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ORGINAL ARTICLE

Superoxide Dismutase and Peroxidase activities under Lead and Cadmium Stresses in *Berberis integerrima* and *Cercis siliquasrum*

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ABSTRACT
Plants have various biochemical mechanisms to tolerate and adapt to environmental stresses. The study was conducted to evaluate superoxide dismutase (SOD) and peroxidase (POD) activities under Pb and Cd stresses in Berberis integerrima and Cercis siliquasrum. 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-days intervals were selected, and superoxide dismutase (SOD) and peroxidase (POD) were investigated as markers of oxidative stress. Results showed the response of antioxidant enzymes to Cd and Pb treatments was different in both species. Berberis integerrima and Cercis siliquasrum have different response to Cd and Pb treatments that the reason due to different 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: *Berberis integerrima, Cercis siliquasrum, SOD, POD.*

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INTRODUCTION

Soil contamination with heavy metals is one of the most eminent environmental issues around the world [4], [13]. Heavy metals can be remained in the environment with no major changes and bioavailable of these elements are different from other soil components [11]. All heavy metals are considered as pollutant in high concentration [7]. Although heavy metals have adverse impacts on plants, they can live in soils contaminated by high concentration of heavy metals [17]. Plants can remove pollutants in various ways. For instance they can mitigate pollution by absorption, stability and transmission methods [14]. Cadmium (Cd) and lead (Pb) are the most poisonous elements [27] which the first one leads to contaminate soil and prevent the root and stem growth as well as affects plant homeostasis. Antioxidant enzymes can't have their normal activities in high concentration of Cd [18] and also Cd changes cell members by lipid peroxidation and chloroplast metabolism by prevention of chlorophyll biosynthesis [21], Second one (Pb) due to its wide distribution and danger is extremely considered for environment. Hence, Soil contamination with Pb can affect both microorganism activities and physiological parameters of plants [18] Heavy metals have impacts on physiological processes that one of them is the production of reactive oxygen spices (ROS) which damages proteins, lipids and DNA [24]. 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 damaging to cell membranes which results in lipid peroxidation and production of malondialdehyde (MDA) [10]. Urban green space can alleviate the pollution. Therefore, using the trees and shrubs tolerating to pollution is one of the most important principles in creating the efficient green space. Hence, the more information about biochemical responses of plants to pollution stress is essential to choose the most appropriate spices in urban areas. For this purpose, the present study aimed to evaluate the Superoxide dismutase and Peroxidase responses of *Berberis integerrima* and *Cercis siliquasrum*, 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 *Berberis integerrima* and *Cercis siliquasrum* in Alborz nursery belonging to the Research Institute of Forests and Rangelands, Karaj, were cultivated in plastic pots containing approximately8kgof soil. After that, the plants were treated by cadmiumchloride and leadnitrate separately in concentrations of 0, 1000, 2000, 4000, 6000ppm, 100ccperplant in three times at 15-days intervals. Subsequently, the leaves of both spices were sampled in four directions of crown one month after the last treatment.

Superoxide dismutase (SOD)

The method of Giannopolitis and Ries [15] was used to measure SOD activity. The mixture consisted of phosphate buffer 50 mM (pH =7.8), methionine 13 mM, Nitro blue tetrazolium (NBT) 75 µM, EDTA 1/0 m, riboflavin 13 mM and 50 ml of enzyme extract. Then the mixture was under fluorescent light for 15 min and in the dark for 15 min and its absorption was read by spectrophotometer at the wavelength of 560 nm according to Unit / mg protein FW.

Peroxidase (POD)

POD activity was recorded according to the method of [8]. The mixture was contained phosphate buffer 0.1mM (pH = 7.5), Pyrogallol solution 4 mM, hydrogen peroxide3mMand100 ml of enzyme extract. After that, the samples were read by spectrophotometer at wavelength of 240 nm for 20 min based on Unit / mg protein FW.

Statistical analyses

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

SOD activity

As is shown by figure 1, SOD activity was lower than that in control at all treatments of Cd stress for *Cercis siliquasrum*. There was a significant reduction at low levels of stress (1000ppm and2000 ppm) by approximately42%-48%. In addition, the enzyme activity was increased in concentration of 4000ppm and dramatically decreased in 6000ppm (77%) under Cd stress However, The reduction in SOD activityin4000ppm of Pb was not statistically significant and the SOD activity was increased in 6000 ppm. Generally, enzyme activity in *Berberis integerrima* was less than *Cercis siliquasrum,* but plant response to stress levels was different. SOD was significantly increased by increasing Cd stress (64-75%) and there was found a remarkable reduction of SOD at 1000 ppm (47%). SOD exists in all oxygen metabolism cells, and SOD in the cytoplasm, mitochondria and chloroplasts are found to superoxide radicals, hydrogen peroxide converts [2]. Similar results in different plant species about alleviating oxidative stress by increasing antioxdant enzymes have been reported [5], [28]. In some cases increasing stress have resulted in reduction of enzyme activity that this issue can be due to inactivation of the enzyme by enzyme catalysis or non-specific binding of heavy metals to be related enzyme activity center[12].

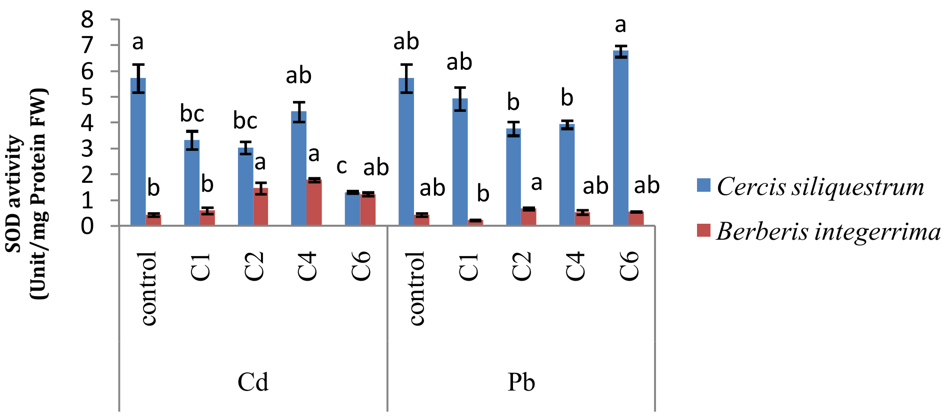

Figure1: The effects of Cd and Pb on SOD activity of *Berberis integerrima* and *Cercis siliquasrum* leaves. C1, C2, C4 and C6 are 1000, 2000, 4000 and 6000 ppm, respectively. Letters indicate significant differences at $p<0.05$.

POD activity

Results of Cd and Pb effects on POD activity are presented in figure 2. There was no significant difference in both *Berberis integerrima* and *Cercis siliquasrum*for various levels of Cd. POD activity in *Berberis integerrima* was more than *Cercis siliquasrum.* However, plant responses to Pb stress were different at different concentrations. There was observed a 42% increase of POD activity at 1000 ppm in *Cercis siliquasrum* in comparison with control, while POD activity in *Berberis integerrima* was less than control and the lowest activity was observed in 1000 and 6000 ppm of Pb. POD has an important role in plant response to stress and it will be active under stress condition [25]. Antioxidant enzyme response to heavy metals varies among different species and even among different organs [20]. This issue can be supported by different POD activity between *Berberis integerrima* and *Cercis siliquasrum* even in same treatments in the present study. Many studies have revealed that increasing POD is in association with heavy metals (Shu et al, 2011; [16], while decreasing POD activity by heavy metals have been reported by some authors (Shu et al, 2011; [19], [22]. In the present study, decreasing the enzyme activity was observed in the high concentrations. The ability of plants appears to be limited to increase the activity of antioxidant enzymes to counteract the effects of stress. Studies have shown increasing the concentrations of heavy metals eventually lead to the loss of all antioxidant enzymes. Enzyme activity at low concentrations of heavy metals increases and it will decrease by increasing heavy metal concentration and finally will decrease after passing threshold, depending on plant [6]. It also induces long-term effects of heavy metals increase the enzyme activity, especially the activity of POD [23].

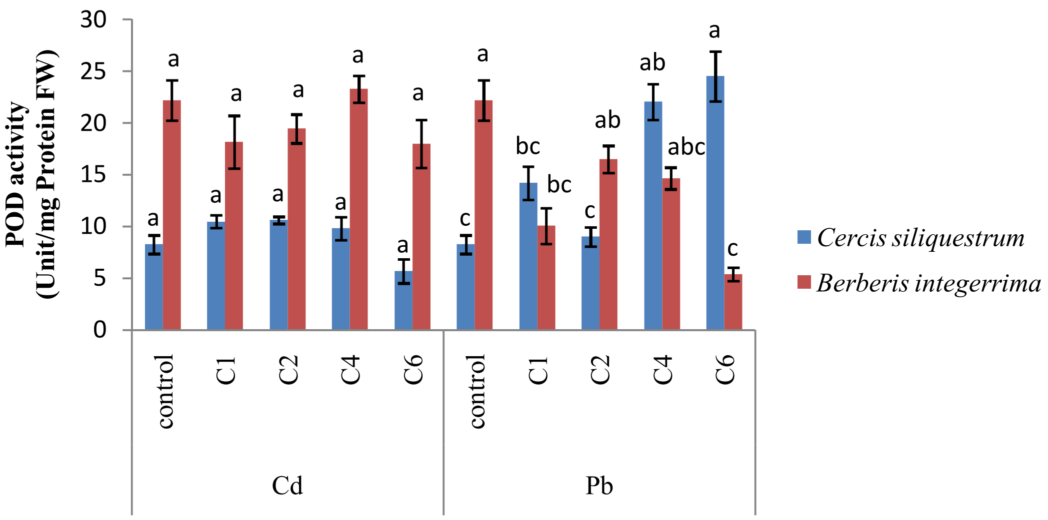

Figure2: The effects of Cd and Pb on POD activity of *Berberis integerrima* and *Cercis siliquasrum* leaves. C1, C2, C4 and C6 are 1000, 2000, 4000 and 6000 ppm, respectively. Letters indicate significant differences at $p<0.05$.

CONCLUSION

The present study has shown the effects of Cd and Pb concentrations on biochemical properties of *Berberis integerrima* and *Cercis siliquasrum* and their response to these metals. Both species have different 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, *Cercis siliquasrum* is more tolerant than *Berberis integerrima.* Therefore, in soils contaminated by Pb ,*Cercis siliquasrum* will be recommended.

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