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RESEARCH ARTICLE

Deciphering the growth, organic acid exudations, and ionic homeostasis of *Amaranthus viridis* L. and *Portulaca oleracea* L. under lead chloride stress

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Abstract Lead (Pb) stress adversely affects *in planta* nutrient homeostasis and metabolism when present at elevated concentration in the surrounding media. The present study was aimed at investigation of organic acid exudations, elemental contents, growth, and lipid peroxidation in two wild plants (*Amaranthus viridis* L. and *Portulaca oleracea* L.), exhibiting differential root to shoot Pb translocation, under Pb stress. Plants were placed in soil spiked with lead chloride ($PbCl_2$) concentrations of 0, 15, 30, 45, or 60 mg Pb/kg soil, in rhizoboxes supplied with nylon nets around the roots. The plant mucilage taken from root surfaces, mirroring the rhizospheric solution, was analyzed for various organic acids. Lead stress resulted in a release of basified root exudates from both plants. Exudates of *P. oleracea* roots showed a higher pH. In both plants, the pH rising effect was diminished at the highest Pb treatment level. The exudation of citric acid, glutamic acid (in both plants), and fumaric acid (in *P. oleracea* only) was significantly increased with applied Pb levels. In both plant species, root and shoot Pb contents increased while nutrients (Ca, Mg, and K) decreased with increasing Pb treatment levels, predominantly in *A. viridis*. At 60 mg Pb/kg soil, shoot Na content of *A. viridis* was significantly higher as compared to untreated control. Higher Pb treatment levels decreased plant fresh and dry masses as well as the quantity of photosynthetic pigments due to enhanced levels of plant H_2O_2 and thiobarbituric acid reactive substances in both species. Photosynthetic, growth, and oxidative stress parameters were grouped into three distinct dendrogram sections depending on their similarities under Pb stress. A positive correlation was identified between Pb contents of studied plants and secretion of different organic acids. It is concluded that Pb stress significantly impaired the growth of *A. viridis* and *P. oleracea* as a result of nutritional ion imbalance, and the response was cultivar-specific and dependent on exogenous applied Pb levels. Differential lipid oxidation, uptake of nutrients (Ca, Mg, and K) and exudation of citric acid, fumaric acid, and glutamic acid could serve as suitable physiological indicators for adaptations of *P. oleracea* to Pb enriched environment. The findings may help in devising strategies for Pb stabilization to soil colloids.

Keywords Organic acids · Pb translocation · Rhizobox · Rhizosphere · Wild plants

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Introduction

According to Environmental Protection Agency (EPA), USA, lead (Pb) is one of the five most toxic heavy metals which is harmful to biotic systems and listed as the major contaminant and great threat to the environment (Wang et al. 2013). It has received great attention of scientists due to its ability to stay in the environment for a prolonged period of time, i.e., 150–

5000 years (Fahr et al. 2013). A major proportion of Pb, present in soil and water bodies, comes from erosion and weathering of rocks (Gadd 2010). In addition, Pb sources include municipality sewage sludge, Pb-supplemented paints, explosives, gasoline, mining/smelting activities, fuel combustion, chemical fertilizers, Pb containing acid batteries, and fusible alloys (Mukai et al. 2001). The soil-Pb concentration varies from 2 to 29 mg/kg of soil in various industrial zones of Pakistan (Malik et al. 2010). Presence of Pb in soil-plant system affects seed germination, seedling growth, root development, chlorophyll synthesis, transpiration rate, biomass, and yield (Pourrut et al. 2011; Bharwana et al. 2014).

High levels of Pb not only inhibit enzymatic activities and membrane permeability in plants but also initiate oxidative stress due to over production of reactive oxygen species (ROS) (Reddy et al. 2005). As the difference between ROS production and elimination increases under Pb stress, it disturbs the cell redox equilibrium and exposes the cells to oxidative stress causing deterioration of biological macromolecules, enhanced lipid peroxidation, and, hence, membrane dismantling (Pourrut et al. 2011). Srivastava et al. (2014) suggested that oxidative stress boosts Pb toxicity in rice (*Oryza sativa* L.).

The arable (wheat, and maize), as well as wild macrophytes (common cotton grass, alpine pennycress, and ryegrass), responds to toxic elements, i.e., Pb by initiating alterations in rhizospheric pH (Stoltz and Greger 2002; Blossfeld et al. 2010; Yang et al. 2011; Javed et al. 2013; Tanwir et al. 2015). The major components which contribute towards fluctuations in the rhizospheric pH are the activity of the proton pumps, imbalance of nutrients and metallic cations uptake, production of respiratory CO_2, and secretion of organic acids and protons by plant roots.

Under heavy metal stress, plant roots secrete low molecular weight organic acids (LMWOAs) and certain other chelating substances which may modulate the bioavailability of metallic cations (Haoliange et al. 2007; Qin et al. 2007; Zeng et al. 2008). The exudation of LMWOAs can probably be interpreted as an adaptation of plants to adverse conditions, especially to toxic Pb concentrations. The dissociated carboxylic acids, holding negative charges, may interact with dissolved cations mainly through formation of metal-carboxylic acid complexes leading to metal immobilization. The composition of exuded LMWOAs may vary from species to species. Moderately high levels of toxic elements induced *Eriophorum angustifolium* roots to release various organic acids, but the exudation was less at higher treatment level (Javed et al. 2013). Lead generally precipitates in soil sediment together with phosphates, sulfates, and rhizospheric chemicals (Blaylock and Huang 2000). Plants control metal uptake and transport by formation of metal-organic acid (in their anionic forms) complexes (Kochian et al. 2005). An increase in Pb uptake was observed after addition of citric acid in *Vicia faba* and *Typha angustifolia* (Muhammad et al. 2009; Shahid et al. 2012). The Pb immobilization in root zone is mainly attributed to its ability to form a complex with histidine, while the translocation to the upper plant organs is due to the formation of its complexes with organic acids (Massaccesi et al. 2014). Furthermore, organic acids may also serve as an alternative source to EDTA in forming complexes with Pb when considering enhanced metal uptake from contaminated soil/sediment (Shakoor et al. 2014).

At normal plant cytosolic pH (7–7.5), organic acids are present as anions and upon release from roots, instead of binding with metallic cations, these anions can take up protons and cause rhizospheric basification. Metal stress suppressed the activity of H^+-ATPase which in turn reduced protons extrusion and caused a rise in external pH (Janicka-Russak et al. 2012). Such basification mechanisms by plants are also likely to cause metal stabilization in the rhizosphere due to reduced metal ions mobility (Javed et al. 2013). The addition of organic acids can also cause soil acidosis depending on the number of carboxylic groups they carry, as well as the initial soil pH (Zhi-An et al. 2008).

Reports exist regarding the impact of Pb toxicity on nutritional ion (Ca^{2+} and K^+) imbalances in various plants (Trivedi and Erdei 1992), e.g., *Brassica napus* grown under Pb stress (Ali et al. 2014). Moreover, Pb can also prevent cation exchange in the roots for K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} (Sharma and Dubey 2005).

Portulaca oleracea L. and *Amaranthus viridis* L. are wild macrophytes distributed in various regions of Pakistan. In an earlier study, both plant species were reported to withstand Pb toxicity and exhibited significant differences in their root to shoot Pb translocation (Malik et al. 2010). However, the mechanisms of Pb tolerance which induced physio-biochemical alterations, as well as the exudation of various organic acids, that enable the adaptation of these wild plants to Pb-polluted rhizosphere, have not been investigated. In the present study, we (a) evaluated the possible role of different organic acids in Pb uptake, distribution, and tolerance in *P. oleracea* and *A. viridis* under Pb stress and (b) assessed the interference of Pb uptake with nutritious ion homeostasis and growth of these plants. For the first time, we investigated and discussed the organic acid exudations of *P. oleracea* and *A. viridis* under Pb stress with reference to their growth and photosynthetic attributes. It was hypothesized that *P. oleracea* and *A. viridis* plants will initiate changes in their rhizospheric environment by secreting different organic acids enabling these macrophytes to tolerate Pb toxicity. Exuded organic acids may modulate Pb uptake and translocation, nutrients, and plant physio-biochemical attributes. Appropriate understanding of physio-biochemical acclimatization, as well as its dependency on organic acids exudations, can assist effective utilization of *P. oleracea* and *A. viridis* for decontamination programs of Pb-polluted soil colloids/sediments.

Materials and methods

Plant acclimatization and rhizobox experiment

Wild grown plants of *Amaranthus viridis* L. and *Portulaca oleracea* L., having uniform growth, were collected from Botanical Garden, located at Government College University, Faisalabad, in flat planes of northeast Punjab, Pakistan (31°24/N, 73°04/E). After washing with distilled water, plants were mounted in Styrofoam plates which covered 1-L black containers having aerated 1% Hoagland solution (Eliasson 1978). There were five to six plants per container, which were placed in a growth chamber at temperature of 22 ± 2 °C having RH of 70% and light/dark conditions of 16 h/8 h. Concentration of Hoagland solution was gradually increased up to 100% in order to acclimatize the plants and was replenished periodically. After acclimatization for 4 weeks, rhizoboxes were used to collect and examine organic acid contents in mucilage which was dissolved from root surface of plants as described by Javed et al. (2013, 2017) and UdDin et al. (2015). *Amaranthus viridis* L. and *P. oleracea* L. plants having uniform size and fully expanded leaves were washed thoroughly and transferred to the rhizoboxes (length = 18 cm, width = 18 cm, thickness = 3 cm) (Greger and Landberg 2008). The pH of the experimental soil was 7.64 with EC of 3.06 dSm^{-1} having Pb concentration of 0.28 mg/kg, available N of 682 mg/kg, available P of 5.2 mg/kg, total Mg of 78.7 mg/kg, total Ca of 730.2 mg/kg, total Fe of 169.8 mg/kg, total K of 256 mg/kg and 1.19% organic matter. Soil was autoclaved and spiked with $PbCl_2$, 8 weeks in advance, to achieve the final concentrations of Pb as 0.28 (natural soil Pb level/control), 15, 30, 45, or 60 mg/kg soil (Malik et al. 2010) before use in rhizoboxes. In each rhizobox, four plants were used where plant roots were placed in a nylon net (25 μm) which prevented direct root-soil contact. For each treatment level, four replicates were maintained.

Collection of root exudates

Two seedlings were taken from rhizoboxes after 72 h and roots were thoroughly rinsed with distilled water. The solution obtained from rinsing of roots was filtered through a filter with 0.45 μm pore size (Millex-HA, Millipore) and was subsequently collected in Eppendorf tubes (Greger and Landberg 2008). Sodium hydroxide solution (0.01 M) was mixed with all the samples in order to analyze the organic acids except the ones used for pH measurements and analysis of oxalic acid (Javed et al. 2013). The collected samples were then freeze-dried with the help of liquid nitrogen and stored at − 80 °C before further analysis of the organic acids.

Analysis of root exudates

Quantitative analysis of organic acids was carried out with high-performance liquid chromatography (HPLC) equipped with Flexer FX-10 UHPLC isocratic pump (PerkinElmer, MA, USA). For this purpose, the freeze-dried root exudate samples were mixed with ethanol (80%) and 20 μl of the solutions was eluded isocratically into a C18 column (Brownlee Analytical C-183 μm; length 150 mm × 4.6 mm^2, USA). The mobile phase of the HPLC was comprised of acidified acetonitrile solution consisting of acetonitrile:H_2SO_4:acetic acid at 15:4:1, respectively, and at pH 4.9. The samples were analyzed for a time period of 10 min at a flow rate of 1.0 ml min^{-1}. The inner column temperature was maintained at 45 °C, and organic acid quantification was executed with the help of a detector (UV-vis Series 200, USA) at 214 nm wavelength based upon the retention times as well as the UV spectra relative to their standards (UdDin et al. 2015).

The pH of the root exudates was recorded after dissolving the freeze-dried samples in redistilled water with the help of micro-pH glass electrode by using a pH meter (ISTEK Model 4005-08007, Seoul, South Korea).

Plant lead and nutritious ion analysis

The plants which were harvested from rhizoboxes after 72 h of Pb exposure for collection of root exudates were subsequently analyzed for Pb as well as Ca, Mg, K, and Na contents. For metal analysis, the plant roots were washed with distilled water twice, quickly dipped in EDTA (20 mM), and thereafter rinsed again with distilled water in order to remove the adsorbed metals from the root surfaces. The plants were separated into the roots and shoots and were oven dried for 24 h at 105 °C. Oven-dried ground material (0.5 g) was then shifted into digestion flasks which were incubated overnight at room temperature after addition of 5 ml conc. H_2SO_4 (Wolf 1982). Afterwards, 0.5 ml H_2O_2 (35%) was poured into the flasks which were placed on a hot plate at temperature of 350 °C until no fume was produced. The digestion flasks were removed from the hot plate and allowed to cool down. The flasks were again placed on a hot plate at temperature of 350 °C after addition of H_2O_2, and this step was repeated until transparent/clear digestion mixtures were obtained. Digested mixtures were diluted in volumetric flasks up to 50 ml and were stored at 4 °C till further analysis. The Pb and nutrient contents (Ca, Mg, K, and Na) of the digested plant samples were analyzed by atomic absorption spectrophotometer (Hitachi, Model 7JO-8024, Tokyo, Japan) by using flame spectrophotometry. Standard reference materials (SRM) and standard solutions were used during metal analysis in order to minimize the matrix affect.

Plant physio-biochemical analysis

Remaining two plants from each rhizobox were used (eight plants for each Pb treatment level) for the measurement of different physio-biochemical attributes.

Plant chlorophyll and carotenoid contents

Method of Arnon (1949) was used to assay plant chlorophyll and carotenoid contents. After homogenization in acetone (80%), fresh plant material was kept at 4 °C overnight. After centrifugation at 3000×*g* for 15 min, the solution was filtered and absorbance of the supernatant was recorded by using a spectrophotometer (Hitachi U-2001, Tokyo, Japan) at wavelengths of 663, 645, and 480 mm. Chlorophyll *a,* chlorophyll *b,* and total chlorophyll as well as total carotenoid contents were calculated by the methods described by Arnon (1949) and Davies (1976), respectively.

Measurement of lipid peroxidation

The level of lipid peroxidation products of fresh leaf tissue was measured according to method of Heath and Packer (1968) in terms of thiobarbituric acid reactive substances (TBARS) assayed by thiobarbituric acid (TBA) reaction. Plant material was homogenized with 0.1% trichloroacetic acid (TCA) by using an ultra-mixer (POLYTRON 2000, Switzerland). The homogenates were centrifuged for 5 min at 10000×*g*, and supernatant was mixed with TCA (20%) and TBA (0.5%) and then heated for 30 min at 100 °C. The tubes were placed on ice to quickly stop the reaction, and samples were again centrifuged at 10000×*g* for 5 min. The absorbance of supernatant was assayed at 532 nm and adjusted for non-specific absorbance at 600 nm by using Ultrospec 3000 (Biochrom Ltd. Cambridge, England). The extinction coefficient was 155 mM cm^{-1}.

Plant's hydrogen peroxide contents

The plant's H_2O_2 content was measured colorimetrically as described by Jana and Choudhuri (1981). Leaf tissues (50 mg) were homogenized in 3 ml of 50 mM phosphate buffer at pH of 6.5, and the homogenate was centrifuged for 25 min at 6000×*g*. Extracted solution (3 ml) was then mixed with 1 ml titanium sulfate (0.1%) in H_2SO_4 (20% *v*/*v*), and the reaction mixture was again centrifuged for 15 min at 6000×*g*. In order to measure H_2O_2 contents, intensity of the yellow-colored supernatant was measured at 410 nm by using Ultrospec 3000 (Biochrom Ltd. Cambridge, England) where the extinction coefficient was 0.28 $mmol^{-1}$ cm^{-1}.

Statistical analysis

Simple regression and analysis of variance (ANOVA) were used for statistical data analysis by a statistical program "R" (version 2.13.0). Tukey's honest significant differences test (HSD-test) was used for recognition of treatment differences at significance levels of $p \leq 0.05$. Prior to statistical analysis, logarithmic or inverse transformations were used to normalize the data, when necessary.

Results

Organic acid contents and pH in root exudates of *A. viridis* and *P. oleracea* under lead stress

In the absence of Pb stress, the pH of 6.88 and 7.12 was observed in the mucilage of *A. viridis* and *P. oleracea*, respectively (Table 1). A consistent increase in pH in the root exudates of *P. oleracea* (up to 45 mg/kg) and in *A. viridis* (up to 30 mg/kg) was observed. However, pH increase at the highest level of Pb was less, where a significant pH decrease was recorded for *A. viridis* at 60 mg/kg Pb treatment.

The organic acids (citric, malic, fumaric, oxalic, and glutamic acids) were an important component of root exudates. The organic acid contents were determined in the two plant species, i.e., *A. viridis* and *P. oleracea* grown with various applied Pb levels. In both plant species, citric acid exudation was enhanced with increase in applied Pb levels. The maximum citric acid secretion was observed, at 60 mg Pb/kg soil, by the roots of *P. oleracea* which was 24% higher than the citric acid released by *A. viridis* (Table 1). The release of all other studied organic acids also followed a similar trend. The roots exudates of *P. oleracea* had 6.75, 21, 1.06, and 7.32% higher malic acid, fumaric acid, oxalic acid, and glutamic acids in comparison to root exudates of *A. viridis* when both species were grown in the presence of 60 mg Pb/kg soil.

Physiological attributes of *A. viridis* and *P. oleracea* under lead stress

The plant fresh and dry weights were decreased when seedlings were grown in the presence of Pb for **72** h. The decrease was associated with applied Pb levels, though Pb-induced effects were more pronounced in *A. viridis* (Table 2). The photosynthetic pigments (chlorophylls *a* and *b* and total chlorophyll) as well as accessory pigments (carotenoids) were also reduced under Pb stress. An increase in H_2O_2 content was observed together with increase in applied Pb stress which was more pronounced in *P. oleracea*. At 60 mg Pb/kg soil, *A. viridis* and *P. oleracea* showed 146 and 95% higher H_2O_2 content, respectively, in comparison to the one exhibited by respective control plants.

Table 1 Organic acid contents in root muciliage of *Amaranthus viridis* L. and *Portulaca oleracea* L. grown in soils spiked with different concentrations of $PbCl_2$ of 0, 15, 30, 45, and 60 mg/kg soil in rhizoboxes, $n = 4$, Mean ± SE

Plant species	$PbCl_2$ treatments (mg/kg)	pH and concentration of organic acids (mg/g root DW) of root exudates					
		pH	Citric acid	Malic acid	Fumaric acid	Oxalic acid	Glutamic acid
Amaranthus viridis L.	0	6.88 ± 0.11^{c}	0.906 ± 0.009^{d}	2.024 ± 0.006^{d}	0.639 ± 0.01^{e}	7.11 ± 0.006^{d}	0.31 ± 0.006^{e}
	15	7.20 ± 0.02^{b}	1.075 ± 0.049^{c}	2.14 ± 0.009^{d}	0.754 ± 0.012^{d}	7.17 ± 0.011^{c}	0.456 ± 0.012^{d}
	30	7.51 ± 0.04^{a}	1.162 ± 0.064^{c}	2.19 ± 0.006^{c}	0.846 ± 0.013^{c}	7.28 ± 0.024^{c}	0.553 ± 0.012^{c}
	45	7.17 ± 0.04^{b}	1.349 ± 0.027^{b}	2.27 ± 0.023^{b}	0.949 ± 0.008^{b}	7.37 ± 0.011^{b}	0.726 ± 0.014^{b}
	60	6.56 ± 0.14^{d}	1.507 ± 0.027^{a}	2.37 ± 0.004^{a}	1.097 ± 0.015^{a}	7.48 ± 0.024^{a}	0.997 ± 0.054^{a}
Portulaca oleracea L.	0	7.12 ± 0.03^{D}	0.926 ± 0.006^{E}	2.002 ± 0.024^{E}	0.647 ± 0.013^{E}	7.10 ± 0.007^{E}	0.33 ± 0.018^{E}
	15	7.37 ± 0.03^{C}	1.158 ± 0.017^{D}	2.21 ± 0.022^{D}	0.801 ± 0.01^{D}	7.19 ± 0.02^{D}	0.523 ± 0.012^{D}
	30	7.70 ± 0.06^{B}	1.329 ± 0.033^{C}	2.32 ± 0.035^{C}	1.096 ± 0.008^{C}	7.36 ± 0.023^{C}	0.64 ± 0.017^{C}
	45	7.82 ± 0.02^{A}	1.613 ± 0.038^{B}	2.40 ± 0.003^{B}	1.225 ± 0.023^{B}	7.46 ± 0.021^{B}	0.853 ± 0.023^{B}
	60	7.16 ± 0.03^{D}	1.860 ± 0.01^{A}	2.53 ± 0.006^{A}	1.318 ± 0.016^{A}	7.56 ± 0.025^{A}	1.07 ± 0.032^{A}

Different letters a–e and A–E represent significant difference in organic acids contents of *Amaranthus viridis* L. and *Portulaca oleracea* L., respectively

An increase in exogenous Pb in soil resulted in higher membrane lipid peroxidation, as evident from TBARS contents at various Pb levels (Table 2). At the highest applied Pb stress, i.e., 60 mg/kg soil, plants of *A. viridis* exhibited a higher lipid peroxidation, as evident from 71% higher TBARS contents (6.07 μmol/g FW), than exhibited by *P. oleracea* (3.55 μmol/g FW).

Root and shoot lead contents

The results showed that the absorption of Pb by the roots was increased in both plant species, i.e., *A. viridis* and *P. oleracea* with increasing applied Pb concentrations (Fig. 1). The two species exhibited differential response and, at 60 mg/kg soil, the former species showed three times higher Pb and the latter one showed two times higher Pb, when compared to respective control group (plants grown without Pb). The maximum shoot Pb content was observed in *A. viridis* (50.96 μg/g DW) followed by *P. oleracea* (31.73 μg/g DW) at 60 mg Pb/kg soil indicating a higher root to shoot translocation in the former plant species.

Root and shoot Ca, K, Mg, and Na contents

The two plant species, *A. viridis* and *P. oleracea*, showed the same Ca uptake when no Pb was applied (control group) (Fig. 2). Exogenous Pb application resulted in a decrease in Ca uptake with the applied Pb level. The maximum decrease in Ca uptake (48% less in comparison to respective control) was exhibited by *A. viridis* at 60 mg Pb/

Table 2 Biomass, photosynthetic pigments, and H_2O_2 and TBARS contents of *Amaranthus viridus* L. and *Portulaca oleracea* L. grown in rhizoboxes having soil spiked with $PbCl_2$ concentrations of 0, 15, 30, 45, or 60 mg/kg soil, $n = 4$, Mean ± SE

$PbCl_2$ treatments (mg/kg soil)	Plant species	Plant fresh weight (g)	Plant dry weight (g)	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoids (mg/g FW)	H_2O_2 (μmol/g FW)	TBARS (μmol/g FW)
0	*Amaranthus*	2.6 ± 0.057^{a}	1.2 ± 0.045^{a}	4.35 ± 0.028^{a}	3.08 ± 0.033^{a}	3.35 ± 0.028^{a}	83.34 ± 1.17^{a}	1.02 ± 0.057^{a}	1.72 ± 0.052^{e}
15	*viridis* L.	2.33 ± 0.033^{b}	0.9 ± 0.057^{b}	3.86 ± 0.036^{b}	2.46 ± 0.040^{b}	3.12 ± 0.033^{b}	67.52 ± 0.92^{b}	1.36 ± 0.036^{b}	2.46 ± 0.040^{d}
30		1.833 ± 0.033^{c}	0.7 ± 0.054^{c}	3.27 ± 0.052^{c}	1.83 ± 0.033^{c}	2.73 ± 0.038^{c}	54.44 ± 0.91^{c}	1.83 ± 0.033^{c}	3.83 ± 0.033^{c}
45		1.46 ± 0.031^{d}	0.56 ± 0.088^{d}	2.82 ± 0.041^{d}	1.45 ± 0.061^{d}	2.45 ± 0.009^{d}	43.81 ± 0.76^{d}	2.12 ± 0.032^{d}	4.45 ± 0.061^{b}
60		1.1 ± 0.057^{e}	0.36 ± 0.066^{e}	3.30 ± 0.026^{c}	1.07 ± 0.037^{e}	2.17 ± 0.04^{e}	37.6 ± 0.61^{e}	2.51 ± 0.030^{e}	6.07 ± 0.037^{a}
0	*Portulaca*	2.26 ± 0.16^{A}	1.06 ± 0.034^{A}	5.55 ± 0.023^{A}	3.61 ± 0.026^{A}	4.56 ± 0.023^{A}	103.5 ± 2.06^{A}	1.05 ± 0.021^{A}	1.62 ± 0.026^{E}
15	*oleracea*	1.8 ± 0.057^{B}	0.83 ± 0.066^{B}	5.08 ± 0.055^{B}	3.28 ± 0.055^{B}	4.49 ± 0.011^{A}	83.98 ± 1.23^{B}	1.16 ± 0.015^{A}	1.92 ± 0.031^{D}
30	L.	1.56 ± 0.088^{C}	0.7 ± 0.047^{C}	4.77 ± 0.063^{C}	3.11 ± 0.023^{C}	4.40 ± 0.028^{B}	81.63 ± 0.98^{B}	1.52 ± 0.023^{B}	2.11 ± 0.023^{C}
45		1.3 ± 0.057^{D}	0.63 ± 0.037^{C}	4.24 ± 0.046^{D}	2.74 ± 0.059^{D}	4.31 ± 0.029^{C}	63.8 ± 1.11^{C}	1.74 ± 0.059^{C}	2.74 ± 0.059^{B}
60		1.16 ± 0.66^{E}	0.5 ± 0.054^{D}	3.85 ± 0.060^{E}	2.55 ± 0.052^{E}	4.16 ± 0.028^{D}	51.69 ± 1.02^{D}	2.05 ± 0.052^{D}	3.55 ± 0.052^{A}

Different letters a–e and A–E represent significant differences for *Amaranthus viridus* L. and *Portulaca oleracea* L., respectively

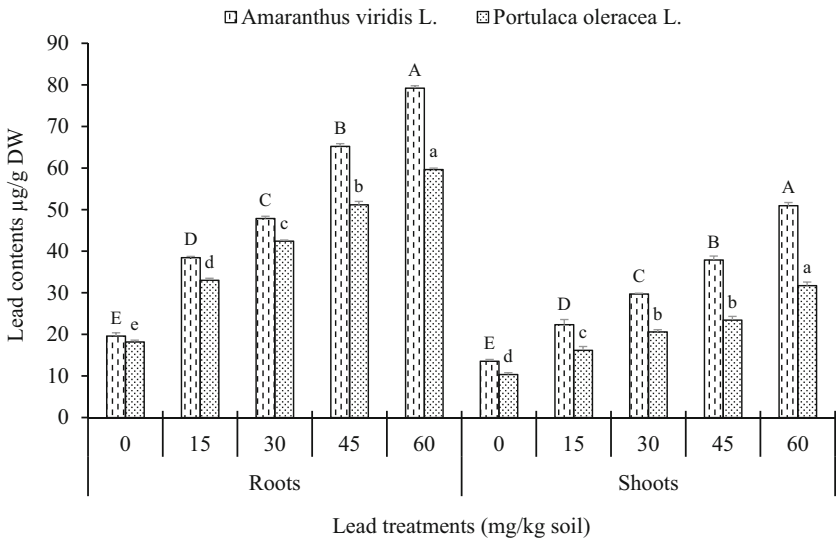

Fig. 1 Lead (Pb) contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. roots and shoots grown with different $PbCl_2$ concentrations of 0, 15, 30, 45, or 60 mg/kg soil in rhizoboxes. The control treatment designated as 0 mg/kg soil Pb was natural soil having Pb level of 0.28 mg/kg soil with no exogenous Pb application. Significant differences in roots and shoots Pb contents of *A. viridis* and *P. oleracia* are represented by letters A–E and a–e, respectively. $n = 4$, Mean ± SE

kg soil while *P. oleracea* showed 37% decrease, with respect to its corresponding control, at the same Pb level. The root to shoot Ca translocation was also negatively affected by Pb and, at 60 mg Pb/kg soil, *A. viridis* showed more reduction in comparison to *P. oleracea*.

The results indicated that the uptake of K by the roots of *A. viridis* decreased with increasing Pb concentration with a maximum decrease at 60 mg Pb/kg soil (Fig. 3). *P. oleracea* roots showed an increase in K uptake (at 15 mg/kg soil), an insignificant change at 30 mg/kg soil, and a significant decrease at 45 and 60 mg/kg soil as compared with respective control. At 60 mg/kg soil, *A. viridis* showed 63.5% decrease while *P. oleracea* roots showed 44% decrease in K uptake, as compared with respective control. A similar trend was observed for root to shoot translocation of K by the two plant species.

The obtained data also revealed that increased Pb levels resulted in decreased Mg uptake by the roots of both plant species, though differences existed between the two macrophytes. *A. viridis* showed more reduction in Mg uptake in comparison to *P. oleracea* as shown in Fig. 4. Root to shoot translocation of Mg was also negatively influenced by Pb, and the two plant species exhibited similar decreasing trend for Mg translocation. *Amaranthus viridis* showed a pronounced reduction as compared to *P. oleracea*, particularly at 60 mg/kg soil.

The uptake of Na by *A. viridis* roots decreased with increase in applied Pb level up to 45 mg/kg soil (Fig. 5).

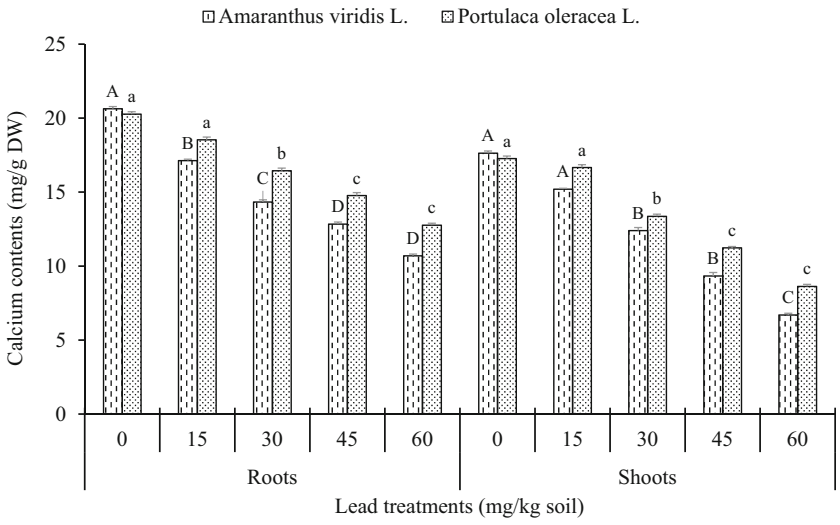

Fig. 2 Calcium (Ca) contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. roots and shoots grown with different $PbCl_2$ concentrations of 0, 15, 30, 45, or 60 mg/kg soil in rhizoboxes. The control treatment designated as 0 mg/kg soil Pb was natural soil having Pb level of 0.28 mg/kg soil with no exogenous Pb application. Significant differences in roots and shoots Ca contents of *A. viridis* and *P. oleracia* are represented by letters A–D and a–c, respectively. $n = 4$, Mean ± SE

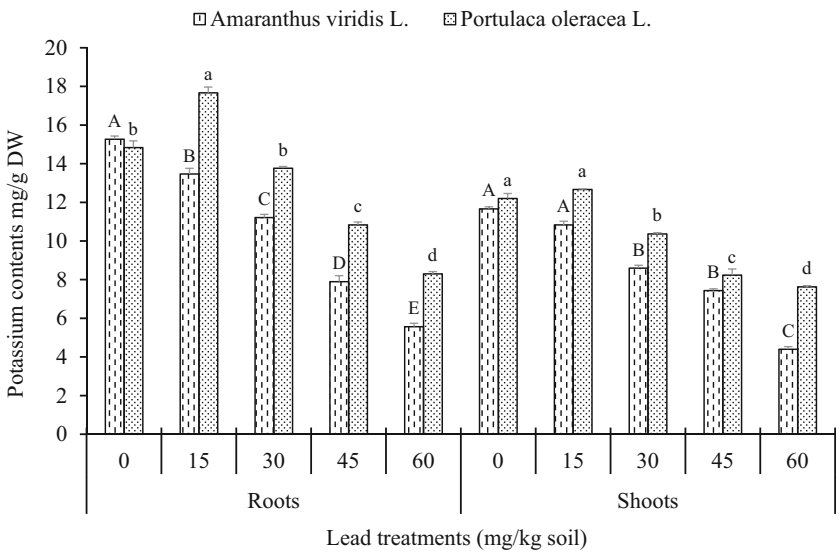

Fig. 3 Potassium (K) contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. roots and shoots grown with different $PbCl_2$ concentrations of 0, 15, 30, 45, or 60 mg/kg soil in rhizoboxes. The control treatment designated as 0 mg/kg soil Pb was natural soil having Pb level of 0.28 mg/kg soil with no exogenous Pb application. Significant differences in roots and shoots K contents of *A. viridis* and *P. oleracia* are represented by letters A–E and a–d, respectively. $n = 4$, Mean ± SE

At highest Pb treatment (60 mg/kg soil), *A. viridis* roots showed increase in uptake of Na in comparison to lower Pb levels (15, 30, or 45 mg/kg soil), though the Na content was less (66.3 mg/g DW) than the control plants (69.5 mg/g DW). The Na uptake by the roots of *P. oleracea* decreased with increase in Pb treatment, and maximum reduction was observed at 60 mg/kg soil. Moreover, *P. oleracea* showed more reduction in Na uptake as compared to *A. viridis*. Root to shoot translocation was also negatively influenced as was evident from reduced shoot Na content in both the plant species, and the reduction was more prominent in *P. oleracea* as compared to *A. viridis*.

Correlation coefficients, principle component analysis, and dendrograms

Significantly positive correlations among Pb contents and the studied organic acids were revealed by Pearson's correlation coefficients, as well as PCA (Table 3, Fig. 6). A significantly positive correlation was identified between root Pb contents and citric acid (0.920***), malic acid (0.906***), fumaric acid (0.901***), oxalic acid (0.946***), and glutamic acid (0.970***). The exudation of citric acid (0.984***), malic acid (0.695***), fumaric acid (0.969***), oxalic acid (0.786***), and glutamic acid (0.956***) was also positively

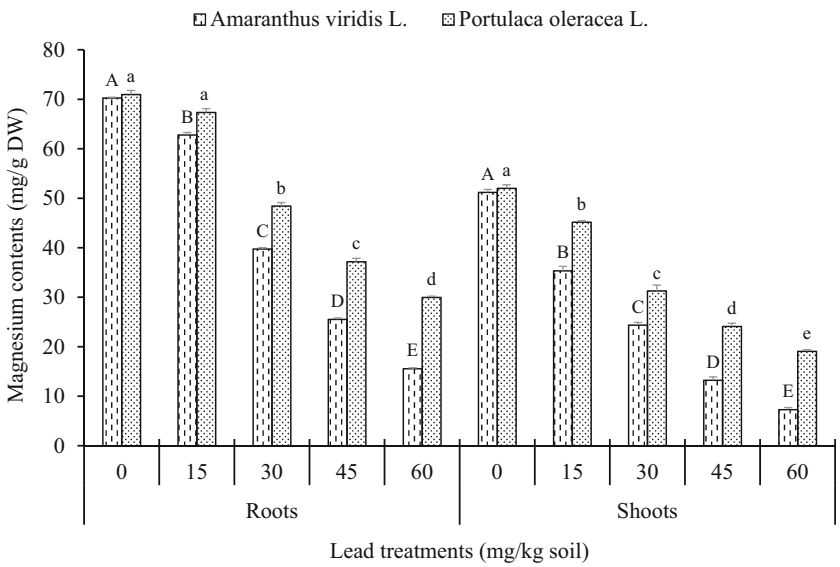

Fig. 4 Magnesium (Mg) contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. roots and shoots grown with different $PbCl_2$ concentrations of 0, 15, 30, 45, or 60 mg/kg soil in rhizoboxes. The control treatment designated as 0 mg/kg soil Pb was natural soil having Pb level of 0.28 mg/kg soil with no exogenous Pb application. Significant differences in roots and shoots Mg contents of *A. viridis* and *P. oleracia* are represented by letters A–E and a–e, respectively. $n = 4$, Mean ± SE

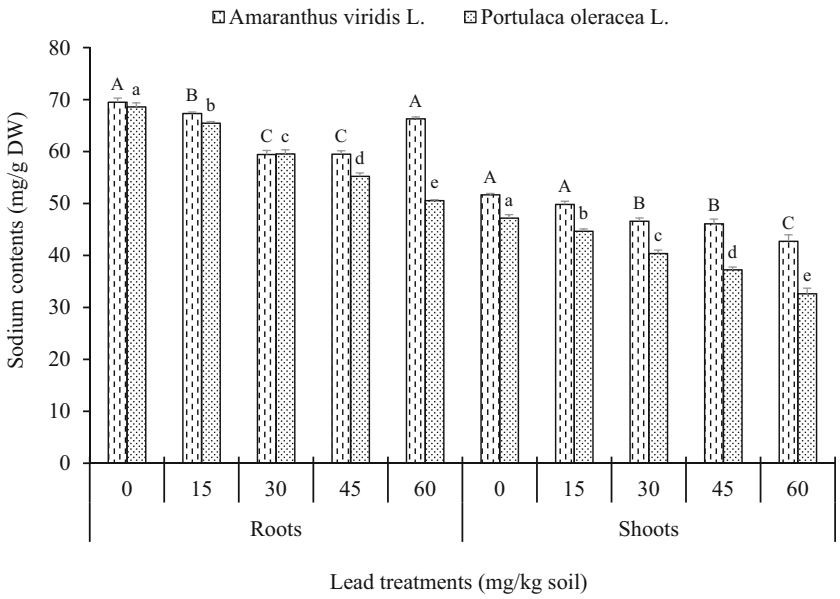

Fig. 5 Sodium (Na) contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. roots and shoots grown with different $PbCl_2$ concentrations of 0, 15, 30, 45, or 60 mg/kg soil in rhizoboxes. The control treatment designated as 0 mg/kg soil Pb was natural soil having Pb level of 0.28 mg/kg soil with no exogenous Pb application. Significant differences in roots and shoots Na contents of *A. viridis* and *P. oleracia* are represented by letters A–C and a–e, respectively. $n = 4$, Mean ± SE

correlated with shoot Pb contents. The organic acids exhibited significantly positive correlations with H_2O_2 and TBARS. However, organic acids were found to be negatively correlated with plant biomass, photosynthetic accessary pigments, and nutrient contents. Dendrogram analysis revealed the groups of plant physiological attributes of *A. viridis* and *P. oleracea*

Table 3 Correlation coefficients (*r*) among organic acids, biomass, photosynthetic pigments, and ionic contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. grown in soils spiked with different concentrations of $PbCl_2$ (0, 15, 30, 45, or 60 mg/kg) in rhizobox system

Variables	Citric acid	Malic acid	Fumaric acid	Oxalic acid	Glutamic acid
Citric acid	1.000***	0.701***	0.971***	0.793***	0.941***
Malic acid	0.701***	1.000***	0.670***	0.941***	0.836***
Fumaric acid	0.971***	0.670***	1.000***	0.769***	0.924***
Oxalic acid	0.793***	0.941***	0.769***	1.000***	0.886***
Glutamic acid	0.941***	0.836***	0.924***	0.886***	1.000***
Plant FW	− 0.808***	− 0.922***	−0.797***	− 0.927***	− 0.871***
Plant DW	− 0.902***	− 0.869***	−0.903***	− 0.889***	− 0.923***
Carotenoids	− 0.936***	− 0.631***	−0.947***	− 0.722***	− 0.900***
H_2O_2	0.972***	0.731***	0.974***	0.830***	0.951***
Chl. *a*	− 0.788***	− 0.327 ns	− 0.829***	− 0.462*	− 0.694***
Chl. *b*	− 0.880***	− 0.357 ns	− 0.899***	− 0.495**	− 0.763***
Total Chl.	− 0.612***	0.043 ns	− 0.646***	− 0.109 ns	− 0.447*
TBARS	0.954***	0.526**	0.956***	0.649***	0.876***
pH	− 0.242 ns	0.193 ns	− 0.181 ns	0.100 ns	− 0.210 ns
Shoot Ca	− 0.954***	− 0.810***	− 0.950***	− 0.897***	− 0.974***
Root Ca	− 0.967***	− 0.787***	− 0.969***	− 0.858***	− 0.961***
Shoot K	− 0.947***	− 0.671***	− 0.936***	− 0.803***	− 0.927***
Root K	− 0.683***	− 0.889***	− 0.655***	− 0.922***	− 0.806***
Shoot Mg	− 0.960***	− 0.760***	− 0.970***	− 0.845***	− 0.942***
Root Mg	− 0.956***	− 0.770***	− 0.966***	− 0.874***	− 0.949***
Shoot Na	− 0.435*	− 0.892***	− 0.402*	− 0.838***	− 0.623***
Root Na	− 0.451*	− 0.814***	− 0.494**	− 0.795***	− 0.584***
Shoot Pb	0.984***	0.695***	0.969***	0.786***	0.956***
Root Pb	0.920***	0.906***	0.901***	0.946***	0.970***

***, **, and * = significant at 0.001, 0.01, and 0.05 levels, respectively

ns = non-significant

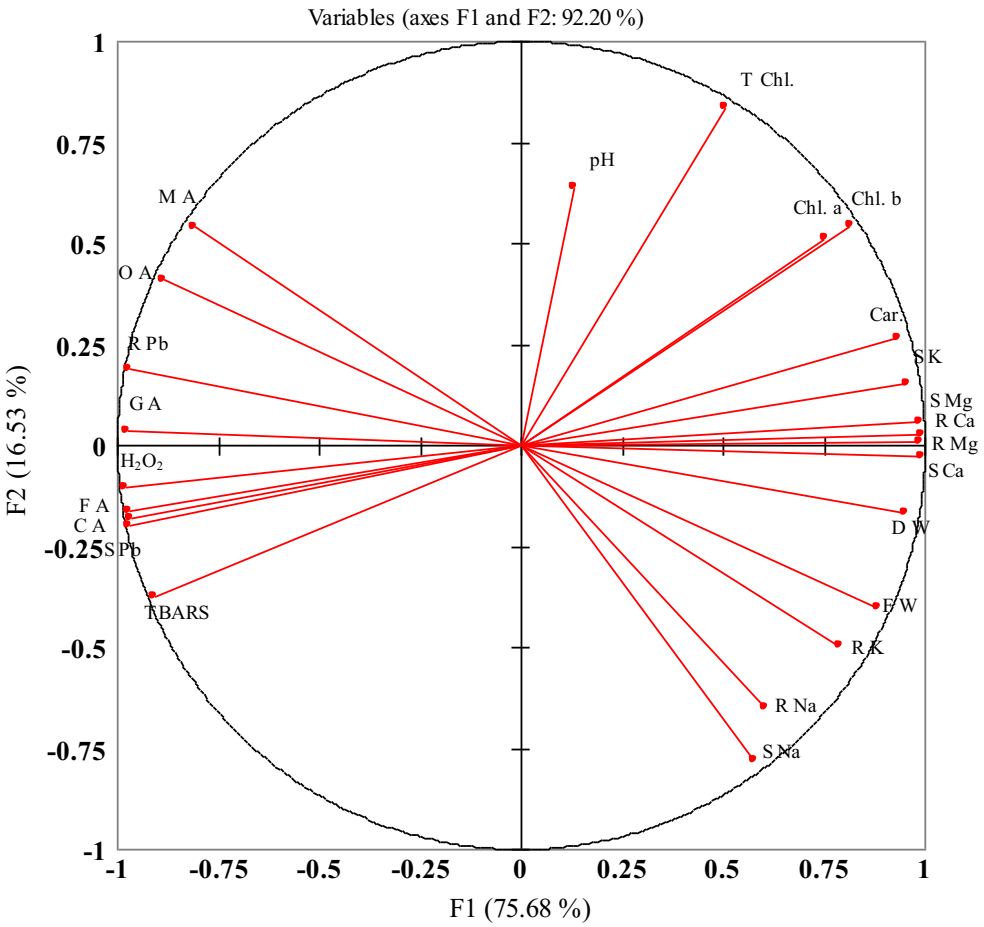

Fig. 6 Principle component analysis (PCA) showing associations among growth, exuded organic acids, and root and shoot elemental contents of *Amaranthus viridis* L. and *Portulaca oleracea* L. CA = citric acid; MA = malic acid; FA = fumaric acid; OA = oxalic acid; GA = glutamic acid; R Pb = root lead; S Pb = shoot lead; R Ca = root calcium; S Ca = shoot calcium; R Mg = root magnesium; S Mg = shoot magnesium; R K = root potassium; S K = shoot potassium; R Na = root sodium; S Na = shoot sodium; H_2O_2 = hydrogen peroxide; Chl. *a* = chlorophyll *a*; Chl. *b* = chlorophyll *b*; T. Chl = total chorophyll; Car = carotenoid; TBARS = thiobarbituric acid reactive substances; DW = dry weight; FW = fresh weight.

which were affected in a similar fashion under Pb stress (Fig. S1). Photosynthetic, growth, and oxidative stress parameters were grouped into three distinct sections depending on their similarities under Pb stress.

Discussion

Organic acid contents and pH in root mucilage of *A. viridis* and *P. oleracea* under Pb stress

The highest pH in the root mucilage was recorded at moderate Pb treatments, i.e., 30 mg/kg soil (for *A. viridis*) and at 45 mg/kg soil (for *P. oleracea*). While, at higher Pb treatment (60 mg/kg soil), the basification response was diminished in both the studied plant species (Table 1). Our results corroborated the findings of Javed et al. (2013) who reported that the roots of *Eriophorum angustifolium* caused rhizospheric basification when stressed by toxic elements (including Pb) at lower treatment level. It can be interpreted that at the highest Pb treatment level, the plants were stressed and lost their pH modulation response. Furthermore, the organic acids are exuded as anions by anion channels (Zhu et al. 2011) and their release remain balanced under metal stress by cations/proton efflux as reported for poplar roots (Qin et al. 2007). In *Cucumis sativus*, metal stress impaired the pumping activities of H^+-ATPase, which caused a decreased efflux of protons (Janicka-Russak et al. 2012). This could explain the rhizospheric basification in the present study under low Pb stress. The pH of root exudates decreased at higher Pb levels which is likely due to the increased contents of LMWOAs, whose carboxyl groups contributed towards the increase of H^+ in the solution as reported for castor (*Ricinus communis* L.) under metal stress (Huang et al. 2016). An investigation with *Eriophorum angustifolium* revealed acidosis of the exudates at higher Pb treatments (Javed 2011). It has been suggested that the pH decrease by organic acids only happened if the initial soil pH was high, as it was in the present study where the starting soil pH was 7.64. Addition of organic acids to soil promotes ammonification which produces protons and leads to a pH decrease

(Paul et al. 2001). Therefore, acidosis of root exudates might also depend on nitrogen conversion triggered by decomposition of the exuded organic acids.

Increasing Pb treatment levels influenced organic acids exudations in root mucilage of *A. viridis* and *P. oleracea* which might be of importance for plant adaptation to Pb-enriched environments (Table 1). Differential root exudation response of *A. viridis* and *P. oleracea* seems to depend on different Pb tolerance levels as proposed previously for *Cynodon dactylon* and *Zea mays* under metal stress (Xie et al. 2014; Javed et al. 2017). Exudation of different organic acids might be due to better nutrient homeostasis of *P. oleracea*, as an increase of K level also caused an elevated exudation in *Phelipanche aegyptiaca* and *Orobanche cumana* (Zhang et al. 2015). It is likely that the better nutrients absorption of K by *P. oleracea* roots and altered cell membrane permeability were related to secretion of organic acids.

The secreted organic acids may protect the plants by reducing acropetal metal transport to shoots due to metal-ion complex formation with organic acid anions in *P. oleracea*, compared to *A. viridis* (Kochian et al. 2005). Therefore, organic acid exudation is a likely mechanism by which *P. oleracea* tolerates Pb stress. In this regard, citric acid, malic acid, fumaric acid, oxalic acid, and glutamic acid are significantly important for *P. oleracea*, because a strong correlation was obtained between root Pb contents and organic acid exudations (Table 2). Despite of *in planta* Pb accumulation, Pb may be non-toxic if bound to organic ligands as compared to free Pb^{2+} ions. The dicarboxylic acids exuded by *A. viridis* and *P. oleracea* hold more negative charges, thus providing more binding sites, as well as higher binding affinity to Pb in comparison to monocarboxylic acids. Our results are in accordance with the finding that exudation of oxalic, malic, and lactic acids was significantly increased by the roots of *Phyllostachys pubescens* under Pb stress which enhanced Pb adsorption to soil colloids (Chen et al. 2016). Therefore, in *P. oleracea*, exudation of citric, glutamic, and fumaric acids could potentially limit the uptake of Pb by enhancing Pb sorption to the soil due to a rise in pH.

Metal stress stimulates organic acids exudation by acting through various mechanisms existing in plants, including both basic (Zhao et al. 2003) and stress-specific processes (Ma et al. 2000). In *A. viridis* and *P. oleracea*, the former mechanism is likely to occur, as their roots exuded various organic acids and Pb stress further activates organic acid exudations. Such an idea corroborated the findings of Javed et al. (2013), who suggested a similar mechanism for common cotton grass when exposed to a mixture of toxic elements including Pb. Reduction in shoot growth of *A. viridis* and *P. oleracea* seems to minimize by increased organic acid production, and the ameliorative effect can be stronger if organic acids are secreted in higher amount.

Effect of Pb stress on growth and photosynthetic pigments of *A. viridis* and *P. oleracea*

Plant fresh and dry weights were significantly decreased with Pb treatment levels in *A. viridis* and *P. oleracea* (Table 2). Increase in plant H_2O_2 contents will in turn increase TBARS levels by initiating a chain reaction in unsaturated lipids, which is likely to negatively affect the growth of *A. viridis* and *P. oleracea*. In both species, a marked reduction of plant dry weights, compared to fresh weights, points out that Pb affects photosynthetic activity of *A. viridis* and *P. oleracea* more than their growth. Earlier works also described significant decrease in dry weight of different plant parts in *Plantago major*, *Sesbania grandiflora*, *Triticum aestivum*, *Lens culinaris*, *Spinacia oleracea*, and *Solanum lycopersicum* under Pb stress (Kosobrukhov et al. 2004; Akinci et al. 2010; Lamhamdi et al. 2013; Malar et al. 2014). In addition, Pb stress in the roots of *Lemna minor* suppressed the cell division and thereby reduced the plant growth (Samar-dakiewicz and Wozny 2005). Dendrogram analysis showed that biomass (fresh weight and dry weight) of the studied plants was affected in a similar way and related parameters were grouped as G-2 (Fig. S1).

Lead (Pb) stress significantly reduced the plant photosynthetic pigments (chlorophylls *a* and *b* and total chlorophyll) as well as accessory pigments (carotenoids), and the effect was Pb dose-dependent and plant-specific. In *A. viridis*, a significant reduction in chlorophyll *b* contents was observed which resulted in decrease of total chlorophyll contents as compared to *P. oleracea* which exhibited marked reduction in chlorophyll *a* under Pb stress. These results point out that Pb stress differentially interferes with the photosynthetic machinery in *A. viridis* and *P. oleracea.* Our results are in line with Shahid et al. (2014) who observed a reduction in photosynthetic pigments in *Vicia faba* after applying different levels of Pb stress. Earlier studies show that lead-induced reduction of chlorophyll arises from diminution in the number of thylakoids/grana, chloroplast disorganization, direct inhibition of chlorophyll biosynthesis, and alterations in chlorophyll structure due to Pb replacement of nutrients like Mg and Cu (Haider et al. 2006; Akinci et al. 2010). *Phaseolus mungo* and *L. culinaris* plants were reported to undertake adaptive mechanisms to protect photosynthetic machinery by increased number of trichomes and stomata which enabled these macrophytes to sustain the efficiency of photosystem II and to minimize water evaporation losses under Pb stress (Azmat et al. 2009). In the present study, Pb-induced increase in H_2O_2 is likely to interfere with electron transport chain of the studied plants and, thus, affects the plant pigment contents, ultimately reducing the growth.

Lead-induced reduction in carotenoid contents might result from altered K uptake by the roots as reported by Elloumi et al. (2014). Under Pb stress, plants generate reactive

oxidative species (ROS) which, in turn, significantly modulate the physiology and morphology of plants. The reduced carotenoid content is also likely due to over production of ROS which damages the photosynthetic pigments, as well as other macromolecules (Yadav 2010). Dendrogram analysis reveals that photosynthetic pigments, i.e., chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids are affected collectively and are grouped as G-1 (Fig. S1).

Lipid peroxidation of *A. viridis* and *P. oleracea* under Pb stress

The increase in exogenous Pb results in higher H_2O_2 (ROS) contents and membrane lipid peroxidation predominantly in *A. viridis* (Table 2). Excessive H_2O_2 production causes oxidative stress, as reported for rice under Pb treatment (Srivastava et al. 2014), and is likely to be commenced by molecular oxygen excitation (O_2) to generate singlet oxygen or by electron transfer to O_2 and genesis of free radicals, i.e., O^{2-} and OH^- (Shigeoka et al. 2002). Higher levels of cellular H_2O_2 might be attributed to their enhanced generation and/or due to reduced scavenging capacity. Reactive oxygen species (ROS), if not efficiently scavenged, reduce plant growth and development by reacting with important metabolites and macromolecules (Richards et al. 2015). Therefore, increased H_2O_2 levels seem to interfere with electron transport chain of the studied plants and thus affect the plant pigment contents, ultimately reducing their growth. Under lead stress, the higher levels of Kreb's cycle intermediates (organic acids) could be reflective of elevated mitochondrial activities which ultimately produce more reducing agents and ATP. Increased metabolic activity thereby results in oxidative stress, as is seen here under lead stress by the positive correlation between organic acids and H_2O_2 as well as TBARS (Table 3). A Pb-induced higher level of ROS, lipid peroxidation, and reduction of plant dry mass with increasing Pb concentration in nutrient solution have previously been reported for the *Vicia faba* root (Pourrut et al. 2011). Oxidative stress (hydrogen peroxide and TBARS contents) parameters exhibit a similar pattern and are grouped as G-3 (Fig. S1). Our results reveal that *P. oleracea* plants maintain lower levels of H_2O_2 and TBARS under Pb stress as compared to *A. viridis* and thereby have a higher adaptability to Pb-polluted environment.

Pb uptake of *A. viridis* and *P. oleracea* under different Pb treatment levels

Lead compartmentalization in the roots and shoots of *A. viridis* and *P. oleracea* was significantly increased with increasing Pb treatment levels where root Pb accumulation was higher than the accumulation in shoots (Fig. 1). Our results corroborates the findings of Malik et al. (2010) who also reported that Pb mainly sequesters in the roots of *A. viridis* and *P. oleracea* and both the species exhibit differential root to shoot Pb translocation. Plant endodermis possesses casparian strips which restricts Pb transport to areal parts of plants (Verma and Dubey 2003). The observed difference in Pb uptake between *A. viridis* and *P. oleracea* might be attributed to higher capacity of *P. oleracea* to resist Pb-induced oxidative stress as reported earlier (Lamhamdi et al. 2013). The lower root to shoot Pb translocation in *P. oleracea* points out its better adaptation to Pb-polluted environment and might be dependent upon exodermis lignification/suberization which in turn restricts root Pb accumulation (Cheng et al. 2015). These authors suggested that exodermal lignification/suberization also can alter Pb availability by initiating changes in rhizospheric environment. In addition, Pb^{2+} binds to carboxyl groups which are possibly pectin compounds of the root cell walls (Inoue et al. 2013). Release of citric acid, glutamic acid, and fumaric acid in root exudates was increased at higher Pb treatment levels which decreased the root Pb accumulation particularly in *P. oleracea*. At normal plant cytosolic pH, organic acids are present as anions which when released may bind to protons resulting in rhizospheric basification. Such plant-induced basification mechanisms may result in stabilization of heavy metals due to their reduced mobility in the rhizosphere (Javed et al. 2013).

Uptake of Ca, K, Mg, and Na by *A. viridis* and *P. oleracea* under Pb stress

With increasing Pb treatments levels, calcium (Ca), magnesium (Mg), and potassium (K) contents of *A. viridis* and *P. oleracea* roots and shoots were decreased significantly, though differentially in both plant species. In all Pb treatment levels, *P. oleracea* appears less disturbed than *A. viridis* regarding studied macronutrients. The K contents in *A. viridis* and *P. oleracea* were significantly lower as compared to corresponding control plants except for the roots of *P. oleracea* at 15 mg Pb/kg soil where K contents increased (Fig. 3). A similar inverse relationship between Pb and K concentrations was found in *Pisum sativum*, *Z. mays*, and *Solanum lycopersicum* (Paivoke 2002; Malkowski et al. 2002; Akinci et al. 2010). The decrease in carotenoid contents of *A. viridis* and *P. oleracea* in the present study might result from alterations in K uptake under Pb stress (Elloumi et al. 2014) or due to enhanced level of H_2O_2 which can damage plant carotenoid contents (Shahid et al. 2014; Hossain et al. 2012). Parallel conclusions can also be made for Ca and Mg contents in *P. oleracea* which appear to be more resistant against Pb toxicity than *A. viridis* (Fig. 2 and Fig. 4). A similar inverse relationship between Pb and Ca ion accumulation has also been reported and might be attributed to ionic similarity of two elements that

permits Pb to replace Ca during specific physiological functions (Azmat et al. 2009).

Our results corroborate the findings of Ali et al. (2014) who reported diminished uptake of nutrients in *B. napus* under Pb stress. Such Pb-induced nutrient ion imbalance varies from species to species (Lamhamdi et al. 2013). The uptake of nutrient ions inhibited by Pb stress blocks the entry of various ions to the absorption sites on the plant roots (Godbold and Kettner 1991). However, in the present study, very large reductions of plant ionic content can rarely arise from just an ion uptake inhibition, but is also likely due to extra ion leakage from root surface due to increased TBARS contents. This could have happened in the present study based on the fact that the release of organic acid anions from the plant root cells must be balanced by an efflux of cations (Ryan et al. 2001). Furthermore, proton pumping ability of H^+-ATPase at the plasmalemma of plant cell decreases under metal stress. Therefore, nutritious cations must be co-released with organic acid anions under Pb toxicity and result in major loss of nutrient ions (Kochian et al. 2005). Potassium ions were reported to be co-released with organic acid anions in poplar roots under metal stress (Qin et al. 2007).

Lead treatment also reduced the uptake of sodium (Na) in the roots and shoots of *A. viridis* and *P. oleracea* (Fig. 5). Interestingly, root Na contents of *A. viridis* significantly increased at 60 mg/kg soil Pb treatment. Sodium is a micronutrient for plants, and higher Na uptake or accumulation in the roots of *A. viridis* could result from competition among the cations. Under metal stress, higher accumulation of monovalent cations has also been reported by Rhodes and Hanson (1993). Thus, sodium together with Pb should affect plant mineral nutrition.

Conclusions

It can be concluded that the roots of *A. viridis* and *P. oleracea* respond to moderately elevated Pb stress by release of root exudates with slightly basic pH, while the basicity of root exudates is diminished at higher Pb levels. The Pb stress triggers the exudation of various organic acids by the roots of *A. viridis* and *P. oleracea*, where the latter species produce organic acids at higher concentration. Lead stress reduces the growth and photosynthetic pigments of the studied plants irrespective of genotypic differences by inducing oxidative damage. Citric acid, malic acid, oxalic acid, fumaric acid, and glutamic acid exudations are positively correlated with root Pb contents, which may maintain optimum Ca, Mg, and K contents in *P. oleracea*. The efficient organic acids exudation and better uptake of nutrient ions enable the adaptations of *P. oleracea* to Pb-polluted environment. The present study focused on short-term Pb effects on organic acid exudations of *A. viridis* and *P. oleracea*. Therefore, investigations during long term with respect to plant root exudates, plant survival and function should be the subject of future investigations. A better understanding of root exudation patterns of *A. viridis* and *P. oleracea* under Pb stress may provide effective approaches to optimize plant-based remediation strategies of metal-contaminated environments.

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Contributions MTJ and MSA designed the experiments. HG and KT performed the experiments and the physiological/biochemical analysis with assistance of MTJ. NH, QA, and NKN performed the statistical analysis of the data and reviewed the manuscript. NI contributed to data discussion. All the authors approved the final manuscript.Funding informationWe gratefully acknowledge the Higher Education Commission (HEC), Pakistan, for provision of funds (Grant No: PD-IPFP/HRD/HEC/2013/3021) to Dr. M. Tariq Javed for the execution of the reported work.

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