



Fate and bioavailability of ^{14}C -pyrene and ^{14}C -lindane in sterile natural and artificial soils and the influence of aging

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ABSTRACT

Soil organic matter is used to extrapolate the toxicity and bioavailability of organic pollutants between different soils. However, it has been shown that other factors such as microbial activity are crucial. The aim of this study was to investigate if sterilization can reduce differences in the fate and bioavailability of organic pollutants between different soils. Three natural soils with increasing total organic carbon (TOC) content were collected and three artificial soils were prepared to obtain similar TOCs. Soils were sterilized and spiked with ^{14}C -pyrene and ^{14}C -lindane. Total ^{14}C radioactivity, HPCD extractability, and bioaccumulation in *Eisenia fetida* were measured over 56 days. When compared to non-sterile soils, differences between the natural and artificial soils and the influence of soil-contaminant contact time were generally reduced in the sterile soils (especially with middle TOC). The results indicate the possibility of using sterile soils as “the worst case scenario” in soil ecotoxicity studies.

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1. Introduction

Soils and sediments are sinks for a major proportion of persistent organic pollutants (POPs) and nowadays represent a secondary source of contamination in the environment. Persistent organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs), have accumulated in soils and sediments as a result of their high lipophilicity, chemical stability, sequestration and low levels of degradation. POPs are characterized by high toxicity and the tendency to accumulate in biota and biomagnify up through food chains. As a result of these characteristics, soil associated POPs pose a serious risk within the environment.

Soil, pollutant and biotic properties, as well as soil-pollutant contact time, are known to have considerable influence on the bioavailability of POPs. It has been shown that the bioavailability, sorption and toxicity of POPs in the soil are mostly driven by the amount of soil organic matter (OM) and its character (Amorim et al., 2002; Hofman et al., 2008; Peters et al., 2007; Pignatello, 1998). Other soil properties, such as pH, cation exchange capacity (CEC), particle size, or clay content also affect directly or indirectly the behavior of POPs in soils (Grathwohl, 1990; Nam et al., 1998;

Sun and Li, 2005). The contact time between pollutants and soil (aging) and microbial activity are also important in controlling the fate and behavior of POPs in soil (Alexander, 2000; Palomo and Bhandari, 2006; Rhodes et al., 2008).

To allow better comparison of studies between different laboratories, standardized bioassays are often used, with many involving artificial soil. Artificial soil was first developed for acute toxicity tests with earthworms (OECD, 1984; ISO, 1993) and has been used as a reference soil in other bioassays (acute and reproduction tests with soil invertebrates). However, the properties of artificial and natural soils may vary greatly, e.g. OM content in artificial soils is considered unrealistically high (Amorim et al., 2002). As a result, the data arising from studies using artificial soils may lead to wrong conclusions and mean that any risk assessment may be erroneous due to the different fate, behavior, and effects of POPs (Amorim et al., 2005). OM content has been suggested for normalization of the toxicological endpoints (NOECs and L(E)C50s) of hydrophobic organic compounds between different soils (European Commission, 2003; van Gestel and van Diepen, 1997), because soil OM is considered to be the most important factor controlling the bioavailability of these compounds. However, many studies have shown that the fate and bioavailability of hydrophobic organic compounds (HOCs) are not necessarily related to organic matter content and direct extrapolation is not always applicable or possible (Hofman et al., 2008; Lock et al., 2002; Vlčková and Hofman, 2012). Differences in HOCs

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fate and bioavailability in soils with similar OM content are assigned to different OM characteristics, such as polarity and aromaticity (Xing et al., 1994) and/or the degree of humification (Grathwohl, 1990). It has been suggested that older and more condensed OM would form stronger and more irreversible associations with organic pollutants than would younger, amorphous, soft (rubbery) carbon (Peters et al., 2007; Sun et al., 2010; Xing and Pignatello, 1997).

Indigenous microorganisms present in soil may interact with pollutants (Guthrie and Pfaender, 1998; Macleod and Semple, 2000; Semple et al., 2007). The rate at which microbial cells can convert chemicals during biodegradation depends on two factors: (i) the rate of uptake and metabolism (the intrinsic activity of the cell) and (ii) the rate of transfer to the cell (mass transfer). Microbial communities differ among soils and it can be expected that this leads to differences in pollutant behavior among soils and limits the possibility of extrapolating data between soils. It can also be expected that the microbial communities originating from peat in artificial soils will be different from those of natural soils. This will increase the lack of comparability between natural and artificial soils.

In previous studies, differences in the fate and bioavailability of POPs in artificial and natural soils with similar TOC contents were observed and the different microbial communities were hypothesized to be a possible reason (Hofman et al., 2008; Rhodes et al., 2008; Vlčková and Hofman, 2012). The working hypothesis for this study was that the persistence, extractability, and uptake of POPs by earthworms will be similar in the sterile artificial and natural soils, which contain similar amounts of organic carbon. To test this hypothesis, an experimental approach similar to those employed in the studies mentioned above was used, i.e. three artificial soils were prepared containing similar TOC contents to three natural soils with increasing TOC content (1.2, 3.5, and 10.2%); however, in our study, the soils were sterilized prior to experiments. The soils were spiked with ^{14}C -labeled pyrene or lindane, which were used as model non-chlorinated and chlorinated POPs, respectively. Total ^{14}C radioactivity, ^{14}C -extractability with hydroxypropyl- β -cyclodextrin (HPCD) and ^{14}C -uptake by the earthworm *Eisenia fetida* were measured over a 56-day soil-contaminant contact period. A shake extraction using HPCD was chosen because of its ability to predict the bioavailability of organic pollutants to microbes (Doick et al., 2006; Reid et al., 2000, 2004).

2. Materials and methods

2.1. Chemicals

Pyrene (chemical purity >95%), [9- ^{14}C]pyrene (specific radioactivity 10–30 mCi mmol $^{-1}$), lindane (chemical purity >95%), and [9- ^{14}C]lindane (specific radioactivity 27.9 mCi mmol $^{-1}$) were purchased from Sigma–Aldrich, UK. The hydroxypropyl- β -cyclodextrin (HPCD) was produced by Acros Organics (Morris Plains, NJ, USA) and its purity was 97%. Acetone was obtained from Fisher Scientific, UK. Sample oxidizer cocktails (Carbosorb-E $^{\text{TM}}$ and Permafluor-E $^{\text{TM}}$) were produced by

PerkinElmer Life Sciences, Boston, MA, USA. Combustion aiding solution (Combustaid $^{\text{TM}}$) was purchased from PerkinElmer Inc., Waltham, USA. The GoldStar multipurpose liquid scintillation cocktail was obtained from Meridian, UK.

2.2. Experimental soils

Three natural soils (NS1, NS2, NS3), identified as cambisols (loamy sands), were collected from the Czech Republic in July 2010 at sites with different vegetation cover to ensure different organic matter content (low TOC in soil from abandoned arable land, middle TOC in soil from orchard grassland, high TOC in mixed forest soil). The soils were air-dried, sieved to 2 mm, and stored ~1 month prior to experimental work. Artificial soils (AS) were prepared according to OECD (OECD, 1984) using kaolin clay powder (Sigma–Aldrich, CZ; product code 18672), fine quartz sea sand (Hornbach, CZ; particles 0.1–0.5 mm), and Sphagnum peat (Agro CS, CZ). Sphagnum peat (26.3% \pm 0.4 TOC) was air-dried, finely ground, sieved to 2 mm and defaunated (frozen to $-20\text{ }^{\circ}\text{C}$ and thawed three times) prior to mixing. The amount of clay was kept constant at 20% (based on dry weight) but 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. Properties of the NS and AS soils were measured (Table 1). All soils were sterilized by 25 kG radiation (Bioster a.s., CZ) approximately 3 weeks prior to experimental work and stored in closed airtight bags in darkness. Before the experiment, soil pH was measured and adjusted in artificial soils to levels found in the natural soils by CaCO_3 addition. All soils were adjusted to 50% of water holding capacity (WHC) by adding distilled water and then stored within sealed kilner jars at $25\text{ }^{\circ}\text{C}$ in darkness for 3 days prior to spiking to establish equilibrium between the soil and the soil pore water phase (OECD, 2010).

2.3. Soil spiking and experimental design

Two experiments (for pyrene and lindane separately) were conducted to study the differences in extractability and bioavailability between the natural and artificial soils. Each soil (equivalent of 920 g soil dry weight) was spiked using the method described by Doick et al. (2003). Non-labeled pyrene was dissolved in acetone and ^{14}C -pyrene was added to achieve a nominal concentration of 10 mg $_{\text{PYR}}$ kg $^{-1}$ and ~164.2 kBq kg $^{-1}$ soil dry weight in each soil. The same method was used to prepare soils amended with lindane and ^{14}C -lindane to achieve a nominal concentration of 1 mg $_{\text{LIN}}$ kg $^{-1}$ and 169.6 kBq kg $^{-1}$ soil dry weight. After spiking, the solvent was evaporated in a fume hood overnight and lost water (checked gravimetrically) was replenished. Spiked soils, as well as non-spiked controls, were stored in sealed kilner jars in darkness at room temperature for 1, 14, 28, and 56 days. A moisture check at each time point showed that water loss was negligible during the experiment and no water was added to the experimental soils. At each time point, several methods were conducted to measure: (i) total content of ^{14}C -pyrene or ^{14}C -lindane associated radioactivity in the soils, (ii) extractability by HPCD, and (iii) bioaccumulation in earthworms *Eisenia fetida*.

2.4. Total ^{14}C -pyrene and ^{14}C -lindane associated radioactivity in soils

The total ^{14}C -pyrene and ^{14}C -lindane associated radioactivity was determined via the combustion of approximately 1.5 g of soil randomly taken from the jar using a Packard 307 sample oxidizer at each sampling point in triplicate for each soil. The combustion process was conducted over 3 min, aided by the addition of Combustaid (0.2 ml). The resultant $^{14}\text{CO}_2$ was trapped in Carbosorb-E and Permafluor-E and quantified by liquid scintillation counting (LSC, Canberra Packard Tri-Carb 2300 TR). The results were related to 1 g soil dry weight and corrected against the results obtained for non-spiked controls (blanks).

2.5. Extractability of ^{14}C -pyrene and ^{14}C -lindane associated radioactivity from soils

The extractability of ^{14}C -pyrene and ^{14}C -lindane was determined by hydroxypropyl- β -cyclodextrin (HPCD) shake extraction. HPCD extraction is a mild extraction able to predict biodegradability to microbes very accurately (Doick et al., 2006;

Table 1

Main properties of the natural (NS1, NS2, and NS3) and artificial (AS1, AS2, and AS3) soils used in the 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} . Values are based on a dry weight.

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	1.2	18	16	13	53	172	0.54	10.7	1.13	6.0
NS2	3.5	17	13	13	58	505	0.85	12.3	1.53	7.0
NS3	10.2	ND ^a	ND ^a	ND ^a	ND ^a	357	1.74	19.2	1.46	5.6
AS1	1.5	22	1	1	77	63	0.52	32.2	1.19	6.2
AS2	3.9	25	2	2	70	117	0.77	54.6	1.68	6.4
AS3	10.8	ND ^a	ND ^a	ND ^a	ND ^a	366	2.01	41.4	1.4	4.9

^a Not possible to determine because of high organic carbon content.

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