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Short Communication

Invasive plant-derived biochar inhibits sulfamethazine uptake by lettuce in soil

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HIGHLIGHTS

- Sulfamethazine (SMT) uptake by lettuce was studied in the presence of biochar.
- Biochar showed highly aromatic and hydrophobic nature.
- Addition of biochar decreased SMT toxicity and increased growth of plant.
- Biochar retained SMT and reduced its uptake by plant.

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ABSTRACT

Veterinary antibiotics are frequently detected in soils posing potential contamination of food crops. Sulfamethazine (SMT) uptake was investigated by lettuce (*Lactuca sativa* L.) grown in the soils treated with/without biochar derived from an invasive plant, burcucumber (*Sicyos angulatus* L.) (BBC700). Soils were contaminated with SMT at 5 and 50 mg kg^{-1}, and treated with/without 5% BBC700 (w w^{-1}). The lettuces were harvested after 5 weeks of cultivation and were analyzed for SMT by a high performance liquid chromatography–tandem mass spectrometry after solid-phase extraction. With 5% BBC700, the uptake of SMT was reduced by 86% in the soil spiked with 5 mg kg^{-1} SMT compared to the control whereas a 63% reduction was observed in the soil spiked with 50 mg kg^{-1} SMT. Application of BBC700, into soils effectively reduced the SMT uptake by lettuce.

1. Introduction

Since their discovery in the early 1900s, antibiotics have been used to treat infectious diseases in humans and animals (Kumar et al., 2005). A significant amount of antibiotics is also used as a feed supplement to promote growth of livestock (Ok et al., 2011; Awad et al., 2014). Antibiotics are designed to act at low doses and to be completely excreted from the body after a short time (Thiele-Bruhn, 2003). Only a fraction of ingested antibiotics is metabolized in an animal's gut; hence, 17–90% of the treated antibiotic is excreted via urine and feces, and eventually releases into the surrounding environments (Kümmerer, 2009; Kim et al., 2010). As a result, antibiotics are recognized as emerging micro-contaminants of manure, soil, vegetables, and the aquatic environment due to their high frequency of detection (Dolliver et al., 2007; Hu et al., 2010).

Among the different sources, the dominant pathway of antibiotics released into the terrestrial environment is via applications of animal manure and biosolids to agricultural lands as fertilizers (Kim et al., 2012). Antibiotics can also be introduced through reclaimed wastewater irrigation, such as raw/treated sewage wastewater (Ok et al., 2011). In some cases, antibiotics are released directly into the environment through leachates from

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animal carcass sites (Kim and Kim, 2012). Released antibiotics in the soil can be taken up by plants, which may pose a threat to human health by consumption of such antibiotic-contaminated plants (Kumar et al., 2005; Dolliver et al., 2007). Therefore, it is necessary to understand the potential risk of antibiotic uptake by food crops.

Sulfonamide (SA) antimicrobials, considered one of the most commonly used antibiotics in human therapy, livestock production, and aquaculture, include a class of synthetic SA derivatives. The consumption of SAs has accounted for 11–23% of that of all veterinary antibiotics used in the European Union (Ok et al., 2011). Sulfamethazine (SMT), one of the most commonly used SAs, is a priority pollutant due to its low molecular weight and low sorption capacity to soil particles (Gao and Pedersen, 2005); therefore, it may facilitate plant uptake. SMT has also received a great deal of interest due to its frequent detection in soils (Awad et al., 2014), surface water (Hu et al., 2010; Li et al., 2012), drinking water (Hu et al., 2010), and food crops (Dolliver et al., 2007; Herklotz et al., 2010; Eggen et al., 2011). Several studies have focused on remediating antibiotic-polluted soils using different amendments such as carbon nanotubes (Ji et al., 2009) and clay minerals (Gao and Pedersen, 2005). Among the sorbents, this study hypothesized that biochar would effectively adsorb and retain SMT because of its unique properties.

Biochar is produced from different biomass wastes in a pyrolysis reactor under low oxygen and moderate temperature conditions. It has a potential to improve soil fertility and sequester carbon in soils (Awad et al., 2012). The hydrophobic surface of the polyaromatic sheets, together with the micro-porosity created by the turbostratic arrangement of the graphene micro-crystallites, renders a potentially strong adsorbent of organic compounds that may contribute to the overall sorption of incidental, natural, or applied chemicals in soils (Ahmad et al., 2012, 2014b; Mohan et al., 2014). Several studies have reported the use of biochar to remove antibiotics from soil and aqueous media (Teixidó et al., 2011). However, antibiotic uptake by plants in the soils exposed to biochar has not been studied.

2. Materials and methods

2.1. Chemicals

Analytical-grade SMT (99%) and chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC)-grade water, methanol, acetonitrile, acetone, hexane, hydrochloric acid, and formic acid were obtained from LiChrosolv Co. (Merck, Darmstadt, Germany). Costar® Spin-X® centrifuge tubes and 0.22 μm cellulose acetate filters were obtained from Corning Inc. (Corning, NY, USA). Solid-phase extraction (SPE) cartridges (3 mL/60 mg of hydrophilic–lipophillic–balanced [HLB]) were purchased from Waters Oasis Co. (Milford, MA, USA). Stock solution (100 mg L^{-1} SMT) was prepared with 1% methanol in amber color bottles for preventing degradation of SMT and then stored at 4 °C.

2.2. Soil collection and characterization

Soil was collected from an agricultural land located in Wonju-si, Gangwon province, Korea. After air drying, the soil was passed through a 2.0 mm sieve and was characterized for selected physicochemical parameters. Soil texture was determined by the hydrometer method (NIAST, 2000), and pH and electrical conductivity (EC) were measured electrometrically in 1:5 soil/water suspension. Exchangeable cations (Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+}) were extracted using 1 M ammonium acetate (pH 7.0) and an inductively coupled plasma optical emission spectrometry (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA).

2.3. Biochar production and characterization

Biochar was obtained from invasive burcucumber plant (*Sicyos angulatus* L.) feedstock. Burcucumber is a widespread invasive species in Korea (Kil et al., 2006). As burcucumber poses a severe threat to biodiversity, the Korea Ministry of the Environment has adopted regulations for its control. Invasive plant species could potentially be an effective feedstock for biochar manufacturing and bring additional ecosystem benefits (Ahmad et al., 2014a). Burcucumber plants were collected and oven-dried overnight at 60 °C. The dried biomass was then crushed and sieved to obtain <1.0 mm particle size. The powdered biomass was pyrolyzed at 700 °C in a muffle furnace (N11/H Nabertherm, Germany) with a heating rate of 7 °C min^{-1} under limited oxygen conditions, thereby producing burcucumber biochar (BBC700). The elemental composition (CHON) of BBC700 was determined by dry combustion (EA1110, CE Instruments, Milan, Italy). Atomic ratios of H/C and O/C were calculated to evaluate BBC700's aromaticity and hydrophobicity, respectively. The surface functional groups of BBC700 were characterized by a Fourier transform-infrared spectroscopy (FT-IR) (Bio-Rad Excalibur 3000MX spectrophotometer; Hercules, CA, USA). Brunauer–Emmett–Teller specific surface area, total pore volume, and pore diameter of BBC700 were also determined with a gas sorption analyzer (NOVA-1200; Quantachrome Corp., Boynton Beach, FL, USA).

2.4. Plant cultivation and antibiotic analysis

Homogenized soil samples were placed in polyvinyl chloride pots. The treatments were employed as follows: (1) control soil, (2) control soil + 5% (w w^{-1}) BBC700, (3) 5 mg kg^{-1} SMT spiked soil (4) 5 mg kg^{-1} SMT spiked soil + 5% (w w^{-1}) BBC700, (5) 50 mg kg^{-1} SMT spiked soil, and (6) 50 mg kg^{-1} SMT spiked soil + 5% (w w^{-1}) BBC700. SMT was applied to the soil as an aqueous solution (from 100 mg L^{-1} which was prepared in 1% methanol), reaching to final concentrations of 0 (control), 5 and 50 mg kg^{-1}. SMT was spiked at the top of the pot and then soil was mixed thoroughly. Triplicate samples were prepared. Water content of the experimental units was maintained at 70% water holding capacity. Five lettuce seeds were planted per each pot. Treated pots were kept in a growth chamber (GC300, Lab Companion, Seoul, Korea) at 24 °C with 70% humidity. Artificial lighting was maintained at 16 h light/8 h dark rotation. After complete germination of the seeds in the pots, the conditions of chamber were changed to a controlled photoperiod with 12 h light/12 h dark rotation. Any application of fertilizers was not allowed during cultivation.

Antibiotic concentrations in the plants were measured after 5 weeks following the methodology of Dolliver et al. (2007). First, 500 mg of air-dried, crushed plant material was extracted in dark using 8 mL methanol: HCl (95:5), manually shaken for 5 min and centrifuged for 15 min, and then the supernatants were collected. The residue was extracted again with 5 mL of acetone using the same procedure. The supernatants were mixed and dried under N_2 gas. The residue was re-suspended in 5 mL methanol: nanopure water (50:50) and defatted three times with 5 mL hexane. Hexane (the upper layer) was removed each time after liquid–liquid partitioning. The remaining liquid was concentrated under N_2 gas to 2.5 mL for SPE using an OASIS HLB 60 mg cartridge. The sample was passed through the preconditioned cartridge at a flow rate of 2 mL min^{-1}, the SPE cartridge was washed twice with 3 mL nanopure water, dried under depression for 5 min, and SMT was eluted from the cartridge twice with 2.5 mL methanol. After elution, 40 μL of the internal standard (1 mg L^{-1} simetone) was added to the methanol extract, which was dried under N_2 gas at 40 °C until a volume of 150 μL was achieved. The volume was brought up to 400 μL by adding 250 μL of the mobile phase A (0.1% formic acid in nanopure water). The solution was mixed and filtered through a 0.22 μm cellulose acetate filter (Kim and Carlson, 2007). A Thermo Finnigan LCQ Duo ion traps mass spectrometer (MS; Thermo, Woburn, MA, USA) equipped with a heated capillary interface and electrospray ionization (ESI) was used to perform the MS analysis. ESI was used to detect the target antibiotic, and a positive mode was adapted. A C18 column (Xterra, 2.1 × 50 mm, 2.5 μm) and a C18 guard column (Phenomenex, Torrance, CA, USA) were used. Mobile phase A was composed of HPLC grade water and formic acid (99.9:0.1 v v^{-1}), and mobile phase B was HPLC grade acetonitrile and formic acid (99.9:0.1 v v^{-1}). The gradient was ramped from 96% mobile phase A and 4% mobile phase B to 70% mobile phase A and 30% mobile phase B in the first min and held isocratic for 29 min. Injection volume was 20 μL. Spray voltage was set to 4.5 kV, and the capillary voltage was autotuned to 3500 V. Drying gas temperature was set to 350 °C. The precursor ion ($[M + H]^+$ (m z^{-1})) value and fragment ion value for SMT were 279 and 204. Selective ion monitoring mode was used to detect simetone and SMT. Freshly prepared standard solutions were used to calibrate HPLC. Three runs were done and the average concentration of the individual antibiotics was calculated.

3. Results

3.1. Soil and biochar characteristics

The physicochemical properties of the soil and BBC700 are presented in Tables S1 and S2, respectively. The soil was loam with 43.3% sand and 25.8% clay (Table S1) with pH 7.06. Exchangeable Ca^{2+} (1.82 $cmol_{(+)}$ kg^{-1}), Mg^{2+} (0.64 $cmol_{(+)}$ kg^{-1}), and K^+ (0.14 $cmol_{(+)}$ kg^{-1}) of the soil were lower than typical values (5.0, 1.5, and 0.5–6.0 $cmol_{(+)}$ kg^{-1}, respectively) of a Korean upland soil (Jo and Koh, 2004). BBC700 was strongly alkaline (Table S2). The presence of mobile matter indicates an organic material supply for soil microorganisms (Ahmad et al., 2014a). Resident or fixed matter in the biochar is the proportion corresponding to its stability in the soil. The considerable amount of resident matter in BBC700 (42.39%) reflects its ability to act as a C sink in the soil because of its slow chemical transformation and microbial decomposition. BBC700 had obvious pores and channel structures as shown in images by a scanning electron microscopy (data not shown). The molar O/C ratio indicated a less hydrophilic nature (Ahmad et al., 2014a) (Table S2). The H/C of 0.22 indicated that the BBC700 was highly carbonized and exhibited a highly aromatic structure. This observation was further supported by the FT-IR data (Fig. 1). Aromatic C was detected at peaks between 885 and 750 cm^{-1} due to the out-of-plane deformations of aromatic C–H (Ahmad et al., 2012).

3.2. SMT uptake by lettuce

The methods of SMT determination including extraction and screening resulted in a good separation of both SMT and simetone (the internal standard) without any interference with the lettuce matrix components, as shown in Fig. S1. Simetone (198 > 124 m z^{-1} transition) was used as the internal standard to evaluate matrix suppression and to correct run-to-run variations (Kim and Carlson, 2007). Furthermore, the cultivation experiment revealed that lettuce grown in soils contaminated with 5 mg kg^{-1} SMT took up about 6% (0.32 mg kg^{-1}) of the applied SMT. However, the uptake amount was reduced to 0.8% (0.05 mg kg^{-1}) with the 5% BBC700 application. Thus, the uptake amount of SMT by lettuce was reduced by 86% with the 5% BBC700 application compared to the control. This reduction of SMT uptake can be due to the sorption capacity of BBC700 (Vithanage et al., 2014). A recent study on BBC700 mixed in different soils has shown a high potential in adsorbing SMT and reducing its transport through a soil (Vithanage et al., 2014). Lettuce grown in the soils contaminated with 50 mg kg^{-1} SMT showed about 1.2% (0.62 mg kg^{-1}) SMT uptake; however, BBC700 reduced its uptake to 0.4% (0.22 mg kg^{-1}). The overall reduction of SMT uptake in the BBC700 treated soil spiked with 50 mg kg^{-1} SMT was not greater than in the same soil spiked with 5 mg kg^{-1} SMT; however, a 63% reduction of SMT uptake was observed (Fig. 2b). The reduction of SMT uptake at a high concentration may be due to a decrease in growth resulting from its high toxicity. Migliore et al. (1996) reported a reduction of root, stalk, and leaf growth in several crops. Sartorius et al. (2009) also found the decreases in growth of plants due to the toxic alteration effects of sulfonamides on root morphology. However, plant growth depends on the antibiotic exposure level (Ferro et al., 2010; Michelini et al., 2012)

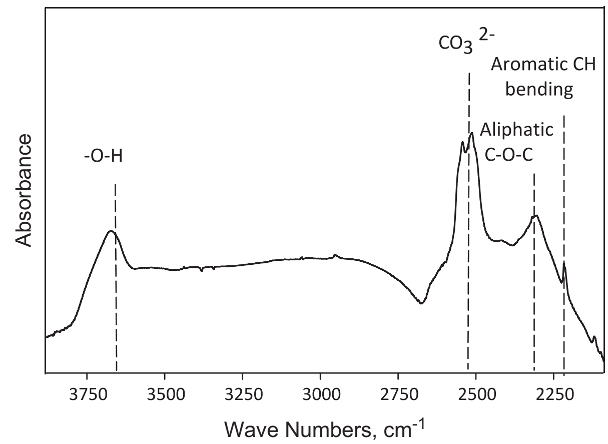

Fig. 1. Fourier transform infrared spectrum of the burcucumber biochar.

A slight increase of lettuce fresh weight was observed in the control and BBC700 treated soils whereas no change was found in the BBC700 treated/untreated soils with 5 mg kg^{-1} SMT contamination (Fig. 2a). A marked difference of fresh weight was determined for soils spiked with 50 mg kg^{-1} SMT whether they had been treated with BBC700 or not. The reduction of fresh weight in the soils spiked with SMT was estimated to be up to 90.05% (10.51–1.05 g) whereas this reduction was decreased by up to 80.05% with the application of BBC700 (10.55–2.10 g). It can be explained with SMT toxicity and the effectiveness of BBC700 against SMT. Our results are in agreement with previous studies showing the inhibitory effects of antibiotics on plant growth (Dolliver et al., 2007). Due to the hydrophilic nature of the SMT molecule (logorithmic octanol water partition coefficient, $\log K_{ow} = 0.27$), it is transferred between roots and foliage, and is delivered to the different plant compartments (Kumar et al., 2005; Dolliver et al., 2007).

A decrease of SMT uptake by lettuce after applying BBC700 is due to adsorption of SMT onto BBC700's surface and reduction of SMT bioavailability. The primary reason behind this retention might be due to electrostatic cation exchange and the π–π electron donor–acceptor interaction of the protonated aniline ring of the SMT molecule with the π-electron rich graphene surface of the BBC700 (Teixidó et al., 2011). Similarly, the diffusion of SMT into the pore spaces of the BBC700 may also be occurred (Teixidó et al., 2011). Our results clearly showed a decrease of SMT concentration in the lettuce grown in the soils treated with BBC700. There

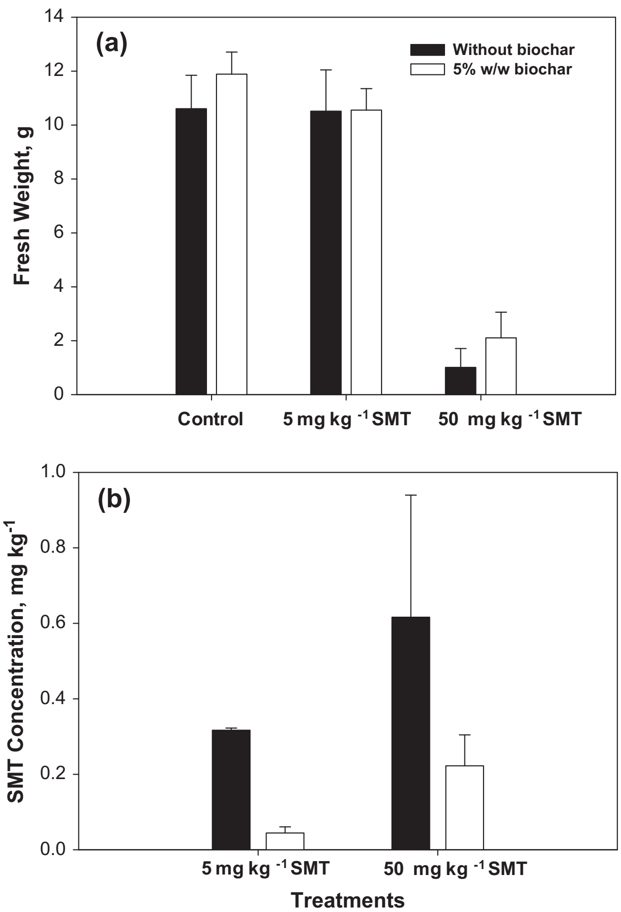

Fig. 2. (a) Fresh weight of lettuce samples under the different treatments. (b) The concentration of antibiotic recorded in the lettuce samples under the different treatments (SMT, sulfamethazine) based on dry weight.

is only limited understanding in the interaction between antibiotics and soil/plants, which may depend upon different factors such as antibiotic concentration in soil solution, soil physiochemical properties, chemical dynamics of antibiotics, and crop type or physiology (Dolliver et al., 2007). Further studies should be undertaken for a better understanding in the inhibition mechanism of biochars on antibiotics' uptake or release in consideration with environmental factors.

4. Conclusions

Biochar derived from burcucumber showed high aromatic and hydrophobic nature. A decrease of SMT uptake by lettuce was observed in the soils treated with biochar. Application of biochar enhances adsorption of SMT on its surface and leads a reduction of SMT bioavailability. This study suggests that biochar can be a practical strategy to control the antibiotics' uptake by plants. However, further studies are recommended to access the responses of plants to antibiotics in the presence of biochars.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.04.040.

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