South African Journal of Botany 150 (2022) 1011–1021

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Physiological responses to lead and PEG-simulated drought stress in metallicolous and non-metallicolous *Matthiola* (Brassicaceae) species from Iran

Behrooz Salehi-Eskandari[a,*], Mina Shahbazi Gahrouei[b], Robert S. Boyd[c], Nishanta Rajakaruna[d,e], Rasoul Ghasemi[a]

[a] *Department of Biology, Payame Noor University, Tehran, Iran*
[b] *Faculty of Biology, University of Isfahan, Isfahan 81746-73441, Iran*
[c] *Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA*
[d] *Biological Sciences Department, California Polytechnic State University, One Grand Avenue, San Luis Obispo, CA 93407, USA*
[e] *Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa*

ARTICLE INFO

Article History:
Received 29 October 2021
Revised 23 August 2022
Accepted 7 September 2022
Available online xxx

Edited by: Prof S. Barnard

Keywords:
Antioxidant enzyme
Co-tolerance
Drought stress
Pb
Matthiola flavida
Osmotic adjustment

ABSTRACT

Plants growing on quarry tailings at the Irankouh Pb/Zn mine encounter both drought stress and high levels of Pb. To better understand the role of drought and Pb in plant adaptation to Pb/Zn quarry tailings, we compared effects of drought stress (simulated by polyethylene glycol - PEG) and Pb, individually and in concert, to determine how these stressors affected two plant species: the metallicolous species *Matthiola flavida* (M) and the non-metallicolous congener, *M. incana* (NM). Plants were exposed to Pb ($Pb(NO_3)_2$) and three levels of PEG-6000 (0, -0.25, and -0.75 MPa) in a complete factorial design. Pb had non-significant effects on growth and oxidative stress but enhanced levels of osmoprotectants and phenol compounds in the M species *M. flavida*, whereas in *M. incana* the effect of Pb was non-significant on the same variables (except for anthocyanins and the osmoprotectants, proline and glycine betaine). Compared to *M. incana*, the M species *M. flavida* was tolerant of Pb, showing strongly reduced root-to-shoot translocation and enhanced Pb accumulation in the root (especially when under drought stress), which reduced toxic effects of Pb in the shoot. Tolerance of Pb by the M species *M. flavida* was aided by the accumulation of reducing sugars and phenolic compounds, as well as by greater catalase activity.

1. Introduction

Metal mine quarry wastes are a challenging substrate for plant growth because they cause simultaneous stresses: these often include drought stress as well as stress from elevated soil metal levels (Ernst, 1988; Mansfield et al., 2014). Co-tolerance, which allows a plant to withstand simultaneous stresses, has been widely studied (de Silva et al., 2012; Nabi et al., 2019; Von Wettberg et al., 2014), but not often in the context of the stresses created by mine quarry wastes. Although tolerance and adaptation to high soil concentrations of heavy metals by plants is metal-specific, and usually those plants cannot tolerate more than one or a few heavy metals (Boyd and Rajakaruna, 2013; Konečná et al., 2020; Singh et al., 2016), the stimulation of general stress response pathways by heavy metals may promote co-tolerance of other stresses such as drought (Von Wettberg et al., 2014; Selby et al., 2014; Selby and Willis, 2018).

One of the most abundant and toxic heavy metals is Pb, which can have lethal effects on plants, animals, and humans (Fahr et al., 2015; Li et al., 2016; Wani et al., 2015). Pb pollution stems from its widespread uses in industrial activities, including in metal mining/smelting and burning of coal; in energy production (in gasoline, emissions from power plants, and batteries); from agricultural activities (in pesticides and fertilizers); as an ingredient in ammunition, explosives, and Pb-containing dyes; and its presence in sewage sludge and wastewaters (Gupta et al., 2013; Kumar et al., 2012; Mahdavian et al., 2016).

Pb is a potentially toxic element to plants and produces many phytotoxic effects, including harm to morphological, biochemical, and physiological processes (Pourrut et al., 2013; Zouari et al., 2018; Zulfiqar et al., 2019). Some plant species, termed metallophytes by

---

Abbreviations: PEG, polyethylene glycol of molecular weight 6000; Pb, lead; M, metallicolous; NM, non-metallicolous; RDW, root dry weight; SDW, shoot dry weight; RWC, relative water content; Pb R, Pb concentration in roots; Pb S, Pb concentration in shoots; Pb S/R, Pb root-to-shoot translocation; Total chl, total chlorophyll; Chl a/b, chlorophyll a/b ratio; Car, carotenoids; Pro, proline; GB, glycine betaine; RS, reducing sugar; Anth, anthocyanins; Phenol, total phenolic concentrations; CAT, catalase; APX, ascorbate peroxidase

[*] Corresponding author.
E-mail address: behsalehi@pnu.ac.ir (B. Salehi-Eskandari).

https://doi.org/10.1016/j.sajb.2022.09.016

Baker (1987), can survive and reproduce on the intensely metal-enriched soils found on metal mine sites. Quarry tailings present around a mining area often are severely polluted by heavy metals (such as Pb) and are characterized by other stresses such as limited water holding capacity, deficiencies of essential nutrients, and low amounts of soil organic matter, and as a consequence are often poorly vegetated (Ghasemi et al., 2018; Mansfield et al., 2014).

The Irankouh Pb/Zn mine is in the semiarid region of Central Iran at 51°42’ E and 32°30’ N. Therefore, plants in this mine area encounter not only heavy metal stress but also drought stress. Drought stress reduces plant growth by affecting various physiological and biochemical processes, such as water relations, nutrient uptake, leaf chlorophyll levels, photosynthesis, water content, and others (Farooq et al., 2012; Okunlola et al., 2017). Plants can ameliorate drought stress through osmotic adjustment, which is promoted by the production and accumulation of soluble products (e.g., sucrose, proline, glycine betaine) and other solutes that help maintain cell turgor (Cvikrová et al., 2013; Salehi-Eskandari et al., 2017). Drought stress, like other stresses, stimulates reactive oxygen species (ROS) generation, which causes oxidative damage to cellular components and photoinhibition (Agrawal et al., 2016; Ashraf and Harris, 2013; Cruz de Carvalho, 2008; You and Chan, 2015). As protection from oxidative stress, plants possess antioxidant scavenger systems which can be classified into two broad categories: antioxidant enzymes, such as catalase (CAT), superoxide dismutases (SOD), and ascorbate peroxidase (APX), as well as non-enzymatic antioxidants such as glutathione, ascorbate, proline, flavonoids, phenolics, α-tocopherol, and carotenoids (Kısa et al., 2016; Salehi-Eskandari et al., 2017).

Matthiola flavida Boiss. (Brassicaceae) is a perennial herb that grows on dry rocky hills and sandy plains in Iran, Pakistan, Kashmir, Afghanistan, and Turkmenistan (Mohtadi, 2014; Sarwar and Qaiser, 2012) and is a potential candidate for phytoremediation of soils contaminated with Pb (Mohtadi et al., 2012a, b). It has been demonstrated that, by adding Fe (Heidari Dehno and Mohtadi, 2018) and the synthetic and biodegradable chelator (S,S)-N,N′-ethylenediamine disuccinic acid (EDDS), hydroponically grown *M. flavida* can accumulate even more Pb in its shoot tissue (Mohtadi et al., 2013). Despite the well-known lethal effect of Pb on plants (Pourrut et al., 2011), Pb hyper-tolerance mechanisms and the combined effects of drought stress with Pb stress on plant physiology need further investigation. We studied Pb stress and drought stress induced by polyethylene glycol (PEG) to better understand their combined effects on the metallicolous (M) species *M. flavida* and its congeneric non-metallicolous (NM) species *M. incana* (L.) R.Br. The species were compared for Pb uptake and translocation, plant growth (dry weight [DW] biomass and relative water content [RWC]), pigments (carotenoids, chlorophylls a and b), osmotic molecules (proline, glycine betaine, reducing sugars), phenolic compounds (anthocyanin, total phenols), and activities of two antioxidant enzymes, catalase (CAT) and ascorbate peroxidase (APX), under varying levels of PEG-induced drought stress. We hypothesized that the M species *M. flavida* should be more Pb tolerant than *M. incana* and that Pb tolerance in *M. flavida* may enhance its resistance to drought.

2. Materials and methods

2.1. Plant materials and treatments

About 5000 seeds of the M species *M. flavida* were collected from a population of about 300 plants growing at the Irankouh Pb/Zn mine site, and seeds of *M. incana* were provided from a commercial supplier (Pakan Bazr Co., Isfahan, Iran). *M. incana* was selected as a comparative NM species because of its close phylogenetic affinity to *M. flavida* (Jaén-Molina et al., 2009). Seeds of both species were surface-sterilized using sodium hypochlorite (bleach with 1% active chlorine) for 15 min, rinsed twice with distilled water, and germinated on Perlite wetted with deionized water. After three weeks, seedlings were transferred to hydroponic culture in 450 ml light-proof vessels (two plants per vessel) containing a modified one-fourth-strength Hoagland’s solution composed of 1.5 mM $Ca(NO_3)_2$, 0.75 mM $MgSO_4$, 1.25 mM KNO_3, 0.28 mM KH_2PO_4, 10 μM Fe(Na)-EDTA, 1 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 5 μM $MnSO_4$, 25 μM H_3BO_3, 0.1 μM Na_2MoO_4, and 50 μM KCl. Final pH was adjusted to 5.8 and nutrient solutions were renewed twice a week. Experiments were performed in a greenhouse with a day/night temperature of 25/20 °C and 16 h photoperiod (light intensity 200 μmol m^{-2} s^{-1}).

Because the species differed in Pb tolerance, the Pb-added treatment for each species was selected as the concentration of Pb to which exposure induced Pb toxicity symptoms (reduced growth, chlorosis, etc.) for each species. Based on prior experiments, 75 mg L^{-1} and 150 mg L^{-1} were selected as the concentrations of $Pb(NO_3)_2$ sufficient to produce toxicity symptoms for the NM species *M. incana* and the M species *M. flavida*, respectively. After 45 days for *M. incana*, and 60 days for *M. flavida*, half of the vessels for each species received nutrient solution amended with $Pb(NO_3)_2$. A longer time was used for *M. flavida* because this species has a slower growth rate compared to that of *M. incana.* To avoid precipitation of Pb phosphate and formation of Pb-EDTA resulting from Pb replacing Fe from the EDTA complex in the Pb-added solutions, the KH_2PO_4 and Fe(Na)-EDTA were removed. They were also removed from the nutrient solutions of the non-Pb-added treatments to make the solutions as similar as possible.

After seven days, all vessels were additionally treated with 0%, 13.8%, or 23.3% of polyethylene glycol 6000 (PEG, w/v), equivalent to osmotic potentials of 0, −0.25, and −0.75 MPa, respectively (Money, 1989). To avoid osmotic shock, PEG was added in −0.25 MPa steps at 6 h intervals until the desired concentration was reached. Nutrient solutions were aerated constantly and replaced every three days during the 14-day treatment period.

Each combination of Pb and drought stress treatments had six vessels (replicates) for each species for a total of 36 vessels/species and 72 vessels for the entire experiment. Three replicates of each species for each treatment combination were harvested for measurements of plant growth and Pb accumulation, and the other three were used to provide plant material for measuring physiological parameters.

2.2. Plant growth and Pb accumulation

After harvesting, plants were separated into root and shoot portions and rinsed three times in deionized water before fresh weight (FW) was measured. Dry weight (RDW, root dry weight, and SDW, shoot dry weight) was measured after drying samples at 70 °C for 48 h in an oven. Plant water content (WC) was calculated as percent water using the FW and dry weight data. Pb concentrations were determined in shoots and roots by digesting 0.1 g of dried ground plant material in a 1/4 (v/v) mixture of 37% (v/v) HCl and 65% (v/v) HNO_3, and Pb was determined using a flame atomic absorption spectrophotometer (AAS, Shimadzu model 6200). Pb translocation factors (TFs) were calculated as Pb concentration in shoots divided by Pb concentration in roots (Salehi-Eskandari et al., 2018).

2.3. Photosynthetic pigments measurement

The youngest mature leaves (4–5th leaf from the apex) of plants in replicates used for physiological measurements were extracted in 80% (v/v) acetone. Total chlorophyll, as well as chlorophyll a, chlorophyll b, and carotenoids contents, were measured by spectrophotometry (Shimadzu model UV-160) according to Lichtenthaler and Wellburn (1983).

2.4. Proline, glycine betaine, and total reducing sugar measurements

Free proline was assessed using the method of Bates et al. (1973). One g fresh weight of leaves was homogenized in 3% Sulfo salicylic acid and the suspension was centrifuged at 13,000 rpm for 10 min at 4 °C. Two ml of this extract was mixed with 2 ml of Ninhydrin reagent and 2 ml of glacial Acetic acid and the final solution boiled for 1 h in a water bath. Samples were cooled in ice, 4 ml of toluene was added, and then samples were mixed strongly with a vortex mixer for 20–25 s. Finally, sample absorbance was measured at 520 nm by spectrophotometry (Shimadzu model UV-160).

Glycine betaine content was determined following a modified method of Grieve and Grattan (1983). Dry and finely powdered leaves (50 mg) were shaken with 2 ml of deionized water for 48 h at 25 °C. Extracts were diluted with 1:1 with 2 N H_2SO_4. Aliquots of 0.5 ml were placed in test tubes and mixed with cold KI-I_2 reagent (0.2 ml) and then cooled in ice water for 1 h. Tubes were stored at 0–4 °C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was aspirated and the remaining crystals were dissolved in 0.5 ml of 1,2-dichloroethane before absorbance was measured at 365 nm (Shimadzu model UV-160).

Reducing sugar (RS) content was assessed using the method of Somogyi (1952) with some modification. Fresh leaves were homogenized with 80% ethanol and heated to 70 °C, then remained at 5 °C until extraction of carbohydrates occurred. Homogenates were centrifuged at 10,000 rpm for 10 min and supernatants used to determine reducing sugars using a standard glucose curve.

2.5. Measurement of anthocyanins and total phenolic contents

Anthocyanins were extracted from fresh leaves from replicates used for physiological measurements with acidified methanol (methanol: HCl, 99:1 v/v). Anthocyanins were measured spectrophotometrically and contents calculated using an extinction coefficient of 33,000 mol^{-1} cm^{-1} (Wanger, 1979). Total phenolic contents were determined using Folin–Ciocalteu reagent according to the method of Velioglu et al. (1998).

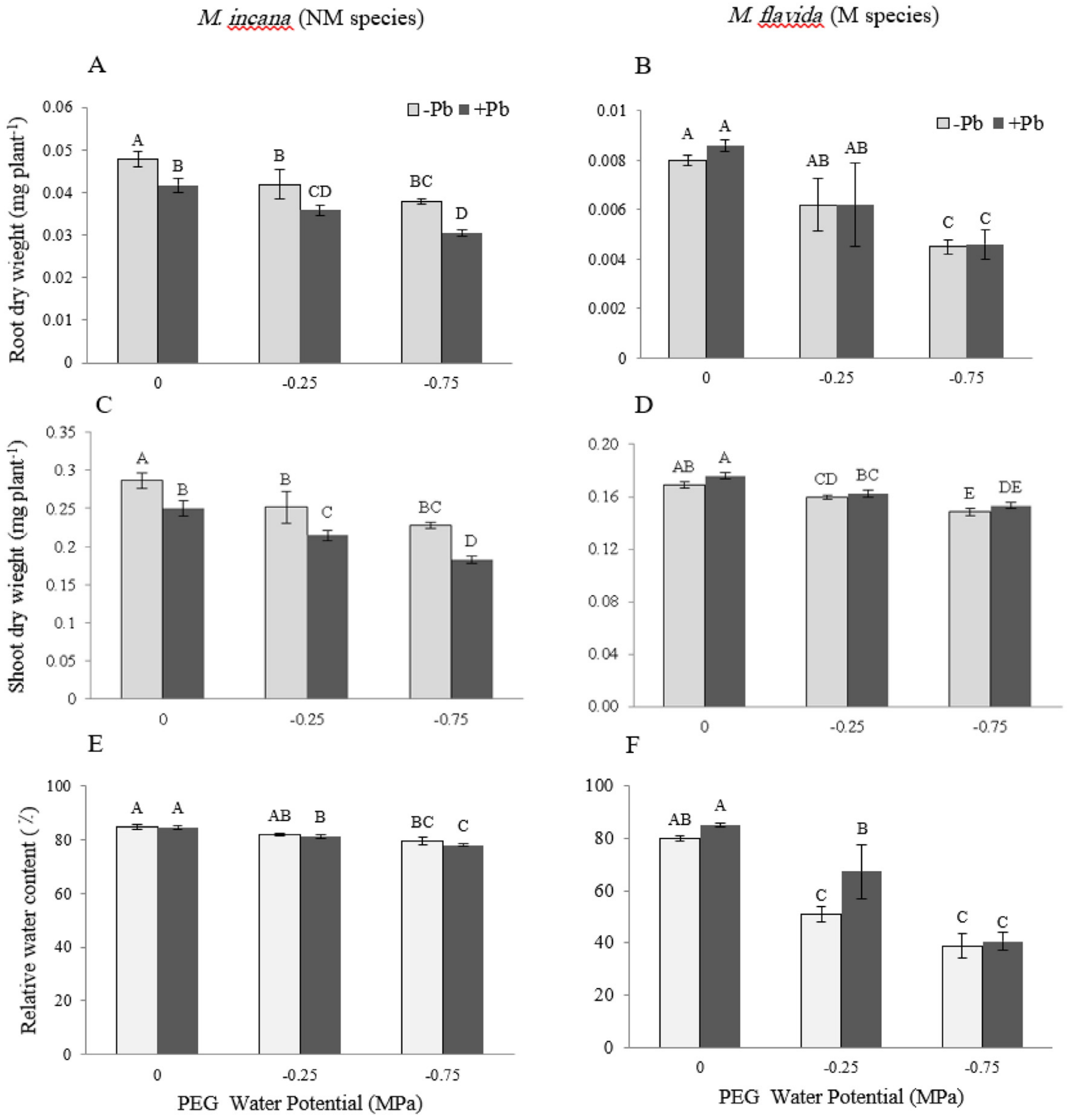

Fig. 1. Effect of drought stress induced by PEG on root dry weight, shoot dry weight, and relative water content (%) in *Matthiola incana* (A, C, E) and *M. flavida* (B, D, F), exposed to no-Pb added (gray bars) or Pb added as $Pb(NO_3)_2$ (black bars)treatments for 14 days. Significant differences between means ($P < 0.05$) are indicated by different letters.

2.6. Enzyme assays

One g of fresh leaf was homogenized in 3 ml of cold 50 mM potassium phosphate buffer (pH 7.8), including 0.2 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP), in an ice bath. The homogenate was centrifuged at 13,000 rpm at 4 °C for 20 min and the supernatant used for enzyme activity assays. CAT activity was determined and expressed using the UV spectrophotometric method of Aebi (1984). Ascorbate peroxidase activity was determined based on the method of Asada and Takahashi (1987).

2.7. Statistical analysis

The experiment used a randomized complete block design with three replications. Results were analyzed using three-way ANOVA, with Pb, PEG, and species as fixed main factors. Individual means were compared using Duncan's test at $P < 0.05$. SPSS software (version 16) was used for statistical analysis of the data and results were expressed as mean ± standard error (SE). To determine which variables were altered the most under the main factors explored in the experiment (M and NM species, PEG, and Pb), a principal component analysis (PCA) was conducted using SPSS.

3. Results

3.1. Plant growth and shoot relative water content

RDW of the NM species *M. incana* in −0.25 MPa was significantly decreased in comparison with the control but, in *M. flavida*, there was no significant difference in RDW in comparison with the 0 MPa treatment (Fig. 1A, B). In the highest PEG treatment (−0.75 MPa), values of RDW for both species were reduced in comparison with the 0 MPa treatment by 43.8% for *M. incana* and 20.5% for *M. flavida*. PEG decreased SDW in both species albeit in distinct concentration-dependent patterns in the absence of Pb (Fig. 1C, D). RDW and SDW of the NM species *M. incana* consistently decreased in all treatments of PEG with Pb in contrast to *M. flavida.* RWC was significantly decreased by PEG in the highest PEG treatment (−0.75 MPa) in the NM species *M. incana* but it was decreased in the −0.25 MPa PEG treatment in *M. flavida.* There were no significant effects of Pb on RWC for both species in all treatments of PEG, except for a positive effect on RWC in the −0.25 MPa treatment, where RWC increased compared to the same treatments without Pb (Fig. 1E, F).

3.2. Pb concentrations

Concentrations of root Pb, and the Pb root-to-shoot translocation factor, were marginally but significantly affected by PEG in the M species *M. flavida* (Fig. 2B, D) whereas there was no significant effect on these parameters for *M. incana* (Fig. 2A, C). In *M. flavida*, root Pb concentration significantly increased in PEG treatments of −0.25 and −0.75 MPa in comparison with the 0 MPa treatment by 19.6% and 26.5%, respectively. However, the translocation factor in the −0.75 MPa-PEG treatment significantly decreased by 35% in comparison with the 0 MPa treatment. Pb concentrations in *M. flavida* were consistently greater in roots (20.6-, 23.1-, 30.1-fold) and shoots (10.5-, 11-, 9.4-fold) at PEG treatments of 0, −0.25, and −0.75 MPa

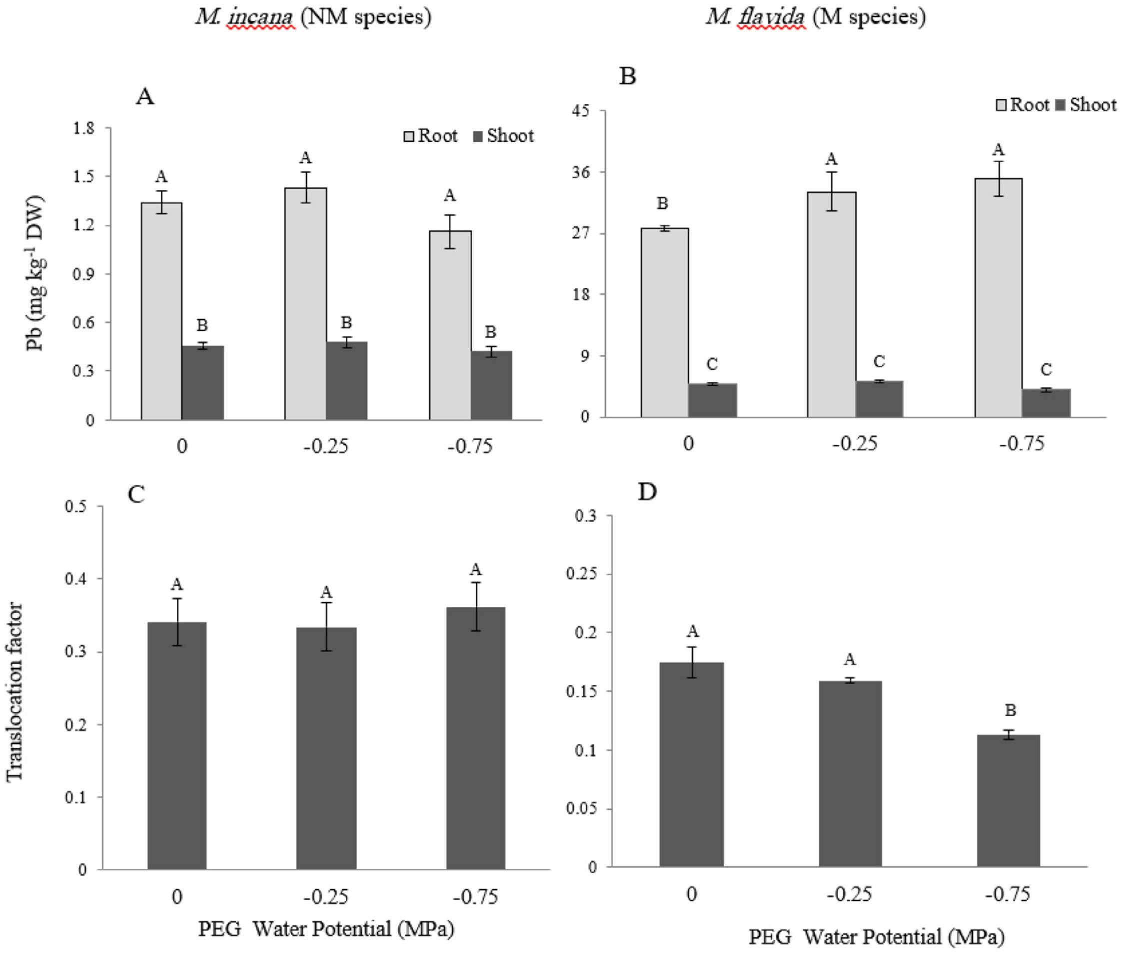

Fig. 2. Effect of drought stress induced by PEG on Pb concentration in roots (gray bars in A, B) and shoots (black bars in A, B) and Pb translocation factors (C, D) in *Matthiola incana* and *M. flavida* exposed to the Pb-added treatment for 14 days. Significant differences between means ($P < 0.05$) are indicated by different letters.

compared to those in the NM species *M. incana* (Fig. 2C, D), resulting in an approximately 2.4-fold difference in the root-to-shoot translocation factor between the species.

3.3. Photosynthetic pigments

Total chlorophyll concentration decreased with increasing PEG treatments in both species (Fig. 3A, B). Pb decreased total chlorophyll in the NM species *M. incana* (except in the −0.75 MPa treatment) and strongly decreased it for all three PEG treatments in *M. flavida*.

Chlorophyll a/b ratios in the NM species *M. incana* were greater than in *M. flavida* at 0 and −0.25 MPa PEG treatments (Fig. 3C, D). Also, PEG significantly decreased the chlorophyll a/b ratio in the NM species *M. incana* but not for *M. flavida*. Pb increased the chlorophyll a/b ratio approximately 1.2-fold only for the 0 MPa treatment in both species.

Effects of PEG and Pb on carotenoids concentration mostly followed the patterns observed for total chlorophyll in both species (Fig. 3E, F). Pb reduced carotenoids concentrations for all PEG treatments in the M species *M. flavida* but only for the 0 PEG treatment for *M. incana*.

3.4. Proline, glycine betaine, and total reducing sugars

Proline concentrations were significantly enhanced by PEG in both species but the concentrations of leaf proline in the M species *M. flavida* responded less strongly to PEG than in *M. incana* (Fig. 4A, B). Pb enhanced proline concentrations in both species, except for the 0 PEG treatment in both species and the −0.25 MPa PEG treatment in the M species *M. flavida*. Proline concentrations in the NM species *M. incana* exposed to −0.25 and −0.75 MPa PEG treatments and Pb were increased more than in *M. flavida* for similar treatments.

Glycine betaine was increased by PEG in both species (Fig. 4C, D). However, it increased less in the NM species *M. incana* exposed to −0.25 and −0.75 MPa PEG treatments than in *M. flavida*. Glycine betaine concentrations increased more in the NM species *M. incana* in −0.25 and −0.75 MPa PEG treatments with Pb, compared to similar treatments for *M. flavida*.

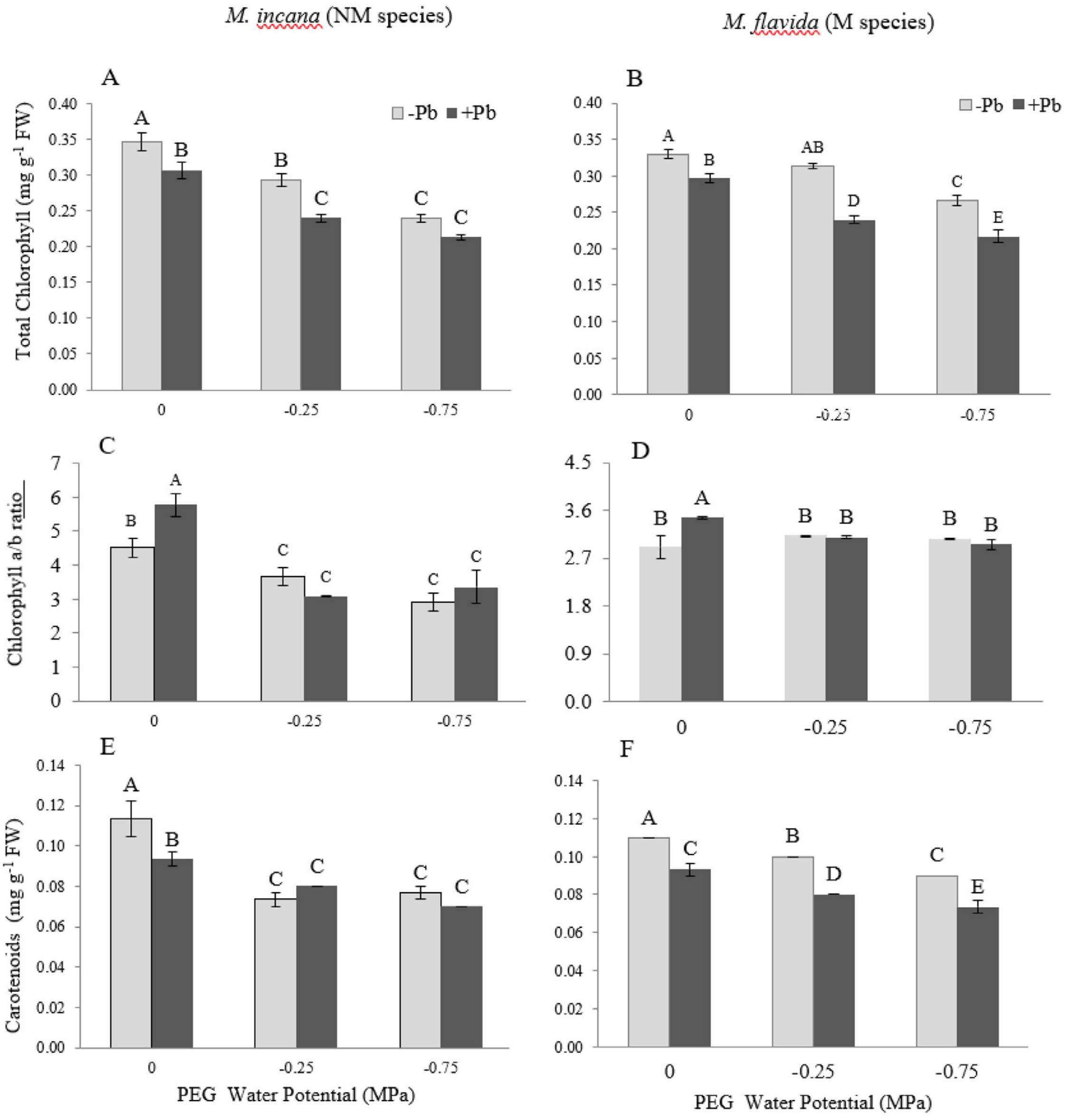

Fig. 3. Effect of drought stress induced by PEG on concentration of total chlorophyll (A, B), chlorophyll a/b ratio (C, D), and concentration of carotenoids (E, F) in *Matthiola incana* and *M. flavida* exposed to no Pb-added (gray bars) or Pb-added as $Pb(NO_3)_2$ (black bars) treatments for 14 days. Significant differences between means ($P < 0.05$) are indicated by different letters.

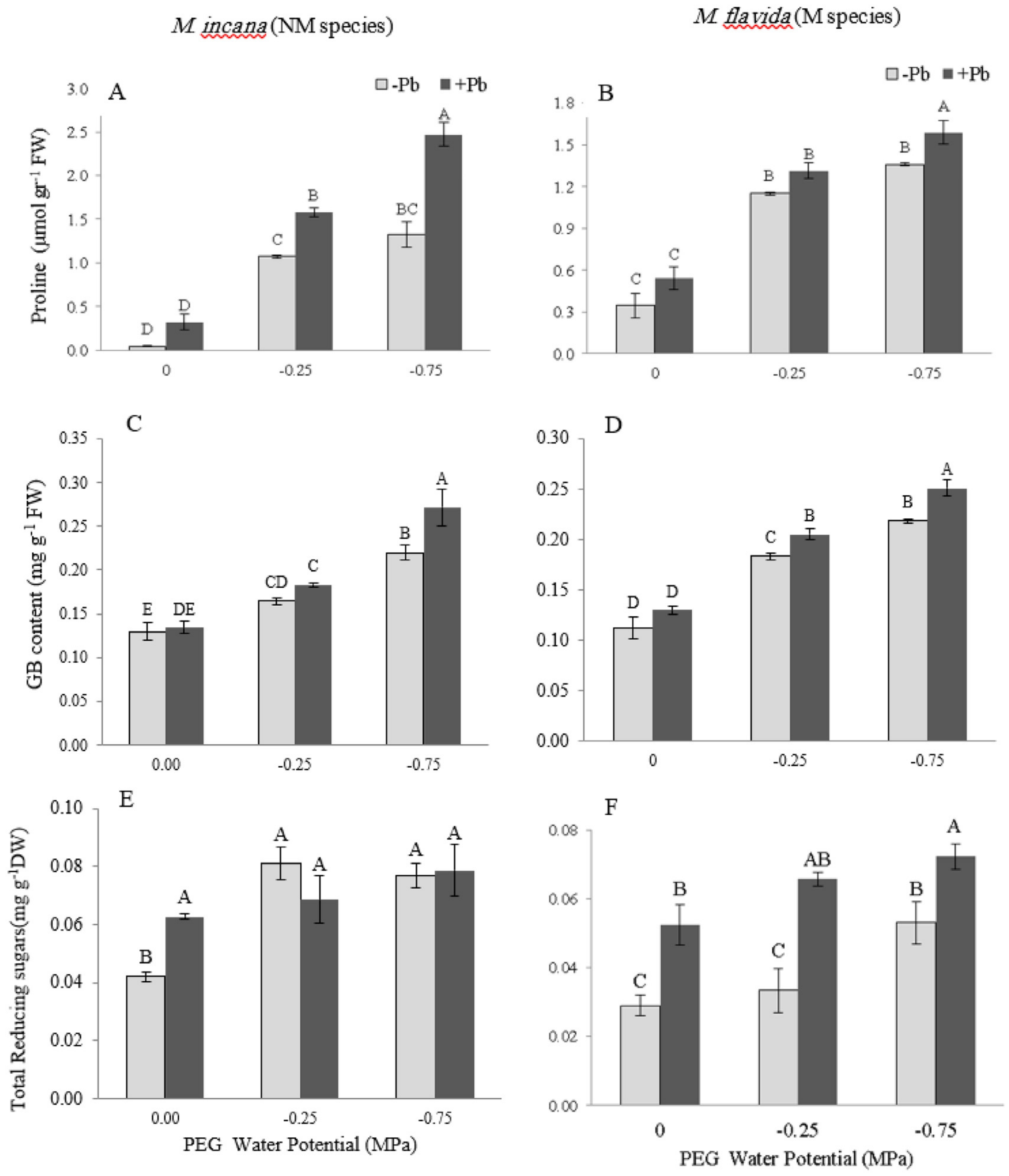

Fig. 4. Effect of drought stress induced by PEG on proline, glycine betaine (GB), and Total Reducing Sugars (TRS) concentrations in *Matthiola incana* (A, C, E) and *M. flavida* (B, D, F) exposed to no Pb-added (gray bars) or Pb-added as $Pb(NO_3)_2$(black bars)treatments for 14 days. Significant differences between means ($P < 0.05$) are indicated by different letters.

Reducing sugar (RS) concentrations significantly increased with increased PEG concentrations in both species, but they increased in concentration-dependent ways between species (Fig. 4E, F). Pb increased RS concentrations for all PEG treatments for the M species *M. flavida*, but did so only for the control PEG treatment in *M. incana*. In general, RS concentrations in *M. flavida* treated with Pb increased more than for *M. incana* when compared to the same treatments in the absence of Pb.

3.5. Anthocyanin and total phenolic contents

Anthocyanin concentrations were greater in the M species *M. flavida* than in *M. incana* and generally increased with increasing PEG concentration in both species (Fig. 5A, B). For the NM species *M. incana*, anthocyanin concentrations were relatively similar across all PEG treatments, yet they increased relative to the control for *M. flavida* in the −0.25 and −0.75 MPa PEG water potential treatments by 9.7% and 8.1%, respectively. Pb significantly increased anthocyanin concentrations only in the −0.75 MPa PEG water potential treatment for both species.

Total phenolic content increased with increasing PEG concentration in both species in concentration-dependent ways (Fig. 5C, D). In the NM species *M. incana*, values for both the −0.25 and −0.75 MPa treatments were increased more than in *M. flavida* compared to 0 MPa plants.

Pb significantly increased total phenolic content only in the −0.75 MPa PEG water potential treatment in the M species *M. flavida*, a 14% increase compared to the same treatment without Pb (Fig. 5D).

3.6. Antioxidant enzymes

CAT activity showed no significant changes for the PEG treatments in both species (Fig. 6A, B). Pb significantly decreased CAT activity of the NM species *M. incana* in the 0, −0.25 and −0.75 MPa PEG

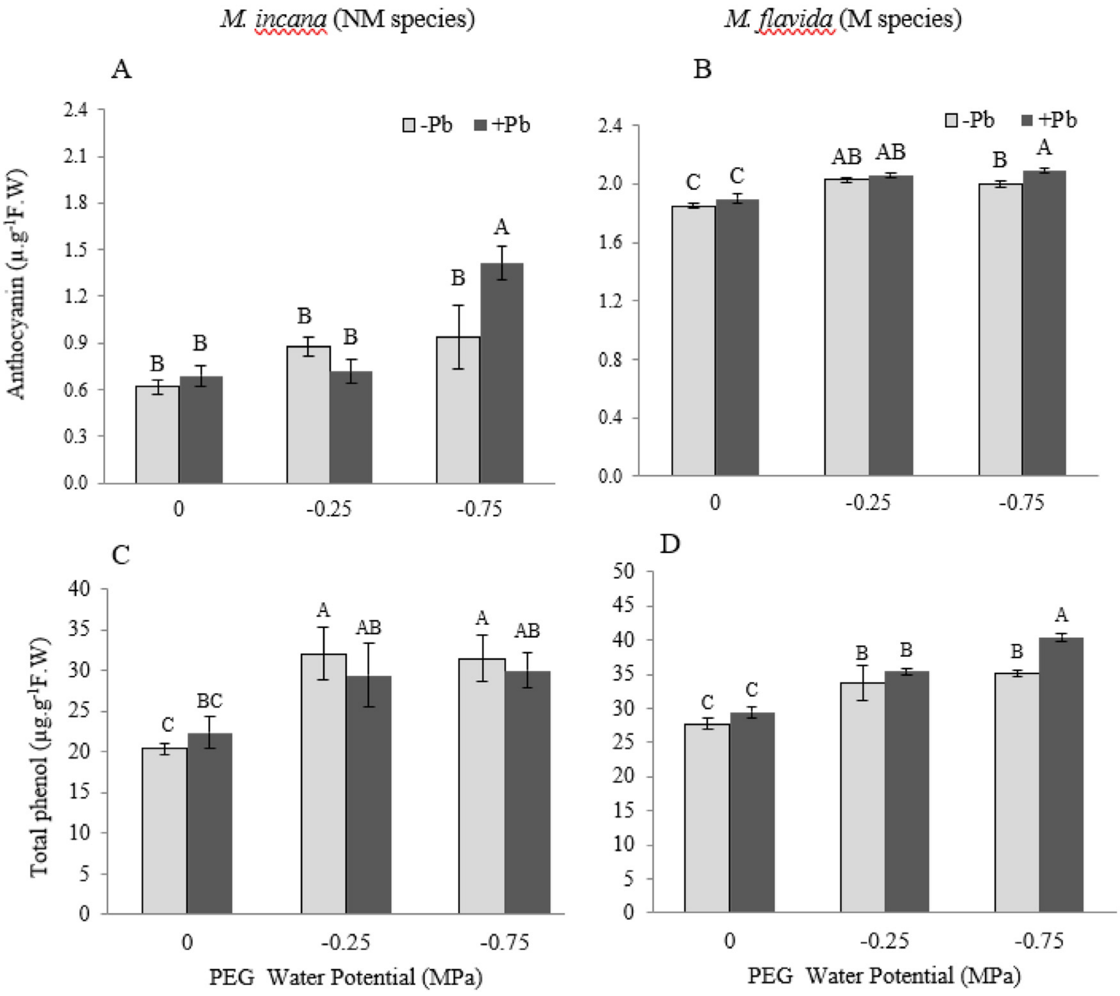

Fig. 5. Effect of drought stress induced by PEG on anthocyanins and total phenolic contents in *Matthiola incana* (A, C) and *M. flavida* (B, D) exposed to no Pb-added (gray bars) or Pb-added as $Pb(NO_3)_2$ (black bars) treatments for 14 days. Significant differences between means ($P < 0.05$) are indicated by different letters.

treatments by 51.6%, 30% and 56.4%, respectively, compared to the same treatments without Pb. In contrast, for NM species (*M. incana*), *M. flavida,* all PEG treatments with Pb added to the nutrient solution had no effect on CAT activity; it only significantly increased (compared to control plants) in the −0.75 MPa PEG treatment.

APX activity of the NM species *M. incana* significantly decreased only in the −0.75 MPa PEG treatment as compared to the control treatment (Fig. 6C, D), but PEG did not significantly affect APX activity of *M. flavida*. Pb did not change APX activity of both species compared to the same treatments without Pb in all PEG treatments.

3.7. Overall effects of PEG and Pb on growth and physiology

Growth, physiological, and antioxidant parameters measured under PEG and Pb differentially influenced the species as illustrated by the PCA (Fig. 7) and the influence of the variables on the axis loadings in Table 1. Principal component 1 (PCA1) described 60.17% and PCA2 35.29% of the variation.

Chlorophyll a/b ratio, SDW, RDW, and RWC were most altered in the NM species *M. incana*, whereas proline and glycine betaine were the most linked physiological variables with Pb. Total chlorophyll and carotenoids were the most altered parameters due to drought. Anthocyanin, phenol, and ascorbate peroxidase were most altered in the M species *M. flavida*. However, the position of reducing sugar suggested that this parameter was somewhat affected by Pb in *M. incana* as well. Catalase was also affected by drought in the M species *M. flavida*.

4. Discussion

Drought stress in arid and semiarid habitats can severely reduce plant growth, change plant chemical composition, and reduce seed quantity and plant survival (Osakabe et al., 2014; Zhang et al., 2018). A common adverse effect of drought stress on plants is reduction of fresh and dry biomass due to decreased numbers and sizes of leaves, which results from reduced plant water potential, increased oxidative stress, and limited photosynthesis (Sharma et al., 2020; Wang et al., 2018). These growth responses help reduce water loss from plants under drought stress (Anjum et al., 2011; Farooq et al., 2009; Hajihashemi and Sofo, 2018). Indeed, any factor that stimulates drought resistance in plants will increase fitness under conditions of low water availability. Our results showed that growth indices (RDW, SDW, and RWC) decreased gradually with increased PEG concentration in both species (Fig. 1). In the presence of Pb, growth indices for the M species *M. flavida* generally were unchanged by PEG treatments compared to the same treatments without Pb, except that RWC was increased for *M. flavida* by treatment with −0.25 MPa PEG and Pb (Fig. 1). In contrast, Pb consistently decreased growth indices in the NM species *M. incana* compared to the same PEG treatments without Pb, except for RWC (Figs. 1, 7).

Mohtadi et al. (2012b) reported *M. flavida* as a Pb hyperaccumulator that grows on metalliferous soils and can contain > 1 mg g^{-1} (0.1% dry weight) of Pb in the shoot without suffering phytotoxic effects (Rascio and Navari-Izzo, 2011). In our results (Fig. 2), Pb concentration was consistently greater in *M. flavida* than in the NM

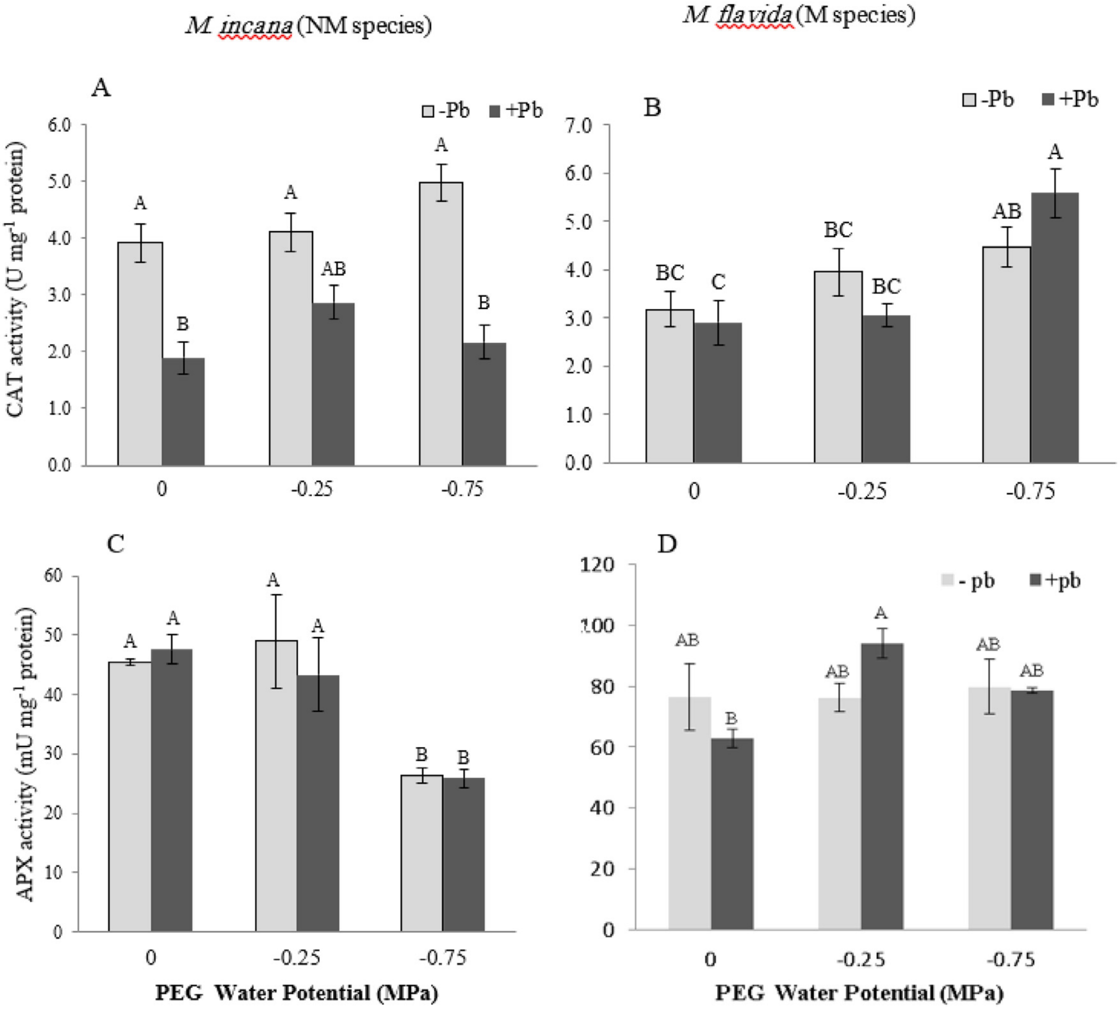

Fig. 6. Effect of drought stress induced by PEG on activities of catalase (CAT) and ascorbate peroxidase (APX) in *Matthiola incana* and *M. flavida* exposed to no Pb-added (gray bars) or Pb-added as $Pb(NO_3)_2$ (black bars) treatments for 14 days. Significant differences between means ($P < 0.05$) are indicated by different letters.

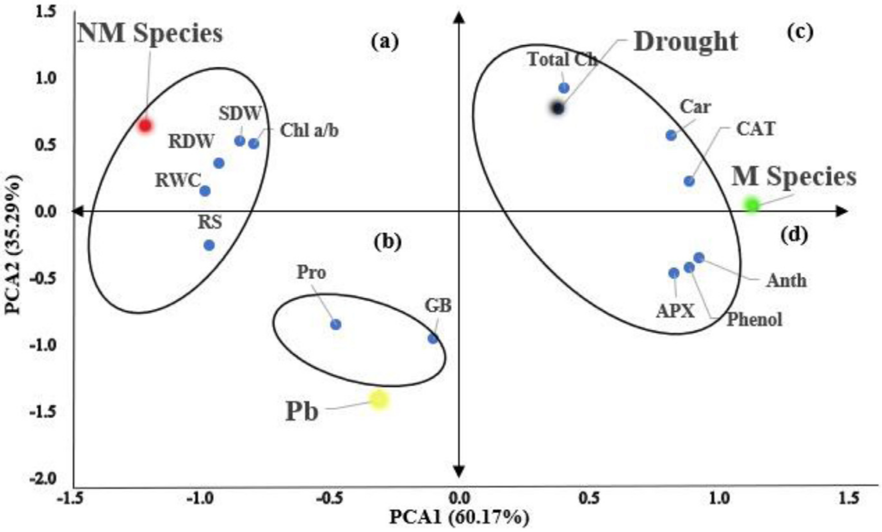

Fig. 7. Results of biplot principal components 1 and 2 (PCA1, 2) analysis obtained for growth, physiological and antioxidant parameters for non-metallicolous species (NM species) and metallicolous species (M species) under PEG and Pb treatments.

Table 1

Loadings of the growth, physiological, and antioxidant variables for both the M species *M. flavida* and the NM species *M. incana* under PEG and Pb treatments as shown on the PCA axes in Fig. 7. Abbreviations: root dry weight (RDW), shoot dry weight (SDW), relative water content (RWC), total chlorophyll (Total chl), chlorophyll a/b ratio (Chl a/b), carotenoids (Car), proline (Pro), glycine betaine (GB), reducing sugar (RS), anthocyanins (Anth), total phenolic concentrations (Phenol), catalase (CAT), and ascorbate peroxidase (APX).

		PCA1	PCA2			PCA1	PCA2
NM Species		−1.21	+0.63	**Drought**		+0.38	+0.76
	Chl a/b	−0.79	+0.50		Total Chl	+0.40	+0.91
	SDW	−0.85	+0.52		Car	+0.82	+0.57
	RDW	−0.93	+0.35		CAT	+0.89	+0.22
	RWC	−0.98	+0.14	**M Species**		+1.14	+0.03
	RS	−0.96	−0.26		Phenol	+0.89	−0.43
Pb		−0.31	−1.42		Anth	+0.93	−0.35
	Pro	−0.47	−0.86		APX	+0.83	−0.47
	GB	−0.10	−0.96				

species *M. incana*. In both species, Pb concentration in the root was several-fold higher than in the shoot, showing that most Pb was absorbed by roots and excluded from transport to shoots. Restricting metal to roots has been reported as a mechanism to avoid Pb toxicity in several plant species (Ginn et al., 2008; Krzesłowska et al., 2010; Mahdavian et al., 2016; Zheng et al., 2012). On the other hand, root Pb concentration was significantly increased by PEG, and the Pb translocation factor (Fig. 2C, D) was reduced in the −0.75 MPa PEG treatment in the M species *M. flavida* but not in *M. incana*. In *M. incana*, there were adverse additive effects of Pb and PEG treatments on growth parameters (Fig. 1). Phytotoxicity of Pb can be due to its interference with plant water balance and uptake of nutrients (such as Mg, Fe, Cu, Zn, Mn, and P), possibly due to blocked influx of these ions by binding to ion transporters (Ekmekçi et al., 2009; Hakeem et al., 2019; Singh et al., 2015), resulting in reduced cell division, elongation, and differentiation (Alamri et al., 2018; Dalla Vecchia et al., 2005; Mondal et al., 2015). De Silva et al. (2012) measured xylem conductivity properties and observed similar responses to heavy metal toxicity and drought stress, concluding that heavy metal toxicity and drought both reduced water uptake and transport capacity.

Why *M. flavida* Pb concentrations were less than hyperaccumulation levels (Van der Ent et al., 2013) in hydroponic cultivation needs additional study. It will be important to investigate if the plants will hyperaccumulate Pb if grown for longer than the 14-day period used in the current study. Our results indicate that *M. flavida* preferentially accumulates Pb in the root and thus acts as an excluder despite being able to translocate and detoxify Pb in the shoot. It is unclear if, given more time than in our 14-day experiment, it can reach hyperaccumulation levels for Pb as previously shown by Mohtadi et al. (2012a) considering its low TF in this study. Sequestration of toxic metals in hyperaccumulator leaves has been suggested as a mechanism for protecting sensitive roots from metal toxicity, and TF $>$ 1 has been proposed as a defining feature of hyperaccumulation (Kazakou et al., 2008). Pb is extremely toxic compared to most metals, especially for shoots, and all plants restrict translocation of Pb so that it is mostly (95%) accumulated in the roots and only a small amount (5%) is transported to the shoot (Gupta et al., 2013; Huang et al., 2012; Zhou et al., 2016). Our study shows that even the metallophyte and previously documented hyperaccumulator *M. flavida* (Mohtadi et al., 2012a) can have a low TF ($\sim$ 0.15) and can restrict translocation even further under Pb stress.

PEG and Pb had additive adverse effects on photosynthetic pigments in both species (Fig. 3). Drought stress can enhance degradation of photosynthetic pigments because of oxidative stress (Basal et al., 2020; Hajihashemi and Sofo, 2018). In our experiment, total chlorophyll and carotenoid pigments were strongly affected by drought stress (Fig. 7). Pb prevents the function of chlorophyll synthase, increases chlorophyll degradation, and reduces chloroplast stromal volume by displacing beneficial divalent ions (Mg^{2+}, Fe^{2+}, and Ca^{2+}), thereby inducing both ion deficiency and elevated ROS production (Hadi and Aziz, 2015; Kumar and Prasad, 2018; Li et al., 2016; Piotrowska et al., 2009). Pb significantly increased the chlorophyll a/b ratio in the control (0 MPa) PEG treatment for both species, suggesting that chlorophyll b appears to be more sensitive to Pb than chlorophyll a (Hou et al., 2018; Stiborova et al., 1986; Vodnik et al., 1999).

Accumulation of solutes such as soluble sugars, glycine betaine, proline, etc. under water stress not only helps maintain turgor pressure but also protects the quaternary structure of macromolecules from adverse effects of ROS (Cechin et al., 2006; Miri and Armin, 2013). Measured solute concentrations generally were increased by PEG but were more strongly enhanced by Pb in both species (Fig. 4), except for reducing sugars, which only increased in response to combinations of Pb and PEG in the M species *M. flavida.* Pb stress promotes proline accumulation by activating r-glutamyl and ornithine-δ-aminotransferase kinase enzymes (Kumar and Prasad, 2018). Accumulation of solutes due to Pb stress contributes to an osmotic balance that allows a plant to minimize storage reserves in order to support basal metabolism under Pb-stressed conditions (Hakeem et al., 2019).

Phenolic compounds (including anthocyanins) have carboxyl and hydroxyl groups that bind heavy metals and are responsible for ROS scavenging in stressed plants (Kısa et al., 2016; Michalak, 2006). Phenolic compounds were increased by PEG treatments in both species (Fig. 5). Pb also increased phenolics in the M species *M. flavida* but only in the −0.75 MPa PEG treatment, which could be due to the greater Pb tolerance of *M. flavida* in comparison to *M. incana* (Houda et al., 2016). In the PCA (Fig. 7), phenolic compounds were mostly associated with the M species *M. flavida.*

CAT and APX are scavengers of H_2O_2, which is produced in peroxisomes, chloroplasts, and the cytosol (Sekmen et al., 2014). Activities of CAT and APX (Fig. 6) increased with increasing PEG concentration in the absence and presence of Pb in the M species *M. flavida*, but *M. incana* showed a different Pb response, with lower CAT activity in treatments of PEG with Pb compared to treatments without Pb. A result similar to *M. flavida* was reported for the Cd/Zn accumulator *Sedum alfredii*, which had increased CAT and APX activity in roots exposed to Pb (Huang et al., 2012). Inducing or inhibiting the activity of antioxidant enzymes under Pb stress could depend on the concentration of treatments or their duration as well as plant species (Singh et al., 2010). ROS have a central role in response to stresses, both in signaling and through direct deleterious effects, and there are intimate tolerance responses to metals and drought. In a recent study involving overexpression of Glutathione s-transferase, Srivastava et al. (2019) observed a dual role for a gene responsible for detoxification of heavy metals and in producing glutathione peroxidase (GPX)-like activity, both of which are essential for heavy metal and drought stress tolerance. Indeed, we suggest that other antioxidant enzymes also act in a coordinated response to different stresses. For example, CAT was associated with PEG-induced drought stress in the M species *M. flavida* (Fig. 7).

Finally, regulatory networks that respond to simultaneous stresses could have additive effects. For example, metal stress combined with drought may aggravate water stress and make plants more vulnerable to drought, often resulting in reductions in hydraulic conductance (de Silva et al., 2012).

5. Conclusions

Growth responses showed that the M species *M. flavida* displayed greater Pb tolerance than *M. incana* and consistently showed greater Pb accumulation in both roots and shoots. We also found that Pb could partly inhibit the negative effect of drought stress in *M. flavida* through accumulation of greater amounts of reducing sugars and phenolic compounds, as well as induction of CAT activity. Joint response elements, such as common and specific transcription factors, or response sequences, need to be determined in both species to identify interactive pathways that address different environmental abiotic stresses.

Declaration of Competing Interest

The authors declare that they have no conflict of interest. We confirm that the manuscript: "Physiological responses to lead and PEG-simulated drought stress in metallicolous and non-metallicolous *Matthiola* (Brassicaceae) species from Iran" complies to the Ethical Rules applicable for this journal. We certify that the submission is an original work is not under review at any other publication.

Acknowledgments

We would like to thank the Graduate School of University of Isfahan for providing research facilities. We also thank our deceased colleague, Dr. Seyed Majid Ghaderian (Faculty of Biology, University of Isfahan, Isfahan, Iran), for contributing to the intellectual foundation of this research project.

References

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol. 105, 121–126.

Agrawal, L., Gupta, S., Mishra, S.K., Pandey, G., Kumar, S., Chauhan, P.S., Chakrabarty, D., Nautiyal, C.S., 2016. Elucidation of complex nature of PEG induced drought-stress response in rice root using comparative proteomics approach. Front. Plant Sci. 7, 1466.

Alamri, S.A., Siddiqui, M.H., Al-Khaishany, M.Y., Nasir Khan, M., Ali, H.M., Alaraidh, I.A., Mateen, M., 2018. Ascorbic acid improves the tolerance of wheat plants to lead toxicity. J. Plant Interact. 13, 409–419.

Anjum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., Lei, W., 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6, 2026–2032.

Asada, K., Takahashi, M., Kyle, D.J., Osmond, B., Arntzen, C.J., 1987. Production and scavenging of active oxygen in photosynthesis. (Eds.) Photoinhibition. Elsevier, Amsterdam, pp. 227–287.

Ashraf, M., Harris, P.J., 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 51, 163–190.

Baker, A., 1987. Metal tolerance. New Phytol. 106, 93–111.

Basal, O., Szabó, A., Veres, S., 2020. Physiology of soybean as affected by PEG-induced drought stress. Curr. Plant Biol., 100135.

Bates, L.S., Waldren, R.P., Teare, I., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.

Boyd, R.S., Rajakaruna, N., Gibson, D., 2013. Heavy metal tolerance. (Ed.) Oxford Bibliographies in Ecology. Oxford University Press, New York.

Cechin, I., Rossi, S., Oliveira, V., Fumis, T.d.F., 2006. Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. Photosynthetica 44, 143–146.

Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav. 3, 156–165.

Cvikrová, M., Gemperlová, L., Martincová, O., Vanková, R., 2013. Effect of drought and combined drought and heat stress on polyamine metabolism in proline-over-producing tobacco plants. Plant Physiol. Biochem. 73, 7–15.

Dalla Vecchia, F., La Rocca, N., Moro, I., De Faveri, S., Andreoli, C., Rascio, N., 2005. Morphogenetic, ultrastructural and physiological damages suffered by submerged leaves of *Elodea canadensis* exposed to cadmium. Plant Sci. 168, 329–338.

de Silva, N.D.G., Cholewa, E., Ryser, P., 2012. Effects of combined drought and heavy metal stresses on xylem structure and hydraulic conductivity in red maple (*Acer rubrum* L.). J. Exp. Bot. 63, 5957–5966.

Ekmekçi, Y., Tanyolaç, D., Ayhan, B., 2009. A crop tolerating oxidative stress induced by excess lead: maize. Acta Physiol. Plant. 31, 319–330.

Ernst, W., Salomons, W., Förstner, U., 1988. Response of plants and vegetation to mine tailings and dredged materials. (Eds.) Chemistry and Biology of Solid Waste 54–69 .

Fahr, M., Laplaze, L., El Mzibri, M., Doumas, P., Bendaou, N., Hocher, V., Bogusz, D., Smouni, A., 2015. Assessment of lead tolerance and accumulation in metallicolous and non-metallicolous populations of *Hirschfeldia incana*. Environ. Exp. Bot. 109, 186–192.

Farooq, M., Hussain, M., Wahid, A., Siddique, K., Aroca, R., 2012. Drought stress in plants: an overview. (Eds) Plant Responses to Drought Stress. Springer, Berlin, Heidelberg, pp. 1–33.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S., 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29, 185–212.

Ghasemi, Z., Ghaderian, S.M., Monterroso, C., Kidd, P.S., 2018. Improving the growth of Ni-hyperaccumulating plants in serpentine quarry tailings. Int. J. Phytoremediat. 20, 699–708.

Ginn, B.R., Szymanowski, J.S., Fein, J.B., 2008. Metal and proton binding onto the roots of *Fescue rubra*. Chem. Geol. 253, 130–135.

Grieve, C., Grattan, S., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70, 303–307.

Gupta, D., Huang, H., Corpas, F., 2013. Lead tolerance in plants: strategies for phytoremediation. Environ. Sci. Pollut. Res. 20, 2150–2161.

Hadi, F., Aziz, T., 2015. A mini review on lead (Pb) toxicity in plants. J. Biol. Life Sci. 6, 91–101.

Hajihashemi, S., Sofo, A., 2018. The effect of polyethylene glycol-induced drought stress on photosynthesis, carbohydrates and cell membrane in *Stevia rebaudiana* grown in greenhouse. Acta Physiol. Plant. 40, 142.

Hakeem, K.R., Alharby, H.F., Rehman, R., 2019. Antioxidative defense mechanism against lead-induced phytotoxicity in *Fagopyrum kashmirianum*. Chemosphere 216, 595–604.

Heidari Dehno, A., Mohtadi, A., 2018. The effect of different iron concentrations on lead accumulation in hydroponically grown *Matthiola flavida* Boiss. Ecol. Res. 33, 757–765.

Hou, X., Han, H., Cai, L., Liu, A., Ma, X., Zhou, C., Meng, F., 2018. Pb stress effects on leaf chlorophyll fluorescence, antioxidative enzyme activities, and organic acid contents of *Pogonatherum crinitum* seedlings. Flora 240, 82–88.

Houda, Z., Bejaoui, Z., Albouchi, A., Gupta, D.K., Corpas, F.J., 2016. Comparative study of plant growth of two poplar tree species irrigated with treated wastewater, with particular reference to accumulation of heavy metals (Cd, Pb, As, and Ni). Environ. Monit. Assess. 188, 99.

Huang, H., Gupta, D., Tian, S., Yang, X.-e., Li, T., 2012. Lead tolerance and physiological adaptation mechanism in roots of accumulating and non-accumulating ecotypes of *Sedum alfredii*. Environ. Sci. Pollut. Res. 19, 1640–1651.

Jaén-Molina, R., Caujapé-Castells, J., Reyes-Betancort, J.A., Akhani, H., Fernández-Palacios, O., de Paz, J.P., Febles-Hernández, R., Marrero-Rodríguez, Á., 2009. The molecular phylogeny of *Matthiola* R. Br. (Brassicaceae) inferred from ITS sequences, with special emphasis on the Macaronesian endemics. Mol. Phylogenet. Evol. 53, 972–981.

Kazakou, E., Dimitrakopoulos, P.G., Baker, A.J., Reeves, R.D., Troumbis, A.Y., 2008. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. Biol. Rev. Camb. Philos. Soc. 83, 495–508.

Kısa, D., Elmastaş, M., Öztürk, L., Kayır, Ö., 2016. Responses of the phenolic compounds of *Zea mays* under heavy metal stress. Appl. Biol. Chem. 59, 813–820.

Konečná, V., Yant, L., Kolář, F., 2020. The evolutionary genomics of serpentine adaptation. Front. Plant Sci. 11, 2004.

Krzesłowska, M., Lenartowska, M., Samardakiewicz, S., Bilski, H., Woźny, A., 2010. Lead deposited in the cell wall of *Funaria hygrometrica* protonemata is not stable—a remobilization can occur. Environ. Pollut. 158, 325–338.

Kumar, A., Prasad, M., Sytar, O., 2012. Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically. Chemosphere 89, 1056–1065.

Kumar, A., Prasad, M.N.V., 2018. Plant-lead interactions: transport, toxicity, tolerance, and detoxification mechanisms. Ecotoxicol. Environ. Saf. 166, 401–418.

Li, Y., Zhou, C., Huang, M., Luo, J., Hou, X., Wu, P., Ma, X., 2016. Lead tolerance mechanism in *Conyza canadensis*: subcellular distribution, ultrastructure, antioxidative defense system, and phytochelatins. J. Plant Res. 129, 251–262.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.

Mahdavian, K., Ghaderian, S.M., Schat, H., 2016. Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous *Peganum harmala* L. under Pb exposure. Environ. Exp. Bot. 126, 21–31.

Mansfield, M.R., Pope, N.S., Mittelhauser, G.H., Rajakaruna, N., 2014. Diversity and soil-tissue elemental relations of vascular plants of Callahan Mine, Brooksville, Maine, USA. Rhodora 116, 283–322.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol. J. Environ. Stud. 15, 523–530.

Miri, H.R., Armin, M., 2013. The interaction effect of drought and exogenous application of glycine betaine on corn (*Zea mays* L.). Eur. J. Exp. Biol. 3, 197–206.

Mohtadi, A., 2014. Heavy Metal Tolerance and Accumulation in *Matthiola flavida*, a Novel Iranian lead Hyperaccumulator. Vrije Universiteit, Amsterdam. Dissertation.

Mohtadi, A., Ghaderian, S.M., Schat, H., 2012a. A comparison of lead accumulation and tolerance among heavy metal hyperaccumulating and non-hyperaccumulating metallophytes. Plant Soil 352, 267–276.

Mohtadi, A., Ghaderian, S.M., Schat, H., 2012b. Lead, zinc and cadmium accumulation from two metalliferous soils with contrasting calcium contents in heavy metal-hyperaccumulating and non-hyperaccumulating metallophytes: a comparative study. Plant Soil 361, 109–118.

Mohtadi, A., Ghaderian, S.M., Schat, H., 2013. The effect of EDDS and citrate on the uptake of lead in hydroponically grown *Matthiola flavida*. Chemosphere 93, 986–989.

Mondal, N.K., Das, C., Datta, J.K., 2015. Effect of mercury on seedling growth, nodulation and ultrastructural deformation of *Vigna radiata* (L) Wilczek. Environ. Monit. Assess. 187, 241.

Money, N.P., 1989. Osmotic pressure of aqueous polyethylene glycols: relationship between molecular weight and vapor pressure deficit. Plant Physiol. 91, 766–769.

Nabi, R.B.S., Tayade, R., Hussain, A., Kulkarni, K.P., Imran, Q.M., Mun, B.G., Yun, B.W., 2019. Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environ. Exp. Bot. 161, 120–133.

Okunlola, G.O., Olatunji, O.A., Akinwale, R.O., Tariq, A., Adelusi, A.A., 2017. Physiological response of the three most cultivated pepper species (*Capsicum* spp.) in Africa to drought stress imposed at three stages of growth and development. Sci. Hortic. 224, 198–205 Amsterdam.

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.-S.P., 2014. Response of plants to water stress. Front. Plant Sci. 5, 86.

Piotrowska, A., Bajguz, A., Godlewska-Żyłkiewicz, B., Czerpak, R., Kamińska, M., 2009. Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). Environ. Exp. Bot. 66, 507–513.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E., 2011. Lead uptake, toxicity, and detoxification in plants. Rev. Environ. Contam. Toxicol. 213, 113–136.

Pourrut, B., Shahid, M., Douay, F., Dumat, C., Pinelli, E., Gupta, D., Corpas, F., Palma, J., 2013. Molecular mechanisms involved in lead uptake, toxicity and detoxification in higher plants. (Eds.) Heavy Metal Stress in Plants. Springer, Berlin, Heidelberg, pp. 121–147.

Rascio, N., Navari-Izzo, F., 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci. 180, 169–181.

Salehi-Eskandari, B., Ghaderian, S.M., Schat, H., 2017. The role of nickel (Ni) and drought in serpentine adaptation: contrasting effects of Ni on osmoprotectants and oxidative stress markers in the serpentine endemic, *Cleome heratensis*, and the related non-serpentinophyte, *Cleome foliolosa*. Plant Soil 417, 183–195.

Salehi-Eskandari, B., Ghaderian, S.M., Schat, H., 2018. Differential interactive effects of the Ca/Mg quotient and PEG-simulated drought in *Alyssum inflatum* and *Fortuynia garcinii*. Plant Soil 428, 213–222.

Sarwar, G.R., Qaiser, M., 2012. Distribution pattern, ecology and endemism of family Crassulaceae in Pakistan and Kashmir. Pak. J. Bot. 44, 2055–2061.

Sekmen, A.H., Ozgur, R., Uzilday, B., Turkan, I., 2014. Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum*) cultivars under combined drought and heat induced oxidative stress. Environ. Exp. Bot. 99, 141–149.

Selby, J.P., Jeong, A.L., Toll, K., Wright, K.M., Lowry, D.B., Rajakaruna, N., Boyd, R.S., Harris, T.B., 2014. Methods and discoveries in the pursuit of understanding the genetic basis of adaptation to harsh environments in *Mimulus*. (Eds.) Plant Ecology and Evolution in Harsh Environments. Nova Science Publishers Inc, New York, NY.

Selby, J.P., Willis, J.H., 2018. Major QTL controls adaptation to serpentine soils in *Mimulus guttatus*. Mol. Ecol. 27, 5073–5087.

Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Sidhu, G.P.S., Bali, A.S., Handa, N., Kapoor, D., Yadav, P., Khanna, K., 2020. Photosynthetic response of plants under different abiotic stresses: a review. J. Plant Growth Regul. 39, 509–531.

Singh, R., Tripathi, R.D., Dwivedi, S., Kumar, A., Trivedi, P.K., Chakrabarty, D., 2010. Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. Bioresour. Technol. 101, 3025–3032.

Singh, S., Srivastava, P.K., Kumar, D., Tripathi, D.K., Chauhan, D.K., Prasad, S.M., 2015. Morphoanatomical and biochemical adapting strategies of maize (*Zea mays* L) seedlings against lead and chromium stresses. Biocatal. Agric. Biotechnol. 4, 286−29.

Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci. 6, 1143.

Somogyi, M., 1952. Notes on sugar determination. J. Biol. Chem. 195, 19–23.

Srivastava, D., Verma, G., Chauhan, A.S., Pande, V., Chakrabarty, D., 2019. Rice (*Oryza sativa* L.) tau class glutathione S-transferase (OsGSTU30) overexpression in *Arabidopsis thaliana* modulates a regulatory network leading to heavy metal and drought stress tolerance. Metallomics 11, 375–389.

Stiborova, M., Doubravova, M., Brezinova, A., Friedrich, A., 1986. Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley (*Hordeum vulgare* L.). Photosynthetica 20, 418–425 (Praha).

Velioglu, Y., Mazza, G., Gao, L., Oomah, B., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113–4117.

Vodnik, D., Jentschke, G., Fritz, E., Gogala, N., Godbold, D., 1999. Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings. Physiol. Plant. 106, 75–81 (København. 1948).

Van der Ent, A., Baker, A.J., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362 (1), 319–334.

Von Wettberg, E., Ray-Mukherjee, J., D'Adesky, N., Nesbeth, D., Sistla, S., Rajakaruna, N., Boyd, R.S., Harris, T., 2014. The evolutionary ecology and genetics of stress resistance syndrome (SRS) traits: revisiting Chapin, Autumn and Pugnaire (1993). (Eds.) Plant Ecology and Evolution in Harsh Environments. Nova Science Publishers, New York, pp. 201–226.

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H., Mei, L., 2018. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. Biol. Open 7.

Wanger, G., 1979. Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplasts. Plant Physiol. 64, 88–93.

Wani, A.L., Ara, A., Usmani, J.A., 2015. Lead toxicity: a review. Interdiscip. Toxicol. 8, 55–64.

You, J., Chan, Z., 2015. ROS regulation during abiotic stress responses in crop plants. Front. Plant Sci. 6, 1092.

Zhang, C., Shi, S., Wang, B., Zhao, J., 2018. Physiological and biochemical changes in different drought-tolerant alfalfa (*Medicago sativa* L.) varieties under PEG-induced drought stress. Acta Physiol. Plant. 40, 25.

Zheng, L., Peer, T., Seybold, V., Lütz-Meindl, U., 2012. Pb-induced ultrastructural alterations and subcellular localization of Pb in two species of *Lespedeza* by TEM-coupled electron energy loss spectroscopy. Environ. Exp. Bot. 77, 196–206.

Zhou, C., Huang, M., Li, Y., Luo, J., Cai, L.P., 2016. Changes in subcellular distribution and antioxidant compounds involved in Pb accumulation and detoxification in *Neyraudia reynaudiana*. Environ. Sci. Pollut. Res. 23 (21), 21794–21804.

Zouari, M., Elloumi, N., Labrousse, P., Rouina, B.B., Abdallah, F.B., Ahmed, C.B., 2018. Olive trees response to lead stress: exogenous proline provided better tolerance than glycine betaine. S. Afr. J. Bot. 118, 158–165.

Zulfiqar, U., Farooq, M., Hussain, S., Maqsood, M., Hussain, M., Ishfaq, M., Ahmad, M., Anjum, M.Z., 2019. Lead toxicity in plants: impacts and remediation. J. Environ. Manag. 250, 109557.