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Abstract

In recent decades, stormwater management has developed to allow stormwater to infiltrate directly into the soils instead of being collected and routed to sewer systems. However, during infiltration, stormwater creates a sediment deposit at the soil surface as the result of high loads of suspended particles (including pollutants), leading to the settlement of sedimentary layers prone to colonization by plants and earthworms. This study aims to investigate the earthworm communities of a peculiar infiltration basin and investigate the influence of edaphic conditions (water content, organic matter content, pH, height of sediment) and of persistent organic pollutants (POPs: PCBs, PCDDs and PCDFs) on these earthworms. Attention was paid to their age (juveniles or adults) and their functional group (epigeic, endogeic, anecic). We found that the earthworm abundance was mostly driven by edaphic conditions, with only a slight impact of POPs, with a significant negative impact of PCBDLno for juveniles and endogeic, and PCDDs for epigeic. On the contrary, the height of the sediment and the water content are beneficial for their presence and reproduction. Furthermore, POPs contents are also linked to physicochemical parameters of the sediment. Bioaccumulation was clearly revealed in the studied site but does not differ between juveniles and adults, except for PCDDs. Conversely, BAF values seemed to vary between functional groups, except for PCBDL non-ortho. It strongly varies with the family types (PCBs versus PCCD/Fs) and between congeners within the same family, with specific strong bioaccumulation for a few congeners.

Key words: Adults/juveniles; earthworms; epigeic/endogeic; edaphic conditions, PCBs; PCDD/Fs; stormwater sediment

1) Introduction

Stormwater management is a significant issue for urban areas, as urbanization artificializes soils thus increasing runoff, and as it carries large amounts of suspended solids and pollutants. An alternative to pipes, normally used in France, is the use of non-artificialized areas where stormwater may infiltrate instead of being stocked at surface (Fletcher et al., 2015). In some cases, infiltration basins are coupled with infiltration ponds to allow suspended matter and pollution to deposit before being infiltrated into infiltration basins. Despite their presence, part of these particles may reach the infiltration basin. In that case, pollutants and suspended solids deposit at the soil surface to form a sedimentary layer (Badin et al., 2009; El-Mufleh et al., 2014; Winiarski et al., 2006) above the soil surface, clogging and decreasing its infiltration capacity (Lassabatere et al., 2010). These places can be rich of biodiversity, from vegetation (Bedell et al., 2021, 2013; Saulais et al., 2011) to invertebrates like earthworms – which are of interest here.

Earthworms have a great place in the trophic chain as a food source for other species, their ability to concentrate and decompose the matter as ecosystem engineers (Babu Ojha and Devkota, 2014; Le Bayon et al., 2017), and to integrate soil chemical pollution (Fründ et al., 2011). Thus, they are bioindicators of the quality of polluted sites and soils, useful for the monitoring of soil's quality and biology and for the assessment of ecosystems risks (Edwards and Bater, 1992; Fried et al., 2019; Pelosi et al., 2014). There are two categories of earthworms, depending on their age: the juveniles, which are small and transparent, and the adults which are bigger, coloured and with a clitellum (Baker et al., 1997; Bouché, 1972). The adults can also be separated in three functional groups: the epigeic which live in the firsts five centimetres of the soil, in the hummus; the endogeic which live in the first ten to fifteen centimetres and create horizontal galleries; and finally the anecic which live deeper and create vertical galleries to bring their food from the ceil to the bottom of their habitat (Bouché, 1972). Nowadays, some studies on earthworms in polluted sites and soils exist (Butt and Quigg, 2020; Coelho et al., 2018; Shang et al., 2013) but a very little on urban artificial mediums, without voluntary colonisation.

Earthworms have already been investigated regarding their interactions with pollutants (Datta et al., 2016; Espinosa-Reyes et al., 2019; Yasmin and D'Souza, 2010). Most studies focusing on earthworm abundance, diversity, resilience and adaptation to chemicals has been mostly performed for cultivated soils and with the objective to use earthworms to improve the soils (Decaëns et al., 2008; Givaudan et al., 2014; Rodriguez-Campos et al., 2014). The study of bioconcentration in earthworms in urban or peri-urban soils has been the subject of a few recent studies and mainly restricted to the study of metal trace element (MTE) (Nannoni et al., 2014). Thus, Nannoni et al. (2014) showed that the uptake and accumulation of Cd, Cu, Pb, Sb and Zn by earthworms were affected by some physicochemical properties of the soil, such as carbon and carbonate contents. In a recent study, Coelho et al. (2018) studied the impact of MTE in an infiltration pond and clearly proved the transfer of metals to earthworms, using the species *Eisenia fetida.* Then, earthworms have the capacity to accumulate organic (and inorganic) contaminants present in soils (Morrison et al., 2000). Specifically, studies have demonstrated that several earthworm species are able to accumulate persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), brominated flame retardants**,** pharmaceuticals, detergent metabolites, polycyclic aromatic hydrocarbons, and pesticides (Carter et al., 2014; Kinney et al., 2008). Since the beginning of the 21st century, some POPs are classified in the Stockholm Convention's Annexes in order to encourage governments to eliminate or reduce their production which is a risk for the environment and health, as we still find them everywhere, at significant concentrations, and as they bioaccumulate through the trophic chain (Boethling et al., 2009; Bruce-Vanderpuije et al., 2019; González-Mille et al., 2019; Nadal et al., 2015). POPs are thus persistent, bioaccumulative, easily transported by the air and the water in the vadose zone and the groundwater and through the trophic chains by animals. POPs are also well known for their toxicity and adverse effect on ecosystems and human health (Ashraf, 2017).

Earthworms can accumulate POPs passively by dermal absorption, and actively through soil ingestion. If the relative importance of dermal and dietary exposure depends on the individual contaminant, some results highlighted that the importance of the dietary pathway increases with the hydrophobicity of the contaminant (Jager et al., 2003; Ma et al., 1998). Vijver et al. (2005) evaluated the importance of different pathways for metal uptake in *Lumbricus rubellus*. They concluded that the main route to metal accumulation was dermal absorption because ingestion via pore water uptake represented only a small contribution. Regardless the bioaccumulation pathways, Ville et al. (1995) observed the accumulation of PCBs in all earthworm tissues for several earthworms species (*Eisenia andrei*, *Eisenia fetida*, *Eisenia hortensis* and *Lumbricus terrestris*), proving that once in the earthworm flesh, the MTE may affect all organs and thus have a toxic effect.

The question of earthworms in stormwater infiltration basin has never been raised and treated into details. In this study, we investigated an infiltration basin that receives the stormwater from an industrial catchment. We questioned the link between POPs, edaphic conditions and earthworms' communities in that kind of artificial system. We expect quite significant levels of POPs and related consequences on earthworms' communities, at least to enhance bioaccumulation. More specifically, the aim of this study is: (i) to attest the presence and assess the quantity of earthworms in such infiltration devices; (ii) to characterize the influence of the biotope (pollutants and edaphic conditions) on earthworms' abundance and (iii) to assess the impact of pollutants on earthworms' bioaccumulation. Every part is assessed specifying the groups: total, age (juveniles and adults) and functional group (epigeic, endogeic, anecic).

2) Material and methods

a) Study site

The studied infiltration basin is located in the industrial area of Chassieu (69680, France) and is referred to as the Django Reinhardt basin (45°44'09.1"N 4°57'27.0"E). The basin is 2 hectares in area and is preceded by a retention pond. This latter artificial system allows the decantation of most of the suspended solids carried by the stormwater. When stormwater reaches a given threshold, water overflows and enters the infiltration basin. Then, stormwater runs over the soil surface and infiltrates into the soil. As the removal of suspended solids is never complete, some particles carried by the water enter the infiltration basin and tend to accumulate at the soil surface when water infiltrates into the soil. Then, a sedimentary layer settles down at the surface and participates in the formation of the upper horizon, which can be colonized by plants and fauna. Due to the topography of the site, sediment deposition is not homogeneous and leads to the regionalization of flow pathways at the surface (**Figure 1**). In the following, we use the term "sediment" to mention this upper horizon that separates the fluvio-glacial substrate to the atmosphere, and that host most of the fauna and flora (Badin et al., 2009).

b) Physicochemical characterization of the sediments

The sediment samples were also taken at each plot over its entire height, close to the harvest zone, and were stored in a cold chamber at +4°C in glass jars without drying or sieving. The sediment was characterized in terms of height and physico-chemical properties. The sediment height was measured manually by coring the sediment till its subbase and measuring the depth between the subbase and the sediment surface. The organic matter was determined by loss-on-ignition at 550°C during 4 hours (NF EN 15935). The water content was determined by heating the soil at 105°C for 24 hours (NF ISO 11465) and by differentiating the weight before and after drying, leading to the determination of the weight water content as follows:

$$w = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (1)$$

where m_{wet} and m_{dry} refer to the weight of the wet and dry matrices. The pH was determined after two hours of agitation and five hours of resting by the norm NF X31-103 (Badin et al., 2009).

For the pollution, we focused on the POPs. We considered the following families of pollutants with their related congeners:

- Polychlorinated dibenzodioxins (PCDDs), gathering the following 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, OCDD.
- Polychlorinated dibenzofurans (PCDFs), gathering the following 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, OCDF.
- Polychlorinated biphenyl non-ortho-substituted (PCBno), gathering the following congeners: PCB 77, PCB 81, PCB 126 and PCB 169.
- Polychlorinated biphenyl mono- or di-ortho-substituted (PCBmdio), gathering the following congeners: PCB 105, PCB 114, PCB 116, PCB 123, PCB 156, PCB 157, PCB 167 and PCB 189.
- Polychlorinated biphenyl non-dioxin like (PCBNDL), gathering the following congeners: PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180.

The POPs contents were determined by congeners and gathered also by family. These were determined by the laboratory LABERCA in Nantes (La Chantrerie - Route de Gachet, 44307 Nantes, France) for all the solid matrices (including sediment, see Annex 1, and dry earthworms, see Annex 2). For these pollutants, we study the contents for each congener plus for the sum of congeners. The others pollutants (e.g., metallic trace element) were also determined but are not shown since they do not interfere with the results presented in this study.

c) Harvest and identification of earthworms

The harvests were carried out in April 2013 over a period of fourteen days. Fourteen one-meter-square plots were delimited in the basin in order to represent the diversity of edaphic conditions and biocenosis. In particular, we sampled three specific water pathways at the soil surface (**Figure 1**) to get observations representative of the observed spatial variability. These fourteen plots are named DJ + a number (**Figure 1**).

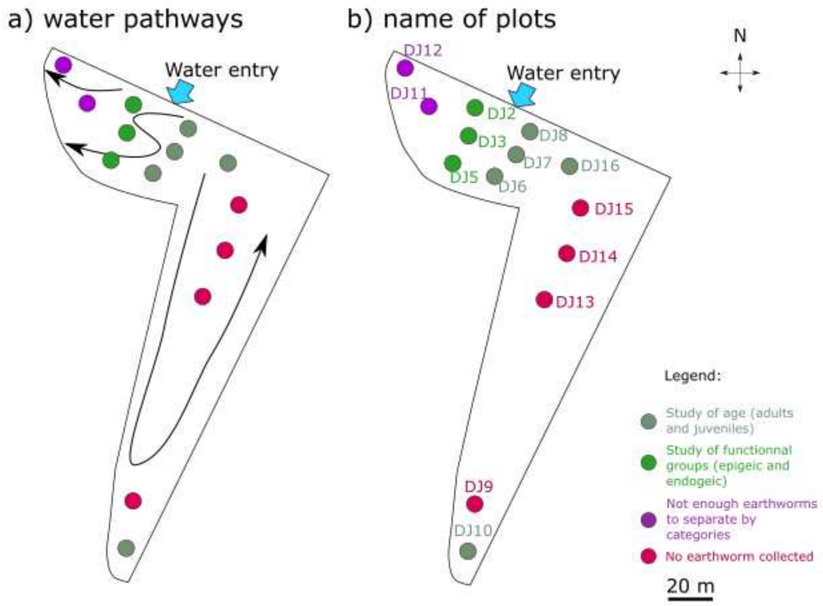

Figure 1 : Representation of a) the water pathway at the soil surface (where water infiltrates and evaporates), and b) the 14 plots on the infiltration pond Django Rheinhardt. In the red plots (DJ9, DJ13, DJ14, DJ15), no earthworms were collected. In the purple ones (DJ11, DJ12), the data were only used for the first part of the study: the analysis of abundance. The green ones, more (DJ6, DJ7, DJ8, DJ10, DJ16) or less (DJ2, DJ3, DJ5) dark, separate the plots which were used to analyze the pollutants contents according to the age or functional group.

The earthworms were sampled according to the method developed by the OPVT[1] (*Observatoire Participatif des Vers de Terre, Participative Observatory of Earthworms*) from the University of Rennes (Andrade et al., 2021). The samplings were made under the shadow of a tent to prevent from the soil exposure to the sun and related heating. The vegetation was removed manually before spreading a stinging solution (300 g of mustard Amora Fine et Forte® diluted in 10 litres of water). The solution was disposed a first time on the surface of the plot. Then, the earthworms coming out of the ground were collected and put in a water bowl. After approximately 15 minutes, the protocol was reiterated between three or four times until no more earthworm appear. This protocol had already proved efficient in most cases and soils (Andrade et al., 2021). However, note that only living earthworms are collected with that method.

Afterwards, the earthworms were classified by age and functional group for each plot. Firstly, they were separated between juveniles and adults. Then, when possible, the adults were separated by functional groups between epigeic, endogeic, anecic (Bouché, 1972; OPVT). For every plot, the quantities of juveniles, adults, epigeic, endogeic, anecic and unclassified earthworms were determined, depending on the collections. In some cases, because of too few earthworms, some of the groups could not be determined.

d) POPs contents in earthworms and bioaccumulation

The contents of POPs in earthworms were determined as follows. The collected earthworms were gathered by groups before being rinsed with deionized water and placed in moist filter paper to disgorge their gastrointestinal tract for 24 hours (OECD, 2010). After that, the individuals corresponding to the replicates done per sample where put together, weighted and frozen (at -20°C). Then, the samples of dry matter were sent to the laboratory LABERCA for the determination of pollutant contents (mass of pollutant per unit mass of dry matter, see Annex 2). In some cases, some of the groups (juveniles, adults, epigeic, endogeic, and anecic) could not be properly characterized

[1] https://ecobiosoil.univ-rennes1.fr/OPVT_accueil.php

because of the lack of earthworms in the sent sample. Consequently, the questions were adapted to the plots according to the earthworm collections. Five plots were thus tested for pollutant contents as a function of juveniles and adults (DJ6, DJ7, DJ8, DJ10 and DJ16). Only three allowed to characterize pollutant contents as a function of the functional groups (DJ2, DJ3 and DJ5). In the end, we obtained one single value per group and per plot (e.g., "DJ7 juveniles'', "DJ7 adults", "DJ3 epigeic", "DJ3 endogenic") for each congener and each family (sum of congeners). Most of the values could be determined because only 2,2% of the values were under the threshold of detection.

We also determined the bioaccumulation factor (BAF) that quantifies the ratio of the pollutant content in the organisms to the pollutant content in the medium (i.e., the sediment). The BAF is similar to the factor of bioconcentration in Amutova et al. (2021) and allows the detection of bioaccumulation, i.e. when the organism concentrates the pollutants in its body. The BAF is computed for each congener but also for the families by summing POPs contents among congeners. As for DJ2, DJ3, DJ5, DJ6, DJ7, DJ8, DJ10 and DJ16 there was enough earthworms collected to separate them in two groups (adults and juveniles or epigeic and endogeic), the "total BAF" per plot was averaged over the groups. However, there is only one value of BAF for the families in DJ11 and DJ12 (**Figure 1**).

e) Statistical analyses

The statistical analyses were performed in three stages, using the R software©. Principal component analysis (PCA) were performed to see the potential correlations between parameters. Pearson correlation tests (Pearson, 1920) were also performed to test correlation between variables. We also verified that the required conditions were fulfilled: i) residues independency (Durbin Watson test), ii) residue normality (Shapiro-Wilk test), and iii) residue of homogeneity (Breusch-Pagan test). Spearman correlation tests were also realized, with its single condition of use: the Durbin Watson test. Only correlation factors higher than 0,6 or lower than -0,6 were considered. The differences in medians between groups were checked with a Wilcoxon test. For all the tests, we rejected the null hypothesis (no correlation or no difference between means) for p-value below the threshold of 0,05. For the graph part, the boxplots were created with the ggplot package in R©.

3) Results and discussions

a) Sediment physicochemical characterization

The sediment features exhibit a great variability (**Figure 2**). The physicochemical properties show the following trends: the sediment thickness varies from 4 to 19 cm, the water content from 30 to 71%, the pH from 6,52 to 7,51, and the organic matter content from 15 to 36% (w/w). Our findings are in line with previous findings. For instance, Badin et al. (2009) found similar values of pH, but slightly lower values of OM contents with 14.3±0.4 % and of water contents with values around 18.6±0.7 %, in the same infiltration basin. However, the order of magnitude and the spatial gradients were respected. The higher values of sediment heights reveal the sedimentation of a thicker layer close to the entry. The higher water contents close to the entry are also logical since the stormwater enters the basin at that location, thus providing large amounts of water. Conversely, the OM content and the pH seem less variable.

The POPs contents strongly vary between families (**Figure 3**). The POPs contents sort as follows by increasing order: PCDFs contents range from 147 to 302 ng/kg of dry weight (dw); PCDDs contents from 870 to 1438 ng/kg dw; the PCBDLno contents from 864 to 3228 ng/kg dw; PCBDLmdio contents from ≈ 20000 to almost 60000 ng/kg dw, and PCBNDL contents from ≈ 300000 to almost 800000 ng/kg dw. The difference between those concentrations is statistically significant (Wilcoxon test's p-values << 0,05 for all pairs of values, between each family). As POPs are hydrophobic pollutants (Ashraf, 2017), PCBDLno and PCDF contents were significantly negatively linked to the water content. Differences in POPs content between different plots may also depend on the organic matter content but also on the sediment height. We may expect this last correlation to reflect the influence of the local hydraulic conditions. Indeed, the organic particles are expected to deposit where the flow rates are lower, i.e., in the parts of the basin prone to sedimentation. However, the observed differences were statistically different. Our findings compare well to previous studies (Annex3): considering the sum PCBNDL + PCB 118, we obtained an average value of 560 ng/kg dw, which corresponds to the orders found by Liber et al. (2019) and Mourier et al. (2014) in sediments from the Rhone river, in the same region. The content of six PCBNDL congeners + PCB 118 in an infiltration basin close to our study site was 418000 ng/kg dw (Datry et al., 2003). Regarding PCDDs and PCDFs, smaller contents than our values by one or two orders of magnitude were found in river banks (Coelho, 2019). These contents are significant and then expected to have toxicological effects. Heavy metals were also found on the site, but we chose to focus on the POPs potential risk in that study.

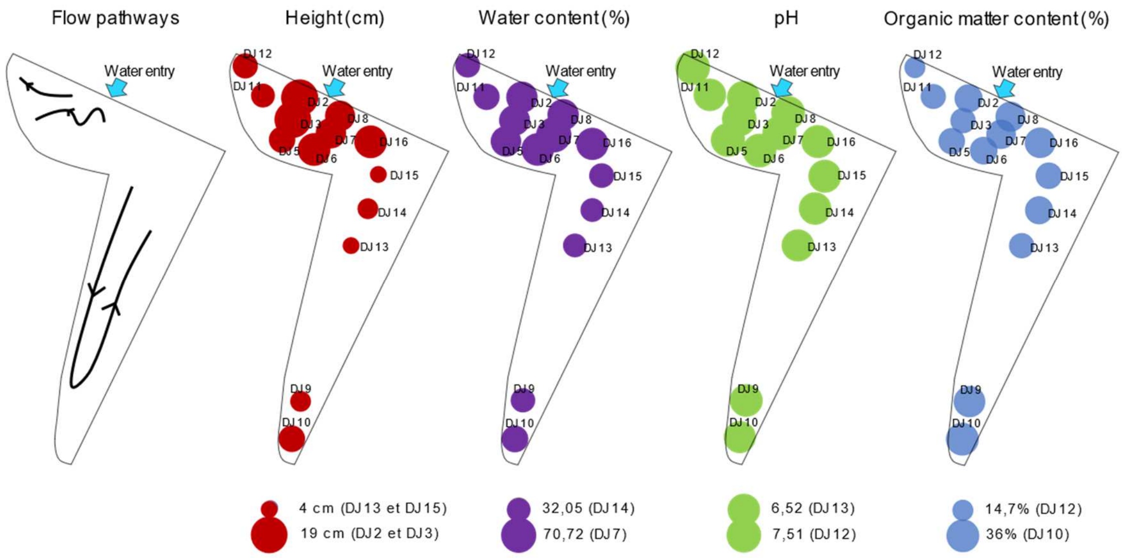

Figure 2 : Physicochemical characterization of the sediment from left to right: flow pathways, height (cm, one value per plot), water content (%, mean of 2 values per plot), pH (mean of 3 values per plot), organic matter content (%, mean of two values per plot).

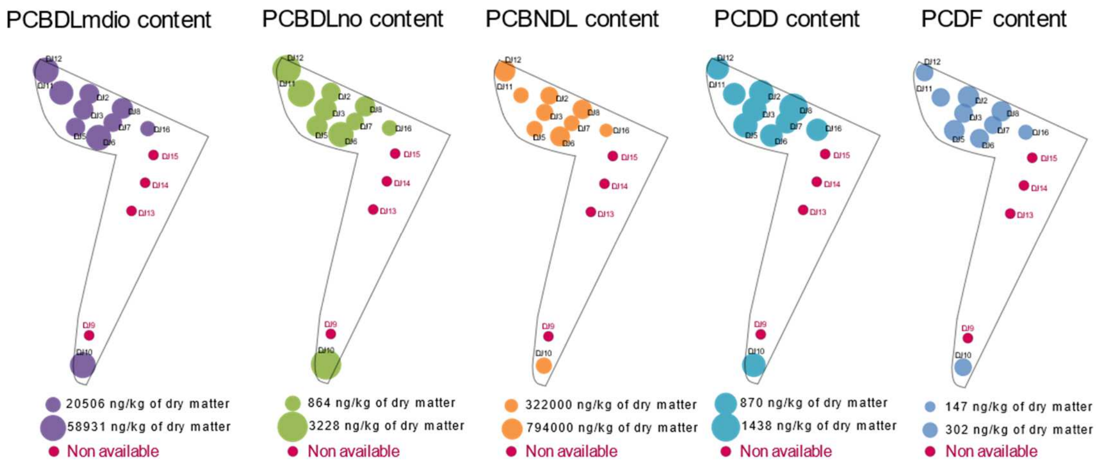

Figure 3 : Contents of POPs in the sediment (one value per plot). Classified by family of POPs, from left to right: PCBDLmdio, PCBDLno, PCBNDL, PCDDs, PCDFs.

b) Total abundance

In total, 1361 earthworms were collected, with a large variation depending upon the considered plots. Earthworm densities ranged from 0 to 246 earthworms.m^{-2}. Four plots (DJ9 and DJ13-15) had no earthworms at all (**Figure 4**). Our values were in line with the regular order of magnitude for urban soils. Butt and Quigg (2020) found a mean of 208 earthworms.m^{-2} in an artificialized old steelwork's soil, Baker et al. (1997) more than 140 earthworms m^{-2} in Australian pastures and orchards versus more than 50 earthworms m^{-2} in cropping soils. In engineered and urban grasslands soils, 200 to 500 earthworms. m^{-2} were collected (Maréchal et al., 2021).

The adults constituted 38% of the total population on average. Most of the adults had their functional group identified, apart from a few exceptions (**Figure 4,** DJ12 and DJ6). No anecic were found. We found only endogeic (*Allolobophora rosea rosea*, *Microscolex sp.*, and *Allolobophora icteria*) and epigeic (*Aporrectodea* sp. and *Lumbricus rubellus*) species with relative proportions of 51% and 49%. The absence of anecic is assumed to result from the too small height of sediments (≤ 19 cm). Our results strongly contrast with Butt and Quigg (2020) who found 5% of epigeic and 39% of anecic. However, these authors explained the small fraction of epigeic by the large decrease in water contents and the fact that the main epigeic species are semi-aquatic. In the infiltration basin, water contents are quite high, as the result of large volumes of stormwater entering the basin. These conditions may have promoted the colonization by epigeic species.

The juveniles were present in large proportions in most plots (62% in average). However, the spatial variability was very strong. While the juveniles predominated in spots DJ2-DJ8 and DJ16, they were absent from several spots (DJ9, DJ13, DJ14, and DJ15) and had very small proportions in the others (12% in DJ10; 16% in DJ11; 25% in DJ12). In a study at seven different study sites in Slovakia, earthworm density, body biomass, and diversity in relation to land use (arable land, permanent grasslands), management, and selected abiotic (soil chemical, physical, climate-related) and biotic (arthropod density and biomass, ground beetle density, carabid density) indicators were analyzed (Kanianska et al., 2016). These authors observed that the percentage of earthworm juveniles within the community was only slightly higher in arable land (80%) than in permanent grasslands (72.4%), and they obtained a positive correlation between earthworm density and biomass with soil moisture in arable land (Kanianska et al., 2016). Our study observed an excellent linear relation between juvenile presences/number and sediment height (R2=0.63; data not shown). The prevailing water potential in the soil had already been correlated to A. caliginosa cocoon production, cocoon development, and growth of juveniles under laboratory conditions (Holmstrup, 2001). Moreover, soil moisture may have an important influence on food availability. Indeed, the ingestion of soil food can be more accessible when soil moisture is high. Consequently, the observed effect of water content on adult biomass and the juvenile number may be due to its direct impact and the combination of such an impact with the adverse impact of water on food intake. Another correlation is also established between earthworm abundance and juvenile percentage (p-value<<0,05, r=0,94), with the greatest percentages of juveniles at the places with the highest earthworm abundances. We then assume that when conditions are favourable to the earthworms, juveniles expand, explaining the link between abundance and juveniles. The proper edaphic conditions (water content, sediment height, and organic content) will then promote, at the same time, earthworms abundance and the expansion of juveniles, explaining the correlation between abundance and juveniles.

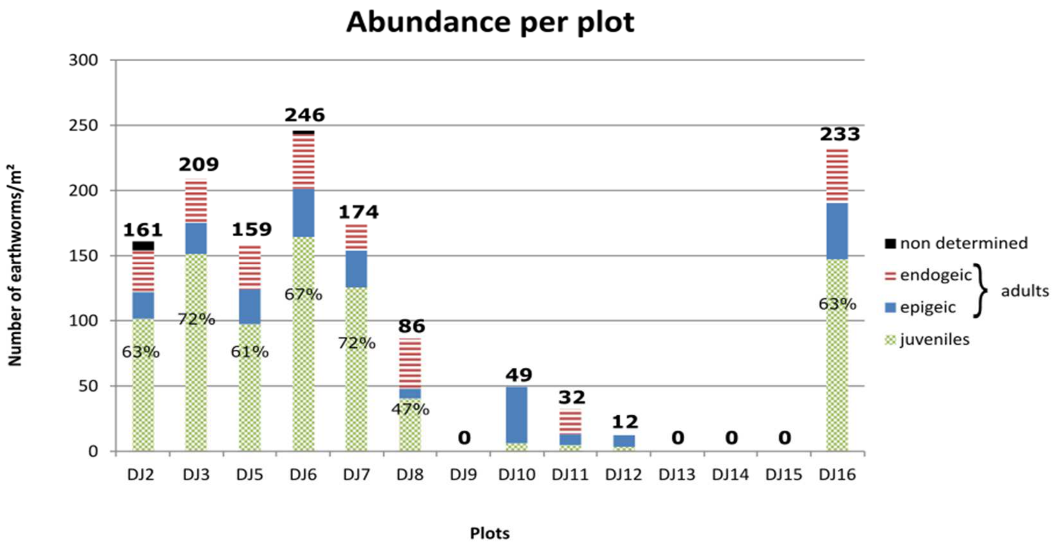

Figure 4 : Abundance per plot of earthworms. The percentage of juveniles is indicated in the green grid histograms. The number in bold is the total number of earthworms collected per plot.

Statistical analyses were performed to correlate the earthworms' abundance and age with edaphic and physicochemical conditions (sediment height, water content, pH, OM, and POPs contents). The total abundance proved strongly correlated to water content and sediment thickness, regardless of age and functional groups. The correlation tests validate the positive correlation between the water content and every category of earthworms (p-values<<0,05, r≃0,9). We assume that above a given threshold (approximately 80%), the sediment becomes too water-saturated to allow any chance of survival for earthworms. Below this threshold, the water content promotes earthworms' colonisation, and the correlation remains, the earthworms preferring wet conditions. Regarding the sediment height, we found the same positive correlation with very high values of coefficients of correlation (p-values<<0,05, r≃0,9) for all categories of earthworms (juveniles, adults, epigeic and endogeic). Logically, the height of sediments allows the earthworms to move in any direction, and the earthworms do not colonize the soil below, this being mostly made of mineral deposits with low organic content (Badin et al., 2009; Lassabatere et al., 2010). We thus found the lowest quantities of earthworms in DJ9-DJ15 because the height of sediment and the water content are also the lowest. These poor edaphic conditions could explain the lack of juveniles in DJ10-DJ12 (**Figure 4**) with stress decreasing reproduction.

Earthworm abundance and age seemed also positively impacted by **pH**, but to a lesser extent, with low values of correlation coefficient (r≈0,5). Our findings align on Vergnes et al. (2017) who showed a positive relation between earthworms' abundance and pH in anthroposols. The total content in organic matter (OM) showed no clear impact as well. Regarding pollution, the total abundance was only significantly linked to the PCBDLno contents in sediments (Pearson: p-value<0,05, r=-0,67). The negative correlations proved the negative and potential toxic effect of PCBDLno. For the other POPs, no clear effect could be detected on the total abundance. However, some trends appeared when the effects were tested per group. Significant (negative) effects were found for the following cases: PCDDscontents in the sediment on epigeic (Spearman, p < 0.50, r=-0,69) and PCBDLno contents in sediment on juveniles and endogeic (Pearson r=-0,73). We conclude that the POPs may have negative impacts on earthworm abundance, as already put forward in industrial and urban soils (Espinosa-Reyes et al., 2019), and for others pollutants in artificial soils as MTEs (Coelho, 2019) and pentachloronitrobenzene (Li et al., 2019). However, the trends are quite tiny here, with more effect of PCBDLno.

c) POPs contents in earthworms and bioaccumulation

The contents of POPs in the earthworms (**Figure 5)** were studied for 8 plots: DJ2, DJ3, DJ5, DJ6, DJ7, DJ8, DJ10 and DJ16 (see green plots in **Figure 1**). Indeed, the mass of earthworms was not enough for the POPs detection in the others plots.

The POPs contents in the earthworms are in the same order than contents in sediments (**Figure 5**): PCDFs (24 ng/kg dw) << PCDDs (98 ng/kg dw) << PCBDLno (432 ng/kg of dw) << PCBDLmdio (11483 ng/kg dw) << PCBNDL (143990 ng/kg dw). These differences in POPs contents are significant (Wilcoxon test's p-value<<0,05**, Figure 5**) and remain the same regardless of the age (juveniles versus adults) and the functional group (endogeic versus epigeic). For one family of POPs, no difference of content is found between adults and juveniles. Conversely, we noticed higher contents in the epigeic groups than in the endogeic ones. However, the difference was not statistically significant. This absence of statistical significance was supposed to result from the too small size of the samples (n = 2 groups*5 plots for the functional groups).

POPs content in earthworms and sediments do not seem to be correlated, but to have a linear relation, as if the content in earthworms already have reach a saturation threshold. In that case, the most contaminated sites do not imply necessarily the biggest POPs content in earthworms.

We also tried to correlate the contents of POPs in earthworms with the characteristics of the sediments at the different plots. The contents in earthworms of PCDDs and of PCBDLmdio were significantly linked to the OM content of the sediment (Pearson r=-0,83 and Pearson r=-0,66, respectively). No other correlation could be found for the other cases.

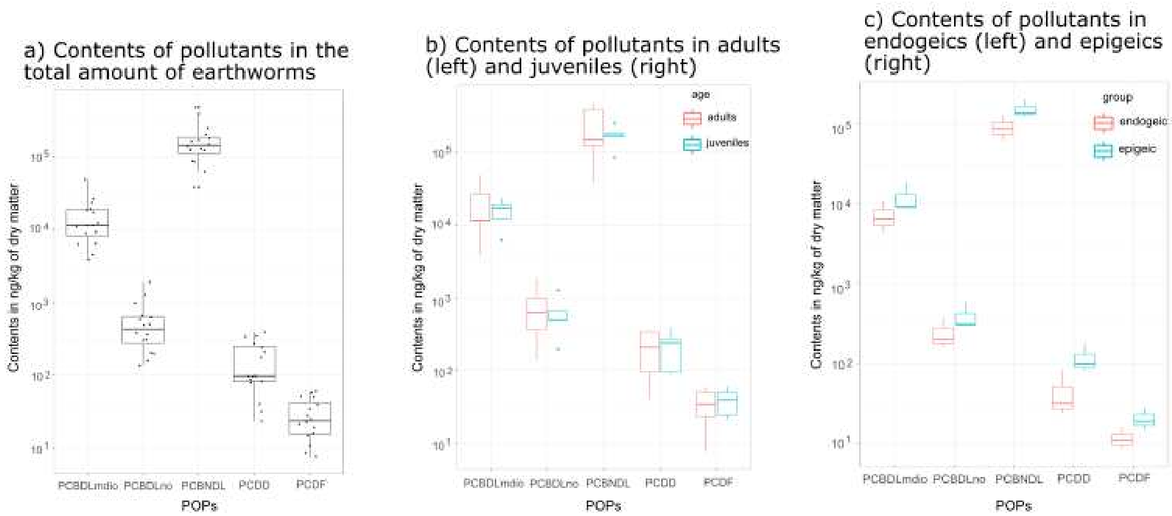

Figure 5: Contents of pollutants in earthworms according to each family of POPs. (a) There are two points per plot (one for juveniles, one for adults or one for epigeic, one for endogeic) for all the eight plots considered here (D2, DJ3, DJ5, DJ6, DJ7, DJ8, DJ10 and DJ16). Each point, per family, is the sum of all the congeners for one category on one plot. (b) Idem with adults and juveniles separated. (c) Idem with epigeic and endogeic separated.

The observed contents are in line with previous studies. In Japan, the analysis of earthworms in rice fields showed tissue levels of 150 µg.kg^{-1} fresh weight of PCBDL (Nakamura et al., 2007). In East China, *E. fetida* and *Allolbophora caliginoa* trapezoides species collected in a typical e-waste dismantling area, showed PCB accumulation in tissues at levels of 1.17 up to 78.6 µg.kg^{-1} dw with PCDDs, and PCDFs accumulation between 0.13 and 0.59 µg.kg^{-1} dw (Shang et al., 2013). Henriksson et al. (2017) also demonstrated the accumulation of PCDDs and PCDFs in *E. fetida* tissues at concentrations of 1.5 to 15000 µg.kg^{-1} dw in Swedish contaminated soils. The accumulation of PCDDs and PCDFs in the tissues of two others earthworms' species, *Allolobophora catiginosa* and *Lumbricus rubellus*, were also observed by Nakamura et al. (2007) who reported concentrations of 0.9 µg.kg^{-1} dw of PCDDs and PCDFs in earthworms taken from rice fields. After a 28-days exposure to contaminated soils, Coelho (2019) measured the PCBs and PCDD/Fs contents in *Eisenia fetida* and concluded to no difference between adults and juveniles and contents ranking as follows: PCBNDL >>> PCBmdio >> PCBno >>> PCDDs >> PCDFs. Our higher observed contents of some POPs in earthworms (*i.e.*, PCBNDL and PCBDL) are thus in agreement with previous studies.

d) Bioaccumulation Factor (BAF)

The bioaccumulation factor (BAF) was computed from the ratios of POPs contents in the earthworms to those in the sediments to quantify the potential mobility of POPs and the risk of bioaccumulation. Effective bioaccumulation occurs for a BAF>1 (Shang et al. 2013). In the following, we do not consider the values from DJ11 and DJ12, in which we had not enough values to separate adults and juveniles, epigeic and endogeic. Then, we only consider the plots DJ2-DJ8, DJ10 and DJ16. We also discussed the differences between the two clusters, (i) PCB cluster including PCDBLno, PCBDLmdio, PCBNDL, and (ii) PCD cluster including PCDDs and PCDFs. We computed BAF for each congener before gathering them by POPs family. Indeed, previous treatment showed that averaging over congeners lowered the values of BAF, resulting in low values of BAF per POP family. **Figure 6** then illustrated BAF per congener, per earthworm group (adults, juveniles, epigeic and endogeic) and per experimental plot (for the plots considered).

The BAF differed between POPs families with differences between the two clusters PCB and PCDD/Fs (**Figure 6**). PCB exhibited higher values than PCDD/Fs, with some very large values, indicating very strong bioaccumulation in some cases. Note that those highest values of BAF are outliers and thus are separated from the main distribution that remains lower than unity (**Figure 6a**, points indicating outliers). Thus, bioaccumulation remained the exception. For PCDD/Fs, no value of BAF exceeds unity, indicating no bioaccumulation at all. Shang et al. (2013) reported that the strong affinity of PCD for organic matter sorption sites may explain its low bioavailability resulting in low bioaccumulation factors.

Globally, the bioaccumulation did not depend on the earthworm age (**Figure 6b**). Wilcoxon's tests indicated similar values between adults and juveniles (tested on DJ2, DJ3 and DJ5), except for PCDDs. Conversely, BAF values seemed to vary between functional groups (tested on DJ6, DJ7, DJ8, DJ10 and DJ16, **Figure 6c**). Wilcoxon tests indicated significant differences between endogeic and epigeic for all POPs families, except for PCBDLno with p-values close to the limit. We may conclude that regarding bioaccumulation, earthworm species count more than age. We also tested the influence of the sediment characteristics (water content, OM, pH) on BAF. The OM content was proved significantly correlated to the BAF for PCDDs (Pearson r=0,70) and PCDFs (Pearson r=0,75; Spearman r=0,67). For the others physicochemical parameters (pH and water content) we cannot conclude to any specific trend. In a study with *Eisenia andrei*, the uptake kinetics of four hydrophobic organic pollutants (pyrene, lindane, p,p'-DDT, and PCB 153) in aged laboratory-contaminated natural soils showed different uptake behavior by earthworms (Svobodová et al., 2020). In the case of p,p'-DDT and PCB 153, BAFss (calculated on the first day of the steady-state) were between 8.2 to 3.1 according to the soil, and lowest than the BAF21 (calculated after 21-day exposure) with a range between **11.5 to 5** (Svobodová et al., 2020). For *Lumbricus rubellus,* an anecic typology of earthworm, Vermeulen et al. (2010) showed BAF for ΣPCBs between 1.09 to 2.76. In a similar experimental design as ours, with also *Eisenia fetida*, Coehlo (2019) obtained BAFS for adults and juveniles with four different soils on

Casier Girardon Peyraud (-PK 61.500, in the Rhone River margin; France) (Table Annex 4). Thus, our study's values for PCDD and PCDFs are lowest and more or less in the same range for both adults and juvenile earthworms. But for PCBs, we observed lower trends for all categories and ages in relation to different contamination levels and other physiochemical characteristics (Table Annex 4).

We zoomed in the cases of bioaccumulation (BAF >1). The highest values of BAF were found for PCBNDL and PCBDLmdio, with values up to almost 5 (**Figure 6**). Some functional and age groups, and some congeners, were particularly involved (**Figure 7)**. For the family of PCBDLno: PCB 77, PCB 81 and PCB 126 had respectively one, three and one occurrences. For the family of PCBDLmdio: PCB 105, PCB 114 and PCB 118 had two, six and three occurrences, respectively. Finally, for the PCBNDL family, PCB 28, PCB 52, PCB 101, and PCB 153 had nine, four, and twice one occurrences, respectively. These results point at the congeners that may be more involved in bioaccumulation and thus in ecotoxicity, *i.e.*, congeners PCB 114 and PCB 28 that seem involved in most of the cases of bioaccumulation. **Figure 7** also show that all the earthworms may be concerned by bioaccumulation. Thus, the bioaccumulation strongly depends on congeners and may concern every category: juveniles, adults, epigeic and endogeic.

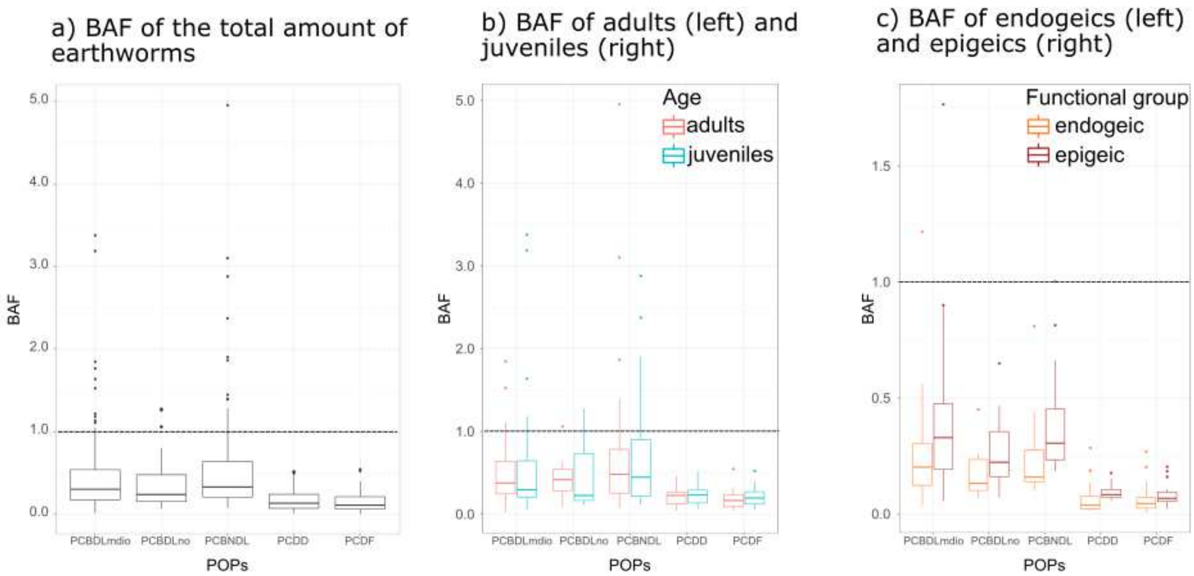

*Figure 6 : Bioaccumulation factor according to the families of POPs with all values for each congener (number of plots*2 categories (adults/juveniles or epigeic/endogeic)*number of congeners per family) for (a) all earthworms, (b) adults and juveniles, (c) endogeic and epigeic.*

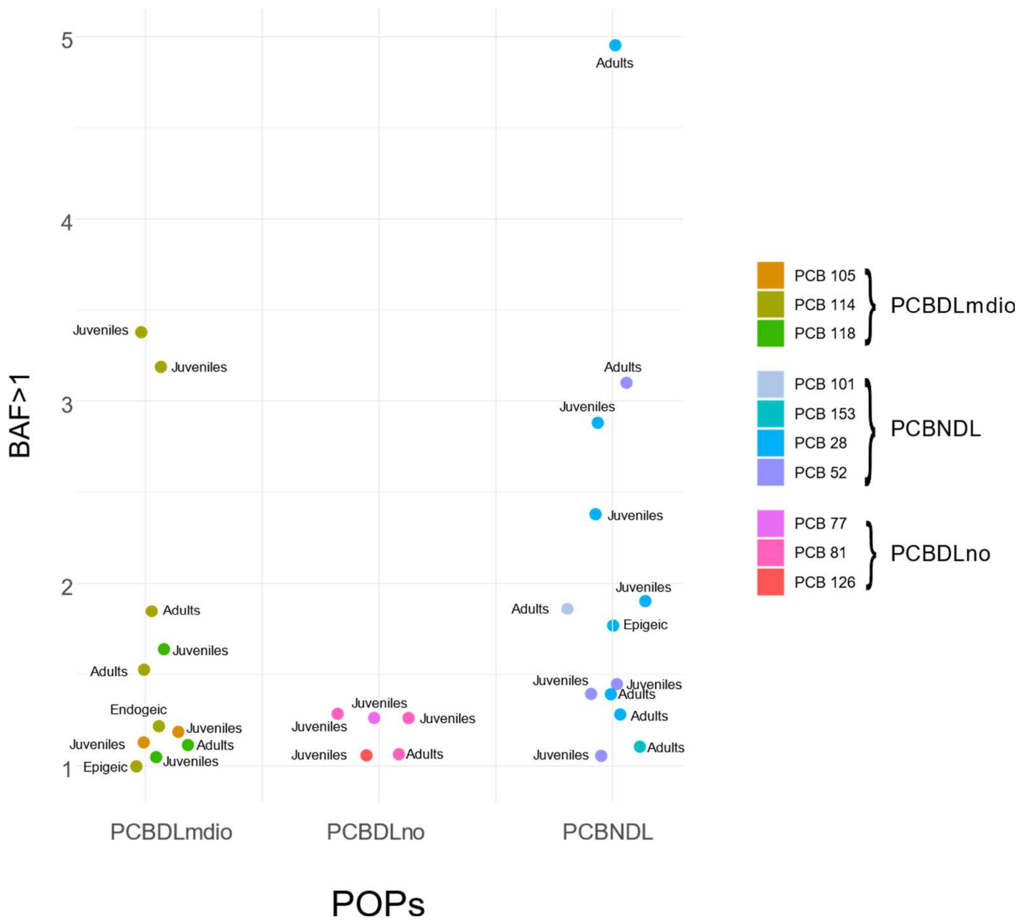

Figure 7 : BAF higher than one per family of POPs. The colors precise the congeners, and the categories (juveniles, adults, epigeic, endogeic) are given.

e) Key factors of POP transfers into earthworms

Different factors could affect the transfers of POPs into earthworms: the environment, the earthworms, and the POPs themselves (**Figure 8**). Shang et al. (2013) outlined sediment parameters can affect the bioavailability of pollutants and thus modify the bioaccumulation risks. However, our results above show that the degree of contamination (POP content) and most of the sediment physico-chemical parameters did not impact the values of BAF, except OM content that favored bioaccumulation. Amutova et al. (2021) discussed the transfers in pollutants according to four physiological steps: absorption, depending on the specie and the pollutants; metabolization, different according to the organs and steps; distribution through blood; and finally, excretion. Thus, the variations of transfers potentially change according to the functional groups, as epigeic and endogeic do not live in the same part of the sediment (leaves/litter versus first centimeters of the sediment).

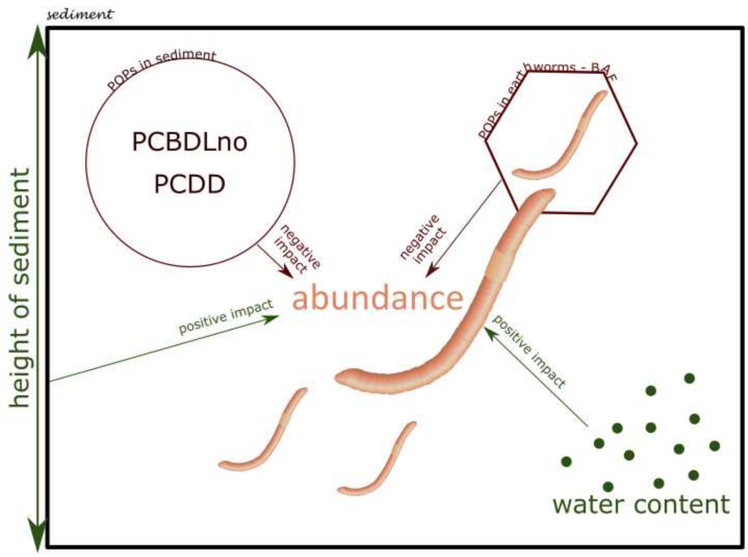

Figure 8: Graphical abstract

The hydrophobicity, a characteristic of POPs, could have an impact on their transfer. We split hydrophobicity considering log(K_{ow}) in four clusters of congeners: from the minimum to the first quartile (Q1), from Q1 to the median, from the median to the third quartile (Q3) and from Q3 to the maximum. The medians are significantly different between the first two and the last two groups. The hydrophobicity is significantly linked to the BAF of PCBNDL (Pearson r=-0,88; Spearman r=-0,94) and of PCBDLmdio (Spearman r=-0,79): the higher the hydrophobicity, the smaller the BAF, at least for PCBDLmdio and PCBNDL. We cannot conclude for the other POPs. Hydrophobicity can explain bioaccumulation, as POPs tend to adsorb on lipids, but only to a certain extent: here only for two families of POPs. However, the choice of log(K_{ow}) as the good descriptor for hydrophobicity has already questioned (Baker et al., 2000). In its review of previous works, Sabljic (2001) insisted on the diversity of descriptors for hydrophobicity, including solubility, parachor, or molecular conductivity. It is clear that the molecular size may also have an impact on bioaccumulation, as reported by Shang et al. (2013), and could be a good candidate for descriptor. In any case, as demonstrated above with our experimental results, each congener has their own properties and should be investigated when bioaccumulation is at stake. Assessing bioaccumulation at the scale of the POP family is a nonsense, and the study of the proportion of congeners per family should be considered.

In addition to the need to account for the chemical features, environmental conditions including the soil features should be considered. POPs bioaccumulation is directly linked to POPs adsorption onto soil organic matter, this last being more properly described by the value of the K_{oc}. This parameter quantifies organic molecules adsorption onto soil organic matter. K_{oc} was often linked to the value of K_{ow}, but such a link was already proved to be matrix dependent (Sabljic et al., 1995; Sabljic et al., 1997). Adsorption of POPs onto the soil OM will depend on the nature of OM particles and its features. The diversity of adsorption mechanisms and their dependency upon chemical and environmental conditions (*e.g.*, pH) may explain contrasting patterns of POPs adsorption and thus bioavailability.

In addition to chemical mechanisms resulting from the POPs and the soil features, POPs' BAF may strongly depend on the earthworm metabolism. Earthworms bioaccumulate POPs passively through dermal absorption, and actively through ingestion. The dietary pathway to bioaccumulation is facilitated by the lipid content of the gut and body wall. The greater the lipid and protein content in earthworms, the higher the bioaccumulation potential, but several different processes (such as uptake, depuration, metabolism and isomerization) also play a role in the final bioaccumulation of contaminants. The phenomenon of bioaccumulation is very complex, with a species-specificity (for dermal composition, metabolization and/or depuration, behaviour) that is controlled by physicochemical properties of both the contaminants and the soil. Put all together, bioaccumulation depends on the concentration and speciation of the contaminants, the type and characteristics of the soil, the temperature, the duration of exposure, the bio-accessibility/mobility of the contaminants, and their interactions with the other contaminants present in the soil. Thus, the pathways of exposures coupled with earthworm metabolism may rule the quantity of POPs adsorbed onto earthworms. Consequently, the age and the functional group of earthworms, that impact earthworm metabolism, are expected to influence bioaccumulation.

4) Conclusion

In this study, we investigated the effects of edaphic conditions and POPs contents on earthworm abundance and bioaccumulation factors, in an artificialized site, where their presence was proved. We observed several species, and most of them were adults and from the functional group endogeic. No anecic were detected. Those artificial systems that collect and infiltrate stormwater may thus be colonized by earthworms as any soils. The operating conditions along with potential contamination (risk of water ponding conditions and presence of urban pollutants) are not enough to prevent from earthworm colonization.

Two families of POPs have a significant and negative impact on some earthworms' groups abundance: PCBDLno on juveniles and endogeic; PCDDs on epigeic. On the contrary, the height of the sediment and the water content are beneficial for their presence and reproduction. Furthermore, POPs contents are also linked to physicochemical parameters of the sediment, especially OM content. Thus, we clearly demonstrated that earthworm abundance was mainly driven by edaphic conditions.

Lastly, we determined that the bioaccumulation of PCDD/Fs families is significantly different of the bioaccumulation of PCB families and depends, for the former, on the OM content in the sediment. Moreover, some congeners seem to bioaccumulate more than others. Finally, bioaccumulation is not different between juveniles and adults, except for PCDDs, but is significantly different between epigeic and endogeic, except for PCBDLno. And then, bioaccumulation was clearly revealed with some large values for certain pollutants and groups of earthworms, demonstrating the effect of pollution on the earthworms.

In terms of advice for sampling properly and investigating POPs bioaccumulation, we advise to collect more earthworms at different periods for each plot in order to assess the potential variability in time of the risk. The potential threshold of POPs contents in earthworms could also be assessed. Sampling should be done to maximize the number of earthworms in order to strengthen statistical tests. Indeed, in our study, some species were determined but not enough to assess statistically the links between species and all the factors we saw here. Lastly, measuring lipid content of each earthworms' sample should be performed in order to link the pollutants accumulation to the lipid content, that is known to favour POPs adsorption onto earthworms.

Moreover, in risk assessments, BAFs are used to estimate and predict the potential trophic transfer of contaminants from soil to wildlife. Therefore, in order to obtain a better understanding of the environmental fate of POPs, their accumulation, dispersion, or in order to model their fluxes, further studies would be useful and should be encouraged. Due to the increasing levels of these organic compounds in terrestrial ecosystems, it is important to study the occurrence, fate and transfer processes of POPs in earthworms, as well as the potential phenomenon of trophic biomagnification. These studies are essential to evaluate and manage the risks posed by organic pollutants, such as PCBs, PCDDs, or PCDFs, to ecosystems and human health, and should be linked with ETMs and other pollutants which could also play a role in the abundance of earthworms in such systems.

In terms of perspectives, these results could be also confronted with further studies accounting for other parameters such as enzymatic activities linked to detoxification in earthworms or their energetic reserves.

5) Annexes

Annex 1: POPs extraction in sediment

As explained in details in Coelho (2019), the Accelerated Solvent Extraction procedure with a SpeedExtractor (Buchi) was used for an aliquot of the samples from each plot. At 100 bar and 120°C, toluene/acetone [70/30, v/v] were injected three times for 5 minutes. The organic phase extracted was dried, weighed, dissolved in 15 ml hexane and added to 13C corresponding to standards. PCB were separated from PBDE with three cleaning steps, using acidic silica, Florisil® and celite/carbon columns. The POPs were quantified by gas chromatography and high-resolution mass spectrometry (GC-HRMS, 7890A (Agilent) / JEOL 800D (JEOL, Tokyo, Japan)). Two µl were injected in splitless mode with helium as a carrier gas (1 ml/min). The GC program differs for the families of POPs: 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 PCB; 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 for PCD. HRMS focused on two abundant ions, using Single Ion Monitoring mode. Quality assurance and quality control were made.

Annex 2: Determination of the lipid content.

As explained in details in (Coelho, 2019), based on (Smedes, 1999), the lipid content is determined gravimetrically, carrying an extraction with 2-propanol and cyclohexane (1g tissue with 1.6 ml of 2-propanol and 2.0 ml cyclohexane). At least 1g of dry tissue followed then the same steps as the sediment, two times for each sample. The obtained value is then divided by the lipid content.

Annex 3: Comparison of POPs content in different studies, in sediments and earthworms

POPs families	Content (ng/kg dw)	Matrix and localisation	Source
PCBNDL + PCB 118 (= PCB indicators)	560	Infiltration basin's sediment	*here*
	670-234 400	Sediments of the Rhone River	Liber et al., 2019
	18 700 (mean after 2007)	Sediments of the Rhone River	Mourier et al., 2014
	418 000	Infiltration basin's sediment	Datry et al. 2003
PCDD	870-1 438	Infiltration basin's sediment	*here*
	100-760	River banks	Coelho, 2019
PCDF	147-302	Infiltration basin's sediment	*here*
	30-100	River banks	Coelho, 2019
PCBDL	432-11 483	Earthworms from infiltration basin	*here*
	150 000	Earthworms from rice fields (Japan)	Nakamura et al., 2007
PCB	432-143 990	Earthworms from infiltration basin	*here*
	1 170-78 600	Earthworms from e-waste area	Shang et al., 2013
PCDD/F	24-98	Earthworms from infiltration basin	*here*
	130-590	Earthworms from e-waste area	Shang et al., 2013
	1 500-15 000 000	Swedish contaminated soils	Henriksson et al., 2017
	900	Earthworms from rice fields (Japan)	Nakamura et al., 2007

Annex 4: Values of mean BAF for all earthworms, juveniles, and earthworms.

POPs families	PCDD	PCDF	PCBDLno	PCBDLmdio	PCBNDL
Our study	0.21	0.18	0.44	0.45	0.40
POPs families	**PCDD juveniles**	**PCDF juveniles**	**PCBDLno juveniles**	**PCBDLmdio juveniles**	**PCBNDL juveniles**
Our study	0.22	0.19	0.49	0.48	0.36
Coehlo 2019	0.19-0.33	0.23-0.45	1.45-2.92	0.43-5.11	2.47-3.58
POPs families	**PCDD adults**	**PCDF adults**	**PCBDLno adults**	**PCBDLmdio adults**	**PCBNDL adults**
Our study	0.20	0.16	0.40	0.43	0.43
Coehlo 2019	0.28-0.45	0.44-0.62	5.8-10.5	1.39-16.8	8.16-14.6

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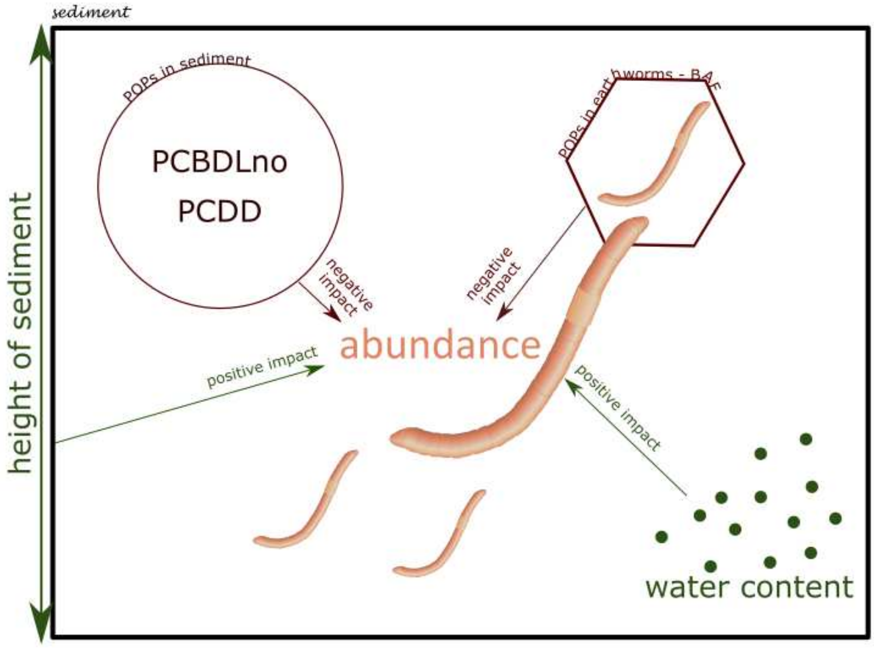

Graphical abstract