Phytoremediation ability and oxidative enzymes activity of Persian clover (*Trifolium resupinatum* L.) in the presence of nickel

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Abstract

Phytoremediation is a relatively new method in agricultural science for removing soil contaminants to some extent through cultivating some plants with the least cost. This research was conducted to examine the role of nickel as a heavy metal in catalase and peroxidase activities and also to study uptake and transfer ability of nickel in the organs of Persian clover. Experiment was done as randomized complete blocks design with four treatments. Treatments were consumption of Ni $(NO_3)_2$ at four levels of 0, 100, 200, 300 ppm. The plants were treated for 10 days. The results of research showed that the increase in nickel concentration reduced LWCA and the fresh weights of shoot and root; however, it had no effect on the dry weight of shoots, RGR, and leaf area. Moreover, nickel concentration in aerial organ was higher than in roots. The results showed that catalase and peroxidase activity increased by increase of nickel in the nutrient solution. The results of this research indicated that protein content decreased as nickel increased. TF was greater than 1 but BCA and BCF were less than 1 and with increase in soil Ni so did ratios value. Therefore, Persian clover is not suggested as a suitable plant for phytoremediation.

Keywords: Catalase, Peroxidase, Pullotion, Heavy metal

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Introduction

Heavy metals make the soil environmental conditions inappropriate and lead to its inefficiency for agricultural activities. They remove soil organisms (Clemente et al., 2007) followed by contamination of surface and underground waters, entering into food chain and penetration into the bodies of organisms and especially man (Bergkvist et al., 2005). Increase in nickel content of soil can be a threat for the environment and human health (Hussain et al., 2010). Signs of toxicity with nickel can be found in growth, photosynthesis, germination, glucose transport (Leon et al., 2005; Ahmad et al., 2009; Ali et al., 2009), and it also causes chlorosis, necrosis, and dryness (Madhava Rao and Sresty, 2000; Pandey and Sharma, 2002).

Heavy metals play an important role in the environment. They are result of human activity and cause toxicity in high concentrations (Ngayila et al., 2008; 2009). Plants show various

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defense mechanisms by antioxidants during accumulation of heavy metals. Antioxidants are important enzymes that are capable of protecting the organisms against free radical-induced oxidative stress (Canadanovic-Brunt et al., 2005).

Phytoremediation of heavy metals of soil is a cost-effective method (Mahmood et al., 2005; El-Gendy, 2008; Chaney et al., 2008). Planting fast-growth plants and trees is a very good technique for the establishment of agricultural land and performing large-scale clean-up operation (Ghosh and Singh, 2005). Phytoremediators are used to collect heavy metals such as aluminum, cadmium, chromium, copper, mercury, nickel, and zinc (Ndimele, 2010). Phytoremediation has been increasingly used in recent years as a cost-effective and environmental-friendly procedure to use the plants to treat the soils contaminated by organic and inorganic materials of xenobiotics (Macek et al., 2004; Eapen and D'Souza, 2005; Cherian and Oliveria, 2005; Eapen et al., 2007; Doty, 2008; Macek et al., 2008).

Persian clover with the scientific name of *Trifolium respinatum* L. is a plant belonging to Asteraceae family. This native Iranian plant with high biomass was used in the present research to examine the possibility of soil refining capability. Plant enzymatic reaction was also assessed as a physiological response.

Materials and Methods

The research was conducted in The Research Farm Greenhouse of The Faculty of Agriculture of Islamic Azad University, Saveh Branch in the agricultural year 2011-12. This study was carried out as randomized complete blocks design with four treatments. Treatments were four levels of nickel nitrate $Ni(NO_3)_2$ consumption, namely, 0, 100, 200, and 300 ppm.

Seeds were first disinfected for cultivation by sodium hypochlorite 5% for 10 minutes and then were washed three times with distilled water. The seeds were placed on Whatman filter papers. The seeds were planted in vases with 12 cm in diameter and a height of 10 cm with cocopeat and peat moss substrates. Seeding depth was 2 cm. Irrigation was carried out after planting (Electrical conductivity of the used water was 0.5 ds/m^2). Until the 9th day of cultivation, Hoagland solution was added. From the 10th day until the 20th day of cultivation, 0.1, 0.2, and 0.3 g/l of Nickel (II) Nitrate Hexahydrate from Applichem Company were added to Hoagland solution. Depending on the plant condition, a daily amount of 150 m/l of nutrient was used.

Level of catalase activity was measured by Pereira method (Pereira et al., 2002). Koroi method (Koroi, 1989) was used to determine peroxidase activity. Moreover, Bradford method (Bradford, 1976) was used to calculate protein content.

Transport factor (TF), Biological Concentration Factor (BCF), and Biological Accumulation Coefficient (BAC) were determined in the study. TF shows the amount of transport of heavy metals from the root to aerial organ of the plant (Marchiol et al., 2004). TF amount, higher than 1 indicates the transport of heavy metals from the root to aerial organ (Jamil et al., 2009) while TF amount, lower than 1, indicates transport to the plant root. BCF is determined using nickel concentration ratio of plant roots to soil (Yoon et al., 2006). BAC is defined as nickel ratio of shoots to soil. Translocation Factor (TF) is nickel concentration ratio of plant shoot to plant root (Cui et al., 2007; Li et al., 2007).

The leaves were put in a canon scanner (Lide 210) and leaf area was calculated with Measuring Leaf Area software. Relative growth rate (RGR) and leaf water content area (LWCA) was calculated by the following formula:

$$RGR = \frac{L_n W_2 - L_n W_1}{t_2 - t_1}$$

$$LWCA = \frac{LFW - LDW}{L}$$

W2 and W1= Dry weigh,
LFW= Leaf fresh weigh,
LDW=Leaf dry weigh, and
L= Leaf

Statistical calculations and analysis of data variance were done using SAS software. Comparison of means was conducted by using *Duncan*'s Multiple Range test (P<0.05).

Results

Results of the research showed that increase in nickel had no effect on the dry weight of shoot (Fig. I) while it decreased fresh weights of shoot and root (Fig. II and III). Increased in nickel also had no effect on RGR and Leaf area, but it decreased LWCA, so that in the presence of 300 mg nickel obtained least LWCA (Table 1).

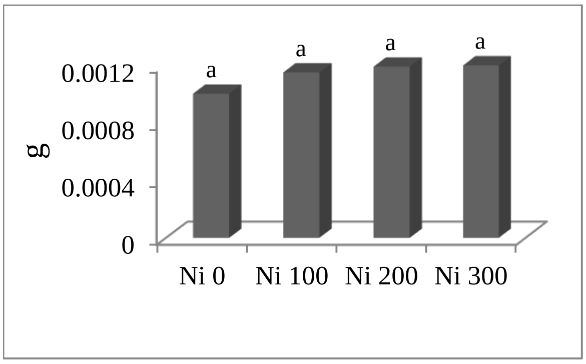

Fig. I: Effects of nickel levels on shoot dry weight .

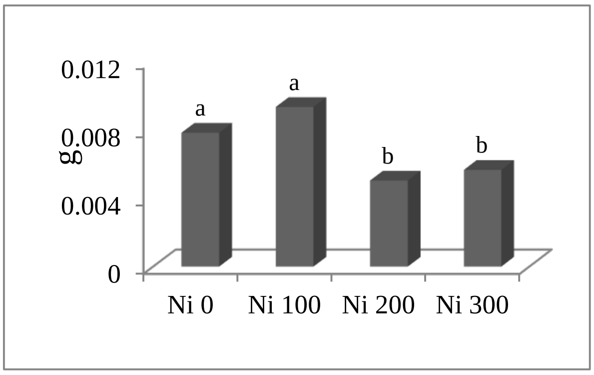

Fig. II: Effects of nickel levels on shoot fresh weight.

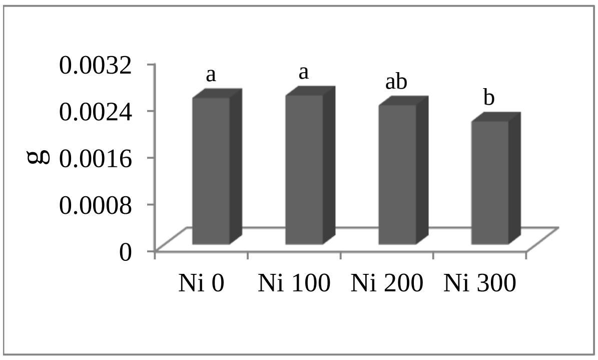

Fig. III: Effects of nickel levels on root fresh weight.

The results of recent research indicated that with increasing the nickel concentration in nutrient solution, nickel accumulation in plant organs also increased. Nickel concentration in aerial organs (Fig. V) was higher than root (Fig. IV). Therefore, it can be concluded that nickel transport in this plant was conducted well and Persian clover is suitable for phytoremediation.

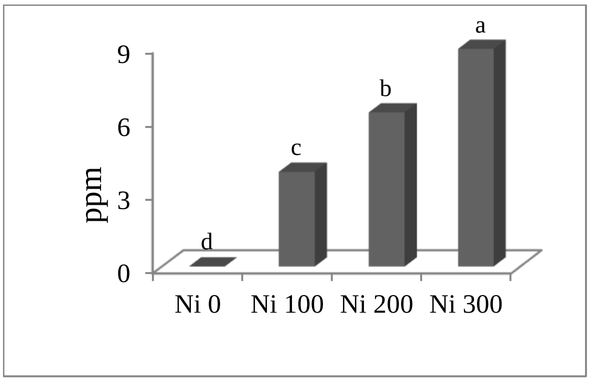

Fig. IV: Effects of nickel levels on nickel concentration root.

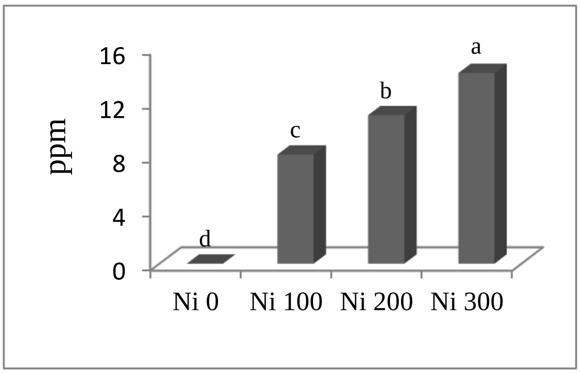

Fig. V: Effects of nickel levels on nickel concentration in shoot.

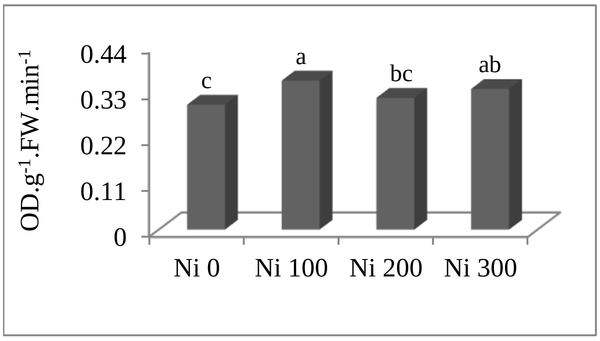

Fig. VI: Effects of nickel levels on catalase activity

Table 1
Effect of Nickel on growth indices in Persian clover

Treatment	RGR	LWCA	LA
Control	0.088 a	0.058 a	0.89 a
100 ppm	0.091 a	0.050 ab	0.85 a
200 ppm	0.077 a	0.046 b	0.799 a
300 ppm	0.088 a	0.034 c	0.86 a

Table 2
TF, BCA and BCF of Ni in Persian clover

Treatment		Root	Shoot	TF	BCA	BCF
Ni (ppm)	100	5.08	11.78	2.31	0. 112	0.051
	200	10.96	19.17	1.74	0.096	0.055
	300	12.94	23.16	1.78	0.0772	0.043

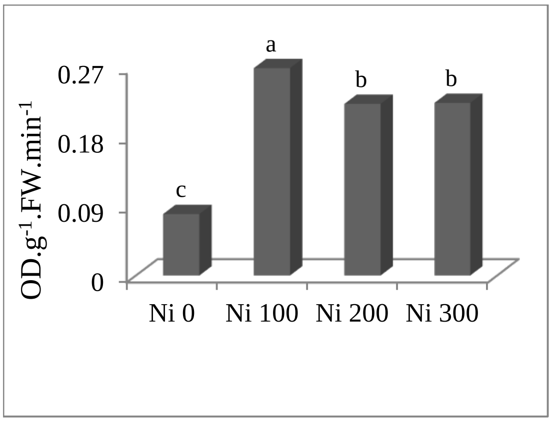

Fig. VII: Effects of nickel levels on peroxidase activity

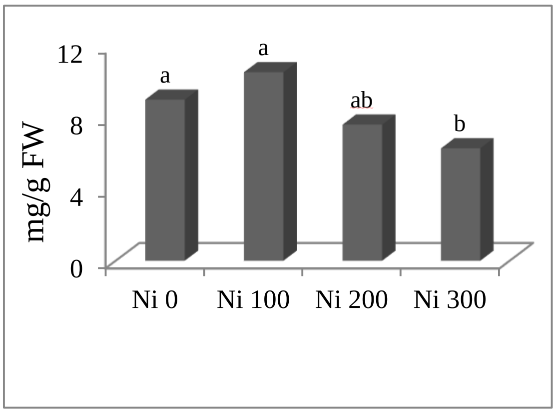

Fig. VIII: Effects of nickel levels on protein concentration

Table 2 shows that transport factor at all levels of consuming nickel is higher than 1. Also, transport capability or transport factor decreased with the increase in nickel concentration.

BAC and BCF were less than 2 and decreased with increase in soil nickel amount. In all conditions BAC was larger than BFC and this shows nickel transmission from root to shoot is better than that of soil to root (Table 1).

Increasing nickel concentration in the environment led to the increase in catalase activity (Fig.VI). The results of research also showed that increasing soil nickel concentration increased peroxidase activity (Fig.VII).

Nickel contamination in the environment decreased the protein amount in the seedling (Fig. VIII). Nevertheless, only at fourth level of nickel, a significant decrease was observed as compared to the control.

Discussion

In this study, dry weight of root was not influenced by heavy metals. Moreover, dry weight of stem was not influenced upon increase in nickel concentration in the nutrition solution. The short treatment period (10-day) may be the reason for this finding. Results of this study are similar to the ones obtained by Martin et al. (2007). In the plants which are under the stress of heavy metals such as nickel, reduction of water absorption and nutrients, reduction of water transmission, disturbance of water balance, inhibition of enzymes activity, reduction of cellular metabolism, reduction of photosynthesis, respiration and transpiration, lack of nitrogen and phosphorus, inhibition of growth, accelerating aging, and even plant death was reported (Sanita and Gabbrielli, 1999). Studies conducted by Molas (2002) and Gonnelli et al. (2001) indicated that the toxicity of high concentration of nickel can affect the contents of chlorophyll, plant growth, and physiological and metabolic processes especially photosynthesis. Heavy metals reduce viscosity and flexibility of cellular wall, longitudinal growth and wet and dry weights of stem (Wright and Apadhaya, 1996).

This study showed that nickel concentration increased in the root and shoot with its increase in the soil. Previous studies showed that the concentration of nickel in the plant is directly related to its concentration in the soil and environment (Huang and Cunnighm, 1985). This indicates the increase of their absorption by the plant. It has been reported that continuous absorption of metals by the plant depends on its capability to absorb, transmit, and accumulate as well as high resistance against large amounts of heavy metals during the plant growth (Abbott and Robson, 1999). Peroxidase is one of the most important enzymes whose activity increases in response to environmental stresses and plays a role in removing H_2O_2 (Pandolfini et al., 1992). H_2O_2 as programmed cell-mediated signal is often used in membrane lipid peroxidation (Cakmak and Horst, 1991). Moreover, H_2O_2 increases the mechanical power and reduces the developmental capability of plant cell wall (Schopfer, 1996). The reports indicate that growth of radish and peroxidase activity in root increased under copper stress (Chen and chen, 2002). In this study, peroxidase activity increased with increase in concentration of heavy metals which is consistent with the results of research conducted on beans (Shaw, 1995), barley (Erdie et al., 2002), tomato (Quiroga and Guerrero, 2000), peanuts (Li and Feng, 2001), radish (Chen and Chen, 2002), and sunflower (Laspina and Groppa, 2005). One of the reasons for low reaction of plant organs to the increased concentration of nickel in the soil might be the increased enzyme activity in the plant and modification of the effect of heavy metals on it. Researchers (Boominatham and Doran, 2002; Pandey and Sharma, 2002; Saeidi-Sar et al., 2007) showed that the activity of catalase enzyme in the leaves increased upon increase in nickel concentration. Singh and Pandey (2011) showed that the amount of protein in lower nickel concentrations (0.01 to 0.1 ppm) and higher nickel concentrations (1.0 and 10 ppm) increased and decreased, respectively.

In general, the results of the present research suggest that Persian clover is not a hyper accumulator because it accumulated less than 1000 mg/kg Ni (Baker and Brooks, 1989). Based on BCF and BAC value, this plant was not suitable for phytoremediation because the ratios were less than 1. However, based on TF value, Persian clover can transfer Ni from soil and root to shoot and reduce soil contamination. Riffat et al (2010) reported BAC and BCF for Ni were less than 1 in 17 plants and this is similar to the findings of this study. The present study showed that despite a transmission factor larger than one, nickel is capable to be transmitted through the root to other plant organs.

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