Journal of Geochemical Exploration 182 (2017) 157–165

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/gexplo

Characteristics of different types of biochar and effects on the toxicity of heavy metals to germinating sorghum seeds

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ARTICLE INFO

Article history:
Received 15 July 2016
Revised 21 November 2016
Accepted 18 December 2016
Available online 23 December 2016

Keywords:
Biochar
Copper
Lead
Cadmium
Seed germination
Sorghum bicolor
PAHs
Sorption

ABSTRACT

Soils contaminated with heavy metals are often notably nutrient poor and unsuitable for plant growth. The addition of biochar can significantly improve soil properties. In this study, the contents of organic compounds and inorganic elements in biochar and the effect of biochar on plant germination were examined. The PAH content of biochar from four different sources was examined, and naphthalene, phenanthrene, fluoranthene and pyrene were identified as primary compounds. The most abundant inorganic elements were potassium, calcium, magnesium, sodium, aluminium, iron and manganese, with strontium and barium also being significantly elevated. The pH of biochar from all sources was strongly alkaline. The sorption characteristics for heavy metals (Cd, Cu and Pb) were also tested for the different types of biochar. Adsorption data were well-described by a Langmuir isotherm with maximum Cd (II), Cu (II) and Pb (II) adsorption capacities of 20.16, 7.83 and 70.92 mg/g for bamboo-derived biochar; 18.80, 13.85 and 200 mg/g for rice husk-derived biochar; 11.63, 20.08 and 123.46 mg/g for ash tree-derived biochar; and 15.11, 10.86 and 196.08 mg/g for beech tree-derived biochar, respectively. The effect of biochar on the toxicity of heavy metals was measured by the inhibition of sorghum seed germination. With biochar, the toxicity of cadmium, copper and lead was reduced. Bamboo-derived biochar was less efficient in reducing the toxicity of cadmium and copper compared with the other types of biochar. For lead, the rice husk-derived biochar was the least efficient in reducing the toxicity.

1. Introduction

For humans and the environment, heavy metals are a significant stress factor. Therefore, reducing the concentrations of toxic metals to a natural level is important. Phytoremediation is a method that relies on the accumulation of metals in plants and their associated microorganisms to remove heavy metals from the environment (Pilon-Smits, 2005). The advantage of this method is that interference with the environment is minimal; however, the effectiveness of phytoremediation is limited when the concentrations of contaminants are high and the characteristics of contaminated soils are poor for plant growth.

Biochar increases the effectiveness of phytoremediation by reducing the mobility (Beesley et al., 2011) and the phytotoxicity (Beesley et al., 2010) of certain organic and inorganic pollutants in soils. Biochar is a product of biomass conversion under a sufficiently high temperature (300–600 °C) and limited air or without air (Lehmann and Joseph, 2009). Through pyrolysis, the chemical properties of carbon fixed in biomass are changed, and as a result, biochar is more resistant to microbial decomposition (Lehmann and Joseph, 2009). The residence time in soils for carbon bound in biochar is many centuries to millennia (Steinbeiss et al., 2009). Because of the long residence time in soil, biochar could be used for carbon capture and storage, leading to a reduction of CO_2 concentration in the atmosphere and slowed climate change (Lehmann and Joseph, 2009). However, the primary soil application of biochar is to improve soil fertility and increase crop yields (Lehmann and Joseph, 2009; Steinbeiss et al., 2009). The application of biochar to improve soil fertility is not new and was used in some ancient cultures (particularly in the humid tropics); in these cultures, biochar was deliberately added to the soil, which created black soil that was not exhausted within a few years of deforestation. Many of these soils, Terra Preta (black soil), are found in the Amazon, with ages that exceed a thousand years (Ennis, 2012). Biochar, because of the porosity, increases soil moisture retention and positively influences aeration. In addition to an increase in water holding capacity, biochar fixes nutrients or fertilizers added to soils (N, P, and K), thereby decreasing the leaching and erosion of nutrients and the consequent eutrophication of local waters. Moreover, biochar forms complexes with minerals, including those that create humus. With a huge internal surface, biochar provides a substrate for abundant microbial colonization (Steinbeiss et al., 2009) and also serves as a refuge for recolonization by microorganisms. All the nutrients contained in the original biomass remain in biochar and are

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http://dx.doi.org/10.1016/j.gexplo.2016.12.013
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slowly released, and unlike ash, which retains only alkali (i.e., K, Ca, and Mg), biochar also contains phosphorus and sulphur. For nitrogen, biochar contains half that in the original biomass. Because of these properties, biochar is also used to mitigate the effects of pollutants through sorption and sequestration (Bornemann et al., 2007; Chen and Yuan, 2011; Soudek et al., 2014) and to remediate contaminated soils and sediments (Beesley et al., 2010). However, information on the application of biochar to improve the efficiency of phytoextraction and phytostabilisation is currently very limited.

Therefore, the aims of this study were to characterize the biochar from different sources and to evaluate the effect of these biochar on metal toxicity, as measured by the germination of sorghum (*Sorghum bicolor* L.) seeds.

2. Materials and methods

2.1. Plant material and chemicals

Seeds (SEED SERVICE s.r.o., Czech Republic) of three cultivars of *S. bicolor* L. (Expres, Honey Graze BMR and Nutri Honey) were used in the germination test. Heavy metal ions (Cd^{2+}, Cu^{2+}, and Pb^{2+}) were obtained from the salts $Cd(NO_3)_2 \cdot H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, and $Pb(NO_3)_2$. The source of the biochar was ash tree (OFFICIO P.S. Ltd., Czech Republic), beech tree (EKOGRILL Ltd., Czech Republic), rice husks or bamboo (Dr Jing Song, Nanjing, China). The substances used in the germination test were dissolved in double-distilled water containing 2 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.8 mM $NaHCO_3$, and 0.08 mM KCl (according to ČSN EN ISO 7346) (the chemicals were all from Penta [http://www.pentachemicals.eu]). The pH was adjusted to 7.6 with the addition of 0.1 M NaOH. For the adsorption experiments, double-distilled water was also used.

2.2. Heavy metal determination by ICP-MS

Element concentrations in solutions were determined with quadrupole-based inductively coupled plasma mass spectrometry (ICP-MS, X Series 2; Thermo Scientific, Thermo Fisher Scientific, USA) under the conditions given in Table 1. To ensure the quality of the analytical data, the procedure was verified using standard reference materials, i.e., NIST 1640 (Trace Elements in Natural Water) and acid digests of NIST 2711 (Montana Soil). The differences between the measured and standard values did not exceed 5% relative standard deviation (RSD). Reagent blanks and unexposed filters mineralized in the identical acid mixture were used at the start of batch of analyses.

Table 1
Operating conditions used for ICP-MS measurements of elements.

Instrument	X Series 2, Thermoscientific
Plasma RF power	1400 W
Reflected power	<1 W
Plasma gas flow rate	13.5 L min^{-1} (Ar)
Auxiliary gas flow rate	0.95 L min^{-1} (Ar)
Nebulizer gas flow rate	0.75 L min^{-1} (Ar)
Cones	Nickel
Sensitivity (solutions)	~3×10^4 cps per ng In mL^{-1}
Nebuliser	Meinhard type
Measurement mode	Peak jumping
Points per peak	1
Dwelltime	10.0 ms
Acquisition time	3 × 67 s
Detector voltage	2986 V (pulse count) –1680 V (analogous)
Measured isotopes	^{7}Li, ^{9}Be, ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{44}Ca, ^{45}Sc, ^{47}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{69}Ga, ^{72}Ge, ^{75}As, ^{82}Se, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{95}Mo, ^{103}Rh, ^{105}Pd, ^{111}Cd, ^{115}In, ^{118}Sn, ^{121}Sb, ^{125}Te, ^{133}Cs, ^{137}Ba, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu, ^{178}Hf, ^{182}W, ^{185}Re, ^{195}Pt, ^{202}Hg, ^{205}Tl, ^{208}Pb, ^{209}Bi, ^{232}Th, ^{238}U
Internal standards	^{74}Ge, ^{103}Rh, ^{187}Re

2.3. Heavy metal determination by AAS

Standards of Cd, Cu and Pb (Analytika Ltd., Czech Republic) were used as reference analytes for the quantitative estimation of heavy metals and to ensure accurate calibration and quality assurance for each analyte. Standard stock solutions (1.0 g/L) were diluted to obtain working standard solutions that ranged from 0.2 to 4 μg/mL for Cd, from 0.5 to 4 μg/mL for Cu and from 2 to 40 μg/mL for Pb; solutions were stored at 4 °C. In all solutions, acidity was maintained with 0.1% nitric acid. A calibration curve was plotted between measured absorbance and concentration (μg/mL). All samples were analyzed in triplicate using a flame atomic absorption spectrophotometer (SensAA, GBS, Australia) with GBS Avanta software version 2.02.

2.4. Sample preparation

Approximately 0.25 g of dry biochar was predigested in 5 mL of a mixture of $HNO_3/HClO_4$ at a ratio of 7:1 (v/v) overnight at room temperature. Then, 3 mL of the acid mixture was added to clean the walls of tube, and the contents of the closed Teflon vessel were digested in a gradient to 100% power after 15 min and at 100% power for an additional 25 min. Digestion was performed in a Multiwave reaction system (Multi-wave PRO, Anthon Paar GmbH, Austria). The cooling required an additional 20 min. The volume of samples was filled to 10 mL and they were analyzed.

2.5. Biochar sample preparation

In order to keep the biochar particles size uniformity, eliminate the interference of other substances and microbial interference in biochars, the original samples were treated as follows: the particle size of the biochar was 0.4–0.8 mm after grinding. The microbial interference was excluded by 30 min UV irradiation.

2.6. pH measurement

The pH was determined on a 1:5 biochar/0.01 M $CaCl_2$ or biochar/distillate water suspension after 30 min of settling. Values of pH were determined using an UltraBasic pH meter (Denver Instrument Company, CO, USA).

2.7. Total carbon and nitrogen determination

For carbon and nitrogen analysis the Skalar Primacs SCN analyzer (Skalar Analytical, Breda, The Netherlands) was used. Total carbon was determined by heating the sample in the presence of a cobalt oxide catalyst at 1050 °C in pure oxygen and IR determination of evolved CO_2 at 4.2 μm. Total nitrogen was determined by analysis, which uses the Dumas method of combusting all nitrogen to NO_x.

2.8. PAH extraction and determination

Biochar (4 g) and anhydrous sodium sulphate (1 g) were placed in Erlenmeyer flasks and extracted with 40 mL of dichloromethane at 25 °C for 24 h.

The dichloromethane solution was evaporated to near dryness using a rotary evaporator (400 mbar, 70 rpm, water bath temperature 35 °C) and then dissolved in 2 mL of cyclohexane. Then, 1 mL of the cyclohexane was cleaned by silica gel column and eluted with acetone/hexane (1:1, v/v). Approximately 4 mL of eluent was collected and subsequently evaporated to dryness under nitrogen gas. The PAHs were dissolved in 1 mL of dichloromethane before HPLC analysis.

Table 2
Biochar contents in N and C and chemical properties related to acidity. Values represent the means ± standard errors. n = 3.

	Ash tree	Bamboo	Beech tree	Rice husks
C_{total} [%]	68.00 ± 6.40	42.71 ± 3.14	43.73 ± 4.59	30.30 ± 2.12
N_{total} [%]	0.38 ± 0.22	0.86 ± 0.14	0.49 ± 0.12	0.37 ± 0.16
C/N ratio	179	50	89	82
pH_{H_2O}	9.79 ± 0.31	8.99 ± 0.26	9.20 ± 0.40	10.00 ± 0.66
pH_{CaCl_2}	8.20 ± 0.42	8.09 ± 0.55	8.02 ± 0.67	8.83 ± 0.31

2.9. Heavy metal adsorption experiment

The metal adsorption experiments were performed using a batch equilibration technique. Stock solutions (1 mg/mL) of Cd^{2+}, Cu^{2+}, and Pb^{2+} were prepared by dissolving analytical grade $Cd(NO_3)_2 \cdot H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, and $Pb(NO_3)_2$ in distilled water. Adsorption isotherms were determined by mixing 0.25 g of biochar with 5 mL of solution in 15 mL falcon tubes at a constant pH of 5. For these experiments, the initial concentrations of Cd^{2+}, Cu^{2+}, and Pb^{2+} ranged from 0.001 to 1.0 mg/mL. The mixtures were agitated on a reciprocating shaker at room temperature (24 °C) at 150 rpm and then centrifuged (20 min at 4000 rpm); the samples were filtered with Whatman No. 1 filter paper. The filtrate solutions were analyzed for residual heavy metal concentrations using AAS.

The effect of solution pH on metal sorption was investigated with the identical approach, but the initial pH values of the solutions were adjusted to values that ranged from 1.0 to 8.0 with the addition of either 1 M NaOH or 1 M HNO_3. The different types of biochar (0.5 g) were added to 15 mL falcon tubes and 5.0 mL of 2.0 mM Cd^{2+}, Cu^{2+}, and Pb^{2+} was added. The mixtures were agitated on a reciprocating shaker at room temperature (24 °C) at 150 RMP and then centrifuged on Universal 32R Hettich Zentrifugen (20 min at 2706 ×g); the samples were filtered with Whatman No. 1 filter paper following incubation. The metal concentrations in filtrates were measured using AAS.

The amount of metal adsorbed by the biochar was the difference between the initial and final ion concentrations of the solutions.

2.10. Semichronic toxicity test

Seeds of three cultivars of *S. bicolor* L. were used for the germination tests. The test concentrations of each metal (Cd^{2+}, Cu^{2+}, and Pb^{2+}) were 0.05, 0.1, 0.5, 1 and 5 mM. All substances were dissolved in double-distilled water containing 2 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.8 mM $NaHCO_3$, and 0.08 mM KCl. The pH was adjusted to 7.6. The seeds were placed in plastic dishes (10-cm diameter) with a layer of a filter paper on the bottom. To half of the Petri dishes, 0.25 g of biochar was added. Seventeen seeds were equally spaced in each dish on the surface of the filter paper, and 5 mL of heavy metal aqueous solution was added. Each treatment had four replicates. The seeds were exposed for 72 h in the dark at 25 °C. Root lengths were measured, and the values for the inhibition of root elongation were calculated with the following Eq. (1):

$$I = (Dc - Dt)/Dc \tag{1}$$

where I is the inhibition of root elongation as a %; Dc is the average length of root under control conditions (i.e., without heavy metal treatment) [mm]; and Dt is the average length of root grown under the tested metal concentration [mm].

2.11. EC_{50} calculation

EC_{50} is the effective concentration at which 50% of tested organisms have a significant response to a tested compound. Nonlinear regression with lower and upper maximums (0 and 100, respectively) was used to calculate the EC_{50}. The software GraphPad Prism (GraphPad, San Diego, CA, USA) was used to process the data, with the output sent to MS Excel.

Table 3
Elemental composition [$\mu g\ g^{-1}$] of the biochars from four different sources. Values represent the means ± standard errors. n = 4.

	Ash tree	Bamboo	Beech tree	Rice husks		Ash tree	Bamboo	Beech tree	Rice husks
Li	0.2 ± 0.01	0.9 ± 0.05	1.0 ± 0.07	0.6 ± 0.14	Sn	0.16 ± 0.005	0.26 ± 0.012	0.43 ± 0.022	0.20 ± 0.009
Be	< 0.003	0.05 ± 0.004	0.07 ± 0.003	0.02 ± 0.001	Sb	0.96 ± 0.045	0.72 ± 0.031	1.6 ± 0.07	0.57 ± 0.018
Na	121 ± 5	111 ± 6.0	193 ± 12	246 ± 12	Te	<0.001	<0.001	0.01 ± 0.001	0.01 ± 0.001
Mg	1405 ± 38	1818 ± 117	2293 ± 144	2091 ± 60	Cs	<0.04	0.10 ± 0.004	<0.04	0.06 ± 0.003
Al	150 ± 11	914 ± 25	1047 ± 76	1067 ± 37	Ba	30 ± 1.8	152 ± 4	178 ± 7	62 ± 2.5
K	10,356 ± 431	7861 ± 220	8768 ± 250	16,757 ± 314	La	0.08 ± 0.003	1.0 ± 0.04	1.3 ± 0.06	0.54 ± 0.019
Ca	13,618 ± 669	30,880 ± 1657	31,100 ± 1104	4607 ± 101	Ce	0.15 ± 0.005	2.1 ± 0.12	2.8 ± 0.11	1.1 ± 0.05
Sc	0.25 ± 0.021	0.43 ± 0.032	0.52 ± 0.041	1.2 ± 0.02	Pr	0.03 ± 0.001	0.32 ± 0.012	0.39 ± 0.020	0.18 ± 0.009
Ti	4.8 ± 0.18	34 ± 2.5	53 ± 3.0	25 ± 1.4	Nd	0.10 ± 0.004	1.1 ± 0.05	1.4 ± 0.06	0.66 ± 0.029
V	<0.16	<0.16	1.1 ± 0.03	1.1 ± 0.05	Sm	0.02 ± 0.001	0.23 ± 0.010	0.28 ± 0.011	0.13 ± 0.004
Cr	7.5 ± 0.32	11 ± 0.39	11 ± 0.4	4.7 ± 0.28	Eu	0.01 ± 0.001	0.06 ± 0.002	0.07 ± 0.003	0.03 ± 0.002
Mn	35 ± 1.5	1194 ± 52	1527 ± 31	689 ± 27	Gd	0.02 ± 0.001	0.23 ± 0.010	0.27 ± 0.010	0.12 ± 0.005
Fe	159 ± 3	1868 ± 93	2408 ± 150	1214 ± 37	Tb	0.003 ± 0.001	0.03 ± 0.001	0.04 ± 0.002	0.02 ± 0.001
Co	0.16 ± 0.004	0.87 ± 0.041	1.1 ± 0.07	0.32 ± 0.008	Dy	0.02 ± 0.001	0.18 ± 0.007	0.21 ± 0.012	0.09 ± 0.004
Ni	5.2 ± 0.22	8.7 ± 0.34	9.6 ± 0.31	2.3 ± 0.19	Ho	0.003 ± 0.001	0.03 ± 0.001	0.04 ± 0.001	0.02 ± 0.001
Cu	4.7 ± 0.20	7.3 ± 0.36	11 ± 0.30	8.8 ± 0.27	Er	0.01 ± 0.001	0.09 ± 0.004	0.10 ± 0.004	0.05 ± 0.002
Zn	13 ± 0.7	15 ± 0.9	53 ± 3.0	25 ± 1.7	Tm	0.001 ± 0.001	0.01 ± 0.001	0.02 ± 0.004	0.01 ± 0.001
Ga	0.27 ± 0.021	2.0 ± 0.08	2.6 ± 0.12	0.93 ± 0.050	Yb	0.01 ± 0.001	0.08 ± 0.003	0.09 ± 0.003	0.05 ± 0.002
Ge	0.02 ± 0.001	0.04 ± 0.002	0.11 ± 0.007	0.18 ± 0.005	Lu	0.001 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
As	9.2 ± 0.37	8.7 ± 0.41	8.2 ± 0.44	5.1 ± 0.25	Hf	0.01 ± 0.001	0.09 ± 0.004	0.13 ± 0.005	0.04 ± 0.001
Se	29 ± 1.8	27 ± 1.5	24 ± 1.1	12 ± 0.5	Ta	<0.013	<0.013	<0.013	<0.013
Rb	9.6 ± 0.45	9.6 ± 0.41	15 ± 0.5	16 ± 0.5	W	0.04 ± 0.001	0.07 ± 0.003	0.11 ± 0.004	0.09 ± 0.004
Sr	65 ± 3.5	76 ± 2.8	89 ± 5.5	16 ± 0.6	Re	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001
Y	0.07 ± 0.004	0.70 ± 0.030	0.83 ± 0.031	0.37 ± 0.019	Pt	<0.0012	<0.0012	<0.0012	<0.0012
Mo	0.96 ± 0.034	1.1 ± 0.06	0.77 ± 0.018	0.74 ± 0.034	Hg	<0.023	<0.023	<0.023	<0.023
Rh	<0.001	<0.001	<0.001	<0.001	Tl	0.01 ± 0.001	0.02 ± 0.001	0.04 ± 0.001	0.04 ± 0.002
Pd	0.01 ± 0.002	0.03 ± 0.002	0.03 ± 0.002	0.01 ± 0.001	Pb	0.86 ± 0.039	1.9 ± 0.08	3.7 ± 0.09	2.4 ± 0.08
Ag	<0.002	<0.002	<0.002	<0.002	Bi	<0.027	<0.027	<0.027	<0.027
Cd	0.26 ± 0.019	0.18 ± 0.005	0.36 ± 0.005	0.14 ± 0.001	Th	0.02 ± 0.001	0.18 ± 0.007	0.19 ± 0.005	0.14 ± 0.006
In	<0.003	<0.003	<0.003	<0.003	U	0.02 ± 0.001	0.06 ± 0.003	0.07 ± 0.003	0.07 ± 0.002

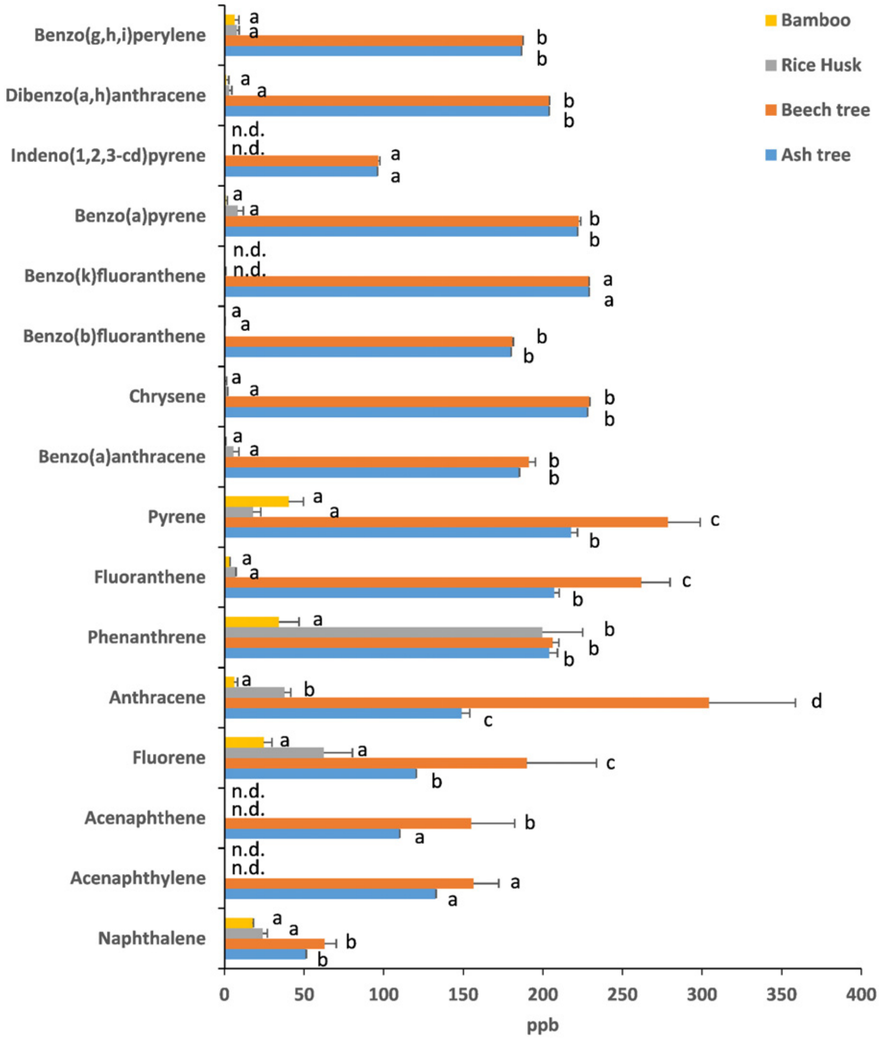

Fig. 1. PAH content in biochar samples. Statistically insignificant values for each measured compound separately on the level of probability P < 0.05 are indicating by the same symbol above column. Abbreviation n.d. instead of column indicates undetectable concentration. n = 3.

2.12. Statistics

The data are the means of four replicates and are expressed as the mean ± SD. Data were analyzed with one-way ANOVAs. Identical symbols above columns in the figures indicate statistically insignificant values at probability P < 0.05. Statistical analyses were performed using STATISTICA (StatSoft, Inc., Tulsa, OK, USA). Error bars indicate the standard deviation of analyzed contents.

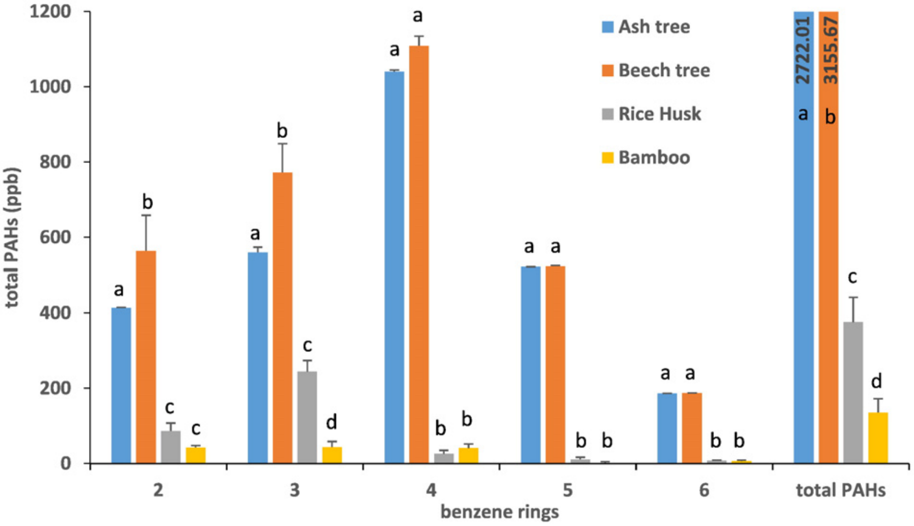

Fig. 2. Total PAH content on the base of benzene rings number in biochar samples. Statistically insignificant values for each number of benzene rings separately on the level of probability P < 0.05 are indicating by the same symbol above column. n = 3.

Table 4
Inhibition coefficient (I) for sorghum cultivar Expres tested on solution supplemented by beech tree derived biochar. Values represent the means ± standard errors. n = 6.

g	I (%)
0.25	11.16 ± 14.45
0.5	14.42 ± 19.73
1	34.42 ± 12.46

3. Results and discussion

The sorption characteristics of biochar reduce the mobility and the phytotoxicity of certain organic and inorganic pollutants in soils, thereby eliminating some limitations of phytoremediation. However, information on the application of biochar to improve the efficiency of phytoextraction and phytostabilisation is currently very limited.

The four types of biochar were strongly alkaline with pH values in the range of 8–10, with the values affected by different feedstock materials (Table 2). But the big differences between softwood (bamboo), hardwood (ash tree and beech tree) and vegetable residues (rice husk) was not found as mentioned in paper of Allaire et al. (2015). Gai et al. (2014) mentioned increasing of biochar pH with higher pyrolysis temperature. For our biochars this effect was not observed. As nitrogen (N) is generally volatilized during pyrolysis, the N concentration in all tested biochars are very low. Content of carbon (C), which is the major constituent of the biochars, increased in order rice husks < bamboo ≤ beech tree < ash tree. The high C/N ratio can be also attributed to the high pyrolysis temperature in case of ash tree derived biochars.

Based on the elemental analyses, the lowest total content was in ash tree-derived biochar and the highest was in beech tree-derived biochar. Thus, the plant species most likely determined the elemental content of the biochar and not the region in which the plants grew (e.g., Czech Rep. or China) or the type and temperature of the pyrolisation. Potassium, calcium, magnesium, sodium, aluminium, iron and manganese were the most abundant inorganic elements, with the contents of strontium and barium also significantly elevated (Table 3). The concentrations of some rare earth elements (REE, i.e., Pr, Gd, Sm, Dy, Ce, Y, La, and Nd) were also high. Moreover, the elevated REE concentrations were found not only in biochar from Chinese materials, assumed to be from the extraction and processing of REEs, but also in the charcoal from beech trees. Additionally, the titanium content was significantly elevated compared with that of, for example, ubiquitous sodium.

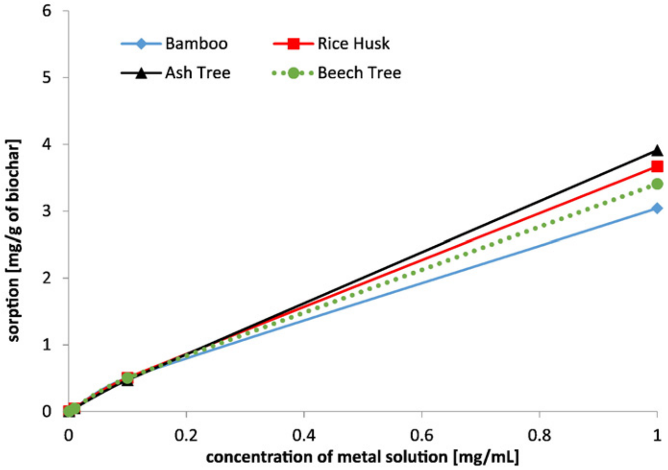

Fig. 4. Effect of initial metal ion concentration and type of biochar on Cu(II) sorption capacity. Error bars are not shown, all standard deviations are lower than 1% of measured values. n = 3.

The contents of polyaromatic hydrocarbons (PAHs) were also different for the different types of biochar. Beech tree and ash tree derived biochars has the highest contents of PAHs (Fig. 1). The most represented PAHs were naphthalene and phenanthrene, with less fluorantrene and pyrene. The contents of other PAHs were minor. The bamboo and rice husks derived biochars were represented mostly by two and three benzene rings PAHs. In contrast with ash tree and beech tree derived biochars which contained predominantly three and four benzene rings PAHs (Fig. 2). According to the literature, the quantity of PAHs is highly dependent on the conditions during pyrolysis of plant material. Devi and Saroha (2015) show that PAH content is strongly dependent on temperature, with the highest contents found in the temperature range 400–500 °C. The results of Luo et al. (2014) are similar, and they found the highest PAH content in corn stalk-derived biochar produced at 300–500 °C and in sewage sludge-derived biochar at 500 °C. The most abundant hydrocarbons in biochar include naphthalene, in addition to anthracene, fluoranthene and pyrene (Freddo et al., 2012). In our experiments, the ash tree- and beech tree-derived biochar produced in charcoal piles at a temperature of approximately 350 °C had high

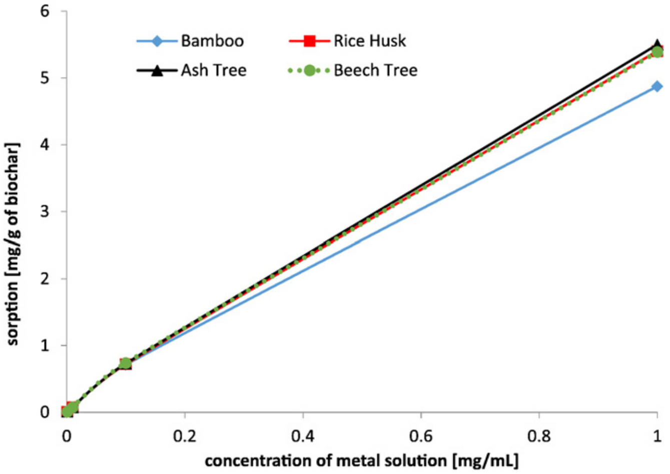

Fig. 3. Effect of initial metal ion concentration and type of biochar on Cd(II) sorption capacity. Error bars are not shown, all standard deviations are lower than 1% of measured values. n = 3.

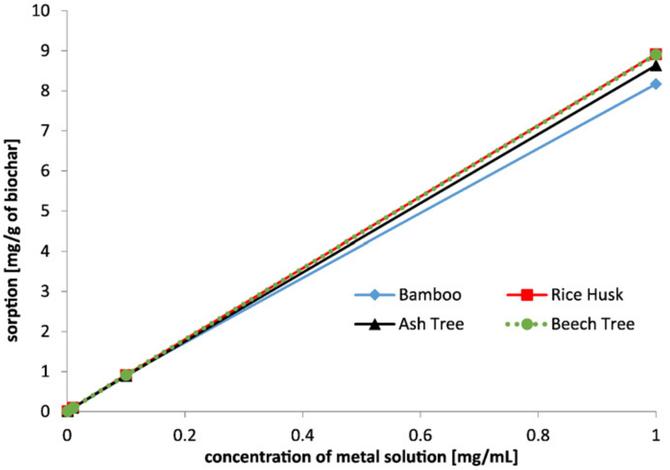

Fig. 5. Effect of initial metal ion concentration and type of biochar on Pb(II) sorption capacity. Error bars are not shown, all standard deviations are lower than 1% of measured values. n = 3.

Table 5
Parameters of Langmuir equation for the adsorption of Cd(II). Cu(II) and Pb(II) by different biochars. (Q_m - complete monolayer adsorption capacity. K_L - Langmuir adsorption constant. R^2 - correlation coefficients).

	Cd			Cu			Pb		
	Q_m [mg/g]	K_L [mL mg^{-1}]	R^2	Q_m [mg/g]	K_L [mL mg^{-1}]	R^2	Q_m [mg/g]	K_L [mL mg^{-1}]	R^2
Bamboo	13.76	0.5489	0.9985	7.83	0.6395	0.9726	70.92	0.1302	0.9989
Rice husks	18.80	0.4030	0.9961	13.85	0.3617	0.9477	200.00	0.0466	0.5965
Ash tree	20.20	0.3745	0.9974	20.08	0.2416	0.9647	123.46	0.0750	0.6490
Beech tree	18.66	0.4069	0.9981	10.86	0.4593	0.9509	196.08	0.0475	0.6162

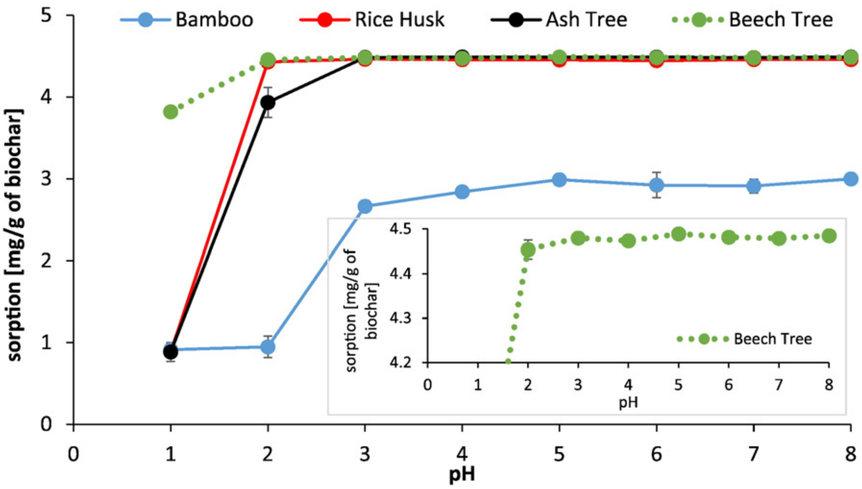

Fig. 6. Effect of pH and type of biochar on Cd(II) sorption capacity at initial concentration of solution 2 mM of cadmium nitrate. Small figure show the data for beech tree derived biochar in higher resolution. Standard deviation is represented as ±S.D. n = 3.

contents of PAHs. By comparison, the PAH concentrations were lower by tenfold in the rice husk- and bamboo-derived biochar from China that was produced at temperatures of 400 °C and 600 °C, respectively.

The PAHs in biochar were assumed to cause toxicity to plants. According to the data (Table 4), the toxicity of biochar increased slightly with the amount of biochar in the medium. The EC_{50} value was calculated as 20.8 g of biochar per 5 mL of aqueous solution. Therefore, the toxicity of biochar was very low or negligible most likely because the solubility of most PAHs was low in an aqueous solution and potential toxicity is summa of many factors.

In the experiment that tested the sorption of metals by biochar, the sorption characteristics for bamboo-derived biochar were different compared with those of biochar from other sources (Figs. 3–5). Sorption (binding) sites for all three tested heavy metals were much less available in the biochar from bamboo, and these binding sites were accessible are only at high concentrations of metals. Adsorption data were well-described by a Langmuir isotherm with maximum Cd (II), Cu (II) and Pb (II) adsorption capacities of 13.76, 7.83 and 70.92 mg/g for bamboo-derived biochar; 18.80, 13.85 and 200 mg/g for rice husk-derived biochar; 20.20, 20.08 and 123.46 mg/g for ash tree-derived biochar; and 18.66, 10.86 and 196.08 mg/g for beech tree-derived biochar, respectively (Table 5).

The adsorption of heavy metals (Cd, Cu and Pb) onto biochar was significantly affected by the pH (Figs. 6–8). Generally, the adsorption was less efficient at very low pH values. Although biochar from beech trees adsorbed nearly all of the metal at the lowest pH (pH = 1)

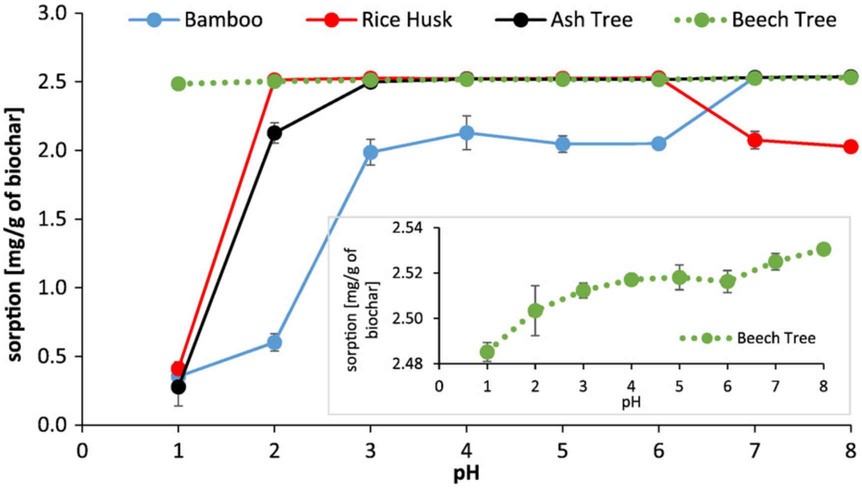

Fig. 7. Effect of pH and type of biochar on Cu(II) sorption capacity at initial concentration of solution 2 mM of copper nitrate. Small figure show the data for beech tree derived biochar in higher resolution. Standard deviation is represented as ±S.D. n = 3.

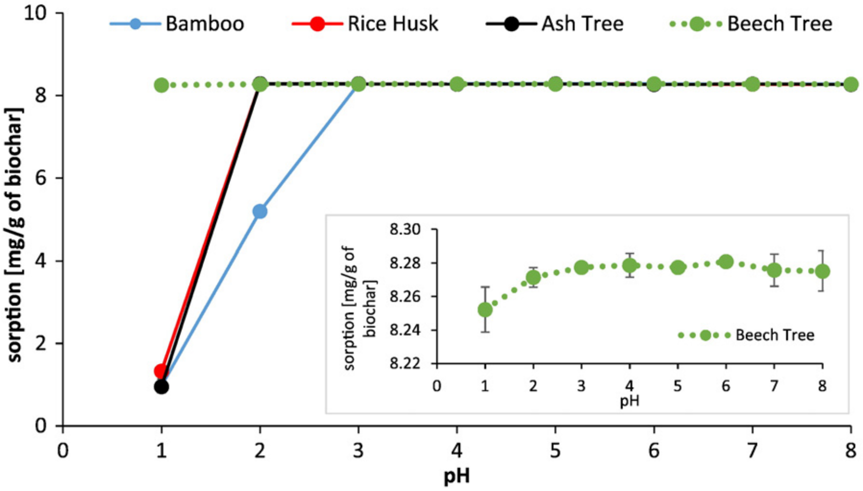

Fig. 8. Effect of pH and type of biochar on Pb(II) sorption capacity at initial concentration of solution 2 mM of lead nitrate. Small figure show the data for beech tree derived biochar in higher resolution. Standard deviation is represented as ±S.D. n = 3.

compared with biochar from other sources, the bamboo-derived biochar absorbed much less of the heavy metals (particularly cadmium and copper). Lead was adsorbed at similar levels with the different types of charcoal, which might be a result partly influenced by the precipitation of lead compounds at low pH, as indicated by the concentrations in controls without biochar at various pH values. Differences in sorption of metals by different biochar types at different pH cannot be explained on the basis of the measured parameters and will aim for further research.

Based on the root-length measurements, EC_{50} values were calculated for the three cultivars of sorghum with the four types of biochar exposed to three heavy metals (Cd, Cu and Pb) (Figs. 9–11). The sorghum cultivars were selected on the base of their different tolerance to abiotic stress. Nutri Honey cultivar contain high amount of carbohydrates and it is tolerant to soil salinity. Honey Graze BMR cultivar is characterized by reduced lignin content (about 40–60%). Express cultivar is tolerant to cold and unfavorable weather and its tannin deficient. Cadmium, copper and lead toxicity was reduced with all biochar types. For the types of biochar, differences in the affinity of ions were found. The reduction in the toxicity of copper and cadmium was the lowest with bamboo-derived biochar, whereas the reduction in lead toxicity was the lowest with rice husk-derived biochar. The EC_{50} values increased tenfold, for some values, of cadmium and copper with biochar, and although biochar lowered lead toxicity, the EC_{50} values were only threefold higher than those of the controls without biochar. The absorption of ions onto the active surfaces of biochar led to the reduction in toxicity. Thus, with the reduced concentration of dissolved, unbound ions, the effect of heavy metal toxicity on the sprouts of germinated seeds was also reduced. Beesley et al. (2010) also describe this reduced availability, and with biochar, a tenfold reduction in the concentration of cadmium in

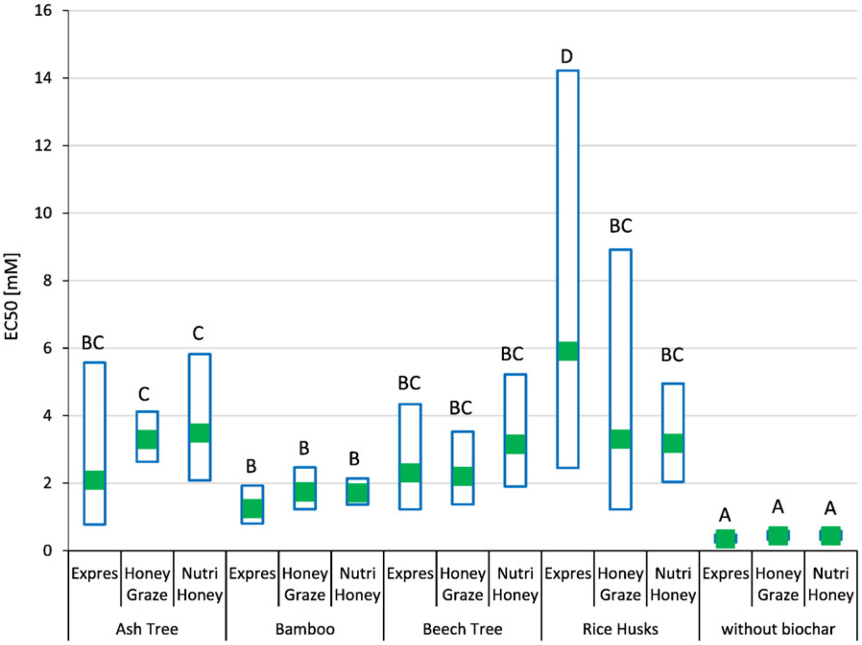

Fig. 9. EC_{50} values for different tested sorghum cultivars in solution supplemented by cadmium ions. (Green square represents average value and blue box surrounding the maximum and minimum EC_{50} value). Statistically insignificant values of EC_{50} on the level of probability $P < 0.05$ are indicating by the same symbol above blue box. n = 4.

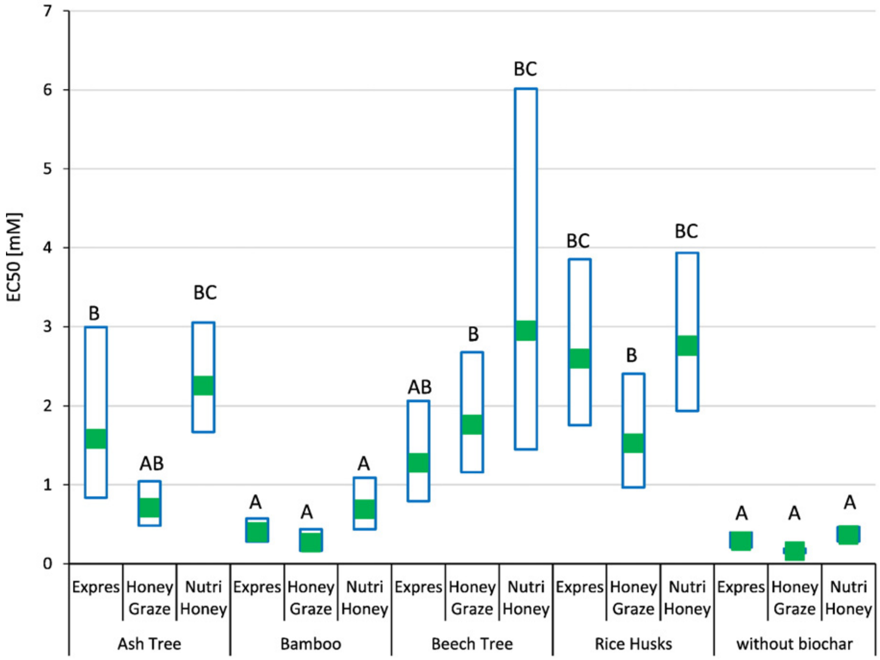

Fig. 10. EC_{50} values for different tested sorghum cultivars in solution supplemented by copper ions. (Green square represents average value and blue box surrounding the maximum and minimum EC_{50} value). Statistically insignificant values of EC_{50} on the level of probability $P < 0.05$ are indicating by the same symbol above blue box. $n = 4$.

pore water was detected; however, the concentrations of copper in pore water increased after biochar addition. To explain this difference, these authors detected a strong correlation between the copper concentrations in pore water and elevated concentrations of soluble organic carbon (DOC) from biochar (see also Bernal et al., 2009). The differences in behaviors between copper and cadmium and those of lead might be because of the different physical-chemical properties of these metals. As determined in many studies (Cao et al., 2009; Karami et al., 2011; Park et al., 2016; Beesley and Marmiroli, 2011), the possible mechanism of adsorption is highly dependent on the type of cation. The adsorption might be caused by the complexation of heavy metals with different biochar functional groups and physical adsorption (Buss et al., 2012). Additionally, inactivated plant biochar inadvertently increases dissolved lead and copper concentrations in sandy, low TOC soils when used to stabilise other contaminants (Uchimiya and Bannon, 2013), which could explain the relatively high lead toxicity with biochar.

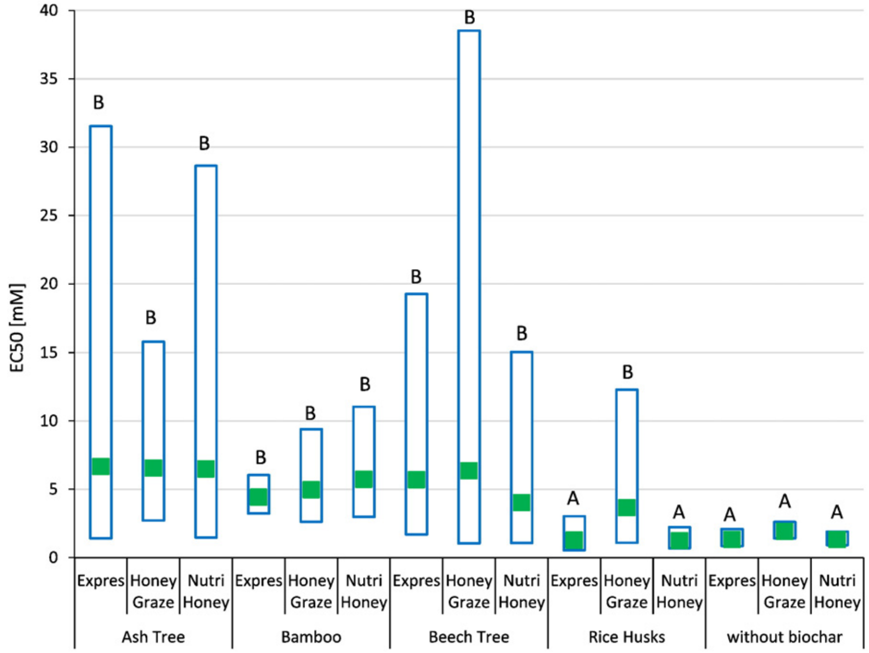

Fig. 11. EC_{50} values for different tested sorghum cultivars in solution supplemented by lead ions. (Green square represents average value and blue box surrounding the maximum and minimum EC_{50} value). Statistically insignificant values of EC_{50} on the level of probability $P < 0.05$ are indicating by the same symbol above blue box. $n = 4$.

4. Conclusions

In this study, the characteristics of biochar from different sources were highly diverse, and the different types of biochar had different effects on germinated seeds. With the different types of biochar, we found significant differences in the toxicity of heavy metals to sorghum cultivars. The contents of PAHs, carbon and nitrogen content, inorganic substances and pH values were also different for biochar from different sources. The application of all biochar types decreased the toxicity of heavy metals to plants. The results demonstrate that it is possible to use biochar to improve soil characteristics of contaminated sites because the application of biochar lowered actual concentrations of heavy metals by adsorption, which reduces abiotic stress on plants. Thus, with the application of biochar, plants have time to adapt to soil contamination, survive and successfully grow and in the process, accumulate heavy metals; these are the plants that are suitable for phytoremediation and soil restoration.

Acknowledgment

This work was supported by project COST TD1107 (project No. LD13029).

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