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Antioxidant Enzymes Functions of Vetiveria zizianoides During the Absorption of Cadmium in Soil

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Research Article

Abstract

Given the importance of cadmium in the ecosystem pollution, the remediation of soils contaminated with this heavy metal in particular through phytoremediation is necessary and inevitable. This research was aimed to investigate the toxicity effects of Cadmium Chloride on the function of antioxidant enzymes in *Vetiveria zizianoides.* The experiment was performed in plastic pots in the Baghou nursery, affiliated to the Department of Natural Resources. At the beginning of the experiment, irrigation was done two times a day and then, due to the moisture in the environment, irrigation was administered once daily. Treatments included 0, 20, 40 and 60 mg/l Cadmium Chloride, arranged in a randomized complete blocks design with four treatments and five replications. The root growth of the plant is high; therefore, after the initial growth of the plant, they were transferred to the field and irrigated with the treatments for two months. At the end of the period, samples were taken and Cd content in root, stem and leaves and the activity of antioxidant enzymes were measured. According to the obtained results, with increasing concentration of Cadmium Chloride, a significant increase was observed for the enzyme activity of Catalase, Peroxidase, Superoxide dismutase, Glutathione reductase, Polyphenol oxidase, Ascorbate peroxidase and Guaiacol peroxidase. In addition, Cd absorption and accumulation was higher in roots as compared to the shoots. The results clearly showed the high capability of Vetiver for the remediation of soils contaminated with Cd. Thus, this plant could be considered as one of the suitable candidates for cultivation in industrial areas.

Keywords: Cadmium; Anti-oxidant enzymes; *Vetiveria zizianoides*.

1. Introduction

Heavy metals at concentrations above the threshold are among the environmental pollutants found especially in the soils of all parts of industrial and agricultural communities [1]. Toxicity of heavy metals and their accumulation in food chains is one of the main environmental and health problems of modern societies [2]. However, these heavy metals contaminated soils can be purified by chemical, physical and biological methods [3]. Investigating the history of research indicates that some cultivars such as barley, alfalfa, mustard, radish, sunflower, peanut, castor, corn and...are modifying the contaminated soils. Certain plant species can transfer heavy metals to the limb [4]. Therefore, harvesting of heavy metals from polluted sites can be effective in extracting heavy metals from the soil without any high costs such as landing, transport and extraction of surface soils from the area [5].

Phytoremediation is a low and simple technology for depleting soil from heavy metals that has been considered in recent years. This technology is used by plants to remove pollutants from soil, water and sediment as a relatively new technology through root refinement, stabilizing plant, absorbent plant, substrate and degrading plant, which causes removal, decomposition or blockage of pollutants [6]. Cadmium is a heavy metal, usually found in the form of anionic compounds, hydrated ions, or complex compounds such as carbonate, hydroxide, chloride, sulfate and organic compounds with humic acid [1]; Due to its high motility and soil absorption by the plant, significant toxicity and biological half-life of about 20 years and the complications of liver and kidney failure, cardiovascular disease, bone, pulmonary, and other diseases in humans are very important [7]. According to Mishra et al. [8] the cadmium content of the plant is 1 to 0.1 mg/L. Most non-contaminated soils contain cadmium less than 1 mg/L [9]. The use of sewage sludge, urban waste and chemical fertilizers containing cadmium (such as phosphorus fertilizers) increases the concentration of cadmium in the soil [10]. When the concentration of cadmium in soil is high, the processes that microorganisms do in the soil are disrupted and the whole ecosystem of the soil is in danger. In the meantime, as the examples of its effects mention, plants are completely exposed to pollution due to lack of mobility. Therefore, they are

more vulnerable to pollutants and other environmental stresses than other living organisms [10]. High concentrations of cadmium lead to a reduction in the absorption of nutrients, preventing enzymatic activity and inducing oxidative stress, which includes alterations in the enzymes of the antioxidant defense system [10,11]. It has also been reported that the cadmium content of 3 mg/kg of plant growth will be stopped; Photoconductive pigments have an inhibitory effect on the electron transfer system and interferes with ATP synthase and NADH oxidase enzymes [4]. Cadmium causes leaf tubing, chlorosis and reduced root and stem growth [12]; he process of germination and growth of seedlings can be limited [13]. Also, acute toxicity to cadmium may cause death of animals and birds and severe poisoning in aquatic animals [14]. Therefore, given the importance of cadmium in contamination of ecosystems, providing methods to reduce contamination, especially contaminated soil contamination, is unavoidable [7]. Early studies indicate that cadmium absorption is different among genotypes of plants. Therefore, it is possible to identify low-cadmium absorbing varieties and species. Differences in root cadmium uptake and the rate of transmission and accumulation in aerial parts are the main factor in explaining the differences between different genotypes in tolerance to cadmium toxicity. *Vetiveria zizanioides* is a forage germinaceae, a herb that is used to repair and regenerate lands that have been degraded; It has a lot of talents to absorb soluble elements such as nitrogen, phosphorus and significant absorption capacity of heavy and toxic metals and solutions in contaminated waters [15]. In this study, the function of this plant in absorbing soil Cd in different organs (leaves, stems, roots) and enzymatic changes caused by cadmium uptake in plants were investigated.

Das et al. [16] showed that cadmium affects cells division and growth, overall growth of plant, meristematic zone cell division and regulating plant growth and development and its impact varies depending on the type of plant. Vitória et al. [17] showed that after 13 h of exposure to cadmium, the activity of catalase and glutathione reductase in the roots and leaves of radish increased. Xu et al. [18] showed that cadmium and other heavy metals caused GSH depletion and suppressed the GR (glutathione reductase) activities. Polle et al. [19] during the autopsy through superoxide dismutase - ascorbate-glutathione, showed that cadmium inhibits the activity of antioxidant enzymes such as catalase, glutathione peroxidase and ascorbate peroxidase by glutathione depletion in the plant. According to Lamattina et al. [20], GSH and its metabolism enzymes provide an effective protection against the damages of ROS through chelating heavy metals and ward off toxicity. Bergmann [21] investigated the role of integrating signals in the development of plant stomata and showed that cadmium stress reduced the number of stomata on the upper and lower surface of leaves. According to Kumar et al. [22], the activity of catalase and peroxidase in wheat was increase against oxidative stress. Tegelberg et al. [23] showed that there was a relationship between the amount of phenol present in the plant and polyphenol oxidase activity so that polyphenol oxidase activity increased by increasing of total concentration of soluble phenol. Furthermore, Ashraf et al. [24] indicated that cadmium inhibited enzymes activities directly by reaction with -SH groups or indirectly by disrupting the balance of ions at the cellular level.

2. Materials and Methods

2.1 Study area

All cultivation operations were performed at a five-hectare nursery, called Baghou, affiliated to the Department of Natural Resources, Hormozgan Province.

2.2 Preparation and planting method

Vetiveria zizanioides, a forage species belonging to the Gramineae family, grows naturally in many parts of the world. Vetiver grass has a tendency to social life and lives in groups. It is a fast-growing species used for restoration of degraded lands due to the specific features in roots, shoots and leaves. Plant roots were obtained from the Department of Natural Resources, Hormozgan province. It is worth mentioning that plant roots have been imported from the Genetic Research Center of the UAE. The experiment was performed in plastic pots in the Baghou nursery, affiliated to the Department of Natural Resources Hormozgan province. Overall, 100 pots were planted of which 85 pots were selected and 15 pots were excluded from the experiment in which planting was unsuccessful for unknown reasons including climate factors or root infection.

Early planting was in April and the initial plant growth reached normal by June. In the first two weeks, irrigation was done two times a day and then, due to the moisture in the environment, irrigation was administered once daily. Since the root growth of the study species is high, they were transferred to the field and the study was performed with the same statistical method expressed. The plants were irrigated with four treatments (0, 20, 40 and 60 mg/L cadmium chloride) for two months and they were harvested eight weeks later.

2.3 Treatments

Treatments included 0, 20, 40 and 60 mg/L cadmium chloride, arranged in a randomized complete blocks design. Each treatment was randomly applied to 21 pots from 15 June. Experiments were conducted at two stages.

In the first stage of the experiment, soil analysis was performed at planting time (15 April). Physical and chemical properties of soil were determined as follows: pH, electrical conductivity (EC), soil organic matter content, Cd concentration, extractable by Diethylene Diamine Penta-acetic acid (DTPA). At the

second stage, On 15 August, cadmium content in soil, roots, stems and leaves with 5 replications per treatment was again measured randomly.

In total, 20 samples of soil, roots, stems, and leaves were transferred to the research lab of Bandar Abbas Branch, Islamic Azad University. All samples were read by atomic absorption. Different plant organs including roots, stems and leaves were dried at 80°C (for 48 h) to be prepared for biochemical and physiological measures.

After harvesting and removing the shoots from the roots, five replicates of each treatment were kept in the freezer of the Research Laboratory at -80°C for the experiments requiring fresh tissue.

2.4 Soil physical and chemical properties

Soil physical and chemical properties were determined in two stages (15 March-15 August) as follows: Soil pH and EC were measured using pH meter and EC-meter in the saturation extract. Soil organic matter content was determined by Walkley and Black (1934) method. The hydrometer method also was used to determine soil texture based on the percentage of clay, silt, and sand.

2.5 Determining Cd concentration in the plant

To measure extractable cadmium in plant tissues (roots, stems and leaves), DTPA-TEA method was used Lindsay and Norvell (1978).

2.6 Measurement of antioxidant enzymes

For the enzymatic extraction, at first, 0.25 g of powdered leaves and roots was immediately weighted by liquid nitrogen and was poured in 1.5 ml Eppendorf. Then, one ml of 50 mM potassium phosphate buffer (pH=7.5), containing 11% triton was added to each Eppendorf. All stages of extraction were performed on the ice. The samples were then placed in the refrigerator for one hour. Extracts were centrifuged for 15 min at 15000 g and 4°C.

The supernatant was used to measure enzyme activity. Measurement of Peroxidase and Polyphenol oxidase activity was performed using the Kar et al. [25] method. Measurement of Catalase activity was performed by the method of Aebi [26]. Superoxide dismutase, APX, and Guaiacol peroxidase activity were assayed by the Gianopolitis et al. [27], Nakano et al. [28] and Updhyaya et al. [29] methods, respectively. Glutathione reductase activity was assayed by oxidation of NADPH at a wavelength of 340 nm [30].

2.7 Data analysis

The experiment was conducted in a completely randomized design with four treatments and five replications. Data were analyzed using SAS 10.3 statistical software. For all data, means and standard errors were calculated and ANOVA was used to compare the significance of changes in the experimental group with the control group at $P<0.05$ and $P<0.01$. Additionally, the mean comparison of data was performed at $P \leq 0.01$ and $P \leq 0.05$ using LSD test. Means with at least one common letter designation are not different, at $P<0.05$.

3. Results

3.1 Results of soil analysis

Soil analysis at the start of planting (15 April) and harvesting (15 August) showed that the pH value was fixed at 6.5, EC increased from 2.6 to 2.7 ds/m and the concentration of cadmium increased from 0.13 to 5.7 Mg/kg of soil. The soil texture was loam-loamy clay (Table 1).

3.2 Cadmium content of leaf, shoot and root in vetiver

The leaf cadmium content was calculated to be 0.90 ± 0.27, 1.72 ± 0.3, 3.80 ± 0.37 and 6.36 ± 0.29 mg/kg dw with increasing concentrations of Cd chloride (0, 20, 40 and 60 mg/L), respectively. As well, the root and shoot cadmium content was calculated to be 1.25 ± 0.06, 3.68 ± 0.25, 8.25 ± 0.34 and 13.38 ± 1.20 mg/kg dw and 0.08 ± 0.06, 0.53 ± 0.08, 1.84 ± 0.15 and 2.52 ± 0.18 mg/kg dw with increasing concentrations of Cd chloride, respectively (Table 2).

The results of measuring Cd content in leaves, shoots and roots showed that the uptake and accumulation of Cd in the plant increased with

Table 1. Soil analysis during the experiment.

Properties / Experiment stage	pH	EC (ds/m)	Soil organic matter	Soil Cd (mg/kg DW)	Soil Texture (%)		
					Clay	Sand	Silt
Planting (15 April)	6.5	2.6	9.29	0.13	21	36	42
Harvesting (15 August)	5.7	2.7	9.4	5.7	20	35	42

Table 2. Mean comparison of traits under the effect of different concentrations of cadmium chloride.

Plant Organs Cd (mg/kg dw)	Cadmium Chloride Concentration (mg/l)			
	0 (control)	20	40	60
Leaf	0.90 ± 0.27^{c}	1.72 ± 0.23^{c}	3.8 ± 0.37^{b}	6.36 ± 0.29^{a}
Root	1.25 ± 0.06^{c}	3.68 ± 0.25^{c}	8.25 ± 0.34^{b}	13.38 ± 1.2^{a}
Shoot	0.08 ± 0.06^{c}	0.53 ± 0.08^{c}	1.84 ± 0.15^{b}	2.52 ± 0.18^{a}

increasing concentrations of Cd chloride, and Cd concentration in roots was more than that of leaves and shoots (Figure 1). According to the LSD test, there is a significant difference at a concentration of 60 mg/L of cadmium chloride (P<0.01).

3.3 Analysis of antioxidant enzyme activities

Table 3 shows mean comparison of antioxidant enzymes under the effect of different concentrations of cadmium chloride.

According to the Table 3, the Catalase enzyme content was increased with increasing concentrations of cadmium chloride, showing a significant difference at a concentration of 60 mg/L compared to the control (P<0.01). However, there were no significant differences between treatments of 20 and 40 mg/L (P<0.05) (Figure 2).

Mean comparison of root and shoot Peroxidase showed that the enzyme content was increased with increasing concentrations of Cd chloride, showing a significant difference at a concentration of 60 mg/L compared to the control (P<0.01). However, no significant differences were found for root and shoot peroxidase content at concentrations of 20 and 40 mg/L and 40 and 60 mg/L Cd chloride even at 5% level of probability (Figure 3). The same results were found for Superoxide dismutase (Figure 4), Glutathione reductase (Figure 5) and Polyphenol oxidase (Figure

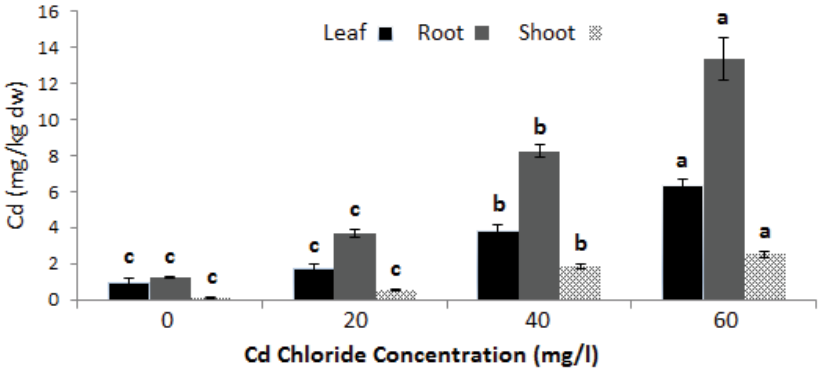

Figure 1. Mean comparison of leaf, root and shoot cd content under the effect of different concentrations of cadmium chloride.

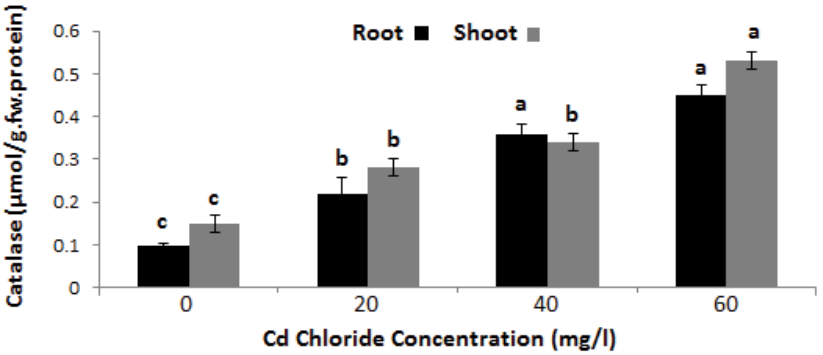

Figure 2. Mean comparison of root and shoot catalase content under the effect of different concentrations of cadmium chloride.

Table 3. Mean comparison of antioxidant enzymes under the effect of different concentrations of cadmium chloride.

Trait (µmol/g.fw.protein)	Cadmium Chloride Concentration (mg/l)			
	0 (control)	20	40	60
Shoot Catalase	0.1 ± 0.004^{c}	0.22 ± 0.037^{b}	0.36 ± 0.024^{a}	0.45 ± 0.022^{a}
Root Catalase	0.15 ± 0.02^{c}	0.28 ± 0.02^{b}	0.34 ± 0.02^{b}	0.53 ± 0.02^{a}
Root peroxidase	0.6 ± 0.08^{b}	0.78 ± 0.05^{ab}	0.84 ± 0.04^{ab}	1.01 ± 0.13^{a}
Shoot peroxidase	0.44 ± 0.02^{b}	0.6 ± 0.04^{b}	0.89 ± 0.05^{a}	0.82 ± 0.06^{a}
Leaf superoxide dismutase	0.15 ± 0.02^{c}	0.28 ± 0.04^{b}	0.36 ± 0.02^{b}	0.49 ± 0.02^{a}
Leaf glutathione reductase	0.011 ± 0.001^{b}	0.038 ± 0.003^{a}	0.049 ± 0.006^{a}	0.042 ± 0.003^{a}
Leaf polyphenol oxidase	0.19 ± 0.01^{c}	0.32 ± 0.006^{bc}	0.46 ± 0.013^{b}	0.72 ± 0.066^{a}
Leaf ascorbate peroxidase	0.2 ± 0.03^{d}	0.46 ± 0.01^{c}	0.62 ± 0.06^{b}	0.92 ± 0.02^{a}
Leaf guaiacol peroxidase	0.28 ± 0.01^{b}	0.3 ± 0.02^{b}	0.42 ± 0.02^{a}	0.47 ± 0.02^{a}

Note: Columns having at least one common letter are not significantly different according to LSD at the 1% level of probability

6). Mean comparison of leaf Ascorbate peroxidase and Guaiacol peroxide showed that the enzyme content was increased significantly (P<0.01) with increasing concentrations of cadmium chloride (Figures 7 and 8).

The ANOVA analysis showed a highly significant difference between the leaf, root and shoot Cd content (P<0.01) and all antioxidant enzymes (Table 4). Moreover, according to the correlation coefficients among the study traits, a significant positive correlation was found between the leaf cadmium content and root and shoot cadmium, root and shoot Catalase, root and shoot Peroxidase, leaf Superoxide dismutase, leaf Glutathione reductase, leaf Polyphenol oxidase, leaf Ascorbate peroxidase and leaf Guaiacol peroxidase. Actually, by increasing or decreasing leaves cadmium content, the values of above-mentioned traits are increased or decreased, respectively.

4. Discussion

The results of cadmium measurements in leaves, stems and roots of Vetiver plant during four treatments showed an increase in the concentration of cadmium chloride; cadmium accumulates more in the root than leaves and stems of the plant. These findings are similar to the results of research on wheat, cucumber, sorghum and cereals [31].

Studies show that cadmium uptake and its

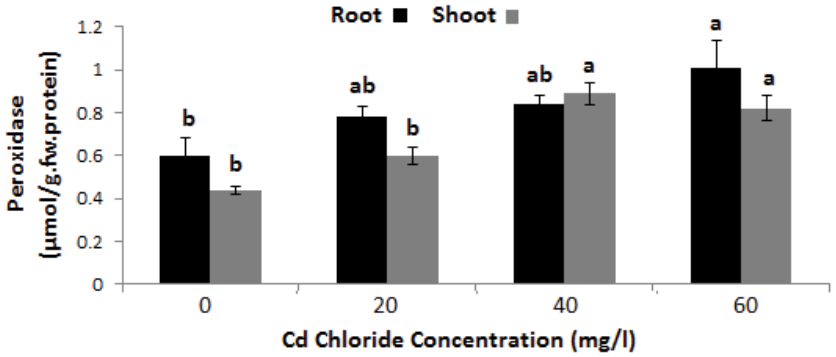

Figure 3. Mean comparison of root and shoot peroxidase content under the effect of different concentrations of cadmium chloride.

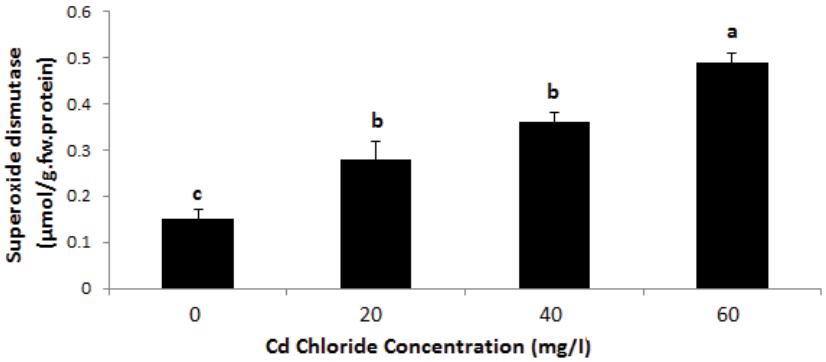

Figure 4. Mean comparison of leaf superoxide dismutase content under the effect of different concentrations of cadmium chloride.

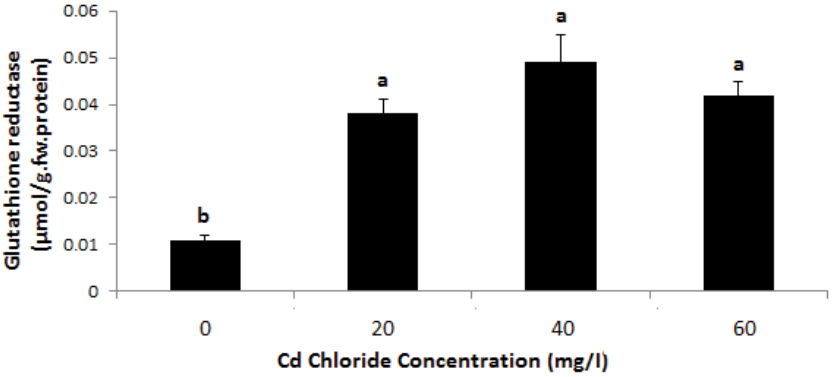

Figure 5. Mean comparison of leaf glutathione reductase content under the effect of different concentrations of cadmium chloride.

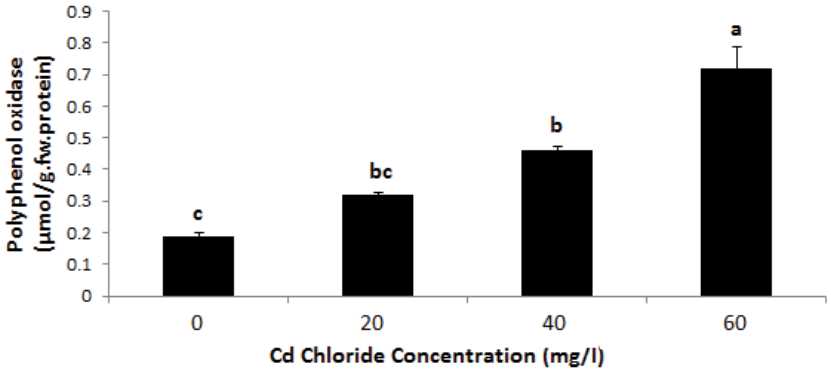

Figure 6. Mean comparison of leaf Polyphenol oxidase content under the effect of different concentrations of cadmium chloride.

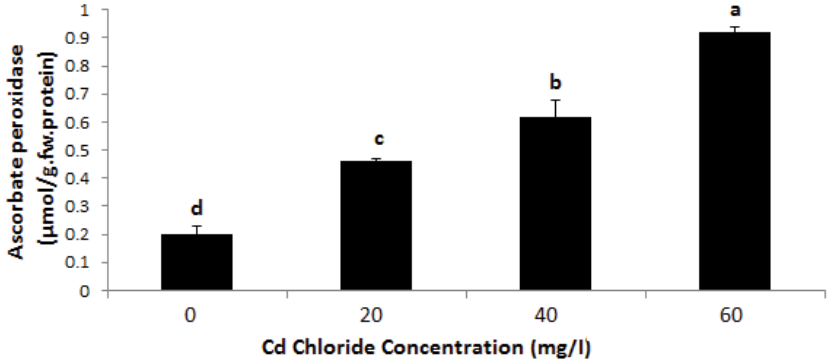

Figure 7. Mean comparison of leaf ascorbate peroxidase content under the effect of different concentrations of cadmium chloride.

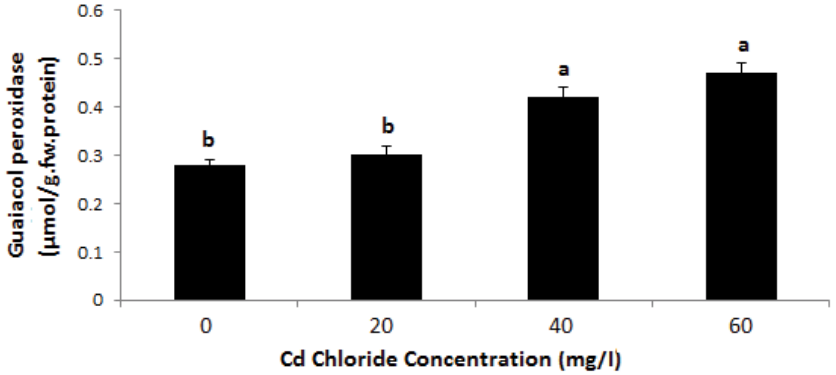

Figure 8. Mean comparison of leaf guaiacol peroxidase content under the effect of different concentrations of cadmium chloride.

Table 4. Analysis of variance of the effect of different concentrations of cadmium chloride on the study traits.

S.V.	df	MS											
		L Cd	R Cd	S Cd	R Ca	S Ca	R Pe	S Pe	L Su	L Gl	L Po	L As	L Gu
Cadmium chloride concentration	3	29.73**	143.0**	6.41**	0.122**	0.126**	0.143**	0.216**	0.099**	0.00136**	0.263**	0.459**	0.0409**
Error	16	0.44	2.02	0.08	0.003	0.002	0.034	0.01	0.004	0.00007	0.006	0.006	0.0016
CV (%)		20.8	21.4	22.8	19.9	14.7	22.9	14.4	19.1	23.5	18.1	14.3	10.8

Note: L Cd=Leaf Cd, R Cd=Root Cd, S Cd=Shoot Cd, R Ca=Root Catalase, S Ca=Shoot Catalase, R Pe=Root Peroxidase, S Pe=Shoot Peroxidase, L Su=Leaf Superoxide dismutase, L Gl=Leaf Glutathione reductase, L Po=Leaf Polyphenol oxidase, L As=Leaf Ascorbate peroxidase, L Gu=Leaf Guaiacol peroxidase,*=significant at P<0.05, **=significant at P<0.01

concentration in the plant depend on environmental conditions, physiological conditions and biochemical factors. The roots usually show more cadmium content than the airframe; because the first organs that are associated with cadmium and prevent as much as possible the movement of cadmium ion to

the air [32]: Therefore, they play a very important role in the deactivation of metals [33]. In plants, the transfer of ions from the cell membrane is mediated by proteins called transporters. These transporters (ion carriers) carry a special ion and operate in a special way. Of the total ions located around the root, only a small amount of plant absorption occurs. Most of these ions are physically absorbed by the cell wall. In the cell wall, a part that is negatively pregnant and called the Co-site is responsible for cell surface absorption. The ions that attach to this part can not enter the cell and can not be transferred to the plant's airspace. Another reason for increasing the amount of cadmium in the roots of the investigated plants may be their accumulation in vacuoles. The accumulation of this element in cellular vacuoles prevents them from transmitting to the aerial parts of the plant, which is why the amount of this element in the root is far more than the air organs. A condition that may have occurred for the Vetiver plant. Therefore, if the growing conditions for this plant are provided, it can be used in soils contaminated with heavy metal cadmium as a treatment plant. Gill et al. [33] in evaluating the effects of cadmium on *Lepidium sativum* showed that with increasing cadmium concentration, the accumulation of this element in the root and leaves increases; So that its concentration in the root at a concentration of 100 mg/kg soil reaches 700 mg/kg. In one study the effects of cadmium on *Solanum nigrum* showed that increased cadmium concentration increases the accumulation of this element in its root and stem. Similar results were obtained in studies on the effect of cadmium on the *Swietenir macrophylum* species, as well as Nikolic et al. [34] on the effect of cadmium on hybridisation and cadmium accumulation responses in spruce seedlings. Studies show that soil pH is the most important factor in the absorption of cadmium by root drying. Cadmium adsorption has been reported to increase with decreasing pH of the culture medium [36]. The results of this study showed that due to the accumulation of a significant amount of cadmium in Vetiver root can be used in cadmium contaminated soils as a stabilizing plant.

4.1 Activity of catalase and peroxidase enzymes

According to the results, the comparison of the mean of the traits studied in this study showed a significant increase in the activity of catalase and peroxidase enzymes in the root and shoot organs of the vetiver, with increasing cadmium chloride concentration; This increase in both enzymes in the treatment of 60 mg/L of cadmium chloride showed a significant difference compared with control; However, there was no significant difference between treatments (aerial catalase and root peroxidase). Among the plant's responses to these stresses is the activity of enzymes such as catalase (CAT) and peroxidase (POX) that neutralizes the activity of reactive oxygen species produced in cells; the production of reactive oxygen species in vegetable juices stimulates and enhances the activity of these enzymes [37].

Cadmium, unlike metals such as copper and iron, produce oxidative stress through a reducing cycle such as fenton or Haber-Wiese reactions. Through indirect mechanisms such as intervention in defense systems, the destruction of the electron transport chain and the induction of fat peroxidation can damage the cell [32]. High concentrations of cadmium cause toxicity in the plant and therefore cause oxidative stress. Oxidative stresses damage the production of oxygen free radicals, such as superoxide radicals (O^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) to plant cells; and free radicals hydroxyl react initially, which causes fat peroxidation [38]. The activity of antioxidant enzymes such as catalase and peroxidase increases in order to reduce and eliminate various active oxygen species and avoid oxidative damage in plants [39]. Hameed et al. [40] showed that cadmium chloride and mercury chloride reduce the activity of catalase enzymes in okra, which is apparently due to inhibitory enzymatic synthesis. It seems that peroxidases are commonly used as enzymes for poisoning and deactivating active oxygen species. Therefore, with increasing levels of activity of these enzymes, the plant is less invasive of reactive oxygen species, which is consistent with the results of the present study. Because catalase and peroxidase enzymes are known to be the main enzymes that destroy H_2O_2 [41]. Peroxides are among the enzymes that play a very important role in responding to a variety of stresses. Peroxides are responsible for the removal of excess amounts of hydrogen peroxide, including proteins induced during host plant defense against stress. Increasing catalase levels by cadmium treatment can help reduce respiration and reduce the CO_2 refraction point. According to researcher the accumulation of H_2O_2 results in the production of reactive oxygen species and increased activity of the enzyme superoxide dismutase in the cell. Research has shown that the presence of heavy metals in the cell leads to the accumulation of reactive oxygen species [42]. ROS, such as single oxygen (O_2), hydrogen peroxide (H_2O_2) and radical hydroxyl (OH), damage biological molecules (DNA, RNA and proteins). H_2O_2 is a productive component of plant oxidation and metabolism, which is considered to be oxidative chloroplast and peroxisomal reactions. Also, increased levels of H_2O_2 *in vivo* can lead to aging and lipid peroxidation in plants [38]. Vegetables have a mechanism for protecting enzymes and non-enzymatic mechanisms for purifying reactive oxygen species (ROS) and reducing their harmful effects. Oxidant enzymes can be considered as an important defense system in plants against oxidative stresses caused by metals [43]. In the present study, CAT activity was significantly increased in roots compared with the cadmium treated plants of the plants, which probably indicates the decomposition of H_2O_2 and toxic peroxides by cadmium accumulation by CAT. The effect of increasing the activity of CAT enzyme in cadmium treated plants has also been reported in similar studies on coffee by Gomes et al. [44] and tomato by Chamseddine et al. [45]. Based

on researches done on beans, increased activity of catalase and peroxidase enzymes was due to metals such as copper, zinc and copper [43]. Also, the decrease in catalase activity due to intense environmental stresses such as salinity, drought, cold and heavy metals reported.

4.2 Superoxide dismutase (SOD)

In this study, with increasing cadmium chloride concentration, the activity of enzyme superoxide dismutase increased significantly in Vetiver leaves, but did not show any significant difference in treatment with 60 mg/L of cadmium chloride compared to control. Superoxide dismutase is the first enzyme involved in the process of eliminating poisoning, converting-O_2 into hydrogen peroxide, decreasing the accumulation of hydrogen peroxide by catalase and peroxidase enzymes, and reducing the amount of this radical in the cellular organelles. These enzymes convert hydrogen peroxide to oxygen and water [46]. The accumulation of H_2O_2 is the result of the production of active oxygen radicals and increased activity of superoxide dismutase in the cell and as a key enzyme on free radicals, it converts hydrogen peroxide produced by catalase and peroxidase into water and oxygen. Increasing the activity of these enzymes in environmental stresses increases plant resistance to stress conditions. Enzymes such as superoxide dismutase provide a defense system for the survival of aerobic organisms. As it is seen, the increase in enzyme activity is consistent with the results of the present study. Also, the enzyme's activity has been reported by two copper and leaded metals in the *Lathyrus sativus* [47]. Also, the results indicate that There is a negative correlation between the amount of chlorophyll pigments with the superoxide dismutase enzyme, which, with a decrease in the chlorophyll content of this enzyme, is likely to increase in response to the production of free radical oxides due to the effect of lead and copper [47]; which is consistent with the results of this study.

4.3 Glutathione reductase (GR)

Regarding the results and the comparison of the mean of the traits studied, with increasing cadmium chloride concentration, glutathione reductase activity increased significantly in vetiver leaf, which is a significant increase in treatment with 40 mg/L of cadmium chloride compared to control some researcher examined the effect of cadmium on corn and the results showed that glutathione reductase (NADPH) was catalyzed by glutathione oxidized reactions, which significantly increased with cadmium treatment finds. Therefore, antioxidant enzymes such as glutathione reductase are stimulated as key enzymes in response to excess cadmium toxicity and increased to eliminate injuries caused by cadmium stress, which is consistent with the results of the present study.

4.4 Polyphenol oxidase

Increasing the concentration of cadmium chloride showed a significant increase in the activity of polyphenol oxidase in vetiver leaves, which showed a significant increase in treatment with 60 mg/L of cadmium chloride compared to control. But among other treatments, this increase was not significantly different. Polyphenol oxidase is found in most of the plants known as catechol oxidase, catecholazos trinosinate, in the presence of oxygen, two kinds of hydroxylation reactions of the phenol compounds and their conversion into quinine, and its main function is a kind of catalyst Quinone is from phenols adjacent to the oxygen molecule. Among its main roles are enzymes, its effect on root formation and root development [48]. Peroxidase and polyphenol oxidase enzymes in meta polyphenols play a role in bolism [49]. It will show that the amount of phenol in the plant and the activity of polyphenol oxidase. This relationship exists, and when the total phenol concentration of the plant increases, the activity of polyphenol oxidase will also increase [23]. In this study, by increasing the phenolic compounds in the vetiver plant, increased activity of the peroxidase and polysaccharide enzymes Phenol oxidase is also observed.

4.5 Ascorbate peroxidase (APX)

Increasing of $CdCl_2$ concentration significantly increased the activity of the ascorbate peroxidase enzyme in vetiver leaves, which showed a significant difference in all treatments of cadmium chloride compared to control. Ascorbate peroxidase as another H_2O_2 sweetener was investigated in this study. Increasing the activity of this enzyme in Cadmium-treated plants actually plays a key role in the response of the plant to increasing H_2O_2 accumulation. Corticosteroid peroxidase is mainly used in chloroplast, cytosolic and other Intracellular organelles are produced and required to maintain regeneration in the cells [29]. Reports on the increase of BET peroxidase enzyme in plants such as caudium chloride by Gomes et al. [44] and peas by Groppa et al. [50], which was consistent with the present study. It is also reported by other researchers that the activity of this enzyme will decrease in high concentrations of $CdCl_2$ (800 ppm). This is because of the high concentrations of Cd in the inactivation of this enzyme by excessive production of reactive oxygen species (ROS), degradation or degradation of non-specialized enzymes, or the transplantation of unnecessary heavy metals such as cadmium to the site of enzyme activity is related [51].

4.6 Guiacol peroxidase (GPX)

The activity of enzyme guiacol peroxidase in Vetiver leaves showed a significant increase in the concentration of cadmium chloride. This increase in 60 mg/L of cadmium chloride was significantly different from the control. The results of this study indicate that the enzyme acts as a protective device in the resistance to induce oxidative and cadmium damage, as well as reduces the amount of hydrogen peroxide to water, fatty acids or hydroxides into the

gaseous peroxidase activity due to the stress of cadmium chloride. Alcohols catalyze. The induction of the activity of guiacol peroxidase in plants on other heavy metals such as aluminum and zinc (Chaoui et al. [52]), iodine and copper (Chamseddine et al. [45]) have also been reported.

5. Conclusion

Our results clearly showed the high capability of *Vetiveria zizianoides* for the remediation of soils contaminated with Cd. According to the results, it is concluded that the uptake and accumulation of cadmium in the roots was higher compared to the shoots of Vetiver, and by increasing the activity of antioxidant enzymes, this plant can be used in the process of phytoremediation mechanisms and reduction of environmental pollution caused by Cd. Evaluation of different varieties of this plant in response to cadmium and other heavy metals under different environmental conditions can complete the results of this study. This study also confirms that as the concentration of heavy metals reaches toxic levels, it causes the physiologically irreversible change in the cell.

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