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Conventional and novel techniques for the determination of Hg uptake by lettuce in amended agricultural peri-urban soils

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Abstract

Peri-urban agriculture provides environmental benefits to the nearby urban areas. However, domestic and industrial infrastructures can be sources of pollution that can affect agricultural production. In this work, the Diffusive Gradient in Thin film (DGT) technique was used to assess the bioavailability of mercury (Hg) in organic-amended agricultural soils, and uptake by lettuce. Two different amendments were studied individually in three different sets using a wood-based biochar at two rates (3% and 6%, w/w), and compost at one rate (30% w/w). The effect of the amendments on Hg bioavailability, mobility and uptake was investigated by means of both DGT analyses and accumulation of Hg by lettuce. DGT manufactured in-house devices with polyacrylamide gel using both open and restricted diffusive layers (ODL and RDL, respectively) were used to determine organic and inorganic Hg labile species in soils, respectively. The Hg concentration in lettuce leaves and roots were analyzed and compared with DGT measurements to predict the uptake of Hg from the different organic-amended soils and the non-amended soils. Results show that the application of biochar reduces the bioavailability of Hg in soil and, in consequence, the Hg uptake by lettuce. Inorganic Hg species were predominant in all the different sets of the experiment (62-97%), although the addition of the different amendments reduced the free ionic species in soil.

Keywords: Diffusive Gradient in Thin Film; DGT; restricted gel; agricultural soils; biochar; compost.

1. Introduction

Mercury (Hg) pollution is an increasing environmental problem worldwide because of its toxicity, persistence in the environment, and bioaccumulation in biota. The world-soil average of Hg is 0.07 mg kg^{-1} (Kabata-Pendias, 2011), but this concentration can be increased by anthropogenic sources, such as gold mining (Marrugo-Negrete et al., 2017), industry (Hang et al., 2017), and atmospheric deposition (de Lima et al., 2017). In addition, Hg can be introduced into agricultural sites from different amendments such as pesticides, fertilizers or poor quality urban compost (Cozzolino et al., 2016). The maximum level permissible in soils according to the EU Directive 86/278/EEC is 1-1.5 mg kg^{-1} (European Commission, 1986).

Different factors that influence the bioavailability of Hg in soil include soil pH, interaction with other metals, cation exchange capacity (CEC), organic matter (OM) content, and sulphur compounds (Hang et al., 2017). It is well known that Hg strongly interacts with OM (humic and fulvic acids) forming stable complexes (Janowska et al., 2017). One method for remediation of soil is through amendments that are directed towards retaining the metal in the soil, and reducing availability to plants. Many studies demonstrated the efficacy of both biochar (Gilmour et al., 2018; H. Li et al., 2017; O'Connor et al., 2018; T. Wang et al., 2018) and compost (Janowska et al., 2017; Restrepo-Sánchez et al., 2015; Smolinska, 2015) to change the chemistry and binding properties of Hg in soil, and, hence, decreasing the amount of Hg available to different crops. It has also been demonstrated that the addition of OM for phytoremediation may increase the Hg bioavailable to plants (Smolinska, 2015).

Understanding Hg bioavailability in agricultural soils is the key for determining plant uptake and implications for food safety. Mobility, bioavailability, and toxicity of Hg depends on its chemical form. Metal bioavailability in soil has been assessed by chemical extractions (Li et al., 2007), but it is well known these methods are insufficient for determining Hg impacts on plants. To discriminate the different compounds or how Hg is bound sequential extractions have been done in many studies (Issaro et al.

2009). However, these techniques did not account for the depletion at the root-soil and pore- solid interfaces (Muhammad et al., 2012). Given this, a better approach to study the bioavailability of metals and to mimic the processes in the rhizosphere can be accomplished by the use of the Diffusive Gradient in Thin Film (DGT) (Davison and Zhang, 1994). This technique has been used mainly in natural waters (Turull et al., 2017), in sediment (Wang et al., 2016) and soil (Hlodák et al., 2015) for the *in situ* determination of kinetically labile metal species (Fernández-Gómez et al., 2014). Moreover, certain studies have demonstrated their effectiveness to predict metal uptake by plants (S. Wang et al., 2018; Zhang et al., 2004). In recent research (Turull et al., 2019), we successfully used open (ODL) and restricted diffusive layers (RDL) to distinguish between inorganic and organic bioavailable Hg compounds in soil.

In this study, an agricultural soil was selected to evaluate the effectiveness of different amendments (biochar and compost) on the mobility of different Hg compounds. To this aim, homemade manufactured DGT samplers using both open and restricted diffusive layers were used to determine different bioavailable of Hg compounds. A sequential extraction procedure was also performed to compare the results with the DGT devices. Lastly, roots and leaves of lettuce were also analysed to correlate Hg concentrations measured by DGT.

2. Materials and methods

2.1 Soil, biochar and compost preparation

The soil sample used was taken from an agricultural site located in the peri-urban area of Barcelona (Spain). The sample was obtained from a mixture of 5 x 10 sub-samples taken from an area of 100 m^2 with a depth soil horizon of 0-25 cm. Air dried soil was sieved (<2 mm) to homogenize the sample and to remove large stones, roots, or

waste. Afterwards, the soil sample was mixed with a wood-based biochar at two rates and compost at one rate.

The compost used in this study was manufactured and packaged by NUBA floris (Borstel, Germany), and mainly consisted of from yellow peat derived from *Sphagnum* moss and was sieved to < 2 mm. Biochar was produced by Bodegas Torres (Vilafranca del Penedès, Barcelona, Spain) from vineyards by pyrolysis at 400-600ºC. The biochar was crushed and sieved to particle sizes between 0.12 and 2 mm (Hurtado Cervera, 2017). The electrical conductivity (EC), pH, and Hg concentration were 2.16±0.05 mS cm^{-1}, 9.82±0.04, and 29±11 ng g^{-1} for biochar and 7.36±0.24 mS cm^{-1}, 6.65±0.35, and 39±7 ng g^{-1} for the compost, respectivey.

After preparing the soil and the amendments, 4 sets of different soil samples were chosen for the experiment: (1) the selected agricultural soil (AS), (2) AS with 3% w/w of biochar (BC3), (3) AS with 6% w/w of biochar (BC6), and (4) AS with 30% w/w of compost (CP30). These application rates were established on the basis of previous research with compost (Fuchs, 2002) and biochar (Hurtado et al, 2017). The time of incorporation of the amendments in soil was 72h before planting the seedlings following the protocol from Trupiano et al. (2017).

All the properties of the soil with the different amendments used for the study, as well as Hg concentration, are shown in the Table 1.

2.2 Plant growth, sampling and analyses

Figure 1 shows the experimental design of this study. Briefly, lettuces (*Lactuca sativa* L. cv. Batavia) were planted in 2.5 L cylindrical pots (17 x 15.5 cm) filled with 2 kg of air-dried soil. One lettuce seedling was planted per pot, bringing a total of 5 replicates per set. During plant growth, the temperature and the amount of light was controlled (Smolinska, 2015), and lettuce plants were irrigated manually every day with Tarssan nutritive solution (50-75 mL per pot, depending on the humidity).

After 48 days of growth, when lettuce reached commercial size, the leaves and roots were harvested separately, and the fresh weight (fw) was determined along with the length of both. Then, leaves and roots were washed off with deionized water to remove any surface contamination, and were dried in an oven at 55ºC (R. Li et al., 2017).

Soil pH and conductivity were determined at the end of the experiment with deionized H_2O at a 1:2 solid: liquid ratio using a previous calibrated pH meter Crison GLP22 and a previous calibrated Hach CDC401, respectively (Gramlich et al., 2018).

The OM was analyzed as loss on ignition using 2.0 g of soil sample in an oven (Carbolite CWF 1300) at 550ºC for 1 h with a previous drying period at 90-100ºC for 1 h (Dean, 1974).

Concentration of nitrates in the soil solution was determined spectrophotometrically as nitrate nitrogen (NO_3-N) using a Hach Lange DR 1900. The extraction was carried out using 50 mL of KCl in a 5 g of soil sample and shaken for 1h (Mulvaney et al., 2016).

All the Hg analysis were performed using and Advanced Mercury Analyzer, model AMA-254. For details of the analysis and certified materials employed see the Supplementary Information.

2.3 Diffusive Gradient in Thin Films (DGT)

2.3.1 Hydrogels and binding layers preparation

The ODL hydrogels were prepared according to Clarisse and Hintelmann (2006) and RDL hydrogels were made as stated in Shiva et al. (2015) with modifications. Details of the preparation and DGT assembly can be found in the Supplementary Information.

2.3.2 Determination of Hg in soils by DGT

A Petri dish with 40 g of soil sample was used and maintained to 100% maximum water holding capacity (MWHC) with deionized water at 25ºC for 48 h, to ensure the equilibrium between fractions and soil (Yao et al., 2016). The water/soil mass phase

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ratio was 0.64 mL g^{-1}, 0.75 mL g^{-1}, 0.77 mL g^{-1} and 0.76 mL g^{-1} for AS, BC3, BC6 and CP30, respectively. Triplicates for every set and per different type of DGT (e.g. ODL and RDL) (n=24) were done.

One DGT device was pressed onto the surface of the soil slurries in one Petri dish for 24 h at 25ºC. To avoid t potential contamination, and to ensure a stable moisture content, the top of the Petri dish was used to cover the DGT device. After retrieval, the filter membrane was carefully washed with deionized water to remove any particles from the surface.

DGT technique follows Fick's First Law. Eq.1 was applied to calculate the concentration of the metal in the matrix (C) :

$$C = \frac{M\Delta g}{DAt} \quad (1)$$

where D is the diffusion coefficient of the Hg in the diffusive layer, M is the mass of Hg accumulated by the resin, Δg is the thickness of the diffusive layer, A is the exposure surface area, and t is the deployment time.

For the analysis of the pore water, after retrieving DGT samplers, soil slurries were centrifuged at 3000 rpm for 20 min followed by filtration through 0.45 µm filter. Prior to analyses, soil solutions were acidified with 1M HNO$_3$ and stored at 4ºC (Ridošková et al., 2017).

It should be noted that the DGT accumulates metal on the resin gel continuously that causes a depletion of the metal concentration in the soil solution (C$_{soln}$), because there is no resupply. Comparing the C$_{DGT}$ and C$_{soln}$ (Eq.2) provides a ratio (R) indicating the depletion of solution concentration at the device interface depending on the adsorption-desorption kinetics within the soil (Sochaczewski et al., 2007):

$$R = \frac{C_{DGT}}{C_{soln}} \quad 0 < R < 1 \quad (2)$$

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To interpret DGT measurements, C_{DGT} can be converted to an effective concentration (C_E) using Eq.3 (Zhang et al., 2004):

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

where the C_E is the metal concentration effectively available from the soil solution and the solid phase, and thus, the concentration found in the rhizosphere of the plants. R_{diff} is the ratio of the theoretical metal concentration in the diffusion only case (which means no resupply from the solid phase) and it is calculated by 2D DIFS model (DGT induced fluxes in sediments) (Sochaczewski et al., 2007). Input parameters used to calculate R_{diff} were the particle concentration (P_c), soil porosity (φ) and the diffusion coefficient in the soil (D_s) that can be calculated using Eqs. 4 to 6 (Zhang et al., 2004):

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

$$\varphi = \frac{d_p}{(P_c + d_p)} \qquad (5)$$

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

where m is the mass of the soil particles, v is the volume of the pore water, D_0 is the diffusion coefficient in water and d_p is the density of the soil particles (commonly assumed 2.65 g cm^{-3} for soils).

2.4 Sequential extraction

In this case, a five-step sequential extraction (SE) procedure (Bloom et al., 2003; Pinedo-Hernández et al., 2015) with some modification was used to develop an understanding of Hg compounds and binding in the soil. Water-soluble (F1), human stomach acid soluble fraction (F2), organo-complexed fraction (F3), elemental mercury

(F4), and residual fraction (F5) were investigated. Details of the SE can be found in the supplementary material.

2.5 Bioconcentration factor (BCF)

The bioaccumulation factor is the index of the capacity of the plant to accumulate Hg in comparison with its concentration in the soil. BCF is calculated as Korzeniowska & Stanislawska-Glubiak, 2015:

$$BCF = \frac{Hg \text{ in roots } (g \, kg^{-1})}{Hg \text{ in soil } (g \, kg^{-1})} \qquad (7)$$

2.6 Statistical Analysis

Analysis of Hg in the different soils, lettuce, roots, and DGT devices was performed as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to evaluate the results for parametric values and Kolmogorov-Smirnov for nonparametric values. Relationships between the parameters were performed using Pearson correlation. Statistical significance was declared when p value was equal to or <0.05. IBM SPSS statistics 25 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

3. Results and discussion

3.1. Mobility and bioavailability of Hg in soils

The concentration of Hg in biochar (29±11 ng g^{-1}) and compost (39±7 ng g^{-1}) added to AS did not exceed the legislation RD 865/2010 for application to agricultural soils (400 ng g^{-1}). The concentrations of Hg in the mobile (F1 and F2), semi-mobile (F3), and non-mobile (F4 and F5) fractions are plotted in Figure 1. The Hg analyzed directly from the soil sample in comparison with the Hg summed from the different fractions (90-97%) demonstrate the effectiveness of the SE procedure. The Hg concentration in the different fractions is ranged in the following order: F4 (78-83%) > F5 (14-16%) > F3 (1-

5%) > F1 (1-2%) > F2 (0-1%). Our findings on the compounds of Hg in soil match with farmland soils contaminated by a chemical plant in China (Zhao et al., 2018).

Hg compounds present in soil depends on many factors, such as physical and chemical soil properties, seasonal temperature, downstream transport of Hg from source locations, or the time since Hg deposition, among others (Gilli et al., 2018). These factors may drastically change Hg chemistry in soil and, thereby, modify its mobility, bioavailability, and toxicity. For example, a study executed in Russia (Gordeeva et al., 2017) demonstrated the difference between soils from the Hg electrolysis shop, where contamination was due to atmospheric deposition (F3 as the highest fraction), and soils from technogenic dumps (F4 and F5 as the highest part).

In this regard, the Hg aging process in soils should be considered, since a decrease of inorganic mercury and/or methylmercury extraction occurs over time (Ma et al., 2015). Soil organic content could play an important role in the Hg aging process, reducing the association rate with stronger binding sites.

Significant differences (p<0.05) were observed between AS and BC3, with a reduction in bioavailability of 60%. The SE showed a reduction in the mobile phases (i.e. F1, F2) and, on the contrary, the amount of Hg in F4 that is not mobile increased. In the case of BC6, the trend is similar, but the percentage in F2 increased with respect to AS (12%). However, no significant differences were obtained. In contrast, the bioavailability of Hg is significantly higher when compost is used as amendment in the soil. There was a 21% increase in F1, and a 3% reduction in F2 (16% more bioavailable than AS).

Results using biochar in the soil show that the pH slightly increased with an additional amount of biochar (Table 1). Moreover, previous studies have shown that biochar pH increased with increased pyrolysis temperature (Nguyen et al., 2010; Zhang et al., 2015; Yuan et al., 2011). As expected, according to the pyrolysis temperature of the used biochar (400-600 ºC), we found a neutral to alkaline biochar (H. Li et al., 2017; Zhang et al., 2015). On the other hand, when compost is added to the soil, pH is

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reduced due to its slightly acidic property. The concentration of H^+ added to the soil from the compost could explain the depletion of pH (do Carmo et al., 2016) that is related with the extremely high EC in comparison with the other sets. With the addition of compost, EC increased, because of the concentration of free ions and nutrients increased. Nitrate increased from 9.2 to 11.4 mg kg^{-1} (Table 1). Significant inverse correlation (R=-0.68, p<0.05) between pH and F1 was observed but, on the contrary, no significant correlation exists with F2. Looking at EC, significant correlation was found with F1 but not with F2 (Table 2).

Focusing on Hg bound to organic matter and sulfides (i.e. F3), significant differences from the control for all treatments were observed. Both BC amendments reduced Hg concentration in the organo-complexed fraction, and for CP30 this is the only fraction that was reduced. As can be seen in Table 2, the OM is highly and significantly (p>0.05) correlated only with F3. It is well known that the OM controls the biogeochemistry of Hg in soils, immobilizing Hg due to the high affinity of this element with the organic matter (Liu et al., 2007); however, dissolved OM in the pore water could be complexed with Hg in the soil and desorb it. The main reason is OM forms soluble metal-organic complexes, increasing Hg availability (Smolinska, 2015). Besides that, organic amendments are sometimes applied to improve the natural phytoextraction by hyperaccumulators, increasing the amount of Hg bioavailable. Moreover, Zhu et al. (2015) determined that, apart from the effect of organic fertilizers to reduce the phytoavailability of Hg in soil, when it is used in excess, the dissolvable fraction of humic acid could increase mercury desorption from the soil.

3.2 Accumulation of Hg from soil to lettuce

Results showed that Hg concentrations in the leaves of lettuce are not significantly different (p<0.05) between all the sets (Table 3). Several studies (Ericksen and Gustin, 2004; Frescholtz and Gustin, 2004; Leonard et al., 1998; Gustin et al., 2004) have shown that plants are a sink for atmospheric Hg. The Hg concentration in the leaves

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was same for all treatments (p>0.05). Because of this, Hg concentration in leaves are not useful for understanding the effect of Hg amendments to the soil, and Hg in roots were chosen to compare the effectiveness of the different amendments.

Roots have been shown to be an important role in the accumulation of Hg in plants, acting as a barrier to the incorporation of the metal, and affecting its translocation to aerial plant tissues (Shahid et al., 2017). In rice plants, the development of apoplastic barriers in the endodermis, the secretion of different organic acids to retain Hg in soil, and the effect of Fe to sequester the metal reduce its bioavailability (Wang et al., 2015). Therefore, the BCF for Hg in plants is low in comparison with other trace metals (Chang et al., 2014).

Results showed that Hg concentrations in the roots of lettuce are significantly different (p<0.05) between AS and all amendments (Table 3) No statistical differences were found between the amendments. Moreover, a significant inverse correlation exists between the Hg concentration found in roots and the addition of OM (R=-0.60, p<0.05) (data not shown).

Using biochar amendments, the accumulation of Hg in the roots was lower than the roots of lettuces growing in AS. In fact, the percentage of uptake with respect to AS is reduced by 35% and 37% in BC3 and BC6, respectively. Although BC3 had significant differences because of the low deviation between lettuce (i.e. 0.70) in the same set, the BCF in both cases has the same value (i.e. 0.017). These results are comparable to those from Shu et al. (2016) where the amount of MeHg in rice decreased between 49 and 92% adding biochar to Hg-contaminated soils. Furthermore, biochar combined with nitrate also reduced MeHg in plant due to nitrate inhibition of the activity of sulfate reducing bacteria that are responsible of the production of MeHg (Zhang et al., 2018). Apart from its effectiveness, it is important to note that biochar is low-cost compared to other technologies (Inyang et al., 2016), and would be a good choice for large surfaces.

When compost was used as amendment, results showed that Hg uptake by roots from CP30 was 29% lower than in AS; however, it is important to note that Hg concentration in CP30 was initially lower (10%) than AS. Despite these dissimilarities, no statistical significant differences (p>0.05) were found in BCF between non-amended soil and the soil amended with compost (0.020 vs 0.022). These results showed that the application of compost does not significantly reduce the concentration of Hg in the lettuce roots or reduce the BCF value of the plants. There is some controversy between studies claiming that the concentration in roots is reduced adding compost and others that confirm the ability to increase the Hg absorption in the plant. For example, Restrepo-Sánchez et al. (2015) showed reduction of Hg transfer to plants using Hg-doped compost in the agricultural soil. While, Zhu et al. (2015) demonstrated that the addition of organic fertilizer could decrease Hg phytoavailability and Hg methylation. and when an excess of organic fertilizer was added Hg desorption from soil increased. Cozzolino et al. (2016) investigated the combination of OM with the inoculation of arbuscular mycorrhizal fungi and showed its effectiveness to reduce Hg uptake for lettuce plants. In contrast, Muddarisna and Siahaan (2014) and Smolinska (2015) found an increase in Hg content in roots and leaves when applying compost in Hg-contaminated soils.

3.3 Bioavailability and mobility of Hg in soil

Concentrations of Hg by DGT were calculated using the diffusion coefficients determined by Turull et al., (2019) (3.08×10^{-6} cm^2 s^{-1} for ODL and 1.79×10^{-6} cm^2 s^{-1} for RDL) for AS, BC3 and BC6 (Table 4). When compost was added, the dissociation of the exchange reaction between Hg-OM and DGT was not fast enough to allow for determination of the Hg concentration available for the plant.. Thus, D from a synthetic solution with compost calculated by Cattani et al. (2009) (6.70×10^{-7} cm^2 s^{-1}) was used to calculate Hg concentration in CP30. In the case of the roots, a significant inverse correlation existed between ODL values and OM (R=-0.848, p<0.01), because of the

capacity of Hg to form strong ion complexes with the functional groups of OM such as sulphur or carboxylic acid groups (Smolinska and Szczodrowska, 2017).

The R value is the ratio between the labile Hg concentration obtained by DGT devices and the soil solution that represents the presence of labile species in the solid phase and the speed of Hg re-supply from the solid phase to the soil solution. In Table 4, ratio measured from the program 2D DIFS (R_{diff}) is shown. R_{diff} value was calculated considering different parameters such as the porosity, the diffusive coefficient or the particle concentration. Also, this value was used to calculate the effective concentration (Ce) using Eq. 3. The values applied to calculate R_{diff} are summarized in Table S1. Therefore, C_E can be compared with the values from lettuce roots. The differences between concentration of Hg in roots and DGT values in the sets AS, BC3, BC6 and CP30 were -3, 14, -1 and -4%, respectively. These values demonstrate the effectiveness of this technique to predict metal concentration in roots. Besides that, the best significant correlation was found between DGT measurements and roots from *Lactuca sativa* (R=0.703) in comparison with the other values such as the sequential extraction (R=0.467) or C_{soln} (R=-0.314). Our results are in agreement with other studies correlating the values of Hg from DGT measurements with different crops. Ridošková et al. (2017) demonstrated the effectiveness of DGT method to predict the root uptake of Hg by C_E where its Hg correlation with *Taraxacum officinale* was 0.84. Also, Liu et al. (2012) predicted the amount of MeHg in *Oryza sativa* by DGT measurements and it was well correlated with the rice roots (R=0.85).

The amount of low molecular species is indicative of the fraction of the inorganic Hg in the soil solution (Table 4). Using DGT with a pore size <1 nm (i.e. RDL), the concentration of free metal species in soil could be estimated. It is important to know the fraction of soluble inorganic Hg species, because of ease of transport by natural processes, is considered a substrate for the Hg methylation (Han et al., 2003). The amount of low molecular species (as %) for AS is 97%, that was progressively reduced

by the addition of organic matter in the soil. Therefore a significant inverse correlation (R=-0.586, p<0.05) was observed between values from RDL and the percentage of OM. In the case of biochar, adding 3% and 6%, the percentage decreased 16 and 20%, respectively. Furthermore, adding compost, the amount of inorganic species decreased by 35%.

Conclusions

In the present study, Hg concentration in an agricultural peri-urban soil from Barcelona occured in an inert form, and only a small part (1-3%) was bioavailable for lettuce. The application of biochar reduced the labile fraction of Hg. In contrast, the use of compost increased the bioavailable Hg for the plants. The concentration used to predict Hg uptake by plants, C_E, can be calculated applying the DIFS program, considering the kinetical properties of each soil. Furthermore, DGT method with a smaller pore size (RDL) was used for the determination of inorganic Hg species in the studied agricultural soils. The results showed that the soil without any amendment had the highest value of free Hg ion, whereas the amount was reduced with increased the OM content in the sample by up to 62%.

Acknowledgments

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Tables

Table 1. Chemical characteristics of the different studied sets. AS: agricultural soil, BC3: AS with 3% w/w of biochar, BC6: AS with 6% w/w of biochar, and CP30: AS with 30% w/w of compost.

Parameter	Unit	AS	BC3	BC6	CP30
OM	%	6.52^a	9.19^b	11.34^c	12.90^d
Nitrate	mg kg^{-1}	9.16^a	9.66^{ac}	9.07^a	11.40^{bc}
pH	-	7.29^a	7.38^b	7.48^c	7.19^d
EC	mS cm^{-1}	1.73^a	1.74^a	1.57^b	5.23^c
Hg	mg kg^{-1}	0.640^a	0.628	0.619	0.578^b

*Different letters in the columns indicate a significant difference p<0.05 between treatments

Table 2. Correlation coefficients between percentage of Hg in each fraction in the soils and the percentage of organic matter (OM), pH, nitrates and electrical conductivity (EC). Sequential extraction fractions: F1: water-soluble, F2: human stomach acid soluble, F3: organo-complexed, F4: elemental mercury, and F5: residual.

	F1	F2	F3	F4	F5
OM (%)	0.390	0.470	-0.866**	0.547	0.052
pH	-0.685*	0.149	-0.331	0.710**	-0.965**
Nitrates	0.547	-0.044	-0.278	-0.109	0.678*
EC	0.748**	0.180	-0.264	-0.208	0.745**

*p<0.05

**p<0.01

Table 3. Concentrations of Hg in lettuce root and leaves, and bioaccumulation factor BCF (n=5). Different letters indicate significant differences within different columns. AS: agricultural soil, BC3: AS with 3% w/w of biochar, BC6: AS with 6% w/w of biochar, and CP30: AS with 30% w/w of compost.

	Hg concentration (ng g^{-1} in fw^a)		
	Roots	**Leaves**	**BCF**
AS	14.08 ± 1.60^a	5.24 ± 0.85^a	0.022^a
BC3	10.40 ± 0.70^b	4.90 ± 0.56^a	0.017^b
BC6	10.24 ± 5.33^{ab}	4.86 ± 0.75^a	0.017^{ab}
CP30	10.96 ± 2.49^{ab}	4.87 ± 0.59^a	0.020^{ab}

^{a}fw: fresh weight

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Table 4. Comparison of different concentration values in soil from the different agricultural soils studied. Concentration of Hg in soil solution (C_{soln}), Hg concentration in soil using open (C_{DGT}-ODL) and restricted (C_{DGT}-RDL) diffusive layers, the ratio of the theoretical concentration if the DGT device is supplied solely by diffusion of solutes from the soil solution (R_{diff}), the calculated effective concentration (C_E) and low molecular species (LMS). AS: agricultural soil, BC3: AS with 3% w/w of biochar, BC6: AS with 6% w/w of biochar, and CP30: AS with 30% w/w of compost.

Line	C_{soln} (µg L^{-1})	C_{DGT}-ODL (µg L^{-1})	C_{DGT}-RDL (µg L^{-1})	R_{diff}	C_E (µg L^{-1})	LMS (%)
AS	0.244±0.014	1.616±0.170	1.567±0.649	0.118	13.696	97
BC3	0.356±0.017	1.113±0.171	0.905±0.783	0.092	12.108	81
BC6	0.218±0.059	1.122±0.071	0.863±0.276	0.111	10.104	77
CP30	0.076±0.003	0.999±0.127	0.623±0.018	0.095	10.489	62

LMS (%): (C_{DGT}-RDL/C_{DGT}-ODL)x100

Figure 1. Schematic of the experimental design for the experiment

Figure 2. Speciation of Hg in the different soil samples. AS: agricultural soil, BC3: AS with 3% w/w of biochar, BC6: AS with 6% w/w of biochar and CP30: AS with 30% w/w of compost Fractions from sequential extraction procedure: water-soluble (F1), human stomach acid soluble (F2), organo-complexed (F3), elemental mercury (F4), and residual (F5).

Highlights:

- We tested whether DGT devices could be used to predict plant Hg uptake in amended soils
- Open and restricted gels were used to discriminate Hg labile species in soils
- DGT Hg was significant correlated with Hg in lettuce roots
- Biochar and compost applications had contrasting effects on plant uptake of Hg
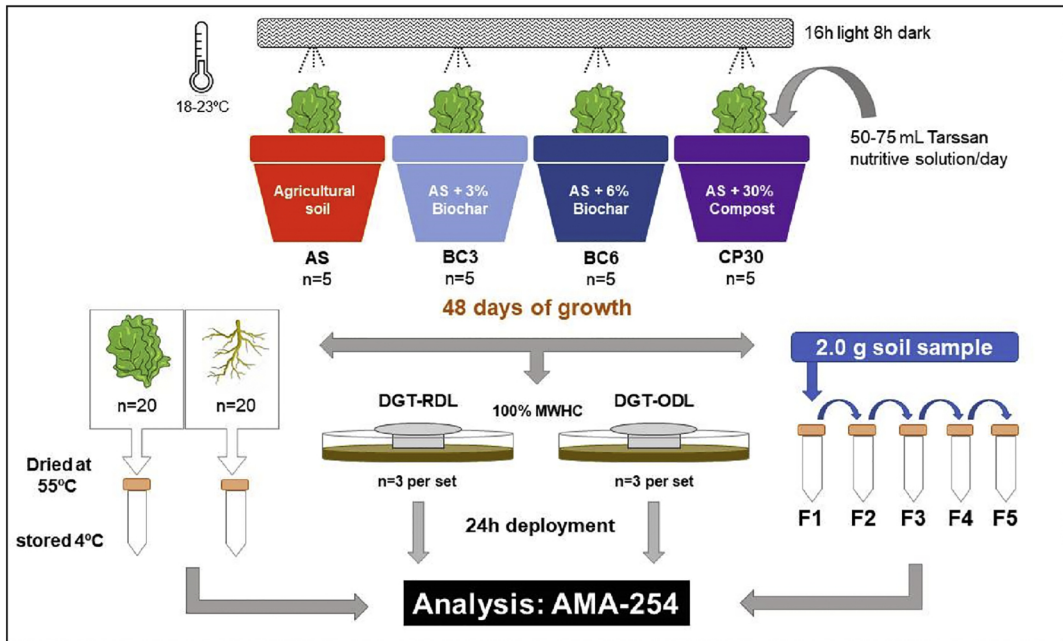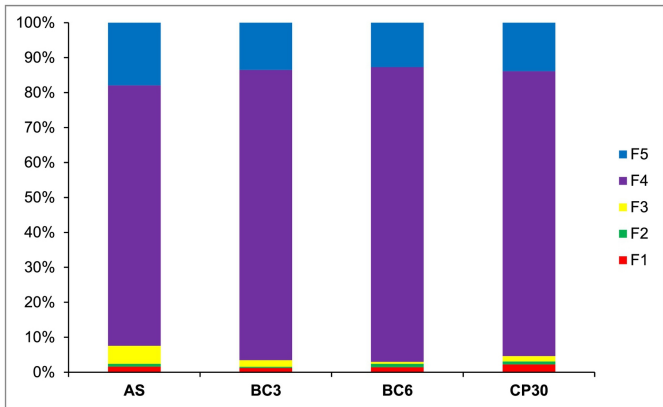- Biochar reduced bioavailability of Hg (60%) and Hg uptake by lettuce (35%)

Figure 1

Figure 2