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Faculty of Natural Resources and Agricultural Sciences

The bioavailability of perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs) in soil to *Eisenia fetida* and *Cucurbita pepo*

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Abstract

The bioavailability of perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs) in soil to *Eisenia fetida* and *Cucurbita pepo*

Masoumeh Moshfeghi Mohammadi

Persistent organic pollutants (POPs) are organic chemicals of great concern because of their persistency, bioaccumulation and toxic effects both in the environment and to humans. Two groups of organic chemicals that are partly listed as POPs are perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs). PFASs are man-made organic chemicals that became of concern in recent decades due to their presence in wildlife and humans. PAHs are organic chemicals that are produced unintentionally as a result of incomplete combustion.

The two main purposes of this master thesis were 1) to investigate the bioavailability of PFASs and PAHs in soil to earthworm (*Eisenia fetida)* and zucchini (*Cucurbita pepo)*, and 2) to study the influence of PFASs on the availability of PAHs. Two different groups of soils (field-contaminated and spiked soils) were studied. The earthworm experiment was done in 7 weeks and the plant experiment in 12 weeks. Chemical analysis was done by liquid chromatography coupled to mass spectrometry (LC-MS\MS) and gas chromatography coupled to mass spectrometry (GC-MS\MS) for PFASs and PAHs, respectively. Bioaccumulation factors (BAFs) and biota-soil accumulation factors (BSAFs) were calculated for the uptake in *E. fetida* while bioconcentration factors (BCFs) were calculated for the uptake in *C. pepo*. The BAFs for PFASs were generally larger than BAFs for PAHs; therefore it can be said that PFASs are more bioaccumulative than PAHs. The BSAFs of PFASs in earthworms increased when the perfluorocarbon chain length increased, but the chain length had an inverse effect on BCF in zucchini, showing a decreased BCF when the chain length increased. The effect of the functional group of the PFASs on BSAF and BCF was studied, and no significant differences between the compounds with the same chain length but different functional groups could be observed. Moreover, BSAFs of PAHs had a slight tendency to increase with an increase in log K_{OW}. The bioavailability of PAHs in two soils, one without PFASs and one with PFASs, was compared and it was shown that the PAH availability increased in the presence of PFASs.

Keywords: PFASs, PAHs, bioavailability, BAF, BSAF, BCF, earthworm, zucchini

Popular science description

The bioavailability of perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs) in soil to *Eisenia fetida* and *Cucurbita pepo*
Masoumeh Moshfeghi Mohammadi

Persistent organic pollutants (POPs) are organic chemicals that are difficult to degrade, accumulative in living organisms and have toxic effects on human health and wildlife. Two chemical groups that can be considered as POPs are perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs). PFASs are man-made organic chemicals which have been produced since the 1950's. They have been used extensively in different industries such as for coating food packages, in paper and textiles (for example water-proof jackets), lubricants, paints and firefighting foams. The PFASs consist of a long chain of carbon atoms with attached fluorine atoms. This makes them very stable and difficult to degrade, and also hydrophobic, which means that they are not very soluble in water. On the other hand, at the end of the carbon chain there is a functional group that is hydrophilic, and therefore has an affinity for water. The combination of these properties makes the PFASs surfactants; they tend to end up at the interface between for instance oil and water. Since the 2000s, the toxicity and environmental effects of PFASs have become of increasing concern. The most investigated PFASs are perfluoroalkyl carboxylic acids (PFCAs) which have carboxylic acid as a functional group, and perfluoroalkyl sulfonic acids (PFSAs) which have sulfonic acid as a functional group.

The PAHs are also organic chemicals that are widely present in environment. Their main production is from the incomplete combustion of fossil fuels, woods, and solid wastes. However, PAHs are also produced by natural sources such as forest fires and biochemical processes in the environment. Sixteen PAHs are considered as especially important pollutants, and seven of these are classified as carcinogenic. The PAHs are hydrophobic and lipophilic (compounds with an affinity for lipids); therefore, they tend to sorb to soil particles. Those pollutants that sorb strongly to soil particles are not available for uptake by animal or plants, that is, they are not bioavailable. This is an important factor controlling the fate of contaminants.

The two main purposes of this master thesis were 1) to investigate the bioavailability of PFASs and PAHs in soil to earthworm (*Eisenia fetida)* and zucchini (*Cucurbita pepo)*, and 2) to study the influence of PFASs on the availability of PAHs. Since PFASs are surfactants they can increase the solubility of hydrophobic substances in water. So, they may increase the mobility of PAHs. Two different groups of soils were studied: field-contaminated and spiked soils. Field-contaminated soils were sampled at polluted areas in the environment, while spiked soils were contaminated intentionally in the laboratory by adding PFASs. The reason for spiking the soil was to increase the concentration of contaminants to easier detect if they were taken up by earthworms and plants. The earthworm experiment was done in 7 weeks and the plant experiment in 12 weeks. Chemical analysis was done by liquid chromatography coupled to mass spectrometry (LC-MS\MS) for PFASs and gas chromatography coupled to mass spectrometry (GC-MS\MS) for PAHs. To evaluate the bioavailability of PFASs and

PAHs, the bioaccumulation in earthworm and the bioconcentration in zucchini were measured. Bioaccumulation is the process in which the concentration of a chemical in an organism is increased over the concentration of the chemical in the surrounding environment by several uptake routes, including through diet. Bioconcentration is the same process, without uptake through diet. Bioaccumulation is expressed by bioaccumulation factors (BAFs) or biota-to-soil-accumulation factor (BSAFs) and bioconcentration is expressed by bioconcentration factors (BCFs). These factors show the ratio of the chemical concentration in the organism to the surrounding environment.

The BAFs for PFASs were generally larger than BAFs for PAHs. Therefore, it can be concluded that PFASs are more bioaccumulative than PAHs. The BSAFs of PFASs in earthworms increased when the chain length of the PFAS increased. On the other hand, the BCFs in plants decreased when the chain length increased. The effect of the functional group of the PFASs on BSAF and BCF was studied, but there were no significant differences in uptake caused by differences in the type of functional group. Moreover, BSAFs of PAHs had a tendency to increase with an increase in hydrophobicity. Finally, the bioavailability of PAHs increased in the presence of PFASs, which indicates that attention needs to be given to places that are contaminated with both PFASs and other compounds.

Abbreviations

Abbreviation	Name
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BSAF	Biota-soil acculation factor
C.pepo	*Cucurbita pepo*
E. fetida	*Eisenia fetida*
PAHs	Polycyclic aromatic hydrocarbons
PFASs	Perfluoroalkyl substances
PFCAs	Perfluoroalkyl carboxilic acids
PFSAs	Perfluoroalkyl sulfonic acids
POPs	Persistent organic pollutants
REACH	Registration, Evaluation, Authorization and restriction of CHemical substances
PFASs	
PFBA	Perfluorobutanoic acid
PFPeA	Perfluoropentanoic acid
PFHxA	Perfluorohexanoic acid
PFHpA	Perfluoroheptanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoDA	Perfluorododecanoic acid
PFTriDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFHxDA	Perfluorohexadecanoic acid
PFOcDA	Perfluorooctadecanoic acid
PFBS	Perfluorobutane sulfonic acid
PFHxS	Perfluorohexane sulfonic acid
PFOS	Perfluorooctane sulfonic acid
PFDS	Perfluorodecane sulfonic acid
FOSA	Perfluoro-1-octanesulfonamide
N-MeFOSA	*N*-methylperfluoro-1-octansulfonamide
N-EtFOSA	*N*-ethylperfluoro-1-octansulfonamide
FOSAA	Perfluoro-1-octanesulfonamidoacetic acid
N-MeFOSAA	*N*-methylperfluoro-1-octanesulfonamidoacetic acid
N-EtFOSAA	*N*-ethylperfluoro-1-octanesulfonamidoacetic acid
N-MeFOSE	*2.(N*-methylperfuoro-1-1octanesulronamido)-methanol
N-EtFOSE	2-(*N*-ethylperfluoro-1-octanesulfonamido)-ethanol
PAHs	
Nap	Naphthalene
Acy	Acenaphthylene
Ace	Acenaphthene
Flu	Fluorene
Phen	Phenanthrene
Ant	Anthracene
Fluo	Fluoranthene
Pyr	Pyrene
B(a)A	Benz[a]anthracene
Chry	Chrysene
B(b)F	Benz[b]fluoranthene
B(k)F	Benz[k]fluoranthene
B(a)P	Benz[a]pyrene
IP	Indeno[1,2,3-c,d]pyrene
BghiP	Benzo[g,h,i]perylene
DBA*	Dibenzo[a,h]anthracene

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1. Introduction

1.1 Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are organic chemicals that are persistent in the environment, bioaccumulative in organisms and have toxic effects on human health and wildlife. Moreover, they have the potential for long-range transport (Lohmann et al., 2007). In 2001, 91 countries and the European Community signed a United Nations treaty called the Stockholm Convention (SC). Under the SC, parties agreed to reduce or eliminate the production, use or release of POPs that are listed under the convention. The original list consisted of 12 POPs, called "the dirty dozen". Later, new POPs have been added to the SC list that means that their production or use should be phased out or restricted. Two chemical groups which can be considered to be POPs, but which are only partially listed in the SC, are perfluoroalkyl substances (PFASs) and polycyclic aromatic hydrocarbons (PAHs).

1.2 Perfluoroalkyl substances (PFASs)

PFASs are organic chemicals that have been synthesized and applied extensively in different industries and commercial products since the 1950's (Kannan, 2011). Perfluoroalkyl substances are compounds for which all hydrogen atoms are substituted with fluorine atoms. Carbon-fluorine bonds are one of the strongest chemical bonds (Buck et al., 2011) and have high thermal stability. PFASs consist of a hydrophobic carbon chain and hydrophilic functional groups (such as carboxylic acids and sulfonic acids); therefore they are both lipophobic, hydrophobic and hydrophilic (Buck et al., 2011; Lindstrom et al., 2011). Because of this combination of physicochemical properties, PFASs are good compounds for water and oil repellents and surfactants (Lindstrom et al., 2011). They have therefore been used extensively in different industries and products, such as coating food packages, paper and textiles, lubricants, paints and firefighting foams (Benskin et al., 2012; Buck et al., 2011; Lindstrom et al., 2011). They can be found in high concentration in water (e.g., drinking water, lakes, rivers and oceans), food, air, aquatic biota and humans (Kannan, 2011; Lindstrom et al., 2011). PFASs are toxic and very resistant to degradation in the environment (Lindstrom et al., 2011). In addition, they are bioaccumulative and can remain in humans or animals for a long time due to their long half-lives (Lindstrom et al., 2011). The environmental occurrence and toxicity of PFASs have received increased attention since the PFASs were found in human blood serum and in the environment in the early 2000s (Kannan, 2011; Lindstrom et al., 2011). Perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) are the most investigated PFAS groups (Conder et al., 2008). Perfluoroctane sulfonate (PFOS), a PFSA, has been listed as an Annex B[1] substance in the Stockholm Convention in 2009 (Kannan, 2011; Lindstrom et al., 2011; Vierke et al., 2012). In addition, perflurooctanoic acid (PFOA), a PFCA, is a candidate to be listed in Annex XVII[2]

[1] Annex A (elimination): the production and use of chemicals listed under this annex must be eliminated by parties. Annex B (restriction): the production and use of chemicals listed under this Annex must be restricted by parties considering any applicable and acceptable purpose.
[2] Annex XVII is a list in REACH which regulates "restrictions of the manufacture, placing on the market and use of certain dangerous chemical substances, mixtures and articles"

of REACH (Registration, Evaluation, Authorization and restriction of CHemical substances, the European Union regulation for chemicals) (Vierke et al., 2012). In years 2001-02, the main manufacture company for fluorinated polymers (3M Co.) stopped the production of PFOS (Kannan, 2011; Lindstrom et al., 2011). Additionally, United States Environmental Protection Agency (USEPA) and eight main manufacturers made an agreement to reduce 95% of emissions of PFOA, its precursors and long-chain PFASs by 2010 and eliminate them by 2015 (Kannan, 2011; Lindstrom et al., 2011; Vierke et al., 2012). However, many other companies continue to produce PFASs worldwide, especially in developing countries (Kannan, 2011; Lindstrom et al., 2011).

To understand the distribution and fate of contaminants in the environment, it is necessary to know how they partition to different phases, which can be evaluated based on the compound's physicochemical properties. The physicochemical properties of PFASs are not well investigated (Kannan, 2011); however, some estimates of the physicochemical properties have been obtained by experiments and modeling studies (Table 1).

Table 1. Physicochemical properties of PFASs (Wang et al,. 2013). S_W: water solubility, K_{OW}: octanol-water partition coefficient, NA: not available.

Abbreviation	Chemical name	Formula	Molecular weight	Log Sw (mol L^{-1})	Log K_{ow}
Perfluorinated carboxylate acids (PFCAs)					
PFBA	Perfluorobutanoic acid	$C_3F_7CO_2H$	214	0.42	2.82
PFPeA	Perfluoropentanoic acid	$C_4F_9CO_2H$	264	-0.37	3.43
PFHxA	Perfluorohexanoic acid	$C_5F_{11}CO_2H$	314	-1.16	4.06
PFHpA	Perfluoroheptanoic acid	$C_6F_{13}CO_2H$	364	-1.94	4.67
PFOA	Perfluorooctanoic acid	$C_7F_{15}CO_2H$	414	-2.73	5.30
PFNA	Perfluorononanoic acid	$C_8F_{17}CO_2H$	464	-3.55	5.92
PFDA	Perfluorodecanoic acid	$C_9F_{19}CO_2H$	514	-4.31	6.50
PFUnDA	Perfluoroundecanoic acid	$C_{10}F_{21}CO_2H$	564	-5.13	7.15
PFDoDA	Perfluorododecanoic acid	$C_{11}F_{23}CO_2H$	614	-5.94	7.77
PFTriDA	Perfluorotridecanoic acid	$C_{12}F_{25}CO_2H$	664	-6.59	8.25
PFTeDA	Perfluorotetradecanoic acid	$C_{13}F_{27}CO_2H$	714	-7.42	8.90
PFHxDA	Perfluorohexadecanoic acid	$C_{15}F_{31}CO_2H$	814	NA	NA
PFOcDA	Perfluorooctadecanoic acid	$C_{17}F_{35}CO_2H$	914	NA	NA
Perfluorinated sulfonic acids (PFSAs)					
PFBS	Perfluorobutane sulfonic acid	$C_4F_9SO_3H$	300	-1.00	3.90
PFHxS	Perfluorohexane sulfonic acid	$C_6F_{13}SO_3H$	400	-2.24	5.17
PFOS	Perfluorooctane sulfonic acid	$C_8F_{17}SO_3H$	500	-3.92	6.43
PFDS	Perfluorodecane sulfonic acid	$C_{10}F_{21}SO_3H$	600	-5.39	7.66
Potential PFSA and PFCA precursors					
FOSA	Perfluoro-1-octanesulfonamide	$C_8F_{17}SO_2NH_2$	499	-5.05	5.62
***N*-MeFOSA**	*N*-methylperfluoro-1-octansulfonamide	$C_8F_{17}SO_2N(CH_3)CH_2COH$	555	-6.35	6.07
***N*-EtFOSA**	*N*-ethylperfluoro-1-octansulfonamide	$C_8F_{17}SO_2N(CH_2CH_3)CH_2COH$	569	-6.97	6.71
FOSAA	Perfluoro-1-octanesulfonamidoacetic acid	$C_8F_{17}SO_2NH(CH_2CO_2H)$	557	NA	NA
***N*-MeFOSAA**	*N*-methylperfluoro-1-octanesulfonamidoacetic acid	$C_8F_{17}SO_2N(CH_3)CH_2CO_2H$	571	NA	NA
***N*-EtFOSAA**	*N*-ethylperfluoro-1-octanesulfonamidoacetic acid	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2H$	585	NA	NA
***N*-MeFOSE**	2.(*N*-methylperfuoro-1-1octanesulronamido)-methanol	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$	569	-6.22	6.00
***N*-EtFOSE**	2-(*N*-ethylperfluoro-1-octanesulfonamido)-ethanol	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$	583	-6.73	6.52

1.3 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are well-known aromatic organic chemicals that consist of two or more fused benzene rings. The main anthropogenic sources of PAHs are incomplete combustion of fossil fuels (e.g., coal and petroleum), woods, and solid wastes, while natural sources like forest fires, organic matter's diagenesis and biochemical synthesis are less important (Banger et al., 2010). PAHs are pollutants that are available in the environment ubiquitously. 16 PAHs has been listed as priority pollutants by USEPA and seven of these are classified as carcinogenic (Banger et al., 2010; Parrish et al., 2006). In contrast to the PFASs, which are both hydrophobic and hydrophilic, the PAHs are hydrophobic and lipophilic. They have low water solubility and high tendency to sorb to soil particles (Banger et al., 2010). The different PAH substances have different molecular structure and physicochemical properties (Table 2); therefore, the fate of PAHs varies in the environment (Parrish et al., 2006).

Table 2. Physicochemical properties of PAHs (Ma et al., 2009; * Lundstedt et al., 2007), S_W: water solubility, K_{OW}: octanol-water partition coefficient.

Abbreviation	Chemical Name	Formula	Molecular weight	Log S_W (mol L^{-1})	Log K_{ow}
Nap	Naphthalene	$C_{10}H_8$	128	-1.51	3.40
Acy	Acenaphthylene	$C_{12}H_8$	152	-1.80	3.85
Ace	Acenaphthene	$C_{12}H_{10}$	154	-2.41	3.95
Flu	Fluorene	$C_{13}H_{10}$	166	-2.77	4.11
Phen	Phenanthrene	$C_{14}H_{10}$	178	-2.94	4.47
Ant	Anthracene	$C_{14}H_6$	178	-4.37	4.51
Fluo	Fluoranthene	$C_{16}H_{10}$	202	-3.59	4.97
Pyr	Pyrene	$C_{16}H_{10}$	202	-3.87	5.01
B(a)A	Benz[a]anthracene	$C_{18}H_{12}$	228	-5.03	5.83
Chry	Chrysene	$C_{18}H_{12}$	228	-5.70	5.01
B(b)F	Benz[b]fluoranthene	$C_{20}H_{12}$	252	-5.82	5.86
B(k)F	Benz[k]fluoranthene	$C_{20}H_{12}$	252	-6.10	5.86
B(a)P	Benz[a]pyrene	$C_{20}H_{12}$	252	-5.79	6.05
IP	Indeno[1,2,3-c,d]pyrene	$C_{22}H_{12}$	276	-6.72	6.57
BghiP	Benzo[g,h,i]perylene	$C_{22}H_{12}$	276	-6.59	6.63
DBA*	Dibenzo[a,h]anthracene	$C_{22}H_{14}$	278	-5.60	6.75

1.4 Bioavailability of contaminants

Soil is a major sink for organic compounds. Several factors such as soil characteristics, contaminant physicochemical properties (e.g. molecular structure, polarity, water solubility, hydrophobicity, lipophilicity and volatility), and environmental factors (e.g. temperature and precipitation) can control and affect the fate and behavior of organic compounds in soil, as well as their bioavailability (Reid et al., 2000; Stokes et al., 2005; Lanno et al., 2004; Semple et al., 2003). According to a report by the US National Research Council (2002), the bioavailability is defined as "the individual physical, chemical and biological interactions that determine the exposure of organisms to chemicals associated with soils and sediments" (Stokes et al., 2005). Therefore, the bioavailability depends on interactions between soil,

compounds and organisms (Bergknut et al., 2007; Lanno et al., 2004; Reid et al., 2000), and can vary between species (Reid et al., 2000; Stokes et al., 2005). Organic compounds can be sequestered within soil minerals and organic matters (Semple et al., 2003), and this sorption to soil particles lowers the compound bioavailability (Reid et al., 2000; Semple et al., 2003). To understand and evaluate the bioavailability, two concepts are important, bioaccumulation and bioconcentration (Conder et al., 2008, Gobas et al., 2009). Bioaccumulation is the process in which the concentration of a chemical in an organism is increased over the concentration of the chemical in the surrounding environment by several uptake routes, including through diet. Bioconcentration is the same process, but dietary uptake is not included as an uptake route. Bioaccumulation and bioconentration are expressed by bioaccumulation factors (BAFs) and bioconcentration factors (BCFs), respectively. These show the ratio between the chemical concentration in the organism and in the surrounding environment.

1.5 Objectives and hypotheses

The objective of this master thesis was to investigate the bioavailability of PFASs and PAHs in soil to an animal (earthworm; *Eisenia fetida*) and a plant (zucchini; *Cucurbita pepo*). Earthworms are soil inhabitants, constantly in contact with soil. Moreover, they consume large amounts of soil material, can be found in most soil types and horizons, and can take up contaminants by dermal contact and by ingestion (Lanno et al., 2004; Reid et al., 2000). *Eisenia fetida* are easily kept in cultures in the laboratory (Lanno et al., 2004) and a protocol of standardized toxicity testing is available (OECD, 2010a). Last but not least, they are reliable, sensitive and cheap to assess the bioavailability of organic chemicals such as PAHs or PFASs (Stroo et al., 2000). Plants belonging to the family *Cucurbitaceae* have been found to be good at accumulating hydrophobic organic compounds (HOCs) (Parrish et al., 2006). Although HOCs with log $K_{ow} > 3.5$ generally are not accumulated in plants, the zucchini species *Cucurbita pepo* have shown different results (Parrish et al., 2006). They are able to take up POPs from soil and accumulate them in their above ground tissues, for example they have been found to accumulate weathered PAHs by several orders of magnitude more than other species (Parrish et al., 2006). *C. pepo* was therefore chosen to evaluate the bioavailability of PFASs and PAHs to plants.

To investigate the bioavailability, both field-contaminated and spiked soils were used. The earthworm experiment was conducted during 7 weeks and the plant experiment during 12 weeks. For biota, bioaccumulation factors (BAFs) and biota-soil accumulation factors (BSAFs) were calculated to evaluate the contaminant bioavailability and the influence of soil characteristics on the bioavailability. For plants, bioconcentration factors (BCFs) were calculated. Moreover, the effects of PFAS chain length and functional group, and of PAH K_{ow} values, on the bioaccumulation were studied. Another objective was to investigate if the presence of PFASs in the soil would influence PAH bioavailability.

2. Materials and methods

2.1 Chemicals and materials

2.1.1 PFAS standards

Twenty five PFASs were analyzed (Table 1). The native standards were purchased from Wellington Laboratories, Ontario, Canada. As internal standard (IS), a mixture consisting of $^{13}C_2$ PFHxA, $^{13}C_4$ PFOA, $^{13}C_5$ PFNA, $^{13}C_2$ PFDA, $^{13}C_2$ PFUnDA, $^{13}C_2$ PFDoDA, $^{18}O_2$ PFHxS, $^{13}C_4$ PFOS, M_8 FOSA, d_3-N-MeFOSAA, d_5-N-EtFOSAA, d-N-MeFOSA, d-N-EtFOSA, d-N-MeFOSE, and d-N-EtFOSE was used (Wellington, Ontario, Canada, 50 pg µl$^{-1,}$ 100 µL added to each sample). As recovery standard, $^{13}C_8$ PFOA was used (Wellington, Ontario, Canada, 200 pg µl^{-1}, 10 µL added to each sample). Additionally, nine different PFASs were added as spiking solution to some of the experiment soils (i.e. PFOS, PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFDoDA, Sigma-Aldrich, St Louis, MO, USA, 2.5 mg mL^{-1}).

2.1.2 PAH standards

Sixteen PAHs were analyzed (Table 2). To construct calibration curves, solutions of native compounds (Wellington, Ontario, Canada) were used. As internal standards, corresponding mass-labeled compounds were used (all compounds except Acy, for which the internal standard for Ace was used; Wellington, Ontario, Canada; 500 pg µl^{-1}, 40 µl added to each sample). As recovery standard, Mirex (Cambridge Isotope Laboratories, England; 10 000 pg µl^{-1}, 10 µL added to each sample) was used.

2.1.3 Other chemicals

Sodium hydroxide, hydrochloric acid, methanol (Hypergrade for LC-MS), acetic acid, acetone (Suprasolv), *n*-hexane (Suprasolv), isooctane (Suprasolv) and silica gel 60 (0.063-0.200 mm) were from Merck, Darmstadt, Germany. Sodium sulfate, ethylacetate and diethylether were from Sigma Aldrich, Germany. ENVI-carb (Supelco, Mesh 120/400, Belafonte, PA, USA) and Millipore water (Milli-Q Advantage A10) were used during the experiment. All glassware and plastic equipment was solvent-rinsed before use.

2.2 Soil sampling, characteristics and preparation

2.2.1 Soil sampling

Six soils presumably contaminated with PFASs were sampled at an old airport site in Riksten, south of Stockholm, Sweden. Two soils presumably contaminated with PAHs were sampled at Älvängen, close to Gothenburg, Sweden. The soils were surface soils (down to 1 m) and they were sieved by 2 mm mesh before use.

2.2.2 Soil characterization

All soil samples were characterized in terms of pH, dry weight, organic matter (OM) and water holding capacity (WHC) at the OMK laboratory, while particle size distribution and the

content of total organic carbon (TOC) and black carbon (BC) were determined at external laboratories (Agrilab, Uppsala, Sweden and the Norwegian Geotechnical Institute, Oslo, Norway).

2.2.2.1 pH

To measure the pH of each soil, 5 g of soil was mixed with 10 mL distilled water (1:2) and shaken for 30 minutes on a horizontal shaker. Then, the pH was determined using a Sontron pH meter type Argus.

2.2.2.2 Dry weight and OM

To determine soil dry weight, wet soils were dried in an oven at 105°C for 24 hours. The dry weight was determined as the mass of dry soil divided by the mass of wet soil (in %). The OM content was determined as the loss-on-ignition at 450°C for 4 hours.

2.2.2.3 Water holding capacity (WHC)

To determine the water holding capacity, Hilgard soil cups with Whatman glass microfiber filter (GF/F) added to the bottom were used. Soils were put in the cups and weighed. Thereafter, they were placed in a pan of water for at least 24 hours until the soils became saturated with water from bottom to surface. Then, they were removed from the pan, drained by gravity for 24 hours, and reweighed. The water holding capacity (in %) was determined by dividing the mass of water in the soil after draining with the mass of the saturated soil.

2.2.3 Soil preparation

A screening of the contaminant levels in the soils was carried out before the bioavailability experiments. The PFAS analysis was done at the OMK lab at the Department of Aquatic Sciences and Assessment (SLU, Uppsala), while the screening of PAH levels was done at a commercial laboratory (ALS Scandinavia, Täby, Sweden). Based on the results from the screening, soils were selected for the experiments and in some cases mixed in order to have sufficient material for the bioavailability experiments. Two PAH-contaminated soils were mixed to form a single, PAH-contaminated, sample (soil S11). Similarly, two non-contaminated soils were mixed to form a single soil sample for PFAS experiments. Then, this soil was divided into two batches: one batch was used as control sample (S3) and the other was spiked with a PFAS-mix to form a highly PFAS-contaminated sample (S8). Similarly, another non-contaminated soil was divided into control (S4) and PFAS-spiked sample (S9). The reasons for spiking soils were to investigate a wider range of PFAS compounds, since not all investigated PFASs were detected in the field-contaminated soil, and to compare the results from aged and freshly contaminated soil. To spike the soils with PFASs, a standard was made by mixing nine PFASs (PFOS, PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFDoDa) at a concentration of 2.5 mg mL^{-1}. Soils S8 and S9 were spiked separately with 3 and 1.7 mL of the PFAS spiking solution (diluted with Millipore water) respectively, and mixed thoroughly in steel buckets. Soils were then kept at +4 °C for one week. Every day, the closed soil buckets were stirred carefully for 10 minutes to be homogenized. Finally, in order to investigate if the presence of PFASs affected the bioavailability of PAHs, spiked PFAS-contaminated soil (S8) and PAH-contaminated soil (S11) were mixed (1:1) to form a single soil (S10).

2.3 *Eisenia fetida* experiments

E. fetida were obtained from Wexthuset, Enhörna, Sweden. They were maintained in a mixture of clean soil and horse manure in the laboratory until use (approximately 12 days). The experiments were done in 1000 mL glass beakers with 500 g soil (dry weight), with the moisture of the soil held at 60% of WHC during the experiment. The beakers with soil were placed in the climate room (20°C) for 24 hours before adding *E. fetida*. Worms were selected from the laboratory culture based on test No. 317, OECD guidelines (OECD, 2010b). For example, mature *E. fetida* with clitellum should be selected. However, since there were insufficient numbers of mature *E. fetida*, also worms without clitellum were used. Worms were taken gently with soft tweezers from soil, washed by dipping in tap water and placed in glass jars with wet filter paper for 20 hours to depurate their guts before addition to the experimental beakers. After depuration, 10 *E. fetida* were weighed to monitor weight loss/gain during the experiment and added to each beaker (10 worms per 500 g soil). Beakers were covered with aluminum foil with aeration holes to retain moist and *E. fetida* in the beakers. Every week the beakers were weighed and additional Millipore water was added if required. For most soils, the experiment was terminated after a 28 days exposure period. Soils S3, S5, S6 and S9 were performed in two replicates; S7, S8, S10 and S11 in three replicates; and S4 in one replicate due to lack of soil. For two of the soils (S7 and S10), the uptake and elimination kinetics of the PFASs and PAHs were studied. Therefore, during the uptake phase, *E. fetida* were sampled at days 2, 6, 13, 21 and 28 in these soils. Thereafter, *E. fetida* were placed in clean soil in separate beakers for the elimination phase and sampled at days 30, 34, 41 and 49. The sampling of the earthworms was performed as follows: Worms were taken gently from the soil, washed by Millipore water in a sieve, and weighed to compare with weights at the start of the experiment. Then they were placed on a filter paper until they were dry (no longer wetting the filter paper) and re-weighed to obtain a proper wet weight determination. After this, they were placed in 50 mL polypropylene tubes (PP-tubes) for PFAS analysis and glass scint vials for PAH analysis. All samples were kept in freezer at -18ºC prior to extraction.

To determine worm dry weight and lipid content, two separate 2000 mL beakers with 900 g non-contaminated soil were prepared and 18 worms were transferred to each beaker (10 g worm per 500 g soil). They were sampled at the end of uptake phase (day 28) and end of elimination phase (day 48). Samples were also taken at the start of the experiment (day 0). The sampling was the same as mentioned above and worms were kept in aluminum foil in plastic bags at -18°C prior to extraction.

2.4 *Cucurbita pepo* experiments

The zucchini species Golden rush (*Cucurbita pepo ssp. pepo*) were used for the experiment. Seeds were obtained from Olssons frö AB, Helsingborg, Sweden, and kept in water overnight prior to planting. Soils (6.7 kg on dry weight basis) were placed in 12 L steel buckets. Soils S5, S6 and S7 had two replicates; S8, S9 and S10 three replicates; and S2 one replicate. There was no sample of S4 due to lack of soil. Two seeds were planted in each bucket. The soils

were irrigated every other day by 100-400 mL water (depending on the moisture of the soil). Plant samples were grown for approximately 12 weeks (83 days). They were first kept in a climate chamber for 4 weeks (T = 20°C), but transferred to a south-facing-window for the remaining growth period (T ≈ 20°C). The plant stems were attached to sticks to avoid contact with contaminated soils. Moreover, water was sprayed on leaves and stems to remove soil particles. Some of the samples wilted and had to be re-planted (in one replicate of samples S3, S6, S9 and all the replicates of S8). This could be a result of insufficient water or light; however, a toxic effect of contaminated soil on the growth cannot be excluded. Two replicates of S9 and one replicate of S10 had no plants at the end of the experiment. To sample the plants at the end of the experiment, they were sprayed with water for cleaning and cut a few centimeters above the soil, then weighed. The mix of stems and leaves for each sample was transferred to PP-tubes for PFAS analysis and amber glass jars for PAH analysis. PFAS samples were freeze-dried prior to analysis while PAH samples were kept in freezer at -18°C prior to analysis.

2.5 POP analysis

2.5.1 PFAS analysis

2.5.1.1 Soil extraction

Soil extraction and clean-up methods were based on Powely et al. (2005). First, soil samples were freeze-dried for 48 hours and mortared. 5 g of dried soil were placed in a 50 mL PP-tube. 2 mL of 100 mM NaOH in 80%/20% MeOH/Millipore water was added and samples were soaked for 30 minutes. 20 mL of MeOH and 100 µL of the IS mixture (20 ng mL^{-1}) were added. Samples were placed in a horizontal shaker at 230 rpm for 30 minutes. Thereafter, samples were centrifuged (Eppendorf, model 5810, Hamburg, Germany) at 3000 rpm for 15 minutes and the supernatant was decanted into another 50 mL PP-tube. The extraction was repeated a second time but using only half of the amount of solvents as in the first extraction. After the second extraction step, 0.1 mL 4 M HCL was added to the tube with supernatants. It was shaken by hand and centrifuged again for 5 minutes. Then, 1/8 of the solution (≈ 4.15 mL) was transferred to a 15 mL PP-tube. The samples were concentrated to 1 mL by N_2 blow down.

2.5.1.2 E. fetida extraction

E. fetida were mortared before extraction. The extraction was performed using 8 mL of MeOH followed by addition of 100 µL of the IS mixture (20 ng mL^{-1}). The samples were shaken using a horizontal shaker for 30 minutes and then centrifuged at 3000 rpm for 15 minutes. The supernatant was transferred to a new 50 mL PP-tube. The extraction was repeated using half of the extraction volume (4 mL). After this, half of the solution (6 mL) was transferred to 15 mL PP-tube and concentrated to 1 mL by N_2 blow down.

2.5.1.3 Plant extraction

Plants were freeze-dried for 72 hours and mortared. The extraction generally followed the same method as for soil samples, but only 2 g of the plant material was used for extraction.

Therefore, after the second extraction, 1/4 (8.30 mL) of the supernatant was transferred to a 15 mL PP-tube and concentrated to 1 mL.

2.5.1.4 Clean-up

The clean-up was the same for all samples. The samples (1 mL) were added to 1.7 mL PP-tubes with 25 mg ENVI-Carb and 50 µL glacial acetic acid, mixed with vortex and centrifuged at 3000 rpm for 20 minutes. 0.5 mL of the supernatant was transferred to an auto-injector vial and 10 µL of the recovery standard was added prior to analysis by liquid chromatography coupled with mass spectrometry (LC-MS/MS).

2.5.1.5 Instrumental analysis

The sample analysis by LC-MS/MS was done by the supervisors according to previous works (Ahrens et al., 2009).

2.5.2 PAH analysis

All the samples (soil, *E. fetida* and *C. pepo*) were mortared with anhydrous sodium sulfate (5 times the weight of the samples) before the extraction. The reason for using sodium sulfate is that it adsorbs the moisture of the samples and also helps destroy cell walls. Samples were not freeze-dried to avoid the loss of volatile PAHs.

2.5.2.1 Soil and C. pepo extraction

Soxhlet was used as extraction method for both soil and plant samples. Prior to extraction, the Soxhlet apparatus and extraction thimbles were cleaned with approximately 250 mL acetone/ hexane (1/1 v/v) overnight. Then the samples were put into the extraction thimbles, 40 µL of PAH IS was added and glass wool was placed on top. The samples were extracted with acetone/hexane (1/1 v/v, 250 mL) for 4 hours for plant samples and 16 hours for soil samples. Then, samples were concentrated using rotavapor to 2 mL.

2.5.2.2 E. fetida extraction

E.fetida samples in sodium sulfate were packed in glass columns (17 mm) with glass wool at the bottom. The columns were rinsed with acetone prior to use. 40 µl of PAH IS was added on top of the sample, followed by 1 cm of sodium sulfate. Samples were then eluted with 100 mL of acetone/hexane (5/2 v/v) followed by 100 mL hexane/diethylether (9/1 v/v). After that, samples were concentrated by rotavapor to 2 mL.

2.5.2.3 Clean-up

The clean-up method was the same for soil, plant and worms. Glass columns were packed with glass wool followed by 10 g silica deactivated with 10% water. 1 cm of activated sodium sulfate was added on top and the column was rinsed with 40 mL hexane/ethylacetate (1/1). Thereafter, samples were put carefully on top of the column and eluted with 50 mL hexane/ethylacetate (1/1). Afterwards, samples were evaporated using rotavapor to 1 mL, and transferred to autoinjector vials. The flasks were rinsed with 3 * 0.5 mL isooctane and evaporated again by N_2 to a final volume of 0.5 mL. Prior to instrumental analysis, 10 µl recovery standard (RS) were added.

2.5.2.4. Instrumental analysis for PAHs

PAH samples were analyzed by the supervisors using gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS), in the form of an Agilent 7890A GC coupled to an Agilent 7000 GC-QQQ. The GC was equipped with a DB5 capillary column (60 m × 250 µm × 0.25 µm; J&W Scientific), and sample aliquots of 1 µL was injected into the GC with the injector operated in splitless mode at 250ºC. The GC oven temperature program started at 70 ºC for 2 min, followed by an increase of 30 ºC/min up to 125 ºC, and an increase of 5 ºC/min up to 310 ºC, which was held for 30 minutes. The total run time was 70 minutes. Helium at a flow of 2 mL/min was used as carrier gas. Electron ionization was used to ionize the analytes. The MS/MS was operated in multiple reactions monitoring (MRM) mode, monitoring various parent and fragment ion masses to identify the different analytes.

2.5.3 Analysis of *E. fetida* dry weight and lipid content

To determine the dry weight of the *E. fetida*, samples were heated to 60 ºC for 24h. To analyze the lipid content, the same extraction process as for the PAH analysis was used, but without adding internal standards or performing a clean-up step. Instead, after concentrating to approximately 1 mL, the samples were put on pre-weighed aluminum foil boats, and the solvent was evaporated by heating. The remaining substance was lipid.

2.6 Quality assurance

Internal standard, which was isotopically labeled chemicals, was added to the samples at the start of extractions to correct for any loss of analytes during preparation and analysis. The recovery of IS was calculated by relating the ratio between the chromatographic peak areas of IS and recovery standard (RS) in the sample, to the ratio between IS and RS in the reference standard (*refstd)*:

$$\boldsymbol{Ri}(\%) = \frac{\boldsymbol{Ai}_{\boldsymbol{Is}(\boldsymbol{sample})}}{\boldsymbol{Ai}_{\boldsymbol{IS}(\boldsymbol{refstd})}} * \frac{\boldsymbol{Ai}_{\boldsymbol{RS}(\boldsymbol{refstd})}}{\boldsymbol{Ai}_{\boldsymbol{RS}(\boldsymbol{sample})}} \boldsymbol{*100} \qquad \textbf{Equation 1}$$

Where R_i is the recovery of the internal standard (%), Ai $_{IS(refstd)}$ is the peak area of internal standard in the reference standard, Ai $_{IS(sample)}$ is the peak area of internal standard in the sample, and Ai $_{RS(refstd)}$ and Ai $_{RS(sample)}$ are the peak area of the recovery standard in the reference standard and sample, respectively. The recoveries of PFASs and PAHs calculated by Equation 1 are presented in Tables A1 and A2 in the Appendix.

To calculate the method detection limit (MDL) for PFASs, the average of the concentration in the blanks for each material was calculated (Appendix I, Table A3). If it was zero, MDL was the lowest detectable concentration. If the blank concentration was not zero, MDL was calculated as:

$$\boldsymbol{MDL} = \boldsymbol{average\ concentration\ in\ blank} + \boldsymbol{3} * \boldsymbol{SD} \qquad \textbf{Equation 2}$$

For PAH samples, a blank was analyzed for each matrix and samples were corrected for levels found in blanks.

2.7 Data analysis and calculations

To evaluate the bioavailability of the soil contaminants to *E. fetida,* bioaccumulation factors (BAFs) and biota-soil-accumulation-factors (BSAFs) were determined. If steady-state was reached, BAFs and BSAF were obtained as follows:

$$BAF_{ss} = \frac{C_a}{C_s} \qquad \textbf{Equation 3}$$

Where C_a is the concentration of the contaminant in the worm (ng g^{-1} dw), C_s is the concentration in soil (ng g^{-1} dw) and BAF_{ss} is the bioaccumulation factor at steady state (g_{soil} g^{-1}_{worm}).

$$BSAF = BAF_{SS} * \frac{f_{oc}}{f_{lip}} \qquad \textbf{Equation 4}$$

Where f_{oc} is the fraction of organic carbon in soil and f_{lip} is the fraction of lipids in worms.

If the accumulation in *E. fetida* did not reach steady state during the experiment, kinetic BAFs (BAF_{kk}) and BSAFs ($BSAF_{kk}$) were determined, which can be done if the uptake and elimination kinetics are investigated. To determine the BAF_{kk}, the elimination rate constant (k_d) and uptake rate constant (k_s) is needed. The elimination rate constant was obtained by fitting the elimination phase data using a nonlinear regression (in Microsoft Excel 2010):

$$C_a = C_0 e^{-k_d t} \qquad \textbf{Equation 5}$$

Where C_a is the concentration of compounds in earthworm (ng g^{-1} dw) at time t, C_0 is the concentration of compounds in earthworm (ng g^{-1} dw) at t=0 (beginning of elimination phase), k_d is the elimination rate constant (d^{-1}) and t is the time (days).

The uptake rate constant (k_s) was calculated using the following equation OECD guidelines No. 317 (OECD, 2010b).

$$C_a = \frac{k_s}{k_d} C_s (1 - e^{-k_d t}) \qquad \textbf{Equation 6}$$

Where C_a and C_s are the concentration of the contaminant in worm and soil, respectively, at time t (ng g^{-1} dw), k_s is the uptake rate constant (g_{soil} g^{-1}_{worm} d^{-1}), k_d is the elimination rate constant (d^{-1}), and t is time (day). The determined k_d and k_s are presented in Table A7 in Appendix.

The kinetic bioaccumulation factor is then obtained from the uptake and elimination rate constants:

$$\boldsymbol{BAF_{kk} = \frac{k_s}{k_d}}$$ **Equation 7**

The kinetic BSAF is determined from the kinetic BAF by:

$$\boldsymbol{BSAF = BAF_{kk} * \frac{f_{oc}}{f_{lip}}}$$ **Equation 8**

For plant samples, the bioconcentration factor (BCF) was determined as follows:

$$\boldsymbol{BCF = \frac{C_a}{C_s}}$$ **Equation 9**

Where C_a is the concentration of compounds in plant (ng g^{-1} ww) and C_s is the concentration of compounds in soil (ng g^{-1} dw).

3. Results and discussion

3.1 Soil characteristics

All the soils were sandy, with the sand content ranging from 89.3% to 99.3% (Table 3). The pH ranged from 4.9 to 8.0 and organic matter (OM) from 1.8 to 12.6% (dw). Total organic carbon (TOC) is a part of soil OM; therefore, they show the same trend. The content of TOC ranged from 0.71% to 8.76% and the BC content was between 0.03% and 4.69% (dw). The WHC was between 31% and 53%.

Table 3. Characteristics of soil samples. S3 and S4 are the same soils as S8 and S9, respectively. (%) are based on dry weight for TOC, BC and OC and wet weight for WHC.

		S3	S4	S5	S6	S7	S10	S11
Particle size distribution	**Silt**	4.4	6.8	2.8	9.3	0.7	4.0	3.6
	Sand	94.4	94.5	96.7	89.3	99.3	95.9	96.8
	Clay	1.3	1.1	0.8	1.4	0.2	1.1	0.1
	pH	4.9	5.9	4.5	4.4	5.6	7.7	8.0
	TOC (%)	1.37	1.00	1.89	8.76	0.71	3.33	4.69
	BC (%)	0.05	0.07	0.07	0.23	0.03	0.65	2.76
	OM (%)	3.57	3.20	4.31	12.60	1.83	5.07	5.80
	WHC (%)	32.6	31.6	35.4	52.9	24.8	31.1	35.9

Water passes quickly through soils containing predominantly sand particles, while a soil containing more silt and clay particles, or organic matter, is good at retaining water. Therefore, the lowest WHC was found in S7, and the highest WHC in S6. The former has a high percentage of sand particles, low percentage of silt and clay particles, and a low OM content. S6, on the other hand, has less sand particles, more silt and clay particles, and a high OM content.

3.2 POP concentrations in soil

3.2.1 PFASs

Soil samples were analyzed at the start of the bioavailability experiment (Table 4). The highest levels were, not surprisingly, found in soils that had received additional contaminants i.e. S8 (ΣPFASs=1141 ng g^{-1} dw), S9 (ΣPFASs=1275 ng g^{-1} dw) and S10 (ΣPFASs=570 ng g^{-1} dw). PFNA displayed the highest concentrations in the spiked soils (S8-S10). Compounds not included in the spike solution were not detected or detected at low concentrations in soils S8-S10 (PFTeDA, PFUnDa, PFTriDa, PFBS, PFDS and FOSA). In the field-contaminated soils, S5 had the highest ΣPFASs (146 ng g^{-1} dw). PFOS was the compound found at the highest concentration. S6 had the lowest concentration of PFASs (ΣPFASs=15.56 ng g^{-1} dw), apart from the two soils used as controls (S3 and S4). PFHxDA, PFOcDA and four PFASs precursors (MeFOSE, EtFOSE, FOSAA, MeFOSAA, EtFOSAA) were not detected in any

soil samples. Therefore, they were not included in the further analysis of biota and plant samples.

Table 4. Concentration of POPs in soil samples (ng g^{-1} dw); ND: not detected, NA: not analyzed.

	S3	S4	S5	S6	S7	S8	S9	S10	S11
PFASs									
PFBA	ND	ND	ND	ND	ND	113	117	56.7	NA
PFPA	ND	ND	0.59	0.26	0.64	144	152	72.2	NA
PFHxA	ND	ND	1.43	1.19	1.03	156	172	78.0	NA
PFHpA	ND	0.06	0.17	0.22	0.2	124	151	62.0	NA
PFOA	0.08	ND	0.29	1.19	0.51	111	156	55.7	NA
PFNA	ND	ND	0.04	0.22	0.09	181	233	90.7	NA
PFDA	ND	ND	0.02	0.29	0.07	119	128	59.4	NA
PFUnDA	ND	ND	ND	0.39	0.08	0.21	ND	0.1	NA
PFDoDA	ND	ND	ND	0.29	ND	124	75	62.1	NA
PFTriDA	ND	ND	ND	0.14	0.05	0.07	0.05	0.04	NA
PFTeDA	ND	ND	ND	0.04	ND	0.05	ND	0.02	NA
PFHxDA	ND	ND	ND	ND	ND	ND	ND	ND	NA
PFOcDA	ND	ND	ND	ND	ND	ND	ND	ND	NA
PFBS	ND	ND	0.1	ND	0.21	ND	ND	ND	NA
PFDS	ND	ND	ND	ND	0.43	ND	ND	ND	NA
PFHxS	ND	0.11	6.66	1	6.33	ND	ND	ND	NA
PFOS	ND	0.34	146	9.64	90.3	66.9	91	33.5	NA
FOSA	ND	ND	ND	0.93	1.29	ND	ND	ND	NA
MeFOSA	ND	ND	0.08	ND	ND	ND	ND	ND	NA
EtFOSA	ND	ND	0.06	ND	ND	ND	ND	ND	NA
MeFoSE	ND	ND	ND	ND	ND	ND	ND	ND	NA
EtFOSE	ND	ND	ND	ND	ND	ND	ND	ND	NA
FOSAA	ND	ND	ND	ND	ND	ND	ND	ND	NA
MeFOSAA	ND	ND	ND	ND	ND	ND	ND	ND	NA
EtFOSAA	ND	ND	ND	ND	ND	ND	ND	ND	NA
ΣPFAS	0.08	0.52	155	15.82	101	1141	1275	570	NA
PAHs									
Nap	NA	NA	NA	NA	NA	NA	NA	10.3	113
Acy	NA	NA	NA	NA	NA	NA	NA	7.83	95.1
Ace	NA	NA	NA	NA	NA	NA	NA	1.29	15.2
Flu	NA	NA	NA	NA	NA	NA	NA	2.97	64.9
Phen	NA	NA	NA	NA	NA	NA	NA	63.7	635
Anth	NA	NA	NA	NA	NA	NA	NA	13.2	145
Fluo	NA	NA	NA	NA	NA	NA	NA	175	1169
Pyr	NA	NA	NA	NA	NA	NA	NA	145	1024
B(a)A	NA	NA	NA	NA	NA	NA	NA	55.3	353
Chry	NA	NA	NA	NA	NA	NA	NA	118	454
B(k+j)F	NA	NA	NA	NA	NA	NA	NA	137	648
B(b)F	NA	NA	NA	NA	NA	NA	NA	104	594
BaP	NA	NA	NA	NA	NA	NA	NA	67.5	492
IP	NA	NA	NA	NA	NA	NA	NA	119	466
DBA	NA	NA	NA	NA	NA	NA	NA	16.5	47.6
BghiP	NA	NA	NA	NA	NA	NA	NA	148	564
ΣPAH	NA	NA	NA	NA	NA	NA	NA	1183	6879

3.2.2 PAHs

Two PAH-contaminated soils (S10, S11) were analyzed prior to bioavailability experiments (Table 4). S11 had the highest concentration of PAHs (ΣPAHs=6879 ng g^{-1} dw). The total concentration of PAHs in S10 (PFAS- and PAH–contaminated soil) was 1183 ng g^{-1} dw. In both soil samples, the highest concentration was found for Fluo, with 175 ng g^{-1} dw in S10 and 1169 ng g^{-1} dw in S11, while Ace had the lowest concentration, with 1.29 ng g^{-1} dw in S10 and 15.2 in S11 (Table 4).

3.3 *E. fetida*

3.3.1 *E. fetida* lipid content and dry weight

Lipid content and dry weight were determined for *E. fetida* at three time points: in the beginning of the worm experiment (day 0), at the end of the uptake phase (day 28) and at the end of the elimination phase (day 48). Lipid content was analyzed in three replicates for day 0, two replicates for day 28 (due to lost samples) and two replicates for day 48. Dry weight was determined in three replicates for all time points. As apparent from Figure 1 and 2, there was a tendency to a decrease in lipid content and increase in dry weight over time, but this was not statistically significant according to a regression test (Anova in Excel 2010, α=0.05). Consequently, the average of all replicates was used for lipid content (8.14±0.37% on dry weight basis) and dry weight (18.2±1.34 %) in calculation of BAFs and BSAFs.

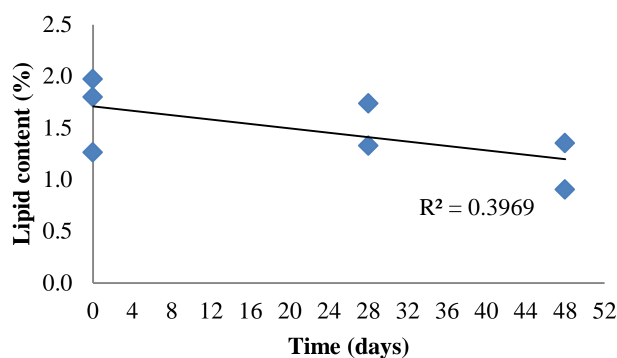


Figure 1. Lipid content of *E. fetida* versus time.

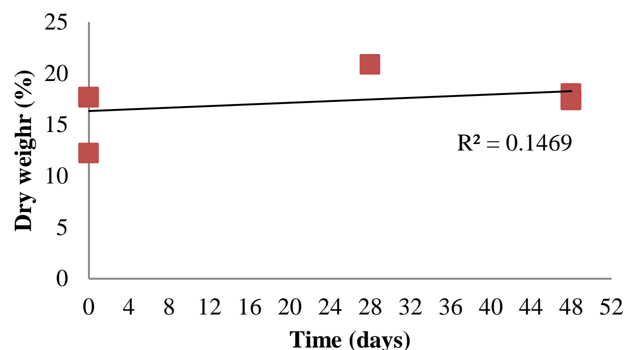


Figure 2. Dry weight of *E.fetida* versus time.

3.3.2 PFASs in *E. fetida*

Results showed that 15 of 18 PFASs were detected in worms that were exposed to field-contaminated as well as spiked soils (Appendix, Table A4).

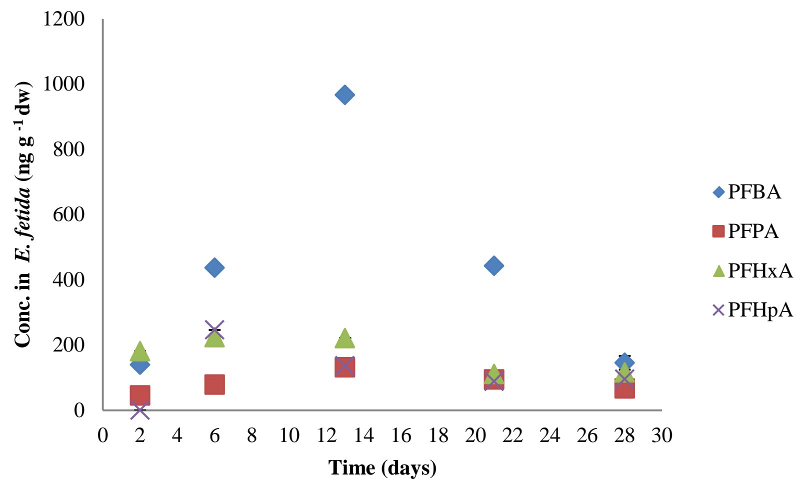
For two soils the uptake and elimination phases were monitored (S7, a field-contaminated soil containing PFASs, and S10, a mixture of a soil spiked with PFAS and a PAH-contaminated soil). The concentrations of PFASs in the worms during the uptake and elimination are available in Appendix, Tables A5 and A6. As expected, during the uptake phase the concentrations in *E. fetida* from both soils increased (Figures 3-6) and during the elimination phase it decreased (Appendix, Figures A1 and A2). For the spiked soil (S10), the PFAS concentrations in *E. fetida* reached steady-state (i.e. no longer increased) before day 28 (Figures 3-4). For PFBA, the concentration was highest on day 13, followed by a sharp decrease; the reason for this is unclear (Figure 3). Overall, it can be said that most of the PFASs reached a steady-state in concentrations in earthworms already by day 13 for the short-chain PFCAs ($C < 8$) and by day 21 for the long-chain PFCAs ($C > 8$) as well as for PFOS.

On the other hand, for worms exposed to the field-contaminated soil (S7), 28 days were enough for some compounds (PFHxA, PFOA, PFDA, PFUnDA, PFBS) to reach steady-state (Figures 5 and 6). However, other compounds (PFPA, PFTriDA, PFDS, PFOS and FOSA) did not reach steady-state by the end of the experiment (Figure 5 and 6). One reason for the longer time required to reach steady-state for the field-contaminated soil compared to the spiked soil could be aging, which has an effect on bioavailability (Reid et al., 2000). Since S7 was a field-contaminated soil, compounds had time to bind to soil particles, and were consequently less available for uptake than in S10, in which contaminants had less time to bind to soil particles and therefore could have been present in the pore water to a higher extent.

BAFs were calculated for all compounds and soils using Equation 2, which assumes that steady-state has been reached in POP concentrations in worms (Table 5). In addition, kinetic BAFs (Equation 6) were calculated for the compounds in S7 that did not reach steady state (PFPA, PFTriDA, PFDS, PFOS and FOSA).

BAFs of the different compounds in the field-contaminated soils (S5, S6 and S7) tend to be lowest in S6 (Table 5). S6 has the highest OM among soil samples (Table 3); therefore, a low BAF can be expected for compounds that bind to the OM of the soils. For the spiked soils (S8, S9 and S10), BAFs of compounds that were included in the spike solution are variable (Table 5). For example, although the BAFs of short chain PFCAs (PFBA, PFPA, PFHxA) are similar in S9 and S10, they are higher in S8. On the other hand, some long chains PFCAs (PFOA, PFNA and PFDA) have similar BAFs in all spiked soils, but somewhat higher in S10. Generally BAFs increased with length chain, which will be discussed later.

a)



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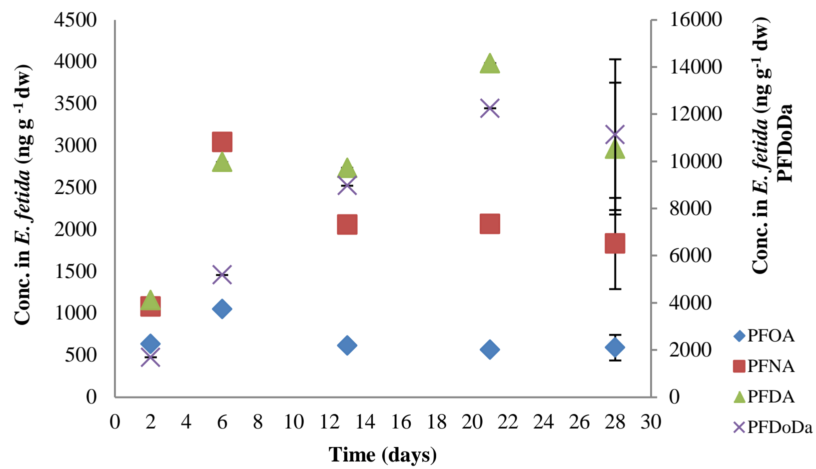

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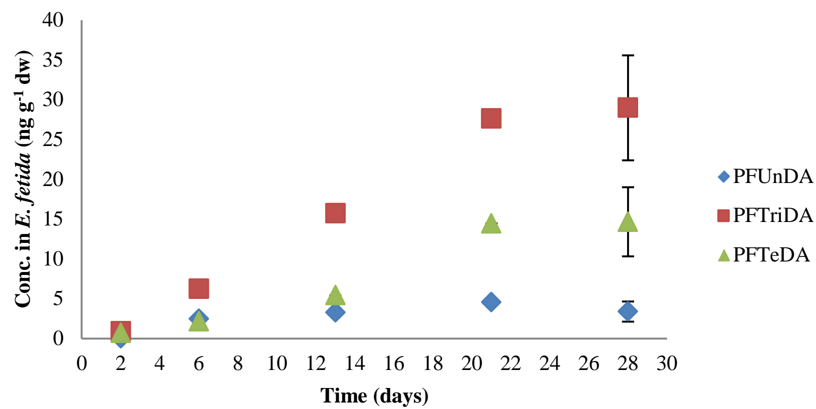


Figure 3 a- c. Concentration of PFCAs in *E.fetida* exposed to S10 during the uptake phase. Error bars represent the standard deviation at day 28 (*n*=2).

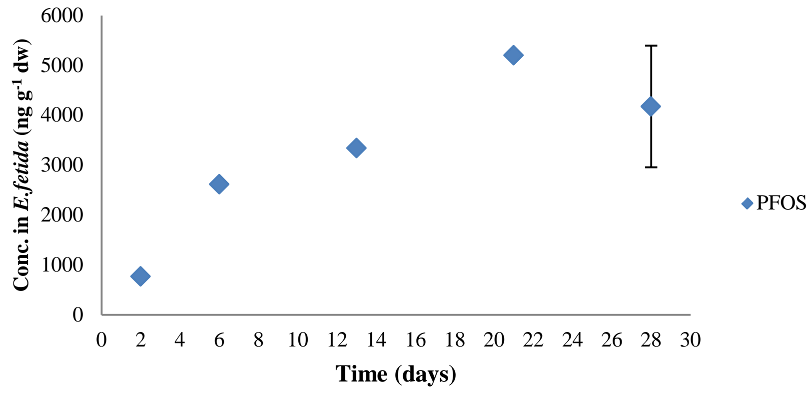


Figure 4. Concentration of PFOS in *E. fetida* exposed to S10 during the uptake phase. Error bars represent the standard deviation at day 28 (*n*=2).

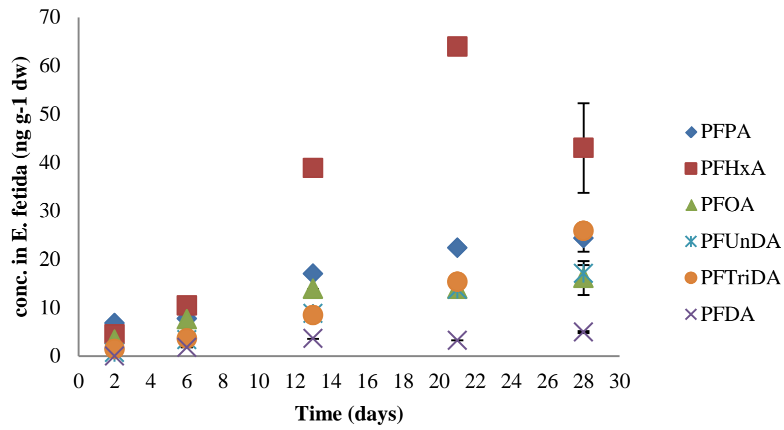


Figure 5. Concentration of PFCAs in *E.fetida* exposed to S7 during the uptake phase. Error bars represent the standard deviation at day 28 (*n*=3).

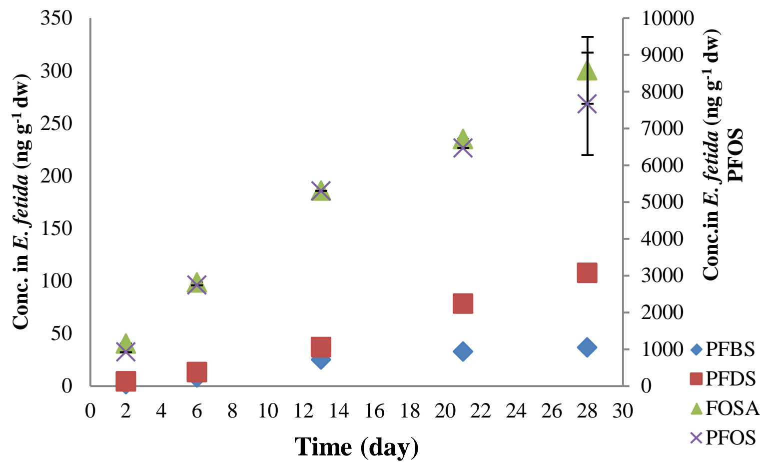


Figure 6. Concentration of PFSAs in *E. fetida* exposed to S7 during the uptake phase. Error bars represent standard deviation at day 28 (*n*=3).

Table 5. BAF ± SD ($g_{soil}\ g^{-1}_{worm}$ dw) of PFASs after 28 days of exposure to different soils (S5-S10). Numbers in parenthesis show BAF_{kk}. * = compounds which are included in the spike solution. ND: not detected.

	S5	S6	S7	S8	S9	S10
PFBA*	ND	ND	ND	49.4±8.18	3.22±0.54	3.47±1.60
PFPA*	27.7±7.02	26.0±11.8	38.8 ±4.24 (66.7)	5.42±0.95	0.74±0.03	0.92±0.23
PFHxA*	14.7±1.62	3.98±2.32	41.8±8.96	8.97±1.63	3.14±0.37	2.03±0.96
PFHpA*	ND	ND	ND	ND	ND	3.04±2.60
PFOA*	11.1±3.26	0.74±0.49	31.9±6.87	12.12±1.39	17.54±2.93	16.8±11.0
PFNA*	ND	5.18±4.17	25.9±9.78	24.25±4.08	22.2±3.71	30.5±18.3
PFDA*	71.9±43.2	7.41±4.10	67.8±2.86	39.63±6.35	33.17±1.32	49.9±13.2
PFUnDA	ND	1.69±0.90	224±22.7	29.97±5.69	ND	32.4±12.1
PFDoDA*	ND	13.6±1.50	ND	60.59±24.2	60.9±6.48	179±51.5
PFTriDA	ND	26.7±16.0	486 ±7.73 (2124)	142.82±48.6	72.7±11.6	784±170
PFTeDA	ND	38.3±15.0	ND	89.41±30.7	42.3±3.19	627±186
PFBS	ND	ND	174±15.2	ND	ND	ND
PFDS	ND	ND	250 ±12.8 (240)	ND	ND	ND
PFOS*	52.6±0.43	2.47±1.11	84.9 ±15.4 (90.9)	52.16±3.32	44.3±4.36	125±36.5
FOSA	ND	7.28±1.90	233 ±24.7 (297)	ND	ND	ND

Interestingly, a higher OM content in spiked soils does not have the same effect as in field contaminated soils. BAFs of longer chain PFCAs (C>8) and PFOS are higher in the soil with higher OM content (S10). This difference might be explained by the interaction time between compounds and soil. In the spiked soils, contaminants have had less time to bind to OM and are more available for uptake by worms. However, this should not lead to higher BAFs in OM-rich soils, only to less clear differences between soils with different OM content. Based on the observations of BAFs in field-contaminated soils (Table 5), OM has a role in decreasing the bioavailability of PFASs, as has been proposed by others (Reid et al., 2002, Trapp et al., 1994).

Most POPs are expected to sorb to organic matter in soil and to lipid in biota (Higgins et al., 2007), which decrease their bioavailability. Therefore, BSAF is used instead of BAF. To determine BSAF, the soil organic carbon content and the biota lipid content are taken into account (see equation 4). In principal, BSAF>1 shows that worms are not in equilibrium with the soil; on the contrary, they have accumulated the compounds to high extent. However, lipid is not suggested as a reservoir for PFASs (Conder et al., 2008). In a study by Higgins et al. (2007), both lipid normalized and non-lipid normalized BSAF were determined and the non-

lipid normalized BSAF values were closer to the expected BSAFs. Accordingly, non-lipid normalized BSAF instead of lipid-normalized BSAF were used for PFASs in this master project (Table 6).

Table 6. BSAFs ± SD (g $_{soil}$ *foc*/g $_{worm}$) of PFASs from different soils (S5-S10). Numbers in parenthesis show calculation based on BAF$_{kk}$. * compounds which are included in spike solution. ND: not detected.

	S5	S6	S7	S8	S9	S10
PFBA*	ND	ND	ND	0.68±0.11	0.03±0.01	0.12±0.05
PFPA*	0.52±0.13	2.28±1.04	0.27± 0.03 (0.47)	0.07±0.01	0.0074±0.0003	0.03±0.01
PFHxA*	0.28±0.03	0.35±0.20	0.30±0.06	0.12±0.02	0.031±0.004	0.07±0.03
PFHpA*	ND	ND	ND	ND	ND	0.10±0.09
PFOA*	0.21±0.06	0.06±0.04	0.23±0.05	0.17±0.02	0.18±0.03	0.56±0.36
PFNA*	ND	0.45±0.37	0.18±0.07	0.33±0.08	0.22±0.04	1.01±0.61
PFDA*	1.36±0.82	0.65±0.36	0.48±0.02	0.54±0.09	0.33±0.01	1.66±0.44
PFUnDA	ND	0.15±0.08	1.59±0.16	0.41±0.08	ND	1.08±0.40
PFDoDA*	ND	1.19±0.13	ND	0.83±0.33	0.61±0.06	5.97±1.71
PFTriDA	ND	2.34±1.40	3.45±0.05 (15.1)	1.96±0.67	0.73±0.12	26.1±5.94
PFTeDA	ND	3.36±1.32	ND	1.22±0.42	0.42±0.03	20.9±6.19
PFBS	ND	ND	1.23±0.11	ND	ND	ND
PFDS	ND	ND	1.77±0.09 (1.70)	ND	ND	ND
PFOS*	0.99±0.01	0.22±0.10	0.60±0.11 (0.65)	0.71±0.05	0.44±0.04	4.15±1.21
FOSA	ND	0.64±0.17	1.65± 0.18 (2.11)	ND	ND	ND

The BSAF values show that short chain PFCAs (PFBA, PFPA, PFHxA, PFHPA) are not bioaccumulative neither from field contaminated soils (S5-S7) nor spiked soils (S8-S10). PFCAs with longer chain (PFOA, PFNA, PFDA, PDUnDA, PFDoDA, PFTriDA, PFTeDA) have more potential of bioaccumulation. This is consistent with what Conder et al. (2008) reported, that PFCAs with seven or less carbon chain length are not bioaccumulative in aquatic organisms. The BSAF values of PFSAs from S7 show that, except PFOS, they have bioaccumulation potential. PFOS, on the other hand, has BSAF<1 from all soils except S10. There is no clear reason for that the lower BSAF for PFOS.

To illustrate the influence of carbon chain length on the BSAFs, it was plotted in Figure 7. Generally, the BSAF of PFASs increased with increasing chain length (C>8). Therefore, it can be said that chain length has an effect on the bioaccumulation of PFASs from soil to biota as has been stated in previous works (Martin et al., 2003, Conder et al., 2008, Zhao et al., 2013). Additionally, to see whether the functional group of the PFASs has an effect on the

bioaccumulation of compounds from soil to biota, BSAF values of PFNA and PFOA was compared. PFNA and PFOS have the same perfluorocarbon chain length (C=8) but PFNA has a carboxylic functional group while PFOS has a sulfonate functional group. A paired t-test (Anova, Excel 2010, α=0.05) was done to compare BSAFs of PFNA and PFOS. There were no significant difference in BSAF between these compounds (p=0.33). This result differs from others that claimed that the bioaccumulation of PFOS is higher than PFNA (Martin et al., 2003, Conder et al., 2008 and Zhao et al., 2013). To fully investigate this, more samples and more species need to be studied.

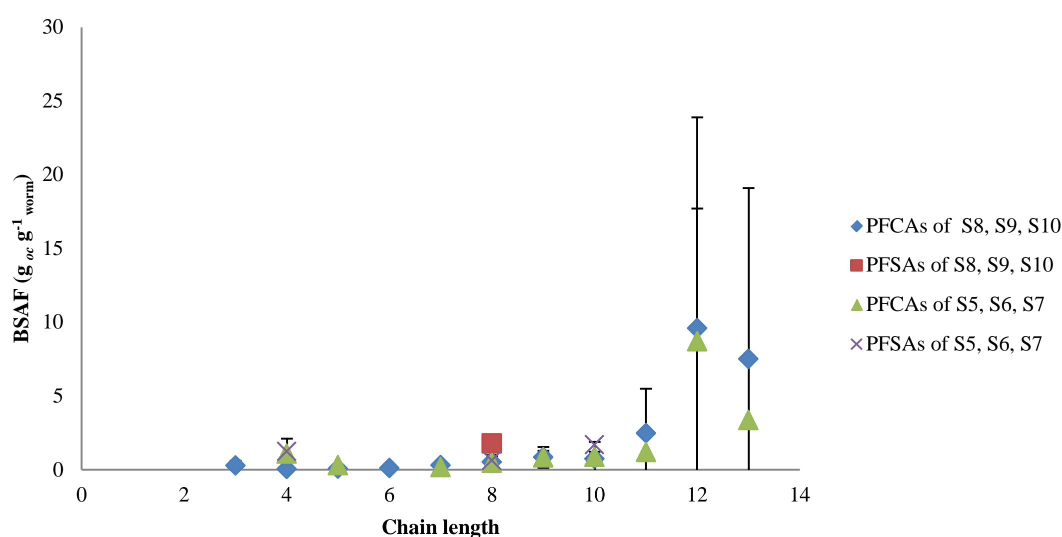


Figure 7.BSAF vs Chain length of PFASs in field contaminated soils (S5, S6 and S7) and Spiked soils (S8, S9 and S10). Error bars represent standard deviation (generally *n*=3).

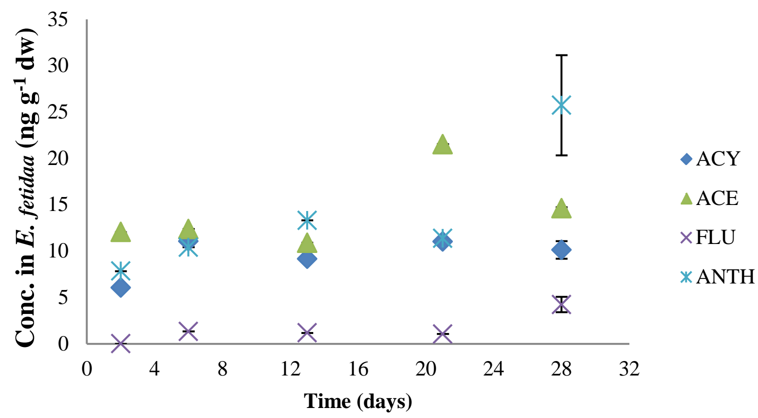
3.3.3 PAHs in *E. fetida*

E. fetida were exposed to two different PAH field-contaminated soils (S10 and S11). All PAHs were detected in worm samples (Appendix, Table A8). NAP was detected in blanks and initial worm samples, most likely due to the volatility of this compound. Therefore, it was excluded from further calculations. For S10 the uptake and elimination phase were monitored.

All the compounds appeared to reach steady state at day 6 (i.e. Acy, Ace, Flu, Anth, Fluo, Pyr, B(a)A and Chry or day 21 (i.e. Phen, B(K+j)F, B(b)F, BaP, DBA, IP and BghiP), but then concentrations increased again at day 28 for many of the PAHs (Figure 6). There is no clear explanation for this. It might be due to errors such as contamination in the lab or student error during sampling and extraction; however, it is unlikely that this would only affect the result at day 28.

Based on the results from the uptake phase (Figure 6), it was presumed that all the compounds reached equilibrium before or at day 28. Therefore, BAFs at steady state were determined using Equation 3. Moreover, BSAF were calculated by Equation 4.

a)



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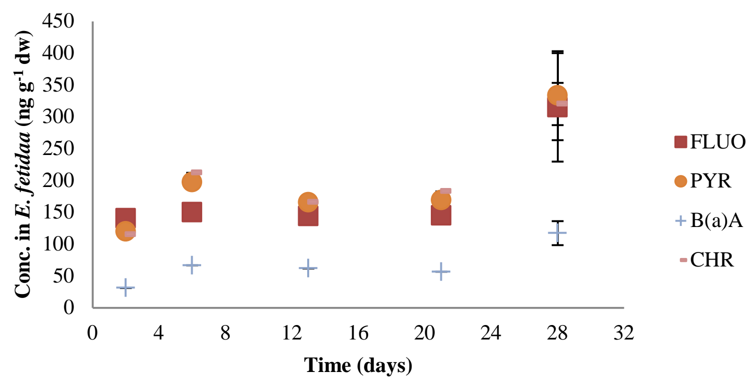

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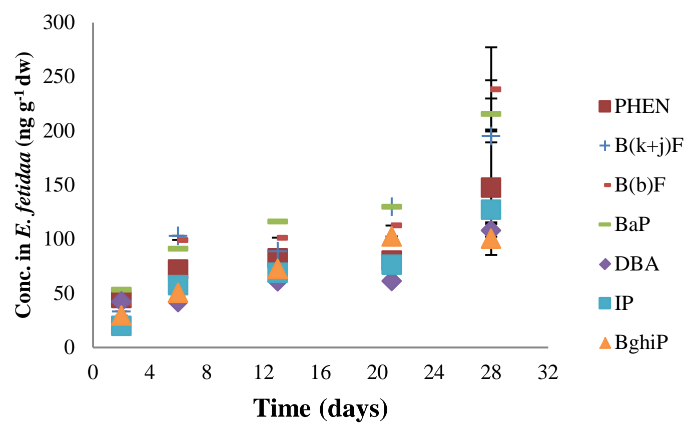

Figure 8. Concentration of PAHs in *E. fetida* exposed to S10 during the uptake phase. Error bars represent standard deviation of compounds at day 28 (*n*=3)

Table 7. BAF ($g_{soil}\ g^{-1}{}_{worm}$) and BSAF ($g_{oc}\ g^{-1}{}_{lip}$) of PAH from S10 and S11. All the calculations are based on dry weight.

	S10		S11	
	BAF ± SD	**BSAF ± SD**	**BAF ± SD**	**BSAF ± SD**
Acy	1.29 ± 0.13	0.51 ± 0.05	0.19 ± 0.02	0.10 ± 0.01
Ace	11.3 ± 0.12	4.43 ± 0.03	0.93 ± 0.34	0.51 ± 0.19
Flu	1.42 ± 0.08	0.56 ± 0.11	0.11 ± 0.04	0.06 ± 0.02
Phen	20.9 ± 0.28	8.18 ± 2.31	0.30 ± 0.05	0.16 ± 0.03
Anth	1.94 ± 5.90	0.76 ± 0.16	0.27 ± 0.06	0.15 ± 0.03
Fluo	1.80 ± 0.41	0.71 ± 0.19	0.54 ± 0.13	0.30 ± 0.07
Pyr	2.31 ± 0.49	0.90 ± 0.19	0.62 ± 0.08	0.34 ± 0.04
B(a)A	2.12 ± 0.48	0.83 ± 0.13	0.79 ± 0.13	0.43 ± 0.07
Chry	2.72 ± 0.34	1.06 ± 0.11	1.79 ± 0.33	0.99 ± 0.18
B(k+j)F	1.43 ± 0.28	0.56 ± 0.15	0.76 ± 0.16	0.42 ± 0.09
B(b)F	2.30 ± 0.38	0.90 ± 0.15	0.75 ± 0.13	0.41 ± 0.07
BaP	3.19 ± 0.21	1.25 ± 0.08	0.65 ± 0.08	0.36 ± 0.04
IP	1.07 ± 0.14	0.42 ± 0.06	0.44 ± 0.10	0.24 ± 0.05
DBA	6.57 ± 0.35	2.57 ± 0.14	1.59 ± 0.43	0.87 ± 0.24
BghiP	0.68 ± 0.10	0.27 ± 0.04	0.23 ± 0.02	0.13 ± 0.01

BAF values from S10 (a mixture of PFAS and PAH contaminated soils) generally exceed 1, while BAFs in S11 (PAH-contaminated soil) are between 0.11 and 1.79 ($g_{soil}\ g^{-1}{}_{worm}$). According to Tables 5 and 7 it can be said that the BAFs of PAHs are lower than the BAFs of PFASs. The BSAFs are generally low for both soils (Table 7; BSAF ≤ 1). This indicates that PAHs are not very available to organisms or that they are metabolized. PAHs have low water solubility and strong tendency to bind to soil particles (as expressed by a high K_{OW}; Table 2), therefore they are less available. Furthermore, the soil samples had been contaminated for a long time; therefore, aging could be another reason of a low availability of PAHs to worms. However, some of the PAHs (Ace, Phen, Chry, DBA) have BSAFs>1 in S10 which indicate that these compounds have a potential of bioaccumulation. Parrish et al. (2006) reported accumulation of fluoranthene, pyrene, benzo[a]anthracene and chrysene by *E.fetida*.

BSAFs were plotted against log K_{OW} in Figure 7 to see if there is any relationship between K_{OW} and BSAF. As apparent, except some compounds that have a high value of BSAF and a high standard deviation, the BSAFs of compounds had a tendency to increase with an increase in log K_{OW}.

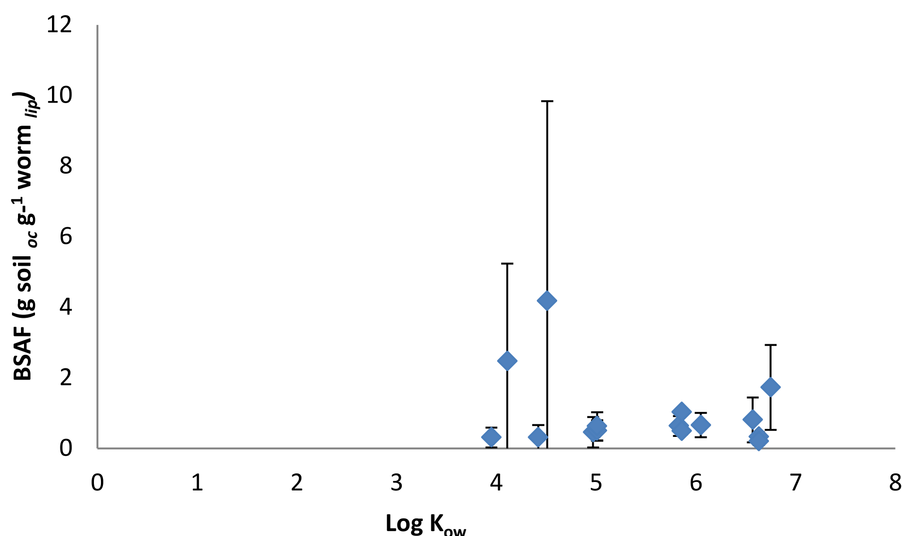

Figure 9. BSAF (average from S10 and S11) vs log K_{OW} of PAHs. Error bars represent standard deviation (n=2).

3.3.4 Potential influence of PFASs on PAHs bioavailability to *E. fetida*

To study the effect of PFASs on the availability of PAHs, BSAFs of PAHs from two different soils (S11 = PAH contaminated soils and S10 = PAH and PFAS contaminated soils) were compared (Figure 10). The results show that all PAHs were more accumulated in worms from S10 compared to S11. The presence of PFASs in S10 may thus enhance the solubility of PAHs and their availability to organisms. This observation is supported by studies that claimed that two anionic PFASs (lithium perfluorooctanesulfonate (LiFOS) and ammonium perfluorooctanoate (APFO)) could increase the solubility of PAHs (An et al., 2001, An et al., 2002). However, these studies were not applied to accumulation from soils. They investigated the influence of LiFOS and APFO on the solubility of PAHs in solution. The soil characteristics differ somewhat between S10 and S11. S11 has a higher content of TOC and BC (Table 3), and these can influence on the availability of compounds to biota. Nonetheless, the PAH contaminated soil that was mixed to form S10 is the same as S11 and the PAHs should thus be similarly bound to the TOC and BC in S10 as they are in S11. Therefore, it is likely that the presence of PFASs has an effect on the availability of PAHs to worms. Since this finding is important for contaminated sites with a mixture of contaminants (PFASs and others), more investigations are needed

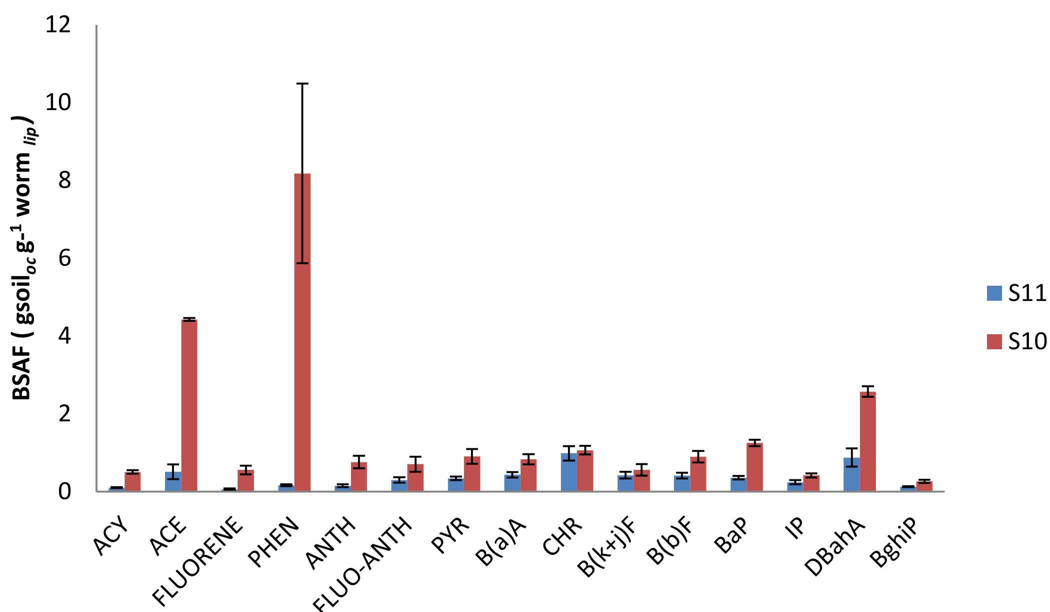

Figure 10. Comparison of BSAFs of PAHs from S11 and S10. Error bars represent standard deviation (n=3)

3.4 *Cucurbita pepo*

3.4.1 PFASs

Most of the investigated PFCAs (i.e. PFPA, PFHxA, PFHpA, PFOA, PFNA and PFDA) were detected in plant samples from field-contaminated (S5, S6 and S7) and spiked soils (S8, S9 and S10), while PFSAs were generally not detected or detected in negligible amounts (Appendix, Table A10). The bioconcentration factor (BCF) was calculated using Equation 9, and results are available in Table 8.

Table 8. BCF of PFASs in *c.pepo* of different soils (S5-S10). *: Compounds which are included in the spike solution. ND: not detected.

	S5	S6	S7	S8	S9	S10
PFBA*	ND	ND	ND	2.94 ± 1.24	8.25	1.49 ± 0.14
PFPA*	0.14 ± 0.01	0.25 ± 0.07	0.59 ± 0.15	0.72 ± 0.15	1.1	0.321 ± 0.001
PFHxA*	0.082 ± 0.004	0.08 ± 0.01	0.43 ± 0.12	0.65 ± 0.12	0.73	0.19 ± 0.04
PFHpA*	ND	ND	0.43 ± 0.10	0.36 ± 0.05	0.47	0.14 ± 0.05
PFOA*	0.04 ±0.01	0.01	0.0035 ± 0.002	0.11 ± 0.01	0.2	0.10 ± 0.04
PFNA*	0.06 ± ND	ND	0.055 ± 0.001	0.03 ± 0.001	0.05	0.028 ± 0.002
PFDA*	0.25 ± ND	ND	0.07 ± ND	0.020 ± 0.005	0.02	0.0108± 0.0001
PFDoDA*	ND	ND	ND	0.003 ± 0.002	0.001	ND
PFBS	0.17 ± 0.02	ND	0.09 ± 0.04	ND	ND	ND
PFHxS	0.05 ± 0.02	ND	0.04 ± 0.01	ND	0.09	ND
PFOS*	0.006 ± 0.003	0.0007 ±0.0001	0.004 ± 0.001	0.010 ± 0.002	0.01	0.024 ± 0.003

BCF values show that most of PFASs (PFBA, PFHpA, PFNA, PFDA, PFDoDA, PFBS, PFHxS) are not taken up by plants from S6. Since S6 has a high OM content, it can influence the availability of PFASs to plants, similarly as for *E. fetida*. In general, BCF values of the field-contaminated soils (S5, S6 and S7) are slightly lower than for spiked soils (S8, S9 and S10), but there are variations within each group.

To see if there is a relationship between BCF and compound chain length, the BCFs were plotted against PFCA and PFSA perfluorocarbon chain length (Figure 8). The BCF of PFCAs shows a strong relationship (r^2= 0.97) with chain length in samples from spiked soils (S8, S9 and S10), in that the BCF are higher for short-chain compounds. A similar trend (r^2= 0.99) is apparent for samples from field-contaminated soils (S5, S6 and S7). It can be concluded that chain length has an effect on the uptake of PFASs by plants; BCF decreases as chain length increases. This is similar to results from previous works (Lechner & Knapp, 2011; Lee et al., 2013; Stahl et al., 2009; Yoo et al., 2011). This may be as a result of the route of uptake. The main uptake routes of contaminants from soil to plants is through the pore water, therefore shorter chain compounds which are more water soluble are more available to plants. Since the BCF data for PFSAs was limited in this experiment, the effect of functional group on BCF could not be well investigated. However, PFOS and PFNA with the same carbon length shown no significant difference in BCF (p=0.1) according to a paired t-test (ANOVA, Excel 2010, α=0.05).

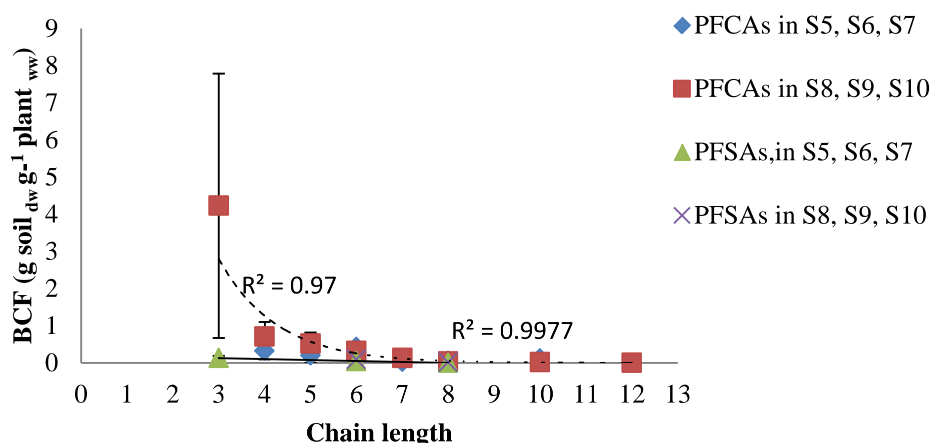

Figure 11. BCF of PFASs in *C. pepo* versus chain length. Error bars represent standard deviation (n=3)

3.4.2 PAHs

Since the mass of plant samples were low and compounds co-extracted from the matrix disturbed the instrumental analysis, the detection of PAHs was difficult. A more time-consuming clean-up would have been needed to improve the chromatography. Consequently, analysis of PAHs in *C.pepo* has been removed from this master thesis.

4. Conclusions

In this master thesis, the bioavailability of PFASs and PAHs from two different groups of soils (field-contaminated and spiked soils) to earthworms and plants were investigated. The results show that most of the PFASs from both groups of soils are available to earthworms, but that the availability varied due to soil characteristics and chemical properties. OM has an important role on the bioavailability of PFASs in field-contaminated soils. Furthermore, most PFASs from field-contaminated and spiked soils were detected in plants, except in samples from a soil with high OM. There is a relationship between chain length and BSAFs of PFASs in worms: when the chain length increases, the BSAF is increased. It can be stated that longer chain compounds have more potential of bioaccumulation than shorter chain. On the other hand, the BCF displays an inverse relationship with chain length in plants, in that short chain compounds has higher BCF than compounds with longer chain. Therefore, it can be argued that the long chain compounds are mainly a concern for biota while the short chain compounds seem to be a concern for plants. One reason might be the different routes of uptake for biota and plants. The uptake in biota occurs both from pore water and by ingestion of soil particles while uptake from pore water is the main uptake route in plants. As a result, the more soluble compounds are more available to plants. The functional group of the PFASs did not have effect on BSAF (in earthworm) and BCF (in zucchini); nonetheless, more studies with more samples are recommended for a more certain evaluation of the effect of functional group.

BAFs of PAHs were higher in a mixture of PFAS and PAH contaminated soil compared to a soil contaminated with only PAHs. BSAF values of PAHs were lower than 1, thus it can be said that they are not bioaccumulative. Finally, the impact of PFASs on the bioavailability of PAHs was investigated. The results show that PFASs may enhance the solubility of PAHs that has an influence on its bioavailability to earthworms.

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Appendix

Table A5. The recovery (%) of PFAS internal standards in soil, *E. fetida, and C. pepo* samples.

	Soil	*E. fetida*	*C. pepo*
C06 PFHxA IS	93	83	171
C08 PFOA IS	150	146	244
C09 PFNA IS	91	114	182
C10 PFDA IS	109	149	198
C11 PFUnDA IS	96	136	196
C12 PFDoDA IS	115	129	189
EtFOSA IS	117	62	85
EtFOSAA IS	130	205	279
EtFOSE IS s	102	51	86
FOSA IS	111	108	171
MeFOSAA IS	131	152	221
MeFOSA IS	32	78	75
MeFOSE IS	105	241	92

Table A6. The recovery (%) of PAHs internal standard in soil and *E. fetida* samples.

	Soil	*E. fetida*
Nap	41	79
Acy	48	90
Ace	48	90
Flu	69	86
Phen	68	91
Anth	60	97
Fluo	76	82
Pyr	70	81
B(a)A	59	74
Chry	42	39
B(k+j)F	29	29
B(b)F	34	41
BaP	53	52
IP	82	76
DBA	104	86
BghiP	76	89

Table A7. Method detection limit (MDL) for PFASs in soil, *E. fetida* and *C. pepo* samples.

	E. fetida			*C. pepo*			soil		
	Blank	MDL		Blank	MDL		Blank	MDL	
	ng absolute	ng absolute	ng/g dw	ng absolute	ng absolute	ng/g dw	ng absolute	ng absolute	ng/g dw
PFBA	ND	0.25	0.05	ND	0.25	0.98	ND	0.25	0.05
PFPA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFHxA	0.03	0.16	0.03	0.10	0.61	2.40	ND	0.05	0.01
PFHpA	0.01	0.05	0.01	0.33	1.15	4.50	ND	0.05	0.01
PFOA	ND	0.05	0.01	0.09	0.43	1.69	0.06	0.33	0.07
PFNA	0.01	0.07	0.01	0.01	0.05	0.20	ND	0.05	0.01
PFDA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFUnDA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFDoDA	0.04	0.22	0.04	0.05	0.28	1.11	ND	0.05	0.01
PFTriDA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFTeDA	ND	0.05	0.01	0.03	0.18	0.71	ND	0.05	0.01
PFHxDA	0.02	0.10	0.02	ND	0.05	0.20	ND	0.05	0.01
PFOcDA	0.03	0.10	0.02	ND	0.05	0.20	ND	0.05	0.01
PFBS	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFDS	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFHxS	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
PFOS	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
FOSA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
MeFOSA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
EtFOSA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
MeFOSE	ND	0.25	0.05	ND	0.25	0.98	ND	0.25	0.05
EtFOSE	ND	0.25	0.05	ND	0.25	0.98	ND	0.25	0.05
FOSAA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
MeFOSAA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01
EtFOSAA	ND	0.05	0.01	ND	0.05	0.20	ND	0.05	0.01

Table A4. Concentration of PFASs in *E. fetida* (ng g^{-1} dw) from all soils (S3-S11) at day 28; ND: not detected, NA: not analyzed.

	S3	S4	S5	S6	S7	S8	S9	S10	S11
	Conc.±SD	**Conc.**	**Conc.±SD**	**Conc.±SD**	**Conc.±SD**	**Conc.±SD**	**Conc.±SD**	**Conc.±SD**	**Conc.**
PFBA	ND	ND	ND	ND	ND	5605 ± 927	376 ± 62	197 ± 90.7	NA
PFPA	ND	ND	16.3 ± 4.12	6.78 ± 3.08	24.3 ± 2.71	783 ± 138	112 ± 4.37	66.7 ± 16.9	NA
PFHxA	2.12 ± 1.04	5.86	21.1 ± 2.31	4.74 ± 2.76	43.03 ± 9.24	1398 ± 254	541 ± 64.3	158 ± 74.8	NA
PFHpA	ND	ND	ND	ND	ND	ND	ND	188 ± 161	NA
PFOA	0.60 ±0.09	0.60	3.21 ± 0.94	0.88 ± 0.59	16.2 ± 3.48	1349 ± 155	2727 ± 456	937 ± 610	NA
PFNA	0.97 ± 0.35	1.53	ND	1.13 ± 0.91	2.42 ± 0.91	4401 ± 740	5174 ± 866	2765 ± 1659	NA
PFDA	0.85 ± 0.07	2.90	1.48 ± 0.89	2.16 ± 1.19	4.98 ± 0.21	4708 ± 754	4252 ± 170	2962 ± 786	NA
PFUnDA	ND	ND	1.44 ± 0.20	0.65 ± 0.35	17.1 ± 1.73	6.29 ± 1.19	ND	3.40 ± 1.27	NA
PFDoDA	2.42 ± 0.16	4.23	3.75 ± 0.90	3.96 ± 0.44	ND	7522 ± 3007	4589 ± 488	11129 ± 3194	NA
PFTriDA	ND	ND	ND	3.74 ± 2.24	25.9 ± 0.41	10.6 ±3.60	3.14 ± 0.50	29.0± 6.60	NA
PFTeDA	ND	ND	ND	1.57 ± 0.62	ND	4.18 ± 1.44	1.05 ± 0.08	14.7 ± 4.35	NA
PFBS	ND	ND	ND	ND	36.7 ± 3.21	ND	ND	ND	NA
PFDS	ND	ND	ND	ND	108 ± 5.52	ND	ND	ND	NA
PFOS	ND	17.26	7667 ± 63.0	23.9 ±10.7	7670 ± 1393	3492 ± 222	4040 ± 396	4177 ± 1221	NA
FOSA	ND	ND	ND	6.81 ± 1.77	301 ± 31.8	ND	ND	ND	NA

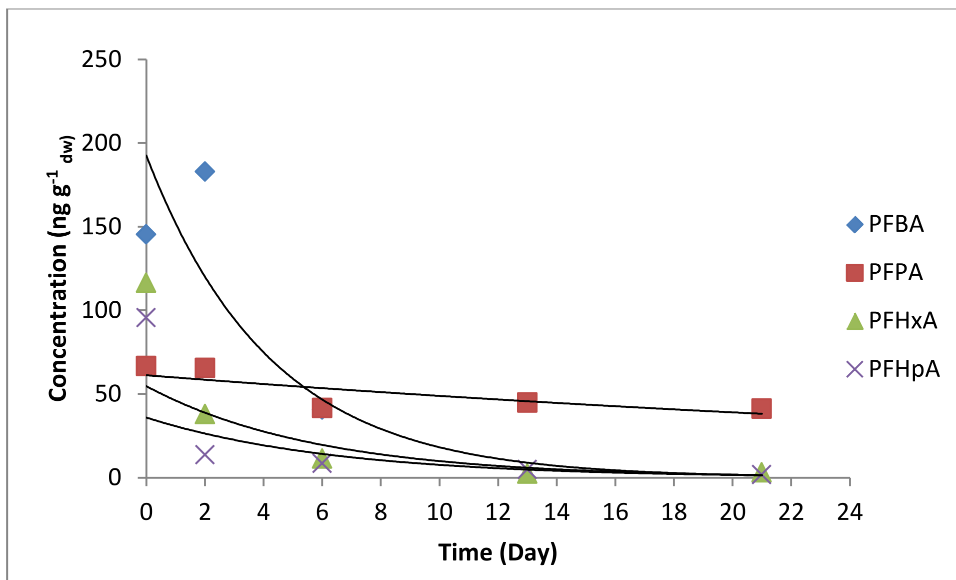
Table A5. Concentration of PFASs in *E. fetida* (ng g^{-1} dw) in uptake and elimination phase (from S7); * the time point which is end of uptake phase (day 28) and beginning of elimination phase (day 0); ND: not detected.

	Uptake Phase					Elimination Phase			
Time (day)	**2**	**6**	**13**	**21**	**28***	**2**	**6**	**13**	**21**
	Conc.	**Conc.**	**Conc.**	**Conc.**	**Conc.±SD**	**Conc.**	**Conc.**	**Conc.**	**Conc.**
PFPA	6.84	7.74	17.0	22.4	24.3 ± 2.71	15.9	12.4	10.3	9.48
PFHxA	4.53	10.4	38.8	63.9	43.0 ± 9.24	8.38	2.66	2.08	ND
PFOA	3.40	7.58	13.9	13.9	16.2 ± 3.48	5.85	2.36	1.72	1.51
PFNA	ND	ND	ND	ND	2.42 ± 0.91	ND	ND	ND	ND
PFDA	ND	1.80	3.63	3.27	4.98 ± 0.21	2.99	1.30	0.59	0.42
PFUnDA	0.70	3.35	8.79	13.8	17.1 ±1.73	15.1	7.43	3.75	1.09
PFTriDA	1.42	3.63	8.48	15.4	25.9 ± 0.41	26.1	30.6	30.4	21.8
PFBS	1.64	8.07	24.9	32.7	36.7 ± 3.21	46.8	23.9	14.8	7.88
PFDS	4.47	13.3	36.9	78.4	1088 ± 5.52	124	88.7	72.8	57.9
PFOS	922	2742	5306	6471	7670 ± 1393	8380	4732	2830	1578
FOSA	40.5	98.6	186	235	301 ± 31.8	298	174	124	113

Table A6. Concentration of PFASs in *E. fetida* (ng g^{-1} dw) in uptake and elimination phase (from S10); * the time point which is end of uptake phase (day 28) and beginning of elimination phase (day 0); ND: not detected.

	Uptake Phase					Elimination phase			
Time (days)	**2**	**6**	**13**	**21**	**28***	**2**	**6**	**13**	**21**
	Conc.	**Conc.**	**Conc.**	**Conc.**	**Conc.±SD**	**Conc.**	**Conc.**	**Conc.**	**Conc.**
PFBA	140	437	966	443	145± 21.2	183	40.6	ND	ND
PFPA	45.3	79.3	132	94.9	66.7 ± 16.9	65.6	41.6	44.8	41.1
PFHxA	181	225	221	111	158 ± 74.8	38.0	11.3	2.4	3.1
PFHpA	ND	246	137	88.4	188 ± 161	13.5	8.37	4.83	1.72
PFOA	633	1051	616	566	937 ± 610	155	110	65.5	14.5
PFNA	1080	304	2059	2065	2765 ± 1659	619	304	37.6	15.4
PFDA	1155	2805	2734	3982	2962 ± 786	1383	565	118	44.6
PFUnDA	ND	2.47	3. 26	4.55	3.40 ± 1.27	ND	ND	1.56	ND
PFDoDa	1690	5178	8970	12242	11129 ± 3194	15660	10467	7268	5571
PFTriDA	0.89	6.25	15.7	27.6	29.0 ± 6.60	40.8	28.7	26.5	24.4
PFTeDA	0.71	2.16	5.44	14.5	14.7 ± 4.35	25.3	19.3	18.1	16.7
PFOS	767	2616	3343	5202	4176 ± 1221	3631	1780	913	401

a)



b)

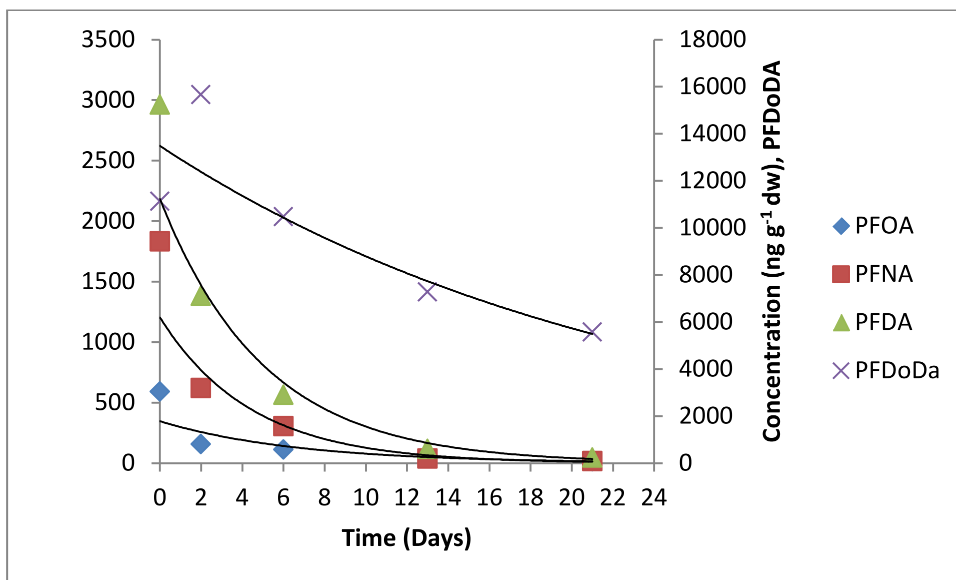


Figure A1. Concentration of PFCAs in *E. fetida* of S10, elimination phase; a) short chain PFCAs (C < 8) b) long chain (C >8).

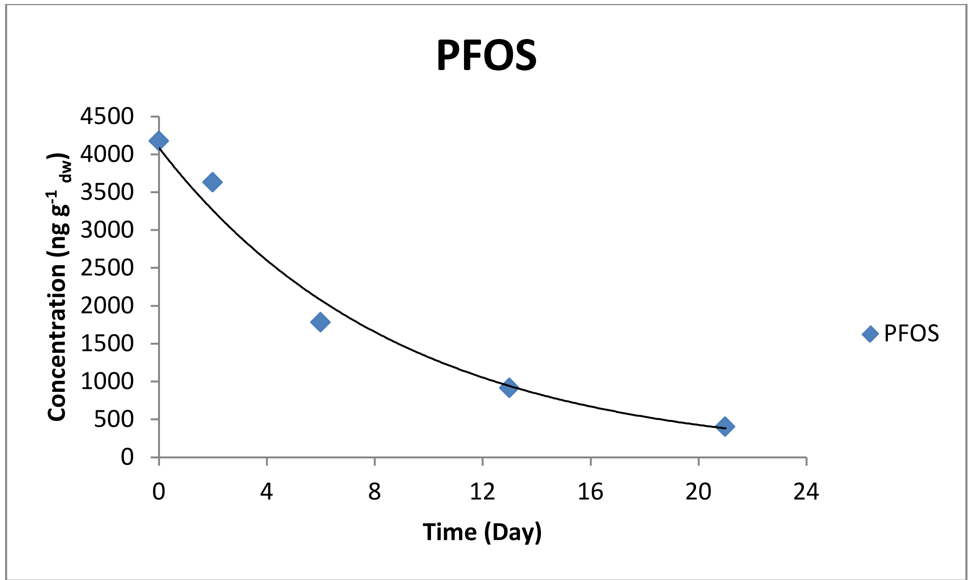
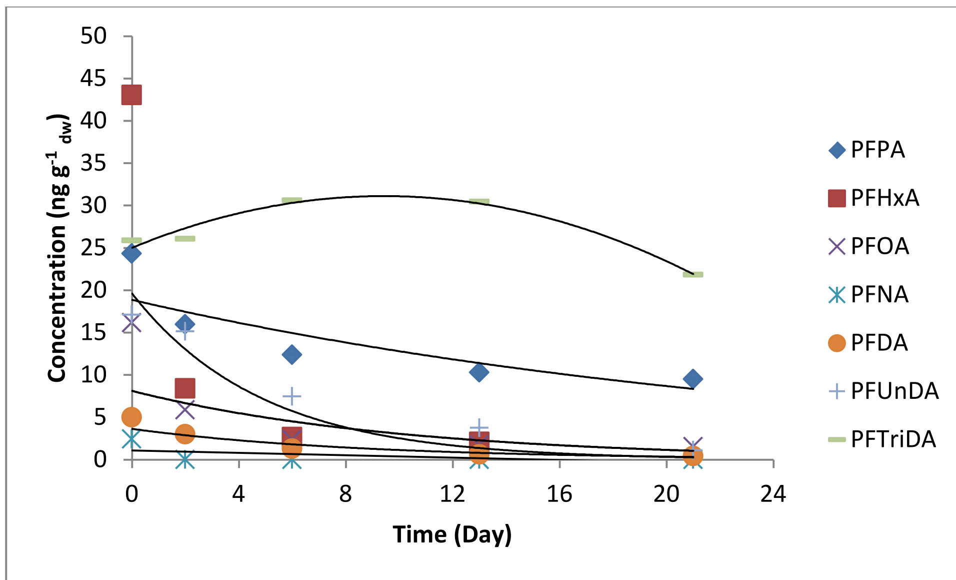


Figure A2. Concentration of PFOS in *E. fetida* of S10, elimination phase.

a)



b)

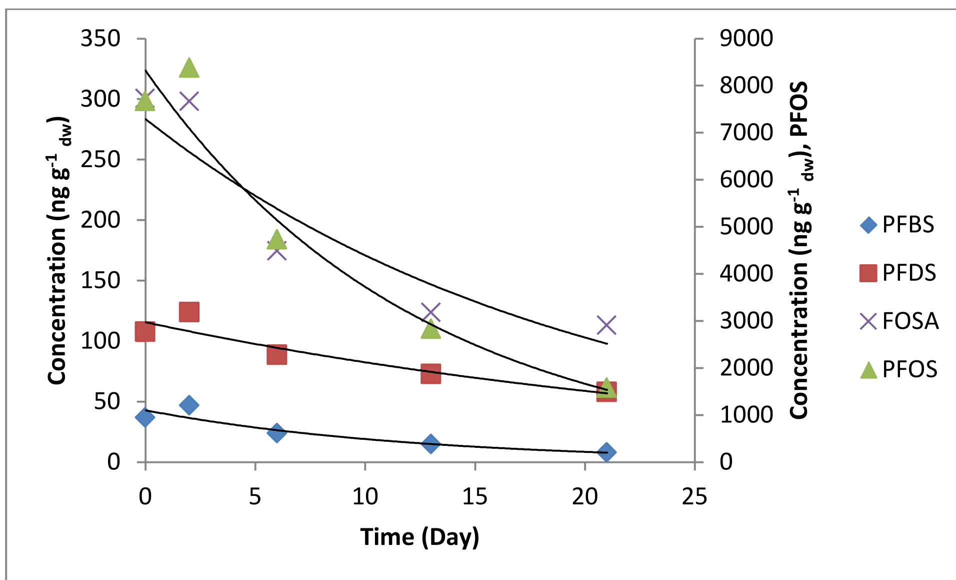


Figure A3. Concentration of PFASs in *E. fetida* of S7, elimination phase; a) PFCAs b) PFSAs.

Table A7. Uptake rate constant (k_s) (g_{soil} g^{-1}_{worm} d^{-1}) and elimination rate constant (k_d) (d^{-1}) for PFASs (S7); NA: not available.

	k_s	k_d
PFPA	2.60	0.04
PFHxA	NA	NA
PFOA	NA	NA
PFDA	NA	NA
PFUnDA	NA	NA
PFTriDA	12.7	0.01
PFBS	NA	NA
PFDS	8.14	0.03
PFOS	7.27	0.08
FOSA	15.2	0.05

Table A8. Concentration of PAHs in *E. fetida* (ng g^{-1} dw) from S10 and S11; NA: S3-S9 were not analyzed.

	S10	S11
	Conc.±SD	**Conc.±SD**
Acy	10.1 ± 0.94	17.7 ± 2.27
Ace	14.6 ± 0.11	14.1 ± 5.15
Flu	4.23 ± 0.83	6.90 ± 2.47
Phen	148 ± 41.7	188 ± 31.0
Anth	25.7 ± 5.41	39.9 ± 9.19
Fluo	315 ± 85.3	636 ± 153
Pyr	333 ± 70.0	634 ± 80.0
B(a)A	117 ± 18.7	278 ± 46.1
Chry	320 ± 33.1	811 ± 152
B(k+j)F	195 ± 51.4	495 ± 102
B(b)F	238 ± 39.0	446 ± 77.8
BaP	215 ± 14.4	318 ± 39.7
IP	127 ± 16.7	204 ± 45.7
DBA	108 ± 5.77	98.4 ± 75.4
BghiP	100 ± 15.1	143 ± 129

Table A9. Concentration of PAHs in *E. fetida* (ng g^{-1} dw) in uptake and elimination phase (from S10); * the time point which is end of uptake phase (day 28) and beginning of elimination phase (day 0); ND: not detected.

	Uptake phase					Elimination phase			
Time (days)	**2**	**6**	**13**	**21**	**28***	**2**	**6**	**13**	**21**
	Conc.	**Conc.**	**Conc.**	**Conc.**	**Conc.±SD**	**Conc.**	**Conc.**	**Conc.**	**Conc.**
Acy	6.04	11.1	9.14	11.0	10.1 ± 0.94	8.89	6.32	7.32	7.95
Ace	12.0	12.4	10.9	21.5	14.6 ± 0.11	11.2	7.58	10.0	9.36
Flu	ND	1.3	1.17	1.05	4.23 ± 0.83	ND	ND	0.23	0.81
Phen	45.6	71.9	82.0	80.0	148 ± 41.7	74.0	29.4	28.6	67.4
Anth	7.83	10.4	13.3	11.4	25.7 ± 5.41	6.30	3.3	2.65	4.89
Fluo	141	150	144	145	315 ± 85.3	64.1	27.5	17.9	35.2
Pyr	120	197	166	169	333 ± 70.0	73.3	34.8	15.8	25.6
B(a)A	31.3	66.8	62.0	56.6	117 ± 18.7	31.9	18.5	7.09	5.97
Chry	115	212	166	183	32033.1	133	73.4	38.1	41.4
B(k+j)F	33.2	103	88.8	130	195 ± 51.4	58.5	25.8	10.0	ND
B(b)F	41.0	99.1	101	113	238 ± 39.0	78.2	34.4	7.08	0.34
BaP	53.1	90.9	116	130	215 ± 14.4	103	68.0	57.0	45.6
IP	19.9	57.5	68.9	76.2	127 ± 16.7	52.0	43.7	24.3	ND
DBA	42.2	41.8	61.0	61.2	108 ± 5.77	52.2	46.4	31.7	46.1
BghiP	29.3	50.3	72.3	102	100 ± 15.1	89.9	34.6	26.0	33.3

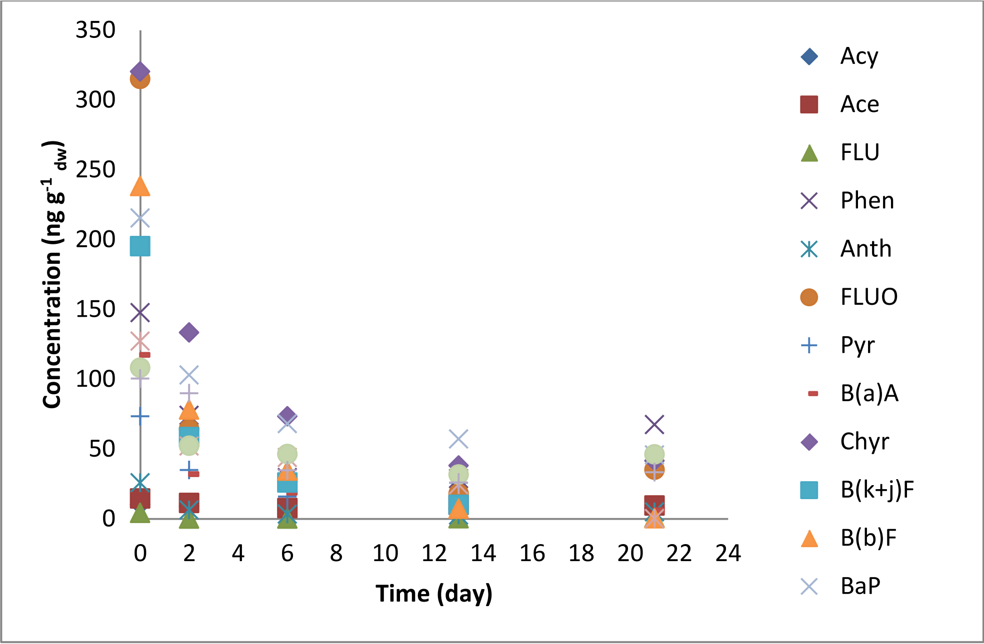


Figure A4. Concentration of PAHs in *E. fetida* of S10, elimination phase.

Table A10. Concentration of PFASs in *C.pepo* (ng g^{-1} dw) from all soil samples (S5-S10); ND: not detected.

	S5	S6	S7	S8	S9	S10
	Conc±SD	Conc±SD	Conc±SD	Conc±SD	Conc	Conc±SD
PFBA	ND	ND	ND	8203 ± 2296	19181	1656 ± 6.47
PFPA	6.95 ± 2.27	5.10 ± 2.15	19.5 ±7.65	2630 ± 350	3328	455 ± 42.8
PFHxA	9.67 ± 2.01	7.58 ± 2.41	23.2 ± 9.45	2551 ± 114	2520	290 ± 38.9
PFHpA	ND	ND	4.44 ± 1.68	1143 ± 88.6	2409	170 ± 42.9
PFOA	0.84 ± 0.07	0.25 ± 0.36	0.92 ± 0.19	302 ± 48.0	621	112 ± 36.8
PFNA	0.28 ± 0.16	ND	0.26 ± 0.03	136 ± 25.3	222	49.7 ± 2.08
PFDA	0.37 ± 0.03	0.23 ± 0.33	0.02 ± 0.07	62.3 ± 23.3	62.7	12.5 ± 1.12
PFDoDa	ND	ND	ND	9.11 ± 7.16	1.21	ND
PFBS	1.46 ± 0.56	ND	1.03 ± 0.56	ND	ND	ND
PFHxS	27.8 ± 4.60	ND	14.7 ± 5.08	ND	ND	ND
PFOS	68.9 ±18.0	0.52 ± 0.03	18.2 ± 6.36	18.0 ± 6.67	16.3	15.5 ± 0.45