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Biochar from Different Carbonaceous Waste Materials: Ecotoxicity and Effectiveness in the Sorption of Metal(loid)s

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Abstract In this study, biochar produced by pyrolysis of urban pruning wood (Bpw) and sewage sludge (Bss) were characterized and investigated as adsorbents for the removal of Cu(II), Pb(II), Zn(II), and As(V) from contaminated solutions. Both types of biochars showed different physical-chemical properties and metal(loid) content. In Bss, Cu, Zn, and Pb concentrations exceeded the upper limit of the common ranges in soils. However, when they were tested for their effect on soil invertebrates, neither of the biochar was expected to exert negative effects as long as the dose applied as an amendment was ≤ 4.8 t ha^{-1}. For an assessment of the effectiveness of biochar in the immobilization of metal(loid)s, three contaminated solutions with acidic pH and different pollutant concentrations were added to both types of biochar. Precipitation as oxy-hydroxides and the formation of complexes with active functional groups of the organic matter were the main mechanisms of metal(loid) fixation by the biochar, with increased precipitation and a rising pH. Both types of biochar were effective at immobilizing Pb and Cu, while Zn showed less effectiveness in this regard and As the least. The high P content of the biochar from sewage sludge favored Pb fixation, presumably forming complexes with phosphates, while competition between phosphate and arsenate ions decreased As adsorption by Fe compounds. The metal(loid)s immobilized by biochar from urban pruning wood were more bioavailable than those fixed by biochar from sewage sludge.

Keywords Organic waste · Biochar · Amendment · Risk assessment · Pollutant immobilization

1 Introduction

Pyrolysis is conducted by heating organic material in a closed container with little or no available oxygen. Pyrolysis of carbonaceous materials results in biochar, characterized by a neutral or alkaline pH, low bulk density, high carbon content, high surface area and pore volume, and high ion-exchange capacity (Lehmann and Joseph 2009). These properties, which vary considerably depending on the heating temperature and the carbonaceous material used (Ahmad et al. 2014), enable biochar to be used for diverse purposes such as carbon sequestration (Lehmann et al. 2008), soil fertility improvement (Glaser et al. 2002), pollution remediation (Beesley and Marmiroli 2011; Park et al. 2013), and waste recycling (Vithanage et al. 2016). The soil application of some biochars might degrade soil quality. Some authors suggest a need to demonstrate both the benefits of biochar to soil health and lack of detrimental effects to the environment (Verheijen et al. 2010). Bioassay-based approaches, similar to what has been

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proposed for characterizing wastes in the EU (Moser and Römbke 2009), may complement the physicochemical characterization for the quality assessment of biochars.

Different types of biochar are emerging as compounds capable of reducing the bioavailability of contaminants in the environment (Sohi 2012). In addition, biochar from waste biomass, compared to other biomass, has the advantages of offering cost-effectiveness and contributing to food security (Brick 2010), while at the same time serving for waste recycling and reuse, this being one of the main objectives of the Sixth Environmental Action Plan 2002 (The European Parliament and the Council of the EU 2002). The effectiveness of biochar at remediating polluted water and soils is based on two of its fundamental properties such as its alkaline pH and relatively high ion-exchange capacity. The rise in pH encourages the precipitation or co-precipitation of heavy metals (Xu et al. 2002; Simón et al. 2005) while also increasing the cation exchange capacity as a consequence of a higher degree of dissociation of the –OH and –COOH groups of organic matter (Evangelou 1998; Mohan et al. 2007; Tong et al. 2011). The result is lower solubility and bioavailability of heavy metals due to greater precipitation or adsorption by organic matter. In contrast, the solubility of metalloids such as As tends to increase with rising pH values (Fitz and Wenzel 2002), probably due to the dominance of more negatively charged arsenate species under basic conditions ($HAsO_4^{-2}$) and the stronger repulsion of the negatively charged surface of the organic matter (González et al. 2012). The stronger competition of OH^- by the anion exchange sites could be another mechanism that would explain the lower adsorption of As with a rising pH (Thanabalasingan and Pickering 1986). Although the negative charges on the surface of the biochar are dominant, some biochar produced at high temperatures may also show positive charges with the ability to adsorb anions such as arsenates (Gai et al. 2014; Mukherjee et al. 2011).

On the other hand, certain constituents of the biochar could also affect the adsorption of metal(loid)s. Thus, iron oxy-hydroxides have a high affinity for As (Fritzsche et al. 2011), with which they form inner-sphere surface complexes (Sherman and Randall 2003) that decrease As solubility and bioavailability, while phosphate ions compete very effectively with the arsenates for the anion exchange sites reducing As adsorption (Adriano 2001) but effectively precipitating Pb as Pb-phosphate minerals (Cao et al. 2002). Calcium and calcium carbonate are other possible constituents of the biochar that can affect

the immobilization of metal(loid)s as a result of their ability to raise the pH (Cao and Harris 2010). Bothe and Brown (1999) indicated that As mobility diminishes from the formation of As-Ca complexes, whereas Moon et al. (2004) revealed that As immobilization augmented as the Ca/As molar ratio increased. In the case of $CaCO_3$, Pokrovsky and Scott (2002) indicated that arsenate can be adsorbed on $> Ca^+$ sites present on the surface of $CaCO_3$, whereas Alexandratos et al. (2007) showed the formation of inner-sphere complexes between AsO_4 tetrahedra and surface CaO_6 octahedra. Simón et al. (2015) indicated that As was sorbed onto $CaCO_3$ grains forming outer-sphere and inner-sphere complexes as well as forming Pb-Ca-arsenate crystals on the surface of $CaCO_3$.

According to the above, the immobilization mechanisms of metal(loid)s by the biochar can be varied, while biochar produced from different feedstocks and at varying temperatures can differ in physical and chemical properties (Ahmad et al. 2014). Both factors imply that the results of the pollutant immobilization by biochar can be diverse and even contradictory, especially when the experimental conditions vary. Different studies (Hartley et al. 2009; Namgay et al. 2010; Zheng et al. 2012; Beesley and Marmiroli 2011) note the need to expand knowledge of the mechanisms by which different types of biochar immobilize diverse pollutants. In this study, we investigate the physical-chemical properties of two biochars from waste carbonaceous materials, evaluating their potential risk to soil invertebrates and their sorption ability of metal(loid)s. The mechanisms responsible for contaminant removal were elucidated at different metal concentrations.

2 Materials and Methods

2.1 Biochar Characterization

Sewage sludge previously dried at 110 °C and urban pruning wood (Bss and Bpw, respectively) were heated in a pyrolytic furnace at a heating rate of 10 °C min^{-1} up to 500 °C, a temperature that was maintained for 30 min. Both types of biochar were ground and sieved at 2 mm. The $CaCO_3$-equivalent content was estimated manometrically (Williams 1949). The specific surface area (SA) and the volume of micropores (Vmicro) were determined using a six-point N_2-BT method (ASAP 2020 – Physisorption Analyzer). The bulk density

(BD) was estimated by the cylinder of a known volume method (Blake and Hartge 1986). The pH was measured in a 1:5 solid-water suspension. A saturation extract of each biochar was prepared (US Salinity Laboratory Staff 1954), the liquid phase was vacuum pumped, and the electrical conductivity (EC) was measured. The cation exchange capacity (CEC) was determined with 1 M Na-acetate at pH 8.2 (Rhoades 1982). The organic carbon (OC) content was analyzed by the wet oxidation method (Mingorance et al. 2007), and the nitrogen content was determined by the Kjeldahl method (Jones 1991). Humic substances were extracted with $Na_4P_2O_7 \cdot 10H_2O$ and NaOH to pH 13, and the solution was acidified with H_2SO_4 until pH 1, separating humic acids (HA) that precipitate and fulvic acids (FA) that remain soluble (Kononova 1966). The organic carbon of the HA and FA was determined according to Mingorance et al. (2007). The atomic percentage at the surface of the biochar (~ 1 nm thick) was determined by R-X Photoelectron Spectroscopy (XPS) in a Kratos Axis Ultra-DLD spectrometer. The total content of major elements was measured by X-ray fluorescence (XFR) in a Philips Magix Pro PW-2440 spectrometer, and the total concentration of metal(loid)s was performed by strong acid ($HNO_3 + HF + HCl$) digestion and a subsequent analysis by inductively coupled plasma-mass spectrometry (ICP-MS) in a Thermo Fisher XSERIES 2 ICP-MS spectrometer. The ICP–MS operating conditions included three replicates of each measurement. All standards were prepared from ICP single-element standard solutions (Merck, Darmstadt, Germany) after dilution with 10% HNO_3. For calibration, two sets of multi-element standards containing all the analytes of interest at five different concentrations were prepared using rhodium as the internal standard. Procedural blanks that were used for the estimated detection limits ($3*\sigma$; $n = 6$) were < 0.21 ppb for As, < 2.68 ppb for Zn, < 0.12 ppb for Cu, and < 0.23 ppb for Pb. The analytical precision was better than ± 6% in all cases.

2.2 Effect on Soil Biota

For an estimate of the biochar ecological risk, the predicted no-effect concentration (PNEC) was used, this being defined as concentration below which an ecosystem is not expected to suffer unacceptable (Domene et al. 2008), and *Enchytraeus crypticus* was selected for this study. The biochars were mixed with

uncontaminated Lufa 2.2 natural soil reach biochar concentrations of 500, 250, 125, 62.5, and 31.3 g/kg, and effects on survival (LC_{50}) and reproduction (EC_{50} and EC_{20}) were recorded after 21 days in all treatments at each dilution concentration (OECD 220 2004), three replicates. The EC_{20} (concentration causing a 20% reduction of the reproduction) was considered to be a reliable and realistic value of maximum tolerable inhibition (Hoekstra and Van Ewijk 1993; Jager et al. 2006). The PNEC was calculated from EC_{20}, considering an ideal soil with a 20-cm plow layer and a density of 1.25 g cm^{-3} and applying a assessment factor of 100 to the EC_{20} (ECHA 2008).

2.3 Pollutant Solutions

For an evaluation of biochar effectiveness for immobilizing metal(loid)s at different concentrations, as in the case of acid saline drainage from sulfide mining characterized by extreme acidity and high salinity and concentrations of pollutants (Sánchez España et al. 2005), three acidic standard solutions in nitric acid were prepared from stock solutions (1000 mg L^{-1}, reagent-grade, Merck). Solution S1 containing 300 mg L^{-1} of Cu, 300 mg L^{-1} of Zn, 100 mg L^{-1} of As, and 100 mg L^{-1} of Pb, with EC = 121 dS m^{-1} and pH = 1.0, represented the highest degree of contamination. Solution S2 containing 150 mg L^{-1} of Cu, 150 mg L^{-1} of Zn, 50 mg L^{-1} of As, and 50 mg L^{-1} of Pb, with EC = 62 dS m^{-1} and pH = 1.3, represented intermediate contamination. Solution S3 containing 75 mg L^{-1} of Cu, 75 mg L^{-1} of Zn, 25 mg L^{-1} of As, and 25 mg L^{-1} of Pb, with EC = 32 dS m^{-1} and pH = 1.5, represented relatively low contamination.

2.3.1 Preparation of the Suspensions and Liquid-Phase Analysis

Two grams of each biochar was placed in contact with 25 cm^3 of each acidic solution (three replicates) for a total of 18 suspensions. In addition, 25 cm^3 of each acid solution was not in contact with the biochar (three replicates). The suspensions and the acidic solutions without biochar were shaken periodically and after 3 days were passed through cellulose filters (0.45-μm pore size). The pH levels and total concentrations of As, Pb, Zn, and Cu in the liquid phases were measured by ICP-MS immediately after filtering. The amounts of immobilized metal(loid)s by the different types of biochar were calculated by

the difference between the concentrations in the filtered acidic solution without biochar (S1, S2, and S3) and in the filtered liquid phase of the suspensions (hereafter E1, E2, and E3, respectively).

2.3.2 Solid-Phase Analysis

In the solid phase of the suspensions, a five-step sequential extraction of the pollutants was performed, modifying the method of Wenzel et al. (2001): step 1, extraction with 0.05 M $(NH_4)_2SO_4$, 20 °C/4 h to estimate non-specifically sorbed pollutants (fraction S); step 2, extraction with 0.05 M $NH_4H_2PO_4$, 20 °C/16 h to estimate specifically sorbed pollutants (fraction P); step 3, extraction with 0.11 M acetic acid, 20 °C/16 h to estimate acid-soluble pollutants presumably precipitated as oxy-hydroxides and bound to carbonates (fraction A); step 4, extraction with 0.2 M NH_4^+-oxalate buffer + ascorbic acid pH 3.25, 96 °C/0.5 h to estimate the reducible phase of the pollutants presumably bound to hydrous oxides of Fe and Al (fraction O); and step 5, strong acid (HNO_3 + HF + HCl) digestion to estimate the residual fraction (fraction R). The concentrations of metal(loid)s in the extracts were measured by ICP-MS, and control samples were used to check the matrix effect of each extraction.

2.4 Statistical Methods

The data distributions in the different treatments were established by calculating the mean values and the standard deviations. The differences between the individual means were compared using Tukey's test ($p < 0.05$). The results were submitted to a correlation analysis, and the coefficient of determination (r^2) and p value were computed. The SPSS (PASW Statistics 20) software package was used for all statistical analyses.

3 Results

3.1 Properties of the Biochar

The properties of the two types of biochar clearly differed (Table 1). Biochar from sewage sludge (Bss) had notably higher bulk density (BD) and higher content in N and humic acids (HA), while biochar from urban pruning wood (Bpw) registered higher values of surface area (SA), micropore volume (Vmicro), electrical conductivity (EC), cation exchange capacity (CEC), and pH, and had a

greater content of organic carbon (OC), calcium carbonate, and fulvic acids (FA). In any case, the percentage of humic substances (HA + FA) in relation to the total OC was similar in both types of biochar (17% in Bss and 20% in Bpw), being constituted almost exclusively by fulvic acids. Although the cation exchange capacity per unit weight (CEC) was clearly higher in Bpw, per unit area, it was almost five times higher in Bss ($0.50 \pm 0.01 \times 10^{-3}$ $cmol_c$ m^{-2}) than in Bpw ($0.11 \pm 0.01 \times 10^{-3}$ $cmol_c$ m^{-2}). The atomic percentage at the surface of both types of biochar estimated by XPS also showed clear differences, with a greater percentage of O, N, Ca, P, and Si on the Bss surface. Only the percentage of C was higher on the Bpw surface. The content of major elements estimated by XFR (S, P, Al, Fe, Si, Mg, and Na) was clearly greater in Bss, and only the content of Ca and above all K was greater in Bpw. The concentrations of potentially toxic trace elements estimated by ICP-MS were also clearly higher in Bss, especially Zn, Pb, and Cu. Arsenic concentration, although higher in Bss, was relatively low in both types of biochar.

3.2 Biochar Risk Assessments

After 21 days of exposure (Table 2), in the two biochars studied, the estimated value of LC_{50} exceeded the highest concentration tested (500 g kg^{-1} soil). Regarding enchytraeid reproduction, biochar from sewage sludge (Bss) showed no effect, with an estimated EC_{50} value higher than the top concentration tested. However, biochar from urban pruning waste (Bpw) reduced the number of juveniles to 50% with respect to the control soil at an application rate of 260 g biochar kg^{-1} soil (200–330 g kg^{-1}, 95% confidence intervals). The estimated EC_{20} values for these biochars were 462 g kg^{-1} (109–518 g kg^{-1}, 95% c.i.) in the case of Bss and 193 g kg^{-1} (143–260 g kg^{-1}, 95% c.i.) in the case of Bpw. The calculated PNEC values were 11.5 and 4.8 t dry mass ha^{-1} for Bss and Bpw, respectively.

3.3 Properties of the Suspensions

The pH of each suspension (E1, E2, and E3) clearly rose relative to the contaminant solution (S1, S2, and S3) from which it came (Fig. 1a). In the E1 suspensions, the increase of the pH in the suspensions with Bss (pH = 2.5) was greater than in those with Bpw (pH = 1.3), while in the E2 and E3 suspensions, the highest pH values was reached in the presence of Bpw. The EC values (Fig. 1b)

Table 1 Mean and standard deviation of the selected properties of biochar from sewage sludge (Bss) and urban pruning wood (Bpw)

Biochar			Biochar		
Properties	Bss	Bpw	Properties	Bss	Bpw
BD (g cm^{-3})	0.63 ± 0.04	0.32 ± 0.02	XFR (g kg^{-1})		
SA (m^2 g^{-1})	50.5 ± 0.8	337 ± 5	S	18.2 ± 0.92	1.65 ± 0.14
Vmicro (cm^3 kg^{-1})	20.4 ± 0.4	134 ± 2	P	58.6 ± 4.7	5.80 ± 0.42
EC (dS m^{-1})	0.18 ± 0.03	0.52 ± 0.04	Al	25.2 ± 2.3	2.06 ± 0.20
CEC (cmol$_c$ kg^{-1})	25.4 ± 0.6	36.9 ± 1.4	Fe	3.73 ± 0.30	0.51 ± 0.08
pH	8.2 ± 0.1	9.8 ± 0.4	Si	54.7 ± 5.3	7.38 ± 0.63
CaCO$_3$ (g kg^{-1})	70.7 ± 0.31	84.5 ± 0.24	Ca	162 ± 7	229 ± 16
OC (g kg^{-1})	354 ± 26	656 ± 33	Mg	11.9 ± 0.4	5.62 ± 0.38
N (g kg^{-1})	4.56 ± 0.33	0.68 ± 0.07	Na	3.65 ± 0.35	nd
HA (g kg^{-1})	1.22 ± 0.08	0.15 ± 0.03	K	9.22 ± 0.32	31.9 ± 1.6
FA (g kg^{-1})	59.3 ± 3.8	128 ± 9	ICP-MS (mg kg^{-1})		
XPS (% atoms)			Cu	435 ± 14	39.5 ± 3.0
O	19.4 ± 0.1	13.1 ± 0.3	Zn	1043 ± 64	58.1 ± 5.5
C	67.3 ± 0.1	85.0 ± 0.5	As	5.83 ± 0.60	2.87 ± 0.23
N	5.47 ± 0.09	nd	Pb	126 ± 7	7.50 ± 0.52
Ca	3.27 ± 0.11	1.63 ± 0.05			
P	2.46 ± 0.09	nd			
Si	1.75 ± 0.10	nd			

BD bulk density, *SA* surface area, *Vmicro* micropore volume, *EC* electrical conductivity, *CEC* cation exchange capacity, *OC* organic carbon, *HA* humic acids, *FA* fulvic acid, *XPS* R-X photoelectron spectroscopy, *XFR* X-ray fluorescence, *ICP-MS* inductively coupled plasma-mass spectrometry, *nd* no detected

increased as the pH values decreased, and both parameters were significantly related by an exponential equation:

$$EC\left(dS\ m^{-1}\right) = 60.56\ e^{-0.316pH}\quad r^2 = 0.923\quad p > 0.001 \tag{1}$$

3.4 Precipitation of the Metal(loid)s

The precipitated Cu and Pb (CuP and PbP, respectively) showed similar trends in all suspensions (Fig. 2a). In

Table 2 Effect on survival (LC$_{50}$) and reproduction (EC$_{50}$ and EC$_{20}$) in Lufa 2.2 natural soil (95% confidence interval) and calculated PNEC value for each biochar

Biochar	LC$_{50}$ (g kg^{-1})	EC$_{50}$ (g kg^{-1})	EC$_{20}$ (g kg^{-1})	PNEC (t DM ha^{-1})
Bss	> 500	> 500	462 (109–518)	11.5
Bpw	> 500	260 (200–330)	193 (143–260)	4.82

suspensions with Bss, the highest precipitation occurred in the most contaminated suspensions (E1), progressively declining towards less contaminated suspensions (E2 and E3). Conversely, in the suspensions with Bpw, the lowest precipitation occurred in E1 suspensions, surging abruptly in E2 suspensions and abating in E3 suspensions. The percentage of CuP and PbP in relation to the total added to each suspension showed greater differences (Fig. 2b). Thus, PbP was approximately 100% in all suspensions, except in E1 suspensions with Bpw, in which it precipitated less than 10%, while the percentage of CuP was significantly related to the pH of the suspension through a logarithmic equation:

$$CuP(\%) = 60.75\ Ln\ pH - 5.82\quad r^2 = 0.947\quad p < 0.001 \tag{2}$$

The trends of Zn and As precipitates (ZnP and AsP, respectively) differed from those of Cu and Pb. Zinc did not precipitate in E1 suspensions of both types of biochar (pH ≤ 2.5), while in E2 suspensions, it precipitated
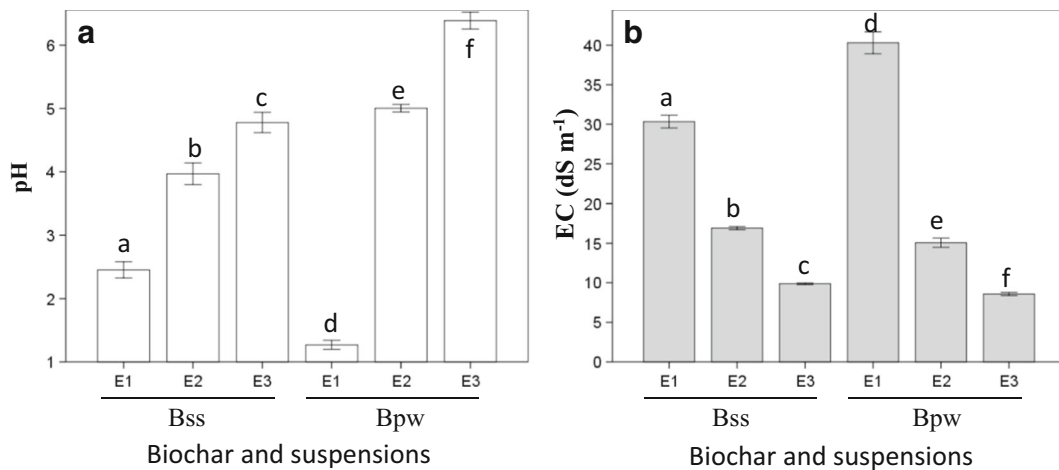
Fig. 1 Mean and standard deviation of pH (**a**) and EC (**b**) in the suspensions of each type of biochar. Mean values followed by the same letter do not significantly differ (Tukey's test $p < 0.05$)

between 0.50 and 0.55 mg g^{-1} (approximately 35% of total Zn added) and between 0.64 and 0.66 mg g^{-1} in E3 suspensions (between 70 and 80% of total Zn added). The pollutant that precipitated least was As, with clear differences depending on the type of biochar. In the case of Bss, AsP was significantly related to pH through an inverse equation:

$$\text{AsP} \left(\text{mg g}^{-1}\right) = 0.239 \, \text{pH}^{-1} - 0.012 \quad r^2 = 0.933 \quad p < 0.001 \tag{3}$$

However, no suspension precipitated more than 0.1 mg As g^{-1} in Bss, and in all suspensions, the percentage precipitated was less than 20% of the total As added. By contrast, in the case of Bpw, AsP was significantly and linearly related to pH through the equation:

$$\text{AsP} \left(\text{mg g}^{-1}\right) = 0.036 \, \text{pH} - 0.056 \quad r^2 = 0.890 \quad p < 0.001 \tag{4}$$

Thus, As did not precipitate in E1 suspensions with a pH of approximately 1.5, registered greater precipitation in E2 suspensions (pH ~ 5) up to approximately 0.1 mg g^{-1} (~ 20% of the total As added), and increased again in E3 suspensions (pH ~ 6.5) up to approximately 0.2 mg g^{-1} (~ 75% of the total As added).

3.5 Sequential Extraction of the Metal(loid)s

The sequential extraction of metal(loid)s in the solid fraction of the suspensions (Fig. 3a) showed that, especially in suspensions with Bss, the major

fraction of the metals was the residual (R). In the case of Cu and Pb, the R fraction was ≥ 80% (Fig. 3b), followed by the O fraction (5–20%). The fractions A (1–5%) and P and S (< 1%) were minor. In suspension with Bpw, the differences with respect to the Bss were mainly a decrease of the R fraction (40–60%) and an increase of the O (30–40%) and A (5–15%) fractions, especially in the case of Cu, whereas the P and S fractions continued as minor fractions (< 2%).

Like Cu and Pb, Zn was predominantly present in the R fraction (c. 75%) for suspensions with Bss, followed by the O (c. 15%) and A (5–15%) fractions. The P and S fractions were minor (< 1%). In contrast, in Bpw, the extracted Zn fractions showed clearly in dissimilar trends relation to Bss. Thus, the R (30–40%) and O (~ 10%) fractions clearly decreased, and the A (30–40%), P (2–10%), and S (5–28%) fractions increased.

The extracted As fractions were clearly different from the extracted fractions of metals, with a decline in the R fraction in most of the suspensions. In suspensions with Bss, the R faction decreased (25–50%), while the O (20–35%), A (10–20%), P (10–15%), and S (5–10%) fractions increased considerably compared to the percentage of precipitated metals in these fractions (Fig. 3b). In suspensions with Bpw, in contrast to suspensions with Bss, there was a sharp fall in the O fraction (< 5%) and spike in the P (15–30%) and S (25–30%) fractions. In the E1 suspensions from Bpw, As was detected only in R fraction (Fig. 3a).
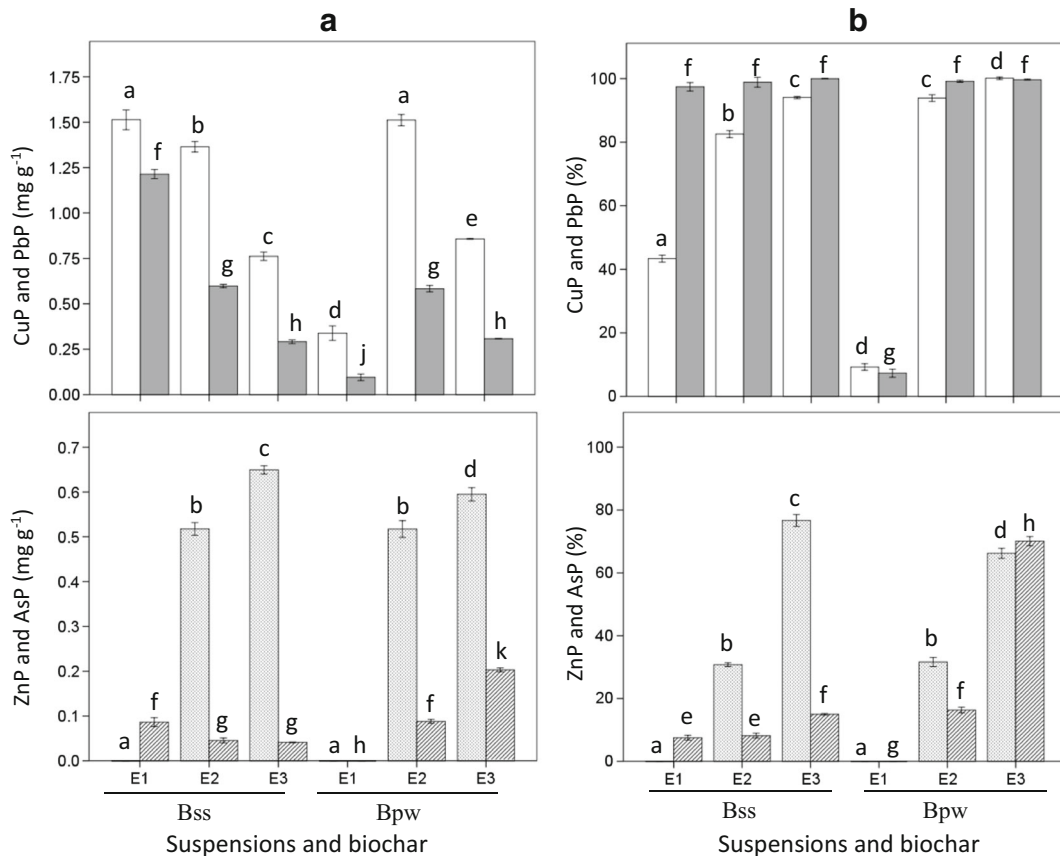
Fig. 2 **a** Mean and standard deviation of Cu (white), Pb (gray), Zn (dotted), and As (striped) precipitated (mg g^{-1}) in the suspensions of each type of biochar. **b** Mean and standard deviation of the percentage precipitate of these elements (%) in relation to the total added. For each element, the mean values followed by the same letter do not significantly differ (Tukey's test $p < 0.05$)

## 4 Discussion

4.1 Biochar

The properties of both types of biochar were similar to those reported by Pituello et al. (2015) for biochar from similar feedstocks and temperatures. In both types of biochar, the humic substances were dominated largely by the less polymerized fraction (FA), although the most polymerized fraction (HA) increased slightly in Bss.

According to Cheng et al. (2008), the higher percentage of O atoms and lower percentage of C atoms on the surface of Bss (Table 1) revealed a higher concentration of carboxylic and phenolic functional groups, a higher concentration of negative charges, and a lower concentration of positive charges compared to that of Bpw. In addition, it also suggests that its half-life is less than that of Bpw (Spokas 2010). The higher CEC per unit area of Bss confirmed the higher density charges on its surface.

However, because SA and Vmicro were almost sevenfold higher in Bpw, although the concentration of negative charges was lower on its surface, the total of these charges per unit weight was higher, as confirmed by the higher CEC per unit weight (Table 1). Wastewater from the treatment plant was relatively rich in nutrients, as revealed the higher percentage of N and P atoms on the surface of Bss. The higher concentration of Ca atoms on the surface of Bss suggests that it was adsorbed by the active functional groups (–COO$^-$ and –O$^-$) in presumably an exchangeable form.

The total content of major elements was also related to the feedstock of each biochar, confirming that the wastewater was rich not only in macronutrients but also in other elements such as S, Si, Fe, Al, Mg, and Na. Ca and especially K were the only major elements whose concentrations were higher in Bpw. In the case of K, it registered high values because it tends to concentrate in plant tissues, and in the case of Ca because, as suggested

Fig. 3 Partitioning of metal(loid)s among the six fractions extracted in the solid phase of the suspensions: R fraction (black), O fraction (striped), A fraction (gray), P fraction (gray light), and S fraction (white), expressed as mean concentration (**a**) and as a percentage of each fraction in relation to the total extracted (**b**)
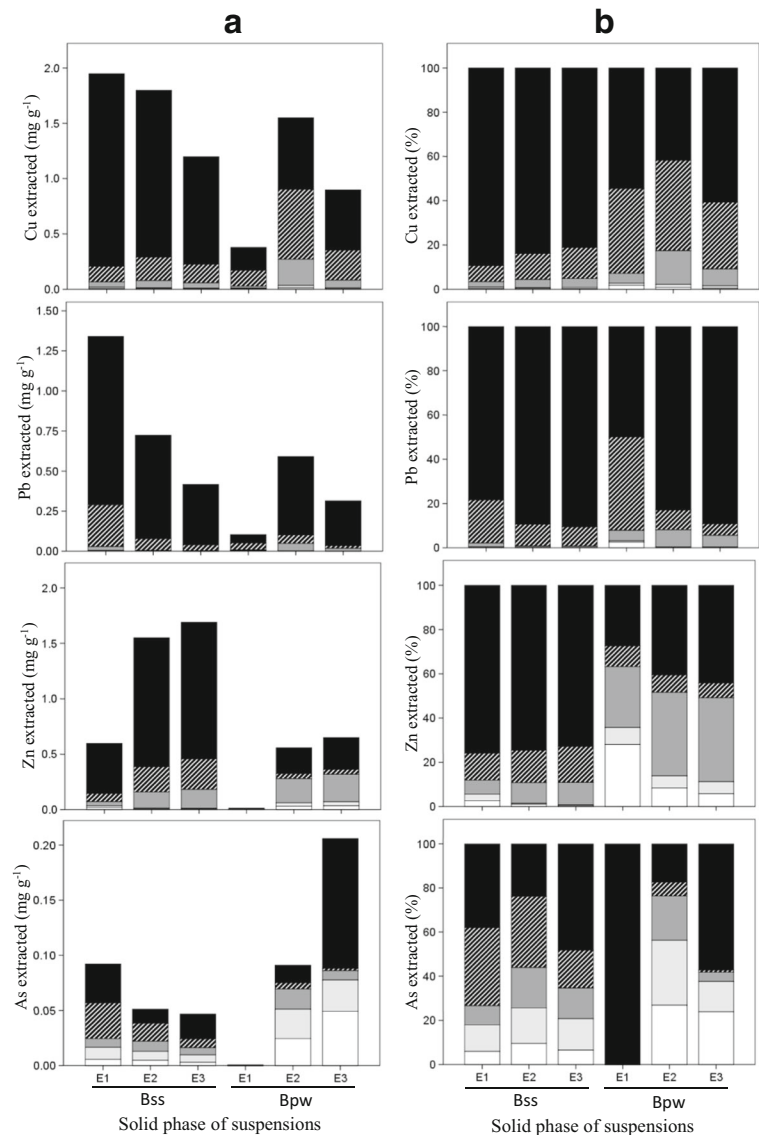
above, it would tend to be adsorbed by the functional groups of the organic matter whose content per unit of weight was higher in Bpw.

The content of trace elements (Table 1) also clearly differed in the two types of biochar, revealing that the sewage was highly contaminated. Thus, the contents of Zn, Cu, As, and Pb were clearly higher in Bss, especially Cu and Zn, which exceeded the upper limit (100 mg Cu kg^{-1} and 300 mg Zn kg^{-1}, Pais and Benton 2000) of the common ranges in soils by approximately fourfold, while the Pb and As concentrations were below the upper limit (200 mg Pb kg^{-1} and 40 mg As kg^{-1}) of the common ranges in soils. In Bpw, the Zn, Cu, As, and

Pb concentrations were relatively low and within the common ranges in soils. These results were also similar to those of Pituello et al. (2015).

Although Bss showed higher Zn, Pb, and Cu concentrations, the results from ecotoxicological test with enchytraeids (PNEC, Table 2) revealed that the harmful effects of Bss would not be expected when the application rate as an amendment was ≤ 11.5 t ha^{-1}, clearly exceeding the application rate of Bpw, which was ≤ 4.8 t ha^{-1} (PNEC). Therefore, the negative effects of Bpw on invertebrates were stronger than those observed for Bss. These results suggest that the metal(loid) concentration would not be the only factor that conditions

the negative effects of biochar on soil biota. The release of organic pollutants by biochar such as phenol, PAHs, and dioxins (Qadeer et al. 2017), as well as other indirect effects such as pH, humidity, aeration, or other factors related to the ecological tolerance range of the exposed species (Didden and Rombke 2001), should also be considered. However, the PNEC value used in this study was estimated from a single ecotoxicity test and applying an assessment factor (ECHA 2008), when a test battery would offer a more complete and realistic scenario. Thus, the risk assessment performed in this study has its limitations and should be used only for comparative purposes between the two different types of biochars investigated.

4.2 Characteristics of Suspensions

The pH buffering capacity of each biochar was different (Fig. 1). Under conditions of high pollution and acidity (E1 suspensions), this capacity was higher in Bss (pH = 2.5) than in Bpw (pH = 1.3). By contrast, under conditions of less marked acidity and pollution (E2 and E3 suspensions), it was Bpw that registered a higher pH buffering capacity, as revealed by the higher pH of both suspensions in relation to the same suspensions from Bss (Fig. 1). The significant relationship between EC and pH (Eq. 1) reveals that the liquid phases of the suspensions, like acidic mine drainage, were saline-acidic.

4.3 Cu and Pb Precipitation

The immobilization of Cu and Pb could be due mainly to precipitation as oxy-hydroxides (Lindsay 2001) and to the formation on the biochar surface of complexes with active functional groups (Tong et al. 2011). Both the higher oxy-hydroxide precipitation and the greater deprotonation of the functional groups by the decreasing H^+ concentration (Szabó et al. 2006) would explain the higher percentage of CuP and PbP by the rise in pH (Ippolito et al. 2012). The significant relationship between the percentage of CuP and pH (Eq. 2) showed that at pH ≥ 5.8, both immobilization mechanisms were able to precipitate 100% of the Cu, whereas at pH < 1.5, the performance of these mechanisms was weak, precipitating less than 10% of the Cu. The different pH buffering capacities of each biochar would account for the greater effectiveness of Bss to precipitate Cu in extremely acid solutions, whereas Bpw was more effective under less

extreme acidic conditions. In the case of Pb, according to Lu et al. (2012) and Cao et al. (2011), it could also precipitate as lead-phosphate-silicate ($5PbO \cdot P_2O_5 \cdot SiO_2$) and as hydroxypyromorphite ($Pb_5(PO_4)_3(OH)$). Because of the formation of these insoluble Pb precipitates in Bss, with a relatively high P and Si content, PbP was close to 100% even in the most acidic and contaminated suspensions (E1). In Bpw, where P and Si atoms were not detected on the surface and the total contents of Si and P were clearly lower, these precipitates either did not form or did so in negligible quantities, and Pb would be adsorbed mainly by the active functional groups or precipitated as oxy-hydroxides, which would explain the low precipitation of Pb in the most acidic suspensions (< 10%) and the strong increase (up to 100%) in suspensions with pH > 5.0.

The sequential extraction of Cu and Pb showed that these elements were adsorbed by Bss in a non-bioavailable form, with the R and O fractions representing more than 95%, whereas fractions potentially extractable by ion exchange mechanisms and H^+ excretion by roots (A, P, and S fractions) were minor. These results suggest that both Cu and Pb complexes with active carboxyl and hydroxyl functional groups of biochar as the precipitated compounds were very stable so that Cu and Pb could stay irreversibly bound to the biochar. In Bpw, the increase in the A fraction could be related to the greater precipitation of Cu and Pb in the form of oxy-hydroxides as a result of the higher pH of the suspensions (Lindsay 2001). Since the Fe and Al contents of Bpw were relatively small, the increase of Cu in the O fraction does not appear to be due to the extraction of Cu bound to Fe and Al, but rather could be attributed to the fact that the reagent used in this extraction (NH_4^+-oxalate + ascorbic acid) was able to partially extract the Cu precipitated or complexed by the active functional groups of this type of biochar, which had not previously been extracted with $(NH_4)_2SO_4$, $NH_4H_2PO_4$, or acetic acid.

4.4 Zn and As Precipitation

As observed above for Cu, Zn could precipitate as an oxy-hydroxide or be adsorbed by the active functional groups of the biochar (Lindsay 2001; Beesley and Marmiroli 2011). Both mechanisms of precipitation would account for both the non-precipitation of Zn in the more acidic suspensions (E1) and the increase of ZnP with rise in the pH. However, ZnP and pH were not

significantly related, probably because the adsorption capacity of Zn by the active functional groups of each biochar was different. Thus, in E2 and E3 suspensions from Bss, with pH values significantly lower that the same suspensions from Bpw, ZnP was similar or even higher, suggesting that the functional groups of Bss had greater affinity for Zn than those of Bpw. The sequential Zn extraction revealed that, as in the case of Cu and Pb, Bss fixed Zn in a much more stable and presumably less bioavailable form than Bpw. Remarkably, the E1 suspensions of both types of biochar did not precipitate Zn (Fig. 2a), suggesting that the Zn extracted (Fig. 3a) would be the one that contained the biochar. Comparing the results of the Zn extraction in E1 suspensions with those extracted in E2 and E3 suspensions, it is emphasized that the Zn contained in the biochar tends to be more exchangeable (increase of the S and P fractions) than the Zn precipitated during the experiment, especially in Bpw.

The contaminant showing the lowest precipitation rate was As, although it varied depending on the type of biochar. Comparing the two types of biochar, the lower content in functional groups as a consequence of its lower SA and Vmicro, lower pH, and higher Fe content in Bss suggests that the As was adsorbed mainly by Fe compounds that have a high affinity for As (Hartley et al. 2004). However, its high P content should reduce As adsorption (Adriano 2001), which would account for the low amount of adsorbed As ($<$ 0.1 mg g^{-1}). In addition, the decline in AsP as the pH rose would be explained by a decrease in the positive charges of the iron compounds (Jain et al. 1999). In Bpw, with a low Fe content and higher SA, Vmicro, and CEC, As should be adsorbed primarily by the formation of bridges with the residual electrostatic charge of the divalent cations adsorbed by active functional groups (Mukherjee et al. 2011). This adsorption mechanism would explain why in the more acid suspensions (E1), As would not precipitate and the AsP would augment at higher pH values of the suspension.

Arsenic extraction would confirm the above results. Thus, the As extracted with NH_4^+-oxalate buffer + ascorbic acid (O fraction) was relatively abundant in Bss and tended to decline with higher pH values. By contrast, in Bpw, the O fraction was minor, whereas the sum of the fractions S and P (non-specific and specifically adsorbed) tended to clearly increase with rising pH values (Shelmerdine et al. 2009). Therefore, the different adsorption mechanisms of As in both types

of biochar would justify the different relationships between pH and AsP, an inverse relationship in Bss (As adsorbed on iron compounds) and a direct relationship in Bpw (As adsorbed by active functional groups).

5 Conclusions

Biochar properties varied considerably depending on the feedstock, which affected the metal(loid) immobilization mechanisms and potential harm to biota. The harmful consequences of biochar from urban pruning wood on invertebrates were higher than effects of biochar from sewage sludge, with estimated "safe" application rate of 4.8 and 11.5 t dry biochar ha^{-1}, respectively. Precipitation as oxy-hydroxides and formation of complexes with active functional groups of organic matter were the main mechanisms of metal fixation in both types of biochar, increasing the percentage precipitated by raising the pH. In biochar from sewage sludge, in addition to the above mechanisms, adsorption by Fe compounds and precipitation in the form of complex phosphates must have occurred. Both types of biochar could be a remediation option in Pb- and Cu-contaminated soil as long as it is applied at safe application rates. The effectiveness of both types of biochar to fix Zn was clearly lower than to fix Cu and Pb. The mechanisms of As fixing were presumably different in both types of biochar, being linked primarily to the iron compounds in biochar from sewage sludge and to the functional groups in biochar from urban pruning wood. The metal(loid)s fixed by biochar from urban pruning wood were more bioavailable than those fixed by biochar from sewage sludge.

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