Environmental fate of sulfonated-PCBs: Soil partitioning properties, bioaccumulation, persistence, and mobility

Jessica Palladini [a], Elisa Terzaghi [a], Renzo Bagnati [b], Alice Passoni [b], Enrico Davoli [b], Angelo Maspero [a], Giovanni Palmisano [a], Antonio Di Guardo [a],*

[a] *Department of Science and High Technology (DiSAT), University of Insubria, Via Valleggio 11, 22100 Como, Italy*
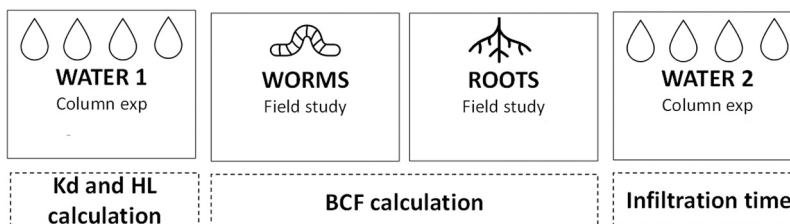[b] *Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche "Mario Negri" IRCCS, Via Mario Negri 2, 20156 Milan, Italy*

HIGHLIGHTS

- Environmental fate features and water analysis for sulfonated PCBs are presented.
- These chemicals have smaller soil/water partition coefficients than corresponding PCBs.
- Degradation in soil of these new metabolites appears to be as slow as PCBs.
- Sulfonated and OH-sulfonated PCBs can accumulate in plant roots.
- These chemicals move fast with soil water possibly reaching the aquifer.

GRAPHICAL ABSTRACT

STUDIES ON SULFONATED-PCBs and OH-SULFONATED-PCBs

WATER 1 — Column exp | WORMS — Field study | ROOTS — Field study | WATER 2 — Column exp

Kd and HL calculation | BCF calculation | Infiltration time

ARTICLE INFO

ABSTRACT

Two new classes of PCB metabolites were recently discovered: sulfonated-polychlorinated biphenyls (sulfonated-PCBs) and hydroxy-sulfonated-polychlorinated biphenyls (OH-sulfonated-PCBs). These metabolites, originating from PCB degradation, seem to possess more polar characteristics than their parent compounds. However, no other information, such as their chemical identity (CAS number) or their ecotoxicity or toxicity, is available so far, although more than about one hundred different chemicals were observed in soil samples. In addition, their physico-chemical properties are still uncertain since only estimations are available. Here we show the first evidence on the fate of these new classes of contaminants in the environment, producing results from several experiments, to evaluate sulfonated-PCBs and OH-sulfonated-PCBs soil partition coefficients, degradation in soil after 18 months of rhizoremediation, uptake into plant roots and earthworms, as well as a preliminary analytical method to extract and concentrate these chemicals from water. The results give an overview of the expected environmental fate of these chemicals and open questions for further studies.

1. Introduction

PCB metabolites were object of lot of attention in the past 40 years, especially the methoxylated-PCBs [3,37], the hydroxy-PCBs [14,17,19, 34], and the sulfate-PCBs [15,16,6]. Metabolic reactions in animals were mainly investigated, although plant role in the interconversion of different forms was recently studied [26,50,51]. Other categories, for example the methyl-sulfone derivatives were studied for their chirality

---

[11], while the hydroxy-PCB were explored as occupational markers of exposure as well as for their toxicity [35,38]. Among PCB metabolites, sulfonated-polychlorinated biphenyls (sulfonated-PCBs) and hydroxy-sulfonated-polychlorinated biphenyls (OH-sulfonated-PCBs) are two recently discovered classes of environmental contaminants, originating from PCBs [1]. Sulfonated-PCBs were identified for the first time by Liu and coworkers [27] in polar bear serum. It was postulated that PCBs could reach the Arctic foodweb through long-range transport, being then accumulated and transformed to sulfonated metabolites. Sulfonated-PCBs (together with OH-sulfonated-PCBs) were also recently identified by Bagnati and coworkers [1], in agricultural soil samples collected from the National Priority Site (SIN) for Remediation SIN Brescia-Caffaro, located in Northern Italy [7,9]. These agricultural areas received irrigation water contaminated by PCBs, discharged by Caffaro chemical plant, a former PCB producer. Since no measured physico-chemical properties were available, [1] estimated these properties, which revealed a general more polar nature than the corresponding same-chlorinated parent compounds. At present, no information exists on their environmental formation, including the organism(s) responsible for their formation. However, Li and coworkers [25] investigated the importance of the intestinal microbiome in PCB biotransformation by feeding mice with an environmental PCB mixture and analyzing their feces. The authors observed that for sulfonated-PCBs, the levels were higher in mice with a microbiome than in the germ-free ones, suggesting that microorganisms could play a role in sulfonated-PCB formation. It was also observed that the biotransformation of PCBs into sulfonated-PCBs could also occur as a detoxification process through a P450-mediated oxidation and the activity of some enzymes, including the Glutathione-*S*-transferase [27]. Therefore, further studies are needed to investigate which metabolic pathway leads to the formation of sulfonated and OH-sulfonated-PCBs and the organisms responsible. For example, earthworms were recently shown to be able to bioaccumulate these metabolites from soil [32]. However, no information on PCB metabolization in these organisms is available yet.

The objective of this work is to show the first evidence on the environmental fate of these new classes of contaminants in the environment. Here the results of several experiments are presented: the evaluation of soil partitioning, degradation, uptake into organisms, as well as a preliminary analytical methods for extracting sulfonated and OH-sulfonated-PCBs from water. Some of these activities were possible thanks to a sulfonated PCB standard, recently synthesized by our group [29]. The results allow to shed some light on their properties and the environmental fate, to address further studies, such as model their fate with multimedia models, at different scales [13,30,43].

2. Materials and methods

2.1. Chemicals and reagents

Dichloromethane (ACS reagent. $\geq$ 99.8%) was obtained from Sigma-Aldrich, Ammonia (28%) from VWR Chemicals and Methanol from Riedel-de Haen (Seelze, Germany). HPLC solvents and reagents were of pesticide or LC-MS grade: water (in house Milli-Q apparatus), acetonitrile, acetone, formic acid and ammonium acetate (Carlo Erba Reagents). Standards of OH-PCBs (4-hydroxy-2,3,3′,4′,5 Pentachlorobiphenyl, OH-PCB-107; 4-hydroxy-2,2′,3,4′,5,5′,6-Heptachlorobiphenyl, OH-PCB-187; 3-hydroxy-2,2′,3′,4,4′,5-Hexachlorobiphenyl OH-PCB-138 and $^{13}C_{12}$-4-hydroxy-2,2′,3,4′,5,5′,6-Heptachlorobiphenyl, $^{13}C_{12}$-OH-PCB-187) were purchased from Wellington Laboratories (Guelph, ON, Canada). A hexa-sulfonated-PCB standard (corresponding to sulfonated-PCB 155, or 2,2′,4,4′6,6′-hexachloro-[1,1′-biphenyl]−3-sulphonic acid) was synthesized by our group and described in a companion paper [29], whereas Supelcosil LC-NH₂ and Supelclean ENVI-18 3 mL cartridges were purchased from Merck (Darmstadt, Germany).

2.2. Analysis of sulfonated-PCBs and OH-sulfonated-PCBs in environmental matrices

Sulfonated-PCBs were extracted from earthworm, soil and root samples using 4 mL of a solution acetone:water (95:5% v/v), containing 1% formic acid, for 30 min using a sonicator. The same procedure was repeated using 4 mL of dichloromethane. Then, samples were reconstituted with 600 μL of acetonitrile and analyzed with an HPLC-HRMS using a LC system coupled to an Orbitrap Q Exactive mass spectrometer. More details on the extraction and the analytical procedure are reported in [32]. Water samples from all experiments but the leaching behaviour one (Section 2.9), were directly injected (100 μL) after centrifugation. These samples were analyzed with LC-HRMS using an UHPLC Thermo Fisher Vanquish coupled to a Thermo Fisher Orbitrap Exploris 120 mass spectrometer at 60,000 resolution.

2.3. Extraction of the synthesized sulfonated-hexa-PCB from water

For the leaching behaviour experiment (see Section 2.9), two types of samples (deionized water (2 replicates per cartridge) and tap water added with dissolved organic carbon (DOC) (one replicate per cartridge) were extracted with a solid phase extraction (SPE) technique using LC-NH₂ and ENVI-C18 cartridges (TEXT SI-1 and Table SI-1). The ENVI-C18 cartridges were used to preferentially adsorb the undissociated sulfonated and OH-sulfonated-PCBs. On the other hand, the LC-NH₂ was used as an ion exchange phase to capture the ionized species from the aqueous phase. Samples extracted with the ENVI-C18 were first acidified with 0.1% formic acid and then the cartridge was eluted with 10 mL of methanol and 10 mL of cyclohexane. Replicate water samples extracted with the LC-NH₂ were eluted as is using 20 mL of a solution methanol: water (60:40% v/v) containing 0.03% NH3 to reach a pH = 10 or 0.02% NH₃ to obtain a pH = 12. Before the extraction, each sample was spiked with 100 ng of the synthesized hexa-sulfonated-PCB to evaluate the recovery of the method. Then, the extracts were concentrated using a vacuum rotary evaporator up to 1 mL. The synthesized-sulfonated-hexa-PCB was then quantified with an HPLC-HRMS using an Agilent-1200 LC system (Agilent Technologies) coupled to a Thermo Fisher Orbitrap Q Exactive mass spectrometer, operating in ESI negative ion full scan MS (120 −1200 u) at 35,000 resolution and in data-dependent MS2 at 17,500 resolution (see TEXT SI-1 for more details). The recovery was determined by comparing the peak area of the hexa-sulfonated-PCB spiked to samples before extraction (A) and the peak area of the same analyte spiked to samples after extraction (B) and calculated as follows: % Rec = B/A x 100.

2.4. Potential for rhizoremediation/degradation in soil

In the current study, soil samples from a previous rhizoremediation greenhouse experiment [45] were analyzed for sulfonated and OH-sulfonated-PCBs to evaluate the potential for biodegradation. More specifically, the experiment [45] was performed to select the best plant species and soil management for the bioremediation of weathered PCBs, using the contaminated soil of the national priority site for remediation (SIN) "Brescia-Caffaro" in Italy [7,9]. To evaluate the persistence in soil, here soil samples from pots cultivated with *Festuca arundinacea* together with *Cucurbita pepo* ssp. *pepo* and *Medicago sativa* alone were selected for the evaluation of sulfonated-PCB metabolite biodegradation. These soils were selected since these plant species showed the best effect on rhizoremediation of PCBs [42,45].

2.5. Determination of soil-water partition

In the current study, samples from previous studies on contaminated soils [1] and infiltration water [46] in a soil column experiment, were analyzed for sulfonated-PCB metabolite determination. In this latter experiment, the soil sample column C-T0 (unplanted soil) and the

corresponding leaching water concentrations, were used to calculate the soil-water partition coefficient. The columns were leached with tap water and samples were collected after 7 days of soil/water contact.

2.6. Bioaccumulation in plants and earthworms

In the current study, data on earthworm accumulation of sulfonated-PCBs from [32] on *Aporrectodea caliginosa* Savigny specimens were used to compare the bioaccumulation of sulfonated and OH-sulfonated-PCBs in earthworms and plant roots. Briefly, grassland topsoil (0–30 cm) and earthworms were collected in a highly contaminated agricultural field located in the SIN Brescia-Caffaro and analyzed for sulfonated-PCBs and OH-sulfonated-PCBs. The Bioconcentration Factor (BCF) was then calculated. In the same agricultural field, a root sample, and an additional soil sample (0–10 cm) were collected, measured, and used to calculate the Root Concentration Factor (RCF) for sulfonated and OH-sulfonated-PCBs. More specifically, samples were collected in a polyphyte permanent meadow [7] where the predominant species were *Medicago sativa* and *Festuca arundinacea*.

2.7. Calculation of partition coefficients and bioconcentration factors

Soil-water partition coefficients (Kd) were first calculated for the different chlorination families which were detected (for sulfonated-PCBs: from tetra to hepta chlorinated congeners; for OH-sulfonated-PCBs: for tetra and penta chlorinated congeners) using respective soil and water concentrations from [1,46]. Kd was also calculated for the synthesized hexa-sulfonated-PCB and for all the PCB chlorination families, as illustrated in [32]. The organic carbon-water partition coefficient (K_{OC}) was then calculated as $K_{OC} = Kd/f_{OC}$ where f_{OC} is fraction of organic carbon in experimental soil, corresponding to 0.017 [45]. K_{OC} was also estimated for all PCB congener homologues families using the Karickhoff equation [22]. Root Concentration Factors (RCFs) were calculated by dividing the measured concentration in fresh roots by the freely dissolved concentration in water (see Table SI-2 for details), while for PCBs the RCF was estimated using the equation developed in a previous work for *Festuca arundinacea* [44]. Similarly, BCFs were calculated for earthworms as ratio of concentration in earthworms to freely dissolved concentration in water (see [32] for more details) (Table SI-2).

2.8. Characterization of leaching behaviour

A column experiment was performed to investigate the mobility of sulfonated and OH-sulfonated-PCBs in soil using a sulfonated-hexa-PCB standard (sulfonated PCB-155), newly synthesized by our group [29]. A glass column (ID: 2.66 cm; length: 50 cm) with a 250 mL reservoir at the top and a Teflon stopcock at the bottom, was filled (from bottom to top) with: 2 g of glass wool, 25 g of sand, 54 g of background soil, 25 g of PCB contaminated soil (from the SIN Brescia-Caffaro) spiked with 645 µg of sulfonated-PCB-155 dissolved in 1 mL of deionized water and additional 25 g of sand. Soil total length in the column was 14.5 cm, and volume was 81 cm^3 (Fig. 1). The soil was wetted with about 10 mL of tap water to reach field capacity conditions. Three days after the spike, tap water was added to the reservoir and water samples were collected at specific incremental times (0, 1, 2, 4, 24, 25, 28, 29, 30, 31, 35 and 37 days).

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

As analytical standards are not available yet for all these chemicals, we used the signal intensity of the internal standards (OH-PCBs) to evaluate the robustness of the analysis, considering acceptable a coefficient of variation (CV %) within 15% between different batches.
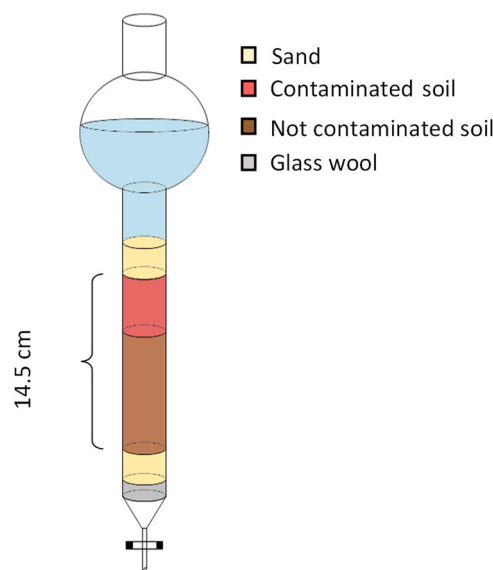

Fig. 1. Sketch of column layering (from bottom to top): glass wool (grey), sand (yellow), background soil (brown), PCB contaminated soil (from the SIN Brescia-Caffaro) (red).

2.10. Statistical analyses

All regression analyses were performed with R Studio v.4.2.1 [36].

3. Results and discussion

While much information exists for PCBs fate in the environment [18, 28,48,7], little is known about the fate of PCB metabolites in the environment [32,40,41,49] since metabolites are often measured in organisms after exposure to parent PCBs [16,23,31,33]. For this reason, it is important to characterize the occurrence and fate of PCB metabolites to evaluate their environmental behaviour, persistence as well as the bioaccumulation and toxicological features.

3.1. Extraction of sulfonated-PCBs from water

The analytical method presented here is the first one dealing with the extraction of sulfonated and OH-sulfonated-PCBs from an aqueous phase. Analytical methods available in the literature regard the extraction of sulfonated-PCBs from biological matrices (serum and feces) [25, 27], soil and earthworms [1,32].

Given the generally low pKa of sulfonated-PCBs (0.48–0.56) [32] these chemicals are expected to be dissociated at environmental pHs. Therefore, it is peculiar to evaluate the dissociation status when extracting these chemicals from water to select the proper cartridge phase and extraction conditions. The best result, with a complete recovery of the sulfonated-PCB 155 (139% ± 15%), was obtained using the ENVI-C18 SPE cartridge, after sample acidification. When using the LC-NH$_2$ cartridge, recovery was considerably lower and more variable among replicates (52% ± 29%). In particular, the recovery at pH 10 was only 27%, while at pH 12 was much higher (46–84%). (TEXT SI-2 and Table SI-1). For these reasons it is recommended, when extracting sulfonated-PCBs from water, to use C18 cartridges using the specified procedure.

3.2. Adsorption to soil

Log K_{OC} was plotted against the number of chlorine atoms (Fig. 2, top chart), Log K_{OW} (Fig. 2, middle chart) and Log D (Fig. 2, bottom chart). The regression lines generally show an increasing trend with the
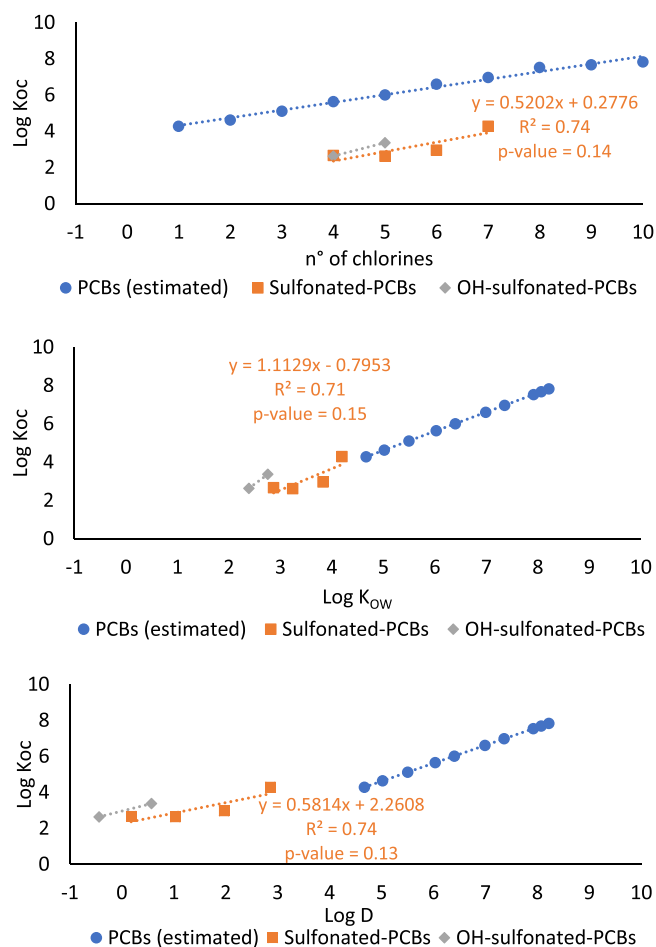
Fig. 2. Linear regressions between Log K_{OC} and n° of chlorine atoms (top), Log K_{OW} (middle) and Log D (bottom). Log K_{OC} was estimated using Karickhoff's equation for PCBs, whereas for sulfonated and hydroxy-sulfonated-PCBs it was calculated from measurements.

increase of number of chlorine atoms (although not statistically significant) for both sulfonated and hydroxy-sulfonated-PCBs, similarly to what happens for the parent compounds, PCBs. However, K_{OC}s for PCB metabolites are about a hundred times smaller than the corresponding PCBs with the same number of chlorine atoms, highlighting the higher polarity of metabolites, their reduced partitioning to soil and, therefore, the enhanced affinity for the water phase. Another indication of the lower hydrophobicity of sulfonated PCBs is given by the regression lines in Fig. 2 (middle chart), where Log K_{OC}s correspond to lower values of Log K_{OW}. In the last chart (Fig. 2), Log K_{OC} was also plotted against Log D, which is the apparent pH dependent Log K_{OW} (calculated as the ratio of the sum of the concentrations of all forms of the compound, ionized and un-ionized in each of the two phases) [32]. This value coincides with Log K_{OW} for non-dissociated chemicals such as PCBs.

The results shown here are obtained at chlorination family level and are of course to be used as such. As soon as more single congeners of these chemicals will be available at mg level, it would be possible to measure, directly or indirectly, the physical chemical properties such as Kow, solubility in water, etc., and estimate their variability with temperature, to gain insight on the chemical behaviour in realistic environmental conditions [12]. However, the results obtained here show a relatively lower affinity for soil compared to PCBs [7], as hypothesized in a previous paper [1].

3.3. Degradation in soil

Concentrations obtained for sulfonated and hydroxy-sulfonated-PCBs in soils from a greenhouse experiment [45] are shown in Fig. 3. The graphs report the chemical species in columns (sulfonated- vs. OH-sulfonated-PCBs) while from top to bottom concentrations at the different conditions (control vs. species cultivated in each pot) and times (from time = 0 to time = 18 months) are reported. "Festuca + Cucurbita soil" and "Medicago soil" are samples of soil cultivated for 18 months with different plant species, *Festuca arundinacea* in consociation with *Cucurbita pepo* spp. *pepo* and *Medicago sativa*, respectively. Being a long-time contaminated soil, sulfonated and OH-sulfonated PCBs were already present in the control soil, at the beginning of the experiment. However, the temporal trends of the different metabolite families for both samples, compared to the control, suggest a limited or very slow degradation of sulfonates, having shown no apparent decrease in 18 months, neither in the control at t = 18 months, nor in the plant-treated soil. Therefore, despite sulfonated and hydroxy-sulfonated-PCBs are metabolites, their long persistence in soil appears to be similar to that of their parent compounds [42,45,47], information which triggers the need of further and specific studies on the halflives of these new classes to evaluate and model their fate.

3.4. Accumulation in plant roots

Log RCFs were plotted against the number of chlorines (Fig. 4, top chart), Log K_{OW} (Fig. 4, middle chart) and Log D (Fig. 4, bottom chart) and were compared to those estimated for PCBs. Although bioconcentration values of sulfonated and hydroxy-sulfonated-PCBs were lower than those estimated for PCBs by a factor of 100–200, respectively, they also increased with the increase of hydrophobicity.

Previous studies showed that the translocation of chemicals into plants is generally restricted to chemicals with a Log K_{ow} between 1 and 3 [4], while more hydrophobic chemicals are generally just accumulated in roots. It is therefore possible that some lower chlorinated or dissociated sulfonated- and OH-sulfonated-PCBs could be translocated to aerial parts of the plants, such as lower chlorinated OH-PCBs [39]. This information is crucial because PCBs are known for being accumulated in roots [44] but no or little translocation is expected to aerial part of the plants due to the high Kow [4], with the exception of plants belonging to Cucurbitaceae family [20,21]. If sulfonated- and OH-sulfonated-PCBs are translocated into plants from soil, an additional burden for the terrestrial (and human) food chain is to be expected. More investigations are needed to verify this condition.

3.5. Accumulation in earthworms

Bioaccumulation of PCBs and their metabolites was investigated in a recently published paper [32]. To show some results from this experiment, we selected the earthworm *Aporrectodea caliginosa*, since it is an endogeic species, typically living within the first 20 cm of soil [10,2,24] and therefore representing metabolite accumulation in top soil. Results were reported (Figure SI-1) to compare the accumulation of PCB sulfonates in earthworms to that in roots. Log BCFs for PCBs and PCB metabolites were plotted against the number of chlorine atoms (Figure SI-1, top chart) Log K_{OW} (Figure SI-1, middle chart) and Log D (Figure SI-1, bottom chart). Unlike the uptake of sulfonated-PCBs in roots, bioconcentration in earthworms seems to be independent of hydrophobicity. In particular, the hydroxy-sulfonated-PCBs show a statistically significant (p-value=0.023). decreasing bioaccumulation trend with K_{OW}. is When the Log BCF is related to the Log D, the decreasing trend for this class is maintained albeit with a smaller slope. On the contrary, sulfonated-PCBs show a reverse trend, which increases with the Log D. This behaviour might perhaps be explained by an additional mechanism of partitioning: [27] showed the presence of sulfonated-PCBs (together with other chemicals) in polar bear serum. This may suggest a potential
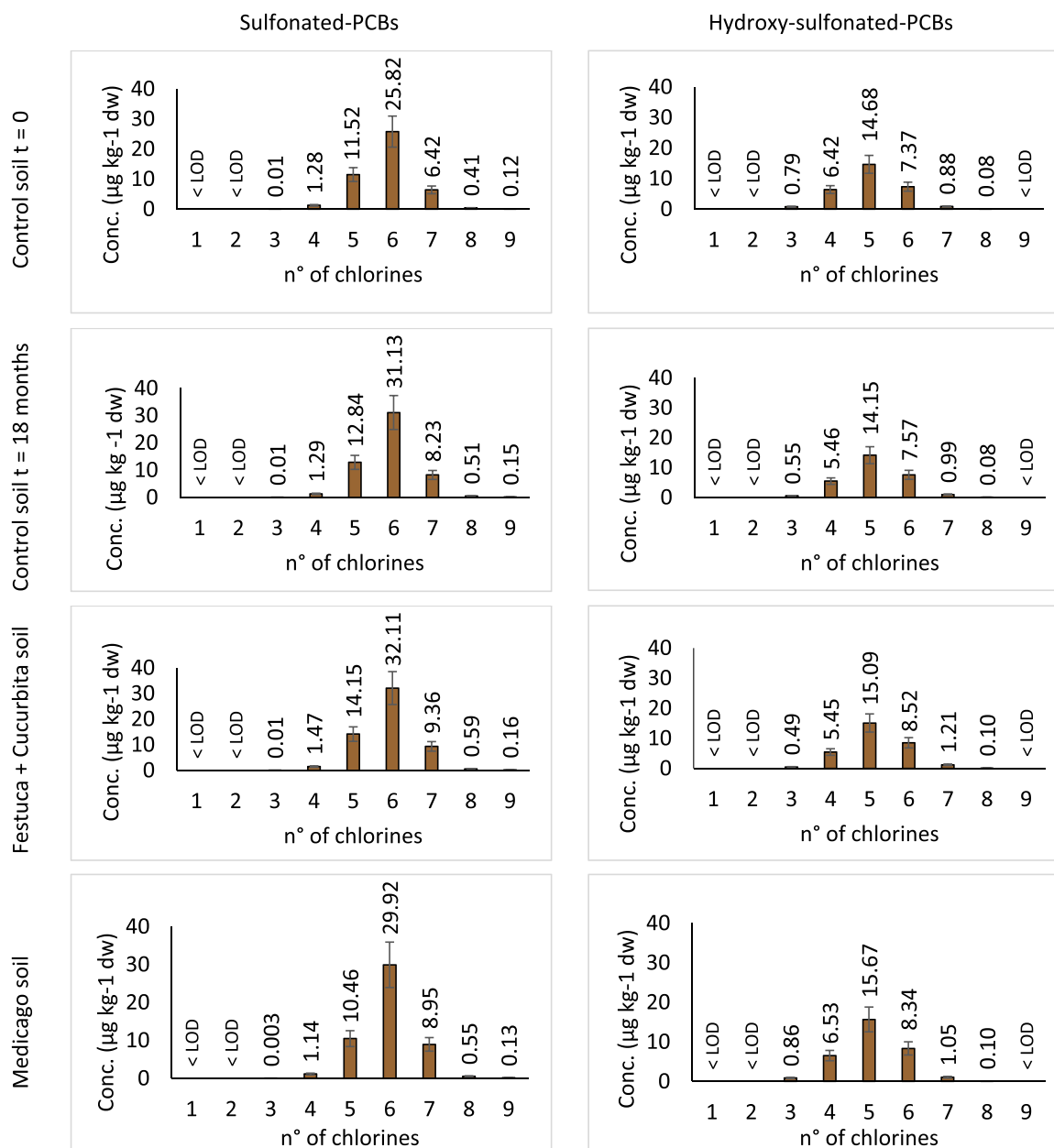
Fig. 3. Concentrations (µg/kg dw) of four samples: Control soil t = 0 (soil collected at the beginning of the experiment) [1], Control soil t = 18 months (soil collected at the end of the experiment), Festuca + Cucurbita soil (soil treated with *Festuca arundinacea* in consociation with *Cucurbita pepo* spp. *pepo* for 18 months) and Medicago soil (soil treated with *Medicago sativa* for 18 months). Bars show the analytical error. LOD = limit of detection.

adsorption and transport mediated by blood proteins, which could be due by the interaction of the sulfonated group with proteins. However, more studies are needed to evaluate the mechanisms of bioaccumulation of these metabolites and their potential role in toxicity.

3.6. Leaching behaviour

Having shown in the previous experiments the lower affinity for soil and the long persistence of these metabolites, it was important to evaluate their behaviour in a soil column with mostly uncontaminated soil to have a grasp of the potential leachability of these chemicals in topsoil. This is possible using the hexachlorinated-sulfonated PCB (sulfonated-PCB-155) as a model compound. The conditions simulated are those of a soil initially at field capacity, which receives rainfall water. When water saturates the porosity, it starts to move vertically (gravitational water), carrying dissolved chemicals through the column. The experiment

showed that the highest concentrations in water leaving the column were measured within the first 32 h of water flow, during which about 43% of the initial amount in soil was collected (Fig. 5). Then, concentrations level started to decrease, probably due to equilibration with soil. After 115 h of water flow, about 30% of the added synthesized sulfonated-hexa-PCB was still inside the soil column.

The results of this experiment confirm the high leachability of sulfonated PCBs compared to the parent compounds, as desumed by the higher Kow and therefore higher Koc and Kd [22] of the corresponding PCB chlorination families.This also shows the capacity to move vertically in soil even after a long equilibration time; although peak concentrations were about 0.3 mg/L after 26 h, after a period of 790 h (about a month) and 21 L of percolating water, concentrations were still around 0.1 µg/L. This much higher mobility of sulfonated-PCBs (and possibly also of the OH-sulfonated-PCBs, more polar because of the additional OH- group) in water could be responsible of potential
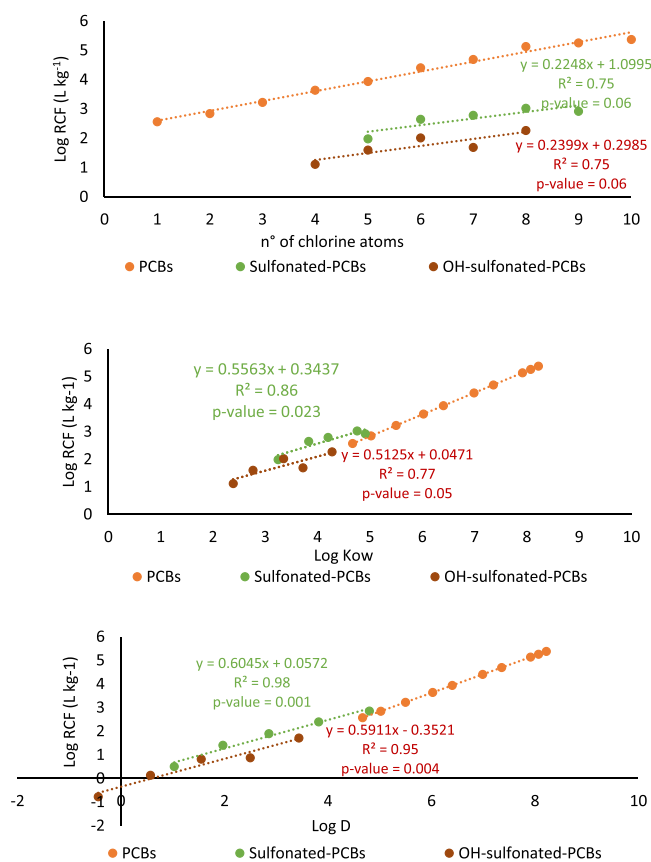
Fig. 4. Linear regressions between Log RCF and n° of chlorine atoms (on the top), Log K_{OW} (in the middle) and Log D (on bottom). The predominant species composing plant roots were *Medicago sativa* and *Festuca arundinacea*. "Log RCF was estimated using Terzaghi et al. [44] equation for PCBs, whereas it was calculated with the measured data obtained for sulfonated and hydroxy-sulfonated-PCBs.

contamination of groundwater and surface water at appreciable levels, especially in contaminated areas, such as the agricultural areas of the SIN Brescia-Caffaro [8,9] or of other areas worldwide.

4. Conclusions

The findings reported here allow us to gain some insight on the environmental fate and properties of these sulfonated PCB metabolites. These chemicals, although similarly chlorinated as the parent compounds, due to the presence of one or more polar groups do change their properties and mobility in soil. Their bioaccumulation in plant roots is much lower than PCBs and may also be reflected in potential for translocation in the upper parts, especially for the lower chlorinated counterparts. Their biodegradability, in an 18 months rhizoremediation experiment in soil, does not seem to be very different from PCBs. This fact, together with the relatively high vertical mobility in soil during water leaching, confirms that these chemicals could be potential candidates for groundwater and surface water contamination. Although some evidence on their environmental behaviour is present, much is still to be done on the identification of single congeners in the two classes. While tens of these metabolites [1] were found in soil, we do not precisely know how many they are and their chemical identification information such as CAS number, EINECS, etc. Additionally, although some physical-chemical properties were estimated [1,32], experimental measurements of Log Kow, Log D, pKa, solubility at different pHs, etc., are not yet available. Additionally, the organism(s) responsible for their formation in the environment and the halflives in the different environmental phases were not identified. Finally, information on the world general environmental contamination is still scarce and above all no information exists on their toxicological and ecotoxicological properties. This is particularly important also considering the recent evidence on the toxicity, especially chronic, of other polar metabolites of PCBs, such as the hydroxy-PCBs [5,38].

Environmental implication

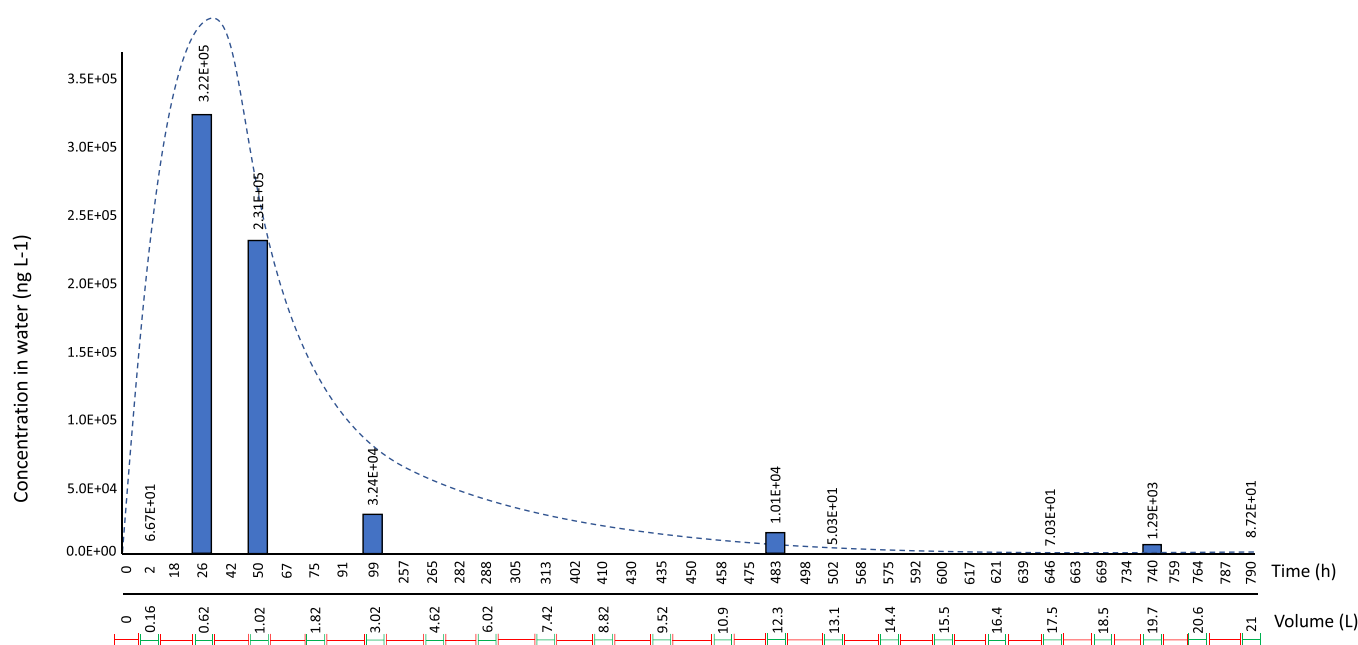In this paper we present the first evidence on the environmental fate

Fig. 5. Concentration ng L^{-1} of the synthesized sulfonated-hexa-PCB in water samples represented as a function of the time (h) (X-axis above) and the volume (L) (X-axis below) of water that was flushed through the column. Both volume and time are cumulative. Where the amount is not shown, water was flushed but not collected. Green and red bars represent the opening (green) and closing (red) of the soil column in terms of time (h): the column was opened for some hours to flush the added water and then closed until the next water addition.

of sulfonated-polychlorinated biphenyls and hydroxy-sulfonated-polychlorinated biphenyls, two new classes of contaminants, recently discovered in soil by our group. These chemicals (more than a hundred counted so far) are truly candidate for being hazardous materials with potentially negative ecotoxicological and toxicological properties, at least comparable to other PCB metabolites (such as OH-PCBs). These are the first results ever on the environmental behaviour of sulfonated- and OH-sulfonated PCBs chemicals. In a companion paper we produced a new standard (a sulfonated-PCB 155) which was employed in this paper to study mobility in soil.

CRediT authorship contribution statement

Jessica Palladini: Investigation, Writing- Original draft preparation, Reviewing and Editing, **Elisa Terzaghi**:, Investigation, Writing-Reviewing and Editing, **Renzo Bagnati**: Investigation, Resources, Data curation, Writing- Reviewing and Editing, **Alice Passoni**: Investigation, Data curation, Writing- Reviewing and Editing, **Enrico Davoli**: Resources, Writing- Reviewing and Editing, **Angelo Maspero**, Writing-Reviewing and Editing, **Giovanni Palmisano**: Investigation, Data curation, Writing- Reviewing and Editing, **Antonio Di Guardo**: Investigation, Funding, Resources, Data curation, Writing- Reviewing and Editing, supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131853.

References

[1] Bagnati, R., Terzaghi, E., Passoni, A., Davoli, E., Fattore, E., Maspero, A., et al., 2019. Identification of sulfonated and hydroxy-sulfonated polychlorinated biphenyl (PCB) metabolites in soil: new classes of intermediate products of pcb degradation. Environ Sci Technol 53, 10601–10611. https://doi.org/10.1021/acs.est.9b03010.
[2] Bart, S., Amossé, J., Lowe, C.N., Mougin, C., Péry, A.R.R., Pelosi, C., 2018. Aporrectodea caliginosa, a relevant earthworm species for a posteriori pesticide risk assessment: current knowledge and recommendations for culture and experimental design. Environ Sci Pollut Res 25, 33867–33881. https://doi.org/10.1007/s11356-018-2579-9.
[3] Bergman, Å., Klasson Wehler, E., Kuroki, H., Nilsson, A., 1995. Synthesis and mass spectrometry of some methoxylated PCB. Chemosphere 30, 1921–1938. https://doi.org/10.1016/0045-6535(95)00073-H.
[4] Briggs, G.G., Bromilow, R.H., Evans, A.A., Williams, M., 1983. Relationships between lipophilicity and the distribution of non-ionised chemicals in barley shoots following uptake by the roots. Pestic Sci 14, 492–500. https://doi.org/10.1002/ps.2780140506.
[5] Dhakal, K., Gadupudi, G.S., Lehmler, H.-J., Ludewig, G., Duffel, M.W., Robertson, L.W., 2018. Sources and toxicities of phenolic polychlorinated biphenyls (OH-PCBs). Environ Sci Pollut Res 25, 16277–16290. https://doi.org/10.1007/s11356-017-9694-x.
[6] Dhakal, K., He, X., Lehmler, H.-J., Teesch, L.M., Duffel, M.W., Robertson, L.W., 2012. Identification of sulfated metabolites of 4-chlorobiphenyl (PCB3) in the serum and urine of male rats. Chem Res Toxicol 25, 2796–2804. https://doi.org/10.1021/tx300416v.
[7] Di Guardo, A., Raspa, G., Terzaghi, E., Vergani, L., Mapelli, F., Borin, S., et al., 2020. PCB vertical and horizontal movement in agricultural soils of a highly contaminated site: Role of soil properties, cultivation history and PCB physico-chemical parameters. Sci Total Environ 747, 141477. https://doi.org/10.1016/j.scitotenv.2020.141477.
[8] Di Guardo, A., Raspa, G., Terzaghi, E., Vergani, L., Mapelli, F., Borin, S., et al., 2020. PCB vertical and horizontal movement in agricultural soils of a highly contaminated site: Role of soil properties, cultivation history and PCB physico-chemical parameters. Sci Total Environ 747, 141477. https://doi.org/10.1016/j.scitotenv.2020.141477.
[9] Di Guardo, A., Terzaghi, E., Raspa, G., Borin, S., Mapelli, F., Chouaia, B., et al., 2017. Differentiating current and past PCB and PCDD/F sources: The role of a large contaminated soil site in an industrialized city area. Environ Pollut 223, 367–375. https://doi.org/10.1016/j.envpol.2017.01.033.
[10] EC, 2010. European atlas of soil biodiversity, Atlas Series. Published by the Publications Office of the European Union, Luxembourg.
[11] Eljarrat, E., Guerra, P., Barceló, D., 2008. Enantiomeric determination of chiral persistent organic pollutants and their metabolites. TrAC Trends Anal Chem Adv MS Anal Metab Degrad Prod - I 27, 847–861. https://doi.org/10.1016/j.trac.2008.08.010.
[12] Finizio, A., Di Guardo, A., 2001. Estimating temperature dependence of solubility and octanol-water partition coefficient for organic compounds using RP-HPLC. Chemosphere 45, 1063–1070. https://doi.org/10.1016/S0045-6535(01)00105-9.
[13] Ghirardello, D., Morselli, M., Semplice, M., Di Guardo, A., 2010. A dynamic model of the fate of organic chemicals in a multilayered air/soil system: Development and illustrative application. Environ Sci Technol 44, 9010–9017.
[14] Gilroy, È.A.M., Muir, D.G.C., McMaster, M.E., Darling, C., Campbell, L.M., Solla, S. R., et al., 2012. Polychlorinated biphenyls and their hydroxylated metabolites in wild fish from wheatley Harbour Area of Concern, Ontario, Canada. Environ Toxicol Chem 31, 2788–2797. https://doi.org/10.1002/etc.2023.
[15] Grimm, F.A., Lehmler, H.-J., He, X., Robertson, L.W., Duffel, M.W., 2013. Sulfated metabolites of polychlorinated biphenyls are high-affinity ligands for the thyroid hormone transport protein transthyretin. Environ Health Perspect 121, 657–662. https://doi.org/10.1289/ehp.1206198.
[16] Grimm, F.A., Lehmler, H.-J., Koh, W.X., DeWall, J., Teesch, L.M., Hornbuckle, K.C., et al., 2017. Identification of a sulfate metabolite of PCB 11 in human serum. Environ Int 98, 120–128. https://doi.org/10.1016/j.envint.2016.10.023.
[17] Guvenius, D.M., Hassanzadeh, P., Bergman, A., Norent, K., 2002. Metabolites of polychlorinated biphenyls in human liver and adipose tissue. Environ Toxicol Chem 21, 2264–2269. https://doi.org/10.1002/etc.5620211102.
[18] Hashmi, M.Z., Chen, K., Khalid, F., Yu, C., Tang, X., Li, A., et al., 2022. Forty years studies on polychlorinated biphenyls pollution, food safety, health risk, and human health in an e-waste recycling area from Taizhou city, China: a review. Environ Sci Pollut Res 29, 4991–5005. https://doi.org/10.1007/s11356-021-17516-0.
[19] Hovander, L., Malmberg, T., Athanasiadou, M., Athanassiadis, I., Rahm, S., Bergman, Å., et al., 2002. Identification of hydroxylated PCB metabolites and other phenolic halogenated pollutants in human blood plasma. Arch Environ Contam Toxicol 42, 105–117. https://doi.org/10.1007/s002440010298.
[20] Huelster, A., Mueller, J.F., Marschner, H., 1994. Soil-plant transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans to vegetables of the cucumber family (Cucurbitaceae). Environ Sci Technol 28, 1110–1115.
[21] Inui, H., Wakai, T., Gion, K., Kim, Y.-S., Eun, H., 2008. Differential uptake for dioxin-like compounds by zucchini subspecies. Chemosphere 73, 1602–1607. https://doi.org/10.1016/j.chemosphere.2008.08.013.
[22] Karickhoff, S.W., 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10, 833–846. https://doi.org/10.1016/0045-6535(81)90083-7.
[23] Koh, W.X., Hornbuckle, K.C., Thorne, P.S., 2015. Human serum from urban and rural adolescents and their mothers shows exposure to polychlorinated biphenyls not found in commercial mixtures. Environ Sci Technol 49, 8105–8112. https://doi.org/10.1021/acs.est.5b01854.
[24] König, H., Varma, A. (Eds.), 2006. Intestinal Microorganisms of Termites and Other Invertebrates, Soil Biology. Springer, Berlin; New York.
[25] Li, X., Liu, Y., Martin, J.W., Cui, J.Y., Lehmler, H.-J., 2021. Nontarget analysis reveals gut microbiome-dependent differences in the fecal PCB metabolite profiles of germ-free and conventional mice. Environ Pollut 268, 115726. https://doi.org/10.1016/j.envpol.2020.115726.
[26] Li, Y., Bako, C.M., Saktrakulkla, P., Lehmler, H.-J., Hornbuckle, K.C., Schnoor, J.L., 2021. Interconversion between methoxylated, hydroxylated and sulfated metabolites of PCB 3 in whole poplar plants. Sci Total Environ 785, 147341. https://doi.org/10.1016/j.scitotenv.2021.147341.
[27] Liu, Y., Richardson, E.S., Derocher, A.E., Lunn, N.J., Lehmler, H.-J., Li, X., et al., 2018. Hundreds of unrecognized halogenated contaminants discovered in polar bear serum. Angew Chem Int Ed 57, 16401–16406. https://doi.org/10.1002/anie.201809906.
[28] Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Global fate of POPs: Current and future research directions. Environ Pollut 150, 150–165. https://doi.org/10.1016/j.envpol.2007.06.051.
[29] Maspero, A., Vavassori, F., Penoni, A., Galli, S., Palmisano, G., Bagnati, R., et al., 2023. Synthesis of a new sulfonated-hexachlorobiphenyl standard for environmental analysis, ecotoxicological, and toxicological studies. Sci Total Environ 882, 163445. https://doi.org/10.1016/j.scitotenv.2023.163445.
[30] Morselli, M., Vitale, C.M., Ippolito, A., Villa, S., Giacchini, R., Vighi, M., et al., 2018. Predicting pesticide fate in small cultivated mountain watersheds using the DynAPlus model: Toward improved assessment of peak exposure. Sci Total Environ 615, 307–318. https://doi.org/10.1016/j.scitotenv.2017.09.287.

[31] Norström, K., Czub, G., McLachlan, M.S., Hu, D., Thorne, P.S., Hornbuckle, K.C., 2010. External exposure and bioaccumulation of PCBs in humans living in a contaminated urban environment. Environ Int 36, 855–861. https://doi.org/10.1016/j.envint.2009.03.005.

[32] Palladini, J., Bagnati, R., Passoni, A., Davoli, E., Lanno, A., Terzaghi, E., et al., 2022. Bioaccumulation of PCBs and their hydroxy and sulfonated metabolites in earthworms: Comparing lab and field results. Environ Pollut 293, 118507. https://doi.org/10.1016/j.envpol.2021.118507.

[33] Park, J.-S., Kalantzi, O.I., Kopec, D., Petreas, M., 2009. Polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) in livers of harbor seals (Phoca vitulina) from San Francisco Bay, California and Gulf of Maine. Mar Environ Res 67, 129–135. https://doi.org/10.1016/j.marenvres.2008.12.003.

[34] Purkey, H.E., Palaninathan, S.K., Kent, K.C., Smith, C., Safe, S.H., Sacchettini, J.C., et al., 2004. Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: rationalizing rodent PCB toxicity. Chem Biol 11, 1719–1728. https://doi.org/10.1016/j.chembiol.2004.10.009.

[35] Quinete, N., Schettgen, T., Bertram, J., Kraus, T., 2014. Occurrence and distribution of PCB metabolites in blood and their potential health effects in humans: a review. Environ Sci Pollut Res 21, 11951–11972. https://doi.org/10.1007/s11356-014-3136-9.

[36] R Core Team, 2022. R: A language and environment for statistical computing. [WWW Document]. R Foundation for Statistical Computing, Vienna, Austria. URL ⟨https://www.r-project.org/⟩ (accessed 2.24.23).

[37] Rezek, J., Macek, T., Mackova, M., Triska, J., Ruzickova, K., 2008. Hydroxy-PCBs, methoxy-PCBs and hydroxy-methoxy-PCBs: metabolites of polychlorinated biphenyls formed in vitro by tobacco cells. Environ Sci Technol 42, 5746–5751. https://doi.org/10.1021/es800445h.

[38] Rodriguez, E.A., Vanle, B.C., Doorn, J.A., Lehmler, H.-J., Robertson, L.W., Duffel, M.W., 2018. Hydroxylated and sulfated metabolites of commonly observed airborne polychlorinated biphenyls display selective uptake and toxicity in N27, SH-SY5Y, and HepG2 cells. Environ Toxicol Pharmacol 62, 69–78. https://doi.org/10.1016/j.etap.2018.06.010.

[39] Sun, J., Pan, L., Chen, J., Li, K., Zhu, L., 2018. Uptake, translocation, and metabolism of hydroxylated and methoxylated polychlorinated biphenyls in maize, wheat, and rice. Environ Sci Pollut Res 25, 12–17. https://doi.org/10.1007/s11356-016-7724-8.

[40] Sun, J., Zhu, L., Pan, L., Wei, Z., Song, Y., Zhang, Y., et al., 2016. Detection of methoxylated and hydroxylated polychlorinated biphenyls in sewage sludge in China with evidence for their microbial transformation. Sci Rep 6, 29782. https://doi.org/10.1038/srep29782.

[41] Tehrani, R., Van Aken, B., 2014. Hydroxylated polychlorinated biphenyls in the environment: sources, fate, and toxicities. Environ Sci Pollut Res 21, 6334–6345. https://doi.org/10.1007/s11356-013-1742-6.

[42] Terzaghi, E., Alberti, E., Raspa, G., Zanardini, E., Morosini, C., Anelli, S., et al., 2021. A new dataset of PCB half-lives in soil: Effect of plant species and organic carbon addition on biodegradation rates in a weathered contaminated soil. Sci Total Environ 750, 141411. https://doi.org/10.1016/j.scitotenv.2020.141411.

[43] Terzaghi, E., Morselli, M., Semplice, M., Cerabolini, B.E.L., Jones, K.C., Freppaz, M., et al., 2017. SoilPlusVeg: An integrated air-plant-litter-soil model to predict organic chemical fate and recycling in forests. Sci Total Environ 595, 169–177. https://doi.org/10.1016/j.scitotenv.2017.03.252.

[44] Terzaghi, E., Raspa, G., Zanardini, E., Morosini, C., Anelli, S., Armiraglio, S., et al., 2022. Life cycle exposure of plants considerably affects root uptake of PCBs: Role of growth strategies and dissolved/particulate organic carbon variability. J Hazard Mater 421, 126826. https://doi.org/10.1016/j.jhazmat.2021.126826.

[45] Terzaghi, E., Vergani, L., Mapelli, F., Borin, S., Raspa, G., Zanardini, E., et al., 2019. Rhizoremediation of weathered PCBs in a heavily contaminated agricultural soil: Results of a biostimulation trial in semi field conditions. Sci Total Environ 686, 484–496. https://doi.org/10.1016/j.scitotenv.2019.05.458.

[46] Terzaghi, E., Vitale, C.M., Salina, G., Di Guardo, A., 2020. Plants radically change the mobility of PCBs in soil: Role of different species and soil conditions. J Hazard Mater 388, 121786. https://doi.org/10.1016/j.jhazmat.2019.121786.

[47] Terzaghi, E., Zanardini, E., Morosini, C., Raspa, G., Borin, S., Mapelli, F., et al., 2018. Rhizoremediation half-lives of PCBs: Role of congener composition, organic carbon forms, bioavailability, microbial activity, plant species and soil conditions, on the prediction of fate and persistence in soil. Sci Total Environ 612, 544–560. https://doi.org/10.1016/j.scitotenv.2017.08.189.

[48] Tong, X., Mohapatra, S., Zhang, J., Tran, N.H., You, L., He, Y., et al., 2022. Source, fate, transport and modelling of selected emerging contaminants in the aquatic environment: Current status and future perspectives. Water Res 217, 118418. https://doi.org/10.1016/j.watres.2022.118418.

[49] Zhai, G., Gutowski, S.M., Lehmler, H.-J., Schnoor, J.L., 2014. Enantioselective transport and biotransformation of chiral hydroxylated metabolites of polychlorinated biphenyls in whole poplar plants. Environ Sci Technol 48, 12213–12220. https://doi.org/10.1021/es503443e.

[50] Zhai, G., Hu, D., Lehmler, H.-J., Schnoor, J.L., 2011. Enantioselective biotransformation of chiral PCBs in whole poplar plants. Environ Sci Technol 45, 2308–2316. https://doi.org/10.1021/es1033662.

[51] Zhai, G., Lehmler, H.-J., Schnoor, J.L., 2013. Sulfate metabolites of 4-monochlorobiphenyl in whole poplar plants. Environ Sci Technol 47, 557–562. https://doi.org/10.1021/es303807f.