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Biochars immobilize soil cadmium, but do not improve growth of emergent wetland species *Juncus subsecundus* in cadmium-contaminated soil

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Abstract
Purpose An addition of biochar mixed into the substrate of constructed wetlands may alleviate toxicity of metals such as cadmium (Cd) to emergent wetland plants, leading to a better performance in terms of pollutant removal from wastewater. The objective of this study was to investigate the impact of biochars on soil Cd immobilization and phytoavailability, growth of plants, and Cd concentration, accumulation, and translocation in plant tissues in Cd-contaminated soils under waterlogged conditions.
Materials and methods A glasshouse experiment was conducted to investigate the effect of biochars derived from different organic sources (pyrolysis of oil mallee plants or wheat chaff at 550 °C) with varied application amounts (0, 0.5, and 5 % *w*/*w*) on mitigating Cd (0, 10, and 50 mg kg^{-1}) toxicity to *Juncus subsecundus* under waterlogged soil condition. Soil pH and $CaCl_2$/EDTA-extractable soil Cd were determined before and after plant growth. Plant shoot number and height were monitored during the experiment. The total root length and dry weight of aboveground and belowground tissues were recorded. The concentration of Cd in plant tissues was determined.
Results and discussion After 3 weeks of soil incubation, pH increased and $CaCl_2$-extractable Cd decreased significantly with biochar additions. After 9 weeks of plant growth, biochar additions significantly increased soil pH and electrical conductivity and reduced $CaCl_2$-extractable Cd. EDTA-extractable soil Cd significantly decreased with biochar additions (except for oil mallee biochar at the low application rate) in the high-Cd treatment, but not in the low-Cd treatment. Growth and biomass significantly decreased with Cd additions, and biochar additions did not significantly improve plant growth regardless of biochar type or application rate. The concentration, accumulation, and translocation of Cd in plants were significantly influenced by the interaction of Cd and biochar treatments. The addition of biochars reduced Cd accumulation, but less so Cd translocation in plants, at least in the low-Cd-contaminated soils.
Conclusions Biochars immobilized soil Cd, but did not improve growth of the emergent wetland plant species at the early growth stage, probably due to the interaction between biochars and waterlogged environment. Further study is needed to elucidate the underlying mechanisms.

Keywords Biochar · Cadmium · Constructed wetland · *Juncus subsecundus* · Waterlogging

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1 Introduction

Wastewater has been used for agricultural irrigation in arid and semiarid regions due to a lack of clean freshwater. However, as one of unexpected side effects, large areas of soils were contaminated by heavy metals such as cadmium (Cd) because of the common practice to discharge a large volume of wastewater either untreated or after minimal preliminary treatments (Sun et al. 2009). Hence, it is necessary to treat wastewater before use due to potential deleterious impacts on human and environment health associated

with the use of wastewater containing pollutants such as metals.

Constructed wetlands for treating wastewater are an important phytoremediation technology around the world (Tel-Or and Forni 2011). The presence of wetland plants is one of the most conspicuous features of constructed wetlands in contrast to soil-only filters (Vymazal 2011). Wetland plants can enhance metal removal and/or stabilization (Weis and Weis 2004). Hence, successful phytoremediation using constructed wetlands depends on the tolerance of wetland plants to the contaminants in wastewater and/or substrate. Although the removal of pollutants may be satisfactory in constructed wetlands, some pollutants such as metals (e.g., Cd) may accumulate in the substrate when wetlands are exposed to wastewater over long periods of time (Marchand et al. 2010). The presence of metals in the substrate could influence growth of wetland plants and pollutant removal in the constructed wetlands (Zhang et al. 2011b, 2012b).

Amendments are used to reduce the bioavailability of metals in the soils and waters by immobilizing them into stable forms (Kumpiene et al. 2008). Organic materials are popular amendments because they are derived from biological matter and often require little pretreatment before they are applied directly to soil (Park et al. 2011b). Biochar is the product of pyrolysis, whereby organic materials of either plant or animal origin are heated (>250 °C) in a low or no oxygen environment (Antal and Grønli 2003). The properties of biochars produced from different feedstocks and by a variety of processes can vary widely (Brewer et al. 2011; Meyer et al. 2011; Schimmelpfennig and Glaser 2012). The application of biochar has attracted tremendous research interest in sequestering carbon in the form of thermally stabilized biomass (Sohi et al. 2010; Meyer et al. 2011). Recently, the benefits associated with soil application of biochars beyond their high carbon (C) content, such as their soil conditioning properties, have been reported (Glaser et al. 2002; Libra et al. 2011; Sohi et al. 2010). Biochars have demonstrated a clear potential for the remediation of a variety of organic (e.g., polynuclear aromatic hydrocarbons) and inorganic pollutants (e.g., metals) present in soils and waters (Barrow 2012; Beesley et al. 2011; Fellet et al. 2011; Jones et al. 2011; Rakowska et al. 2012). Depending on the feedstock and pyrolysis conditions used to produce biochar, biochar-induced changes in soil chemistry can provide additional benefits, such as metal immobilization and stabilization (Uchimiya et al. 2012a, b). However, little is known about biochar role in reducing the phytoavailability of heavy metals and phytotoxicity to plants in metal-polluted soils (Beesley et al. 2011).

The emergent wetland species such as *Juncus subsecundus* N.A. Wakef. (family Juncaceae) are often used in constructed wetlands (Zhang et al. 2011a, b). In our previous studies, the growth and biomass of *J. subsecundus* were significantly decreased by Cd additions (Zhang et al. 2011b, 2012b). Some studies have found that an addition of biochar decreased the soil Cd phytoavailability (Namgay et al. 2010; Park et al. 2011a) and decreased Cd uptake and translocation in rice (Cui et al. 2011). However, no previous study has quantified the influence of biochars on plant growth, Cd uptake, and translocation in emergent wetland plant species used in constructed wetlands. Hence, the objectives of this study were to investigate (1) the impact of biochars derived from different sources (pyrolysis of oil mallee or wheat chaff at 550 °C) at different application rates on soil Cd immobilization and phytoavailability and (2) the effect of biochars on wetland plant growth, Cd concentration, accumulation, and translocation in *J. subsecundus* in Cd-contaminated soils under waterlogged conditions.

2 Materials and methods

2.1 Biochar characterization

Biochars made from oil mallee (whole plants) or wheat chaff (Pacific Pyrolysis Pty Ltd, NSW, Australia) were assessed for basic characteristics (Table 1). The pH and electrical conductivity (EC) of biochar were measured in water at 1:5 (*w*/*v*) ratios. The pH was also measured in 0.01 M $CaCl_2$ at 1:5 (*w*/*v*) ratio. A subsample of biochar was finely ground before total carbon and nitrogen contents were determined by dry combustion analysis using an elementar (vario MACRO CNS; Elementar, Germany). Cation exchange capacity (CEC) was determined as described by Gillman and Sumpter (1986). The ammonium (NH_4^+) and nitrate (NO_3^-) contents were determined by extracting with 0.5 M K_2SO_4 and analyzing the extract colorimetrically for NH_4^+ (Krom 1980; Searle 1984) and NO_3^- (Kamphake et al. 1967; Kempers and Luft 1988) on an automated flow injection Skalar auto-analyzer (Skalar San *Plus*). The biochars were out-gassed under vacuum at 105 °C for 8 h using VacPrep before measuring their pore volumes using BET (Micromeritics, Gemini). Proximate analysis (i.e., determination of moisture, volatile matter, fixed carbon, ash, and other properties by prescribed methods) was used to assess ash content by heating biochar to 750 °C for 6 h according to ASTM International (2007). Total content of the elements in ash was quantified by ICP-AES (Spectro CIROS, CCD, Germany) after digestion in HNO_3.

2.2 Preparation of contaminated soil

Soil without detectable Cd was collected from Gingin, Western Australia (31°46′ S, 115°86′E), air-dried, and sieved through a 2-mm mesh. This soil, used as media in constructed wetlands for treatment of stormwater (Zhang et al. 2011a, b, 2012b), was sandy loam, containing coarse sand (200–2,000 μm) 873 gkg^{-1}, fine sand (20–200 μm)

Table 1 Characteristics of the two biochars used in the study

Properties	Oil mallee	Wheat chaff
Pyrolysis temperature (°C)	550	550
pH (H_2O)	7.51	9.00
pH ($CaCl_2$)	7.20	8.49
Carbon (%)	67.01	56.63
Nitrogen (%)	0.54	2.1
C/N ratio	125	27
NH_4–N (mg kg^{-1})	<0.1	1.9
NO_3–N (mg kg^{-1})	<0.2	<0.1
CEC (m.e./100 g C)	11.25	30.43
EC (mS cm^{-1})	0.852	7.66
Pore volume (m^2 g^{-1})	14.40	190.08
Proximal analysis (%)		
Ash	8.6	16.2
Silicon (SiO_2)	38.6	53.3
Aluminum (Al_2O_3)	4.5	2.33
Iron (Fe_2O_3)	3.0	2.44
Calcium (CaO)	34.7	5.2
Magnesium (MgO)	8.8	3.91
Sodium (Na_2O)	1.5	0.27
Potassium (K_2O)	6.3	21.8
Titanium (TiO_2)	0.24	0.16
Manganese (Mn_3O_4)	0.68	0.22
Phosphorus (P_2O_5)	0.94	7.8
Sulfur (SO_3)	1.3	2.32
Strontium (SrO)	0.26	0.04
Barium (BaO)	0.06	0.08
Zinc (ZnO)	0.05	0.04
Vanadium (V_2O_5)	0.01	<0.02

79 gkg^{-1}, silt (2–20 μm) 19 gkg^{-1}, and clay (<2 μm) 29 g kg^{-1}. Soil chemical properties were: pH_{water} 6.9, pH_{CaCl2} 5.9, EC 0.034 dS m^{-1}, total organic carbon 3.9 gkg^{-1}, total nitrogen 0.22 gkg^{-1}, total phosphorus 0.12 gkg^{-1}, total Cd <0.0007 mg kg^{-1}, total copper (Cu) 1.3 mg kg^{-1}, total lead (Pb) 1.8 mg kg^{-1}, and total zinc (Zn) 1.7 mg kg^{-1}.

Cadmium (as $CdCl_2 \times 2$ 1/2 H_2O, analytical grade, Ajax Chemicals, Sydney, Australia) was dissolved in Milli-Q water and added to soil at concentrations of 0, 10, or 50 mg Cd kg^{-1}. The basal nutrients in solution were added to all treatments at the following rates (in milligrams per kilogram of soil): 33.3 nitrogen (N), 20.5 phosphorous (P), 88.7 K, S 34.2, Ca 41.0, Cl 72.5, Mg 3.95, Mn 3.26, Zn 2.05, Cu 0.51, B 0.12, Co 0.11, and Mo 0.08 and were mixed uniformly.

Biochars derived from oil mallee or wheat chaff were passed through a 2-mm sieve after drying at 70 °C for 5 days in a forced-air cabinet. The biochars were uniformly mixed into soils at the rate of 0.5 or 5 % (*w*/*w*), in addition to a non-biochar treatment. The amended soils were placed in plastic bags and equilibrated in a dark room at 25 °C for 3 weeks. The soils were mixed twice every week, and the moisture content was kept at 10 % (*w*/*w*). The soil samples were collected after equilibration and analyzed for soil pH and extractable Cd.

2.3 Experimental setup

The experiment was set up in a complete randomized block design (three Cd treatments × five biochar additions) with three replicates. Based on the previous experiments (Zhang et al. 2011b, 2012b), the emergent wetland plant species *J. subsecundus* was selected for this study conducted in a glasshouse at The University of Western Australia (31°58′ S, 115°49′ E) with controlled day/night temperatures of 25/20 °C under natural light conditions from mid-October to mid-December, 2011. The seedlings of *J. subsecundus* were collected from the local nursery and transplanted (with initial plant fresh weight 10.5±0.4 g per pot) into 3.8-L pots (170 mm in diameter at the top and 180 mm in height) containing 3 kg of soil per pot. The pots were watered with deionized water to achieve a water layer of 15 mm above the soil surface and maintained by refilling twice a week.

2.4 Sampling and measurements

The shoot number and the longest shoot height were measured weekly after plant establishment. The plants were harvested after 9 weeks of growth. The soil samples from the pots were collected and analyzed for pH and extractable Cd. Shoots were cut just above the soil surface and their base was washed with deionized water. Each pot was then excavated, and the roots (including rhizomes) were separated from soil by washing with running tap water over a mesh and rinsing with deionized water three times. The subsamples of roots were collected for root length analysis, which were measured using a gridline intercept method (Newman 1966). All samples were dried to a constant weight at 70 °C for 5 days in a forced-air cabinet, weighed for dry weight (DW) biomass, and ground to pass a 0.75-mm mesh. The concentration of Cd in plant tissues was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 5300DV, PerkinElmer, Shelton, USA) after digesting plant material in a heated mixture of concentrated nitric and perchloric acids (Bassett et al. 1978).

The pH_{CaCl2} and EC (5:1 solution/soil) were determined by a Thermo Scientific Orion Star Series meter (Thermo Fisher Scientific Inc., USA). The concentration of the extractable Cd in soil samples was measured by ICP-OES after extraction and filtration. Briefly, soil (4 g) was shaken with 0.01 M $CaCl_2$ (20 mL) for 4 h or 0.05 M EDTA (pH 7.0,

20 mL) for 1 h at 25 °C on an end-over-end shaker (McGrath 1996). Extract was filtered through a 0.45-μm membrane Acrodisc® syringe filter.

2.5 Data calculation

The soil Cd immobilization efficiency was calculated as:

$$\text{Cd immobilization efficiency}\,(\%) = (1 - \text{extractable Cd in biochar treatment}/\text{extractable Cd in non} - \text{biochar treatment}) \times 100 \quad (1)$$

The shoot or root concentration factor (SCF or RCF) was calculated as:

$$\text{SCF or RCF} = \text{Cd concentration in shoots or roots}/\text{Cd concentration in soils} \quad (2)$$

The translocation factor (TF) was calculated as:

$$\text{TF}\,(\%) = (\text{Cd concentration in shoot}/\text{Cd concentration in root}) \times 100 \quad (3)$$

Total accumulation of Cd in plants, expressed as microgram per pot, was calculated as:

$$\text{Total Cd accumulation} = (\text{Cd concentration in shoots} \times \text{shoot DW}) + (\text{Cd concentration in roots} \times \text{root DW}) \quad (4)$$

The percentage removal of Cd by plants was calculated as:

$$\text{Removal of Cd by plants}\,(\%) = (\text{total Cd accumulation in plants}/\text{total added Cd in soil}) \times 100 \quad (5)$$

2.6 Statistical analyses

Statistical analyses were carried out using IBM® SPSS® version 19. Two-way analysis of variance was used to detect significant effects of Cd and biochars on all measured parameters. Least significant difference (LSD) was applied to test significance between means. The significant Pearson's correlations between the soil pH or Cd concentrations and measured parameters of plant tissues after 9 weeks of *J. subsecundus* growth were tested.

3 Results

3.1 Initial pH and extractable Cd in soil after incubation

The soil pH increased significantly with the biochar additions, but was not significantly influenced by Cd treatments after 3 weeks of soil incubation (Fig. 1). The highest pH was in oil mallee biochar at the high application rate, followed by wheat chaff biochar at the high application rate, then biochars at the low application rates and the non-biochar amendment.

The $CaCl_2$-extractable soil Cd was significantly influenced by the interaction of Cd and biochar additions (Table 2). More than 96 % of $CaCl_2$-extractable soil Cd was significantly immobilized in the biochar treatments at the high application rate regardless of biochar type and the rate of Cd addition. At the low biochar application rate, the $CaCl_2$-extractable soil Cd immobilization was significantly higher with oil mallee biochar than wheat chaff biochar regardless of Cd treatments (Fig. 2).

3.2 Plant growth

The longest shoot length, total shoot number, and root length were significantly influenced by different Cd and biochar additions as well as their interactions after 9 weeks

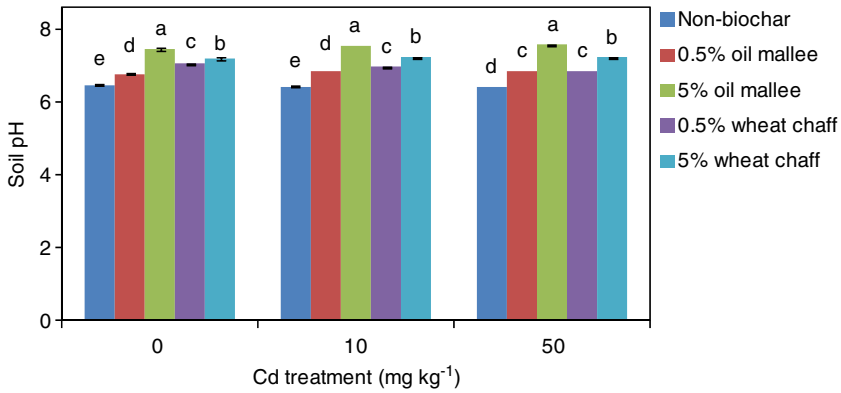

Fig. 1 Soil pH_{CaCl2} influenced by different Cd and biochar additions after 3 weeks of soil incubation. *Bars* (means±SE, $n=3$) with different letters within the same Cd treatments are significantly different based on LSD ($p \leq 0.05$)

of *J. subsecundus* growth (Fig. 3). In non-Cd-contaminated soil, the total shoot number and total root length significantly decreased with biochar treatments at the high application rate compared to the control (without addition of Cd or biochar), but the longest shoot length was not significantly influenced by biochar additions. In the treatment with the low rate of Cd addition, the longest shoot length and shoot number significantly increased with biochar treatments at the high application rate (except for shoot number in the wheat chaff biochar) compared to the low-Cd-contaminated soil without biochar, but no significant difference was detected in the high-Cd-contaminated soil (see Fig. 3).

3.3 Plant biomass

The aboveground, belowground, and total biomass of plants was significantly influenced by different Cd and biochar additions as well as their interactions after 9 weeks of *J. subsecundus* growth (Fig. 4). The biomass significantly decreased with Cd additions. In non-Cd-contaminated soil, the biomass was significantly decreased with biochar additions (except for the low rate of wheat chaff biochar) compared to the control, with more decrease at the high application rate of biochar derived from wheat chaff compare to oil mallee, but the result was opposite at the low application rate (see Fig. 4). In Cd-contaminated soil, the total biomass did not significantly increase with biochar additions compared to the Cd-contaminated soil without biochar (see Fig. 4).

3.4 Concentration, accumulation, and translocation of Cd in plants and removal by plants

The concentration, accumulation, and translocation of Cd in plant tissues and Cd removal by plants were significantly influenced by different Cd and biochar additions as well as their interaction (except for Cd removal by plants) after 9 weeks of *J. subsecundus* growth (Tables 3 and 4). The concentration of Cd in shoots and roots significantly increased with Cd additions and significantly decreased with the biochar additions in the high-Cd treatment, but not in the low-Cd treatment (except for Cd concentration in shoots at the high biochar application rate of wheat chaff biochar and in roots at the high application rate of either biochar). The SCF, RCF, and TF were significantly reduced by the biochar additions (except for TF at the low-Cd treatment) compared

Table 2 The $CaCl_2$-extractable soil Cd influenced by different Cd and biochar additions after 3 weeks of soil incubation and 9 weeks of plant growth

Cd treatment (mg kg^{-1})	Biochar treatment (% *w*/*w*)				
	Non-biochar	0.5 % oil mallee	5 % oil mallee	0.5 % wheat chaff	5 % wheat chaff
After 3 weeks of soil incubation					
0	Not detectable	Not detectable	Not detectable	Not detectable	Not detectable
10	0.68±0.06 a	0.05±0.01 b	Not detectable	0.27±0.06 b	Not detectable
50	11.56±0.35 a	3.36±0.23 c	0.23±0.04 d	5.86±0.13 b	0.40±0.16 d
After 9 weeks of plant growth					
0	Not detectable	Not detectable	Not detectable	Not detectable	Not detectable
10	0.95±0.02 a	0.31±0.01 b	0.04±0.002 b	0.52±0.02 a, b	0.05±0.001 b
50	11.51±0.52 a	3.44±0.21 c	0.47±0.01 d	5.99±0.13 b	0.65±0.08 d

Means (± SE, $n=3$) with different letters within the rows are significantly different based on LSD ($p \leq 0.05$)

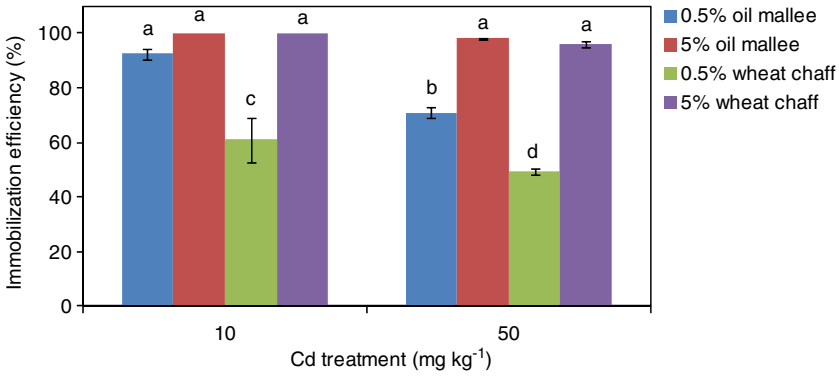

Fig. 2 Soil Cd immobilization efficiency (based on $CaCl_2$-extractable Cd) influenced by different Cd and biochar additions after 3 weeks of soil incubation. The extractable Cd in biochar treatments was assumed to be zero when non-detectable using ICP-OES. *Bars* (means±SE, n=3) with different letters are significantly different based on LSD ($p \leq 0.05$)

to non-biochar treatment (see Table 3). The total Cd accumulation in plants and removal by shoots or whole plants significantly decreased in the biochar treatments (except for wheat chaff biochar at the low application rate in the low-Cd treatment) compared to the non-biochar treatment (see Table 4).

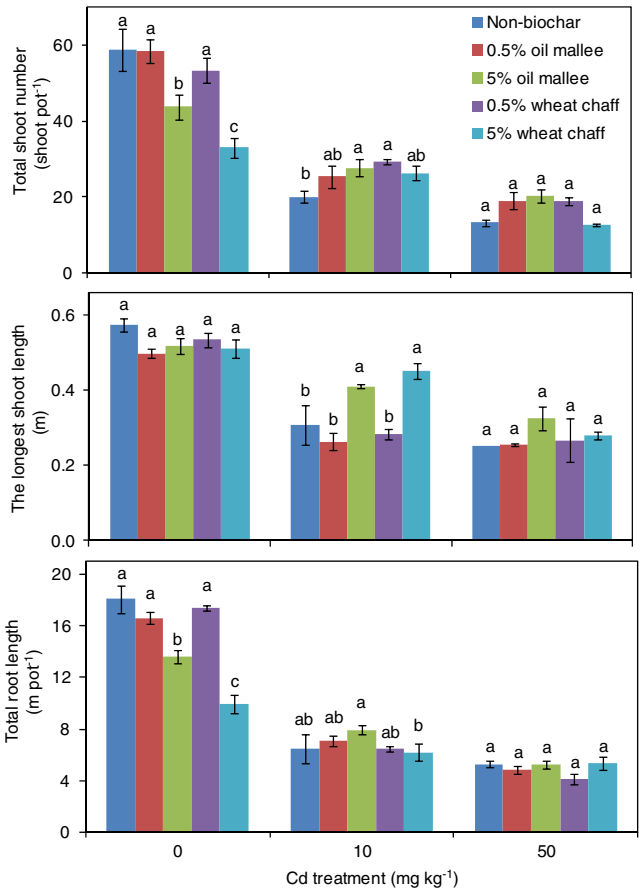

Fig. 3 The total shoot number, longest shoot length, and total root length influenced by different Cd and biochar additions after 9 weeks of *J. subsecundus* growth. *Bars* (means±SE, n=3) with different letters within the same Cd treatments are significantly different based on LSD ($p \leq 0.05$)

3.5 pH, EC, and extractable Cd in soil after plant growth

The pH, EC, and extractable Cd in soil were significantly influenced by different Cd and biochar additions as well as their interaction (except for soil EC) after 9 weeks of *J. subsecundus* growth. The soil pH and $CaCl_2$-extractable Cd after 9 weeks of *J. subsecundus* growth (Fig. 5 and Table 2) almost mirrored those after 3 weeks of soil incubation (see Fig. 1 and Table 2). The soil EC significantly increased with

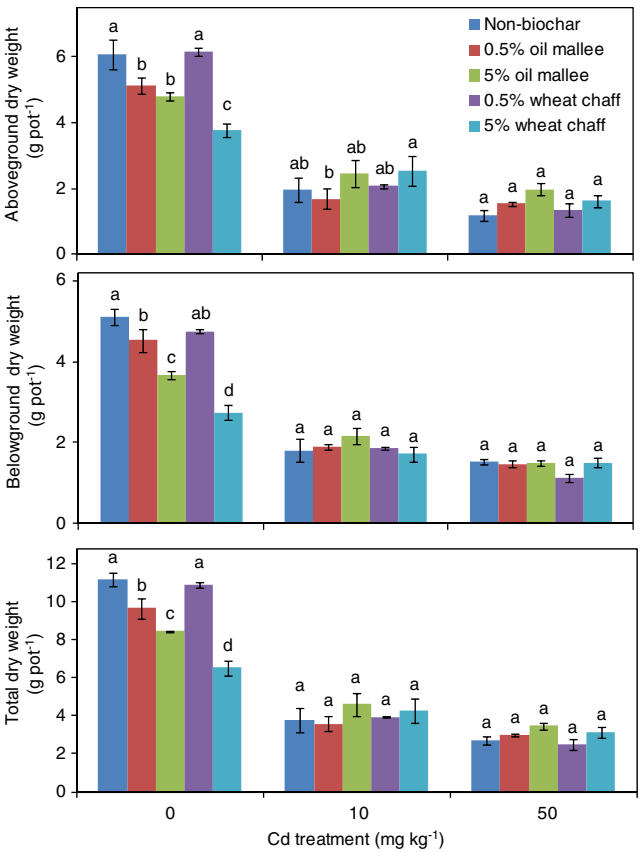

Fig. 4 Aboveground, belowground, and total plant biomass influenced by different Cd and biochar additions after 9 weeks of *J. subsecundus* growth. *Bars* (means±SE, n=3) with different letters within a Cd treatment are significantly different based on LSD ($p \leq 0.05$)

Table 3 The concentration of Cd in plant tissues, shoot or root concentration factors (SCF or RCF), and translocation factors (TFs) influenced by different Cd treatments and biochar additions after 9 weeks of *J. subsecundus* growth

Biochar treatment (% *w/w*)	Shoot (mg kg^{-1})			Root (mg kg^{-1})			SCF		RCF		TF (%)	
	C0[a]	C1	C2	C0	C1	C2	C1	C2	C1	C2	C1	C2
Non-biochar	Not detectable	46.8±5.5 a	267.7±20.5 a	0.2±0.1 a	76.9±6.9 a	390.4±26.7 a	4.7±0.6 a	5.4±0.4 a	7.7±0.7 a	7.8±0.5 a	60.8±4.9 b	68.6±2.0 a
0.5 % oil mallee	Not detectable	35.0±4.2 a, b	98.5±8.5 c	0.3±0.1 a	64.5±4.6 a, b	205.4±5.7 c	3.5±0.4 b	2.0±0.2 b	6.5±0.5 b	4.1±0.1 b	53.9±2.9 b	47.9±3.6 b
5 % oil mallee	Not detectable	29.0±3.3 a, b	25.4±3.7 d	0.1±0.2 a	43.3±0.6 b, c	88.6±5.3 d	2.9±0.3 c	0.5±0.1 c	4.3±0.1 c	1.8±0.1 c	67.2±8.4 b	29.2±5.3 c
0.5 % wheat chaff	Not detectable	40.2±2.4 a	127.2±11.6 b	0.4±0.3 a	60.4±4.2 a, b	271.2±20.7 b	4.0±0.2 b	2.5±0.2 b	6.0±0.4 b	5.4±0.4 b	67.6±7.9 b	47.0±2.6 b
5 % wheat chaff	Not detectable	17.8±1.3 b	19.2±1.1 d	0.2±0.1 a	16.3±0.9 c	46.6±0.8 e	1.8±0.1 d	0.4±0.02 c	1.6±0.1 d	0.9±0.02 c	109.0±3.0 a	41.2±2.2 b, c

Means (± SE, n=3) followed by the same lowercase letter within columns are not significantly different according to LSD ($p \leq 0.05$)

[a] Cd treatments: C0, C1, and C2 represent, respectively, 0, 10, and 50 mg Cd kg^{-1}

Table 4 Cadmium accumulation in plants and percentage removal by plants influenced by different Cd treatments and biochar additions after 9 weeks of *J. subsecundus* growth

Biochar treatment (% *w/w*)	Shoot (μg pot^{-1})		Root (μg pot^{-1})			Total plant (μg pot^{-1})		Removal by shoot (%)		Removal by whole plant (%)	
	C1[a]	C2	C0	C1	C2	C1	C2	C1	C2	C1	C2
Non-biochar	94±27 a	315±22 a	1±0.3 a	141±29 a	593±18 a	235±52 a	907±9 a	0.3±0.1 a	0.2±0.02 a	0.8±0.2 a	0.6±0.01 a
0.5 % oil mallee	57±3 b, c	150±9 b	1±0.6 a	121±4 a	302±14 b	178±2 b	452±18 b	0.2±0.01 b	0.1±0.01 b	0.6±0.01 b	0.3±0.01 b
5 % oil mallee	69±6 a, b, c	49±4 c	0.2±0.6 a	94±10 b	132±3 c	163±15 b	181±2 c	0.2±0.02 b	0.03±0.002 c	0.5±0.1 b	0.1±0.001 c
0.5 % wheat chaff	83±3 a, b	168±23 b	2±1 a	113±6 a	305±9 b	196±5 a, b	473±20 b	0.3±0.01 a	0.1±0.02 b	0.7±0.02 a, b	0.3±0.01 b
5 % wheat chaff	45±7 c	32±6 c	1±0.2 a	28±2 c	70±6 d	73±10 c	102±12 d	0.1±0.03 c	0.02±0.004 c	0.2±0.03 c	0.1±0.01 c

Means (± SE, n=3) followed by the same lowercase letter within columns are not significantly different according to LSD ($p \leq 0.05$)

[a] Cd treatments: C0, C1, and C2 represent, respectively, 0, 10, and 50 mg kg^{-1} of Cd

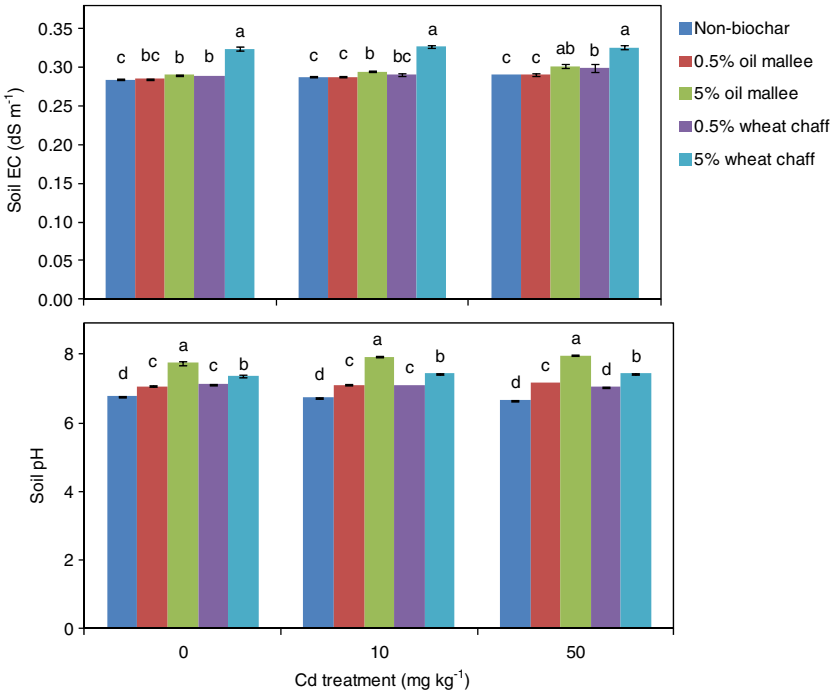

Fig. 5 Soil pH_{CaCl2} and EC influenced by different Cd and biochar additions after 9 weeks of plant growth. *Bars* (means± SE, n=3) with different letters within a Cd treatment are significantly different based on LSD ($p \leq 0.05$)

biochar additions (see Fig. 5). The EDTA-extractable soil Cd significantly decreased with biochar treatments (except for oil mallee biochar at the low application rate) in the high-Cd-contaminated soil, but not in the low-Cd-contaminated soil regardless of biochar type and application rate (Fig. 6).

3.6 Correlations between soil pH or soil extractable Cd and plant parameters

Significant Pearson's correlation coefficients (r) were detected between the soil extractable Cd concentrations and all measured parameters of plants after 9 weeks of *J. subsecundus* growth (Table 5). The $CaCl_2$-extractable soil Cd was highly correlated with either the concentration or accumulation of Cd in plants, whereas the EDTA-extractable soil Cd was highly correlated with plant growth and biomass. Significant Pearson's correlation coefficients (r) were also detected between the soil pH and concentration or accumulation of Cd in plant tissues (see Table 5).

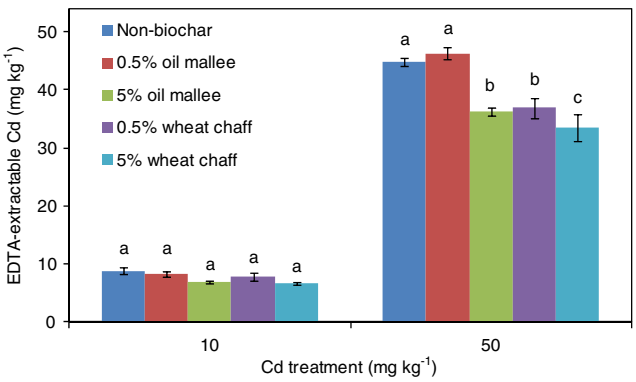

Fig. 6 The EDTA-exchangeable soil Cd influenced by different Cd and biochar additions after 9 weeks of plant growth. *Bars* (means±SE, n=3) with different letters within the same Cd treatments are significantly different based on LSD ($p \leq 0.05$)

4 Discussion

The immobilization of metals such as Cd by addition of biochars has been reported in the recent literature (cf. Beesley et al. 2011). For example, the addition (5 % *w*/*w*) of biochar derived from chicken manure and green waste significantly immobilized NH_4NO_3-extractable Cd in a metal-contaminated soil, with a higher immobilization rate of Cd in chick manure biochar than green waste biochar (Park et al. 2011a). In the present study, the biochar additions significantly immobilized $CaCl_2$-extractable soil Cd (see Table 2), with higher efficiency of immobilization at the high application rate (5 %) than the low application rate (0.5 %), and in the oil mallee biochar than wheat chaff biochar treatments at the low application rate (see Fig. 2). Those results indicated that biochars derived from different feedstocks, produced by different methods, and applied at different rates could show a varied potential of metal immobilization. Hence, the findings are not easily transferable to other biochar sources.

Metal ions are expected to interact with the biochar organic carbon component via various mechanisms such as (a) electrostatic interactions between metal cations and the negatively charged biochar surface and (b) ionic exchange between ionizable protons at the biochar surface, metal

Table 5 Pearson's correlation coefficients (r) between the soil pH or Cd concentrations and measured parameters of plant tissues after 9 weeks of *J. subsecundus* growth

Measured plant parameter	$CaCl_2$-extractable Cd	EDTA-extractable Cd	pH_{CaCl2}
Shoot Cd concentration	0.97***	0.71***	−0.60***
Root Cd concentration	0.95***	0.81***	−0.54***
Shoot Cd accumulation	0.93***	0.73***	−0.61***
Root Cd accumulation	0.95***	0.79***	−0.57***
Aboveground biomass	−0.47**	−0.70***	0.42**
Belowground biomass	−0.41**	−0.68***	0.17^{NS}
Total biomass	−0.45**	−0.69***	0.35*
The longest shoot height	−0.50***	−0.74***	0.48**
Total shoot number	−0.47**	−0.72***	0.29^{NS}
Total root length	−0.42**	−0.69***	0.19^{NS}

NS not significant

*$p\leq0.05$, significant; **$p\leq0.01$, significant; ***$p\leq0.001$, significant

cations, etc. (cf. Uchimiya et al. 2012a, b). However, the efficiency of metal immobilization/stabilization in biochar-amended soils strongly depends on the release of native inorganic components by the soils and biochars and the influence of the biochar amendment on the soil pH and dissolved organic carbon (Beesley et al. 2010). The increased soil pH after biochar additions in the present study (see Figs. 1 and 5) could partly account for Cd immobilization. However, it remains unclear to what extent pH may influence Cd partitioning among different soil pools.

There is no agreement in the literature as to which extractant most accurately estimates the phytoavailability of trace metals in soils (Menzies et al. 2007). The use of various extractants such as neutral salt solutions (i.e., $CaCl_2$ and NH_4NO_3) and complexing agents (i.e., EDTA and diethylene triamine pentaacetic acid (DTPA)) could result in extraction of different chemical fractions of metals. For example, the $CaCl_2$-extractable soil Cd significantly decreased with additions of biochar produced from wheat straw in a contaminated paddy soil. The DTPA-extractable Cd was not significantly changed in the first year after application of biochar, but significantly reduced in the second year after application in a 2-year paddy field experiment compared to non-biochar (Cui et al. 2011). In the present study, the $CaCl_2$-extractable soil Cd significantly decreased (see Fig. 2) and the EDTA-extractable Cd significantly decreased with biochar additions (except for oil mallee biochar at the low application rate) in the high-Cd treatment, but not in the low-Cd treatment (see Fig. 6).

A comprehensive study by Menzies et al. (2007) regarding the evaluation of a single extractant for estimation of the phytoavailable metals in soils emphasized that neutral salt solution tended to provide the best relationship between soil extractable metal and plant tissue accumulation, whereas complexing agents or acid extractants (e.g., HCl) were generally poorly correlated to plant uptake. Although $CaCl_2$- and EDTA-extractable Cd were both significantly correlated to plant Cd uptake and plant growth in the present study, $CaCl_2$-extractable Cd was better correlated to plant Cd uptake and EDTA-extractable Cd was better correlated with plant growth and biomass (see Table 5). The EDTA extraction was tentatively proposed to measure the pool of a metal that can be released from the soil solid phase into solution through forming chelates, which is generally accepted as indicative of accessibility to plant root uptake (Quevauviller 2002). Accordingly, higher EDTA extractability refers to a smaller fraction of bounded metals. In the present study, the concentration of Cd in shoots and roots significantly decreased with the biochar additions in the high-Cd treatment, but mostly not in the low-Cd treatment (see Table 3), which was reflected in the EDTA-extractable soil Cd that was not significantly different in the low-Cd-contaminated soil regardless of the presence or absence of biochar additions (see Fig. 6). Accordingly, the SCFs and RCFs were significantly lower in the biochar treatments than without, whereas the Cd TFs were similar in the low-Cd treatment (except for the high wheat chaff biochar), but lower in the high-Cd treatment compared to the non-biochar treatment (see Table 3), indicating that the addition of biochars influenced plant uptake, but less so Cd translocation in plants, at least in the relatively low-Cd-contaminated soils.

Plants could mediate the transformation, mobility, and bioavailability of metals, especially in the rhizosphere due to plant–soil–microbe interactions (Park et al. 2011b). In our previous study with the same wetland species and Cd-contaminated soil as in this study, the pH was significantly lower in the rhizosphere than non-rhizosphere and significantly decreased with the Cd additions in the rhizosphere after 10 weeks of *J. subsecundus* growth (Zhang et al. 2012b). The pH changes in the rhizosphere could influence Cd speciation and bioavailability in media, possibly resulting in varied Cd uptake by plants. Nevertheless, recent studies have indicated that the transformation of metals in soils is a dynamic process, meaning phytoavailability may change with time (Rao et al. 2008) and is therefore difficult to measure (Moreno-Jiménez et al. 2010).

A plant growth response to biochar-amended soil has been variable, with both positive and negative results reported in field and greenhouse studies (Lehmann and Joseph 2009), but a meta-analysis showed a small positive overall effect on plant growth (Jeffery et al. 2011). Even though studies on the effect of biochar on plant growth were mostly conducted in greenhouses (e.g., Buss et al. 2011; Namgay et al. 2010; Park et al. 2011a; Solaiman et al. 2012) and field sites (e.g., Jones et al. 2012; Solaiman et al. 2010) with dryland crops, few studies have been carried out in the field with wetland plants (e.g., Asai et al. 2009; Zhang et al. 2010, 2012a). For instance, the amendments of biochar produced by pyrolysis of the wheat straw at 350–550 °C increased rice (*Oryza sativa*) yield regardless of fertilization with N in a paddy field at Tai Lake plain, China (Zhang et al. 2010). The application of biochar produced from wood residues (e.g., teak and rosewood) by the earth mound method increased rice grain yields in paddy fields with low P availability and improved the response to N and NP chemical fertilizer treatments, but the biochar decreased grain yields in soils with a low indigenous N supply (Asai et al. 2009). In the present study, the addition of biochars, especially at the high application rate, had a significant negative effect on growth of an emergent wetland species in waterlogged soil without Cd addition (see Figs. 3 and 4). These results indicated that the effect of biochar application on plant growth could highly depend on soil conditions such as pH, EC, moisture, organic matter, and fertility as well as plant species.

The soil pH (see Figs. 1 and 5) and EC (see Fig. 5) significantly increased with the addition of biochars in the present study, but those increases did not influence plant growth. The nutrient availability in soil could probably be excluded as a reason because the basal nutrients were added to the soil in the present study. Furthermore, no significant difference in the concentrations of N and P in plant shoots was detected between control (no Cd or biochar addition) and biochar treatments without Cd (data not shown). However, the redox status (Eh) effect in the waterlogged soil might not be totally excluded, particularly in the early stage of plant development, even though wetland plants can transport oxygen from top tissues to roots. The addition of biochars might decrease the Eh in the media due to high organic C in biochars (see Table 1) in which labile components of biochar could be decomposed (Zhang et al. 2010).

The negative effect of the biochar additions on plant growth was possibly related to the volatile organic compounds in biochars because the presence of individual volatile organic compounds in soil system can trigger various plant and microbial responses (Spokas et al. 2011). The studies conducted by Deenik et al. (2010, 2011) have indicated that relatively high concentrations of volatile matter in biochar had a negative effect on plant growth, but this effect would decrease or disappear with time (i.e., after the first year of cropping). Nevertheless, the mechanism behind the decrease of plant growth by biochar addition is still unknown. Hence, further studies are needed to clarify whether volatile organic compounds in biochars are more effective on plant growth in waterlogged than dryland soils.

Recent studies have observed that the addition of biochars could mitigate metal toxicity to plants, resulting in a decrease in metal uptake and an increase in plant growth (e.g., Ahmad et al. 2012; Buss et al. 2011; Park et al. 2011a). For example, application of a high-temperature (600–800 °C) biochar produced from forest green waste significantly increased the young quinoa (*Chenopodium quinoa*) performance under Cu stress. At the 4 % *w*/*w* biochar application rate, the plants with 200 mg kg^{-1} Cu reached almost the same biomass as in the control after 3 weeks of Cu treatments. Less Cu entered the plant tissues in the presence than absence of the biochar (Buss et al. 2011). The biochar derived from pyrolysis of oak wood at 400 °C at 5 % (*w*/*w*) was the most effective in decreasing the bioavailability of Pb among the amendments (mussel shell, cow bone, and biochar) and increasing lettuce (*Lactuca sativa*) seed germination by 360 % and root length by 189 % in the contaminated military shooting range soil compared to the unamended soil (Ahmad et al. 2012). In the present study, however, an addition of biochars significantly reduced Cd uptake by plants, but did not improve plant growth in Cd-contaminated soils, as also found by others (e.g., Cui et al. 2011; Namgay et al. 2010). For instance, the concentration and accumulation of Cd in rice significantly decreased with additions of biochar produced from wheat straw, but rice grain yield was not significantly different between biochar and non-biochar applications in Cd-contaminated paddy soil (Cui et al. 2011). The concentrations of Cd in shoots significantly decreased with the additions of wood bluegum (*Eucalyptus saligna*) biochar, especially at the high-Cd treatment, but the biomass of maize (*Zea mays*) was not significantly influenced by the additions of biochar in either 10 or 50 mg kg^{-1} Cd-contaminated soils after 10 weeks of growth (Namgay et al. 2010). These results indicated that there was a significant interactive effect of metals and biochar additions on plant growth and metal uptake, varying with different plant species, application rates, and the nature of biochars as well as soil contamination with either single or mixed metals.

5 Conclusions

Although biochar amendment significantly immobilized soil Cd and reduced its phytoavailability, there was little growth response of the wetland species in Cd-contaminated soil during 9 weeks, probably due to the interaction between biochars and waterlogged environment. Currently, the

scarcity of data limits evaluation of the potential of biochars as an amendment in metal-contaminated substrates of constructed wetlands. Nevertheless, influence of biochars on wetland plant growth and metal uptake is likely to be complicated. Hence, further studies on different plant species, varied sources, and application rates of biochars and metal-contaminated levels are required to elucidate the underlying mechanisms before practical application can be considered.

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References

Ahmad M, Soo Lee S, Yang JE, Ro HM, Han Lee Y, Sik Ok Y (2012) Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and biotoxicity in military shooting range soil. Ecotoxicol Environ Saf 79:225–231

Antal MJ, Grønli M (2003) The art, science and technology of charcoal production. In Eng Chem Res 42:1619–1640

Asai H, Samson BK, Stephan HM, Songyikhangsuthor K, Homma K, Kiyono Y, Inoue Y, Shiraiwa T, Horie T (2009) Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. Field Crops Res 111:81–84

ASTM International (2007) D1762-84: Standard test method for chemical analysis of wood charcoal. ASTM Int., West Conshohocken, PA

Barrow CJ (2012) Biochar: potential for countering land degradation and for improving agriculture. Appl Geogr 34:21–28

Bassett J, Denney RC, Jeffery GH, Mendham J (1978) Vogel's textbook of quantitative inorganic analysis including elementary instrumental analysis, 4th edn. Longman, New York

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

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

Brewer CE, Unger R, Schmidt-Rohr K, Brown RC (2011) Criteria to select biochars for field studies based on biochar chemical properties. Bioenerg Res 4:312–323

Buss W, Kammann C, Koyro HW (2011) Biochar reduces copper toxicity in *Chenopodium quinoa* Willd. in a sandy soil. J Environ Qual 40:1–9

Cui L, Li L, Zhang A, Pan G, Bao D, Chang A (2011) Biochar amendment greatly reduces rice Cd uptake in a contaminated paddy soil: a two-year field experiment. BioResources 6:2605–2618

Deenik JL, McClellan T, Uehara G, Antal MJ, Campbell S (2010) Charcoal volatile matter content influences plant growth and soil nitrogen transformations. Soil Sci Soc Am J 74:1259–1270

Deenik JL, Diarra A, Uehara G, Campbell S, Sumiyoshi Y, Antal MJ Jr (2011) Charcoal ash and volatile matter effects on soil properties and plant growth in an acid ultisol. Soil Sci 176:336–345

Fellet G, Marchiol L, Delle Vedove G, Peressotti A (2011) Application of biochar on mine tailings: effects and perspectives for land reclamation. Chemosphere 83:1262–1267

Gillman GP, Sumpter EA (1986) Modification to the compulsive exchange method for measuring exchange characteristics of soils. Aust J Soil Res 24:61–66

Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol Fert Soils 35:219–230

Jeffery S, Verheijen FGA, van der Velde M, Bastos AC (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agr Ecosyst Environ 144:175–187

Jones DL, Edwards-Jones G, Murphy DV (2011) Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biol Biochem 2011:804–813

Jones DL, Rousk J, Edwards-Jones G, DeLuca TH, Murphy DV (2012) Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol Biochem 45:113–124

Kamphake LJ, Hannah SA, Cohen JM (1967) Automated analysis for nitrate by hydrazine reduction. Water Res 1:205–216

Kempers AJ, Luft AG (1988) Re-examination of the determination of environmental nitrate as nitrite by reduction with hydrazine. Anal 113:1117–1120

Krom MD (1980) Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. Anal 105:305–316

Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—a review. Waste Manage 28:215–225

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

Libra JA, Ro KS, Kammann C, Funke A, Berge ND, Neubauer Y, Titirici M-M, Fühner C, Bens O, Kern J, Emmerich K-H (2011) Hydrothermal carbonization of biomass residuals: a comparative review of the processes and applications of wet and dry pyrolysis. Biofuels 2:89–124

Marchand L, Mench M, Jacob DL, Otte ML (2010) Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ Pollut 158:3447–3461

McGrath D (1996) Application of single and sequential extraction procedures to polluted and unpolluted soils. Sci Total Environ 178:37–44

Menzies NW, Donn MJ, Kopittke PM (2007) Evaluation of extractants for estimation of the bioavailable trace metals in soil. Environ Pollut 145:121–130

Meyer S, Glaser B, Quicker P (2011) Technical, economical, and climate-related aspects of biochar production technologies: a literature review. Environ Sci Technol 45:9473–9483

Moreno-Jiménez E, Esteban E, Fresno T, López de Egea C, Peñalosa JM (2010) Hydroponics as a valid tool to assess arsenic availability in mine soils. Chemosphere 79:513–517

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

Newman EI (1966) A method of estimating the total length of root in a sample. J Appl Ecol 3:139–145

Park JH, Choppala GK, Bolan NS, Chung JW, Chuasavathi T (2011a) Biochar reduces the bioavailability and biotoxicity of heavy metals. Plant Soil 348:439–451

Park JH, Lamb D, Paneerselvam P, Choppala G, Bolan N, Chung JW (2011b) Role of organic amendments on enhanced bioremediation of heavy metal(oid) contaminated soils. J Hazard Mater 185:549–574

Quevauviller P (2002) Operationally-defined extraction procedures for soil and sediment analysis. part 3: new CRWs for trace-element extractable contents. Trends Anal Chem 21:774–785

Rakowska MI, Kupryianchyk D, Harmsen J, Grotenhuis T, Koelmans AA (2012) In situ remediation of contaminated sediments using carbonaceous materials. Environ Toxicol Chem 31:693–704

Rao C, Sahuquillo A, Lopez Sanchez J (2008) A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. Water Air Soil Poll 189:291–333

Schimmelpfennig S, Glaser B (2012) One step forward toward characterization: some important material properties to distinguish biochars. J Environ Qual. doi:10.2134/jeq2011.0146

Searle PL (1984) The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen—a review. Anal 109:549–568

Sohi SP, Krull E, Lopez-Capel E, Bol R (2010) A review of biochar and its use and function in soil. Adv Agron 105:47–82

Solaiman ZM, Blackwell P, Abbott LK, Storer P (2010) Direct and residual effect of biochar application on mycorrhizal root colonisation, growth and nutrition of wheat. Aust J Soil Res 48:546–554

Solaiman Z, Murphy D, Abbott L (2012) Biochars influence seed germination and early growth of seedlings. Plant Soil 353:273–287

Spokas KA, Novak JM, Stewart CE, Cantrell KB, Uchimiya M, DuSaire MG, Ro KS (2011) Qualitative analysis of volatile organic compounds on biochar. Chemosphere 85:869–882

Sun Y, Zhou Q, An J, Liu W, Liu R (2009) Chelator-enhanced bioextraction of heavy metals from contaminated soil irrigated by industrial wastewater with the hyperaccumulator plant (*Sedum alfredii* Hance). Geoderma 150:106–112

Tel-Or E, Forni C (2011) Bioremediation of hazardous toxic metals and organics by photosynthetic aquatic systems. Plant Biosyst 145:224–235

Uchimiya M, Bannon DI, Wartelle LH (2012a) Retention of heavy metals by carboxyl functional groups of biochars in small arms range soil. J Agric Food Chem 60:1798–1809

Uchimiya M, Cantrell KB, Hunt PG, Novak JM, Chang S (2012b) Retention of heavy metals in a Typic Kandiudult amended with different manure-based biochars. J Environ Qual 41:1138–1149

Vymazal J (2011) Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674:133–156

Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for bioremediation and restoration. Environ Int 30:685–700

Zhang A, Cui L, Pan G, Li L, Hussain Q, Zhang X, Zheng J, Crowley D (2010) Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. Agr Ecosyst Environ 139:469–475

Zhang Z, Rengel Z, Liaghati T, Antoniette T, Meney K (2011a) Influence of plant species and submerged zone with carbon addition on nutrient removal in stormwater biofilter. Ecol Eng 37:1833–1841

Zhang Z, Rengel Z, Meney K, Pantelic L, Tomanovic R (2011b) Polynuclear aromatic hydrocarbons (PAHs) mediate cadmium toxicity to an emergent wetland species. J Hazard Mater 189:119–126

Zhang A, Bian R, Pan G, Cui L, Hussain Q, Li L, Zheng J, Zhang X, Han X, Yu X (2012a) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles. Field Crops Res 127:153–160

Zhang Z, Rengel Z, Chang H, Meney K, Pantelic L, Tomanovic R (2012b) Bioremediation potential of *Juncus subsecundus* in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs). Geoderma 175–176:1–8