Combining grass and legume species with compost for assisted phytostabilization of contaminated soils

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COMBINING GRASS AND LEGUME SPECIES WITH COMPOST FOR ASSISTED PHYTOSTABILIZATION OF CONTAMINATED SOILS

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Abstract:	Assisted phytoremediation, i.e. the combination of amendment and plant cultivation to remove potentially toxic elements (PTE) from soil, or to reduce their mobility and toxicity, can represent an effective gentle remediation option for the recovery of PTE-contaminated soils. The aim of this study was to evaluate the suitability of different grass and legume species, such as Arundo donax L., Hordeum vulgare L. and Lupinus albus L., in assisted phytoremediation programs of PTE-contaminated soils in combination with a municipal solid waste compost (MSWC) used at 2 and 4% rates. The soil was heavily contaminated by different PTE, i.e. Pb (15,383 $mg \cdot kg^{-1}$), Zn (4,076 $mg \cdot kg^{-1}$), Sb (109 $mg \cdot kg^{-1}$), Cd (67 $mg \cdot kg^{-1}$) and As (49 $mg \cdot kg^{-1}$). The selected plant species were able to grow in the contaminated soil, and their biomass production was significantly influenced by the compost either positively (e.g. A. donax) or negatively (e.g. H. vulgare roots). Compost addition significantly decreased or did not influence the PTE uptake and bioaccumulation factors of A. donax and H. vulgare roots and shoots, while it increased those of L. albus (particularly in roots) with respect to As, Sb, Pb and Cu. Finally, MSWC increased the PTE removal efficiency of A. donax (and partially of L. albus but not by H. vulgare), i.e. its ability to bioaccumulate PTE in the below ground organs, especially when grown in soils amended with 4% MSWC. The results indicated that A. donax, and in selected cases L. albus, can be used in combination with MSWC for the phytostabilization of PTE-contaminated soils.
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Response to Reviewers:	Ms. Ref. No.: Ms. No. ETI-D-20-00614 Title: COMBINING GRASS AND LEGUME SPECIES WITH COMPOST FOR ASSISTED PHYTOSTABILIZATION OF CONTAMINATED SOILS Journal: Environmental Technology & Innovation Dear Prof. Ravi Naidu Editor in chief Environmental Technology & Innovation below you will find our responses to the reviewers’ comments and suggestions. As you will see, all the requested or recommended changes have been applied to the revised manuscript. We would like to thank the anonymous reviewers since we feel that their observations and suggestions, contributed significantly to improve the manuscript. Reviewer #1 The revisions suggested by the Reviewer#1 have been incorporated in the revised version of the manuscript and shown as yellow text. - The title clearly reflect the contents. Thank you. - The aims of the paper is poorly written. It must be improved accordingly. As suggested by the review we improved the aims of the paper (Lines 20-21, 121-125). - Introduction: in the present form the introduction is not enough described This conflicts with the Reviewer#2, for which the Introduction is “interesting and quite well written, but too long”. According with the Reviewer#2, we deleted some parts of the “Introduction” and we better explained the aim of the study (as also required by the Reviewer#1), as well as the novelty and the originality of the research (Lines 121-130). - Material and methods: The methodology is well thought through. The description of materials and methods is informative to allow replication of the experiment. Thank you. - The originality and novelty of the paper need to be further clarified. What progress against the most recent state-of-the-art similar studies was made in this study? As suggested by the Review#1 we better underlined the originality and novelty of the paper (Lines 125-130). Reviewer #2 All the revisions suggested by the Reviewer#1 have been incorporated in the revised version of the manuscript and shown as green text. The authors provide information about plant analyses, but data regarding soils and compost characteristics are only vaguely commented and referred to previous publications. Data related to soil and compost characteristics (before plant growth) have been added in this revised version as supplementary material (i.e. Table S1). Moreover, an additional figure (Fig. S1), showing the mobility of PTE after soil incubation with compost (i.e. before plant growth), has been also added as supplementary material. Now the reader will be able to easily understand compost and soil features just before planting. In fact, soil analysis seem to have been performed only in soils after the incubation period with the compost, but not after the development of the pot experiment and the growth of the plants in those. This, as the authors comment in the paper, may have

influenced the soluble fraction of the PTEs in the soils and cannot be omitted. This is possibly the main concern of this manuscript, which has been generally very well written, but may still be thoroughly revised to avoid repetition and vague/imprecise/speculative comments.
We thank the reviewer for this observation. We better explained in the “Material and methods” that the pot experiment started immediately after the incubation period (Lines 184). In addition, according with the Reviewer observation, we carried out a number of additional chemical analyses on soils collected after plant growth. In particular, we determined the main soil chemical parameters (Table S2) as well as the labile fraction of PTE (water-soluble and exchangeable) therein (Fig. S2). The respective materials and methods employed have been added in the revised text (Lines 199-212). Finally, the text has been revised accordingly and, hopefully, vague/imprecise/speculative comments have been significantly reduced (if not completely eliminated).

Abstract: the conclusion in the abstract is contradictory and a bit confusing, as the authors say that 'removal efficiency' of A. donax was increased and that this made it suitable for phytostabilizing PTEs in soils. This must be rewritten in a clearer way.
We agree, we clarified this part, thank you (Lines 31-33).

Introduction: interesting and quite well written, but too long. In addition, the aim of the work may better reflect the novelty of the results and the originality of the research performed.
We thank the reviewer for his comment, we deleted some parts of the Introduction, in addition we better explained the aim, novelty and originality of the research carried out (Lines 68, 80, 82, 121-130).

Materials and Methods: a complete description of the characteristics of the original materials would be of interest, even if only in the supplementary information of the manuscript.
As required, we added as Supplementary Materials a table (Table S1) and one figures (Fig. S1) reporting soil and compost characteristics and PTE mobility after the incubation period and before plant growth. In addition, we added in Table S2 and Fig. S2 selected chemical parameters and labile PTE in soils collected after plant growth (Lines 199-212).

The interest of the different rations included in the plant analysis part is frequently arguable. A precise description of the data used for their calculation (e.g., which soil data were used, or if plant and soil concentrations and amounts were expressed or used in a per pot basis) would provide this data some robustness.
We tried to make clearer the calculation of the different indexes mentioned in the text (Lines 236-239). Moreover, we would like to say that these indexes (i.e. BAF, TF, RF, mineralomasses) are widely used in phytoremediation studies since they provide useful information on a plant suitability for PTE stabilization or extraction.

Results and discussion: this section must be thoroughly revised and corrected, as at the moment is a bit repetitive and focuses mainly on qualitative results (if values were higher or lower for a species or another, with or without compost, etc.). The real interest of the results comes from their quantitative analysis, as this would really provide useful and contrastable information to other readers. For instance, discussion about BAF (and most of TF and RF) simply mirror the discussion of PTE concentrations in the different plant parts and is therefore of little relevance. What is really relevant from those ratios etc. is their value, if those values can be considered to be appropriate for stabilization or extraction, etc. Otherwise the discussion can result a bit ambiguous.
In principle, we understand the reviewer comment and we applied some revisions in this sense (Lines 413-417, 433-436, 447-450, 470-471, 481-484). However, we would like to say that quantitative information can be abundantly found in tables, figures and supplementary materials and that mentioning those values also in the text would not provide additional information (which is already there) nor improve the manuscript. We also believe that text reading should be accompanied by that of tables and figures and that readers can now benefit of both quantitative and qualitative information. Moreover, the aim of our study was to evaluate the suitability of three different plant species, in combination with MSWC, for the recovery of a soil contaminated by different PTE. In this sense, values higher or lower for a species or another, with or without compost, is

what we were interested. Finally, and most importantly, we would like to say that BAF and TF cannot be used in any way to identify plants suitable for PTE stabilization or extraction as they only highlight the attitude (or potential) of a given plant to concentrate PTE from soil to plant tissues (BAF) and to transfer them from roots to shoots. Plant growth, PTE uptake and mineralomasses (and therefore RF) are certainly most relevant in this sense as highlighted in the text.

In addition, part of the discussion can be considered to be somewhat speculative and not fully based on the results presented in the manuscript nor supported by appropriate close studies (e.g., lines 265-274, 318-319, 335-336, 348-349, 360-361, 365-373, 377-378, 451-453, 461-463, 479-480, 481-483).
As suggested by the reviewer we deleted some parts of the discussion (Lines 281, 372, 481, 487), and we better explained some aspects (which appeared rather speculative), following the results obtained of soil analyses carried out after plant growth (i.e. Table S2 and Fig. S3) (Lines 325-328, 341-343, 356-361, 376-378, 385-388, 421-423, 432-433, 447-450, 453-455, 470-471, 481, 483-484).

Also, the fact that A. donax is a well known invasive species and in that way has many detractors may be considered/mentioned in the text.
We mentioned this aspect in the text, thank you (Lines 94-97).

SPECIFIC COMMENTS
Delete too general comments in lines 90-91 and 427-434.
Done (Lines 82, 442).

Line 223: what 'soil mass' was used for this calculations? Per pot? How can that values be compared with results from other experiments?
For the calculation of RF, the weight of soil in the pot (kg) was used (i.e. 3 kg d.m. for A. Donax and 1 kg for lupin and barley), as reported by Moameri and Khalaki (2019). In this way it is possible to compare the results obtained in one pot with those of the other pots. We better explained what "soil mass" means in the manuscript (Lines 236-239).

Lines 258-259: soil values after incubation or after the pot experiment?
We better specified this point (Lines 272).

Lines 318-319 and 335-336: labile PTE concentrations in the soils from the pots were not measured or at least are not shown or described in the manuscript.
As suggested by the reviewer, labile PTE concentrations in soils before and after the pot experiment were reported as supplementary material (Fig. S1-S2; Lines 325-328, 341-343).

Lines 348-349: how was this determined?
(Lines 356-361) As suggested by the reviewer we determined the concentration of labile PTE (i.e. the PTE fraction extracted with $(NH_4)_2SO_4$) and we reported the results in the Supplementary Figure 3. We described in the "Materials and methods" section how the labile fraction of PTE after the plant growth was determined (Lines 199-212).

Lines 365-366: Cu and, especially, Pb concentrations were very high in this soils, which makes this comment unexpected.
We revised the text. In particular, we mentioned some results which have been added in Fig. S1 and are related to PTE mobility in soil before plating. Fig. S1 clearly shows that most Cu and Pb in soil (before planting) was extracted with EDTA (Lines 378-380).

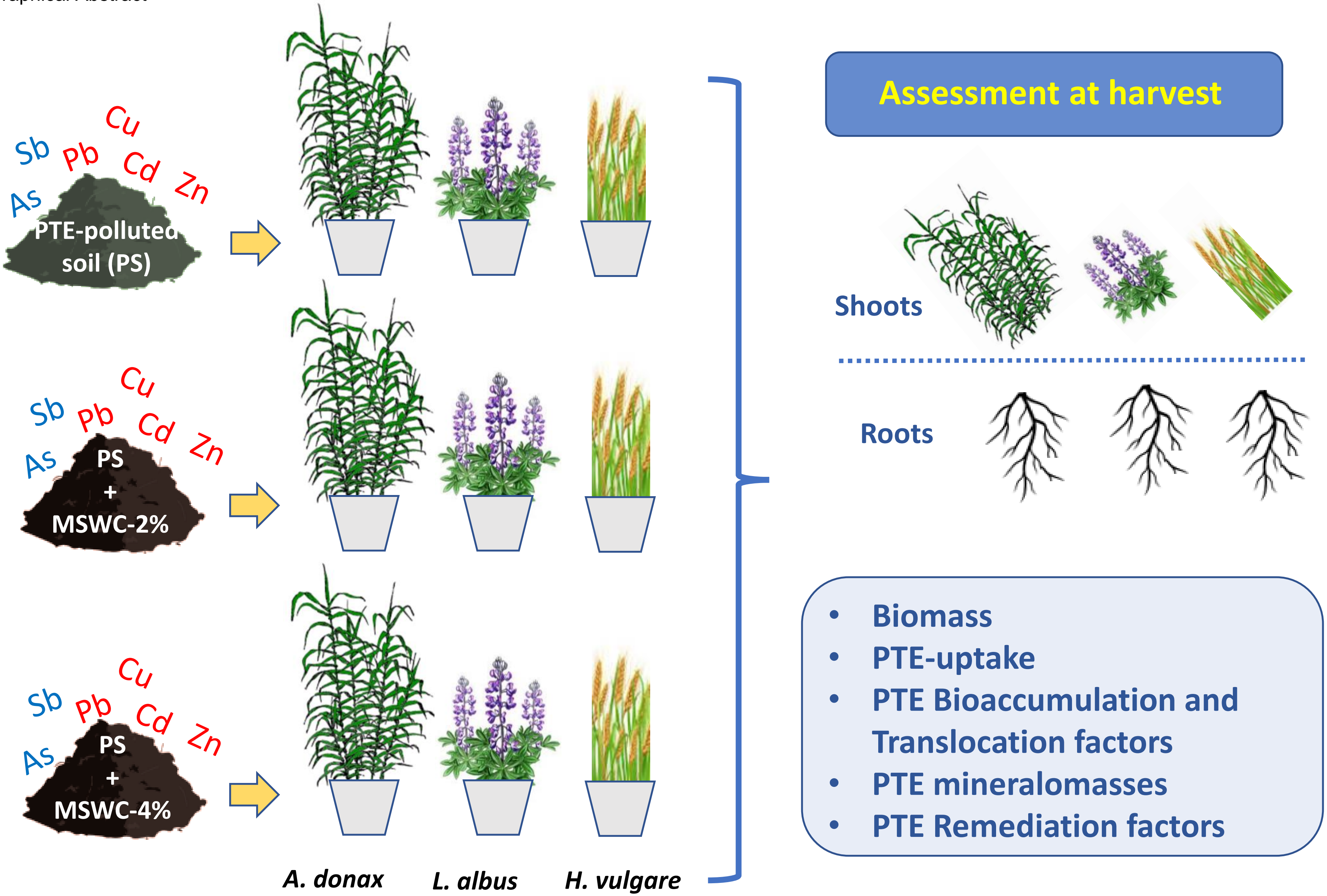
Lines 377-378: that is quite unlikely (quantitatively) and those data were not determined/shown in the manuscript.
Our statement is now supported by the PTE mobility data determined after plant growth, lupin in this case (Fig. S2). (Lines 392-393).

Lines 402-404: an example of repeated information.
We deleted the sentence, thank you (Lines 418).

Lines 481-483: that is possibly just a consequence of a dilution effect (higher biomass with the same accumulation capacity).

	We mentioned this possibility in the revised manuscript, thank you (Lines 490-492). Figure 1: are those data per pot? Please clarify this. The data in Figure 1 are expressed as root and shoot dry weight (g plant-1). We better clarified this aspect in the y-axis of the Figure 1, thank you. Metal concentrations in plant roots (especially for Pb and Zn in H. vulgare and L. albus) seem too high, even for soils with such a with PTE concentrations. Were those data contrasted with previous results/experiments with those species in other contaminated soils? As highlighted by the review, Pb and Zn concentrations in plant roots (especially for white lupin and H. vulgare) are very high. However, Martìnez-Alcalá et al. [Chemical and biological properties in the rhizosphere of Lupinus albus alter soil heavy metal fractionation. Ecotoxicology and Environmental Safety 73 (2010) 595–602] detected more than 6000 mg kg-1 of Zn in roots of lupin plants grown in an acidic soil containing 364 mg kg-1 of Zn. Moreover, Ebbs et al. [Phytoextraction of zinc by Oat (Avina sativa), Barley (Hordeum vulgare), and Indian Mustard (Brassica juncea). Environmental Science & Technology 32 (1998) 802-806] detected about 650 mg kg-1 of Zn in shoots of barley plants grown in contaminated soil containing 3100 mg kg-1 of Zn, Andrey et al. [The role of biochar-microbe interaction in alleviating heavy metal toxicity in Hordeum vulgare L. grown in highly polluted soils. Appl. Geochemistry 104 (2019) 93-101] also found that in a soil heavily contaminated, especially by zinc (6232 mg kg-1), barley plants showed 4372 and 2659 mg kg-1 of Zn in roots and shoots respectively. This suggests that the peculiarity of each contaminated soil (i.e. specific origin, texture, pH, point of zero charge, organic matter content, relative and absolute PTE abundance and speciation, and so on) and of each plant species, produce site-specific results sometimes difficult to compare with others obtained in apparently similar contexts (Lines 345-347, 373).

Graphical Abstract



COMBINING GRASS AND LEGUME SPECIES WITH COMPOST FOR ASSISTED PHYTOSTABILIZATION OF CONTAMINATED SOILS

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Abstract

Assisted phytoremediation, i.e. the combination of amendment and plant cultivation to remove potentially toxic elements (PTE) from soil, or to reduce their mobility and toxicity, can represent an effective gentle remediation option for the recovery of PTE-contaminated soils. The aim of this study was to evaluate the suitability of different grass and legume species, such as *Arundo donax* L., *Hordeum vulgare* L. and *Lupinus albus* L., in assisted phytoremediation programs of PTE-contaminated soils in combination with a municipal solid waste compost (MSWC) used at 2 and 4% rates. The soil was heavily contaminated by different PTE, i.e. Pb (15,383 mg·kg^{-1}), Zn

($4{,}076\ mg \cdot kg^{-1}$), Sb ($109\ mg \cdot kg^{-1}$), Cd ($67\ mg \cdot kg^{-1}$) and As ($49\ mg \cdot kg^{-1}$). The selected plant species were able to grow in the contaminated soil, and their biomass production was significantly influenced by the compost either positively (e.g. *A. donax*) or negatively (e.g. *H. vulgare* roots). Compost addition significantly decreased or did not influence the PTE uptake and bioaccumulation factors of *A. donax* and *H. vulgare* roots and shoots, while it increased those of *L. albus* (particularly in roots) with respect to As, Sb, Pb and Cu. Finally, MSWC increased the PTE removal efficiency of *A. donax* (and partially of *L. albus* but not by *H. vulgare*), i.e. its ability to bioaccumulate PTE in the below ground organs, especially when grown in soils amended with 4% MSWC. The results indicated that *A. donax*, and in selected cases *L. albus*, can be used in combination with MSWC for the phytostabilization of PTE-contaminated soils.

Keywords: Potentially toxic elements; Gentle remediation options; Organic amendments; PTE-uptake; Bioaccumulation factors

1. Introduction

Soil contamination by potentially toxic elements (PTE) represents one of the main global environmental problems, as nowadays too many anthropogenic activities release PTE in different environmental compartments (Radziemska et al., 2017). In particular, mining activities represent a source of severe pollution of their surrounding environment due to the progressive PTE release by unstable waste rocks and tailings (Castaldi et al., 2005; Wong, 2003). This commonly leads to the loss of land suitable for crop cultivation (and/or grazing) and can pose serious health concerns given the potential entry of PTE into the food chain (Radziemska et al., 2017; Duri et al., 2018; Wong, 2003).

The soils in the proximity of mining sites are often characterized by low pH values, high concentrations of labile (i.e. soluble and easily bioavailable) PTE, poor soil structure and low level of nutrients due to reduced organic matter inputs (Bacchetta et al., 2015; Castaldi et al., 2005; Eijsackers, 2010; Garau et al., 2017; Manzano et al., 2016; Wong, 2003). Moreover, PTE contamination can have a detrimental influence on soil microbial abundance, diversity and activity (Abou Jauode et al., 2020; Diquattro et al., 2020; Garau et al., 2017), which in turn can negatively affect plant growth (Castaldi et al., 2018). For these reasons, both the securing of such areas and the reduction of PTE spreading have become pressing environmental concerns worldwide.

The so called Gentle Remediation Options (GRO), i.e. those approaches or strategies aimed at preserving or improving soil functions while limiting the environmental impact of PTE, have emerged in the last decade as promising alternatives for the recovery of contaminated soils (Abou Jaoude et al., 2020; Cundy et al., 2015). Among GRO, the use of plants for the *in situ* stabilization of PTE or their extraction (i.e. phytostabilization and phytoextraction respectively) has been increasingly investigated in the last years (e.g. Castaldi et al. 2018; Garau et al., 2014; Kumpiene et al., 2014), along with the influence of chemical additives or organic-based amendments on such processes (i.e. assisted phytoremediation; Cundy et al., 2015).

Plants growing in PTE-contaminated soils are exposed to several stresses and their ability to overcome these harsh conditions can vary depending on plant genotype, soil physico-chemical properties and labile PTE concentration. On the other hand, the amendment addition can substantially affect the plant adaptation and its growth performance in such PTE-contaminated soils. Plants can absorb and accumulate PTE in roots or hypogeal organs, blocking them belowground, but they can also translocate the contaminants into the aerial part (Barbosa and Fernando, 2018). Therefore, an accurate plant selection is necessary in order to achieve specific phytoremediation objectives, e.g. phytostabilization or phytoextraction (Sánchez-Pardo and Zornoza, 2014; Wong, 2003). Apart from this, such plants should be able to grow in nutrient-poor soils and in the presence of high concentrations of PTE (Sánchez-Pardo and Zornoza, 2014; Wong, 2003).

Different native species, in particular grasses and legumes, could successfully restore soil fertility and promote ecological recovery (Wong, 2003). This is due to their capacity to develop an extensive fibrous root system, which improves soil physical structure, attenuates erosion and enhances soil organic matter content (Ying et al., 2008). In this context, barley (*Hordeum vulgare* L.) could be useful for phytostabilization purposes given its ability to accumulate PTE in roots (Dago et al., 2014; Ruiz-dı et al., 2010). This crop, characterized by low input requirements, is able to develop specific defense mechanisms against PTE, e.g. the production of phytochelatins (small cysteine-rich peptides) which are accumulated in the cell vacuole after PTE binding (Dago et al., 2014). Likewise, studies conducted on giant reed (*Arundo donax* L.) highlighted the ability of this grass helophyte to grow in acidic soils contaminated by different PTE (i.e. As, Cd, Cu, Pb and Zn), which were mainly accumulated in below ground tissues (roots and rhizomes), and poorly translocated to aerial parts (Castaldi et al., 2018). Even if this grass is recognised as an invasive species able to form dense monotypic stands in habitats where it establishes (e.g. Hardesty-

Moore et al., 2020), it should be considered as a potential resource for the recovery of polluted soils where plant growth and establishment is severely affected.

Also legumes proved to be useful for the remediation of PTE-contaminated soils (Castaldi et al., 2005; Wong, 2003). For instance, white lupin (*Lupinus albus* L.), which is able to grow in adverse soil conditions such as acidic pH, poor fertility and salinity (Carpena et al., 2003; Castaldi et al., 2005; Sánchez-Pardo and Zornoza, 2014; Ximénez-Embún et al., 2001), was effectively employed for the phytoremediation of PTE-contaminated soils (Fumagalli et al., 2014; Zornoza et al., 2018).

In this context, the addition of organic-based amendments to contaminated soils, e.g. municipal solid waste compost, manure, sludge, or biochar, was recognized to facilitate plant growth and establishment. This can be due to a reduction of labile (and bioavailable) PTE, to a stimulation of microbial abundance and diversity, and to an improved mineral content and biogeochemical cycling in the amended soils (Castaldi et al., 2005, 2018; Garau et al., 2014, 2017; Wong, 2003; Zornoza et al., 2018). Although previous studies showed that *A. donax, H. vulgare* and *L. albus* can be able to accumulate PTE, and different amendments were tested to enhance their bioaccumulation abilities (Barbafieri et al., 2017; González et al., 2019; Mâșu et al., 2012; Zornoza et al., 2018), nonetheless their effectiveness as phytostabilizing plants needs further study, especially in multi-PTE-contaminated soils. Moreover, the role of organic amendments (e.g. compost) on the phytostabilizing ability of these plant species has been rarely addressed before.

Although the use of compost in assisted phytoremediation proved in some instances to be a promising GRO (Abou Jaoude et al., 2020; Manzano et al., 2016), such approach needs careful case-by-case assessment and optimization due to the great variability of soil and environmental conditions, PTE type and concentration, and selected plant species (Marchand et al., 2010). Accordingly, the aim of the present study was to evaluate the suitability of three different plant species (i.e. *A. donax, H. vulgare* and *L. albus*), in combination with a low-cost and sustainable amendment (i.e. a municipal solid waste compost: MSWC), for the recovery of a mining soil contaminated by different PTE such has As, Sb, Pb, Cd and Zn. Research on this issue is particularly crucial, especially considering that the influence of MSWC on PTE phytostabilizing abilities of *A. donax, H. vulgare* and *L. albus* was rarely (if never) considered before. Likewise, this is the first study (to our knowledge) comparing the (assisted) PTE phytostabilizing performances of *A. donax, H. vulgare* and *L. albus* in the same contaminated soil. To this end, plants growth was investigated in a polluted amended (and unamended) mining soil, and the PTE uptake and partitioning in roots (and rhizome) and shoots were quantified. The bioaccumulation and translocation factors were also determined for each plant species along with the respective PTE removal efficiency, i.e. remediation factors.

2. Materials and methods

2.1. Experimental set-up, soil and compost characteristics

The contaminated soil was collected from an ex-mining area located in the Southwestern Sardinia (Italy, N 39°40′29.71″; E 8°37′17.97″, Montevecchio, Guspini), where galena (PbS) and sphalerite (ZnS) were the main ores extracted (Castaldi et al., 2005; Wanty et al., 2013). Soil samples (10) were randomly collected from the upper soil layer (0-25 cm) in a site which extended for about 1 ha. The collected soil samples were pooled in the laboratory to obtain a composite soil that was thoroughly characterized from a physico-chemical point of view, and then used for the plant-growth experiment. The main features of such soil were previously reported (Garau et al., 2019a; Table S1). Briefly, the soil was a sandy clay loam (USDA classification) with sub-acidic pH (i.e. 5.93), substantial content of organic matter (OM, ~3.2%), and high cation exchange capacity (CEC, 36.86 $cmol_{(+)}\cdot kg^{-1}$). The content of the main mineral elements was in general suitable for crop cultivation (total N 1.3 $g\cdot kg^{-1}$, Olsen-P ~23 $mg\cdot kg^{-1}$, exchangeable Ca, Mg and K ~23, 11 and 1 $cmol_{(+)}\cdot kg^{-1}$ respectively). The total concentrations of Pb (15,383 $mg\cdot kg^{-1}$), Zn (4,076 $mg\cdot kg^{-1}$), Cu (181 $mg\cdot kg^{-1}$), Sb (109 $mg\cdot kg^{-1}$), Cd (67 $mg\cdot kg^{-1}$) and As (49 $mg\cdot kg^{-1}$) exceeded the threshold levels established by the Italian legislation for potentially contaminated sites (D.lgs. 152/2006).

The composite soil (~ 120 kg) was divided in 3 subsamples (~ 40 kg each) which randomly received one of the following treatments: no treatment, i.e. polluted untreated soil (Control); 2 % (w/w) MSWC (MSWC-2%); 4 % (w/w) MSWC (MSWC-4%). The MSWC used derived from municipal and green waste composting and was provided by the Facility Plant Secit S.p.A. Consorzio Zir (Chilivani-Ozieri, Italy). Before addition to the polluted soil, MSWC was dried at 105 °C for 48 hours, finely ground and sieved to < 2 mm. The main characteristics of the MSWC were previously reported by Diquattro et al. (2018) (Table S1). Briefly, the MSWC had a sub-alkaline pH (i.e. 7.93) with 3.3 $mS\cdot cm^{-1}$ electrical conductivity (EC), the pH_{PZC} was 5.6, and the OM content was 27.3 %. The MSWC had a high cation exchange capacity (CEC, 92.3 $cmol_{(+)}\cdot kg^{-1}$), dissolved organic carbon (DOC, 0.82 $mg\cdot kg^{-1}$) and humic acids content (14.24 %). After amendment addition, treated and untreated soils were mixed, moisture content raised to 60 % of their water holding capacity, and then incubated for 4 months at 20 °C. During this time, they were mixed twice a week and their water content maintained constant.

After the 4-months of contact, soil sub-samples from each treatment were air-dried and chemical analyses were repeated to evaluate the influence of MSWC on the different soil chemical parameters. A detailed description of the analyses carried out was previously reported (Garau et al., 2019a) while the main soil chemical parameters (after incubation with MSWC) are here resumed. Briefly, the addition of MSWC at 2 and 4% rates resulted in an increase of soil pH (6.5 and 6.8 respectively) and exchangeable Ca (26 and 28 $cmol_{(+)} \cdot kg^{-1}$ respectively) and K (1.5 and 2.0 $cmol_{(+)} \cdot kg^{-1}$ respectively), while a significant decrease of exchangeable Mg was detected. Moreover, increases of 5.8 and 14.3 % of CEC, 127 and 336 % of DOC, and 4 and 5 % of OM was observed in MSWC-2% and MSWC-4% respectively, compared to the control soil.

2.2. Plant-growth experiment and plant analysis

Immediately after the incubation period, the soil from each treatment was used to fill 36 plastic pots (19 cm diameter, 17 cm height) each containing some 3 kg of soil, which were separately planted with seeds of *H. vulgare* and *L. albus* and clonal ramets of *A. donax*. These plant species were selected because of their potentials as phytostabilizing plants and their tolerance to harsh conditions, e.g. poor fertility, acidic soils, salinity, low water availability (Barbafieri et al., 2017; Castaldi et al., 2005, 2018; González et al., 2019; Mâșu et al., 2012; Zornoza et al., 2018).

A. donax was collected from a natural site in Southwestern Sardinia (Italy) and vegetatively propagated (Castaldi et al. 2018). Six barley (cv. Arda) and white lupin (cv. Multitalia) seeds and three giant reed rhizome cuttings were separately planted in 4 replicated pots from each treatment. A total of 36 pots were prepared (4 replicated pots x 3 treatments x 3 plant species). Barley and white lupin were grown over 6 months, from October 2018 to March 2019, while giant reed was grown over 18 months, from October 2017 to March 2019, in a naturally-lit greenhouse at an average temperature of 20-25 °C and 60-70% relative humidity.

At harvest, root-adhering soil collected from plants subjected to the same treatment was bulked together, sieved to < 2 mm and soil pH, EC, total organic C, N and DOC were determined (Gazzetta Ufficiale, 1992; Table S2). Moreover, the mobility of Cd, Cu, Pb and Zn (i.e. their water-soluble and exchangeable fraction) was determined in root-adhering soil by treating soil samples (1 g) with 25 mL of a 0.5 M $Ca(NO_3)_2$ solution and shaking for 16 h at 20 °C (Basta and Gradwohl, 2000). Likewise, the mobility of As and Sb in root-adhering soil was assessed by treating soil samples (1 g) with 25 mL of a 0.05 M $(NH_4)_2SO_4$ solution and shaking for 4 h at 20 °C (Wenzel et al., 2001). Following each extraction, soil samples were centrifuged at 3500 rpm for 15 min and filtered (Whatman 41 filter) to separate the liquid and solid phases. PTE concentrations in filtered solutions were determined using a Perkin Elmer Analyst 200 flame atomic absorption spectrometer (AAS) (for Zn quantification) or a Perkin Elmer AAnalyst 400-HGA 900 atomic adsorption spectrometer equipped with a graphite furnace (for As, Sb, Cd, Pb and Cu quantification).

At harvest, shoots were separated from roots. In the case of *A. donax* roots and rhizomes were pooled together (hereafter mentioned as roots). Shoot and roots were accurately washed with deionised water and oven dried at 55 °C for 72 h to determine the respective dry weight. The PTE content (i.e. Pb, Cd, Zn, Cu, As and Sb) in shoots and roots was determined after mineralization of plant tissues with H_2O_2 and a mixture of 69% HNO_3 and ultrapure H_2O (ratio 1:1), in a Microwave Milestone MLS 1200 (EPA Method 3052). After mineralization, the total concentration of PTE was determined using a Perkin Elmer AAnalyst 200 flame atomic absorption spectrometer (for Zn quantification) or a Perkin Elmer AAnalyst 400-HGA 900 atomic adsorption spectrometer equipped with a graphite furnace (for As, Sb, Cd, Pb and Cu quantification). Peach leaf was used as standard reference material (NIST-SRM 1547).

The PTE bioaccumulation (BAF), translocation (TF) and remediation (RF) factors were then calculated (Bonanno and Vymazal, 2017; Moameri and Khalaki, 2019) for each plant species grown in each soil along with the respective mineralomasses (MM) (Lebrun et al. 2018):

- BAFr was calculated as the ratio between the PTE concentration in roots and that present in soil;
- BAFs was calculated as the ratio between the PTE concentration in shoots and that present in soil;
- TF was calculated as the ratio between the PTE concentration in shoots and that present in roots;
- MMr was calculated as the root biomass x PTE concentration in roots;
- MMs was calculated as the shoot biomass x PTE concentration in shoots;
- RFr was calculated as (root biomass in the pot x PTE concentration in root) / (soil mass in the pot x PTE concentration in soil) x 100;

- RFs was calculated as (shoot biomass in the pot x PTE concentration in shoot) / (soil mass in the pot x PTE concentration in soil) x 100.

2.3. Data analysis

For each plant species, root and shoot biomass and PTE concentration in root and shoot were determined in triplicate, and mean values ± standard errors were reported in tables and figures. Likewise, BAF, TF, MM, and RF indexes were calculated and expressed as mean values ± standard errors. For each plant species, a one-way Analysis of Variance (One-way ANOVA) was carried out to investigate the effects of treatments on the different parameters analyzed. Prior to the ANOVA, the homogeneity of variance was tested by the Cochran's test (Winer, 1971) and data were transformed whenever necessary. Where significant *P*-values were obtained ($P < 0.05$), differences between individual means were compared using the post-hoc Fisher's least significance difference test (LSD, $P < 0.05$). Statistical analyses were carried out using the NCSS 2007 Data Analysis software (v. 07.1.21; Kaysville, Utah).

3. Results and discussion

3.1. Influence of MSWC on plant growth

The amendment rate influenced significantly the biomass production of *A. donax*, (Fig. 1), i.e. the growth of this helophyte species followed the order: MSWC-4% > MSWC-2% > Control. In particular, the root biomass increased more than 1.5- and 3.9-

fold for MSWC-2% and -4% respectively compared to control plants. Moreover, the shoot biomass increased by 2.3- and 4.5-fold for plants grown on MSWC-2% and -4% respectively, compared to control plants (Fig. 1). Compost had a different effect on *H. vulgare* growth, i.e. root biomass decreased by 1.4- and 1.7-fold in plants grown on MSWC-2% and -4% respectively, while shoot biomass increased by 1.2- and 1.3-fold (Fig. 1). Despite the average shoot and root yield of *L. albus* decreased at increasing compost addition, such differences were not significant (Fig. 1).

These results clearly showed that MSWC had a quite different influence on plant growth in the contaminated soil. The significant increase of shoot and root biomass of giant reed in amended soils can be likely due to the enhanced soil fertility attributes recorded in such soils after incubation with compost, e.g. OM, total N, available P, CEC, exchangeable K and Ca, and DOC followed the order MSWC-4% > MSWC-2% > control soil (Table S1; Garau et al., 2019a). Moreover, the concentration of labile PTE was significantly lower in amended soils due to a PTE-immobilizing capability of MSWC (Garau et al., 2019a), and this could have favored plant growth and biomass production as observed by other studies (Castaldi et al., 2018; Mâșu et al., 2012; Zornoza et al., 2018). A similar response was observed in the case of barley shoots, but not roots whose dry weight declined at increasing compost rate. Such increased root yield of barley in control soil could be explained by a possible PTE influence on the cytokinin level. Cytokinins, a class of phytohormones, can modulate plant growth and development, and their contents in plant tissues can change upon PTE stress (Pál et al., 2018).

Differently from giant reed and barley, the addition of MSWC showed a non significant effect on white lupin roots and shoots (Fig. 1). This could be explained by considering that this plant grows well in acidic soils (pH ~5.0) and its biomass, particularly root, commonly decreases as the pH raises (e.g. Kerley, 2000; Tang et al., 1995). In this context, the pH increase recorded in MSWC-amended soils could be responsible for the observed phenomena and could mask a possible positive impact of MSWC on plant growth due to PTE immobilization in soil and increased fertility attributes.

The results highlighted an overall ability of the selected plants, when combined with MSWC, to grow satisfactorily on a PTE heavily contaminated soil (see for comparison yield data from Gorovtsov et al., 2019; Fumagalli et al., 2014; Yang et al., 2012). However, given the inconsistent influence of compost on shoot and root growth, the actual effectiveness of the tested assisted phytoremediation approach needs further investigation. In this context, the estimation of PTE uptake by the plants, bioaccumulation, translocation and mineralomasses can be useful to select plant species to combine with MSWC for the phytoremediation of PTE-contaminated soils.

3.2. Influence of MSWC on PTE uptake in plant tissues

With the exception of white lupin, MSWC addition reduced significantly or did not affect the PTE uptake by shoot and root of the selected plants (Fig.s 2-4). Furthermore, as a general trend, much higher PTE concentrations were detected in roots compared to shoots, thus supporting the phytostabilizing potential of the plant species under study (Castaldi et al., 2005, 2018; Fumagalli et al., 2014; Gorovtsov et al., 2019).

While MSWC addition mostly reduced the PTE uptake in giant reed and barley plants, compost rate did not show a consistent effect on PTE uptake as instead was observed for plant growth. In particular, As and Sb concentration in roots of *A. donax* decreased by 1.2- and 1.3-fold for As, and by 1.2-fold for Sb in MSWC-2% and -4% plants respectively, compared to control roots (Fig. 2). Compost did not affect significantly the Sb concentration in roots of barley, while the As concentration decreased by 1.7- and 1.5-fold in MSWC-2% and -4% roots respectively, compared to control roots. Arsenic in *A. donax* and *H. vulgare* shoots was not detected, while reduced Sb concentrations were detected in shoots of both grasses when MSWC-2% was added to the soil (Fig. 2). On the contrary, MSWC addition significantly increased both As and Sb uptake by roots and shoots of white lupin: (e.g. As uptake in roots increased by more than 2.7- and 2.3-fold, and Sb uptake increased by ~14- and 7-fold in MSWC-2% and -4% plants respectively; Fig. 2).

These results confirmed that *A. donax* and *H. vulgare* are able to retain most of As and Sb in below ground organs (Castaldi et al., 2018; Zeng et al., 2019), and indicated that MSWC addition did not modify the overall partitioning of these PTE between roots and shoots, while reducing (in most cases) their concentrations. However, this was not the case of *L. albus* (Fig. 2). All this clearly indicated that the interaction between MSWC and plant species had a significant influence on PTE mobilization and uptake , and that this latter aspect is hardly predictable based on the sole knowledge of PTE labile concentrations in soil before planting. For instance, it was previously shown that MSWC addition to this soil significantly reduced labile Sb, while labile As (which was present at concentrations < 1 mg·kg^{-1} soil) remained unchanged (Fig. S1; Garau et al., 2019a). However, Sb and As uptake did not follow such trend, with the only exception of Sb in giant reed roots (Fig. 2), whereas in the other cases PTE uptake was either reduced in MSWC-treated soils (e.g. As in barley roots) or greatly stimulated (e.g. Sb and As in white lupin roots). This latter case was remarkable and could be explained with the ability of *L. albus* to release great amounts of organic anions, such as citrate and malate, from its typical proteoid roots (e.g. Yan et al., 2002). Such organic anions, whose metal complexing abilities increase as the pH raises (Quin et al., 2004), as it was the case in MSWC-treated soils (Table S1), could be responsible for the partial solubilization of Fe/Al oxy-hydroxides, which represent the largest sink of both PTE in the soil under study (Garau et al., 2019a), with the consequent release of labile As and Sb in amended soils (Castaldi et al. 2013). However, after plant growth, labile As and Sb significantly decreased in amended soils compared to control ones (Fig. S2) likely reflecting the highest uptake of these PTE in MSWC-treated soils (Fig. 2).

Differently from As and Sb, a more uniform trend across plant species was recorded for Cd and Zn uptake (note that the concentration of this later PTE was very high in roots of control barley and lupin as also recorded in other studies, e.g. Martìnez-Alcalá et al., 2010; Gorovtsov et al. 2019). Significant reductions were highlighted in shoots and roots of plants grown on amended soils, with the exception of Cd in shoots of white lupin (Fig. 3), while an inconsistent influence of compost rate was also observed. For instance, the concentration of Cd in roots decreased by ~2- and 3-fold in *A. donax*, 4- and 2-fold in *H. vulgare* and 4- and 7-fold in *L. albus* grown in MSWC-2% and -4% respectively, compared to control roots (Fig. 3). Interestingly, Cd uptake in shoots was generally limited compared to that absorbed by roots in all species under study. This is noteworthy, since Cd is generally highly translocated and bioaccumulated in shoots (Castaldi et al., 2005; Llugany et al., 2012; Sánchez-Pardo et al., 2015). These uptake data confirmed the role of MSWC in reducing the labile Cd and Zn in soil (Fig. S1; Garau et al., 2019a) and suggested that the interactions between MSWC, plant and soil microbial community had a poor influence on the re-mobilization of these PTE. Importantly, this was supported by the PTE mobility data recorded in soil after plant growth, i.e. significantly lower concentrations were detected in MSWC-treated soils compared to unamended controls (Fig. S2).

This was also the case of Pb and Cu uptake, whose trend was similar for *A. donax* and *H. vulgare*: the addition of compost significantly reduced (or left unchanged) the uptake of these PTE. However, Pb and Cu uptake by the roots of *L. albus* was substantially increased in MSWC-amended soils (Fig. 4). For instance, Pb concentration in roots decreased by ~ 23% in giant reed and 69% in barley grown in MSWC-4%, compared to control roots. On the other hand, Pb and Cu concentrations in white lupin roots increased up to ~ 2.4- and 1.5-fold respectively for plants grown on MSWC (Fig. 4).

The reduced Pb and Cu uptake, which was observed for *A. donax* and *H. vulgare* after soil amendment, followed the labile concentrations of these PTE in amended and unamended soils before planting (Fig. S1; Garau et al., 2019a). The increased Pb and Cu uptake by white lupin roots in MSWC-amended soils (particularly relevant for Pb) further supported the specificity of this plant compared to both grasses and could be explained by an increased mobilization of these PTE in the lupin rhizosphere in the presence of MSWC. However, labile (i.e. water-soluble and exchangeable) Cu and Pb decreased in MSWC-soils after lupin growth (Fig. S2), probably as a result of the high root uptake of these PTE. Differently from Cd and Zn, the majority of Cu and Pb in the contaminated soil (before planting) was extracted after EDTA treatment (Fraction 3, Fig. S1; Garau et al., 2019a), suggesting a substantial presence (and accessibility) in soil of sparingly soluble Cu and Pb precipitates, e.g. metal (Me) oxi-hydroxides and/or Me phosphates (Basta and Gradwohl, 2000). As earlier mentioned, organic compounds (e.g. citrate and malate) released by white lupin roots (Yan et al., 2002) in compost-amended soils could be responsible for a partial re-solubilization of such sparingly soluble precipitates, which determined an increase of labile Cu and Pb and a consequent higher uptake of these PTE (Dinkelaker et al., 1989; Ma et al., 2016; Quin et al., 2004; Yan et al., 2002). However, as previously mentioned, labile Cu and Pb significantly decreased in MSWC-amended soils after lupin growth (Fig. S2).

Taken together, the obtained results highlighted an overall decrease of PTE concentration in shoots and roots of *A. donax* and *H. vulgare* in amended soils, while substantial increase of As, Sb, Pb and Cu uptake was noticed for *L. albus* roots (Fig.s 2, 4). In this latter case, PTE immobilization in roots should be regarded as positive since it is contributing to reduce labile PTE in soil (Fig. S2). This finally suggests that all the selected plant species can be eligible as potential candidates for assisted phytostabilization in combination with MSWC.

3.3. Influence of MSWC on PTE bioaccumulation (BAF), translocation (TF), mineralomasses (MM) and remediation (RF) factors

Plant species suitable for phytoremediation programs are primary selected based on their ability to grow satisfactorily in contaminated soils, as well as to preferentially accumulate the contaminants in roots (phytostabilizing plants) or to translocate them in the above ground organs (phytoextracting plants; Sinhal et al., 2015). In order to evaluate the respective phytoremediation potential of *A. donax, H. vulgare* and *L. albus,* PTE bioaccumulation factors (i.e. BAFr and BAFs), translocation factor (TF), mineralomasses (i.e. MMr and MMs) and remediation factors (i.e. RFr and RFs) were determined.

3.3.1. Bioaccumulation factors (BAFr and BAFs)

The bioaccumulation factors BAFr and BAFs, which quantify the plant efficiency to uptake and accumulate the selected PTE in roots or shoots (Bonanno and Vymazal, 2017) were mostly <1 irrespectively of the plant species and MSWC addition (i.e. 0.06 10^{-3} - 0.81). The only exceptions were Zn and Cd BAFr in control plants of barley and lupin (e.g. 1.51 and 2.90 for Zn and Cd in control plants of lupin; Tables 1-3). Overall, this indicated much lower concentrations of PTE in plant tissues than those recorded in soil as well as the low extractive capacity of the selected plants.

The BAF decrease observed in the former plants was attributed to a reduced PTE uptake of plants grown on compost-amended soils. This supported a stable immobilization of the contaminants by MSWC and a negligible influence of the root activity of *A. donax* and *H. vulgare* on PTE re-mobilization phenomema, as highlighted by PTE mobility data recorded after plant growth (Fig. S2). This is relevant as such re-mobilization phenomena are not rare in assisted phytoremediation experiments (e.g. Castaldi et al., 2009). However, significant BAF increases were previously reported for *A. donax* growing on different soils mainly contaminated by As and amended with the same MSWC used in this study (Castaldi et al., 2018). This likely indicates that soil components, including microbial community, can play a major role in mobilization/immobilization phenomena resulting from the combined action of organic-based amendments and root activity. Furthermore, this emphasizes the need of a case by case, or soil by soil, evaluation of the most suitable phytoremediation practices.

Significant increases of As, Sb, Pb and Cu BAF factors (especially BAFr) were recorded for white lupin grown on MSWC-amended soils. This was attributed to the solubilization ability of lupin roots towards these PTE in amended soils. However, it is important to underline the very low BAF values (always <1) detected for all plants which somehow limit the environmental relevance of such increases. An opposite trend was noticed for Cd and Zn (Table 3): the decrease of Cd and Zn BAFr in amended soils (e.g. 2.9 and 1.5 in control in plants vs 0.5 in MSWC-4%) was remarkable as these PTE are commonly very mobile in soil (Adriano et al., 2001).

3.3.2. Translocation factor (TF)

Overall, all TFs were < 1 for control plants (Tables 1-3) indicating that *A. donax*, *H. vulgare* and *L. albus* can be considered as potential phytostabilizing species as previously pointed out by other authors (Bonanno, 2013; Bonanno and Vymazal, 2017; Castaldi et al., 2005, 2018). Moreover, compost had a very limited influence on PTE translocation from roots to shoots, apart from Cd in *A. donax* grown on MSWC-4% soil, where TF increased from 0.51 to 1.17 (Table 1). In particular, TF values remained unchanged (e.g. Cu, Zn) or mostly decreased for *A donax* grown on MSWC-4% soils (e.g. Sb, Pb). However also PTE concentrations in plant tissues were decreased in this plant (Fig. 2), which made these increases irrelevant. TF values in *H. vulgare* and *L. albus* were either slightly increased (e.g. Pb, Cu for barley and As, Cd, Zn for lupin), decreased (e.g. Cd, Zn for barley and Pb and Cu for lupin) or remained unvaried (e.g.

Sb for barley and lupin) (Tables 1-3). These results reflected the different bioaccumulation of PTE in the different parts of the plants grown in the amended and unamended soils.

Taken together, the results obtained suggest that the addition of compost did not substantially influence the PTE partitioning between shoots and roots, with these latter remaining the main sinks of PTE. Despite this, TF values suggested different PTE translocation abilities between plant species, e.g. Pb in *A. donax* vs *H. vulgare*, or Cd in *L. albus* vs *H. vulgare*, which could depend on the species considered, growth stage and root physiology, as well as on distinct rhizosphere microbial communities.

3.3.3. PTE mineralomasses and remediation factors (RF)

PTE mineralomasses (MMr and MMs) quantify the actual PTE amounts bioaccumulated by the plants (Lebrun et al., 2018) and as such they can be useful to estimate the contribution of each species in PTE immobilization. In this sense, also the remediation factors (RFr and RFs) can be helpful, as it indicates how effectively PTE are absorbed by belowground organs.

Although MM values cannot be used to discriminate phytostabilizing from phytoextracting plants, irrespective of plant species, MMr values of control plants were generally higher than MMs ones confirming that PTE were preferentially stored within the root system (Tables 1-3). From an environmental point of view, this can be considered favorable since PTE immobilization belowground limits the risk of contaminants spreading and thus their entry in the food chain. This finally confirms the suitability of giant reed, barley and white lupin as PTE phytostabilizers, while rules out their relevance as phytoextractants.

The addition of MSWC did not change this trend, with the exception of Cd for which MMs > MMr. However, compost addition (especially MSCW-4%) significantly increased both MM of *A. donax* (Table 1) as a likely result of its improved growth in the amended soil. RF values were very low in all plants (Moameri and Khalaki, 2019), however MSWC addition increased the RF of *A. donax*, particularly in plants grown in MSWC-4%, clearly indicating that PTE were extracted more efficiently by *A. donax* grown in compost treated soil.

As regards to *H. vulgare*, MSWC addition mostly reduced MMr and RFr values (Table 2). These results could be due to the reduced root biomass detected in compost-amended plants (Fig. 1). In addition, the biostimulation effect of MSWC on barley shoots was not always accompanied by a parallel MMs and RFs increase (Table 2), thus pointing out to a negligible (or even repressive) effect of MSWC on PTE mobilization and absorption by barley (Fig. S2). However, what observed could be the consequence of a dilution effect, i.e. higher biomass in barley plants grown in the amended soil with the same PTE accumulation capacity of control plants. These results seem to indicate that the MSWC used in this study is likely not the best amendment to combine with barley if increased PTE mineralomasses (MMr in particular) are desired.

Compost addition mostly increased As, Sb and Pb MM values in *L. albus*, while Cu, Cd and Zn MM were mainly reduced, even if they were similar to control plants (Table 3). Also the RF values in MSWC-amended soils varied depending on the PTE considered, i.e. they increased for As, Sb and Pb while decreased for Zn, Cu and Cd.

These data could suggest a better suitability of white lupin, when combined with MSWC, as phytostabilizer for selected PTE, such as As, Sb and Pb.

4. **Conclusions**

The results obtained showed that *A. donax*, *H. vulgare* and *L. albus* were able to grow in a PTE-polluted soil, and that MSWC amendment had different effects on plant growth, i.e. both stimulating and repressive ones. When grown in the presence of MSWC, all plant species but barley improved their PTE phytostabilizing capabilities, overall showing significantly greater PTE bioaccumulation in roots, as well as low translocation from roots to shoots. This suggests that the use of the tested MSWC should be avoided if barley is selected as phytostabilizing plant. On the contrary, MSWC substantially improved the white lupin bioaccumulation performance with respect to selected elements (i.e. As, Sb and Pb). For this reason, *L. albus* could be recommended as phytostabilizer together with MSWC in those cases where As, Sb and Pb are the main soil contaminants, as it was in our case. Finally, *A. donax* which showed consistently higher mineralomasses and lower BAF factors in amended soils, appeared as the best choice for the assisted phytostabilization of the PTE-contaminated soil. The perennial nature of such helophyte grass and its great adaptability to harsh environmental conditions makes this plant as one of the best candidates for MSWC-assisted phytoremediation of PTE contaminated soils.

Credit Author Statement

Matteo Garau: Investigation, Methodology, Formal analysis, Writing - Original draft preparation. **Paola Castaldi:** Conceptualization, Formal analysis, Resources, Writing - Review & Editing. **Stefania Diquattro:** Methodology, Formal analysis. **Maria Vittoria Pinna:** Methodology, Formal analysis. **Caterina Senette:** Methodology. **Pier Paolo Roggero:** Conceptualization, Resources, Project administration. **Giovanni Garau:** Conceptualization, Formal analysis, Writing - Review & Editing.

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References

Abou Jaoude, L., Castaldi, P., Nassif, N., Pinna, M.V., Garau, G., 2020. Biochar and compost as gentle remediation options for the recovery of trace elements-contaminated soils. Sci. Total Environ. 711, 134511.

Adriano, D.C., 2001. Bioavailability of trace metals. Trace Elements in Terrestrial Environments, 2nd ed. Springer, New York, pp. 61–89.

Bacchetta, G., Cappai, G., Carucci, A., Tamburini, E., 2015. Use of native plants for the remediation of abandoned mine sites in mediterranean semiarid environments. Bull. Environ. Contam. Toxicol. 94, 326–333.

Barbafieri, M., Pedron, F., Petruzzelli, G., Rosellini, I., Franchi, E., Bagatin, R., Vocciante, M., 2017. Assisted phytoremediation of a multi-contaminated soil: Investigation on arsenic and lead combined mobilization and removal. J. Environ. Manage. 203, 316–329.

Barbosa, B., Fernando, A.L., 2018. Aided Phytostabilization of Mine Waste, in: Prasad, M.N.V., de Favas, P.J.C., Maiti, S.K. (Ed.), Bio-Geotechnologies for mine site rehabilitation. Elsevier, Amsterdam, pp. 147–157.

Basta, N., Gradwohl, R., 2000. Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soils by a sequential extraction procedure. J. Soil Contam. 9, 149–164.

Bonanno, G., 2013, Comparative performance of trace element bioaccumulation and biomonitoring in the plant species *Typha domingensis*, *Phragmites australis* and *Arundo donax*. Ecotox. Environ. Safe. 97, 124–130.

Bonanno, G., Vymazal, J., 2017, Compartmentalization of potentially hazardous elements in macrophytes: insights into capacity and efficiency of accumulation. J. Geochem. Explor. 181, 22–30.

Carpena, R.O., Vázquez, S., Esteban, E., Fernández-Pascual, M., Rosario, M., Felipe, D., Zornoza, P., 2003. Cadmium-stress in white lupin: effects on nodule structure and functioning. Plant Physiol. Biochem. 41, 911–919.

Castaldi, P., Santona, L., Melis, P., 2005. Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. Chemosphere 60, 365–371.

Castaldi, P., Silvetti, M., Manzano, R., Brundu, G., Roggero, P.P., Garau, G., 2018. Mutual effect of *Phragmites australis*, *Arundo donax* and immobilization agents on arsenic and trace metals phytostabilization in polluted soils. Geoderma 314, 63–72.

Castaldi, P., Silvetti, M., Mele, E., Garau, G., Deiana, S., 2013. Arsenic mobilization by citrate and malate from a red mud–treated contaminated soil. J. Environ. Qual. 42, 774–781.

Castaldi, P., Melis, P., Silvetti, M., Deiana, P., Garau, G., 2009. Influence of pea and wheat growth on Pb, Cd, and Zn mobility and soil biological status in a polluted amended soil. Geoderma 151, 241–248.

Cundy, A., Bardos, P., Puschenreiter, M., Witters, N., Mench, M., Bert, V., Friesl-Hanl W., Müller, I., Weyens, N., Vangronsveld, J., 2015. Developing effective decision support for the application of "gentle" remediation options: The GREENLAND project. Remediation J. 25(3), 101–114.

Dago, À., González, I., Ariño, C., Díaz-cruz, J.M., Esteban, M., 2014. Chemometrics applied to the analysis of induced phytochelatins in *Hordeum vulgare* plants stressed with various toxic non-essential metals and metalloids. Talanta 118, 201–

209.

Diquattro, S., Garau, G., Castaldi, P., 2018. Municipal solid waste compost as a novel sorbent for antimony(V): adsorption and release trials at acidic pH. Environ. Sci. Pollut. Res. 25, 5603–5615.

Diquattro, S., Garau, G., Mangia, N.P., Drigo, B., Lombi, E., Vasileiadis, S., Castaldi, P., 2020. Mobility and potential bioavailability of antimony in contaminated soils: Short-term impact on microbial community and soil biochemical functioning. Ecotox. Environ. Safe. 196, 110576.

Dinkelaker, B., Romheld, V., Marschner H., 1989. Citric-acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.) Plant Cell Environ. 12(3), 285-292.

Dlgs. 152/2006. Decreto legislativo 3 aprile 2006, n. 152. Norme in materia ambientale, G.U. n. 88 del 14 aprile 2006.

Domínguez, M.T., Montiel-Rozas, M.M., Madejón, P., Diaz, M.J., Madejón, E., 2017. The potential of native species as bioenergy crops on trace-element contaminated Mediterranean lands. Sci. Total Environ. 590–591, 29–39.

Duri L.G., Fiorentino N., Cozzolino E., Ottaiano, L., Agrelli, D., Fagnano M., 2018. Bioassays for evaluation of sanitary risks from food crops cultivated in potentially contaminated sites. Ital. J. Agron. 13, 45–52.

Eijsackers, H., 2010. Earthworms as colonisers: Primary colonisation of contaminated land , and sediment and soil waste deposits. Sci. Total Environ. 408, 1759–1769.

Fresno, T., Moreno-Jiménez, E., Zornoza, P., Peñalosa, J.M., 2018. Aided phytostabilisation of As- and Cu-contaminated soils using white lupin and combined iron and organic amendments. J. Environ. Manage. 205, 142–150.

Fumagalli, P., Comolli, R., Ferrè, C., Ghiani, A., Gentili, R., Citterio, S., 2014. The rotation of white lupin (*Lupinus albus,* L.) with metal-accumulating plant crops : A strategy to increase the benefits of soil phytoremediation. J. Environ. Manage. 145, 35–42.

Garau, G., Silvetti, M., Castaldi, P., Mele, E., Deiana, P., Deiana, S., 2014. Stabilising metal(loid)s in soil with iron and aluminium-based products: microbial, biochemical and plant growth impact. J. Environ. Manage. 139, 146–153.

Garau, M., Garau G., Diquattro S., Roggero P.P., Castaldi, P., 2019a, Mobility, bioaccessibility and toxicity of potentially toxic elements in a contaminated soil treated with municipal solid waste compost. Ecotox. Environ. Safe. 186, 109766.

Garau, G., Diquattro, S., Lauro, G.P., Deiana, S., Castaldi, P., 2019b. Influence of Pb(II) in the sorption of As(V) by a Ca-polygalacturonate network, a root mucilage model. Soil Sci. Plant Nutr. 65(3), 305–315.

Garau, G., Silvetti, M., Vasileiadis, S., Donner, E., Diquattro, S., Deiana, S., Lombi, E., Castaldi, P., 2017. Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal (loid)s. Soil Biol. Biochem. 111, 25–35.

Gazzetta Ufficiale, 1992, Metodi ufficiali di analisi chimica dei suoli. DM 11 maggio 1992, suppl. G.U. 121, 25maggio 1992 [Official Gazette of the Italian Republic. Official methods for chemical.

González, Á., García-gonzalo, P., Gil-díaz, M.M., Alonso, J., Lobo, M.C., 2019. Compost-assisted phytoremediation of As-polluted soil. J. Soil. Sediment. 19, 2971–2983.

Gorovtsov, A., Rajput, V., Minkina, T., Mandzhieva, S., Sushkova, S., Kornienko, I., Tatiana, V.G., Chokheli, V., Aleshukina, I., Zinchenko, V., Fedorenko, E., Movsesyan, H., 2019. The role of biochar-microbe interaction in alleviating heavy metal toxicity in *Hordeum vulgare* L. grown in highly polluted soils. Appl. Geochem. 104, 93–101.

Gulías, J., Melis, R., Scordia, D., Cifre, J., Testa, G., Cosentino, S.L. and Porqueddu, C., 2018. Exploring the potential of wild perennial grasses as a biomass source in semi-arid Mediterranean environments. Ital. J. Agron. 13, 937.

Kerley, S., 2000. Changes in root morphology of white lupin (*Lupinus albus* L.) and its adaptation to soils with heterogeneous alkaline/acid profiles. Plant Soil 218, 197–205.

Kumpiene, J., Bert, V., Dimitriou, I., Eriksson, J., Friesl-Hanl, W., Galazka, R., Herzig, R., Janssen, J., Kidd, P., Mench, M., Müller, I., Neu, S., Oustriere, N., Puschenreiter, M., Renella, G., Roumier, P.H., Siebielec, G., Vangronsveld, J., Manier, N., 2014. Selecting chemical and ecotoxicological test batteries for risk assessment of trace element-contaminated soils (phyto)managed by gentle remediation options (GRO). Sci. Total Environ. 496, 510–522.

Hardesty-Moore, M., Orr, D., McCauley, D.J., 2020. Invasive plant Arundo donax alters habitat use by carnivores. Biol. Invasions 22, 1983–1995.

Lebrun, M., Miard, F., Nandillon, R., Hattab-hambli, N., Scippa, G., Bourgerie, S., Morabito, D., 2018. Eco-restoration of a mine technosol according to biochar particle size and dose application: study of soil physico-chemical properties and phytostabilization capacities of *Salix viminalis*. J. Soil Sediment 18, 2188–2202.

Liu, Y., Guo, Z., Xiao, X., Wang, S., Jiang, Z., Zeng, P., 2017. Phytostabilisation potential of giant reed for metals contaminated soil modified with complex organic fertiliser and fly ash : A field experiment. Sci. Total Environ. 576, 292–302.

Llugany, M., Miralles, R., Corrales, I., Barceló, J., Poschenrieder, C., 2012. *Cynara cardunculus* a potentially useful plant for remediation of soils polluted with cadmium or arsenic. J. Geochem. Explor. 123, 122–127.

Ma, Y., Oliveira, R.S., Freitas, H., Zhang, C., 2016. Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. Front. Plant Sci. 7, 918.

Manzano, R., Silvetti, M., Garau, G., Deiana, S., Castaldi, P., 2016. Influence of iron-rich water treatment residues and compost on the mobility of metal(loid)s in mine soils. Geoderma 283, 1–9.

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

Martìnez-Alcalá, I., Walker, D.J., Bernal, M.P., 2010. Chemical and biological properties in the rhizosphere of Lupinus albus alter soil heavy metal fractionation. Ecotox. Environ. Safe. 73, 595–602.

Mâșu, S., Dragomir, N., Morariu, F., 2012. The bioaccumulation of heavy metals in barley (Hordeum vulgare L) cultivated on fly ash dump mixed with compost and natural zeolite materials. Sci. Pap. Anim. Sci. Biotechnol. 45, 237–241.

Moameri, M., Khalaki, M.A., 2019. Capability of Secale montanum trusted for phytoremediation of lead and cadmium in soils amended with nano-silica and municipal solid waste compost. Environ. Sci. Pollut. Res. 26, 24315–24322.

Nehnevajova, E., Ramireddy, E., Stolz, A., Gerdemann-Knörck, M., Novák, O., Strnad, M., Schmülling, T., 2019. Root enhancement in cytokinin-deficient oilseed rape causes leaf mineral enrichment, increases the chlorophyll concentration under nutrient limitation and enhances the phytoremediation capacity. BMC Plant Biology 19, 83.

Pál, M., Janda, T., Szalai, G., 2018. Interactions between plant hormones and thiol-related heavy metal chelators. Plant Growth Regul. 85, 173-185.

Qin, F., Shan, X-Q., Wei, B., 2004. Effects of low-molecular-weight organic acids and residence time on desorption of Cu, Cd, and Pb from soils. Chemosphere 57, 253–263.

Radziemska, M., Gusiatin, Z.M., Bilgin, A., 2017. Potential of using immobilizing agents in aided phytostabilization on simulated contamination of soil with lead. Ecol. Eng. 102, 490–500.

Ruiz-dı, B., Ferna, M., Puertas-mejı, M.A., 2010. Effect of cadmium ion excess over cell structure and functioning of *Zea mays* and *Hordeum vulgare*. Biochem. Syst. Ecol. 38, 285–291.

Sánchez-Pardo, B., Zornoza, P., 2014. Ecotoxicology and environmental safety mitigation of Cu stress by legume–Rhizobium symbiosis in white lupin and soybean plants. Ecotox. Environ. Safe. 102, 1–5.

Silvetti, M., Demurtas, D., Garau, G., Deiana, S., Castaldi, P., 2017. Sorption of Pb, Cu, Cd, and Zn by municipal solid waste composts: metal retention and desorption mechanisms. Clean-Soil Air Water 45, 1600253.

Sinhal, V.K., Srivastava, A., Singh, V.P., 2015. Phytoremediation: a technology to remediate soil contaminated with heavy metals. Intern. J. Green Herbal Chem. 4, 439–460.

Tang, C., Robson, A.D., Longnecker, N.E., Buirchell, B.J., 1995. The growth of Lupinus species on alkaline soils. Aust. J. Agric. Res. 46, 255-68.

Wanty, R.B., Podda, F., De Giudici, G., Cidu, R., Lattanzi, P., 2013. Zinc isotope and transition-element dynamics accompanying hydrozincite biomineralization in the Rio Naracauli, Sardinia, Italy. Chem. Geol. 337-338, 1–10.

Wenzel, W.W., Kirchbaumer, N., Prohaska, T., Stingeder, G., Lombi, E., Adriano, D.C., 2001. Arsenic fractionation in soils using an improved sequential extraction procedure. Anal. Chim. Acta 436, 309-323.

Winer, B.J., 1971. Statistical principles in experimental design (2nd edition). McGraw Hill, New York.

Wong, M.H., 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50, 775–780.

Wu, F., Xu, F., Ma, X., Luo, W., Lou, L., Wong, M.H., 2018. Do arsenate reductase activities and oxalate exudation contribute to variations of arsenic accumulation in populations of Pteris vittata? J. Soil. Sediment. 18, 3177–3185.

Ximénez-Embún, P., Madrid-Albarrán, Y., Cámara, C., Cuadrado, C., Burbano, C., Múzquiz, M., 2001. Evaluation of lupinus species to accumulate heavy metals from waste waters. Int. J. Phytoremediat. 3, 369–379.

Yan, F., Zhu, Y., Muller, C., Zorb, C., Schubert, S., 2002. Adaptation of H^+-pumping and plasma membrane H^+ ATPase activity in proteoid roots of white lupin under phosphate deficiency. Plant Physiol. 129(1), 50–63.

Yang, M., Xiao, X.-Y., Miao, X.-F., Guo, Z-H., Wang, F-Y., 2012. Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead Trans. Nonferrous Met. Soc. China 22, 1462-1469.

Ying, T., Yong-Ming, L., Chang-Yong, H., Jian, L., Zhen-Gao, L., Christie, P. 2008.

Tolerance of grasses to heavy metals and microbial functional diversity in soils contaminated with copper mine tailings. Pedosphere 18, 363–370.

Zeng, P., Guo, Z. Xiao, X., Peng, C., Huang, B., Feng, W., 2019. Ecotoxicology and environmental safety complementarity of co-planting a hyperaccumulator with three metal(loid)-tolerant species for metal(loid)-contaminated soil remediation. Ecotox. Environ. Safe. 169, 306–315.

Zornoza, P., Pe, J.M., Fresno, T., Moreno-Jim, E., 2018. Aided phytostabilisation of As- and Cu-contaminated soils using white lupin and combined iron and organic amendments. J. Environ. Manage. 205, 142–150.

Figure captions

Fig. 1. Roots and shoots dry weight of *A. donax, H. vulgare* and *L. albus* grown on the unamended and amended soils. For each part of plant species, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Fig. 2. Concentrations of As and Sb in roots and shoots of *A. donax, H. vulgare* and *L. albus* grown on the amended and unamended soils. For each plants part, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Fig. 3. Concentrations of Cd and Zn in roots and shoots of *A. donax*, *H. vulgare* and *L. albus* grown on the amended and unamended soils. For each plants part, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Fig. 4. Concentrations of Pb and Cd in roots and shoots of *A. donax*, *H. vulgare* and *L. albus* grown on the amended and unamended soils. For each plants part, mean values followed by different letters denote statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Fig. S1. PTE released after sequential extraction of soils before plant growth. For each PTE and within each fraction, bars with different letters denote statistically significant differences at $P < 0.05$ according to the post-hoc Fisher's least significant difference test. Graphs are from Garau et al. (2019) Mobility, bioaccessibility and toxicity of potentially toxic elements in a contaminated soil treated with municipal solid waste compost. Ecotoxicology and Environmental Safety 186 (2019) 109766. https://doi.org/10.1016/j.ecoenv.2019.109766

Fig. S2. Labile (i.e. water-soluble and exchangeable) PTE extracted from the different soils after plant growth (mean values ± standard deviations; n = 3). For each PTE and plant species, bars with different letters indicate statistically significant differences according to the Fisher's least significant difference test ($P < 0.05$).

Figure

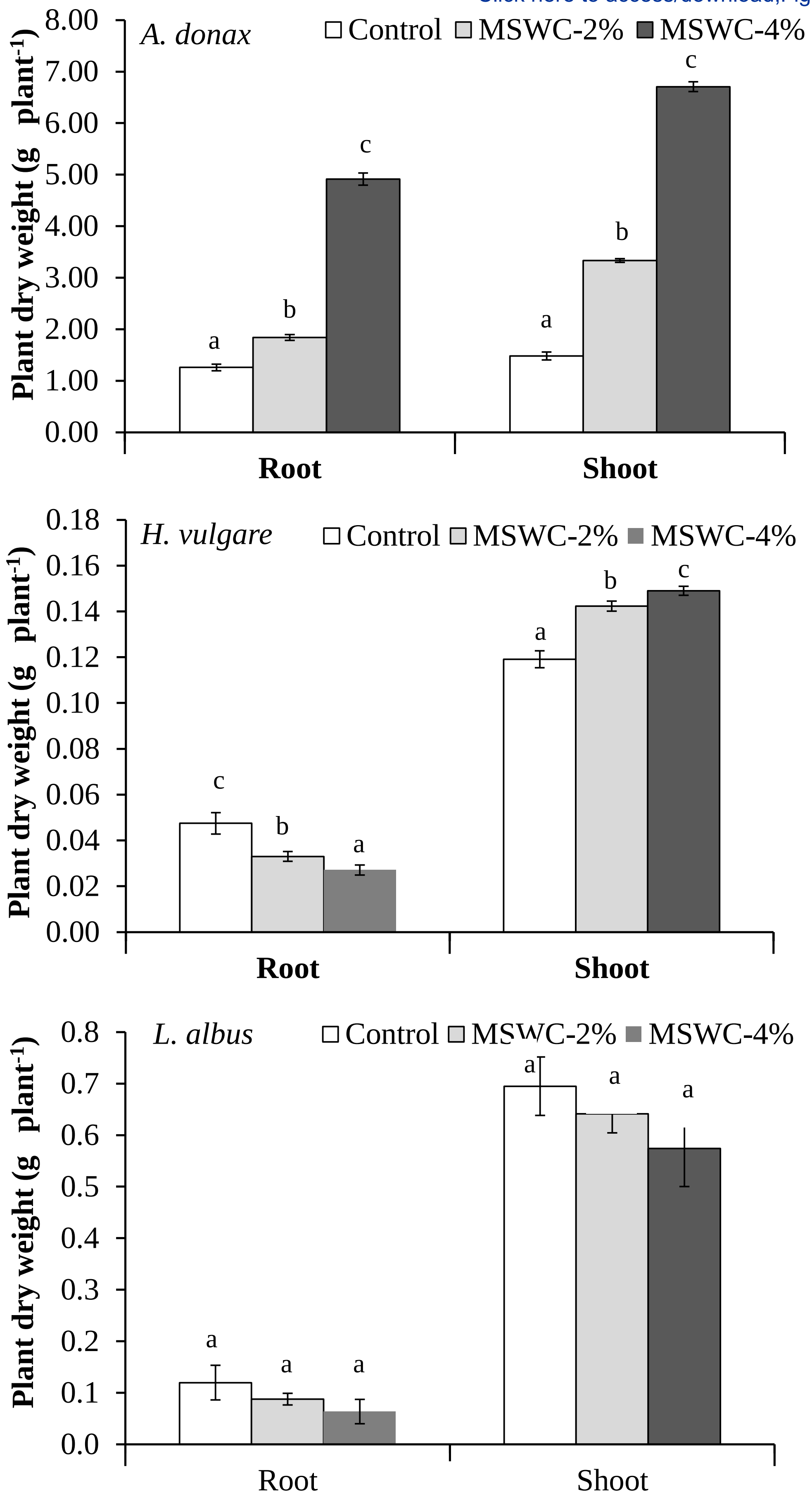
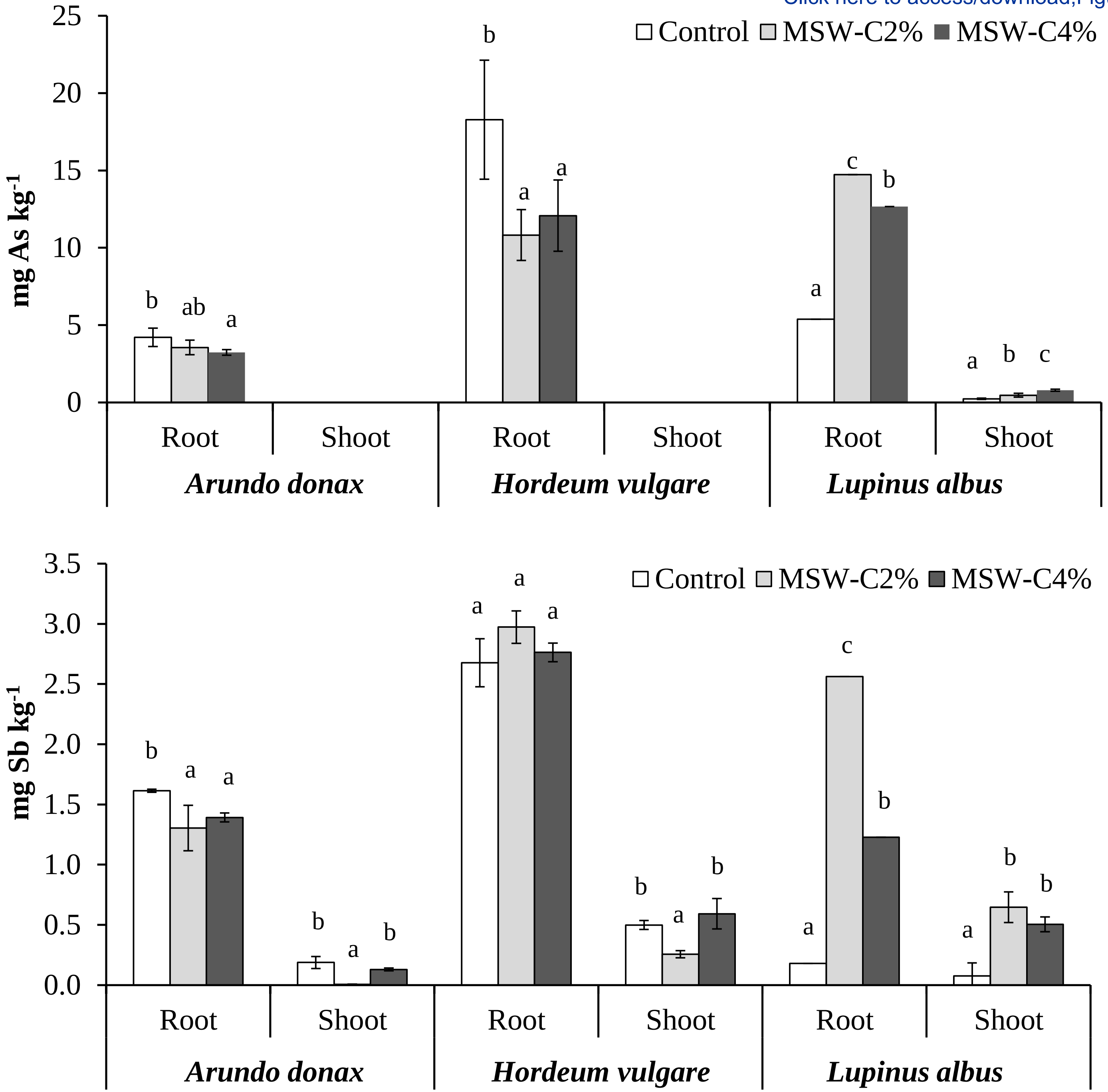


Figure 1


mg As kg^{-1}
25
20
15
10
5
0
Control
MSW-C2%
MSW-C4%
b
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a
b
a
a
a
c
b
a
b
c
Root
Shoot
Root
Shoot
Root
Shoot
Arundo donax
Hordeum vulgare
Lupinus albus
mg Sb kg^{-1}
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
MSW-C2%
MSW-C4%
b
a
a
b
a
b
a
a
a
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a
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Root
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Arundo donax
Hordeum vulgare
Lupinus albus

Figure 2

Figure

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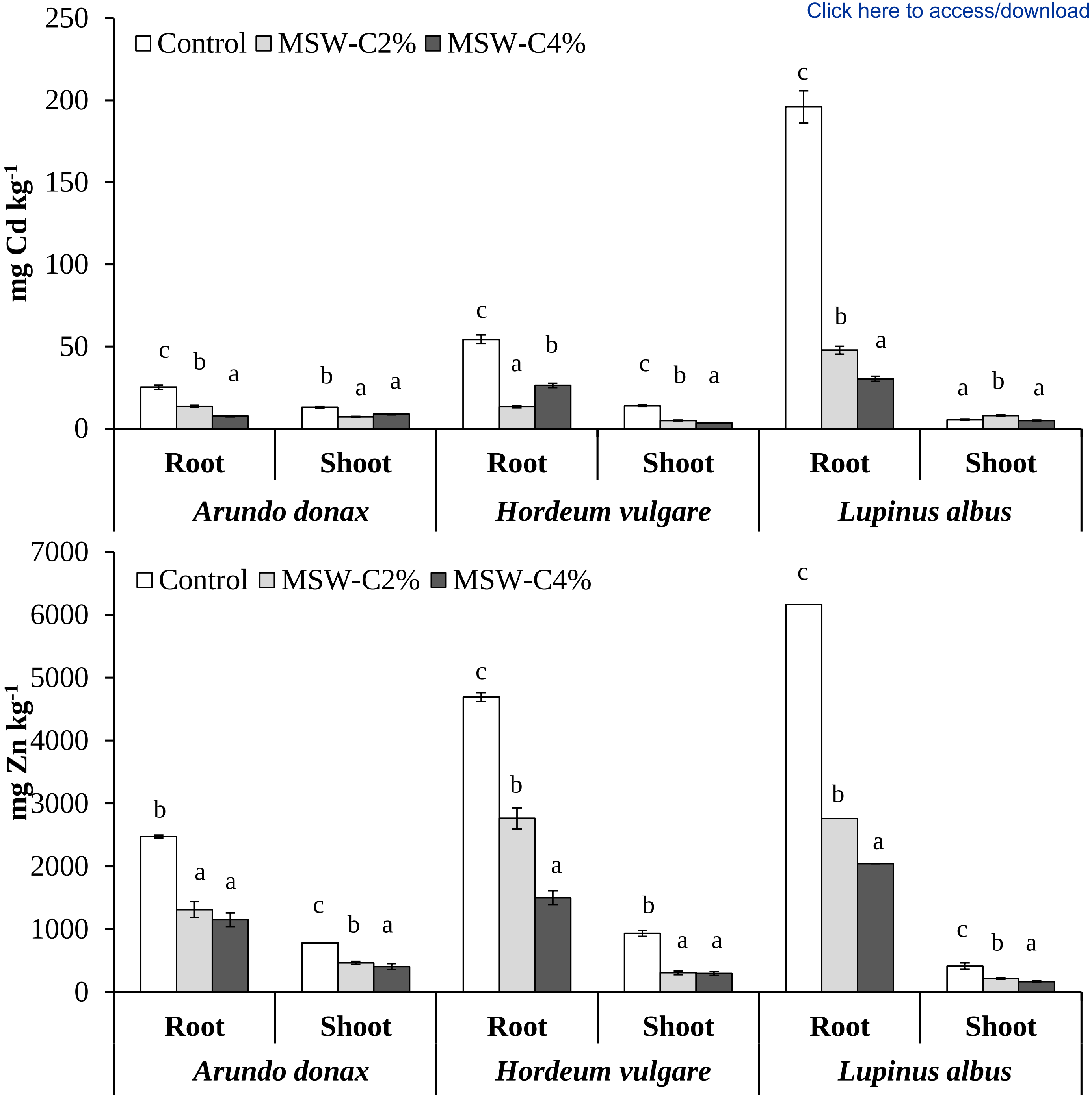

Figure 3

Figure

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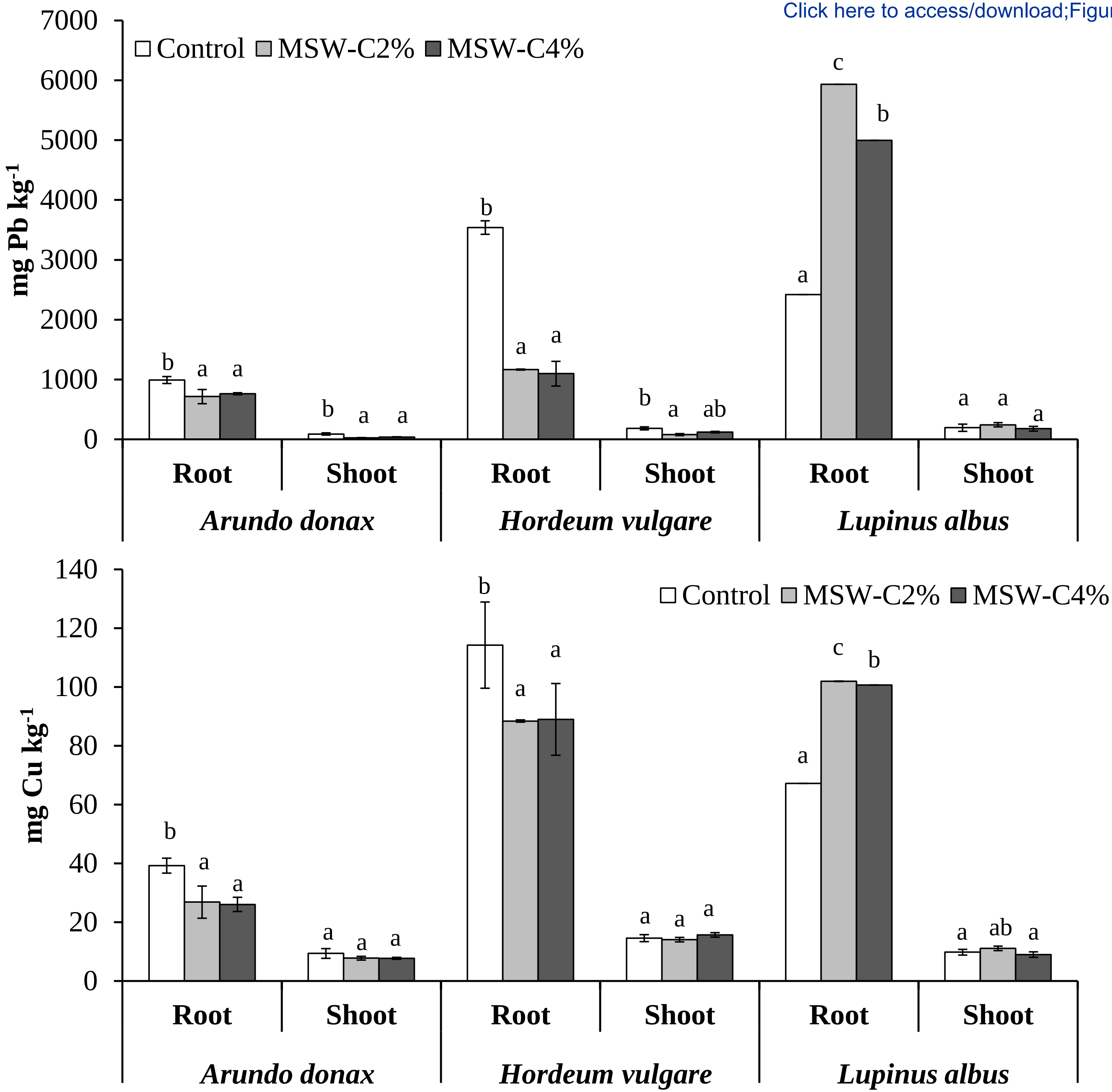
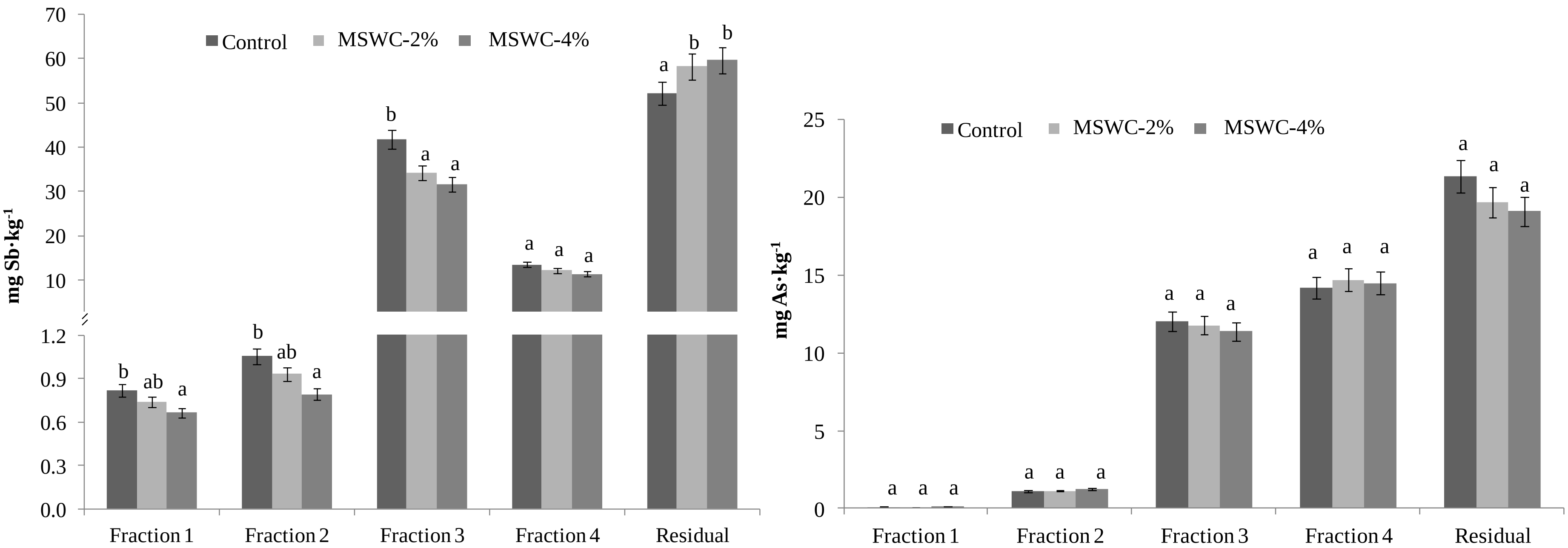


Figure 4



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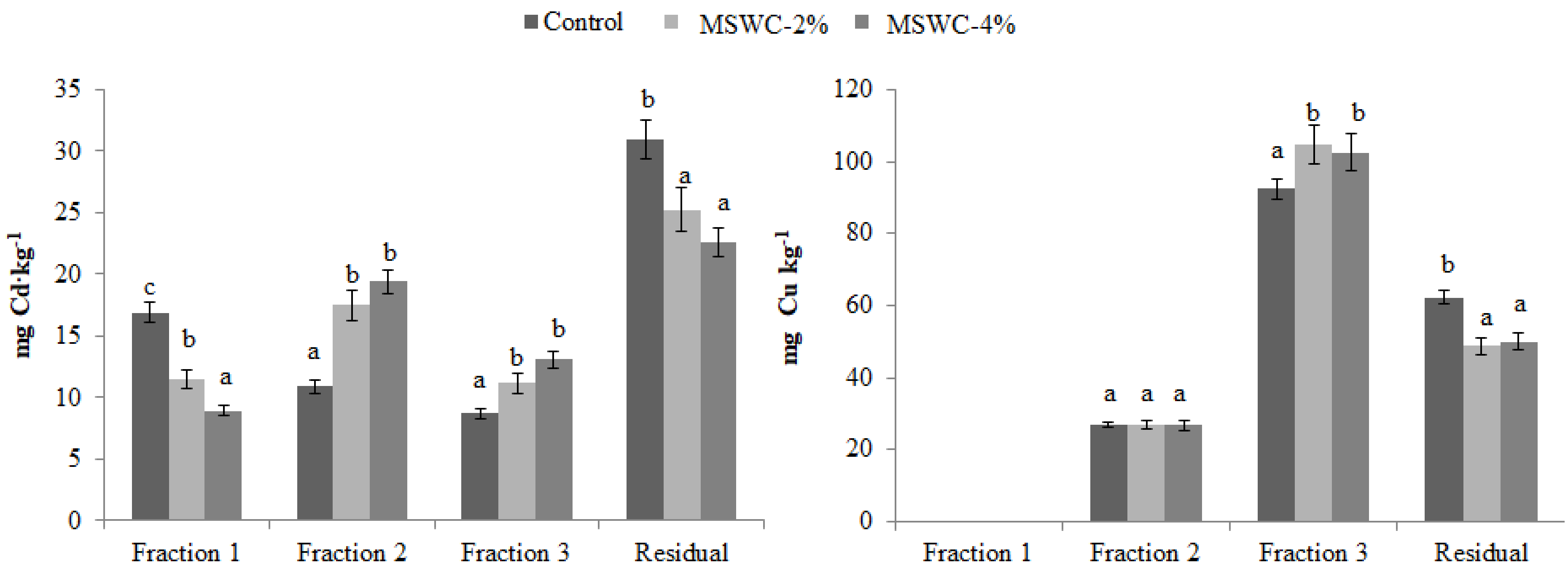
Fraction 1. Water soluble and exchangeable As and Sb extracted with 0.05M $(NH_4)_2SO_4$
Fraction 2. Specifically sorbed As and Sb extracted with 0.05M $NH_4H_2PO_4$
Fraction 3. As and Sb associated with amorphous Fe and Al hydrous oxides extracted with 0.2M NH_4^+-oxalate buffer solution
Fraction 4. As and Sb associated with crystallized Fe and Al hydrous oxides extracted with 0.2M NH_4^+-oxalate buffer + 0.1M ascorbic acid solution
Residual. As and Sb released after acid digestion with H_2O_2 and HNO_3 + HCl (3:1 ratio).
All the relevant details on the sequential extraction applied can be found at https://doi.org/10.1016/j.ecoenv.2019.109766.



Fraction 1. Water soluble and exchangeable Cd and Cu extracted with 0.5M $Ca(NO_3)_2$
Fraction 2. Cd and Cu forming weak surface complexes extracted with 1M NaOAc solution at pH 5.0
Fraction 3. Surface complexed and precipitated Cd and Cu extracted with 0.1M Na_2EDTA solution
Residual. Cd and Cu released after acid digestion with H_2O_2 and HNO_3 + HCl (3:1 ratio).

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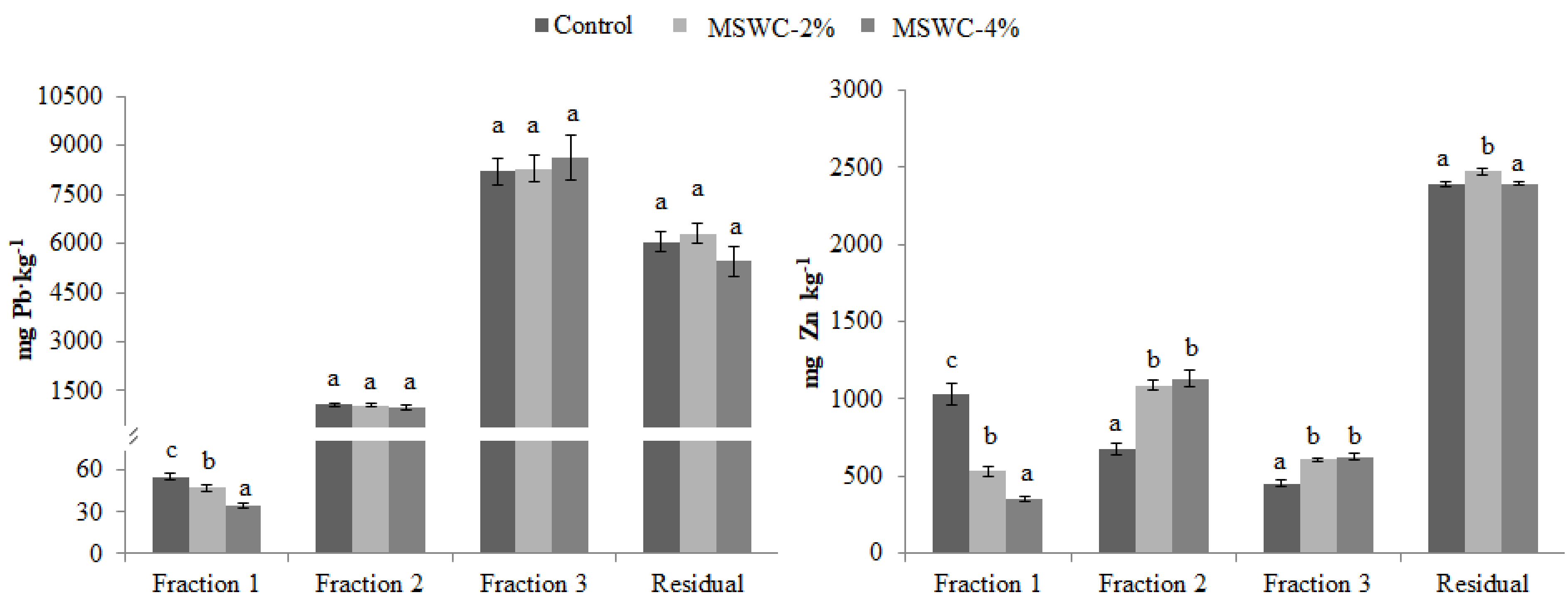
Figure S1

Continued



Fraction 1. Water soluble and exchangeable Pb and Zn extracted with 0.5M $Ca(NO_3)_2$
Fraction 2. Pb and Zn forming weak surface complexes extracted with 1M NaOAc solution at pH 5.0
Fraction 3. Surface complexed and precipitated Pb and Zn extracted with 0.1M Na_2EDTA solution
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Figure S1

Figure

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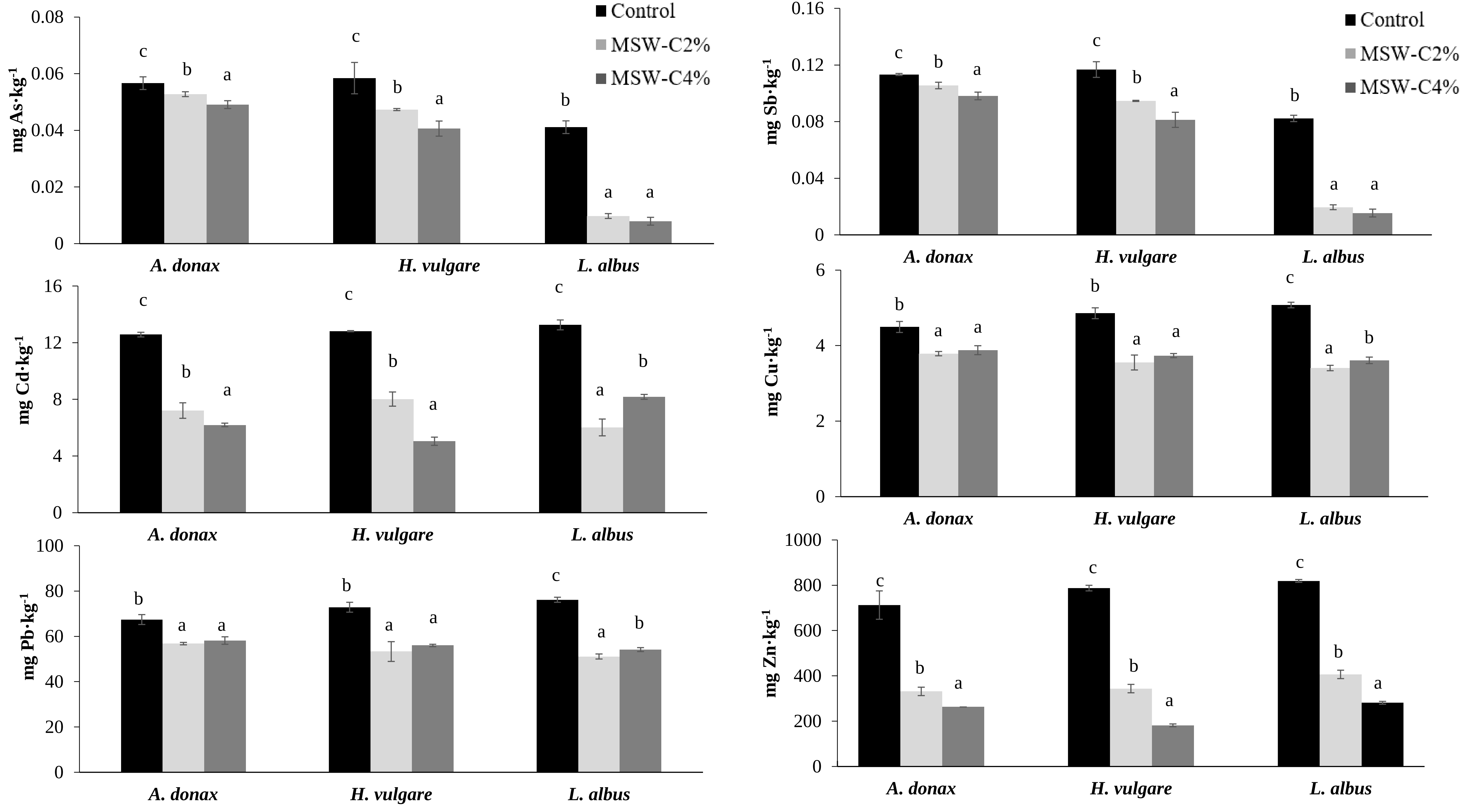

Figure S2. Labile (i.e. water-soluble and exchangeable) PTE extracted from the different soils after plant growth (mean values ± standard deviations; n = 3). For each PTE and plant species, bars with different letters indicate statistically significant differences according to the Fisher's least significant difference test ($P < 0.05$).

Figure S2

Table 1

Bioaccumulation (BAFr and BAFs), translocation (TF) and remediation (RFr and RFs) factors and mineralomasses (MMr and MMs) of PTE in *A. donax*.

		Arundo donax						
		BAFr	BAFs	TF	MMr	MMs	RFr	RFs
As	Control	0.09^{a}	-	-	$5.30\cdot10^{-3\,a}$	-	$3.62\cdot10^{-3\,a}$	-
	MSWC-2%	0.08^{a}	-	-	$6.53\cdot10^{-3\,a}$	-	$4.60\cdot10^{-3\,a}$	-
	MSWC-4%	0.07^{a}	-	-	$15.90\cdot10^{-3\,b}$	-	$11.44\cdot10^{-3\,b}$	-
Sb	Control	$14.74\cdot10^{-3\,b}$	$1.71\cdot10^{-3\,b}$	0.12^{c}	$2.03\cdot10^{-3\,a}$	$0.28\cdot10^{-3\,a}$	$0.62\cdot10^{-3\,a}$	$0.08\cdot10^{-3\,b}$
	MSWC-2%	$12.28\cdot10^{-3\,a}$	$0.06\cdot10^{-3\,a}$	$4.51\cdot10^{-3\,a}$	$2.40\cdot10^{-3\,a}$	$0.02\cdot10^{-3\,a}$	$0.75\cdot10^{-3\,a}$	$0.01\cdot10^{-3\,a}$
	MSWC-4%	$13.38\cdot10^{-3\,ab}$	$1.24\cdot10^{-3\,a}$	0.09^{b}	$6.84\cdot10^{-3ab}$	$0.87\cdot10^{-3\,b}$	$2.19\cdot10^{-3\,b}$	$0.28\cdot10^{-3\,c}$
Cd	Control	0.37^{c}	0.19^{b}	0.51^{a}	$31.72\cdot10^{-3\,b}$	$19.25\cdot10^{-3\,a}$	$15.68\cdot10^{-3\,a}$	$9.52\cdot10^{-3\,a}$
	MSWC-2%	0.21^{b}	0.11^{a}	0.52^{a}	$25.04\cdot10^{-3\,a}$	$23.68\cdot10^{-3\,a}$	$12.74\cdot10^{-3\,a}$	$12.07\cdot10^{-3\,a}$
	MSWC-4%	0.12^{a}	0.14^{a}	1.17^{b}	$37.39\cdot10^{-3\,c}$	$58.95\cdot10^{-3\,b}$	$19.45\cdot10^{-3\,b}$	$30.66\cdot10^{-3\,b}$
Zn	Control	0.61^{b}	0.19^{b}	0.32^{a}	3.11^{b}	1.16^{a}	0.03^{a}	$9.45\cdot10^{-3\,a}$
	MSWC-2%	0.31^{a}	0.11^{a}	0.36^{a}	2.41^{a}	1.54^{a}	0.02^{a}	$12.08\cdot10^{-3\,a}$
	MSWC-4%	0.28^{a}	0.10^{a}	0.35^{a}	5.65^{c}	2.70^{b}	0.05^{b}	$22.06\cdot10^{-3\,b}$
Pb	Control	0.06^{b}	$5.77\cdot10^{-3\,c}$	0.09^{b}	1.25^{a}	0.13^{a}	$2.71\cdot10^{-3\,a}$	$0.28\cdot10^{-3\,ab}$
	MSWC-2%	0.05^{a}	$1.87\cdot10^{-3\,a}$	0.04^{a}	1.32^{a}	0.10^{a}	$2.79\cdot10^{-3\,a}$	$0.21\cdot10^{-3\,a}$
	MSWC-4%	0.05^{a}	$2.23\cdot10^{-3\,b}$	0.04^{a}	3.74^{b}	0.23^{b}	$8.26\cdot10^{-3\,b}$	$0.50\cdot10^{-3\,b}$
Cu	Control	0.22^{b}	0.05^{b}	0.24^{a}	0.05^{a}	$13.81\cdot10^{-3\,a}$	$9.06\cdot10^{-3\,a}$	$2.54\cdot10^{-3\,a}$
	MSWC-2%	0.15^{a}	0.04^{a}	0.30^{a}	0.05^{a}	$25.87\cdot10^{-3\,a}$	$9.12\cdot10^{-3\,a}$	$4.78\cdot10^{-3\,b}$
	MSWC-4%	0.14^{a}	0.04^{a}	0.30^{a}	0.13^{b}	$51.39\cdot10^{-3\,b}$	$23.73\cdot10^{-3\,b}$	$9.55\cdot10^{-3\,c}$

For each PTE, BAF, TF and MM values followed by different letters indicate statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Table 2

Bioaccumulation (BAFr and BAFs), translocation (TF) and remediation (RFr and RFs) factors and mineralomasses (MMr and MMs) of PTE in *H. vulgare*.

		Hordeum vulgare						
		BAFr	BAFs	TF	MMr	MMs	RFr	RFs
As	Control	0.29 ^{a}	-	-	0.87·10-3 ^{b}	-	$1.19·10^{-3}$ ^{b}	-
	MSWC-2%	0.23 ^{a}	-	-	0.29·10-3 ^{a}	-	$0.50·10^{-3}$ ^{a}	-
	MSWC-4%	0.26 ^{a}	-	-	0.40·10-3 ^{a}	-	$0.47·10^{-3}$ ^{a}	-
Sb	Control	0.02 ^{a}	$4.55·10^{-3}$ ^{b}	0.19 ^{b}	$0.13·10^{-3}$ ^{b}	$0.06·10^{-3}$ ^{b}	$0.08·10^{-3}$ ^{c}	$0.04·10^{-3}$ ^{b}
	MSWC-2%	0.03 ^{a}	$2.41·10^{-3}$ ^{a}	0.09 ^{a}	$0.08·10^{-3}$ ^{a}	$0.04·10^{-3}$ ^{a}	$0.06·10^{-3}$ ^{b}	$0.02·10^{-3}$ ^{a}
	MSWC-4%	0.03 ^{a}	$5.69·10^{-3}$ ^{b}	0.21 ^{b}	$0.09·10^{-3}$ ^{a}	$0.09·10^{-3}$ ^{c}	$0.05·10^{-3}$ ^{a}	$0.06·10^{-3}$ ^{b}
Cd	Control	0.81 ^{c}	0.21 ^{c}	0.26 ^{b}	$2.58·10^{-3}$ ^{c}	$1.66·10^{-3}$ ^{c}	$2.55·10^{-3}$ ^{c}	$1.64·10^{-3}$ ^{b}
	MSWC-2%	0.20 ^{a}	0.08 ^{b}	0.37 ^{c}	$0.36·10^{-3}$ ^{a}	$0.70·10^{-3}$ ^{b}	$0.45·10^{-3}$ ^{a}	$0.71·10^{-3}$ ^{a}
	MSWC-4%	0.41 ^{b}	0.05 ^{a}	0.13 ^{a}	$0.87·10^{-3}$ ^{b}	$0.52·10^{-3}$ ^{a}	$0.74·10^{-3}$ ^{b}	$0.54·10^{-3}$ ^{a}
Zn	Control	1.15 ^{c}	0.23 ^{b}	0.20 ^{b}	0.22 ^{c}	0.11 ^{b}	$3.64·10^{-3}$ ^{c}	$1.82·10^{-3}$ ^{b}
	MSWC-2%	0.65 ^{b}	0.07 ^{a}	0.11 ^{a}	0.07 ^{b}	0.04 ^{a}	$1.43·10^{-3}$ ^{b}	$0.68·10^{-3}$ ^{a}
	MSWC-4%	0.37 ^{a}	0.07 ^{a}	0.21 ^{b}	0.05 ^{a}	0.04 ^{a}	$0.66·10^{-3}$ ^{a}	$0.72·10^{-3}$ ^{a}
Pb	Control	0.23 ^{b}	$12.00·10^{-3}$ ^{b}	0.05 ^{a}	0.17 ^{b}	0.02^{b}	$0.73·10^{-3}$ ^{b}	$0.10·10^{-3}$ ^{b}
	MSWC-2%	0.07 ^{a}	$5.04·10^{-3}$ ^{a}	0.07 ^{b}	0.03 ^{a}	0.01^{a}	$0.16·10^{-3}$ ^{a}	$0.05·10^{-3}$ ^{a}
	MSWC-4%	0.07 ^{a}	$8.05·10^{-3}$ ^{ab}	0.11 ^{c}	0.04 ^{a}	0.02^{ab}	$0.13·10^{-3}$ ^{a}	$0.08·10^{-3}$ ^{ab}
Cu	Control	0.63 ^{b}	0.08 ^{a}	0.13 ^{a}	$5.42·10^{-3}$ ^{b}	$1.73·10^{-3}$ ^{a}	$1.99·10^{-3}$ ^{b}	$0.64·10^{-3}$ ^{a}
	MSWC-2%	0.49 ^{a}	0.08 ^{a}	0.16 ^{ab}	$2.39·10^{-3}$ ^{a}	$2.00·10^{-3}$ ^{b}	$1.08·10^{-3}$ ^{a}	$0.74·10^{-3}$ ^{b}
	MSWC-4%	0.50 ^{a}	0.09 ^{a}	0.18 ^{b}	$2.94·10^{-3}$ ^{a}	$2.33·10^{-3}$ ^{c}	$0.90·10^{-3}$ ^{a}	$0.87·10^{-3}$ ^{c}

For each PTE, BAF, TF and MM values followed by different letters indicate statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Table 3.

Bioaccumulation (BAFr and BAFs), translocation (TF) and remediation (RFr and RFs) factors and mineralomasses (MMr and MMs) of PTE in *L. albus*.

		Lupinus albus						
		BAFr	BAFs	TF	MMr	MMs	RFr	RFs
As	Control	0.17^{a}	$4.72\cdot10^{-3\ a}$	0.04^{b}	$0.64\cdot10^{-3\ a}$	$0.16\cdot10^{-3\ a}$	$0.44\cdot10^{-3\ a}$	$0.11\cdot10^{-3\ a}$
	MSWC-2%	0.31^{b}	$9.69\cdot10^{-3\ b}$	0.03^{a}	$1.29\cdot10^{-3\ c}$	$0.29\cdot10^{-3\ b}$	$0.91\cdot10^{-3\ c}$	$0.21\cdot10^{-3\ b}$
	MSWC-4%	0.27^{ab}	$17.07\cdot10^{-3\ c}$	0.06^{c}	$0.80\cdot10^{-3\ b}$	$0.45\cdot10^{-3\ c}$	$0.58\cdot10^{-3\ b}$	$0.33\cdot10^{-3\ c}$
Sb	Control	$1.65\cdot10^{-3\ a}$	$0.70\cdot10^{-3\ a}$	0.43^{b}	$0.02\cdot10^{-3\ a}$	$0.05\cdot10^{-3\ a}$	$0.01\cdot10^{-3\ a}$	$0.02\cdot10^{-3\ a}$
	MSWC-2%	0.02^{c}	$6.08\cdot10^{-3\ b}$	0.25^{a}	$0.22\cdot10^{-3\ c}$	$0.41\cdot10^{-3\ c}$	$0.07\cdot10^{-3\ c}$	$0.13\cdot10^{-3\ c}$
	MSWC-4%	0.01^{b}	$4.84\cdot10^{-3\ b}$	0.41^{b}	$0.08\cdot10^{-3\ b}$	$0.29\cdot10^{-3\ b}$	$0.02\cdot10^{-3\ b}$	$0.09\cdot10^{-3\ b}$
Cd	Control	2.90^{c}	0.08^{a}	0.03^{a}	$23.41\cdot10^{-3\ c}$	$3.72\cdot10^{-3\ ab}$	$11.57\cdot10^{-3\ c}$	$1.84\cdot10^{-3\ a}$
	MSWC-2%	0.73^{b}	0.12^{b}	0.17^{b}	$4.20\cdot10^{-3\ b}$	$5.09\cdot10^{-3\ b}$	$2.14\cdot10^{-3\ b}$	$2.59\cdot10^{-3\ b}$
	MSWC-4%	0.47^{a}	0.08^{a}	0.16^{b}	$1.92\cdot10^{-3\ a}$	$2.83\cdot10^{-3\ a}$	$1.00\cdot10^{-3\ a}$	$1.47\cdot10^{-3\ a}$
Zn	Control	1.51^{c}	0.10^{b}	0.07^{a}	0.74^{c}	0.29^{c}	$6.03\cdot10^{-3\ c}$	$2.33\cdot10^{-3\ c}$
	MSWC-2%	0.65^{b}	0.05^{a}	0.08^{b}	0.24^{b}	0.14^{b}	$1.90\cdot10^{-3\ b}$	$1.07\cdot10^{-3\ b}$
	MSWC-4%	0.50^{a}	0.04^{a}	0.08^{b}	0.13^{a}	0.09^{a}	$1.06\cdot10^{-3\ a}$	$0.76\cdot10^{-3\ a}$
Pb	Control	0.16^{a}	0.01^{a}	0.08^{b}	0.29^{a}	0.14^{ab}	$0.63\cdot10^{-3\ a}$	$0.29\cdot10^{-3\ ab}$
	MSWC-2%	0.38^{c}	0.02^{a}	0.04^{a}	0.52^{c}	0.16^{b}	$1.11\cdot10^{-3\ c}$	$0.33\cdot10^{-3\ b}$
	MSWC-4%	0.33^{b}	0.01^{a}	0.04^{a}	0.32^{b}	0.10^{a}	$0.70\cdot10^{-3\ b}$	$0.23\cdot10^{-3\ a}$
Cu	Control	0.37^{a}	0.05^{a}	0.15^{b}	$8.03\cdot10^{-3\ b}$	$6.79\cdot10^{-3\ b}$	$1.47\cdot10^{-3\ b}$	$1.25\cdot10^{-3\ b}$
	MSWC-2%	0.57^{c}	0.06^{b}	0.11^{a}	$8.95\cdot10^{-3\ b}$	$7.10\cdot10^{-3\ b}$	$1.65\cdot10^{-3\ c}$	$1.31\cdot10^{-3\ b}$
	MSWC-4%	0.56^{b}	0.05^{a}	0.09^{a}	$6.39\cdot10^{-3\ a}$	$5.13\cdot10^{-3\ a}$	$1.19\cdot10^{-3\ a}$	$0.95\cdot10^{-3\ a}$

For each PTE, BAF, TF and MM values followed by different letters indicate statistically significant differences according to the Fisher's Least Significant Difference (LSD) test ($P < 0.05$).

Table S1.

Characteristics of MSWC, untreated polluted (control) and MSWC-amended soils (dry matter basis) before plant growth (mean values ± standard deviations; n = 3). For each row, and only for soils, mean values followed by different letters indicate statistically significant differences according to the Fisher's least significant difference test ($P < 0.05$).

	MSWC	Control soil	MSWC-2%	MSWC-4%
pH	7.93	5.93^{a}	6.53^{b}	6.78^{c}
Electric Conductivity (EC, mS cm^{-1})	3.26	1.34^{a}	1.75^{b}	2.07^{c}
Total organic matter (%)	47.45	3.24^{a}	3.99^{b}	4.85^{c}
Total N (%)	2.18	0.13^{a}	0.17^{b}	0.23^{c}
Dissolved organic carbon (DOC, mg·g^{-1})	0.817	0.11^{a}	0.25^{b}	0.48^{c}
P available (mg·kg^{-1})	62.24	22.62^{a}	32.83^{b}	44.82^{c}
Cation Exchange capacity (CEC, cmol$_{(+)}$·kg^{-1})	92.30	36.86^{a}	39.01^{ab}	42.04^{b}
Exchangeable Na (cmol$_{(+)}$·kg^{-1})	-	0.44^{a}	0.79^{b}	1.24^{c}
Exchangeable K (cmol$_{(+)}$·kg^{-1})	-	1.03^{a}	1.55^{b}	2.09^{c}
Exchangeable Ca (cmol$_{(+)}$·kg^{-1})	-	22.84^{a}	25.91^{ab}	27.92^{b}
Exchangeable Mg (cmol$_{(+)}$·kg^{-1})	-	10.88^{a}	9.72^{a}	9.95^{a}
Total PTE (mg·kg^{-1})				
As	n.d.	48.75^{a}	47.29^{a}	46.31^{a}
Cd	n.d.	67.45^{a}	65.43^{b}	64.08^{b}
Cu	19.24	181.45^{a}	180.34^{a}	179.44^{a}
Pb	3.72	15,383^{a}	15,690^{a}	15,116^{a}
Sb	n.d.	109.48^{b}	106.20^{a}	104.01^{a}
Zn	30.52	4,076^{a}	4,261^{a}	4,082^{a}

n.d. = not detected.

Data are from Garau et al. (2019) Mobility, bioaccessibility and toxicity of potentially toxic elements in a contaminated soil treated with municipal solid waste compost. Ecotoxicology and Environmental Safety 186 (2019) 109766. https://doi.org/10.1016/j.ecoenv.2019.109766.

Table S2

Main chemical characteristics of the untreated polluted (control) and MSWC-amended soils (dry matter basis) after plant growth (mean values ± standard deviations; n = 3). For each row mean values followed by different letters indicate statistically significant differences according to Fisher's least significant difference test ($P < 0.05$).

	Plant	Control	MSW-C2%	MSW-C4%
pH_{H2O}	*A. donax*	6.35±0.01 a	6.89±0.01 b	7.21±0.02 c
	H. vulgare	6.37±0.02 a	6.98±0.02 b	7.27±0.02 c
	L. albus	6.09±0.03 a	6.82±0.04 b	7.01±0.01c
EC (mS cm^{-1})	*A. donax*	0.75±0.02 a	0.77±0.02 a	0.88±0.02 b
	H. vulgare	1.49±0.00 b	1.51±0.01 b	1.33±0.02 a
	L. albus	1.05±0.06 b	0.73±0.08 a	1.56±0.05 c
Total organic matter (%)	*A. donax*	3.24±0.04 a	3.80±0.04 b	4.59±0.08 c
	H. vulgare	3.42±0.06 a	3.97±0.1 b	4.64±0.01 c
	L. albus	3.84±0.07 a	4.31±0.11 b	4.91±0.06 c
Total N (%)	*A. donax*	0.12±0.01 a	0.15±0.00 b	0.21±0.00 c
	H. vulgare	0.11±0.00 a	0.16±0.00 b	0.20±0.00 c
	L. albus	0.14±0.00 a	0.18±0.00 b	0.22±0.00 c
DOC (mg·g^{-1})	*A. donax*	0.05±0.00 a	0.09±0.00 b	0.09±0.00 b
	H. vulgare	0.07±0.00 c	0.04±0.00 b	0.02±0.00 a
	L. albus	0.05±0.00 a	0.14±0.00 b	0.26±0.00c

Declaration of interests

x The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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