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Phytostabilization of As, Sb and Pb by two willow species (*S. viminalis* and *S. purpurea*) on former mine technosols

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Abstract

It is becoming increasingly popular to use phytoremediation methods for the reclamation of mine sites containing metal(loid)s. This study aims to assess the phytostabilization ability of two willow species (*Salix viminalis* and *Salix purpurea*) on technosols from a former gold mining site presenting polymetallic contamination (As, Sb and Pb). Different reconstituted soils using a mixture of the litter and the technosol collected on this previously mined site and a non-contaminated control garden soil were investigated in a

laboratory mesocosm. The physico-chemical soil parameters and the total metal(loid)s content in pore water were determined. After 45 days of growth, roots, leaves and stems from the two willow species were harvested. The biomass, metal(loid) concentrations and the mineral mass were determined for the different plant organs. Both *Salix* species were able to develop root systems on the different reconstituted soils and a variable aboveground biomass according to the soil composition. As, Sb and Pb accumulated mainly in the *Salix* rhizosphere. *S. purpurea* was more efficient in accumulating As in the plant's upper parts than *S. viminalis*. *S. viminalis* showed an ability to transfer Pb and Sb to its shoots whereas *S. purpurea* did not translocate these elements.

Abbreviations:

G: Garden soil

T: Technosol

L: Litter

PTTE: Potentially Toxic Trace Elements

SPW: soil pore water

1. Introduction

At the interface with the atmosphere, biosphere, hydrosphere and lithosphere, soil is important for the flora, for the fauna and for human activities (concept of "critical zone," Brantley et al., 2006). Since the beginning of industrialization in the 19th century, the development of human activities has induced soil contamination by metal(loid)s, thereby

generating both sanitary and ecotoxicological risks. Mining activities and metal processing industry are great concerns, generating potentially toxic trace elements (PTTE) rich-wastes (Shu et al., 2001; Schuwirth et al., 2007). In particular, the ore extraction process is accompanied by rock extraction which contains mineral phases potentially rich in metals and metalloids (Davies 1983). One of the resulting effects is the formation of mining wastes stored as tailings. In the case of gold mining, metal extraction is often associated with arsenic-rich arsenopyrites tailings which can induce potential acid drainage in the environment.

The potential transfer of these PTTE to vegetation (phytoavailability) or any dispersion in the environment could be limited and better managed. In a context of ecological risk assessment of former mining and industrial sites, it is essential to develop knowledge (i) of the behavior of metal(loid)s in various abiotic and biotic compartments of contaminated technosols, (ii) to define the mechanisms inducing their transfers and their ability to bioaccumulate, and *in fine* (iii) their toxic and ecotoxicological effects at the different biological integration levels (Morel, 1997; Hazrat et al., 2013). Mobility is defined as the ability for an element to move from a soil compartment to another one (exchangeable forms, minerals, oxides / hydroxides, organic molecules) and the bioavailability of an element corresponds to its ability to move from the soil compartment to a living organism (Juste, 1988). Moreover even if the total content of a metal(loid) in a soil is high, its bioavailability could be limited. Therefore knowledge of the quantity of metal(loid)s and their physical and chemical forms is paramount to understanding the transfer to and the accumulation within living species. Many factors can influence the metal(loid)s' mobility in soil such as pH and organic composition and concentration (Bolan et al., 2014). When metal(loid)s are easily bioavailable, they can be absorbed by crops and disseminate along the food chain, ultimately damaging health by causing mutagenic and carcinogenic effects (Hazrat et al., 2013).

To remediate metal(loid) contaminated technosols, there are several available approaches notably physico-chemical or thermal techniques which are difficult to implement, expensive and ultimately disrupting for the ecosystem and soil fertility balance (Hazrat et al., 2013). In contrast phytoremediation appears to be an environmentally friendly and inexpensive alternative technology (Chaney 1983) to reclaim contaminated soils. Phytoremediation takes advantage of the specific processes and metabolism of plants. Water and chemicals are taken up from soil and soil pore water to leaves by root absorption and leaf transpiration (pump function, using solar energy). In addition, root exudates released into the soil lead to remobilization of contaminants, allowing them to be phytoextracted or phytostabilized (Kidd et al., 2009). Moreover, phytoremediation restores wildlife and can be used on a large area and does not require maintenance once implemented, as well as enhancing soil fertility (Mench et al., 2009). Its purpose is either to limit metal(loid) dispersion into the environment or to allow the extraction of soil contaminants to the above ground plant parts (Vangronsveld et al., 2009). An efficient plant for phytoremediation has to present: (i) a high biomass production, (ii) a facility for cultivation and (iii) a high ability to tolerate, translocate, accumulate metal(loid)s in its biomass or to exclude these elements from living organs (Hazrat et al., 2013; Vangronsveld et al., 2009).

Currently around 500 plants are known to be metal(loid) hyperaccumulators. For example the *Brassicaceae* family is well known for its ability to tolerate Ni and Zn (Kumar et al., 1995; Baker et al., 1992; Van der Ent et al., 2013). However this family presents a low biomass production; thus an alternative to overcome this low biomass production is the use of woody species which demonstrate a high biomass production and a large capacity to store metal(loid)s into the woody organs, this process is called dendroremediation (Gonzalez-Oreja et al., 2008). Furthermore, these species are often able to extend their root systems over a large volume of soil, which potentially allows a better phytoextraction and/or

phytostabilisation. Among these trees, willows (*Salix* sp) are good candidates (Malá et al., 2010) because they can be used either in Short Rotation Coppice (SRC) or in Very Short Rotation Coppice (VSRC) (Algreen et al. 2013; Karp and Shield, 2008; Ceulemans et al. 1996) allowing the formation of an important biomass.

About 450 species of willow have been described (Argus 1997) and among them only around ten have been investigated for their phytoremediation ability. In this study we selected two species: *Salix viminalis* and *Salix purpurea*, which have proved to be efficient in accumulating metal(oid)s (Mleczek et al., 2010; Cosio et al., 2006) and have contrasting stational requirements. Experiments to assess the tolerance and metal(loid)s distribution in willow organs have been mainly made in hydroponic conditions using monometallic stresses (Wang and Greger, 2004; Dos Santos Utmazian et al., 2007; Purdy and Smart 2008; Zhivotovsky et al., 2010). To be closer to field conditions, it is important to develop experimental approaches using real soils collected from contaminated sites.

The aim of this study was to test the ability of two willow species to develop a root system and an aerial biomass on a metal contaminated technosol. We tested the potential advantage of an organic amendment to improve phytoremediation process. This work focused on using a garden soil as an amendment and also as a control soil. The concentration of metal(loid)s was determined for bulk soil and soil pore water, in order to follow their bioavailability and thus their potential uptake by willows.

This study was conducted on mine technosols highly contaminated with As, Sb and Pb. Different soil mixtures were prepared using the technosol mineral horizon and its associated litter and a control garden soil. Non rooted cuttings of two willow species (*Salix viminalis* and *Salix purpurea*) were placed on these reconstructed soils in the laboratory and allowed to grow for 45 days. The physico-chemical soil parameters and the total concentrations of metal(loid)s in the soil mixtures and in the soil pore water (SPW) were

determined. Then, the willow's biomass was quantified, thereby allowing an assessment of the accumulation of metal(loid)s in the different organs. Finally, the phytoremediation potential of these two species according to the amendments applied was discussed and compared in terms of metal(loid) tolerance.

2. Material and methods

2.1 Field site and soil sampling

Soils used for this study came from a settling basin of a former gold mine, located at "La Petite Faye", 60 km north of Limoges, France (GPS coordinates: 01°34'23"E; 46°08'37"N). The mine has not been in use since 1964 and produced approximately 34,000 tons of mine wastes that were dumped into a settling basin of about 12,000 sqm, and with a depth greater than five meters. The site studied currently has vegetation as described by Wanat at al. (2014) consisting mainly of grasses, ferns (*Pteridium aquilinum*) and giant horsetails (*Equisetum telmateia*). The main PTTE present in soils are As, Pb and Sb (Wanat et al., 2013, 2014). Due to the mining process, the site was divided into two areas, depending on the granulometry of materials inducing two levels of metal(loid)s content. Our study focused on a technosol collected in the least contaminated area, the same used in Qasim and Motelica-Heino (2014).

2.2 Preparation of the soil mixtures

Nine different soil mixtures (Table 1) were prepared using two soil horizons collected in the settling basin and a garden soil:

- Litter (L), the organic substrate above the technosol, between 0 and 5 cm depth,

- Technosol (T), the mineral horizon derived from the mining waste from the tailings pond, between 5 and 25 cm depth,

- Garden soil (G), soil collected between 5 and 25 cm deep in the park of Orléans University, France, used as a control soil.

For each reconstituted soil (Table 1), the mixtures were prepared using a volume/volume ratio from the different soils tested (L, T and G) to obtain a final volume of 2 liters. Four replicates were prepared for each treatment.

2.3 Plant material

Two willow species were studied (*Salix viminalis* L. and *Salix purpurea* L.) over a 45-day period. Experiments were performed using unrooted cuttings that were 15 cm long and 0.8 cm in diameter. For each repetition, 12 cuttings were used per species. Pots were maintained to field capacity daily using tap water. The temperature in the mesocosm was maintained at $20 \pm 2°$ C. The day light was set at 16 h per day using photoflood lamps (1000 $\mu moles.m^{-2}.s^{-1}$).

2.4 Physico-chemical parameters and metal(loid)s concentrations of the studied soils

Before adding the cuttings to the different soil mixtures to be tested, 50 grams of soil samples were taken randomly from each pot (n = 4), dried at 60°C for 72 hours and then sieved to obtain a 1 mm diameter maximum particle size.

Total metal(loid) concentrations in soils were determined by HR-ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Thermo Finnigan Element 2XR, Thermo Scientific, Waltham, MA, USA). Previously, two hundred mg of sieved soil were treated using an acid mixture (3 ml of 37% hydrochloric acid and 6 ml of nitric acid 65%) and mineralized using a

microwave (Multiwave ECO Anton Paar, Graz, Austria). The digested samples were adjusted to 50 ml with deionized water and filtered by a HAWP Millipore membrane 0.45µm.

2.5 Physico-chemical parameters and concentrations of metal(loid)s in the soil pore water (SPW).

All measurements were carried out before the introduction of cuttings from 20 ml of SPW which was sampled from the soils studied using porous plugs rhizons ™ (porosity = 0.15µm) (Rhizosphere, Wageningen , The Netherlands).

The water content of the soils was calculated using the formula: $W = \dfrac{Mw}{Ms}$, with Mw = weight of water in the soil and Ms = dry weight of the soil.

pH measurements of the SPW were performed using a pH meter (Cyberscan 500, Eutech instruments).

Measurements of DOC (dissolved organic carbon) were performed using a DOC analyzer (Shimadzu Corporation, Kyoto, Japan). Quantifications of major cations and anions were performed by ion chromatography (Columns: CS16 and AS17, and ICS900 ICS1100, Dionex, Sunnyvale, CA, USA). The ionic strength was calculated from the data obtained after the quantification of major cations and anions. Measurement of total dissolved concentrations of metal(loid)s was performed according to the protocol adopted for bulk soils (cf. above paragraph) after adding 50 µl of 67% nitric acid to each sample.

2.6 Biomass and metal(loid)s measurements in willows organs

After 45 days of growth on the different tested soils, organs of each plant were sampled and separated (leaves, stems and roots formed during the experiment). The samples were dried in an oven at 60°C for 72 hours. Dry weights of roots, stems and leaves were measured. Dry samples were finely ground (propeller mill, IKA, Staufen, Germany) before

mineralization, and metal(loid) concentrations were measured in the different organs, using the same protocol as the one used for soil samples. The mineral mass of the different metal(loid)s was calculated as follows:

$$\text{Mineral mass (µg)} = [\text{metal(loid)s}] \times \text{organ dry matter}$$

The stocks of metal(loid)s in different parts of *S. viminalis* and *S. purpurea* were calculated by multiplying metal(loid) concentrations in each compartment by its biomass. Stocks of elements in vegetation are expressed in µg per pot.

2.7 Statistic analysis

All statistical analyzes were performed with R Version 2.10 (Development Core Team, 2009) software. The tests used are non-parametric because of the non-normality of the data (tested by the Shapiro-Wilk test). The samples were compared to the control condition which corresponded to the garden soil (G). To test the treatment effect (t) and the genotype effect (g) on different variables, the Kruskal-Wallis test was used. To test the treatment effect x genotype (txg), the adonis test was used.

3. RESULTS

The garden soil (G) was used as a reference soil (Control). Results for the different treatments were compared to G modality results.

3.1 Physico-chemical properties of constructed soil from soil pore waters (SPW) investigations

Physico-chemical parameters of the SPW are displayed in Table 2. The water content of the garden soil (G) corresponds to 13% whereas for technosol (T) this value was twice as

high. For the litter (L) as well as for the different LT mixtures, water contents were three times higher than the value of G. The calculated values for the GT mixtures were close to the control soil (G). The pH of the garden soil SPW was 7.4 ± 0.1, whereas the Technosol (T) and Litter (L) pH were significantly more acidic, with values of 6.9 and 5.6 respectively. The addition of 12.5% of garden soil to the technosol (T) and the litter (L) increased the pH to 7.5, which makes them significantly equivalent to the values of the garden soil. A higher addition of soil garden (25 and 50%) to T and L provided no significant changes in pH, which stabilized around 7.5. Finally, for the LT mixtures we observed that the addition of 12.5% of Technosol to L did not change the pH compared to L. However the addition of 25 and 50% of Technosol increased the SPW pH gradually by 0.6 unit to reach 6.6 for the LT50 mixture which stayed significantly lower than G.

The Dissolved Organic Carbon concentration (DOC) in the SPW showed no significant differences between G and T. However L had a DOC concentration of 74.1 ± 9 mg.l^{-1}, which was about twice as high the values for the T and G soils. Thus the addition of litter to the technosol (T) significantly increased the DOC.

The analysis of concentrations of major cations and anions in the SPW showed a higher concentration of K^+, NO_3^-, SO_4^{2-}, and PO_4^{3-} in the litter (L). For the litter and for these four ions, the concentrations were 2.8, 6, 2.4 and 22 times higher than in the control garden soil respectively. Ca^{2+}, NO_2^-, NO_3^- and PO_4^{3-} concentrations in technosol were lower than the one calculated in G. They were 1.9, 68, 22.5 and 12 times lower respectively.

The GT mixture had major cation and anion concentrations close to the values measured in the soil garden. In contrast the LT mixtures had always K^+, Mg^{2+}, NO_3^-, SO_4^{2-}, and PO_4^{3} concentrations higher than for garden soil.

3.2 Metal(loid)s concentrations in the bulk soils and soil pore waters.

3.2.1 Metal(loid)s total concentrations in bulk soils

As, Sb and Pb concentrations in the soil and SPW are shown in Table 3. Total metal(loid) concentrations in the garden soil G were 45.5, 0 and 35.1 mg.kg^{-1} for As, Sb and Pb respectively. For the technosol T, the concentrations of As and Pb were respectively 35 times and 6 times higher (1593 and 210 mg.kg^{-1} respectively; Table 3) than those measured for the control soil (G). Sb concentration in the technosol was 66.6 mg.kg^{-1} whereas in the garden soil no Sb was observed.

Arsenic concentration in the litter (L) was not significantly different from G. Pb concentrations were 10 times lower than those observed in G. However we noted in the litter the presence of Sb at low concentration (0.18 mg.kg^{-1}). Concentrations measured in the reconstituted soils were related to the ratio in the prepared mixtures.

3.2.2 Metal(loid) concentrations in the soil pore water

For all studied soils, pure (T or L) and mixture (GT or LT), total dissolved Pb concentrations in the SPW were not different to those observed in the control soil G (0.088 µg.L^{-1}). As concentrations of litter (L) were about 1000 times higher than those of G. For T, this value was 1200 times higher than the one observed for G. For GT12.5, GT25 and GT50 mixtures, As concentrations were respectively 216, 524 and 970 times higher than for the control soil G. For LT mixtures, regardless of the volume of litter, concentrations were about 2100 times higher than in the garden soil (G). Sb concentrations in SPW of T were 680 times higher than those measured in L. Technosol addition to the garden soil at 12.5, 25 and 50% induced an increase in Sb concentrations which reached 60.5, 82.5 and 122.1 µg.l^{-1} respectively. Moreover the addition of technosol to the litter at 12.5, 25 and 50% led to an increased in Sb concentration which reached 0.92, 16.1 and 43.7 µg.l^{-1} respectively.

3.3 Biomass production

Figure 1 shows the biomass produced by *Salix viminalis* and *Salix purpurea* for the different soils tested. *S. viminalis* (Figure 1A) showed a biomass production on the litter twice as high as the biomass of the control plants, while the biomass of the plants grown on the T technosol were 3 times lower than that of the G garden soil. *S. purpurea* biomass production on G (Figure 1B) was twice higher than that of *S. viminalis* (Figure 1A).

The decrease of biomass observed for *S. purpurea* on the substrate T was similar to the one observed in *S. viminalis* under the same conditions. In contrast, the growth of *S. purpurea* on the litter decreased by 5 times compared to the plants grown on G, whereas for *S. viminalis*, the litter soil increased the biomass production by 2 fold in comparison to garden soil. GT mixtures did not induce any reduction of biomass production in *S. purpurea* (Figure 1B), whereas for *S. viminalis*, the progressive supply of technosol induced progressive reduction of biomass, approximately by a factor 3, for the GT50 mixture (Figure 1A). For the LT mixtures, the biomass produced by *S. viminalis* was the same as that for garden soil, whereas for *S. purpurea*, they were systematically three times lower compared to G.

3.4 Metal(loid)s concentrations in the different organs of the two willow species.

3.4.1 Arsenic

Figure 2(A-B) shows the metal(loid) concentrations in the different organs of the two willow species which grew on the different soil mixtures. A higher concentration of As, whatever the treatment or the species was found in the root system. For *S. purpurea* (Figure 2B), T and L soils showed a 10-fold increase in the concentration of As in roots compared to G. The GT and LT treatments also showed a significant As increase in roots: 3.5 to 10 times compared to G. For *S. viminalis* (Figure 2A), we observed the same As concentration

variation as the one observed for *S. purpurea*. In *S. purpurea* (Figure 2B) As concentrations in aerial organs of plants growing on GT mixtures showed an increase by three fold compared to G. On the contrary, in *S. viminalis,* As concentration in the upper organs (Figure 2A) did not show an increase on GT mixtures compared to G. However, for this species, As concentration in the aerial parts was doubled for the all LT soils. Conversely this increase was not observed for *S. purpurea* (Figure 2B).

3.4.2 Antimony

For the two *Salix* species the highest concentrations of Sb were found in the roots (Figure 2C-D). The highest concentrations in these organs were obtained for the technosol. The concentrations for this element reached 70 mg.kg^{-1} for *S. viminalis* and 120 mg.kg^{-1} for *S. purpurea*. We did not measure Sb in the aerial parts of *S. purpurea* (Figure 2D) for any of the soil mixtures tested. For both species when grown on T, Sb concentrations in the aerial parts reached 2 mg.kg^{-1}. Moreover when *S. viminalis* was placed on GT mixtures the Sb concentrations in the aerial parts reached also around 2 mg.kg^{-1}.

3.4.3 Lead

Similarly to observations made for As and Sb and for both species the highest Pb concentrations were measured in the root system (Figure 2E-F). Indeed, in *S. purpurea* (Figure 2F), Pb concentrations reached 400 mg.kg^{-1} on T and L, whereas for G the Pb concentrations were 27 times lower (15 mg.kg^{-1}). For *S. viminalis* (Figure 2E) and for T and L, Pb concentrations in the roots were about 2.8 times lower than for *S. purpurea* (Figure 2F). In *S. viminalis* and for all treatments Pb concentrations in stems and leaves were between 2 and 15 mg.kg^{-1}. For *S. purpurea* (Figure 2F) and for all treatments, Pb concentrations in aerial

parts were not at detectable levels except for T in which Pb concentration in stems reached 2.5 mg.kg^{-1}.

3.5 Mineral mass and stocks

3.5.1 *Arsenic*

Mineral mass (Figure 3A) and stocks were calculated to determine the total amount of metal(loid)s accumulated in various organs of *S. viminalis* and *S. purpurea. S. viminalis* generally had a higher amount of As in its roots compared to the shoots whatever the treatment. On the contrary, *S. purpurea* showed a higher amount of As in its shoots than in its roots for the GT mixture. Indeed, the amounts of As in the upper organs reached 100 µg for *S. purpurea*, whereas for *S. viminalis* they did not exceed 30 µg.

3.5.2 *Antimony*

For *S. viminalis*, the highest calculated Sb mineral mass in leaves were found for the GT and T treatments (Figure 3B) and were in the range of 0.2 µg. For *S. purpurea*, only plants placed on the T treatment presented Sb in the upper organs (0.4 µg). The highest amounts of Sb were found in roots for this species.

3.5.3 *Lead*

S. purpurea only accumulated Pb in its roots (Figure 3C), up to 12 µg for the T technosol. In contrast for *S. viminalis*, Pb was found in both roots (up to 6 µg on litter) and aerial parts (from 0.5 to 2 µg).

4. DISCUSSION

The presence of anthropogenic metal(loid)s in soils at concentrations that exceed trace levels is a concern for the environment and human health. Indeed, the mobility of these PTTE within an ecosystem is a risk because of their potential propagation to all trophic levels. To prevent metal(loid)s (i) lateral and vertical transfer, (ii) runoff into surface water, (iii) infiltration into the groundwater or (iv) export by erosion, the use of willows as a tool for the *in-situ* stabilization of contaminants was investigated in this work. The first goal of this study was to characterize soils and SPW of reconstituted technosols from a former mining site. These characterizations were used to determine the physico-chemical parameters and PTTE concentrations in soil and in SPW in contact with the rhizosphere of the two willow species studied (*S. viminalis* and *S. purpurea*). In plants metal(loid)s accumulation is associated to uptake of metals by roots from the soil pore water. This absorption and accumulation into the vacuole is improved by the plant exudation of a class of organic compounds able to enhance the bioavailability of metal(loid)s. Whereas the transport across a cellular membrane is mediated by proteins with transport functions such as ZIP proteins family before chelation and transport into the vacuole for sequestration (Clemens, 2002).

4.1 Characterization of original soils and mixtures

Among the different studied soils, L was the substrate which retained the greatest amount of water, which was the most acidic and had the highest amount of organic matter and nutrients, particularly in terms of nitrogen, phosphate and potassium. In the lower horizon, the technosol had a pH close to neutral and the lowest nutrient levels. As expected, nitrate content in this soil horizon was under the level observed in the garden soil. Technosols studied present low fertility properties. The addition of L to the technosol allowed the increase of the nutrient contents of the SPW. Thus, in such conditions the litter addition could contribute to

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improving *Salix* growth. Moreover, the litter addition allowed the increase of the water content of the technosol from 27% to 47% when no litter or 50% of litter was added respectively. This increase of water retention produced by the litter addition is an important point considering the necessity for *Salix* species to grow under moist soil conditions to create a greater amount of biomass (Aasamaa et al., 2010; Toillon et al., 2013). The addition of G to the technosol allowed the recovery of nutrient contents close to that of G.

The metal(loid) concentrations determined in SPW (Table 3) correspond approximately to the PTTE concentrations potentially available for the root system, although dependent on metal(loid)s speciation. As, Sb and Pb concentrations in SPW were not proportional to total measured concentrations in the corresponding soils, and the same results have been observed by Qasim and Motelica-Heino (2014). However, metal(loid) mobility in a soil and soil pore water is able to change during time, depending on several factors, such as pH, water content, mineral and organic content (Bolan et al., 2014). pH is one of the main factors affecting the transfer of metal(loid)s from the solid to the liquid phase (Leschber et al., 1984; Gandois et al., 2010), the availability of metal cations increasing in acidic conditions whereas anion availability (e.g. As and Sb) increases when the pH increases (Bolan et al., 2014). Subsequently for GT mixtures, which had a slightly basic pH (7.5), the highest total dissolved concentrations of Sb in SPW was observed (except in technosol). Moreover, in LT mixtures Sb concentrations were lower because of the acidity brought by the litter. However As concentrations in SPW were higher for the LT mixtures (until 13506.8 µg.L^{-1}) compared to the technosol (7243 µg.L^{-1}). This was due to the acidity of the LT soil produced by the addition of L and because of the higher concentrations of organic matter (DOC), probably competing with As for sorption sites such as those on iron-oxide surfaces, causing increased As mobility (Redman et al., 2002). On the other hand, strong content of soluble P and fulvic acids could displace As from organic/inorganic binding sites (Beesley et al., 2010; Businelli et

al., 2009; Kunhikrishnan et al., 2012; Wang and Mulligan, 2006). Pb concentration in technosol was about 210 mg.kg^{-1}, which is within the range of concentrations commonly found in the case of strong natural anomalies values (Baize, 1997). However, concentrations in SPW for all studied soil mixtures were less than 1.83 µg.l^{-1}, a value which was significantly lower than the EU Directive 98/83, which tolerates a maximum concentration of this element in drinking water at 10 µg.l^{-1} level. We conclude that this element, though present in large quantities in the soil, is probably not mobile and does not represent a proven health risk.

For the LT mixture, the mobility of Sb corresponding to the concentration of this metal(loid) in the SPW was lower compared to the technosol and on the other hand As mobility was increased by the addition of litter. We can conclude that the addition of amendments in a phytoremediation strategy to improve the development of plants can produce different effect on metal(loid) bioavailability. Indeed, the contribution of litter when mixed with technosol promotes the growth of *S. viminalis* but at the same time induces a strong release of As in the soil pore water.

4.2 Behavior of Salix viminalis and Salix purpurea toward organs PTTE accumulation

None of the willow plants during the experiment period showed visible signs of toxicity. For both *Salix* species tested, the biomass production dropped on the technosol in comparison to the control garden soil. This was probably due to the high content of metal(loid)s in SPW, especially As (7243 µg.L^{-1}) and Sb (554.71 µg.L^{-1}) and the lower content of nutrients. The biomass production on litter increased for *S. viminalis* whereas it decreased for *S. purpurea.* These contrasting observations on the biomass production for these two species are mainly in relation to the high nitrate levels observed in litter and are consistent with the results obtained by tree producers in nurseries for these two species when growing on a soil containing high

nitrate content. Indeed, it is well known that *S. viminalis* is recommended for planting on rich soil whereas *S. purpurea* is a poor soil species. Indeed, we observed for GT mixtures that *S. purpurea* was better at dealing with lower nitrate concentrations in SPW even with an increase of metal(loid) elements concentration. In contrast, for *S. viminalis*, a decrease in biomass production was observed in the GT mixtures with a higher amount of technosol. This is likely to be related to a decrease of nitrate concentrations in the SPW, and an increase in the concentration of metal(loid) elements. On the LT mixture, *S. viminalis* was able to produce the same biomass as on garden soil G thanks to high level of nutrients brought by litter while As concentration in SPW was higher. Though As concentration measured on SPW was very high (until 13506.8 $\mu g.L^{-1}$), As could be chelated by fulvic and humic acids, which could reduce the toxic effects of As on the plants (Saada et al., 2003; Redman et al., 2002). Although, humic acid (HA) and fulvic acid (FA) have mainly negative charges, they have a few positively charged functional groups, such as sulfhydryl and amine, which form the oxyanions -OM complexes (Saada et al., 2003). Moreover, As can be associated with FA and HA by metal cation bridges (Redman et al., 2002).

These results are in agreement with data from previous literature (Sander and Ericsson, 1998; Meers et al., 2007; Otones et al., 2011), indeed *S. viminalis* and *S. purpurea* both accumulate significant amounts of As in roots and in the aerial parts. For several species of willow, including hybrid *S. viminalis x S. miyabeana*, it was shown that As accumulation was higher in plants growing on a soil with high phosphorus concentrations (Puckett et al., 2012; Purdy and Smart, 2008). In our case, on the LT mixture, which has the highest concentrations of phosphorous (provided by the litter, Table 2), we observed the highest As accumulations in all the organs for *S. viminalis*. However, the same result was not observed for *S. purpurea* on LT mixture. Therefore levels of available phosphate have different effects depending on the species. Indeed, Puckett et al., (2012) showed that the increase of As accumulation in hybrids

of *S. viminalis* was linked to an overexpression of the phosphate transporter (PHT), which also supports As. For GT mixtures, which are the easiest to implement in *in situ* revegetation strategies, *S. purpurea,* presents a greater accumulation of As without loss of biomass. Conversely, a drop in the biomass production and a lower accumulation of arsenic were noted for *S. viminalis*. For *S. purpurea*, Pb and Sb were detected only in the root system, whatever the soil treatment. Consequently, *S. purpurea* could be considered as an excluder for these two elements. Conversely, *S. viminalis* transferred some Pb and Sb to its aerial parts, and therefore allows them to be extracted. These results had already been shown for Pb during experiments in hydroponics with *S. viminalis* (Zhivotovsky et al., 2010). However for Sb previous literature had no data about the phytoremediation ability of willow species in real conditions as far as we know.

5. CONCLUSION

Despite high concentrations of metal(loid)s in technosol notably As, both willow species tested were able to develop a root system and aboveground biomass. However, we note that only *S. purpurea* is really efficient in terms of vegetalization of the mining site when comparing the biomass produced in the mixture control garden soil and technosol (GT) compared to the control garden soil. In addition, this species showed a strong accumulation of As, which was four times higher than that observed for *S. viminalis*. However, given metal(loid) concentrations measured on the site, using an SRC, thousands of years would be necessary to extract all the metal(loid)s present in this technosol. Given the biomass production of *Salix purpurea* on the GT mixture and its high accumulation of metal(loid)s in its root systems this species can be used as a successful phytostabilization plant in such situations.

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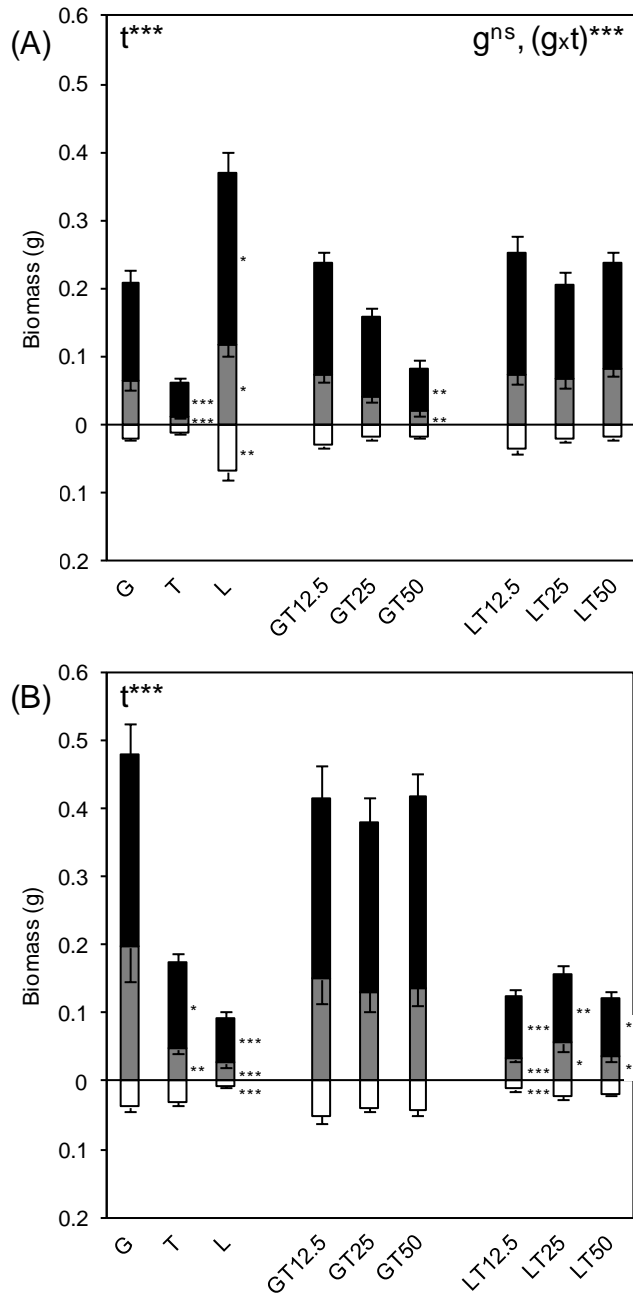
Fig. 1. Biomass (dry weigth) of the different organs (■ leaves, ■ stems, □ roots) of *S. viminalis* (A) and *S. purpurea* (B) after 45 days of growth on the different soils, (n=12, ± SE). t= treatment effect, g= genotype effect, (g$_\times$t)= genotype x treatment effect. Significantly different values by comparison with the garden soil (G), * p <0.05; ** p <0.01; *** p <0.001.
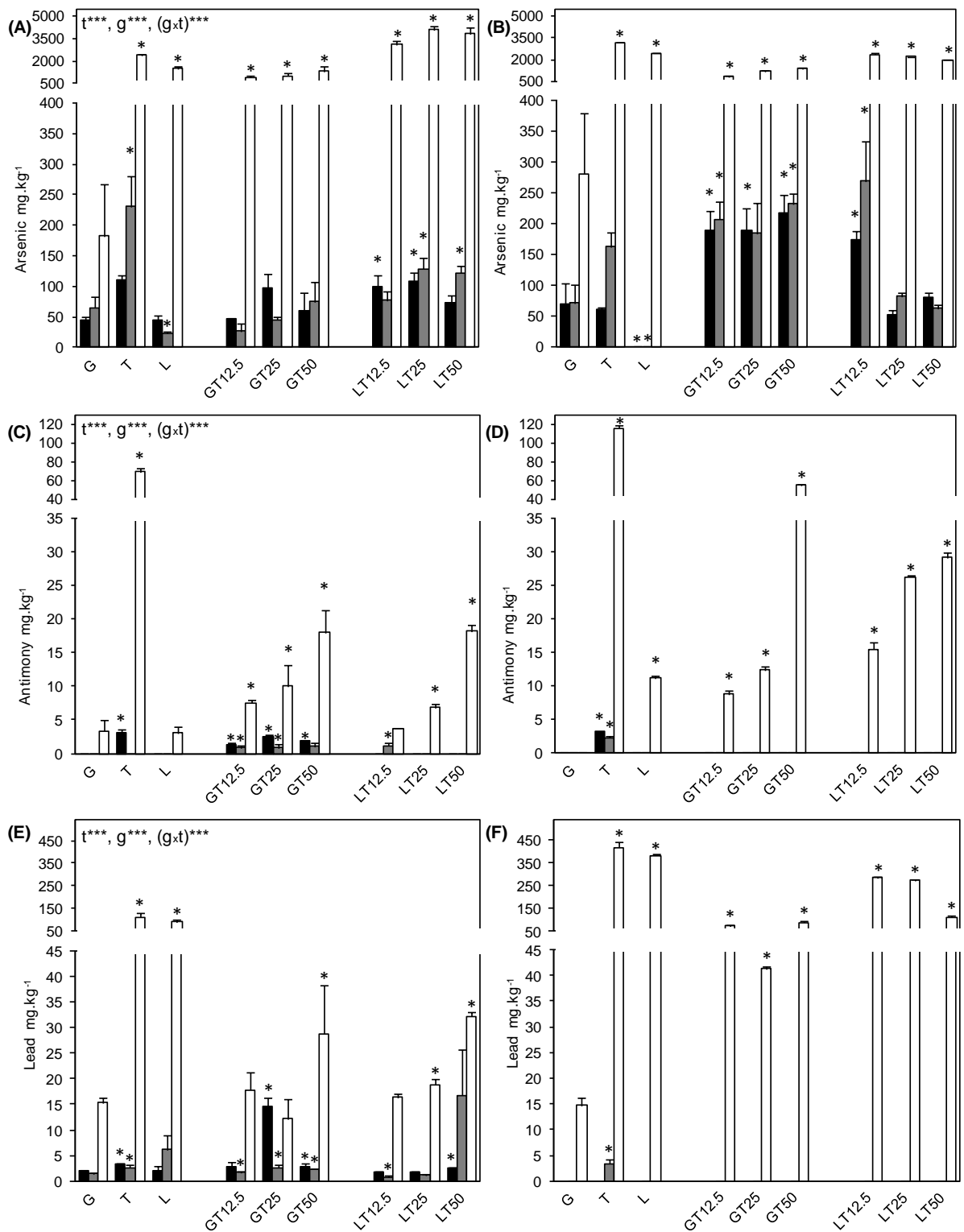
Fig. 2. Concentration of metal(loid)s (A and B = As, C and D= Sb, E and F = Pb) in the different organs (■ leaves,▨ stems,□ roots) of the two willow species (A, C and E= *Salix viminalis*. B, D and F= *Salix purpurea*) after 45 days of growth on the different soils, (n=4, ± SE). t= treatment effect, g= genotype effect, (gxt)= genotype x treatment effect. Significantly different values by comparison with the garden soil (G), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig. 3. Mineralomass of metal(loid)s (A and B= As, C and D= Sb, E and F= Pb) in the different organs (■ leaves,▨ stems,☐ roots) of the two willow species (A, C and E = *Salix viminalis*. B, D and F= *Salix purpurea*) after 45 days of growth on the different soils, (n=4, ± SE). t= treatment effect, g= genotype effect, (gxt)= genotype x treatment effect. Significantly different values by comparison with the garden soil (G), * p <0.05; ** p <0.01; *** p <0.001.

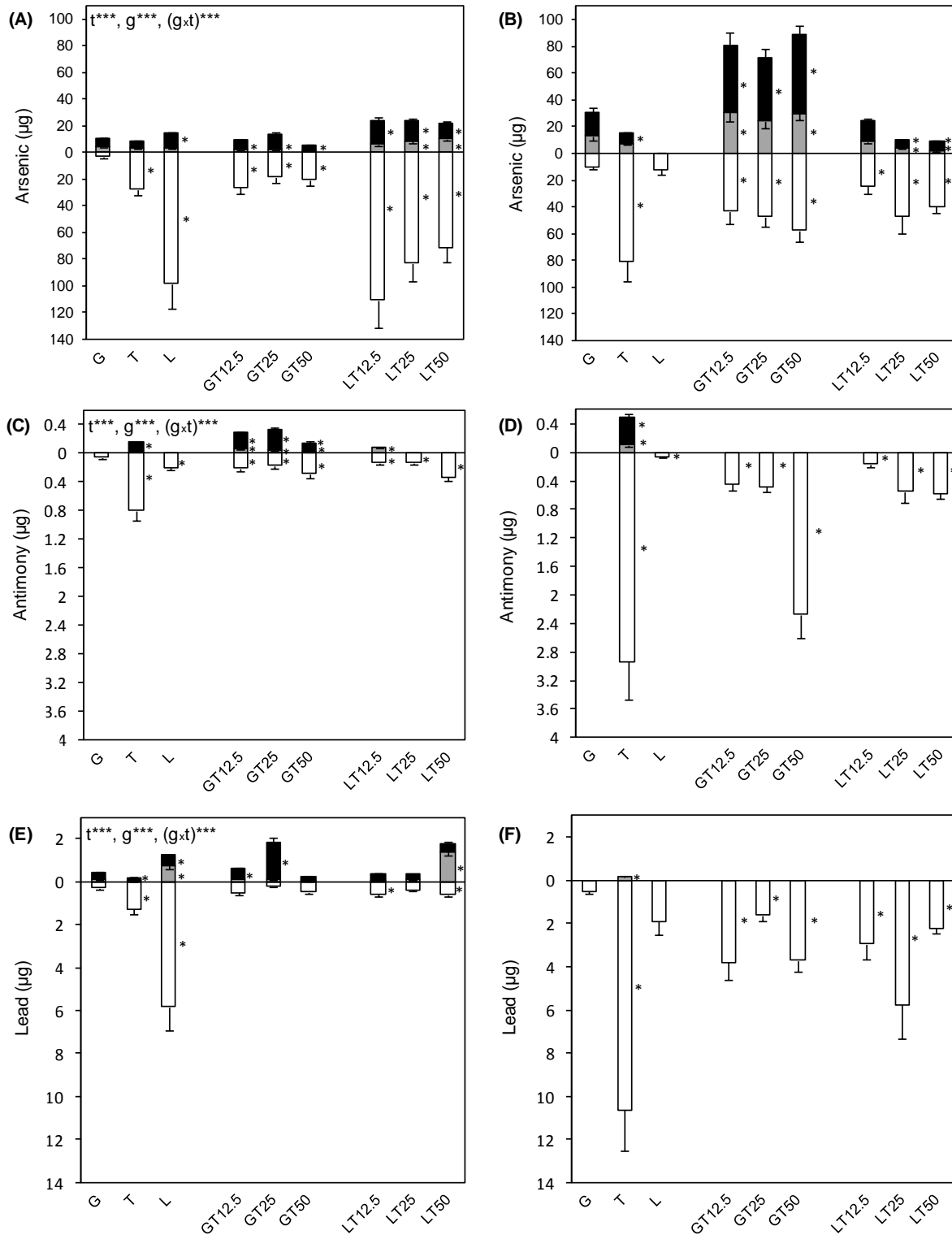**Table 1**

Codes and volumes (%) of the different reconstructed soils. L = litter, T = technosol, and G = garden soil. Final volume = 2 Liters.

Code	% garden soil	% technosol	% litter
G	100		
T		100	
L			100
GT12.5	87.5	12.5	
GT25	75	25	
GT50	50	50	
LT12.5		12.5	87.5
LT25		25	75
LT50		50	50

Table 2

Physico-chemical parameters of the different soil pore water solutions (n=4, ± SE). W = water content of soil mass (%), pH, DOC= Dissolved Organic Carbon (mg.l^{-1}) , major cations and anions (mg.l^{-1}). Significant difference with garden soil (G) at level * p<0.05; ** p<0.01; ***p<0.001. t = treatment effect.

	G	T	L	GT12.5	GT25	GT50	LT12.5	LT25	LT50
w (t***)	13 ± 7	27 ± 0.7*	43 ± 3*	14 ± 0.4	16 ± 0.6	18 ± 0.6*	47 ± 6*	42 ± 2*	39 ± 2*
pH (t***)	7.36 ± 0.11	6.91 ± 0.07*	5.6 ± 0.02*	7.59 ± 0.03	7.57 ± 0.02	7.5 ± 0.03	5.44 ± 0.02*	6.03 ± 0.04*	6.59 ± 0.1*
DOC (t***)	42.59 ± 3.12	33.39 ± 6.66	74.1 ± 9*	31.61 ± 2.08*	45.01 ± 5.61	39.52 ± 7.61	61.23 ± 3.68*	55.79 ± 2.07*	47.8 ± 4.42
Cations									
Na^+ (t*)	31.7 ± 4.92	21.55 ± 2.42	32.69 ± 7.11	26.83 ± 4.12	26.66 ± 2.7	23.07 ± 1.19	40.67 ± 1.97	37.12 ± 3.48	29.81 ± 0.09
K^+ (t***)	17.25 ± 1.46	26.33 ± 1.78*	47.58 ± 8.55*	16.15 ± 2.18	20.23 ± 1.94	23.4 ± 0.35*	130.72 ± 3.66*	112.84 ± 12.57*	85.51 ± 9.65*
NH_4^+ (t**)	0.87 ± 0.32	1.79 ± 0.14	1.34 ± 1.03	1.44 ± 0.15	1.67 ± 0.38	1.78 ± 0.06*	0.3 ± 0.15	0.49 ± 0.2	0.61 ± 0.13
Mg^{2+} (t***)	20.26 ± 1.74	15.92 ± 1.68	27.92 ± 3.45	19.56 ± 1.23	20.40 ± 2.24	17.51 ± 0.27	45.86 ± 0.82*	38.05 ± 4.62*	32.63 ± 3.87*
Ca^{2+} (t***)	125.23 ± 13.39	66.35 ± 8.53*	133.16 ± 3.95	116.83 ± 8.97	109.07 ± 13.09	83.9 ± 2.19*	224.86 ± 25.17*	110.9 ± 5.55	91.83 ± 3.43
Anions									
Cl^- (t*)	95.58 ± 22.84	64.85 ± 3.76	60.67 ± 5.82	68.89 ± 11.45	68.7 ± 3.87	64.51 ± 5.48	80.24 ± 2.84	80.67 ± 5.21	66.94 ± 2.68
NO_2^- (t***)	4.75 ± 1.52	0.07 ± 0.02*	0*	1.03 ± 0.18*	0.75 ± 0.28	0.48 ± 0.13*	0*	0.1 ± 0.02	0.09 ± 0
NO_3^- (t***)	69.27 ± 27.88	3.07 ± 0.71*	416.92 ± 27.36*	9.14 ± 2.14*	10.77 ± 4.86	10.09 ± 3.35*	584.75 ± 37.95*	336.58 ± 24.95*	218.1 ± 33.89*
SO_4^{2-} (t***)	35.83 ± 6.98	38.26 ± 3.01	84.59 ± 7.17*	27.01 ± 1.48	42.21 ± 5.64	35.89 ± 3.03	193.23 ± 5.12*	137.35 ± 7.97*	114.25 ± 2.66*
PO_4^{3-} (t***)	2.09 ± 0.37	0.17 ± 0.04*	46.35 ± 25.2*	1.65 ± 0.2	1.54 ± 0.13	0.86 ± 0.09*	27.73 ± 1.05*	14.52 ± 0.55*	5.23 ± 5.67*

Table 3

Total concentrations of metal(loid)s in soils (mg.kg^{-1}), n = 4 ± SE and total dissolved concentrations of metal(loid)s in the soil pore water (spw), (µg.l^{-1}), n = 4 ± SE. Significant difference with garden soil (G) at level * p<0.05; ** p<0.01; ***p<0.001. t = treatment effect. ns = no significant.

	G	T	L	GT12.5	GT25	GT50	LT12.5	LT25	LT50
[As] (t***)	45.5 ± 18.1	1593 ± 280.3**	24.6 ± 14.5	212.4 ± 59.4	440.9 ± 18.8*	561 ± 32.4***	949.3 ± 39.7**	1119.7 ± 46.8***	1436.1 ± 130.6**
[As]$_{spw}$ (t***)	5.9 ± 0.9	7243 ± 1785*	5431 ± 2334.1*	1279.4 ± 143.6*	3093.3 ± 600.9*	5719 ± 1044.9*	13506.8 ± 737.1*	13780.1 ± 752.3*	10298.9 ± 809.5*
[Sb] (t***)	0	66.57 ± 1.45**	0.18 ± 0.004**	15.33 ± 3.82**	25.22 ± 2.05**	34.92 ± 1.99**	46.47 ± 2.53**	62.05 ± 2.14**	83.04 ± 2.53*
[Sb]$_{spw}$ (t***)	0	554.71 ± 88.15*	0.81 ± 0.81	60.51 ± 16.94*	82.56 ± 37.48	122.16 ± 25.05*	0.92 ± 0.55	16.1 ± 2.26*	43.7 ± 6.52*
[Pb] (t***)	35.1 ± 3.3	210.6 ± 45.5*	3.6 ± 0.8**	80 ± 20.9	101.2 ± 5.7*	109.9 ± 5.9*	169 ± 4.6*	199.1 ± 7.2**	221 ± 27.9**
[Pb]$_{spw}$ (t^{ns})	0.088 ± 0.084	1.17 ± 0.39	1.67 ± 0.28	0.23 ± 0.14	0.32 ± 0.15	0.79 ± 0.2	1.8 ± 0.75	1.4 ± 0.59	1.83 ± 1.45