Chemosphere 179 (2017) 222–231

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Bioavailability of five hydrophobic organic compounds to earthworms from sterile and non-sterile artificial soils

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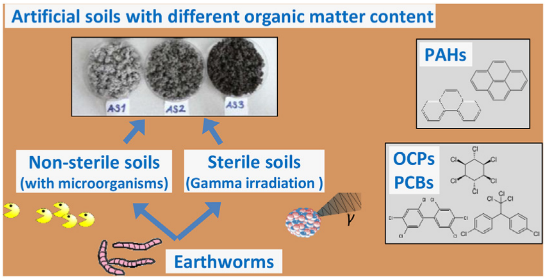
HIGHLIGHTS

- Microorganisms' role in the bioavailability of hydrophobic organic contaminants was studied.
- Total PHE concentrations in non-sterile soils decreased, in sterile soils were constant.
- Bioaccumulation of PAHs (pyrene and phenanthrene) was highly affected by sterilization.
- Bioavailability of chlorinated compounds (DDT, PCB, HCH) was unaffected by sterilization.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 January 2017
Received in revised form
24 March 2017
Accepted 27 March 2017
Available online 28 March 2017

Handling Editor: I. Cousins

Keywords:
Bioaccumulation factors
Artificial soil
Sterilization
Organic carbon content
Aging

ABSTRACT

Bioaccumulation factors (BAFs) of organic pollutants to soil biota, often required by risk assessment, are mostly obtained in non-sterile laboratory-contaminated artificial soils. However, microbial degradation has been indicated by many authors to influence the fate of hydrophobic organic compounds (HOCs) in soils. A question arises if the microbial community of peat which is used for artificial soil preparation affects the measured values of BAFs. In this study the effect of soil microorganisms on bioavailability of HOCs was studied and a portion of each soil was sterilized by gamma irradiation. Results indicated that the sterilization process significantly affected the fate of polycyclic aromatic hydrocarbons (PAHs; phenanthrene and pyrene) and increased bioavailability of these compounds to earthworms with BAFs several times higher in the sterile soils compared to their non-sterile variants. This suggests that sterilization of soils can be used as the "worst-case scenario" for laboratory tests of toxicity or bioaccumulation of biodegradable HOCs such as PAHs. It represents a situation of limited microbial degradation resulting in higher bioavailable fractions to other organisms (e.g. invertebrates). This may be the case in soils where microbial communities face stresses caused by contamination or land management. The bioavailability of chlorinated HOCs (lindane, 4,4′-DDT and PCB 153) was not affected by sterilization, as their BAFs were similar in the sterile and non-sterile soils during the experiment.

1. Introduction

Artificial soil has been developed to achieve a standardized "soil-like" medium, better representative of natural soils than filter paper, any solution or agar, but less variable than natural soils (Heimbach and Edwards, 1983; Edwards, 1984). The composition of artificial soil is defined as a mixture of 70% fine quartz sand, 20% kaolin clay, and 10% finely ground sphagnum peat (OECD, 1984; ISO, 1998). It was optimized to provide the "worst-case scenario" of

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http://dx.doi.org/10.1016/j.chemosphere.2017.03.117

exposure, because it was designed to resemble natural loamy soil with low retention for metals and polar pesticides related to the high sand amount and low cation exchange capacity of the clay used (Edwards, 1984).

However, the "worst-case scenario" of exposure is not guaranteed for HOCs, as artificial soil has a relatively high amount of organic matter that strongly binds these contaminants (Hofman et al., 2008; Vlčková and Hofman, 2012; Amorim et al., 2005). Moreover, the fate, sorption, bioavailability, and toxicity of HOCs in artificial soils have been repeatedly reported to be significantly different from those in natural soils even if the organic matter content was comparable (Hofman et al., 2008; Vlčková and Hofman, 2012; Bielská et al., 2014). One of the possible reasons for this could be the microbial community of peat, which strongly influences the behavior of HOCs (Šmídová and Hofman, 2014; Šmídová et al., 2012; Hofman et al., 2014). Šmídová et al. (2012) suggested that sterilization could bring the fate and behavior of HOCs in artificial soil closer to natural soils (if the organic carbon content is similar). Sterile artificial soil could then be consider as the "worst case scenario" and represent a situation of higher bioavailable fraction caused by limited microbial degradation.

Earthworms may constitute up to 80% of the total biomass of the soil fauna (Kabata-Pendias, 2010) and contribute substantially to soil formation, soil quality maintenance, and nutrient cycling in terrestrial ecosystems. As they are able to accumulate various contaminants, including organic pollutants and heavy metals (Ma et al., 1995; Nahmani et al., 2007; Vermeulen et al., 2010), they are frequently used in the monitoring of contaminated soils (Shang et al., 2013; Suthar et al., 2008), and in laboratory testing (ISO, 2008; OECD, 2010). The fate and behavior of HOCs in soils, as affected either by soil physicochemical properties or soil microorganisms, are closely linked to their bioavailability to earthworms. Šmídová and Hofman (2014) studied the bioaccumulation of different HOCs in earthworms in six natural soils and after initial accumulation they found a decrease in the concentration in the organisms (i.e. peak-shaped accumulation curves), probably caused by microbial degradation resulting in depletion of compounds in soil and consequent elimination of them from earthworms. The results indicate that when a bioavailable fraction of a compound is degraded by microorganisms and its desorption from the soil is not fast enough to replenish the degraded mass in soil pore-water, the bioavailability to earthworms can be seriously underestimated compared to situation when the equilibrium between the soil and the pore-water phase is stable.

Comparing the results of Šmídová and Hofman (2014) with sterile soils and Šmídová et al. (2012) with non-sterile soils it can be hypothesized that peat microorganisms play a significant role in reducing the bioavailability of degradable HOCs to earthworms. However, the results of these two studies cannot be compared directly because of the different methods used for the measurement of HOCs concentrations: In the study Šmídová et al. (2012) ^{14}C-analysis was used, which does not allow the ^{14}C radioactivity of parental compounds and that of possible degradation products to be distinguished, while the GC/MS analysis used by Šmídová and Hofman (2014) was addressed to defined POPs like pyrene and lindane only. The fate and bioaccumulation in earthworms of HOCs in sterile and non-sterile artificial soils have never been compared directly in one experiment and this was taken as the main goal of the present study.

The hypotheses of our study were as follows: (i) the fate of HOCs in contaminated artificial soil is different in sterile and non-sterile variants, (ii) the uptake of HOCs by earthworms increases by soil sterilization and this is time-dependent, and (iii) the increasing organic matter content decreases the bioavailability of HOCs in both sterile and non-sterile soils. These hypotheses were investigated by means of two polycyclic aromatic hydrocarbons (PAHs; phenanthrene and pyrene) and three chlorinated HOCs (lindane, 4,4'-DDT, and PCB 153) in three artificial soils of differing organic matter content.

2. Materials and methods

2.1. Test organisms

Eisenia andrei earthworms have been cultured in a mixture of garden substrate, granulated cattle manure and *Sphagnum* peat (50:40:10 w:w:w; all constituents from Argo CS, CZ) at the Research Centre for Toxic Compounds in the Environment (Brno, Czech Republic). The water content of the substrate for the culture was approximately 80% of the water-holding capacity (WHC) and the pH was adjusted to 6–7 with $CaCO_3$. The earthworms were fed with granulated cattle manure (Argo CS, CZ) and the culture was maintained at 20 ± 1 °C in the dark. HOC levels in the earthworms from the culture were negligible. Only adults with a well-developed clitellum and a weight of at least 300 mg were used in this study.

2.2. Experimental soils

Artificial soils (AS1, AS2, and AS3) were prepared according to the OECD guideline (OECD, 1984) using three constituents: fine quartz sea sand (Hornbach, CZ; particles 0.1–0.5 mm), kaolin clay powder (Sigma-Aldrich, CZ; product code 18672), and *Sphagnum* peat (Agro CS, CZ). The peat was air-dried, finely ground and sieved to 2 mm. The total organic carbon (TOC) content of the peat was measured by LiquiTOC II (Elementar Analysensysteme GmbH, Germany) as 51.5 ± 1.3%. The clay amount was kept always at 20% (based on dry weight) to avoid possible effects of its variable content on the results. On the other hand, varying quantities of the peat were used for AS1, AS2 and AS3 to achieve their low, middle and high organic matter content, respectively, as in our previous studies focused on comparison of artificial soils with natural soils (Vlčková and Hofman, 2012; Šmídová et al., 2012). The sand additions corresponded to peat amounts to get the final desired mass of soils (equivalents to 5.3 kg_{dw} for AS1, 4.6 kg_{dw} for AS2, and 2.6 kg_{dw} for AS3, respectively). Measured values of TOC were 1.6, 3.4 and 15.3% in AS1, AS2, and AS3, respectively, which roughly corresponds to a scale of 1:2:10. The values are close to typical arable, grassland and forest soils of the Czech Republic. The soil pH, determined by 1 M KCl solution in a 1:5 ratio, was adjusted to a level of 6.0 ± 0.5 by addition of $CaCO_3$ (OECD, 2010). Other physico-chemical properties were analyzed in an accredited company (Laboratoř Morava, CZ). WHC was measured according to the ISO guideline 11268-2 (ISO, 1998). The properties of the soils are presented in Table 1. A half amount of each soil was packed in an airtight glass vessel and sterilized by 25 kGy gamma irradiation (Bioster a.s., CZ) (the variants were labelled s-AS1, s-AS2, and s-AS3, respectively). Nonsterile portions of each soil were stored in airtight glass vessels for about a week in the dark at room temperature (21 ± 2 °C) until the experiment started. Freshly prepared deionized water was added to all soils to achieve 50% WHC 48 h before application of the contaminants (OECD, 2010).

2.3. Preparation of contaminated soils

Phenanthrene, pyrene, and γ-hexachlorocyclohexane (lindane) were obtained from Sigma Aldrich, UK. PCB 153 was obtained from Dr. Ehrenstorfer GmbH (Germany) and 4,4′-DDT from Supelco, UK. Selected characteristics of the studied compounds, such as the CAS number, purity, molecular weight, solubility, and log K_{ow} are presented in Table 2. The spiking procedure was derived from Doick