



A comparison of POPs bioaccumulation in *Eisenia fetida* in natural and artificial soils and the effects of aging

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ABSTRACT

The close relationship between soil organic matter and the bioavailability of POPs in soils suggests the possibility of using it for the extrapolation between different soils. The aim of this study was to prove that TOC content is not a single factor affecting the bioavailability of POPs and that TOC based extrapolation might be incorrect, especially when comparing natural and artificial soils. Three natural soils with increasing TOC and three artificial soils with TOC comparable to these natural soils were spiked with phenanthrene, pyrene, lindane, p,p'-DDT, and PCB 153 and studied after 0, 14, 28, and 56 days. At each sampling point, total soil concentration and bioaccumulation in earthworms *Eisenia fetida* were measured. The results showed different behavior and bioavailability of POPs in natural and artificial soils and apparent effects of aging on these differences. Hence, direct TOC based extrapolation between various soils seems to be limited.

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1. Introduction

Persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs), have accumulated in soils as a result of their chemical stability, high lipophilicity, sequestration and low ability to degrade. Soils are a sink for the major portion of POPs, whether recently or historically discharged, and their contamination poses nowadays serious threats to soil organisms, plants and whole terrestrial ecosystems. The bioavailability of POPs in soils has often been discussed in recent years as a key process affecting their hazard level in ecosystems – toxicity, tendency to accumulate in biota, and transfer to food chains. However, factors affecting POPs bioavailability and mechanisms responsible for its changes in soils still need deeper investigation.

The bioavailability of POPs is affected by properties of compounds, organisms, and soils. The wide variability of these properties increases uncertainties in making general conclusions from individual studies. However, it was shown that soil organic matter plays the most important role in POPs sorption and bioavailability (e.g. Belfroid et al., 1996; Pignatello, 1998; Amorim et al., 2002). Other soil characteristics which may be important are particle size, pore size and quantity, clay content and character, soil structure, pH and CEC, moisture, and temperature. The bioavailability of POPs is also significantly influenced by the duration of soil-contaminant contact, so-called

aging (e.g. Alexander, 2000; Northcott and Jones, 2001). The decrease of bioavailability with aging has usually been explained by slow diffusion and entrapment with small (nano-) pores in soil aggregates, or the formation of strong chemical bonds to soil constituents (Pignatello and Xing, 1996; Gevao et al., 2000; Ehlers and Loibner, 2006).

The close relationship between soil organic matter and the bioavailability of POPs in soils suggests the possibility of using it for the extrapolation of bioavailability (and consequently toxicity and bioaccumulation) between different soils. van Gestel and van Diepen (1997) suggested the normalization of toxicological endpoints of hydrophobic organic contaminants in soils based on organic matter content. This was used as a basis for the extrapolation of toxicity results between different soils in the European Technical Guideline on Risk Assessment (European Commission, 2003). This approach assumes that all soil organic matter behaves similarly irrespectively of its type, composition or characteristics. However, some studies showed that the relationship between the amount of soil organic matter in the sediment or soil and the reduction in bioavailability and adsorption is not linear (Weston, 1990; Cuypers et al., 2002). Differences between the sorption behavior of different organic materials were attributed to differences in organic matter polarity and aromaticity (Xing et al., 1994) and the degree of humification and particle sizes of organic matter (Grathwohl, 1990). In addition, factors other than organic matter (clay content, moisture content, presence of non-aqueous phase liquids or metals...) have been reported to affect (at least partially) the behavior of organic contaminants in soils (Belfroid et al., 1996;

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Table 1
Main properties of natural (NS1, NS2, and NS3) and artificial (AS1, AS2, and AS3) soils used in this study: total organic carbon content (TOC), particle size analysis, cation exchange capacity (CEC), water holding capacity (WHC), organic carbon to total nitrogen ratio (C:N), ratio of humic acids to fulvic acids (HA:FA) in sodium pyrophosphate extract, and pH_{KCl} .

Soil	TOC (%)	Particle size analysis (%)				CEC (meq/100 g)	WHC (mL/g)	C:N	HA:FA	pH_{KCl}
		<10 μm	10–50 μm	50–100 μm	0.1–2 mm					
NS1	1.8	23	15	11	51	169.5	0.38	3.3	0.79	5.2
NS2	5.6	16	16	17	51	559.8	0.74	9.2	0.77	6.3
NS3	12.6	20	23	18	39	241.2	1.11	20.1	1.42	5.1
AS1	1.9	33	56	6	5	58.7	0.40	16.4	3.11	5.6
AS2	5.4	25	70	2	3	130.3	0.63	34.4	3.11	6.8
AS3	12.4	21	77	1	1	231.3	1.31	24.5	3.24	5.0

Xing and Pignatello, 1997; Hofman et al., 2008). Indigenous microorganisms present in natural soils may interact with organic contaminants as well as they can change their fate and bioavailability (Guthrie and Pfaender, 1998; Macleod and Semple, 2003). According to these uncertainties, direct extrapolation according to organic matter content should be verified.

Artificial soil was developed in order to achieve a standardized “model soil”, which can be used to produce reproducible and comparable data. However, properties of artificial and natural soils often vary and may lead to substantial differences in the fate, behavior, and effects of certain contaminants. Although this medium is more representative than filter paper, solutions or agar used in previous soil ecotoxicity tests, some recent studies have already indicated that artificial soil might not be able to produce the same results as natural soils in tests measuring the toxicity of heavy metals (Lock and Janssen, 2001; Amorim et al., 2005a), pesticides (Amorim et al., 2002, 2005b), and organic compounds (Hofman et al., 2008; Lock et al., 2002; Rhodes et al., 2008).

The aim of this study was to test if organic carbon content is a single factor affecting the bioavailability of POPs and enabling direct extrapolation of their toxicity and bioaccumulation between various soils as supposed in the European Technical Guideline on Risk Assessment (European Commission, 2003), especially when artificial soils are used for testing, and extrapolation from them to natural soils is required. Two PAHs (phenanthrene and pyrene) and three chlorinated organic contaminants (lindane, p,p'-DDT, and PCB 153) were chosen as model POPs with different environmental properties, and their bioavailability to earthworms *Eisenia fetida* was investigated in three natural soils with increasing TOC content (1.8, 5.6, and 12.6%) and in three artificial soils, which were prepared to contain the same TOC content as these natural soils. Total concentration and bioaccumulation in earthworms was measured after 0, 14, 28 and 56 days to see how soils differ with prolonging the contaminant-soil contact time (aging).

2. Materials and methods

2.1. Test organisms

Earthworms (*E. fetida*) were cultured at the laboratories of the Research Centre for Toxic Compounds in the Environment (Brno, Czech Republic) in a mixture of garden substrate (50%), granulated cattle manure (40%) and Sphagnum peat (10%). The water content of the substrate was approximately 80% WHC (water holding capacity) and pH was adjusted to 6–7 with CaCO_3 . The earthworms were fed with granulated cattle manure and the culture was maintained at 20 ± 1 °C in darkness. For the experiments, only adults with a well-developed clitellum and a weight of at least 300 mg were used.

2.2. Experimental soils

Natural soils (NS1, NS2 and NS3) which were identified as cambisols, were collected from the Czech Republic in spring 2008. To cover possible differences in organic matter characteristics (and not only differences in its total content) NS1 was arable soil, NS2 was grassland soil and NS3 was forest soil. The soils were air-dried, sieved to 2 mm, and stored in black plastic bags until the experimental start. The TOC of the soils was measured by Liqui TOC II (Elementar, Analysensysteme GmbH,

Germany) and was 1.8, 5.6 and 12.6% for NS1, NS2, and NS3, respectively. The properties of the soils are displayed in Table 1. Their texture was loamy sand.

Artificial soils (AS1, AS2 and AS3) were prepared according to OECD (OECD, 1984) from three constituents: fine quartz sea sand (Hornbach, CZ), kaolin clay powder (Sigma–Aldrich, CZ; product code 18672) and Sphagnum peat (Agro CS, CZ). The sphagnum peat was air-dried, finely ground and sieved to 2 mm. Its TOC was measured as $40.1\% \pm 1.9$. The amount of clay was kept constant at 20% (based on dry weight) but the amounts of peat and sand in AS1, AS2, and AS3 were modified to achieve the same TOC contents as measured in NS1, NS2, NS3, respectively. The final properties of the mixed soils were measured and are presented in Table 1.

In addition, soil pH was measured and CaCO_3 was added to the artificial soils in order to achieve pH values similar to those found in natural soils. Water holding capacity was measured for all soils and dH_2O was added to achieve 50% WHC moisture. All soils were then stored in glass jars at 25 °C in darkness for approximately 1 week prior to experimental work to stabilize the soil structure and chemistry.

2.3. Preparation of contaminated soils

Phenanthrene, pyrene and lindane (γ -hexachlorocyclohexane) were obtained from Sigma–Aldrich (CR). PCB 153 was obtained from Dr. Ehrenstorfer GmbH (Germany) and p,p'-DDT from Supelco (UK). The chemical purity of all compounds was >97%. Selected characteristics of these compounds (molecular weight, MW; solubility in water, S_w ; and logarithm of the octanol/water partition coefficient, $\log K_{ow}$) are presented in Table 2. Dichloromethane (DCM), hexane and chloroform (all > 95% purity) for experiments were purchased from Lach-Ner (CR) and acetone (99.8% purity) from Chromservis (CR). One spiking solution (350 mL) for all soils was prepared as a mixture of all five compounds dissolved in 1:3 DCM:acetone (v/v) solution (because of the low solubility of some compounds in 100% acetone). Final concentrations were measured as 753 ± 10 $\mu\text{g/mL}$ for Phe, 683 ± 11 $\mu\text{g/mL}$ for Pyr, 79 ± 2 $\mu\text{g/mL}$ for Lin, 112 ± 2 $\mu\text{g/mL}$ for p,p'-DDT, and 73 ± 1 $\mu\text{g/mL}$ for PCB 153. Each soil moistened at 50% WHC in amount corresponding to 3100 g dry weight was spiked according to the recommendations of Doick et al. (2003). Thus, $\frac{1}{4}$ of the soil was well mixed with 50 mL of the spiking solution in a stainless steel bowl with a stainless steel spoon and then, this amount was well mixed with the rest of the soil. After spiking, the solvent was evaporated in a fume hood for 4 h and lost water (checked by weighing) was replenished. After that, soils were put into 3 L glass jars and kept overnight at laboratory temperature in darkness to stabilize the contamination. The next morning (i.e. day 0 of the experiment), total soil concentration in each soil was measured in five independent samples randomly taken from the jar. The low variability (coefficients of variance were 0.6–13.6% for all soils) verified the homogenous distribution of compounds in the whole amount of soil. Average initial (0 d) concentrations (range for all six soils) were: 6122.4–7311.5 ng/g soil dry weight for phenanthrene, 4387.5–5714.8 ng/g soil dry weight for pyrene, 591.2–976.3 ng/g soil dry weight for lindane, 310.3–540.4 ng/g soil dry weight for p,p'-DDT, and 499.6–688.9 ng/g soil dry weight for PCB 153. Similar levels were often used in studies focused on behavior of POPs in soils (Benimeli et al., 2008; Kelsey et al., 2010; Rhodes et al., 2008).

2.4. Experimental design

Soils were stored in closed jars in darkness at laboratory temperature for the whole experimental period. The first sampling was performed on day 0 (i.e. the day after the contaminated soils were prepared), followed by samplings on day 14,

Table 2
Name, CAS number, and selected characteristics of compounds used in this study.

	Phenanthrene	Pyrene	Lindane	p,p'-DDT	PCB 153
CAS	85-01-8	129-00-0	58-89-9	50-29-3	35065-27-1
MW (g/mol)	178.23	202.26	290.83	354.49	360.88
S_w (mg/L)	1.15	0.07	7.3	0.025	0.0009
$\log K_{ow}$	4.45	4.88	3.72	6.91	8.35

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