

Contrasting effects of organic amendments on phytoextraction of heavy metals in a contaminated sediment

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ABSTRACT

Background and aims Soil amendments are often added to polluted soils to increase phytoremediation efficiency. Here we investigated the potential of a range of organic amendments for phytoextraction of heavy metals in a contaminated sediment.

Methods Two experiments compared adsorption and phytoextraction of heavy metals by a Cd-hyperaccumulator *Carpobrotus rossii* grown in the contaminated sediment amended with six organic amendments.

Results The adsorption capacity as measured by Langmuir adsorption maximum followed the order of Cr > Zn > Cu > Cd, and the effect of organic amendments followed the order of chicken manure > cow manure > brown coal > golden-wattle biochar > blue-gum biochar > radiata-pine biochar. The addition of amendments increased the adsorption of heavy metals, with brown coal resulting in the lowest concentrations of water-extractable Cd, Cu and Zn. Two manures resulted in the highest concentrations of these water-extractable heavy metals in the rhizosphere soil of *C. rossii*. Furthermore, brown coal resulted in higher shoot accumulation of these heavy metals than three wood-derived biochars, whilst the manures generally had the lowest accumulation of Cd and Cu although they increased shoot biomass.

Conclusions The addition of brown coal decreased whereas manure addition increased the mobility (water-extractable fraction) of heavy metals in rhizosphere soil. Phytoextraction of Cd and Cu was greater with brown coal than with biochars or manures. Brown coal is suitable for enhancing phytoextraction of these heavy metals because it could increase their accumulation in shoots of *C. rossii* and decrease the risk of leaching of these heavy metals into groundwater.

Keywords: animal manures • biochars • brown coal • *Carpobrotus rossii* • Langmuir model • phytoremediation

Introduction

In order to increase the efficiency of phytoremediation of heavy metals, various soil amendments are often added to polluted soils. These amendments include synthetic chelators, organic wastes and biochars. However, some soil amendments may result in increased risk of heavy metal leaching into groundwater through the formation of soluble metal complexes (Bolan et al. 2014). Furthermore, some synthetic chelates like EDTA as soil amendments can cause secondary pollution due to their long persistence in the environment, and toxic effects on plants and soil organisms (Grčman et al. 2001). Thus, it is important to identify soil amendments that can increase phytoremediation efficiency, without increasing the risk of leaching loss of heavy metals from polluted soils.

Some organic wastes such as manures and composts have been used to change physical, chemical, nutritional and biological soil properties to increase plant growth. These amendments are generally rich in nutrients and organic matter, which facilitate plant growth (Bolan et al. 2014; Chaney 1983). Recent studies have shown that the addition of organic amendments decreases leaching of heavy metals mainly through increasing adsorption of heavy metals, particularly for acid soils (Beesley et al. 2014). The addition of organic materials has been shown to enhance the solubility of some heavy metals (e.g. Cu and metalloid As) in soils due to high amounts of dissolved organic carbon in these materials (Beesley et al. 2010; Houben et al. 2012; Park et al., 2011a). Dissolved organic carbon can form complexes with heavy metals (Bolan et al. 2003; Vaca-Paulin et al. 2006), and hence increase their mobility (Bolan et al. 2003a). Therefore, ideal soil amendments should contain low concentrations of toxic pollutants and also minimize heavy metal leaching.

Compared to waste organic materials like manures, biochars contain recalcitrant organic carbon (Lehmann et al. 2006) with much lower dissolved organic carbon especially when pyrolyzed at temperature > 600 °C. Biochars generally have strong adsorption capacity of heavy metals due to their high surface area and porosity (Bolan et al. 2014). Most studies either focus on the adsorption behaviours of biochars in polluted waters or the use of biochars for minimizing metal accumulation in crop plants. However, little work has been reported investigating the effects of adsorption behaviours of biochars on metal accumulation and mobility in the phytoextraction process.

The impacts of organic amendments on the behaviours of heavy metals in soils and plants are specific to the metal element (including metal state), metal concentration and soil type. This is likely due to the highly variable nature of the amendments (Uchimiya et al. 2011b). For example, the application of a hardwood-derived biochar to a sandy soil increased concentrations of KH_2PO_4 -extractable Zn but did not give consistent effects on Cu and Cd across a range of application rates (Namgay et al. 2010), whereas the application of another hardwood-derived biochar increased Cu concentration, but decreased Cd and Zn concentrations in soil pore water (Beesley et al. 2010). Additionally, the solubility and phytoavailability of heavy metals are often affected by many other factors, such as affinity of heavy metals to organic materials, levels of heavy metals in soils and content of dissolved organic matter (Bolan et al. 2003).

This study used six organic amendments including three types of biochars, and two commercially available animal manures. A brown coal was also used to make comparison because of its low pH and relatively recalcitrant carbon. Two experiments were conducted to compare adsorption capacities of heavy metals among the six amendments, and to examine the effects of organic amendments on phytoextraction of Cd along with Cr, Cu and Zn using an Australian native succulent halophyte *Carpobrotus rossii* (Haw.) Schwantes (Aizoaceae) grown on a polluted sediment. Our previous studies showed that *C. rossii* was able to hyperaccumulate Cd (Zhang et al. 2014) and was tolerant to multiple heavy metals. It was hypothesised that animal manures with higher pH would result in greater immobilization of heavy metals in the sediment and thus lower phytoextraction efficiency than biochars. In contrast, acidic brown coal would increase phytoavailability of heavy metals in the sediment and lead to phytotoxicity in plants.

Materials and methods

Materials

Six organic materials were used; three wood-derived biochars differing in pH, CEC, and N and O contents, two animal manures and a brown coal. The three biochars were derived from radiata pine (*Pinus radiata* D. Don), blue gum (*Eucalyptus globulus* Labill) and golden wattle (*Acacia pycnantha*). Chicken manure and cow manure were obtained from a nursery. Morwell brown coal was sourced from a mine in Victoria. The basic properties of these amendments are shown in Tables 1 and 2. The concentration of Cr in three biochars and two manures ranged from 9.0 to 24.0 mg kg⁻¹ (Table 2), which is higher than the toxic level threshold (5 mg kg⁻¹) (Muhammad et al. 2012). The concentration of Cu and Zn in cow and chicken manures was 47 and 90 mg kg⁻¹ Cu, 1161 and 1575 mg kg⁻¹ Zn (Table 2), respectively, which is near (> 100 mg kg⁻¹ Cu) or above (> 300 mg kg⁻¹ Zn) (Kachout et al. 2012; Levy et al. 1999) the toxic level thresholds. [The high levels of heavy metals in the manures had probably resulted from the use of metal-containing additives in animal feeds.](#)

The sediment was collected from a landfill site in Beaufort, Victoria, Australia (37°36'S, 143°35'E). The sediment in the landfill originated from drainage systems and rivers in the area. The sediment had a field capacity of 18.5% and contained 37.1% sand, 41.1% silt and 21.8% clay. Other properties are shown in Table 1. The concentration of Cr in the sediment was > 49 mg kg⁻¹ which was above the toxic level threshold (Muhammad et al. 2012). [The high Cr concentration in the sediment might be due to the use of metals including Cr by a number of timber factories in the area and possible contaminations of these metals to the drainage and water systems.](#)

Carpobrotus rossii (Aizoaceae) was collected from the same landfill site. Uniform cuttings were used for propagation in a heated sand bed. Fertilization was provided by mixing the sand with Osmocote fertilizer (10 g kg⁻¹, Scotts Australia Pty Ltd), and irrigation (tap water) was made with an auto-watering sprayer (Zhang et al. 2014). After one month the root systems of cuttings were well developed and the seedlings were then transplanted to the experiment pots.

Experiment one: adsorption isotherm curves

An adsorption isotherm experiment was conducted, following previously published protocols, using a series of increasing concentrations of Cd (0.05-1.6 mM), Cr (0.4 –50 mM), Cu (0.1 –3.2 mM) and Zn (0.25 –8 mM) in 0.01 M NaNO₃ (Chen et al. 2011). The four metals were added individually as Cd(NO₃)₂, Na₂Cr₂O₇, Cu(NO₃)₂ and Zn(NO₃)₂, respectively. The shaking time was set as 24 h, which has been reported as the equilibrium time in most adsorption isotherm experiments (Uchimiya et al. 2011b).

Each concentration level of the heavy metal solutions was adjusted to pH 5.5 with HNO₃ or NaOH to simulate acidic soil pH (Foy et al. 1978). Then, each organic material (0.05 g) was mixed with 25 mL of heavy metal solution. The suspension was shaken in an end-over-end shaker at 25°C and then filtered through Whatman No. 1 filter paper. Each treatment was in duplicates. The equilibrium concentrations of Cd, Cr, Cu and Zn in the filtrates were determined using inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima 8000 ICP-OES). Blanks (0.01 M NaNO₃ alone) without heavy metals and controls without amendments were also included in every batch. The standards covered the range of concentration in the samples. This allows for increased precision and confidence of the measured value of low metal concentrations. The pH of equilibrium solution was determined using a Thermo Orion 720 pH meter (Thermo Electron Corporation, USA).

The experimental data of adsorption were fitted using the Langmuir adsorption model:

$$q_e = \frac{S_{max} \times K_L \times c}{1 + K_L \times c}$$

where q_e (mmol g⁻¹) is the adsorbed amount, S_{max} (mmol g⁻¹) is a fitting parameter for the maximum

monolayer adsorption capacity which reflects the number of reactive surface adsorption sites (Song et al. 2014), c is the equilibrium concentration (mmol L^{-1}), and K_L (L mmol^{-1}) is the Langmuir adsorption constant which is a measure of the affinity (intensity) of heavy metals for the surface (Song et al. 2014), or related to the binding energy of metals on absorbents (Appel and Ma 2002).

Experiment two: plant treatments

The experiment consisted of eight treatments in four replicates. The eight treatments were control (without Cd or amendments being added), Cd, Cd+radiata pine biochar, Cd+blue gum biochar, Cd+golden wattle biochar, Cd+brown coal, Cd+chicken manure, and Cd+cow manure.

Sediment (1.5 kg with plants and 0.75 kg for bulk sediment without plants) was weighed into each transparent plastic bag (28×40 cm), and spiked with 20 mg Cd kg^{-1} [as $\text{Cd}(\text{NO}_3)_2$] (except for the no-Cd control). The basal nutrients were then added as a solution in the following composition (mg kg^{-1} soil) 150 KNO_3 , $21 \text{ MgSO}_4 \cdot \text{H}_2\text{O}$, $150 \text{ KH}_2\text{PO}_4$, $236 \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$, $0.67 \text{ H}_3\text{BO}_3$, $0.15 \text{ Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 90 urea. Considering there were high concentrations of Cu, Mn and Zn in the sediment, basal nutrients did not include these elements. After thoroughly mixing with basal nutrients and Cd, the sediments were incubated at 25°C and 70% of field capacity for 4 weeks. Six organic amendments were separately added at a rate of 20 g kg^{-1} to the designated treatments, mixed and incubated for one more week (Park et al. 2011b). Bags were shaken to mix the sediment daily during the whole incubation.

After incubation, the treated sediments were transferred into 1.5-L pots lined with plastic bags. Two seedlings were transplanted into each pot. The pots were irrigated with deionized water to maintain 80% of field capacity every two days and fertilized with 20 mg kg^{-1} urea every two weeks from the 3rd week after transplanting. The plants were grown in a naturally lit glasshouse with minimum and maximum temperatures of 19 and 33°C , respectively. An additional set of pots containing 0.75 kg sediment without plants were used as bulk sediment. Pots were randomized within replicates. Re-randomization was conducted every two days when watering.

Plants were harvested 56 days after transplanting. Shoots were separated from the roots at the soil surface. The oven-dried plant samples were ground ($< 0.5 \text{ mm}$) with a stainless steel mill (ZM200, Retsch Technology GmbH, Germany) and then digested by microwave digestion (Multiwave 3000, Anton Paar GmbH, Austria) using HNO_3 . Concentrations of Cd, Cr, Cu and Zn in the digests were determined using ICP-OES (Perkin Elmer Optima 8000, MA, USA).

Rhizosphere sediment was collected by gently shaking off the sediment adhering to roots. The sediments were air-dried and sieved through a 2-mm mesh. Concentrations of H_2O - or CaCl_2 -extractable Cd, Cr, Cu and Zn in treatment sediments were measured according to methods of Ayoub (2003). Sediment pH was measured. Detailed procedures were described previously (Zhang et al. 2014).

Statistical analysis

All results were presented as the mean \pm standard error of two or four independent replicates. Statistical analysis was conducted using SPSS statistics 17.0 software package (SPSS, Chicago, Illinois, USA). The Fisher LSD test was used to compare statistical difference of means between treatments at $p = 0.05$.

Results

Experiment one: Effects of adsorption on pH in solution

Overall, compared to the pH (5.5) of the initial metal solution, the addition of organic materials decreased the pH in the equilibrium solution; the pH decreased with increasing concentrations of heavy metals and then reached a constant value (Fig. 1). One exception was a slight pH increase with increasing Cr concentration in the brown coal treatment (Fig. 1B). The decreasing extent of pH

for all heavy metals generally followed the order of brown coal (except for Cr) > cow manure > chicken manure > golden wattle biochar > blue gum biochar > radiata pine biochar. Among the four heavy metals, the extent of pH decrease followed an order of Cu > Zn > Cr > Cd in the three biochars and two manures, but Cu > Zn > Cd > Cr in the brown coal treatment (Fig. 1).

Experiment one: Adsorption isotherm curves

The Langmuir model provided a strong fit of the experimental data ($r^2 = 0.810$ to 0.991) except for Cd with golden wattle biochar ($r^2 = 0.683$) (Table 3). In almost all treatments, the amount of adsorbed heavy metals increased sharply with the increasing concentrations of heavy metals and then showed approximate saturation of adsorption at highest concentrations except for radiata pine and blue gum biochars in which the adsorption of Cr, Zn and Cu was not close to saturation (Fig. 2). Among the four heavy metals, the values of adsorption maxima S_{\max} showed the order of $\text{Cr}^{6+} > \text{Zn}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+}$. However, the values of K_L showed the other order of $\text{Cd}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Cr}^{6+}$.

In comparison, two manures had higher S_{\max} of Cd, Cu and Zn than the three biochars, which in turn had higher S_{\max} than the brown coal (Table 3). The brown coal and chicken manure had higher Cr S_{\max} than the three biochars, while the cow manure had the least.

Experiment two: Effects of organic amendments on sediment pH

The pH (CaCl_2) of the rhizosphere sediment was 4.10 in the no-Cd control, significantly lower than pH (4.30 – 4.94) in other treatments (data not presented). Compared to the Cd-alone treatment, the addition of organic amendments increased pH of bulk and rhizosphere sediments except for a decrease in bulk sediment and a similar value in rhizosphere sediment in the brown coal treatment. The pH values of rhizosphere sediments were increased more in the two manure treatments than in the three biochar treatments. On average, rhizosphere pH was 0.41 units lower than bulk sediment pH.

Experiment two: Concentrations of extractable heavy metals in sediment

The concentrations of H_2O -extractable Cr, Zn and Cu were lower in bulk than in rhizosphere sediments, and did not generally differ between the control and Cd-alone treatment (Fig. 3). The concentration of H_2O -extractable Cd was also lower in bulk than in rhizosphere sediments in three biochars and two manures treatments, but higher in bulk sediments in Cd-alone and brown coal treatments (Fig. 3A).

Compared to the Cd-alone treatment, the addition of manures and blue gum biochar increased but the addition of brown coal decreased the concentration of H_2O -extractable Cd in rhizosphere sediment. In comparison, the addition of three biochars did not affect the concentrations of H_2O -extractable Cr and Cu in rhizosphere sediments. The addition of two manures decreased the concentration of H_2O -extractable Cr but increased the concentration of Zn in rhizosphere soil (Fig. 3).

The addition of organic materials, especially of two manures, decreased the concentrations of CaCl_2 -extractable Cd and Zn. The addition of biochars and brown coal, but not the two manures decreased the concentration of CaCl_2 -extractable Cr in rhizosphere sediments. The concentration of CaCl_2 -extractable Cu was under the detection limit except for the two manure treatments which increased Cu concentration (Fig. 4).

Compared to the H_2O extraction, CaCl_2 extraction resulted in higher concentrations for Cd and Zn but lower concentrations for Cr in all treatments, and lower concentrations for Cu in the two manure treatments (Figs. 3-4).

Experiment two: Biomass production

In comparison with the control, the addition of Cd decreased root and shoot biomass by an average of 14% (Fig. 5). Compared to the Cd-alone treatment, the root and shoot biomass were not affected by three biochars, decreased by brown coal but increased by two manures (Fig. 5A-B). Among the three biochars, the biomass was slightly higher in the golden-wattle biochar than in other two biochar treatments.

Experiment two: Accumulation of heavy metals in plants

Compared to the Cd-alone treatment, the concentration of Cd in shoots was not affected by radiata-pine biochar and blue-gum biochar, decreased by golden-wattle biochar and two manures, but increased by brown coal (Fig. 6). The concentrations of Cr and Zn in shoots were decreased in all organic material treatments except for an increase of Zn concentration in the brown coal treatment, while the concentration of Cu were not affected by radiata-pine and blue-gum biochars, decreased by two manures and golden-wattle biochar, but increased by brown coal.

Total uptake of Cd, Cr, Cu and Zn in shoots followed the same trend as their concentrations except that total uptake of Cu was the highest in the control (Fig. 6).

The concentrations of Cd in shoots correlated positively with concentrations of CaCl₂-extractable Cd ($r^2=0.74$, $p < 0.001$) but negatively with water-extractable Cd in rhizosphere sediment ($r^2 = 0.61$, $p < 0.001$) and rhizosphere pH ($r^2 = 0.79$, $p < 0.001$) (Fig. 7).

Discussion

Comparison of adsorption capacity among six organic materials

In the present study, two manures generally had higher adsorption ability of heavy metals than three biochars and brown coal, as indicated by the higher S_{\max} in two manures obtained from the Langmuir model (Fig. 2 and Table 3). The higher adsorption ability of the manures may be explained by three reasons. The first reason was that two manures had a larger number of adsorption sites or functional groups such as carboxylic, lactonic, phenolic, aromatic and amino-groups (Choppala et al. 2013b; Fletcher and Beckett 1987), which was reflected by their higher CEC values (Table 1). The second reason was the higher pH of the two manures than the other organic materials (Table 1). High pH not only causes increasing negative charges of organic materials which would facilitate adsorption, but also leads to the formation of metal-hydroxy complexes which is a preferential form of adsorption (Naidu et al. 1994). A further reason was that the manures contained higher concentrations of Ca and P (Table 2). The high P content would promote precipitation or co-precipitation of heavy metals (Liu et al. 2009), enhancing in the presence of Ca (Lu et al. 2012).

The adsorption of heavy metals onto organic materials like biochars and manures involves several mechanisms such as electrostatic interactions, ion exchange (including protons), and/or complexation via chemical ligand binding (Uchimiya et al. 2012). Ion exchange may be a prevalent mechanism of adsorption for organic materials which are attributed to various functional groups (Borchard et al. 2012; Uchimiya et al. 2011a). This helps to explain the decreasing pH during adsorption processes (Fig. 1). At the higher metal concentrations, the insignificant change in pH might be attributable to near saturation of binding sites for ion-exchange on the surface of organic materials (Naidu et al. 1994), and/or the increasing positive surface charges of organic materials due to decreased pH, thereby resulting in fewer negatively charged binding sites. In addition, nitrogen-containing functional groups such as amide groups (-NH₂) have also been shown a good coordination affinity to some heavy metals like Cd and Cu (Park et al. 2011a). This was confirmed in the present study that there were strong positive correlations between nitrogen content in organic materials and adsorption capacity (S_{\max}) of Cd and Cu.

The higher adsorption of heavy metals with the brown coal than the three biochars (Fig. 2 and Table 3) was associated with greater pH decreases (Fig. 1). The results suggest that ion-exchange adsorption was the predominant process because brown coals generally contain high oxygen with high proportions of carboxylic and phenolic hydroxyl functional groups (Karabulut et al. 2000).

Comparison of adsorption behaviour among four heavy metals

As expected, the four metals differed greatly in their adsorption onto organic materials. The adsorption capacity (S_{\max}) followed the order of $\text{Cr} > \text{Zn} > \text{Cu} > \text{Cd}$ in organic amendments except for cow manure (Table 3). It has often been observed that organic amendments adsorb higher amounts of Cu and Zn than Cd. Other studies showed that the adsorption capacity of Zn and Cd was negatively related to their hydrolysis constants (Pagnanelli et al. 2003), which is in agreement with the present study. The highest adsorption of Cr may partly be attributed to organic amendment-induced reduction of Cr^{6+} to Cr^{3+} and subsequent immobilization of Cr^{3+} .

The small change in pH with Cr in the adsorption experiment (Figs. 1-2) suggests that ion exchange was not the main reason for Cr adsorption on organic amendments, especially the acidic brown coal. The adsorption of the oxyanion $\text{Cr}_2\text{O}_7^{2-}$ on organic materials like biochars would be decreased with increasing pH due to the increased adsorption of OH^- by biochars (Deveci and Kar 2013) and resultantly the less positive charge of biochars (Choppala et al. 2013a). This may explain why the brown coal with the lowest pH had the greatest adsorption of Cr (Fig. 2B and Table 3). As discussed above, organic amendments are rich in functional groups which provide electrons for the reduction of Cr^{6+} to Cr^{3+} (Choppala et al. 2013a; Hsu et al. 2009). For example, Hsu et al. (2009) found that Cr^{6+} was first adsorbed on the surface of crop-residue-derived black carbon, due to the electrostatic attraction between oxyanion Cr^{6+} (mainly HCrO_4^- at $0.86 < \text{pH} < 6.51$) and positive charge surface of biochar at pH lower than its point of zero charge. It was then reduced to Cr^{3+} mainly by phenolic groups, and finally the resulting Cr^{3+} was bound to the surface of black carbon through complexation and/or precipitation (Deveci and Kar 2013; Hsu et al. 2009). Therefore, the reduction of Cr^{6+} to Cr^{3+} might influence the correlation between CEC and adsorption, as supported by the small change in K_L values (from 0.037 to 0.070) (Table 3).

Effects of organic amendments on solubility and phytoavailability of heavy metals

The addition of organic amendments generally decreased the solubility (H_2O -extractable) and phytoavailability (CaCl_2 -extractable) of heavy metals in the sediment (Figs. 3 and 4). This has been attributed mainly to two reasons. The first reason is the strong adsorption of organic materials as indicated in adsorption isotherm curves (Fig. 2 and Table 3). Except for the two manures, the strong negative correlation between solubility of heavy metals (Cd, Cu and Zn) and adsorption capacity (S_{\max}) confirms that the adsorption of heavy metals by organic amendments was responsible for the decreased solubility of these metals in the sediment. Similarly, the phytoavailability of Cd and Zn correlated negatively with the adsorption capacity (S_{\max}) or CEC of organic amendments (Cd: $r = -0.85$, $p = 0.03$; Zn: $r = -0.89$, $p = 0.02$), indicating that the adsorption capacity of the amendments and associated CEC play important roles in phytoavailability of these metals.

The second reason was ascribed to the increased sediment pH due to the addition of organic materials. The addition of organic amendments often has a liming effect on acidic soils (Tang et al. 2015), which was also observed with the acidic sediment in the present study. This might partly be responsible for the decreased solubility of Cd and Zn (Fig. 3). Increased pH would favor the precipitation of metals, and increase negative charges of the surface of soil colloids (Naidu et al. 1994), which further increases adsorption. Additionally, rhizosphere alkalisation is positively correlated with nitrogen contents in the organic amendments ($r = 0.84$, $p = 0.036$), suggesting that nitrogen-containing groups might also be responsible for metal adsorption via the complexation with amines or amino acids, as occurs with Cu^{2+} at higher pH (McBride 1998). This is consistent with the relationship between nitrogen content and adsorption capacity of Cd and Cu as discussed above. It is worth noting that the lowest phytoavailability of Cd and Zn in the two manure

treatments might be partly related to higher P contents (Table 2), which in turn favored Cd/Zn-phosphate precipitation (Loganathan et al. 2012) and/or co-precipitation of Cd/Zn-phosphate with Ca (Basta et al. 2001) due to the use of CaCl_2 .

The greater increase in the water solubility of Cd, Cu and Zn (Fig. 3) and phytoavailability of Cu and Cr (Fig. 4) by animal manures than wood-derived biochars might be ascribed to the higher content of dissolved organic carbon (DOC) in the manures (Table 1) and resultantly greater formation of soluble organic-metal complexes (Bolan et al. 2003; Vaca-Paulin et al. 2006). However, the DOC in the sediment solution can be influenced by sediment pH. The water extract with higher pH could result in greater increase in DOC in the sediment solution than the CaCl_2 -extract. Park et al. (2011b) also observed that the concentration of Cu in soil pore water increased in biochar treatments although the concentration of MgCl_2 -extractable Cu decreased. The results were similar to those observed in other studies with Cd, Zn and Pb (Houben et al. 2012). Thus, the high DOC concentrations resulting from high pH were partly responsible for the higher solubility of Cd, Cu and Zn in the two manure treatments (Fig. 4). In addition, higher concentrations of Cu, Zn and Cd in the two manures (Table 2) further contributed to the high solubility of these metals.

The brown coal had little effects on the phytoavailability of Cd and Zn (Fig. 4), likely due to the lack of liming effect of the brown coal, although it had an intermediate Cd adsorption capacity among the six organic materials (Fig. 2 and Table 3). Furthermore, the addition of brown coal did not increase the solubility of Cd and Zn; the solubility was lower than those in the biochar treatments (Fig. 3) although the brown coal treatment had the lowest pH. The lower solubility and higher phytoavailability further confirm that non-specific adsorption of heavy metals might be predominant in controlling the behaviour of heavy metals in the brown coal treatment as discussed above. Thus, the addition of brown coal may not increase the leaching risk of heavy metals into groundwater.

The decrease in potential Cr phytoavailability in the bulk sediment by the organic amendments might be attributed to the effects of organic amendments on adsorption capacity of Cr (S_{max}). Moreover, the higher concentrations of H_2O -extractable Cr than those of CaCl_2 -extractable fraction are consistent with the study of Gupta and Sinha (2006) who observed that concentrations of NaNO_3 -extractable Cr were higher than those of CaCl_2 -extractable Cr. It appears that lower concentrations of CaCl_2 -extractable than H_2O -extractable Cr in the present study were ascribed to both lower pH of CaCl_2 extracts than H_2O extracts, and resultantly higher adsorption of Cr on solid organic matter due to the decreased DOC. Furthermore, under acidic soil conditions like in the present study, soluble Cr^{6+} is easy to reduce to insoluble Cr^{3+} (Wittbrodt and Palmer 1997) and to be adsorbed by soil (Griffin et al. 1977).

It was not expected that the concentration of CaCl_2 -extractable Cu would be under the detection limit except for very low concentrations of Cu in the two manure treatments (Fig. 4C). Zhou et al. (2005) also observed very low concentrations of NH_4NO_3 -extractable Cu ($0.18 - 0.81 \text{ mg kg}^{-1}$) in rhizosphere soil when a garden soil was amended with manures, although the total Cu contents in their treatments ranged from 48.7 to 180.9 mg kg^{-1} , which are higher than those ($13 - 14.8 \text{ mg kg}^{-1}$) in the present study. Thus, the low concentrations of CaCl_2 -extractable Cu might result from both low total Cu content in sediments and strong adsorption of Cu on organic and/or clay colloids. Cu^{2+} has a stronger affinity to both organic matter and clay than Ca^{2+} (Cavallaro and McBride 1980). In contrast, despite the low values, Cu was detected in the H_2O extracts of all treatments, which was attributable to the formation of soluble Cu-organic complexes (Bolan et al. 2003).

Effects of organic amendments on phytoextraction

The decreased phytoextraction by organic amendments as indicated by total metal uptake in shoots except for Cd, Cu and Zn in the brown coal treatment (Fig. 6), and for Cd in the pine and blue gum biochars, is consistent with most studies (Li et al. 2006; Liu et al. 2009). The low phytoextraction of Cd in the treatments of golden-wattle biochar and two manures was attributed to three aspects. First, the amendments lowered CaCl_2 -extractable Cd and thus decreased Cd availability for plant uptake.

Second, the amendments favored the formation of soluble organic-metal complexes (Sauve et al. 2000) which are less available for plants than free Cd^{2+} (Bolan et al. 2003). This is evidenced by the results that the concentrations of Cd in shoots positively correlated with the concentrations of CaCl_2 -extractable Cd but negatively with water-soluble Cd (Fig. 7) as well as dissolved organic carbon ($r^2 = 0.52$, $p < 0.001$) in rhizosphere sediment. Third, the amendments increased rhizosphere pH and hence decreased Cd phytoavailability. Furthermore, The highest shoot biomass in the manure treatments (Fig. 5A) could also be due to the great supply of nutrients (Table 2).

Interestingly, the addition of brown coal led to the highest phytoextraction of Cd, Cu and Zn (Fig. 6) although it decreased shoot biomass due to the toxic concentrations of Cd in shoots ($232 \mu\text{g g}^{-1}$). The high phytoextraction of Cd and Zn was attributed to high phytoavailability in sediments (Fig. 4) and resultantly high concentrations of heavy metals in shoots, whilst the high phytoextraction of Cu were mainly due to the higher translocation ability (data not shown). Further work is needed to investigate why the brown coal increased the translocation of Cd, Cu and Zn since the concentrations of these heavy metals in roots were lower than those in the other treatments and the control in the present study (data not shown).

Conclusions

The addition of organic amendments generally decreased the solubility and phytoavailability of heavy metals in the sediment, which was attributed mainly to the increased adsorption and pH. Among the organic amendments, the brown coal resulted in greater phytoextraction of metals, especially for Cd, and lower mobility of heavy metals (except for Cr and Zn), implying that the use of brown coal for phytoextraction would have the lower leaching risk of heavy metals but greater phytoextraction of heavy metals.

In comparison, the addition of manures decreased the metal uptake by *C. rossii* from the contaminated sediment, and thus the use of manures may not be suitable for phytoextraction. However, manures can enhance plant growth partly because they can immobilize heavy metals in soils and hence decrease the uptake of heavy metals. In addition, with greater contents of recalcitrant carbon and adsorption of heavy metals, the application of biochars and brown coal should provide long-term benefits on soil properties. It would also be of interest to explore whether the combined application of biochars, brown coals and manures increases the phytoextraction but decreases the risk of leaching loss of heavy metals, and provides better long-term benefits.

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References

- Appel C, Ma L (2002) Concentration, pH, and surface charge effects on cadmium and lead sorption in three tropical soils. *J Environ Qual* 31: 581-589
- Ayoub AS, McGaw BA, Shand CA, Midwood AJ (2003) Phytoavailability of Cd and Zn in soil estimated by stable isotope exchange and chemical extraction. *Plant Soil* 252: 291-300
- Basta NT, Gradwohl R, Snethen KL, Schroder JL (2001) Chemical immobilization of lead, zinc, and cadmium in smelter-contaminated soils using biosolids and rock phosphate. *J Environ Qual* 30: 1222-1230
- Beesley L, Inneh OS, Norton GJ, Moreno-Jimenez E, Pardo T, Clemente R, Dawson JJC (2014) Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. *Environ Pollut* 186: 195-202
- Beesley L, Moreno-Jimenez E, Gomez-Eyles JL (2010) Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. *Environ Pollut* 158: 2282-2287
- Bolan N, Adriano D, Mani S, Khan A (2003) Adsorption, complexation, and phytoavailability of copper as influenced by organic manure. *Environ Toxicol Chem* 22: 450-456

- Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soils - To mobilize or to immobilize? *J Hazard Mater* 266C: 141-166
- Borchard N, Prost K, Kautz T, Moeller A, Siemens J (2012) Sorption of copper (II) and sulphate to different biochars before and after composting with farmyard manure. *Europ J Soil Sci* 63: 399-409
- Cavallaro N, McBride MB (1980) Activities of Cu^{2+} and Cd^{2+} in soil solutions as affected by pH. *Soil Sci Soc Am J* 44: 729-732
- Chaney RL (1983) Plant uptake of inorganic waste constituents. In: Parr JF, Marsh PD, Kla JM. Park Ridge, NJ (eds), *Land Treatment of Hazardous Wastes*. Noyes Data Corporation, pp:50-76
- Chen XC, Chen GC, Chen LG, Chen YX, Lehmann J, McBride MB, Hay AG (2011) Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresour Technol* 102: 8877-8884
- Choppala G, Bolan N, Kunhikrishnan A, Skinner W, Seshadri B (2013a) Concomitant reduction and immobilization of chromium in relation to its bioavailability in soils. *Environ Sci Pollut Res* 20: 1-10
- Choppala G, Bolan N, Seshadri B (2013b) Chemodynamics of chromium reduction in soils: implications to bioavailability. *J Hazard Mater* 261: 718-724
- Deveci H, Kar Y (2013) Adsorption of hexavalent chromium from aqueous solutions by bio-chars obtained during biomass pyrolysis. *J Indust Engineer Chem* 19: 190-196
- Fletcher P, Beckett PHT (1987) The chemistry of heavy-metals in digested sewage-sludge-II. Heavy-metal complexation with soluble organic matter. *Water Res* 21: 1163-1172.
- Foy CD, Chaney RL, White MC (1978) Physiology of metal toxicity in plants. *Annu Rev Plant Phys* 29: 511-566
- Grčman H, Velikonja-Bolta Š, Vodnik D, Kos B, Leštan D (2001) EDTA enhanced heavy metal phytoextraction metal accumulation, leaching and toxicity. *Plant Soil* 235: 105-114
- Griffin RA, Au AK, Frost RR (1977) Effect of pH on adsorption of chromium from landfill - leachate by clay minerals. *J Environ Sci Health Part A: Environ Sci Engineer* 12: 431-449
- Gupta AK, Sinha S (2006) Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: Selection of single extractants. *Chemosphere* 64: 161-173
- Houben D, Pircar J, Sonnet P (2012) Heavy metal immobilization by cost-effective amendments in a contaminated soil: Effects on metal leaching and phytoavailability. *J Geochem Explor* 123: 87-94
- Hsu NH, Wang SL, Lin YC, Sheng GD, Lee JF (2009) Reduction of Cr(VI) by crop-residue-derived black carbon. *Environ Sci Technol* 43: 8801-8806
- Kachout SS, Ben Mansoura A, Mechergui R, Leclerc JC, Rejeb MN, Ouerghi Z (2012) Accumulation of Cu, Pb, Ni and Zn in the halophyte plant *Atriplex* grown on polluted soil. *J Sci Food Agric* 92: 336-342
- Karabulut S, Karabakan A, Denizli A, Yürüm Y (2000) Batch removal of copper(II) and zinc(II) from aqueous solutions with low-rank Turkish coals. *Separation Purification Technol* 18: 177-184
- Lehmann J, Gaunt J, Rondon M (2006) Bio-char sequestration in terrestrial ecosystems-a review. *Mitigation and Adaptation Strategies for Global Change* 11: 403-427
- Levy DB, Redente EF, Uphoff GD (1999) Evaluating the phytotoxicity of Pb-Zn tailings to big bluestem (*Andropogon gerardii* Vitman) and switchgrass (*Panicum virgatum* L.). *Soil Sci* 164: 363-375
- Li S, Liu RL, Wang M, Wang XB, Shan H, Wang HT (2006) Phytoavailability of cadmium to cherry-red radish in soils applied composted chicken or pig manure. *Geoderma* 136: 260-271
- Liu L, Chen H, Cai P, Liang W, Huang Q (2009) Immobilization and phytotoxicity of Cd in contaminated soil amended with chicken manure compost. *J Hazard Mater* 163: 563-567
- Loganathan P, Vigneswaran S, Kandasamy J, Naidu R (2012) Cadmium sorption and desorption in soils: A review. *Crit Rev Env Sci Tec* 42: 489-533
- Lu H, Zhang W, Yang Y, Huang X, Wang S, Qiu R (2012) Relative distribution of Pb^{2+} sorption mechanisms by sludge-derived biochar. *Water Res* 46: 854-862
- McBride MB (1998) Soluble trace metals in alkaline stabilized sludge products. *J Environ Qual* 27: 578-584
- Muhammad I, Puschenreiter M, Wenzel WW (2012) Cadmium and Zn availability as affected by pH manipulation and its assessment by soil extraction, DGT and indicator plants. *Sci Total Environ* 416: 490-500
- Naidu R, Bolan NS, Kookana RS, Tiller KG (1994) Ionic-strength and pH effects on the sorption of cadmium and the surface-charge of soils. *Europ J Soil Sci* 45: 419-429
- Namgay T, Singh B, Singh BP (2010) Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (*Zea mays* L.). *Aust J Soil Res* 48: 638-647
- Pagnanelli F, Esposito A, L. Toroa, Veglio F (2003) Metal speciation and pH effect on Pb, Cu, Zn and Cd biosorption onto *Sphaerotilus natans*: Langmuir-type empirical model. *Water Res* 37: 627-633
- Park JH, Choppala GK, Bolan NS, Chung JW, Chuasavathi T (2011a) Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil* 348: 439-451
- Park JH, Panneerselvam P, Lamb DT, Choppala G, Bolan NS, Chung JW (2011b) Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils, *Journal of Hazardous Materials* 185: 549-574

- Sauve S, Norvell WA, McBride M, Hendershot W (2000) Speciation and complexation of cadmium in extracted soil solutions. *Environ Sci Technol* 34: 291-296
- Song Z, Lian F, Yu Z, Zhu L, Xing B, Qiu W (2014) Synthesis and characterization of a novel MnOx-loaded biochar and its adsorption properties for Cu²⁺ in aqueous solution. *Chem Engineer J* 242: 36-42
- Tang X, Li X, Liu X, Hashmi MZ, Xu J, Brookes PC (2015) Effects of inorganic and organic amendments on the uptake of lead and trace elements by *Brassica chinensis* grown in an acidic red soil. *Chemosphere* 119C: 177-183
- Uchimiya M, Cantrell KB, Hunt PG, Novak JM, Chang S (2012) Retention of heavy metals in a Typic Kandudult amended with different manure-based biochars. *J Environ Qual* 41: 1138-1149
- Uchimiya M, Klasson KT, Wartelle LH, Lima IM (2011a) Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cations. *Chemosphere* 82: 1431-1437
- Uchimiya M, Klasson KT, Wartelle LH, Lima IM (2011b) Influence of soil properties on heavy metal sequestration by biochar amendment: 2. Copper desorption isotherms. *Chemosphere* 82: 1438-1447
- Vaca-Paulin R, Esteller-Alberich MV, Lugo de la Fuente J, Zavaleta-Mancera HA (2006) Effect of sewage sludge or compost on the sorption and distribution of copper and cadmium in soil. *Waste Manag* 26: 71-81
- Wittbrodt P, Palmer C (1997) Reduction of Cr(VI) by soil humic acids. *Europ Soil Sci* 48: 151-162
- Zhang C, Sale PW, Doronila AI, Clark GJ, Livesay C, Tang C (2014) Australian native plant species *Carpobrotus rossii* (Haw.) Schwantes shows the potential of cadmium phytoremediation. *Environ Sci Pollut Res* 21: 9843-9851
- Zhou DM, Hao XZ, Wang YJ, Dong YH, Cang L (2005) Copper and Zn uptake by radish and pakchoi as affected by application of livestock and poultry manures. *Chemosphere* 59: 167-175

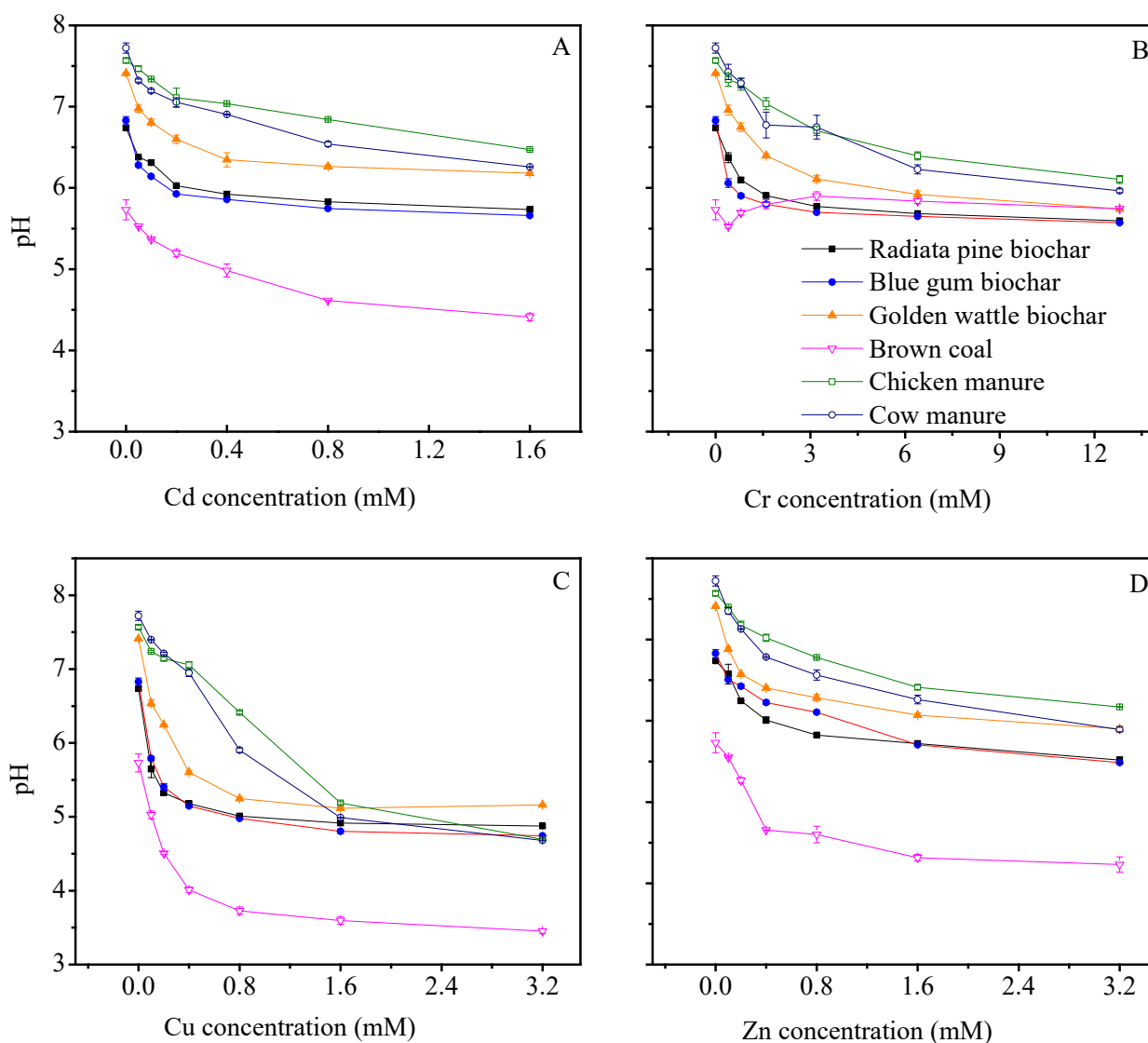


Figure 1. The pH changes as a function of concentration of Cd (A), Cr (B), Cu (C) and Zn (D) after shaking with six organic amendments for 24 h (0.05 g in 25 mL metal solution, $I = 0.01$ M NaNO_3). The initial pH of metal solutions was adjusted to 5.5 prior to addition of organic amendments. Vertical bars indicate standard errors ($n = 2$) where they are larger than symbols.

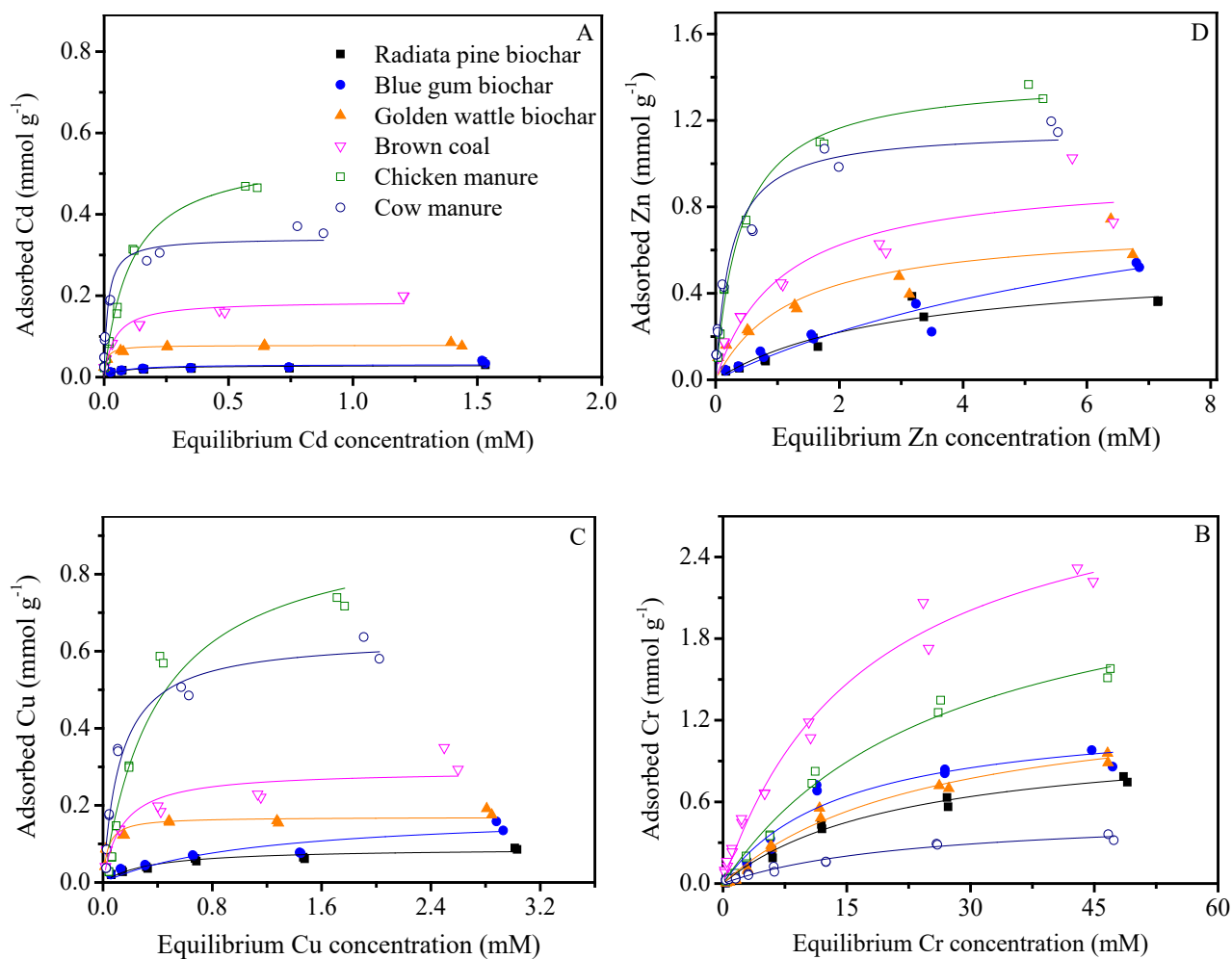


Figure 2. Langmuir adsorption isotherm curves of Cd (A), Cr (B), Cu (C) and Zn (F) in six organic materials (0.05 g in 25 mL metal solution, $I = 0.01$ M NaNO_3 , 24-h reacting time). The pH of initial metal solutions was adjusted to 5.5 prior to mixing with organic materials. The curves were fitted on two replicates.

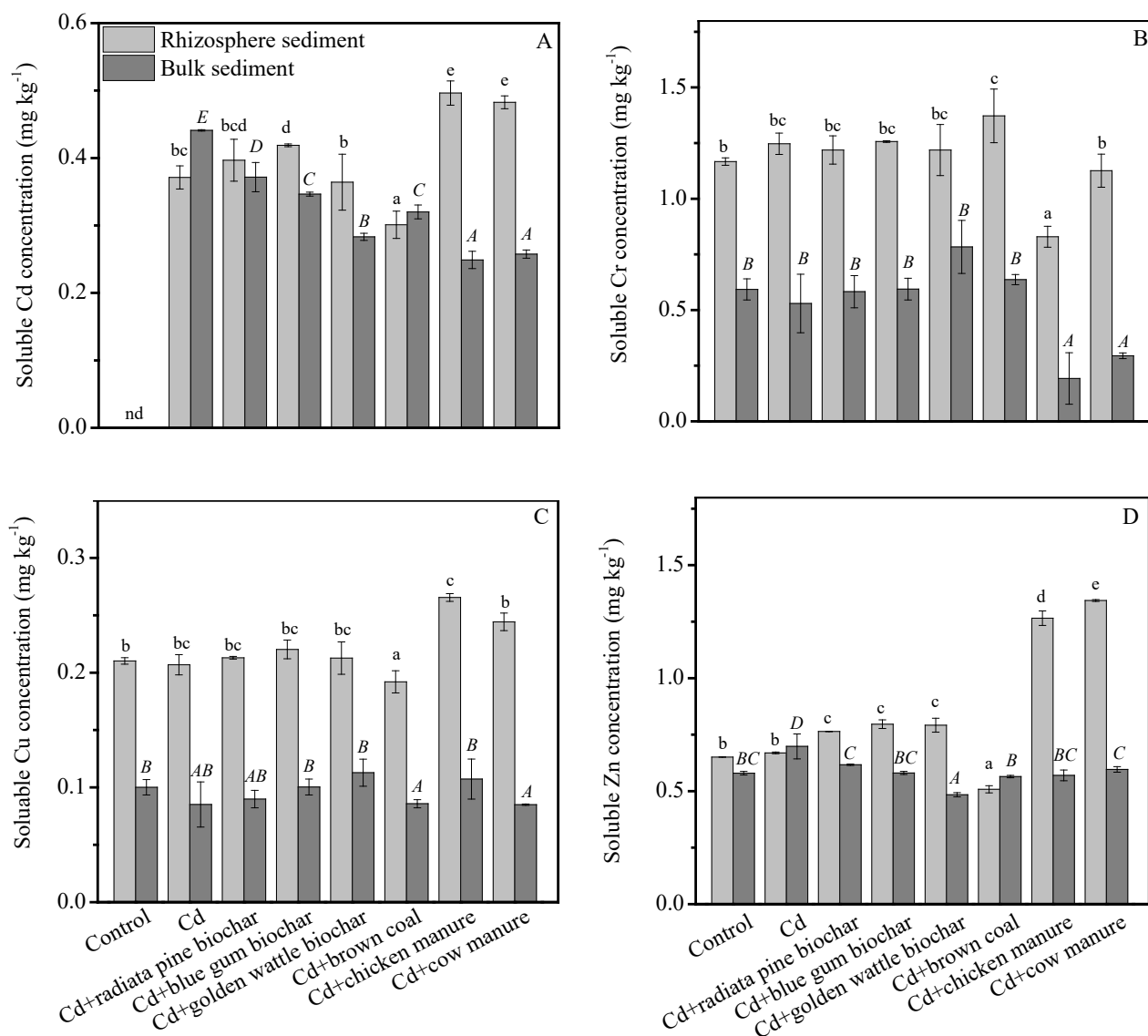


Figure 3. Concentrations of H₂O-extractable heavy metals in bulk and rhizosphere sediment after plants of *Carpobrotus rossii* were grown for 56 days in a sediment spiked with 20 mg Cd kg⁻¹ amended with six organic amendments at an addition rate of 20 g kg⁻¹. The values are mean ± standard errors (n = 4). Different letters indicate the significant difference among treatments ($p = 0.05$), lowercase for rhizosphere and italic uppercase for bulk sediment ($p = 0.05$). nd, under the detection limit.

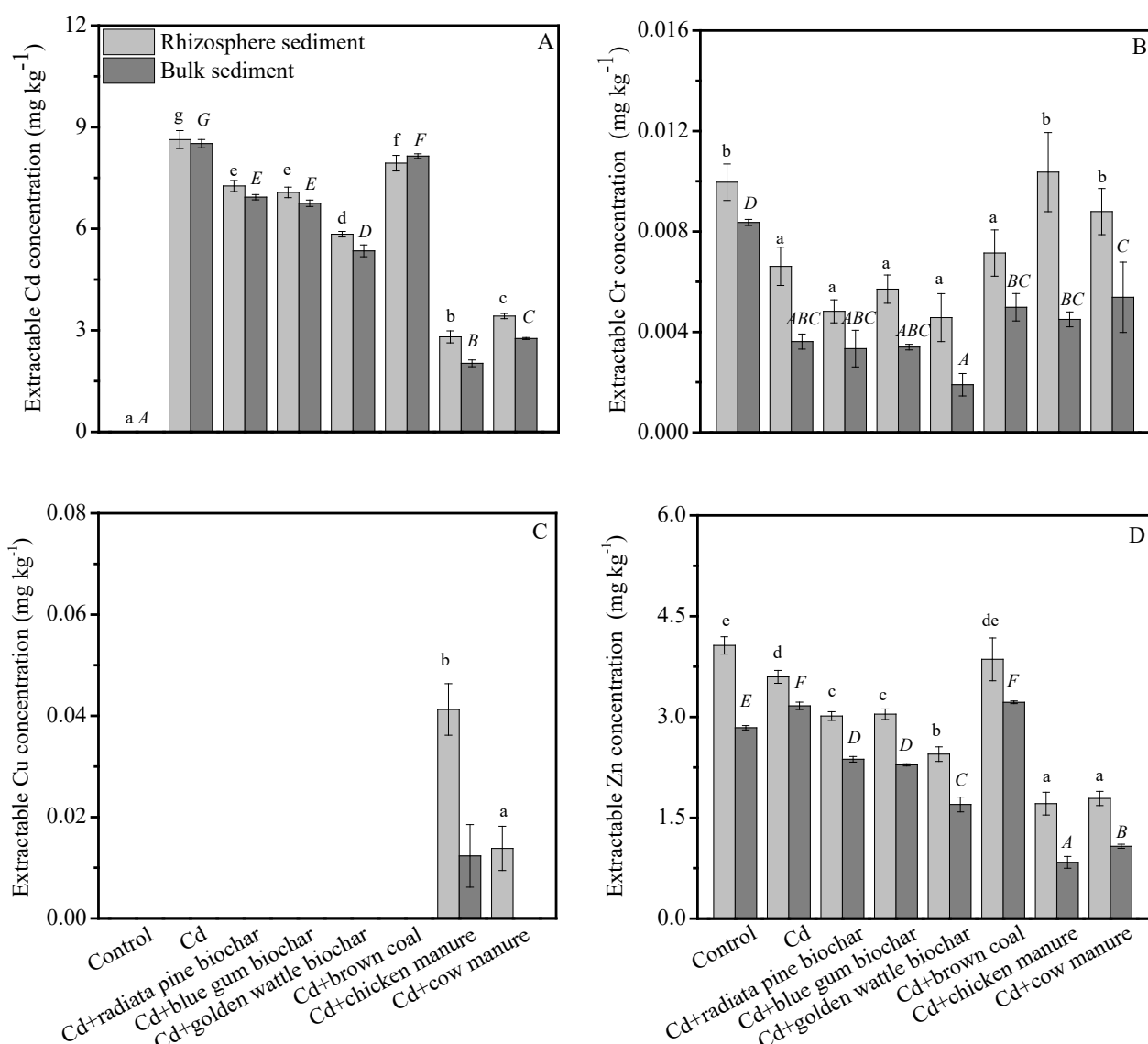


Figure 4. Concentrations of CaCl₂-extractable heavy metals in bulk and rhizosphere sediment after plants of *Carpobrotus rossii* were grown for 56 days in a sediment spiked with 20 mg Cd kg⁻¹ and amended with six organic amendments at an addition rate of 20 g kg⁻¹. The values are mean ± standard errors (n = 4). Different letters indicate the significant difference among the treatments (*p* = 0.05), lowercase for rhizosphere and italic uppercase for bulk sediment. The data of Cu in some treatments were under the detection limit.

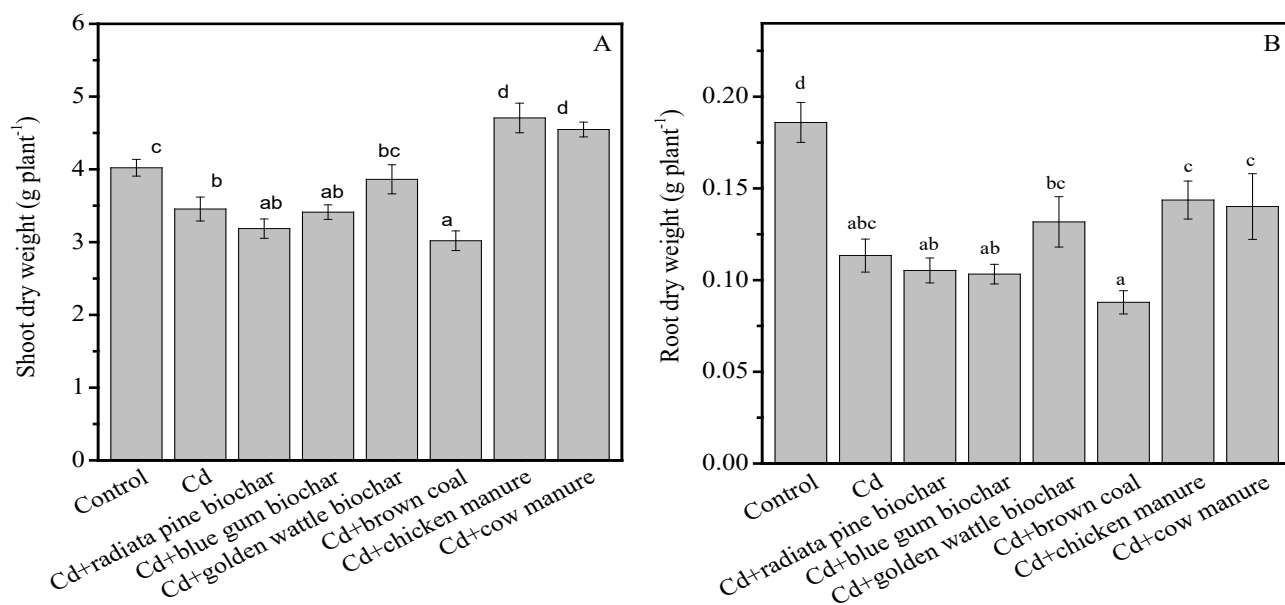


Figure 5. Dry weights of shoots (A) and roots (B) of *Carpobrotus rossii* grown for 56 days in a sediment spiked with 20 mg Cd kg⁻¹ and amended with six organic amendments at an addition rate of 20 g kg⁻¹. The values are mean \pm standard errors (n = 4). Different letters indicate the significant difference among treatments ($p = 0.05$).

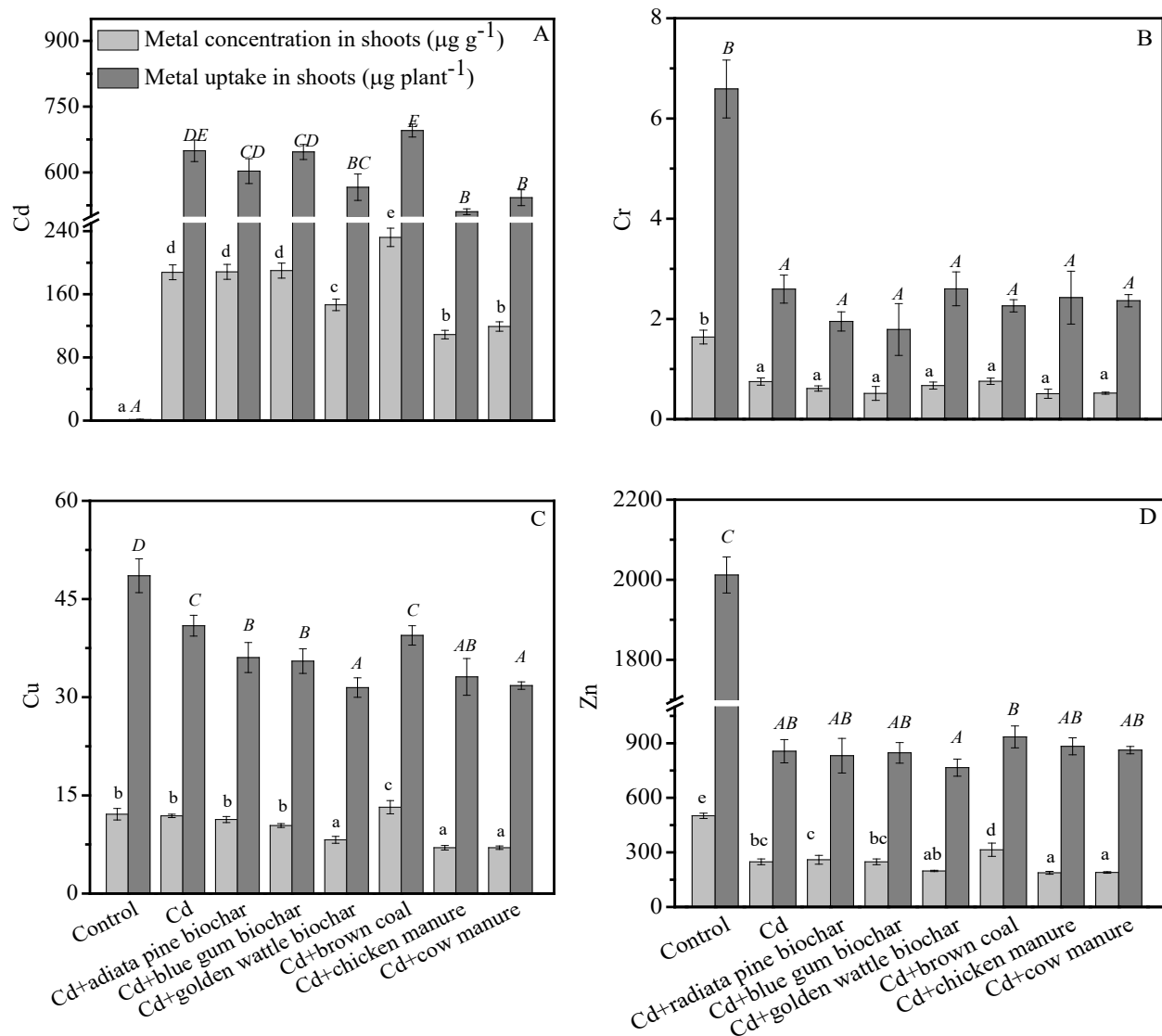


Figure 6. Effect of organic amendments on concentrations and uptake of heavy metals in shoots of *Carpobrotus rossii* grown for 56 days in a sediment spiked with 20 mg Cd kg⁻¹. The values are mean ± standard errors (n = 4). Different letters indicate the significant difference among treatments ($p = 0.05$), lowercase for metal concentration and italic uppercase for uptake in shoot, respectively.

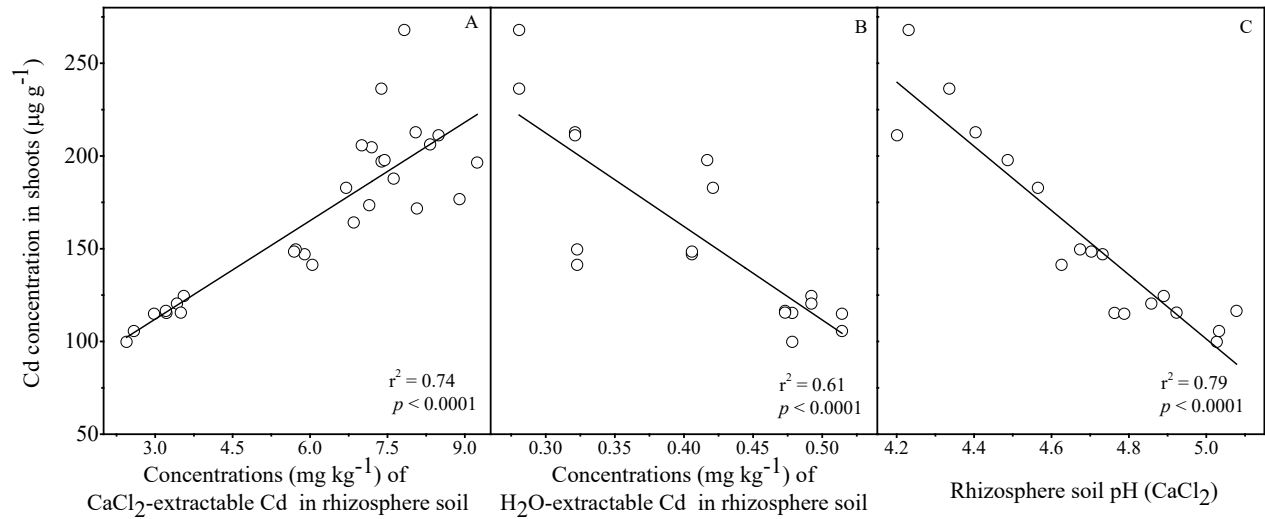


Figure 7. The relationships between Cd concentrations in shoots and concentrations of CaCl_2 - (A), H_2O -extractable (B) Cd in rhizosphere sediments, and rhizosphere pH (C) of *Carpobrotus rossii* grown for 56 days in a sediment spiked with 20 mg Cd kg^{-1} and amended with six organic materials at an addition rate of 20 g kg^{-1} .

Table 1. Concentrations of C and N, atomic ratio of C/N, cation exchange capacity (CEC), pH, electrical conductivity (EC) and dissolved organic carbon (DOC) of three wood-derived biochars, a brown coal, two animal manures and the sediment used in the study

Amendments	Concentration (g kg ⁻¹)		CEC (cmol _c kg ⁻¹)	pH		EC (dS m ⁻¹)	DOC (μg g ⁻¹)
	C	N		CaCl ₂	H ₂ O		
Radiata pine biochar	677	0.3	16	6.3	6.6	0.28	95
Blue gum biochar	649	0.4	23	6.1	6.3	0.34	102
Golden wattle biochar	604	7.8	41	7.0	7.2	1.13	105
Brown coal	494	5.0	23	4.9	4.8	0.22	33
Chicken manure	218	23.5	89	7.4	7.8	8.21	9925
Cow manure	180	16.3	85	7.4	7.6	10.61	6318
Sediment	23	1.0	10	4.8	5.9	98.30	96

Note: C and N were determined using an elemental analyzer (Perkin Elmer 2400 Series II CNHS/O System, USA).

CEC was determined using 1 M NH₄OAc extraction without pre-wash, thus CEC for two manures is over-estimated.

pH was determined using 1:5 soil: 0.01 M CaCl₂ or H₂O.

EC was determined using 1:5 soil: H₂O.

DOC was extracted in 0.5 M K₂SO₄.

Table 2. Total concentrations of heavy metals (mg kg⁻¹) and Ca, K, Na and P (g kg⁻¹) in organic amendments and sediment.

Amendments	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Ca	K	Na	P
Radiata pine biochar	0.04(0.00)	13.1(1.1)	6.5(0.3)	227(9)	4.0(0.1)	nd	23(2)	3.99	1.61	1.08	6.06(0.06)
Blue gum biochar	nd	11.7(0.4)	5.6(0.1)	147(2)	4.3(0.1)	nd	10(0)	5.24	2.95	1.55	5.88(0.00)
Golden wattle biochar	nd	9.0(0.3)	32.3(9.2)	175(20)	3.8(0.2)	nd	118(7)	9.20	4.76	2.15	4.50(0.17)
Brown coal	0.46(0.01)	0.8(0.0)	4.3(0.2)	41(0)	5.4(0.0)	2.4(0.2)	35(1)	4.34	0.57	0.43	0.01 (0.00)
Chicken manure	0.55(0.07)	24.2(0.8)	90.2(3.0)	582(5)	15.4(0.1)	nd	1575(12)	5.41	12.75	4.09	13.75(0.72)
Cow manure	0.66(0.02)	24.0(0.1)	46.9(0.5)	322(4)	10.1(0.0)	nd	1161(21)	5.70	12.49	8.95	8.17(0.02)
Sediment	1.20(0.10)	49.0(4.3)	13.0(1.1)	197(22)	22.0(1.4)	8.7(0.3)	51(1)	-	-	-	-

Note: Concentrations of total metals were determined through reverse aqua regia digestion (concentrated HNO₃:HCl = 3:1 by v/v). nd, under the detection limit. The values in the parentheses are standard errors of duplicate measurements. -, not measured.

Table 3. Comparisons of the parameters S_{\max} (mmol g⁻¹, the maximum of adsorbed heavy metal), K_L (L mmol⁻¹, the affinity of adsorption), as determined by Langmuir equation, of four heavy metals reacted for 24 h with six organic materials. r^2 represents the fitting coefficient. Values are mean (standard error) (n = 2).

Heavy metal	Parameter	Radiata pine biochar	Blum gum biochar	Golden wattle biochar	Brown coal	Chicken manure	Cow manure
Cd	S_{\max}	0.029(0.002)	0.031(0.003)	0.078(0.005)	0.187(0.010)	0.556(0.019)	0.342(0.020)
	K_L	16.328(5.973)	15.940(5.973)	103.031(45.210)	23.889(5.923)	9.415(0.882)	66.902(21.331)
	r^2	0.829	0.885	0.683	0.927	0.991	0.929
Cr	S_{\max}	1.100(0.061)	1.258(0.121)	1.421(0.118)	3.176(0.188)	2.518(0.170)	0.517(0.045)
	K_L	0.046(0.006)	0.070(0.017)	0.040(0.007)	0.057(0.008)	0.037(0.005)	0.043(0.008)
	r^2	0.989	0.952	0.982	0.985	0.989	0.974
Cu	S_{\max}	0.089(0.006)	0.183(0.033)	0.170(0.007)	0.291(0.022)	0.938(0.081)	0.637(0.031)
	K_L	2.882(0.644)	0.894(0.374)	29.070(6.208)	7.344(2.395)	2.480(0.532)	7.770(1.291)
	r^2	0.909	0.838	0.906	0.872	0.947	0.962
Zn	S_{\max}	0.542(0.079)	1.132(0.309)	0.718(0.091)	0.958(0.106)	1.403(0.034)	1.158(0.059)
	K_L	0.340(0.109)	0.122(0.053)	0.798(0.299)	0.931(0.313)	2.435(0.212)	4.095(0.929)
	r^2	0.919	0.939	0.845	0.886	0.991	0.951