavailable in soils and can be accumulated in earthworm tissues, presenting an exposure pathway into the terrestrial food web, as suggested by Law et al. (2006a).

Table 35 shows several BAF values calculated for PCBs and PBDEs in two earthworm species.

The uptake and bioaccumulation of contaminants in earthworm tissues cannot be generalized between species due to differences in biological, ecological characteristics and environmental factors.

Table 35 – BAF values obtained for *E. fetida* in the present study and those reported in two studies for two other earthworm species for Σ PCBs and Σ PBDEs exposed in laboratory conditions.

Specie	Habitat	Σ PCBs	Σ PBDEs	Reference
<i>E. fetida</i> (adults)	Epigeic	1.39 – 165	0.01 – 6.28	Present study
<i>E. fetida</i> (juveniles)		0.43 – 78.1	0.03 – 7.92	
<i>Lumbricus rubellus</i>	Anecic	1.09 – 2.76	1.99 – 5.67	Vermeulen et al., 2010

Concerning ecological grouping and species habitat preference, *E. fetida* is an epigeic earthworm meaning that they usually live at the soil surface and feeding on leaf litter. *Lumbricus rubellus* is an anecic species meaning that they live and burrow in the deeper soil but comes to the soil surface to feed on leaf litter or mixtures of litter and soil particles. In environmental conditions, since *E. fetida* stays near the surface it can be more exposed to POPs contamination than the other species that come to the surface sporadically. The three species considered present the same reproduction mode but the times of cocoon incubation time vary from around 18 days (*E. fetida*) to approximately 90 days (*Lumbricus rubellus*) (Butt, 1991; Domínguez, 2004). Also, maturity time is highly variable among earthworm species, from 28 to 112 days for *E. fetida* and *Lumbricus rubellus*, respectively (Svendsen et al., 2002; Domínguez, 2004). These differences in cocoon incubation and adult versus sexual maturation time can also explain differences in the POPs uptake rates and accumulated levels.

The differences in BAF values for the three species considered shown in Table 35, for the specific case of PCBs and PBDEs, can also be explained by the fact that *E. fetida* presents a much higher lipid content than the other two species. This observation had already been reported in a study performed by Carter et al. (2014). The higher lipid content, combined with the higher lipophilic nature and higher log K_{ow} values of the PCBs and PBDEs (8.5×10^{-7} to 0.92 and 5.9 to 10, respectively) favor uptake into lipids and thus the higher BAF values observed for *E. fetida*. Based on the idea that an increase in object size leads to a decrease of the surface area to volume ratio, since *E. fetida* is the smallest earthworm species of the three considered, it would present a higher potential for the diffusion of POPs through its tissues than the two larger species considered.

Concerning the POPs dynamics, in soils with high OM contents (the case of the anthroposoils considered in the present study with OM contents between 18.2 and 41.5 g.kg⁻¹ dw) hydrophobic POPs are essentially retained near the soil surface and not leached

(Chiou, 2002). Therefore, the uptake by upper soil species (*E. fetida*) may occur on a larger scale than for the other species that are preferentially in deeper zones.

The BAF values are quite high for some of the POPs analyzed (up to 154), especially for nBFRs. For some of the POPs quantified, the BAFs reported in the present study were lower than 10, but despite that the transfer of these POPs to higher trophic levels and the subsequent potential toxicity effects caused can occur. POPs properties and soil parameters along with habitat preference and biological differences can affect earthworm uptake of contaminants in the natural environment differently. To better understand and validate the results obtained it would be necessary to carry out more detailed studies.

4.8.1.2. BAF for plants

The BAFs were calculated for plants collected *in situ* belonging to the species Common nettle (*Urtica dioica*) and Japanese knotweed (*Fallopia japonica*). BAFs were also calculated for the three plant species: alfalfa (*Medicago sativa*), watercress (*Nasturtium officinale*) and white mustard (*Sinapis Alba*) cultivated in the anthroposoils considered.

Table 36 presents the BAFs calculated of the POPs measured in the plant species collected *in situ* in anthroposoils PEY Ic and PEY IIIa.

The BAFs for POPs for *in situ* plant species are in general below 1, except for the sum of the Σ_7 PBDEs in Japanese knotweed leaves and for the sum of HBCDs isomers in the leaves and roots of Japanese knotweed plants (Table 36).

Table 36 –BAFs calculated of POPs quantified in the plants collected *in situ* in soils PEY Ic and PEY IIIa (AP= aerial parts; R = Roots).

	Common nettle (PEY Ic)		Japanese knotweed (PEY IIIa)		
	Aerial parts	Roots	Leaves	Stems	Roots
Σ PCBs-dl-cop	0.02	0.02	0.80	0.16	0.20
Σ PCBs-dl-noncop	0.02	0.04	0.62	0.10	0.43
Σ PCBs-ndl	0.03	0.07	0.68	0.12	0.40
Σ_7 PCDDs	0.00	0.00	0.03	0.01	0.07
Σ_{10} PCDFs	0.00	0.01	0.03	0.01	0.02
Σ_7 PBDEs	0.12	0.11	4.99	0.71	0.69
Σ_8 PBDEs	0.01	0.00	0.55	0.13	0.59
Σ HBCDs	0.02	0.01	1.38	0.97	10.3

Note: BAF values >1.00 are shown in bold type.

Table 37 presents the calculated BAFs of the POPs measured in the plants cultivated in the anthroposoils EST G and PEY Ic.

According to the data shown in Table 37, the greatest difference is that the BAFs for both aerial parts and roots are significantly higher in the plant species exposed to anthroposoil EST G (mostly higher than to 1) than the BAFs corresponding to plants exposed to PEY Ic. On the contrary, the BAFs for both the aerial parts and roots of plant exposed to PEY Ic are below 1 with only very few exceptions. For Σ_7 PCDDs, only the roots of cress cultivated in EST G showed a BAF higher than 1. For Σ_{10} PCDFs, only the roots of the three plant species cultivated in EST G showed BAFs higher than 1. In the case of PCBs, all the BAFs for the three plant species cultivated in EST G were significantly higher than 1, clearly showing the occurrence of accumulation of PCBs in both the aerial parts and roots of the plants. The BAFs for PCBs in plants cultivated in anthroposoil PEY Ic are all lower than 1, except in alfalfa and mustard roots for PCBs-ndl and in cress roots for PCBs-dl-noncop and PCBs-ndl. For Σ_7 PBDEs, all the BAFs of plants cultivated in EST G were significantly higher than 1 as well as higher than 1 for the roots of mustard and cress cultivated in PEY Ic. But when BDE-209 is considered (Σ_8 PBDEs), only mustard cultivated in EST G presents BAFs higher than 1. Regarding the HBCDs, BAFs higher than 1 were verified only for the roots of mustard and cress cultivated in anthroposoil EST G.

Table 37 –BAFs Calculated of POPs quantified in the plants cultivated in anthroposoils EST G and PEY Ic (AP= aerial parts; R = Roots).

	EST G						PEY Ic					
	ALFALFA		MUSTARD		CRESS		ALFALFA		MUSTARD		CRESS	
	AP	R	AP	R	AP	R	AP	R	AP	R	AP	R
Σ PCBs-dl-cop	13.8	2.22	5.94	3.30	10.9	3.18	0.17	0.47	0.15	0.68	0.23	0.72
Σ PCBs-dl-noncop	10.0	3.31	5.11	5.06	10.4	5.26	0.20	0.87	0.19	0.87	0.28	1.29
Σ PCBs-ndl	5.06	4.36	2.58	7.03	5.13	6.42	0.23	1.17	0.20	1.08	0.30	1.81
Σ_7 PCDDs	0.11	0.69	0.08	0.96	0.11	1.48	0.03	0.24	0.05	0.46	0.07	0.60
Σ_{10} PCDFs	0.36	1.03	0.21	1.57	0.50	1.86	0.05	0.29	0.07	0.56	0.10	0.76
Σ_7 PBDEs	13.6	3.33	9.25	3.84	16.1	5.16	0.60	0.88	0.49	1.03	0.99	1.58
Σ_8 PBDEs	0.41	0.39	1.29	2.00	0.61	0.70	0.09	0.28	0.05	0.23	0.11	0.32
Σ_3 HBCDs	0.08	0.89	0.12	2.46	0.06	3.87	0.05	0.15	0.06	0.97	0.60	0.79

Note: BAF values >1.00 are shown in bold style.

The BAFs obtained for the plants tested clearly indicate a considerable difference in the availability of the contaminants in both soils considered. Although the levels of POPs were significantly higher in PEY Ic than in EST G, it seems that the POPs were more available to be taken up by plants in the anthroposoil EST G. This difference in quantified POPs availability may be due to the physico-chemical parameters of the soil, namely the nature of OM, TOC and the slightly acidic pH of the EST G anthroposoils.

In a study on the phytoremediation of PCBs in contaminated soils, Pinsker (2011) obtained BAFs ranging from 1.9 to 4.2 for cucurbits roots and BAFs lower than 1 in shoots. In the referred study, the BAF of tall fescue (*Festuca arundinacea*) and sedge (*Cyperaceae*) was highest for shoots at around 1.5, and less than 1 in roots. Also the black mustard (*Brassica nigra*) and white heath aster (*Symphotrichum ericoides*) had the highest BAFs for shoots with 10 and 6.45 and the BAF for roots was also lower than 1, like the other plant species tested. The results of the present study are in the same range as those verified in the study referred to.

According to Wang et al. (2011a), when comparing with PCBs with the same number of halogen-substitutions, the accumulation tendencies are generally higher for PBDEs rather than for PCBs, a characteristic that can be attributed to their higher hydrophobicity. This trend is also verified in the results presented in Tables 36 and 37, where the BAFs for both the plants collected *in situ* and those cultivated in laboratory conditions were higher for PBDEs than for PCBs. The BAFs obtained in both the *in situ* and in laboratory conditions plants sampled and tested are in same range as the data found in the literature. Wang et al. (2011b), in a study with different edible vegetables species, obtained BAFs from 0.08 to 7.18 for PBDE accumulation. The BAFs obtained in the present study are in the same range.

The differences found in the entry and accumulation of the POPs considered in the 3 plant species tested in the present study are linked to the chemical and physical properties of each compound, such as hydrophobicity, water solubility, and vapor pressure. Environmental conditions such as temperature and soil organic content also play an important role in the availability and accumulation of POPs. Plant species differ from each other in terms of physiological characteristics that control their growth and development, and the accumulation of POPs. Root type also plays an important role since roots provide anchorage and energy storage and they are the plant part that undergoes physical and chemical interaction with the soil (Day et al., 2010). Moreover, roots are inserted in the rhizosphere where a huge population of bacteria and fungi are nourished

by organic nutrients commonly exuded from roots and some of these micro-organisms contribute actively to plant development and health. They are also responsible for modifications in soil acidity, adding chelating agents and expanding the effective absorption area, resulting in increased water and nutrient uptake.

It is important to carry out further studies using a wider range of soil types and different families of POPs, and a greater number of different plant species to permit more specific and accurate conclusions on the differences in the fate, bioavailability, uptake and bioaccumulation of POPs.

In general, the BAFs obtained for *E. fetida* and the plant species tested were higher for most of the POPs quantified. Despite the limitations of BAFs (already mentioned in this discussion), it is useful for understanding the extent of entry and accumulation of the contaminants in the organisms, considering each contaminant in an individual approach.

4.8.2. Determination of SET, ERITME and ERITME-POP

The SET index can be used to indicate the anomalous transfer of a pollutant while the ERITME index allows evaluating the real ecotoxicological risk due to the anomalous transfer evidenced by the SET calculation. These indexes were calculated considering PCBs, PCDDs, PCDFs, PBDEs, PBBs and HBCDs.

Figure 35 presents a schematic representation of the SET and ERITME calculation methods. The first step for the calculation of the SET and ERITME indexes was to define the C_{IRef} value that corresponds to the reference internal concentration of a certain contaminant in the organisms studied. After that, a coefficient of accumulation (AQ) was also determined for each contaminant considering the C_{IRef} value and the POPs levels found in *E. fetida* tissues. The values obtained for C_{IRef}, AQ and AQ-1 are presented in Annex III, Table III.1. In the present study, C_{IRef} values corresponds to the contaminant level found in the tissues of *E. fetida* specimens exposed to the control soils (ISO EST and ISO PEY). AQ values corresponds to the ratio between the levels found in the tissues of *E. fetida* exposed to the studied soils and the C_{IRef} value. AQ values higher than 1 indicates the existence of contaminant transfer.

Regarding the AQ values obtained, no excess transfer was observed in the case of PCBs-dl-cop, PCDDs and PCDFs. For all the samples studied, excess transfer was observed for PCBs-ndl and in the case of PCBs-dl-noncop the excess transfer occurred only in the PEY samples. The transfer of PBDEs mainly occurred in the PEY samples, while only lower

brominated congeners (BDE-28, -47, -99 and -100) were transferred in excess in the EST samples. It was also verified that there was no excess transfer in any of the samples for PBBs.

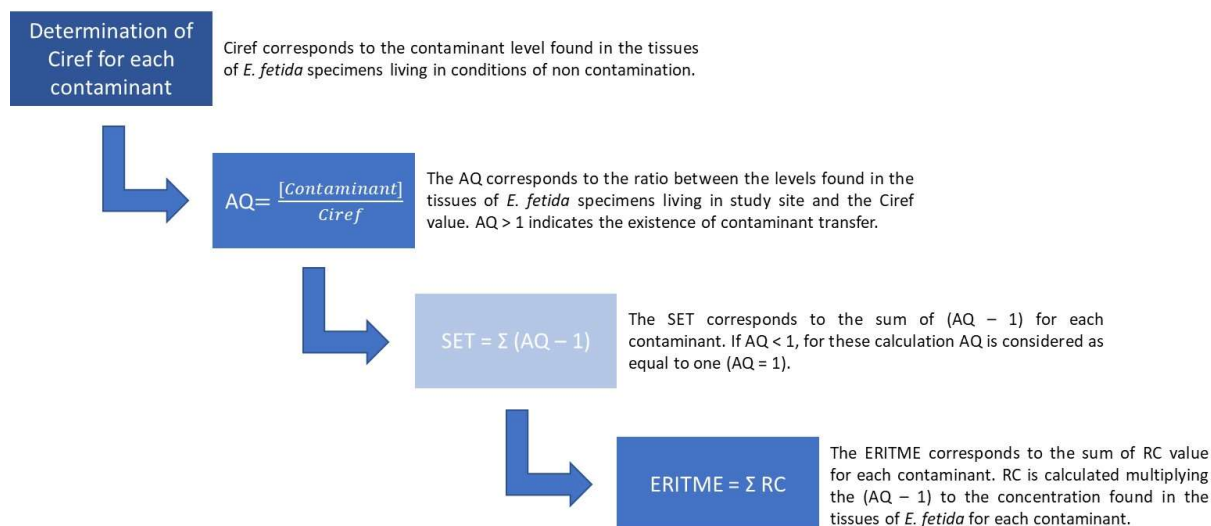


Figure 11 – Flow chart representation of the SET and ERITME calculation method (adapted from Baures (2018), based on Pauget and De Vaufleury (2015)).

The SET index gives a global idea of the excess transfer for all the contaminants in the matrices studied. The values obtained for SET (presented in Table 39) are in agreement with the trend shown by the AQ values. In general, the excess transfer and accumulation of POPs is much more significant in the PEY samples, which can be explained by the abnormal transfer of PCBs-dl-noncop.

The ERITME index allows evaluating the possible inherent global environmental risk associated with the excess transfer of the contaminants considered. The values obtained for ERITME-POP are presented in Table 38.

Table 38 – SET and ERITME-POP indexes calculated for each of the anthroposoils studied for the POPs concentrations measured.

	EST C	EST G	EST K	EST L	PEY Ic	PEY IIb	PEY IIIa	PEY IVa
SET	12.96	15.57	12.72	14.60	101.26	96.57	14.25	66.30
ERITME-POP	5220	6095	5392	5859	50886	45695	7784	24907

Considering, the ERITME-POP values the matrices studied can be classified in an apparent increasing order of toxicity:

PEY Ic > PEY IIb > PEY Iva > PEY IIIa > EST G > EST L > EST K > EST C.

This order of toxicity is in accordance with the POPs levels quantified in soils and with the concentrations found in the exposed *E. fetida*, alfalfa, cress and mustard, where in general the higher values were recorded in the PEY samples and especially in PEY Ic followed by PEY IIb. In the case of the EST samples, the values were in general always lower than those recorded in the PEY samples, with Anthroosoil G being the most contaminated and the tissues of the organisms exposed to this anthroosoil those that exhibited higher levels in their tissues when compared with the other three EST samples. MTE data on the EST samples had already been published in Coelho et al. (2018). In general, the BAFs calculated obtained for MTEs in both the EST and PEY samples were lower than 1, and despite high values for Cd and Cu, Zn presenting the highest BAFs. The relatively high values obtained for Zn were probably related to the high concentrations verified for Zn both in soils and sediments as well as with relatively high values for Zn exchangeable and/or bioavailable fractions. Despite this and regarding the AQ values (the coefficient of accumulation considered in the SET and ERITME calculations), excess transfer was observed for Fe, Ni and Pb in exposed *E. fetida*. This antagonistic trend between the BAF values and the SET/ERITME indexes was also found in a study by Mariet et al. (2017), who used snails as bioindicators to assess the environmental risk posed by the presence of MTEs. In the present study, the authors found that the BAF values were higher than 1 for Cd, Cu and Zn, but despite this no excess transfer from soils to snails was observed in the case of Cu, as Cd and Zn were transferred in low concentrations (Mariet et al., 2017). The BAFs obtained for Ag, As and Pb were lower than 1, suggesting a smaller transfer but the concentrations found in the snail tissues were anomalous, thus indicating the occurrence of excess transfer (Mariet et al., 2017). Despite this and when considering the AQ values, the results do not indicate the same trend shown by BAFs as the AQ values indicate that no excess transfer occurred for PCBs (PCBs-dl-cop), PCDDs, PCDFs and PBBs. The transfer from the anthroosoils to *E. fetida* tissues only occurred for PCBs-ndl, PCBs-dl-noncop and PBDEs.

The present study showed that POPs accumulation is a complex process that cannot be predicted by measuring the available fraction of contaminants alone. The BAFs, SET and ERITME-POP indexes are useful tools for characterizing the risks posed by contaminated soils and sediments. Since BAF is dependent on the initial contaminant concentrations in the matrix, there is a certain tendency to under or over-estimate the real risk for the organisms exposed. Both SET and ERITME-POP indexes are useful in risk evaluation as

they are based on the comparison of the matrices studied with others that have already been analyzed. These indexes are independent of soil characteristics and are based on the internal concentrations of contaminants measured in the organisms exposed and on the real bioavailable fractions of the contaminants in the matrices. The calculation of these quotients allows us to infer a global toxicity. The SET and ERITME-POP indexes provided a global evaluation of the level of toxicity of each sample studied, considering the abnormal excess transfers of contaminants from the matrix to the exposed *E. fetida*. The values obtained for the calculated indexes and the effects observed, especially in the reproduction rates, seem to be linked. To date, no studies have used SET and ERITME calculations to analyze their results of POPs accumulation in living organisms, so comparison with the results obtained by other authors is impossible. Associating the calculation of these two indexes with the determination of a certain toxic effect is useful in the definition of limit values as well as in the evaluation of the risks using a more realistic method of considering the real complexity of ecotoxicological processes. This approach can be enhanced if the exposure time of the organisms to the test matrices is long enough to reach the equilibrium state and thus calculate the SET and ERITME indexes with the equilibrium concentrations.

MAIN RESULTS

- ✓ The BAFs values calculated clearly show the potential of the POPs analyzed to accumulate in *E. fetida* tissues
- ✓ Adult and juvenile *E. fetida* can be considered as deconcentrators of PCDDs, PCDFs, PBDEs and PBBs (BAF<1) but macroconcentrators of PCBs, nHBB, nPBB, nPBT and PBEB (BAF>2), with exceptions in the case of a few samples.
- ✓ For some of the POPs quantified, the BAFs for *E. fetida* reported in the present study were lower than 10, which suggests the existence of a potential of transfer of these POPs to higher trophic levels and the subsequent potential toxicity effects caused.
- ✓ The BAFs for POPs for *in situ* plant species are in general below 1, except for the sum of the Σ_7 PBDEs in Japanese knotweed leaves and for the sum of HBCDs isomers in the leaves and roots of Japanese knotweed plants.
- ✓ BAFs for both the aerial parts and roots of the cultivated plant species are significantly higher in the plant species exposed to anthroposoil EST G (most of all superior to 1) than the BAFs corresponding to plants exposed to PEY Ic
- ✓ The BAFs obtained for the plants tested clearly indicate a significant difference in the availability of the contaminants in both the soils considered. Although the levels of POPs were significantly higher in PEY Ic than in EST G, it seems that the POPs were more available for uptake by plants in the anthroposoil EST G.
- ✓ Considering the ERITME-POP values, the matrices studied can be classified in an apparent increasing order of toxicity: PEY Ic > PEY Iib > PEY Iva > PEY IIIa > EST G > EST L > EST K > EST C.
- ✓ POPs accumulation is a complex process that cannot be predicted by measuring only the available fraction of contaminants. The BAFs, SET and ERITME indexes are useful tools for characterizing the risks posed by contaminated soils and sediments.
- ✓ The SET and ERITME-POP indexes provided a global evaluation of the degree of toxicity of each sample studied, considering the abnormal excess of transfers of contaminants from the matrix to the *E. fetida* exposed. The values obtained for the calculated indexes and the effects observed, especially in the reproduction rates, seems to

Chapter 5. Conclusions and Future perspectives

5.1. Conclusions

Nowadays, the assessment of environmental contamination has changed from the classical total concentration-based approach to a more complete method that considers the real risk posed by chemical contamination on a case-by-case evaluation. Great attention was given to the fate of the contaminants in soils and sediments as well as to the hazard and real risk to living organisms, by evaluating potential contaminant mobility and bioavailability. Monitoring of soil ecosystems is essential to assess levels of contamination and determine the potential impacts of these contaminations on soil sustainability.

The present work focused mainly on BFRs, which belong to the POPs family. These compounds represent a large group of major industrial chemicals whose production and use has increased significantly in recent decades. Despite its considerable use in fire prevention, concerns are rising continuously due to the persistence, bioaccumulation and potential toxicity of these compounds for both animals and humans. The current lack of knowledge of BFRs and data on them, leads to questions that must be answered. It is of great importance to perform more systematic environmental monitoring regarding certain environmental compartments and living species to understand how and where BFRs are being released into the environment, and what happens after their entry into environmental compartments, in order to fully ascertain the risks posed.

The aim of the present work was to answer the following questions (in bold type) that correspond to the main objectives of the present thesis:

1) Do the anthroposoils selected show detectable POPs concentrations? Which POPs families are present in these anthroposoils?

The 8 anthroposoils studied are quite different in terms of physico-chemical characteristics and inorganic and organic contamination.

Among all the POPs quantified in the anthroposoils studied, those present in considerable levels were PCBs, PCDDs, PCDFs, PBDEs and HBCDs. The PBBs and the four nRBFRs analyzed (nHBB, nPBB, nPBT and PBEB) were detected in very low concentrations. When comparing the two sites studied, the EST samples presented lower levels of the POPs analyzed than the PEY samples.

Despite their noticeable presence, the levels of PCBs, PCDDs, PCDFs, PBDEs and HBCDs were in general lower than those found in the literature for sites with similar contamination source types and land use in Europe.

The nBFRs values obtained are not comparable with most of the few data found in the literature that usually deals with highly contaminated sites in Asian countries (usually e-waste manufacturing and/or disposal sites). There is a considerable lack of studies on the presence, distribution and fate of nBFRs in terrestrial ecosystems.

2) Are POPs available for accumulation by earthworms and plants?

Several entire specimens of common nettle and Japanese knotweed plants were collected *in situ* from the two sampling points PEY Ic and PEY IIIa. Among the POPs quantified in the aerial parts and roots of the two plants analyzed, PCBs-ndl were those that presented higher levels in plant tissues, followed by the sum of the 8 PBDEs. Similar levels of PCBs-noncop and HBCDs were recorded. On the contrary, PCDDs and PCDFs occurred in only very low concentrations in plants. The availability of POPs to be taken up from soil and the occurrence of POPs transfer from soil to plant parts in the natural environment was shown by the levels detected in plant parts for most of the POPs analyzed. In the case of BFRs, the levels measured in the plants collected *in situ* were lower when compared with the other POPs measured.

Bioaccumulation laboratory tests were performed with *E. fetida* and three plant species (alfalfa, cress and white mustard). The data obtained clearly revealed the occurrence of bioaccumulation of the POPs quantified in both adult and juvenile *E. fetida* tissues, even at very low concentrations in the case of the nBFRs. Moreover, in the case of PCBs, PCDDs, PCDFs and PBDEs, clear strong correlations were observed between the levels quantified in *E. fetida* tissues and the levels found in the anthroposoils for these POPs. Despite the existence of such good correlations, these relations were not visible in the PCA. The levels of all the POPs quantified were higher in *E. fetida* exposed to PEY anthroposoils and higher in juveniles than in adults exposed to both the anthroposoils tested. The levels of POPs quantified in juvenile *E. fetida* tissues may have been due to the occurrence of POPs transfer from adults to juveniles or may only be the result of new accumulation of POPs from the anthroposoils. Moreover, the burrowing activity of adults as well as different lifestyle and feeding habits may favor higher bioavailability of POPs for juveniles and their subsequent higher accumulation in tissues.

The POPs concentration measured in the tissues of plants cultivated on EST G anthroposoil were lower than the levels measured in the plants cultivated on the PEY Ic anthroposoil (Table 28). This fact was expected since the levels of POPs in the PEY soils were much higher than those detected in the EST anthroposoils. Despite this general trend, HBCDs were an exception as the levels measured in the different plant parts were higher in the case of those cultivated on the EST anthroposoils tested. Another point that was expected and verified was that the roots accumulated higher POPs quantities than the aerial parts for all the POPs considered.

In conclusion, the POPs present in these anthroposoils were available for accumulation by soil organisms and plants.

3) In the case of bioaccumulation, what are the toxic effects caused by the POPs levels in the animal and plant species investigated?

Some toxic effects were observed for *E. fetida* and for the three plant species cultivated in the anthroposoils studied, in particular concerning:

- ***E. fetida* reproduction rate.** At the end of 28 days exposure, the mortality rate was very low in the anthroposoils studied. Among the EST samples, a large number of juveniles were observed in the ISO soil while a lower number were found in soil G. Soils C and L presented numbers of juveniles close to those observed for ISO soil. Concerning the PEY samples, no great differences were observed. It should be noted that a larger number of juveniles occurred in PEY IVa which may be related to the high OM content that served as feed for the earthworms and thus helped their development. The number of juveniles produced in the PEY samples were in general lower than those from the EST samples, probably due to the inhibition effects caused by the sandy texture that negatively affects the earthworm's normal biological activities and by the higher MTEs and POPs levels found in the PEY anthroposoils.

- ***E. fetida* body mass.** Some differences were noticed in the weight loss or gain of earthworms, considering the mean values of adult body weight at the beginning and at the end of the exposure time. The highest mass losses were found in *E. fetida* exposed to ISO soils, probably due to the low nutrient quantities in this "control" sample. Mass gains were observed for *E. fetida* exposed to EST G and

EST K anthroposoils and only a slight loss of mass was observed for individuals exposed to anthroposoils EST C and EST L. This is probably related to the relatively high OM contents in EST anthroposoils. In all the PEY samples, mass losses in *E. fetida* were observed even when these anthroposoils were rich in OM content and the pH values were within the range of ideal values for the good growth and development of *E. fetida*. Probably, the sandy character of these anthroposoils and the presence of relatively high concentrations of MTEs and POPs inhibited its growth and physiological development.

- **plant seed germination rate.** The germination rate was higher for the control (ISO soil), EST G, PEY Ic and PEY I Ib, compared to the other soils tested for all the three plants tested. This good germination can be associated with the relatively high, but not toxic, levels of some MTEs found in these anthroposoils. The plants cultivated in the anthroposoils EST C, EST K and PEY IVa showed very low germination rates for the three plant species tested, which may be due to the compactness and/or presence of contaminants (both organic and inorganic) in these three anthroposoils.
- **maximal height of plant aerial parts.** The mean height of aerial parts was higher for the control, EST G, EST C, PEY Ic and PEY I Ib compared to the other soils tested, for all the three plants tested. Nevertheless, in the case of alfalfa and cress the anthroposoils played an important role in the maximum height but in the case of the mustard a higher aerial part height was expected when compared with the two other species tested due to the plant's characteristics. In the case of anthroposoil EST L, the low plant height can be linked to the slightly acid pH (4.75), lower OM content and the noticeable Pb concentration.

Thus, it can be assumed that toxic effects were caused mainly by the mix of contaminants present in the anthroposoils tested, with in some cases a slight influence of certain physico-chemical parameters (namely sand content, pH, OM content).

4) What is the degree of bioaccumulation of POPs in the species tested?

The traditional approach for the risk assessment of chemical substances is usually linked to the direct effects of the chemicals on the organisms and based in a comparison between

the concentration in the environmental compartment and the presence or absence of effects in the organisms living in, or in close contact with, the soil compartment (Jager, 1998). The chemical accumulated can pass through the food chain and predators can be largely exposed via food ingestion. *E. fetida* and the plant species used as test organisms in the present work, form part of the basis of the terrestrial food chain, and due to their abundance they are a large part of the diet of many invertebrate and vertebrate species. The assessment of this secondary potential contamination level and the degree of bioaccumulation of POPs in the species tested is usually expressed through the calculation of BAFs. For the *E. fetida* adults and juveniles, the BAFs were higher than 1 for the PCBs. In the case of PCDDs, PCDFs and PBDEs the BAFs were generally lower than 1 for all the samples. For PBDEs, only the adult *E. fetida* exposed to anthroposoil PEY IIIa presented a BAF higher than 1. This fact can be linked to differences in lipid contents, 1.73% in juveniles and 4.07% in adults, leading to easy entry and accumulation/chelation in adults that present higher lipid contents in their tissues. BAFs for PBBs were generally zero or close to zero for all the samples. On the contrary, nBFRs presented BAFs higher than 1, for most of the samples considered. Attention is required due to the fact that the analytical technique used to quantify these compounds presented certain limitations in the detection and quantification of the POPs considered in the *E. fetida* tissues. Regarding the PBDEs, data already exists in the literature but information on the presence and accumulation of nBFRs in earthworms is very scarce. Moreover, the present study seems to be one of the first to have attempted to assess the presence and quantify the levels of nBFRs in such terrestrial organisms. The BAF values clearly showed the potential of the POPs analyzed to accumulate in *E. fetida* tissues. Earthworms in general are a major food source for many animals (birds, reptiles and small mammals), thus the presence of POPs in their tissues can be a significant vector of POPs transfer into higher trophic levels through biomagnification.

BAFs were also calculated for plants collected *in situ* and for the three plant species cultivated in two of the anthroposoils studied (EST G and PEY Ic). The BAFs for POPs for *in situ* plant species were in general below 1, except for the sum of the Σ_7 PBDEs in Japanese knotweed leaves and for the sum of HBCD isomers in the leaves and roots of Japanese knotweed plants. The BAF values calculated for plants collected *in situ* and for both aerial parts and roots are significantly higher in the plant species exposed to anthroposoil EST G (mostly higher than 1) than the BAFs corresponding to plants exposed to PEY Ic. The BAFs obtained for the plants tested clearly indicate a considerable

difference in the availability of the contaminants in both the soils considered. Although the levels of POPs were significantly higher in PEY Ic than in EST G, it seems that the POPs were more available for plant uptake in anthroposoil EST G. This difference in quantified POPs availability may be due to the soil's physico-chemical parameters, namely the nature of OM, TOC and the slightly acidic pH of EST G anthroposoils.

BAF calculation is usually required and included in evaluation and risk assessment models for contamination prediction but it presents certain limitations. BAF comprises a cumulative effect, considering each contaminant individually so it is not representative of the real risk posed by the mix of contaminants in the environmental matrices. To overcome this shortcoming, more complete indexes that take into account the concentrations of the contaminants and the weight of toxicity points can be used to better evaluate and predict the real risk.

5) Concerning the evaluation of the real risks, what do the indexes calculated such as BAF, SET and ERITME express?

The SET index can be used to indicate the anomalous transfer of a pollutant while the ERITME index is used to evaluate the real ecotoxicological risk due to the anomalous transfer evidenced by the SET calculation. The SET index gives a global idea of the excess transfer of all the contaminants in the matrices studied. The ERITME index is used to evaluate the possible inherent global environmental risk associated with the excess transfer of the contaminants considered.

Analysis of the ERITME-POP values makes it possible to classify the matrices studied by increasing order of toxicity:

PEY Ic > PEY Iib > PEY Iva > PEY IIIa > EST G > EST L > EST K > EST C.

This order of toxicity is in accordance with the levels of POPs quantified in the soils and with the concentrations found in the *E. fetida*, alfalfa, cress and mustard exposed, where in general the higher values were recorded in the PEY samples and especially in PEY Ic, followed by PEY Iib. In the case of the EST samples, the values were in general always lower than those recorded in the PEY samples. For anthroposoil G, which was the most contaminated, the tissues of the organisms exposed to this anthroposoil exhibited higher levels in their tissues when compared with the other three EST samples.

The BAF values calculated for *E. fetida* adults and juveniles were higher than 1 in the case of PCBs and nBFRs, while the BAF values were lower than 1 for the PCDDs,

PCDFs, PBDEs and PBBs. This suggests that a transfer of PCBs and nBFRs had occurred from the matrices to the tissues of the *E. fetida* exposed and that no transfer had occurred for the PCDDs, PCDFs, PBDEs and PBBs. Despite this and when considering the AQ values (the accumulation coefficient considered in the SET and ERITME calculations), the results do not indicate the same trend shown by BAFs since the AQ values indicate that no excess transfer occurred for certain PCBs (PCBs-dl-cop), PCDDs, PCDFs and PBBs. Transfer from the anthroposoils to *E. fetida* tissues only occurred for PCBs-ndl, PCBs-dl-noncop and for the PBDEs.

6) Are these reference indexes useful in the Environmental Risk Assessment approach?

In the present study, the BAF values calculated for *E. fetida* were higher than 1 in the case of PCBs and nBFRs, suggesting that the transfer of PCBs and nBFRs occurred from the matrices to the tissues of exposed *E. fetida*. When considering the AQ values (the accumulation coefficient considered in the SET and ERITME calculations), the results do not indicate the same trend as shown by the BAFs since the AQ values indicate that the excess transfer occurred only for the PCBs-ndl, PCBs-dl-noncop and PBDEs.

It has already been clearly demonstrated in the literature, and in the present study, that the soil physico-chemical parameters can affect the bioavailability of contaminants to living organisms. Assessing the total concentrations of the contaminants in an environmental compartment is not enough to efficiently predict the risk posed by them to the living community. The traditional approach suggests using the BAF calculation which is usually required for evaluating and assessing contamination risk, however questions can arise regarding the quality and correctness of the BAF values obtained. BAFs present certain limitations, since they do not comprise a cumulative effect, because they treat each contaminant individually and thus in a less global way.

Thus, the SET and ERITME approaches were developed to overcome this shortcoming as they are based on real measures in organisms. These indexes clearly show that an unexpected abnormal bioaccumulation of contaminants can occur in slightly contaminated soils and even exceed the limits tolerated for human consumption. Therefore, these more realistic indexes have proven useful for risk evaluation and managing contaminated sites. Thus, in the present study, where the anthroposoils studied were characterized by a mixture of different families of POPs and MTEs, with some of them present in low concentrations, it appeared more appropriate to apply the SET and

ERITME indexes as they take into account the sum of all the contaminants, and not one by one as in the BAF.

5.2. Future Perspectives

To take the present work further, it would be interesting to see whether the use of different soils with different characteristics, OM typologies and POPs levels, and their bioavailability, leads to significant differences in the PCA results and relations between the variables. This would entail:

- Increasing the number of samples as well as considering soils with different typologies in order to identify the influence of the different parameters of POPs mobility and transfer to organisms. This can be done by implementing the same approach in other contaminated sites with different physico-chemical properties and contamination levels;
- Selecting and studying other anthroposoils with lower or higher C/N ratios and sand content to determine whether any difference is noticeable in the PCA;
- Performing tests on soil OM separation and analyzing the different typologies of OM (such as humic acids), and determine whether certain POPs are potentially linked or present in these different separated fractions;
- Performing more detailed granulometric analyses to determine the speciation and distribution of contaminants in the different soil fractions;
- Evaluating POPs bioavailability through various chemical extractions. Since DTPA+EDTA extraction did not extract POPs from the anthroposoils studied, tests on the aging and/or lixiviation of soil litter, for example, could also be done.

To better understand the bioaccumulation process as a dynamic one, it would be interesting to perform kinetic studies for the different POPs considered and find links in the differences in levels of the POPs found in adult and juvenile *E. fetida* tissues. Moreover, it is important to establish a relation between the physiological characteristics and processes involved in the bioaccumulation of POPs by earthworms. To answer these questions, the following tasks could be performed:

- Chemical kinetics studies to understand the accumulation rates and variations over the time of exposure of the organisms to contaminated matrices, since the equilibrium state of certain contaminants is probably not reached during the usual exposure period of 56 days;
- Expose physiologically distinct earthworms and plants as well as other test species to better understand how their feeding and burrowing habits signify possible species-specific differences in the accumulation and distribution of contaminants;
- Conduct long term exposure tests to assess the effects of a contaminated soil on the various generations of the organisms tested to better understand the differences in sensitivity of adults and juveniles to contamination;
- Explore distribution at the organ level to better understand the internal toxicity effects in the organisms and the presence of potential transformation products (especially in the case of compounds with low stability in soils) – perform studies on cellular toxicity and genotoxicity in earthworms and evaluate the enzymatic activity (Superoxide dismutase (SOD), Catalase) and the perturbation of photosynthetic activity in plants;
- Use marked compounds, such as radioactive C₁₄ or others, to better understand the mechanisms associated with, and/or responsible for, the mobility and transfer of certain POPs families, namely the BFRs since their behavior and transfer to terrestrial organisms remain poorly documented in the literature.

It is important to carry out further studies using a larger number of different earthworm species to permit more specific and accurate conclusions on uptake and bioaccumulation differences. Firstly, we could begin with the collection of earthworms (notably in terms of ecological groups such as epigeic, endogeic and acenic) to evaluate transfer *in situ*. In addition to oligochaetes, other invertebrates from the terrestrial compartment such as springtails, insects and snails as well as primary predators and herbivores could be used to elucidate how species characteristics and habits can affect the fate, bioavailability, uptake and accumulation of different families of POPs. Also, testing with different species of different trophic levels allows understanding how and to what extent biomagnification occurs. Finally, tests could be carried out on models in which the data obtained is introduced to infer dispersion and biomagnifications in the trophic chain. The model TerraSys^{MC} developed by Sanexen (Canada) for the ecological risk evaluation of contaminated lands could be tested as it combines all the parameters used for risk analysis

in both the terrestrial and aquatic compartments. TerraSys^{MC} has already been widely tested for MTEs (Hayet, A., 2010) but it could be interesting to test this model for POPs, to infer the transfer between organisms from immediate trophic levels (earthworms and birds or moles, for example).

Finally, these additional studies will allow better understanding, prediction and risk assessment of contaminant (namely the POPs) accumulation in living communities in terrestrial ecosystems and the potential for contaminants to enter the food chain and be subject to biomagnification.

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Annex I

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Annex II

Table II.1 – Correlation matrix (Pearson) for anthroposoils physico-chemical properties, metallic trace elements concentrations and organic contaminants levels in anthroposoils and in *Eisenia fetida* tissues (significance level $\alpha = 0.95$; coefficients higher than 0.7 are marked in bold).

Variables	Sand	Silt	Clay	OM	TOC	NT Dumas	C/N	Carb tx	CEC Metson	pH H ₂ O	pH KCl	CaO	K ₂ O	MgO	P Olsen
Sand	1														
Silt	-0,995	1													
Clay	-0,837	0,776	1												
OM	-0,659	0,603	0,832	1											
OC	-0,659	0,603	0,832	1,000	1										
NT Dumas	-0,830	0,777	0,953	0,917	0,917	1									
C/N	0,812	-0,805	-0,696	-0,445	-0,445	-0,740	1								
Carbonates tx	-0,602	0,520	0,923	0,737	0,737	0,861	-0,574	1							
CEC Metson	-0,859	0,822	0,893	0,857	0,857	0,948	-0,735	0,747	1						
pH H ₂ O	-0,624	0,547	0,917	0,725	0,725	0,886	-0,679	0,981	0,786	1					
pH KCl	-0,637	0,562	0,916	0,743	0,743	0,902	-0,709	0,970	0,801	0,997	1				
CaO	-0,596	0,514	0,916	0,709	0,709	0,850	-0,589	0,995	0,726	0,985	0,974	1			
K ₂ O	-0,740	0,697	0,828	0,759	0,759	0,902	-0,759	0,807	0,917	0,851	0,862	0,786	1		
MgO	-0,693	0,619	0,954	0,787	0,787	0,916	-0,642	0,987	0,839	0,979	0,972	0,977	0,881	1	
P Olsen	-0,378	0,355	0,431	0,523	0,523	0,599	-0,555	0,411	0,727	0,525	0,551	0,391	0,786	0,507	1
Cd tot	-0,593	0,529	0,823	0,619	0,619	0,726	-0,483	0,818	0,771	0,788	0,772	0,779	0,744	0,849	0,507
Cu tot	-0,578	0,528	0,738	0,473	0,473	0,646	-0,567	0,677	0,753	0,716	0,705	0,667	0,658	0,720	0,595
Cr tot	-0,698	0,626	0,948	0,785	0,785	0,884	-0,580	0,931	0,867	0,913	0,904	0,907	0,830	0,957	0,521
Ni tot	-0,704	0,636	0,931	0,757	0,757	0,858	-0,566	0,901	0,864	0,879	0,868	0,873	0,816	0,933	0,519
Pb tot	-0,683	0,626	0,855	0,532	0,532	0,684	-0,468	0,826	0,694	0,768	0,737	0,809	0,643	0,839	0,232
Zn tot	-0,708	0,644	0,915	0,706	0,706	0,832	-0,585	0,872	0,864	0,863	0,851	0,850	0,802	0,909	0,540
ΣPCDDs soil	-0,433	0,382	0,626	0,434	0,434	0,535	-0,388	0,624	0,641	0,606	0,594	0,575	0,614	0,662	0,530
ΣPCDDs EF	-0,446	0,386	0,680	0,531	0,531	0,626	-0,425	0,732	0,685	0,711	0,700	0,683	0,735	0,765	0,589
ΣPCDFs soil	-0,512	0,446	0,769	0,400	0,400	0,579	-0,453	0,796	0,567	0,757	0,731	0,783	0,534	0,779	0,216
ΣPCDFs EF	-0,472	0,411	0,709	0,361	0,361	0,547	-0,448	0,776	0,495	0,737	0,712	0,769	0,535	0,752	0,170
ΣPCBs cop soil	-0,492	0,442	0,663	0,492	0,492	0,560	-0,349	0,610	0,678	0,582	0,566	0,562	0,581	0,655	0,480
ΣPCBs cop EF	-0,445	0,406	0,567	0,477	0,477	0,511	-0,319	0,492	0,589	0,478	0,473	0,456	0,480	0,533	0,400
ΣPCBs non-cop soil	-0,395	0,338	0,625	0,516	0,516	0,603	-0,416	0,697	0,671	0,684	0,677	0,642	0,760	0,737	0,660
ΣPCBs non-cop EF	-0,258	0,216	0,435	0,418	0,418	0,470	-0,330	0,517	0,493	0,519	0,522	0,476	0,610	0,547	0,551
Σ ₆ PCBs NDL soil	-0,443	0,385	0,669	0,543	0,543	0,622	-0,412	0,701	0,704	0,683	0,674	0,647	0,729	0,742	0,626
Σ ₆ PCBs NDL EF	-0,333	0,287	0,515	0,447	0,447	0,510	-0,352	0,563	0,555	0,557	0,555	0,521	0,616	0,596	0,537
Σ ₇ PBDEs soil	-0,438	0,379	0,670	0,742	0,742	0,757	-0,482	0,712	0,781	0,730	0,745	0,661	0,854	0,767	0,803
Σ ₇ PBDEs EF	-0,543	0,472	0,818	0,718	0,718	0,807	-0,548	0,876	0,743	0,869	0,869	0,852	0,804	0,887	0,531
Σ ₈ PBDEs soil	-0,409	0,350	0,647	0,664	0,664	0,696	-0,444	0,700	0,742	0,706	0,713	0,645	0,816	0,750	0,763
Σ ₈ PBDEs EF	-0,335	0,310	0,406	0,276	0,276	0,373	-0,397	0,389	0,336	0,387	0,399	0,367	0,341	0,396	0,179
Σ PBBs soil	-0,520	0,450	0,792	0,557	0,557	0,690	-0,490	0,841	0,710	0,815	0,799	0,806	0,734	0,854	0,499
Σ PBBs EF	-0,137	0,109	0,264	0,219	0,219	0,279	-0,222	0,357	0,321	0,354	0,352	0,319	0,455	0,381	0,428
PBEB soil	-0,555	0,505	0,711	0,398	0,398	0,604	-0,569	0,750	0,396	0,741	0,731	0,794	0,446	0,700	-0,077
PBEB EF	-0,234	0,275	-0,035	-0,022	-0,022	0,003	-0,111	-0,144	0,057	-0,159	-0,153	-0,160	0,071	-0,086	-0,040
nPBT soil	-0,455	0,421	0,551	0,346	0,346	0,406	-0,241	0,438	0,557	0,401	0,380	0,397	0,379	0,482	0,307
nPBT EF	-0,477	0,429	0,641	0,437	0,437	0,567	-0,497	0,645	0,514	0,631	0,632	0,626	0,518	0,645	0,253
nHBB soil	0,021	-0,054	0,159	0,179	0,179	0,253	-0,231	0,395	0,236	0,402	0,406	0,354	0,571	0,406	0,545
nHBB EF	-0,467	0,419	0,634	0,404	0,404	0,556	-0,510	0,666	0,466	0,650	0,649	0,656	0,510	0,653	0,186
nPBB soil	-0,457	0,392	0,716	0,646	0,646	0,721	-0,481	0,786	0,753	0,781	0,780	0,737	0,838	0,824	0,706
nPBB EF	-0,511	0,455	0,714	0,541	0,541	0,668	-0,555	0,741	0,583	0,733	0,738	0,723	0,619	0,739	0,324

Table II.2 – Correlation matrix (Pearson) for metallic trace elements concentrations in anthroposoils and organic contaminants levels in anthroposoils and in *Eisenia fetida* tissues (significance level $\alpha = 0.95$; coefficients higher than 0.7 are marked in bold).

<u>Variables</u>	<u>Cd tot</u>	<u>Cu tot</u>	<u>Cr tot</u>	<u>Ni tot</u>	<u>Pb tot</u>	<u>Zn tot</u>
Cd tot	1					
Cu tot	0,894	1				
Cr tot	0,951	0,847	1			
Ni tot	0,970	0,869	0,996	1		
Pb tot	0,910	0,810	0,902	0,919	1	
Zn tot	0,969	0,920	0,983	0,992	0,927	1
ΣPCDDs soil	0,950	0,882	0,818	0,855	0,783	0,866
ΣPCDDs EF	0,939	0,804	0,854	0,874	0,772	0,865
ΣPCDFs soil	0,913	0,854	0,865	0,880	0,933	0,892
ΣPCDFs EF	0,783	0,684	0,770	0,772	0,834	0,771
ΣPCBs cop soil	0,951	0,899	0,835	0,874	0,820	0,890
ΣPCBs cop EF	0,727	0,695	0,671	0,695	0,618	0,703
ΣPCBs non-cop soil	0,912	0,761	0,816	0,836	0,702	0,821
ΣPCBs non-cop EF	0,604	0,465	0,562	0,565	0,417	0,540
Σ₆PCBs NDL soil	0,955	0,834	0,856	0,882	0,762	0,875
Σ₆PCBs NDL EF	0,716	0,599	0,655	0,667	0,543	0,652
Σ₇PBDEs soil	0,796	0,645	0,798	0,794	0,529	0,757
Σ₇PBDEs EF	0,808	0,658	0,870	0,852	0,711	0,818
Σ₈PBDEs soil	0,852	0,698	0,810	0,816	0,589	0,786
Σ₈PBDEs EF	0,450	0,364	0,430	0,433	0,371	0,411
Σ PBBs soil	0,987	0,873	0,933	0,946	0,885	0,942
Σ PBBs EF	0,477	0,354	0,398	0,410	0,317	0,393
PBEB soil	0,352	0,291	0,546	0,498	0,583	0,481
PBEB EF	-0,103	-0,178	-0,111	-0,092	-0,034	-0,103
nPBT soil	0,848	0,859	0,707	0,759	0,777	0,795
nPBT EF	0,676	0,572	0,677	0,675	0,627	0,656
nHBB soil	0,363	0,139	0,307	0,297	0,145	0,249
nHBB EF	0,608	0,491	0,638	0,628	0,605	0,603
nPBB soil	0,913	0,758	0,873	0,879	0,705	0,855
nPBB EF	0,694	0,564	0,734	0,720	0,633	0,689

Table II.3 – Correlation matrix (Pearson) for organic contaminants levels in anthroposoils and in *Eisenia fetida* tissues (significance level $\alpha = 0.95$; coefficients higher than 0.7 are marked in bold).

Variables	Σ PCDDs soil	Σ PCDDs EF	Σ PCDFs soil	Σ PCDFs EF	Σ PCBs cop soil	Σ PCBs cop EF	Σ PCBs non-cop soil	Σ PCBs non-cop EF	Σ_6 PCBs NDL soil	Σ_6 PCBs NDL EF	Σ_7 PBDE s soil	Σ_7 PBDE s EF	Σ_8 PBDE s soil	Σ_8 PBDE s EF	Σ PBBs soil	Σ PBBs EF	PBEB soil	PBEB EF	nPBT soil	nPBT EF	nHBB soil	nHBB EF	nPBB soil	nPBB EF	
Σ PCDDs soil	1																								
Σ PCDDs EF	0,939	1																							
Σ PCDFs soil	0,850	0,803	1																						
Σ PCDFs EF	0,688	0,781	0,870	1																					
Σ PCBs cop soil	0,981	0,898	0,851	0,668	1																				
Σ PCBs cop EF	0,731	0,804	0,633	0,720	0,759	1																			
Σ PCBs non-cop soil	0,933	0,965	0,737	0,646	0,872	0,645	1																		
Σ PCBs non-cop EF	0,608	0,792	0,437	0,594	0,541	0,755	0,725	1																	
Σ_6 PCBs NDL soil	0,975	0,971	0,799	0,673	0,941	0,706	0,986	0,686	1																
Σ_6 PCBs NDL EF	0,723	0,871	0,570	0,695	0,678	0,870	0,780	0,972	0,770	1															
Σ_7 PBDE s soil	0,770	0,856	0,534	0,476	0,717	0,578	0,909	0,721	0,880	0,732	1														
Σ_7 PBDE s EF	0,685	0,795	0,706	0,746	0,654	0,609	0,774	0,690	0,767	0,706	0,808	1													
Σ_8 PBDE s soil	0,853	0,914	0,614	0,536	0,797	0,621	0,963	0,736	0,941	0,766	0,987	0,800	1												
Σ_8 PBDE s EF	0,445	0,370	0,451	0,343	0,411	0,143	0,429	0,049	0,439	0,071	0,397	0,495	0,418	1											
Σ PBBs soil	0,944	0,948	0,928	0,813	0,918	0,690	0,930	0,629	0,956	0,727	0,801	0,825	0,861	0,469	1										
Σ PBBs EF	0,514	0,687	0,346	0,507	0,441	0,645	0,616	0,940	0,572	0,890	0,561	0,485	0,593	-0,014	0,508	1									
PBEB soil	0,094	0,196	0,539	0,600	0,097	0,103	0,117	0,086	0,124	0,109	0,127	0,512	0,093	0,247	0,388	-0,005	1								
PBEB EF	-0,117	-0,022	-0,179	0,053	-0,108	0,119	-0,074	0,129	-0,098	0,074	-0,076	0,024	-0,085	0,167	-0,142	0,199	-0,054	1							
nPBT soil	0,901	0,754	0,804	0,590	0,957	0,724	0,701	0,370	0,805	0,539	0,496	0,469	0,594	0,340	0,792	0,304	0,020	-0,093	1						
nPBT EF	0,618	0,702	0,688	0,809	0,593	0,701	0,596	0,606	0,618	0,650	0,537	0,776	0,565	0,690	0,695	0,525	0,443	0,254	0,499	1					
nHBB soil	0,388	0,560	0,173	0,264	0,233	0,123	0,674	0,615	0,540	0,519	0,678	0,491	0,683	0,209	0,450	0,573	0,010	0,033	-0,025	0,256	1				
nHBB EF	0,518	0,518	0,654	0,555	0,484	0,263	0,525	0,282	0,532	0,289	0,472	0,598	0,491	0,827	0,641	0,290	0,547	0,109	0,379	0,766	0,275	1			
nPBB soil	0,888	0,950	0,724	0,648	0,834	0,637	0,982	0,733	0,965	0,776	0,958	0,842	0,984	0,434	0,928	0,595	0,220	-0,093	0,638	0,616	0,670	0,557	1		
nPBB EF	0,602	0,660	0,682	0,713	0,567	0,515	0,630	0,498	0,636	0,517	0,622	0,874	0,629	0,807	0,721	0,353	0,524	0,156	0,431	0,928	0,346	0,825	0,677	1	

Annex III

RC OCs PEY IIb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RC OCs PEY IIIa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RC OCs PEY IVa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CIRef and [C] *Ef* are in in ng.kg⁻¹ dw. N.B.: Toxicity point values from ASTDR (Agency for Toxic Substances & Disease Registry), 2019. Minimal Risk Levels (MRLs) for Hazardous Substances. <https://www.atsdr.cdc.gov/mrls/pdfs/ATSDR%20MRLs%20-%20June%202019-H.pdf>.

Table III.2 - Data used to SET and ERITME indexes calculation for studied organic contaminants – PCB congeners.

	PCB 77	PCB 81	PCB 126	PCB 169	PCB 105	PCB 114	PCB 118	PCB 123	PCB 156	PCB 157	PCB 167	PCB 189	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
CIRef	452.65	12.32	34.92	3.17	2250.87	97.35	3747.43	133.56	1071.76	198.98	567.84	229.07	0.42	0.90	3.51	13.22	15.35	13.36
[C] EfEST C	39.04	2.22	7.43	0.81	957.59	46.52	3598.75	35.41	435.79	54.27	226.17	27.28	0.76	4.16	11.46	6.47	14.57	4.23
[C] EfEST G	43.98	2.62	9.69	1.15	1074.66	56.40	3945.38	50.78	643.46	79.42	311.18	58.83	0.74	4.74	12.91	9.34	16.36	6.23
[C] EfEST K	33.43	2.27	5.18	0.51	957.61	48.56	3681.75	35.48	445.04	56.03	221.98	29.03	0.74	4.83	11.64	6.60	12.39	4.27
[C] EfEST L	39.17	2.56	7.22	0.70	1023.30	53.97	3955.35	38.00	540.79	69.58	268.23	34.41	0.77	4.91	12.29	7.65	14.07	5.12
[C] EfPEY Ic	493.57	36.96	97.18	11.23	9650.23	534.38	20916.43	790.96	5909.54	1238.20	3766.87	1132.38	0.66	5.59	26.74	92.38	138.21	83.95
[C] EfPEY IIb	165.94	15.88	44.92	5.41	11.049.08	474.75	32064.96	534.37	7138.15	1166.63	3554.88	980.79	0.41	4.72	25.01	81.03	122.39	74.19
[C] EfPEY IIIa	92.50	9.28	33.77	4.42	2164.70	146.86	5840.25	156.80	1714.94	309.88	938.96	269.71	0.51	4.02	11.24	21.55	34.88	19.68
[C] EfPEY IVa	235.01	11.93	37.37	4.89	4153.00	191.78	12690.08	217.50	2489.65	352.53	1293.89	422.28	1.14	7.98	20.61	30.38	50.83	30.11
AQ EST C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.81	4.60	3.26	1.00	1.00	1.00
AQ EST G	1.00	1.00	1.00	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.00	1.00	1.76	5.23	3.67	1.00	1.07	1.00

RC OCs EST C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	486.29	2158.10	1357.64	0.00	0.00	0.00
RC OCs EST G	0.00	0.00	0.00	0.00	0.00	0.00	31.69	0.00	0.00	0.00	0.00	0.00	0.00	454.22	2539.70	1604.82	0.00	39.42	0.00
RC OCs EST K	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	456.78	2602.12	1387.35	0.00	0.00	0.00
RC OCs EST L	0.00	0.00	0.00	0.00	0.00	0.00	33.29	0.00	0.00	0.00	0.00	0.00	0.00	501.60	2653.57	1498.40	0.00	0.00	0.00
RC OCs PEY Ic	54.25	1200.54	1069.70	1527.50	1972.40	2639.55	2748.92	2953.20	2708.31	3133.71	3380.23	2366.05	339.39	3103.12	3966.17	3593.43	4800.81	3169.09	
RC OCs PEY IIb	0.00	173.89	171.87	425.38	2345.29	2325.99	4533.91	1800.52	3396.11	2917.90	3156.24	1969.01	0.00	2531.77	3669.95	3078.13	4182.75	2731.09	
RC OCs PEY IIIa	0.00	0.00	0.00	237.59	0.00	305.11	335.08	104.41	360.07	334.42	439.69	106.45	132.88	2063.18	1318.89	378.38	763.23	283.80	
RC OCs PEY IVa	0.00	0.00	42.15	326.74	507.04	582.01	1431.80	377.08	793.77	463.04	767.18	506.10	1021.56	4691.94	2918.89	779.25	1386.45	752.01	

CIRef and [C] *Ef* are in $\text{ng.kg}^{-1} \text{ dw}$. N.B.: Toxicity point values from ASTDR (Agency for Toxic Substances & Disease Registry), 2019. Minimal Risk Levels (MRLs) for Hazardous Substances. <https://www.atsdr.cdc.gov/mrls/pdfs/ATSDR%20MRLs%20-%20June%202019-H.pdf>.

AQ PEY Ic	5.28	4.13	4.16	9.47	1.77	4.57	1.00	1.00	1.59	2.10	2.07	1.00	1.00	1.00
AQ PEY IIb	3.19	3.55	5.70	12.43	3.15	6.99	1.00	1.00	1.74	1.77	1.36	1.00	1.00	1.00
AQ PEY IIIa	1.55	1.00	1.00	2.16	1.00	1.10	1.00	1.00	1.00	1.49	1.00	1.00	1.00	1.00
AQ PEY IVa	16.42	3.91	5.40	8.69	2.46	4.36	1.00	1.00	1.39	2.75	1.00	1.00	1.00	1.00
[AQ-1] EST C	5.89	0.14	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00
[AQ-1] EST G	6.59	0.54	0.30	0.28	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00
[AQ-1] EST K	4.90	0.23	0.10	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
[AQ-1] EST L	6.21	0.29	0.23	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
[AQ-1] PEY Ic	4.28	3.13	3.16	8.47	0.77	3.57	0.00	0.00	0.59	1.10	1.07	0.00	0.00	0.00
[AQ-1] PEY IIb	2.19	2.55	4.70	11.43	2.15	5.99	0.00	0.00	0.74	0.77	0.36	0.00	0.00	0.00
[AQ-1] PEY IIIa	0.55	0.00	0.00	1.16	0.00	0.10	0.00	0.00	0.00	0.49	0.00	0.00	0.00	0.00
[AQ-1] PEY IVa	15.42	2.91	4.40	7.69	1.46	3.36	0.00	0.00	0.39	1.75	0.00	0.00	0.00	0.00
Toxicity Point	178.00	178.00	178.00	178.00	178.00	178.00	178.00	178.00	600.00	600.00	600.00	178.00	178.00	178.00
RC OCs EST C	1048.81	25.28	000	4.57	0.00	0.00	0.00	0.00	0.00	138.87	0.00	0.00	0.00	0.00
RC OCs EST G	1172.82	95.59	53.11	49.30	0.00	0.00	0.00	0.00	54.17	0.00	0.00	0.00	0.00	0.00
RC OCs EST K	871.97	41.09	17.23	15.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RC OCs EST L	1106.12	50.89	40.32	11.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RC OCs PEY Ic	762.62	556.64	561.86	1507.63	136.80	635.99	0.00	0.00	354.58	660.17	644.96	0.00	0.00	0.00

RC OCs PEY IIb	390.15	454.30	836.53	2034.83	382.89	1065.74	0.00	0.00	441.65	461.74	217.67	0.00	0.00	0.00
RC OCs PEY IIIa	98.42	0.00	0.00	207.04	0.00	18.45	0.00	0.00	0.00	296.77	0.00	0.00	0.00	0.00
RC OCs PEY IVa	2745.26	517.80	783.00	1369.00	259.07	598.67	0.00	0.00	236.48	1051.08	0.00	0.00	0.00	0.00

CIRef and [C] *Ef* are in in ng.kg⁻¹ dw. N.B.: Toxicity point values from ASTDR (Agency for Toxic Substances & Disease Registry), 2019. Minimal Risk Levels (MRLs) for Hazardous Substances. <https://www.atsdr.cdc.gov/mrls/pdfs/ATSDR%20MRLs%20-%20June%202019-H.pdf>.