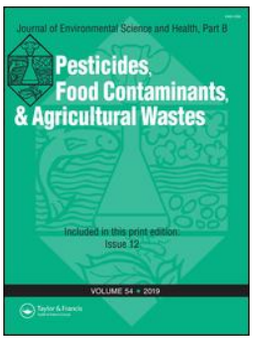

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Impact of biochar on plant growth and uptake of ciprofloxacin, triclocarban and triclosan from biosolids

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ABSTRACT

Application of municipal biosolids in agriculture present a concern with potential uptake and bioaccumulation of pharmaceutical compounds from biosolids into agronomic plants. We evaluated the efficacy of biochar as a soil amendment to minimize uptake of antimicrobial agents (ciprofloxacin, triclocarban, and triclosan) in lettuce (*Lactuca sativa*) and carrot (*Daucus carota*) plants. Biochar reduced the concentration of ciprofloxacin and triclocarban in lettuce leaves and resulted in a 67% reduction of triclosan in carrot roots. There was no substantial difference in pharmaceutical concentrations in carrot and lettuce plant matter at low (2.0 g kg^{-1} soil) and high (20.4 g kg^{-1} soil) rates of applied biochar. The co-amendment of biochar and biosolids increased soil pH and nutrient content which were positively correlated with an increase in lettuce shoot biomass. Our results demonstrate the potential efficacy of using walnut shell biochar as a sorbent for pharmaceutical contaminants in soil without negatively affecting plant growth.

Abbreviation: HPLC: high pressure liquid chromatography; LC/MS: Liquid Chromatography Mass Spectrometry

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KEYWORDS
Contaminant; antimicrobial; plant accumulation; translocation; soil amendment

Introduction

Antibiotics and other pharmaceuticals are used intensively in human medicine for the prevention and treatment of numerous diseases and ailments. Although prescription doses are based on amounts needed to treat specific medical conditions, a significant portion of the chemical is not metabolized by the body and ends up excreted in human waste, in some cases up to 90% of the administered dose.[1] Consequently, many pharmaceuticals have been detected in treated wastewater and biosolids from wastewater treatment facilities.[2–4] With increasing interest in the agricultural application of biosolids as a soil amendment, concerns have arisen regarding the potential environmental and ecological effects of high concentration biosolid-associated pharmaceuticals in both their original or partially degraded, yet still highly active, forms. Environmental exposure and contamination could adversely affect wildlife, disrupt processes of soil microbial communities, produce detrimental human health effects from long-term exposure to trace levels of pharmaceuticals, and cause the proliferation of antibiotic resistant bacteria.[5,6]

Biosolids use in agriculture has many benefits. Fertilization with biosolids can be an economically viable way to provide essential nutrients to crops, reducing fossil fuel based fertilizers, while adding value to a waste material. Demonstrated benefits to soils and crops include improvement of soil structure, water-holding capacity, nutrient holding capacity, aeration, carbon for microbial communities, and provision of nutrients for plant growth.[7] Concerns raised regarding the use of biosolids as a soil amendment include the presence of inorganic and organic contaminants and pathogens in addition to the attraction of disease vectors. While wastewater treatment facilities use various technologies to process biosolids, and United States (US) federal regulations require testing for specific metals, contaminants, and pathogens prior to agricultural use or disposal, there are no US regulations currently in place for acceptable pharmaceutical inclusion level thresholds.[8] Thus, the presence of high levels of pharmaceuticals could reduce the agricultural benefits associated with biosolids amendment.

To mitigate potential risks associated with pharmaceuticals in biosolids, it would be beneficial to co-amend biosolids with a highly sorbing material that would preferentially bind the pharmaceutical contaminants, decreasing their bioavailability to soil microbes and limiting subsurface leaching and groundwater exposure. Biochar, a co-product of biofuel production, has the potential to stabilize biosolid carbon (C), nitrogen (N), and phosphorus (P) in soil, increase soil fertility and crop yield, reduce greenhouse gas emissions, and attenuate agrochemicals.[9–11] Although biochars vary based on feedstock and production methods,[12] similarities in their chemical composition, high degree of aromaticity, and hydrophobic nature make many biochars favorable

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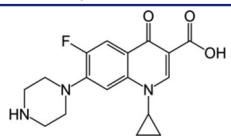
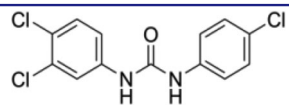
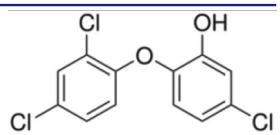
Table 1. Chemical structures and selected properties of antibiotics used in the study.[5,27,31,40]

Pharmaceutical	Ciprofloxacin	Triclocarban	Triclosan
Chemical Structure			
pKa	6.18, 8.76	12.7	7.90
Log K_{ow}	0.28	4.90	4.76

sorbents for a variety of natural and synthetic organic chemicals. The addition of biochar to soil has shown to increase the sorption of a range of heavy metals, pesticides, herbicides and pharmaceuticals.[13–15]

The feedstock used for biochar production has a substantial impact on the physiochemical properties of the biochar. Multiple studies have demonstrated that differences and similarities between biochars can be identified on the basis of knowledge of the feedstock from which it is made.[12,16] Biochars produced from woody biomass (trees, and other woody materials) tend to exhibit greater aromaticity, surface area and C/N ratio, with lower ash contents than biochars from non-woody biomass (agricultural crops and residues, animal waste, urban and industrial solid wastes, etc.).[12,17] Selection of feedstock/biochar can enhance the effectiveness of the intended purpose or function for biochar application.

In addition to mitigating the concentration of pharmaceuticals in the soil, the addition of biochar could reduce the potential for pharmaceutical bioaccumulation and toxicity in plants. Previous studies have shown uptake and accumulation of pharmaceutical compounds from soils into agronomic plants.[18–22] For example, chlortetracycline was detected in green onions, cabbage, and corn grown in spiked soils or in soils treated with spiked pig manure.[23] Triclosan, triclocarban, and carbamazepine were found in aboveground tissues, and diphenhydramine and fluoxetine in root tissues, in soybean plants grown in soils treated with reclaimed water and biosolids.[20] Pharmaceuticals can also affect plant growth and development. A significant decrease in plant development, root and leaf length, and number of leaves was demonstrated in lettuce, bean, cucumber and radishes grown in soil spiked with enrofloxacin (5 mg L^{-1}).[24] These and other studies have shown that the potential for and rate of plant uptake is affected by the concentration and chemical composition, primarily log K_{ow}, chemical charge, and half-life, of each compound.[21,25,26]

Ciprofloxacin, triclocarban, and triclosan have been classified as "contaminants of emerging concern" by the US Environmental Protection Agency (EPA), a designation for substances present in concentrations that are very low but with potentially detrimental ecotoxicological effects.[27] Ciprofloxacin, an antibiotic in the fluoroquinolone family, is used extensively for a wide range of infections of the respiratory, urinary and gastrointestinal tracts, as well as for skin and soft tissue infections.[28] The World Health Organization (WHO) has defined ciprofloxacin as a critically important antibacterial class for human medicine.[29] With an octanol-water partition coefficient (log K_{ow}) of 0.28, ciprofloxacin also demonstrates high sorption to soil.[30,31] Triclocarban and triclosan are bacteriostatic agents that are added to many commonly-used household cleaning products and personal care products such as bar soap, toothpaste, liquid soap, and cosmetics. Triclocarban and triclosan are hydrophobic, with log K_{ow} values of 4.9 and 4.8,[32] respectively, and therefore have a high tendency to sorb to organic matter and particles in sludge.[33–35] In a survey of 84 wastewater treatment facilities in the US, ciprofloxacin, and triclocarban were found in treated sewage sludge (biosolids) of all sites tested with maximum concentrations of 47.5 mg kg^{-1} and 441 mg kg^{-1}, respectively.[36] In the same study, triclosan was detected in 92.4% of sites tested with a maximum concentration of 133 mg kg^{-1}. The elevated concentrations and high levels of occurrence of these antimicrobials in treated biosolids warrant the investigation of mitigating their uptake into agronomic plants.

Our objectives were to examine the potential for biochar co-amendments added to biosolids-amended soil to minimize plant uptake of three antimicrobial agents (ciprofloxacin, triclocarban, and triclosan) commonly detected in municipal biosolids. We hypothesized that biochar would preferentially bind the pharmaceutical contaminants and reduce their bioavailability and uptake. Thus we, expected to observe lower concentrations of ciprofloxacin, triclocarban, and triclosan in plants grown in soil amended with biochar. Lettuce and carrot growth and vigor in response to biochar, biosolid and antimicrobial treatments to soil were also determined.

Materials and methods

Chemicals

Analytical grade ciprofloxacin, triclocarban, and triclosan were purchased from Sigma-Aldrich (St. Louis, Missouri (MO), USA). Selected chemical and physical properties of the pharmaceuticals are listed in Table 1. Enrofloxacin (Sigma-Aldrich) and labeled forms of triclocarban (TCC-d_7) and triclosan (TCC-$^{13}C_6$) (Cambridge Isotope Laboratories, Inc, Andover, Massachusetts (MA), USA) were used as recovery surrogates in the chemical analyses. Optima grade methanol, methyl-tert butyl ether (MTBE), and acetonitrile were purchased from Fisher Scientific (Waltham, MA, USA).

Soil, biosolids, and biochar

The surface horizon (0–15 cm) of a loamy sand soil was collected from the United States Department of Agriculture-

Table 2. Selected properties of the soil, biosolids, and biochar used in the study.

Property	Soil	Biosolids	Walnut shell biochar
pH (1:5H_2O)	(1:1) 5.32	7.70	9.86
Electrical conductivity (1:5H_2O), dS m^{-1}	(1:1) 0.58	3.44	31.8
Sand, %	82.5	–	–
Silt, %	1.5	–	–
Clay, %	6	–	–
Total nitrogen, %	0.06	1.01	0.47
NH_4-N, mg kg^{-1}	2.54	500.4	–
NO_3-N, mg kg^{-1}	0.48	6.84	–
Extractable phosphorus, mg kg^{-1}	7.9	176	–
Extractable potassium, mg kg^{-1}	66	612	–

Natural Resources Conservation Service (USDA-NRCS) Lockeford Plant Materials Center (Lockeford, California (CA), USA). The soil is classified as an Aquic Xerofluvent according to the USDA Soil Taxonomy Classification system.[37] The soil was air-dried and sieved to ensure uniformity (<2 mm). Biosolids produced using anaerobic digestion followed by extended storage in stabilization basins were provided by the Woodland Wastewater Treatment Plant (Woodland, CA, USA). The biosolids were sun-dried, mechanically ground and subsequently passed through a 2 mm sieve. Biochar was obtained from Dixon Ridge Farms (Winters, CA, USA). The biochar was made from walnut shells (*Juglans californica*) using a Biomax 50 downdraft gasifier at 900 °C. Previous experiments with walnut shell biochar have demonstrated its efficacy in binding a range of contaminants, including ciprofloxacin, triclocarban and triclosan.[13] Selected properties of the soil, biosolids, and biochar are given in Table 2. A full chemical and physical characterization of the walnut shell biochar can be found in Mukome et al.[12]

Experimental setup

The experiment was carried out at the Hoagland Hall Greenhouse Facility at the University of California, Davis. The greenhouse was maintained at 25/14 °C day/night temperature and 16-h photoperiod. A leaf crop (lettuce, *Lactuca sativa L.*) and root crop (carrot, *Daucus carota*) were planted from seed in one-gallon plastic pots. The treatments consisted of a factorial design of 3 levels of biochar (0, 2.0, 20.4 g kg^{-1} soil, equivalent to 10 and 100 t ha^{-1} soil to a depth of 12 in) combined with 2 biosolid rates (0, 63.1 g kg^{-1} soil). The application rate of biosolids was based on plant demand for nitrogen, calculated on total N content in biosolids.[38] The equation and explanation for determining the biosolids application rate can be found in the Appendix. The treatments were labeled as No BC + BS, Low BC + BS, High BC + BS, No BC -BS, Low BC -BS, High BC -BS, according to the presence and concentration of biochar and biosolids, and with a C or L before each treatment name designating carrot or lettuce crop. Each treatment contained 5 replicates. The soil treatments were premixed in five-gallon buckets.

The nonbiosolids-amended treatments received 44.6 mg N kg-1 soil in mineral fertilizer. Based on the 16-16-16 fertilizer (Lilly Miller Brands, Walnut Creek, CA, USA) used, 19.5 mg kg^{-1} phosphorus, 37.0 mg kg^{-1} potassium and 13.9 mg kg^{-1} sulfur were also applied. The pharmaceuticals were spiked into the air-dried biosolids in methanol (with 0.1% formic acid) at concentrations of 100 mg kg^{-1} ciprofloxacin, 500 mg kg^{-1} triclocarban and 200 mg kg^{-1} triclosan. The spike concentrations for ciprofloxacin, triclocarban, and triclosan are 52.5, 11.8 and 33.5% greater, respectively, than the maximum concentrations that were detected for these pharmaceuticals in a survey of contaminants in biosolids from wastewater treatment plants in the United States.[36] The methanol from the pharmaceutical spikes was allowed to evaporate before the biosolids were mixed into the soil treatments. Soil moisture content was maintained throughout the experiment at 60–70% water-holding capacity with deionized water. To prevent the potential loss of pharmaceuticals through leaching, saucer drip trays were placed under each pot to collect leachate. If any leaching occurred, all leachate was reapplied to the soil. Five seeds of lettuce or carrots were sown in each pot, and the germinated plants were later thinned to one plant per pot by selecting the largest seedling.

After nine weeks of growth, the above and belowground biomass of the lettuce and carrot plants were harvested, thoroughly rinsed twice with deionized water, and lyophilized. The aboveground biomass and below ground biomass are identified as shoots and roots, respectively. The dry matter yield was recorded and the dried samples were ground to powder using a coffee grinder with stainless steel blades and grinding chamber (KitchenAid, St. Joseph, Michigan (MI), USA) and subsequently stored at −80 °C until extraction.

Pharmaceutical analysis

The antibiotics were extracted from the plant material using methyl-tert butyl ether (MTBE) and acetonitrile following a multi-residue sonication extraction procedure.[39] Due to the small size of some of the plants, the ground samples of all five replicates from each treatment were combined and divided into three subsets for analysis. First, 0.2 grams of plant material was added into 50 mL Pyrex centrifuge tubes with Teflon-lined septum caps. Recovery surrogates were spiked (25 μL, 10 mg L^{-1} methanol) into the plant material followed by adding 20 mL of MTBE. The centrifuge tubes were vortexed for 30 sec at 2300 rpm and then ultrasonicated for 20 min using a Bransonic 12 ultrasonic cleaner (Branson Ultrasonic cleaner, Shelton, Connecticut (CT), USA). The tubes were then centrifuged for 20 min at 1000 RCF. The supernatant was decanted into 60 mL amber vials and the residue was extracted one more time with 20 mL acetonitrile. The combined extracts were evaporated just to dryness using a nitrogen evaporator (Meyer N-evap analytical evaporator, Organomation Association, South Berlin, MA, USA) at 40 °C. The dried extracts were reconstituted in 1 mL of 0.001% formic acid in methanol (v/v) and diluted with 20 mL ultrapure water. The aqueous sample solutions were loaded onto 3 mL solid phase extraction cartridges (Oasis HLB), set on a Supelco Visiprep vacuum manifold, which were preconditioned with 6 mL 0.001% formic acid in methanol followed by 6 mL of ultrapure water. The

cartridges were left to dry and subsequently eluted under gravity with 7 mL 0.001% formic acid in methanol (v/v). The eluates were dried under nitrogen, reconstituted in 0.5 mL 0.001% formic acid in methanol (v/v), and filtered through a PTFE 13 mm syringe filter (0.2 µm) (Fisherbrand, Fisher Scientific, Waltham, MA, USA). The filtered sample was injected into a HPLC MS/MS for detection. The spike recoveries for ciprofloxacin, triclocarban and triclosan were 72.4 (±11%, n = 3), 76.1 (±9%, n = 3) and 79.8 (±13%, n = 3), respectively.

Chemical analysis

High-pressure liquid chromatography tandem mass spectrometry (HPLC MS/MS) analysis was performed using an Agilent series 1200 HPLC and 6320 Ion Trap mass spectrometer detector (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was carried out on the reverse-phase Agilent Zorbax Eclipse Plus C18 (100 × 2.1 mm, 3.5 µm) analytical column, with a guard column with the same stationary phase (12.5 × 4.6 mm, 5 µm) (Agilent Technologies, Palo Alto, CA, USA). The column temperature was 40 °C and the autosampler temperature was 4 °C. The mobile phase consisted of 0.001% formic acid in water (v/v, solvent A), and 0.001% formic acid in methanol (v/v, solvent B). The flow rate was 0.5 mL min^{-1}. The following gradient program (with respect to mobile phase B) was used: 0–2 min 20%, 2–5 min 20–95%, 5–7 min held at 95%. Calibration curve standards (0.005–0.5) of the pharmaceuticals were freshly prepared in 0.001% formic acid in methanol (v/v) for each run. The MS used positive electro-spray ionization (ESI) MS/MS mode for ciprofloxacin, negative ESI MS/MS mode for triclocarban and negative ESI MS mode for triclosan. Nebulizer temperature was 350 °C, nebulizer pressure was 50 psi, and the drying gas flow rate was 10.0 L min^{-1}. Compounds were quantified using m/z 287 for triclosan, the transition m/z 332→ m/z 288 for ciprofloxacin and the transition m/z 313→ m/z 160 for triclocarban.

Soil analysis

Two grams of 2 mm-sieved air-dried soil were measured in 50 mL glass centrifuge tubes with Teflon-lined caps. The soil was extracted with 30 mL water in an ultrasonicating bath for 30 min and centrifuged in the same tubes at 1000 RCF for 20 min using a Beckman GS-6R centrifuge. The supernatant was filtered in Buchner funnels using Whatman GF/F, 70 mm, glass fiber filters into 55 mL borosilicate glass culture tubes (VWR, Radnor, Pennsylvania (PA), USA), and the filtrate was acidified to pH 2 using HCl (1 mol L^{-1}). The sample was concentrated using solid phase extraction (Waters Oasis HLB 6 mL cartridges) following the method protocol in Guidice and Young.[40] Each cartridge was conditioned with 5 mL 75:25 ethyl acetate:acetone. Eluates were evaporated to dryness in glass vials with nitrogen using a TurboVap at ambient temperature and a pressure of 10 psi.

Data analysis

Data analysis was performed using R (v. 3.2.1; R Core Team, 2014). Overall effect of treatment was obtained based on an F test ($\alpha = 0.05$) using Dunnet's test and applying the glht function from the multcomp package.[41] Each treatment was compared to the control, No BC -BS (no application of biochar or biosolids), with the exception of root, shoot and soil ciprofloxacin (CIP), triclocarban (TCC) and triclosan (TCS), for which the control is No BC + BS. Data analysis on the effect of each treatment containing biosolids and its composition (with or without biochar) was also conducted using Tukey's HSD test ($\alpha = 0.05$) to observe significant differences across biosolids containing treatments (glht function from the multcomp package[41]).

Results and discussion

Pharmaceuticals in soil and plant material

A water extraction method was used to measure plant-available pharmaceutical concentrations in soil at the end of the experiment. Ciprofloxacin and triclosan were not detectable in the soils in the biosolids treatments in both the lettuce

Table 3. Concentrations of triclocarban (TCC), ciprofloxacin (CIP) and triclosan (TCS) in biosolids-amended soil and lettuce roots and shoots with varying biochar treatments.[1]

	Treatments		
Item	L No BC + BS	L Low BC + BS	L High BC + BS
Root TCC (mg kg^{-1})	3.95 (0.192)	3.43 (0.044)**	2.10 (0.070)***
Shoot TCC (mg kg^{-1})	1.37 (0.119)	0.91 (0.071)**	0.76 (0.186)**
Soil TCC (mg kg^{-1})	0.80 (0.198)	0.96 (0.318)	0.68
Root CIP (mg kg^{-1})	0.77 (0.164)	0.75 (0.098)	0.79 (0.058)
Shoot CIP (mg kg^{-1})	0.75 (0.114)	0.54 (0.170)	0.19 (0.022)**
Soil CIP (mg kg^{-1})	<0.09	<0.09	<0.09
Root TCS (mg kg^{-1})	<0.04	<0.04	<0.04
Shoot TCS (mg kg^{-1})	<0.04	<0.04	<0.04
Soil TCS (mg kg^{-1})	<0.04	<0.04	<0.04

[1]Values in parenthesis represent the standard deviation of its respective treatment (n = 3 for root and shoot concentrations and n = 5 for soil concentrations of TCC, CIP and TCS). Statistical significance is based on comparisons of each group to the control (Dunnet's test): $^{\dagger}P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 4. Concentrations of triclocarban (TCC), ciprofloxacin (CIP) and triclosan (TCS) in biosolids-amended soil and carrot roots and shoots with varying biochar treatments.[1]

	Treatments		
Item	C No BC + BS	C Low BC + BS	C High BC + BS
Root TCC (mg kg^{-1})	2.79 (0.547)	3.01 (0.229)	2.29 (0.059)
Shoot TCC (mg kg^{-1})	0.951 (0.137)	0.716 (0.212)	0.437 (0.109)*
Soil TCC (mg kg^{-1})	1.04 (0.225)	0.77 (0.176)	0.73
Root CIP (mg kg^{-1})	0.163 (0.033)	0.137 (0.062)	0.115 (0.016)
Shoot CIP (mg kg^{-1})	0.570 (0.152)	0.295 (0.035)	0.444 (0.101)
Soil CIP (mg kg^{-1})	<0.09	<0.09	<0.09
Root TCS (mg kg^{-1})	2.07 (0.498)	0.690 (0.265)**	0.509 (0.400)**
Shoot TCS (mg kg^{-1})	8.63 (0.59)	14.70 (3.21)*	9.14 (1.22)
Soil TCS (mg kg^{-1})	<0.04	<0.04	<0.04

[1]Values in parenthesis represent the standard deviation of its respective treatment (n = 3 for root and shoot concentrations and n = 5 for soil concentrations of TCC, CIP and TCS). Statistical significance is based on comparisons of each group to the control (Dunnet's test): $^{\dagger}P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

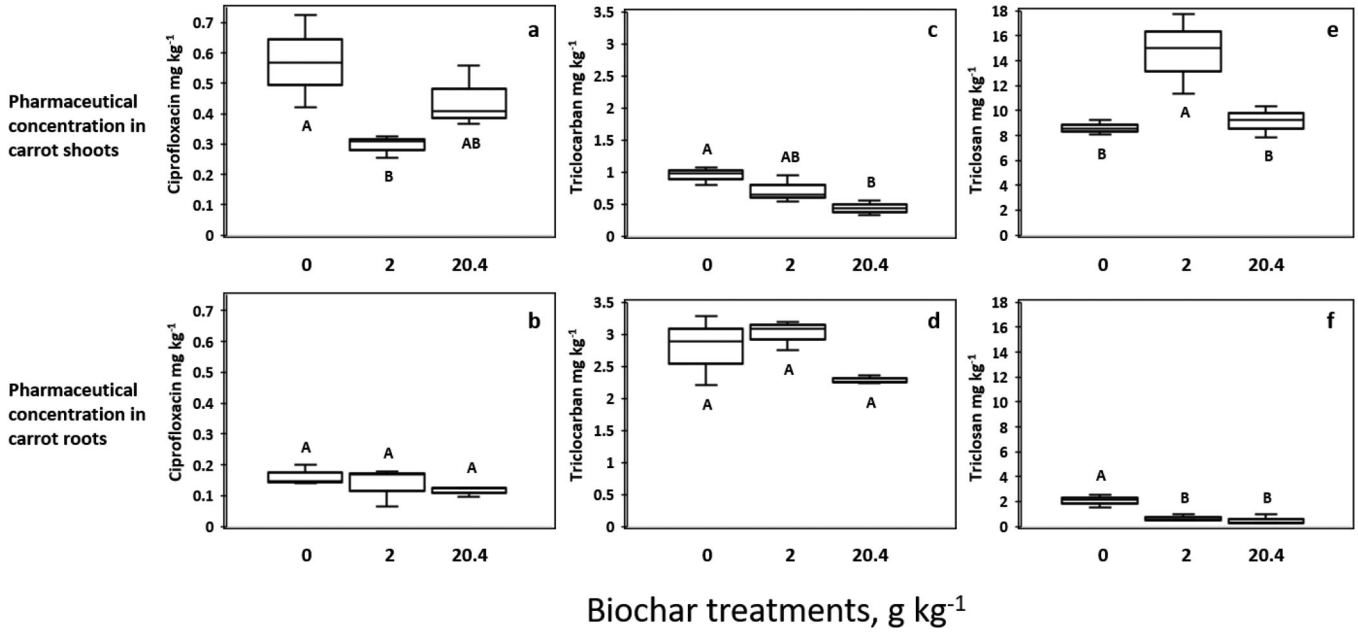


Figure 1. Uptake of ciprofloxacin, triclocarban, and triclosan into carrot shoots (top) and roots (bottom) after plants were grown from seed in pharmaceutically spiked biosolids amended to soil (63.1 g kg^{-1}). Average concentrations (dry weight) provided with error bars representing the standard error (n = 3). Differing letters designate significant differences between treatments, $P < 0.01$.

and carrot trials following water extraction (Tables 3 and 4). Triclocarban was detected in the soil at 1.04 mg kg^{-1} in the carrot biosolids only treatment (C No BC + BS) and at 0.77 and 0.73 mg kg^{-1} in the mixed treatments of low biochar/biosolids (C Low BC + BS) and high biochar/biosolids (C High BC + BS), respectively. Although there was a trend of decreasing triclocarban concentrations in soil with increasing walnut shell biochar content ($P < 0.1$) in the carrot treatments, neither concentration was significantly different from the biosolids only treatment (C No BC + BS) (Fig. 1c,d). The water-extracted pharmaceutical concentrations detected in the soil were low in comparison to the initial spiked concentrations, likely due to a combination of strong sorption to soil and biological degradation. Triclocarban and triclosan with a log K_{ow} of 4.90 and 4.76, respectively, have a strong affinity to soil. Although ciprofloxacin has a low K_{ow} (0.22) it has been shown to bind favorably to soils over a wide pH range due to its zwitterionic nature.[42] Wu et al. estimated the half-life of triclosan and triclocarban under aerobic soil conditions from 20 to 58 days for triclosan and from 87 to 231 days for triclocarban,[43] while 40–75% of ciprofloxacin degraded in a china cambisol within 40 days.[44]

Ciprofloxacin

The solvent extracted concentrations of ciprofloxacin in carrot roots ranged from 0.12 to 0.16 mg kg^{-1} dry mass (Table 4). No significant difference was observed among the biochar treatments. Ciprofloxacin concentrations in carrot shoots were more than two times the root concentrations, suggesting translocation of the compound within the carrot plant (Fig. 1a,b). Nonvolatile contaminants with low K_{ow} values have shown to accumulate in the leaves of plants. These contaminants, taken up by plants with soil pore water, are more easily translocated to the leaves, but accumulate in the leaves due to their low volatility as the plant transpires.[45] The concentration of ciprofloxacin in the carrot shoots of the C Low BC + BS treatment was significantly different ($P < 0.05$) than the biosolids only treatment (C No BC + BS). At the higher rate of application of biochar with biosolids (C High BC + BS) there was a reduction in ciprofloxacin concentration, although this difference was not significant.

Addition of biochar did not significantly change ciprofloxacin concentrations in lettuce roots (Fig. 2a,b). There was also no difference in the concentrations in the lettuce roots and shoots of the biosolids only treatment (L No BC + BS). The biochar treatments showed a decreasing trend in ciprofloxacin concentrations with increasing biochar application, but only the high biochar application (L High BC + BS) was significantly different ($P < 0.01$) from the biosolids only treatment (L No BC + BS) (Table 3). Biochar may only indirectly affect the movement of ciprofloxacin in lettuce given that the concentrations in the roots are similar, whereas the shoot concentrations decrease with increasing biochar content. The L High BC + BS treatment had a significant increase in shoot biomass, which may have contributed to an increase in the translocation of ciprofloxacin.

Triclocarban

There was no significant difference between the concentrations of triclocarban in carrot roots among biochar treatments with biosolids (Fig. 1c,d). The maximum concentration of triclocarban detected was 3.01 mg kg^{-1} dry mass (Table 4). Concentrations of triclocarban were greater in roots than shoots by a factor $\geq$ 2.9. A trend of decreasing triclocarban with increasing biochar content was seen in carrot shoots (Table 4; Fig. 1c,d). The higher rate of biochar application with biosolids (C High BC + BS) was significantly different

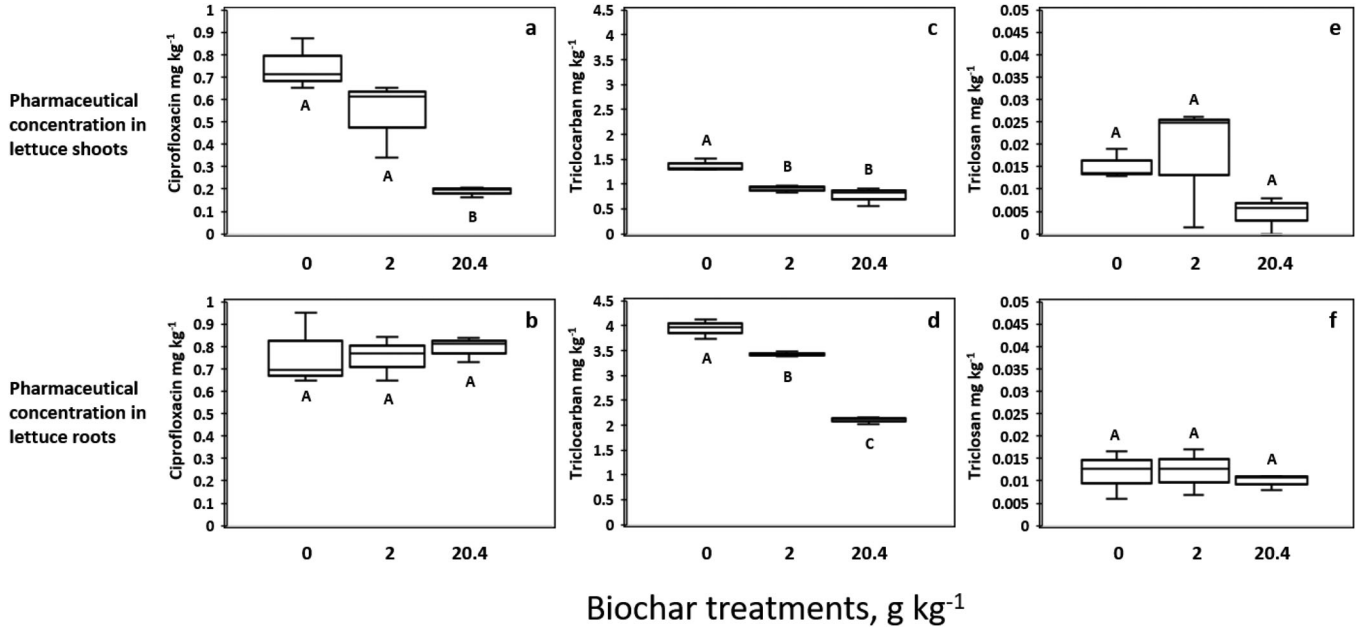


Figure 2. Uptake of ciprofloxacin, triclocarban, and triclosan into lettuce shoots (top) and roots (bottom) after plants were grown from seed in pharmaceutically spiked biosolids amended to soil (63.1 g kg^{-1}). Average concentrations (dry weight) provided with error bars representing the standard error (n = 3). Differing letters designate significant differences between treatments, $P < 0.01$.

($P < 0.05$) from the biosolids only treatment (C No BC + BS). Addition of biochar had a significant impact on uptake of triclocarban into lettuce as evidenced by decreasing concentrations of triclocarban in the roots and shoots with increasing biochar applications (Fig. 2c,d). The maximum concentration of triclocarban in lettuce roots was detected in the biosolids only treatment at 3.95 mg kg^{-1} (Table 3). The triclocarban concentrations decreased significantly with the Low BC + BS and the High BC + BS treatments with concentrations of 3.43 mg kg^{-1} ($P < 0.01$) and 2.10 mg kg^{-1} ($P < 0.001$). The concentrations of triclocarban were greater in the roots than in the shoots by a factor of ≥2.8. Concentrations in the shoots decreased from 1.37 mg kg^{-1} in the biosolids only treatment (No BC + BS) to 0.91 and 0.76 mg kg^{-1} in the 2.0 and 20.4 g kg^{-1} biochar treatments, respectively. Both biochar treatments were significantly ($P < 0.01$) different from the biosolids only treatment.

Triclocarban was found in higher concentrations in the roots than in the shoots in both lettuce and carrots. This follows contaminant uptake models of roots and root crops where contaminants with high log K_{ow} values are found in higher concentrations in the roots than in aboveground biomass.[45] The movement of triclocarban (K_{ow} 4.90) in the plant is limited due to its hydrophobicity.

Triclosan

The concentrations of triclosan in carrot roots ranged from 0.51 to 2.07 mg kg^{-1} (Table 4). The concentrations decreased significantly ($P < 0.01$) with biochar addition. At 2.0 g kg^{-1} soil, there was a 67% reduction of triclosan in the carrot root. Although there was a decreasing trend, there was no significant difference between the high (C High BC + BS) and low (C Low BC + BS) biochar applications (Fig. 1e,f). There were considerably higher concentrations of triclosan in the carrot shoots than the roots, by a factor ≥ 4.2. The higher concentrations of triclosan in the carrot shoots contrasted with results for triclocarban, where higher concentrations were found in roots. There was no significant difference between the C High BC + BS treatment and the biosolids only treatment (C No BC + BS). Triclosan concentrations in the C Low BC + BS treatment were significantly higher than the other treatments.

Prosser et al.[46] investigated the concentration of triclosan in tissues of radish, carrot and soybeans grown in potted soil amended with biosolids. Triclosan was found in greater concentrations in the leaves of radish plants than in the roots demonstrating the ability for plants to uptake and bioconcentrate triclosan in aboveground biomass. For carrots, the concentration of triclosan was greater in the shoots than the roots during the growing season. However, by the end of the growing period the shoots and roots had equal concentrations of triclosan.[46] The higher concentrations of triclosan in carrot shoots than roots and the contrasting results for triclocarban in this study can be the result of continual accumulation of triclosan in the carrot shoots throughout the growing season and the metabolism of triclosan in the carrot root. Macherius et al.[47] demonstrated the ability for carrot root cells to metabolize triclosan after uptake. They also indicated that in contrast to triclosan, triclocarban was not metabolized in the carrot root cells due to the lack of a phenolic group for direct conjugation reactions.

Effect of biochar

Concerns have been raised about the potential human exposure to pharmaceuticals due to agricultural application of biosolids as a soil amendment. Prior results from hydroponic and greenhouse studies concluded there is significant

Table 5. Effects of biochar and biosolids treatments on romaine lettuce biomass and number of leaves.[1]

	Treatments					
Item	L No BC -BS	L No BC + BS	L Low BC -BS	L Low BC + BS	L High BC -BS	L High BC + BS
Shoot biomass (g)	15.1 (0.339)	15.7† (0.949)	16.1** (0.331)	15.8* (0.252)	15.9* (0.384)	16.2*** (0.559)
Root biomass (g)	12.6 (0.233)	12.6 (0.068)	13.2* (0.108)	12.7 (0.239)	13.2† (0.225)	12.9** (0.232)
Number of leaves	15.6 (1.14)	21.0*** (2.00)	18.6† (1.67)	21.2*** (2.04)	18.6† (2.07)	21.6*** (2.07)

[1]Values in parenthesis represent the standard deviation of its respective treatment (n = 5). Statistical significance is based on comparisons of each group to the control: †$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

potential for uptake of pharmaceutical compounds into plants,[2,23,48] though other studies have demonstrated the potential for exposure would be very low.[46,49,50] Acceptable daily intake (ADI) values for ciprofloxacin, triclocarban, and triclosan are much greater than exposure estimates based on concentrations detected in the lettuce and carrot plant biomass in our study. For example, to exceed the ADI for triclosan, one would have to consume 22 kg of lettuce per day based on the concentrations found in the lettuce shoots. Although not directly toxic, these pharmaceuticals may present other concerns such as possible endocrine disruption, antibiotic resistance or adverse health effects from long-term low-dose exposure, and thus research on this topic must continue.[50,51]

The concentrations of the pharmaceuticals detected in plants grown in soils amended with biosolids, but lacking biochar, were consistent with previous studies on uptake of antimicrobials by agronomic plants.[46,48,49,52,53] The addition of biochar as a co-amendment reduced the concentration of triclocarban in both the roots and shoots of lettuce and ciprofloxacin in the lettuce shoots. Walnut shell biochar addition also reduced the concentration of triclosan in carrot roots as seen when comparing the low and high application rates of biochar; however, the relationship was not linear. The higher biochar application rate of 20.4 g kg^{-1} would not be expected to be economically feasible in a typical agricultural setting, but was selected to represent an upper bound for contaminant sorption and reduction in plant uptake. Surprisingly, though, there was little difference in the impact on lettuce of the high and low doses of biochar (Fig. 2). Therefore, the agronomically feasible application of 2.0 g kg^{-1} walnut shell biochar may be sufficient to mitigate the uptake of these pharmaceuticals in carrots and lettuce when biosolids are used as a soil amendment.

Plant growth

Lettuce and carrot biomass

Higher lettuce dry shoot biomass was observed in all biochar and biosolids treatments than in unamended soils, with significant differences among all biochar (with and without biosolids) treatments ($P \leq 0.05$) (Table 5). The addition of biochar to biosolids treatments did not increase the biomass of the lettuce and carrots compared to the biosolids only treatment. Lettuce root and shoot biomass was not significantly different between the biosolids only treatment (L No BC + BS) and the control but the total plant biomass was on average 0.6 grams higher ($P < 0.1$). An increase in lettuce root biomass, although not significant ($P < 0.1$), was measured in the L High BC -BS biochar treatment. There were no significant differences, however, in root or shoot carrot biomass between the biosolids and biochar treatments and the control, though a trend of increased carrot shoot biomass ($P < 0.1$) was evident in the combined biosolids and biochar applications (C Low BC + BS and C High BC + BS) and carrot root biomass in the C Low BC + BS treatment. Lettuce shoot biomass was significantly higher in all treatments indicating that biochar and biosolids enhanced lettuce leaf growth. Previous studies have shown that the physical and chemical properties of biochar and biosolids (e.g. higher porosity, surface area, charge density, cation exchange capacity, plant available nutrient content and pH) can improve soil fertility.[54–57] These beneficial properties could have increased the productivity of the nutrient-limited sandy soil used in our study. Carrot shoot biomass was not significantly different between the control and any of the treatments (Table 6), though this may have been a result of insufficient growth time or small pot size, which could have limited carrot growth across all treatments.

The addition of biochar has been shown to promote root growth in a variety of agronomic crops.[58,59] Prendergast-Miller et al.[59] demonstrated that roots in sandy soil will grow preferentially toward biochar fragments or particles due to greater nutrient and water availability in the biochar. This preferential growth likely extended the rhizosphere in the biochar amended soil, resulting in increased root biomass. The addition of biochar to soil in our study also demonstrated increased lettuce root biomass. The increased biomass we observed in the biochar treatments could have resulted from increased lettuce root growth in search of nutrients bound to biochar as biochars produced at higher temperatures (>600 °C) have been shown to sorb nutrients in soil solution.[60–62] This phenomenon was not observed in the carrot root biomass of the biochar only treatments.

Visual differences (plant vigor, color, brightness) in biosolids vs. non-biosolids treatments were evident (Fig. 3a–c). Measurement of crop nutritional value was not within the scope of our study; however, because these visual differences may reflect differences in plant nutrition, this is worthy of further research.

The biosolids treatments demonstrated a highly significant ($P < 0.001$) increase in the number of leaves in the lettuce plants, with an average of five more leaves per plant in biosolids treatments than in the control (Table 5). The biochar only treatment (L No BC + BS), although not significantly different from the control ($P < 0.1$), also showed an increase of three more leaves compared to the control. The leaf color of the biosolids treatments was considerably darker than in all other treatments. The plants of the

Table 6. Carrot root and shoot biomass with different application rates of biochar and biosolids to soil.[1]

	Treatments					
Item	C No BC -BS	C No BC + BS	C Low BC -BS	C Low BC + BS	C High BC -BS	C High BC + BS
Shoot biomass (g)	12.9 (0.421)	13.1 (0.302)	13.1 (0.213)	13.3† (0.202)	13.1 (0.195)	13.3† (0.220)
Root biomass (g)	12.4 (0.365)	12.8 (0.502)	12.7 (0.448)	13.2† (0.702)	12.8 (0.481)	12.9 (0.229)

[1]Values in parenthesis represent the standard deviation of its respective treatment (n = 5). Statistical significance is based on comparisons of each group to the control: $^{\dagger}P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Figure 3. Photographs of carrot and lettuce plants at time of harvest: a. carrot plants from C No BC + BS (left) and control (right) treatments, b. lettuce plants from C No BC + BS (left) and control (right) treatments, c. and d. lettuce plants from L Low BC + BS (left) and L Low BC -BS (right) treatments, e. lettuce showing leaf necrosis on lower leaves (L No BC + BS).

Table 7. Soil pH and electrical conductivity (EC) with different application rates of biochar and biosolids.[1]

	Treatments					
Item	No BC -BS	No BC + BS	Low BC -BS	Low BC + BS	High BC -BS	High BC + BS
Soil pH	5.32 (0.016)	7.29*** (0.037)	5.63*** (0.053)	7.30*** (0.026)	7.60 *** (0.113)	7.60*** (0.016)
Soil EC (dS m^{-1})	0.58 (0.051)	2.00*** (0.204)	0.54 (0.038)	1.93*** (0.141)	0.86* (0.136)	2.09*** (0.128)

[1]Values in parenthesis represent the standard deviation of its respective treatment (n = 4). Statistical significance is based on comparisons of each group to the control: $^{\dagger}P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

biochar only treatments, on the other hand, were visually chlorotic (Fig. 3d), though quantitative color measurements were not taken.

The significant increase in the number of lettuce leaves was not always consistent with an increase in leaf shoot biomass. This was especially apparent in the biosolids treatments where the average weight per leaf was 0.75 g $leaf^{-1}$ compared to the control of 1.03 g $leaf^{-1}$. Increased nitrogen fertilizer levels significantly affect numbers[63] and dry weight of leaves in romaine lettuce, though the increase was minimal during the first stages of growth or stem elongation.[64] The lower average weight per lettuce leaf in the biosolids treatments could be attributed to the lettuce plants not having reached full growth. Boroujerdnia and Ansari[63] harvested their lettuce plants after 156 days, in contrast to the harvest date of 90 days in our study. Also, although the non-biosolids treatments did receive an equivalent amount of total nitrogen as mineral fertilizer, the availability of the nitrogen likely differed between the two nutrient sources.

Soil pH

The addition of both biochar and biosolids significantly affected soil pH ($P < 0.001$) (Table 7). The low biochar treatment, Low BC -BS, slightly increased the pH by 0.3 units while the high biochar treatment increased the pH by more than two units. Biosolids alone also increased soil pH by about two units. The Low BC + BS treatment had a pH of 7.30 and was largely unaffected by the biochar. The mixture of soil, biosolids, and biochar appeared to buffer the pH at 7.6 as the presence of biosolids made little difference in the high biochar treatments.

Soil electrical conductivity

Soil electrical conductivity (EC) was significantly higher in all treatments compared to the soil control, except for the low application rate of biochar, Low BC -BS (Table 7). Treatments with biosolids had highly significant effects ($P < 0.001$), with the EC levels around 2 dS m^{-1} compared to the control, 0.58 dS m^{-1}. The elevated levels were likely responsible for the leaf burn (necrosis) that was visible on the edges of the older leaves of the lettuce plants in the biosolids treatments and especially noticeable in the biosolids only treatment (No BC + BS) (Fig. 3e). The EC levels of the Low BC -BS treatments were similar to the control, though the EC levels of the high application of biochar (High BC -BS) did increase significantly ($P < 0.05$). Application of biosolids was the greatest contributor to higher EC levels in the treatments and could be a cause for concern when used as a fertilizer. Careful consideration is needed in determining biosolids application rates that balance plant nutrient needs without excessively increasing the EC level of the soil. EC levels were only elevated with the high biochar application rate (High BC -BS); however, given that this application rate is not agronomically feasible there is little risk of this being a problem.

Conclusions

Both carrot and lettuce plants can uptake and translocate the antibiotic ciprofloxacin and the antimicrobial agents triclocarban and triclosan from biosolid amended soil. The addition of walnut shell biochar (900 °C) as a co-amendment reduced the concentration of triclocarban and ciprofloxacin in lettuce leaves and reduced uptake of triclosan in carrot roots slightly. There was no substantial difference in pharmaceutical concentrations in carrot and lettuce plant matter between the low and high application rates of biochar, therefore 2.0 g kg^{-1} biochar may be sufficient to reduce the uptake of these pharmaceuticals with the application of biosolids. The co-amendment of biochar and biosolids improved soil fertility by increasing soil pH and nutrient content, which were correlated with an increase in lettuce shoot biomass; however, the high EC resulting from biosolids application likely caused necrosis of the outer edges on the older leaves of the lettuce plants. Our results suggest that this walnut shell biochar has the potential to be used as a co-amendment to mitigate uptake of pharmaceutical contaminants from biosolids without negatively affecting plant growth.

Appendix

Determination of biosolids application rate

Biosolids were provided by the Woodland Wastewater Treatment Plant (Woodland, CA), and nutrient data were determined by the combustion method for total N (AOAC Official Method 972.43) and a KCl extraction for NO_3-N and NH_4-N[65] (Table 2). The application rate of biosolids used was calculated based on plant demand for nitrogen, at a rate of 200 kg N ha^{-1}. This was calculated by determining the plant available nitrogen (PAN) in the biosolids[38]:

$$\mathrm{PAN} = \mathrm{NO_3 - N} + \mathrm{K_{vol}(NH_4^{+} - N)} + \mathrm{K_{min}(Org - N)},$$

where PAN is lbs. of plant-available N per dry ton biosolids; NO3-N, NH4-N, and Org-N are pounds nitrate, ammonium, and organic nitrogen per dry ton biosolids, respectively; K_{vol} is the volatilization factor, or plant-available fraction of NH_4-N; and K_{min} is the mineralization factor, or

plant-available fraction of organic nitrogen. Using the N data from the biosolids (Table 2), and assuming K_{vol} = 100% and K_{min} = 0.42, the PAN was calculated to be 707 mg N kg^{-1} biosolids, which corresponded to an application rate of 63.1 g biosolids kg^{-1} soil.

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References

[1] Calamari, D.; Zuccato, E.; Castiglioni, S.; Bagnati, R.; Fanelli, R. Strategic Survey of Therapeutic Drugs in the Rivers Po and Lambro in Northern Italy. *Environ. Sci. Technol.* **2003**, *37*, 1241–1248. DOI: 10.1021/es020158e.

[2] Jones-Lepp, T. L.; Stevens, R. Pharmaceuticals and Personal Care Products in Biosolids—Sewage Sludge: The Interface between Analytical Chemistry and Regulation. *Anal. Bioanal. Chem.* **2007**, *38*, 1173–1183.

[3] US EPA. *Chapter 2: Land Application of Biosolids*; US EPA: Washington DC, **2008**.

[4] Castiglioni, S.; Bagnati, R.; Fanelli, R.; Pomati, F.; Calamari, D.; Zuccato, E. Removal of Pharmaceuticals in Sewage Treatment Plants in Italy. *Environ. Sci. Technol.* **2006**, *40*, 357–363. DOI: 10.1021/es050991m.

[5] Halling-Sørensen, B.; Nors Nielsen, S.; Lanzky, P. F.; Ingerslev, F.; Holten Lützhøft, H. C.; Jørgensen, S. E. Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment-A Review. *Chemosphere* **1998**, *36*, 357–393. DOI: 10.1016/s0045-6535(97)00354-8.

[6] Kümmerer, K. The Presence of Pharmaceuticals in the Environment Due to Human Use-Present Knowledge and Future Challenges. *J. Environ. Manage.* **2009**, *90*, 2354–2366. DOI: 10.1016/j.jenvman.2009.01.023.

[7] Verheihen, F. G. A.; Jeffery, S.; Bastos, A. C.; Van Der Velde, M.; Diafas, I. Biochar Application to Soil: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN; Office for the Official Publications of the European Communities: Luxembourg, **2009**, 149pp.

[8] Shammas, N. K.; Wang, L. K. Land Application of Biosolids. In *Handbook of Environmental Engineering*; Lawrence K. Wang, Nazih K. Shammas, Yung-Tse Hung Eds.; Humana Press: Totowa, New Jersey, **2007**; Vol. 6, pp 705–745.

[9] Lehmann, J. Bio-Energy in the Black. *Front. Ecol. Environ.* **2007**, *5*, 381–387. DOI: 10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2.

[10] Rondon, M.; Lehmann, J.; Ramírez, J.; Hurtado, M. Biological Nitrogen Fixation by Common Beans (Phaseolus vulgaris L.) Increases with Bio-Char Additions. *Biol. Fertil. Soils* **2007**, *43*, 699–708. DOI: 10.1007/s00374-006-0152-z.

[11] Sombroek, W. G.; Nachtergaele, F. O.; Hebel, A. Amounts, Dynamics and Sequestering of Carbon in Tropical and Subtropical Soils. *Ambio* **1993**, *22*, 417–426.

[12] Mukome, F. N. D.; Zhang, X.; Silva, L. C. R.; Six, J.; Parikh, S. J. Use of Chemical and Physical Characteristics to Investigate Trends in Biochar Feedstocks. *J. Agric. Food Chem.* **2013**, *61*, 2196–2204. DOI: 10.1021/jf3049142.

[13] Bair, D. A.; Mukome, F. N. D.; Popova, I. E.; Ogunyoku, T. A.; Jefferson, A.; Wang, D.; Hafner, S. C.; Young, T. M.; Parikh, S. J. Sorption of Pharmaceuticals, Heavy Metals, and Herbicides to Biochar in the Presence of Biosolids. *J. Environ. Qual.* **2016**, *45*, 1998–2006. DOI: 10.2134/jeq2016.03.0106.

[14] Uchimiya, M.; Lima, I. M.; Thomas Klasson, K.; Chang, S.; Wartelle, L. H.; Rodgers, J. E. Immobilization of Heavy Metal Ions (CuII, CdII, NiII, and PbII) by Broiler Litter-Derived Biochars in Water and Soil. *J. Agric. Food Chem.* **2010**, *58*, 5538–5544. DOI: 10.1021/jf9044217.

[15] Wang, D.; Mukome, F. N. D.; Yan, D.; Wang, H.; Scow, K. M.; Parikh, S. J. Phenylurea Herbicide Sorption to Biochars and Agricultural Soil. *J. Environ. Sci. Health B* **2015**, *50*, 544–551. DOI: 10.1080/03601234.2015.1028830.

[16] Hassan, M.; Liu, Y.; Naidu, R.; Parikh, S. J.; Du, J.; Qi, F.; Willet, I. R. Influences of Feedstock Sources and Pyrolysis Temperature on the Properties of Biochar and Functionality as Adsorbents: A Meta-Analysis. *Sci. Tot. Environ.* **2020**, *744*, 140714. DOI: 10.1016/j.scitotenv.2020.140714.

[17] Jafri, N.; Wong, W. Y.; Doshi, V.; Yoon, L. W.; Cheah, K. H. A Review on Production and Characterization of Biochars for Application in Direct Carbon Fuel Cells. *Process Saf. Environ. Prot.* **2018**, *118*, 152–166. DOI: 10.1016/j.psep.2018.06.036.

[18] Herklotz, P. A.; Gurung, P.; Vanden Heuvel, B.; Kinney, C. A. Uptake of Human Pharmaceuticals by Plants Grown under Hydroponic Conditions. *Chemosphere* **2010**, *78*, 1416–1421. DOI: 10.1016/j.chemosphere.2009.12.048.

[19] Lillenberg, M.; Litvin, S. V.; Nei, L.; Roasto, M.; Sepp, K. Enrofloxacin and Ciprofloxacin Uptake by Plants from Soil. *Agron. Res.* **2010**, *8*, 807–814.

[20] Christou, A.; Karaolia, P.; Hapeshi, E.; Michael, C.; Fatta-Kassinos, D. Long-Term Wastewater Irrigation of Vegetables in Real Agricultural Systems: Concentration of Pharmaceuticals in Soil, Uptake and Bioaccumulation in Tomato Fruits and Human Health Risk Assessment. *Water Res.* **2017**, *109*, 24–34. DOI: 10.1016/j.watres.2016.11.033.

[21] Colon, B.; Toor, G. S. A Review of Uptake and Translocation of Pharmaceuticals and Personal Care Products by Food Crops Irrigated with Treated Wastewater. In *Advances in Agronomy*; Sparks, D. L., Ed.; Academic Press: Oxford UK, **2016**; Vol. 140, pp 75–100.

[22] Wu, C.; Spongberg, A. L.; Witter, J. D.; Fang, M.; Czajkowski, K. P. Uptake of Pharmaceutical and Personal Care Products by Soybean Plants from Soils Applied with Biosolids and Irrigated with Contaminated Water. *Environ. Sci. Technol.* **2010**, *44*, 6157–6161. DOI: 10.1021/es1011115.

[23] Kumar, K.; Gupta, S. C.; Baidoo, S. K.; Chander, Y.; Rosen, C. J. Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure. *J. Environ. Qual.* **2005**, *34*, 2082–2085. DOI: 10.2134/jeq2005.0026.

[24] Migliore, L.; Cozzolino, S.; Fiori, M. Phytotoxicity to and Uptake of Enrofloxacin in Crop Plants. *Chemosphere* **2003**, *52*, 1233–1244. DOI: 10.1016/S0045-6535(03)00272-8.

[25] Mathews, S.; Henderson, S.; Reinhold, D. Uptake and Accumulation of Antimicrobials, Triclocarban and Triclosan, by Food Crops in a Hydroponic System. *Environ. Sci. Pollut. Res.* **2014**, *21*, 6025–6033. DOI: 10.1007/s11356-013-2474-3.

[26] Ben Mordechay, E.; Tarchitzky, J.; Chen, Y.; Shenker, M.; Chefetz, B. Composted Biosolids and Treated Wastewater as Sources of Pharmaceuticals and Personal Care Products for Plant Uptake: A Case Study with Carbamazepine. *Environ. Pollut.* **2018**, *232*, 164–172. DOI: 10.1016/j.envpol.2017.09.029.

[27] Lozano, N.; Rice, C. P.; Ramirez, M.; Torrents, A. Fate of Triclosan in Agricultural Soils after Biosolid Applications. *Chemosphere* **2010**, *78*, 760–766. DOI: 10.1016/j.chemosphere.2009.10.043.

[28] Hooper, D. C. Expanding Uses of Fluoroquinolones: opportunities and Challenges. *Ann. Intern. Med.* **1998**, *129*, 908–910. DOI: 10.7326/0003-4819-129-11_Part_1-199812010-00015.

[29] Angulo, F. J.; Collignon, P.; Powers, J. H.; Chiller, T. M.; Aidara-Kane, A.; Aarestrup, F. M. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies for the Use of Antimicrobials in Food Production Animals. *Clin. Infect. Dis.* **2009**, *49*, 132–141. DOI: 10.1086/599374.

[30] Wu, C.; Spongberg, A. L.; Witter, J. D. Sorption and Biodegradation of Selected Antibiotics in Biosolids. *J. Environ. Sci. Health A* **2009**, *44*, 454–461. DOI: 10.1080/10934520902719779.

[31] Uslu, M.; Yediler, A.; Balcıoğlu, I.; Schulte-Hostede, S. Analysis and Sorption Behavior of Fluoroquinolones in Solid Matrices. *Water. Air. Soil Pollut.* **2008**, *190*, 55–63. DOI: 10.1007/s11270-007-9580-0.

[32] Halden, R. U.; Paull, D. H. Co-Occurrence of Triclocarban and Triclosan in U.S. water Resources. *Environ. Sci. Technol.* **2005**, *39*, 1420–1426. DOI: 10.1021/es049071e.

[33] Singer, H.; Müller, S.; Tixier, C.; Pillonel, L. Triclosan: Occurrence and Fate of a Widely Used Biocide in the Aquatic Environment: Field Measurements in Wastewater Treatment Plants, Surface Waters, and Lake Sediments. *Environ. Sci. Technol.* **2002**, *36*, 4998–5004. DOI: 10.1021/es025750i.

[34] Heidler, J.; Halden, R. U. Mass Balance Assessment of Triclosan Removal during Conventional Sewage Treatment. *Chemosphere* **2007**, *66*, 362–369. DOI: 10.1016/j.chemosphere.2006.04.066.

[35] Heidler, J.; Sapkota, A.; Halden, R. U. Partitioning, Persistence, and Accumulation in Digested Sludge of the Topical Antiseptic Triclocarban during Wastewater Treatment. *Environ. Sci. Technol.* **2006**, *40*, 3634–3639. DOI: 10.1021/es052245n.

[36] US EPA. Targeted National Sewage Sludge Survey, Statistical Analysis Report. **2009**. U.S. Environmental Protection Agency, Office of Water: Washington DC, Report #: EPA-822-R-08-018.

[37] Soil Survey Staff. *Keys to Soil Taxonomy*, 12 th ed.; USDA-Natural Resources Conservation Service: Washington DC, **2014**.

[38] Haering, K. C.; Evanylo, G. K.; Abaye, A. *The Mid-Atlantic Nutrient Management Handbook*. Mid-Atlantic regional Water Program; **2006**.

[39] Wu, X.; Conkle, J. L.; Gan, J. Multi-Residue Determination of Pharmaceutical and Personal Care Products in Vegetables. *J. Chromatogr. A* **2012**, *1254*, 78–86. DOI: 10.1016/j.chroma.2012.07.041.

[40] Giudice, B. D.; Young, T. M. Mobilization of Endocrine-Disrupting Chemicals and Estrogenic Activity in Simulated Rainfall Runoff from Land-Applied Biosolids. *Environ. Toxicol. Chem.* **2011**, *30*, 2220–2228. DOI: 10.1002/etc.631.

[41] Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous Inference in General Parametric Models. *Biom. J.* **2008**, *50*, 346–363. DOI: 10.1002/bimj.200810425.

[42] Vasudevan, D.; Bruland, G. L.; Torrance, B. S.; Upchurch, V. G.; MacKay, A. A. pH-Dependent Ciprofloxacin Sorption to Soils: Interaction Mechanisms and Soil Factors Influencing Sorption. *Geoderma* **2009**, *151*, 68–76. DOI: 10.1016/j.geoderma.2009.03.007.

[43] Wu, C.; Spongberg, A.; Witter, J. Adsorption and Degradation of Triclosan and Triclocarban in Soils and Biosolids-Amended Soils. *J. Agric. Food Chem.* **2009**, *57*, 4900–4905. DOI: 10.1021/jf900376c.

[44] Cui, H.; Wang, S.-P.; Fu, J.; Zhou, Z.-Q.; Zhang, N.; Guo, L. Influence of Ciprofloxacin on Microbial Community Structure and Function in Soils. *Biol. Fertil. Soils* **2014**, *50*, 939–947. DOI: 10.1007/s00374-014-0914-y.

[45] Trapp, S.; Legind, C. N. Uptake of Organic Contaminants from Soil into Vegetables and Fruits. In *Dealing with Contamintad Sites*; Swartjes F. A. Ed.; Springer: Dordrecht, **2011**; pp 369–408.

[46] Prosser, R. S.; Lissemore, L.; Topp, E.; Sibley, P. K. Bioaccumulation of Triclosan and Triclocarban in Plants Grown in Soils Amended with Municipal Dewatered Biosolids. *Environ. Toxicol. Chem.* **2014**, *33*, 975–984. DOI: 10.1002/etc.2505.

[47] Macherius, A.; Eggen, T.; Lorenz, W.; Moeder, M.; Ondruschka, J.; Reemtsma, T. Metabolization of the Bacteriostatic Agent Triclosan in Edible Plants and Its Consequences for Plant Uptake Assessment. *Environ. Sci. Technol.* **2012**, *46*, 10797–10804. DOI: 10.1021/es3028378.

[48] Wu, C.; Spongberg, A.; Witter, J.; Fang, M.; Czajkowski, K.; Ames, A. Dissipation and Leaching Potential of Selected Pharmaceutically Active Compounds in Soils Amended with Biosolids. *Arch. Environ. Contam. Toxicol.* **2010**, *59*, 343–351. DOI: 10.1007/s00244-010-9500-y.

[49] Sabourin, L.; Duenk, P.; Bonte-Gelok, S.; Payne, M.; Lapen, D. R.; Topp, E. Uptake of Pharmaceuticals, Hormones and Parabens into Vegetables Grown in Soil Fertilized with Municipal Biosolids. *Sci. Total Environ.* **2012**, *431*, 233–236. DOI: 10.1016/j.scitotenv.2012.05.017.

[50] Boxall, A. B. A.; Johnson, P.; Smith, E. J.; Sinclair, C. J.; Stutt, E.; Levy, L. S. Uptake of Veterinary Medicines from Soils into Plants. *J. Agric. Food Chem.* **2006**, *54*, 2288–2297. DOI: 10.1021/jf053041t.

[51] Jjemba, P. K. The Potential Impact of Veterinary and Human Therapeutic Agents in Manure and Biosolids on Plants Grown on Arable Land: A Review. *Agr. Ecosys. Environ.* **2002**, *93*, 267–278. DOI: 10.1016/S0167-8809(01)00350-4.

[52] Holling, C. S.; Bailey, J. L.; Vanden Heuvel, B.; Kinney, C. A. Uptake of Human Pharmaceuticals and Personal Care Products by Cabbage (Brassica Campestris) from Fortified and Biosolids-Amended Soils. *J. Environ. Monit.* **2012**, *14*, 3029–3036. DOI: 10.1039/c2em30456b.

[53] Carter, L. J.; Harris, E.; Williams, M.; Ryan, J. J.; Kookana, R. S.; Boxall, A. B. A. Fate and Uptake of Pharmaceuticals in Soil–Plant Systems. *J. Agric. Food Chem.* **2014**, *62*, 816–825. DOI: 10.1021/jf404282y.

[54] Glaser, B.; Lehmann, J.; Zech, W. Ameliorating Physical and Chemical Properties of Highly Weathered Soils in the Tropics with Charcoal – A Review. *Biol. Fertil. Soils* **2002**, *35*, 219–230. DOI: 10.1007/s00374-002-0466-4.

[55] Dharmakeerthi, R. S.; Chandrasiri, J. A. S.; Edirimanne, V. U. Effect of Rubber Wood Biochar on Nutrition and Growth of Nursery Plants of Hevea brasiliensis Established in an Ultisol. *SpringerPlus* **2012**, *1*, 84–84. DOI: 10.1186/2193-1801-1-84.

[56] Speir, T. W.; Horswell, J.; van Schaik, A. P.; McLaren, R. G.; Fietje, G. Composted Biosolids Enhance Fertility of a Sandy Loam Soil under Dairy Pasture. *Biol. Fertil. Soils* **2004**, *40*, 349–358. DOI: 10.1007/s00374-004-0787-6.

[57] Diacono, M.; Montemurro, F. Long-Term Effects of Organic Amendments on Soil Fertility. A Review. *Agron. Sustain. Dev.* **2010**, *30*, 401–422. DOI: 10.1051/agro/2009040.

[58] Brennan, A.; Jiménez, E.; Puschenreiter, M.; Alburquerque, J.; Switzer, C. Effects of Biochar Amendment on Root Traits and Contaminant Availability of Maize Plants in a Copper and Arsenic Impacted Soil. *Plant Soil* **2014**, *379*, 351–360. DOI: 10.1007/s11104-014-2074-0.

[59] Prendergast-Miller, M. T.; Duvall, M.; Sohi, S. P. Biochar–Root Interactions Are Mediated by Biochar Nutrient Content and

Impacts on Soil Nutrient Availability. Eur. J. Soil *Sci.* **2014**, *65*, 173–185. DOI: 10.1111/ejss.12079.

[60] Knowles, O. A.; Robinson, B. H.; Contangelo, A.; Clucas, L. Biochar for the Mitigation of Nitrate Leaching from Soil Amended with Biosolids. *Sci. Total Environ.* **2011**, *409*, 3206–3210. DOI: 10.1016/j.scitotenv.2011.05.011.

[61] Laird, D.; Fleming, P.; Wang, B.; Horton, R.; Karlen, D. Biochar Impact on Nutrient Leaching from a Midwestern Agricultural Soil. *Geoderma* **2010**, *158*, 436–442. DOI: 10.1016/j.geoderma.2010.05.012.

[62] Yao, Y.; Gao, B.; Zhang, M.; Inyang, M.; Zimmerman, A. R. Effect of Biochar Amendment on Sorption and Leaching of Nitrate, Ammonium, and Phosphate in a Sandy Soil. *Chemosphere* **2012**, *89*, 1467–1471. DOI: 10.1016/j.chemosphere.2012.06.002.

[63] Boroujerdnia, M.; Ansari, N. A. Effect of Different Levels of Nitrogen Fertilizer and Cultivars on Growth, Yield and Yield Components of Romaine Lettuce (lactuca sativa L.). Middle East. *Russ. J. Plant Sci. Biotechnol.* **2007**, *1*, 47–53.

[64] Tei, F.; Benincasa, P.; Guiducci, M. Effect of Nitrogen Availability on Growth and Nitrogen Uptake in Lettuce. *Acta Hortic.* **2000**, *533*, 385–392. DOI: 10.17660/ActaHortic.2000.533.47.

[65] Knepel, K. Determination of Nitrate in 2M KCl Soil Extracts by Flow Injection Analysis. *QuikChem Method* **2003**, *12*, 107–104.