Science of the Total Environment 595 (2017) 132–140

Contents lists available at ScienceDirect

Science of the Total Environment

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

Bioavailability and toxicity of pyrene in soils upon biochar and compost addition

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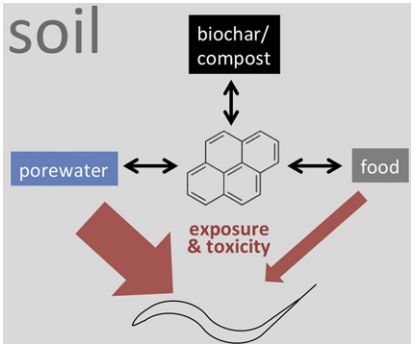
HIGHLIGHTS

- Compost (10%) and biochar (5%) amendment to soil is not toxic to *C. elegans*.
- $EC50_{Reproduction}$ for pyrene were 14 and 31 mg/kg (soil dry weight) for two different soils, respectively.
- Combined addition of compost and biochar most effectively reduced porewater concentrations and toxicity of pyrene.
- The porewater concentration predicted 52% of pyrene toxicity to nematodes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form 24 March 2017
Accepted 25 March 2017
Available online 3 April 2017

Editor: Ajit Sarmah

Keywords:
Biochar
Compost
Nematodes
Pyrene toxicity
Solid-phase microextraction
Sorption

ABSTRACT

The study investigates the role of biochar and/or compost in mitigating the toxic effects of pyrene in soils using reproduction of nematodes and porewater concentration as measures of pyrene toxicity and bioavailability, respectively. Two soils were spiked with increasing levels of pyrene to achieve a concentration-response relationship for the reproduction of *Caenorhabditis elegans*. The observed EC50 values (pyrene concentration causing 50% inhibition of reproduction) were 14 mg/kg and 31 mg/kg (dry mass) for these soils, corresponding to equilibrium porewater concentrations of 37 μg/L and 47 μg/L, respectively. Differences in organic carbon content were not sufficient to explain the variability in toxicity between the different soils. Soils causing a significant inhibition of reproduction were further amended with 10%-compost, 5%-biochar, or both, and the effects on reproduction and porewater concentration determined. Combined addition of compost and biochar was identified as the most effective strategy in reducing pyrene concentration in soil porewater, which was also partly reflected in soil toxicity. However, porewater concentrations predicted only 52% of pyrene toxicity to nematodes, pointing to particle-bound or dietary exposure pathways.

Capsule: Amending pyrene-spiked soil with biochar and compost effectively reduced pyrene porewater concentrations and toxicity to nematodes, which were significantly related.

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http://dx.doi.org/10.1016/j.scitotenv.2017.03.230
0048-9697/

1. Introduction

Soil pollution is an important factor restraining the sustainable development of agriculture that can threaten food security. To control risks associated with soil contamination as well as to improve soil quality and fertility, various management strategies are applied such as the application of compost (Huang et al., 2016) and biochar (Kuppusamy et al., 2016). Compost is a product of composting, i.e. the process of humification and stabilization of organic wastes that is rich in nutrients, humic matter, and microorganisms (Luo et al., 2016). Biochar is a pyrolysis product obtained when biomass is heated under oxygen limited conditions (Sun et al., 2014). It combines a number of important advantages such as i) carbon sequestration (Windeatt et al., 2014), ii) neutralization of soil acidity (Obia et al., 2015), iii) reduction of greenhouse gases emissions (N_2O and CH_4) (Martin et al., 2015), iv) prevention of nutrient leaching (Troy et al., 2014), and v) pollutant immobilization (Xie et al., 2015).

While an intensive research has been devoted to the area of remediation and sorption (Ahmad et al., 2014), less attention has been paid to the effects of compost and biochar on pollutant bioavailability and toxicity to soil biota (Lehmann et al., 2011). With the application of biochar and compost, physicochemical properties of soil are being influenced including the changes in physical structure, fertility, microbial activity, crop growth and biomass, and bioavailability of nutrients and toxic compounds (Calleja-Cervantes et al., 2015; Pérez-de-Mora et al., 2006). Soil biota can be potentially both negatively and positively affected by these changes, depending on the properties of the amended soil, application doses of amendments and their properties, sensitivity of soil organisms and many other factors. Studies on this topic have been rather observational, and report somewhat unclear and contrasting results, especially regarding biochar (Lehmann and Joseph, 2015), thus adding limited understanding of the effects of biochar on soil organisms and linked ecosystem processes. Moreover, of the variety of soil organisms, earthworms have been investigated most often while, yet, other soil organisms, such as springtails and nematodes, have been studied far less (Lehmann et al., 2011).

In this study, the toxicity of the polycyclic aromatic hydrocarbon (PAH) pyrene in soil amended with biochar, compost, or both was investigated at increasing pyrene concentrations to provide a dependent effect on nematode reproduction rates as an endpoint. The nematode *Caenorhabditis elegans* was used as a model organism and representative of soil micro-fauna. Nematodes are the most abundant and species rich metazoans in soil ecosystems and possess key positions in soil food webs (Ferris et al., 2001), considerably influencing nutrient cycling (Beare, 1997). The toxicity test with *C. elegans* is suitable for testing soil samples (Höss et al., 2009), is readily standardized (ISO, 2010) and has shown to be well reproducible and repeatable if using growth and reproduction as toxicity endpoints (Höss et al., 2012).

In the first step, the dose-response relationship was established between the concentration of pyrene in soils and the effects on nematode reproduction. In the following step, soils at pyrene levels causing 30%–95% inhibition of reproduction in comparison to pyrene-free soil were amended with 5%-biochar, 10%-compost, and both of these materials and the effects on reproduction as well as pollutant bioavailability assessed via standard toxicity testing (ISO 10872) and a passive sampling technique of solid-phase microextraction (SPME), respectively (Lu et al., 2011). The application dose of biochar corresponds to doses applied in other remediation studies with biochar (Beesley et al., 2016). For compost, the applied dose fell within the range (i.e., <15%) proved non-toxic for seed germination and plant growth (Someus et al., 2015).

The purpose of this study was to provide information on the remediation efficiency of biochar/compost at low to high toxic doses of pyrene and to compare between reproduction rates of nematodes and the porewater concentrations providing a measure of pyrene bioavailability (Mayer et al., 2014). Moreover, secondary toxic effects of biochar and compost resulting from their properties and potential presence of toxic chemicals in themselves (such as PAHs in biochar; Stefaniuk et al., 2016) and heavy metals in compost (Huang et al., 2016) were assessed using controls, i.e. contaminant-free soils amended with these materials. The study provides a better insight on pyrene toxicity in soils treated with compost, biochar and with both compost-biochar, the latter allowing consideration of the potential biochar and compost interactions. To our knowledge, a very limited number of studies have been devoted to the assessment of toxicity to nematodes other than parasitic nematodes (Ebrahimi et al., 2016) under the single and combined applications of compost and biochar.

2. Material and methods

2.1. Chemical and materials

Solvents for soil spiking and for extractions (hexane and acetone) were purchased from Sigma-Aldrich, Germany. Pyrene and *p*-terfenyl used as an instrumental standard for analysis were purchased from Sigma-Aldrich, Germany. Distilled water was used for experiments. NaCl and KCl were purchased from Carl Roth GmbH (Karlsruhe, Germany). Ludox silica suspension (Ludox TM50) and 12 well-plates were purchased from Sigma-Aldrich (Munich, Germany) and VHR International GmbH (Darmstadt, Germany), respectively. Standard soil Lufa St. 2.2 soil was purchased from Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer (LUFA; Speyer, Germany).

2.2. Characteristics of experimental soils, compost, and biochar

Experimental soils were sampled in summer 2015 from two agricultural field sites in Austria: from Eschenau (Lower Austria) and from Kaindorf (Styria). Eschenau soil was a planosol while Kaindorf soil was a cambisol (Kloss et al., 2014). After sampling, the two soils were dried, sieved (<2 mm mesh) and stored at 20 °C and in the dark prior to further use. Compost was kindly provided by fk Agrar- und Umweltservice Ges.m.b.H (Pixendorf, Austria). A standard biochar produced from *Miscahnthus* at a pyrolysis temperature of 550 °C was purchased from the UK biochar research center. Prior to the physicochemical analysis, representative portions of soils and compost were sieved through a <2 mm mesh, whereas a representative portion of biochar was crushed and sieved through a <250 μm mesh. The elemental composition of materials was determined using an elemental analyzer (C%, H% and N%, Elementar Vario MACRO). The total organic carbon content was determined using a carbon analyzer equipped with a solid-state infrared detector (LECO RC-612). Mineral content was measured by weighting samples before and after heating at 750 °C for 6 h (American Society for Testing and Materials, method D-1762-84). The oxygen content was estimated by mass balance: O = 100 - (C + H + N + mineral, all weight in %). In addition, the total concentrations of metals in the biochar were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (Perkin Elmer Optima 5300 DV), after microwave assisted acid digestion with nitric acid and hydrogen peroxide (Microwave 3000-Anton Paar, Kah et al., 2016). The specific surface area and pore volume of biochar were determined via gas-physisorption of N_2 and CO_2 after sample degassing at 105 °C for 14 h.

2.3. Soil spiking with pyrene

After pyrene addition using hexane as a carrier solvent, soils were mixed thoroughly and left in the fume hood overnight to let the solvent residues fully evaporate. The following day after spiking, soils were sampled (aliquots of 3 g in triplicates) and subjected to a hot-solvent extraction (hexane:acetone, 50 mL, 2 h). The extracts were cleaned with silica gel column chromatography and subjected to the gas chromatography analysis with mass spectrometry detection (GC-MSD, GC 7890B