



Refining properties of lavender (*Lavandula spica* L.) in cadmium contaminated environments

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Abstract

Considering the ever-increasing state of pollution in different aspects of the environment, ornamental plants and herbs such as *Lavandula* have become the center of attention mostly due to their availability and use in urban green spaces. Phytoremediation is a modern and economical method which can be applied to metropolises for decreasing metal contaminations in soil, water, and atmosphere. This study examined the contamination resistance of *Lavandula* against cadmium through 3 completely randomized block design experiments. The treatments included cadmium at 2 concentrations (0 and 100 mg) and DTPA at 2 concentrations (1 and 2 mM). Results suggested that heavy metal stress decreased morphologic properties under study and by adding DTPA the cadmium absorption decreased in *Lavandula*.

Keywords: *Lavandula*; heavy metals; phytoremediation; DTPA

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Introduction

Pollutants are considered as disrupting factors of the ecosystem. Amongst them, heavy metals are especially important due to the fact that they are resistant to decomposition and have great physiological damaging effects on organisms at low concentrations (Alloway, 1990). Iran, being a vast country and similar to others located on the arid belt of the earth, suffers from water scarcity and therefore, its large cities such as Tehran turn to urban and industrial wastewater to compensate for this shortage.

Long-term application of wastewater

which is generally used in vegetable cultivation results in the concentration of heavy metals in soil and transferring to plants on a high and dangerous level (Yargholi et al., 2007). Phytoremediation is a simple and economic technology in which plants such as forage, woody plants, and shrubs are used to remove, maintain, and neutralize environmental pollutants such as heavy metals, rare elements, organic oil compounds, and radioactive materials from soil and water. Such plants are able to absorb, transfer and stabilize heavy metals through their organs and decompose them (Kling, 1997).

Recently, many researchers have taken interest in using plants to remove heavy metals from contaminated soil (Chaney et al., 1997,

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McGrath et al., 1993, Raskin et al., 1994). Some species of plants are able to transfer heavy metals to their aerial organs. Removing heavy metal rich aerial organs from contaminated environments can be useful in removing heavy metals from soil with minimum cost compared to excavating and transferring surface soil from the site (Blaylock et al., 1997). Hyperaccumulators are plants which can concentrate heavy metals in their aerial organs on an extra ordinary level (Shen et al., 2002, Chaney et al., 1997).

Two methods are suggested to increase the solubility of heavy metals such as lead in soil and ultimately, to increase the absorption level of plants. These are decreasing the pH level of soil using various acids and increasing the solubility of the element through various chelates such as NTA (Nitrilo triacetic acid), EDTA (Ethylen ediaminetetra acetic acid), HEDTA (hydroxyl ethylene diamine triacetic acid), and DTPA (diethylene triamine penta acetic acid) (Blaylock et al., 1997). Acids and chelates are used in greenhouses and farms to increase the absorption level of heavy metals in soil by plants (Huang et al., 1997).

Lavandula is a perennial, frutescent (shrubby) and lush (succulent) plant. Its flowers are dark purple and in the form of compact Hyacinths. It is shaped as long oval tetrahedrons with big violet bracts at its head. The fruit is composed of 4 nutlets, each gaining an oval shape and transparent brown color after ripening. Lavandula has long and thin leaves with reversed edges and is covered with cotton-like lint on both sides. It has multiple square stems, each between 30 to 60 cm tall (Omidbeygi, 2000).

The main objectives of this study were to examine and recognize the level of absorption of cadmium by Lavandula in contaminated soils, to use an economic and non-destructive method for refining soil and solving current issues of refining and extracting heavy metals and to decrease expenses. Additionally, the study tried to determine the impact of cadmium on morphology and phytochemistry properties of Lavandula as a hazardous pollutant for the health of human and other organisms.

Materials and Methods

In order to examine the ability of Lavandula in phytoremediation of cadmium from soil, experiments were carried out in the greenhouse of Mellat Park using pots and also at the Razi lab. Of Islamic Azad University, Tehran Science and Research Branch. The tests were done through 3 completely randomized block design experiments. The treatments included different levels of cadmium (0 and 100 mg) and DTPA (0,1 and 2 mM) in the form of solution spraying at pre-determined times. To apply the treatments, 100 mg of cadmium was mixed with soil and held for 3 weeks in sealed bags to stabilize the metal in the soil. Then the soil was transferred to the pots to cultivate Lavandula in them. After the completion of the experiment period (4-5 weeks) the bushes were removed from the soil and transferred to the laboratory. At the time of removal, the height of aerial organs and the length of the roots were recorded. During the next stage, the roots and aerial organs were weighed using a digital scale and their wet weight was recorded. To record the dry weight of the root and aerial organs, they were kept for 48 hours at 70 °C inside an oven. After 2 days, the root and aerial organs were removed from the oven and weighed using the digital scale. The dried plants were then powdered for recording their details. The plant extract was prepared using the digestion and dry burning methods and the concentration of Cadmium was determined using atomic absorption spectroscopy. Then, the content of the elements were measured. SOD activity measurement was done using the Minami and Yoshikawa (1979) method. The Bates (1973) method was used for Proline measurement. Data was analyzed using Spss Software and diagrams were prepared using Excel. Means were compared using the Duncan test at 1% and 5% probability levels.

Results

The variance analysis (Table 1) showed a significant difference between the treatments regarding the amount of cadmium absorption at 1% level. The minimum amount of cadmium absorption at roots and aerial organs was 2 mM

Table 1
Variance Analysis

Source of Variation	Mean Square							
	Degree of Freedom	Wet Weight of Aerial Organs	Dry Weight of Aerial Organs	Wet Weight of Root	Dry Weight of Root	Plant Height	Total Leaf chlorophyll	Leaf proline
Treatment	8	575.940**	11.204**	17.367**	1.902**	92.754**	0.384**	1.812**
Experimental Error	-	0.098	0.029	0.064	0.017	0.172	0.005	0.004
Variation Coefficient (%)	-	12.86	13.06	12.54	12.75	13.8	11.98	13.69

Source of Variation	Mean Square			
	Degree of Freedom	Stem Lead	Root Lead	
Treatment	5	108.402**	93.769**	
Experimental Error	-	0.021	0.021	
Variation Coefficient (%)	-	11.78	12.90	

** , * , ns respectively mean significance in the 1 and 5 % probability level and insignificance

DTPA while the maximum amount was recorded as 100 ppm of cadmium. Also, there was a significant difference between the treatments regarding the amount of leaf proline at 1% level in a way that the minimum proline content of DTPA 2 mM was 0.23. There was a significant difference between the treatments regarding the amount of leaf chlorophyll at 1% level, the maximum amount with 2 mM DTPA being 1.5467 mg of chlorophyll. The highest wet and dry weights of the roots under 2 mM DTPA were 15.43 gm and 3.80 gm, respectively. The highest dry weight of shoots under 2 mM DTPA was 7.23 gm. Also, the highest wet weights of shoots under 2 mM and 1 mM DTPA were 30.47 and 29.80 mg, respectively. The amount of absorbed cadmium was higher for aerial organs compared to the roots.

The effect of cadmium and DTPA concentrations on dry and fresh weights of roots and shoots

The weight of the dry matter of *Lavandula* and total absorption of cadmium by the aerial organs of the plant shows the effects of DTPA on phytoremediation and cadmium extraction by *Lavandula* during experimental treatments. As it can be seen, cadmium decreased the plant's dry material, but increasing DTPA levels led to an increase in the dry matter and in general, the amount of cadmium

extraction from the soil was improved during DTPA treatments. Accordingly, the minimum and maximum dry and wet weights were recorded under 100 ppm of cadmium and 2 mM DTPA treatments, respectively. By increasing the amount of DTPA, so did the dry and wet weights of the plant. On the other hand, under cadmium-only treatment, the wet weight decreased significantly confirming the toxicity of cadmium

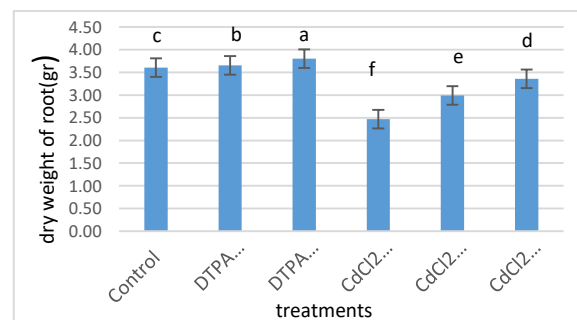


Fig. I. The effect of various cadmium and DTPA treatments on the dry weight of roots

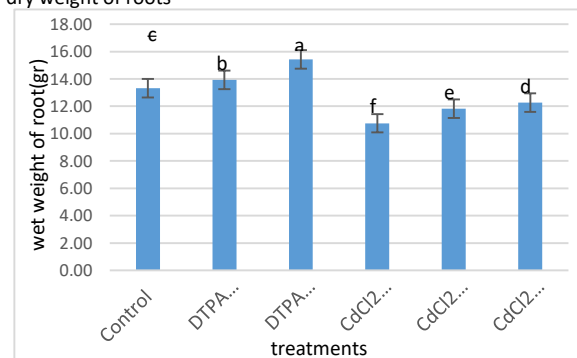


Fig. II. The effect of various cadmium and DTPA treatments on the wet weight of roots

on the growth of *Lavandula* (Figs. I, II, III, IV).

The effect of cadmium concentrations on the chlorophyll content of *Lavandula*

During this study the amount of chlorophyll under 100 ppm cadmium treatment experienced a decline compared to the control. At 100 ppm treatment, adding DTPA caused a gradual increase in the chlorophyll content. Accordingly, the highest amount of decline in the chlorophyll content of the plants under the 1 mM DTPA + 100 mg cadmium and 2 mM DTPA + 100 mg cadmium treatments were 0.9500 mg per gram wet weight and 0.9567 mg per gram wet weight, respectively (Fig. V). It is assumed that high concentrations of cadmium affects morphologic properties and causes a decline in the amount of chlorophyll, and upon adding DTPA the absorption of heavy metals such as cadmium decreases. One reason for the decline in chlorophyll content is heavy metals such as Lead acting as biosynthesis inhibitors.

The effect of Cadmium and DTPA concentrations on the accumulation of Cadmium in roots and shoots of *Lavandula*

Increasing the amount of cadmium in treatments led to an increase in cadmium accumulation in roots. The highest amount of cadmium accumulation in the root of the plants was recorded under the 100 ppm cadmium treatment which was approximately 12 mg per kg of the dry weight. Comparing the amount of cadmium accumulated in shoots and roots of *Lavandula* under similar treatments suggested the significant ability of the root for storing and accumulating more cadmium. Examining the amount of cadmium accumulation in aerial organs of *Lavandula* in response to different treatments showed an increase so that the highest amount was recorded under 100 ppm concentration of cadmium treatment (Figs. VI and VII).

The effect of cadmium concentrations on the proline content of *Lavandula*

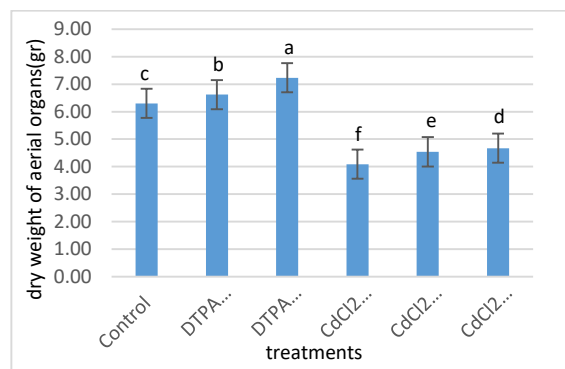


Fig. III. The effect of various cadmium and DTPA treatments on the dry weight of aerial organs

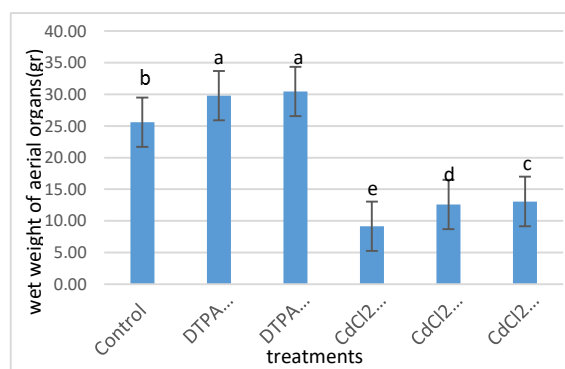


Fig. IV. The effect of various Cadmium and DTPA treatments on the wet weight of aerial organs

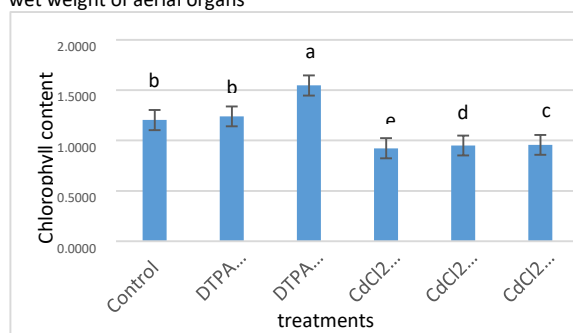


Fig. V. The effect of various cadmium and DTPA treatments on the chlorophyll content

Proline is a fundamental factor in the important process of plant growth under environmental stress. Comparing the proline level of *Lavandula* under different cadmium treatments to control plants (Fig. VIII) showed that by increasing the cadmium concentration in the growth environment of the plant, its proline content increased significantly. Results obtained from comparing means showed a significant difference between control and cadmium treatments. In other words, the 100 ppm cadmium treatment affected the proline level of *Lavandula*. Also, there was a significant difference

in DTPA treatments. The highest and lowest proline levels were observed in the 100 ppm cadmium and 2 mM DTPA treatments, respectively. Adding DTPA to cadmium also caused a decline in the proline content level (Fig. VIII).

The effect of cadmium concentrations on Superoxide dismutase (SOD) enzyme activity

Increase in the cadmium treatment caused an increase in the Superoxide dismutase enzyme activity so that the highest and lowest levels of activity were observed in 100 mg cadmium and 2 mM DTPA treatments, respectively and one can assume that the amount of Cadmium caused changes in the activities of this enzyme in plants under study (Fig. IX).

The effect of cadmium concentrations on root height and length of Lavandula

Analysis of variance showed a significant difference ($p \leq 0.01$) in the height and length of Lavandula roots among the treatments in such a way that the highest height belonged to the 2 mM DTPA treatment showing a significant difference compared to the control plants. The longest roots again belonged to the 2 mM DTPA which was significantly different from the control plants. This suggests that Cadmium had a negative effect on the length of the root and the height of the plant, preventing the growth of both organs (Figs. X and XI).

Discussion

Aminolevulinic acid dehydrogenase and protochlorophyllide reductase enzymes and act as biosynthesis inhibitors for chlorophyll. These metals inhibit the synthesis of gamma aminolevulinic acid and the formation of the protochlorophyllide reductase and substrate enzyme complex. The mutual interaction of the heavy metal with the sulfhydryl group enzymes is considered as the most important mechanism of such inhibitors (Khatibi et al., 2008). In addition to heavy metals acting as biosynthesis inhibitors for chlorophyll, such metals also cause the biodegradation of chlorophyll. Another effect of

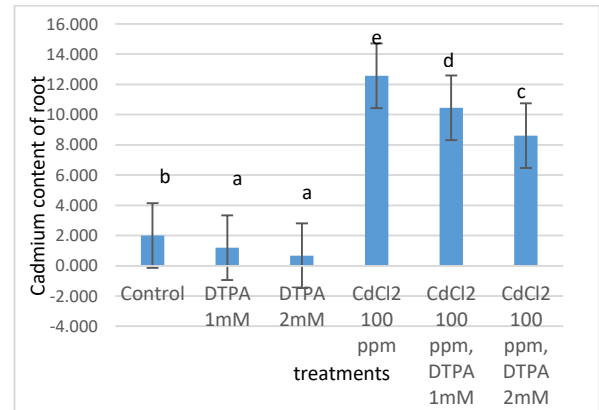


Fig. VI. The effect of various cadmium and DTPA treatments on the cadmium content of roots

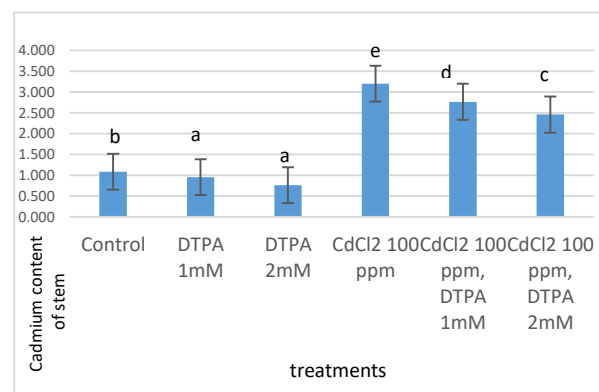


Fig. VII. The effect of various cadmium and DTPA treatments on the cadmium content of stems

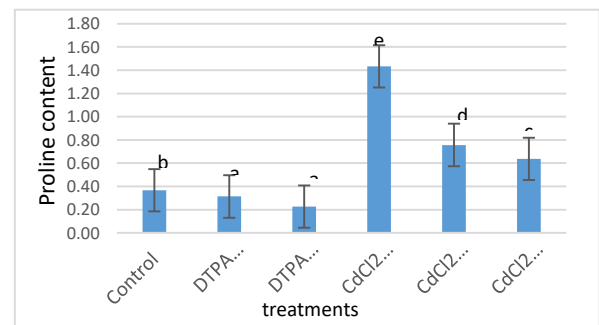


Fig. VIII. The effect of various cadmium and DTPA treatments on proline content

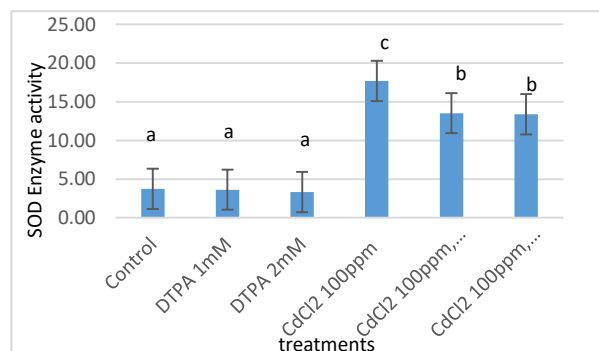


Fig. IX. The effect of various cadmium and DTPA treatments on the SOD Enzyme activity

heavy metals on the biosynthesis of chlorophyll includes replacing the central magnesium in chlorophyll which results in the decline of absorbing light by chlorophyll and therefore a decline in photosynthesis (Sharma and Dubey, 2005). Decline of chlorophyll can be described as the habituation of plants to the stress since it decreases the possibility for further damage to the photosynthesis system by forming free radicals of oxygen when extra energy is available (Jung, 2004). Cadmium is in constant competition with Iron which causes the root of the plant to absorb cadmium instead and finally replacing magnesium in the chlorophyll molecules and lead to the decline of chlorophyll synthesis (Kupper et al., 1998). Our study showed the effect of cadmium on *Lavandula* which resulted in a significant decline in the chlorophyll content compared to the control plants which proves the other results of the experiment. The presence of cadmium resulted in a decline in growth, evaporation, perspiration, and ion absorption by the plant and the decline in water absorption and ion concentration prevents the root activity (Veselo et al., 2003). The decrease of transfer of cadmium from root to aerial organs can be a result of the inactiveness of the element in the cell walls or the joining of cadmium to the organic compounds available in the root (Phytochelatins) (Sanitadi Toppi and Gabrielli, 1999). This finding was the same as (Mohammad Pour et al., 2012).

Proline is an important osmotic regulator for many halophyte and non-halophyte species such as Alfalfa and Sorghum. Accumulation of proline in rice is a sign of salt stress injury, and is a cause of the increase in Ornithine aminotransferase and its precursor, glutamic (Steward and Lee, 1974). Inside plant cells, proline plays a role in osmotic balance of vacuoles (Flores et al., 2002). Additionally, proline acts as a container for carbon and nitrogen. It also protects the plant from the damages of free radicals. The decline of proline consumption in protein synthesis during stress might be the reason for proline accumulation (Turhan and Ayaz, 2004). We can associate proline accumulation with water shortage due to the effect of heavy elements in *silene vulgaris* (Schat et al, 1998). The results from *Groenlandia densa* plant showed a gradual increase of proline

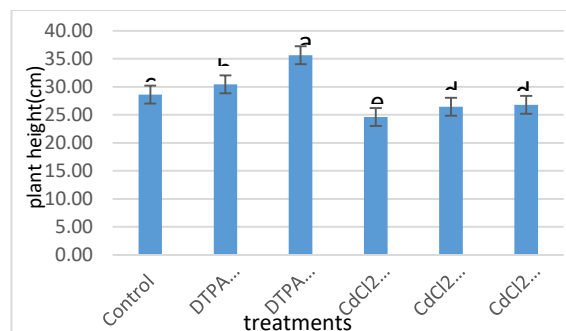


Fig. X. The effect of various cadmium and DTPA treatments on plant height

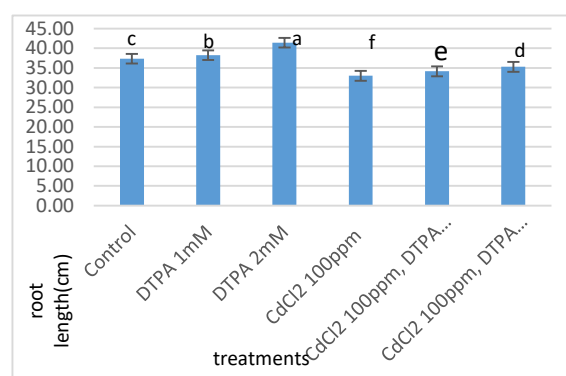


Fig. XI. The effect of various cadmium and DTPA treatments on root length

content under the effect of cadmium (Parlak and Yilmaz, 2011). Dinakar et al. (2008) showed that the proline content increased under the effect of cadmium in roots and leaves of *Arachis hypogaea* which is consistent with the results of this study.

Superoxide dismutase (SOD) include metalloenzymes that catalyze the disproportion of superoxide free radicals to hydrogen peroxide and oxygen and they seem to have an important role in protecting cells against the indirect damaging effects of such radicals (Luis et al., 1983). Studies show that high concentrations of heavy metals result in the decline of antioxidant enzymes activity rather than their increase (Schutzendubel and Polle, 2002). Gosh (2005) reported that cadmium prevents the activities of some enzymes and the sedimentation of essential elements or metabolites and therefore causes cell damage. This finding is inconsistent with the results of (Mozafari et al., 2010). Schutzendubel et al. (2001) reported that cadmium causes delay in plant growth. Saberi et al. (2009) reported that various cadmium concentrations do not significantly affect the rate

and speed of sprouting and copper sulphate does not significantly affect the sprouting rate of *A. lentiformis*. But the increase in lengths of radicle, plumule, and plantlet and also the seed vigor index were significant at ($p \leq 0.01$) under the influence of the treatments which confirms the results of this study. Morifah, (2008) stated that cadmium causes a decrease in the weight and measurement of aerial organs, root length, and leaf area. The presence of cadmium leads to the decline in the growth, evaporation, perspiration and ion absorption by the plant and the decline in water absorption and ion concentration prevents the root activity (Veselov et al., 2003).

Conclusion

Cadmium resulted a change (increase or decrease) in all evaluated properties and by adding the DTPA chelate, the absorption level of this heavy metal by *Lavandula* decreased significantly.

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