


Physiological and biochemical responses of *Melissa officinalis* L. to nickel stress and the protective role of salicylic acid

Elahe Soltani Maivan, Tayebeh Radjabian, Parvaneh Abrishamchi & Daryush Talei


To cite this article: Elahe Soltani Maivan, Tayebeh Radjabian, Parvaneh Abrishamchi & Daryush Talei (2017) Physiological and biochemical responses of *Melissa officinalis* L. to nickel stress and the protective role of salicylic acid, *Archives of Agronomy and Soil Science*, 63:3, 330-343, DOI: [10.1080/03650340.2016.1207241](https://doi.org/10.1080/03650340.2016.1207241)

To link to this article: <http://dx.doi.org/10.1080/03650340.2016.1207241>

 View supplementary material 

 Accepted author version posted online: 29 Jun 2016.
Published online: 15 Jul 2016.

 Submit your article to this journal 

 Article views: 39

 View related articles 

 View Crossmark data 

Physiological and biochemical responses of *Melissa officinalis* L. to nickel stress and the protective role of salicylic acid

Elahe Soltani Maivan^a, Tayebeh Radjabian^a, Parvaneh Abrishamchi^b and Daryush Talei^c

^aDepartment of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran; ^bDepartment of Biology, Faculty of Basic Sciences, Ferdowsi University, Mashhad, Iran; ^cMedicinal Plants Research Center, Shahed University, Tehran, Iran

ABSTRACT

The present study investigated the mediatory effects of salicylic acid (SA) in alleviating nickel (Ni) toxicity in *Melissa officinalis* L. One-month-old plants were exposed to different levels of Ni and SA concentrations in sand culture under greenhouse conditions. Excess Ni significantly inhibited the growth indices and dramatically increased accumulation of Ni in the leaves and roots. Exogenously SA applications (1.0 mM) led to a substantial improvement in the shoot and root fresh and dry weights. Foliar application of SA mitigated the deleterious effects of Ni and decreased its transport to the shoots. The results showed a significant loss in chlorophylls and carotenoids contents only at 500 µM of Ni. The impact of SA was not significant in terms of chlorophyll contents, while carotenoid contents of the Ni-stressed plants were significantly affected by SA. Exposure to Ni did not modify proline accumulation. Hydrogen peroxide accumulation was observed under Ni stress, while lipid peroxidation significantly decreased at the same conditions. Application of SA caused a significant decrease in electrolyte leakage of Ni-stressed plants. Due to the high potential for Ni accumulation in the roots and translocation factor values lower than 1, *M. officinalis* could be introduced as an excluder medicinal plant.

ARTICLE HISTORY

Received 13 July 2015
Accepted 26 June 2016

KEYWORDS


Excluder plant; lipid peroxidation; malondialdehyde; *Melissa officinalis* L.; nickel stress; salicylic acid; translocation factor

Introduction

Lemon balm (*Melissa officinalis* L.) is an important medicinal plant in the family of Lamiaceae. This medicinal herb is native to the Eastern Mediterranean regions, central and southern Europe and Asia Minor. In Iran, this plant grows wildly or is cultivated in the north, northwest and western parts of the country (Anon 2002). The leaves of plant contain three major groups of phytochemical compounds, namely protocatechuic acid, caffeic acid and rosmarinic acid. The herb exhibited a wide scope of pharmaceutical properties such as sedative, carminative, antispasmodic, antibacterial, antiviral, antifungal, anti-inflammatory and antioxidative. Furthermore, *M. officinalis* is used to treat Graves', Alzheimer's and thyroid diseases (Kim et al. 2010; Weitzel & Petersen 2011).

Salicylic acid (SA) is considered as a hormone-like substance, influences various physiological and biochemical functions in plants. It can act as a potent signalling molecule and has diverse effects on tolerance to biotic and abiotic stresses (Saeidnejad et al. 2012). SA is involved in the plant's responses to many types of abiotic stresses such as salinity, drought, heat, cold and

CONTACT Tayebeh Radjabian ✉ rajabian@shahed.ac.ir Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran; Daryush Talei ✉ d.talei@shahed.ac.ir Medicinal Plants Research Center, Shahed University, Tehran, Iran

 Supplemental data for this article can be accessed [here](#).

herbicide stress (Radwan et al. 2010). Furthermore, SA can ameliorate the injurious effects of heavy metals in plants. SA pretreatment has alleviated the adverse effects of Cd in pea seedlings (Popova et al. 2009) and Mn toxicity in cucumber (Shi & Zhu 2008) and deleterious effects of nickel (Ni) on growth of *Catharanthus roseus* L. plants (Idrees et al. 2013). Protective effects of SA including upregulation of antistress processes and recovery of growth processes have been reported (Ahmad et al. 2011). Exogenous SA could regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresses (Yusuf et al. 2012). SA effects on stress adaptation and damage development of plants depend on plant species and concentration, method and time of SA application (Kazemi et al. 2010).

Ni, one of the important heavy metal pollutants, is of considerable concern because its concentration is rapidly increasing in soils of different parts of the world (Kaur et al. 2012). Ni is an essential metal ion which is required in traces amounts for growth and development of higher plants; however, it is strongly phytotoxic at high concentrations for the majority of plant species and alters their various physiological processes (Kazemi et al. 2010). The most common symptoms of Ni toxicity are damages on the photosynthetic apparatus and thereby decreasing chlorophyll content (Yusuf et al. 2012). Ni also causes inhibition of seed germination, increase in ion leakage, disorder in mineral nutrition, sugar transport, water relations and reduction in root development in plants (Seregin & Kozhevnikova 2006; Maheshwari & Dubey 2009; Dubey & Pandey 2011). At cellular level, high concentrations of Ni stimulate the production of reactive oxygen species (ROS) such as $O_2^{\cdot-}$ and H_2O_2 and oxidative stress would arise if the balance between ROS generation and elimination were broken. Therefore, plant cells contain protective and repair systems that, under normal circumstances, minimize the occurrence of oxidative damage (Yan et al. 2008; Kazemi et al. 2010). Therefore, plant cells dispose of complex defence mechanisms against toxic concentrations of heavy metals, including enzymatic and non-enzymatic antioxidants, ion complexation and transport processes and proline accumulation (Smeets et al. 2008; Lu et al. 2010b; Yusuf et al. 2011). In the last years, some studies have revealed the existence of different plant metal transporter families such as heavy metal-associated transporters, natural resistance-associated macrophage proteins, cation diffusion facilitators and ZIP family of metal transporters, as well as metallothioneins and phytochelatins that regulate metal homeostasis, root to shoot metal translocation and also probably they are involved in mediating metal-resistant and metal-hyperaccumulating characters (Ovečka & Takáč 2014; Park & Ahn 2014). Because of the drastically increasing heavy metal contaminations of agricultural soils, it is obvious that safety of the plants as medicinal herbs or food is important for human health. Due to the presence of essential oils, phenolic acids and flavonoids and consequently high antioxidant capacity, many studies have proved that the aerial parts of *M. officinalis* are used as tea to reduce oxidative stress in human (Rashidi et al. 2014). As Ni is toxic for humans (>1 mg/day), so contamination of plant preparation with high concentration of Ni can cause health problems (Stanojkovic-Sebic et al. 2015). However, a number of recent studies have shown that foliar application of Ni could provide beneficial effects on the chemical composition and yield of essential oil and herbage of some aromatic and medicinal plants that are used for pharmaceutical and edible purposes (Teixeira da Silva et al. 2012).

Since some plant species are able to uptake and accumulate large amounts of heavy metals without showing any severe toxic symptoms, therefore further studies are needed to evaluate the tolerance level of medicinal plants against heavy metals, as well as to determine the amounts of heavy metals in different plant tissues (Hajar et al. 2014).

M. officinalis is cultivated as a medicinal plant in many countries, and aerial parts of the plant are used as a source for medicinal preparations. As for *M. officinalis*, little information concerning the physiological and biochemical mechanisms and defence responses of this plant to heavy metals stress is available. The present study was designed to evaluate the changes in some morphological, physiological and biochemical traits of Ni-exposed *M. officinalis* plants by

exogenous application of SA; also, Ni accumulation/translocation was assessed in order to determine its tolerance to the applied ion metal doses. The hypothesis tested was that SA may protect the plants from the stress, generated by Ni.

Materials and methods

Plant materials and growth conditions

The seeds of *M. officinalis* were collected from the Pakan Bazr Company, Isfahan, Iran. The uniform and healthy-mature seeds were surface sterilized with 70% ethanol for 1 min and then 0.125% solution of sodium hypochlorite for 10 min and thoroughly rinsed with distilled water. The sterilized seeds were transferred into each plastic pot containing well-washed sand under glass-house conditions. Mean day temperature was $28^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and night temperature was $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$, with photon flux density $400\text{--}600 \mu\text{M m}^{-2} \text{s}^{-1}$ (PAR), 16/8 light/dark photoperiod and average relative humidity $35.9\% \pm 6.5\%$ during the entire growth period. When the seedlings emerged from the sand, they were thinned to seven per pot. Hoagland's nutrient solution half-strength (Hoagland & Arnon 1950) was supplied to all pots for 30 days.

Experimental design

We carried out a factorial experiment based on randomized complete block design with two factors and three replicates. The factors were different concentrations of Ni solutions with seven levels (0, 25, 50, 75, 100, 250 and 500 μM) and SA with two levels (0 and 1.0 mM). The plants at four-leaf stage were placed in different Ni ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) levels on Hoagland solution. The Ni treatment solutions were applied every alternate day after leaching well the metal and nutrient solution already present in the sand. To maintain the moisture content of the sand, 100 mL of distilled water was applied to each pot every day. The SA (1.0 mM) mixed with tween-20 (a surfactant) was sprayed in the evening of the same day with a manual sprayer (10 mL/plant). Treatments were continued during 1 month after sowing to assess various morphological, physiological and biochemical parameters. After 30 days Ni treatment, all plants were harvested and the shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid contents, hydrogen peroxide (H_2O_2), lipid peroxidation and electrolyte leakage were measured.

Determination of pigment contents

The chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents were measured according to the Lichtenthaler (1987) method. Fresh leaves (0.03 g) were homogenized with 10 mL 80% chilled acetone. Extract was centrifuged at $2000 \times g$ for 10 min. Absorbance of supernatant was estimated spectrophotometrically at 663, 645 and 480 nm using spectrophotometer (Shimadzu UV-Vis 1201). The contents of pigments were expressed in terms of mg g^{-1} FW of tissue.

Determination of proline content

Proline content in the samples was determined according to the method described by Bates et al. (1973). The leaf and root tissues (0.25 g) were extracted with 10 mL 3% sulphosalicylic acid. For each sample, 2 mL extracts were held for 1 h in boiling water by adding 2 mL ninhydrin and 2 mL glacial acetic acid, after which 4 mL cold toluene was added. The absorbance of supernatant was read at 520 nm and content calculated against standard proline.

Determination of H₂O₂ content

The H₂O₂ levels were determined according to Singh et al. (2007). Fresh leaf and root tissues (0.5 g) were homogenized with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) using precooled pestle and mortar. The extract was centrifuged at 12,000 × *g* for 15 min at 4°C. Subsequently, 0.5 mL of the supernatant was added to 0.5 mL of potassium phosphate buffer (10 mM, pH 7.0) containing 1 mL of 1M potassium iodide (KI) solution. The absorbance of each supernatant was measured at 390 nm, and the content of H₂O₂ was calculated using molar extinction coefficient 0.28 μM⁻¹ cm⁻¹.

Determination of lipid peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation according to Heath and Packer (1968). Fresh leaf and root samples (0.9 g) were homogenized in 5 mL of TCA (0.1% w/v). The extracts were centrifuged at 13,000 × *g* for 10 min at 4°C and then 1 mL of the supernatant fractions was mixed with 4 mL of 0.5% thiobarbituric acid in 20% TCA. The mixture was heated at 95°C for 30 min and cooled in an ice bath for 10 min. Samples were centrifuged at 10,000 × *g* for 10 min at 15°C. The absorbance of the supernatant was measured at 532 nm and the non-specific absorbance at 600 nm was subtracted. Results were expressed as MDA equivalents in μM g⁻¹ FW and calculated using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Determination of electrolyte leakage

Electrolyte leakage was used to assess membrane permeability as described by Dionisio-Sese and Tobita (1998) method. Twenty leaf discs (2–3 mm²) were prepared from young leaves using a puncher device, placed in a closed vial containing 10 mL double-distilled H₂O and used to measure the electrical conductivity of the solution (EC₀). Subsequently, samples were heated at 50°C and 60°C for 25 min in a water bath and then electrical conductivity of the solutions was measured (EC₁) using an electrical conductivity meter (WTW inoLab 720 conductivity meter, Germany). Finally, the vials were heated at 100°C for 10 min and allowed to cool at room temperature, and the electrical conductivity (EC₂) was then determined. The electrolyte leakage was calculated as follows: electrolyte leakage (%) = (EC₁ – EC₀)/(EC₂ – EC₀) × 100.

Determination of Ni accumulation and of translocation factor

Dried shoots and roots tissues (0.5 g) were placed into a crucible in an electrical furnace at 500°C for 4 h to get a white ash. The white ash sample was dissolved in 2.5 mL 20% HCl and then distilled water was added to make volume of the solution to 10 mL. The total concentration of Ni in samples was determined by inductively coupled plasma emission spectroscopy (Varian Vista-PRO simultaneous CCD ICP-OES, Australia). Translocation factor (TF) or mobilization ratio of Ni from roots to shoots was calculated according to the equation (Ni shoot content/Ni root content) proposed by Stoltz and Greger (2002).

Statistical analysis

At first, the raw data were tested for normality distribution by using the SPSS software version 22 (IBM SPSS Advanced Statistics 22, IBM Corp., Armonk, NY, USA) and then the main data analysed for measured traits using analysis of variance and Duncan's multiple-range test ($P \leq 0.01$). The GraphPad Prism software version 6 (San Diego, CA, USA) was used for drawing the graphs. The values are means of three replications.

Results

Effects of Ni and SA levels on growth traits

The results showed that Ni levels and SA significantly affected morphological traits ($P \leq 0.01$) (Table S1). The interaction of Ni \times SA was not significant in terms of the growth traits except for RFW and RDW (Table S1). All the studied growth traits varied with Ni levels and SA. Really, a negative, significant trend, independently by the presence of SA, is observable for SDW and SFW, but not for roots. Also, data on shoot and root fresh and dry weights of the treated plants indicated that growth rates were negatively correlated to the substrate concentration of Ni ($P \leq 0.01$). The highest decreases in the FW and DW of roots (34.4% and 41.5%) and shoots (27.5% and 36.5%) were recorded in the plants treated with 250 and 500 μM Ni, respectively, as compared to control (Figure 1(a,b)). Plants grown at the low levels of Ni reached relatively higher total DWs and did not imply toxicity symptoms. However, their total DWs were significantly reduced at higher levels of Ni (500 μM) and they indicated the symptoms of Ni toxicity as growth depression (Figure 1(c)). For SL and RL, the results were similar to those obtained for DW and FW. No significant changes were detected for SL and RL of the treated plants with lower concentrations (25 and 50 μM) of Ni, when compared to plants grown under control conditions and the highest decrease for these growth indices was observed only at 500 μM of Ni (Figure 1(d)). The SDW/RDW ratio as the other important characteristic trait determining growth was measured in the stressed plants. It was revealed that excluding the 500 μM , in other treatments an increase in shoot/root ratio was observed in parallel with the enhancement of Ni concentration (Figure 1(e)). Exposure of plants to excess Ni (500 μM) was accompanied by decreases in the shoot/root ratios; however, the observed differences were not statistically significant, compared with the control group. Conversely, exogenously SA applications (1.0 mM) led to a substantial improvement in the SFW and RFWs, as well as the SDW and RDW (Figure 2(a,b)).

Effect of Ni and SA levels on chlorophyll and carotenoid contents

The analysis of variance revealed that only chlorophyll *a* and carotenoid contents of the treated plants were significantly ($P \leq 0.01$) affected by Ni concentration (Table S2). The results indicated that there were no significant differences among SA levels on photosynthetic pigment contents, including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents. The interaction of Ni \times SA was not significant in terms of chlorophyll contents, while carotenoid contents of the Ni-stressed plants were significantly affected by SA (Table S2). Although, only a significant decrease was established in the chlorophyll *a* and carotenoid contents of the plants exposed to 500 μM Ni in comparison with the control plants (Figure 3(a,b)). The highest chlorophyll *a* and carotenoid contents were observed at 100 and 250 μM Ni, respectively, while treatment of plants with Ni at 500 μM resulted in a slight decrease in the chlorophyll *a* (15.2%) and carotenoid (15.7%) contents, in comparison with the control. The exogenous application of SA (1.0 mM) at the highest concentration of Ni (500 μM) significantly decreased carotenoid content (Figure 3(b)). Additionally, treatment of plants with different concentrations of Ni alone and/or in combination with SA did not significantly affect the chlorophyll *a/b* ratio of the leaves.

Effect of Ni and SA levels on proline contents

The results showed that Ni levels significantly affected proline content ($P \leq 0.01$), while there were no considerable changes in proline content of the treated plants after application of SA (Table S2). Variations in the proline content were highly significant due to changes in the Ni levels ($P \leq 0.01$). The

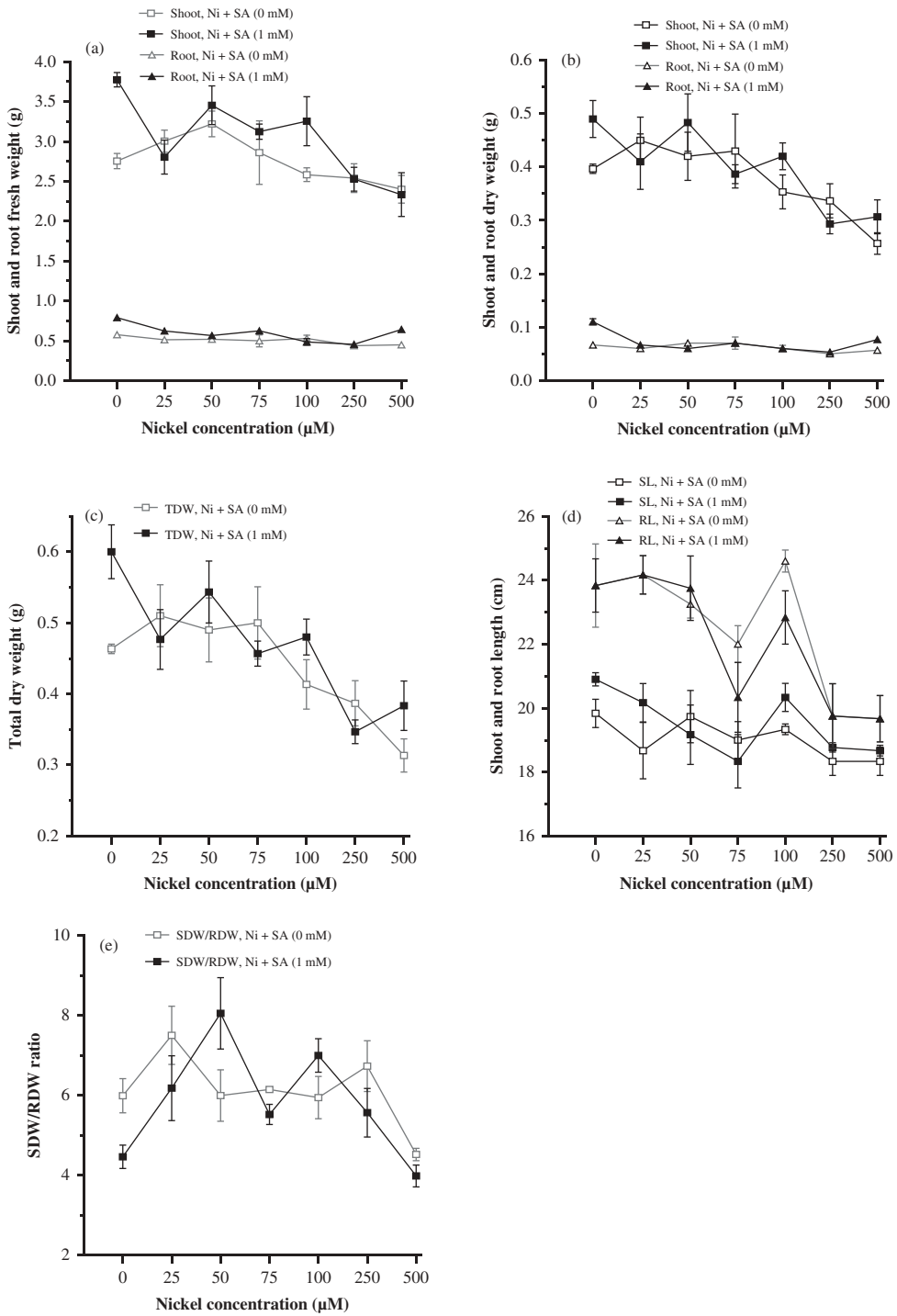


Figure 1. The interaction effect of SA and Ni on shoot and root fresh weights (a), shoot and root dry weights (b), total dry weight (c), shoot length (SL) and root length (RL) (d) and SDW-RDW ratio (e) in *M. officinalis*. Mean values \pm SEM are from three independent replicates.

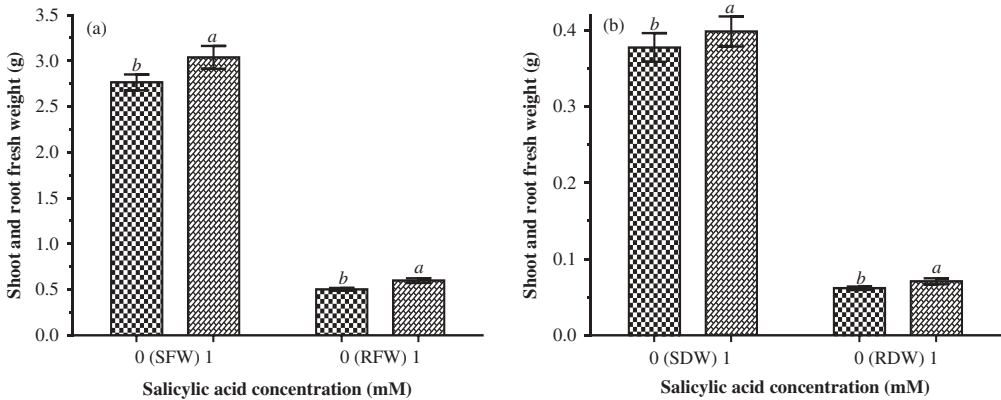


Figure 2. Effect of exogenous application of SA on shoot and root fresh weights (a), and shoot and root dry weights (b) in *M. officinalis* under Ni stress. Mean values \pm SEM are from three independent replicates, and values superscripted by different letters are significantly different ($P \leq 0.01$).

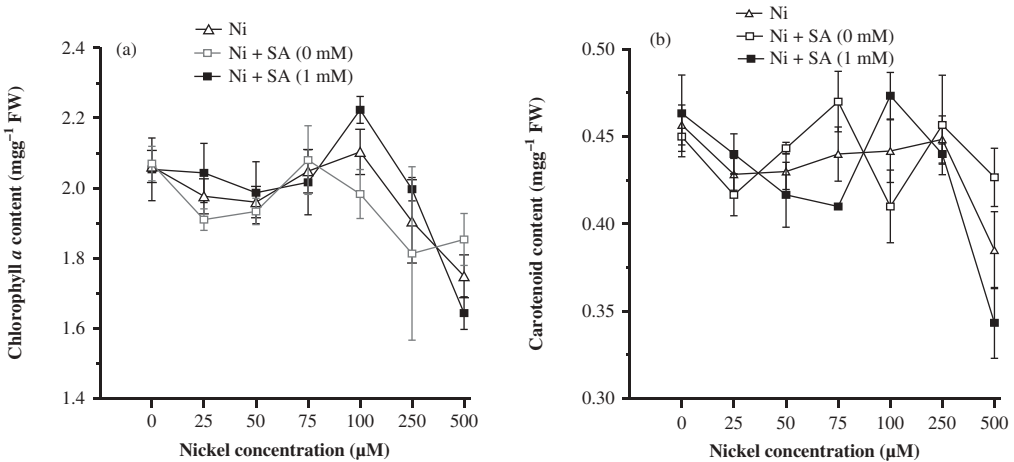


Figure 3. The interaction effect of SA and Ni on chlorophyll *a* (a) and carotenoid content (b) in *M. officinalis*. Mean values \pm SEM are from three independent replicates.

interaction of Ni \times SA was not significant in terms of proline contents (Table S2). In both leaves and roots of *M. officinalis* plants after exposure to Ni, decrease of free proline was observed, but in the case of leaves decrease in the content of this amino acid was greater than in roots. The highest reduction in proline content of roots (50.72%) and leaves (56.44%) of treated plants was obtained at 250 μM Ni as compared to control (Figure 4). The exogenous application of SA decreased accumulation of proline in the leaves, while it caused an increase in the content of proline in the roots of treated plants.

Effect of Ni and SA levels on H₂O₂, lipid peroxidation contents and electrolyte leakage

The results revealed that Ni stress enhanced the production of H₂O₂ in *M. officinalis* plants and H₂O₂ level was markedly higher in the roots as compared to the leaves (Figure 5(a)). The highest amounts of H₂O₂ were accumulated in the leaves (1.15 $\mu\text{M g}^{-1}$ FW) and roots (1.67 $\mu\text{M g}^{-1}$ FW) of plants treated with 100 and 500 μM Ni, respectively. Lipid peroxidation was appraised in terms of MDA

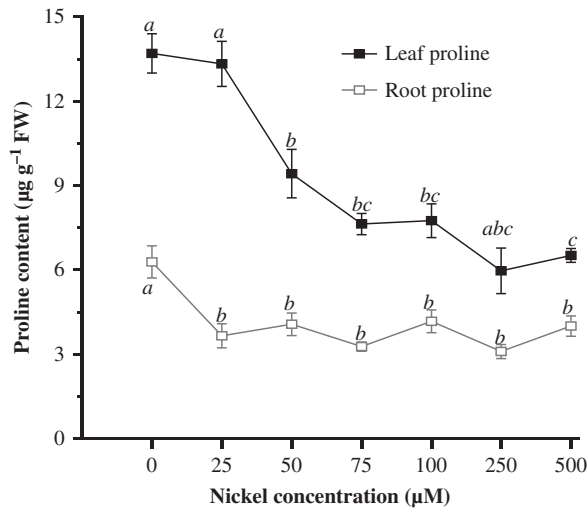


Figure 4. Effect of different concentrations of Ni levels on proline contents in *M. officinalis*. Mean values \pm SEM are from three independent replicates, and values superscripted by different letters are significantly different by Duncan's multiple-range test ($P \leq 0.01$).

content. The results showed that Ni and SA levels significantly affected the MDA content and the electrolyte leakage of the leaf samples at $P \leq 0.01$ (Table S2). Relative to the roots, leaves of Ni-treated plants exhibited a higher rate of lipid peroxidation (Figure 5(b)). The lowest accumulation of H_2O_2 , MDA and electrolyte leakage was observed in the leaves of plants exposed to 75 μM Ni as compared to control (Figure 5(a–c)).

Exogenous application of SA to the Ni-stressed plants reduced H_2O_2 production in the leaves, while it had no significant effects on H_2O_2 content of the roots (Figure 5(a)). The application of SA under Ni stress induced the accumulation of H_2O_2 in the leaves, so the greatest increase was observed at 100 μM concentration of Ni. Under Ni stress, the exogenous application of SA decreased significantly the MDA contents of leaves and roots; this effect was more pronounced at high Ni concentrations ($>75 \mu M$) (Figure 5(b)). Treatment of plants with Ni increased in electrolyte leakage in the leaves. Conversely, application of SA reduced electrolyte leakage in the Ni-stressed plants; however, this effect was not significant when compared to Ni-exposed groups alone (Figure 5(c)).

Effect of Ni and SA levels on Ni accumulation and TF

Nickel accumulation in the leaves and roots of plants and TF factor were significantly ($P \leq 0.01$) influenced by the concentration of Ni in the growth media (Table S2). In Ni-treated plants, the amount of Ni accumulation in the roots relatively was higher than in the leaves. The highest content of Ni in the leaves (411.83 $\mu g g^{-1}$ DWs) and roots (358.53 $\mu g g^{-1}$ DWs) of treated plants were found at 500 μM Ni (Figure 6(a)). The results showed that application of SA significantly affected the Ni accumulation in the leaves, while application of SA had no significant effect on Ni accumulation in the roots. Exogenous application of SA greatly decreased Ni content in the leaves and increased Ni accumulation in the roots under excess Ni concentrations as compared to Ni treatment alone (Figure 6(a)). The TF/mobilization ratio of Ni from root to shoot has been estimated. The highest (1.09) and lowest (0.35) amount of TF in the treated plants were found at control and 75 μM Ni, respectively. The interaction of Ni \times SA was significant in terms of Ni accumulation in the leaves and roots and Ni TF. Although Ni TF was increased with increasing of Ni

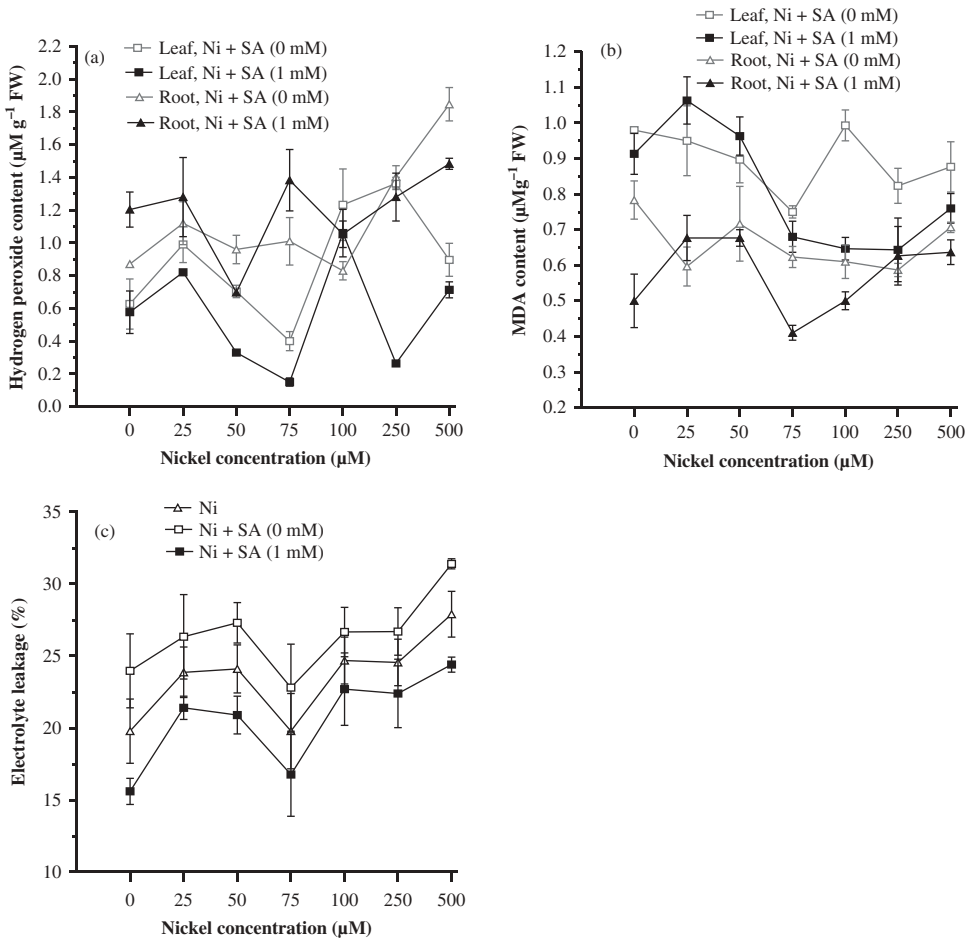


Figure 5. The interaction effect of SA and Ni on H_2O_2 content (a), MDA content (b) and leaf electrolyte leakage per cent (c) in *M. officinalis*. Mean values \pm SEM are from three independent replicates.

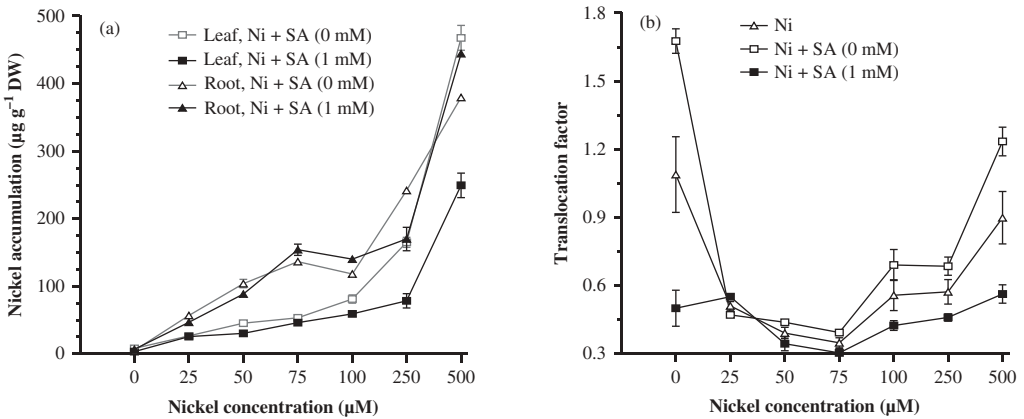


Figure 6. The interaction effect of SA and Ni on Ni accumulation (a) and Ni translocation factor (b).

concentration from 75 to 500 μM in the culture media, but the application of SA caused a decrease in TF of the Ni-stressed plants compared to the control (Figure 6(b)).

Discussion

In higher plants, Ni toxicity like other heavy metals is generally associated with growth inhibition and the decline in biomass production. Plant growth inhibition by Ni and other heavy metals is mainly due to metabolic disorders and instant inhibition of both cell elongation and cell division (Seregin & Kozhevnikova 2006; Liu et al. 2009; Siddiqui et al. 2009; Yusuf et al. 2011). Ni is an essential element for normal growth and development of all plants and serves as a cofactor for many physiological processes, but is toxic at higher levels (Seregin & Kozhevnikova 2006; Yusuf et al. 2011). Also, the beneficial effects of foliar applications of Ni on herbage, chemical composition and essential oil yield of some medicinal and aromatic plants have been showed (Teixeira da Silva et al. 2012). The present study indicated that Ni stress reduced the growth indices of *M. officinalis*. Since roots are the primary target of metal anions, their growth is usually more severely affected than that of the aerial parts of the plant. Therefore, in excluder species which accumulate Ni, root tests are widely used for evaluating the toxicity of various toxicants including heavy metals (Yusuf et al. 2011). Our results matched up well with the findings of Gajewska and Skłodowska (2008), and Duman and Ozturk (2010), who showed that heavy metal toxicity inhibited the growth of *M. officinalis*. The results showed that SA application alone increased the growth, FW and DW of the shoots and roots. In agreement with the reports of Kováčik, Gruz, et al. (2009) in *Matricaria chamomilla* plants, and Wang et al. (2009) in *Zea mays*, the findings of this study indicated that a substantial improvement in root FW and DW was noticed due to exogenously applied SA under 500 μM concentrations of Ni. Also, consistent with the results of Kováčik, Klejdus, et al. (2009) on *M. chamomilla*, the present study revealed that non-significant changes were observed in the chlorophylls and carotenoid contents of *M. officinalis*-treated plants with different concentrations of Ni, except at 500 μM , as compared to the control. Decrease in the chlorophyll content associated with heavy metals could be mostly due to their effects on chlorophyll synthesizing and degrading enzymes, replacement of Mg ion from chlorophylls and interfering with the uptake of some essential elements for photosynthetic apparatus (Sharma & Dubey 2005; Yusuf et al. 2012). Carotenoids as antioxidant molecules play a key role in protecting chlorophyll pigments under stress conditions (Kenneth et al. 2000). So, in this study increase in the carotenoid contents in plants under stress with increasing of the Ni concentration in the culture medium was expected for protecting the chlorophyll molecules and photosynthetic apparatus.

It has been reported in many studies that exogenous application of SA could mitigate toxicity effect of heavy metals on photosynthetic pigment contents and net photosynthetic rate (Kranterev et al. 2008; Popova et al. 2009; Wang et al. 2009; Kazemi et al. 2010; Guo et al. 2013; Saidi et al. 2013). The evidence suggests that the beneficial effects of SA on photosynthetic apparatus might be due to its direct effects as a potent antioxidant to scavenge heavy metal-induced ROS (Popova et al. 2009; Ahmad et al. 2011). However, some studies have shown that SA might indirectly improve resistance of plants to heavy metal stress through upregulating the capacity of antioxidant defence system (enzymatic or non-enzymatic antioxidants) in chloroplasts (Kranterev et al. 2008; Shi & Zhu 2008; Popova et al. 2009; Wang et al. 2009; Yusuf et al. 2012; Guo et al. 2013; Idrees et al. 2013; Saidi et al. 2013). It has also been shown that exposure to SA could reduce the inhibitory effects of heavy metals on the absorption and transport of nutrients which are essential for chlorophylls and carotenoid biosynthesis in the chloroplasts (Saidi et al. 2013). Based on our findings, the interaction effects of different concentrations of SA and Ni were not statistically significant on chlorophylls (Chl *a*, Chl *b*, Chl *a + b*, chlorophyll *a/b* ratio) and carotenoid contents; however, a significant decrease was only observed in the carotenoid content of the treated plants with 500 μM Ni after exposure to 1.0 mM SA. As it has been mentioned in many reports, the

stimulatory or inhibitory effect of SA on the concentrations of photosynthetic pigments might be dependent on the species (Arfan et al. 2007).

MDA is a product of lipid peroxidation when plants suffer an oxidative stress, being used as an indicator of the degree of oxidative stress (Lin & Kao 2000). In accordance with the results recorded for Ni-treated chamomile plants (Kováčik, Klejduš, et al. 2009), in wheat leaves (Gajewska & Skłodowska 2007) and *Halogeton glomeratus* under Ni- and Cu-polluted sites (Lu et al. 2010b), our results showed that MDA content in the root tissues of Ni-treated *M. officinalis* plants remained substantially unchanged after treatment with different levels of Ni, although the content of H₂O₂ was elevated in the roots with increasing of the Ni concentration in the medium. There was a positive correlation between H₂O₂ levels and inhibition of root growth in treated plants. In this context, inhibition of *M. officinalis* root growth under Ni stress was related to H₂O₂ production but not to lipid peroxidation. In comparison to the leaves, MDA contents in the roots of *M. officinalis* plants under Ni stress were may be not due to increased antioxidative enzyme activities, which reduced H₂O₂ levels and membrane damage (Zhang et al. 2007). Our results indicated that *M. officinalis* plants could protect themselves from the oxidative damages through rapid modulation of antioxidative systems. Interaction of SA with Ni decreased MDA accumulation, indicating its involvement in protection of treated plants against oxidative damage. These results are in agreement with those reported by Krantev et al. (2008), Kazemi et al. (2010) and Ahmad et al. (2011). In this study, the content of proline in the leaves and roots decreased markedly in response to Ni application, which is in agreement with other reports on the effect of Cu in *Nicotiana benthamiana* leaf discs (Ku et al. 2012) and in aquatic plant *Spirodela polyrrhiza* (L.) Schleid by excess iron and copper (Xing et al. 2010). The decline of proline content may be due to the inhibition of the expression of pyrroline-5-carboxylate synthetase and ornithine aminotransferase by excess Ni ions. Therefore, the extreme sensitivity of the synthesis and degradation pathways of proline is important for the regulation of its metabolic processes which may be adversely affected by stress (Borah & Devi 2012). Recent proteomic, genomic and metabolomic studies have revealed that proline can be accumulated in plant tissues under stressful conditions and act as an antioxidant, an activator of ROS detoxification pathways, a free radical scavenger, and a cell redox balancer and a stabilizer of membranes. Also due to the chelating ability, it has been suggested that proline might protect plants against heavy metal toxicity (Hossain et al. 2014). It has been also demonstrated that proline accumulation did not occur until the damage had been caused by metal toxicity (Alia & Matysik 2001; Singh & Sinha 2005; Sinha & Saxena 2006). However, many researchers believe that the proline accumulation is only an indicator for heavy metal-induced damages and is not involved in protection against metal-induced oxidative damages (Schat et al. 1997; Akbulut & Çakır 2010). In this study, decrease in proline accumulation in the treated plants with increase in the concentration of Ni in the culture media might be due to the involvement of other antioxidative mechanisms.

TF or shoot/root metal concentration ratio is one of the most important approaches to express metal accumulation and metallophyte classification of medicinal plants. Based on the ratio between shoot:root metal concentration, plants can be classified into two groups of metal excluders and metal non-excluders (indicators, hyperaccumulators). Generally, TF values for accumulators or hyperaccumulators are >1 (Masarovičová et al. 2010). Our results indicated that Ni accumulation in the leaves and roots was dependent on the Ni concentration in the media cultures, and Ni contents in the roots were higher than those of shoots. Moreover, with application of SA, even at high doses of Ni, there was very little transport to the leaves, which are the parts, consumed by humans. Furthermore, TF values in the treated plants at different concentrations of Ni alone or with application of SA were below 1, indicating that *M. officinalis* did not have significant phytoremediation potential. Low TF values showed high phytostabilization potential of Ni in roots. In spite of high content of Ni in *M. officinalis* plants (over 400 µg g⁻¹ DW at 500 µM Ni in culture medium), only a small extent of damage occurred in plants exposed to Ni. Similar results have been obtained by Zheljzakov et al.

(2008) for *M. officinalis* in contaminated soils with Cd, Zn and Pb. It may be therefore concluded that these medicinal plant species actually show remarkable tolerance to some heavy metals including Ni and can be grown as an alternative high-value crop in metal-polluted agricultural soils.

Tolerance to toxic levels of heavy metals in plants can be attributed to the development of various exclusion processes in them that restrict metal movement to the roots or improvement of their protective metabolic mechanisms against those metals. Ni-tolerant plant species accumulate high contents of Ni in their roots by two main mechanisms of sequestration and/or translocation (Singh et al. 2010). Consistent with the results of Kováčik, Klejdus, et al. (2009) on *M. chamomilla* and Lu et al. (2010a) on *Zygophyllum xanthoxylon*, preferential Ni accumulation in the roots and leaf Ni content lower than 1000 $\mu\text{g g}^{-1}$ DW supports classification of *M. officinalis* as an Ni excluder not as a hyperaccumulator plant. Based on our findings, cultivation of this medicinal plant under natural condition for pharmaceutical uses should be extremely ensured.

Conclusion

In conclusion, the results of the present study revealed that Ni at different concentrations was not toxic to *M. officinalis* and exogenous SA exerted significant beneficial effects, while SA-treated plants accumulated considerably higher levels of Ni in the roots as compared to those in the leaves. SA also could alleviate the oxidative damages caused by Ni through modulating antioxidant defence system, which might have the effective strategies for protection against Ni stress. Since no discernible phytotoxic symptoms such as chlorosis or necrosis lesions were observed at high concentrations of Ni, *M. officinalis* appears to be a tolerant medicinal plant species. Lipid peroxidation was also not affected by Ni stress even at high concentrations of this metal. Overall, roots were more responsive to Ni stress mainly based on growth parameters. Taken together, preferential Ni accumulation in the roots, and leaf Ni content substantially lower than 1000 $\mu\text{g g}^{-1}$ DW, supports classify of *M. officinalis* as an Ni excluder and not as a hyperaccumulator plant to Ni stress.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Ahmad P, Nabi G, Ashraf M. 2011. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *S Afr J Bot.* 77:36–44.
- Akbulut M, Çakır S. 2010. The effects of Se phytotoxicity on the antioxidant systems of leaf tissues in barley (*Hordeum vulgare* L.) seedlings. *Plant Physiol Biochem.* 48:160–166.
- Alia M, Matysik J. 2001. Effect of proline on the production of singlet oxygen. *Amino Acids.* 21:195–200.
- Anon N. 2002. Iranian herbal pharmacopoeia. Vol. 1. Tehran: Iranian Ministry of Health and Medical Education Publications; p. 114–121.
- Arfan M, Athar HR, Ashraf M. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J Plant Physiol.* 164:685–694.
- Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil.* 39:205–207.
- Borah M, Devi A. 2012. Effect of heavy metals on *Pisum sativum* Linn. *Int J Adv Biol Res.* 2:314–321.
- Dionisio-Sese ML, Tobita S. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135:1–9.
- Dubey D, Pandey A. 2011. Effect of nickel (Ni) on chlorophyll, lipid peroxidation and antioxidant enzymes activities in black gram (*Vigna mungo*) leaves. *Int J Sci Nat.* 2:395–401.
- Duman F, Ozturk F. 2010. Nickel accumulation and its effect on biomass, protein content and antioxidative enzymes in roots and leaves of watercress (*Nasturtium officinale* R. Br.). *J Environ Sci.* 22:526–532.
- Gajewska E, Skłodowska M. 2007. Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. *Biometals.* 20:27–36.

- Gajewska E, Skłodowska M. 2008. Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. *Plant Growth Regul.* 54:179–188.
- Guo Q, Meng L, Mao P-C, Jia Y-Q, Shi Y-J. 2013. Role of exogenous salicylic acid in alleviating cadmium-induced toxicity in Kentucky bluegrass. *Biochem Syst Ecol.* 50:269–276.
- Hajar EWI, Sulaiman AZB, Sakinah A. 2014. Assessment of heavy metals tolerance in leaves, stems and flowers of *Stevia rebaudiana* plant. *Procedia Environ Sci.* 20:386–393.
- Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys.* 125:189–198.
- Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. *Circular Calif Agric Exp Sta.* 347:1–32.
- Hossain MA, Hoque A, Burritt DJ, Fujita M. 2014. Chapter 16 - proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. In: Ahmad P, editor. *Oxidative damage to plants*. San Diego (CA): Elsevier Academic Press; p. 477–522.
- Idrees M, Naeem M, Aftab T, Khan M. 2013. Salicylic acid restrains nickel toxicity, improves antioxidant defence system and enhances the production of anticancer alkaloids in *Catharanthus roseus* (L.). *J Hazard Mat.* 252–253:367–374.
- Kaur G, Singh HP, Batish DR, Kohli RK. 2012. Growth, photosynthetic activity and oxidative stress in wheat (*Triticum aestivum*) after exposure of lead to soil. *J Environ Biol.* 33:265–269.
- Kazemi N, Khavari-Nejad RA, Fahimi H, Saadatmand S, Nejad-Sattari T. 2010. Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. *Sci Hort.* 126:402–407.
- Kenneth E, Pallet K, Young J. 2000. Carotenoids. In: Ruth, GA, Hess, JL, editors. *Antioxidants in higher plants*. Boca Roton (FL): CRC Press; p. 60–81.
- Kim S, Yun EJ, Bak JS, Lee H, Lee SJ, Kim CT, Lee JH, Kim KH. 2010. Response surface optimised extraction and chromatographic purification of rosmarinic acid from *Melissa officinalis* leaves. *Food Chem.* 121:521–526.
- Kováčik J, Gruz J, Hedbavny J, Klejdus B, Strnad M. 2009. Cadmium and nickel uptake are differentially modulated by salicylic acid in *Matricaria chamomilla* plants. *J Agric Food Chem.* 57:9848–9855.
- Kováčik J, Klejdus B, Kaduková J, Bačkor M. 2009. Physiology of *Matricaria chamomilla* exposed to nickel excess. *Ecotox Environ Saf.* 72:603–609.
- Krantev A, Yordanova R, Janda T, Szalai G, Popova L. 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *J Plant Physiol.* 165:920–931.
- Ku H-M, Tan C-W, Su Y-S, Chiu C-Y, Chen C-T, Jan F-J. 2012. The effect of water deficit and excess copper on proline metabolism in *Nicotiana benthamiana*. *Biol Plantarum.* 56:337–343.
- Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* 148:350–382.
- Lin CC, Kao CH. 2000. Effect of NaCl stress on H₂O₂ metabolism in rice leaves. *Plant Growth Regul.* 30:151–155.
- Liu D, Zou J, Meng Q, Zou J, Jiang W. 2009. Uptake and accumulation and oxidative stress in garlic (*Allium sativum* L.) under lead phytotoxicity. *Ecotoxicology.* 18:134–143.
- Lu Y, Li X, He M, Wang Z, Tan H. 2010a. Nickel effects on growth and antioxidative enzymes activities in desert plant *Zygophyllum xanthoxylon* (Bunge) Maxim. *Sci Cold Arid Reg.* 2:436–444.
- Lu Y, Li X, He M, Zhao X, Liu Y, Cui Y, Pan Y, Tan H. 2010b. Seedlings growth and antioxidative enzymes activities in leaves under heavy metal stress differ between two desert plants: a perennial (*Peganum harmala*) and an annual (*Halogeton glomeratus*) grass. *Acta Physiol Plantarum.* 32:583–590.
- Maheshwari R, Dubey R. 2009. Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings. *Plant Growth Regul.* 59:37–49.
- Masarovičová E, Kráľová K, Kummerová M. 2010. Principles of classification of medicinal plants as hyperaccumulators or excluders. *Acta Physiol Plantarum.* 32:823–829.
- Ovečka M, Takáč T. 2014. Managing heavy metal toxicity stress in plants: Biological and biotechnological tools. *Biotechnol Adv.* 32:73–86.
- Park W, Ahn S-J. 2014. How do heavy metal ATPases contribute to hyperaccumulation? *J Plant Nutr Soil Sci.* 177:121–127.
- Popova LP, Maslenkova LT, Yordanova RY, Ivanova AP, Krantev AP, Szalai G, Janda T. 2009. Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. *Plant Physiol Biochem.* 47:224–231.
- Radwan DEM, Fayez KA, Mahmoud SY, Lu G. 2010. Modifications of antioxidant activity and protein composition of bean leaf due to Bean yellow mosaic virus infection and salicylic acid treatments. *Acta Physiol Plantarum.* 32:891–904.
- Rashidi M, Malekiran AA, Abdollahi M, Habibollahi S, Dolatyari N, Narimani M. 2014. The effect of tea-cinnamon and *Melissa officinalis* L. aqueous extraction, on neuropsychology distress, biochemical and oxidative stress biomarkers in glass production workers. *Health.* 6:2592–2601.
- Saeidnejad AH, Mardani H, Naghibolghora M. 2012. Protective effects of salicylic acid on physiological parameters and antioxidants response in maize seedlings under salinity stress. *J Appl Environ Biol Sci.* 2:364–373.
- Saidi I, Ayouni M, Dhieb A, Chtourou Y, Chaïbi W, Djebali W. 2013. Oxidative damages induced by short-term exposure to cadmium in bean plants: protective role of salicylic acid. *S Afr J Bot.* 85:32–38.

- Schat H, Sharma SS, Vooijs R. 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. *Physiol Plant*. 101:477–482.
- Seregin I, Kozhevnikova A. 2006. Physiological role of nickel and its toxic effects on higher plants. *Russ J of Plant Physiol*. 53:257–277.
- Sharma P, Dubey RS. 2005. Lead toxicity in plants. *Braz J Plant Physiol*. 17:35–52.
- Shi Q, Zhu Z. 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environ Exp Bot*. 63:317–326.
- Siddiqui S, Meghvansi MK, Wani MA, Jabee F. 2009. Evaluating cadmium toxicity in the root meristem of *Pisum sativum* L. *Acta Physiol Plantarum*. 31:531–536.
- Singh HP, Batish DR, Kohli RK, Arora K. 2007. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regul*. 53:65–73.
- Singh R, Singh D, Kumar N, Bhargava S, Barman S. 2010. Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. *J Environ Biol*. 31:421–430.
- Singh S, Sinha S. 2005. Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. *Ecotox Environ Saf*. 62:118–127.
- Sinha S, Saxena R. 2006. Effect of iron on lipid peroxidation, and enzymatic and non-enzymatic antioxidants and bacoside-A content in medicinal plant *Bacopa monnieri* L. *Chemosphere*. 62:1340–1350.
- Smeets K, Ruytinx J, Van Belleghem F, Semane B, Lin D, Vangronsveld J, Cuypers A. 2008. Critical evaluation and statistical validation of a hydroponic culture system for *Arabidopsis thaliana*. *Plant Physiol Biochem*. 46:212–218.
- Stanojkovic-Sebic A, Pivic R, Josic D, Dinic Z, Stanojkovic A. 2015. Heavy metals content in selected medicinal plants commonly used as components for herbal formulations. *Tarim Bilim Derg*. 21:317–325.
- Stoltz E, Greger M. 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. *Environ Exp Bot*. 47:271–280.
- Teixeira da Silva JA, Naeem M, Idrees M. 2012. Beneficial and toxic effects of nickel in relation to medicinal and aromatic plants. *Med Aromat Plant Sci Biotechnol*. 6:94–104.
- Wang H, Feng T, Peng X, Yan M, Tang X. 2009. Up-regulation of chloroplastic antioxidant capacity is involved in alleviation of nickel toxicity of *Zea mays* L. by exogenous salicylic acid. *Ecotox Environ Saf*. 72:1354–1362.
- Weitzel C, Petersen M. 2011. Cloning and characterisation of rosmarinic acid synthase from *Melissa officinalis* L. *Phytochemistry*. 72:572–578.
- Xing W, Huang W, Liu G. 2010. Effect of excess iron and copper on physiology of aquatic plant *Spirodela polyrrhiza* (L.) Schleid. *Environ Toxicol*. 25:103–112.
- Yan R, Gao S, Yang W, Cao M, Wang S, Chen F. 2008. Nickel toxicity induced antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. cotyledons. *Plant Soil Environ*. 54:294–300.
- Yusuf M, Fariduddin Q, Hayat S, Ahmad A. 2011. Nickel: an overview of uptake, essentiality and toxicity in plants. *Bull Environ Contam Toxicol*. 86:1–17.
- Yusuf M, Fariduddin Q, Varshney P, Ahmad A. 2012. Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (*Brassica juncea*) through an improved antioxidant system. *Environ Sci Pollut Res*. 19:8–18.
- Zhang F-Q, Wang Y-S, Lou Z-P, Dong J-D. 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorhiza*). *Chemosphere*. 67:44–50.
- Zheljazkov VD, Jeliakova EA, Kovacheva N, Dzhurmanski A. 2008. Metal uptake by medicinal plant species grown in soils contaminated by a smelter. *Environ Exp Bot*. 64:207–216.