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Short communication

Reduced bioaccumulation of PAHs by *Lactuca satuva* L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar

Sardar Khan [a,b], Ning Wang [a], Brian J. Reid [c], Alessia Freddo [a,c], Chao Cai [a,*]

[a] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China
[b] Department of Environmental Science, University of Peshawar, Pakistan
[c] School of Environmental Sciences, University of East Anglia, Norwich, UK

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ABSTRACT

The influence of sewage sludge (SS) and sewage sludge biochar (SSBC) upon biomass yield and the bioaccumulation of PAHs into lettuce plants grown in contaminated soil ($\sum$16PAH 20.2 ± 0.9 mg kg^{-1}) is presented. All SSBC amendments (2, 5 and 10%) and the 2% SS amendment significantly ($P < 0.01$) increased lettuce biomass. Both SS and SSBC amendments significantly reduced ($P < 0.01$) the bioaccumulation of PAHs at all application levels; with reduction in $\sum$16PAH concentration ranging between 41.8 and 60.3% in SS amended treatments and between 58.0 and 63.2% in SSBC amended treatments, with respect to the control. Benefits in terms of biomass production and PAHs bioaccumulation reduction were greatest where SSBC was used as a soil amendment. At high application rates (10%) SSBC reduced bioaccumulation of PAHs by between 56% and 67%, while SS reduced bioaccumulation of PAHs by less than 44%.

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1. Introduction

Industrialization and urbanization have dramatically increased the volume of sewage sludge produced by wastewater treatment plants throughout the world. In China, approximately 30 million tons of sewage sludge was generated in 2010 (Yu, 2011). Agricultural application of sludge has increased dramatically following the passing of the Ocean Dumping Act (1988). In China, 44% of sewage sludge is used in the agriculture sector (this compares with: 71% in UK; 54% in Germany; 54% in Spain; 65% in France; and 60–65% in the USA (Spinosa, 2011; Yu, 2011; Eljarrat et al., 2008)).

Application of sewage sludge to agricultural land delivers well recognized benefits in terms of nutrient addition, increased soil organic matter content (Benckiser and Simarmata, 1994), benefits to soil structure (Richards et al., 2000) and as a consequence benefits for crop yield (El-Motaium and Abo El-Seoud, 2007). However, negative issues relating to sewage sludge application to agricultural land also exist. It is well documented that over application of sewage sludge can adversely affect soil biota (Andrés et al., 2011). In addition, sewage sludge has the potential to introduce pathogens into the soil (Kelley et al., 1984). The transfer of these pathogens on to food and ultimately into the human food chain has also been established (Reilly, 2001).

Biochar is carbon rich material produced through the process of pyrolysis under limited oxygen conditions (Cao et al., 2011). When applied to soil, biochar increases soil cation exchange and water-holding capacities (Glaser et al., 2002; Bélanger et al., 2004; Keech et al., 2005; Liang et al., 2006). In addition, biochar is rich in nutrients (e.g. P, K) and other microelements (e.g. Mg, Ca, Mn) (Neary et al., 1999). Biochar has also been shown to immobilize metals and reduce the bioavailability of hydrophobic organic compounds (HOCs) due to its sorptive capacity (Wang et al., 2011). It is suggested that the application of biochar to soils that have elevated burdens of metals and HOCs may abate problems associated with their toxicity and their transfer onto and into food and ultimately into the human food chain. This scenario is significant in China where rapid urbanization has lead to elevated levels of metals and HOCs (particularly, polycyclic aromatic hydrocarbons (PAHs)) in peri-urban soils that are extensively used for crop production. This research provides a direct comparison of sewage sludge (SS) and sewage sludge biochar (SSBC) influence upon crop yield and mitigation of PAH bioaccumulation into lettuce plants grown in contaminated soil.

* Corresponding author.
E-mail address: ccai@iue.ac.cn (C. Cai).

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2. Materials and methods

2.1. Chemicals

All solvents were HPLC/spectro grade purchased from Tedia Company Inc, USA. Silica gel, alumina and sodium sulfate were purchased from Sinopharm Chemical Reagent Co Ltd., China. Surrogate standards (PAH-Mix 24 deuterated, LA20950024HE) and reference materials (PAH-Mix 9, XA20950009CY) were purchased from the laboratory of Dr. Ehrenstorfer, Augsburg, Germany.

2.2. Soil sampling

Soil samples (upper horizon, 0–20 cm) contaminated with PAHs were collected from different locations around Sanming Steel Refinery (Fujian Province, China). Soil samples were sieved (2 mm mesh) and homogenized to provide a composite sample. Sub-samples were freeze-dried at −50 °C and 123 ± 2 Pa and stored at −20 °C in paper sacks for future analyses. Properties of soil such as pH, electrical conductivity (EC), loss on ignition (LOI), carbon (C), nitrogen (N), sulfur (S), porosity and surface area are given Table S1.

2.3. Biochar preparation

Sewage sludge obtained from Xiamen Yundang wastewater treatment plant was air dried. SSBC was prepared from SS by pyrolysis at 500 °C for 6 h in a high performance automatic controlled furnace (GWL-1200, Henan, China), under a continuous flow of nitrogen. A cooling chamber, with water, was used for passing the off-gas to allow condensation of heavy tars. The biochar was then cooled inside the furnace to room temperature in the presence of nitrogen gas. Properties of SS and SSBC such as pH, EC, LOI, C, N, S, porosity and surface area are given Table S1.

2.4. Experimental design

PAH contaminated soil was amended with SS or SSBC ($n = 4$) at application rates of 2, 5 and 10% (defined as SS2, SS5, SS10 or SSBC2, SSBC5, SSBC10, respectively) (dry weight basis). An un-amended control soil was also prepared ($n = 4$). Treatments had a total mass of 2 kg. Six uniform seedlings of lettuce (*Lactuca satuva* L.) were transplanted to each treatment pot and then thinned to 4 after one week (Khan et al., 2008). The experiment was conducted in a greenhouse under natural light (12 h) with day temperature of 30 ± 3 °C and night temperature of 24 ± 3 °C. Soils were irrigated with deionized water to maintain the moisture content (60% field capacity). The pots were randomized at regular interval to compensate for light and temperature differences inside the greenhouse. Plants were harvested after 8 weeks following their initial transplanting, and separated into shoots and roots. Shoots were rinsed briefly with deionized water, while roots were first washed with tap water and then with deionized water to remove adhering soil particles. After drying with tissue paper, shoots and roots were freeze-dried at −50 °C and 123 Pa and dry weights recorded.

2.5. PAH extraction and quantification

Lettuce, soil, SS and SSBC samples (2 g) were extracted with dichloromethane (DCM) and acetone (1:1 ratio) using accelerated solvents extraction (ASE, Dionex-350) (Freddo et al., 2012). The extracts were evaporated to 1 mL using a rotary evaporator and purified using silica chromatography columns prepared with silica gel, Al_2O_3 and capped with Na_2SO_4 (all activated before use; see Khan et al., 2008). Thereafter, the columns were washed with hexane. The concentrated extracts, then were loaded to columns to separate the PAHs from other polar interfering compounds. These columns were eluted with 60 mL mixture of hexane and DCM (7:3), the eluted fractions were again evaporated up to 1 mL using rotary evaporator and transferred to Kuderna-Danish concentrator and rinsed with 10 mL of n-hexane (Khan et al., 2008). Thereafter, the eluted fraction was again reduced to 1 mL under nitrogen flow and transferred to a vial capped with a Teflon-lined septum for analysis of PAHs. The final concentrated extracts analyzed using gas chromatograph mass spectrometry (GC–MS, Agilent Technologies 5975C) (see Supporting information). The GC–MS was equipped with an inert XL MSD with a triple axis detector and used under the selected ion monitoring mode. An HP-5 silica fused capillary column (60 m × 0.25 mm inner diameter × 0.25 μm film thickness) was used with helium as the carrier gas at a constant flow rate of 1 ml min^{-1}. The GC oven temperature was programmed to ramp from 50 °C to 200 °C at 10 °C min^{-1}, then to 300 °C at 10 °C min^{-1} and to then hold for 8 min at this temperature. The injector and detector temperatures were 280 °C and 300 °C, respectively. Mass spectra were acquired at the electron ionization mode, while selected ion monitoring (SIM) mode was carried out using the molecular ions selective for individual PAHs.

The efficiency of ASE extraction and silica column purification for PAH recovery from soil, sludge, biochar, plant samples and sample blanks was checked using surrogate PAH-deuterated standards (acenaphthene d10, chrysene d12, naphthalene d8, perylene d12 and phenanthrene d10). The results showed satisfactory recovery, with the average recovery ranging from 83.6 ± 8.2% to 96.5 ± 6.4%.

2.6. Data analysis

The data were statistically analyzed using the statistical package SPSS 11.5. The measures were expressed in terms of mean, while the figures presented the mean values and standard deviation of four replicates. Statistical significance was computed using Duncan's multiple range test and paired-samples *t*-test, with a significance level of $P < 0.01$.

3. Results and discussion

3.1. PAHs in sludge, biochar and soil

The total PAH concentrations in SS, SSBC and soil were, respectively: 2.95 ± 0.10, 4.35 ± 0.33 and 20.2 ± 0.22 mg kg^{-1} (Table S2). The PAH concentrations in sewage sludge were found to be within permissible limits ($\sum$9PAHs, 6 mg kg^{-1}) as set by Council of the European Community (CEC, 2000) for sludge application (5 tons of dry weight per ha) to agricultural land. The PAH concentrations in SSBC were below those recently recommended by the International Biochar Initiative (IBI, 2012) (between 6 and 20 mg kg^{-1}) and below those reported by Freddo et al. for a range of dissimilar biochars (2012). Comparison of $\sum$16PAH concentration in the control soil (20.21 ± 0.22 mg kg^{-1}) with those in the SS10 (18.5 ± 1.0 mg kg^{-1}) and SSBC10 (18.6 ± 1.0 mg kg^{-1}) revealed a reduction in PAH concentration of 10%. This result could be explained by the reduced amount (10%) of contaminated soil present in SS10 and SSBC10. This 'dilution' effect will have contributed to the bioaccumulation reductions discussed below but, it is stressed, that bioaccumulation reductions are of far greater magnitude that can be attributed to dilution alone.

3.2. Plant biomass

The root biomass production in the SS and SSBC treatments followed a similar pattern of changes as those observed for shoots (Fig. 1). In all SSBC treatments, the biomass of shoots was significantly higher ($P \leq 0.01$) than in the control soil (Fig. 1). These three treatments appreciably improved shoot biomass yields when compared to the control, showing increases of: 71%, 93% and 46% in SSBC2, SSBC5 and SSBC10 treatments, respectively. Shoot biomass in the SS2 treatment was also significantly ($P \leq 0.01$) increased (an increase of 83%) with respect to the control soil (Fig. 1). It was noted

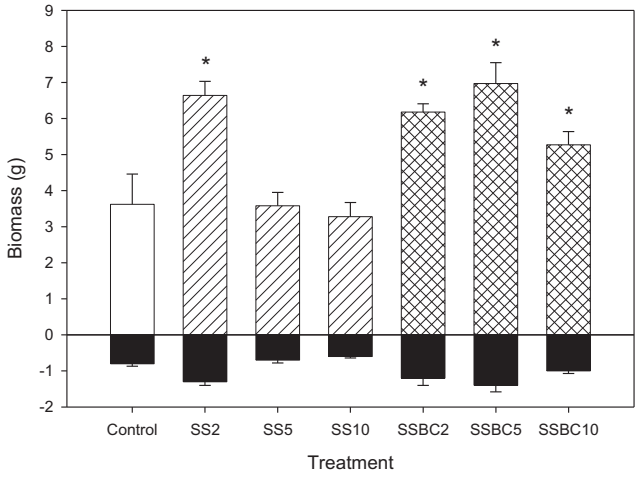

Fig. 1. Shoot (white) and root (black) biomass (g dry weight/pot) in the control (unhatched), sewage sludge amended treatments (SS; hatched) and sewage sludge biochar treatments (SSBC; cross-hatched) at application rates of 2%, 5% and 10%. Error bars indicate ±1 standard deviation. Asterisks indicate significantly higher whole plant biomass ($P \leq 0.01$) with respect to the control.

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