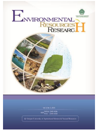

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Phytoremediation of cadmium and nickel using *Vetiveria zizanioides*

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Abstract
Phytoremediation is a well known heavy metals remediation technique for contaminated soils and water. The present study was aimed to evaluate the uptake and dry weight response of vetiver grass (*Vetiveria zizanioides* L.) subjected to different levels of cadmium (Cd) (0, 3, 6 and 12 mg/kg soil) and nickel (Ni) (0, 50, 100 and 200 mg/kg soil) stress. The experiment was conducted in pots using a completely randomized design with four replications for three months. Concentration of Ni and Cd was determined using atomic absorption spectrophotometer. The dried weight of aerial and underground parts of Vetiver grass individuals at the end of the experiment were used to compare plant weight response to different stresses. Statistical analysis was performed using version 18 of SPSS software and analysis of variance (ANOVA) and mean comparisons were completed through Tukey method. No restrictions on Cd uptake was observed in root and shoot of Vetiver grass, but in the case of Ni, its concentration in shoots of vetiver grass decreased with higher metal levels.The highest transfer factor (TF) among Ni treatments was observed at the lowest concentration (50 mg/L) and the highest TF among Cd treatments was observed at the highest concentration (12 mg/L). Results revealed that Ni and Cd had a significant ($p<0.05$) positive effect on shoot and root dry weight of Vetiver grass. Our results suggest capability of this plant for use in phytoextraction of Ni and phytostabilisation of Cd contaminated soils.

***Keywords*: Contamination, Dry weight, Heavy metals, Remediation, Translocation factor**

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Introduction

Soil contamination is a serious worldwide concern, therefore effective remediation approaches are necessary. Phytoremediation is eco-friendly and solar-driven technology with good community acceptance. It is an emerging green technology that is used for the uptake of vast quantities of heavy metals from soil and storage of these in a harvestable (removable) component. According to Baker and Brooks (1989), hyper-accumulating plants are those that accumulate more than 100 mg kg^{-1} dry weight of cadmium, or more than 1000 mg kg^{-1} dry weight of nickel, in their shoots when grown on metal rich soils.

Excessive entry of heavy metals such as Ni and Cd by air or water causes soil pollution (Lasat, 2002*,* Morel and Hering, 1993). This was considered one of the greatest environmental threats (Kimbrough *et al.*, 1999) and have negative effects on the ecology of the soil, land fertility, the quality of crop production and water resources and finally creates serious problems for the health of humans and animals (Thawornchaisit and Polprasert, 2009). Adriano (1986) declared that industrial activities and mining, fuel combustion, agricultural pesticides and fertilizers are the main sources of heavy metals. These metals are seen in different soils as a free metal ions, metal soluble complexes, metal exchangeable ions, and metals linked with organic materials, precipitated or insoluble compounds as oxides, carbonates and hydroxides or it may form part of silicate materials (Leyval *et al.*, 1997). Most heavy metals are among the micronutrients and a low amount of them is needed for plant growth. Generally, the presence of excessive heavy metals, reduce photosynthesis and decrease plant growth (Gajewska *et al.*, 2006). Unlike organic pollutants, metal contaminants are not biodegradable and decontamination of soils poluted by these metals is a necessity (Lasat, 2003). Soil pollution with heavy metals can be resolved by physical, chemical and biological methods (Mc Eldowney *et al.*, 1993). Physical and chemical methods have irreversible effects on soil properties, destroy biodiversity and are very costly. Phytoremediation is a low-cost technology that can bring out toxic metals without reducing the soil fertility (Singh *etal.*, 2003*,* Raskin *et al.*, 1997). Hyperaccumulator plants are of great importance in phytoremediation (Shen *et al.,* 1997). Although Ni is an essential nutrient, the requirement for this element of non-hyperaccumulator plant is so low that Ni deficiency has never been reported in field-grown crops. Since these metals are water soluble, they are easily bioavailable and bioaccumulatable.

The effects of different concentrations of cadmium on root length of *Picea abies* demonstrated that root length decreased with Cd concentration increasing (Goldbold and knetter, 1991). Ma *et al.* (1997) have mentioned that the reduction of root length in high concentration of heavy metals is probably because of a reduction in the viscosity and flexibility of root cell walls. Ghaderian and Pakdaman (2014) revealed that Ni concentration in the root of *Pistacia vera*is is about ten times higher than its aerial parts and its dry weight reduce with increase in Ni concentration. Investigation on the ability of *Glycyrrhiza glabra* (var. glabra and var. glandulifera) for phytoremediation, tolerance to cadmium and its accumulation within plant demonstrated that Ca causes a decrease in seed germination. The dry weight of root in both variety and the length of root in var. *glandulifera* decreases significantly in proportion to the control group. Whereas, the dry and fresh weight of shoot, the length of shoot and the fresh weight of root in both variety and the length of root in var. *glabra* didn’t change significantly. No metal-toxicity symptoms such as burning and redness of the tissue were observed in Cd treatment (Sarmadi *et al.,* 2011). The effect of various concentration of cadmium and nickel (0, 5, 10, 20, 40 and 50 mg/L) on the growth of sunflower (*Helianthus annuus*) revealed that the studied measures were significantly affected by these metals at higher concentration of 40 and 50 mg/L. So, the lower concentration of heavy metals (5 to 20 mg/L) were observed to be stimulating the root and shoot length and increase biomass of the sunflower plant (Chhotu *et al.,* 2008). Besalatpour *et al.,* (2008) stated that *Agropyron smithii* L. and *Festuca arundinacea* L. have high capability for remediation of contaminated soils. Comparing the phytoremediation capability of *Lepidum sativum* and *Brassica olerace* demonstrated that *Brassica olerace* has higher capability than *Lepidum sativum* (Davari *et al.*, 2010). *V. zizanioides* is a multiple use grass that acts also as heavy metals phytoextractor from contaminated soils (Shu and Xia., 2003). It has been reported that some phytoremediator plants such as sunflower use different methods for different soil contaminants. It may remediate Cd, Ni, Zn and Cu contaminated soils through phytostabilization, while may remediate Pb contaminated soils through phytoextraction. (Sewalem *et al.,* 2014, Chhotu *et al.,* 2008). Soil chloride (Cl^{-1}) concentration and the concentration of heavy metals are critical in Cd accumulation in plants, so, Cd absorbtion by plants increase in salty environment or the environment containing higher concentration of Cd (Hattori *et al.*, 2006, Ozkutlu *et al.,* 2007, Tallio *et al.,* 2003). Different heavy metal accumulator plants have different capability of heavy metal accumulation. Fattahi-Kiasari *et al.*, 2010, stated that *Zea mays* L. has higher capability than *Gossypium hirsutum* L. and *Helianthus annuus* L.

This work investigates the potential of *V. zizanioides* for phytoremediation purposes. The aim is to determine the accumulation of heavy metals, namely Ni and Cd in different sections – roots and shoots – of *V. zizanioides*.

Materials and methods

Vegetation experiment

Plant accumulation capacity was tested in a three-month pot experiment. Pots containing uncontaminated soil with clay silt texture (Table 1) were placed outdoors and were irrigated by contaminated water using $Nicl_2$ and $Cdcl_2$ in 4 different concentrations (0, 50, 100 and 200 mg/L for Ni and 0, 3, 6 and 12 mg/L for Cd) twice a week. There were 4 replicates for each treatment of studied species, so totally 32 pots were used in this study.

Table 1. Physico-chemical properties of the used soil

Soil texture	Soil Depth (cm)	Bulk Density (gr/cm3)	Salinity (dsm^{-1})	pH	Organic Carbon (%)	Total Nitrogen (%)
Silty clay	0-20	1.37	0.25	8.39	0.99	0.09

Plant analysis

Entire plants – four replicates for each treatment – were washed with de-ionnized water, after which they were separated in roots and shoots, oven dried at 90 ◦C for 24h, grinded, and sieved to <1mm. The resulting samples were digested at high temperature (up to 205 ◦C) with a mixture of concentrated nitric acid, perchloric acid and sulfuric acid (40:4:1). Metal (Ni and Cd) ions concentration were determined using atomic absorption spectrophotometer (Varian 220 SpectrAA model).

The aerial and underground parts of Vetiver grass individuals were clipped manually, with special scissors at the end of the experiment and their oven dried weight was used to compare plant weight response to different stress.

Statistical Analysis

Analysis of variance (ANOVA) was implemented using version 18 of SPSS software, followed by Tukey's post-hoc test between the means of treatments to determine the significant difference.

Results and Discussion

Extraction of Ni and Cd from roots and shoots of V. zizanioides

Ni and Cd concentration found in roots of *V. zizanioides* L. significantly increased with increasing metal treatments. The mean of Ni accumulation in roots of *V. zizanioides* for 50, 100 and 200 mg/L Ni in irrigation water were 16.6, 22.79 and 36.00 mg/kg, respectively, and the mean of Cd accumulation for 3, 6 and 12 mg/L Cd in irrigation water were 1.29, 2.09 and 2.91

mg/kg respectively. The initial concentration of metals in the soil solution is considered as an effective factor on its accumulation in the root (Mahar *etal.*, 2016).

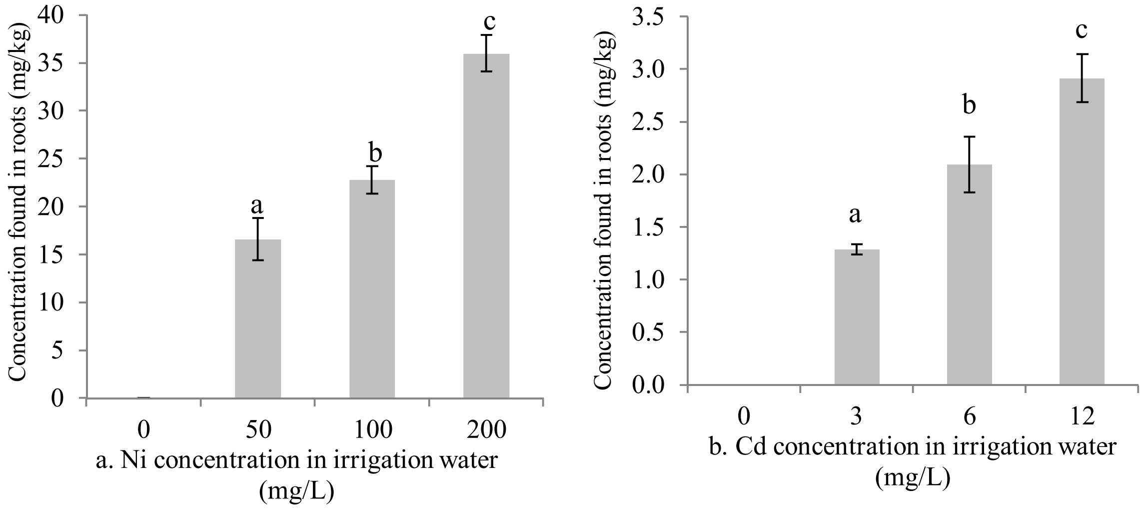


Figure 1. Metals concentration found in roots of *V. zizanioides*

The highest Ni concentration in shoots of *V. zizanioides* (36.67 mg/kg) was observed in the pots irrigated with the water containing 50 mg/L Ni, and its concentration significantly decreased at higher metal levels (27.87 and 31.32 mg/kg Ni for the pots irrigated with 100 and 200 mg/L Ni). Contrary, significant increase in Cd concentration was observed in shoots of *V. zizanioides* with increasing Cd levels in irrigation water. The mean of Cd accumulation in shoots of *V. zizanioides* for 3, 6 and 12 mg/L Cd in irrigation water were 0.49, 0.76 and 2.38 mg/kg respectively.

The mean of total Ni accumulation of *V. zizanioides* for 50, 100 and 200 mg/L Ni in irrigation water were 53.27, 50.67 and 67.32 mg/kg respectively, and in the pots irrigated with 200 mg/L Ni, significantly higher accumulation was observed as compared to other treatments. Significant increase in the mean of total Cd accumulation of *V. zizanioides* was observed with increasing Cd levels in irrigation water. So, its mean accumulation for 3, 6 and 12 mg/L Cd in irrigation water were 1.78, 2.86 and 5.29mg/kg, respectively. It has been suggested that higher activities of antioxidant enzymes may help metal accumulator plants to survive under metal stress.

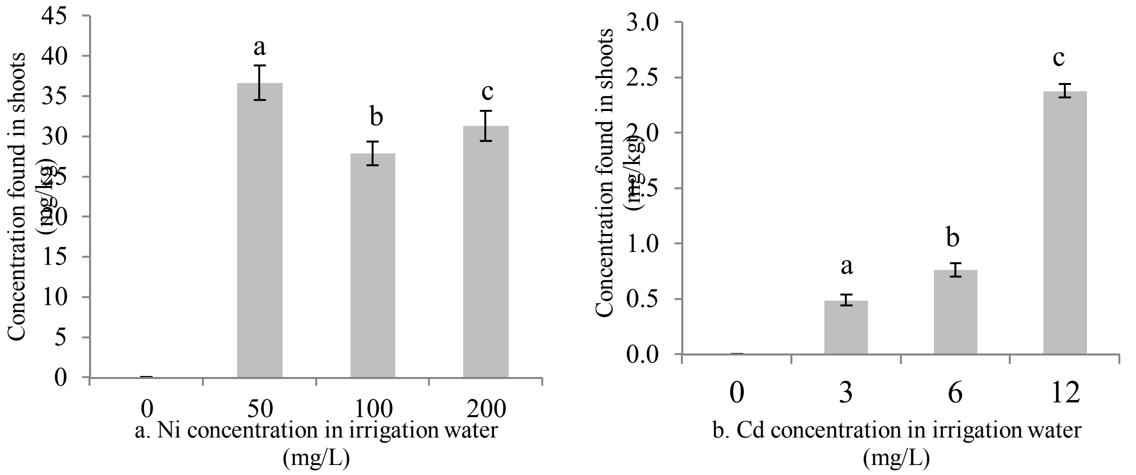


Figure 2. Metals concentration found in shoots of *V. zizanioides*

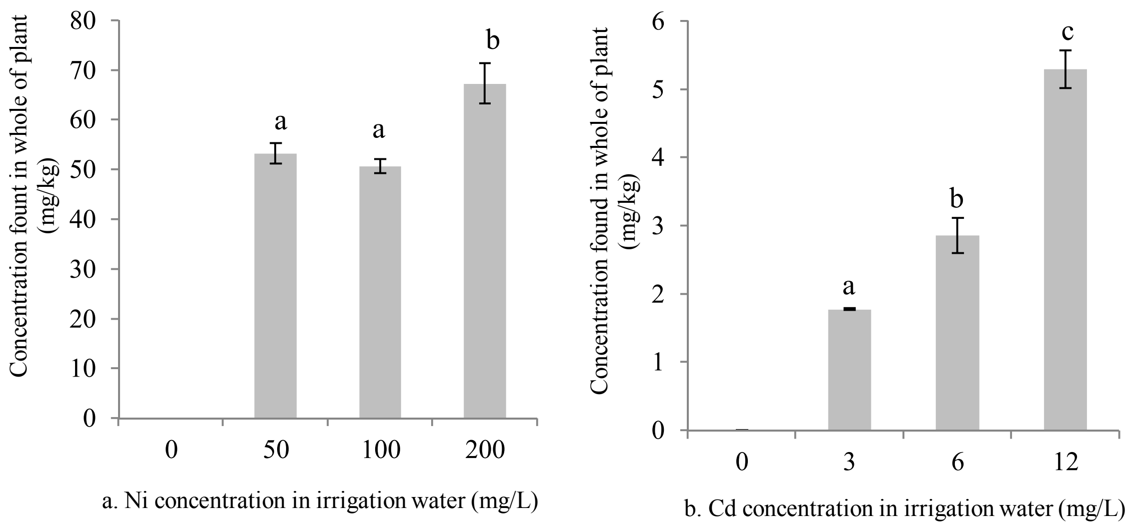


Figure 3. Metals concentration found in the whole *V. zizanioides*

The concentration of Cd was higher in roots than shoots and the concentration of Ni was higher in shoots than roots except the pots irrigated with 200 ml/L Ni. Plants treated with Ni increased Ni uptake more than 2.0 times in roots in 200 mg/L treatment as compared to 50mg/L treatment. Plants treated with Cd increased Cd uptake by 2.25 times in roots and 4.85 times in shoots in 12mg/L treatments as compared to 3mg/L treatment, respectively.

Our results are in agreement with those that claom generally the cadmium concentration in the plant roots is higher than its concentrations in the organs of plant (Sêkara *et al.,*2005*,* Boominathan and Doran, 2003). It has been suggested that Cd-binding peptides in roots may prevent the transmission of Cd to young organs (McKenna *et al.,* 1993).

There was a significant difference in the transfer factor among Ni treatments, so the highest shoot-to-root ratio among them was observed at concentration 50 mg/L and decreased significantly in higher Ni stress. According to the results, 31.16%, 45%, and 53.48% of the Ni absorbed by *V. zizanioides* for 50, 100 and 200 mg/L Ni in irrigation water is retained in the roots. Ni in the roots most likely settles in the vascular cylinder rather than in the cortex (Riesen and Feller, 2005). TF reduction might be due to increase in Ni concentration in the cortex of *V. zizanioides*. Studying cellular compartmentation of Ni in some hyperaccumulator plants revealed that major part of Ni in the epidermal cells is present in the vacuoles and the smaller one is present in the cell wall (Kupper *et al.,* 2001). It is suggested that Ni immobilization in the root's vacuoles and its sequestration in the walls of xylem parenchyma cells increase with increasing Ni stress (Seregin and Kozhevnikova, 2006).

In the plants treated with Cd, the highest transfer ratio was observed at concentration 12mg/L Cd and it was significantly higher than other treatments. According to the results, 72.47%, 73.33%, and 55.01% of the Cd absorbed by *V. zizanioides*for 3, 6 and 12 mg/L Cd in irrigation water is retained in the roots.

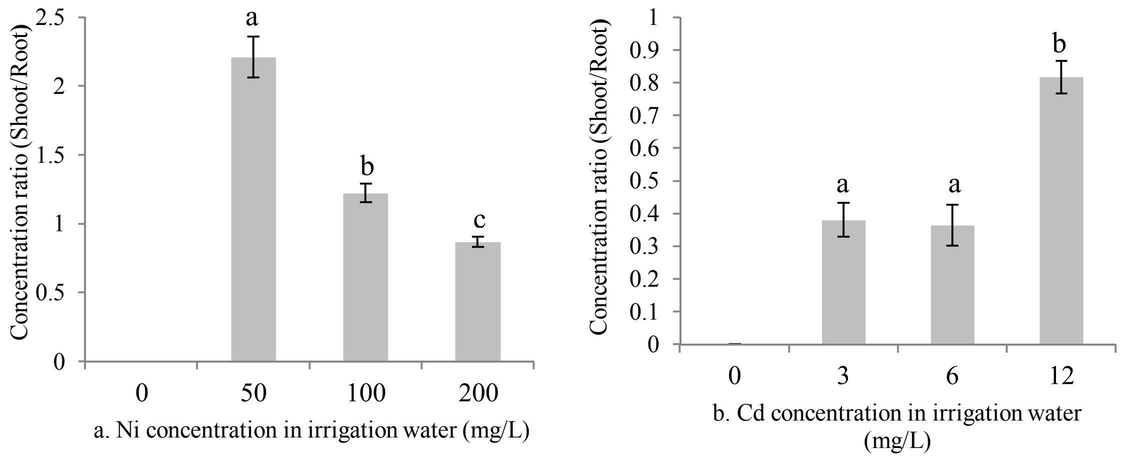
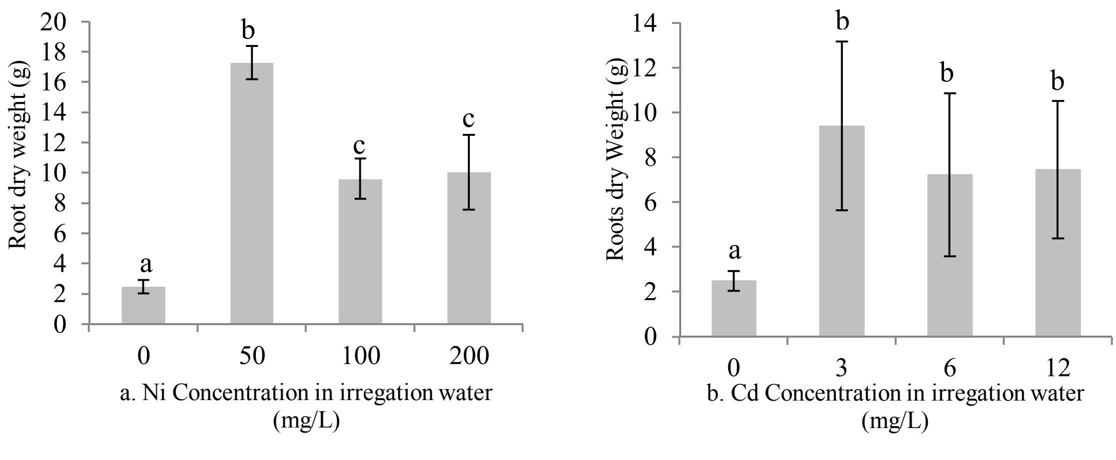


Figure 4. The transfer factor (Shoot/Root ratio) of *V. zizanioides*

Effects of heavy metals on roots and shoots dry weight

Treatments of both metals significantly affected the dry weight of roots and shoots, so in both roots and shoots, dry weights were significantly higher under metal treatments as compared to the respective untreated control. There was a significant difference among roots dry weight of Ni treated plants, so the highest dry weight was observed at lowest Ni stress (50 mg/L) and at higher Ni stress it decreased but was significantly higher compared to the control treatment. The highest shoot dry weight among Ni treated plants and the highest roots and shoots dry weight among Cd treated plants was observed at 50 mg/L and 3 mg/L levels of metals, respectively. However, there was no significant difference among treatments. There was significant difference among total dry weight of Ni treated plants, so the highest total dry weigh was observed at the lowest Ni stress (50 mg/L) and at higher Ni stress it decreased but was significantly higher compared to the control. No significant difference was observed among total dry weight of Cd treated plants, but was significantly higher than control plants. Liu *et al.* (2008) reported that growth and biomass of *Calendula officinalis* and *Althaea rosea* increased at the Cd level of 30 mgkg^{-1} of soil and decreased with higher Cd levels. In contrast, the opposite results were obtained for *Linum usitatissimum* (Amna *et al.,*2015). An increase in metal concentrations along with increase in biomass might be due to increase in the activities of antioxidant enzymes under metal stress (Tauqeer *et al.,* 2016).



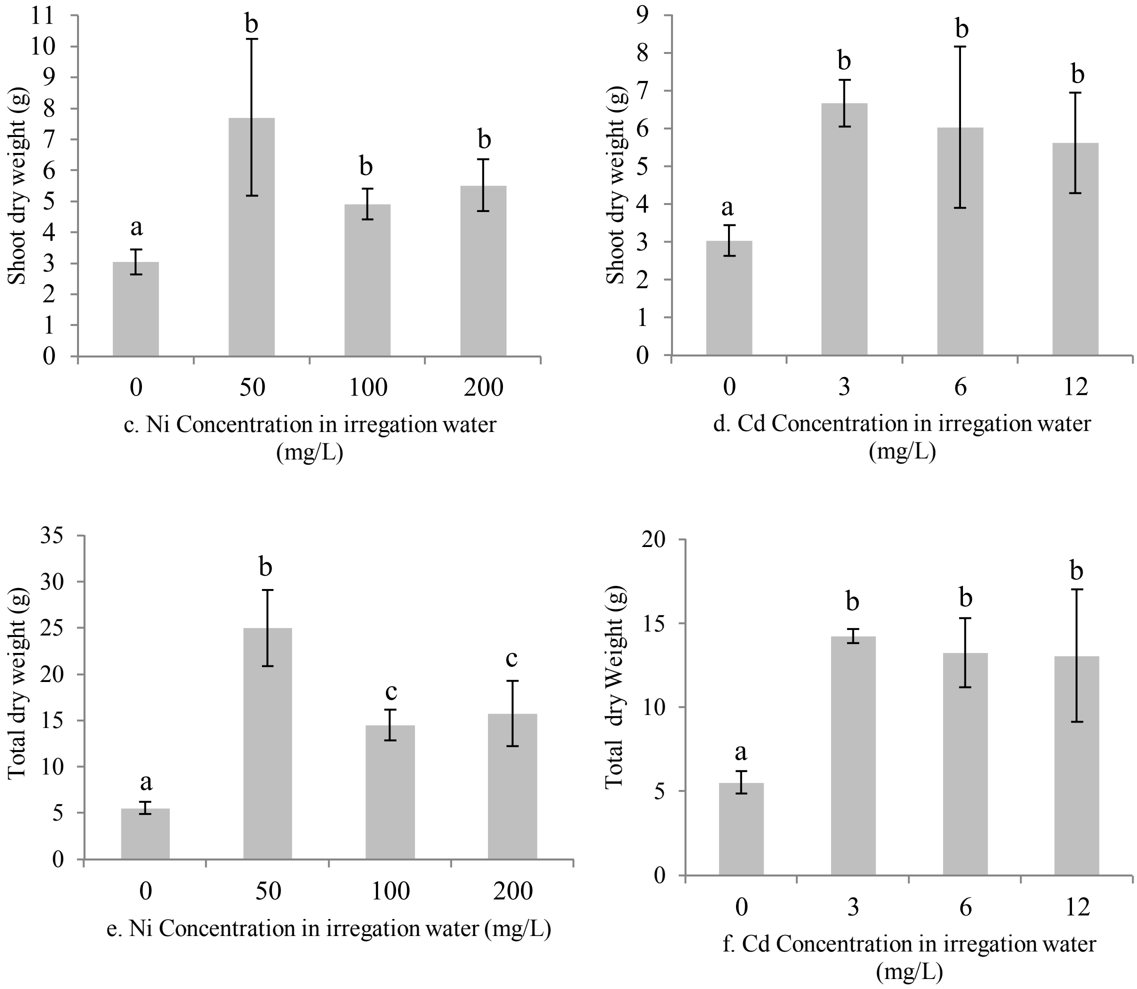


Figure 5. Effect of various Ni (0,50,100 and 200 mg/L) and Cd (0,3,6 and 12mg/L) treatments on root, shoot and total dry weight of *V. zizanioides*

Conclusion

Our results revealed that roots and shoots dry weight of *V. zizanioides*is increased under Ni and Cd stress. The stimulating effect of Ni and Cd was stronger on roots compared to shoots. The positive effect of Ni and Cd on root and shoot dry weight indicated a large metal tolerance ability of *V. zizanioides*. Distribution of adsorbed metal by a plant in its tissues is critical in efficiency of soil cleaning by a plant. Regarding concentrations of Ni and Cd in shoots of *V. zizanioides*, (lower than 1000 $mgkg^{-1}$ and 100 $mgkg^{-1}$, respectively), the plant can not be labeled as hyperaccumulator species. However, high Ni tolerance and TF (>1) demonstrated by *V. zizanioides* suggest capability of this plant for application in phytoextraction of Ni contaminated soils. In addation, high Cd tolerance and low TF (<1) demonstrated by *V. zizanioides* as well as its large and fast-growing root system, signify usefulness of applying *V. zizanioides* in phytostabilisation of Cd contaminated soils.

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