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Short Communication

Antioxidant enzymes functions of *Vetiveria zizianoides* during the absorption of cadmium in soil

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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 Cd 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 Cd Chloride, arranged in a randomized complete blocks design with four treatments and five replications. The root growth of 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 Cd 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, cadmium 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 Cadmium. Thus, this plant could be considered as one of the suitable candidates for cultivation in industrial areas.

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

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INTRODUCTION

Heavy metals in concentrations above the threshold are environmental pollutants found especially in soils throughout the industrial and agricultural societies (Lasat, 2002). Heavy metals toxicity and their accumulation in the food chains is one of the main environmental and health problems in today's societies (Adriano, 2001). However, the soils contaminated with heavy metals can be remediated by chemical, physical and biological techniques (McEldowney *et al*., 1993).

According to the previous studies, some crop species like barley, alfalfa, mustard, radish, sunflower, peanut, castor bean, corn and so forth are soil amendments. Some of the specific species are able to transfer heavy metals to their shoots (Alia, *et al*., 2001). Therefore, harvesting the shoots rich in heavy metals from contaminated areas could be effective in removing heavy metals from soil without the high costs like excavation and transferring soil surface from the region (Blaylock *et al*., 1997). Phytoremediation is a low cost and simple technology to remove heavy metals from soil which in recent years has been taken into consideration. Phytoremediation, as a relatively new technology, causes to removal, degradation or trapping of contaminants (Lombi *et al*., 2001).

Cadmium is a heavy metal, generally found in the form of anionic compounds, hydrated ions or complex mineral compounds such as carbonate, hydroxide, chloride, sulfate and organic compounds with humic acid (Adriano, 2001). Cadmium has particular importance due to the high mobility and dynamics in soil, absorption by plants, significant toxicity, and a biological half-life of twenty years as well as complications including liver and kidney failure

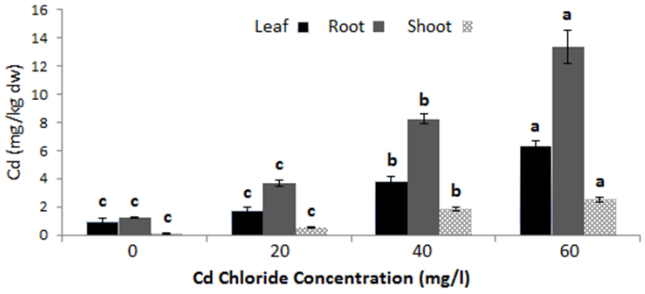

Figure 1. Mean comparison of leaf, root and shoot cadmium content

cardiovascular disease, bone, lung, etc. in humans (Mauskar, 2007). According to (Mishra and Choudhuri, 1999), the amount of cadmium in the plant ranges between 1 to 0.1 milligrams per liter. Most non-contaminated soils have cadmium less than one milligram per liter (Alloway, 1990). The use of sewage sludge, municipal waste and fertilizers containing cadmium (such as phosphate fertilizers) increases the concentration of cadmium in soils (Majer *et al*., 2002).

When cadmium concentrations in soils are high, the processes done by micro-organisms in the soil are disrupted and the whole soil ecosystem is exposed at risk. As examples mentioned above, plants are fully exposed to contamination due to inactivity. Therefore, their susceptibility to contaminants and other environmental stresses is higher than that of other living creatures(10).Very high concentrations of cadmium results in decreased absorption of nutrients (Agrawal and Sharma, 2006), preventing enzymatic activities and induction of oxidative stress including changes in enzymes related to the antioxidant defense system (Majer *et al*., 2002). It is also reported that cadmium in an amount of 3 mg per kg of soil stops the growth of plants with an inhibitory effect on photosynthesis pigments as well as disrupting ATP synthase and NADH oxidase enzymes (Alia *et al*., 2001).

Preliminary analysis showed that cadmium uptake in plants differs among genotypes. Therefore, it is possible to detect varieties or species with low ability to absorb cadmium. The difference in uptake of cadmium by roots and the rate of accumulation in the shoots is the main factor in explaining the differences among different genotypes tolerant to cadmium toxicity. Cadmium causes leaf-rolling, chlorosis, reduced roots and shoots growth (Smeets *et al*., 2005), and limits the process of germination and seedling growth (Rascio *et al*., 1993). As well, the acute toxicity of cadmium may cause death of animals and birds and cause severe poisoning in aquatic animals (Kabata-Pendias and Pendias, 2001). Therefore, given the importance of cadmium in the ecosystem pollution, providing ways to reduce pollution in particular remediation of contaminated soils is necessary and inevitable (Mauskar, 2007).

Vetiver, a forage species belonging to the Gramineae family, is used for restoration and reclamation of degraded lands. It has a high potential to absorb soluble elements such as nitrogen, phosphorus, as well as significant absorption of heavy metals soluble in contaminated water (Pais *et al*., 1997). In this study, the function of this plant in absorbing soil cadmium in different organs (leaves, stems, roots) and enzymatic changes caused by cadmium uptake in plants were investigated.

Das *et al*. (1997) 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*. (2001) showed that after 13 hours in exposure to cadmium, the activity of catalase and glutathione reductase in the roots and leaves of radish increased. Xu *et al*. (2010) showed that cadmium and other heavy metals caused GSH depletion and suppressed the GR (glutathione reductase) activities. Polle (2001) 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*. (2003) GSH and its metabolism enzymes provide an effective protection against the damages of ROS through chelating heavy metals and ward off toxicity. Bergmann (2004) 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 and Prasanna (2004), the activity of catalase and peroxidase in wheat was increase against oxidative stress. Tegelberg *et al*. (2004) 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 and Harrish, (2004) indicated that cadmium inhibited enzymes activities directly by reaction with -SH groups or indirectly by disrupting the balance of ions at the cellular level.

MATERIALS AND METHODS

Study area

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

Preparation and planting method

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.

Table 1. 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}

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 per liter cadmium chloride) for two months and they were harvested eight weeks later.

Treatments

Treatments included 0, 20, 40, and 60 mg/l cadmium chloride, arranged in a randomized complete block design. Each treatment was randomly applied to 21 pots from 15 June. Experiments were conducted at two stages. In the first stage of experiment, soil analysis was performed at planting time (15 April). At the second stage (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 hours) 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.

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.

Determining cadmium concentration in the plant

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

Measurement of antioxidant enzymes

For the enzymatic extraction, at first, 0.25 grams of powdered leaves shoot/roots was weighed and immediately liquid nitrogen was added in 1.5-ml eppendorfs. Then, one ml of 50 mM potassium phosphate buffer (pH = 7.5), containing 11% triton was added to each eppendorfs. All stages of extraction were performed in 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 and Mishre, (1976) method and CAT activity by Aebi (1984). SOD, APX and Guaiacol peroxidase activity was assayed by the Gianopolitis and Ries (1977); Nakano and Asada (1981); Updhyaya *et al*. (1985) methods, respectively. Glutathione reductase activity was assayed by oxidation of NADPH at a wavelength of 340 nm (Dalton *et al*., 1986).

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 $P<0.01$. Means with at least one common letter designation are not different, at $P<0.05$.

RESULTS

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.

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 cadmium chloride (0, 20, 40 and 60 mg/l), respectively. As well, the root and shoot

Table 2. 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
Cd chloride concentration	3	29.73±3**	143.0±8**	6.41±1**	0.122±0.01**	0.126±0.01**	0.143±0.02**	0.216±0.03**	0.099±0.02**	0.00136±0.003*	0.263±0.09**	0.459±0.05**	0.0409±0.04**
Error	16	0.4±0.14	2.02±0.01	0.08±0.0001	0.003±0.0001	0.002±0.0001	0.034±0.0002	0.01±0.001	0.004±0.0001	0.00007±0.00000001	0.006±0.0001	0.006±0.0001	0.0016±0.002
CV (%)		20.8±1.2	21.4±4	22.8±3	19.9±3	14.7±2	22.9±3	14.4±1.1	19.1±4	23.5±4	18.1±3	14.3±2	10.8±1.2

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

cadmium content were 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±0.18mg/kg dw with increasing concentrations of cadmium chloride, respectively.

The results of measuring cadmium content in leaves, shoots and roots showed that the uptake and accumulation of cadmium in the plant increased with increasing concentrations of cadmium chloride, and cadmium 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).

Analysis of antioxidant enzyme activities

According to the Table 1, 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).

Mean comparison of root and shoot peroxidase showed that the 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, 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 cadmium chloride even at 5% level of probability. The same results were found for SOD, glutathione reductase and polyphenol oxidase. 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.

The ANOVA analysis showed a highly significant difference between the leaf, root and shoot cadmium content (P<0.01) and all antioxidant enzymes (Table 2). 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 CAT, root and shoot peroxidase, leaf SOD, 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.

DISCUSSION

According to the obtained results, with increasing the concentration of cadmium chloride, more cadmium was accumulated in roots as compared with the leaves and stems of vetiver. These findings are similar with the results of studies on wheat, cucumber, sorghum and grain (Youn-Joo, 2004). Studies showed that the cadmium uptake by plants and its concentration depends on environmental conditions, physiological and biochemical factors (Soti *et al*., 2015). The roots usually contain more cadmium than the shoots since they are the first organs associated with cadmium and prevent cadmium ion mobility to shoots as much as possible (Benavides *et al*., 2005). Therefore, roots play a crucial role in inactivating metals (Gill *et al*., 2012).

In plants, ion transport is mediated by proteins called transporters through the cell membrane (Buchanan *et al*., 2015). Only a small part of the ions around the roots is absorbed by the plant (Britto and Kronzucker, 2015). Most of these ions are physically absorbed into the cell wall (Duca, 2015).

In the cell wall, the part that is negatively charged, called Coo- site, is responsible for the adsorption in the cell wall (Vale *et al*., 2016). The ions attached to this area cannot enter cells and also cannot be transported to the aerial parts of the plant (Clemens and Ma, 2016). Accumulation in vacuoles may be another reason for the increased levels of cadmium in the roots of the study plant. The accumulation of these elements in cell vacuoles prevents their transmission to the shoots and for this reason the amount of this element in roots is much more than shoots. This may also have occurred for vetiver grass. Therefore, if growing conditions for the plant is provided, it can be used in the soils contaminated with cadmium as phytoremediation.

Gill *et al*. (2012) studied the effects of cadmium on *Lepidium sativum* and showed that the accumulation of cadmium in the roots and leaves was increased with increasing concentration of cadmium, so that the cadmium content in the root reached to 700 mg/kg at a concentration of 100 mg/kg of soil. Furthermore, (Al Khateeb and Al-Qwasemeh, 2014) investigated the effect of cadmium on *Solanum nigrum* and showed that increasing the concentration of cadmium led to the increased accumulation of this element in roots and shoots. Similar results were reported on the effects of cadmium on *Swietenir macrophyl* (Liu *et al*., 2015) and hybrid poplar species as well as cadmium accumulation responses in one-year-old seedlings of poplar (Nikolic *et al*., 2008).

New studies showed that soil pH is the most important factor in cadmium uptake by roots (Dahlin *et al*., 2016). It is reported that cadmium uptake increases with decreasing pH of medium (Singh *et al*., 2003). The results of this study also showed that due to the accumulation of significant amounts of cadmium in the roots of vetiver, this plant can be used for phytoremediation in soils contaminated with cadmium.

Catalase and peroxidase activity

According to the results, the activity of catalase and peroxidase in the roots and shoot of vetiver showed a significant increase with increasing concentrations of cadmium chloride. This increase in both enzymes showed a significant difference to the control group in the treatment of 60 mg/l of cadmium chloride. However, this increase did not show significant differences among the treatments (shoot catalase and root peroxidase).

The activity of enzymes such as catalase (CAT) and peroxidase (POX) is among plant responses against such stresses, caused to neutralize reactive oxygen species produced in the cells (Manivannan *et al*., 2014). The production of reactive oxygen species in the plant cell stimulates and increases the activity of the mentioned enzymes (Mishra *et al*., 2009). Unlike metals such as copper and iron causing oxidative stress through reducing cycle like Fenton or Haber-Weiss reactions, cadmium causes damage to the cells through indirect mechanisms such as intervention in defense systems, electron transport chain destruction and induction of lipid peroxidation (Benavides *et al*., 2005).

High concentrations of cadmium may cause toxicity and oxidative stress (Nair *et al*., 2015). Oxidative stresses damage the plant cells through producing oxygen free radicals including superoxide radical (O^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), and hydroxyl free radicals starts reactions leading to lipid peroxidation (Chen *et al*., 2007). To reduce and eliminate reactive oxygen species and avoid oxidative stress in plants, the activity of anti-oxidative enzymes such as catalase and peroxidase increases (Smeets *et al*., 200). Hameed *et al.* (2011) showed that cadmium chloride and mercuric chloride led to the reduced activity of catalase in the okra plant, which is apparently due to inhibition of enzyme synthesis. On the other hand, it seems that peroxidases generally act as detoxification enzymes of reactive oxygen species (Dubrovskaya *et al*., 2016). Therefore, with increasing levels of activity of these enzymes, plant is less attacked by reactive oxygen species, which is consistent to the results of current study. Basically, catalase and peroxidase are known as the main enzymes destroying H_2O_2 (Tewari *et al*., 2005). Peroxidases include the enzymes having a very important role in response to a variety of stresses. Peroxidase is responsible for removing excess hydrogen peroxide including induced proteins in the host plant defense against stress. Increased levels of catalase due to the cadmium treatment leads to lower photo-respiration and reduced CO_2 compensation point (47).

According to Tewari *et al*. (2005), accumulation of H_2O_2 is resulted from the production of reactive oxygen radicals and increased activity of superoxide dismutase in the cell. Research has shown that the presence of heavy metals in the cells leads to the accumulation of reactive oxygen species (Radotić *et al*., 2000). ROS such as singlet oxygen (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) damage biomolecules (DNA, RNA and proteins). Increased production of H_2O_2 in the corn is reported, indicating cadmium-induced oxidative in this plant. H_2O_2 is a component of plant oxidative metabolism, considered as chloroplast oxidative reactions. Also, increased levels of H_2O_2, leads to induction of aging and lipid peroxidation in the plants (Chen *et al*., 2007).

Plants have protective enzymes and non-enzymatic mechanisms to clean up Reactive Oxygen Species (ROS) and reduce their harmful effects (Buchanan *et al., 2015*). In this study, CAT activity was significantly increased in the roots compared to shoots treated with cadmium, indicating the decomposition of H_2O_2 and toxic peroxides by CAT due to the accumulation of cadmium. The increased activity of the CAT enzyme is also reported in similar researches on coffee (Gomes-Junior *et al*., 2006) and tomato (Chamseddine *et al*., 2009) treated with cadmium. Increased activity of catalase and peroxidase is also reported for beans due to the effect of metals such as copper, zinc and lead (Weckx and Clijsters, 1996). Also, the decline in catalase activity due to harsh environmental stresses such as salinity, drought, cold and heavy metal stress is reported (Pasala *et al*., 2016).

Superoxide dismutase

In this study, the activity of superoxide dismutase in the leaves of vetiver showed a significant increase with increasing concentration of cadmium chloride. This increase showed a significant difference to the control group in the treatment of 60 mg/l of cadmium chloride. However, no significant differences were observed among the treatments.

Super-oxide-dismutase, the first enzyme involved in the process of detoxification, converts O^{2-} to hydrogen peroxide and reduces the concentration of hydrogen peroxide by catalase and peroxidase, leading to the reduced amount of this radical in cell organelles. These enzymes convert the hydrogen peroxide into oxygen and water (Zhang *et al*., 2009).

Concentration of H_2O_2, resulted from production of reactive oxygen radicals and increased SOD activity in cells, affects free radicals as a key enzyme, and the hydrogen peroxide produced is converted to oxygen and water by catalase and peroxidase (Kusvuran *et al*., 2016). Increased activity of these enzymes in environmental stresses, increases plant resistance to stress conditions. Enzymes such as superoxide dismutase provide defense system for the survival of aerobic organisms. As can be seen, increased enzyme activity corresponds with the results of

current study. Also, increased activity of this enzyme by copper and lead is reported in *Lathyrus* (Estrella-Gómez *et al*., 2009). The results also showed a negative correlation between chlorophyll pigments and superoxide dismutase, so that the activity of this enzyme increases by reducing the amount of chlorophyll, which is likely in response to oxygen free radical production caused by lead and copper (Estrella-Gómez *et al*., 2009) corresponding with the results of this study.

Shaw *et al*. (2004) indicated that aluminum treatment in soybean increased the amount of superoxide dismutase and malondialdehyde. (Benavides *et al*., 2005) showed that high concentrations of cadmium cause toxicity in plant, subsequently causing oxidative stress. It was also shown that oxidative stresses by producing of oxygen free radicals including superoxide radicals (O^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) damaged the plant cells. Gomes-Junior *et al*. (2006) indicated that in the coffee plant, the stress caused by cadmium increased the amount of catalase, superoxide dismutase, polyphenol oxidase and peroxidase, especially in high concentrations of cadmium chloride, and increased the amount of APX at 200 ppm cadmium chloride. According to Shah (2007), antioxidative enzymes activities under the influence of cadmium stress depend on the concentration of metal, which can be inhibiting or stimulating.

This study showed that plants having high antioxidant activity were more resistant to stress. Dismutation of superoxide anion by superoxide dismutase, followed by increased activity of catalase, causes the regulation of peroxidases activity in cell. This study also indicated that cadmium toxicity increased the activity of superoxide dismutase to combat free radicals caused by cadmium stress. Wang *et al*. (2008) stated that cadmium disrupted nitrogen metabolism through inhibiting the activity of enzymes such as glutamine synthase, glutamate synthase, and nitrate reductase, as well as nitrate reduction caused to reduced protein production and stopped plant growth. In *Arabidopsis*, increased concentration of proline and glutathione was reported with increasing cadmium (Xu *et al*., 2010). Jianpeng *et al*. (2010) reported the inhibition of transfer of radiolabeled iron in the plant organs treated with cadmium. It seems that generally peroxidase acts as the scavenger enzymes in reactive oxygen species.

Glutathione reductase

The activity of glutathione reductase in the leaves of vetiver showed a significant increase with increasing concentration of cadmium chloride. This increase showed a significant difference to the control group in the treatment of 40 mg/l of cadmium chloride. However, no significant differences were observed among the treatments. According to the results of cadmium effects on maize, glutathione reductase, catalyzing the NADPH of oxidized glutathione reactions, increased significantly with treatment of cadmium (Nahar *et al.,* 2016). Antioxidant enzymes such as glutathione reductase, as the key enzymes, are stimulated in response to additional cadmium toxicity and are increased to mitigate the damage caused by cadmium stress (Kusvuran *et* al., 2016).

Polyphenol oxidase

The activity of polyphenol oxidase in the leaves of vetiver showed a significant increase with increasing concentration of cadmium chloride. This increase showed a significant difference to the control group in the treatment of 60 mg/l of cadmium chloride. However, no significant differences were observed among the treatments.

Polyphenol oxidase is found in most spermatophytes and called catechol oxidase, catecholase and tyrosinase (McCluskey *et al*., 2015). Polyphenol oxidase shows two types of reactions in the presence of oxygen including hydroxylation of mono-phenolic compounds and converting them into quinine (Sulaiman *et al*., 2015). Adventitious root formation and root organization and development are among the main role of this enzyme (Yilmaz and Parlak, 2011). Peroxidase and polyphenol oxidase are involved in the metabolism of polyphenols (Bajguz and Hayat, 2009).

Evidences showed that there is a relationship between phenol content in the plant and its polyphenol oxidase activity, so that the activity of this enzyme is increased by increasing the concentration of total phenols (Tegelberg *et al*., 2004). In this study, increased peroxidase and polyphenol oxidase activity was also observed with

increasing phenolic compounds in the vetiver grass.

Ascorbate peroxidase

The activity of ascorbate peroxidase in the leaves of vetiver showed a significant increase with increasing concentration of cadmium chloride. This increase showed a significant difference to the control group in all treatments of cadmium chloride. Ascorbate peroxidase, as another enzyme sweeping H_2O_2, was investigated in this research. Such studies indicated that increased activity of this enzyme in the plants treated with cadmium is a key plant response to H_2O_2 accumulation (Chiang *et al*., 2015).

Ascorbate peroxidase is mainly produced in the chloroplast, cytosol and other intracellular organelles, needed for the maintenance of reduction in cell (Updhyaya *et a*., 1985). An increase of APX is also reported in plants such as coffee (Gomes-Junior *et al*., 2006) and peas (Groppa *et al*., 2007) under the toxicity of cadmium chloride, corresponding to the results of this study. It is also reported that in high concentrations of cadmium chloride (800 ppm), the activity of this enzyme is decreased, related to inactivation of this enzyme by overproduction of ROS, enzyme non-specified gradation or bonding of non-essential heavy metals such as cadmium to the site of action of enzymes (Filek *et al*., 2008).

Guaiacol peroxidase

The activity of guaiacol peroxidase enzyme in the leaves of vetiver showed a significant increase with increasing concentration of cadmium chloride. This increase showed a significant difference to the control group in the treatment of 60 mg/l of cadmium chloride. Results of increased activity of guaiacol peroxidase, in response to cadmium chloride, showed that this enzyme acts as a defense mechanism to resist cadmium-induced oxidative damage. This enzyme also catalyzes the reduction of hydrogen peroxide to water and reduction of fat or hydroxides to alcohol (Monferrán *et al*., 2009). Induction of guaiacol peroxidase in plants is also reported for other heavy metals like zinc (Chaoui *et al*., 1997) and copper (Chamseddine *et al*., 2009).

CONCLUSION

Our results clearly showed the high capability of *V. zizianoides* for the remediation of soils contaminated with cadmium. 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 cadmium. 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 physiologically irreversible change in the cell.

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