JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Immobilization of Chlorobenzenes in Soil Using Wheat Straw Biochar

Yang Song,[†,‡] Fang Wang,[†,‡] Fredrick Orori Kengara,[†,§] Xinglun Yang,[†] Chenggang Gu,[†] and Xin Jiang*,[†]

[†]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, P. R. China

[§]Department of Chemistry, Maseno University, Maseno 40105, Kenya

Supporting Information

ABSTRACT: Biochar has shown great potential for immobilizing organic contaminants in soil. In this study, pentachlorobenzene (PeCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB), and 1,2,4-trichlorobenzene (1,2,4-TCB) artificially spiked soil was amended with wheat straw biochar at 0.1%, 0.5%, 1%, and 2% application rates, respectively. The sorption, dissipation, and bioavailability of chlorobenzenes (CBs) in soil were investigated. The sorption of PeCB by biochar was significantly higher than that of its sorption by both biochar-amended and unamended soil ($p < 0.05$). The dissipation and volatilization of CBs from biochar-amended soil significantly decreased relative to unamended soil ($p < 0.05$). Bioavailability of CBs, expressed as butanol extraction efficiency and earthworm (*Eisenia fetida*) bioaccumulation factor, significantly decreased with increasing aging time and biochar application rate. The effect of biochar content in soil on the bioavailability of CBs was more pronounced for 1,2,4-TCB relative to other CBs. This study suggested that wheat straw biochar, even at low application rates, could effectively immobilize the semivolatile CBs in soil and thus reduce their volatilization and bioavailability.

KEYWORDS: *biochar, bioavailability, butanol extraction, earthworm, POPs, volatilization*

INTRODUCTION

Biochar, a form of charred organic matter, is an increasingly utilized cost-effective soil amendment in agricultural and environmental applications.[1−3] Crop-residue-derived biochar, which has variously been described as a "soil conditioner", can sequester C, reduce the emission of greenhouse gases, and improve soil fertility and thus plant growth.[4−6] Besides these characteristics, biochar has a large surface area and high microporosity, which results in a very high affinity and capacity for sorbing and immobilizing organic contaminants.[7,8] Therefore, biochar lends itself as a good material for contaminant immobilization, a kind of soil remediation strategy.[8]

It is increasingly recognized that the bioavailable concentration, rather than total concentration, of contaminants in soil dominates their potential risks, degradation, uptake by biota, leaching, and other environmental fates.[9,10] Therefore, immobilization, which seeks to reduce the bioavailability and mobility of contaminants in soil by applying different organic/inorganic amendments, has become an increasingly popular in situ soil remediation strategy.[11−13] Meanwhile, a series of chemical extraction methods, such as mild solvent extraction and solid-phase extraction, have been developed to assess the bioavailability of organic contaminants in soil.[10,14,15] Application of crop straw or hardwood-derived biochar, especially the ones pyrolyzed at high temperature, to contaminated soil could reduce the bioavailability of organic contaminants to soil biota.[16,17] For example, plant uptake of chlorpyrifos decreased with increasing biochar addition in soil.[16] The microbial degradation of benzonitrile,[18,19] atrazine,[20] and simazine,[21] etc., decreased in biochar-amended soil. Reduced earthworm accumulations of polycyclic aromatic hydrocarbons (PAHs)[22] and atrazine[11] in biochar-amended soils have also been reported. Most of the above studies were based on immobilizing polar or less volatile organic contaminants with biochar.[16−21] However, reports on whether or not biochar could immobilize semivolatile or volatile compounds are limited.[23]

China is an important producer of chlorobenzenes (CBs) in the world and accounts for more than 50% of the worldwide production.[24] A lot of vegetable fields are close to the CB factories in the suburban areas of China. CBs can be released into air from factories and then enter into soil through dry/wet deposition and wastewater irrigation.[25] It has been reported that all the CB congeners, especially trichlorobenzenes (TCBs) and tetrachlorobenzenes (TeCB), have been detected in the soil of vegetable fields within 1 km from a CB factory.[25] Therefore, it is of great importance to immobilize the detected CBs in soil for safe vegetable production and to reduce their revolatilization from soil into air. Our previous study demonstrated that the bioavailability of hexachlorobenzene (HCB) was significantly decreased by wheat straw biochar addition into soil and established a mild solvent extraction method, butanol extraction, to assess the bioavailability of CBs.[26] However, among CBs congeners, the lower chlorinated CBs are more easily volatilized[27] and less persistent[28] than HCB. The objective of the present study was therefore to investigate whether wheat straw biochar could immobilize the semivolatile CBs in soil thereby reducing their volatilization losses and whether the immobilization efficiency of contaminants by biochar is dependent on the volatility of the chemicals. CBs of different volatilities, pentachlorobenzene (PeCB),

Received: January 28, 2013
Revised: April 6, 2013
Accepted: April 11, 2013
Published: April 11, 2013

1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB), and 1,2,4-trichlorobenzene (1,2,4-TCB), which are listed as priority semivolatile chemicals by US EPA, were selected as model compounds for the study, and the results were compared with existing data on HCB.[26] The immobilization effectiveness was evaluated by determining the residues, volatilization, butanol extraction, and accumulation of the CBs by earthworms. The changes in bioavailability of the CBs with aging time, as well as biochar application rate in soil, were fitted into a prediction model.

■ MATERIALS AND METHODS

Chemicals. The CB standards (>99.5% purity) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The physicochemical properties of HCB, PeCB, 1,2,4,5-TeCB, and 1,2,4-TCB are shown in Table 1.[28] The solvents and all other chemical reagents, purchased from Nanjing Chemical factory (Nanjing, China), were of analytical grade. Anhydrous sodium sulfate was oven-dried at 400 °C for 4 h.

Table 1. Physicochemical Properties of Chlorobenzenes Studied

	melting point (°C)	boiling point (°C)	vapor pressure at 25 °C (Pa)	S^a (mg L^{-1})	H^b (kPa·M^3 mol^{-1})	$K_{ow}{}^c$
1,2,4-TCB	−17.00	213.50	45.30	45.30	0.439	3.98
1,2,4,5-TeCB	139.50	243.60	0.72	2.16	0.261	4.51
PeCB	86.00	277.00	0.29	0.83	0.977	5.03
HCB	230.00	322.00	0.0023	0.02	0.005	6.18

[a]Aqueous solubility at 25 °C. [b]Henry's Law constant. [c]Log octanol/water partition coefficient.

Soil Sampling and Biochar Preparation. An agricultural soil collected from a vegetable field was used in this experiment. The soil was sampled from the upper 20 cm, passed through a 2-mm sieve for the incubation experiment, and air-dried and passed through a 0.15-mm sieve for the sorption experiment. The soil had a pH of 7.56 and a total carbon content of 3.10%, clay of 13.61%, silt of 63.11%, and sand of 23.28%. The wheat straw biochar was produced under anoxic conditions at 500 °C.[26] The biochar had a pH of 10.51, total carbon content of 48.53%, and specific surface area of 4.81 m^2 g^{-1}. Details of soil and biochar properties are available in an earlier publication.[26]

Sorption of PeCB by Soil, Biochar, and Biochar-Amended Soil. Due to the high volatility of 1,2,4,5-TeCB and 1,2,4-TCB, the sorption experiment was conducted with PeCB only. The PeCB solution, in acetone, was added to 10 mL of 0.005 M $CaCl_2$ solution in a 30 mL glass tube, giving initial concentrations ranging from 0.05 to 2 μg mL^{-1}. The acetone concentration was 0.1% by volume to minimize cosolvent effects.[29] A total amount of 50 mg of soil was then amended with 0%, 0.1%, 0.5%, 1%, and 2% each of biochar and added to the tubes. Five milligrams of biochar was also used as a sorbent. The use of these masses was to ensure that 60−80% of the added PeCB was sorbed by the sorbents according to a preliminary experiment. The tubes were immediately closed with Teflon-lined screw caps and rotated on an overhead shaker at 40 rpm for 72 h at 25 °C. After shaking, the suspensions were centrifuged at 4024*g* for 30 min. Then 5 mL supernatants were sampled and extracted twice with equal volumes of *n*-hexane on a vortex shaker for 2 min. The extracts were pooled together, dried with anhydrous sodium sulfate, and then concentrated to 2 mL for further gas chromatographic (GC) analysis. All the treatments were conducted in triplicate.

The sorption isotherms were fitted into the Freundlich model:[29]

$$Q_e = K_f C_e^n \quad (1)$$

where Q_e and C_e are the amounts of PeCB sorbed (μg g^{-1}) and the equilibrium solution concentration (μg mL^{-1}), respectively, n is an empirical exponent indicative of isotherm nonlinearity, and K_f is a Freundlich unit capacity coefficient $[(\mu g\ g^{-1})/(\mu g\ mL^{-1})^n]$.

The solid/water distribution coefficients (K_d, mL g^{-1}) at different concentrations were calculated as:

$$K_d = Q_e/C_e \quad (2)$$

Immobilization of CBs in Soil by Biochar. Artificially contaminated soil was used in this experiment. The mixed CB (PeCB, 1,2,4,5-TeCB, and 1,2,4-TCB) standards were dissolved in acetone and then applied to an aliquot of 15 g (dry weight) of soil in a 50-mL glass beaker. After evaporation of acetone, the 15 g of spiked soil was mixed and then transferred to a 1000-mL glass beaker which already contained 284.7 g (dry weight) of equilibrated soil (20% soil moisture content, 25 °C for 1 week) and 0.3 g of biochar (0.1% amendment) to give a total amount of 300 g (dry weight). This was mixed by stirring carefully and thoroughly with a spatula and transferred to a 1000-mL incubation flask, and the water content was adjusted to 28% soil moisture content. Then 2 g of the spiked soil was sampled to analyze the initial concentrations of CBs in the soil. The remaining soil was compacted to a volume equivalent to 1.3 g cm^{-3} of soil density. The flask was closed tightly with a rubber plug which contained inlet and outlet tubes, and incubated at 25 °C for 24 weeks in the dark. The set-ups for the 0.5%, 1%, and 2% biochar content were conducted in the same way as outlined above for the 0.1% treatment. The unamended treatment, without addition of biochar, was used as control. There were therefore a total of five treatments: 0% biochar, 0.1% biochar, 0.5% biochar, 1% biochar, and 2% biochar, all conducted in triplicates.

During the incubation period, the flasks were aerated once per week for 20 min at an exchange rate of 0.4 L min^{-1}, in a closed laboratory trapping system,[30] to flush out and trap the volatilized CBs. After aeration, 10 g of soil was sampled for CB residues and butanol extraction analysis. For the earthworm bioassay experiment, 25 g soil was sampled after 1 week, and again after 24 weeks, of incubation.

Residues and Volatilizations of CBs in Soil. The residues of CBs in soil were extracted by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) and were expressed as total concentrations of CBs.[31] Briefly, 2 g soil samples were homogenized with 5 g of diatomaceous earth and extracted with hexane/acetone (4:1, v/v) at a temperature of 100 °C and a pressure of 1500 psi. The extracts were rotary evaporated at 45 °C to about 2 mL and then applied to a silica gel/anhydrous sodium sulfate column, followed by elution with 15 mL of hexane/dichloromethane (9:1, v/v). Finally, the eluate was concentrated to 1 mL for subsequent GC analysis (details on GC detection conditions are available in a previous publication[31]).

At the various sampling points, the volatile fractions of CBs were trapped in two trapping bottles, each containing 15 mL of hexane.[30] The two hexane trapping solvents were then pooled together, dried with anhydrous sodium sulfate, and concentrated to 1 mL for subsequent GC analysis.

The dissipation of CBs in soil was fitted into a modified first-order kinetics equation:[26]

$$C = C_0[\lambda + (1 - \lambda)e^{-kt}] \quad (3)$$

where C (μg g^{-1}) and C_0 (μg g^{-1}) are the concentrations of CBs in soil at time t and time 0 (initial concentration), respectively, λ is the coefficient of nonbioavailable fraction of CBs in soil, k is the first-order rate constant (week^{-1}). The data on CBs in this study were compared with existing data on HCB.[26]

Butanol Extraction. To assess the immobilization effectiveness of biochar, butanol extraction[31] was used and expressed as bioavailable fractions. Generally, 2 g of soil was extracted with 15 mL of butanol in a glass centrifuge tube by shaking on an orbital shaker at 200 rpm for 2 h, followed by centrifugation at 1448*g* for 30 min. The butanol supernatants were discarded, and the extracted soil was washed with 10 mL of deionized water followed by exhaustive ASE extraction as described above. The CB concentrations in the butanol extract were calculated by subtracting the concentration in soil after butanol extraction from the total concentration in soil before extraction.

The regression of butanol extraction of CBs with aging time as well as biochar application rate was expressed using the model:

$$B = B_0 e^{-(\alpha t + \beta f)} \quad (4)$$

where B (%) is the butanol extraction efficiency for CBs, B_0 (%) is the butanol extraction at aging time (t) 0 and biochar application rate (f) 0, and α (week^{-1}) and β are constant coefficients. The data on CBs in this study were compared with existing data on HCB.[26]

Earthworm Uptake. The earthworm (*Eisenia fetida*) uptake of CBs in biochar-amended soil was conducted as a bioassay experiment. Briefly, 10 adult worms with a clitellum were exposed to 25 g (dry weight) of soils, adjusted to 30% soil moisture content with deionized water in a 100-mL glass jar, and covered with aluminum foil having several holes. The soils were kept under constant room light at 25 °C for 14 d. After exposure, the worms were rinsed and allowed to purge their gut contents for 48 h on moistened filter papers. The worms were weighed, freeze-dried, and ground with 7 times their weights of anhydrous sodium sulfate and equal weights of quartz sand, followed by ASE extraction and GC analysis using the method for soil described above.

The extent of earthworm accumulation of CBs was expressed using a bioaccumulation factor (BAF):[13]

$$\mathrm{BAF} = C_{\mathrm{worm}}/C_{\mathrm{soil}} \quad (5)$$

where C_{worm} (μg g^{-1}) and C_{soil} (μg g^{-1}) are the concentrations of CBs in earthworms (dry weight) and in soil (dry weight), respectively. The data on CBs in this study were compared with existing data on HCB.[26]

Quality Control and Data Analysis. To estimate the recoveries of PeCB in the sorption experiment, blank samples without sorbents were prepared and analyzed using the same procedure above. The average recovery of PeCB in blank samples was 76.08 ± 6.7%. To estimate the recoveries of CB residues in soil and in earthworms, a recovery study was carried out by spiking CBs (20 ng) to 10 g of soil or 2 g of earthworms. The extraction and purificiation of the samples were performed using the same procedure as described above. The average recoveries for three replicates were 82.46−88.06% in soil and 77.18−87.55% in earthworms. All statistical data analysis was performed with SPSS 17.0, and the significance level was $p < 0.05$.

■ RESULTS AND DISCUSSION

Sorption of PeCB by Soil, Biochar, and Biochar-Amended Soil. The Freundlich sorption isotherms and solid/water distribution coefficients of PeCB to soil, biochar, and biochar-amended soil are shown in Figure 1 and Table 2. On the basis of the K_f and K_d values, the sorption affinity of PeCB to biochar was significantly higher than that to soil ($p < 0.05$).

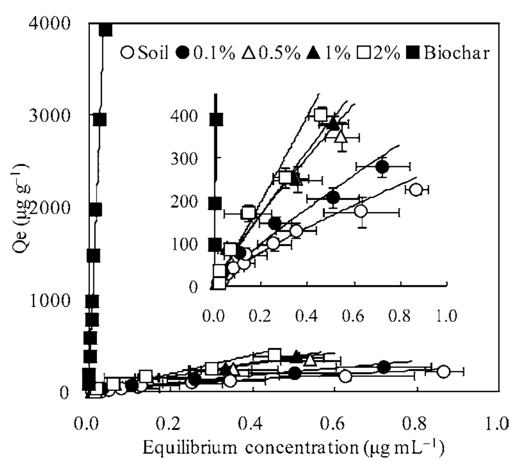

Figure 1. Sorption isotherms of pentachlorobenzene by soil and biochar (dots: measured data; curves: Freundlich model fitted).

Therefore, the amendment of biochar to soil resulted in higher sorption affinity of PeCB to biochar-amended soil than unamended soil, even with 0.1% biochar amendment. Moreover, with increasing biochar content, the coefficient n values increased progressively to 1, indicating a changed in sorption dynamics from nonlinear to linear. This linearization of the process could mean that biochar dominates the sorption of PeCB in soil at high amendment ratios. A linear isotherm has also been observed for sorption of HCB to biochar,[26] and this was ascribed to partitioning of CBs into the noncarbonized organic phase[32] and entering into the micropores of biochar.[29] Therefore, PeCB, and by extension other lower chlorinated CBs which are more volatile and smaller, could more easily enter into the biochar micropores relative to HCB. Therefore, it may be inferred that the effect of biochar on the sorption of CBs to biochar-amended soil is more significant for the lower chlorinated and hence more volatile CBs.

Immobilization of CBs in Biochar-Amended Soil. To check whether biochar could immobilize semivolatile CBs in soil, the changes in residues and volatilization of CBs over time were monitored. As shown in Figure 2, the dissipations of PeCB, 1,2,4,5-TeCB, and 1,2,4-TCB in the biochar-amended treatments were lower relative to the 0% treatment, throughout the whole incubation period, which could be confirmed by the dissipation rate k values (Table S1, Supporting Information). The k values in biochar-amended treatments were significantly lower than in the unamended soil for different CBs ($p < 0.05$). The more biochar was added to soil, the more the residues of CBs were formed. Owing to its higher volatility, higher initial concentration of 1,2,4-TCB - relative to PeCB and 1,2,4,5-TeCB - was used in this study. Nevertheless, 2% biochar addition effectively immobilized this high 1,2,4-TCB concentration, resulting in 75.32% of 1,2,4-TCB residues in soil after 24 weeks of incubation (Table S1, Supporting Information).

The lower the chlorination level of CBs, the more hydrophilic and degradable they are in soil under aerobic conditions.[33] In this study, the percentage of PeCB residues was significantly higher than that of 1,2,4,5-TeCB and 1,2,4-TCB, regardless of treatment (Table S1, Supporting Information). The relative concentrations of CB residues in biochar-amended treatments, compared to the 0% treatment, after 24 weeks of incubation are shown in Figure 4a. The relative concentrations of the four compounds were similar in the 0.1% treatment, increased slightly for HCB and PeCB with increasing biochar content (HCB data source from previous research[26]), but increased greatly for 1,2,4,5-TeCB and 1,2,4-TCB. At biochar content of 2%, the relative concentration was highest for 1,2,4-TCB, followed by 1,2,4,5-TeCB, PeCB, and HCB. This shows that with increasing biochar application rate, the immobilization of CBs becomes more apparent, especially for the lower chlorinated (hence volatile) 1,2,4-TCB. This is not surprising because the higher chlorinated CBs such as HCB are less volatile and therefore their immobilization by biochar would not have such a pronounced effect. It can therefore be inferred that the biochar immobilization effect increased with decreasing chlorination of CBs.

The lower the chlorination of CBs, the faster the volatilization from soil occurs.[28] As shown in Figure 3, the volatilization ratio of CBs was in the order of 1,2,4-TCB > 1,2,4,5-TeCB > PeCB, regardless of treatment. Significantly lower volatilizations of CBs were detected in biochar-amended treatments, indicating that sorption reduced the volatilization. Decreased volatilization of CBs from soil results in lower

Table 2. Freundlich Sorption Parameters and Solid/Water Distribution Coefficient for PeCB

	Freundlich constants			log K_d			
	log K_f (μg g^{-1})/(μg mL^{-1})^n	n	R^2	0.05 μg mL^{-1}	0.5 μg mL^{-1}	1 μg mL^{-1}	2 μg mL^{-1}
soil (0%)	2.45 ± 0.05	0.79 ± 0.04	0.98	2.87 (4.55)[a]	2.76 (4.44)	2.58 (4.26)	2.42 (4.10)
0.1%	2.65 ± 0.12	0.90 ± 0.11	0.94	2.92	2.86	2.76	2.59
0.5%	2.86 ± 0.10	0.87 ± 0.08	0.96	2.93	3.12	3.07	2.81
1%	2.95 ± 0.15	0.96 ± 0.11	0.94	2.93	3.12	3.09	2.87
2%	3.01 ± 0.25	1.01 ± 0.20	0.86	3.08	3.14	3.08	2.94
biochar	5.12 ± 0.09	1.07 ± 0.04	0.99	4.94	5.03	5.06	4.95

[a]Log organic carbon normalized sorption constant (K_{oc}).

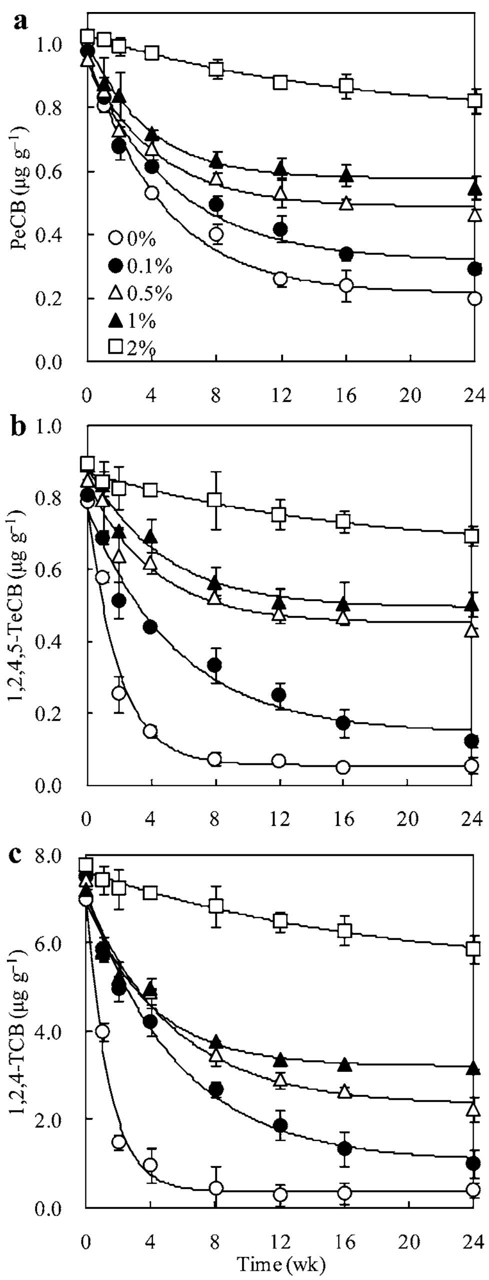

Figure 2. Time course of chlorobenzenes residues in soils amended with different percentages of biochar (a: PeCB; b: 1,2,4,5-TeCB; c: 1,2,4-TCB, dots: measured data; curves: model fitted).

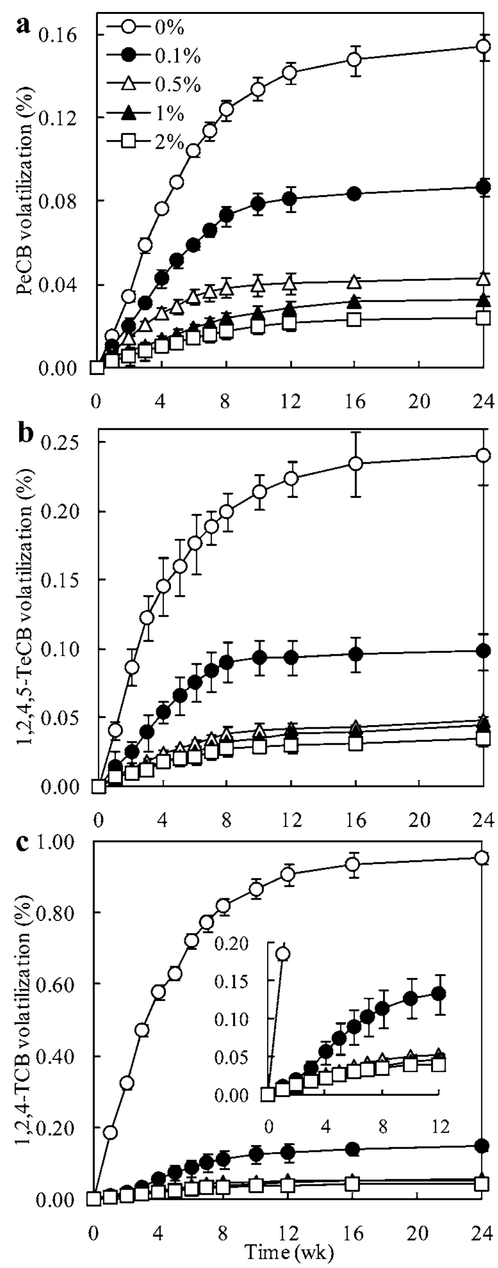

Figure 3. Volatilization of chlorobenzenes from soils amended with different percentages of biochar (a: PeCB; b: 1,2,4,5-TeCB; c: 1,2,4-TCB).

concentrations of CBs in air, and therefore the uptake of CBs by leafy vegetables could be reduced because leaf−air transfer of the semivolatile compounds has been shown to be the main CB accumulation pathway in these crops.[34] The more biochar added to the soil, the lower the volatilization of CBs from the soil. Moreover, the reduction of volatilization in all biochar-

dx.doi.org/10.1021/jf400412p | J. Agric. Food Chem. 2013, 61, 4210−4217

amended treatments, relative to the 0% treatment, was highest for 1,2,4-TCB, followed by 1,2,4,5-TeCB, PeCB, and HCB (Figure 4b) (HCB data source from previous research[26]). Even for the 0.1% biochar amendment, there was nearly a 90% reduction in volatile loss of 1,2,4-TCB (Figure 4b). These results confirmed the hypothesis that biochar amendment is effective at immobilizing more volatile CBs.

Butanol Extractions of CBs from Soil. As shown in Figure 5, the butanol extraction efficiencies of CBs in each treatment decreased with increasing aging period, indicating that aging reduced the bioavailability of CBs in soil. The butanol extraction efficiencies after 24 weeks of incubation decreased to 16−34% of the extraction after 1 week of incubation, among all treatments. With aging, organic compounds can be strongly sequestered by soil organic matter or enter into the nanopores.[9] Therefore, part of the compounds could not be extracted by a mild solvent such as butanol.[10]

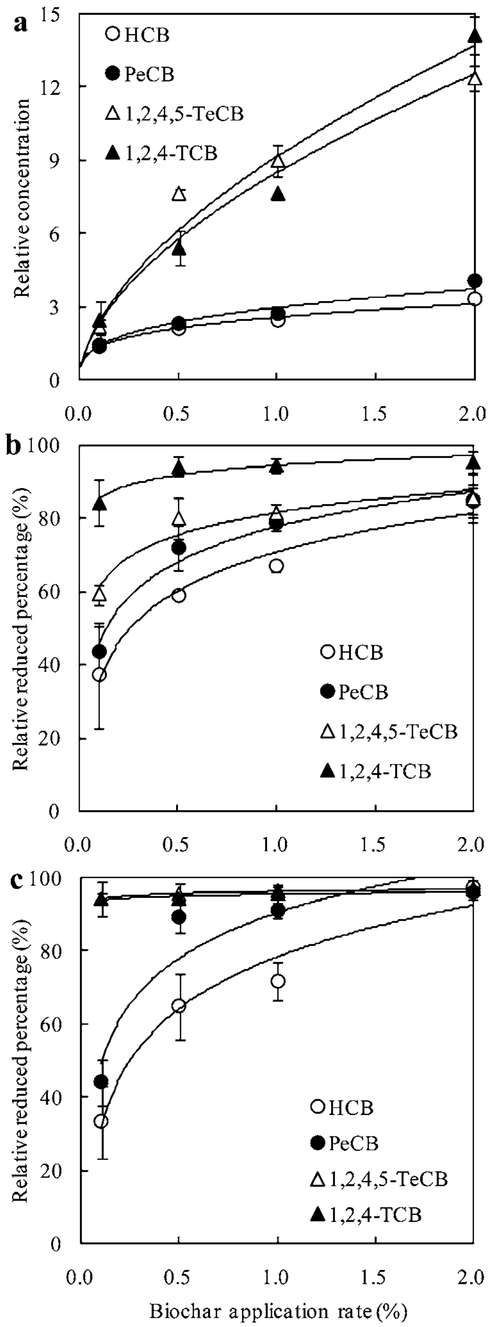

Figure 4. The concentrations of chlorobenzenes (CBs) (a), the reduction of volatilization of CBs (b), and the reduction of earthworm uptake of CBs (c) in biochar-amended treatments relative to 0% treatment after 24 weeks of incubation. (HCB data source from previous research[26]).

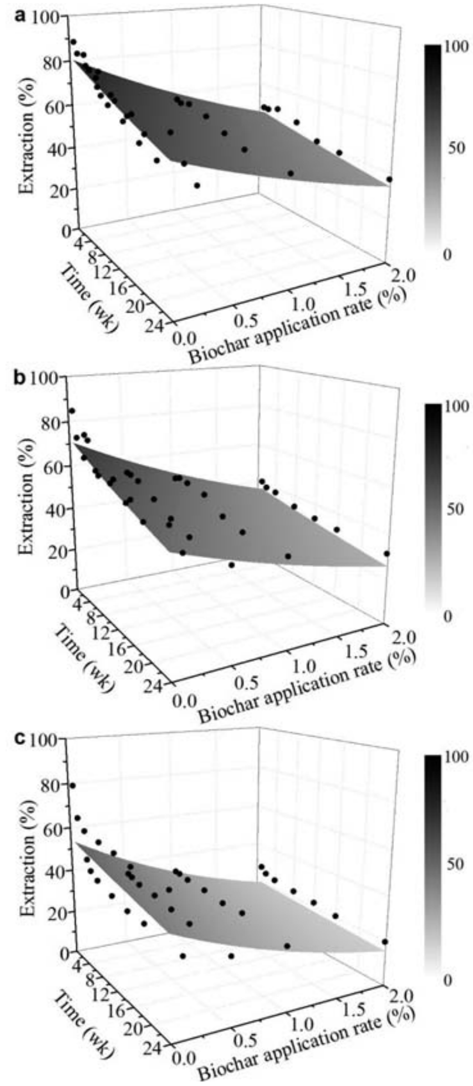

Figure 5. Changes in butanol extraction percentages of chlorobenzenes with incubation time and biochar application rate in biochar-amended soils (a: PeCB; b: 1,2,4,5-TeCB; c: 1,2,4-TCB, dots: measured data; plane: model fitted).

As shown in Figure 5, the butanol extraction efficiencies at each incubation time point decreased with increasing soil biochar content, and this was consistent with the dissipation of CBs from soil. In fact, the initial butanol extraction efficiencies after 1 week were linear correlated to the dissipation ratios of CBs after 24 weeks of incubation (R^2 = 0.60−0.98). The more biochar added to the soil, the lower the CB dissipation rates and the lower the butanol extraction efficiencies that were observed. Moreover, the low butanol extraction efficiencies in biochar-amended treatments lasted throughout the incubation period, indicating that the sorbed CBs may not be released from biochar. It has been reported that biochar aged in soil for 2 years still had a high sorption affinity.[21,35] With aging in soil, biochar could sorb the dissolved organic carbon,[35] which has

Table 3. Regression Curves To Predict the Earthworm Uptake of Chlorobenzenes in Biochar-Amended Soil

	regressions				
	B vs (t and f)[a]	R^2	E vs B[b]	R^2	E vs (t and f)[c]
HCB[d]	$B = 86.87e^{-(0.006t+0.259f)}$	0.93	$E = 0.38B - 16.63$	0.89	$E = 33.01e^{-(0.006t+0.259f)} - 16.63$
PeCB	$B = 82.10e^{-(0.012t+0.288f)}$	0.82	$E = 0.09B - 3.06$	0.78	$E = 7.39e^{-(0.012t+0.288f)} - 3.06$
TeCB	$B = 72.52e^{-(0.016t+0.328f)}$	0.87	$E = 0.06B - 1.88$	0.77	$E = 4.35e^{-(0.016t+0.328f)} - 1.88$
TCB	$B = 55.05e^{-(0.011t+0.501f)}$	0.73	$E = 0.05B - 0.87$	0.83	$E = 2.75e^{-(0.011t+0.501f)} - 0.87$

[a]Regressions between butanol extraction percentages of CBs in soil (B) and aging time (t) and biochar application rate (f). [b]Correlations between earthworm uptake percentages of CBs from soil (E) and butanol extraction (B). [c]Regressions between earthworm uptake percentages of CBs (E) and aging time (t) and biochar application rate (f). [d]HCB data source from previous research.[26]

the most influence on the mobility of hydrophobic organic compounds in soil.[36] Moreover, mineral surfaces may cover the surfaces of biochar over time, and the compounds sorbed in the pore spaces would therefore not be released, resulting in long-lasting reduced bioavailability.[8] Reports in the literature have shown that reduction in the available amount of a pollutant in soil reduces its uptake by both flora and fauna.[10] The effect of this is to reduce the movement of the pollutant in the food chain or food web. Therefore, the reduced bioavailability of CBs in soil, as demonstrated in this study, results in a reduction of uptake by plant roots. This, subsequently, reduces the accumulation of the pollutant by root vegetables such as carrots and other crops.[37]

Because butanol extraction was affected by both aging time and biochar application rate in soil, a model (eq 4) containing the two factors was used to fit the butanol extraction data. The reductions in butanol extraction of CBs fitted into the exponential curve, and this is consistent with other reports.[11] The exponential curve indicates that the rate of reduction in butanol extractable residues slowed with increasing biochar content in soil. However, applying higher amounts of biochar does not necessarily lead to better results; higher rates of biochar amendment may result in decreased soil fertility due to sorption of plant nutrients[38] and increased toxicity to soil organisms due to an abundance of unpalatable substrates in biochar, such as PAHs.[39] From this point of view, low biochar application rates to soil would be better than high application rates where such low amounts could effectively "grab" the lower chlorinated CBs and thereby reduce their volatility.

As shown in Table 3, the B_0 value was on the order of HCB > PeCB > 1,2,4,5-TeCB > 1,2,4-TCB and was positively correlated with the K_{ow} of CBs ($R^2 = 0.80$) (HCB data source from previous research[26]). This could be explained by the fact that butanol is a mild solvent. The higher the K_{ow} of the compound, the higher the butanol extraction efficiencies.[40] The α values were similar for the different CBs, indicating that the effect of aging time on butanol extraction of the different CBs was similar. However, the β values were on the order of 1,2,4-TCB > 1,2,4,5-TeCB > PeCB > HCB and correlated negatively with the K_{ow} of CBs ($R^2 = 0.69$), indicating that the effect of biochar amendment on butanol extraction was more significant for the more volatile lower chlorinated CBs. This result is consistent with the high immobilizing effect of biochar on lower chlorinated CBs (Figure 4) and confirms that the soil biochar content plays an important role in affecting the environmental fate of the semivolatile CBs.

Earthworm Accumulation of CBs in Soil. During the accumulation experiments, no mortality was observed. As shown in Figure 6, the BAFs of CBs to earthworms decreased with increasing biochar content, after 1 week of incubation. After aging for 24 weeks, although there were significantly

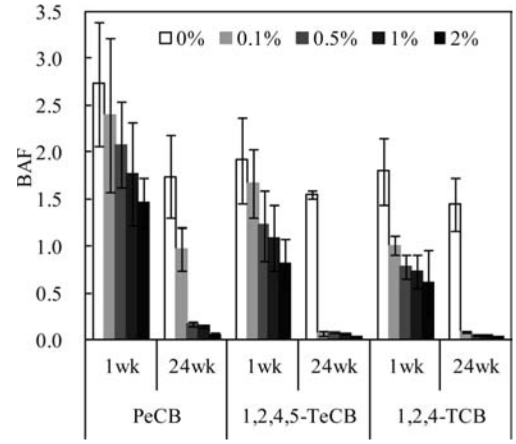

Figure 6. Bioaccumulation factors (BAF) of chlorobenzenes in earthworm from soils amended with different percentages of biochar after 1 week and 24 weeks of incubation.

higher residues of CBs in biochar-amended soils (Figure 2), the BAFs of PeCB, 1,2,4,5-TeCB, and 1,2,4-TCB in biochar-amended treatments were significantly lower than in the 0% treatment ($p < 0.05$) and with a value of less than 1.

The relative percentages of the reduction in earthworm uptake of CBs in biochar-amended soils, relative to the 0% treatment, are shown in Figure 4c. Compared to the 0% treatment, the 0.1% biochar amendment reduced the earthworm uptake of HCB and PeCB by 33% and 44%, respectively (HCB data source from previous research[26]), and by 94% for both 1,2,4,5-TeCB and 1,2,4-TCB. This indicates that a low level of biochar application can effectively reduce the earthworm accumulation of 1,2,4,5-TeCB and 1,2,4-TCB. Moreover, as shown in Figure 4c, the relative reduction in percentages of CBs in each biochar treatment was on the order of 1,2,4-TCB ≈ 1,2,4,5-TeCB > PeCB > HCB. This can be explained two ways: First, earthworm *E. fetida* accumulation of hydrophobic pollutants is an equilibrium process that mainly takes place through the outer epidermis.[40] In fact, the BAF was on the order of HCB > PeCB > 1,2,4,5-TeCB > 1,2,4-TCB and was positively correlated with the K_{ow} of CBs in the different treatments ($R^2 = 0.71-0.99$), indicating that higher chlorinated CBs could be more easily accumulated by earthworms. Second, as discussed above, 1,2,4-TCB could more easily enter into the micropores of biochar, thereby becoming less bioavailable to the earthworms. These arguments are supported by the findings of this study in that (i) biochar effectively immobilized the lower chlorinated and therefore more volatile CBs, and (ii) even a low content of biochar in soil significantly reduced the volatilization and bioavailability of CBs, especially the lower chlorinated ones.

Prediction of Earthworm Uptake of CBs in Soil. Chemical extraction was developed to mimic biota utilization of contaminants in soil, in bioavailability assessment studies, due to its advantages of time-saving, cost-effectiveness etc.[10] Butanol has proved to be a good mimic of the passive uptake of chemicals by organisms through their outer epidermis,[14] such as the accumulation process of *E. fetida*.[40] Therefore, the earthworm uptake of CBs in this study was modeled by butanol extraction. As shown in Table 3, the earthworm uptake of CBs correlated well with butanol extractions. In fact, the trend of earthworm uptake of CBs was consistent with the butanol extraction trend of CBs: HCB > PeCB > 1,2,4,5-TeCB > 1,2,4-TCB (HCB data source from previous research[26]), indicating that butanol extraction is reliable in assessing the bioavailability of a series of CBs in soil.

The earthworm uptake of CBs in biochar-amended soil could therefore be predicted by the different aging times and biochar contents. As shown in Table 3, on the basis of regression analysis, it is possible to calculate how much biochar is needed to effectively immobilize CBs over a certain period in a given soil. However, bioavailability is also soil and compound specific.[10] Therefore, to extrapolate the regression results to different soils and compounds, more systematic research should be conducted.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jiangxin@issas.ac.cn. Phone: +86 25 86881195. Fax: +86 25 86881000.

Author Contributions

[‡]Y.S. and F.W. contributed equally to this paper.

Funding

This study was financially supported by the National Nature Science Foundation of China (41030531, 21277148), the Specific Fund for Agro-Scientific Research in the Public Interest (201203045), and the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-EW-QN403).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CBs, chlorobenzenes; HCB, hexachlorobenzene; PeCB, pentachlorobenzene; 1,2,4,5-TeCB, 1,2,4,5-tetrachlorobenzene; 1,2,4-TCB, 1,2,4-trichlorobenzene; POPs, persistent organic pollutants; PAHs, polycyclic aromatic hydrocarbons; GC, gas chromatography

■ REFERENCES

(1) Beesley, L.; Moreno-Jimenez, E.; Gomez-Eyles, J. L.; Harris, E.; Robinson, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, *159*, 3269−3282.

(2) Schmidt, M. W. I.; Noack, A. G. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Global Biogeochem. Cycles* **2000**, *14*, 777−793.

(3) Sohi, S. P.; Krull, E.; Lopez-Capel, E.; Bol, R. A review of biochar and its use and function in soil. *Adv. Agron.* **2010**, *105*, 47−82.

(4) Atkinson, C. J.; Fitzgerald, J. D.; Hipps, N. A. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil* **2010**, *337*, 1−18.

(5) Steinbeiss, S.; Gleixner, G.; Antonietti, M. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol. Biochem.* **2009**, *41*, 1301−1310.

(6) Woolf, D.; Amonette, J. E.; Street-Perrott, F. A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, *1*, 56−65.

(7) Chun, Y.; Sheng, G. Y.; Chiou, C. T.; Xing, B. S. Compositions and sorptive properties of crop residue-derived chars. *Environ. Sci. Technol.* **2004**, *38*, 4649−4655.

(8) Kookana, R. S. The role of biochar in modifying the environmental fate, bioavailability, and efficacy of pesticides in soils: a review. *Aust. J. Soil Res.* **2010**, *48*, 627−637.

(9) Alexander, M. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ. Sci. Technol.* **2000**, *34*, 4259−4265.

(10) Reid, B. J.; Jones, K. C.; Semple, K. T. Bioavailability of persistent organic pollutants in soils and sediments - a perspective on mechanisms, consequences and assessment. *Environ. Pollut.* **2000**, *108*, 103−112.

(11) Cao, X.; Ma, L.; Liang, Y.; Gao, B.; Harris, W. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. *Environ. Sci. Technol.* **2011**, *45*, 4884−4889.

(12) Udeigwe, T. K.; Eze, P. N.; Teboh, J. M.; Stietiya, M. H. Application, chemistry, and environmental implications of contaminant-immobilization amendments on agricultural soil and water quality. *Environ. Int.* **2011**, *37*, 258−267.

(13) Wen, B.; Li, R. J.; Zhang, S. Z.; Shan, X. Q.; Fang, J.; Xiao, K.; Khan, S. U. Immobilization of pentachlorophenol in soil using carbonaceous material amendments. *Environ. Pollut.* **2009**, *157*, 968−974.

(14) Johnson, D. L.; Jones, K. C.; Langdon, C. J.; Piearce, T. G.; Semple, K. T. Temporal changes in earthworm availability and extractability of polycyclic aromatic hydrocarbons in soil. *Soil Biol. Biochem.* **2002**, *34*, 1363−1370.

(15) Kelsey, J. W.; Kottler, B. D.; Alexander, M. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ. Sci. Technol.* **1997**, *31*, 214−217.

(16) Yang, X. B.; Ying, G. G.; Peng, P. A.; Wang, L.; Zhao, J. L.; Zhang, L. J.; Yuan, P.; He, H. P. Influence of biochars on plant uptake and dissipation of two pesticides in an agricultural soil. *J. Agric. Food Chem.* **2010**, *58*, 7915−7921.

(17) Yang, Y. N.; Sheng, G. Y.; Huang, M. S. Bioavailability of diuron in soil containing wheat-straw-derived char. *Sci. Total Environ.* **2006**, *354*, 170−178.

(18) Zhang, P.; Sheng, G. Y.; Feng, Y. H.; Miller, D. M. Predominance of char sorption over substrate concentration and soil pH in influencing biodegradation of benzonitrile. *Biodegradation* **2006**, *17*, 1−8.

(19) Zhang, P.; Sheng, G. Y.; Wolf, D. C.; Feng, Y. C. Reduced biodegradation of benzonitrile in soil containing wheat-residue-derived ash. *J. Environ. Qual.* **2004**, *33*, 868−872.

(20) Loganathan, V. A.; Feng, Y.; Sheng, G. D.; Clement, T. P. Crop-residue-derived char influences sorption, desorption and bioavailability of atrazine in soils. *Soil Sci. Soc. Am. J.* **2009**, *73*, 967−974.

(21) Jones, D. L.; Edwards-Jones, G.; Murphy, D. V. Biochar mediated alterations in herbicide breakdown and leaching in soil. *Soil Biol. Biochem.* **2011**, *43*, 804−813.

(22) Gomez-Eyles, J. L.; Sizmur, T.; Collins, C. D.; Hodson, M. E. Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. *Environ. Pollut.* **2011**, *159*, 616−622.

(23) Bushnaf, K. M.; Puricelli, S.; Saponaro, S.; Werner, D. Effect of biochar on the fate of volatile petroleum hydrocarbons in an aerobic sandy soil. *J. Contam. Hydrol.* **2011**, *126*, 208−215.

(24) Zhang, M. Y.; Lu, Y. M. Market analysis and development trend of chlorobenzenes. *Chlor-Alkali Industry (In Chinese)* **2005**, *1*, 26−32.

(25) Song, Y.; Wang, F.; Bian, Y.; Zhang, Y.; Jiang, X. Chlorobenzenes and organochlorinated pesticides in vegetable soils from an industrial site, China. *J. Environ. Sci.* **2012**, *24*, 362−368.

(26) Song, Y.; Wang, F.; Bian, Y.; Kengara, F. O.; Jia, M.; Xie, Z.; Jiang, X. Bioavailability assessment of hexachlorobenzene in soil as affected by wheat straw biochar. *J. Hazard. Mater.* **2012**, *217−218*, 391−397.

(27) Kengara, F. O.; Doerfler, U.; Welzl, G.; Ruth, B.; Munch, J. C.; Schroll, R. Enhanced degradation of ^{14}C-HCB in two tropical clay soils using multiple anaerobic-aerobic cycles. *Environ. Pollut.* **2013**, *173*, 168−75.

(28) Malcolm, H. M.; Howe, P. D.; Dobson, S. Chlorobenzene other than hexachlorobenzene: environmental aspects. *Concise International Chemical Assessment Document 60*, World Health Organization, 2004.

(29) Wang, X.; Xing, B. Sorption of organic contaminants by biopolymer-derived chars. *Environ. Sci. Technol.* **2007**, *41*, 8342−8348.

(30) Schroll, R.; Brahushi, F.; Dörfler, U.; Kuhn, S.; Fekete, J.; Munch, J. C. Biomineralisation of 1,2,4-trichlorobenzene in soils by an adapted microbial population. *Environ. Pollut.* **2004**, *127*, 395−401.

(31) Song, Y.; Wang, F.; Yang, X.; Liu, C.; Kengara, F. O.; Jin, X.; Jiang, X. Chemical extraction to assess the bioavailability of chlorobenzenes in soil with different aging periods. *J. Soils Sediments* **2011**, *11*, 1345−1354.

(32) Cao, X.; Ma, L.; Gao, B.; Harris, W. Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environ. Sci. Technol.* **2009**, *43*, 3285−3291.

(33) Field, J. A.; Sierra-Alvarez, R. Microbial degradation of chlorinated benzenes. *Biodegradation* **2008**, *19*, 463−480.

(34) Yang, X.; Jiang, X.; Yu, G.; Yao, F.; Bian, Y.; Wang, F. Leaf-air transfer of organochlorine pesticides from three selected vegetables. *Environ. Pollut.* **2007**, *148*, 555−561.

(35) Yang, Y. N.; Sheng, G. Y. Pesticide adsorptivity of aged particulate matter arising from crop residue burns. *J. Agric. Food Chem.* **2003**, *51*, 5047−5051.

(36) Tao, S.; Xu, F. L.; Liu, W. X.; Cui, Y. H.; Coveney, R. M. A chemical extraction method for mimicking bioavailability of polycyclic aromatic hydrocarbons to wheat grown in soils containing various amounts of organic matter. *Environ. Sci. Technol.* **2006**, *40*, 2219−2224.

(37) Yang, X. L.; Wang, F.; Gu, C. G.; Jiang, X. Tenax TA extraction to assess the bioavailability of DDTs in cotton field soils. *J. Hazard. Mater.* **2010**, *179*, 676−683.

(38) Rondon, M. A.; Lehmann, J.; Ramirez, J.; Hurtado, M. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol. Fertil. Soils* **2007**, *43*, 699−708.

(39) Hilber, I.; Blum, F.; Leifeld, J.; Schmidt, H. P.; Bucheli, T. D. Quantitative determination of PAHs in biochar: A prerequisite to ensure its quality and safe application. *J. Agric. Food Chem.* **2012**, *60*, 3042−3050.

(40) Gomez-Eyles, J. L.; Collins, C. D.; Hodson, M. E. Relative proportions of polycyclic aromatic hydrocarbons differ between accumulation bioassays and chemical methods to predict bioavailability. *Environ. Pollut.* **2010**, *158*, 278−284.