Heavy Metals Effects on Brassica Oleracea and Elements Accumulation by Salicylic Acid

Mina Taghizadeh*a, Mousa Solgia, Maryam Karimib, Mohammad Hosein Sanatib, Shohreh Khoshbin

*aDepartment of Horticultural Science, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran.
bDepartment of Medical Biotechnology, National Institute of genetic engineering and biotechnology, Tehran, Iran.

*aCorrespondence should be addressed to Dr. Mina Taghizadeh. Email: m-taghizadeh@araku.ac.ir

Background

Soil pollution with heavy metals such as lead, copper, zinc and cadmium is an increasingly phenomenon caused by human activities such as industrialized wastes materials and agricultural inputs applications (urban solid waste, sewage sludge, pesticide and fertilizer). The existence of these heavy metals in atmosphere, soil and water can cause risk to all of the components of the ecosystem even at low concentrations (1,2). The presence of heavy metals in the soil as one of the major environmental stresses may cause retardation of plant growth and produce reactive oxygen species (3). Lead (Pb) as a heavy metal is one of the greatest important pollutants of land ecosystem. In addition to natural processes, lead can be produced through artificial sources (sooty exhaust from cars, factories, tank batteries, pesticides, etc.) These activities continuously may increase the level of heavy metals in the ecosystem (4). Lead toxicity effects in plants usually appears in concentrations higher than 30 µg g⁻¹ and cause reducing the growth and decreasing the chlorophyll synthesis (5). Lead toxicity causes many difficulties such as inhibition in growth and yield levels, yellowing of young leaves,
reduction in absorption of essential elements such as iron and reduction in the rate of photosynthesis (6). Zinc (Zn) is one of the essential metals with low consumption that affects many metabolic processes of plants (6). Zn plays a significant role in many biological processes but excessive levels of this element can cause toxic effects (7). Toxicity levels in various plants are different due to plant genotype, ecological conditions and soil conditions. Higher levels of zinc compete with plant uptake of iron, phosphorus, copper and manganese and can cause their deficiencies in plant tissue and reduce the quality and quantity of production. Many problems may occur if a person takes too much Zn. Symptoms may include nausea, abdominal pain and digestive spasms. The intake of zinc in the range of approximately 100–300 mg per day may cause cell anemia and copper deficiency (8). High concentrations of Zn in the soil have a negative effect on plant metabolic activities, such as retarded growth of the entire plant due to the lack of cell division and cell elongation, disruption of cell membrane and organelles, chromatin condensation and increase in the number of nucleolus (9). In addition, high concentrations of Zn can be the cause of chlorosis young leaves which gradually spreads to the developed leaves (10). Other physical symptoms by Zn toxicity include red brown color due to reduced absorption of phosphorus (11). Phytoremediation is defined as the process by which plants remove, degrade, or immobilize soils, sediment and surface water contaminated with organic and mineral contaminants, including toxic metals (12). After sufficient plant growth and metal accumulation, the above-ground parts of the plant are harvested, a process referred to as phytomining. Phytomining is a “green” technology that uses metal-hyperaccumulating plant species to extract metals from soil, harvest the biomass and burn it to produce bio-ore (13,14). The Brassicaceae family includes many plants of economic importance that used for foods, animal forage, oil manufacture, condiments and biofuel (15). Several genus of Brassicaceae are a main source of essential nutrients with antioxidative characteristic and other compounds such as some glucosinolates that are documented as valuable for human wellbeing (16). Members of the Brassicaceae are promising crop for phytoremediation of toxic heavy metals (17). Some of the Brassica species are reported to be suitable for heavy metals accumulation such as Pb, Cd, Zn, Cu and Ni (14). Some of these plants can uptake relatively high amounts of poisonous heavy metals, without visible signs, leads to potential pollution of the food chain (17) and this has to be taken into reason in any phytoremediation procedure (18).

Salicylic acid (SA) belongs to a group of phenolic compounds that known as an important molecule to moderate plant responses to environmental stresses (19). Also, this acid plays a key role in plant growth and development of buds, membrane permeability, mitochondrial respiration, stomata closure, transfer of materials, photosynthesis, attract of ions and plant growth rate. The effect of SA on promoting plant performance under biotic (20) and abiotic stresses has been demonstrated (21). SA could affect most of metabolic reactions and change them in the plants (22). These changes are often performed for modulating plant responses in different environmental conditions (23).

**Aims of the study:** A field study was conducted in sites in Arak city, where wide industrial practices are undertaken. It is anticipated that soil is polluted with heavy metals (24). In the present study, Brassica oleracea var. acephala was used to evaluate the heavy metal (Zn and Pb) phytoextractive ability and effect of Salicylic acid on phytoextraction potential.

**Materials & Methods**

The genetic homogeneity of Brassica oleracea var. acephala seeds, commonly known as...
Ornamental kale were used for this study. Seeds were disinfected by 70% solution of commercial bleach (5% active ingredient) for 1 min, followed by rinses with sterilized distilled water for three times. The seeds treated at different concentrations of Salicylic acid at two concentrations (200 and 300 mg L\(^{-1}\)) at 25 °C for 6 h. Distilled water sterilized was used as control, too. After 6 hours, all the seeds were washed with distilled water. Before cultured seeds, all vessel culture containing the medium, were sterilized in autoclave and 7 g L\(^{-1}\) agar medium, heavy metals factor including \(\text{Pb(NO}_3\text{)}_2\) in different concentrations (0, 50, 100 and 200 mg L\(^{-1}\)) and \(\text{ZnSO}_4\) in (0, 50, 100 and 200 mg L\(^{-1}\)) were used. The treated seeds were transferred a growth chamber (temperature at 24±2°C and 16/8 h light/darks of photoperiod). Germinated seeds were counted daily. Other traits were measured through the following formulas (25).

1. **Final Germination Percentage (FGP):** This parameter represents the germination rate that was calculated using the below formula for each replication of the treatment:

\[
\text{FGP} = 100 \times \frac{\text{Ng}}{\text{Nt}}
\]

Where \(\text{Ng}\) is the total number of germinated seeds; \(\text{Nt}\) is the total number of evaluated seeds.

2. **Germination Rate Index (GRI):** This parameter is the average daily germination that was calculated based on the following equation:

\[
\text{GRI} = \frac{\text{G1}}{1} + \frac{\text{G2}}{2} + \ldots + \frac{\text{Gi}}{i}
\]

Where \(\text{Gi}\) is germination percentage in day \(i\).

3. **Coefficient of velocity of germination (CVG):** The CVG gives a suggestion of the rapidity of germination:

\[
\text{CVG} = \frac{\sum \text{Ni}}{\sum \text{Nti}} \times 100
\]

Where \(\text{N}\) is the number of seeds germinated on day \(i\), and \(\text{Ti}\) is the number of days from sowing.

4. **Mean Daily Germination (MDG):** This index shows the speed and acceleration of germination:

\[
\text{MDG} = \frac{\sum \text{Nt}}{\sum \text{N}}
\]

Where \(\text{N}\) is the number of germinated seeds, \(\text{t}\) is the number of days from the beginning of germination and \(\sum \text{N}\) is the total number of seeds germinated.

5. **Average Value Germination (AVG):** An index of germination rate that was calculated using the next formula:

\[
\text{AVG} = \frac{\sum \text{Nt}}{\sum t}
\]

Where \(\text{Nt}\) is the total number of germination seeds in a time and \(t\) is the total number of days until the maximum of germination.

6. **Seed Vigor index (SV):**

Where \(\text{GP}\) is the germination percent, \(\text{PL}\) is the length of plumule and \(\text{RL}\) is the length of radicle:

\[
\text{SV} = (\text{PL} + \text{RL}) \times \text{GP}
\]

After 14 days, fresh weight (g), width of leaves, length of radicle and plumule (mm) were measured. The seedlings were placed in oven for 48 h at 72°C, after that the dry weight were measured. At the end of the experiment, relative chlorophyll content index was measured using a chlorophyll meter (HansatechI SPAD- CL-10). To estimate the total heavy metals in the seedling (26), the samples were milled and then dried in a furnace. Then about 1 g of dried samples was digested in 10 ml of concentrated nitric acid for 24h. The solution was boiled until light fumes were given off. Then, the solution was brought to 50 ml and was passed through filter paper. Finally these solutions were used for heavy metal concentration measurements, using the flame atomic absorption method (Varian, Spectra aa 220 - Australia). This experiment was accompanied as factorial in completely randomized design with three replications. Analysis of variance was done by Statistical Analysis Software (SAS). Simple and interactive effects in response to type and concentration of heavy metals and Salicylic acid on seed germination, growth and tolerance characteristics Brassica oleracea of seedlings were conducted using Duncan Multiple Range Test (DMRT).
Increased concentrations of metals had the highest inhibitory effect on all seedling morphological characteristics. At the higher concentrations of heavy metals, growth inhibition was observed in seedlings while in lower concentrations, normal growth was observed. Generally, by increasing in heavy metal concentrations, seed germination and seedling growth decreased. But this decrease due to zinc was very lower compared to the lead. At higher concentrations of lead, seed germination was stopped and seedling showed abnormality symptoms (Fig 1).

The interaction effect between heavy metal and concentration on germination of ornamental kale showed that lead metal with 200 mg L\(^{-1}\) had the lowest final germination percentage (FGP) (40) and the control had the highest rate (100). With increasing concentrations of lead in the medium, the germination rate index (GRI) decreased and with 100 mg L\(^{-1}\) of zinc in medium, the highest germination rate index was observed. The highest daily mean germination (MDG) was obtained (5.58) in 200 mg L\(^{-1}\) of lead in environment. The lowest coefficient of velocity of germination (CVG) was observed in 50 mg L\(^{-1}\) of zinc (194.7) and 200 mg L\(^{-1}\) of lead (138.9), respectively. On the other hand, the lowest average value germination (AVG) (1.88) was achieved by the application of lead 200 mg L\(^{-1}\) concentration (Table 1).

Interaction between concentration of two factors (SA and heavy metals) showed that SA at 200 mg L\(^{-1}\) concentration without heavy metal had the highest rate of chlorophyll (46.6). In each level of salicylic acid, with increasing in metal concentration, the chlorophyll content was decreased significantly. This trend was observed in fresh weight, too. The highest fresh weight (0.58 g) was observed in the control and the lowest (0.09 g) was observed in 200 mg L\(^{-1}\) of acid salicylic. Results showed that different metal concentrations had no different significant effects on dry weight in the absence of seed priming with SA. On the other hand, seedling dry weight decreased severely in seeds which treated by SA in the presence of high metal concentrations.

The highest amount of dry weight (0.17 g) was obtained when 300 mg L\(^{-1}\) SA with 50 mg L\(^{-1}\) of metal were used and the lowest amount (0.03 g) was obtained when 200 mg L\(^{-1}\) of metal in combination with two concentrations of SA (200 and 300 mg L\(^{-1}\)) were used. Increasing the metal concentrations caused decreasing the radicle length and leaf width at all levels of SA. Therefore the lowest width of leaf (0.3 cm) was detected with 200 mg L\(^{-1}\) metal concentration additional 200 or 300 mg L\(^{-1}\) SA treatment. The highest root length (8.1 cm) has been observed using SA at 200 and 300 mg L\(^{-1}\) concentration without heavy metal. Increasing concentrations of metal from 0 to 50 mg L\(^{-1}\) at all levels of SA treatments increased the root/shoot length and then reduce it. Interaction effects between different concentrations of metals and SA were significant at the ratio of root to shoot (P≤0.01). Among treatments, the highest of this trait (0.58 cm) was obtained using 300 mg L\(^{-1}\) of SA and at the presence of 50 mg L\(^{-1}\) of metals (Table 2).
ZnSO₄  
Pb(NO₃)₂

Figure 1) Comparison of seedlings growth ornamental kale in different concentrations (mg/l) of ZnSO₄ and Pb(NO₃)₂

Table 1) Effects of interaction between metals type and concentrations on the seed germination characteristics in ornamental kale

| Metal | Concentration(mg/L) | FGP  | GRI  | CVG  | MDG  | AVG  |
|-------|---------------------|------|------|------|------|------|
| Zn    | 0                   | 100  | 220  | 287  | 3.45 | 2.87 |
|       | 50                  | 94   | 192  | 194  | 4.59 | 3.59 |
|       | 100                 | 98   | 238  | 266  | 3.54 | 2.92 |
|       | 200                 | 85   | 202  | 265  | 3.64 | 2.61 |
| Pb    | 0                   | 97   | 199  | 192  | 4.47 | 3.64 |
|       | 50                  | 93   | 180  | 178  | 4.8  | 3.72 |
|       | 100                 | 95   | 217  | 254  | 3.76 | 3.01 |
|       | 200                 | 40   | 68   | 138  | 5.58 | 1.88 |

Mean values followed by different letters are significantly different (a, b, c, …)

Table 2) The effects different concentrations of lead and zinc and salicylic acid treatment on characteristics of ornamental kale seedlings

| SA( mg/L) | Metal (mg/L) | Chlorophyll | Fresh weight (g) | Dry weight (g) | Root/Shoot (cm) | Root (cm) | Leaf width (cm) |
|-----------|--------------|-------------|------------------|----------------|-----------------|-----------|-----------------|
| 0         | 0            | 42.1        | 0.43             | 0.11           | 4.2             | 7.1       | 0.91            |
|           | 50           | 36.6        | 0.55             | 0.11           | 7.6             | 5.3       | 0.77            |
|           | 100          | 35.5        | 0.46             | 0.1             | 5.5             | 4.05      | 0.7             |
|           | 200          | 17.4        | 0.32             | 0.14           | 2.2             | 1.5       | 0.58            |
| 200       | 0            | 46.6        | 0.53             | 0.16           | 6.1             | 8.1       | 0.88            |
|           | 50           | 39.8        | 0.45             | 0.12           | 8.1             | 5.4       | 0.72            |
|           | 100          | 29.8        | 0.28             | 0.07           | 4.8             | 3.1       | 0.43            |
|           | 200          | 13.6        | 0.09             | 0.03           | 2.5             | 1.19      | 0.34            |
| 300       | 0            | 45.5        | 0.58             | 0.14           | 7.8             | 8.1       | 0.96            |
|           | 50           | 42.1        | 0.43             | 0.17           | 8.9             | 5.8       | 0.69            |
|           | 100          | 20.5        | 0.24             | 0.08           | 4.3             | 2.3       | 0.38            |
|           | 200          | 11          | 13.0             | 0.03           | 1.5             | 1.1       | 0.34            |

Mean values followed by different letters are significantly different (a, b, c, …)

Comparing the means indicated that increasing the concentration of metals had the highest inhibitory effect on all germination indices. However, the most inhibition of germination indices observed in lead treatment. Exceptionally, increasing concentrations of heavy metals increased these two characteristics of MDG and AVG. Seed
pretreatment with SA couldn’t improve germination in response to metals stress. According to the results, germination indices decreased lower than 300 mg L$^{-1}$ SA, compared to the control. It is remarkable that combination of zinc and 200 mg L$^{-1}$ SA increased GRI, MDG and AVG traits. Generally, pretreatment with SA couldn’t improve germination in response to metals stress.

Table 3) The effects of different concentrations lead, zinc and salicylic acid treatment on the characteristics of ornamental kale

| AS concentration (mg/L) | Metal | concentration (mg/L) | FGP | GRI | CVG | MDG | AVG | SV |
|-------------------------|-------|----------------------|-----|-----|-----|-----|-----|----|
|                         |       | 0                    | 0   | 100 | 246.3$^{abc}$ | 295.5$^{abc}$ | 3.33$^{cd}$ | 2.77$^{abcde}$ | 99.98$^{c}$ |
|                         |       | 50                   | 100 | 208.7$^{bc}$ | 198.3$^{def}$ | 4.36$^{cde}$ | 2.63$^{abc}$ | 36.37$^{abcd}$ |
|                         |       | 100                  | 100 | 197.9$^{cdef}$ | 175.8$^{fg}$ | 4.76$^{cde}$ | 3.97$^{ab}$ | 35.92$^{cdef}$ |
|                         |       | 200                  | 93.3$^{abc}$ | 189.8$^{ef}$ | 207.9$^{bdefg}$ | 4.32$^{cde}$ | 3.38$^{ab}$ | 16.6$^{efg}$ |
|                         |       | Zn                   | 0   | 96.6$^{a}$ | 232.4$^{a}$ | 266.7$^{a}$ | 3.54$^{def}$ | 2.36$^{abcd}$ | 66.58$^{a}$ |
|                         |       | 50                   | 80  | 229.6$^{bc}$ | 330.08$^{a}$ | 3.03$^{f}$ | 2.27$^{th}$ | 3.53$^{def}$ |
|                         |       | 100                  | 80  | 186.1$^{ef}$ | 282.1$^{gh}$ | 3.61$^{def}$ | 2.44$^{fgh}$ | 27.93$^{cdef}$ |
|                         |       | 200                  | 33.3$^{a}$ | 34.1$^{h}$ | 171.5$^{gh}$ | 5.05$^{abc}$ | 1.44$^{i}$ | 6.92$^{g}$ |
|                         |       | Pb                   | 0   | 100 | 241.7$^{abc}$ | 267.3$^{abcdef}$ | 3.70$^{df}$ | 3.03$^{bdef}$ | 95.52$^{a}$ |
|                         |       | 50                   | 100 | 198.9$^{cdef}$ | 186.14$^{ef}$ | 4.56$^{bdef}$ | 3.80$^{abc}$ | 55.9$^{c}$ |
|                         |       | 100                  | 96.6 | 225.2$^{a}$ | 255.18$^{a}$ | 3.82$^{def}$ | 3.05$^{cdef}$ | 34.33$^{edef}$ |
|                         |       | 200                  | 96.6 | 200.3$^{a}$ | 194.9$^{fgh}$ | 4.43$^{cde}$ | 3.59$^{abcd}$ | 13.49$^{fg}$ |
|                         |       | Pb                   | 0   | 100 | 238.8$^{abcd}$ | 257.86$^{abcdef}$ | 3.63$^{def}$ | 3.02$^{bdefg}$ | 63.43$^{b}$ |
|                         |       | 50                   | 100 | 212.3$^{a}$ | 220.22$^{abcd}$ | 4.06$^{def}$ | 3.36$^{bcdef}$ | 37.2$^{def}$ |
|                         |       | 100                  | 90  | 208.9$^{a}$ | 242.04$^{abcd}$ | 3.81$^{def}$ | 2.80$^{cdef}$ | 17.09$^{efg}$ |
|                         |       | 200                  | 40  | 3.67$^{h}$ | 122.9$^{b}$ | 5.83$^{a}$ | 1.94$^{hi}$ | 5.9$^{g}$ |
|                         |       | Zn                   | 0   | 100 | 253.42 | 299.06$^{a}$ | 3.33$^{ef}$ | 2.77$^{gh}$ | 89.32$^{a}$ |
|                         |       | 50                   | 100 | 192.02$^{def}$ | 192.72$^{def}$ | 4.48$^{cde}$ | 3.50$^{abcd}$ | 54.74$^{bcd}$ |
|                         |       | 100                  | 86.6 | 154.4 | 153.1 | 5.18$^{abc}$ | 3.75$^{abcd}$ | 23.27$^{defg}$ |
|                         |       | 200                  | 90  | 151.3$^{a}$ | 133.1$^{h}$ | 5.65$^{ab}$ | 4.19$^{a}$ | 12.91$^{fg}$ |
|                         |       | Pb                   | 0   | 100 | 244.3$^{abc}$ | 276.16$^{abcd}$ | 3.46$^{def}$ | 2.88$^{cdefg}$ | 96.10$^{a}$ |
|                         |       | 50                   | 96.6 | 208.3 | 214.2 | 4.2$^{cdef}$ | 3.38$^{abcde}$ | 38.78$^{cde}$ |
|                         |       | 100                  | 86.6 | 213.1 | 272.6 | 3.50$^{et}$ | 2.55$^{gh}$ | 21.8$^{defg}$ |
|                         |       | 200                  | 46.6 | 73.99 | 122.4 | 5.86$^{a}$ | 2.27$^{ghi}$ | 5.04$^{g}$ |

Mean values followed by different letters are significantly different (a, b, c, …)

In this study, the accumulation of lead and zinc in Brassica oleracea pretreatment seeds on SA different concentration was investigated. The treatment of 200 mg L$^{-1}$ metals was waivered for the analysis, because of abnormality in seed germination and very low growth in this treatment. In this evaluation two important characteristics include concentration and the accumulation (uptake) of heavy metals related to dry matter was calculated. Increasing concentrations of metal (especially in 50 and 100 mg L$^{-1}$ treatments) significantly increased the metal accumulation in plant tissues compared to the control (Figure 2. a and b). The results indicated that the highest uptake of lead (8500 mg L$^{-1}$) was observed at 300 mg L$^{-1}$ SA...
with 100 mg L\(^{-1}\) metal. When lead concentration was increased, the amount of concentration of lead in plant tissues was significantly increased (Figure 2.a). But this increasing due to lead was too much in comparison to zinc concentration in ornamental kale. About metal concentration in seedling, there were significant differences among 50 and 100 mg L\(^{-1}\) zinc compared to the control. SA had no effect on accumulation of metals in plant tissue. The maximum effect of concentration of zinc in tissues was observed in 300 mg L\(^{-1}\) SA, and 100 mg L\(^{-1}\) zinc (Figure 2.b).

Uptake of lead in plant tissue was increased by the cumulative concentration of Pb in the medium. Thus the extreme of accumulation of lead (187 mg L\(^{-1}\)) was observed at seedling of treatment by 300 mg L\(^{-1}\) AS and 100 mg L\(^{-1}\) lead in media (Figure 3.a). Different concentrations of zinc (50 and 100 mg L\(^{-1}\)) and pretreatment with SA had no major effect on uptake of metal by seedling. But lowest uptake of zinc was observed in the control. The maximum accumulation of zinc was observed in 300 mg L\(^{-1}\) SA and 100 mg L\(^{-1}\) zinc in media (Figure 3.b).

**Figure 2**) Effect of salicylic acid on lead (a) and zinc (b) concentration in the seedling and media. Mean values followed by different letters are significantly different

**Figure 3**) Accumulation of lead (a) and zinc (b) in *Brassica oleracea* seedling pretreated with salicylic acid. Mean values followed by different letters are significantly different
Discussion

Seed germination process is unique of the important and critical phases in plant growth (27). The results showed that increasing the concentration of metals decreased the germination rate index, final germination percentage, coefficient of seed vigor index and velocity of germination of ornamental kale. The results relevant inhibition seedling growth by lead has the greatest effect in comparison with zinc in media. Similar outcomes were reported by researchers about the adverse effect of Ni and cobalt on germination seed (28). Reduction in seed germination could be because of the heavy metals on contravention compounds in the seeds (29). Outcomes of this study presented that when metal concentration was increased, germination percentage was significantly declined. Since heavy metals disrupt the hormonal balance of the plant. Metal toxicity -due to numerous aspects of its behavior- mimics calcium metabolism and inhibits the activities of many enzymes such as malate dehydrogenase and glucose-6-phosphate dehydrogenase (29). Other researchers reported that inhibitory effects of silver nanoparticles on Seed germination of Bermudagrass (Cynodon dactylon) may be due to the nanoparticles penetrate into cells, destruction of genetic material and interruption of cell function (30). The results indicated that with increasing the concentrations of lead in the growth media, the root length reduced and subsequently shoot length reduced. More sensitivity of root and shoot length could be described by high accumulation of lead in root and lignification (31) and interaction with cell membrane sulfhydryl groups (32). Depending on genotype, environmental stresses can reduce photosynthetic pigments (33;34). Comparing means indicate that the lead negatively affects the chlorophyll content and so decreases the photosynthesis rate in plant. Inhibition of chlorophyll synthesis by lead is due to the Inhibition of synthesis of gamma-aminolevulinic acid dehydrogenase and protochlorophyllide reductase complex formation with the substrate (5). Interaction between heavy metal with sulphydryl group of enzymes is the most important mechanism of this inhibition. Generally, different researchers have reported the reduction of chlorophyll content of different crops by heavy metals toxicity, such as Ni in parsley and cadmium in canola (35,36).

As the results showed, application of 200 mgL⁻¹SA on seed priming, significantly increased chlorophyll content compared to the control. SA significantly increased the chlorophyll content in rice seedlings under lead stress (37). SA, time and concentration has dual effects due to the type of planet, but at appropriate concentrations they can increase chlorophyll and photosynthesis (38). Enhancing the chlorophyll content was probably due to increased antioxidant capacity, improved cell permeability and synthesis of new proteins (36). There are some reports indicating that SA increased photosynthesis in corn (Zea mays L.) and soybeans (39,35). In another report, SA enhances the amount of chlorophyll in Spinach (Spinacia oleracea) (40). Likewise, treatment of barley seedlings with SA prevented toxicity by cadmium (23). In this experiment, it was detected that metal stress reduced dry weight and fresh weight, therefore increasing heavy metals concentrations decreased the dry matter (41). Khodary (2004) suggested that SA enhanced the maize salt tolerance in terms of improving the measured plant growth criteria. It might activate the metabolic consumption of soluble sugars to form new cell constituents as a mechanism to stimulate the growth of maize plants (42). Also, SA treatments prevent the growth depression caused by cadmium. Slices from leaves treated with (200 and 300 mg. L⁻¹) SA for 24 h also showed an increased in the level of tolerance toward high Cadmium concentrations as compared to concentration...
control metal. But the maximum dry weight was perceived in 300 mg L\(^{-1}\) SA and 50 mg L\(^{-1}\) of metal treatment (23). Decreasing leaf area is a common response to heavy metal stress (1). Abiotic stresses such as the existence of heavy metals at toxic levels caused significant decrease in growth and crops by reducing the leaf area. Interaction effects between different concentrations of metals and SA showed that the highest of leaf width (0.9 cm) was obtained by using 300 mg L\(^{-1}\) of SA. There are various reports about the effect of SA on growth parameters. For example SA treated sunflower (Helianthus annuus L.) plants shown an increase in tolerance to copper treatment and grew well (22). Also salicylic acid improved growth in barley (Hordeum vulgare L.), corn (Zea mays L.) and wheat (Triticum spp.) under salinity stress (43,44,45).

In this study, the highest radicle was detected using 200 and 300 mg L\(^{-1}\) SA. In abiotic stresses, SA increased the compatibility of the plant by an effect on abscisic acid (ABA) and the increase of this phytohormone (46). In wheat plant, SA increases plumule and radicle growth and the ratio of root to shoot under salinity stress (47). Interaction effects between different concentrations of metals and SA indicated that the highest amount of the root/shoot length was due to 200 mg L-1 SA and 50 mg L\(^{-1}\) metals concentration. In this study, the absorbed heavy metals by seed inhibited the germination and development of ornamental kale seedling. One mechanism, including metals could be barred from accumulating or entering the roots (48). According to Figure 3, treatment with 100 mg L\(^{-1}\) zinc and lead with 300 mg L\(^{-1}\) SA exhibited the maximum absorption of these elements. Increasing concentrations of metals were observed in accordance with increasing the ratio of metal accumulation (49). Similar effects about the accumulation of heavy metals by plants have been reported (50). According to the results, under different treatments of lead and zinc, the germination and growth of ornamental kale decreased as compared to the control. It is remarkable that this inhibition due to lead was too much in comparison with zinc. Ornamental kale could tolerate 100 mg L\(^{-1}\) of heavy metals and gathered metals in organs during growth. Also the results presented that seed pretreatment with SA couldn’t improve tolerance to heavy metals stress satisfactory, nevertheless the extreme absorption was found at 300 mg L\(^{-1}\) SA and 100 mg L\(^{-1}\) of metal treatment. Also, 300 mg L\(^{-1}\) had a positive effect on metal concentration in seedling stage. Since the hyperaccumulator is the plant that can absorb 1,000 mg of lead in dry weight. In this study the absorption rate of metals was 8500.5 mg Kg\(^{-1}\) dry weights.

### Conclusion

Considering the toxicity of metals on the growth of Brassica oleracea, this species has been shown to accumulate moderate level of lead and zinc. Seedling that can absorb 10,000 mg of zinc in dry weight is considered as phytoremediation plant, the absorption rate of zinc in this study was 1085.1 mg Kg\(^{-1}\) dry weights. The outcomes have revealed that the brassica oleracea var. oleraceae be very promising crop for phytoremediation of site polluted with either biogenic or toxic heavy metals. The ornamental kale would be a high biomass crop that can accumulate the contaminant of lead and zinc in the soil. Thus, this variety and other edible plant in species B. oleracea cultivated in pollution region can be serious risk for human health.

### Footnotes

**Conflict of Interest:**

The authors declared no conflict of interest.

### References

1. Sharma P, Dubey RS. Lead toxicity in plants. Brazilian Journal of Plant Physiology. 2005;17(1): 35-52.
2. Babae Darzia V, Mohammadib MJ, Neisid A, Yarie AR, Takkastanf A, Charkhloog E, Moradih M,
Omidi Khaniabadih Y, Yusefzadeh A. Heavy Metals Removal from Sewage Sludge and Municipal Solid Waste (MSW) by Co-Composting Process. Arch Hyg Sci 2017; 6(3): 276-280
3. Chehregani A, Malayeri B. Removal of heavy metals by native accumulator plants. Internatinal journal of Agriculture and Biology. 2007; 462-465.
4. Yang YY, Jung JY, Song WY, Suh HS, Lee Y. Identification of rice varieties with high tolerance or sensitivity to Lead and characterization of the mechanism of tolerance. Plant Physiology. 2000; 124(3): 1019- 1026.
5. Ruley AT, Nilesh CS, Shrivendra VS. Antioxidant defense in a lead accumulating plants. Sesbania dormancies. Plant Physiology and Biochemistry. 2004; 41: 899-906.
6. Pallav Sh, Rama D. Lead toxicity in plant. Brazilian Journal of Plant Physiology. 2005; 17: 1-6.
7. Cakmak I, Marschner H. Effect of Zn nutritional status on superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. In: Barrow NJ (ed), Plant Nutrition from Genetic Engineering to Field Practice. Kluwer Academic Publishers, Dordrecht. 1993; 133-137.
8. Stanley B. Toxicology metals. Thonwiely of sons. 1987; 658.
9. Chaudhry TM, Hayes WJ, Khan AG, Khoo CS. Phytoremediation focusing on accumulator plants that remEDIATE metal contaminated soils. Australasian J. Ecotoxicol. 2001; (in press).
10. Ebbs SD, Kochian LV. Toxicity of zinc and copper to Brassica species: implications for phytoremediation. Journal of Environmental Quality. 1997; 26: 776-781.
11. Brown SL, Chaney RL, Angle JS, Baker AJM. Zinc and cadmium uptake by hyperaccumulator Th laspi caerulescens grown in nutrient solution. Soil Science society of America journal. 1995; 59: 125–133.
12. Alkorta I. Recent findings on the phytoremediation of soil contaminated with environmentally toxic heavy metals and metalloids such as zinc and cadmium, lead and arsenic*. Reviews in Environmental Science and Bio/Technology. 2004; pp. 71–90.
13. Brooks RP. Geobotany and hyperaccumulators. IN: R.R.Brooks (ed) plants that hyperaccumulate heavy metals. 1998; PP. 55-94.
14. Robinson BH, Banuelos G, Conesa HM, Evangelon WH, Schulin R. The Phyto management of Trace Elements in Soil. Critical Reviews in Plant Sciences. 2009; 28(4): 240-266.
15. Schmidt R, Bancroft I. Genetics and Genomics of the Brassicaceae; Springer: Berlin, Germany. 2010; p. 677.
16. Goncalves EM, Alegria C, Abreu M. Benefits of brassica nutraceutical compounds on human health. In Brassicaceae: Characterization, Functional Genomics and Health Benefits; Lang, M., Ed.; Nova Science Publishers: Hauppauge, NY, USA. 2013; pp. 20-65.
17. Gall JE, Rajakaruna N. The physiology, functional genomics, and applied ecology of heavy metal-tolerant brassicaceae. In Brassicaceae: Characterization, Functional Genomics and Health Benefits; Lang, M., Ed.; Nova Science Publishers: Hauppauge, NY, USA. 2013; pp. 121–148.
18. Neilson S, Rajakaruna N. Roles of rhizospheric processes and plant physiology in applied phytoremediation of contaminated soils using brassica oilseeds. In The Plant Family Brassicaceae; Anjum, N.A., Ahmad, I., Pereira, M.E., Duarte, A.C., Umar, S., Khan, N.A., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 2012; 21: 313–330.
19. Senaratna T, Touchell D, Bunn E, Dixon K. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regulation. 2000; 30: 157-161.
20. Alvarez M. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. Plant molecular Biology. 2000; 44:429-442
21. Tissa S, Darren T, Eric B, Kinsley D. Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regulation. (2000); 30: 157-161.
22. El-tayeb MA. Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regulation. 2005; 45: 215-224.
23. Metwally A, Finkemeier I, Georgi M, Dietz KJ. Salicylic acid alleviates the cadmium toxicity in barley seedling. Plant Physiology. 2003; 272-281.
24. Solgi E, Esmaili-Sari A, Riyahi-Bakhtiari A, Hadipour M. Soil Contamination of Metals in the Three Industrial Estates, Arak, Iran. Bulletin of Environmental Contamination and Toxicology. 2012; 88: 634–638.
25. Bajji M, Kinet JM, Slotts N. Osmotic and ionic effects of NaCl on germination, early seedling growth, and ion content of Atriplex halimus (Chenopodiaceae). Canadian Journal of Botany. 2002; 80: 297-304.
26. Madejon P, Murillo JM, Maranon T, Cabrera F, Soriano MA. Trace element and nutrient accumulation in sunflower plants two years after the Aznalcollar mine spill. The Science of the Total Environment. 2003; 307: 239-257.
27. Almansouri M, Kinet JM, Slotts N. Effect of salt & osmotic stresses on germination in durum Wheat. Plant and soil journal. 2001; 231:234-245.
28. Rahman Khan M, Mahmud Khan M. Effect of varying concentration of nickel and cobalt on the plant growth and yield of chickpea. Australian J. Basic and Applied Science. 2010; 4 (6): 1036–1046.
29. Shafiq M, Iqbal MZ, Athar M. Effect of lead and cadmiumgermination and seedling growth of...
Leucaenaleucopehala. Journal of Environmental Scienceand Management. 2008; 12 (2): 61- 66.
30. Taghizadeh M, Solgi M, Karimi M, Shahcheraghi T, noorayi Z. the effect of heavy metals on seed germination and regeneration of Bermudagrass lawns in vitro culture. Journal of Plant Production. 2013; 5(1): 95-108.
31. Almeida AF, Valle AA, Mielke MS, Gomes FP, Braz J. Tolerance and prospection of phytoremediator woody species of Cd, Pb, Cu and Cr. Plant Physiol. 2007; 19:83-98.
32. Jam M, Alemzadeh A, Mohammad Tale A and Esmaeili-Tazangi S. Heavy metal regulation of plasma membrane H+ -ATPase gene expression in halophyte Aeluropus littoralis. Molecular Biology Research Communications. 2014; 3(2):129-139
33. Manio T, Stentiford EI, Millner PA. The effect of heavy metals accumulation on the chlorophyll concentration of Typha latifolia plants, growing in substrate containing sewage sludge compost and watered with metaliferus water. Ecological Engineering. 2003; 20:65-74
34. Bertrand M, and Poirier I. Photosynthetic organisms and excess of metals. Photosynthetica. 2005; 43 (3): 345-353
35. Khatibi M, Rashed MH, Ganjeali A, Lahooti M. The effects of different nickel concentration on some morpho-physiological characteristics of parsely. Iran. J. Field Crops Research. 2008; 2: 295-302.
36. Popova L, Ananieva V, Ananieva V, Christov Z. Salicylic acid and methyl jasmonate induced protection on photosynthesis to paraquat oxidative stress. Bulgarian Journal of Plant Physiology (Special issue). 2003; 133-152.
37. Jing CH, Cheng Z, Li-ping L, Zhong-yang S, Xue-bo P. Effects of exogenous salicylic acid on growth and H2O2- metabolizing enzymes in rice seedlings under lead stress. Journal of Environmental Science. 2007; 19: 44- 49.
38. Belkhadi A, Hediji H, Abbes Z, Nouairi I, Barhoumi Z, Zrarouk M, Chaibi W, Djebali, salicylic acid pre-treatment on cadmium W. Effects of exogenous toxicity and leaf lipid content in Linum usitatissimum L.. Ecotoxicology and Environmental Safety. 2010; 1-8.
39. Kaydan D, Yagmur M, Okut N. (Effects of salicylic Acid on the Growth and some physiological characters in salt stressed wheat (Triticum aestivum L.) Tarim Bilimleri Dergisi. 2007; 13: 114-119.
40. Eraslan F, Inal A, David J, Gunes A. Interactive effects of salicylic asic and silicon on oxidative damage and antioxidant activity in spinach (Spinacia oleracea L.cv. Matador (grown under boron toxicity and salinity. Plant Growth Regulation. 2008; 55: 207-219.
41. Kabir M, IqbalMZ, Shafigh M, Faroogi ZR. Reduction in germination and seedling growth of Thespesiapopulnea L. caused by lead and cadmium treatments. Pakistan Journal of Botany. 2008; 40 (6): 2419-2426.
42. Khodary S. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed Maize plant. International Journal of Biology. 2004; 6: 5-8.
43. Hussein MM, Balbaa LK, Gaballah MS. Salicylic Acid and Salinity Effects on Growth of Maize plants. Journal of Agriculture and Biology Science. 2004; 3(4): 321-328.
44. Hanan E. Influence of salicylic acid on stress Tolerance during seed germination of Triticum aestivum and Hordeum vulgare. Advances in Biology Research. 2007; 1(1-2): 40-48.
45. Soltani F, Ghorbani M, and Manouchehri-kalantari KH. Effect of cadmium on photosynthetic pigments, sugars and malondialdehyde content in Brassica napus L. Iran. J. Biology. 2004; 2: 136-145.
46. Vishwakarma K, Upadhayay N, Kumar N, Yadav G, Singh J, Mishra RK, Kumar V, Verma R, Upadhayay RG, Pandey M, Sharma S. Abscisic Acid Signaling and Abiotic Stress Tolerance in Plants: A Review on Current Knowledge and Future Prospects. Frontiers in Plant Science. 2017; 8 (16): 1-12
47. Dolatabadian A, Modarres Sanavi S, Etemadi F. Effects of salicylic acid pretreatment on seed germination of wheat (Triticum aestivum L.) in salinity stress, Iranian Journal of Biology. 2008; 21 (4): 692-702.
48. Hall JL. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of endurant plants distributed in an old smeltery, northeast China. Environmental Geology. 2002; 51: 1043–1048.
49. Kosobrukhov A, knyazeva I. Plantago major plants responses to increase content of lead in soil: growth and photosynthesis. Plant Grow Regulation. 2004; 42:145-151.
50. Taghizadeh M, Kafi M, Fatahi Moghadam MR, Ravaghebi Firouzabad GHR, the effect of lead concentrations on seed germination of lawn and evaluation of its absorption to phytoremediation, Journal of Horticultural Science of Iran. 2011; 42 (3).