# Biological removal of cadmium from soil by phytoremediation and its impact on growth parameters, photosynthetic pigments, phenol and malondealdehyde content in *Vetiveria zizianoides*

**Azhir Khalil Aria[1], Hossein Abbaspour [1]*, Sekineh Saeidi Sar[1], Mohsen Dehghani Ghanatghestani[2]**

*1. Department of Biology, Faculty of Science, Islamic Azad University, Damghan Branch, Iran*
*2. Department of Environment, Faculty of Natural Resources, Islamic Azad University, Bandar Abbas Branch, Iran*

## Abstract

Phytoremediation is one of the most widely used methods for removing soil contaminants. In this research, the function of *Vetiveria zizianoides* to remove cadmium from four different treatments with varying amounts of cadmium chloride contamination (including 0, 20, 40, and 60 mg per liter) was investigated and physiological changes caused by the accumulation of this metal in the plant were monitored. According to the obtained results, cadmium accumulation in roots was higher as compared to the shoots. Based on factors such as the amount of biomass and the length of organs, the growth rate was reduced in plants accumulating more cadmium. Our findings also showed that increased concentrations of cadmium chloride in the experimental units caused a significant reduction of photosynthetic pigments compared to control. However, phenolic compounds and malondialdehyde showed a significant increase with increasing concentrations of cadmium chloride. In addition, the cadmium uptake by the plant was increased with increasing concentration of cadmium chloride. Our results clearly showed the high capability of *Vetiveria zizianoides* for biological removal of cadmium from contaminated soil.

***Keywords***: phytoremediation; cadmium; photosynthetic pigments; phenol; malondialdehyde (MDA); vetiver

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## Introduction

Nowadays, a variety of different pollutants from different sources enters into the soil and thereby endangers the health of life species in particular microbial activity (Stirling et al., 2016). Some of these pollutants such as heavy metals are not degraded in nature (Förstner and Wittmann, 2012) and even are accumulated, leading to irretrievable losses for all life species and consequently human (Ashraf et al., 2014). The costs of using this technique is less than five percent of the cost of industry practices and in this respect it is one of the cheapest ways to

*Corresponding author
*E-mail address*: abbaspour75@yahoo.com
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clean up soils contaminated with heavy metals (Vara Prasad and de Oliveira Freitas, 2003). In addition, the use of plants to remove pollutants can have other positive effects such as soil stabilization, preventing leaching of pollutants, green space expansion, and preventing soil erosion (Paz-Alberto and Sigua, 2013). High tolerance to pollutants, fast growth, high biomass, and ease of cultivation are among the characteristics of plant species appropriate for phytoremediation (Portney, 2016). However, several soil factors including pH, organic matter content, soil texture, and content of other organic and inorganic compounds are among the important features affecting phytoremediation (Neilson and Rajakaruna, 2015).

Vetiver Grass *Vetiveria zizanioides*, a forage species belonging to the Gramineae family, grows naturally in many parts of the world (Ghosh et al., 2015). Vetiver grass has a tendency to social life and lives in groups. Moreover, it is a fast-growing species used for restoration of degraded lands due to the specific features in roots, shoots, and leaves (Chen et al., 2004). Vetiver is one of the valuable plant species having the characteristics of a good candidate for phytoremediation (Vargas et al., 2016). For example, the removal of zinc in abandoned mines (Vargas et al., 2016), wastewater purification (Rezania et al., 2015), removal of arsenic from contaminated water (Tiwari et al., 2016), removal of heavy metals such as manganese, iron, lead, zinc, and copper from industrial wastewater (Banerjee et al., 2016) and reclamation of abandoned mines of various metals (Sun et al., 2016) are among the uses of this plant. This species is also highly resistant against heavy metals; however, the high concentration of this element in the plant may cause problems in the growth and physiological processes of the plant (Adigun and Are, 2015).

Vetiver is receiving high attention from environmental scientists as a soil stabilizer species which prevents desertification, so that it is widely used in countries such as Thailand to control soil erosion and produce sediment load (Boonyanuphap, 2013). Since the root penetration in this plant is relatively high, it can be used for cleaning up the pollutants in the deeper part of the soil surface (Saeb et al., 2015). Due to high compatibility of *Vetiveria zizianoides* to drought and humidity, it could be considered as one of the candidates for phytoremediation in wet and dry environments (Tiwari et al., 2016; Vargas et al., 2016).

In this research, the ability of *Vetiveria zizianoides* was investigated to absorb cadmium in various experimental units treated with varying concentrations of cadmium chloride to evaluate some physiological and growth changes of the study plant.

## 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

Plant roots imported from the Genetic Research Center of the UAE, were obtained from the Department of Natural Resources, Hormozgan province. During the spring 2015, 100 pots were planted and were transferred to the experimental units in the field after six weeks. A total of 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 twice a day and then, due to the moisture in the environment, irrigation was administered once daily.

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 Apr.) | 6.5 | 2.6 | 9.29 | 0.13 | 21 | 36 | 42 |
| Harvesting (15 Aug.) | 5.7 | 2.7 | 9.4 | 5.7 | 20 | 35 | 42 |

Before planting vetiver in the plots, cadmium concentration and physical and chemical characteristics of the soil (including pH, EC, organic matter content, and soil texture) were measured. After planting, parts of roots and shoots were separated and processed by atomic absorption spectrometry to analyze the amount of cadmium. The uptake of cadmium in shoots and roots was obtained from multiplying the cadmium concentration by the dry biomass. Treatments included 0, 20, 40, and 60 mg per liter cadmium chloride, arranged in a randomized complete blocks design with five replications.

## Measuring the root and shoot lengths and fresh and dry weights

The root and shoot lengths were measured with a clean ruler for five replications. Fresh weight of shoots, leaves, and roots were measured separately using a digital scale (Sartorius model TE124s) with a precision of 0.001 g. To measure the dry weight, roots and shoots of the plant were separately wrapped in aluminum foil and were dried in an oven at 80° C for 48 hours. After drying the samples, their weights were measured using a digital scale with 0.001 g precision.

## Measuring cadmium content

Assessment of cadmium content in different parts of the study plant (roots, shoots, and leaves) was conducted using the DTPA-TEA method. In this way, 25 ml of di-ethylene diamine penta-acetic acid extractor containing 005/0 M DTPA and 01/0 M triethanolamine TEA and 01/0 M calcium chloride solution with a pH of 7.3 were added to 10 g of dry soil and in a flask 125 mm was centrifuged for two hours (at 180 rpm) as mentioned by Lindsay and Norvell (1978). The extract of centrifuge was assessed by an atomic absorption spectrophotometer.

## Measuring the content of chlorophyll a, chlorophyll b, and carotenoids

The method described by Arnon (1949) and spectrophotometric method were employed to measure the content of chlorophyll a, chlorophyll b and carotenoids. The absorbance was read for chlorophyll a, b, and carotenoids at 663 nm, 645 nm, and 470 nm, respectively.

## Measuring phenolic compounds content

To measure the content of phenolic compounds, the method described by Shehab et al. (2010) was used. In this method, after the preparation of fresh leaf tissue extracts, the absorption percentage of samples was read at a wavelength of 725. Then, the content of phenolic compounds was recorded by using Gallic acid standard curve based on mg/g fresh tissue.

## Measuring malondialdehyde content

The malondialdehyde content, as index of lipid peroxidation, was assayed by the method of Heath and Packer (1968).

## Statistical analysis

In this study, the significance of differences among the groups were assessed by one-way analysis of variance (ANOVA). The mean comparison was performed at $P \leq 0.01$ and $P \leq 0.05$ using LSD test. All data were analyzed by SAS statistical software.

## 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 (Table 1). The results indicate the pH stability during the experiment. Moreover, soil texture was loam - loamy clay.

Table 2
Mean comparison of traits under the effect of different concentrations of Cd 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$^{a}$ |

Table 3
Mean comparison of growth parameters under the effect of different concentrations of cadmium chloride

| Growth parameters | Cadmium Chloride Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 (control) | 20 | 40 | 60 |
| fresh weight of Root (g) | 70.00 ±3.16$^{a}$ | 58.80±3.31$^{b}$ | 37.00±2.00$^{c}$ | 24.20±1.16$^{d}$ |
| dry weight of Root (g) | 12.50 ±1.12$^{a}$ | 12.76±0.58$^{a}$ | 7.60±0.93$^{b}$ | 4.80±0.37$^{b}$ |
| fresh weight of shoot (g) | 23.60 ±1.03$^{a}$ | 17.80±1.02$^{b}$ | 9.50±0.22$^{c}$ | 7.70±0.58$^{c}$ |
| dry weight of shoot (g) | 4.50 ±0.22$^{ab}$ | 5.24±0.49$^{a}$ | 3.60±0.51$^{bc}$ | 2.30±0.20$^{c}$ |
| Root length (cm) | 14.00 ±1.38$^{a}$ | 11.80±0.80$^{ab}$ | 7.80±1.02$^{bc}$ | 5.40±0.68$^{c}$ |
| Shoot length (cm) | 42.00 ±2.55$^{a}$ | 30.00±3.16$^{b}$ | 19.00±2.92$^{c}$ | 9.00±1.18$^{c}$ |

## Cadmium content of leaves, shoots, and roots of 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 dry weight with increasing concentrations of Cd chloride (0, 20, 40, and 60 mg/l), respectively. Moreover, the root and shoot cadmium contents were calculated to be 1.25±0.06, 3.68±0.25, 8.25±0.34, and 13.38±1.20 mg/kg dry weight, and 0.08±0.06, 0.53±0.08, 1.84±0.15, and 2.52±0.18 mg/kg dry weight with increasing concentrations of Cd chloride, respectively (Table 2).

According to the obtained results, the uptake and accumulation of cadmium in the plant was increased with increased concentration of cadmium chloride, showing significant difference in different treatments based on LSD test. The findings also indicated that the highest cadmium accumulation was found in the roots (Fig. I).

## Growth analysis of vetiver

According to the results of this study, the growth rate of vetiver based on shoot length index and fresh and dry weight of organs showed a significant difference so that the lowest growth rate was recorded in the treatment with the

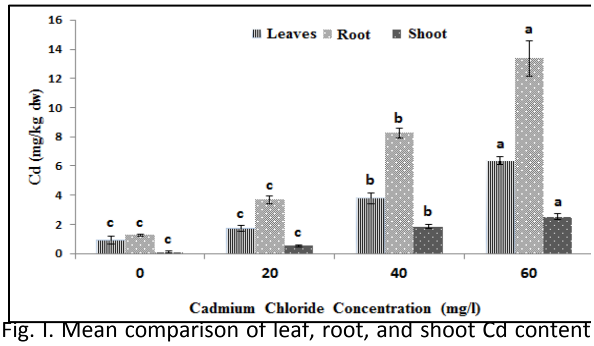

Fig. I. Mean comparison of leaf, root, and shoot Cd content under the effect of different concentrations of Cd chloride

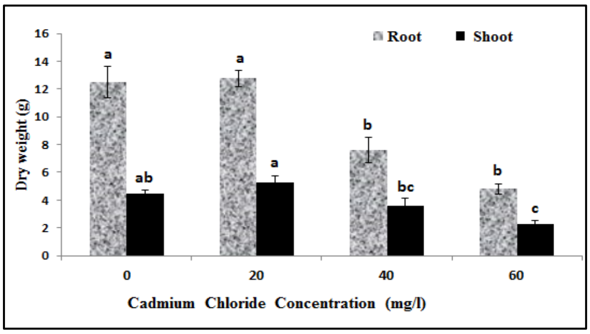

Fig. II. Mean comparison of root and shoot dry weight under the effect of different concentrations of cadmium chloride highest concentration of cadmium (Table 3). The results of LSD test showed that the values of all growth parameters mentioned above were reduced with increased concentration of cadmium chloride (0, 20, 40 and 60 mg per liter). This downward trend showed significant differences (P<0.05) for root fresh and dry weight, shoot fresh and dry weight, root length, and shoot length at 60 mg/l of cadmium chloride compared to control (Figs. 2-4).

Table 4
Mean comparison of photosynthetic pigments under the effect of different concentrations of cadmium chloride

| Photosynthetic pigments (mg/g.fw) | Cadmium Chloride Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 (control) | 20 | 40 | 60 |
| Total Chlorophyll | 1.48 ±0.02$^{a}$ | 1.60±0.14$^{a}$ | 1.60±0.18$^{a}$ | 1.20±0.10$^{a}$ |
| Chlorophyll a | 0.90 ±0.08$^{a}$ | 0.74±0.05$^{ab}$ | 0.52±0.04$^{bc}$ | 0.34±0.02$^{c}$ |
| Chlorophyll b | 0.47 ±0.02$^{a}$ | 0.63±0.04$^{a}$ | 0.40±0.03$^{b}$ | 0.20±0.03$^{c}$ |
| Carotenoids | 0.80 ±0.03$^{a}$ | 0.80±0.03$^{a}$ | 0.68±0.02$^{a}$ | 0.32±0.03$^{b}$ |

Table 5
Mean comparison of MDA and leaf phenol content under the effect of different concentrations of cadmium chloride

| | Cadmium Chloride Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 (control) | 20 | 40 | 60 |
| leaf phenol (mg/g.fw) | 0.076 ±0.005$^{b}$ | 0.122±0.020$^{b}$ | 0.1222±0.010$^{b}$ | 0.218±0.009$^{a}$ |
| Root MDA (nmol/g.fw) | 0.96 ±0.11$^{c}$ | 1.22±0.15$^{c}$ | 2.44±0.18$^{b}$ | 3.23±0.19$^{a}$ |
| Shoot MDA (nmol/g.fw) | 0.40 ±0.03$^{c}$ | 0.52±0.04$^{bc}$ | 0.65±0.05$^{b}$ | 1.30±0.08$^{a}$ |

Same results also were obtained for photosynthetic pigments (Table 4), MDA, and leaf phenol content (Table 5) under the effect of different concentrations of cadmium chloride.

The average content of photosynthetic pigments of vetiver and carotenoids changes in different treatments are shown in Fig. V and Fig. VI, respectively. According to the results of LSD test, with increasing concentration of cadmium chloride (0, 20, 40, and 60 mg/l), no significant differences (P<0.05) were found for the total chlorophyll content; however, this difference was significant for chlorophyll a and chlorophyll b. The content of chlorophyll a and chlorophyll b reduced with increased concentration of cadmium chloride, showing a significant difference at 60 mg/l of cadmium chloride. The average content of carotenoids showed no significant difference (P<0.05) with increased concentration of cadmium chloride at 0, 20, and 40 mg/l; however, this downward trend showed significant differences (P<0.05) at a concentration of 60 mg/l of cadmium chloride. However, no significant difference was found at concentrations of 20 and 40 mg/l of cadmium chloride (P<0.05).

The leaf phenol content increased with increased concentration of cadmium chloride (0, 20, 40, and 60 mg/l), showing highly significant difference at 60 mg/l of cadmium chloride (Fig.VII); however, no significant difference was found in leaf phenol content at 20 and 40 mg/l of cadmium chloride compared to the control group (P<0.05). According to the results of LSD test, no significant differences (P<0.05) were found in the root and shoot MDA content with increasing concentration of cadmium chloride (0, 20, 40, and 60 mg per liter). The root and shoot MDA content showed a significant difference at 60 mg/l of cadmium chloride (P<0.05) compared to the control group (Fig. VIII). However, no significant difference was observed in root MDA content at 20 and 40 mg per liter of cadmium chloride. According to the results of ANOVA, significant differences (P<0.01) were found for the leaf, shoot, and root cadmium content, root fresh and dry weight, shoot fresh and dry weight, root and shoot length, root and shoot chlorophyll a and b, carotenoids, MDA, and leaf phenol content in the vetiver plant; however, no significant difference was found for the total chlorophyll content. The leaf cadmium content showed a significant positive correlation with root and shoot cadmium content, root and shoot MDA content, and leaf phenol content. By increasing or decreasing the content of leaf

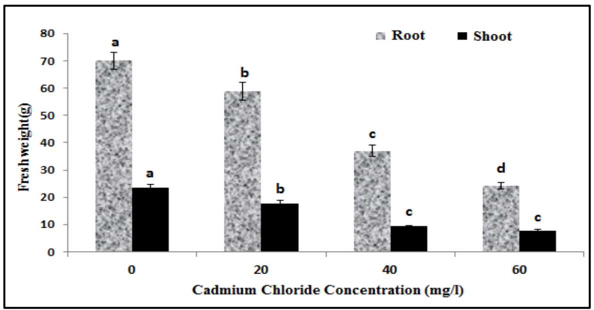

Fig. III. Mean comparison of root and shoot fresh weight under the effect of different concentrations of cadmium chloride

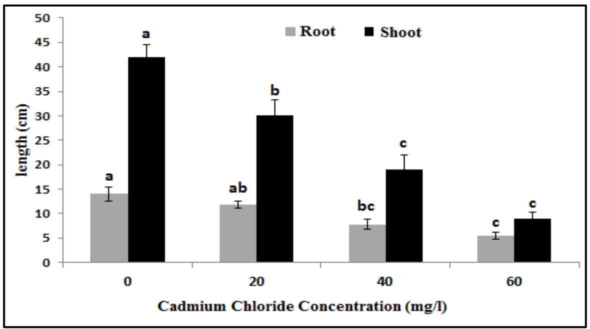

Fig. IV. Mean comparison of root and shoot length under the effect of different concentrations of cadmium chloride

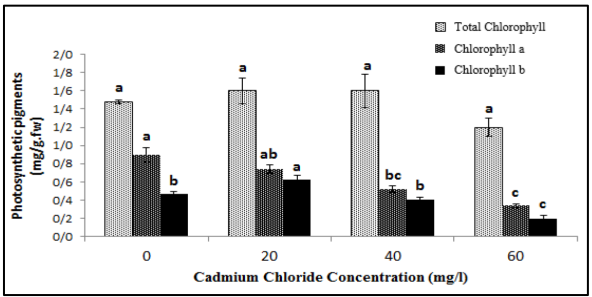

Fig. V. Mean comparison of photosynthetic pigments under the effect of different concentrations of cadmium chloride

cadmium the values of the traits mentioned above are reduced and increased, respectively. On the other hand, the leaf cadmium content showed a significant negative correlation with root and shoot dry and fresh weight, root and shoot length, chlorophyll a, b and carotenoids. This means that by increasing or decreasing the content of leaf cadmium, the values of the traits mentioned above are reduced and increased, respectively.

## Discussion

Our results clearly showed the high capability of *Vetiveria zizianoides* for the remediation of soils contaminated with cadmium. The results of measuring the cadmium content in leaves, stems, and roots of vetiver under four treatments showed that cadmium was more accumulated in the roots compared to leaves and stems. The accumulation of cadmium in the roots of some other plant species such as wheat, cucumber, sorghum, and cereals have also been confirmed (An, 2004). However, the cadmium uptake by plants and its concentration in plant tissues depend on environmental conditions and physiological and biochemical factors (Clemens et al., 2013; Farooq et al., 2013; Roy and McDonald, 2015).

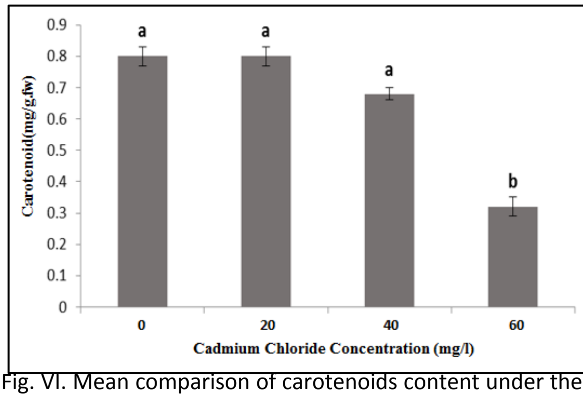

Fig. VI. Mean comparison of carotenoids content under the effect of different concentrations of cadmium chloride

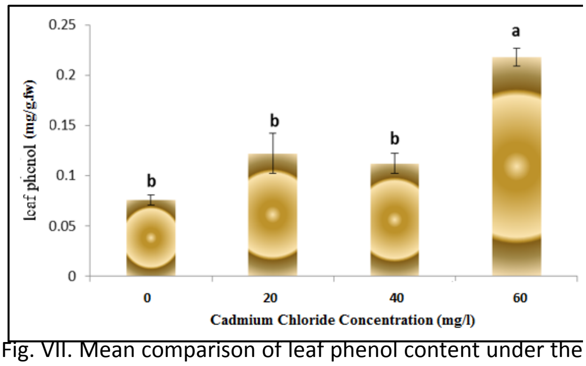

Fig. VII. Mean comparison of leaf phenol content under the effect of different concentrations of Cd chloride

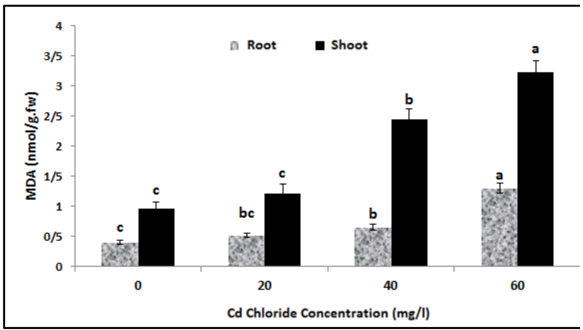

Fig. VIII. Mean comparison of root and shoot MDA content under the effect of different concentrations of Cd chloride

Root is the first organ exposed to contaminants and often prevents the transfer of cadmium ion to shoots (Benavides et al., 2005). The role of roots in deactivation of metal ions has been established in other studies (Gill et al., 2012). In addition, the higher accumulation of cadmium is reported in the root of other plants such as *Lepidium sativum* (Gill et al., 2012), *Swietenir macrophyl* (Fan et al., 2011), and *Populus alba* (Nikolic et al., 2008).

Studies show that the uptake of cadmium increases by reducing the soil pH (Mao et al., 2014; Puga et al., 2015; Rizwan *et al.*, 2012). The results of this study suggest that roots and shoots fresh and dry weight showed a significant decrease with increasing concentrations of cadmium chloride. Furthermore, the root weight loss in vetiver is more severe compared to the shoots. Disruption in photosynthesis, respiration, and nitrogen metabolism due to the accumulation of cadmium and reduced biomass is the cause of this problem (Gouia et al., 2000). However, despite increased uptake of cadmium, increased growth of vetiver is reported by some other researchers (Vo and Le, 2009). In addition, reduced growth rates under different treatments of cadmium are reported in some other plant species such as bean (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*), and wheat (*Triticum sativum*) (Gouia et al., 2000; Lagriffoul et al., 1998). This is related to the inhibitory effects of cadmium in root meristem cell division (Aina et al., 2007).

The inhibitory role of cadmium in reducing plant height has been proven in several studies (Liu et al., 2015; Tran and Popova, 2013). Results of this study suggest that increasing concentrations of cadmium chloride reduced leaf photosynthetic pigments in vetiver. It is also reported for the weeds such as Cyperus and Digitaria (Ewais, 1997).

Transfer of cadmium to the shoots and finally its accumulation in the leaf cells leads to morphological and physiological symptoms in the leaves, of which chlorosis is the most prominent symptoms (Bergmann, 2004). This disease in vetiver leaves was recorded in treatments with maximum concentration of cadmium. It seems that incomplete biosynthesis of chlorophyll is the cause of this problem (John et al., 2008). The content of different types of carotenoids is reduced under cadmium stress, probably due to the non-photochemical suppression of excited chlorophylls (Kalaji et al., 2016). The reduced chlorophyll content in leaves could be due to the inhibition of photosynthetic pigments synthesis by cadmium through inhibiting the absorption of essential nutrients such as iron, manganese, and magnesium (Feng et al., 2010). Increased content of phenol was shown as a result of increasing the cadmium content, which is related to its role in antioxidant protection (Rezazadeh et al., 2012).

Increased malondialdehyde, as a toxic product caused by peroxidation of lipids, represents the amount of free radicals production and damaged tissue (J. Li et al., 2016). Increased content of malondialdehyde shows that cadmium ions could stimulate the production of free radicals in higher plants (Çelekli et al., 2013). Similar results are reported for the effects of other heavy metals such as copper and nickel on increased malondialdehyde in maize (Y. Li et al., 2013; Pandey et al., 2013).

Finally, according to the findings of the study, it is concluded that the uptake and accumulation of cadmium in roots are higher compared to the shoots of vetiver; therefore, this plant can be used in the process of phytoremediation and reducing environmental pollution caused by cadmium. Thus, this plant could be considered as one of the candidates suitable for cultivation in industrial areas of southern Iran. 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.

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