



Application of co-composted biochar significantly improved plant-growth relevant physical/chemical properties of a metal contaminated soil

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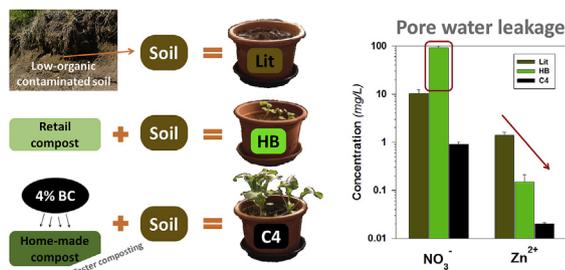
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HIGHLIGHTS

- Co-composting biochar with woody and green waste hastened the maturing of compost
- Resulting compost-char improved moisture and nutrient status when added to soil
- Regardless of biochar, all composts reduced CaCl₂ extractable and pore water metal concentrations
- Plant growth success⁰ was promoted furthest by the addition of co-composted biochar
- Compost-char was shown to outperform retail purchased compost in several key soil and plant parameters

GRAPHICAL ABSTRACT



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ABSTRACT

A woody-biochar was added to waste biomass during a composting process. The resulting compost-char was amended to a metal contaminated soil and two plant species, *L. perenne* and *E. sativa*, were grown in a pot experiment to determine 1) plant survival and stress factors, 2) uptake of metals to plants and, 3) chemical characteristics of sampled soils and pore waters. Compost supplemented with biochar after the composting process were also tested, as well as a commercially available compost, for comparison.

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Co-composting with biochar hastened the composting process, resulting in a composite material of reduced odour, increased maturity, circum-neutral pH and increased moisture retention than compost (increase by 3% of easily removable water content).

When amended to the soil, CaCl₂ extractable and pore water metals were reduced by all compost treatments with little influence of biochar addition at any tested dose. Plant growth success was promoted furthest by the addition of co-composted biochar to the test soil, especially in the case of *E. sativa*. For both tested plant species significant reductions in plant metal concentrations (e.g. 8-times for Zn) were achieved, against the control soil, by compost, regardless of biochar addition.

The results of this study demonstrate that the addition of biochar into the composting process can hasten the stability of the resulting compost-char, with more favourable characteristics as a soil amendment/improver than compost alone. This appears achievable whilst also maintaining the provision of available nutrients to soils and the reduction of metal mobility, and improved conditions for plant establishment.

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1. Introduction

The addition of organic matter to soils can improve its structure, nutrients content and chemical characteristics (Chirakkara and Reddy, 2015; Rahman et al., 2017). The use of compost (the product of spontaneous microbial bio-oxidation of raw wastes to produce a biologically stable, humified organic material; Beesley et al., 2014) can enhance plant growth and increase microbial activity in soils (Chirakkara and Reddy, 2015) by providing nutrients and increasing humic content, thereby increasing water holding capacity, improving soil structure and microbiological activity (Medina and Azcon, 2010). Compost also has properties advantageous to the remediation of metal-contaminated soils because stable complexes are able to form between organic ligands and metals assisted by changes in soil pH (Oustriere et al., 2017), which can render soluble forms of metals less mobile.

For similar reasons the use of biochar as a bio-sorbent for amending contaminated soils has also gained attention in recent years. Biochar is the solid product from pyrolysis of waste biomass residues, under anoxic conditions using temperatures ranging from 350 to 900 °C (Forjan et al., 2018; Novak et al., 2019). The addition of biochar to contaminated soils has been demonstrated to increase cation exchange capacity, and act as an enhanced habitat for microbes and fungi (Rahman et al., 2017) due to the relatively high surface area to volume, in comparison to, for example, composts. For this reason, the use of biochars as bio-sorbents to metal contaminated soils has been demonstrated to reduce metal leaching (Beesley et al., 2011). Three main sorption mechanisms exist by which biochar application to soils can immobilise metals in solution: (i) ion exchange, (ii) metal complexation with free functional groups together with physical adsorption, and (iii) surface precipitation (Trakal et al., 2016).

Several authors have proven that the combination of compost together with biochar may be more suitable than biochar alone to promote immobilization metal(loid) contaminants and buffer nutrient depletion in contaminated soils (Beesley et al., 2014). Compost combined with biochar may also have the added value of further improve total soil C, N, and P, stabilizing soil aggregates, and stimulating microorganism activity in soils (Forjan et al., 2018; Novak et al., 2019; Oustriere et al., 2017; Radin et al., 2018; Rodriguez-Vila et al., 2016; Ye et al., 2019). Potential benefits of using biochar as a compost amendment can be thus summarised as i) increasing microbial activity by potentially enhancing aeration, ii) reducing soil bulk density, iii) increasing compost temperature, iv) decreasing ammonia volatilization, v) enhancing water holding capacity, vi) reducing nutrient losses by leaching, vii) reducing

greenhouse gas (GHG) and odour emissions, and viii) increasing the degree of humification (Wang et al., 2019; Akdeniz, 2019; El-Naggar et al., 2019; Godlewska et al., 2017). As the application of compost has been shown to affect positively the above-mentioned soil properties, the use of biochar as an amendment during composting has the potential to improve these benefits even further (Wang et al., 2019). Therefore, the integration of biochar into the compost at the production stage should result in a matured soil amendment suitable for general soil improvement, with the added value of maximal potential as a bio-sorbent for metals in solution; therefore, the application of this material should prove more favourable than its individual components for application to metal contaminated soils.

The aims of the present study were to demonstrate the efficacy of co-composted biochar compared to biochar subsequently amended with compost, in the context of their amendment to a metal-contaminated soil, specifically examining 1) soil water and nutrients retention; 2) metal(loid)s stabilization (solubility); as well as 3) the consequent performance of two plants species (*Lolium perenne* L. and *Eruca sativa*) attributable to the effects of the tested amendments.

2. Material and methods

2.1. Experimental biochar, composts and soil

The biochar used in the experiment was a mixture of soft wood, mostly spruce made in a CHP plant in Kozomín (Czech Republic) in atmospheric, fixed-bed, multi-stage gasifier, which uses air as a gasifying medium (GP 750). The biochar was heated for 6 h between 500 and 600 °C. Initial biochar characteristics are in Table S1 while its chemical composition is in Table 1.

Compost was prepared on the campus of Czech University of Life Sciences Prague (Suchdol, Prague) from the following materials; woody component from small sticks of maple and oak and green material (freshly cut grass and leaves) at a ratio of 1:5 (vol.). This was done by adding these components to a 200-L spinning plastic drum. Three different drums were operated simultaneously to prepare the following materials: compost only (C0), and compost mixed with 4 and 10%wt. of biochar (C4 & C10). The drums were placed in a greenhouse at approximately 20 °C and spun to mix and aerate 3 times per week during 16 weeks. Moisture content and pH were measured during the composting and co-composting processes from the start until 126 days. Samples were obtained weekly from each treatment (C0, C4 & C10) and 5 g was used to measure pH in a H₂O suspension at 1:5 (w:v) ratio. The remaining sample was

Table 1
Pristine characteristics of the soil and all used amendments.

	Litavka	Biochar	HB	C0	C4	C10
Clay (%)	8.7 ^a	/	/	/	/	/
Silt (%)	34.8 ^a	/	/	/	/	/
Sand (%)	56.5 ^a	/	/	/	/	/
pH (–)	5.90	11.4	6.66	7.59	7.33	7.67
C (g kg ⁻¹)	2.87	868	197	377	451	503
N (g kg ⁻¹)	0.20	5.80	11.7	17.5	19.0	17.0
K (g kg ⁻¹)	6.58	3.15	9.84	14.5	11.8	10.6
Mg (g kg ⁻¹)	0.68	2.82	3.62	2.91	2.43	2.27
Fe (g kg ⁻¹)	37.4	/	8.47	2.47	1.28	2.01
Mn (g kg ⁻¹)	4.28	/	0.26	0.32	0.22	0.35
Cu (mg kg ⁻¹)	71.9	6.86	27.3	14.1	11.8	12.3
Zn (mg kg ⁻¹)	4002	651	167	133	110	249
Pb (mg kg ⁻¹)	3539	12.9	25.87	9.12	13.31	22.6
Cd (mg kg ⁻¹)	39.0	0.13	0.63	0.25	0.12	0.21

^a Values obtained from Jacka et al. (2018)

weighed and dried at 60 °C until constant weight, moisture content was calculated as the difference between wet and dry weight. Further details of the compost preparation can be found in the Supplement. In addition to co-composted amendments, further amendments were prepared whereby compost was mixed with biochar after composting had taken place (ie not co-composted). These amendments had the same amounts of biochar as detailed above (4 & 10% wt.) and are hereafter referred to as FC4 and FC10 respectively.

The soil “Litavka” used for the experiments subsequently detailed was collected from a site located near a lead smelter (Přibram, Czech Republic). The soil used was taken from several sampling locations at various depths up to half a meter deep in order to collect a representative bulk sample. Bulk soil was dried and sieved (<2 mm). Initial soil analyses are presented in Table 1.

2.2. Soil water retention and available water content

Four treatments were used for the measurement of soil water retention (SWRC): control Litavka soil without any addition of organic substrate (Lit.), soil mixture with C0, soil mixture with C4, and soil mixture with FC4 (all amendments in a ratio 1:2 (w/w) to soil). Percentages of biochar are by weight of dry matter. All treatments were filled into sample rings and pre-prepared (fully saturated and later kept at given suction pressure head) for consequent testing according to the preparation procedure introduced in the Supplement.

Thereafter the soil plus amendments were tested: (1) in the Sandbox up to the suction pressure of 1 m (up to pF2, which was used as estimate of field capacity) using the standard method (Eijkelkamp, 2019); (2) in the 5 Bar Ceramic Plate Extractor 1600 (Soilmoisture, United States of America) in suction pressure from 10 to 50 m (pF3 – pF3.7) were measured using the standard method (Soilmoisture, 2008).; and (3) in the 15 Bar Ceramic Plate Extractor 1500 (Soilmoisture, United States of America) in suction pressure by 150 m (pF4.18, wilting point) was measured using the standard method (Soilmoisture, 2015). Available soil water content for plants (AWC) was calculated as the difference between volumetric water content at pF2 and pF4.18. Easily (readily) available water content for plants (EAWC) was calculated as the difference between pF2 and pF3.7.

2.3. Growing pot experiment

One-hundred seeds of yard grass (*Lolium perenne* L.) and arugula (*Eruca sativa* Mill.) were sown directly into 1 L pots containing 1440 g of soil and composts in a ratio 2:1 (w/w). Seven

different treatments were prepared, each in four replicates: Litavka soil without compost (Lit), prepared compost (C0), co-composted biochar (C4, C10), and compost with biochar added later (FC4, FC10). The fourth treatment was retail compost (HB) (Agro Zahradnický Kompost, Agro CS, CZE) included as a control, against which to compare the ‘home-made’ amendments. Each pot was watered by distilled water to 60% WHC (\approx pF2) for germination; this was maintained over the whole period of the experiment (checked and adjusted, if necessary, 2–3 times per week). The growing experiment was conducted inside a greenhouse, with an average temperature of 20 °C and a 12-h period of light, ensured by high-pressure sodium lamps. The plants were grown for a period of 35 days.

2.4. Characterisation of metal (loid)s in amended soil and pore water

After 35 days soil sub-samples were taken from each pot and air dried and sieve at 2 mm. Pseudo total concentration of elements were extracted by microwave-assisted *aqua-regia* extraction, according to EPA 3051A: 0.250 g of sample was digested under microwave oven (Multiwave PRO microwave reaction system SOLV, Anton Paar, Germany), in 9 mL HNO₃ + 3 mL HCl + H₂O₂ in order to enhance the organic matter decomposition. After cooling down, the samples were diluted to 50 mL with demineralised water and filtered using 0.45 µm pore nitro-cellulose syringe filters.

For the directly available metal pool, samples of 4 g of soil were treated with 40 mL of 0.01 M CaCl₂ (Quevauviller, 1998), shaken for 3 h at 300 rpm, centrifuged for 10 min at 3000 rpm and filtered through a 0.45 µm nylon filter (VWR, Germany). The pH was measured after centrifuge using an inoLab® pH metre (pH 7310, WTW, Germany).

Pore water was also collected at 35 days from each pot using 10 cm long rhizons (Eijkelkamp, NED) extracted by removable plastic syringes and stored at 10 °C until analysis. Electric conductivity (EC) and pH in the extractant were measured using a multimeter and pH meter (Multi 3420 and pH 7310 respectively, WTW, Germany). Major inorganic anions were determined using Dionex ICS-5000 ion chromatography system (Dionex, USA) and Total organic/inorganic C was determined using the carbon analyser TOC-L CPH for liquids, both after diluting 1 mL of the extractant into 49 mL of deionized water.

Total concentrations of elements in all the analysed solutions were obtained by inductively-coupled plasma optical emission spectrometry (ICP-OES) (720 ES, Varian Inc., CA, USA).

2.5. Plant analysis

After soil and pore water samples had been taken, leaves of the plants were cut at the base and 3 cuts were made on 3 different leaves at different heights to obtain representative 3 g samples, which were then frozen at –80 °C for further analysis. The precise extraction procedures and analyses are presented in the Supplement (S1).

For the extraction of proteins, frozen plant material was homogenized with a mortar and pestle in cold 0.1 M Tris/HCl buffer (Roth) (supplemented with 5 mM EDTA (Ing. Petr Švec – PENTA Ltd., Czech Republic), 1% PVP K30 (Carl Roth GmbH + Co. KG), 5 mM DTE (Roche), and 1% Nonidet P40 (Roche)) at pH 7.8 (10 mL of extraction buffer per 1 g FW). The homogenate was centrifuged at 20,000 rpm at 4 °C for 30min. The sample was filtered with Miracloth Filter (Calbiochem), and the supernatant volume was measured. For the first precipitation, 40% ammonium sulfate (LachNer, Czech Republic) was added, and the solution was agitated for 30min. The suspension was centrifuged at 20,000 rpm at 4 °C

for 30min using Ultracentrifuge L7-55 (Beckman, USA). The sample was filtered through Miracloth Filter, and the supernatant volume was again measured. For the second precipitation, 80% ammonium sulfate was added, and the solution was agitated for 30min. The suspension was centrifuged at 20,000 rpm at 4 °C for 30min. The pellet was resuspended in 2.5 mL of 25 mM Tris/HCl buffer (pH 7.8), placed in PD 10 columns, eluted with 3.5 mL of 25 mM Tris/HCl buffer (pH 7.8) and stored at -80 °C.

The protein samples were used for the measurement of enzymes. All enzyme assays were performed using a TECAN Infinite M200 microplate reader (Tecan Group Ltd., Switzerland). Peroxidase (POX) activity was detected based on the colour reaction with ABTS or guaiacol substrate (modified from Drotar et al., 1985). Glutathione-S-transferase (GST) activity was detected based on the reaction with five different substrates (DCNB, fluorodifen, CDNB, pNBC or pNBoC) based on a methodology modified from Habig et al., (1974). Catalase (CAT) activity was detected based on the disappearance of hydrogen peroxide (modified from Verma and Dubey, (2003)). Ascorbate peroxidase (APX) activity was detected by a decrease in the absorbance of ascorbate (Vanacker et al., 1998). Superoxid dismutase (SOD) activity was detected by using xanthine-oxidase system based on the methodology of El-Shabrawi et al., (2010). Details of the modifications to the methodologies can be found in supplements (S4).

The enzyme activities were expressed in ncat mg⁻¹ protein. The protein concentration was determined according to the Bradford assay (1976) using bovine serum albumin as a standard (BIO-RAD). All reported values for enzyme activities were the means of the determinations of eight separate extracts for each sampling point. Each extract was prepared from both parts (root, leaf) of the plant.

Leaf frozen samples (10–20 mg FW) were extracted with 10 mL methanol in the dark at 4 °C. The absorbance of methanolic extract was evaluated using 470, 652.4, and 665.2 nm and it was monitored in a Tecan Infinite 200 PRO microplate reader (Tecan, Switzerland) and the chlorophyll and carotenoid contents were calculated using a formula according to Lichtenhaler (1987). All samples were analysed in triplicate.

The remaining plant material was dried at 80 °C and ground. The amount of 0.2 g of dry material was digested in 2:8 mL H₂O₂:HNO₃ at 200 °C in microwave oven, then diluted to 25 mL with deionized water, filtered through a 0.45 µm nylon filter and analysed by ICP-OES for determination of elements. For each set of samples, the reference plant material NCS ZC73018 (Bowen's Kale; IUPAC, 1979) was also analysed.

2.6. Statistics

Analysis of variance (ANOVA) was performed to find differences between treatments and Tukey Honest Significant Difference method was used with confidence level of 0.95, the results are presented for $p < 0.05$. The statistical analyses were performed using the software R 3.5 (The R Foundation for Statistical Computing 2018; under the GNU General Public License).

3. Results and discussion

3.1. Impacts of the co-composting process on amendment characteristics

The biochar used in this study has very high reactive surfaces and porosity as well as carbon purity (e.g. low amount of volatile matter), which deems it suitable for co-composting (due to potentially high capacity to retain water from the fresh biomass during the process). The retail compost (HB) has slightly acidic pH in comparison to all 'home-made' composts with pH = 7.33–7.67.

Total nitrogen content in HB was lower in comparison to all home-made composts. Additionally, C content is significantly lower in HB and the presence of biochar in home-made compost increased C content even further, due to its own high C content (86.6%).

As shown in Fig. S1 the presence of biochar enhanced the composting process. After one month of composting, the grass was still visible in C0 and C4, but hardly present in C10. The C0 variant was clearly the least decomposed and the most pungent in aroma; C4 was less pungent and C10 had no unpleasant smell as the compost materials had broken down the furthest. Co-composting biochar indeed hastened the composting process, confirming the findings of (Fischer and Glaser, 2012; Chen, 2016; Lohri et al., 2016). Both C4 (4% BC) and C10 (10% BC) reached a stable state 3 and 2 weeks earlier than C0 (0% BC), respectively. This could be explained by an increased water and nutrients holding capacity of the biochar presented which logically created better conditions for co-composting process.

Moisture content during co-composting process was kept between 65 and 75% during the first 80 days (Fig. S2). After this period of time the moisture content started decreasing in accordance with the amount of BC in the compost: C0 decreased to 25%, C4 to 52% and C10 remained stable at 67% moisture content at the end of the monitoring period. During the first two weeks of co-composting pH varied considerably between all the treatments and samples, but after the third week it reached neutral values between 6.9 and 7.6 (Fig. S2) in the three prepared composts and remained stable until the end of the sampling. The compost with highest dosage of biochar (C10) showed slightly higher (alkaline) pH in comparison to C0 and C4, respectively.

Based on the observations of the experiment, it can be concluded that the ideal percentage of BC needed to aid decomposition here lies somewhere between 4 and 10%. However, this is also most likely dependent on the initial composition of the feedstock materials for the compost. Co-composting chicken manure, for instance, requires a larger amount of biochar in order to see favourable results; Agegnehu et al. (2017) found a 20% addition of BC decreased nitrogen loss by 52%. Decreasing the time necessary for a stable finished compost has obvious advantages practically and economically, as long as there is no sacrifice in the quality of the material.

3.2. Implications for soil water retention

The quality of the resulting amendments produced by the co-composting process, in terms of suitability as a soil amendment, can be indicated by soil water retention. Fig. 1a shows distinct differences in easily available water content for plants (EAWC) according to soil amendment; the lowest EAWC was observed for control soil. Pure compost addition (C0) significantly increased mean EAWC value from 21% to 23%. Addition of biochar to compost further significantly increased EAWC value from 23% to approx. 26%. This increase was noted independently of the timing of biochar addition (before (C4) or after composting process (FC4)). The increase of EAWC in soil after the compost application is the result of the increased amount of organic matter. When the biochar was added into the compost the amount of easy-available water increased significantly due, in all likelihood, to high biochar porosity and significant swelling effect explained as a loading of H₂O molecules on the biochar surface through the hydrogen bonds (Jacka et al., 2018).

Without amendment control soil (Lit.) also exhibited significantly lower available water content for plants (AWC) than following amendment (see Fig. 1b). Pure compost addition (C0) distinctly increased mean AWC value from 23% for control to 29%. Addition of biochar to compost (independently on timing of

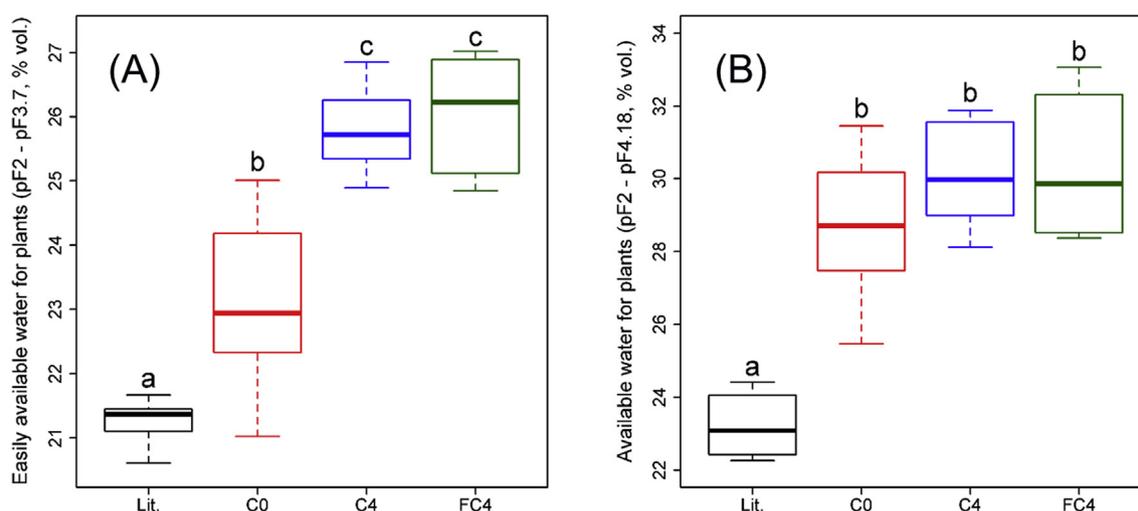


Fig. 1. Boxplot (sample minimum, first quartile, median, third quartile, maximum, $n = 7$) of easily available soil water for plants (A) and available soil water for plants (B): Lit = Litavka soil (Control); C0 and C4 = co-compost with 0%, 4% and 10% BC, respectively; FC4 = compost with addition of 4% BC at the end of composting process. Different letters represent statistical differences by Tukey HSD at $p < 0.05$.

biochar addition) only slightly increased mean value of AWC from 29% to approx. 30% and this difference in mean AWC value was not statistically significant. This suggests that the amount of strongly bonded water (difference between pF 3.7 and 4.18) increased in the compost treatment from 2% to 6%. Conversely, both biochar + compost treatments increased the amount only by 2%. This means that biochar presence in compost enhanced mainly the amount of water ranging between field capacity ($pF = 2.0$) and the drought stress zone ($pF = 3.7$; Mikolajczak et al., 2017). Thus the application of compost and especially, compost-biochar mixture could be beneficial for plants during period(s) of agricultural drought.

3.3. Trace elements and nutrients according to amendments and plants

As expected, the different treatments affected the pseudo-total concentrations of elements in soils as did (Fig. S3). The concentration of K in soil was increased by the co-composting of BC (both, C4 and C10), whilst significantly decreased when treated with retail compost under *E. sativa* growth; for all the other treatments, concentrations of K were higher in soils with *E. sativa* growth. Unlike the pseudo-total concentrations, the CaCl_2 -extractable fraction shows clear influences of the treatments for a wider range of elements, with a general increase of the nutrients and reduction of contaminants (Fig. 2). Rhizosphere soil of *E. sativa* showed higher concentrations of extractable K, Mg, Fe, Mn and organic C compared with that of *L. perenne*. In particular, CaCl_2 -extractable K was the highest for FC10 treatment, mainly in the rhizosphere soil of *E. sativa*, although the same treatment shows statistical differences with C4 and C10 under *L. perenne* growth. Magnesium was the highest under retail compost, and the lowest under C0 treatment. Extractable Fe under *L. perenne* growth did not show differences between different compost treatments, but for *E. sativa* there is a clear increase under C0 treatment. Extractable Mn was significantly decreased due to the effect of home-made compost, while there are no statistical differences between Litavka variant and retail compost (higher for both). Dissolved organic C was the highest for retail compost under *E. sativa* growth (together with FC1 under same specie), and the lowest under *L. perenne* (together with Lit., same species) also under HB treatment. The increase in the

CaCl_2 -extractable concentrations for all amended soils reflects the contribution of the composts to the pool of nutrients in the contaminated soil. This resulted in an increase of the uptake of nutrients by *L. perenne*, which reflects its ability to adapt well to contamination stress (discussed later). At the same time, nutrients are added to the soil through the amendment by compost, resulting in a higher uptake by the plant only when its physiological mechanisms are adapted to overcome stress. Radin et al. (2018) found no differences in nutrients uptake by oil palm under different mixtures of biochar and compost.

Potassium concentration in pore water was the lowest in control soil without amendment, with no statistical differences between the other treatments. Concentration of Mg in pore water was highest under retail compost whereas Mn in pore water was significantly reduced with home-made compost treatments. Total C in pore water was significantly lower under control whilst chloride was the highest under retail compost. Nitrate was considerably greater under retail compost treatment, followed by the control, while significantly reduced under all other treatments. Concentration of sulfate was the only variable in the pore water that was affected by both plant species and by the interaction of treatment with plant species: the highest concentrations were found under retail compost, particularly under *L. perenne*, while *E. sativa* caused a reduction in concentration in pore water. In summary, the HB compost may simply have had more nutrients in available forms easily leached into pore water, because it was aged further than the home-made compost it was compared against here. Although it is not stated on the packaging of HB used in this study, it is also possible that soluble nutrients are artificially added to the compost to improve growing performance, as could be the case for other retail purchased soil amendments.

The uptake of nutrients by plants (Table 3) was lowest for plants grown in control soil (Lit. soil), as expected, but different treatments showed different interactions with nutrient uptake. Potassium in *L. perenne* has lowest concentration for plants in control soil, and there are no meaningful differences between other treatments, while in *E. sativa*, the opposite behaviour appeared: plants under Lit. soil showed the highest concentrations, while treatments C0 and C4 show the lowest. Calcium showed no statistical differences for *E. sativa*, but for *L. perenne* the highest uptake was found under retail compost and Lit. soil, however only the

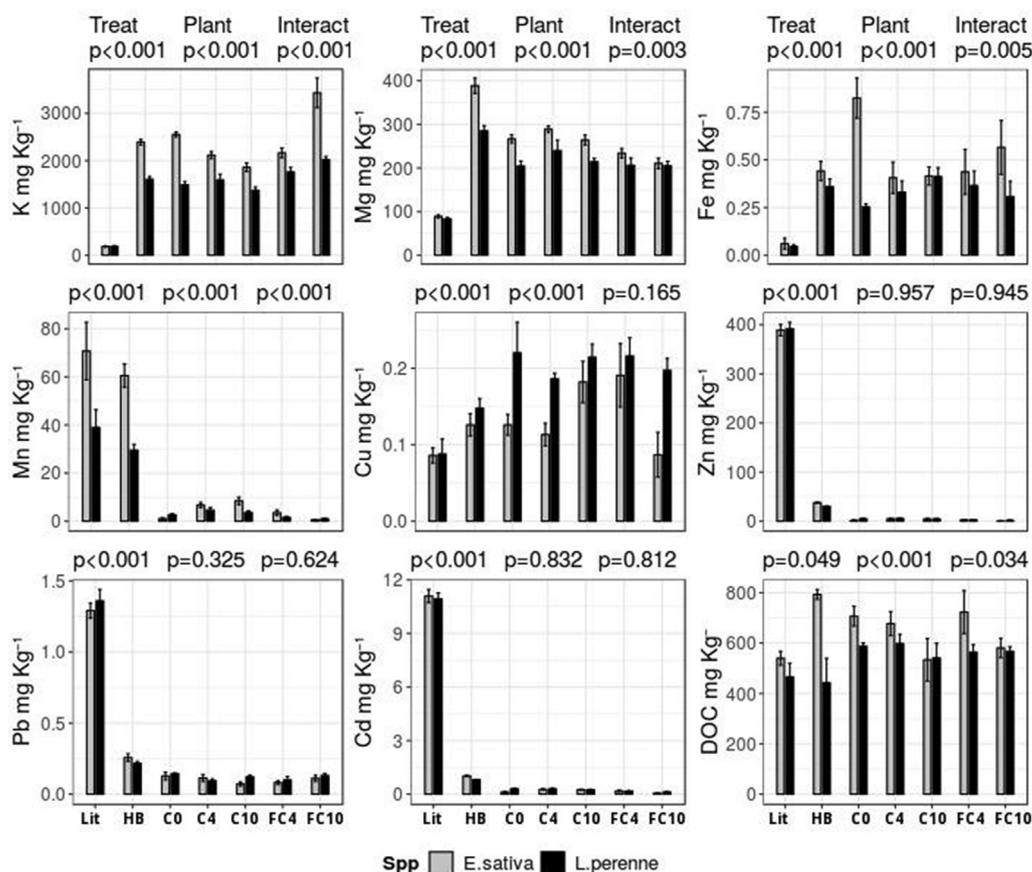


Fig. 2. Mean and standard error values of the concentrations CaCl_2 -extractable elements in soil. **Lit** = Litavka soil (Control); **HB** = retail compost; **C**, **C4** and **C10** = co-compost with 0%, 4% and 10% BC, respectively; **FC4** and **FC10** = compost with addition of 4 and 10% BC at the end of composting process. The **p**-values are provided for effect of treatment (Treat), plant species (Plant) and the interaction of both (Interact).

addition of 10% of BC (both, co-composted and final) considerably reduced the uptake of Ca by the plant. Magnesium showed no meaningful differences for *L. perenne*, but for *E. sativa* there was considerably higher uptake in plants under control soil than plants growing under the addition of compost. Sulphur in *E. sativa* showed the same behaviour as Mg: significantly higher for Lit. soil, and no other differences for the composts. *L. perenne* also showed the highest uptake of S under Litavka treatment, but more interactions are shown in Table 3: the lowest concentration was found under the 10% BC co-composted treatment (C10); retail compost reflects the highest uptake of S, and there is no statistical difference between this and the compost without BC (C0) nor the composts where BC was added at the end (FC4, FC10). Uptake of Mn was also higher in Lit. soil, while no differences were found between treatments for *E. sativa*, for *L. perenne* there is a clear decrease in the plants under C10 treatment. Copper in *L. perenne* showed no differences, while in *E. sativa* was higher only in Lit. soil.

3.4. Metals concentration and availability in soil

Pseudo-total concentrations of Cu, Cd, Zn and Pb in soils were generally higher in the soils under *L. perenne*, whilst the lowest concentrations were that of the retail compost under *E. sativa* growth (Fig. S3). Application of 'home-made' compost and/or biochar to the contaminated soil had also significant effects on the pseudo-total concentration of metals, as can be seen in Fig. S3, where despite of statistical differences of the treatment, the variation between particular amendments for some of the metals is

minor. Ye et al. (2019) logically highlighted that the pseudo-total concentrations of amended soils mirror dilution effects of compost on the soil it is amended to. This is likely to have been the case in the present study, due to the 2:1 ratio, by mass, of soil to composts added. Additionally, Forjan et al. (2018) found that the pseudo-total concentration of Cu, Pb, Ni and Zn increased in contaminated soils treated with compost and biochar, due to the high concentration of these elements in the compost, however the percentage of CaCl_2 -extractable concentrations in relation to the pseudo-total concentration decreased with the application of the amendment due to the ability of the compost and biochar to fix these metals. Rodriguez-Vila et al. (2016) found a decrease in the CaCl_2 -extractable Al, Co, Cu, Fe and Ni after application of compost and biochar to contaminated soils vegetated by *B. juncea*, together with increase of nutrients and pH to neutral values. Such studies highlight the relative insensitivity of assessing the impacts of organic amendments in soils via the use of pseudo-total concentrations only; essentially, they are a 'blunt-instrument' in this situation. The CaCl_2 -extractable fraction of all the contaminants (Zn, Pb and Cd) was significantly reduced under all the treatments in the present study, better reflecting the expected outcome of adding organic amendments to metal rich soil (Fig. 2). Concentrations of Zn and Cd in the pore water showed the same effect (Fig. 3). Concentration of contaminants in both extractions were not affected by plant species, with the only exception of Pb in pore water which was poorly influenced by the different treatments (Fig. 3).

Concentrations of Cd, Pb and Zn in pore water were reduced in all amended soils compared to the control and, according to the

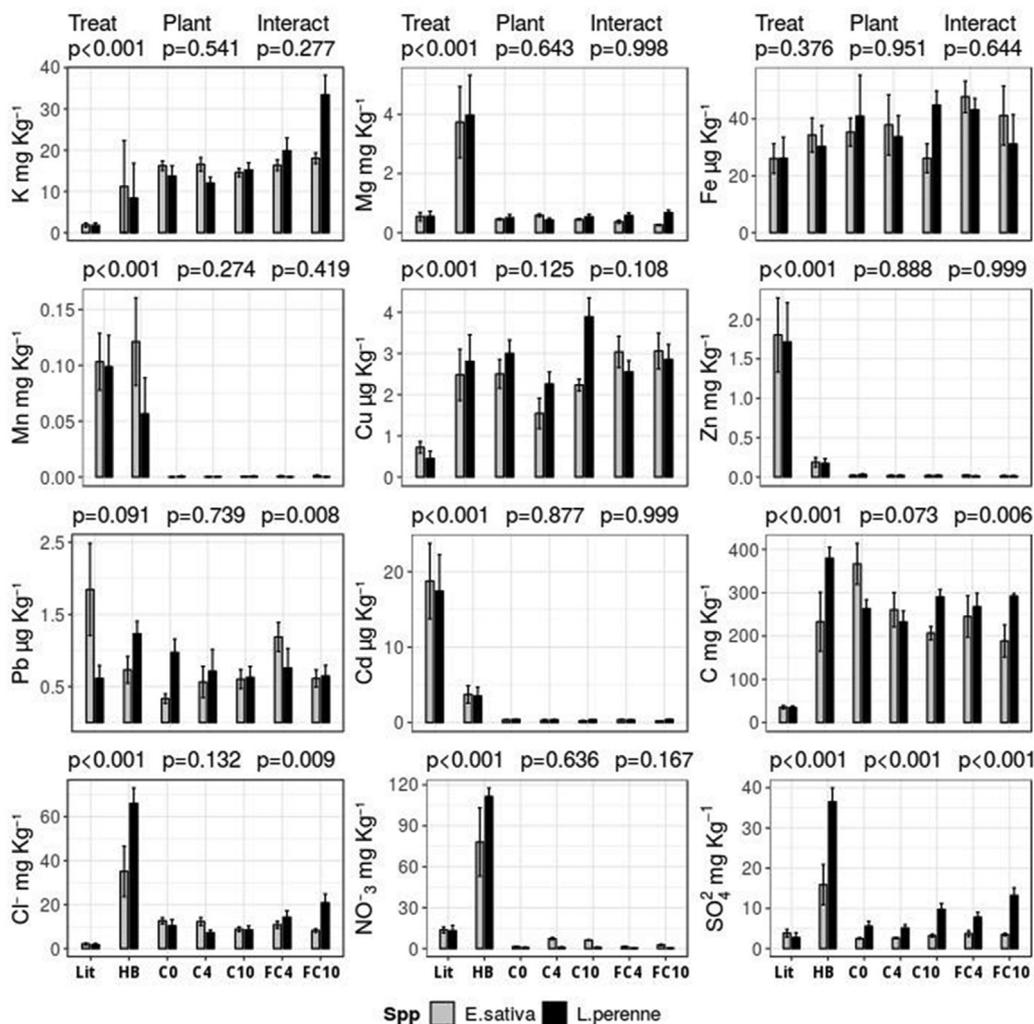


Fig. 3. Concentration of elements and main anions in the pore water (Mean and Standard Error). **Lit** = Litavka soil (Control); **HB** = retail compost; **C**, **C4** and **C10** = co-compost with 0%, 4% and 10% BC, respectively; **FC4** and **FC10** = compost with addition of 4 and 10% BC at the end of composting process. The **p**-values are provided for effect of treatment (Treat), plant species (Plant) and the interaction of both (Interact).

literature, this seems to be a general response when organic amendments with low concentrations of contaminants are applied to contaminated soils (Forjan et al., 2018; Novak et al., 2019; Oustriere et al., 2017; Trakal et al., 2017; Rodriguez-Vila et al., 2016; Yang et al., 2018). Oustriere et al. (2017) found a decrease in the concentration of Cd, Pb and Zn in pore water of soils treated with compost and biochar under different combinations, and concluded that the interactions between biochar-amended soils and metals are complex and it could be due to a combination of the following factors: (1) increase in the pH activates electrostatic interactions with negatively charged surfaces on soils, (2) specific metal-ligand complexation involving surface functional groups of biochars and (3) sorptive interactions between cations and aromatic π electronic systems from C=C bonds of biochars. Novak et al. (2019) found a decrease of H_2O - and $CaCl_2$ -extractable Cd and Zn with addition of compost and biochar at different rates, partly due to an increase of the pH from acidic to more neutral values, but also due to sorption by mineral ash constituents present in the poultry litter biochar used, however, similar to the present study, no differences were found between different treatments (compost and biochar ratios), but only between control and treated soils. This generalised trend has often been seen in studies where biochars have been amended to metal-rich soils, as summarised by Beesley et al. (2015).

3.5. Uptake of metals by plants

Metals (Cd, Pb and Zn) uptake by plants showed similar behaviour as that found for most of the nutrients; plants growing in Lit. soil had greater concentrations of these elements than soil with compost addition (regardless of biochar presence). This is true for Zn in both plant species, and Cd and Pb in *L. perenne*. In *E. sativa*, Pb has no statistical differences regardless of the treatment, while Cd was also higher for plants in Lit. soil, and considerably lower for the soils with 4% of BC (both C4 and FC4).

All treatments decreased the availability of the target contaminants (Cd, Pb and Zn) in the soil, resulting in a decreased of the plant uptake (with the exception of Pb for *E. sativa*), with the home-made compost being more efficient than retail purchased comparator; however no difference between addition or absence of biochar was found, nor between the different combinations. Cadmium and Pb are not essential elements for plant growth, but they are taken up by plants into their tissues nonetheless, resulting in adverse effects in the physiological function of plants when the concentrations are high (phytotoxic). Zinc is considered as a micronutrient important for cellular enzymatic functions, protein production and membrane integrity (Novak et al., 2019), but is phytotoxic at excessive concentrations in plants. Forjan et al. (2018)

found that the effect of applying compost with biochar reduced available concentrations of Ni over time, due to an increase in the pH and organic matter content, aided by the uptake of the element by the roots of the plants. Longer term experiments are necessary to understand and possibly predict the effect of biochar for stabilization of metallic contaminants. Novak et al. (2019) found that, after different combinations of compost and biochar applied to contaminated soils, the lowest Cd concentrations in plant tissue were found in plants growing in soils amended by a mixture of compost and biochar. An additional explanatory factor behind the general reduction in plant metal uptake regardless of amendment type, was the dilution factor of soils by amendments already discussed. At the pot scale, as used here, the concentration of rooting in the confines of the pot will likely exacerbate the effects of amendment addition, compared to field conditions where plant roots could spread. This is especially the case here as the amendments were added at relatively high volumes to the pots, when considering their likely much lower bulk densities compared to soil and the mass added to the soil. The importance of verification of the results seen here in field conditions is worthy of note, especially considering the relative lack of field trials verifying the impacts of biochars and their derivatives on metal polluted soils (Sizmur et al., 2016).

3.6. Plant growth and stress

Visual differences were observed between treatments during the growing period, as shown in Fig. 4. Five weeks after germination *E. sativa* did not germinate in the control-contaminated soil (Lit), while application of HB and FC10 treatments produced small specimens where necrosis was observed; application of home-made compost with and without biochar co-composted produced strong plants growth. For *L. perenne* the response was different, due to its relative insensitivity to high soil metal contents, plants under control established successfully but with poor biomass production. Plants under HB developed better and plants with the application of home-made compost produced the greatest biomass. Only few days before harvest, signs of chlorosis were observed in the plants growing under control (Lit). The poor development of plants growing in the control soil was likely due to phytotoxicity of metals; this was particularly acute with *E. sativa*, where even the addition of retail compost did not help alleviate toxicity stress. Exposure to metals creates reactions with the oxygen in the metabolic system of plants, producing more reactive forms of oxygen which induce alterations in protein expression and

carbohydrate metabolism; to cope with such damage plant cells produce antioxidant enzymes.

Application of compost increased production of total chlorophyll and carotenoids in *L. perenne*, with no statistical difference between the compost treatments (Table 2). Stress-related enzymes produced by plants did not show statistical differences caused by treatments, with the only exception of peroxidase produced by *E. sativa*, where the co-composting with 10% BC (C10) shows the lowest production, while the compost without BC (C0) shows the highest. However, not enough plant material was produced in the control soil to obtain enzymes production, which could suggest differences between control and compost addition, like chlorophyll and carotenoid production. In general, the results showed that the addition of compost improved the growth and development of plant considerably, regardless of the type of compost or BC addition.

Sensitivity in plants to metal stress generally depends upon metal concentration, plant species, exposure time and age (Sidhu et al., 2017). The only difference found under the statistical analysis was POX activity in *E. sativa*, which was higher in the compost without biochar. It has been proved that POX activity increases with the exposure to high concentration of metals in the growing substrate. Drzakiewicz et al. (2004) found significant increase of POX in exposure to Cu, and Sidhu et al. (2017) with exposure to Pb. The difference in the influence of POX activity between *E. sativa* and *L. perenne* observed in our study could be due to photosynthetic oxygen evolution in C_3 plants, which produce large amounts of reactive forms of oxygen in the form of H_2O_2 (Drzakiewicz et al., 2004).

Weili et al. (2019) found that different concentrations of biochar applied in hydroponics resulted in differences of enzymatic activity for *Amarantus mangostanus*, showing a general increase of stress with medium concentrations of the BC ($2.6\text{--}13.3\text{ g L}^{-1}$) and absence of stress (compared to the control) with low (1.3 g L^{-1}) and high (26.6 g L^{-1}) concentrations. It seems clear that different source material for biochar variously affects the physiological response of the plants: the biochar used for the present study appears to be suitable as an amendment for metal-contaminated soils, such as the one tested here.

4. Conclusion

The application of biochar hastened the maturing of compost made of woody and green material. As a consequence of improved moisture and nutrients status of the resulting compost-char hybrid the pool of $CaCl_2$ -extractable nutrients was enhanced upon addition to a soil contaminated by Pb and Zn. This also corresponded with a decrease in metals extractable from amended soil (pore water and $CaCl_2$ fraction). The plant species grown in amended soil reacted differently to the more favourable conditions induced by the amendments; *L. perenne* was more resistant to contamination stress, therefore no physiological differences were observed regardless of the amendment type and dose applied to the soil. On the other hand, *E. sativa* was more sensitive to metal contamination in the tested soil, though oxidative stress (reflected in POX activity) was reduced furthest with the addition of biochar. The addition of biochar co-composted with, or added at the end of composting process, had no effect on the development of the plant (enzymes activity, chlorophyll production, uptake of elements) during the experiment but did positively impact on some characteristics of the soil. Our next studies will focus on 1) testing different application rates of the compost-char to a variety of contaminated soils and 2) conducting small field trials to validate the results of greenhouse and laboratory studies.



Fig. 4. *L. perenne* (A) and *E. sativa* (B) 5 weeks after germination. Left to right: Lit, HB, C0, C4, C10, FC4 and FC10.

Table 2

Mean values \pm standard deviation of enzymes activity (POX, GST, CAT, APOX and SOD) given in ncat mg^{-1} , total chlorophyll (Chlor), and carotenoids (Car), both in mg g^{-1} DW and total protein (Prot) in plant (mg ml^{-1}). **Lit** = Litavka soil (Control); **HB** = retail compost; **C0**, **C4** and **C10** = co-compost with 0%, 4% and 10% BC, respectively; **FC4** and **FC10** = compost with addition of 4 and 10% BC at the end of composting process. The **p**-value obtained after one-way ANOVA is provided in the last column. Different letters represent statistic differences by Tukey HSD at $p < 0.05$.

	Lit.	HB	C0	C4	C10	FC4	FC10	p
<i>L. perenne</i>								
POX	NA	0.29 \pm 0.11	0.29 \pm 0.04	0.46 \pm 0.26	0.29 \pm 0.07	0.57 \pm 0.52	0.4 \pm 0.12	0.511
GST	NA	0.93 \pm 0.78	0.58 \pm 0.43	0.5 \pm 0.19	1.57 \pm 2.62	0.46 \pm 0.24	0.6 \pm 0.4	0.735
CAT	NA	619 \pm 279	472 \pm 225	891 \pm 424	645 \pm 257	873 \pm 485	1018 \pm 410	0.305
APOX	NA	3.05 \pm 0.69	3.02 \pm 0.79	4.86 \pm 2.43	3.29 \pm 1.84	4.94 \pm 2.77	2.54 \pm 0.21	0.275
SOD	NA	48.2 \pm 11.3	58.9 \pm 11.1	45.3 \pm 22.4	39.2 \pm 16.6	58 \pm 15.5	56.7 \pm 12.4	0.395
Chlor	^a 640 \pm 140	^b 1264 \pm 286	^b 1212 \pm 115	^b 1541 \pm 129	^b 1463 \pm 102	^b 1279 \pm 66	^b 1383 \pm 170	<0.005
Car	^a 159 \pm 38	^b 308 \pm 73.3	^b 296 \pm 34.8	^b 389 \pm 30.8	^b 367 \pm 25.1	^b 324 \pm 16.4	^b 339 \pm 55.8	<0.005
Prot	NA	1.71 \pm 0.39	1.22 \pm 0.17	1.66 \pm 0.62	1.78 \pm 0.47	1.3 \pm 0.27	1.28 \pm 0.21	0.190
<i>E. sativa</i>								
POX	NA	NA	^a 0.2 \pm 0.14	^{ab} 0.07 \pm 0.02	^b 0.05 \pm 0.02	^b 0.06 \pm 0.03	NA	0.039
GST	NA	NA	1.05 \pm 0.66	0.88 \pm 0.54	1.61 \pm 1.42	1.68 \pm 2	NA	0.769
CAT	NA	NA	502 \pm 155	461 \pm 438	791 \pm 1089	361 \pm 305	NA	0.779
APOX	NA	NA	3.22 \pm 0.91	4.64 \pm 1.61	3.54 \pm 0.55	2.99 \pm 1.17	NA	0.223
SOD	NA	NA	32.9 \pm 4.8	43 \pm 16.1	34.7 \pm 18.1	37.5 \pm 14.4	NA	0.767
Chlor	NA	810 \pm 312	1116 \pm 132	1487 \pm 72	1585 \pm 728	1311 \pm 110	NA	0.059
Car	NA	197 \pm 91.6	293 \pm 34.1	379 \pm 19.9	394 \pm 186	332 \pm 20.5	NA	0.066
Prot	NA	NA	1.98 \pm 0.07	1.77 \pm 0.6	1.64 \pm 0.19	2.02 \pm 0.53	NA	0.552

Table 3

Mean values and standard deviation of the concentrations of elements in plant tissue, given in mg Kg^{-1} . **Lit** = Litavka soil (Control); **HB** = retail compost; **C0**, **C4** and **C10** = co-compost with 0%, 4% and 10% BC, respectively; **FC4** and **FC10** = compost with addition of 4 and 10% BC at the end of composting process. The **p**-value obtained after one-way ANOVA is provided in the last column. Different letters represent statistic differences by Tukey HSD at $p < 0.05$.

	Lit.	HB	C0	C4	C10	FC4	FC10	p
<i>L. perenne</i>								
K	^a 18198 \pm 3027	^b 37372 \pm 3580	^b 37906 \pm 13,165	^b 35719 \pm 350	^b 32442 \pm 4233	^b 43446 \pm 2632	^b 44214 \pm 1077	<0.005
Ca	^{ab} 2808 \pm 432	^b 3360 \pm 346	^{ac} 2143 \pm 543	^{ac} 2265 \pm 81	^c 1857 \pm 325	^{ac} 2523 \pm 277	^c 2026 \pm 114	<0.005
Mg	1344 \pm 189	1786 \pm 183	2092 \pm 1539	1447 \pm 85	1230 \pm 171	1557 \pm 144	1514 \pm 47	0.519
S	^a 2794 \pm 144	^b 1941 \pm 344	^{bcd} 1723 \pm 296	^{cd} 1420 \pm 138	^c 1187 \pm 171	^{bd} 1954 \pm 254	^{bd} 1931 \pm 83	<0.005
Fe	115 \pm 88.8	41.9 \pm 6.2	36.9 \pm 4.96	200 \pm 272	43.7 \pm 7.69	73.5 \pm 5.86	107 \pm 28.3	0.305
Mn	^a 184 \pm 96.5	^{ab} 154 \pm 20.8	^{ab} 91.85 \pm 97.8	^{ab} 59.91 \pm 7.78	^b 55.92 \pm 10.63	^{ab} 59.72 \pm 8.39	^{ab} 66.11 \pm 8.3	0.013
Cu	6.25 \pm 3.26	7.27 \pm 0.82	8.64 \pm 7.87	5.92 \pm 0.41	5.48 \pm 0.82	7.14 \pm 0.45	7.57 \pm 0.26	0.865
Zn	^a 1668 \pm 155	^b 212 \pm 23	^b 159 \pm 36	^b 133 \pm 11	^b 114 \pm 13	^b 155 \pm 16	^b 133 \pm 4	<0.005
Pb	^a 17.5 \pm 10.71	^b 4.98 \pm 0.32	^b 2.69 \pm 0.63	^b 3.5 \pm 0.21	^b 3.3 \pm 0.8	^b 3.77 \pm 0.54	^b 7.65 \pm 3.87	0.001
Cd	^a 16.76 \pm 3.5	^b 2.36 \pm 0.17	^b 4.85 \pm 6.27	1.32 \pm 0.32	0.84 \pm 0.23	1.41 \pm 0.31	0.94 \pm 0.12	<0.005
<i>E. sativa</i>								
K	^a 62764 \pm 7701	NA	^{bc} 42423 \pm 2129	^{bc} 43208 \pm 8296	^{ab} 46799 \pm 8479	^{abc} 44209 \pm 2084	54,638 \pm NA	0.037
Ca	19,686 \pm 1337	NA	12,915 \pm 3019	12,860 \pm 3833	12,771 \pm 2637	10,865 \pm 1432	8930 \pm NA	0.059
Mg	^a 4706 \pm 667	NA	^b 2671 \pm 501	^b 2735 \pm 641	^b 2780 \pm 451	^b 2465 \pm 291	2280 \pm NA	0.006
S	^a 29972 \pm 2305	NA	^b 11641 \pm 2028	^b 10676 \pm 2625	^b 11067 \pm 1949	^b 12097 \pm 1604	13,240 \pm NA	<0.005
Fe	59.53 \pm 4.21	NA	54.67 \pm 9.2	51.02 \pm 7.31	51.07 \pm 16.45	54.82 \pm 1.85	49.73 \pm NA	0.916
Mn	^a 304.5 \pm 14	NA	^b 21.8 \pm 3.75	^b 25.79 \pm 8.97	^b 28.28 \pm 10.21	^b 25.95 \pm 5.57	24.53 \pm NA	<0.005
Cu	^a 24.7 \pm 4.71	NA	^b 6.14 \pm 1.08	^b 5.39 \pm 1.03	^b 5.99 \pm 2.72	^b 5.85 \pm 0.7	3.64 \pm NA	<0.005
Zn	^a 713 \pm 8	NA	^b 283 \pm 44	^b 199 \pm 48	^b 237 \pm 93	^b 245 \pm 29	243 \pm NA	<0.005
Pb	5.14 \pm 2.3	NA	3.97 \pm 1.71	2.81 \pm 1.67	1.97 \pm 1.23	2.5 \pm 0.2	2.62 \pm NA	0.232
Cd	^a 13.73 \pm 2.01	NA	^{ab} 8.56 \pm 2.01	^b 7.06 \pm 1.73	^{ab} 7.47 \pm 2.91	^b 7.12 \pm 0.96	7.47 \pm NA	0.041

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2019.125255>.

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