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ORIGINAL ARTICLE

Physiological and Biochemical Response of *Berberis integerrima* and *Cercis siliquastrum* to cadmium and lead stress

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ABSTRACT
Plant species have various physiological and biochemical mechanisms to tolerate and adapt to environmental stresses. The present study was aimed to evaluate oxidative stress of Cd and Pb on Berberis integerrima and Cercis siliquastrum. For this purpose, three-year-old seedlings with various concentrations (1000, 2000, 4000 and 6000 ppm) of Cd and Pb for 45 days in 15-day intervals were selected, and photosynthetic pigments content, malondialdehyde (MDA), proline, and catalase(CAT)were investigated as markers of oxidative stress. The results showed that chlorophyll remained unchanged in Cd stress where as in Pb treatment decreased. In addition, Carotenoid increased in both treatments. MDA content and proline also increased in both treatments. The response of antioxidant enzymes to Cd and Pb treatments was different in both species. Berberis integerrima and Cercis siliquastrum have different response to Cd and Pb treatments that the reason due to different physiological and biochemical responses of plants to environmental stresses.The results of this study can be used to choose the most appropriate plant according to the related stress especially under pollution stress.

***Keywords**: Lead, Cadmium, MDA, Berberisintegerrima, Cercis siliquastrum.*

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INTRODUCTION

Soil contamination by heavy metals is one of the most important environmental issues around the world [1].Heavy metals can be remained in the environment without any major change and bioavailable of these elements are different from other soil components [2]. All heavy metals are considered as pollutant in high concentration [3]. Although heavy metals have adverse impacts on plants, they can live in soils contaminated by high concentration of these metals [4]. Plants can remove pollutants in various ways; for example they can reduce the pollution by absorption, stability and transmission methods [5]. Cadmium (Cd) and lead (Pb)are the most poisonous elements[6] that the first one leads to contaminate soil and prevents the root and stem growth as well as affects plant homeostasis. Antioxidant enzymes can't have their normal activities in high concentration of Cd [5] and also Cd changes cell members by lipid peroxidation and chloroplast metabolism by prevention of chlorophyll biosynthesis [7]. Second one, Pb, due to its wide distribution and danger is extremely considered for environment. Hence, soil contamination by Pb can affect both microorganism activities and physiological parameters of plants [5]. Heavy metals have impacts on physiological processes that one of them is producing the large number of reactive oxygen spices (ROS), which damage proteins, lipids and DNA [8]. The activities of antioxidant enzymes, such as SOD, CAT, APX and GR, will be increased in response to increasing the ROS. In addition, in stress conditions, ROS causes damage to cell membranes resulting in lipid peroxidation and production of malondialdehyde (MDA) [9]. Proline due to its significant role in photosynthetic activities, protein stability and cell membrane by removing ROS is an important non-enzyme antioxidant in plants under

stress condition [10].For this purpose, the present study was aimed to evaluate the physiological and biochemical responses of *Berberis integerrima* and *Cercis siliquestrum*, two main ornamental shrubs planting in urban areas of Iran, to Pb and Cd stress.

MATERIAL AND METHODS

Experimental treatments

Three-year-old seedlings of *Berberisintegerrima* and *Cercis siliquastrum* in Alborz nursery belonging to the Research Institute of Forests and Rangelands, Karaj, were cultivated in plastic pots containing approximately 8kgof soil. After that, the plants were treated by cadmium chloride and leadnitrate separately in concentrations of 0,1000,2000,4000,6000ppm, 100ccperplantin threetimesat intervals of 15 days. Subsequently, the leaves of both spices were sampled in four directions of crown one month after the last treatment. Chlorophyll content was measured by Arnon method [10].MDA content in leaves of seedlings was determined according to the method of Stewart and Bewley [11]. Proline concentration was recorded based on the method of Bates *et al* [12].Catalase activity: For this purpose, 100 ml of phosphate buffer1/0 mM and200mlof hydrogen peroxide3% were used to prepare the final buffer. Then10mlof enzyme extract was added to 2 µl of the final buffer. Finally, the samples were read by spectrophotometer at wavelength of240 nm based on Unit/mg protein FW. Data were submitted to CRD and Duncan multiple range test using SAS 9.1. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The content of photogenic pigments

The results showed that although different levels of cadmium caused a slight decrease in chlorophyll and carotenoid in *Berberis integerrima* with respect to control, differences among stress levels were not statistically significant. This value was found for chlorophyll a, b and total chlorophyll in *Cercissi liquastrum,* but increasing the cadmium concentration resulted in a significant increase of 66% for carotenoidin comparison with control at stress level of 6000 ppm. Pb had a greater impact on chlorophyll and carotenoid than Cd (table 1). As is shown by table 1, increasing the Pb concentration reduced chlorophyll a, in both species so that the lowest value was found at 4000 ppm (10% reduction to control) for *Berberis integerrima* and at 6000 ppm (24% reduction to control) for *Cercissi liquastrum*. There was no significant difference among the various levels of Pb in terms of chlorophyll b and total chlorophyll for *Cercissi liquastrum*, but increasing Pb concentration up to 2000 ppm caused a 27-30 % augmentation of carotenoid and this value was not observed in higher stress levels. For *Berberis integerrima*, enhancing Pb had no effect on chlorophyll b, but there was found in Chlorophyll a 18 % reduction and a 12 % increase for total chlorophyll and carotenoid at 6000 ppm, respectively. Chlorophyll content in plants is often measured to assess environmental stresses. Although Cd had been known as a highly toxic metal than Pb for plants [13-15]. Different authors have reported chlorophyll reduction and carotenoid increment under the influence of heavy metals in different plant species [16, 17]. In this research, Pb had a greater impact on chlorophyll content and led to reduce it. Sinha and Shrivastava [16] reported the reduction of chlorophyll a and b in *Brassica juncea*under Pb stress.

Table1. Photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoid) in Cd and Pb treatments

	Treatment (ppm)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Cercis siliquastrum	Cd				
	0	0.00416 a	0.01030 a	0.01472 a	0.00028 c
	1000	0.00383 a	0.00888 a	0.01295 a	0.00036 c
	2000	0.00400 a	0.00992 a	0.01417 a	0.00063 b
	4000	0.00384 a	0.00895 a	0.01302 a	0.00067 b
	6000	0.00397 a	0.00887 a	0.01308 a	0.00081 a
	Pb				
	0	0.00416 a	0.01030 a	0.01472 a	0.00039 b
	1000	0.00414 a	0.01022 a	0.01463 a	0.00053 a
	2000	0.00418 a	0.01022 a	0.01466 a	0.00055 a
	4000	0.00375 b	0.00934 a	0.01332 a	0.00034 b
	6000	0.00409 ab	0.00984 a	0.01419 a	0.00034 b
Berberisintegerrima	Cd				
	0	0.00361 a	0.00757 a	0.01132 a	0.00043 a
	1000	0.00294 a	0.00726 a	0.01038 a	0.00055 a
	2000	0.00339 a	0.00756 a	0.01116 a	0.00047 a
	4000	0.00342 a	0.00755 a	0.01118 a	0.00036 a
	6000	0.00359 a	0.00792 a	0.01173 a	0.00043 a

Pb				
0	0.00361 a	0.00757 a	0.01132 a	0.00043 b
1000	0.00344 a	0.00720 a	0.01084 ab	0.00037 b
2000	0.00328 a	0.00704 a	0.01052 ab	0.00040 b
4000	0.00323 ab	0.00683 a	0.01025 ab	0.00043 b
6000	0.00274 b	0.00644 a	0.00935 b	0.00063 a

MDA content

MDA in both *Berberis integerrima* and *Cercis siliquastrum* was significantly increased with increasing Pb and Cd concentration (Figure 1). The maximum MDA was observed in Cd stress for both species at 6000ppm that it indicates large amount of ROS production in this treatment. However, the highest lipid peroxidation in Pb stress for *Berberis integerrima* and *Cercis siliquastrum* was at 6000 ppm and 4000 ppm, respectively. MDA is an oxidized product of membrane lipids and will be accumulated when plants face oxidative stresses. MDA concentration is often considered as an index of lipid peroxidation in stress levels [18, 19]. In this research, MDA content was increased with increasing stress rate indicating the increase of oxidative stress at high concentrations of Pb and Cd. Similar results have been reported for *Lonicera japonica* under Cd stress [20].

Proline content

There was non-significant change in proline content of *Cercis siliquastrum* in Cd stress, while proline content led to a gradual increase in Pb stress so that the maximum stress level was 6000 ppm (figure2).On the other hand, increasing Cd had more impact on *Berberis integerrima* than *Cercis siliquastrum*. Treatments of 6000ppm and 4000ppm had 49% and 21% increases, respectively, in comparison with control. In addition, proline content increased up to 61% and 55 % in Pbstress treatments of 6000 ppm and 4000 ppm, respectively. Similar results in various plant species regarding increasing proline under Cd and Pb stresses have been obtained [21, 22]. Proline accumulation in response to non-live stresses may be due to de novo synthesis and its decomposition decrease [23].

CAT activity

Different concentrations of Cd in both *Berberisintegerrima* and *Cercis siliquastrum* had a significant effect on CAT activity. Effect of Pb stress on CAT activity was different in two species (figure3). Enzyme activity was increased with increasing stress intensity in *Cercis siliquastrum* (47-56%) and the maximum activity was observed at stress level of 4000 ppm. In contrast, there was approximately found a 41% reduction of CAT activity at 4000 ppm and 6000 ppm with respect to control for *Berberis integerrima*. CAT decomposes hydrogen peroxide to oxygen and water [24, 25]. ROS induced by heavy metals stress leads to inhibit these enzymes activities by attacking the antioxidant enzymes and making the oxidative damages and finally H_2O_2 will be increased by decreasing CAT activity [34]. Decreasing the CAT activity with increasing Cd concentration in some plants due to reduction of plant proteins by metal toxicity and oxidative stress have been reported [26].

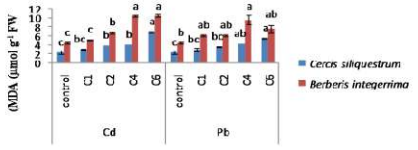

Figure1: The effects of Cd and Pb on MDA content of *Berberis integerrima* and *Cercis siliquastrum* leaves. C1, C2, C4 and C6 are 1000, 2000, 4000 and 6000 ppm, respectively. Numbers followed by same letters indicate significant differences at p<0.05.

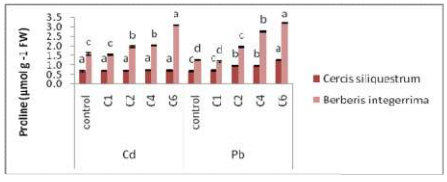

Figure2: The effects of Cd and Pb on proline content of *Berberis integerrima* and *Cercis siliquastrum* leaves. C1, C2, C4 and C6 are 1000, 2000, 4000 and 6000 ppm, respectively. Letters indicate significant differences at p<0.05.

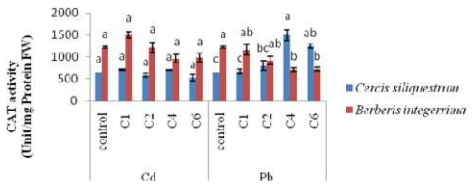

Figure3: The effects of Cd and Pb on CAT activity of *Berberisintegerrima* and *Cercis siliquastrum*l eaves. C1, C2, C4 and C6 are 1000, 2000, 4000 and 6000 ppm, respectively. Letters indicate significant differences at p<0.05.

CONCLUSION

The present study has shown the effects of Cd and Pb concentrations on physiological and biochemical properties of *Berberisintegerrima* and *Cercis siliquastrum* and their response to these metals. Both species have different physiological and biochemical responses to heavy metal stress except to Cd stress, and results showed both species have the same potential for tolerating various concentrations of Cd. In

regarding to Pb stress, *Berberisintegerrima* is more tolerant than *Cercis siliquastrum.* Therefore, in soils contaminated by pb, *Berberisintegerrima* will be recommended.

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