Environ Monit Assess (2018) 190: 218
https://doi.org/10.1007/s10661-018-6592-8

Do biochars influence the availability and human oral bioaccessibility of Cd, Pb, and Zn in a contaminated slightly alkaline soil?

Adeline Janus · Christophe Waterlot · Sophie Heymans · Christophe Deboffe · Francis Douay · Aurélie Pelfrêne

Received: 6 August 2017 / Accepted: 5 March 2018 / Published online: 14 March 2018

Abstract Different remediation techniques have been used to restore metal-contaminated sites, including stabilizing metals by adding amendments to the soils. This study experimented three biochars, made from wood and miscanthus, cultivated on contaminated and uncontaminated soils, used as amendments at a 2% application rate on a metal-contaminated soil for 9 months in laboratory-controlled conditions. The objective was to evaluate whether biochars were able to decrease the availability and human oral bioaccessibility of metals in an alkaline soil. To meet this goal, the modifications of the soil's physicochemical parameters, metal distribution in soil, and human bioaccessibility were evaluated at different sampling times. The results showed that biochar application to the alkaline soil did not always decrease the soil metal availability, which challenges the value of using biochars in already slightly alkaline soils at a low application rate. However, differences in efficiency between the three biochars tested were highlighted. The biochar produced with miscanthus cultivated on uncontaminated soil led to higher soil metal bioaccessibility. Moreover, because of the absence of any increase in soil metal availability with the biochar produced from biomass cultivated on contaminated soil, the use of such biochars can be recommended for the remediation of contaminated soil.

Keywords Biochar · Alkaline soil · Contaminated biomass · Metal distribution · Oral bioaccessibility

A. Janus (✉) · C. Waterlot · F. Douay · A. Pelfrêne
Yncréa Hauts-de-France, Laboratoire Génie Civil et géoEnvironnement (LGCgE), 48 Boulevard Vauban, 59046 Lille Cedex, France
e-mail: adeline.janus@yncrea.fr

S. Heymans · C. Deboffe
Néo-Eco Recycling, 59320 Haubourdin, France

Introduction

Metals in soils are not biodegradable and are slowly depleted by leaching, plant uptake, and erosion (Kabata-Pendias and Pendias 1984; Kobya et al. 2005). These metals can lead to environmental risks by altering ecosystem functioning and soil ecological health (Martin and Ruby 2004). Moreover, through the consumption of contaminated crops, the ingestion, and/or inhalation of contaminated dust/soil particles, the populations located on the contaminated sites can also be exposed, possibly causing health risks.

To restore contaminated sites, different remediation techniques exist, including the stabilization of metals. To enhance this stabilization, organic and inorganic amendments can be added to the soils (Martin and Ruby 2004; Park et al. 2011b). Within these amendments, biochars have received great attention for the past several years, which are defined as the carbon-rich products obtained when biomass (e.g., wood or manure) is heated in a closed container with little or no available air (Lehmann and Joseph 2009). Among the different raw materials that can be used to produce biochars, the use of plant biomass produced on contaminated sites can be a solution to limit the conversion of

land usually used for food production (Evangelou et al. 2014). For example, Břendová et al. (2015) evaluated the metal sorption efficiency of biochars produced on contaminated and uncontaminated soils and demonstrated that the metal sorption efficiency of biochars was similar, with a minimal risk of metal release for the biochar produced on contaminated soils.

Many studies have evaluated and demonstrated the ability of biochars to modify soil characteristics and to decrease the availability of metals in acidic soils, even at low application rates (Namgay et al. 2010; Beesley et al. 2011; Jiang et al. 2012; Houben et al. 2013; Bian et al. 2014; Ehsan et al. 2014; Niu et al. 2015; Rodríguez-Vila et al. 2015). These availability decreases are commonly correlated with the specific surface area (SSA) of biochars, their porosity, pH, or cation exchange capacity (CEC) and therefore depend on the sorption capacity of biochars (Janus et al. 2015). Moreover, the effects of biochars on metal availability are also related to the soil modification following biochar amendment, notably the increase in soil pH, which is one of the main factors affecting soil adsorption sites and therefore metal availability (Pérez-Esteban et al. 2014). However, to our knowledge, few studies have evaluated the effects of biochar addition on metal availability in alkaline soils (Al-Wabel et al. 2015; Zhao et al. 2016; Ahmad et al. 2017; Shen et al. 2018).

To estimate the risk to which the population is exposed to metallic soil contamination in case of soil ingestion, metal oral bioaccessibility can be measured. The oral bioaccessibility of soil contaminant is defined as the fraction that is soluble in the gastrointestinal environment and available for absorption (Ruby et al. 1999). In this context, the BioAccessibility Research Group of Europe (BARGE) developed a unified method (called the unified bioaccessibility method (UBM)) to evaluate oral bioaccessibility through ingestion of soil particles in a reproducible, robust, and defensible manner (Wragg et al. 2011). The impact of biochars on metal oral bioaccessibility has been studied over the last few years (Ahmad et al. 2012; Uchimiya et al. 2012; Cui et al. 2016; Rizwan et al. 2016). However, to our knowledge, no study has evaluated the relation between biochars and oral bioaccessibility using the UBM test.

The aim of this study was to evaluate how the effects of three biochars evolved over 9 months on soil parameters, metal availability, and oral bioaccessibility in an alkaline-contaminated soil sampled near a former lead smelter. The second objective was to compare the efficiency of biochars produced from plant biomass cultivated on metal-contaminated and uncontaminated soils, respectively.

Materials and methods

Soil and biochar preparation

The soil studied was taken in the organic-mineral horizon from a poplar grove planted on a former agricultural parcel, located at Evin Malmaison (northern France) near a former lead and zinc smelter (Metaleurop Nord, France). The soil is highly contaminated with Pb and Zn but also with Cd. The soil presents clay, silt, and sand contents equal to 154, 623, and 223 g kg^{-1}, respectively. Its characteristics are described in Table 1.

Three biochars were tested: the first biochar (called BcM) was produced from *Miscanthus* × *giganteus* plants (a perennial grass), cultivated on agricultural land located near the former lead smelter Metaleurop Nord. The aerial parts of this plant were harvested after senescence (February) and then pyrolyzed at 600 °C for 1 h at La Carbonerie (Crissey, France). The second biochar (called BuM) was also made from *Miscanthus* × *giganteus*, but it was cultivated on an uncontaminated soil and produced by Pyreg GmbH (Dörth, Germany) at 600 °C for 30 min according to the procedure detailed in Houben et al. (2013). The last biochar (BW) was made from hardwood (hornbeam, beech, and oak) at 400 °C for 12 h at La Carbonerie. All the biochars were ground and sieved to particles with sizes ranging from 315 μm to 2 mm.

Biochar characterization

The pH (H_2O) was measured after stirring a mixture of biochar and deionized water (1:5 *v/v*) according to the ISO 10390 standard. The CEC was determined after percolation of ammonium acetate (1 M, pH = 7) into biochar samples followed by an extraction of ammonium ions (NH_4^+) with sodium chloride (1 M) according to the French NF X31–130 standard. The SSA and the carbon and nitrogen contents were measured by the CIRAD Research Center (Montpellier, France). The surface area of biochars was determined from N_2 isotherms using a Micromeritics ASAP2020 analyzer; the data were fit to the Brunauer-Emmet-Teller (BET) equation to calculate the surface area. The total C and N

Table 1 Physico-chemical parameters of the soil studied ($n = 5$)

	pH	CEC ($cmol^+ kg^{-1}$)	Total $CaCO_3$ ($g kg^{-1}$)	Organic matter (%)	Pseudototal concentrations[a] ($mg kg^{-1}$)		
					Cd	Pb	Zn
Mean	7.55	12.40	10.24	6.08	20.6	1025	1476
SD	0.03	0.64	0.55	0.03	0.22	18	20

SD standard deviation, *CEC* cation exchange capacity

[a] Extraction with aqua regia

contents were measured by an elemental analyzer (Variomacrocube, Elementar). The oxygen content was calculated by subtracting the ash, carbon, hydrogen, and nitrogen contents, expressed as a percentage, from 100 (Al-Wabel et al. 2013). The pseudototal Cd, Pb, and Zn concentrations of the biochars were determined by atomic absorption spectrometry (AA-6800, Shimadzu) after digesting the biochar sample with a mixture of hydrogen peroxide and nitric acid (1:1 *v/v*).

Experimental setup

The three biochars were applied to the contaminated soil, initially dried at 40 °C, ground, and sieved to 2 mm, at a 2% (*w/w*) application rate, with 1 g of biochar added to 50 g of soil and placed in amber glass jars. After the amendment, the mixtures were incubated in the laboratory at 20 °C and maintained at 60% field capacity with deionized water. Soil with no amendment was used as control. Each experimental modality described hereafter was performed in three replicates, with no amendment (called CT) or amended with BcM, BuM, or BW. The pot experiment was conducted over 9 months, in the dark to avoid the development of algae. Soil samples were taken at different times: after 3, 6, and 9 months (one set of pots per sample time). A first control (CT_0, unamended soil), which went through the same equilibrium conditions as the amended soils in terms of watering, was sampled after the 5 days of equilibrium to represent the initial time. Thus, both impacts of watering and biochar amendment were evaluated.

Analyses

At 3, 6, and 9 months, the soils were dried at 40 °C, ground, and passed through 2-mm and 250-μm sieves using a mill (ZM200, Retsch). First, the soils were characterized with pH, CEC, and $CaCO_3$ content. The pH and CEC measurements were taken as described above. The $CaCO_3$ content was determined by measuring the CO_2 formed after adding HCl (4 M) to the aliquot according to the ISO 10693 standard.

The pseudototal Cd, Pb, and Zn concentrations were determined after an acidic digestion in aqua regia (HCl/HNO_3, 3/1 *v/v*) using a digestion block at 120 °C for 90 min. For quality assurance, a certified soil was used (ERM-CC141, ERM, Belgium). The samples were then analyzed with a flame atomic absorption spectrometer (AA-6800, Shimadzu). For the certified soil, measured concentrations were equal to 99, 114, and 94% of the expected concentrations for Cd, Pb, and Zn, respectively. The metal fractionation in soil was evaluated with the BCR sequential extraction method according to the modified BCR sequential extraction protocol (Waterlot et al. 2012). Four fractions were obtained, noted F1, F2, F3, and F4, and defined as (F1) water/acid soluble and exchangeable fraction (0.11 mol L^{-1} acetic acid), (F2) reducible fraction (0.5 mol L^{-1} hydroxylammonium chloride), (F3) oxidizable fraction (30% H_2O_2 and 1.0 mol L^{-1} ammonium acetate), and (F4) residual fraction (aqua regia). Quality control was checked using a certified reference material (BCR 701). For all the fractions, the concentrations obtained were similar to the expected concentrations. The sum of each fraction compared to pseudototal concentrations was in the range of 98–109% for Cd, 97–107% for Pb, and 99–112% for Zn. Therefore, the values were expressed for each element as a percentage of the sum of all sequential extraction fractions.

The oral bioaccessibility of Cd, Pb, and Zn was determined using the UBM test (Wragg et al. 2011), previously described in full by Pelfrêne et al. (2011). This protocol consists in two parallel sequential extraction procedures and simulates the chemical processes occurring in the mouth, stomach, and intestine

compartments using synthetic digestive solutions according to physiological transit times. The UBM test provided samples for analysis from both the gastric and gastrointestinal phases. Blank and certified reference material, NIST 2710a (Montana I Soil), was also used to verify the protocol quality. The metal concentrations were 6.2 ± 1.6 mg Cd kg^{-1}, 2681 ± 446 mg Pb kg^{-1}, and 1455 ± 116 mg Zn kg^{-1} in the gastric phase, and equal to 2.0 ± 1.1 mg Cd kg^{-1}, 463 ± 97 mg Pb kg^{-1}, and 256 ± 31 mg Zn kg^{-1} in the gastrointestinal phase.

Statistical analysis

The experimental data were expressed as means and standard deviations and were compared statistically using the Tukey's test at the 5% level. Differences between values at $p > 0.05$ were considered not statistically significant. Moreover, all data were subjected to analysis of variance (ANOVA). All statistical tests were performed using the R software for Windows.

Results and discussion

Biochar parameters

Table 2 presents selected parameters of the biochars made from wood (BW), miscanthus cultivated on uncontaminated soil (BuM), and miscanthus cultivated on contaminated soil (BcM).

The analysis of the three biochars revealed that their metal concentrations were very low, even for the biochar BcM. Moreover, the highest Cd and Pb concentrations were obtained for the biochar BW. However, the metal concentrations in the three biochars are under the threshold values imposed by French legislation on the spreading of organic amendments designed to improve agricultural soil properties (AFNOR 2006).

BuM presented higher pH and lower $CaCO_3$ contents, O/C ratio, and SSA than BcM. These differences are probably explained by the pyrolysis conditions used to produce these two biochars. Indeed, BuM was pyrolyzed for 30 min versus 1 h for BcM, and it has already been demonstrated that the residence time of feedstock in the pyrolyzer has an impact on biochar characteristics, notably on C content, pH, and SSA (Kwapinski et al. 2010; Peng et al. 2011; Yuan et al. 2014; Janus et al. 2015). Moreover, the miscanthus used to produce the two biochars did not have the same origin, which can also explain these differences obtained in biochar characteristics. BW presented different parameters compared to the miscanthus biochars. Indeed, BW had a lower pH and SSA, and presented higher N and $CaCO_3$ contents and O/C ratio. These differences are explained by the feedstock used. Several studies have also revealed the impact of the feedstock used on the biochar characteristics (Antal and Grønli 2003; Novak et al. 2009; Lei and Zhang 2013).

Table 2 Chemical and physical parameters of biochars

		BcM	BuM[a]	BW
pH (H_2O)		9.39	10.24	8.41
C	%	78.2 ± 0.7	77.0 ± 1.1	79.0 ± 2.1
N	%	0.35 ± 0.09	0.49 ± 0.16	0.98 ± 0.04
O/C		0.10 ± 0.01	0.04 ± 0.02	0.13 ± 0.03
$CaCO_3$	g kg^{-1}	14.7	8.6 ± 0.1	21.6 ± 4.1
SSA	m^2 g^{-1}	170.7	150.8	121.6
Cd	mg kg^{-1}	< 0.4	0.1	1.0 ± 0.0
Pb	mg kg^{-1}	< 4.3	nd	24.2 ± 0.1
Zn	mg kg^{-1}	82.2	116	12.6 ± 1.6

[a] pH and Cd/Pb/Zn contents obtained in Houben et al. (2013)

SSA specific surface area, *nd* not detected

Evolution of soil physicochemical parameters

The impacts of biochar application on the soil's pH, CEC, and $CaCO_3$ content are shown in Fig. 1.

The analysis of soil pH revealed no difference between the control and the amended soils but significant differences in soil pH depending on the sampling time and the biochar.

At 3 months, a decrease was observed for the unamended soil by 0.1 units, probably explained by watering the soil with osmosed water, which presented a slightly acidic pH (pH = 5.7). Comparing the three biochar-amended soils and the unamended soil at 3 months revealed no difference, demonstrating that the biochars did not compensate the effect of watering. At 6 and 9 months, time, watering, and the biochar amendments did not modify the soil pH. However, a higher soil pH was always observed with the BW amendment compared to those obtained with the BuM amendment, probably explained by the higher $CaCO_3$ content of this biochar (Table 2). Previous studies also observed no modification of soil pH following biochar

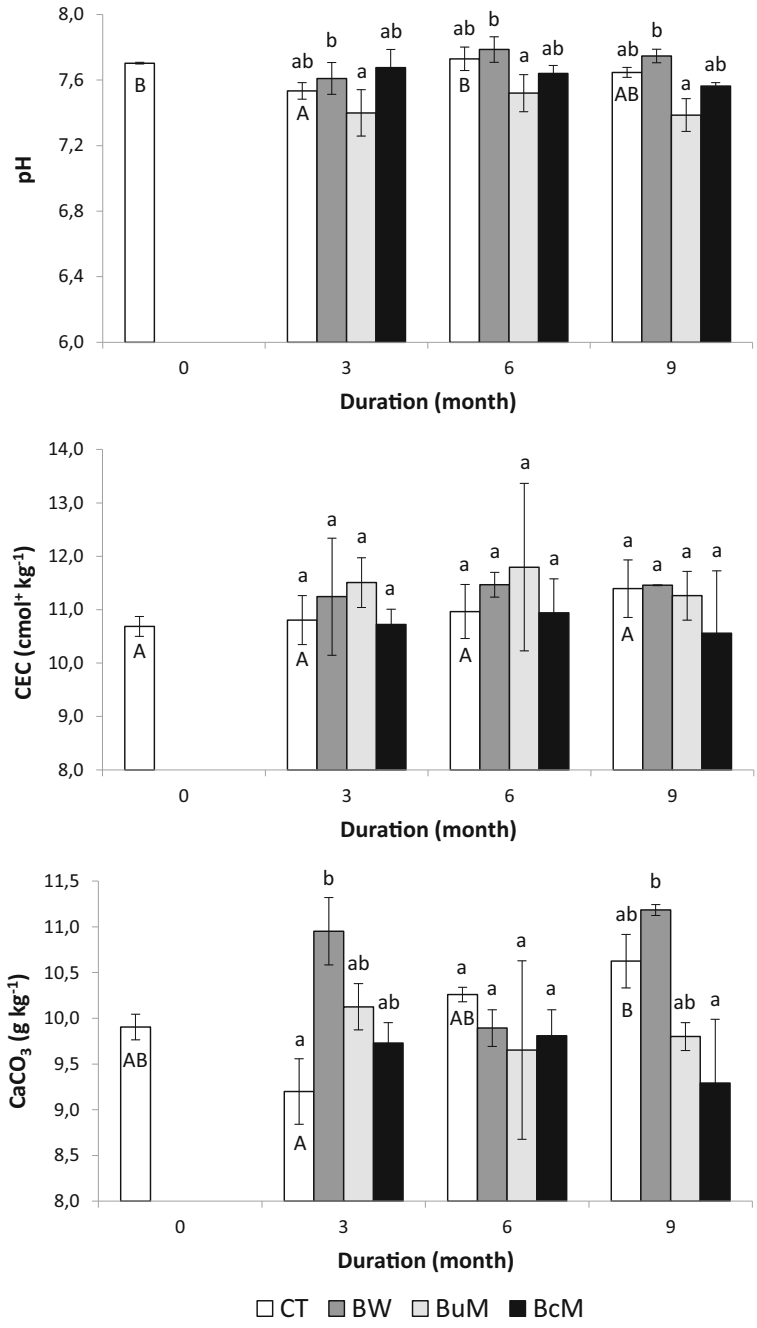

Fig. 1 Evolution of the soil pH, CEC, and $CaCO_3$ content during the experiment for the non-amended soil (CT) and the soils amended with: the woody biochar (BW), the biochar made from *Miscanthus* cultivated on uncontaminated soil (BuM), and the biochar made from *Miscanthus* cultivated on contaminated soil (BcM) ($n = 3$). Capital letters compared the control between the different sampling times. Lowercase letters compared the different treatments at each sampling times. Different letters correspond to values statiscally different ($p < 0.05$)

addition for an application rate lower than 3% (*w*/*w*) in slightly alkaline soils (Méndez et al. 2012; Al-Wabel et al. 2015; Zhang et al. 2016).

The soil CEC did not vary during the experiment, demonstrating that there was no effect of watering and biochar amendment. The absence of an effect on soil CEC was also observed by several authors who experimented a low application rate (Méndez et al. 2012; Houben et al. 2013). Moreover, according to Jha et al. (2010), the effect of biochar addition on soil CEC is dependent on the type of biomass used, possibly explaining why the biochars tested in this study did not modify this parameter.

Lastly, the soil $CaCO_3$ content did not change over time. However, at 3 months, a 19% increase in the $CaCO_3$ content was observed with the BW amendment.

This increase might be explained by the high $CaCO_3$ content of BW compared to the soil $CaCO_3$ content. In contrast, the BcM and BuM biochars presented $CaCO_3$ contents similar to the soil, explaining the absence of an effect for these two amendments. At 6 and 9 months, the three biochars did not modify the soil $CaCO_3$ content. Nevertheless, as for the soil pH, differences between BW and BcM were observed at 9 months with a statistically higher soil $CaCO_3$ content for the BW amendment. This result was probably related to the higher $CaCO_3$ content in BW compared to BcM.

Metal distribution in soil

The distribution of Cd, Pb, and Zn in the F1, F2, F3, and F4 fractions is shown in Tables 3, 4, and 5, respectively. For all the conditions and during the 9 months of the experiment, Cd was mainly present in the water, exchangeable and acid-soluble fraction (F1: 65–68%), and the reducible fraction (F2: 27–29%). In contrast, Pb was mainly present in the reducible fraction (F2: 80–85%) followed by the F1 fraction (8–11%). The Zn distribution showed the highest concentrations in the F1 and F2 fractions (F1: 39–43%; F2: 38–44%), followed by the residual fraction (F4: 10–13%) and the oxidizable fraction (F3: 6–8%).

Concerning Cd distribution, no effect of watering and biochar amendment was observed for the F1, F2, and F3 fractions. However, in the residual fractions, differences between the amendments were detected at 9 months. Indeed, the BcM biochar significantly increased the F4 fraction from 3.1 to 3.9% compared to the unamended soil, indicating lower mobility for Cd following BcM amendment.

For Pb, watering with osmosed water did not affect metal fractionation over time. No effect of biochar amendment compared to the control was observed for the four fractions during the experiment. However, at 9 months, differences between the biochars themselves were observed. A higher percentage was observed with BW in the F3 fraction (6.1%) compared to BcM (3.7%).

At 3, 6, and 9 months, watering with osmosed water affected Zn distribution by decreasing the percentage in the F1 fraction and increasing F2. In the F1 fraction, a decrease from 42.7 to 40.2% was observed at 3 months and remained similar at 6 and 9 months. In contrast, the F2 fraction percentage increased for CT at 3 months from 38.0 to 43.9%. At 6 and 9 months, the increase was lower but remained statistically significant (41.4 and 41.0% at 6 and 9 months, respectively) compared to the initial time. The use of osmosed water during the experiment modified Zn fractionation in soil. These

Table 3 Distribution of Cd (expressed as percent of the sum in all fractions) for each sampling date (0, 3, 6, and 9 months) in the soil without biochar (CT), with woody biochar (BW), with biochar made from *Miscanthus* cultivated on uncontaminated soil (BuM), and with biochar made from *Miscanthus* cultivated on contaminated soil (BcM) (F1: exchangeable, water- and acid-soluble fraction; F2: reducible fraction; F3: oxidizable fraction; F4: residual fraction) ($n = 3$)

		Fractions			
		F1	F2	F3	F4
0 month	CT	67.1 ± 1.0 A	27.5 ± 0.6 A	2.3 ± 0.3 A	3.1 ± 0.3 A
3 months	CT	66.3 ± 1.2 Aa	28.1 ± 1.2 Aa	2.0 ± 0.3 Aa	3.5 ± 0.2 Aa
	BW	66.6 ± 1.2 a	28.2 ± 1.4 a	2.1 ± 0.2 a	3.1 ± 0.3 a
	BuM	65.5 ± 1.3 a	29.1 ± 1.1 a	2.4 ± 0.4 a	3.0 ± 0.1 a
	BcM	67.2 ± 1.1 a	27.6 ± 0.7 a	2.1 ± 0.2 a	3.1 ± 0.4 a
6 months	CT	65.8 ± 2.8 Aa	28.9 ± 3.3 Aa	2.0 ± 0.2 Aa	3.3 ± 0.9 Aa
	BW	65.6 ± 1.1 a	28.7 ± 1.2 a	2.4 ± 0.3 a	3.3 ± 0.2 a
	BuM	65.7 ± 1.3 a	29.1 ± 1.7 a	2.5 ± 0.3 a	2.7 ± 0.4 a
	BcM	67.6 ± 0.8 a	27.5 ± 1.0 a	1.9 ± 0.8 a	3.0 ± 0.4 a
9 months	CT	66.4 ± 0.6 Aa	28.4 ± 0.7 Aa	2.1 ± 0.4 Aa	3.1 ± 0.4 Aa
	BW	65.0 ± 0.1 a	29.2 ± 0.2 a	2.5 ± 0.1 a	3.2 ± 0.2 ab
	BuM	65.7 ± 1.3 a	29.0 ± 0.9 a	2.3 ± 0.2 a	3.0 ± 0.3 a
	BcM	66.0 ± 1.9 a	28.2 ± 2.2 a	2.0 ± 0.3 a	3.9 ± 0.3 b

Capital letters compared the control between the different sample times. Lowercase letters compared the different treatments at each sample times. Different letters correspond to values statiscally different ($p < 0.05$)

Table 4 Distribution of Pb (expressed as percent of the sum in all fractions) for each sampling date (0, 3, 6, and 9 months) in the soil without biochar (CT), with woody biochar (BW), with biochar made from *Miscanthus* cultivated on uncontaminated soil (BuM), and with biochar made from *Miscanthus* cultivated on contaminated soil (BcM) (F1: exchangeable, water- and acid-soluble fraction; F2: reducible fraction; F3: oxidizable fraction; F4: residual fraction) ($n = 3$)

		Fractions F1	F2	F3	F4
0 month	CT	10.1 ± 1.0 AB	80.1 ± 1.7 A	3.7 ± 0.1 A	6.1 ± 1.6 A
3 months	CT	10.6 ± 0.5 Ba	84.4 ± 0.8 Aa	2.7 ± 0.8 Aa	2.3 ± 0.4 Aa
	BW	9.7 ± 1.1 a	83.7 ± 1.3 a	2.7 ± 0.9 a	3.9 ± 0.6 a
	BuM	9.7 ± 1.1 a	84.4 ± 0.7 a	2.6 ± 0.4 a	3.4 ± 1.0 a
	BcM	10.9 ± 1.1 a	83.2 ± 1.2 a	3.3 ± 0.6 a	2.5 ± 0.9 a
6 months	CT	9.4 ± 0.5 Aba	82.9 ± 1.7 Aa	3.5 ± 0.3 Aa	4.2 ± 1.4 Aa
	BW	8.4 ± 0.8 a	85.2 ± 0.2 a	2.9 ± 1.4 a	3.5 ± 1.4 a
	BuM	8.6 ± 1.8 a	83.8 ± 0.9 a	5.2 ± 1.5 a	2.3 ± 0.3 a
	BcM	9.2 ± 0.3 a	83.3 ± 1.3 a	3.9 ± 0.8 a	3.5 ± 0.6 a
9 months	CT	8.5 ± 0.5 Aa	83.1 ± 1.9 Aa	3.8 ± 1.3 Aab	4.6 ± 1.9 Aa
	BW	8.2 ± 0.2 a	82.5 ± 1.6 a	6.1 ± 0.6 b	3.2 ± 1.5 a
	BuM	7.9 ± 0.8 a	84.3 ± 1.1 a	4.1 ± 1.1 ab	3.8 ± 2.3 a
	BcM	9.1 ± 0.3 a	85.0 ± 0.6 a	3.7 ± 0.4 a	2.2 ± 0.7 a

Capital letters compared the control between the different sample times. Lowercase letters compared the different treatments at each sample times. Different letters correspond to values statiscally different ($p < 0.05$)

results contradict those obtained by Waterlot et al. (2011), who observed an increase in the Zn percentage in the F1 fraction and a decrease in the F2 fraction. However, the soil tested in the present study and the soils used in Waterlot et al. (2011) are different in terms of physicochemical parameters (grain size, pH,

Table 5 Distribution of Zn (expressed as percent of the sum in all fractions) for each sampling date (0, 3, 6, and 9 months) in the soil without biochar (CT), with woody biochar (BW), with biochar made from *Miscanthus* cultivated on uncontaminated soil (BuM), and with biochar made from *Miscanthus* cultivated on contaminated soil (BcM) (F1: exchangeable, water- and acid-soluble fraction; F2: reducible fraction; F3: oxidizable fraction; F4: residual fraction) ($n = 3$)

		Fractions F1	F2	F3	F4
0 month	CT	42.7 ± 0.6 B	38.0 ± 0.9 A	8.0 ± 0.2 A	11.4 ± 0.3 A
3 months	CT	40.2 ± 0.6 Aa	43.9 ± 0.4 Ca	6.3 ± 0.5 Aa	9.6 ± 0.5 Aa
	BW	40.3 ± 0.5 a	42.9 ± 0.7 a	6.3 ± 0.3 a	10.5 ± 0.8 a
	BuM	41.8 ± 0.8 a	42.1 ± 0.2 a	5.8 ± 0.5 a	10.4 ± 0.4 a
	BcM	40.7 ± 1.6 a	42.2 ± 1.5 a	5.9 ± 0.7 a	11.1 ± 0.7 a
6 months	CT	40.2 ± 1.1 Aa	41.4 ± 1.8 Bca	7.1 ± 1.4 Aa	11.3 ± 1.5 Aa
	BW	39.2 ± 0.6 a	42.4 ± 0.7 a	6.9 ± 1.7 a	11.5 ± 1.5 a
	BuM	39.8 ± 0.1 a	42.7 ± 2.0 a	7.6 ± 1.7 a	9.8 ± 1.2 a
	BcM	40.2 ± 0.2 a	41.8 ± 2.0 a	8.0 ± 1.4 a	10.0 ± 1.2 a
9 months	CT	40.2 ± 0.7 Aa	41.0 ± 0.4 Ba	7.9 ± 0.7 Aa	10.9 ± 1.2 Aa
	BW	39.3 ± 1.2 a	39.6 ± 1.8 a	8.2 ± 0.2 a	12.8 ± 1.2 a
	BuM	38.6 ± 0.6 a	42.4 ± 1.1 a	7.2 ± 1.9 a	11.9 ± 1.9 a
	BcM	38.7 ± 1.1 a	42.2 ± 2.8 a	7.8 ± 1.7 a	11.3 ± 1.2 a

Capital letters compared the control between the different sample times. Lowercase letters compared the different treatments at each sample times. Different letters correspond to values statiscally different ($p < 0.05$)

CEC, $CaCO_3$ content), and it is known that metal fractionation is dependent on soil parameters (Lasat 2000; Gleyzes et al. 2002). Lastly, the input of biochars did not significantly change the Zn fractionation in the soil over the time.

Several studies evaluated the impacts of biochars on metal distribution and obtained results contradicting those found in this study. A decrease in the metal contents in the F1 fraction was generally observed following biochar amendments (chicken manure- and green waste-derived biochars (5% (*w*/*w*)), rice straw biochar (3–5% (*w*/*w*)), and wine lees biochar (0.5–1% (*w*/*w*))) (Park et al. 2011a; Jiang et al. 2012; Zhu et al. 2015). However, these results were obtained for acidic soils, and therefore, the conversion of the metal exchangeable fraction in less available forms can be for the most part explained by the increase in soil pH following biochar addition. Nevertheless, for alkaline soils, Zhao et al. (2016) also reported a reduction in the Cd F1 fraction following biochar addition at an application rate equal to 5, 10, and 15% (*w*/*w*). In their study, despite an initial alkaline pH (pH = 8.06), an increase in the soil pH following biochar addition at these three application rates was observed, probably explaining the decrease in the F1 fraction percentage. This hypothesis is supported by the study conducted by Zhang et al. (2016), who showed that the Zn and Cd availability in alkaline soil, measured with EDTA mixture, did not decrease following biochar addition. The authors explained these results by the lack of a significant increase in the pH of soils treated with all kinds of biochars. Moreover, the application rates used in their study were much higher than those used in the present study. Since it is known that the application rate has an impact on biochar effects (Houben et al. 2013; Bopp et al. 2016; Zhao et al. 2016), this factor might also be an explanation for the absence of an effect following biochar amendment.

Metal bioaccessibility

The results of Cd, Pb, and Zn bioaccessibility in the gastric (G) and gastrointestinal (GI) phases are presented in Fig. 2.

For the four conditions, in the gastric phase, the percentage of bioaccessibility ranged from 81 to 99% for Cd, 81 to 100% for Pb, and 54 to 62% for Zn. Therefore, the most bioaccessible metals in this soil are Cd and Pb, followed by Zn. The difference of bioaccessibility between the three metals is most likely due to the different chemical forms in which the metals are bound to the soil's constituents (Pelfrêne et al. 2011).

Concerning Cd, an increase in bioaccessibility from 87 to 96% was observed at 3 months compared to the initial time in the unamended soil. At 6 and 9 months, Cd bioaccessibility returned to a value similar to the initial time. The input of biochars modified Cd bioaccessibility in the gastric phase: (1) at 3 months, the BW and BuM biochars decreased the percentages from 96% in the unamended soil to 92 and 89%, respectively, and (2) at 9 months, differences were observed between the three biochars: bioaccessibility was statistically higher with BuM (92%) than with BW and BcM (83 and 84%, respectively).

Bioaccessibility increased from 91 to 99% at 3 months for Pb in the unamended soil and remained on the same order of magnitude at 6 and 9 months. The biochar amendments did not modify Pb bioaccessibility at 3 and 6 months, but a decrease from 98 to 94% was observed at 9 months with BcM compared to the control. Once again, statistical differences were observed between BuM and BcM, with percentages equal to 99 and 94%, respectively.

Lastly, a time effect was observed in the unamended soil at 3 and 9 months by decreasing Zn bioaccessibility from 61% (unamended soil) to 59 and 58%, respectively. The reduction in bioaccessibility might be correlated with the decrease observed in the F1 fraction of the sequential extraction (5.6%) over time. An effect of biochar amendment was observed at 3 months with BW, which decreased the Zn percentage from 59 to 56%. In contrast, at 6 months, BW did not modify Zn bioaccessibility, while BuM and BcM decreased Zn bioaccessibility from 63 to 56 and 57%, respectively. At 9 months, BuM increased the bioaccessibility percentage from 58 to 63% (compared with the unamended soil). Moreover, bioaccessibility was lower with BW (57%) and BcM (55%) than with BuM (63%).

For the gastrointestinal phase in the four conditions, bioaccessibility ranged from 39 to 51%, 12 to 25%, and 13 to 17% for Cd, Pb, and Zn, respectively. As in the gastric phase, the most bioaccessible metal was Cd, following by Pb and Zn.

The Cd bioaccessibility in the gastrointestinal phase significantly decreased from 46 to 42% for the unamended soil at 9 months, highlighting the impact of time and watering on oral bioaccessibility. Moreover, impacts of biochars on bioaccessibility were also demonstrated: (1) at 3 months, with a lower

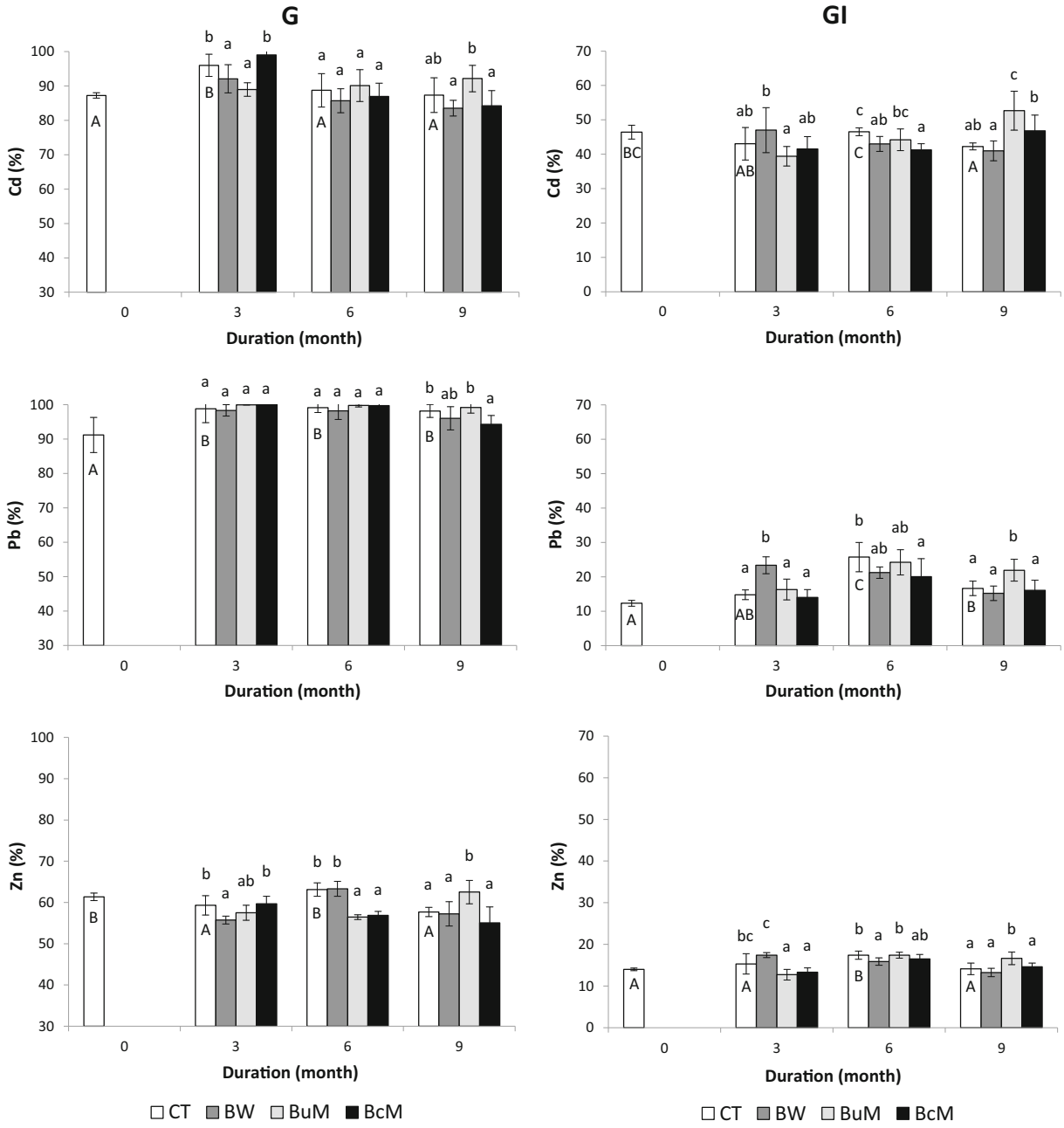

Fig. 2 Cd, Pb, and Zn oral bioaccessibility in the gastric (G) and gastrointestinal (GI) phases (results expressed as the percentage of the pseudototal soil trace element concentrations) for the non-amended soil (CT) and the soil amended with the woody biochar (BW), the biochar made from *Miscanthus* cultivated on uncontaminated soil (BuM), and the biochar made from *Miscanthus* cultivated on contaminated soil (BcM) ($n = 3$). Capital letters compared the control between the different sampling times. Lowercase letters compared the different treatments at each sampling times. Different letters correspond to values statiscally different ($p < 0.05$)

Cd percentage for BuM (39%) compared to BW (47%); (2) at 6 months with lower bioaccessibility for the soil amended with BcM (from 47 to 41%); and (3) at 9 months, where the amendment with BuM (53%) increased the Cd percentages compared to CT (42%), BW (41%), and BcM (47%).

Contrary to Cd, higher Pb percentages were observed at 6 and 9 months for CT, with increases from

12% (0 month) to 26 and 17%, respectively. Concerning the amendments, different trends were observed for the three sampling times. At 3 months, BW increased the Pb bioaccessibility from 15 to 23%. At 6 months, BcM decreased bioaccessibility from 26 to 20%; in contrast, at 9 months, BuM increased bioaccessibility from 17 to 22%.

As for Pb, the time and watering increased Zn bioaccessibility at 6 months from 14 to 17%, but then, at 9 months, the values became similar to the control at the initial time. The two biochars made from miscanthus decreased bioaccessibility at 3 months from 15 to 13%. At 6 months, BW also decreased Zn percentages from 17 to 16%, and at 9 months, BuM increased the percentage from 14% (CT) to 17%.

Concretely, no clear trend was observed on oral bioaccessibility following biochar amendments. These results can be explained by the absence of biochar effects on metal distribution in soil. Several studies evaluated the influence of biochars on metal bioaccessibility. Despite using other oral bioaccessibility tests (i.e., PBET, glycine extraction, and SBET), many studies also found no effect after adding biochar (Ahmad et al. 2012; Uchimiya et al. 2012; Rizwan et al. 2016; Cui et al. 2016). In addition to these hypotheses, the application rate might also be a factor explaining the absence of an effect. In this study, a 2% application rate was used, while the majority of studies used higher application rates (varying between 3 and 20% (*w*/*w*)).

However, differences were obtained between the two biochars made from miscanthus. At 9 months, higher bioaccessibility was always observed with BuM compared to BcM for the three metals and the two phases. There are two characteristics that can differentiate these biochars: the pyrolysis conditions and the origin of the feedstock used to produce these biochars. These two biochars were both pyrolyzed at 600 °C but for 30 min for BuM and 1 h for BcM. Several studies demonstrated the influence of residence time on biochar characteristics, with modification of the ash and volatile matter content, the elemental composition, and even the pore volume in biochar, for example (Peng et al. 2011; Wu et al. 2012; Ronsse et al. 2013; Lin et al. 2016). Therefore, modifying these characteristics might result in different impacts on soil parameters, and consequently on metal retention in soil. Indeed, the influence of biochar characteristics on metal sorption has been proven in several studies (Uchimiya et al. 2011; Kołodyńska et al. 2012; Zhang et al. 2013). Uchimiya et al. (2011) highlighted the influence of the surface functional groups of biochars, related to their pH and oxygen content, on their ability to retain metals (Ni, Cu, Pb, Cd) in soils. Moreover, the diffusion of metals through the macro- and micro-pores of biochars can also reduce their mobility (Kołodyńska et al. 2012; Inyang et al. 2016). In the present study, BcM presented a higher O/C ratio and SSA, which can explain the lower bioaccessibility measured with this biochar compared to BuM. Moreover, for the three metals and the two phases (except for Cd in the gastrointestinal phase), the soil amended with BcM presented metal bioaccessibility values similar to BW, a commercialized biochar. This revealed no additional risks for metal bioaccessibility linked to the use of the biochar produced with miscanthus cultivated on contaminated soil. This highlights the possibility of using contaminated sites to produce biochars for soil remediation and thus ensure income from these lands that are now unusable for food production.

Conclusion

The present study was conducted to determine and compare the efficiency of three biochars produced with different feedstock and pyrolysis processes on environmental availability and oral bioaccessibility of Cd, Pb, and Zn in a slightly alkaline soil. These results showed that biochar application did not necessarily lead to lower metal availability in the alkaline soil at the dose tested. However, differences in efficiency between the three biochars tested were highlighted, especially between the two biochars made with *Miscanthus* × *giganteus*, with lower efficiency for the biochar produced with miscanthus cultivated on uncontaminated agricultural soil. These differences were probably related to the distinct pyrolysis process between the two miscanthus biochars, which led to different biochar properties. Moreover, similar results were obtained between the biochar made with miscanthus cultivated on contaminated soil and the biochar made from wood. These results showed the advantage of using biochars produced with biomass cultivated on contaminated soil, thus showing that these sites, unusable for food production, could possibly be developed. It would therefore be interesting to pay greater attention to the use of biochars made with biomass cultivated on contaminated soil to remediate polluted soil while ensuring their harmless

and nontoxic effects, especially for the soil's living organisms and plants.

Acknowledgments The authors wish to thank the Hauts-de-France Regional Council and Bpifrance for the financial support of this research and the CIRAD for the production and characterization of the biochars. The authors also warmly thank David Houben who provided the biochar made from *Miscanthus* × *giganteus* cultivated on uncontaminated soil.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

AFNOR B (2006) NFU 44–051. Amendements organiques, dénominations, spécifications et marquages. 15p

Ahmad, M., Lee, S. S., Yang, J. E., Ro, H. M., Han Lee, Y., & Sik Ok, Y. (2012). Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. *Ecotoxicology and Environmental Safety, 79*, 225–231. https://doi.org/10.1016/j.ecoenv.2012.01.003.

Ahmad, M., Lee, S. S., Lee, S. E., Al-Wabel, M. I., Tsang, D. C., & Ok, Y. (2017). Biochar-induced changes in soil properties affected immobilization/mobilization of metals/metalloids in contaminated soils. *Journal of soils and sediments, 17*(3), 717–730. https://doi.org/10.1007/s11368-015-1339-4.

Al-Wabel, M. I., Al-Omran, A., El-Naggar, A. H., Nadeem, M., & Usman, A. R. A. (2013). Pyrolysis temperature induced changes in characteristics and chemical composition of biochar produced from *Conocarpus* wastes. *Bioresource Technology, 131*, 374–379. https://doi.org/10.1016/j.biortech.2012.12.165.

Al-Wabel, M. I., Usman, A. R. A., El-Naggar, A. H., Aly, A. A., Ibrahim, H. M., & Elmaghraby, S. (2015). *Conocarpus* biochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. *Saudi Journal of Biological Sciences, 22*, 503–511. https://doi.org/10.1016/j.sjbs.2014.12.003.

Antal, M. J., & Grønli, M. (2003). The art, science, and technology of charcoal production†. *Industrial and Engineering Chemistry Research, 42*, 1619–1640. https://doi.org/10.1021/ie0207919.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J. L., Harris, E., Robinson, B., & Sizmur, T. (2011). A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environmental Pollution, 159*, 3269–3282. https://doi.org/10.1016/j.envpol.2011.07.023.

Bian, R., Joseph, S., Cui, L., Pan, G., Li, L., Liu, X., Zhang, A., Rutlidge, H., Wong, S., Chia, C., Marjo, C., Gong, B., Munroe, P., & Donne, S. (2014). A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. *Journal of Hazardous Materials, 272*, 121–128. https://doi.org/10.1016/j.jhazmat.2014.03.017.

Bopp, C., Christl, I., Schulin, R., & Evangelou, M. W. H. (2016). Biochar as possible long-term soil amendment for phytostabilisation of TE-contaminated soils. *Environmental Science and Pollution Research, 23*, 17449–17458. https://doi.org/10.1007/s11356-016-6935-3.

Břendová, K., Tlustoš, P., & Száková, J. (2015). Can biochar from contaminated biomass be applied into soil for remediation purposes? *Water, Air, and Soil Pollution, 226*, 193. https://doi.org/10.1007/s11270-015-2456-9.

Cui, H., Fan, Y., Fang, G., Zhang, H., Su, B., & Zhou, J. (2016). Leachability, availability and bioaccessibility of cu and cd in a contaminated soil treated with apatite, lime and charcoal: a five-year field experiment. *Ecotoxicology and Environmental Safety, 134*, 148–155. https://doi.org/10.1016/j.ecoenv.2016.07.005.

Ehsan, M., Barakat, M. A., Husein, D. Z., & Ismail, S. M. (2014). Immobilization of Ni and Cd in soil by biochar derived from unfertilized dates. *Water, Air, and Soil Pollution, 225*, 2123. https://doi.org/10.1007/s11270-014-2123-6.

Evangelou, M. W. H., Brem, A., Ugolini, F., Abiven, S., & Schulin, R. (2014). Soil application of biochar produced from biomass grown on trace element contaminated land. *Journal of Environmental Management, 146*, 100–106. https://doi.org/10.1016/j.jenvman.2014.07.046.

Gleyzes, C., Tellier, S., & Astruc, M. (2002). Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. *TrAC Trends in Analytical Chemistry, 21*, 451–467.

Houben, D., Evrard, L., & Sonnet, P. (2013). Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (*Brassica napus* L.) *Biomass and Bioenergy, 57*, 196–204. https://doi.org/10.1016/j.biombioe.2013.07.019.

Inyang, M. I., Gao, B., Yao, Y., Xue, Y., Zimmerman, A., Mosa, A., Pullammanappallil, P., Ok, Y., & Cao, X. (2016). A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. *Critical Reviews in Environmental Science and Technology, 46*, 406–433. https://doi.org/10.1080/10643389.2015.1096880.

Janus, A., Pelfrêne, A., Heymans, S., Deboffe, C., Douay, F., & Waterlot, C. (2015). Elaboration, characteristics and advantages of biochars for the management of contaminated soils with a specific overview on *Miscanthus* biochars. *Journal of Environmental Management, 162*, 275–289. https://doi.org/10.1016/j.jenvman.2015.07.056.

Jha, P., Biswas, A. K., Lakaria, B. L., & Rao, A. S. (2010). Biochar in agriculture-prospects and related implications. *Current Science, 99*, 1218–1225.

Jiang, J., Xu, R., Jiang, T., & Li, Z. (2012). Immobilization of Cu(II), Pb(II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. *Journal of Hazardous Materials, 229–230*, 145–150. https://doi.org/10.1016/j.jhazmat.2012.05.086.

Kabata-Pendias, A., & Pendias, H. (1984). *Trace elements in soils and plants*. Boca Raton: CRC Press.

Kobya, M., Demirbas, E., Senturk, E., & Ince, M. (2005). Adsorption of heavy metal ions from aqueous solutions by activated carbon prepared from apricot stone. *Bioresource*

Technology, 96, 1518–1521. https://doi.org/10.1016/j.biortech.2004.12.005.

Kołodyńska, D., Wnętrzak, R., Leahy, J. J., Hayes, M. H. B., Kwapiński, W., & Hubicki, Z. (2012). Kinetic and adsorptive characterization of biochar in metal ions removal. *Chemical Engineering Journal, 197*, 295–305. https://doi.org/10.1016/j.cej.2012.05.025.

Kwapinski W., Wolfram P., Byrne C., Melligan F., Novotny E. H., Leahy J. J., Hayes M. H. B. (2010) Properties of biochar produced from *Miscanthus* × *giganteus* and its influence the growth of maize (*Zea mays* L.). In Embrapa Solos-Artigo em anais de congresso (ALICE). In: Fifteenth meeting of the international humic substances society, humic substances and the maintenance of ecosystem services - vol. 1, June 27 July 2, 2010. Tenerife, Spain. pp 95–99.

Lasat, M. (2000). Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *Journal of Hazardous Substance Research, 2*, 1–25. https://doi.org/10.4148/1090-7025.1015.

Lehmann, J., & Joseph, S. (2009). *Biochar for environmental management: science and technology*. Sterling: Earthscan, London.

Lei, O., & Zhang, R. (2013). Effects of biochars derived from different feedstocks and pyrolysis temperatures on soil physical and hydraulic properties. *Journal of Soils and Sediments, 13*, 1561–1572. https://doi.org/10.1007/s11368-013-0738-7.

Lin, Y., Yan, W., & Sheng, K. (2016). Effect of pyrolysis conditions on the characteristics of biochar produced from a tobacco stem. *Waste Management and Research, 34*, 793–801. https://doi.org/10.1177/0734242X16654977.

Martin, T. A., & Ruby, M. V. (2004). Review of in situ remediation technologies for lead, zinc, and cadmium in soil. *Remediation Journal, 14*, 35–53. https://doi.org/10.1002/rem.20011.

Méndez, A., Gómez, A., Paz-Ferreiro, J., & Gascó, G. (2012). Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. *Chemosphere, 89*, 1354–1359. https://doi.org/10.1016/j.chemosphere.2012.05.092.

Namgay, T., Singh, B., & Singh, B. P. (2010). Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (*Zea mays* L.) *Australian Journal of Soil Research, 48*, 638–647. https://doi.org/10.1071/SR10049.

Niu, L., Jia, P., Li, S., Kuang, J. L., He, X. X., Zhou, W. H., Liao, B., Shu, W. S., & Li, J. T. (2015). Slash-and-char: an ancient agricultural technique holds new promise for management of soils contaminated by Cd, Pb and Zn. *Environmental Pollution, 205*, 333–339. https://doi.org/10.1016/j.envpol.2015.06.017.

Novak, J. M., Lima, I., Xing, B., Gaskin, J. W., Steiner, C., Das, K. C., Ahmedna, M., Rehrah, D., Watts, D. W., Busscher, W. J., & Schomberg, H. (2009). Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. *Annals of Environmental Science, 3*, 195–206.

Park, J. H., Choppala, G. K., Bolan, N. S., Chung, J. W., & Chuasavathi, T. (2011a). Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant and Soil, 348*, 439–451. https://doi.org/10.1007/s11104-011-0948-y.

Park, J. H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., & Chung, J. W. (2011b). Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. *Journal of Hazardous Materials, 185*, 549–574. https://doi.org/10.1016/j.jhazmat.2010.09.082.

Pelfrêne, A., Waterlot, C., Mazzuca, M., Nisse, C., Bidar, G., & Douay, F. (2011). Assessing Cd, Pb, Zn human bioaccessibility in smelter-contaminated agricultural topsoils (northern France). *Environmental Geochemistry and Health, 33*, 477–493. https://doi.org/10.1007/s10653-010-9365-z.

Peng, X., Ye, L. L., Wang, C. H., Zhou, H., & Sun, B. (2011). Temperature- and duration-dependent rice straw-derived biochar: characteristics and its effects on soil properties of an Ultisol in southern China. *Soil and Tillage Research, 112*, 159–166. https://doi.org/10.1016/j.still.2011.01.002.

Pérez-Esteban, J., Escolástico, C., Moliner, A., Masaguer, A., & Ruiz-Fernández, J. (2014). Phytostabilization of metals in mine soils using *Brassica juncea* in combination with organic amendments. *Plant and Soil, 377*, 97–109. https://doi.org/10.1007/s11104-013-1629-9.

Rizwan, M. S., Imtiaz, M., Huang, G., Chhajro, M., Liu, Y., Fu, Q., Zhu, J., Ashraf, M., Zafar, M., Bashir, S., & Hu, H. (2016). Immobilization of Pb and Cu in polluted soil by superphosphate, multi-walled carbon nanotube, rice straw and its derived biochar. *Environmental Science and Pollution Research, 23*, 15532–15543. https://doi.org/10.1007/s11356-016-6695-0.

Rodríguez-Vila, A., Covelo, E. F., Forján, R., & Asensio, V. (2015). Recovering a copper mine soil using organic amendments and phytomanagement with *Brassica juncea* L. *Journal of Environmental Management, 147*, 73–80. https://doi.org/10.1016/j.jenvman.2014.09.011.

Ronsse, F., van Hecke, S., Dickinson, D., & Prins, W. (2013). Production and characterization of slow pyrolysis biochar: influence of feedstock type and pyrolysis conditions. *GCB Bioenergy, 5*, 104–115. https://doi.org/10.1111/gcbb.12018.

Ruby, M. V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D. E., Casteel, S. W., Berti, W., Carpenter, M., Edwards, D., Cragin, D., & Chappell, W. (1999). Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environmental Science and Technology, 33*, 3697–3705. https://doi.org/10.1021/es990479z.

Shen, Z., Hou, D., Zhao, B., Xu, W., Ok, Y., Bolan, N. S., & Alessi, D. S. (2018). Stability of heavy metals in soil washing residue with and without biochar addition under accelerated ageing. *Science of The Total Environment, 619*, 185–193. https://doi.org/10.1016/j.scitotenv.2017.11.038.

Uchimiya, M., Wartelle, L. H., Klasson, K. T., Wartelle, L. H., & Lima, I. M. (2011). Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *Journal of Agricultural and Food Chemistry, 59*, 2501–2510. https://doi.org/10.1021/jf104206c.

Uchimiya, M., Bannon, D. I., Wartelle, L. H., Lima, I. M., & Klasson, K. T. (2012). Lead retention by broiler litter biochars in small arms range soil: Impact of pyrolysis temperature. *Journal of Agricultural and Food Chemistry, 60*, 5035–5044. https://doi.org/10.1021/jf300825n.

Waterlot, C., Pruvot, C., Ciesielski, H., & Douay, F. (2011). Effects of a phosphorus amendment and the pH of water used for watering on the mobility and phytoavailability of Cd, Pb and Zn in highly contaminated kitchen garden soils. *Ecological*

Engineering, 37, 1081–1093. https://doi.org/10.1016/j.ecoleng.2010.09.001.

Waterlot, C., Bidar, G., Pruvot, C., & Douay, F. (2012). Effects of grinding and shaking on Cd, Pb and Zn distribution in anthropogenically impacted soils. *Talanta, 98*, 185–196. https://doi.org/10.1016/j.talanta.2012.06.068.

Wragg, J., Cave, M., Basta, N., Brandon, E., Casteel, S., Denys, S., Gron, C., Oomen, A., Reimer, K., Tack, K., & van de Wiele, T. (2011). An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. *Science of the Total Environment, 409*, 4016–4030. https://doi.org/10.1016/j.scitotenv.2011.05.019.

Wu, W., Yang, M., Feng, Q., McGrouther, K., Wang, H., Lu, H., & Chen, H. (2012). Chemical characterization of rice straw-derived biochar for soil amendment. *Biomass and Bioenergy, 47*, 268–276. https://doi.org/10.1016/j.biombioe.2012.09.034.

Yuan, H., Lu, T., Wang, Y., Huang, H., & Chen, Y. (2014). Influence of pyrolysis temperature and holding time on properties of biochar derived from medicinal herb (*Radix isatidis*) residue and its effect on soil CO_2 emission. *Journal of Analytical and Applied Pyrolysis, 110*, 277–284. https://doi.org/10.1016/j.jaap.2014.09.016.

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N., Pei, J., & Huang, H. (2013). Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. *Environmental Science and Pollution Research, 20*, 8472–8483. https://doi.org/10.1007/s11356-013-1659-0.

Zhang, G., Guo, X., Zhao, Z., He, Q., Wang, S., Zhu, Y., Yan, Y., Liu, X., Sun, K., Zhao, Y., & Qian, T. (2016). Effects of biochars on the availability of heavy metals to ryegrass in an alkaline contaminated soil. *Environmental Pollution, 218*, 513–522. https://doi.org/10.1016/j.envpol.2016.07.031.

Zhao, B., Xu, R., Ma, F., Li, Y., & Wang, L. (2016). Effects of biochars derived from chicken manure and rape straw on speciation and phytoavailability of cd to maize in artificially contaminated loess soil. *Journal of Environmental Management, 184*, 569–574. https://doi.org/10.1016/j.jenvman.2016.10.020.

Zhu, Q., Wu, J., Wang, L., Yang, G., & Zhang, X. (2015). Effect of biochar on heavy metal speciation of paddy soil. *Water, Air, and Soil Pollution, 226*, 429. https://doi.org/10.1007/s11270-015-2680-3.