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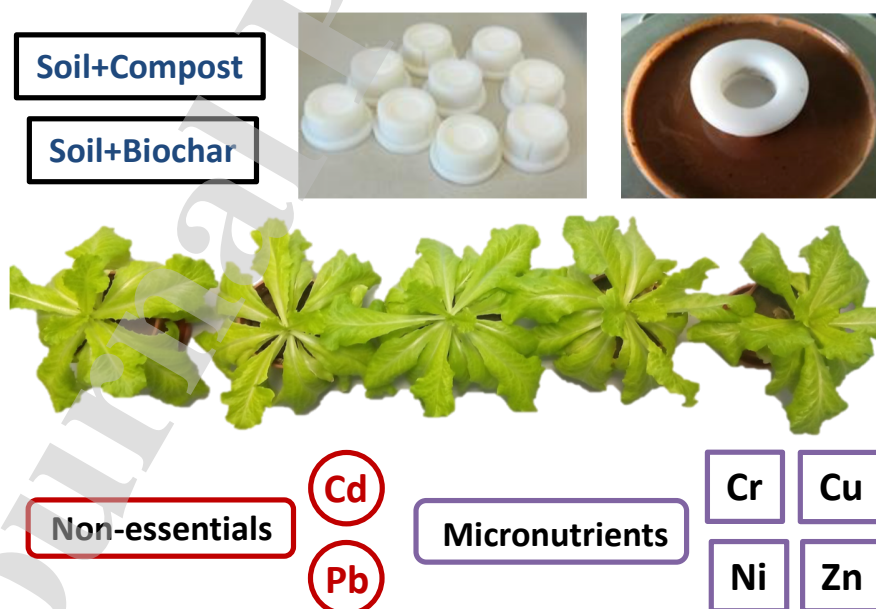
Graphical Abstract

Soil+Compost

Soil+Biochar

Non-essentials

Cd

Pb

Micronutrients

Cr Cu

Ni Zn

Effect of different amendments on trace metal bioavailability in agricultural soils and metal uptake on lettuce evaluated by Diffusive Gradient in Thin Films

Marta Turull[a], Clàudia Fontàs[b] and Sergi Díez[a,*]

[a]Environmental Chemistry Department, Institute of Environmental Assessment and Water Research, IDÆA-CSIC, E-08034, Barcelona, Spain

[b]Department of Chemistry, University of Girona, C/Maria Aurèlia Capmany 69,17003 Girona, Spain

*Corresponding author: E-mail: sergi.diez@idaea.csic.es

Abstract

In this study, we have investigated the effect of two different organic amendments on trace metal transfer from soils to crops. Agricultural soils were mixed with (i) biochar (BC) at two levels (3% and 6%, w/w), and (ii) compost at one level (30%, w/w). Lettuce (*Lactuca sativa* L.) was planted, and at the end of growth, trace metals (Cd, Cr, Cu, Ni, Pb and Zn) were analysed in both plants and soil. To evaluate the bioavailability and mobility of labile trace metals, different methodologies were used, such as the diffusive gradient in thin film (DGT) technique, BCR sequential extraction and pore water extraction. It has been observed that the lettuces grown in soils with added compost, the amounts of Cd, Cr, Cu and Pb was 1.7, 2.4, 1.3 and 3.7 times lower, respectively, than those found in lettuces planted in soils with no amendment. Moreover, the addition of 3% of BC reduced the uptake of Cr (24%) whereas a 6% of BC reduced Cr (55%) and Pb (50%) levels. Using the DGT technique in soils with different organic matter (OM) content, labile fractions of Cr, Cu and Pb were successfully correlated ($p<0.05$) with lettuce total metal concentrations. In conclusion, BCR sequential extraction and DGT method have shown similar efficiency in front other methodologies, although it seems that DGT-methodology is faster and more cost-effective method than sequential extraction for predicting metal uptake by plants.

Keywords: organic amendments; agricultural soil; Diffusive gradient in thin film; DGT; Chelex-100

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1. Introduction

Metal pollution has become a worldwide environmental problem, primarily by anthropogenic sources (i.e. industrial activities, mining, traffic emissions and agricultural activities) (Huang et al., 2018). The need to control the amount of metal content in agricultural soils is important due to the health risk impact to humans attributed to metal uptake by crops. In agricultural soils, trace metals are analysed to evaluate their agricultural ecological risk (Mungai et al., 2016). Total metal concentration is the value commonly used to regulate the trace metal contamination in soil. Nevertheless, it does not represent the bioavailable fraction, which is the fraction absorbed by plants and incorporated into the food chain (Venegas et al., 2016).

Soil metal distribution and mobility depends not only on the chemical composition, it also depends on the physicochemical properties of the soil such as the organic matter (OM), pH and exchangeable capacity (Memoli et al., 2018). Stabilization of metals in soil by the addition of organic amendments (i.e. compost, peat, manures or biosolids) has been commonly used for their immobilization due to the low cost and facility of application in agricultural soils (Gul et al., 2015; Venegas et al., 2016). These amendments cannot significantly decrease the concentration of total trace metals in soil but can reduce their bioavailability for plants. Some studies have reported the effectiveness of compost in reducing trace metal uptake by plants, such as Cd (Kim et al., 2017), Hg (Restrepo-Sánchez et al., 2015), Pb (Tang et al., 2015) and Zn (Pichtel and Bradway, 2008), whereas other studies have shown the opposite (Egene et al., 2018; Riaz et al., 2018a). Differences depend on the characteristics of the soils, and especially on the quantity and chemical characterization of OM of the amendment (Welikala et al., 2018). Nowadays, biochar, which is a carbon-rich component produced by the pyrolysis of an organic material in the absence of oxygen, is used in soil amendment due to its ability to complex metals besides for their abilities to increase soil fertilization, water-holding capacity and microbial activity, among

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other benefits (Abbas et al., 2018; Lahori et al., 2017). Recent studies have reported biochar to effectively retain different trace metals such as Pb (Ahmad et al., 2012), Cd (Abbas et al., 2018), Cr and Zn (Trakal et al., 2017), thereby reducing their bioavailability.

Currently, different methods have been used to study the bioavailability of trace metals. The most employed methods to predict metal uptake by plants are soil solution extraction, which is controlled basically by thermodynamic/kinetic parameters (Kabata-Pendias, 2004), and the BCR sequential extraction method related to the extractable fraction available for plants (Zhang et al., 2017). However, many processes affect the supply of solutes to plants (diffusional and convective transport to the root) and the biological effectiveness of free ion activity are still not clear in predicting root uptake by these methods (Dai et al., 2018; Zhang et al., 2001).

The diffusive gradient in thin films (DGT) technique was demonstrated to assess metal bioavailability in natural waters (Fernández-Gomez et al., 2011, 2012, 2014; Turull et al., 2018), sediments (Wu et al., 2018) and soils (Harper et al., 1998). This technique was developed by Davison and Zhang for the in situ determination of kinetically labile metal species in aquatic systems (Davison and Zhang, 1994). More recently, it has been demonstrated that DGT is a good predictor of metal availability to plants such as Cu (Zhang et al., 2001), Cd and Pb (Ridoskova et al., 2017), Ni (Gao et al., 2018), and Zn (Tandy et al., 2011). Recently, it has been demonstrated that DGT is useful to explain the mobility some trace metals (Pb, Cd and As) in soils amended with biochar (Tang et al., 2019). However, its ultimate potential uptake by plants was not estimated.

In the present study, the effect of the addition of two different amendments (biochar and compost) on the bioavailability, mobility and uptake of Cd, Cr, Cu, Ni, Pb and Zn by lettuces (*Lactuca sativa*) was evaluated. Considering the variation of OM in the soil samples by the addition of the amendments, DGT-labile metal concentrations in soil were determined and

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correlated with metal uptake. Besides that, a three-step sequential extraction for soil, total metal concentration in soil and the analysis of soil solution were also performed to determine which technique is better for predicting plant uptake.

2. Materials and methods

2.1 Site description and experimental design

The agricultural soil samples used in this study were taken from the peri-urban area of Barcelona (Spain) (labelled as AS), and from an agricultural ecological field near Barcelona (labelled as C), selected for the purposes of comparison in a pristine area closed to the selected agricultural area. Both soil sampling sites were sampled to represent an average from a mixture of 5 × 10 sub-samples taken from an area of 100 m^2 with a depth soil horizon of 0–25 cm. To characterize the soil and separate all the large components of the samples (stones, waste, plants…), a fraction of soil with particle size below 2 mm was obtained by sieving the air-dried sample (Kovaríková et al., 2007).

The biochar (BC) amendment was produced by Bodegas Torres (Vilafranca del Penedès, Barcelona, Spain) from vineyards by pyrolysis at 400–600 °C. After that, biochar was crushed and sieved to obtain particle sizes between 0.12 and 2 mm (Hurtado et al., 2017). For biochar, the N_2-B.E.T SA was 387 m^2 g^{-1}, whereas pore volume and pore size were 0.0679 cm^3 g^{-1} and 3.26 nm, respectively. Ultimate analysis of BC resulted in C, H, N and S contents of 62.8, 1.1, 0.3 and less than 0.1%, respectively, and a molar H/C ratio of 0.21. Biochar conductivity was 2158 ± 46 µS cm^{-1} and specific weight was 1.72 ± 0.05, whereas a 1:10 solid:solution ratio with deionized water had a pH of 9.82 ± 0.04.

The compost amendment used in this project was manufactured and packaged by NUBA floris (Borstel, Germany), obtained mainly from yellow peat from *Sphagnum* and OM of

vegetal origin, also sieved to obtain particles smaller than 2 mm. The compost density was 0.42 g cm^{-3}, OM was 39% and pH ranged from 6.4 to 6.9.

After preparing the different soils and the amendments, five sets with different soil composition were chosen for the experiment: (1) a control pot with the soil recollected from the ecological soil as the reference site (C); (2) a peri-urban soil from near Barcelona (AS); (3) AS with 3% (w/w) biochar (BC3); (4) AS with 6% (w/w) biochar (BC6); and (5) AS with 30% (w/w) compost (CP30). The rates of the different amendments were chosen according to previous studies (Fuchs, 2002; Hurtado et al., 2017). The different amendments were incorporated into the soil 72 h before cultivation of the seedlings (Trupiano et al., 2017).

2.2. Plant growth

Lettuce seedlings (*Lactuca sativa* L.) were planted in 2.5 L cylindrical pots (17 cm diameter and 15.5 cm depth) filled with 2 kg of air-dried soil. The lettuces were grown for 48 days. One lettuce was planted per pot, making a total of five replicates per set. During plant growth, the temperature was controlled at ambient temperature between 18 and 23 °C, and the amount of light was controlled by a fluorescent light with an electronic timer (16 h of light and 8 h of night) (Smolinska, 2015). The pots were rotated randomly every week in a different position to avoid the space influence. Moreover, plants were irrigated manually every day with between 50 and 75 mL per pot (depending on the humidity) of a Tarssan nutritive solution (Green Apple, 2019).

2.3. Lettuce analysis

When lettuces reached commercial size, leaves and roots were harvested separately and washed out with deionized water to remove the soil from the samples. Fresh weight of root, stem and leaf for each plant was assessed. Leaf samples were dried in an oven and stored at 4 °C for further analysis. EPA method 200.2 (Martin et al., 1994) was selected for analysis

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of the leaf samples. Briefly, 4 mL of (1+1) HNO_3 and 10 mL of (1+4) HCl were added to a 1.0 g sieved (5-mesh) and dried sample and heated at 95 °C for 30 min in an EvapoClean system. An aliquot of 3–4 mL was evaporated during the process. The sample was transferred to a 100 mL volumetric flash and diluted with Milli-Q water. Prior to analysis, it was centrifuged at 3000 rpm for 20 min and filtrated. All analyses were conducted in triplicate per set. Moreover, determination of chlorophyll every week and measurements of length, width and roots at the end of the experiment were done, as the results are shown in figures S1 and S2 of supplementary data, respectively.

2.4. Soil treatment and DGT measurement

A mixture of soil from all the pots of every set was dried in an oven and stored at 4 °C until DGT deployment and analysis. Thirty grams of air-dried soil was placed in a Petri dish (Zhang et al., 1998), saturated with Milli-Q water according to ISO 11464 (ISO, 2006) and left to equilibrate for 48 h. Then, DGT devices (DGT Research Ltd., UK) based on Chelex-100 as binding layer were placed on each soil sample and left for 24 h (Zhang et al., 2001). Then, the devices were collected and rinsed with deionized water to clean the soil particles. The resin gels were removed from the DGT devices and immersed in 1.5 mL of 1 M HNO_3 for at least 24 h and stored in a refrigerator at 4 °C until analysis.

After DGT deployment, soil solution was centrifuged for 20 min at 3000 rpm at room temperature. Then, the solutions were filtered with a 0.45 μm polysulfone filter, acidified with 1% (v/v) HNO_3 and stored at 4 °C until analysis. Triplicates of blanks were analysed to check the quality control of the analysis.

The average labile metal mass in the resin was calculated using Equation 1.

$$M = \frac{C_a(V_e + V_g)}{f_e} \qquad (1)$$

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where C_a is the concentration of analytes in the eluent (ng mL^{-1}), V_e is the volume of the eluent (mL), V_g is the volume of the binding gel (typically 0.15 mL) and f_e is the elution efficiency for the analyte (commonly used 0.8 for metals).

Based on the Fick's law of diffusion, the concentration in DGT (C_{DGT}) was calculated as follows:

$$C_{DGT} = \frac{M\Delta g}{DAt} \qquad (2)$$

where M is the mass of the analyte (ng) accumulated in the resin, D is the diffusion coefficient (cm^2 s^{-1}), t is the deployment time (s), Δg is the thickness and A is the exposure area (3.14 cm^2) (Zhang et al., 1998).

2.5. Prediction of plant uptake by DGT

The effective concentration (C_E) represents the hypothetical concentration that should be needed to accumulate the measured amount of each element on DGT resin if there was only diffusional supply (Wu et al., 2018; Zhang et al., 2001) and it can be calculated from Equation 3:

$$C_E = \frac{C_{DGT}}{R_{diff}} \qquad (3)$$

where R_{diff} represents the ratio of the mean interfacial concentration determined using a 2D-DIFS modelling program (Harper et al., 1998; Sochaczewski et al., 2007) to simulate the deployment in a diffusion only scenario. For 2D-DIFS program, value of T_c was used as the diffusive case (700 s) (Sochaczewski et al., 2007) and particle concentration (Pc) and soil porosity (ϕ_s) were calculated as follow:

$$Pc = \frac{m}{v} \qquad (4)$$

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$$\Phi_s = \frac{dp}{(Pc+dp)} \qquad (5)$$

$$D_s = \frac{D_0}{1-\ln\Phi_s^2} \qquad (6)$$

where m is the total mass of the particles soil, V is the volume of pore water, dp is the density of the soil particles (commonly assumed to be 2.65 g cm^{-3} in soil) and D_0 is the diffusion coefficient in water (Zhang et al., 2004).

2.6. Sequential extraction

A BCR three-step sequential extraction (SE) procedure (Guevara-Riba et al., 2004; Memoli et al., 2018) was used to analyse the chemical fractionation of every trace metal in the five different sets of the study. First of all, 1 g of dry soil sieved to < 200 μm was used in this process. To determine the extractable fraction (F1, acid-soluble), 40 mL of 0.1 M acetic acid was added to the soil sample and shaken for 16 h at 30 rpm at room temperature in an end-over-end shaker. After that, the soil solution was separated by centrifugation at 4500 rpm for 20 min, filtered with a 0.45 mm filter and stored at 4 °C until analysis. For the reducible fraction (F2, associated with Fe-Mn oxides), 40 mL of 0.5 M hydroxylamine hydrochloride, adjusted to pH 1.5 by adding 2 M HNO$_3$, was added to the residue from F1, and the treatment was the same as in the first step. In the case of the oxidizable fraction (F3, associated with OM content and sulfides), 10 mL of 8.8 M hydrogen peroxide was added to the residue from F2, digested for 1 h at room temperature and then for 1 h more at 85 °C, reducing the volume to <3 mL. Another aliquot of 10 mL H$_2$O$_2$ was added and digested for 1 h at 85 °C, reducing the volume to about 1 mL. Then, the amount was extracted with 50 mL of 1 M ammonium acetate (pH 2, adding HNO$_3$) and shaken for 16 h at 30 rpm at room temperature. Finally, for the residual fraction (F4, associated with primary and secondary well-crystallized minerals), 10 mL of HNO$_3$ concentrate and 10 mL of HClO$_4$ concentrate were added to the

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sample from F3 and digested for 16 h at 135 °C and then 16 h more at 135 °C to reduce the acids. To recuperate the sample, 3 mL of HNO_3 concentrate was added; 1 mL of every sample was diluted with Milli-Q to 25 mL and stored at 4 °C until analysis. After every step except the last one, a washing step is mandatory. The residue was washed with 20 mL of deionized water, shaken for 15 min and centrifuged, discarding the supernatant.

F1 and F2 could be considered bioavailable fractions for living beings, F3 the fraction which was potentially bioavailable and could be absorbed by plants in a strong acid medium state, and F4 not bioavailable.

2.7. Analytical methods

(1) Soil pH and conductivity were determined with deionized water at a 1:2 solid:liquid ratio using a previously calibrated Crison GLP22 pH meter and a previously calibrated Hach CDC401, respectively (Gramlich et al., 2018).

(2) The OM was analysed as the percentage loss of ignition using 2.0 g of soil sample from different sets in an oven (Carbolite CWF 1300) at 550 °C for 1 h after initial drying at 90–100 °C for 1 h (Dean, 1974).

(3) The concentration of nitrates in the soil was analysed spectrophotometrically as nitrate nitrogen (NO3-N) using a Hach Lange DR 3900. For the extraction, 50 mL of KCl was added to a 5 g soil sample and shaken for 1 h (Mulvaney et al., 2016).

(4) Chlorophyll content in lettuce was measured with a chlorophyll meter (CCM200Plus, Opti-Sciences, Hudson, NH, USA) from the absorbance of three leaves from each lettuce. A calibration curve to relate the chlorophyll content to the absorbance previously measured with a chlorophyll content meter was obtained. Then, samples of leaves were extracted with 5 mL of N,N-dimethylformamide and kept in the dark at 4 °C for 48 h. These extracts were

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measured by spectrophotometric determination (at 647 and 664.5 nm), and total chlorophyll was calculated using Inskeep's and Bloom's coefficients (Inskeep and Bloom, 1985; Porra, 2002).

(5) The concentration of trace metals in the soil, soil solutions, SE and lettuce biomass were analysed on an inductively coupled plasma mass spectrometer (Thermo Scientific, XSeries 2 ICP-MS). Standard reference material from spinach leaves (SRM 1570a, National Institute of Standards and Technology, Gaithersburg) and blanks were analysed to check the quality control of the analysis.

2.8. Statistical analysis

Results of the study were reported as the mean ± deviation (SD). Analysis of variance (ANOVA) was used to evaluate the results with IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL, USA) for parametric values and Mann-Whitney for non-parametric values. Pearson's linear correlation was used to establish the relationship between the variables (r). Statistical significance was declared when p value was ≤ 0.05.

3. Results and discussion

3.1. Trace metals in soil

Table 1 shows the physicochemical properties and total metal concentration for each set of experiments as well as the different amendments. The mean concentration of trace metals in the agricultural soils decreased as follows: Zn > Pb > Cu > Cr > Ni > Cd, with values higher than those found in the crustal earth, except for Cr. In addition, concentrations of Cu, Pb and Zn were greater than the regulated values (BOE, 2009). Values of trace metals in biochar and compost did not exceed the maximum levels from regulation RD 865/2010 for application in agricultural soil (BOE, 2010).

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3.1.1. Trace metal mobility and bioavailability

The SE procedure was used to analyse the chemical fractionation of every trace metal. Results for the speciation of trace metals are shown in Fig. 1. In the control soil (C), trace metals are mostly in non-mobile fractions because most of them are bound to silicates and primary minerals. In the case of more polluted sites, these metals are highly mobile and also bound to other phases (Rauret, 1998). The percentage of bioavailable trace metals in C was lower than in AS, which could be an indicative of past anthropogenic pollution.

For Cd, the largest fractions found in SE were the exchangeable (24–47%) and reducible fractions (45–66%). Concentrations from F3 and F4 were below the detection limit in all sets. Significant differences ($p<0.01$) were found in F1 and F2 for the different treatments, where CP30 was the only set for which F1 was reduced and F2 was increased. The largest fraction for Cr was F4 (67–90%), which means that it may have originated from the soil parent material (Yutong et al., 2016). No significant differences ($p>0.05$) were found in F1 between AS and the different treatments, while F2 was significantly reduced ($p<0.01$) in CP30. A similar tendency was observed for Cu and Ni since both trace metals were retained in the residual fraction (35–57% for Cu and 58–64% for Ni), and the exchangeable fraction was very low (1–3% for Cu and 1–5% for Ni). Besides that, on the addition of compost, a great increase in F3 was observed (47% for Cu and 29% for Ni) in comparison with AS. In addition of the different amendments, statistical differences ($p<0.01$) for Cu were found for BC6 and CP30 in bioavailable forms. In the same way, Ni was significantly reduced ($p<0.05$) in CP30 but no differences were observed in BC3 or BC6 in comparison with AS. The largest part observed for Pb was the reducible fraction (67–84%). Fe and Mn hydrous oxides play an important role in Pb mobility because of their facility to be its scavengers in soil (Lu et al., 2018). A significant reduction of Pb percentage in F1 for BC3 and CP30 was observed in comparison with AS. For F2, only CP30 was significantly reduced ($p<0.05$). Most of the Zn

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concentration in soil was found in the reducible (44–52%) and residual fractions (31–41%), and only a small part was found in F1 (5–8%) and F3 (7–10%). A significant increase (p<0.01) was found in F1 for BC6 and CP30 but no significant differences were found in the other fractions.

Therefore, it was demonstrated that, in comparison with AS, the addition of compost could reduce significantly (p<0.05) the bioavailability (F1+F2) of the following trace metals: Cd (20%), Cr (20%), Cu (67%), Pb (27%) and Ni (20%). It should be noticed that Zn is the only one for which bioavailability increased (7%). Zn is very sensitive to pH changes and the slightly decrease of pH in CP30 could explain the increase of Zn bioavailable in soil (Doelsch et al., 2010). On the other hand, biochar significantly reduced (p<0.05) the bioavailability of Cd in comparison with AS. Also, bioavailability was reduced for Ni (5% for BC3 and 6% for BC6) and Zn (3% for BC3), even though not significantly (p>0.05). On the contrary, a slight increase in comparison with AS was found in BC3 for Cu (4.5%) and Pb (26%), whereas it was in BC6 for Cu (14%) Zn (7%).

A Pearson's correlation analysis was applied to study the relationship between the fractionation in soil for each trace metal and the soil properties (Table S1). In the bioavailable fractions, OM and pH were the soil properties which affected most directly the distribution of trace metals. In F3, all metals were positively and significantly correlated (p<0.05) with OM, although Cd was negatively correlated (−0.790, p<0.01), indicating that the amendments could facilitate the transformation from the bioavailable Cd to immobilized fractions (Liu et al., 2020)

3.2. Metal uptake by lettuce

Trace metal concentrations in lettuces are represented in Table 2.

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As it can be observed, significant differences for Cd were found by the addition of 3% of biochar in comparison with AS due to its porosity, functional groups and high pH (Amirahmadi et al., 2020). Besides that, adding compost to the soil decreased Cd uptake by lettuce (89%), although no significant differences were observed. Cd concentration were compliant with the legislation. For Cr and Pb, the uptake by lettuce in BC6 and CP30 sets was significantly reduced ($p<0.05$). These results showed the effectiveness of the different amendments studied to reduce both trace metals mobility because of its increasing of EC and DOC in the soil. In the case of Cu, the addition of compost reduces uptake by lettuces due to the high affinity of Cu for OM that retain significant concentrations of Cu in the soil (Takamatsu, 1983). Ni was the only studied trace metal for which no significant effects were observed for any of the fertilizer sets or even between C and AS. Adding biochar to the soil increased Zn uptake by lettuces. Specifically, the addition of 6% of biochar significantly increased ($p<0.05$) the concentration of Zn in leaves in comparison with AS, possibly due to the Zn concentration from the addition of biochar (the amount of Zn in BC6 is higher than the other pots) (Abbas et al., 2017), whereas no significant increase was detected when adding 3% of biochar. These results match with previous studies. On the contrary, by the addition of compost, no significant reduction of uptake by lettuces was found when adding 3% of biochar or 30% of compost.

3.3. Bioavailability in soil

3.3.1. Concentration of trace metals in pore water

Values of soil pore water trace metals concentration are summarized in Table 3. Metal concentrations were above the limit of detection for all trace metals, except for Cd. As it can be seen, the addition of biochar increased the concentration of Cr, Cu, Pb and Zn in pore water, and decreased it for Ni. Our results match those from previous research (Moreno-Jiménez et al., 2016; Trakal et al., 2017). On the other hand, a significant ($p<0.05$) decrease

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was observed for Pb in pore water and an increase of Zn concentration by the addition of compost, as found in previous studies (Moreno-Jiménez et al., 2016; Riaz et al., 2018b). Moreover, significant correlations (p<0.05) between the concentration of trace metals in pore water and their total concentration in lettuce were found for Cu (r = 0.699, p<0.01), Pb (r = 0.603, p<0.05) and Zn (r = 0.555, p<0.05).

3.3.2. DGT measurements

Comparison of different metal concentrations obtained by DGT measurements from the different studied soils are summarized in Table 4. No data were obtained for Cd since no metal concentration was detected in the DGT devices.

Values of C_E for Cu and Ni were significantly (p<0.05) reduced in BC6 and CP30 in comparison with the non-amended soil. The addition of compost and biochar had no significant effects on C_E for Cr, Pb and Zn although mean values were lower in comparison to AS.

The R value is the ratio calculated by C_{DGT} divided by C_{soln} and is commonly used to determine the capability of metal resupply released from the solid phase to soil solution, fundamentally the desorption rates from solid to solution (Degryse et al., 2009; Yao et al., 2017). In all trace metals, R was < 0.3, indicating a low resupply from the solid phase caused by slow desorption kinetics in the soil (Egene et al., 2018). Values of R for all trace metals were reduced by the addition of 6% of biochar, while only Cu and Zn levels were reduced by the addition of compost.

K_{dl} can be calculated dividing the concentration in the pore water (C_{soln}) by the initial sorbed concentration in soil (C_s) (Lehto, 2016). As it can be seen in Table S2, in the case of Zn, K_{dl} slightly increase by the addition of biochar and slightly decrease adding compost. On the other hand, for the other trace metals, K_{dl} greatly increased by the addition of compost most

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likely due to the amount of OM added to the soil, which had a direct impact in the reduction of trace metals in the soil solution.

3.3.3 Comparison of DGT technique to other methods

To determine which method is better to evaluate the metal uptake in soils with different amount of OM, values of trace metals analysed in DGT (C_{DGT}), C_E values, soil solution concentration (C_{soln}), bioavailable fraction from the SE and total metal concentration in soil were correlated with the trace metal concentration found in lettuce leaves, as it is shown in Table 5.

Generally, C_{DGT} and C_E are very strongly correlated and some studies which correlate both values with the plant uptake did not find any benefit in calculating C_E in the study (Degryse and Smolders, 2017). However, results from our study show a significant correlation between the C_E and uptake by lettuce for Cr ($r = 0.528$, $p<0.05$), Cu ($r = 0.836$, $p<0.01$) and Pb ($r = 0.783$, $p<0.01$), in front of the significant correlation between the C_{DGT} and lettuces for Cu ($r = 0.793$, $p<0.01$) and Pb ($r = 0.786$, $p<0.01$). In contrast with other studies (Agbenin and Welp, 2012; Cornu et al., 2016), Cu and Pb were the trace metals for which a significant correlation ($p<0.01$) was found between C_E and lettuces. To the best of our knowledge, this is the first study that correlates the calculated C_E for Cr and its total content in lettuce. Besides, a significant correlation was found between C_{soln} and metal in leaves for Cu ($r = 0.827$, $p<0.01$) and Cr ($r = 0.645$, $p<0.01$), but not for Pb. These results match those of Zhao et al. (Zhao et al., 2006) for Cu and Agbenin et al. (Agbenin and Welp, 2012) for Pb.

In the present study, no correlation at all were found for Ni, in contrast to some studies (Luo et al., 2014; Zhao et al., 2018), whereas a significant correlation was found with the total concentration in soil ($r = 0.641$, $p<0.05$). Similar to a previous research (Gao et al., 2018), the concentrations measured by DGT devices were very low (range of 0.13–0.35 µg L^{-1}), as

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were R values, the lowest for all the trace metals. It is possible that in the case of Ni, diffusion

is limited at low concentrations (Luo et al., 2014).

Finally, no significant correlation was found between C_E of Zn and lettuces, although some

other studies demonstrated the effectiveness of DGT devices applying Chelex-100 (Cornu

and Denaix, 2006). On the contrary, previous works (Gramlich et al., 2018; Koster et al.,

2005) demonstrated that the DGT method is not useful for predicting Zn uptake by lettuce,

lupine, grass and beans. In our study, a significant correlation was found with the total soil

concentration (r = 0.848, p<0.01) and the bioavailable fraction (r = 0.738, p<0.01),

suggesting that other factors in soil not considered by DGT may dominate Zn uptake by

lettuces, such as the capacity of the solid phase or its desorption kinetics of Zn (Ernstberger

et al., 2005).

4. Conclusions

The mean concentration of trace metals in soils with amendments decreased as follows:

Zn > Pb > Cu > Cr > Ni > Cd. Values for Cu, Pb and Zn levels were above the acceptable

levels as established by regulatory organizations. It has been demonstrated that the

addition of compost to soil can significantly reduce metal uptake by lettuce and, indeed,

the bioavailability of trace metals, except for Ni and Zn, which increased but not

significantly. Similarly, biochar significantly reduced the bioavailability of metals except for

Cu, Ni and Zn only by the addition of 6% of biochar. These values reflect the effectivity of

the addition of OM in agricultural soils to retain trace metals, reducing their bioavailability

for plants. For Cr and Pb, it has been showed that even adding a much lower percentage

of biochar than compost, the metal retention of both metals was similar in comparison with

AS.

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Comparing the different methods to correlate the metal uptake by lettuce with different OM by the addition of two different amendments, our results indicate that DGT measurements and the sequential extraction are more predictive in front of the pore water extraction and total metal concentration in soil. The bioavailable fraction from SE was significantly correlated with Cr, Cu and Zn, whereas DGT-methodology was significantly correlated with Cr, Cu and Pb. In the same way, it should be highlighted that, in comparison with the sequential extraction, DGT is a more practical, fast and cost-effective method, becoming the best choice for metal bioavailability studies.

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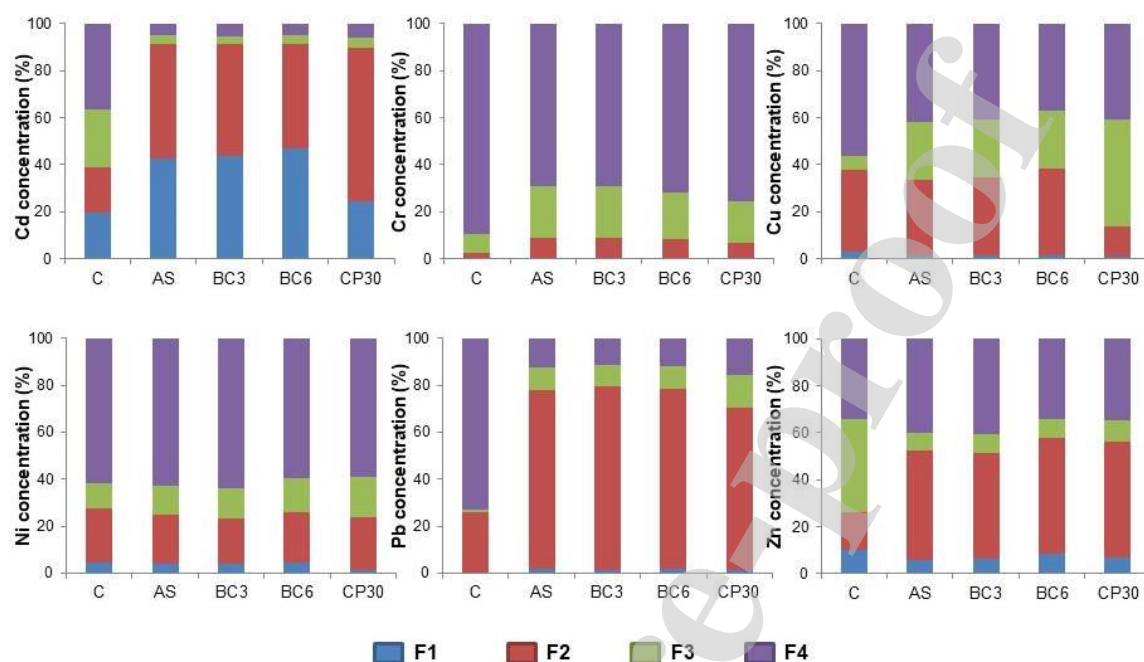
Figure 1. Speciation of trace metals in soil. F1: exchangeable fraction; F2: reducible fraction; F3: organic-bound; F4: residual; C: control; AS: agricultural soil; BC3: AS + biochar (3%); BC6: AS + biochar (6%); CP30: AS + compost (30%).

Table 1. Characterization of the different sets of the experiment, biochar and compost. Legislation of the organic amendments and soil trace metal were also reported. C: control; AS: agricultural soil; BC3: AS + biochar (3%); BC6: AS + biochar (6%); CP30: AS + compost (30%); S: for soil; A: for amendments

	C	AS	BC3	BC6	CP30	Biochar	Compost	Crustal Earth[a]	Legislation
OM (%)	2.72	6.52	9.19	11.34	12.90	-	-	-	-
pH	7.15	7.29	7.38	7.48	7.19	-	-	-	-
EC (mS)	1.14	1.73	1.74	1.57	5.23	-	-	-	-
Nitrates (mg kg^{-1})	9.15	9.16	9.66	9.07	11.40	-	-	-	-
Cd (mg kg^{-1})	0.08	0.58	0.55	0.56	0.47	nd	nd	0.1	2.5 (S)[b] 0.7 (A)[c]
Cr (mg kg^{-1})	14.32	84.75	89.03	89.32	88.00	0.17	6.22	100	400 (III) / 1 (VI) (S)[b] 0.5 (VI) / 70 (total) (A)[c]
Cu (mg kg^{-1})	38.70	108.74	110.52	108.82	86.61	22.52	26.68	55	90 (S)[b] 70 (A)[c]
Ni (mg kg^{-1})	15.42	34.50	34.97	32.45	23.80	nv	15.32	20	45 (S)[b] 25 (A)[c]
Pb (mg kg^{-1})	18.85	116.67	141.26	110.85	90.54	1.11	14.22	15	60 (S)[b] 45 (A)[c]
Zn (mg kg^{-1})	106.60	292.112	296.58	326.166	269.64	26.93	154.26	70	170 (S)[b] 200 (A)[c]

[a]The crustal Earth values were taken from (Kabata-Pendias, 2011); [b]NGR values of trace metals in soil from (BOE, 2009); [c]RD 865/2010 ; nd: non detected

Table 2. Minimum and maximum (in parenthesis) and median concentration (mg kg^{-1} fresh weight) of trace metals in lettuce from different sample sets. C: control; AS: agricultural soil; BC3: AS + biochar (3%); BC6: AS + biochar (6%); CP30: AS + compost (30%).

	C	AS	BC3	BC6	CP30	MVL[1]
Cd	(0.005-0.007) 0.006[a]	(0.050-0.079) 0.067[bd]	(0.091-0.123) 0.102[c]	(0.090-0.102) 0.096[bc]	(0.037-0.061) 0.047[d]	0.2
Cr	(0.018-0.026) 0.021[a]	(0.064-0.081) 0.073[b]	(0.046-0.067) 0.059[bc]	(0.037-0.058) 0.047[cd]	(0.026-0.035) 0.031[ad]	-
Cu	(0.374-0.459) 0.415[a]	(0.475-0.573) 0.512[ab]	(0.583-0.616) 0.596[bc]	(0.605-0.780) 0.683[c]	(0.366-0.418) 0.399[a]	-
Ni	(0.338-0.436) 0.392[ab]	(0.222-0.269) 0.241[a]	(0.204-0.402) 0.294[ab]	(0.392-0.550) 0.468[b]	(0.283-0.457) 0.376[ab]	-
Pb	(0.005-0.024) 0.014[a]	(0.057-0.081) 0.073[b]	(0.081-0.092) 0.085[b]	(0.042-0.055) 0.049[c]	(0.016-0.026) 0.020[a]	0.3
Zn	(1.870-2.329) 2.119[a]	(2.669-3.237) 2.972[b]	(3.510-3.671) 3.587[bc]	(3.806-4.138) 4.012[c]	(3.332-4.081) 3.595[bc]	-

Different letters indicate a significant difference at $p < 0.05$ between treatments.

MVL[1]: Maximum value legislated (European Commission, 2006)

Table 3. Pore water metal concentration (μg L^{-1}). C: control; AS: agricultural soil; BC3: AS + biochar (3%); BC6: AS + biochar (6%); CP30: AS + compost (30%).

	C	AS	BC3	BC6	CP30
Cr	1.72±1.74	2.38±0.08	2.54±0.12	2.78±0.12	1.61±0.06
Cu	40.19±1.02	58.65±1.13	64.08±2.49	54.69±0.83	38.35±2.80
Ni	22.13±1.21	109.79±6.42	95.78±13.55	82.81±3.67	36.19±5.61
Pb	nd	1.45±0.46	2.91±0.82	3.14±0.89	nd
Zn	23.71±20.19	25.74±5.77	19.92±5.39	24.82±4.87	26.53±573

nd: non-detected

Table 4. Trace metal concentrations found in DGT devices (C_{DGT}), DGT-measured effective concentration (C_E) and the ratio (R) calculated by C_{DGT} and C_{soln}. Mean and standard deviation are determined. C: control; AS: agricultural soil; BC3: AS + biochar (3%); BC6: AS + biochar (6%); CP30: AS + compost (30%).

		C	AS	BC3	BC6	CP30
Cr (µg L^{-1})	**C_{DGT}**	0.15±0.05	0.11±0.02	0.15±0.05	0.14±0.05	0.09±0.00
	C_E	0.56±0.02	0.60±0.09	0.68±0.23	0.62±0.21	0.43±0.02
	R	0.217	0.045	0.058	0.049	0.058
Cu (µg L^{-1})	**C_{DGT}**	1.78±0.17	2.78±0.08	2.57±0.29	1.79±0.48	1.66±0.24
	C_E	10.03±0.92	15.5±0.4	14.3±1.7	8.46±0.26	8.62±0.86
	R	0.044	0.047	0.040	0.033	0.043
Ni (µg L^{-1})	**C_{DGT}**	0.10±0.02	0.35±0.02	0.34±0.06	0.17±0.05	0.15±0.05
	C_E	0.69±0.09	1.80±0.26	1.78±0.32	0.91±0.30	0.81±0.28
	R	0.006	0.003	0.004	0.002	0.004
Pb (µg L^{-1})	**C_{DGT}**	nd	0.12±0.03	0.12±0.03	0.10±0.01	0.09±0.01
	C_E	-	0.82±0.19	0.84±0.17	0.70±0.09	0.64±0.04
	R	-	0.083	0.047	0.035	0.178
Zn (µg L^{-1})	**C_{DGT}**	2.22±0.56	4.52±1.79	3.66±0.19	2.75±0.23	2.81±1.26
	C_E	13.2±2.6	24.5±9.6	19.6±1.0	14.8±1.2	15.2±6.9
	R	0.144	0.182	0.194	0.113	0.102

nd: non-detected

Table 5. Simple linear correlations (r) between trace metal concentrations in lettuces in dry weight (dw) and trace metal concentration from DGT (C_{DGT}), C_E values, total trace metal content in pore water (C_{soln}), in soil and percentage of bioavailability (F1+F2) from SE.

Lettuces (dw)	C_{DGT}		C_E		C_{soln}		Bioavailable fraction (%)		Total metal concentration in soil	
	r		r		r		r		r	
Cr	0.456	NSS	0.528	*	0.645	**	0.579	*	0.355	NSS
Cu	0.793	**	0.836	**	0.827	**	0.721	**	0.319	NSS
Ni	0.086	NSS	0.086	NSS	0.061	NSS	0.290	NSS	-0.037	NSS
Pb	0.786	**	0.783	**	0.361	NSS	0.482	NSS	0.641	*
Zn	0.337	NSS	0.221	NSS	0.477	NSS	0.738	**	0.848	**

Statistically significant relationship $(p < 0.05)$* $(p < 0.01)$**
NSS: no statistically significant relationship

Highlights:

- We tested the effect of biochar and compost applications on lettuce uptake of metals
- Uptake of Cd, Cr, Cu and Pb was 2 to 4 times lower with compost than in unamended soils
- Biochar produces a greater weight of leaves, roots and total biomass of lettuces
- Biochar also increases leaf length and enhances significantly leaf chlorophyll content
- The effective concentration of Cr, Cu and Pb was significant correlated with metals in lettuce

Marta Turull: Writing - Original Draft, Investigation, Formal analysis, Methodology. **Clàudia Fontàs**: Supervision, Writing - Review & Editing. **Sergi Díez:** Conceptualization, Supervision, Writing - Review & Editing

Conflict-of-Interest Statement

Manuscript Title:

Effect of different amendments on trace metal bioavailability in agricultural soils and metal uptake on plants evaluated by the Diffusive Gradient in Thin Films

☐ We declare no conflict of interest

☒ We declare the following potential conflict of interest:

Please check the following as appropriate:

    x All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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Author's name	Affiliation
Sergi Díez	IDAEA-CSIC
Marta Turull	IDAEA-CSIC
Clàudia Fontàs	Universitat de Girona