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ENVIRONMENTAL TOXICOLOGY AND RISKS ASSOCIATED WITH HUMAN HEALTH

Lead availability and phytoextraction in the rhizosphere of *Pelargonium* species

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Abstract

Availability of lead (Pb) in soil is a major factor controlling the phytoremediation efficiency of plants. This study was focused on investigating the plant-induced changes in rhizosphere and corresponding effect on bioavailable fraction of Pb and accumulation in different plant parts. For rhizosphere study, special cropping device was designed locally. Two Pb accumulator plants *Stigmatocarpum criniflorum* (L. f.) L. Bolus and *Pelargonium* × *hortorum* L.H. Bailey were grown in cropping device setup containing Pb spiked soil (500, 1000, 1500, and 2000 mg kg^{-1}) for a period of 3 weeks. Further plants were also analyzed for Pb-induced oxidative stress. The results indicated higher ability of soil adjustment for Pb uptake by *P. hortorum*. The soil pH was ($p < 0.05$) decreased ($\Delta pH = -0.22$ pH), and dissolved organic carbon (DOC) content was significantly increased (by 1.7-fold) in rhizosphere of *P. hortorum*. The bioavailable fraction of Pb was twofold higher in rhizosphere of *P. hortorum* than *S. criniflorum* at the same soil Pb concentration (2000 mg kg^{-1}). Maximum Pb concentration in root and shoot of *S. criniflorum* was 755 ± 99 and 207 ± 12 mg Pb/kg DW and for *P. hortorum* was 1281 ± 77 and 275 ± 7 mg Pb/kg DW. *P. hortorum* uptakes more Pb per plant by threefold compared with *S. criniflorum*. The oxidative stress results indicated higher Pb tolerance and suitability of *P. hortorum* for phytoextraction of Pb-contaminated soil.

Keywords Lead · Rhizosphere · Hyperaccumulators · Phytoavailability · Oxidative stress

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Introduction

Lead (Pb) is one of the most abundantly found toxic heavy metals in soils and has no biological function. It is ubiquitously distributed in soil. As a result of anthropogenic activities, 1000-fold increase in Pb soil contamination is observed over the past years (Kushwaha et al. 2018). In Pakistan, Pb concentration in industrial zone of Islamabad and Rawalpindi has been reported up to 1352 mg kg^{-1} (Manzoor et al. 2018). It is potentially toxic to living systems and is considered as a chemical of great concern in the new European REACH regulations. The in situ phytoremediation of Pb-contaminated soils using suitable plants is cost-effective, environmental friendly, and best alternative to old and conventional physico-chemical techniques (Gul et al. 2019; Manzoor et al. 2019a, b, c; Arshad et al. 2016). However, the efficiency of phytoremediation system greatly depends on Pb phytoavailability, which is typically very low (< 2.5%) (Wierzbicka et al. 2007), and only 0.14% of total Pb in soil

is in exchangeable fraction (Jena et al. 2013). Low phytoavailability of Pb in soil limits the field application of phytoremediation system. Generally, Pb is found attached to inorganic anions like HCO_3^-, CO_3^{2-}, SO_4^{2-}, and Cl^- or may be complexed with organic ligands including amino, fulvic, and humic acids. Some faction of Pb may also found adsorbed into soil particles (Fe oxides, organic matter, and clay particles) (Shahid 2010) and strongly bound to organic and colloidal material (Kushwaha et al. 2018).

Different soil and biological factors control Pb speciation and availability in soil. The soil factors that directly interact with Pb speciation include pH, soil texture, structure, organic matter, organic colloids, iron oxides, cation exchange capacity (CEC), Pb concentration, and other chemical additives in soil (Shahid 2010), whereas biological factors include root exudation that alters the soil pH and dissolved organic carbon (DOC) fraction in the rhizosphere soil (Adejumo et al. 2018; Khoshgoftarmanesh et al. 2018). Therefore, investigating the ability of plants to modify its rhizosphere that favors Pb phytoavailability would further increase in developing phytoremediation technology. In our preceding study (Manzoor et al. 2018), *Stigmatocarpum criniflorum* and *Pelargonium hortorum* have been identified for phytoextraction of Pb in study area. The following study have been designed to evaluate in detail the ability of these plants to induce rhizosphere changes that favors Pb mobility and phytoextraction and to investigate plant ability to tolerate oxidative stress induced by Pb.

Material and methods

Soil characterization and Pb spiking

For rhizosphere experiment soil was collected (NUST nursery, Islamabad), air-dried (3 weeks), and sieved through a 2-mm mesh. The soil was then artificially spiked with different concentration of lead (500, 1000, 1500, and 2000 mg kg^{-1}), followed by regular mixing for 15 days to reach metal stabilization. The physicochemical properties of soil are given in Table 1. Soil pH, texture, and organic matter were 7.41, clay loam, and 0.48%, respectively, following methods described by Manzoor et al. (2018).

Table 1 Physiochemical characteristics of soil used in experiment

Parameters	Values
Soil pH	7.41
Soil EC (dS m^{-1})	0.55
Texture	Clay loam
Organic matter carbon (%)	0.48
Total nitrogen content (%)	0.033
Total phosphorus content (mg kg^{-1})	6.37
Potassium (mg kg^{-1})	731
Magnesium (mg kg^{-1})	303
Iron (mg kg^{-1})	2788

Pre-experimental setup and plant acclimatization

For rhizosphere study, two plant species, *S. criniflorum* and *P. hortorum*, were selected based on the previous study for screening of Pb hyperaccumulator plants (Manzoor et al. 2018). Two-month-old seedlings of these plants were obtained from the local nursery (Islamabad Nursery Ltd.). Plants were uprooted from pots, washed in running water carefully, and placed in hydroponic solution for acclimatization and development of new roots. After 2 weeks, plants were transferred to cropping devices designed locally (Fig. S1), modified from earlier reports (Arshad et al. 2016).

The devise helped in separating direct contact of soil and roots. A total of 40 cropping devices containing one plant per device were placed in a container having 15 L of nutrient solution to support plant growth and root-soil contact. Each treatment was replicated four times. The composition of nutrient solution is given in Table S1. The plants were cultured in cropping devices for 2 weeks prior to experiment, for development of root mat. During this period, distilled water was used to maintain the level of nutrient solution. The experimental units were placed in the glasshouse with a photoperiod of 14 h, and the temperature ranged between 26 and 21 °C.

Rhizosphere experiment

After 2 weeks of root development, about 10 g of soil was added in detachable unit of cropping device (diameter 60 mm) to form soil layer of 3 mm thickness after sampling soil for initial chemical properties. During the course of experiment, soils were moistened with nutrient solution via dipped filter paper. The containers were covered with aluminum foil to limit evaporation loss. During this stage, 0.2×strength Hoagland's solution was used to favor Pb uptake by reducing ion competition. Nutrient solution was added regularly to maintain enough level in container to keep filter paper wet. Control soil with similar setting was placed to evaluate pH change. After 3 weeks, plants were harvested and analyzed for Pb concentration in root and shoots. Soil was considered as rhizosphere soil and analyzed for physicochemical parameters.

Soil and plant analysis

Rhizosphere soil was analyzed for pH determination (AFNOR 1994) immediately after harvesting. Soil pH and Pb content were analyzed by extracting soil with 0.01 M $CaCl_2$ (1:5, dry soil/extractant). The soil solution

was stirred for 30 min at 150 rpm, and the pH was determined from supernatant after 30 min. Solutions were then filtered for Pb determination through AAS (Model, GBC 932 Plus, Australia). The DOC was analyzed through TOC analyzer. After harvesting, plants were washed carefully under running tap water, and then roots were immersed in 0.01 N HCL to analyze the amount of adsorbed Pb into root cells ($[Pb]_{ad}$) (Arshad et al. 2016). Plants were then placed in the oven for drying at 65 °C. After 48 h, dry weights were recorded, and plants were grounded to powder form prior to mineralization with HNO_3 and $HClO_4$ (4:1). After complete digestion, clear aliquots were obtained and filtered for Pb determination through AAS. TF and BF were calculated from the results (Manzoor et al. 2018).

Oxidative stress in *P. hortorum* exposed to Pb

Thiobarbituric acid reactive substances (TBARS) were analyzed following method explained by de Oliveira et al. (2017). Briefly, fresh plant material (0.3 g) was homogenized with 1.5 mL of 5% (w/v) trichloroacetic acid (TCA) solution followed by centrifugation at 10,000*g* for 10 min. Afterward, 1 mL of supernatant was taken in appendroff and mixed with 1 mL of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid. The solution was then placed at 95 °C for 30 min followed by quick cooling. Finally, the absorbance was measured at 532 and 600 nm (Shimadzu UVI60U, Columbia, USA). The amounts of TBARS were calculated as (μmol g^{-1} fw), and extinction coefficient used for measurement was 155 mM^{-1} cm^{-1}. The H_2O_2 contents were determined following method described by Junglee et al. (2014). Fresh biomass was grinded using liquid nitrogen. In one sample, 1 mL of control homogenization solution (CHS) and test homogenization solution (THS) was added in pair of samples. Samples were grinded and centrifuged at 12,000*g* for 10 min at 4 °C. Finally, the absorbance was taken at 250 nm by using UV spectrophotometer, and calculations were made from standard curve (0, 0.1, 0.2, 0.5, and 1 nM H_2O_2).

Invitrogen ROS technology was used to further observe ROS localization inside plant tissue as described by (Grzelak et al. 2001). The seeds were surface sterilized and grown on Murashige and Skoog (MS) agar medium supplemented with Pb (0, 20, and 40 mg L^{-1}). After 15 days, the root tips were cut (2 cm root tips) and immersed for 1 h in 1 ml (10×) of DCF (2′, 7′-dichlorofluorescein). The positive controls were also prepared by treating root tips with 1% H_2O_2. The extra loaded dye was removed by washing in distilled water, and roots were kept in 4-(2-125 hydroxyethyl)-1-piperazineethanesulfonic acid (HEPS) buffer. Root tips were then examined under confocal laser scanning microscope (Zeiss LSM 800, Carl Zeiss Iberia, S.L., Spain).

Evans blue uptake assay was performed to examine the membrane integrity by following method of Das et al. (2017). The roots were stained with 0.025% (w/v) Evans blue in 100 mM $CaCl_2$ at pH 5.6 for 10 min followed by washing in 100 mM $CaCl_2$. The samples were then homogenized with 1% (w/v) sodium dodecyl sulfate (SDS) and centrifuged for 10 min. The supernatant were then collected, and the absorbance, showing the extent of plasma membrane damage, was recorded at 600 nm. Membrane integrity index was determined by measuring electrolyte leakage from plant tissues (de Oliveira et al. 2017). Membrane integrity was determined by measuring the electrical conductivity (EC) of root and shoot cell leakage in deionized water at 40 °C and 100 °C, respectively (Das et al. 2017). Integrity index was calculated as C40/C100 × 100.

Statistical analysis

The data was analyzed though analysis of variance (ANOVA). To observe difference among treatments, Duncan's multiple range test (DMRT) was applied at confidence interval of 95%. The results with $p < 0.05$ were taken as significant.

Results and discussion

Root-induced rhizosphere modifications

Soil pH

Soil pH was measured by extracting soil (1:5, soil/extractant ratio) with $CaCl_2$ (0.01 mM) (expressed as pH $CaCl_2$) and presented in Fig. 1a. Changes in pH can be defined in terms of ΔpH and calculated using the following formula:

$$\Delta pH = \text{final pH in treated soil–final pH of the control soil} \quad (1)$$

The negative and positive values of ΔpH indicated acidification and alkalization of the soil. Significant ($p < 0.05$) decrease in soil pH was noticed in case of *P. hortorum* in 1500 and 2000 mg Pb/kg soil compared *S. criniflorum* and control plants at all levels of soil Pb concentration. There was no significant effect of nutrient solution on ΔpH in control soil. Therefore, the change in soil pH can be attributed to root-derived chemical changes in soil.

P. hortorum acidified the soil by − 0.22 pH units $CaCl_2$ extracted soil solutions at 2000 mg kg^{-1} soil Pb concentration. *S. criniflorum* could not significantly change soil pH irrespective of Pb concentrations in soil. Change in soil pH depends on many factors. Plant genotype, amount and composition of root exudates rhizo depositions, mineral and nutrients composition in soil, biogeochemical processes, and other environmental factors account for pH change in soil (Sun et al. 2018). Khoshgoftarmanesh et al. (2018) working with zinc

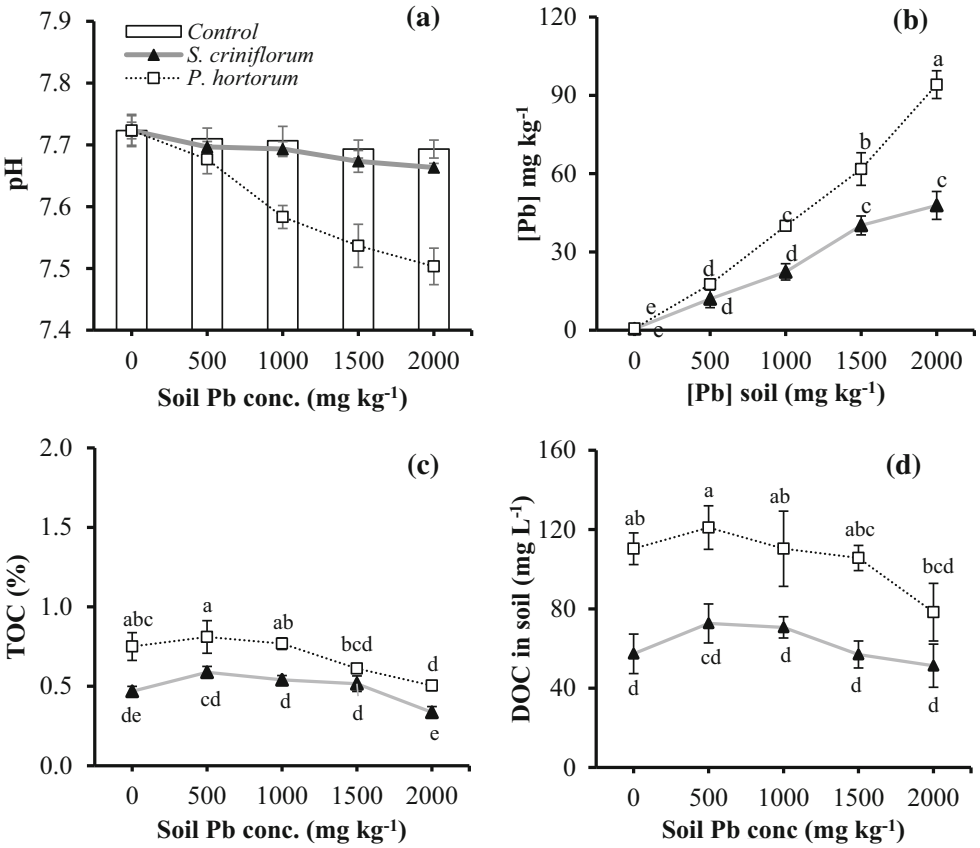

Fig. 1 Root-induced changes in rhizosphere pH (**a**); available Pb fraction (**b**); and TOC (**c**) and DOC content (**d**). Results are significant at $p < 0.05$, as indicated by different letters

fractionation found that rhizosphere pH was lower than that of bulk soil facilitating bioavailability of zinc for uptake in *Triticum aestivum*.

Lead phytoavailable fractions in soils

Soil was extracted with 0.01 M $CaCl_2$ for determination of labile fraction of Pb which is readily available for plant uptake. The ability of plants to modify the labile fraction of Pb in soil is given in Fig. 1b. Results indicate higher ability of *P. hortorum* to increase available fraction of Pb compared with *S. criniflorum* at all levels of soil Pb concentrations. *P. hortorum* increased Pb mobility one to twofold more compared with *S. criniflorum* and control soil. Maximum Pb_{CaCl2} observed for *P. hortorum* was 94 ± 5 mg kg^{-1} at 2000 mg Pb/kg, which is twofold higher than Pb mobilized by *S. criniflorum* at the same soil Pb concentration. Maximum Pb solubility induced by *S. criniflorum* was 48 ± 5 mg kg^{-1} in soil containing 2000 mg Pb/kg soil. Generally, soil acidification directly favors solubility and mobilization of heavy metals. In different studies, it was observed that exchangeable Pb, Cd, and Zn fractions were higher in rhizosphere soil compared with bulk soil (Adejumo et al. 2018; Zhan et al. 2018; Khoshgoftarmanesh et al. 2018). The available fractions were increased due to reduction in rhizosphere pH and exudation of greater amounts of DOC (Zhan et al. 2018). Adejumo et al. (2018) reported genotype effect on increased Pb availability in rhizosphere soil than bulk soil. *Eleusine indica*, capable of lowering soil pH (5.0) and increasing DOC content in rhizosphere, accumulated significantly high Pb in shoot (8030 mg kg^{-1}) compared with *Chromolaena odorata* which accumulated least Pb in shoot (209 mg kg^{-1}) owing to the highest values for rhizosphere pH.

Organic matter pool

Plants are accounted as a major contributor in soil organic matter content through root exudation, cell debris, dry and dead plant tissue, mucilage, and root turnover (Sun et al. 2018). Considerable effect of plants was noticed on TOC and DOC content at varying soil Pb concentrations (Fig. 1c and d). Significant ($p < 0.05$) increase in organic matter derivatives was observed for *P. hortorum* up to 1000 mg Pb/kg soil compared with *S. criniflorum*. Maximum TOC (0.8%) in *P. hortorum* was obtained at 500 mg Pb/kg soil. Similarly, maximum TOC observed in case of *S. criniflorum* was 0.6%, which was 1.4 times lower than TOC obtained *P. hortorum* at same soil Pb concentration (500 mg Pb/kg soil). With increase in Pb concentration, a general decrease in TOC percentage was observed for both plants.

The decrease in TOC content in *S. criniflorum* was 1.8 times, whereas in *P. hortorum* the decrease was 1.6 times

when Pb concentration was increased from 500 to 2000 mg kg^{-1} soil. Similar trends were noticed for DOC content in the soil solution. Highest significant ($p < 0.05$) increase in DOC was obtained at 500 mg Pb/kg soil by *P. hortorum*. Maximum DOC observed in *P. hortorum* cultured soil was 121 ± 11 mg L^{-1} which is 1.7 times more compared with *S. criniflorum* of the same soil Pb concentration. Adejumo et al. (2018) while studying rhizosphere soil found more amount of DOM and carbohydrate functional groups (C–O, 1100–1000; and O–H, 3700–3600) in rhizosphere soil compared with bulk soil (Adejumo et al. 2018; Khoshgoftarmanesh et al. 2018) showing root-induced changes in rhizosphere that favor Pb mobility and uptake by plants (Zhan et al. 2018).

Lead uptake characteristics in plants

Pb concentration in plant tissues

Concentrations of Pb in roots and shoots are given in Fig. 2a. Results indicated that Pb concentrations in both plants significantly differ. Many factors interact and define plant's ability to concentrate Pb in root and shoot tissue that might include genotype, total and bioavailable fraction of Pb in soil medium, plant induce rhizosphere modifications, and other environmental factors (Arshad et al. 2008). Results indicated that with the increase in soil Pb concentration, Pb accumulation in root and shoots of both plants increased significantly, compared with control plants. Among both plants, *P. hortorum* accumulated significantly ($p < 0.05$) high concentrations of Pb in both roots and shoots (1281 ± 77 and 276 ± 7 mg Pb/kg DW) at highest soil Pb concentration level (2000 mg kg^{-1}). These results are in accordance with those reported in screening of ornamental plants for Pb accumulation presented by Manzoor et al. (2018). Maximum Pb concentrations in roots and shoots of *S. criniflorum* were 755 ± 99 and 207 ± 12 and for *P. hortorum* were 1281 ± 77 and 275 ± 7 mg/Pb/kg^{1} DW, respectively. Less shoot accumulation in this study may be due to limited direct root-soil contact in cropping device setup.

Similar trend of lower shoot accumulation by Pb hyperaccumulator, Attar and Concolor, was observed by Arshad et al. (2016) while studying rhizosphere soil in cropping device setup. The maximum Pb_{shoot} accumulation recorded in Attar was 284 mg kg^{-1} at 1500 mg Pb/kg soil after 2 weeks of exposure. Whereas in our case, the maximum accumulation observed in *P. hortorum* was 276 mg kg^{-1} DW at 2000 mg Pb/kg soil. The difference lies in the fact that *P. hortorum* accumulates less amount of Pb in shoot and root compared with Attar when studied on pot and field experiment (Arshad et al. 2008; Manzoor et al. 2018).

Pb uptake

Total Pb uptake by both plants was calculated by multiplying Pb concentration with biomass obtained in

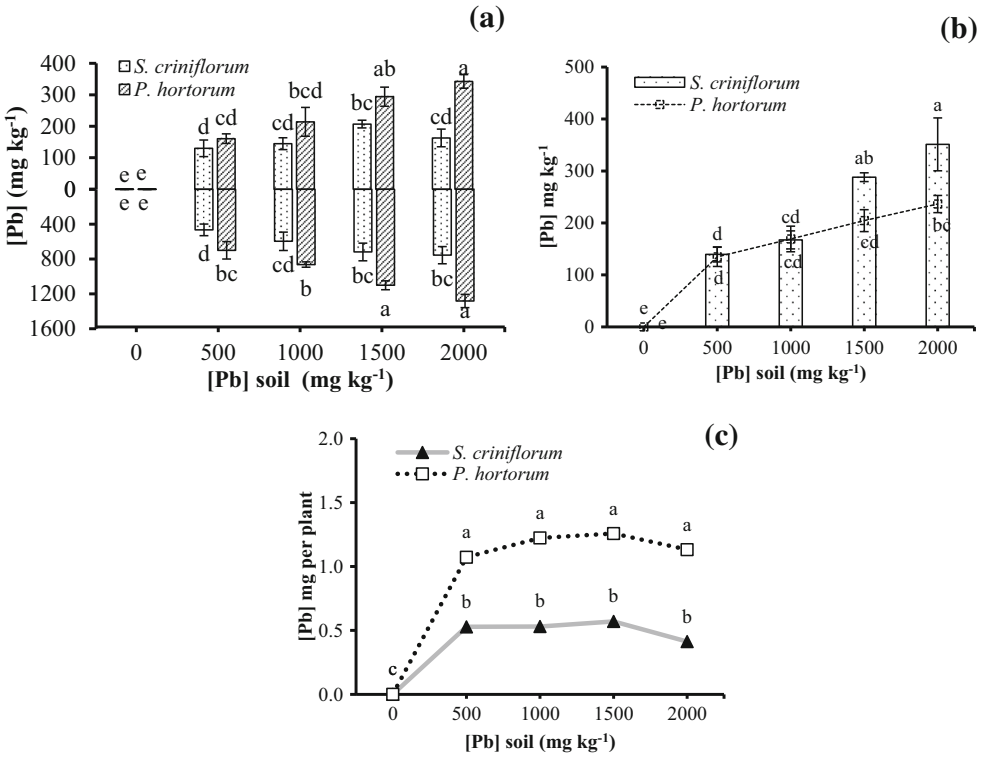

Fig. 2 Lead concentrations in plant parts (**a**); lead uptake (**b**), and adsorption (**c**) by plants after 3 weeks of culture experiment

respective soil Pb concentrations. The formula used for uptake calculation is as follows:

$$\text{Pb uptake(mg)} = ([\text{Pb}_{\text{shoot}}] \times \text{DW}_{\text{shoot}}) + ([\text{Pb}_{\text{root}}] \times \text{DW}_{\text{root}}) \quad (2)$$

Lead uptake by *P. hortorum* was significantly ($p < 0.05$) higher compared with *S. criniflorum* at all levels of soil Pb concentration (Fig. 2b). *P. hortorum* uptake two- to threefold more Pb per plant compared with *S. criniflorum*. The maximum uptake in *S. criniflorum* and *P. hortorum* was 0.6 and 1.3 mg^{-1} plant at 1500 mg Pb/kg. The higher uptake in *P. hortorum* could be due to the influence of different soil and plant factors. Firstly, the acidification of rhizosphere pH by *P. hortorum* was able to lower the soil pH significantly compared with *S. criniflorum* and control soil (Fig. 1a). It is well reported that at lower pH, more Pb is solubilized and available for plant uptake (Zhan et al. 2018). Secondly, it increased labile fraction of Pb for plant uptake (Fig. 1b). Thirdly, *P. hortorum* increased soil organic matter pool (TOC and DOC) (Fig. 1c and d). DOC plays important role in Pb chelation, solubility, and increase Pb uptake by plant (Khoshgoftarmanesh et al. 2018). Last but not the least, *P. hortorum* produced higher biomass (Table 2) and exhibited higher $[\text{Pb}]_{\text{root}}$ and $[\text{Pb}]_{\text{shoot}}$ compared with *S. criniflorum* at all soil Pb concentrations (Fig. 2a), which are important components of uptake calculations.

Pb adsorption on roots

Apart from Pb uptake and accumulation, considerable amount of Pb has been found loosely attached on cell wall of roots (Fig. 2c). This loosely attached Pb also termed as $[\text{Pb}]_{\text{adsorbed}}$ was determined after extracting the adsorbed Pb fraction with 0.01 N HCl solution. Results showed considerable amount of Pb adsorbed on roots of both plants. With increase in soil Pb concentration, significant increase in $[\text{Pb}]_{\text{adsorbed}}$ was observed for both plants. Significantly ($p < 0.05$) higher (1–2 times) Pb was adsorbed on *S. criniflorum* roots compared with *P. hortorum* at 1500 and 2000 mg Pb/kg soil concentrations. Maximum adsorbed Pb concentration for *S. criniflorum* (351 ± 51 mg Pb/kg DW_{root}) and *P. hortorum* (237 ± 17 mg/Pb/kg[1] DW_{root}) was observed at 2000 mg Pb/kg soil. Percentage $\text{Pb}_{\text{adsorbed}}$ was calculated by following formula:

$$\%\text{Pb}_{\text{adsorbed}} = [\text{Pb}_{\text{adsorbed}}]/[\text{Pb}_{\text{root}}] \times 100 \quad (3)$$

Results indicated that Pb adsorbed ranged for 3–18% for *P. hortorum* and 10–48% for *S. criniflorum*. This highest % $\text{Pb}_{\text{adsorbed}}$ and $\text{Pb}_{\text{adsorved}}$ (Fig. 2c) for *S. criniflorum* could be the reason for low Pb accumulation (Fig. 2a), uptake (Fig. 2b), and translocation (Table 2) compared with *P. hortorum*. Lead adsorption can take place on polysaccharides of the rhizodermal cells and carboxyl group of mucilage and uronic acid (Petruzzelli et al. 2015). The binding of Pb to these exchange sites limits the entry in plant tissue.

Biomass

Biomass of both plants after culture experiment is presented in Table 2. *P. hortorum* exhibited twofold more biomass (root and shoot) compared with *S. criniflorum* upon exposure to different concentrations of Pb in soil. *P. hortorum* produced significantly high biomass of root and shoot (1.1 ± 0.1 and 1.6 ± 0.1 g DW) at 500 mg Pb/kg soil treatment. Maximum root and shoot biomass (0.8 ± 0.1 and 1.2 ± 0.1 g DW) of *S. criniflorum* was also obtained at 500 mg Pb/kg soil. With increasing Pb concentration from 500 to 2000 mg Pb/kg soil, a 2.1- and 1.7-fold decrease in root and 1.5- and 1.6-fold decrease in shoot biomass were observed for *S. criniflorum* and *P. hortorum*, respectively. Contrary to this, no decrease in biomass of *Pelargonium* cultivars (Attar and Concolor) was observed, irrespective of soil Pb concentrations (500 and 1500 mg Pb/kg soil) (Arshad et al. 2008) indicating greater potential for Pb phytoextraction. The plant biomass was significantly higher in 500 mg Pb/kg soil level, which indicates that *P. hortorum* is Pb loving plant up to certain level (500 mg Pb kg^{-1} soil); however, with further increase in Pb concentration, decrease in plant dry biomass was observed. The higher Pb uptake with minimum decrease in dry biomass of

Table 2 Biomass and Pb uptake factors after 15 day of culture experiment. Results are significant at $p < 0.05$, as indicated by different letters

Parameters	*S. criniflorum*				*P. hortorum*			
Soil [Pb] mg kg^{-1}	500	1000	1500	2000	500	1000	1500	2000
TF	0.30	0.25	0.30	0.21	0.24	0.23	0.23	0.22
CF_r	0.93	0.60	0.48	0.38	1.40	0.86	0.73	0.64
CF_s	0.26	0.14	0.14	0.08	0.32	0.19	0.17	0.14
Biomass_r (g)	0.78^{bc}	0.58^{d}	0.50^{ed}	0.37^{e}	1.14^{a}	1.08^{a}	0.84^{bc}	0.67^{cd}
Biomass_s (g)	$1.16b^{cd}$	1.20^{bcd}	1.00^{de}	0.76^{e}	1.63^{a}	1.49^{ab}	1.33^{a-d}	1.00^{de}

TF, CF*r*, and CF*s* correspond to translocation factor, root concentration factor, and shoot concentration factor; $\text{biomass}_{r,s}$ corresponds to biomass of root and shoot, respectively

P. hortorum could be due to higher Pb tolerance ability and detoxification of Pb inside a plant tissue (Arshad et al. 2008).

Results of TF and CF are given in Table 2. Values of TF were higher for both plants at 500 mg Pb/kg soil compared with increasing soil Pb concentrations. This indicates lesser translocation to shoots when Pb concentration in the soil was raised. Bio-concentration factors (CF) were calculated separately for root and shoot (CF_r and CF_s, respectively). Results (Table 2) showed that CF_r values were higher than CF_s values which depict that more accumulation was observed in roots of both plants compared with shoot. Reduction in TF, CF_r, and CF_s values in *S. criniflorum* and *P. hortorum* has previously been reported by Manzoor et al. (2018) and can be attributed to several plant and soil factors. At higher soil Pb concentration, blockage of ion channels may occur, or Pb may attach and be fixed on exchange sites; also adsorption or binding of Pb on to carboxyl groups present on cell wall may reduce the overall Pb available and translocation inside plant tissue (Petruzzelli et al. 2015).

Oxidative response of *P. hortorum* upon Pb exposure

a. TBARS and H_2O_2

Production and accumulation of reactive oxygen species is a common phenomenon in plant on exposure to Pb and most commonly determined by estimating lipid peroxidation through production of thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2). Results of TBARS and H_2O_2 production in *P. hortorum* on exposure to Pb are presented in Fig. 3a and b. Both results are coherent in reporting higher oxidative stress in shoot compared with roots. This indicates higher Pb accumulation, and translocation in shoot caused production of more ROS in shoot. The TBARS results show that *P. hortorum* produces no significant oxidative stress at 500 mg Pb/kg soil. A fivefold increase in TBARS was observed in root (16 to 86 μmol kg^{-1} fw) and shoot (21 to 105 μmol kg^{-1} fw) of *P. hortorum* at 2000 mg Pb/kg soil compared with 500 mg Pb/kg soil.

Quantification of H_2O_2 content is also considered as important oxidative stress marker in plant exposed to environmental contaminants, and high production of H_2O_2 may cause cell damage leading to death of cell (Das et al. 2017). It is produced as a result of Pb stress in plant that induces superoxide dismutase (SOD) activity and that liberates H_2O_2 through dismutation of superoxide radicles (O^{2-}) stress (Shahid et al. 2017). Like TBARS, no significant increase in H_2O_2 production was observed in plants at 500 mg Pb/kg soil compared with control plants. At 2000 mg Pb/kg soil, significant increase in H_2O_2 production in root (2 to 4 μmol kg^{-1} fw) and shoot (10 to 27 μmol kg^{-1} fw) was observed, compared with soil with 500 mg Pb/kg soil. Results from H_2O_2 also assay revealed that higher oxidative stress was found in shoot relative to shoot at all levels of soil Pb concentration. H_2O_2 production was sevenfold higher in shoot compared with roots at 2000 mg Pb/kg soil Pb concentration.

Membrane injury (MI) and membrane stability (MS) index

These indices determine the membrane damage and stability after exposure to environmental contaminants. MI index is obtained by estimating electrolyte leakage from cell after disruption of cell membrane considering ion leakage as an indication of cell disruption and death (Das et al. 2017). Results indicated significantly higher electrolyte leakage in root (62%) and shoot (57%) of *P. hortorum* at 2000 mg Pb/kg soil (Fig. 3c). Higher MI was observed in roots, whereas higher membrane stability was observed in shoot. This could be due to the fact that roots are in direct contact with soil containing toxic heavy metals and other contaminants and are highly sensitive organ toward environmental cues (Shahid et al. 2017). Membrane stability index was estimated by measuring the amount of Evans blue dye accumulates inside dead cells (Das et al. 2017). No significant difference in cell damage was observed between root and shoot at all levels except 2000 mg Pb/kg soil (Fig. 4d), whereas 1.4-fold increase in cell damage was observed in shoot, possibly due to increased concentration of Pb in shoot of *P. hortorum* (Manzoor et al. 2018).

ROS localization and imaging

Production of reactive oxygen species (ROS) in plants is common when exposed to heavy metals containing Pb. Most frequently produced ROS are hydrogen peroxide(H_2O_2), singlet oxygen (1/2 O_2), superoxide anion (O_2^-), hydroxyl ($HO^{\cdot}$), alkoxyl ($RO^{\cdot}$), peroxyl ($RO_2^{\cdot}$), and organic hydroperoxide (ROOH) (Shahid et al. 2014). Therefore, in this study, roots were analyzed for all ROC species localization and signaling by Invitrogen technique. The images obtained are presented in Fig. 4. The figure includes both internal and external cells showing ROS signals. Results indicate less ROS in control in both internal and external cells. In positive control (roots dipped in 1% H_2O_2 before imaging), strong ROS signals were observed both in internal and external cells (Fig. 4c and d) compared with control and treatment levels. At 500 mg Pb/kg soil, less ROS signal was obtained in internal and external cells. This is consistent with results of TBARS, H_2O_2, membrane injury, and stability indices, where no significant change in oxidative stress was observed in root and shoots of *P. hortorum*. Similarly, at higher Pb concentration, i.e., at 1500 mg Pb/kg soil, more ROS signal was obtained comparable with positive control showing increased oxidative stress as observed in quantitative assays. Under natural condition, H_2O_2 is produced in root cells and regulates cell division and differentiation (Yu et al. 2016). However, under stress condition, there is more SOD activity that liberates more H_2O_2

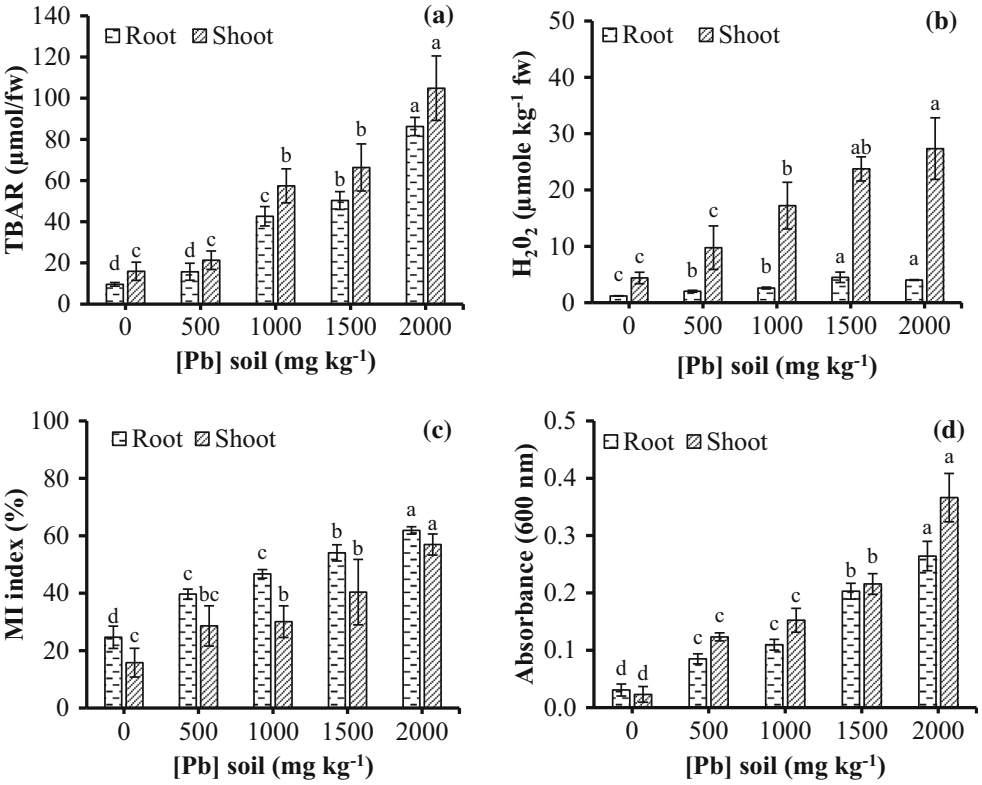

Fig. 3 Oxidative stress. TBARS (**a**); H_2O_2 contents (**b**); membrane injury (MI) and stability index measured by Evans blue uptake (**c**); and electrolyte leakage in root and shoot after growing for 2 months in potting mixture containing Pb after culture experiment (**d**), respectively. The bars are means ± SE of three replicates. Bars bearing same letters are not significantly different at $p < 0.05$

(Shahid et al. 2017; Das et al. 2017). It is documented that at lower concentrations, H_2O_2 helps plants in activating tolerance and defense mechanism against environmental contaminants, whereas in higher concentration, ROS interact with lipids, proteins, enzymes, and DNA causing irreparable metabolic dysfunctions, enzyme inactivation, and membrane

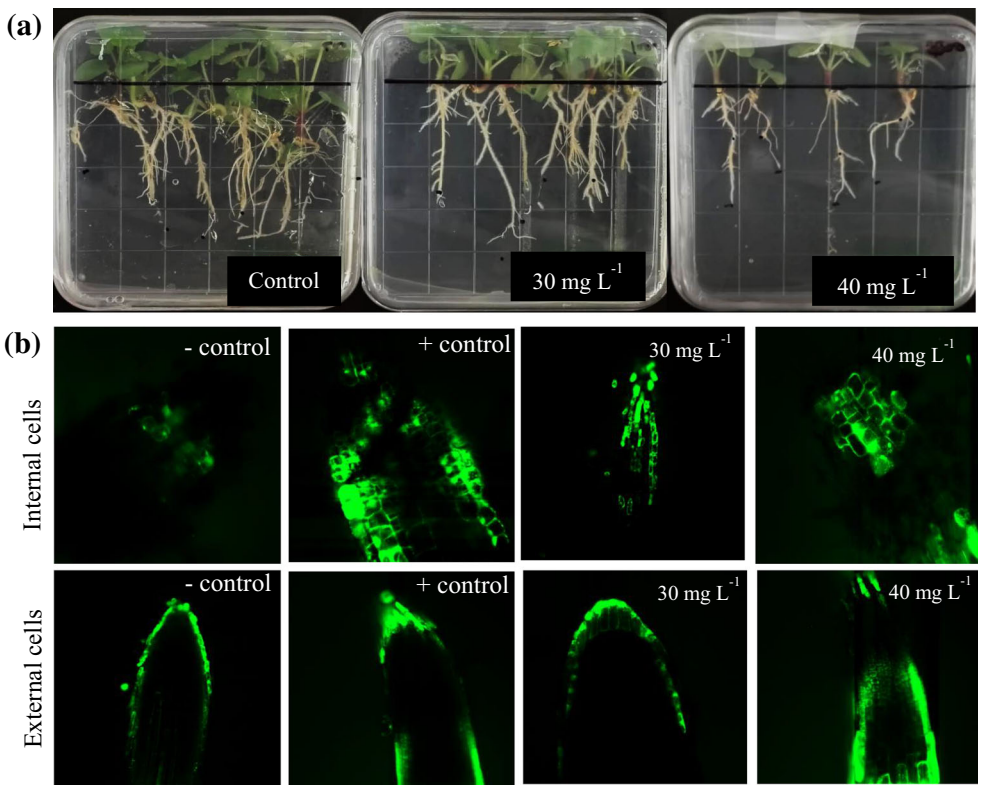

Fig. 4 Growth of *P. hortorum* (**a**). ROS localization (**b**) in root tips after growing for 2 weeks on MS agar supplemented with Pb (0, 30, and 40 mg L^{-1}, respectively)

damage leading to cell death (Shahid et al. 2017; Pourrut et al. 2011). This could be the reason for reduced growth and root shoot length (Table 2; Fig. 4a) of 2000 mg Pb/kg soil. At lower Pb concentration (500 mg Pb/kg), no oxidative damage was observed in *P. hortorum*, which could be due to the ability of this plant to maintain ROS level below threshold by the activation of ROS-scavenging processes as present in many hyperaccumulator plants (Shahid 2010).

Conclusions

The study concluded that both plants have the ability to induce rhizosphere changes during the 3-week culture in cropping device. Results indicated better ability of *P. hortorum* to acidify rhizosphere soil and increase the DOC content in soil that may have influenced Pb mobility in soil solution. Lead accumulation results showed greater uptake in *P. hortorum* than *S. criniflorum* at all levels of Pb in spiked soil. Physiological assays showed ability of *P. hortorum* to tolerance high concentration of Pb, making it a suitable candidate for phytoremediation of Pb-contaminated soils. The study provided useful information, about rhizospheric modifications induced by plants capable of phytoextracting high levels of Pb that will be helpful for better understanding of phytoremediation systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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