Bioaccumulation of emerging organic compounds (perfluoroalkyl substances and halogenated flame retardants) by earthworm in biosolid amended soils

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ABSTRACT

In the present work, the bioaccumulation behavior of 49 target emerging organic compounds (20 perfluoroalkyl substances, PFASs, and 29 halogenated flame retardants, HFRs) was studied in soil invertebrates (*Eisenia andrei*). Multi species soil systems (MS · 3) were used to assess the fate and the effects associated with the application of four biosolids in agricultural soil on terrestrial soil organisms. Biosolid amendment increased concentrations 1.5–14-fold for PFASs, 1.1–2.4-fold for polybrominated diphenyl ethers, PBDEs, and 1.1–3.6-fold for chlorinated flame retardants, CFRs. Perfluorooctanesulfonate, PFOS, (25%) and BDE-209 (60%) were the predominant PFAS and HFR compounds, respectively, in biosolids-amended soils. Total concentrations (ng/g dry weight) in earthworms from biosolid-amended soils ranged from 9.9 to 101 for PFASs, from 45 to 76 for PBDEs and 0.3–32 for CFRs. Bioaccumulation factors (BAFs) were calculated to evaluate the degree of exposure of pollutants in earthworms. The mean BAF ranged from 2.2 to 198 for PFASs, 0.6–17 for PBDEs and 0.5–20 for CFRs. The relationship of PFAS and PBDE BAFs in earthworms and their log K_{ow} were compared: PFAS BAFs increased while PBDE BAFs declined with increasing log K_{ow} values. The effect of the aging (21 days) on the bioavailability of the pollutants in amended soils was also assessed: the residence time affected differently to the compounds studied.

1. Introduction

Perfluoroalkyl substances (PFASs) and halogenated flame retardants (HFRs) as polybrominated diphenyl ethers (PBDEs) and dechlorane plus (DP) have been detected in a variety of wildlife and environment (Giesy and Kannan., 2001; Hansen et al., 2001; De Wit, 2002; De Boer et al., 2003; Shoeib et al., 2004; Higgins et al., 2005; De la Torre et al., 2012a). These compounds have been intentionally incorporated in common consumer and industrial products and their production, use and disposal has led to their widespread distribution in the environment. One potential mechanism for introduction of these compounds to the environment is the application of biosolids to land. The recycling of biosolids to agriculture is the option favored internationally for biosolid management as it contributes to improve soil properties (EEC, 1986, 1991). However, the amendment in agricultural soils has

been demonstrated to cause the contamination of the soil (Sellström et al., 2005; Washington et al., 2010). The migration of pollutants from soil to plants or soil organisms could facilitate a probable entry pathway into the food chain. Organisms can achieve high concentrations of certain organic contaminants relative to concentrations of these substances in the environment they inhabit. Food web analyses have shown that PFASs and HFRs can bioaccumulate and biomagnify in aquatic ecosystems (Law et al., 2003; Houde et al., 2006). In terrestrial ecosystems, earthworms are an important link in transporting environmental contaminants from soil to other organisms in terrestrial food webs (Sellström et al., 2005), being an appropriate model organism to assess bioavailability as it lives in close contact with soil, has thin and permeable cuticle, and also consumes large amounts of soil (Jager et al., 2005). Besides, these invertebrates can change the availability of inorganic and organic pollutants in soils. Zhao et al. (2014) detected that the co-presence of wheat and earthworms enhanced the bioavailability of PFASs in soil. Therefore, it is necessary studies focused on pollutants from soil to better

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understand their bioaccumulation potential in terrestrial environment. The accumulation of chemicals is a dynamic process consisting of uptake, partition, storage, and excretion, which is greatly influenced by biological (target organisms or organs) and geochemical (physicochemical properties of the pollutants and the medium) factors (Wen et al., 2011). Then, due to their different physicochemical properties of PFASs and HFRs, the comparison of the bioaccumulation behavior would be of great interest.

In the present study, multi species soil systems (MS·3) are used to assess the fate and the effects associated with the application of biosolids in agricultural soil on terrestrial soil organisms. MS·3 is a terrestrial microcosm, an artificial assemblage of soil macro-organisms (microorganisms, invertebrates and plants) lying on homogeneous columns of sieved natural soil that allows to simulate agricultural land conditions (Fernández et al., 2004; Carbonell et al., 2009). In this system, a light/dark period and daily irrigation produces a gradient of conditions from the top to the bottom of the column. For this reason, the soil-air interface, water transport and degradation/sorption kinetics are reproduced in a better way than in standard soil bioassays.

The main objective of this work was to determine the transfer and bioaccumulation of selected emerging organic compounds as PFASs, PBDEs, decabromodiphenylethane, DBDPE, Dechloranes (602, 603, 604, and DP), Chlordane Plus (CP) and Mirex from biosolid-amended soil to earthworm (*Eisenia andrei*) using MS·3 systems. To the best of our knowledge, this is the first time to compare the bioaccumulation behavior of PFASs and HFRs in earthworms (*Eisenia andrei*) exposed to biosolids-amended soils.

2. Materials and methods

2.1. Standards and reagents

Analysis of PFASs (perfluorobutanesulfonate -PFBS-, perfluorohexanesulfonate -PFHxS-, perfluorooctanesulfonate -PFOS-, perfluorodecanesulfonate -PFDS-, perfluorobutanoic acid -PFBA-, perfluoropentanoic acid -PFPeA-, perfluorohexanoic acid -PFHxA-, perfluoroheptanoic acid -PFHpA-, perfluorooctanoic acid -PFOA-, perfluorononanoic acid -PFNA-, perfluorodecanoic acid -PFDA-, perfluoroundecanoic acid -PFUdA-, perfluorododecanoic acid -PFDoA-, perfluorotridecanoic acid -PFTrDA-, perfluorotetradecanoic acid -PFTeDA-, perfluorohexadecanoic acid -PFHxDA-, perfluorooctadecanoic acid -PFODA-, perfluorooctanesulfonamide -FOSA-, N-methyl perfluorooctanesulfonamide -N-MeFOSA- and N-ethyl perfluorooctanesulfonamide -N-EtFOSA-), PBDEs (IUPAC congener numbers: BDE-17, -28, -47, -66, -77, -85, -99, -100, -119, -138, -153, -154, -156, -183, -184, -191, -196, -197, -206, -207, -209), DBDPE and DP were performed by isotopic dilution using labelled ^{13}C, ^{18}O or deuterated standard solutions: MPFAC-MXA, N-d3-MeFOSA, N-d5-EtFOSA, ^{13}C$_9$-PFNA, MBDE-MXE and BDE-CVS-EISS were purchased from Wellington Laboratories Inc. (Guelph, Canada) and MDP from Cambridge Isotope Laboratories. Inc. (Andover, MA, USA). Dec 602 (CAS# 31107-44-5), Dec 603 (CAS# 13560-91-4) and Dec 604 (CAS# 34571-16-9) were obtained from Toronto Research Chemical Inc. (Toronto, ON, Canada).

EnviCarb cartridges (500 mg, 6 mL) were provided from Sigma-Aldrich (St. Louis, MO, USA) and Oasis WAX cartridges (500 mg, 6 mL) from Waters (Milford, MA, USA), both used for solid phase extraction (SPE).

Other chemicals used as anhydrous sodium sulphate, copper fine powder, sulphuric acid (95–97%) and solvents (hexane, dichloromethane, ethyl acetate and toluene) for organic trace analysis were obtained from Merck (Darmstadt, Germany). Ammonium acetate, ammonium hydroxide, sodium acetate, siliceous

earth extrapure, acetic acid, methanol, acetonitrile were purchased from Scharlau (Barcelona, Spain).

2.2. Study design

Multi species soil systems (MS·3) were used as rapid tests for assessing in biosolid-amended soil and exposed earthworms the presence of 49 emerging organic compounds: 20 PFASs (4 sulphonates -PFSAs-, 13 carboxilic acids -PFCAs- and 3 sulphonamides), PBDEs (21 congeners from tri to decaBDE), DBDPE, DP, Dec 602, Dec 603, Dec 604, CP and Mirex.

Firstly, a pollutant characterization was conducted in 16 biosolids: 4 municipal solid waste (MSW) compost and 12 wastewater treatment plant (WWTP) biosolids. Samples were collected during 2011 and were kindly provided by Spanish waste management companies and wastewater treatment plants. Then, only four biosolids, with a higher burden of facing contaminants, were selected for MS·3 experiment: an aerobically digested municipal solid waste (MSW) compost (W-1), an anaerobically digested thermal drying sludge (W-2), an aerobically digested composted sewage sludge (W-3) and an anaerobically digested MSW compost (W-4).

MS·3 systems consisted in PVC cylinders (20 cm internal diameter and 30 cm high) covered by a fine nylon mesh at the bottom to avoid soil loss and connected to a leachate collection device. A mixture (8 kg) of each biosolid selected and a control soil was used to fill the microcosm columns. Then, the columns were saturated with spring water and 30 plant seeds (*Triticum aestivum*, *Brassica rapa* and *Vicia sativa*), and 20 earthworms (*Eisenia andrei*) were introduced. During the exposure period (21 days) the MS·3 columns were daily irrigated (100 mL/day) to simulate 1000 mm rainfall/year. The experiments were conducted in a climate room with a light – dark cycle of 16–8 h, air condition of 21 ± 1 °C, and humidity of 55–60%.

The soil used in this study was a typical agricultural soil with known history; pesticides and fertilizers had not been applied at least for the last 10 years. The soil sample was taken within the top 20 cm soil layer, sieved (2 mm mesh), and homogenized before use. Biosolid application rates (0.12–0.56 kg biosolid/treatment) were calculated by considering the nitrogen agronomic requirement of plants sowed. The four treatments and the control were performed in triplicate, although due to small sample size (especially for earthworms) chemical analyses were conducted with pooled samples.

After MS·3 experiment, earthworms were allowed to depurate for 24 h to avoid the presence of soil particulates that could interfere with the bioaccumulation study.

2.3. Sample preparation

PFASs from biosolids and soils were extracted and purified according to the analytical procedure previously described (Navarro et al., 2011). Earthworms spiked with MPFAC-MXA, N-d3-MeFOSA and N-d5-EtFOSA solutions were extracted with acetonitrile, vortex-mixed, shaken for 10 min, ultrasonicated at 40 °C for 30 min and centrifuged for 15 min at 3000 rpm. The extraction process was repeated twice with fresh acetonitrile. Extracts were then combined and evaporated to 2 mL under nitrogen. Then, 100 μL of acetic acid was added before centrifugation for 5 min at 2000 rpm and passed through EnviCarb SPE cartridges. The purified extract was reduced to 140 μL under a gentle stream of nitrogen using TurboVap II evaporator (Vertex, Technics, Madrid, Spain). 240 μL of methanol and 240 μL of 2 mM ammonium acetate in Milli-Q water were added to the final extract spiked with ^{13}C$_9$-PFNA solution prior to HPLC-MS/MS injection.

For HFR determination, samples were processed according to

methods reported previously (De la Torre et al., 2011a, 2011b, 2012b). Briefly, waste and soil samples spiked with MBDE-MXE and MDP were extracted with a mixture of hexane: dichloromethane (1:1 v/v) by pressurized fluid extraction (ASE 200, Dionex, Sunnyvale, CA, USA). Earthworm samples were Soxhlet extracted with hexane/dichloromethane (1:1, v/v) for 24 h. The extracts were solvent exchanged with hexane and digested with 50 mL of concentrated sulphuric acid. Clean up and fractionation steps were accomplished using an automated purification Power Prep™ System (FMS Inc., USA) including acidic silica gel, basic alumina and carbon columns. Fractions were concentrated to dryness under a flow of nitrogen and redissolved in nonane and then spiked with the ^{13}C injection standards solutions (BDE-CVS-EISS) prior to HRGC-LRMS or HRMS analyses.

2.4. Instrumental analysis

PFASs were determined by HPLC-MS/MS (Varian 212 Liquid Chromatograph coupled to a Varian 320 triple cuadrupole MS). The chromatographic separation was carried out in an ACE C18-PFP (50 × 2.1 mm, 3 μm) analytical column. PBDEs were analyzed by LRMS (Agilent 6890 Gas Chromatograph connected to an Agilent 5973 MSD) for biosolids and soils. Earthworms determination required the sensitivity of the HRMS (Micromass Autospec Ultima). In both cases chromatographic separation was performed with a short and narrow capillary column (15 m × 0.25 mm i. d. × 0.10 μm film thickness; DB5 MS from J&W Scientific, Folson CA). Dechlorane compounds were analyzed by ECNI-MS (Agilent 5973MSD) using methane as a reagent gas. Complete details of instrumental method are described elsewhere (De la Torre et al., 2011a, 2011b; Navarro et al., 2011).

2.5. Quality assurance

Some criteria were used to ensure the correct identification and quantification of all analytes: i) $\pm$ 3 s retention time between the analyte and the standard, ii) the ratio of quantifier and qualifier ions had to be within $\pm$ 15% of the theoretical values and iii) signal to noise ratio had to be greater than 3:1. Procedural blanks were conducted with each batch and were extracted, purified and analyzed under the same conditions. In addition, instrumental blanks consisting of methanol (HPLC) and nonane (HRGC) were run before each sample injection to check for memory effects and contamination from analysis systems. Mean recoveries were 63%, 90% and 66% for PFASs, PBDEs and DP, respectively. Limits of detection (LODs), defined as the concentration giving a signal to noise ratio greater than 3 were in the range of 0.01–0.65 ng/g for PFASs, 0.002–8.20 pg/g for PBDEs and 1.00–9.50 pg/g for dechlorane compounds.

3. Results and discussion

3.1. Biosolid characterization

Biosolids from different Spanish WWTPs (n=16) were analyzed to characterize their PFAS and HFR content. Levels found of 9 PFASs and 22 HFRs are illustrated in Table S1. BDE-47, −99, −100, −153, −154, −183, −196, −197, −206, −207, −209, anti-DP, syn-DP and Dec-603 were detected in all samples, followed by BDE-28 and Dec-602 (94%), PFDA (88%), PFOA (75%), BDE-184 (69%) and PFOS (63%). PFOS (3.95–83.5 ng/g. d.w.), PFHxA (4.65–32.2 ng/g d.w.), PFOA (0.99–14.0 ng/g d.w.), PFDA (0.54–18.3 ng/g d.w.), BDE-99 (1.45–17.1 ng/g d.w.), BDE-206 (2.02–38.1 ng/g d.w.), BDE-207 (2.94–41.1 ng/g d.w.), BDE-209 (16.1–1312 ng/g d.w.) and DP (sum of anti- and syn-DP; 2.28 − 51.8 ng/g d.w.) were the compounds with higher contribution to the total pollutant contents. To the best of our knowledge this is the first time PFASs, brominated flame retardants, BFRs, and Dechloranes are measured at the same time in biosolids, however levels of these pollutants are in agreement with previously PFASs (Navarro et al., 2011), BFRs (De la Torre et al., 2011b, 2012b) and DP (De la Torre et al., 2011a) reported concentrations.

From the 16 biosolids analyzed, only four were selected for MS·3 experiment (see Table S2). Most of compounds studied, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, BDE-28, −47, −99, −100, −153, −154, −206, −207, −209 and DP, showed statistically significant differences ($p < 0.05$; Student's t-test) between selected biosolids, which might be of interest so as to check the compound behavior in the MS·3 experiment. Several PFASs were found in the four biosolids selected. Only PFHxS and PFDS were not detected in any sample. PFAS concentrations ranged from 0.21 to 64.4 ng/g d.w., being these ones higher in sewage sludge biosolids (W-2 and W-3) than those obtained in MSW compost (W-1 and W-4). W-2 was the biosolid with the highest PFAS content (120 ng/g d.w.) followed by W-3 (96.8 ng/g d.w.), W-1 (27.3 ng/g d.w.) and W-4 (17.6 ng/g d.w.). PFOS (37%), PFBA (31%), PFOA (11%) and PFDA (10%) were the compounds with higher contribution to the PFAS total content in these four biosolids. Considering halogenated flame retardants (HFRs), only BDE-77, −119, −156, Dec-604 and Mirex were under LOD in the biosolids selected. PBDE concentrations ranged from 0.03 to 347 ng/g d. w, DBDPE from 95.3 to 149 ng/g d.w. and CFRs from 0.003 to 10.7 ng/g d.w. The four biosolids presented a similar HFR pattern: PBDEs > DBDPE > DP > > Dec 603 > Dec 602 and CP, which is in agreement to previous results reported in Spanish biosolids (De la Torre 2011a, 2011b, 2012b). BDE 209 was the predominant PBDE congener (78%) followed by BDE-207 (6%), −206 (5%), −99 (4%) and −47 (2%). Interestingly, relative high contribution of non-aBDEs were found, which evidences a decaBDE commercial mixture origin (La Guardia et al., 2006). The fraction of anti-DP (f_{anti}) was defined as the concentration of anti-DP divided by the total DP. f_{anti} value in biosolids (0.74 $\pm$ 0.02; mean $\pm$ SD) closely resembles the OxyChem DP mixture (f_{anti} = 0.75) (Sverko et al., 2011).

3.2. Biosolid application to soil

Although pesticides or fertilizers had not been applied to the soil used in the experiments for the last 10 years, the presence of other pollutants as PFASs or HFRs were detected in the reference soil. The use of agronomic rates for biosolid application limited the amount of chemical added to the soil, but these rates were required to reproduce agricultural land conditions. However, a slight increase in the concentration of the pollutants due to the amendment was observed, which allowed to evaluate the effect on soil invertebrates.

Total concentrations for PFASs ranged from 0.60 to 0.97 ng/g d. w. in reference or control soils and 1.01–3.72 ng/g d.w. in amended soils (Table S3). Concentrations of these compounds in soils correlated linearly (r=0.597; $p < 0.01$; Spearman test) with concentrations in biosolids. PFOS (25%) was the predominant compound followed by PFBA and PFOA (22%). Biosolid-amended soil/control soil ratios were calculated to assess the contribution of the biosolid application in soil (Fig. S1). Mean values for total PFSAs (6.56 $\pm$ 1.73; mean $\pm$ SD) and PFCAs (1.94 $\pm$ 0.60) were higher than 1, showing an increase in the concentration of PFASs in soil due to the amendment. PFNA present the highest value, 14.04, followed by PFOS (2.88) > PFOA (2.34) > PFDoA (2.27) > PFHxA (2.17). Considering the different amendment treatments, the treatment 2 (T-2) presents higher ratio values (5.20 $\pm$ 7.56). The biosolid used in this treatment, an anaerobically digested thermal drying sludge

(W-2), achieved the highest total PFAS concentration (120 ng/g d. w.), followed by W-3 (96.8 ng/g d.w.), W-1 (27.3 ng/g d.w.) and W-4 (17.5 ng/g d.w.) This tendency found in the biosolids corresponds well with the soil ratios calculated: T-2 > T-3 $(3.79 \pm 6.55) >$ T-1 $(1.99 \pm 0.84) >$ T-4 (1.61 ± 2.04), revealing a dose dependent behavior.

Total concentrations for PBDEs ranged from 7.19 to 11.5 ng/g d. w. in reference or control soils and 7.5–35.2 ng/g d.w. in amended soils, 0.95–1.60 and 0.44–16.4 ng/g d.w. respectively, for DBDPE and 0.27–0.67 and 0.22–1.39 ng/g d.w. respectively for DP and related compounds (Table S3). Concentrations in soils correlated linearly (r > 0.831; $p < 0.01$) with concentrations in biosolids. Slightly higher f_{anti} value (0.80; mean) was obtained in amended soils compared to that found in the biosolids (0.74), which has been interpreted as indicative of the preferential environmental degradation of the syn isomer (Wang et al., 2010; Sverko et al., 2011). BDE-209 was the predominant PBDE congener (75%) followed by −207 (6%), −206 (4%), −99, −196 and −197 (3%). The contribution of PBDEs in the amended soils corresponds well with the tendency found in the biosolids. Biosolid-amended soil/control soil ratios for PBDEs, DP and related compounds were also calculated (Fig. S1). Mean values for total PBDEs $(1.59 \pm 0.71;$ mean $\pm$ SD), DBDPE (3.01 ± 3.63) and DP (1.72 ± 0.56) were lower than calculated for PFASs, but higher than 1, showing as well, an increase in soil due to the amendment. The highest ratio was for syn-DP (3.64) followed by DBDPE > Dec-602 (2.73) > BDE-99 (2.43) > BDE-100 (2.33). The treatment 4 (T-4), which corresponds an anaerobically digested MSW compost, present higher ratio values (2.67 ± 1.78), followed by T-2 $(1.67 \pm 1.11) >$ T-3 $(1.60 \pm 0.79) >$ T-1 (1.47 ± 1.06). In this case, a significant dose dependent tendency is observed by DBDPE, showing ratio values: T-4 (8.36) > T-2 (2.14) > T-3(1.06) > T1 (0.48) that are in consistency with the corresponding concentrations for biosolids: W-4 (149.46 ng/g d.w.) > W-2 (125.83 ng/g d.w.) > W-3 (95.27 ng/g d. w.) > W-1(< LOD). This dose dependent behavior has not been detected for all compounds, which could be easily attributed to the heterogeneity inherent of biosolids.

3.3. Bioaccumulation in earthworms

3.3.1. Concentrations of PFASs and HFRs in earthworms

PFOS and long-chain perfluorinated carboxylic acids, PFCAs, $(C_8–C_{14}:$ PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA) were detected in earthworms. Total PFAS concentrations in earthworms from biosolid-amended soils ranged from 9.88 to 99.86 ng/g d.w. and were higher than concentrations from control ones (1.76 ng/g d.w.) (Table S3, Fig. 1). Concentrations of PFOA, PFUdA, PFTrDA and PFTeDA in earthworms correlated well with levels described in biosolids (r > 0.877; $p < 0.01$). The highest value of total PFAS concentration (99.86 ng/g d. w) corresponds to earthworms from T-2, which showed also the highest PFAS values in biosolids $(120.02 \pm 15.86$ ng/g d.w.) and soils $(2.52 \pm 1.56$ ng/g d.w.). Percentage distributions of PFASs observed in earthworms in the different treatments are shown in Fig. 2. PFOS (39%) was the predominant compound followed by PFDoA and PFTeDA (22%) and PFTrDA (15%). The latter three and PFUdA, were found in biosolids, but PFTrDA and PFTeDA were not detected in soils. Therefore, their presence in earthworms may indicate accumulation phenomena although soil levels were bellow detection limits. PFBS was not detected in earthworms, although concentration in soil in some case (0.19 ng/g d.w.) reached levels similar to PFOS (0.21 ng/g d. w.). This fact suggests low bioaccumulation rates for PFBS in earthworms, which is in agreement with previous studies conducted in biota (Martin et al., 2003; Loi et al., 2011).

Halogenated flame retardants (16 PBDE congeners and 5 CFRs) were also detected in earthworms. As mentioned for PFASs,

pollutant levels above detection limits in the control soil resulted in measurable levels in earthworms obtained in the MS·3. However, a statistically significant PBDE concentration increase was observed in earthworms when we considered biosolid amended soils (from 7.5 up to 76.3 ng/g d.w. for PBDEs and from 0.23 up to 31.4 ng/g d.w. for DP, respectively; Table S3 and Fig. 1.). PBDE concentrations in earthworms correlated well with levels found in biosolids (r=0.715; $p < 0.01$) and soils (r=0.703; $p < 0.01$). Furthermore, PBDE pattern in earthworms also resembles the one described in amended soils, being BDE-209 the predominant congener (81%) followed by − 47 and − 99 (6%), see Fig. 2. CFR concentrations in earthworms correlated linearly with concentrations in biosolids (r=0.845; $p < 0.01$). Concentrations of anti-DP in most of the earthworm samples, as in all biosolids selected and soil ones were notably higher than those of syn-DP. This predominance of anti-DP isomer is in accordance with previous studies in biota (Kang et al., 2010; De la Torre et al., 2012a; Xiao et al., 2013; He et al., 2014). Mean f_{anti} in earthworms (0.66) was lower than corresponding value quantified in the amended soils (0.80). Data suggest an enrichment of syn-DP in earthworms relative to cultivation media, and although it must be viewed with caution regarding sampling size, similar behavior has been reported in aquatic species (Hoh et al., 2006; Tomy et al., 2007; Kang et al., 2010; He et al., 2014) which may support this hypothesis. The chemical structure of the anti-DP isomer should be more susceptible to biological attack than the less sterically hindered syn-DP isomer, which could explain a lower f_{anti} value in biological samples compared to the value of technical DP (Xiao et al., 2013).

3.3.2. Bioaccumulation factors of PFASs and HFRs in earthworms

Bioaccumulation factors (BAFs) are widely used to estimate the degree of exposure and the potential risk in wildlife based on levels of soil contamination. BAFs were determined by division of the amount found in earthworms on a dry weight basis (ng/g d.w.) by concentrations determined in soil on a dry weight basis (ng/g d. w.; mean soil t=0 and final) (Table S3). The mean organic content of the biosolid amended soils was $4.4 \pm 0.4\%$ (mean $\pm$ SD) and the mean lipid content of earthworms was $9.3 \pm 0.3\%$. Due to the very low variability found between treatments, BAF values calculated have been considered comparable. PFAS BAF values in earthworms correlated linearly with total PFAS concentrations in biosolids (r=0.752; $p < 0.01$). The mean BAF of PFASs ranged from 2.2 (PFOA) to 198 (PFDoA). Zhao et al. (2013) also reported the highest BAF value for PFDoA in earthworms exposed to artificially contaminated soils with PFASs. If BAFs for PFOS (21) and PFOA (2.2) are compared, PFASs with the same carbon chain length, values are higher for PFOS. This higher predominance of PFSA BAFs in comparison with PFCA BAFs is in accordance with other studies (Zhao et al., 2013; Wen et al., 2015). A calculation for the PFAS BAF on wet weight basis, for comparison with the literature data, shows that values ranged from 1.1 to 4.1 for PFOS, 0.2–0.4 (PFOA), 0.6–3.4 (PFDA), 1.6 (PFNA), 18 (PFUdA) and 11–115 (PFDoA) and most of them correspond well with data previously reported in worms (1.7–24 for PFOS, 3.2– 8.3 for PFDA and 2.3–6.4 for PFUdA; Loi et al., 2011). BAFs were also calculated based on the concentration of the compounds in soils at the beginning and the end of the experiment (21 days), and some PFAS BAFs declined with the increasing residence time in soil (Fig. 3). The BAFs of PFOA, PFNA, PFDA and PFUdA decreased 26, 34, 58% and 32% respectively, suggesting that the bioavailability of these congeners in soil decreased as the time of their residence in soil increased. The contribution of these compounds to the total concentration of PFASs in soils was from 2 to 22% and 5–13% in earthworms. Nevertheless, the effect of the aging (21 days) on the bioavailability of PFOS, the main PFASs in soils (25%) and earthworms (39%), was not observed. According to Sijm et al. (2000), the
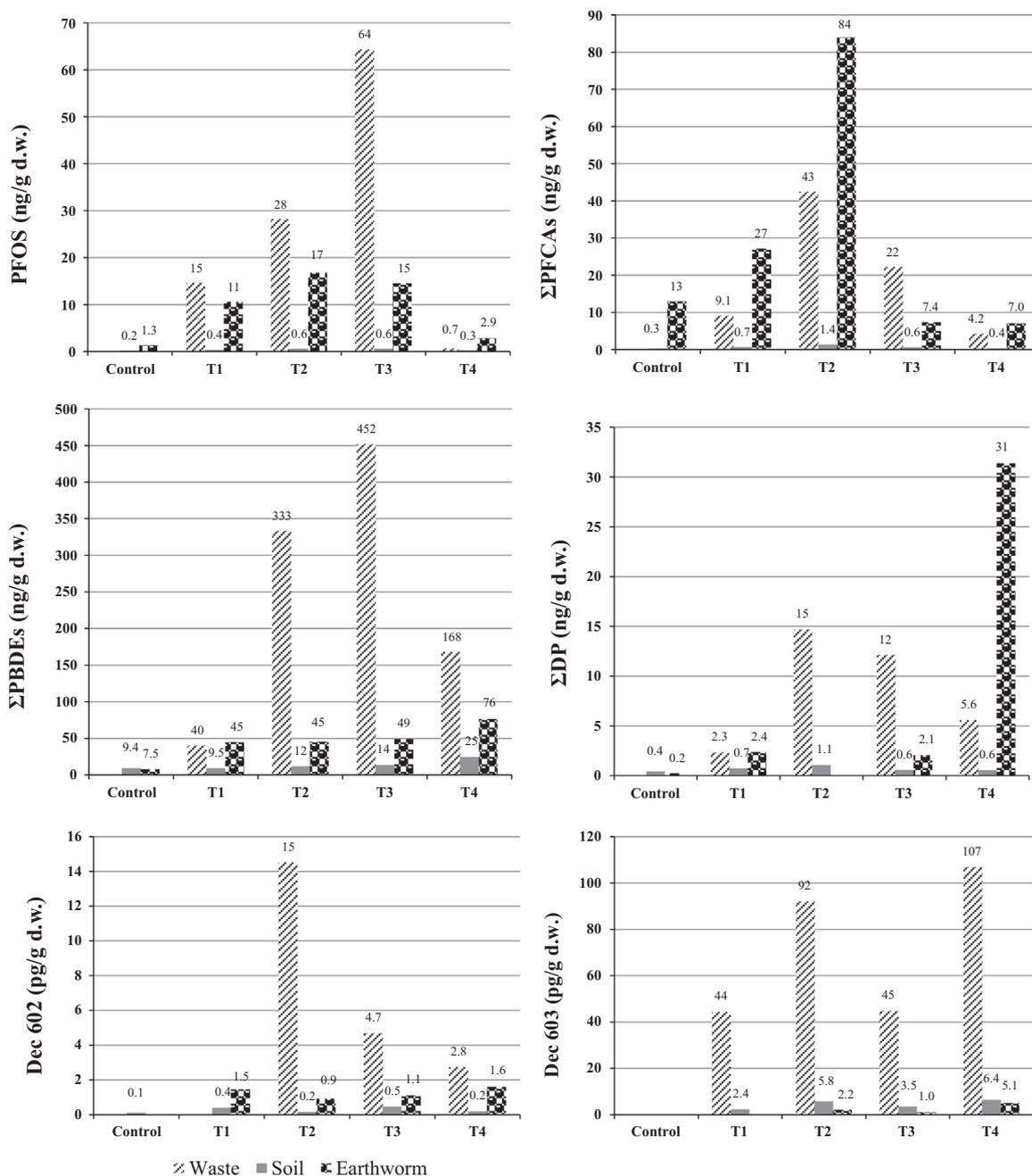
Fig. 1. Concentrations of PFOS, PFCAs (sum of C_8–C_{14}), PBDEs, DP (sum of *anti*- and *syn*-DP), Dec 602, and Dec 603 for the wastes, amended soils, and earthworms studied.

bioavailability of contaminants in soil depends on the physico-chemical properties of the chemicals, the characteristics of soil, the physiology and habitat of organisms and the residence time of the pollutants in soil matrix. By contacting with soil substrates for a long time, hydrophobic organic chemicals diffuse slowly into the micropores or components of solid organic matter in soils and the desorption-resistant fraction increases with time as the chemical remains in the soil (Hatzinger and Alexander, 1995). Once chemicals were entrapped into micropores in the soil, it would become very difficult for them to release into porewater, to be bioavailable and to be taken up by organisms. To explore this hypothesis, the relationship between PFAS BAFs in earthworms and the chemical log K_{ow} was investigated. As shown in Fig. 3, BAFs increased with the log K_{ow} and also with the perfluorinated chain length for PFCAs indicating an effective bioaccumulation of long-chain per-fluorinated carboxylic acids in earthworms. This tendency has

been observed previously (Lasier et al., 2011; Zhao et al., 2013, 2014). Significant linear correlation was observed between Log BAF and Log K_{ow} for PFASs (r= 0.527, $p < 0.01$).

Bioaccumulation factors were also determined for HFRs (Table S3). PBDE bioaccumulation decreases with the bromination degree, which is in agreement to other studies (Sellström et al., 2005; Liang et al., 2010), and higher BAF was obtained for *syn*-DP (20) compared to *anti* isomer (16). Related to BAF values calculated on soil basis at the beginning and the end of the experiment, after aging for 21 days, the BAFs of some PBDE congeners decreased compared to corresponding BAFs from the initial biosolids-amended soil (see Fig. 3), suggesting that the bioavailability of these congeners in soil may decrease with the increasing residence time in soil: decrease in BAFs of BDE-47 (57%) > BDE-196 (37%) > BDE-206 (36%) > BDE-191 (35%). These congeners contributed to the total concentration of PBDEs in soils from 0.2 to 4%
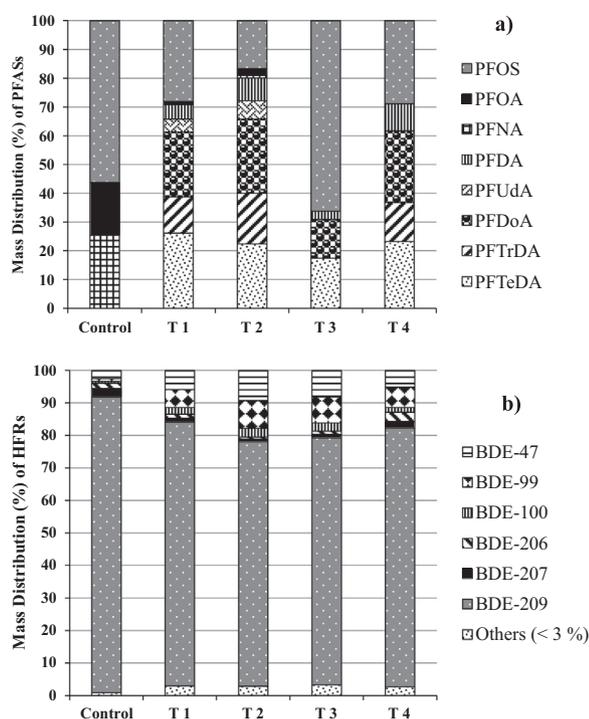
Fig. 2. Mass distribution (percentage of the total amount taken up by the earthworms) of PFASs (a) and PBDEs (b) in different treatments. For clarity, only compounds with contribution higher than 3% are shown; those with < 3% are included as others (BDE-17, BDE-28, BDE-138, BDE-153, BDE-154, BDE-183, BDE-184, BDE-191, BDE-196 and BDE-197).

and 0.1–7% in earthworms. The effect of the aging (21 days) on the bioavailability of the rest of the PBDE and CFRs were not significant. Thus, no differences were found for BDE-209, the major PBDE congener in soil (75%) and earthworm (76%). Liang et al. (2010) observed for earthworms exposed to PBDE artificially contaminated soils that, after 90 days aging, the bioavailability of the PBDE congeners was affected differently.

The relationship of PBDE and CFR BAFs in earthworms and their log K_{ow} is illustrated in Fig. 3. PBDE BAFs declined with increasing log K_{ow} of the congeners, significant linear correlation was found for PBDEs (r= −0.491, $p < 0.01$; Spearman test) and CRFs (r=0.773, $p < 0.01$). PBDEs in soil become less available with their increasing log K_{ow}. These results are similar to findings previously reported (Sellström et al., 2005; Liang et al., 2010). The large molecular size (> 0.95 nm) and high hydrophobicity could limit the ability of PBDEs to penetrate in cell membranes and decrease the bioavailability and consequently, the uptake efficiency of the earthworms from the soil.

The HFR BAFs were found to be lower than PFAS ones, indicating a lower bioaccumulation potential in earthworms for the former compared to the latter. It was reported that concentration of PFASs in organisms positively correlated with protein content, rather than lipid content (Hoff et al., 2003). The PFASs possess a high affinity to proteins and, sometimes, they are referred as proteinophilic. Besides, they are potentially accumulative in biota, especially in protein-rich tissues (Bischel et al., 2010; Zhao et al., 2013; Loi et al., 2011). Nevertheless, PBDEs are lipophilic and bioaccumulate in the fatty tissues of the organisms (Voorspoels et al., 2006). Thus, significant correlations have been found in biota between concentrations of PBDEs and lipid content (Law et al., 2006; Yu et al., 2009; Xiang et al., 2007), and also between DP levels and lipid content (Tomy et al., 2007). The earthworms have high protein content, in the range of 65–67% dry weight, and lower lipid content, 10.9–12.1% (Zhao et al., 2013). The protein/lipid

content in earthworms and the different binding or accumulation affinities of PFASs (proteins) and HFRs (lipids) could explain the differences observed in BAFs and consequently, the different bioaccumulation behavior in earthworms.

3.3.3. Specific congener behavior of PBDEs in earthworms

Uptake efficiencies, metabolism, and bioavailability of the PBDE compounds to living organisms can account for the differences in congener profiles (Luross et al., 2002). It has been reported that the concentration ratio of BDE-99 and BDE-100 could be related to the difference in metabolism of biota in aquatic environment (Voorspoels et al., 2003; Xiang et al., 2007; Yu et al., 2009). Lower concentration ratios of BDE-99 to BDE-100 in biota (from 70:30 to 25:75) compared to those found in sediments (80:20) where they lived in, have been reported to reflect that the specie is capable of metabolizing BDE-99 (Voorspoels et al., 2003; Xiang et al., 2007). Concentration ratios of BDE-153 to BDE-154 were also calculated by Xiang et al., and similar results were found, suggesting that BDE-153 to BDE-154 ratio also reflected the metabolization ability of aquatic species (Xiang et al., 2007). For comparison in terrestrial environment, the concentration ratios of BDE-99 to BDE-100, in the present study, for earthworms were significantly lower ($p < 0.05$; Mann-Whitney U test), with a mean ratio of 72:28, than the ratios in soils (mean 80:20) and biosolids (83:17). Similar behavior was detected for BDE-153 to BDE-154 ratios ($p < 0.01$; Mann-Whitney U test), with a mean concentration of 45:55 for earthworms, 60:40 for soils and 61:31 for biosolids, which may indicate that earthworms are capable of metabolizing BDE-99 and BDE-153. These results were corroborated with calculated BAFs. Bioaccumulation factors for BDE-100 (14) and BDE-154 (4.1) were higher than those for BDE-99 (11) and BDE-153 (2.5). A similar pattern was observed in the aquatic worm *Lumbriculus variegatus*, showing BAFs for BDE-100 (9.9) and − 154 (9.1) higher than for BDE-99 (4.0) and − 153 (4.7) (Ciparis and Hale, 2005). Besides, the depuration rate constants calculated in *L. variegatus* for BDE-100 and − 154 were lower than that for BDE-99 and likely BDE-153, suggesting that ortho-substituted congeners are eliminated more slowly and consequently, are more bioaccumulative than meta-substituted ones (Ciparis and Hale, 2005).

4. Conclusions

Multi species soil systems (MS·3) were used to assess the fate and the effects associated with the application of biosolids in agricultural soil on terrestrial soil organisms. The study of 49 emerging organic compounds, 20 PFASs and 29 HFRs, in biosolids, biosolids-amended soils and earthworms exposed to these soils demonstrates the presence and transfer of these pollutants in the soil-biota system. The biosolid application contributed to the increase of perfluoroalkyl substances and halogenated flame retardants in soil and to their bioaccumulation in earthworms. To the best of our knowledge, this study is the first to compare the bioaccumulation behavior of PFASs and HFRs in earthworms exposed to biosolids-amended soils. Results showed a higher bioaccumulation potential in earthworms for PFASs followed by CFRs and PBDEs. The differences in bioaccumulation of the pollutants in earthworms suggest a different bioavailability of the compounds in the soil. The characteristics of soil, the physiology and habitat of organisms and mainly the physicochemical properties of the chemicals could have an important role in the migration of the compounds through the soil food web.
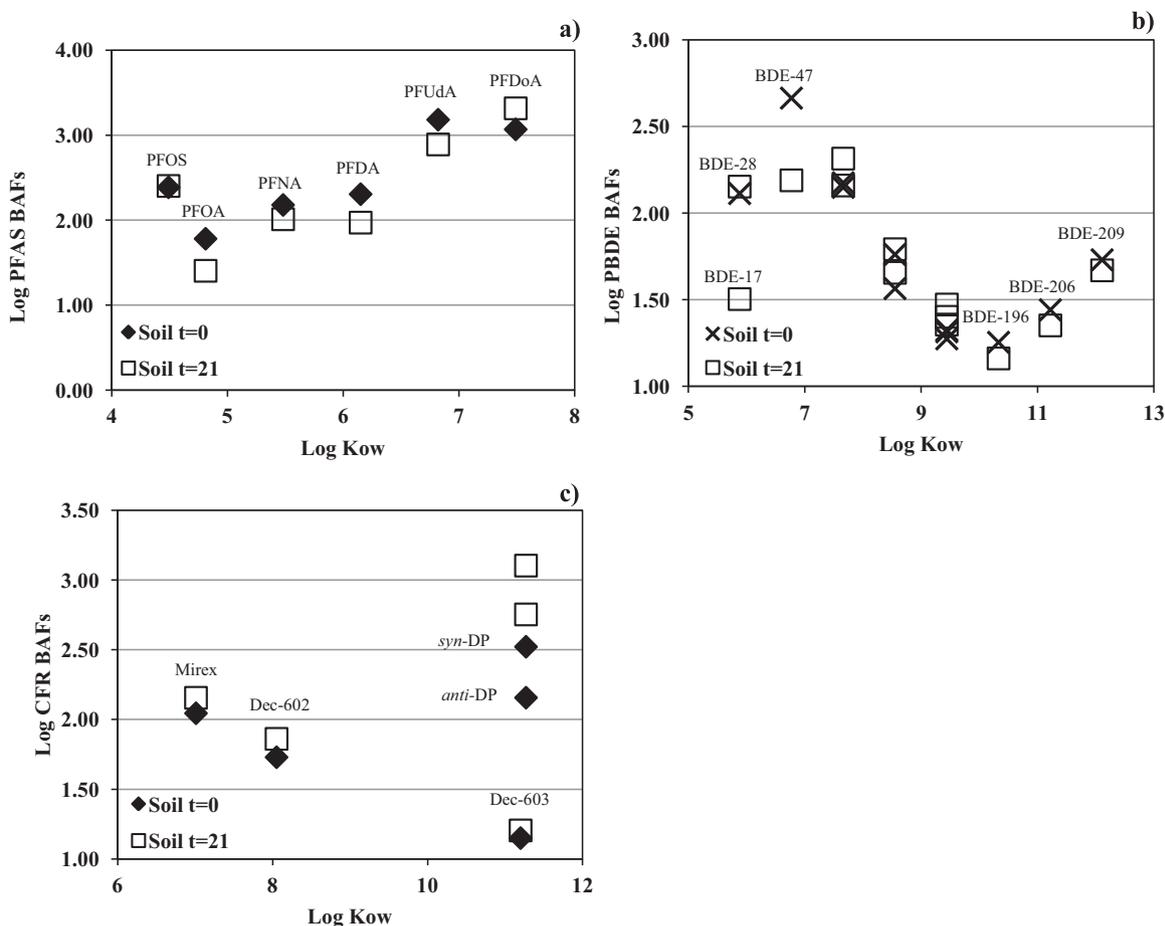
Fig. 3. Relationship of the BAFs of PFASs (a), PBDEs (b) and chlorinated flame retardants, CFRs, (c) in earthworms with their log K_{ow}. Data expressed as Log BAF represent $1+\log(1+\text{BAF})$ and BAF represents mean values. BAFs were calculated as ratio between earthworm concentrations after exposed in the soil for 21 days and soil concentration at the beginning and the end of the experiment (both on a dry weight basis). Log K_{ow} used were calculated by EPISuite 4.1. For clarity, BAF values for BDE-197 and BDE-207 are not shown.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.05.004.

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