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تاثیر فلزات سنگین روی و کادمیم بر رشد و برخی صفات فیزیولوژیک اسفناج

The impact of Zn and Cd heavy metals on the growth and some physiological characteristics of spinach

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چکیده

غلظت بالای فلزات سنگین باعث ایجاد اختلال در فرایندهای متابولیکی رشد گیاهان شده و حتی باعث مرگ آن‌ها می‌شود. گیاهان آستانه تحمل متفاوتی به فلزات سنگین نشان می‌دهند به همین منظور تاثیر غلظت‌های مختلف فلزات سنگین روی و کادمیوم (صفر، ۲ و ٤ میلی‌گرم در لیتر) در دو رقم اسفناج (بذر معمولی و خاردار) بر روی برخی صفات مورفولوژیکی، فیزیولوژیکی و محتوای عناصر، در قالب آزمایش فاکتوریل بر مبنای طرح کاملا تصادفی با سه تکرار مورد بررسی قرار گرفت. نتایج نشان داد برخی صفات مورفولوژیکی گیاه فقط تحت تاثیر مستقل فلزات و نوع رقم قراردارند به طوری که کمترین وزن تر برگ و ارتفاع گیاه در تیمار چهار میلی‌گرم در لیتر کادمیوم و بیشترین مقدار این دو صفت در تیمار روی دو میلی گرم در لیتر مشاهده شد. بالاترین وزن‌خشک گیاه در رقم خاردار و بیشترین ارتفاع گیاه در رقم بذر معمولی ثبت گردید. اثرات متقابل نوع رقم و فلزات سنگین در برخی صفات فیزیولوژیکی مانند محتوای مواد جامد محلول، محتوای کاتالاز و سوپراکسید دیسموتاز معنی‌دار بود. بیشترین فعالیت آنزیم کاتالاز در تیمار ۲ میلی‌گرم در لیتر کادمیوم در بذر معمولی و بیشترین میزان آنزیم سوپراکسید دیسموتاز در تیمار ٤ میلی‌گرم در لیتر روی و ۲ میلی گرم کادمیوم در بذر معمولی مشاهده شد. فلز روی تا دو میلی گرم در لیتر باعث افزایش رشد و نمو اسفناج گردید ولی بیشتر از این مقدار مانند فلز کادمیوم باعث کاهش عملکرد و افزایش مقدار آنزیمهای آنتی اکسیدانی شد. تمامی محتوای عناصر اندازه گیری شده اثر متقابل معنی‌داری نشان دادند. با افزایش کادمیوم جذب کلسیم، پتاسیم، روی ،منگنز و منیزیوم توسط گیاه کاهش یافت به طوری که کمترین مقدار تمامی عناصر اندازه گیری شده در چهار میلی گرم در لیتر کادمیوم در بذر خاردار مشاهده گردید. نتایج بدست آمده از تعیین غلظت زیستی نشان داد که محتوای کادمیوم برگ اسفناج خاردار و معمولی بین ۱٤٥ تا ۱٦۷ میلی‌گرم در کیلوگرم متغییر بود و اسفناج می‌تواند نماینده خوبی برای استفاده در زیست پالایی فلز کادمیوم باشد.

واژه‌های کلیدی

اسفناج،
صفات فیزیولوژیک،
فلز سنگین،
آنزیم

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Introduction

With the development of industrial zones and the huge population growth, many parts of the world especially industrial regions and the places with heavy mining industry and even the areas with prolonged agricultural patterns are facing progressive pollution problems (Liu *et al*. 2010). The concentrations of heavy metals higher than normal threshold in the soils gradually restrict the plant growth, food production and microbial populations in the soil. Heavy metals have direct deteriorative effects on ecosystems. Variations in the biochemical, physiological and metabolism of plants under stressful conditions are frequently reported. Some heavy metals, like Co, Cr, Fe, Mn, Ni, Cu, Cd and Zn are un-substitutable components of pigments or enzymes in plants and vegetation. So, they are essential elements in very trace amounts. (Srivastava *et al*. 2017; Ali *et al*. 2015). The presence of high dosages of Zn and Cd interfere soil organisms at the genes level and checks the enzymes and microbial activity in the soil (Liu *et al*. 2010). Furthermore, heavy metals hinder the metabolic pathways even at the cellular level. Zn-toxicity drastically affects dehydrogenase, urease, and β- glycosidase activity. Meanwhile, entering Zn^{2+} in the human food chains hurts pancreas and disturb proteins metabolism (Zhi-Xin *et al*. 2006). Whereas, Cd toxic levels prevents the activity of alkaline-phosphatase at the soil environment (Zhi-Xin *et al*. 2006). The plant type is the dominant criteria depicting tolerance to heavy metal contamination. Plants intrinsically have several mechanisms to combat and tolerate the heavy metals toxicity; prevention of the minerals entrance into the cells by their attachment to the cell wall, and producing low molecular weight enzymatic antioxidants (Shu *et al*. 2011) superoxide and H_2O_2 (Amaraathared *et al*. 2011). A research conducted on spinach revealed that under Cd high availability, the element accumulation was at the toxic levels in the roots and shoots of plants (Eisazadeh Lazarjan *et al*. 2016). However, with higher-growth rate and higher yields, spinach could be a good phytoremediation candidate for the Cd polluted soils (Eisazadeh Lazarjan *et al*. 2015). Cd influence the growth and physiological response of radish by the impact on growth characteristics and finally yield in main part by the negative effects on proline, anthocyanin and phenolic content (Moridian Pidosti et al. 2018). In *Brassica napus*, Cd inhibited the seed germination by th structural defects caused on seed cells (Ali *et al*. 2015). Moreover, in spinach, the high Zn, Cd, Ni and Cu concentration recorded the growth potential via the antioxidant enzymes deterioration and the toxic effects they have been on the activated CAT and the great increase in the SOD activity (Pandy *et al*. 2009) So, the identification and characterization of plants with the reasonable tolerance for the risky environments is greatly crucial. Compared to physico-chemical amendment procedures, phytoremediation is economic environment friend method to get-ride of heavy metals toxicity in the soil. The aim of the present study was to investigate the effects of Cd and Zn availability in the growing media on the growth of spinach (as a candidate for phytoremediation) in hope to have a detailed idea on the recommendation of this crop for the remedy of Zn- Cd contaminated soils.

MATERIALS AND METHODS

The experiment was conducted during the year 2017 at the Research Greenhouse of Azarbaijan Shahid Madani University, Iran. The light intensity was 400 µmol $m^{-2}s^{-1}$ under a 16: 8, day: night regime, and 20-25^{0C} day/night temperature. Two seed types (common and thorny) were used. The seeds were surface sterilized by sodium hypochlorite 10 % for 30 min and were planted at 5 liter pots filled with medium sized perlite. The plants were nourished by the Hoagland's nutrient solution (Hassanpouraghdam *et al*. 2019). pH of the nutrient solution was adjusted at 5.6. To avoid the salinity setup, the growing media were weekly washed with tap water. After the establishment of plants, heavy metals (Zn and Cd) treatments (0, 2 and 4 mg L^{-1}) were imposed on plants. The plants were sampled for analysis before flowering.

Plant fresh and dry weight: The fresh and dry weight of aerial parts were recorded by a digitl scale (BB141, Boeco, Germany).

Minerals content: Minerals content was quantified by the method of Honarjoo *et al*. (2013).

Enzymes activity: H_2O_2 content was traced by Amaraathared *et al*. (2011) and superoxide dismutase was evaluated by Minami and Yoshilcawa (1997) based on the assay of light dependent nitro- blue-tetra Zolium reduction at 560 nm as well as catalase by the method of Luhova *et al*. (2003).

The assessment of Bio-Concentration Factor (BCF) in the roots and leaves: The trait was assayed as: the concentration added to the growing medium ($mgkg^{-1}$) / the mean elemental concentration at plant tissue (mg kg^{-1}) (Ghosh and Sing, 2005).

Data analysis: The data were analyzed by MSTATC software. Means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Plant fresh and dry weight

The leaves fresh weight was affected by the heavy metals type (Table 1). The highest dry weight of plant was belonged to 2 mg L^{-1} of Zn and the least plant fresh weight was recorded at 4 mg L^{-1} Cd (Table 3). Thorny spinach type attained the highest plant fresh weight (Table 2). Photosynthesis reaction is more sensitive to Cd toxicity and the Cd high-availability greatly reduces the stomatal conductance, chlorophyll content, disturbs photosynthetic enzymes activity, and interferes electron transport in photosystems I and II. It seems that the efficiency of some plants in Cd accumulation is dependent upon the cells detoxification (Nikolic *et al*. 2014). Ghasemi and Shahabi (2011) reported that Cd availability reduced the growth and yield of tomato. The main idea about is lessening cell expansion and/or cell division prevention. Furthermore, Cd interferes photosynthesis and respiration and, by impairing CO_2 influx or by impacting the enzymes of carbon reducing cycle in photosynthetic apparatus reduces the photosynthetic rate and dry matter production (Penge *et al*. 2008). Aravind and Prasad (2004) reported that Cd reduced chlorophyll biosynthesis by the impact on δ-aminolevulinic acid dehydratase. Besides, Cd reduces protochlorophyll reductase by its effects on sulphydryl radicles and by reducing the 5-amino- luvilinic acid (ALA) levels. This later acid is a precursor for the formation of tetrapyrol in chlorophyll structure (Aravind and Prasad, 2004).

Plant height

Table 2 shows that the top height was recorded with common spinach seeds showing 22 % increase compared to thorny seed type. Heavy metals influenced plants height as well, and, the highest data was recorded in 2 and 4 mg L^{-1} of Zn (Table 3). It seems that Zn concentration employed had no negative effects on plant height. Contrarily, in a study conducted on maize, the scientists reported that Zn application improved plant height, aerial parts dry weight, the relative water content and Zn and K content in aerial parts (Karmollachaab and Gharineh, 2013). Zn is a dominant micronutrient having pivotal roles in DNA, RNA, auxin and protein biosynthesis; its deficiency disturbs metabolism and deteriorates macromolecules like cell membranene achored proteins, chlorophylls, nucleic acids and enzymes of IAA biosynthesis pathway and eventually, reduces growth potential and productivity of plants (Cakmak 2000, Broadley *et al*. 2007). Previous studies have verified that Cd modifies the meristematic cell division domains. The low Cd (1-4 µmol) increases cell division and plant growth. However, with the higher concentrations, retard cell division, plant growth and genes expression pattern especially cyclin B1 genes are interfered; the gene which are actively involved in cell division behaviors (Liu *et al*. 2010).

H_2O_2 content

Hydrogen peroxide content was influenced by the interaction effects of cultivar and heavy metal treatment. The highest H_2O_2 content was recorded under 2 mg L^{-1} Cd in common seeds (Table 4). Our results are the same as the reports of Vojodi Mehrabani *et al*. (2017) and Valizadeh *et al*. (2017). Cd stimulates H_2O_2 and subsequently hydroxyl radicals biogenesis and accelerates cell membrance lipids decomposition and peroxidation and hence, speeds up membrane deterioration and cell death (Hsu band Lao 2007). The toxic heavy metals availability stimulates the production of ROS molecules such as; superoxide, hydroxyl and hydrogen peroxide. These destructive molecules invade all types of vital macromolecules and demolish cell membrane leading to membrane breakdown and a very increased membrane leakage (Xiao *et al*. 2008).

Catalase activity

Catalase activity was significantly influenced by the interaction of cultivar and heavy metals level. The most recorded data was in 4 mg L^{-1} Zn with thorny seed (Table 4). Vojodi Mehrabani *et al*. (2017) reported that, with Cd application, catalase and ascorbate peroxidase activities were increased in garden cress. They have reported that the higher SOD activity lead to higher H_2O_2 release and to keep the redox potential, H_2O_2 was decomposed into H_2O and oxygen by the action of catalase and peroxidase. Catalase has an important role in scavenging H_2O_2 into H_2O and O_2. Unyayar *et al*. (2005) reported that even H_2O_2 in high doses is toxic for the cells. However, with low concentration plays as a messenger in gene expression process related to tolerance. Catalase with overcoming on oxidative stress and by scavenging H_2O_2 prevents ROS molecules generation and so prevents protein and nucleic acids deterioration (Unyayar *et al*. 2005).

Table 1- ANOVA for the effects of cultivar and different levels of Zn and Cd on enzymic activity, H_2O_2 content, elemental content and morphological traits of *Spinacia oleracea* L.

Source of variation	de	K content	Ca content	Mg content	Mn content	Cd content	Zn content	Plant height	Arial part dry weight (g)	Arial part fresh weight (g)	Catalase activity	H_2O_2 content	Superoxide dismutase activity
Seed type	1	10^{7**}×40	10^{**}×53	**39463	**1393	**61027	**18820	104^{*}	0.002^{ns}	0.008^{*}	17361^{ns}	0.005^{ns}	0.01^{ns}
Heavy metal treatment	4	10^{6**}×33	10^{6**}×22	724^{ns}	**265	**80195	**87433	30^{*}	0.03^{*}	0.002^{ns}	88302^{ns}	0.007^{*}	0.05^{ns}
Seed type × Heavy metal treatment	4	10^{6**}×13	10^{5**}×51	**1747	**121	**14606	**4666	2.3^{ns}	0.004^{ns}	0.001^{ns}	164009^{*}	0.012^{**}	0.11^{*}
Error	18	2611729.19	10^{3}×16	631	20	176.3	149	7.4	0.0001	0.001	54928	0.002	0.02
c.v(%)		6.3	1.1	1.01	6.8	9.3	2.8	14	6.8	7.9	12.9	6.6	5.9

ns,* and ** show non-significant and significant at $P\leq0.05$ and $P\leq0.01$, respectively.

Table 2- Mean comparison for the effects of cultivar on plant height and fresh weight of *Spinacia oleracea*.

Cultivars	Plant height (cm)	Arial part fresh weight (g)
Thorny seeds	13^{b}	22.4^{a}
Common seed	16.7^{a}	11.3^{b}

Similar letters in the columns are non-significant based on Duncan's test

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Table 3- Mean comparison for the effects of heavy metal type on plant height and arial part fresh weight of *Spinacia oleracea*

Metal type	Plant height (Cm)	Arial part dry weight (g)
Control	11[b]	0.46[b]
2 mg L^{-1} Zn	16[a]	0.56[a]
4 mg L^{-1} Zn	16[a]	0.40[b]
2 mg L^{-1} Cd	11[b]	0.44[b]
4 mg L^{-1} Cd	9.3[c]	0.33[c]

Similar letters in the columns are non-significant based on Duncan's test.

Table 4- Mean comparison for the interaction effects of cultivar and different levels of heavy metals on enzyme activity and H_2O_2 content of *Spinacia oleracea* L.

Cultivar	Heavy metal treatment (mg L^{-1})	H_2O_2 content (absorption changes min^{-1} mg^{-1} protein)	Superoxide dismutase activity (enzyme unit 1 mg^{-1} protein)	Catalase activity (µmol g^{-1}FWt)
Thorny seed	Control	0.06[b]	0.15[ab]	371[b]
Thorny seed	2 mg L^{-1} Zn	0.02[b]	0.10[b]	343[b]
Thorny seed	4 mg L^{-1} Zn	0.03[b]	0.14[b]	855[a]
Thorny seed	2 mg L^{-1} Cd	0.06[b]	0.22[b]	189[b]
Thorny seed	4 mg L^{-1} Cd	0.07[b]	0.26[b]	271[b]
Common seed	Control	0.02[b]	0.08[b]	369[b]
Common seed	2 mg L^{-1} Zn	0.04[b]	0.17[b]	314[b]
Common seed	4 mg L^{-1} Zn	0.3[b]	0.65[a]	302[b]
Common seed	2 mg L^{-1} Cd	0.21[a]	0.36[a]	535[ab]
Common seed	4 mg L^{-1} Cd	0.03[b]	0.12[b]	238[b]

Similar letters in the columns are non-significant based on Duncan's test.

SOD activity

The highest data for SOD activity was recorded in common seed spinach with 2 and 4 mg L^{-1} of Zn and thorny seed × control plants. In thorny seed cultivars, there was no difference in SOD activity considering the studied traits (Table 4). SOD is a metalloenzyme having Fe, Cu, Zn or Mn in its prostatic active site under higher Cd availability, Fe, Zn and Mn content in plant tissue was declined and seems that SOD activity declined reduction in Cd exposed plants is a response to the deficiency of mineral elements which are essential for the activity of the related enzymes (Chamseddine *et al*. 2009). The Same data have been reported by Pandy *et al*. (2009) in spinach. In a research conducted by Sobkowiak and Jaunna (2003), Cd was replaced by Zn. Zinc is a pivotal element for binding the transcription factor in regulating sites of several genes. It seems that higher Cd concentration goes to the reduced transcription factors availability and hence, the declined transcriptomes of SOD and APX. Moreover, Cd modifies the DNA methylation pattern, diminishes RNA biosynthesis or prevents RNA polymerase action in the transcription of the related genes (Pierron *et al*. 2014).

Cd content

The highest Cd content was belonged to thorny seeds×leaf sample of 4 mg L^{-1} of Cd as well as in common seed × leaf sample of 4 mgL^{-1} of Cd (Table 5). In the study of Vojodi Mehrabani *et al*. (2017) with more Cd availability, its amounts were higher in the plants aerial parts. Cd by passing the apoplastic route or by the simplistic pathway enters the root cells and by the xylem sap transfers to the aerial plant parts (Nikolic *et al*. 2014). Cd negatively impacts cell division and enlargement, plant growth and productivity. Furthermore, Cd prevents rubisco enzyme activity; the key enzyme in Calvin cycle, and consequently retards transpiration, minerals uptake and distribution and, N and P metabolism (Manna, 2013). In the sensitive plants, Cd alters the membrane permeability and retards or prevents minerals intake and impedes appropriate concentration of minerals inside cells. Genetic variability in Cd accumulation in plants is related to the physiological aspects of plants such as; the diverse ability of plants roots in Cd

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absorption, diversity in the translocation of Cd and the different ability in Cd accumulation.

Zn content

The highest Zn content was recorded for thorny seed × leaf samples under 4 mgL^{-1} Zn and in common seeds × 2 mg L^{-1} Zn (Table 5). The same results on the reduction of Zn absorption in plants in response to Cd availability have been reported by Yildiz (2005). Alio *et al*. (2015) reported that with increasing heavy metals (Zn and Cd) in plant growing medium the absorption of plant nutrition was decreased in spinach. Furthermore, Pandy *et al*. (2009) noted that the long time exposure to heavy metals of Zn, Cd and Ni retarded the growth of plants and led to chlorosis as well as reduced the ascorbate content of plants. Zn at low concentration is an essential micro-nutrient, but at higher concentration reduce plant growth, induces chlorosis and alters the chlorophylls structure (Ali *et al*. 2015). Moreover, Chaoui *et al*. (1997) noted that, with any Zn availability, protein and some antioxidant enzymes biosynthesis was improved. These protein molecules bind to metal elements and by generating metallothionein (protein metal complexes) neutralize the toxic effects of elements.

Potassium content

The highest K content was documented in thorny seed ×leaf sample of 4 mg L^{-1} Zn, root sample of thorny seed and common spinach root sample × 2 and 4 mg L^{-1} Zn (Table 5). Cd has high affinity with sulfhydryl, hydroxyl and nitrogen containing ligands and by this way inactivates several enzymes. Furthermore, Cd interferes photosynthesis, respiration and other metabolic processes. The major reason in Cd toxicity is it's interaction with essential mineral elements which hinders the balanced uptake and distribution of essential elements and goes to vital elements deficiency (Ciecko 2004). Cd availability reduced the uptake of Ca^{2+}, Mg $^{2+}$ and K^{+} in plant (Vojodi Mehrabani *et al*. 2017; Valizadeh *et al*. 2017).

Calcium content

Thorny seed× leaf sample of control plants hold the highest Ca^{2+} content (Table 5). The diminished Ca^{2+} uptake in response to Cd availability has been reported by Gussarsson et al. (1996) Growth retardation is the first response to the Cd toxicity. Ghasemi and Shahabi (2011) reported that in maize plants, with any Cd concentration increase, the absorption of N, P, K, Zn Cu and Na declined. Similar result about decreasing in elemental content was reported by Alio *et al*. (2015) in spinach. Increasing Ca^{2+} concentration inside plant is an efficient mechanism against Cd toxicity (Ghasemi and Shahabi, 2011).

Mg content

Mg^{2+} had the highest concentration in thorny seeds × leaf sample of control plants and in common seeds × leaf sample of 2 mg L^{-1} Zn treatments (Table 5). Cd toxicity and higher availability declined the absorption of Mn, Fe and Cu in tomato leaves (Ghasemi and Shahabi, 2011). Cd is involved in the movement and translocation of micro nutrients to the leaves. It seems that the major reason for nutrients concentration loss in the Cd rich environment is because of the competition for the binding sites or the differential selectivity of elemental carriers toward the cells. The differences in the potential of Cd uptake by the roots and the subsequent translocation and accumulation in the aerial parts is a pivotal factor in describing the differences between genotypes in their tolerance to the heavy metals. In the research conducted by Nikolic *et al*. (2014), the sufficient mg^{2+} content in plant tissues was essential to compensate the toxic Cd effects.

Mn^{2+} content

The highest Mn^{2+} concentration was recorded in common seed × leaf samples of 2 and 4 mg L^{-1} of Zn (Table 5). Cd availability negatively impacted Mn^{2+} uptake. Rubisco activity is thoroughly sensitive to Cd toxicity. Cd in the photolysis reaction, substitutes Mn^{2+} ion and hinders the water complex breaking (Stobar *et al*. 1985). Mn^{2+} uptake improvement in Marvdasht wheat under 4 mg L^{-1} of Cd could be described to the lower concentration of Cu^{2+}, Zn $^{2+}$ and Fe^{2+}. Cd competes with Mn^{2+} in protein carriers of cell membranes (Penge *et al*. 2008).

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Table 5- Mean comparison for the interaction effects of cultivar and different levels of heavy metal on elemental content (mg Kg^{-1}) of *Spinacia oleracea* L**.**

Cultivar	Heavy metal treatment	Ca content	K content	Zn content	Mn content	Mg content	Cd content
Thorny seeds	Control (leaf)	19.4 ^{a}	23.8 $^{b-d}$	0.30 ^{hi}	0.06 $^{d-g}$	2.5 ^{a}	-
Thorny seeds	2 mg L^{-1} cd in leaf sample	12.5 ^{f}	22.1 $^{b-d}$	0.44 ^{fg}	0.06 $^{d-f}$	2.4 $^{c-f}$	0.33 ^{b}
Thorny seeds	4 mg L^{-1} cd in leaf sample	15.6 ^{d}	18.0 ^{f}	0.26 ^{hi}	0.06 $^{b-d}$	2.5 $^{b-e}$	0.37 ^{a}
Thorny seeds	2 mg L^{-1} Zn in leaf sample	15.3 ^{e}	21.5 $^{b-f}$	0.54 ^{b}	0.06 $^{b-d}$	2.5 $^{b-d}$	–
Thorny seeds	4 mg L^{-1} Zn in leaf sample	5.5 ^{i}	33.0 ^{a}	0.59 ^{a}	0.05 ^{hi}	2.4 $^{c-f}$	–
Thorny seeds	Control (root)	4.8 ^{k}	25.0 ^{b}	0.30 ^{hi}	0.06 $^{e-h}$	2.4 $^{d-g}$	-
Thorny seeds	2 mg L^{-1} cd in root sample	4.82 ^{k}	25.0 ^{b}	0.30 ^{hi}	0.06 $^{e-h}$	2.4 ^{g}	0.09 ^{e}
Thorny seeds	4 mg L^{-1} cd in root sample	6.11 ^{h}	17.7 ^{f}	0.47 ^{de}	0.05 ^{hi}	2.4 $^{d-g}$	0.12 ^{d}
Thorny seeds	2 mg L^{-1} zn in root sample	5.15 ^{j}	30.0 ^{a}	0.46 $^{d-f}$	0.05 $^{g-i}$	2.4 $^{d-g}$	–
Thorny seeds	4 mg L^{-1} zn in root sample	6.25 ^{gh}	20.0 $^{b-e}$	0.44 ^{fg}	0.06 $^{d-g}$	2.4 $^{d-g}$	–
Common seed	Control (leaf)	18.96 ^{b}	21.0 $^{c-f}$	0.29 ^{i}	0.06 $^{c-e}$	2.3 $^{f-h}$	-
Common seed	2 mg L^{-1} cd in leaf sample	12.5 ^{f}	22.0 $^{b-f}$	0.41 ^{g}	0.06 $^{e-g}$	2.4 ^{fg}	0.29 ^{c}
Common seed	4 mg L^{-1} cd in leaf sample	17.03 ^{c}	20.0 $^{d-f}$	0.48 ^{de}	0.05 $^{f-i}$	2.3 $^{f--h}$	0.38 ^{a}
Common seed	2 mgL^{-1} Zn in leaf sample	15.64 ^{d}	18.1 ^{ef}	0.59 ^{a}	0.08 ^{a}	2.5 ^{ab}	-
Common seed	4 mg L^{-1} Zn in leaf sample	15.50 ^{de}	21.0 $^{c-f}$	0.51 ^{c}	0.07 ^{ab}	2.5 $^{b-d}$	-
Common seed	Control (root)	5.44 ^{i}	22.8 $^{b-e}$	0.32 ^{h}	0.05 $^{f-i}$	2.4 ^{fg}	-
Common seed	2 mg L^{-1} cd in root sample	4.95 ^{jk}	24.9 ^{bc}	0.32 ^{h}	0.06 $^{e-h}$	2.4 ^{fg}	0.09 ^{e}
Common seed	4 mg L^{-1} cd in root sample	6.14 ^{gh}	21.8 $^{b-e}$	0.48 ^{cd}	0.04 ^{i}	2.4 ^{fg}	0.12 ^{d}
Common seed	2 mg L^{-1} zn in root sample	5.13 ^{j}	29.0 ^{a}	0.45 ^{ef}	$^{e-i}$ 0.05	2.4 $^{e-g}$	-
Common seed	4 mg L^{-1} zn in root sample	6.43 ^{g}	29.9 ^{a}	0.41 ^{g}	0.06 $^{b-e}$	2.4 $^{d-g}$	–

Similar letters in the columns are non-significant based on Duncan's test.

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Bio-concentration factor

Bio-concentration factor is the potential of plants in accumulation of a special metal element from the containing medium. Variations in bio-concentration factor is dependent upon the bio-accumulation per biomass of a given plant as well as on the element concentration. BFC ≥ 1 means that, the plant is adequately potential in the absorption and translocation of element (Ghosh and Singh, 2005). The plants species have different behaviors in response to heavy metals. Based on the results obtained (Table 6), Cd content of thorny and common spinach leaves was between 145-167 mg kg^{-1}. The least Zn content was recorded in 4 mg L^{-1} of Cd in thorny seed spinach leaves. The results of the present experiment are in line with the findings of Vojodi Mehrabani *et al*. (2016).

Table 6- The means for Bio-Concentration factor (BCF) of Zn and Cd in *Spinacia oleracea* L.

Cultivar	Heavy metal treatment	Zn content (g Kg^{-1})	Cd content (g Kg^{-1})
Thorny seeds	Control (leaf)	0.30	–
Thorny seeds	2 mg L^{-1} cd in leaf sample	0.22	0.16
Thorny seeds	4 mg L^{-1} cd in leaf sample	0.14	0.09
Thorny seeds	2 mg L^{-1} Zn in leaf sample	0.27	–
Thorny seeds	4 mg L^{-1} Zn in leaf sample	0.08	–
Thorny seeds	Control (root)	0.30	–
Thorny seeds	2 mg L^{-1} cd in root sample	0.15	0.04
Thorny seeds	4 mg L^{-1} cd in root sample	0.11	0.03
Thorny seeds	2 mg L^{-1} zn in root sample	0.23	–
Thorny seeds	4 mg L^{-1} zn in root sample	0.11	–
Smooth seed	Control (leaf)	0.29	–
Smooth seed	2 mg L^{-1} cd in leaf sample	0.20	0.14
Smooth seed	4 mg L^{-1} cd in leaf sample	0.12	0.09
Smooth seed	2 mgL^{-1} Zn in leaf sample	0.28	–
Smooth seed	4mg L^{-1} Zn in leaf sample	0.12	–
Smooth seed	Control (root)	0.32	–
Smooth seed	2 mg L^{-1} cd in root sample	0.16	0.04
Smooth seed	4 mg L^{-1} cd in root sample	0.12	0.03
Smooth seed	2 mg L^{-1} zn in root sample	0.22	–
Smooth seed	4 mg L^{-1} zn in root sample	0.10	–

Similar letters in the columns are non-significant based on Duncan's test.

Conclusions

Accumulation rate of heavy metals in plants is quite dependent on species and growing conditions. The overall results indicated the positive effects of Zn on dry weight, plant height, SOD content, as well as on Zn^{2+} and K^{+} content. Cd toxicity stress increased CAT and H_2O_2 content of plants. Overall, for growing spinach as a vegetable, Zn application would be a promising way to improve the growth and yield of plant. Otherwise, for phyto-remediation of Cd polluted areas, spinach would be again a promising candidate owing to the fast-growing habit and multi harvest nature of the plant during a year.

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The impact of Zn and Cd heavy metals on the growth and some physiological characteristics of spinach

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Abstract

High concentrations of heavy metals disrupt the metabolic processes of plant growth and even cause plant death. Plants show different tolerance thresholds to heavy metals, therefore, the effect of different concentrations of heavy metals zinc (Zn) and cadmium (Cd) (zero, 2 and 4 mgL^{-1}) in two varieties of spinach (common seed and Thorny seeds) on some morphological, physiological traits and content of the elements were examined in the factorial experiment based on a completely randomized design with three replications. The results showed that some morphological traits of the plant are affected by metals and cultivar type independently, so that the lowest wet weight of leaves and plant height was observed in four mgL^{-1} of Cd treatment and the highest amount of these two traits was observed in two mgL^{-1} of Zn. The highest dry weight of the plant was recorded in the thorny cultivar and the highest plant height was recorded in the normal seed cultivar. The interaction of the type of cultivar and heavy metals was significant in some physiological traits such as soluble solids content, catalase content, and superoxide dismutase. The highest activity of catalase enzyme was observed in the treatment of 2 mgL^{-1} of Cd in normal seeds and the highest amount of superoxide dismutase was observed in the treatment of 4 mgL^{-1} of Zn and 2 mgL^{-1} of Cd in normal seeds. Zinc metal up to 2 mgL^{-1} increased the growth and development of spinach, but more than this amount, like cadmium metal, reduced yield and increased the amount of antioxidant enzymes. All content of the measured elements showed a significant interaction. As the cadmium increased, the plant's uptake of the Ca, K, Zn, Mn and Mg decreased, so that the lowest amount of all the elements measured in four mgL^{-1} of Cd was observed in thorny seeds. The results of determining the biological concentration in this experiment showed that the cadmium content of thorny and common spinach leaves varied between 145 and 167 $mgKg^{-1}$ and spinach could be a good representative for use in cadmium phytoremediation.

Keywords: Enzyme activity, Heavy metal, Physiological characteristics, *Spinacia oleracea*

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