

Lead uptake, bioaccumulation and tolerance mechanisms in summer savory (*Satureja hortensis* L.)

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Abstract

The objective of this study was to determine the effects of lead (Pb) exposure on bioaccumulation, growth, and tolerance mechanisms in summer savory (*Satureja hortensis* L.). Plants were subjected to different levels of Pb concentrations including 0 (control), 5, 10, 25, 50 and 100 mg L⁻¹ in growing medium. Pb treatment led to significant increase in root and shoot Pb content. Calculation of BF, TF and TC revealed that Pb prefers to be accumulated in roots of *S. hortensis* and root to shoot transport was effectively restricted. Pb toxicity negatively affected plant growth as indicated by significant decrease in plant dry weight as well as root and shoot length. Pb stress resulted in significant decrease in chlorophyll-*a*, chlorophyll-*b* and total chlorophyll content, whereas proline, soluble and reducing carbohydrates and anthocyanin content significantly increased as a result of Pb exposure. Specific activity of antioxidant enzymes including catalase and ascorbate peroxidase continuously increased as concentration of Pb in growing medium elevated. Based on our findings, due to high potential for Pb accumulation in root, *S. hortensis* may offer a feasible tool for phytostabilization purposes in mildly Pb contaminated soils.

Keywords: Antioxidant enzymes, Heavy metals, Lead, Medicinal plants, Phytoremediation, *Satureja hortensis*

Introduction

Natural weathering of minerals accompanied with recent anthropogenic activities have contributed to discharge elevated levels of trace metals into the environment (Zhang *et al.*, 2018). Heavy metals pose a serious threat to human health due to their direct toxic effects and further potential for increasing in the food chain through bio-accumulation (Ghaderian and Ghotbi Ravandi, 2012). Lead (Pb) as a major pollutant of both terrestrial and aquatic ecosystems, ranks second among all toxic heavy metals (Kumar *et al.*, 2012). Mining, smelting, sewage sludge, exhaust fumes of automobiles and power plants, fertilizers and pesticides, Pb containing paints and effluent of battery storages are the main sources of Pb emission to the environment (Sharma and Dubey, 2005; Guagliardi *et al.*, 2015).

Plants are able to absorb and remove metal pollutant including Pb from contaminated environment. Phytoremediation is an eco-friendly, low cost and novel technology that uses plants to reduce concentrations of organic and metal pollutants (Luo *et al.*, 2016). One of the aspects of phytoremediation is risk containment through stabilization pollutants in soils by plant roots (also known as phytostabilization). In this technique,

roots of certain metal tolerant plants, accumulate and immobilize metals in the soil and thus, prevent their entry to groundwater and into the food chain (Ali *et al.*, 2013). These plants can also be used in re-vegetation of metals contaminated areas, as they contain several detoxification and tolerance mechanisms, allowing them to survive on polluted sites (Malar *et al.*, 2014).

Pb has no known function in biological systems and is toxic even at low concentration (Fahr *et al.*, 2013). In addition to be a carcinogen, Pb poisoning can also cause an irreversible damage to nervous system (Hattab *et al.*, 2016). Growing on Pb polluted soils, plants also may be affected by adverse effects of Pb toxicity, as it readily absorbs and accumulates in plants (Auguy *et al.*, 2013). Degree of Pb induced toxicity depends on plant species, developmental stage, Pb concentration in soil, exposure period, soil pH and soil mineral and organic composition (Lamb *et al.*, 2010; Pourrut *et al.*, 2011a).

Pb can promote cellular damage either directly or through generation of reactive oxygen species (ROS) (Sharma and Dubey, 2005; Wang *et al.*, 2010; Pourrut *et al.*, 2011b). Pb interference with other nutrient absorption and translocation can cause nutrient deficiency in plants (Fodor *et al.*, 1996). Furthermore,

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due to chemical similarity to several essential elements, Pb can replace the functionally active metals on active sites of enzymes and inhibit function of many key enzymes in several metabolic pathways such as respiration and photosynthesis (Wang *et al.*, 2010). Besides, distortion of chloroplast ultra-structure, inhibition of chlorophyll synthesis as well as impairment of plastoquinone and electron transfer chain exacerbate reduction in photosynthetic efficiency under Pb stress (Cenkci *et al.*, 2010; Pourrut *et al.*, 2011b; Kumar *et al.*, 2012). Despite being inactive redox metal, Pb can induce oxidative stress and redox imbalance in plant cells due to excessive formation of ROS (Lopez-Orenes *et al.*, 2014; Shahid *et al.*, 2014). Reactive oxygen species promptly damage macromolecules including membrane lipids, proteins, nucleic acids and pigments, leading to irreversible metabolic impairment (Reddy *et al.*, 2005; Maldonado-Magana *et al.*, 2011).

To minimize detrimental effects of ROS, plant cells have evolved a complex network of enzymatic and non-enzymatic antioxidant systems which protects plant cells from oxidative damage by scavenging various types of ROS (Fahr *et al.*, 2013; Hossain and Komatsu, 2013; Singh *et al.*, 2016). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) are among ROS scavenging enzymes that maintain ROS content and cellular redox within certain range (Gupta *et al.*, 2009). Key role of CAT and APX as two enzymes involved in control of Pb-induced H₂O₂ content, have been recently described (Wang *et al.*, 2012; Draghiceanu *et al.*, 2018; Ferrer *et al.*, 2018). Furthermore, toxic metals such as Pb, promote the concentration of non-enzymatic antioxidants such as glutathione, ascorbate and other metabolites such as carbohydrates, anthocyanins and proline which protect plant cells by chelation of metals, scavenging ROS and stabilization of membranes and macromolecules (Szabados and Savoure, 2010; Kumar *et al.*, 2012; Bezerril Fontenele *et al.*, 2017).

The genus *Satureja* belongs to the Lamiaceae family and comprises about 200 species which are mostly spread through Europe, South America, West Asia, Mediterranean regions, North Africa and the Canary Island. *Satureja hortensis* L. is an annual herbaceous plant commonly known as summer savory (Katar *et al.*, 2017; Boroja *et al.*, 2018). It is widely cultivated in various regions of Iran as one of the most commercially valuable Iranian *Satureja* species. Extracts and essential oils of *S. hortensis* are proven to have antispasmodic, anti-diarrheal, anti-inflammatory, antifungal, antibacterial and anti-oxidative effects (Momtaz and Abdollahi, 2010).

In spite of extensive literature focusing on the impact of metal stress on crops, there are still limited reports describing the effects of heavy metals and specially Pb on the medicinal plants (Jezler *et al.*, 2015). In this regard, the aim of this study was to examine the ability of Pb bioaccumulation by *S. hortensis* and provide insights to the physiological response

mechanisms underlying Pb tolerance.

Material and Methods

Chemicals: All chemicals and reagents used for preparation of Hoagland's medium and biochemical experiments were analytical grade and purchased from Merck (Darmstadt, Germany).

Plant material, growing condition and lead treatment: Seeds of *S. hortensis* were obtained from Pakan Seed Company (<http://www.pakanbazr.com/en/>), Isfahan, Iran. Seeds were sterilized in 70% ethanol followed by three washes with distilled water. Seeds were sown in 90 mm diameter plastic pots with 85 mm height filled with perlite. Plants were grown in controlled condition (16 hrs. photoperiod, 16-26 °C night-day, and light intensity approximately of 250 μ photons m⁻² s⁻¹).

Plants were watered with modified Hoagland's solution (Hoagland and Arnon, 1950) containing 1.5 mmol L⁻¹ Ca(NO₃)₂, 0.28 mmol L⁻¹ KH₂PO₄, 0.75 mmol L⁻¹ MgSO₄, 1.25 mmol L⁻¹ KNO₃, 0.5 μ mol L⁻¹ CuSO₄, 1 μ mol L⁻¹ ZnSO₄, 5 μ mol L⁻¹ MnSO₄, 25 μ mol L⁻¹ H₃BO₃, 0.1 μ mol L⁻¹ Na₂MoO₄, 50 μ mol L⁻¹ KCl, 10 μ mol L⁻¹ Fe-EDDHA (ferric ethylene diamine- di-2-hydroxy phenyl acetate) and the solution was exchanged twice every week. In order to prevent leaching Pb or nutrient from pots, plastic trays were placed under each pot.

At 30th day of growth, healthy and uniformly grown plants were exposed to six different concentrations of Pb including 0, 5, 10, 25, 50 and 100 mg L⁻¹ (0, 0.015, 0.03, 0.075, 0.15 and 0.3 mM) in the growing medium in the form of Pb(NO₃)₂. Pb was added through Hoagland's nutrient solution (200 mL of solution per pot) for two weeks. Nutrient solution without Pb served as the control. The pH of all the solutions were monitored every day and maintained at 5.6-5.8.

Growth analysis: At harvest, the length of plant shoots and roots was measured. The collected root and shoot were subsequently dried at 70°C until the constant weight was reached and the dry weight determined. Growth inhibition induced by Pb stress was calculated as follow (Hattab *et al.*, 2016):
Growth inhibition = 1- (dry weight of treated plants/ dry weight of control plants)*100.

Determination of lead accumulation: Pb treated plants were washed thoroughly to remove Pb ions adhering to the surface. Pb content of root and shoot samples was determined according to Ghaderian and Ghotbi Ravandi (2012) using Atomic Absorption Spectrophotometer (AAS-6300, Shimadzu, Kyoto, Japan). The bioaccumulation factor (BF), translocation factor (TF) and transfer coefficient (TC) were calculated based on formulas described by Yousefi *et al.* (2018).

BF = concentration of an element in roots /concentration of element in the medium.

TF = concentration of an element leaves /concentration of element in the root.

TC = concentration of an element in leaves /

concentration of element in the medium.

Physiological measurements: Proline content was determined according to method of Bates *et al.* (1973). Samples (0.5 gr) were homogenized in sulphosalicylic acid (3%, w/v) and mixed with acid ninhydrin and glacial acetic acid. The mixture was boiled at 100°C in water bath for 60 minutes followed by rapid cooling in ice-water bath. After adding toluene to the mixture, absorbance of resulting chromophore was read at 520 nm via UV-Vis spectrophotometer (CARY 300, Agilent, Santa Clara, USA). Proline content was assessed by calibration curve and reported as $\mu\text{mol.g}^{-1}$ FW (fresh weight).

Soluble sugars were extracted from 1 gr of fresh root and shoot samples with 80% ethanol. After centrifugation, 1mL of supernatant was mixed with phenol (5%) and sulphuric acid (98%) followed by water bath (20 mins., 30°C). Optical density of solution was determined at 490 nm via a double beam spectrophotometer (UV-Visible, CARY). Soluble sugar content (mg g^{-1} FW) was calculated based on standard curve of glucose (DuBois *et al.*, 1956). Reducing carbohydrate content was assessed according to Miller (1959). 1 gr of fresh root and shoot samples were homogenized in 80% ethanol and centrifuged at 2000 rpm for 20 minutes. DNSA (3, 5-dinitro salicylic acid) was added to 1 mL of supernatants and mixture was boiled for 20 minutes. Reducing sugar content (mg g^{-1}) was quantified via a double beam spectrophotometer (CARY 300) at wavelength of 515 nm and calculated based on glucose standard curve.

For determination of chlorophyll content in leaves, fresh tissues (0.1 g) were grounded with mortar and pestle under dark and chilled condition. Pigment was extracted with acetone (80%) and filtered through filter papers. Chlorophyll content of samples was determined spectrophotometrically via a double beam spectrophotometer (CARY 300) as described by Arnon (1949) and expressed as mg g^{-1} FW.

In order to determine leaves anthocyanin content, samples were homogenized in 1% methanol-HCl and samples were kept in refrigerator (4°C) for 2 days. After filtration of the extracts, anthocyanin content was assessed spectrophotometrically (CARY 300) as difference between absorbance at 530 nm and 657 nm and expressed as $\mu\text{mol g}^{-1}$ FW (Eryilmaz 2006).

For determination of antioxidant enzymes activity, fresh leaf samples (500 mg) from each treatment were grounded and homogenized in ice cold mortar using 50 mmol L^{-1} phosphate buffer (pH= 7.0) containing 1 mmol L^{-1} EDTA and 1% PVP (poly vinyl pyrrolidone). Homogenates subsequently centrifuged at 12000 rpm for 20 minutes at 4°C and the resulting supernatant was used to enzyme assays. The protein concentration of leaf extracts was assessed according to Bradford (1976). Specific activity of CAT (EC: 1.11.1.6) was determined by method described by Aebi (1974). CAT activity was assayed by monitoring the decrease in absorbance at 420 nm as a consequence of H_2O_2 consumption for 3

minutes. APX (EC: 11.1.11.1) activity was determined by measuring the decrease in absorbance of the oxidized ascorbate at 290 nm, as defined by Nakano and Asada (1987).

2.6. Statistical analysis: The experiments were designed in completely randomized design (CRD) with three independent replicates. All data were presented as mean $\pm$ standard deviation. Statistical analysis was carried out by statistical analysis system (SAS) software (SAS Institute Inc., Cary, NC, USA). One way analysis of variance (ANOVA) followed by Post hoc Tukey test was performed to assess the differences ($P \leq 0.05$) among various means.

Results

Lead accumulation: After 14 days of Pb exposure, Pb content of shoots and roots of *S. hortensis* significantly elevated in response to increasing concentrations of Pb in growing medium (Figure 1).

Pb preferentially localized in roots where more than 90 percent of total absorbed Pb was accumulated in the roots (Figure 2).

BF value which determines the ability of plants to accumulate metals from soil did not significantly change under concentration of 5 and 10 mg L^{-1} Pb in medium. However, exposure to higher concentration of Pb led to significant decrease ($P \leq 0.05$) in BF (Table 1). TF which depicts the ability of plant to translocate metals from root to shoot, significantly ($P \leq 0.05$) reduced in Pb concentrations higher than 5 mg L^{-1} (Table 1). Also, the ability of plants to store metals in aerial parts (TC) was decreased significantly ($P \leq 0.05$) in spite of progressive increase in Pb content in the medium (Table 1).

Effect of lead on plant growth parameters: Biomass, root and shoot length have been widely used as parameters to indicate heavy metal toxicity in plants. Effects of Pb concentrations on shoot and root length of *S. hortensis* are presented in Figure 3. Pb treatment decreased shoot and root length. At highest Pb concentration (100 mg L^{-1}), both shoot and root length decreased approximately 48% compared to the control group.

Pb stress caused a significant decrease ($P \leq 0.05$) in shoot dry matter in *S. hortensis*, while significant reduction in root dry matter occurred in concentrations higher than 5 mg L^{-1} (Figure 4). The highest concentration of Pb in growing medium (100 mg L^{-1}) inhibited the growth of root and shoot by 57% and 61%, respectively, compared to the control group (Figure 4).

Physiological response to lead stress: Effects of Pb exposure on proline content of *S. hortensis* plants are depicted in Fig 5. Pb stress led to significant increase ($P \leq 0.05$) in proline content in both root and shoot. Compared to the control, proline content of root and shoot of plants treated with 100 mg L^{-1} Pb, increased 2.8 and 2.1 fold, respectively.

Changes in soluble and reducing carbohydrates in response to increasing Pb concentrations in growing

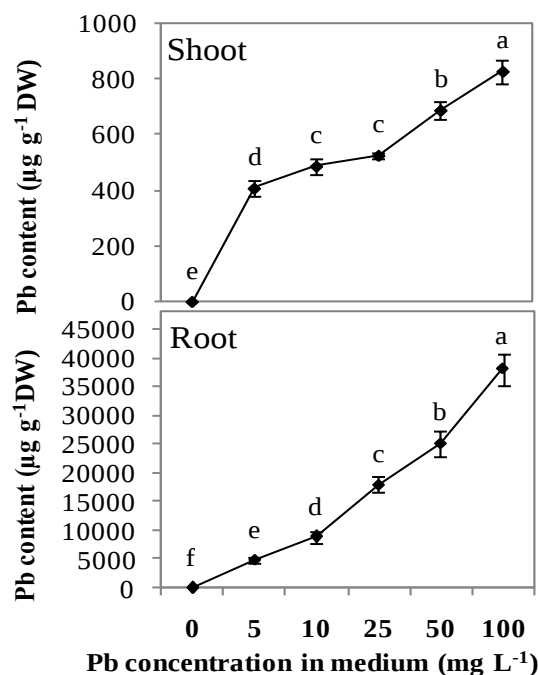


Figure 1. Lead accumulation in shoot and root of *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

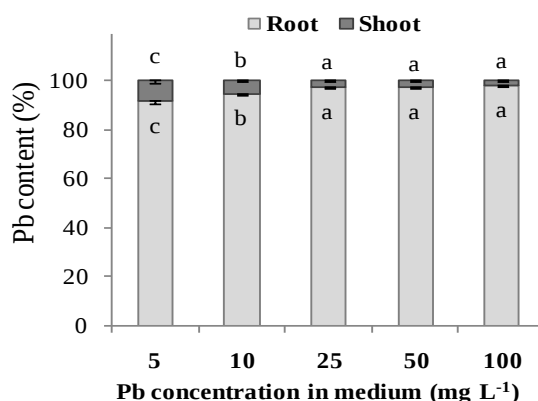


Figure 2. Distribution of absorbed lead in root and shoot *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

medium are presented in Table 2. Compared to the control, concentration of soluble carbohydrates in both root and shoot of *S. hortensis* was found to significantly ($P \leq 0.05$) increased at all Pb treatments except for 5 mg L⁻¹. Similarly, significant increase in reducing carbohydrates in root of *S. hortensis* was only observed in Pb concentrations higher than 5 mg L⁻¹, whereas, in shoot, all Pb concentrations led to significant increase in reducing carbohydrates content compared to the control (Table 2).

S. hortensis plants grown in Pb containing medium exhibited a gradual decrease in chlorophylls content compared to the control group, as Pb concentrations of medium increased (Table 3). Chlorophyll-*b* and total

chlorophylls content significantly ($P \leq 0.05$) declined upon Pb concentrations over 10 mg L⁻¹, whereas significant decrease ($P \leq 0.05$) in chlorophyll-*a* was observed at Pb concentrations of 50 and 100 mg L⁻¹ in the medium.

Changes in anthocyanin content of *S. hortensis* plants, as a result of increasing concentration of Pb in growing medium, are depicted in Figure 6. Significant increase ($P \leq 0.05$) in anthocyanin content was observed in Pb treatments higher than 5 mg L⁻¹. At highest concentration of Pb treatment, anthocyanin content increased more than 2 fold compared to the control.

Figure 7 depicts the specific activity of antioxidant enzymes, CAT and APX, in leaves of *S. hortensis* as a

Table 1. Changes in bioaccumulation factor, translocation factor and transfer coefficient of lead in *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

	Pb Concentration in growing medium (mg L ⁻¹)				
	5	10	25	50	100
Bioaccumulation factor	0.89 $\pm$ 0.09 ^a	0.89 $\pm$ 0.06 ^a	0.72 $\pm$ 0.09 ^a	0.5 $\pm$ 0.05 ^b	0.38 $\pm$ 0.03 ^c
Translocation factor	0.09 $\pm$ 0.007 ^a	0.05 $\pm$ 0.003 ^b	0.03 $\pm$ 0.003 ^c	0.02 $\pm$ 0.004 ^c	0.021 $\pm$ 0.002 ^c
Transfer coefficient	0.08 $\pm$ 0.005 ^a	0.04 $\pm$ 0.002 ^b	0.02 $\pm$ 0.0005 ^c	0.01 $\pm$ 0.0006 ^c	0.008 $\pm$ 0.0004 ^c

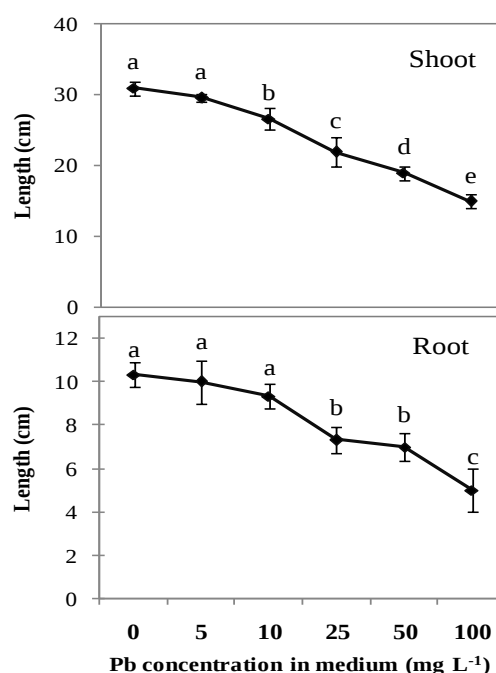


Figure 3. Effects of Lead stress on root and shoot lengths of *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

result of progressive Pb stress. CAT activity exhibited a dose-dependent and significant ($P \leq 0.05$) increase upon exposure to Pb (Figure 7A). Specific activity of APX exhibited a similar pattern and significantly increased ($P \leq 0.05$) as a result of Pb treatment (Figure 7B). At highest Pb concentration in the medium (100 mg L⁻¹), specific activity of CAT and APX increased 135% and 255% compare to the control group, respectively.

Discussion

Pb is one of the most toxic metals and has been considered as a world-wide concern due to its importance in the environmental quality and health. In addition to detrimental effects on plant growth, metabolism and yield, bioaccumulation of Pb by plants can pose a profound threat to animals and human life as it enters the food chains (Shi *et al.*, 2018). Pb concentration in plant organs is a function of Pb content in the soil and the level of accumulation differs between and within species (Luo *et al.*, 2016). In the present

study, Pb content of the root and shoot of *S. hortensis* was significantly increased as Pb levels in growing medium increased (Figure 1). Furthermore, more than 90% of Pb absorbed by *S. hortensis* in all treatment was accumulated in the root (Figure 2). Numerous studies have reported that most of absorbed Pb remains in the roots which make the root barrier for Pb translocation to aerial tissues (Shi *et al.*, 2018). Most of the absorbed Pb in the root is bound to ion exchangeable sites of cell wall or extra-cellularly precipitate as phosphates or carbonates. Unbound Pb can move thorough apoplastic and symplastic (via calcium channels) pathways and accumulates near endodermis, which is partial barrier for Pb transport to xylem tissues (Sharma and Dubey, 2005).

Difference in metal accumulation or localization appears to be a key factor determining plant tolerance. BF, TF, and TC represent simple methods to assess plant potential to accumulate and translocate trace metals for phytoremediation purposes (Eid and Shaltout,

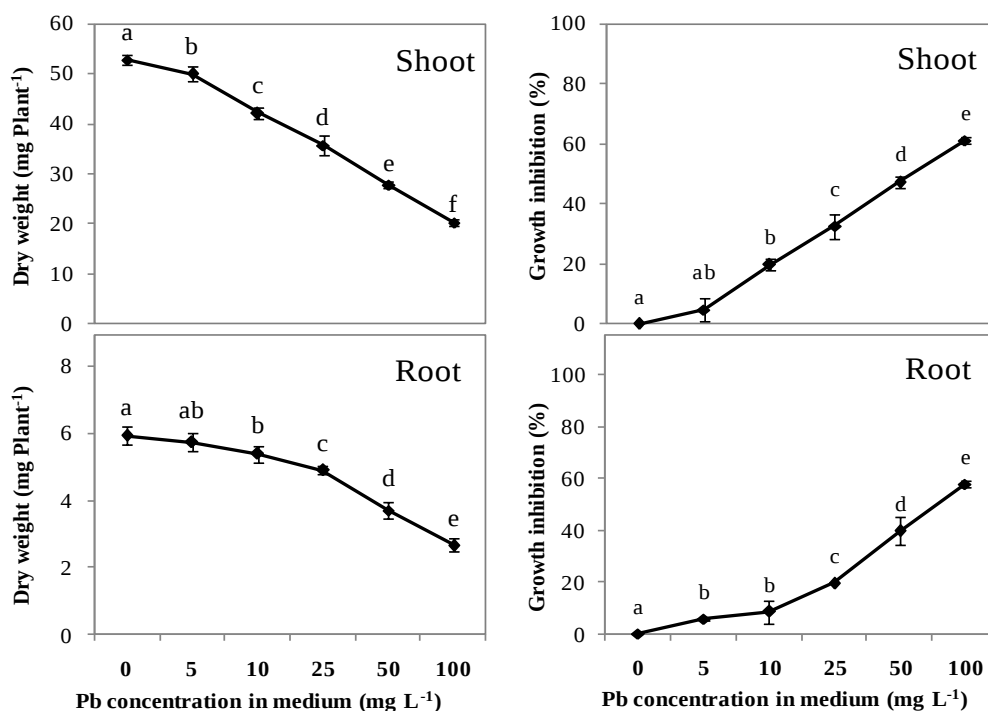


Figure 4. Changes in root and shoot dry weight and growth inhibition in *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

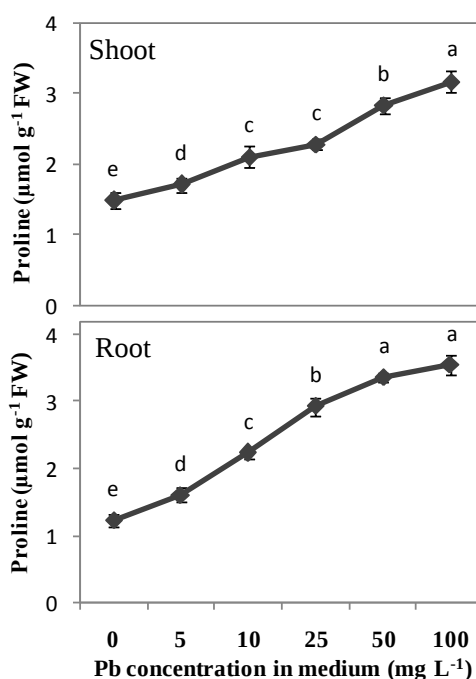


Figure 5. Proline accumulation in shoot and root of *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

2016). BF evaluates the potential of roots to uptake metals from soil. BF values greater than 1.0 ($BF > 1.0$) were reported in metal accumulating plants while the BF values smaller than 1.0 ($BF \leq 1.0$) were found in

metal excluders (Branzini *et al.*, 2012). In our study, BF values were calculated 0.89 in plants treated with low Pb concentrations of 5 and 10 mg L⁻¹ (Table 1). This relatively high value of BF suggests a considerable

Table 2. Changes in reducing and soluble carbohydrates (mg g^{-1} FW) in root and shoot of *Satureja hortensis* L. exposed to different concentrations of lead (0, 5, 10, 25, 50 and 100 mg L^{-1}) 14 days. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

Pb Concentration in growing medium (mg L^{-1})	Reducing carbohydrates (mg g^{-1} FW)		Soluble carbohydrates (mg g^{-1} FW)	
	Shoot	Root	Shoot	Root
0	23.4 $\pm$ 0.9 ^e	18.8 $\pm$ 1.3 ^d	66.8 $\pm$ 2.0 ^c	35.5 $\pm$ 1.9 ^c
5	27.4 $\pm$ 2 ^d	20.5 $\pm$ 1.4 ^{cd}	68.9 $\pm$ 1.7 ^c	39.4 $\pm$ 2.4 ^c
10	28.8 $\pm$ 1.8 ^d	21.5 $\pm$ 1.0 ^{bc}	75.5 $\pm$ 2.5 ^b	48.1 $\pm$ 1.3 ^b
25	33.3 $\pm$ 1 ^c	23.8 $\pm$ 1.7 ^b	76.2 $\pm$ 1.9 ^b	50.8 $\pm$ 2.8 ^b
50	38.2 $\pm$ 0.5 ^b	23.9 $\pm$ 1.3 ^b	82.8 $\pm$ 3.1 ^a	52.9 $\pm$ 1.7 ^{ab}

Table 3. Changes in chlorophyll *a*, chlorophyll *b* and total chlorophylls content (mg g^{-1} FW) of *Satureja hortensis* L. exposed to different concentrations of lead (0, 5, 10, 25, 50 and 100 mg L^{-1}) 14 days. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

Pb Concentration in growing medium (mg L^{-1})	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total Chlorophylls
0	1.88 $\pm$ 0.15 ^a	0.66 $\pm$ 0.013 ^a	2.87 $\pm$ 0.12 ^a
5	1.7 $\pm$ 0.11 ^{ab}	0.64 $\pm$ 0.018 ^{ab}	2.69 $\pm$ 0.11 ^{ab}
10	1.66 $\pm$ 0.15 ^{abc}	0.62 $\pm$ 0.028 ^b	2.58 $\pm$ 0.14 ^b
25	1.64 $\pm$ 0.16 ^{abc}	0.58 $\pm$ 0.01 ^c	2.5 $\pm$ 0.13 ^{bc}
50	1.53 $\pm$ 0.11 ^{bc}	0.53 $\pm$ 0.019 ^d	2.29 $\pm$ 0.06 ^{cd}
100	1.42 $\pm$ 0.10 ^c	0.51 $\pm$ 0.022 ^d	2.18 $\pm$ 0.11 ^d

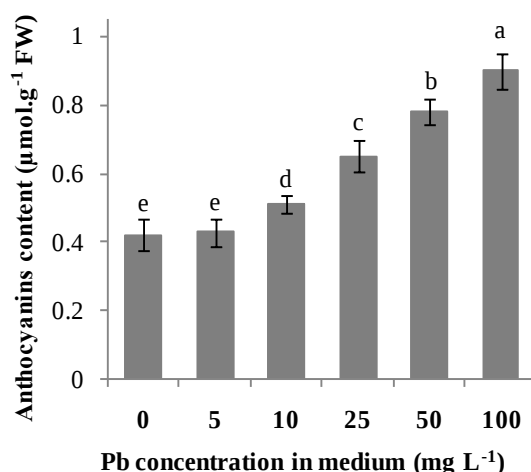


Figure 6. Changes anthocyanin content in leaves of *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L^{-1}) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

potential of *S. hortensis* roots to absorb and remove Pb ions in mildly contaminated soils. The ability of translocating metals from root to shoot is determined by TF (Eid and Shaltout, 2016). TF values higher than 1.0 indicate a great efficiency of plant to translocate metals from root to aerial parts (Alaboudi *et al.*, 2018). Since Pb accumulation was considerably higher in roots of *S. hortensis* compared to aerial parts, measured TF values were lower than one ($\text{TF} < 1.0$). In addition, TF significantly declined as Pb concentration in medium

increased (Table 1). These results suggest that there is a strong inhibition of long distance translocation of Pb in *S. hortensis* to exclude Pb transport from root to shoot. Reduction in TC (ability of plant to store metals in aerial parts) observed in *S. hortensis* with increasing levels of Pb exposure, is mostly as a result of inhibition of root to shoot transport of Pb rather than exclusion of Pb absorption from soil (Table 1). Remarkable ability of Pb accumulation in roots accompanied by low rate of translocation to aerial parts, suggests that *S. hortensis*

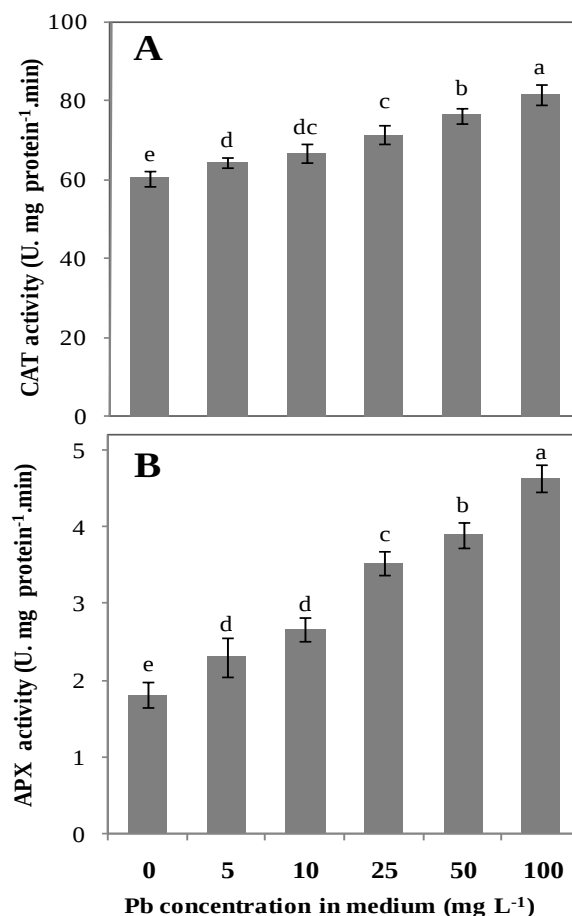


Figure 7. Specific activity of catalase (A) and ascorbate peroxidase (B) in leaves of *Satureja hortensis* L. exposed 14 days to different concentration of Pb (0, 5, 10, 25, 50 and 100 mg L⁻¹) in growing medium. Values are mean $\pm$ SD of three independent replicates. Different letters indicate significant differences ($P \leq 0.05$).

could be considered as reliable candidate for phytostabilization of Pb in contaminated soils.

High concentrations of Pb in plant tissues can cause severe growth inhibition and even plant death (Yang *et al.*, 2008). Plant dry weight as well as lengths of root and shoot is commonly used as an indicator of growth inhibition by toxic metals, such as Pb (Maldonado-Magana *et al.*, 2011). In our study, both root and shoot length negatively affected by exogenous Pb treatment and exhibited approximately 48% reduction under highest Pb exposure (Figure 3). Similarly root and shoot growth was significantly inhibited as a result Pb stress (Figure 4). The inhibition of plant growth is mostly as a consequence of Pb interference with photosynthesis processes and reduction in photosynthetic products (Sharma and Dubey, 2005). Furthermore, reduction in cell water potential (lower turgor) as a result of decrease in membrane permeability can limit cell expansion and contribute to the inhibited elongation of root and shoot under Pb stress (Kumar *et al.*, 2012). In addition, Pb damage to microtubule arrangement and alignment in mitotic spindle is an important component of Pb induced growth retardation in plants (Eun *et al.*, 2008).

Reduction in the chlorophyll content is the foremost bio-indicator of heavy metal toxicity (Dinakar *et al.*,

2008). In the present study, Pb treatment resulted in gradual loss of chlorophyll-*a*, chlorophyll-*b* and total chlorophyll content in *S. hortensis* (Table 3), as found in different plant species including *Chara aculeolata* (Sooksawat *et al.*, 2013), *Zygophyllum fabago* (Lopez-Orenes *et al.*, 2014), *Medicago sativa* (Hattab *et al.*, 2016) and *Vigna unguiculata* (Bezerril Fontenele *et al.*, 2017). The reduction mechanisms in pigment content at molecular level have been partially explained. Pb interacts directly by substituting itself for the divalent ions bound to metalloenzymes such as δ -aminolevulinic acid dehydratase (ALAD), which is a key metalloenzyme for chlorophyll biosynthesis. The activity of ALAD decreases in plant leaves when exposed to heavy metals including Pb. Pb toxicity inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe. Furthermore, increased activity of chlorophyllase enzyme, ROS induced peroxidation in chloroplast membranes and decrease in chloroplast volume induced by Pb ions contribute to declined chlorophyll content in response to Pb exposure (Sharma and Dubey, 2005; Bilal Shakoor *et al.*, 2014; Rodriguez *et al.*, 2015).

Anthocyanins are group of phenolic compounds that contribute to adaptive responses of plants to heavy

metals. Anthocyanins form chelate complexes with metals and transport them into vacuoles for detoxification (Voght, 2010). In our study, Pb concentration higher than 10 mg L⁻¹ in medium resulted in significant increase in anthocyanin content (Figure 6). The results of the present work is supported by findings of Kosyk *et al.* (2017) where cadmium toxicity caused a significant increase in anthocyanin content even after 1 day of exposure. Kumar *et al.* (2012) reported a higher level of anthocyanin content associated by Pb stress in *Talium triangulare*. They postulated that there is a strong correlation between presence of trace metals in environment and leave anthocyanin content. Research conducted by Glinska *et al.* (2007) revealed that anthocyanin-rich extracts of red cabbage, protects the meristematic cell of *Allium cepa* roots against Cd, Cr and Pb. Similarly, application of exogenous anthocyanins increased the chlorophyll and protein content, improved photosynthetic parameters, alleviated ROS- driven reactions and stimulate enzymatic (CAT and APX) and non-enzymatic (proline and ascorbate) antioxidant systems in *Egeria densa* in response to excess Cd and Mn exposure (Maleva *et al.*, 2018).

Numerous studies have confirmed the ability of Pb to induce ROS formation in plant cells which results in interruption of redox homeostasis and oxidative injuries (Reddy *et al.*, 2005; Yadav, 2010; Zhou *et al.*, 2017). The extent of plant tolerance to Pb stress is highly correlated with ROS scavenging capacity of antioxidant machinery (Xu *et al.*, 2009). One of the protective mechanisms adopted by plant to eliminate stress-induced ROS is the enhanced enzymatic antioxidant activity. Antioxidant enzymes such as CAT and APX play a key role in defense against Pb stress and protect plants from oxidative damage induced by H₂O₂ (Ashraf and Tang, 2017). In our work, exposure to Pb triggered the enhancement in activity of antioxidant enzymes (Figure 7). CAT and APX specific activity significantly increased under Pb treatment compared to the control group. This observation is consistent with reports on elevation of antioxidant enzymes activity associated with Pb toxicity in *Macrotyloma uniflorum* and *Cicer arietinum* (Reddy *et al.* 2005), *Vicia faba* (Wang *et al.* 2010), *Triticum aestivum* (Yang *et al.* 2011), *Sesbania grandiflora* (Malar *et al.*, 2014), *Acalypha indica* (Venkatachalam *et al.*, 2017) and *Rhus chinensis* (Zhou *et al.*, 2017). Increased activity of antioxidant enzymes in response to Pb can be due to Pb-induced expression of antioxidant enzyme genes. Hattab *et al.* (2016) reported an increase in expression of SOD, APX and glutathione peroxidase (GP) in alfalfa in response to different concentrations of Pb. Similarly, increased expression of CAT, APX, GP, glutathione peroxidase (GPOX) and glutathione-s-transferase (GST) was observed under Pb treatment in tomato plants (Bali *et al.*, 2019). In addition, post-translational modification of antioxidant enzymes has been reported to increase enzymatic activity in response to heavy metals stresses (Visioli and Marmiroli, 2013).

Proline, as a stress response molecule, accumulates in plant tissues to offset adverse effects caused by unfavorable growth conditions (Verbruggen and Hermans, 2008). Increased expression of genes encoding the proline biosynthetic enzymes, including Δ -pyrroline-5-carboxylate synthase (P5CS) as well as proline transporters have been reported in response to Pb stress (Liu *et al.*, 2009). In addition to osmoprotectant function, proline plays a role in stabilization of proteins, scavenging of reactive oxygen species as well as cell redox homeostasis (Liang *et al.*, 2013). Chaperon properties of proline protects cell against oxidative stress by enhancement and stabilization of redox enzymes. Proline has been found to increase the activity of antioxidant enzymes including SOD, CAT, and APX under metals stress (Islam *et al.*, 2009; Xu *et al.*, 2009). Chelation of metals is another mechanism by which proline involves in the protection of plant cells against trace metals stress. It has been suggested that formation of proline-metal complexes can protect enzymes from inhibition caused by heavy metals. Furthermore, it has been reported that proline is able to directly react with hydrogen peroxide, hydroxyl radicals and singlet oxygen in both free and polypeptide bound forms (Kaul *et al.*, 2008; Szabados and Savoure, 2010; Rejeb *et al.*, 2014). In the present study, the proline content in *S. hortensis* was significantly increased as Pb dosage raised in medium (Figure 5). Similar to our results, Yang *et al.* (2011) reported a significant proline accumulation in two wheat cultivars exposed to short term Pb toxicity. Increased proline content as a result of Pb treatment was also reported in two aromatic rice cultivars (Ashraf and Tang, 2017).

Sugars are another well-characterized osmolytes that accumulate in response to heavy metal stress in plants (Su *et al.* 2017). In our work, exposure to Pb stress resulted in continuous increase in soluble and reducing carbohydrates in both roots and shoots of *S. hortensis* (Table 2). These observations are in coherence with the results of Ashraf and Tang (2017) where the concentrations of soluble carbohydrates increased in roots and shoots of two rice cultivars under Pb treatment. According to Moya *et al.* (1993), reduction in carbohydrate utilization due to deleterious effects of heavy metals on growth, is a main reason of sugar accumulation in plant tissues. Carbohydrates actively contribute to scavenging ROS, redox balance and preservation of macromolecules and membranes structure and thus their accumulation plays an important protective role during stress condition (Kapoor *et al.*, 2016).

Conclusions

Pb pollution as a serious environmental concern, not only affects growth and yield of economically valuable plants, but also poses serious threat to human health. In the present study, we examined the effects of Pb exposure on growth, as well as accumulation and physiological responses in medicinal plant, *Satureja hortensis*. Our results demonstrated that roots of *S.*

hortensis were able to absorb and accumulate considerable amount of Pb from soil and restrict its translocation to shoots as indicated by BF and TC, respectively. Pb accumulation had detrimental effects on plant growth, biomass production and chlorophyll content. The imposed Pb stress triggered the enhancement of response mechanisms including elevated activity of antioxidant enzymes as well as accumulation of anthocyanins, proline and carbohydrates. Considering the priority of Pb accumulation in roots in large quantities followed by limited rate of translocation to shoot in *S. hortensis*, this plant can be presumed as a suitable tool for

phytostabilisation of Pb or alternate crop in mildly Pb-contaminated soils.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Aebi, H. (1974) Catalase. In: Methods of Enzymatic Analysis (ed. Hans, U. B.) Pp. 673–677. Academic Press Inc, New York.
- Alaboudi, K. A., Ahmed, B. and Brodie, G. (2018) Phytoremediation of pb and cd contaminated soils by using sunflower (*Helianthus annuus*) plant. Annals of Agricultural Sciences 63: 123–127.
- Ali, H., Khan, E. and Sajad, M. A. (2013) Phytoremediation of heavy metals-concepts and applications. Chemosphere 91: 869–881.
- Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24: 1–15.
- Ashraf, U. and Tang, X. (2017) Yield and quality responses, plant metabolism and metal distribution pattern in aromatic rice under Lead (pb) toxicity. Chemosphere 176:141–155.
- Auguy, F., Fahr, M., Moulin, P., Brugel, A., Laplaze, L., El Mzibri, M., Filali-Maltouf, A., Doumas, P. and Smouni, A. (2013) Lead tolerance and accumulation in *Hirschfeldia incana*, a mediterranean Brassicaceae from metalliferous mine spoils. Plos One 8: 1–10.
- Bates, L. S., Waldren, R. P. and Teare, I. D. (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205–207.
- Bali, S., Jamwal, V. L., Kaur, P., Kohli, S. K., Ohri, P., Gandhi, S. G., Bhardwaj, R., Al-Huqail, A. A., Siddiqui, m. H., Ahmad, P. (2019) Role of P-type ATPase metal transporters and plant immunity induced by jasmonic acid against Lead (Pb) toxicity in tomato. Ecotoxicology and Environmental Safety 174: 283–294.
- Bezerril Fontenele, N. M., Otoch, M. L. O., Gomes-Rochette, N. F., Sobreira, A. C. M., Barreto, A. A. G. C., de Oliveira, F. D. B., Costa, J. H., Borges, S. D. S. S., do Nascimento, R. F. and Fernandes de Melo, D. (2017) Effect of lead on physiological and antioxidant responses in two *Vigna unguiculata* cultivars differing in pb-accumulation. Chemosphere 176: 397–404.
- Bilal Shakoar, M., Ali, S., Hameed, A., Farid, M., Hussain, S., Yasmeen, T., Najeeb, U., Bharwana, S. A. and Abbasi, G. H. (2014) Citric acid improves lead (pb) phytoextraction in *Brassica napus* L. by mitigating pb-induced morphological and biochemical damages. Ecotoxicology and Environmental Safety 109: 38–47.
- Boroja, T., Katanic, J., Rosic, G., Selakovic, D., Joksimovic, J., Mistic, D., Stankovic, V., Jovicic, N. and Mihailovic, V. (2018) Summer savory (*Satureja hortensis* L.) extract: phytochemical profile and modulation of cisplatin-induced liver, renal and testicular toxicity. Food and Chemical Toxicology 118: 252–263.
- Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248–254.
- Branzini, A., Gonzalez, R. S. and Zubillaga, M. (2012) Absorption and translocation of copper, zinc and chromium by *Sesbania virgata*. Journal of Environmental Management 102: 50–54.
- Cenkci, S., Cigerci, I. H., Yildiz, M., Ozay, C., Bozdog, A. and Terzi, H. (2010) Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. Environmental and Experimental Botany 67: 467–473.
- Dinakar, N., Nagajyothi, P. C., Suresh, S., Daykiran, Y. U. and Damodharam, T. (2008) Phytotoxicity of cadmium on protein, proline and antioxidant enzyme activities in growing *Arachis hypogaea* L. seedlings. Journal of Environmental Sciences 20: 199–206.
- Draghiceanu, O. A., Cristina Soare, L., Fierascu, I., Fierascu, R. C. and Popescu, M. (2018) Lead-induced physiological, biochemical and enzymatic changes in *Asplenium scolopendrium* L. Bulletin of Environmental Contamination and Toxicology 100: 438–443.
- DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350–356.
- Eid, E. M. and Shaltout, K. H. (2016) Bioaccumulation and translocation of heavy metals by nine native plant species

- grown at a sewage sludge dump site. *International Journal of Phytoremediation* 18: 1075–1085.
- Eryilmaz, F. (2006) The relationships between salt stress and anthocyanin content in higher plants. *Biotechnology and Biotechnological Equipment* 20: 47–52.
- Eun, S. O., Youn, H. S. and Lee, Y. (2008) Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiologia Plantarum* 110: 357–365.
- Fahr, M., Laplaze, L., Bendaou, N., Hoher, V., El Mzibri, M., Bogusz, D. and Smouni, A. (2013) Effect of lead on root growth. *Frontiers in Plant Science* 4:175.
- Ferrer, M. A., Cimini, S., Lopez-Orenes, A., Calderon, A. A. and De Gara, L. (2018) Differential pb tolerance in metalcolous and non-metalcolous *Zygophyllum fabago* populations involves the strengthening of the antioxidative pathways. *Environmental and Experimental Botany* 150: 141–151.
- Fodor, F., Sarvari, E., Lang, F., Szigeti, Z. and Cseh, E. (1996) Effects of pb and cd on cucumber depending on the complex in the culture solution. *Journal of Plant Physiology* 148: 434–439.
- Ghaderian, S. M. and Ghotbi Ravandi, A. A. (2012) accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. *Journal of Geochemical Exploration* 123: 25–32.
- Glinska, S., Bartczak, M., Oleksiak, S., Wolska, A., Gabara, B., Posmyk, M. and Janas, K. (2007) Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of *Allium cepa* L. roots treated with heavy metals. *Ecotoxicology and Environmental Safety* 68: 343–350.
- Guagliardi, I., Cicchella, D., De Rosa, R. and Buttafuoco, G. (2015) Assessment of lead pollution in topsoils of a southern Italy area: analysis of urban and peri-urban environment. *Journal of Environmental Sciences* 33: 179–187.
- Gupta, D. K., Nicoloso, F. T., Schetinger, M. R. C., Rossato, L. V., Pereira, L. B., Castro, G. Y., Srivastava, S. and Tripathi, R. D. (2009) Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. *Journal of Hazardous Materials* 172: 479–484.
- Hattab, S., Hattab, S., Flores-Casseres, M. L., Boussetta, H., Dumas, P., Hernandez, L. E. and Banni, M. (2016) Characterisation of lead-induced stress molecular biomarkers in *Medicago sativa* plants. *Environmental and Experimental Botany* 123: 1–12.
- Hoagland, D. R. and Arnon, D. I. (1950) The water-culture method for growing plants without soil. *Circular*. California Agricultural Experiment Station 347:1–32.
- Hossain, Z. and Komatsu, S. (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. *Frontiers in Plant Science* 3: 310.
- Islam, M. M., Hoque, M. A., Okuma, E., Jannat, R., Akhter Banu, M. N., Jahan, M. S., Nakamura, Y. and Murata, Y. (2009) Proline and glycinebetaine confer cadmium tolerance on tobacco bright yellow-2 cells by increasing ascorbate-glutathione cycle enzyme activities. *Bioscience Biotechnology and Biochemistry* 73: 2320–2323.
- Jezler, C. N., Mangabeira, P. A. O., De Almeida, A. A. F., De Jesus, R. M., De Oliveira, R. A., Da Costa Silva, D. and Do Bomfim Costa, L. C. (2015) Pb and cd on growth, leaf ultrastructure and essential oil yield mint (*Mentha arvensis* L.). *Ciencia Rural* 45: 392–398.
- Kapoor, D., Rattan, A., Bhardwaj, R. and Kaur, S. (2016) Photosynthetic efficiency, ion analysis and carbohydrate metabolism in *Brassica juncea* plants under cadmium stress. *Journal of Pharmacognosy and Phytochemistry* 5: 279–286.
- Katar, D., Kacar, O., Kara, N., Aytac, Z., Goksu, E., Kara, S., Katar, N., Erbas, S., Telci, I. and Elmastas, M. (2017) Ecological variation of yield and aroma components of summer savory (*Satureja hortensis* L.). *Journal of Applied Research on Medicinal and Aromatic Plants* 7: 131–135.
- Kaul, S., Sharma, S. S. and Mehta, I. K. (2008) Free radical scavenging potential of L-proline: evidence from in vitro assays. *Amino Acids* 34: 315–320.
- Kosyk, O. I., Khomenko, I. M., Batsmanova, L. M. and Taran, N. U. (2017) Phenylalanine ammonia-lyase activity and anthocyanin content in different varieties of lettuce under the cadmium influence. *The Ukrainian Biochemical Journal* 89: 85–91.
- Kumar, A., Prasad, M. N. and Sytar, O. (2012) Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically. *Chemosphere* 89: 1056–1065.
- Lamb, D. T., Ming, H., Megharaj, M. and Naidu, R. (2010) Relative tolerance of a range of Australian native plant species and lettuce to copper, zinc, cadmium, and lead. *Archives of Environmental Contamination and Toxicology* 59: 424–432.
- Liang, X., Zhang, L., Natarajan, S. K. and Becker, D. F. (2013) Proline mechanisms of stress survival. *Antioxidants and Redox Signaling* 19: 998–1011.
- Liu, T., Liu, S., Guan, H., Ma, L., Chen, Z., Gu, H., Qu, L. J. (2009) Transcriptional profiling of Arabidopsis seedlings in response to heavy metal lead (Pb). *Environmental and Experimental Botany* 62: 377–386.
- Lopez-Orenes, A., Martinez-Perez, A., Calderon, A. A. and Ferrer, M. A. (2014) Pb-induced responses in *Zygophyllum fabago* plants are organ-dependent and modulated by salicylic acid. *Plant Physiology and Biochemistry* 84: 57–66.
- Luo, Z. B., He, J., Polle, A. and Rennenberg, H. (2016) Heavy metal accumulation and signal transduction in herbaceous and woody plants: paving the way for enhancing phytoremediation efficiency. *Biotechnology Advances*

34: 1131–1148.

- Malar, S., Manikandan, R., Favas, P. J. C., Sahi, S. V. and Venkatachalam, P. (2014) Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in *Sesbania grandiflora*: a potential plant for phytoremediation. *Ecotoxicology and Environmental Safety* 108: 249–257.
- Maldonado-Magana, A., Favela-Torres, E., Rivera-Cabrera, F. and Volke-Sepulveda, T. (2011) Lead bioaccumulation in *Acacia farnesiana* and its effect on lipid peroxidation and glutathione production. *Plant and Soil* 339: 377–389.
- Maleva, M., Garmash, E., Chukina, N., Malec, P., Waloszek, A. and Strzalka, K. (2018) Effect of the exogenous anthocyanin extract on key metabolic pathways and antioxidant status of Brazilian elodea (*Egeria densa* (Planch.) Casp.) exposed to cadmium and manganese. *Ecotoxicology and Environmental Safety* 160: 197–206.
- Miller, G. L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31: 426–428.
- Momtaz, S. and Abdollahi, M. (2010) An update on pharmacology of satureja species; from antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation. *International Journal of Pharmacology* 6: 346–353.
- Moya, J. L., Ros, R. and Picazo, I. (1993) Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. *Photosynthesis Research* 36: 75–80.
- Nakano, Y. and Asada, K. (1987) Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant and Cell Physiology* 28: 131–140.
- Pourrut, B., Jean, S., Silvestre, J. and Pinelli, E. (2011) Lead-induced DNA damage in *Vicia faba* root cells: potential involvement of oxidative stress. *Mutation Research Genetic Toxicology and Environmental Mutagenesis* 726: 123–128.
- Pourrut, B., Shahid, M., Dumat, C., Winterton, P. and Pinelli, E. (2011) Lead uptake, toxicity, and detoxification in plants. In: *Reviews of Environmental Contamination and Toxicology* (ed. Whitacre, D. M.) Pp. 113–36. Springer New York, New York.
- Reddy, A. M., Kumar, S. G., Jyothsnakumari, G., Thimmanaik, S. and Sudhakar, C. (2005) Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). *Chemosphere* 60: 97–104.
- Rejeb, K. B., Abdelly, C. and Savoure, A. (2014) How reactive oxygen species and proline face stress together. *Plant Physiology and Biochemistry* 80: 278–284.
- Rodriguez, E., Da Conceicao Santos, M., Azevedo, R., Correia, C., Moutinho-Pereira, J., Ferreira de Oliveira, J. M. and Dias, M. C. (2015) Photosynthesis light-independent reactions are sensitive biomarkers to monitor lead phytotoxicity in a pb-tolerant *Pisum sativum* cultivar. *Environmental Science and Pollution Research* 22: 574–585.
- Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M. and Pinelli, E. (2014) Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. In: *Reviews of Environmental Contamination and Toxicology* (ed. Whitacre, D. M.) Pp. 1–44. Cham: Springer International Publishing.
- Sharma, P. and Dubey, R. S. (2005) Lead toxicity in plants. *Brazilian Journal of Plant Physiology* 17: 35–52.
- Shi, X., Wang, S., Wang, D., Sun, H., Chen, Y., Liu, J. and Jiang, Z. (2018) Woody species *Rhus chinensis* Mill. seedlings tolerance to pb: physiological and biochemical response. *Journal of Environmental Sciences* 78: 63–73.
- Singh, S., Parihar, P., Singh, R., Singh, V. P. and Prasad, S. M. (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Frontiers in Plant Science* 6: 1143.
- Sooksawat, N., Meetam, M., Kruatrachue, M., Pokethitiyook, P. and Nathalang, K. (2013) Phytoremediation potential of charophytes: bioaccumulation and toxicity studies of cadmium, lead and zinc. *Journal of Environmental Sciences* 25: 596–604.
- Su, C., Jiang, Y., Li, F., Yang, Y., Lu, Q., Zhang, T., Hu, D. and Xu, Q. (2017) Investigation of subcellular distribution, physiological, and biochemical changes in *Spirodela polyrhiza* as a function of cadmium exposure. *Environmental and Experimental Botany* 142: 24–33.
- Szabados, L. and Savoure, A. (2010) Proline: a multifunctional amino acid. *Trends in Plant Science* 15: 89–97.
- Venkatachalam, P., Jayalakshmi, N., Geetha, N., Sahi, S. V., Sharma, N. C., Rene, E. R., Sarkar, S. K. and Favas, P. J. C. (2017) Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant *Acalypha indica* L. under lead stress. *Chemosphere* 171: 544–553.
- Verbruggen, N. and Hermans, C. (2008) Proline accumulation in plants: a review. *Amino Acids* 35: 753–759.
- Visioli, G., Marmioli, N. (2013) The proteomics of heavy metal hyperaccumulation by plants. *Journal of Proteomics* 79: 133–145.
- Voght, T. (2010) Phenylpropanoid biosynthesis. *Molecular Plant* 3: 2–20.
- Wang, C., Tian, Y., Wang, X., Geng, J., Jiang, J., Yu, H. and Wang, C. (2010) Lead-contaminated soil induced oxidative stress, defense response and its indicative biomarkers in roots of *Vicia faba* seedlings. *Ecotoxicology* 19: 1130–1139.
- Wang, P., Zhang, S., Wang, C. and Lu, J. (2012) Effects of pb on the oxidative stress and antioxidant response in a pb bioaccumulator plant *Vallisneria natans*. *Ecotoxicology and Environmental Safety*

78: 28–34.

- Xu, Z., Zhou, Q. and Liu, W. (2009) Joint effects of cadmium and lead on seedlings of four Chinese cabbage cultivars in northeastern China. *Journal of Environmental Sciences* 21: 1598–1606.
- Yadav, S. K. (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany* 76: 167–179.
- Yang, R., Yu, G., Tang, J. and Chen, X. (2008) Effects of metal lead on growth and mycorrhizae of an invasive plant species (*Solidago canadensis* L.). *Journal of Environmental Sciences* 20: 739–744.
- Yang, Y., Zhang, Y., Wei, X., You, J., Wang, W., Lu, J. and Shi, R. (2011) Comparative antioxidative responses and proline metabolism in two wheat cultivars under short term lead stress. *Ecotoxicology and Environmental Safety* 74: 733–740.
- Yousefi, Z., Kolahi, M., Majd, A. and Jonoubi, P. (2018) Effect of cadmium on morphometric traits, antioxidant enzyme activity and phytochelatin synthase gene expression (SoPCS) of *Saccharum officinarum* Var. Cp48-103 in vitro. *Ecotoxicology and Environmental Safety* 157: 472–481.
- Zhang, K., Chai, F., Zheng, Z., Yang, Q., Zhong, X., Fomba, K. W. and Zhou, G. (2018) Size distribution and source of heavy metals in particulate matter on the lead and zinc smelting affected area. *Journal of Environmental Sciences* 71: 188–196.
- Zhou, C., Huang, M., Ren, H., Yu, J., Wu, J. and Ma, X. (2017) Bioaccumulation and detoxification mechanisms for lead uptake identified in *Rhus chinensis* Mill. seedlings. *Ecotoxicology and Environmental Safety* 142: 59–68.