

The Absorption and Metabolism of Heavy Metals and Mineral Matters in the Halophyte Plant *Artemisia aucheri*

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Abstract

To our knowledge there has been no research on *Artemisia aucheri* as a halophyte plant, when grown in cadmium (Cd) contaminated soils. We tried to quantify the ability of *A. aucheri* in uptake of Cd and some nutrients in contaminated soil. In a pot culture experiment, five levels of Cd concentration were tested by adding 15, 30, 60, 120 and 240 mg of Cd per kilogram of dried soil. Plants were harvested and analyzed for Cd, N, P, K, Mg, Mn, Cu, Fe and Zn uptake. Depression in biomass production of plants as a result of excess Cd was observed. Cadmium concentration in plant shoots plant increased with increasing Cd supply significantly affecting the content of plant nutrients. Cd excess amounts increased macronutrients and decreased micronutrients concentrations in plant.

Keywords: *Artemisia aucheri*, biomass reduction, cadmium, nutrients

1. Introduction

Heavy metal contamination of soil and groundwater causes major environmental and human health problems. The commonly used methods for extraction of heavy metal from the environment are expensive (Singh et al., 2006). Phytoremediation is one of the effective technologies for this goal. Hence, in this technology testing and introducing new plants are very important. In addition salinity is also common in different parts of the world. Therefore, investigating the survival of salt-tolerant halophytes under heavy metal stress seems pertinent (Shevyakova et al., 2003).

Cadmium is one of the most important environmental contaminants with limited solubility in soils and hence limited availability for plant uptake (McBride, 1994). There has been extensive research regarding the accumulation of heavy metals in different plant species that has made the industry develop phytoextraction strategies to remediate heavy metal contaminated areas (Glass, 2000).

When plants are subjected to the excess amounts of Cd genetical, biochemical and physiological changes are resulted, eventually, leading to plant phyto-toxicity, and hence plant decreased growth (Liao et al., 2005; Nouairi et al., 2006; Siroka et al., 2004). There are interaction effects between heavy metals and essential macro and micronutrients, considerably affecting plant nutrient uptake (Pal et al., 2006).

The plant we chose for our approach is *Artemisia aucheri* from Poaceae family. It is a halophyte plant that grows in saline soils of Iran. To our knowledge, no studies have been reported with *A. aucheri* plants. The aim of our research was to investigate the effects of excess cadmium on biomass production, Cd concentration, and uptake and translocation of some nutrients including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) in *A. littoralis*.

2. Materials and Methods

This study was conducted under glasshouse conditions. The soil was collected from the top 20 cm layer from the arable plots. The soil was air dried and sieved to <2 mm before use and characterized to determine different soil properties including organic carbon (OC, 0.076%), Total N (0.084 %), P_{avail} (17 mg kg⁻¹), K_{avail} (213.75 mg kg⁻¹), Fe_{avail} (5.54 mg kg⁻¹), Cu_{avail} (47.8 mg kg⁻¹), Zn_{avail} (490 mg kg⁻¹), Mg_{avail} (1992.5 mg kg⁻¹), Mn_{avail} (162.5 mg kg⁻¹), Cd (0.25 mg kg⁻¹ soil dry weight). Ten Kg of soil was placed into pots. The soil was contaminated using five levels of Cd (as CdCl₂): 15, 30, 60, 120, 240 mg kg⁻¹ plus a control. Three transplants of two-week old *A. aucheri* were transplanted into each pot. *A. aucheri* is a permanent plant that regrowing from underground parts.

Plant samples were taken at three different stages including 80, 160 and 240 days after planting. After the third harvest, roots were extracted from pots and washed with tap and distilled water.

2.1 Cd and nutrients Analysis

Plant materials of each stage were analyzed for Cd concentration. Plant samples were divided into stems and leaves and washed thoroughly with distilled water. Samples were oven dried at 70 °C for 24 h. The dried plant material was ground and digested with a mixture of concentrated HNO₃ and HClO₄. Analysis of Cd in the stems and leaves was performed by Atomic Absorption Spectrophotometer.

At the third stage, all three stages shoot samples from all three samplings were combined and concentrations of nutrients (N, P, K, Cu, Fe, Cu, Mn, Mg and Zn) were determined. Total N and total P were measured by Berthelot reaction and molybdenum blue method, respectively (Page et al., 1982). Other nutrients were measured by Atomic Absorption Spectrophotometer.

2.2 Statistical Analysis

Analysis of variance (ANOVA) was performed at a level of 95% confidence using SAS software. Means were compared using least significant difference (LSD protected) method at $P = 0.05$.

3. Results

3.1 Dry Matter Production

Increasing concentration of Cd significantly decreased leaf and stem dry matter production of plants ($p < 0.05$) (Figure 1 and Figure 2).

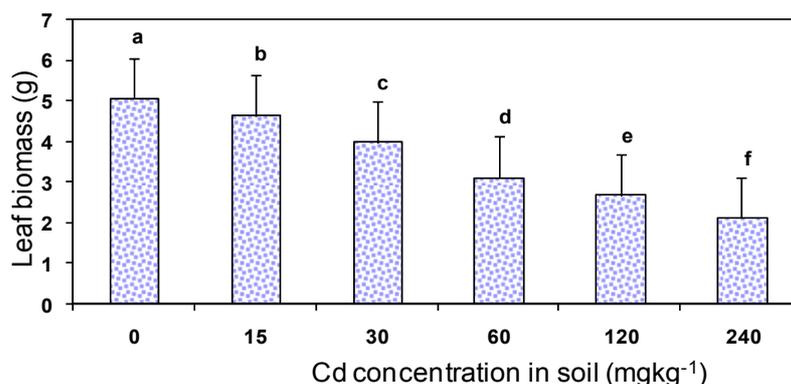


Figure 1. Leaf dry matter of *Artemisia aucheria* after exposure to various Cd concentrations. Mean values within the same column, followed by different letters are significantly different using LSD protected test ($p < 0.05$). Data represent means \pm S.D.

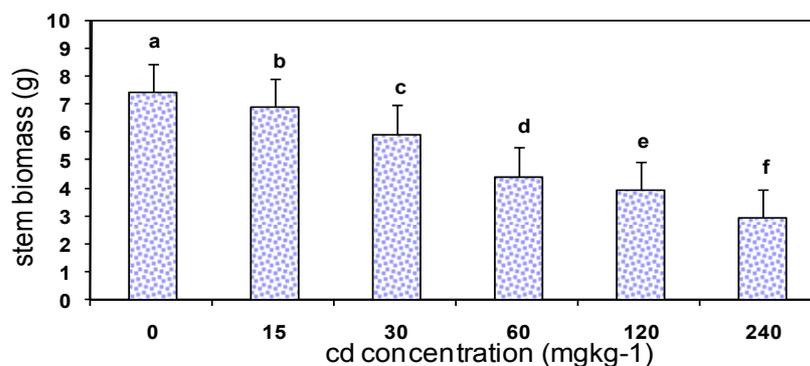


Figure 2. Stem dry matter of *Artemisia aucheria* after treating with various Cd concentrations. Mean values within the same column, followed by different letters are significantly different using LSD protected test ($p < 0.05$). Data represent means \pm S.D.

3.2 Plant Cd Accumulation

Cadmium accumulation in shoots increased markedly with increasing Cd concentrations in soil. The highest concentration of Cd was 77.05 mg kg⁻¹ dry weight ($p < 0.05$) (Figure 3). Cadmium concentrations in roots significantly increased with Cd levels increasing from 15 to 60 mg kg⁻¹, and then significantly decreased as Cd concentrations increased from 60 to 240 mg kg⁻¹. Cd concentrations in shoots were higher than those in roots ($p < 0.05$) (Figure 3).

3.3 N Content of Leaf, Stem and Root

The applied concentrations of Cd significantly influenced the N content of leaf, stem and root (data not presented). In leaves, the concentration of N increased with an increase in Cd supply up to 120 mg kg⁻¹ ($p < 0.05$), then decreased significantly as Cd concentration increased to 240 mg kg⁻¹ dry soil ($p < 0.05$) (Figure 4).

The stem N concentration significantly increased with increasing Cd content ($p < 0.05$) (Figure 4). Nitrogen concentrations in the *A. aucheri* leaves and roots grown in the control and 240 mg kg⁻¹ treatment were significantly lower than the other treatments ($p < 0.05$) (Figure 4).

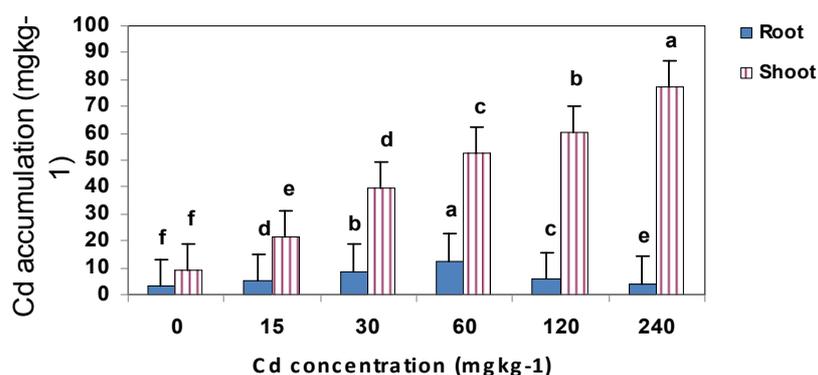


Figure 3. The concentration of Cd in shoots and roots of *Artemisia aucheria* after treating with various Cd concentration. Mean values within the same column, followed by different letters are significantly different using LSD protected test ($p < 0.05$). Data represent means \pm S.D.

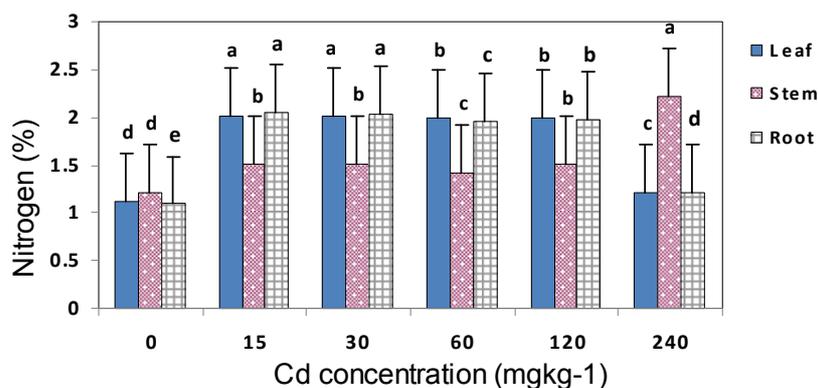


Figure 4. The concentration of N in leaves, stems and roots of *Artemisia aucheria* after treating with various Cd concentration. Mean values within the same column, followed by different letters are significantly different using LSD protected test ($P < 0.05$). Data represent means \pm S.D.

3.4 P Content of Leaf, Stem and Root

Figure 5 shows the concentration of P measured in *A. aucheri* plant tissues. Relative to the other treatments, P content in leaves, stems and roots was significantly higher when Cd concentration ranged from 15-30 mg kg⁻¹. P content of leaf, stem and root decreased significantly when Cd concentration increased from 30 to 120 mg kg⁻¹

dry weight soil. The P content of plant tissues increased again for the 240 mg kg⁻¹ treatment ($p < 0.05$) (Figure 5).

3.5 K Content of Leaf, Stem and Root

K content of plant tissues were significantly affected by different Cd concentrations in soil ($p < 0.05$) (Figure 6). As seen in Figure 6, K concentration in leaves and roots treated by different Cd concentrations followed the same pattern. It was maximal at the concentration of 15 mg kg⁻¹. However, when Cd concentration increased to 120 mg kg⁻¹, the K accumulation in *A. aucheria* leaves and roots reduced from 1661.38 to 1523.4 and from 1656.6 to 1519.62 mg kg⁻¹ dry weight, respectively. Also, an increase in K concentration in leaves and roots was observed in the concentration of 120 mg kg⁻¹ to 240 mg kg⁻¹ ($p < 0.05$) (Figure 6).

The results showed that plants grown in uncontaminated soil had the highest content of K in stems ($p < 0.05$). A decrease in stem K was observed when Cd concentration increased from 15 to 120 mg kg⁻¹, then it increased at 240 mg Cd kg⁻¹ ($p < 0.05$) (Figure 6).

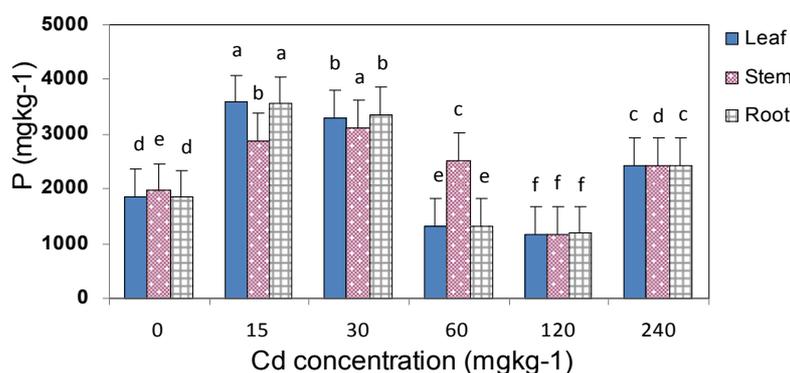


Figure 5. The concentration of P in leaves, stems and roots of *Artemisia aucheria* after treating with various Cd concentration. Mean values within the same column, followed by different letters are significantly different using LSD protected test ($P < 0.05$). Data represent means \pm S.D.

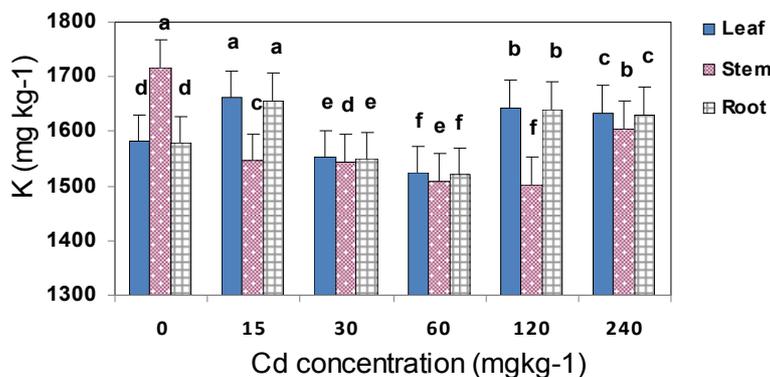


Figure 6. The concentration of K in leaves, stems and roots of *Artemisia aucheria* after treating with various Cd concentration. Mean values within the same column, followed by different letters are significantly different using LSD protected test ($P < 0.05$). Data represent means \pm S.D.

3.6 Shoot Mg and Micronutrients

The Mg concentrations in the shoots increased as Cd levels increased from 0 to 60 mg kg⁻¹, it then markedly decreased at the range of 120-240 mg Cd kg⁻¹ ($p < 0.05$). Mn accumulation in shoots decreased with increasing soil Cd concentrations. The accumulation of Mn was the highest in the shoots of control plants ($p < 0.05$) (Table 1). Copper content of shoots decreased with increasing Cd concentration. The highest Cu concentrations were

related to the control plants ($p < 0.05$) (Table 1). The Fe concentration in *A. aucheri* shoots decreased significantly as Cd concentration increased from 15 to 240 mg kg⁻¹. The Zn concentrations in the control plants were higher than all the other treatments ($p < 0.05$). Zinc concentrations in the shoots were significantly reduced when Cd concentration increased from 15 to 240 mg kg⁻¹. The concentration of Zn in the shoots of *A. aucheri* was maximal at control ($p < 0.05$) (Table 1).

Table 1. Mean comparison of shoot content of Mg, Mn, Cu, Fe and Zn affected by different Cd concentrations

Cd concentration (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)
0	566.40 ^c	51.45 ^a	25.95 ^a	436.10 ^a	29.75 ^a
15	733.20 ^c	43.90 ^b	19.80 ^b	324.70 ^b	28.75 ^b
30	744.38 ^b	28.05 ^c	17.80 ^b	253.60 ^c	21.15 ^c
60	748.30 ^a	22.50 ^d	13.11 ^c	207.80 ^d	18.70 ^d
120	621.40 ^d	21.80 ^d	12.00 ^{cd}	185.80 ^e	16.68 ^e
240	564.50 ^c	20.35 ^e	10.33 ^d	150.30 ^f	14.71 ^f

Mean values within the same column, followed by different letters are significantly different using LSD protected test ($P < 0.05$). Data represent means \pm S.D.

4. Discussion

Chlorosis symptoms appeared on the leaves of *A. aucheri* grown in soil with 240 mg kg⁻¹ Cd. Cadmium toxicity symptoms have been related to interactions between uptake and translocation of nutrients in plants. Leaf chlorosis resulted by Cd addition appeared to be associated with Fe (Foy et al., 1978) or Zn (Turner, 1973) in plants. Our results also indicate that shoot Fe and Zn content was reduced under Cd stress.

Figures 1 and 2 clearly demonstrate the adverse effects of different Cd-levels on plant biomass. The depression in plant growth as a result of excess Cd is similar to the result of Jiang et al. (2004), who reported that plant growth including root dry matter was negatively affected by Cd at 170 mg Cd kg⁻¹ soil dry weight. These results are also in agreement with the findings of Michalska and Asp (2001) and Jiang et al. (2003). Decreased plant growth by excess Cd can be attributed to the inhibition of cell growth (Prasad, 1995), mitosis, the decreased synthesis of cell-wall components, damage to the golgi apparatus, and alteration in the polysaccharide metabolism. However, browning is resulted by suberin storage (Punz & Sieghardt, 1993).

Cadmium content of shoots increased with increasing soil Cd. These results are in accordance with the observations of Jiang et al. (2004) and Zhao et al. (2003). Also, Vogel-Mikus et al. (2005) reported significant hyperaccumulation of Cd in shoots and roots with increasing Cd concentrations. Moreover, their findings showed that Cd concentrations in roots were significantly lower than shoots of *Thlaspi praecox* Wulfen (Brassicaceae). According to our results Cd concentrations in roots were higher than those in shoots (Figure 3). These results are contrary to the findings by Salt et al. (1997) and Jiang et al. (2004).

The highest Cd concentration in roots was found approximately at the 60 mg Cd kg⁻¹ dry weight soil. Thereafter, Cd concentration decreased in the roots (Figure 3). This result are in agreement with the results observed by De Oliveira et al. (1994) testing two soybeans varieties grown in a range of 0.2 to 1 mg Cd L⁻¹. Liu et al. (2006) also showed that the distributive levels of Cd in the roots of *Zea mays* reduced with increasing Cd concentration.

The results presented here indicate that the total N content of *A. littoralis*, growing at different Cd concentrations was higher than control (data not shown). This can be due to reduced plant growth. N concentration in stems and roots (but not in leaves) of sunflower seedlings (V4 stage) was significantly lower at the spilled-affected site (Madejon et al., 2003). Our results disagree with the work of Narwal et al. (1993) who indicated that excess Cd reduced maize N content.

Phosphorus deficiency is the second (after N) major nutrient limitation to plant growth. P concentration in plant tissues decreased with increasing Cd concentration, but it was increased by the 240 mg kg⁻¹ treatment (Figure 5). Some researchers have indicated the adverse effect of Cd on P uptake by sunflower (Madejon et al., 2003) and maize (Narwal et al., 1993), while other researchers did not find changes in P uptake by maize (Nocito et al. 2002). A reduction in P concentration in all parts of cabbage was observed as excess lead was applied by Sinha

et al. (2006). Michalska and Asp (2001) reported that Cd application did not have significant effects on the content of P in three lettuce cultivars.

The results in the present investigation demonstrated that K concentration decreased in different tissues of *A. littoralis*. However, it was increased in roots and leaves at the concentration of 240 mg Cd kg⁻¹ and in stems at the concentration of 120 mg Cd kg⁻¹ (Figure 6). According to the literature, contradictory information can be found in the effects exerted by Cd on plant growth. Accordingly, Jiang et al. (2003) demonstrated that K content in the roots of indian mustard were not affected by Cd concentration ranging from 10 to 150 mg Cd kg⁻¹ soil dry weight. An enhancement was observed when Cd was added at 170 mg kg⁻¹ soil dry weight. Cd content of indian mustard shoot increased at the Cd concentrations ranging from 10 to 110 mg kg⁻¹, and then remained constant as Cd concentration increased from 110 to 190 mg kg⁻¹. Also, an increase in K content was reported by Cieccko et al. (2004). However, Michalska and Asp (2001) and Narwal et al. (1993) observed that K content was decreased when Cd was added to the nutrient solution. This might have been because of the detrimental effects of Cd on the cellular plasma membrane of *Spinacea oleracea* L. root (Sadana & Bijah 1987).

The Mg concentration in the *A. aucherish* shoots increased as Cd concentration increased from 0 to 60 mg kg⁻¹ soil dry weight, and then decreased (Table 1). Increased Mg content was reported by Obata and Umebayashi (1997), however Yang et al. (1996) observed a reduction in Mg content of plant shoot. According to Table 1, Mn accumulation in shoots decreased with increasing soil Cd concentrations. Obata and Umebayashi (1997), Madejon et al. (2003) and Lui et al. (2006) reported reduction in the shoot Mn content. Our finding is also in agreement with the reports by Hernández et al. (1998). Yang et al. (1996) also demonstrated a reduction in the root influx of Mn to plant shoot movement.

Shoot Cu content decreased when Cd was applied to the soil (Table 1). Our results are similar to the results of Breckle and Kahle (1992) and Liu et al. (2006). However, some contrary reports were made by Obata and Umebayashi (1997) and Madejon et al. (2003). The Fe concentration in *A. aucherish* shoots decreased as Cd addition increased from 15 to 240 mg kg⁻¹ (Table 1). Cadmium has been reported to reduce Fe uptake in different plants (Haghiri, 1973; Breckle & Kahle 1992; Madejon et al., 2003). But, Jiang et al. (2004) and Liu et al. (2006) reported an increase in shoot Fe content as Cd concentrations increased.

The Zinc concentration in the shoots was reduced when Cd concentration increased from 15 to 240 mg kg⁻¹ (Table 1). In the research work by Michalska and Asp (2001) Zn showed no clear response to Cd treatment. Reduction of plant Zn content under Cd stress has been indicated by some workers (e.g. Marschner 1995; Turner, 1973; Thomas & Harrison, 1989). However, the findings of Madejon et al. (2003) indicated that shoot Zn content increased. However, Jiang et al. (2004) found that Zn concentrations in shoots of indian mustard was initially increased as Cd concentration increased and then it markedly decreased and remained constant.

5. Conclusion

In conclusion, the pot-culture experiment conducted with excess Cd concentrations demonstrated that Cd stress significantly reduced plant biomass production. It also altered Cd and nutrients uptake and translocation in *A. littoralis*. In this study, low and high Cd concentrations in the roots (12.42 mg kg⁻¹) and shoots (77.05 mg kg⁻¹) was observed indicating that *A. aucherican* be appropriately used for phytoextraction use. Interestingly, Cd excess amounts increased the uptake of plant macronutrient and decreased the uptake of plant micronutrients. In the next research work, we can look for methods, resulting in the enhancement of *A. aucherican* ability to absorb Cd.

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