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

Mina Taghizadeh[a*], Mousa Solgi[a], Maryam Karimi[b], Mohammad Hosein Sanati[b], Shohreh Khoshbin[a]

[a]Department of Horticultural Science, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran.
[b]Department of Medical Biotechnology, National Institute of genetic engineering and biotechnology, Tehran, Iran.

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

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Background & Aims of the Study: The objective of this study was to investigate the seed pre-treatment with salicylic acid (SA) on tolerance and remediation ability of Brassica oleracea var. acephala under lead and zinc stresses.

Materials and Methods: Present study was conducted to evaluate phyto-accumulative ability of Brassica oleracea var. acephala in heavy metal concentrations. After seed disinfection, the seeds were soaked in the solution of SA (0, 200 and 300 mgL^{-1}) for 6 hours and cultured in media with different concentrations $ZnSO_4$ and $Pb(NO_3)$ (0, 50, 100 and 200 mgL^{-1}). Some germination indices such as the shoot to root ratio, leaf width, fresh and dry weight, chlorophyll content and absorption of heavy metal by seedling were investigated, after 14 days.

Results: The results showed that with increasing metal density, almost all of germination indices decreased significantly in comparison with the control. The most heavy metals accumulation was observed in seed pre-treatment with 300 mg L^{-1} SA and 100 mg L^{-1} metals treatment. By increasing the concentration of lead and zinc in the medium, the accumulation of metals was increased significantly in the plants. So, in the treatment of 100 mg L^{-1} of each metal, the accumulation of lead and zinc were 8500.5 and 1085.1 mg kg^{-1} in dry weight respectively.

Conclusions: The results show that that ornamental Kale can be used as a hyperaccumulator plant for lead and zinc in polluted regions in this study. The Ornamental kale would be a high biomass crop that can accumulate the contaminant of lead and zinc in the soil.

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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 causes 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 Brasicaceae 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 homogeny 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 $Pb(NO_3)_2$ in different concentrations (0, 50, 100 and 200 mg L^{-1}) and $ZnSO_4$ 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:

$$FGP = 100 \times \frac{Ng}{Nt} \qquad 1)$$

Where Ng is the total number of germinated seeds; 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:

$$GRI = \frac{G1}{1} + \frac{G2}{2} + \ldots + \frac{Gi}{i} \qquad 2)$$

Where Gi is germination percentage in day i.

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

$$CVG = \frac{\sum Ni}{(\sum NiTi)} * 100 \qquad 3)$$

Where N is the number of seeds germinated on day i, and Ti is the number of days from sowing.

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

$$MDG = \frac{\sum Nt}{\sum N} \qquad 4)$$

Where N is the number of germinated seeds, t is the number of days from the beginning of germination and ∑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:

$$AVG = \frac{\sum Nt}{\sum t} \qquad 5)$$

Where 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 GP is the germination percent, PL is the length of plumule and RL is the length of radicle:

$$SV = (PL + RL) \times GP \qquad 6)$$

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 replicates. 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).

Results

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 ($P \leq 0.01$). 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).

$ZnSO_4$

$Pb(NO_3)_2$

Figure 1) Comparison of seedlings growth ornamental kale in different concentrations (mg/l) of $ZnSO_4$ and $Pb(NO_3)_2$

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^{a*}	220.3^{b}	287.4^{a}	3.45^{c}	2.87^{c}
	50	94.4^{e}	192.5^{cd}	194.7^{b}	4.59^{b}	3.59^{a}
	100	98.9^{b}	238.5^{a}	266.9^{a}	3.54^{c}	2.92^{b}
	200	85.6^{g}	202.7^{bc}	265.6^{a}	3.64^{c}	2.61^{c}
Pb	0	97.8^{c}	199.9^{bcd}	192.4^{b}	4.47^{b}	3.64^{a}
	50	93.3^{f}	180.4^{d}	178.6^{bc}	4.8^{b}	3.72^{a}
	100	95.6^{d}	216.7^{b}	254.8^{a}	3.76^{c}	3.01^{b}
	200	40^{h}	68.4^{e}	138.9^{c}	5.58^{a}	1.88^{d}

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	Root (cm)	Leaf width (cm)
0	0	42.1^{abc*}	0.43^{abc}	0.11^{abc}	4.2^{de}	7.1^{a}	0.91^{ab}
	50	36.6^{bcd}	0.55^{a}	0.11^{abc}	7.6^{abc}	5.3^{b}	0.77^{bc}
	100	35.5^{cd}	0.46^{ab}	0.1^{abc}	∘5.5^{cd}	4.05^{c}	0.7^{cd}
	200	17.47^{ef}	0.32^{bcb}	0.14^{ab}	2.2^{e}	1.5^{ef}	0.58^{d}
200	0	46.6^{a}	0.53^{a}	0.16^{ab}	6.1^{bcd}	8.1^{a}	0.88^{ab}
	50	39.8^{abc}	0.45^{ab}	0.12^{ab}	8.1^{ab}	5.4^{b}	0.72^{cd}
	100	29.8^{d}	0.28^{cd}	0.07^{bc}	4.8^{d}	3.1^{c}	0.43^{e}
	200	13.6^{ef}	0.09^{e}	0.03^{c}	2.5^{e}	1.19^{f}	0.34^{e}
300	0	45.5^{ab}	0.58^{a}	0.14^{ab}	7.8^{ab}	8.1^{a}	0.96^{a}
	50	42.1^{abc}	0.43^{abc}	0.17^{a}	8.9^{a}	5.8^{b}	0.69^{cd}
	100	20.5^{e}	0.24^{de}	0.08^{abc}	4.3^{de}	2.3^{de}	0.38^{e}
	200	11^{e}	13.0^{e}	03.0^{c}	1.5^{d}	1.1^{f}	0.34^{e}

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 increased lead tolerance compared to the control. By increasing the concentration of heavy metals (especially lead), seeds vigor decreased and accelerated this characteristic by increasing the SA concentration. The highest percentage of FGP and SV were related to the control treatments. The highest CVG was obtained from the application of 50 mg L^{-1} of lead and the maximum of AVG and MDG were observed in 200 mg L^{-1} zinc and lead treatment respectively with 300 mg L^{-1} concentrations of SA. Also, the most GRI observed in 300 mg L^{-1} SA (control treatment) (Table 3).

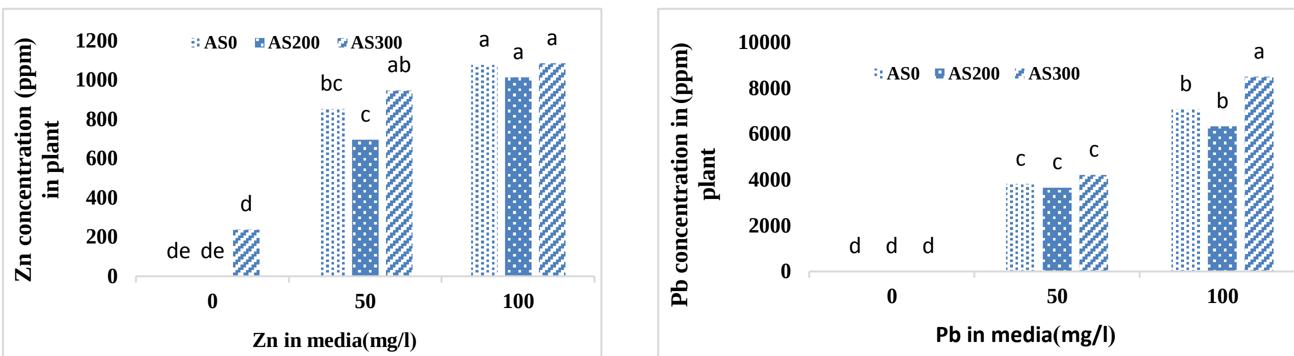
Table 3) The effects of different concentrations lead, zinc and salicylic acid treatment on the characteristics of ornamental kale

AS concentration (mg/L)	Metal	concentration (mg/L)	FGP	GRI	CVG	MDG	AVG	SV
0	**Zn**	0	100 ^{a*}	246.3 ^{ab}	295.5 ^{abc}	3.33 ^{ef}	2.77 ^{defgh}	99.98^{a}
		50	100 ^{a}	208.7 ^{abcde}	198.3 ^{cdefgh}	4.36 ^{cde}	2.63 ^{abcd}	36.37 ^{cdef}
		100	100 ^{a}	197.9 ^{cdef}	175.8 ^{fgh}	4. 76 ^{abcd}	3.97 ^{ab}	35.92 ^{cdef}
		200	93.3 ^{ab}	189.8 ^{efg}	207.9 ^{bcdefgh}	4.32 ^{cde}	3.38 ^{abcdef}	16.6 ^{efg}
	Pb	0	96.6 ^{ab}	232.4 ^{abcde}	266.7 ^{abcdef}	3.54 ^{def}	2.86 ^{cdefgh}	^{b}66.58
		50	90 ^{abc}	229.6 ^{abcde}	330.08 ^{a}	3.03 ^{f}	2.27 ^{ghi}	3.53 ^{cdef}
		100	80 ^{c}	186.1 ^{efg}	282.1 ^{abcd}	3.61 ^{def}	2.44 ^{fgh}	27.93 ^{defg}
		200	33.3 ^{e}	34.1 ^{h}	171.5^{fgh}	5.05 ^{abc}	1.44 ^{i}	6.92 ^{g}
200	**Zn**	0	100 ^{e}	241.7 ^{abc}	267.3 ^{abcdef}	3.70 ^{def}	3.08 ^{bcdefg}	95.52 ^{a}
		50	100 ^{e}	198.9 ^{cdef}	186.14 ^{defgh}	4.56 ^{bcde}	3.80 ^{abc}	55.9 ^{bc}
		100	96.6^{ab}	225.2 ^{abcde}	255.18^{abcdef}	3.82 ^{def}	3.05 ^{bcdefg}	34.33 ^{cdef}
		200	96.6^{ab}	200.3^{bcde}	194.9 ^{defgh}	4.43^{cde}	3.58^{abcd}	13.49^{efg}
	Pb	0	100 ^{e}	238.8^{abcd}	257.86 ^{abcdef}	3.63^{def}	3.02 ^{bcdefg}	63.43 ^{b}
		50	100 ^{e}	212.3 ^{abcde}	220.22 ^{abcdefg}	4.06 ^{cdef}	3.36 ^{abcdef}	3.72 ^{cdef}
		100	90 ^{ebc}	208.9^{abcde}	242.04 ^{abcdefg}	3.81 ^{def}	2.86 ^{cdefgh}	17.09 ^{efg}
		200	40 ^{de}	3.67 ^{h}	122.9 ^{h}	5.83^{a}	1.94 ^{hi}	5.91 ^{g}
300	**Zn**	0	100 ^{e}	253.42 ^{a}	299.06^{ab}	3.33 ^{ef}	2.77 ^{defgh}	89.32 ^{a}
		50	100 ^{e}	192.02^{defg}	192.72 ^{defgh}	4.48 ^{cde}	3.50 ^{abcd}	54.74 ^{bcd}
		100	86.6 ^{bc}	154.4 ^{fg}	153.1 ^{gh}	5.18 ^{abc}	3.75 ^{abcd}	23.27^{defg}
		200	90 ^{abc}	151.3 ^{g}	133.1 ^{h}	5.65 ^{ab}	4.19 ^{a}	12.91 ^{fg}
	Pb	0	100 ^{e}	244.3 ^{abc}	276.16 ^{abcd}	3.46^{ef}	2.88 ^{cdefgh}	96.10 ^{a}
		50	96.6 ^{eb}	208.3 ^{abcde}	214.2 ^{bcdefgh}	4.2 ^{cdef}	3.38 ^{abcdef}	38.78 ^{cde}
		100	86.6 ^{bc}	21.3 ^{abcde}	272.6 ^{abcde}	3.50 ^{ef}	2.52^{efgh}	2.18^{defg}
		200	46.6 ^{d}	73.99 ^{h}	122.4 ^{h}	5.86 ^{a}	2.27 ^{ghi}	5.04 ^{g}

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

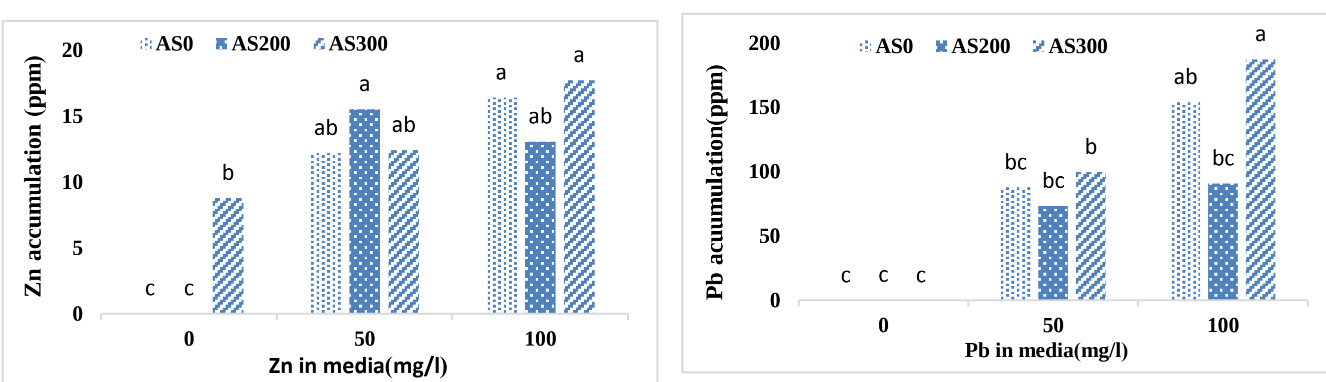
In this study, the accumulation of lead and zinc in Brassica oleracae pretreatmented seeds on SA different concentration was investigated. The treatment of 200 mg L^{-1} metals was waivered for the analysis, because of abnormality in seed germination and very low growth in this treatment. In this evaluation 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).



(b) (a)

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



(b) (a)

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 sulfhydryl 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^{-1}SA 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.

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