Nickel accumulation and its effect on biomass, protein content and antioxidative enzymes in roots and leaves of watercress (Nasturtium officinale R. Br.)

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Abstract

In order to understand its response towards nickel stress, watercress (Nasturtium officinale R. Br.) was exposed to nickel (1–25 mg/L) for 1, 3, 5 and 7 days. The accumulation and translocation of nickel were determined and the influence of nickel on biomass, protein content and enzymatic antioxidants was examined for both roots and leaves. It was determined that N. officinale could accumulate appreciable amounts of Ni in both roots and leaves. Nickel accumulated particularly in the roots of plants. Biomass increased at low nickel concentrations but certain measurable change was not found at high concentrations. Under stress conditions the antioxidant enzymes were up-regulated compared to control. An increase in protein content and enzyme activities was observed at moderate exposure conditions followed by a decline at both roots and leaves. The maximum enzyme activities were observed at different exposure conditions. Our results showed that N. officinale had the capacity to overcome nickel-induced stress especially at moderate nickel exposure. Therefore, N. officinale may be used as a phytoremediator in moderately polluted aquatic ecosystems.

Key words: nickel; accumulation; antioxidative enzymes; biomass; watercress

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Introduction

The contamination of water by heavy metals is one of the most serious problems in the world. Each plant species has different levels of tolerance towards different contaminants, as do morphologically similar species growing in the same area (Siedlecka and Krupa, 2002). In order to identify suitable plants for pollutants removal from the aquatic environment, we require broad knowledge about the physiological and biochemical features of potentially useful species.

Nickel is one of the toxic heavy metals present in raw wastewater due to industries such as electroplating, dye manufacturing, porcelain enameling, and steam-electric power plants (Padmavathy, 2008). For humans, nickel can cause serious health problems such as allergic sensitization (Wilhelm et al., 2007), dermatitis (Bocca et al., 2007), and lung and nervous system damage (Haber et al., 2000). The Ni concentration in surface water was approximately 0.01–0.002 mg/L (Karadede and Unlu, 2000). Although nickel is known to be essential for plants at low concentrations (Gajewska and Sklodowska, 2007; Baccouch et al., 2001), it is phytotoxic at high concentrations (Madhava Rao and Sresty, 2000). The nickel concentration in plants has been shown to range from 0.1 to 5.0 µg/g of dry matter (Mishra and Kar, 1974). The Ni deficiency is rare compared to its excess, which is often caused by metal mining and smelting. It has been reported that the negative effects of Ni on plants are closely related to dose and exposure time (Kováčik et al., 2009; Poulik, 1999). The effect of nickel on plants varies according to plant species and cultivation conditions (Hao et al., 2006).

Antioxidative enzymes such as catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) play a key role in radicals and peroxides control that are produced under conditions of metal stress (Tanyolac et al., 2007). Kumar et al., (2007) demonstrated that moderate nickel treatments (100 µmol/L NiSO₄) also lead to a significant increase in the concentration of H₂O₂ and antioxidant enzyme activities in maize leaves. The molecular mechanism for the generation of reactive oxygen species (ROS) and the factors affecting the synthesis of ROS in Ni-treated plants are, however, largely unknown. Metal accumulation properties of N. officinale have been studied extensively (Zurayk et al., 2001; Aslan et al., 2003; Saygideger and Dogan, 2005), however, little attention has been paid to the ensuing antioxidant responses and resultant effect of accumulation in this plant.

The objectives of this study were to determine the accumulation and translocation properties of Ni, and to determine the effects of Ni concentrations (1, 5, 10, 25 mg/L) on biomass, protein content, CAT, SOD and APX
activities of *N. officinale*. The results may be useful when *N. officinale* is used as a phytoremediator in Ni contaminated water.

1 Material and methods

1.1 Sample collection and cultivation

Watercress, *Nasturtium officinale* R. Br., is an aquatic perennial plant. Its leaves and stems are partially submerged during growth. Cool running water must be available in their habitat year-round. This plant is harvested and consumed as a salad green. As a medicinal plant, watercress has been traditionally considered a diuretic, purgative and tonic.

*N. officinale* seedlings were collected in April, 2008 from the Karasu Stream in Kayseri, Turkey. This location has a semi-arid and very cold Mediterranean climate. The average annual mean temperature in the sampling area is 10.6°C. The maximum mean temperature is 30.5°C in July and August, and the minimum mean temperature is −7.6°C in January. The annual mean precipitation is 422.8 kg/m².

According to the results of preliminary range determining tests (data not shown), the highest nickel exposure concentration for *N. officinale* was selected as 25 mg Ni/L. Collected samples were washed using distilled water and acclimatized for three days in a climate chamber with a water temperature of 15°C, a relative humidity of 70% and photoperiod of 16 hr/8 hr (light/dark). The plants that were in the best condition were selected for subsequent experiments.

1.2 Experimental design

Prior to the experiment, containers were disinfected by immersing in 1% (V/V) NaClO for three to five minutes. Containers were then rinsed three times with distilled water (Hou et al., 2007). Experiments were set up in triplicates, and each replicate contained approximately 4 g of plants. NiSO₄ was used in experiments. Nickel stock solutions were prepared using double distilled water. Plants were treated with different concentrations of Ni (0, 1, 5, 10 and 25 mg/L) maintained in 10% Hoagland’s solution in 400 mL conical flasks (Srivastava et al., 2006). Flasks were placed into the climate chamber for a period of 1, 3, 5 and 7 days. At the end of the exposure experiment, plant samples were collected and sieved with a plastic griddle and were washed with 0.01% Na-EDTA solution; then samples were rinsed with deionized water to remove adsorbed metal from surface of leaves. Plant samples were drained and blotted on paper towel for 2 min, and weighted thereafter. Root and leaf parts were subsequently separated.

1.3 Quantification of nickel

An aliquot of each sample was dried at 70°C. Each sample was then digested with 10 mL of pure HNO₃ using a CEM Mars 5 (CEM Corporation Mathews, USA) microwave digestion system. The digestion conditions were as follows: maximum power was 1200 W, power was 100%, ramp was 20 min, pressure was 1.24 MPa, temperature was 200°C and hold time was 10 min. After digestion, the volume of each sample was adjusted to 25 mL using double deionized water. The total concentration of each metal was determined using Inductively coupled plasma mass spectroscopy (Agilent 7500a, USA). The stability of the device was evaluated every ten samples by examining the internal standard. Reagent blanks were also prepared to detect potential contamination during the digestion and analytical procedure. Peach Leaves (NIST, SRM-1547) were used as a reference material, and all analytical procedures were also performed with this reference material. The samples were analyzed in triplicates.

To determine the Ni translocation properties of *N. officinale*, translocation factor (TF) was calculated as the ratio of the metal concentration in the leaves to the metal concentration in the roots (Stoltz and Greger, 2002).

1.4 Antioxidant enzyme extraction and assay

Plant tissues, both roots and leaves (500 mg), were homogenized in 1 mL of 100 mmol/L chilled potassium phosphate buffer (pH 7.0) containing 0.1mmol/L EDTA and 1% polyvinyl pyrrolidone (PVP, W/V) at 4°C. The homogenate was centrifuged at 15,000 ×g for 15 min at 4°C. The supernatant was used to measure the activities of SOD and APX (Srivastava et al., 2006). The protein content was determined according to the method of Lowry et al., (1951) using bovine serum albumin as the standard protein.

1.5 Superoxide dismutase activity determination

The SOD activity was determined by the method of Beauchamp and Fridovich (1971) by monitoring the photoreduction of nitroblue tetrazolium (NBT). The required cocktail for SOD activity estimation was prepared by mixing 27 mL sodium phosphate buffer (pH 7.8), 1.5 mL methionine (300 mg/mL), 1 mL NBT (14.4 mg/10 mL), 0.75 mL Triton-X-100 and 1.5 mL 2 mmol/L EDTA. To 1 mL of this cocktail, 10 μL riboflavin (4.4 mg in 100 mL) and 50 μg sample protein were added. The test tubes were shaken and placed 30 cm below a 15-W fluorescent lamp. A tube containing sample protein kept in the dark served as a blank, while the control tube did not have the enzyme and was kept under the light. We measured the absorbance at 560 nm. The NBT reduction under illumination was measured both in the absence and presence of the enzyme. The activity of SOD correlates with the amount of NBT reduction in light without protein minus the NBT reduction with protein, and is expressed as units per mg protein.

One unit of activity is the amount of protein required to inhibit 50% of the initial reduction of NBT under light conditions.

1.6 Catalase activity determination

For the measurement of the CAT activity, we performed an extraction step using a buffer containing 50 mmol/L Tris-HCl (pH 7.0), 0.1 mmol/L EDTA, 1 mmol/L PMSF, and 0.3 g/g (fresh weight) PVP. Activity was measured by the method of Aebi (1974). The reaction mixture
comprised 2.5 mL of 50 mmol/L sodium phosphate buffer (pH 7.0), 300 µL of 20 mmol/L H₂O₂ and a suitable aliquot of enzyme. The change in absorbance was measured at 240 nm (extinction coefficient 40 mmol/(L·cm)). Enzyme activity was expressed as units per mg protein.

1.7 Ascorbate peroxidase activity determination

The activity of APX was measured by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mmol/(L·cm)). The reaction mixture (3 mL) contained 50 mmol/L phosphate buffer (pH 7.0), 0.1 mmol/L H₂O₂, 0.5 mmol/L sodium ascorbate, 0.1 mmol/L EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm (Nakano and Asada, 1981) and the enzyme activity was expressed as units per mg protein.

1.8 Statistical analysis

Data were expressed as mean with standard error. The experiments were performed in a randomized order. The Kolmogorov-Smirnov test and Levene’s test were used to ensure the normality assumption and the homogeneity of variances, respectively. Heterogeneity of variance were recognised, data were log transformed ln(x+1) and reevaluated. Significant differences were calculated using Student’s t-test wherever applicable. Analysis of variance (ANOVA) was performed to confirm significant differences among treatments. All pairwise mean comparisons were made using post-hoc analyses. Duncan’s test was used to determine the significant difference between treatments. We used 0.05 as the statistical significance threshold. All statistical analyses were performed with the SPSS 15.0 software package.

2 Results

2.1 Nickel accumulation and translocation in N. officinale

The accumulation of Ni in the roots and leaves of N. officinale was found to depend on both the concentration and duration of exposure. The highest accumulation of Ni (5757.9 µg/g dry weight) was found in the roots of the N. officinale specimens that had been exposed to 25 mg/L Ni for 7 days (Fig. 1). The Ni translocation is significantly higher than that of control plants (Fig. 2). The highest translocation factor (TF) ratio was obtained when plants were exposed to 1 mg/L of Ni. The TF values at all Ni concentrations tested were lower than 1.

2.2 Effect of nickel on plant growth and protein content

We observed that biomass increased in plants exposed to 1 mg/L Ni (Fig. 3). In comparison with the control a significant decrease in biomass was observed only at 25 mg/L Ni for 7 days. Visual changes including chlorosis were observed on the leaves of plants exposed to 25 mg/L Ni for 5 and 7 days. The leaf protein content was higher than the root protein content (P ≤ 0.05). An increment of 155% was observed in the root protein content at 5 mg/L by day 7 (Fig. 3). Leaves showed an increment of approximately 40% at 5 mg/L within 3 days (Fig. 3).

2.3 Response of antioxidant enzymes

In this study, we examined the activities of certain antioxidant enzymes such as SOD, APX and CAT, which showed varying responses with induction at various concentrations and exposure durations.

The SOD activity in the roots of N. officinale showed the highest activity at 5 mg/L Ni on day 3 comparing with control, while in leaves the highest activity was detected at 5 mg/L Ni on day 5 (Fig. 4). After reaching their maximum levels, SOD activity showed a decline.
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The CAT activity of both roots and leaves showed a similar trend (Fig. 5). The maximum level was obtained at 5 mg/L Ni on day 5. The CAT activity showed a decline at all durations in roots and leaves exposed to 10 and 25 mg/L Ni, and the maximum decline rate was recorded as 45% in leaves on day 7 at 25 mg/L.

Ni stress resulted in increased APX activity in both the roots and leaves of *N. officinale* only at 1 mg/L Ni treatment (Fig. 6). At 1 mg/L Ni, the APX activity in roots and leaves reached the highest value on treatment day 3. The maximum decline was determined to be 130% in leaves on day 3 at 5 mg/L.

3 Discussion

In this study, it was shown that *N. officinale* can grow in water contaminated with Ni. Maleva et al. (2009) evaluated the accumulation properties of Ni from an aquatic plant, *Elodea canadensis*, and confirmed that *Elodea* plant tolerated higher concentrations of Ni than the amount normally present in the contaminated areas. Moreover, *N. officinale* accumulated appreciable amounts of Ni in both roots and leaves. Similarly, Kara (2005) examined the bioaccumulation of Cu, Zn and Ni from the wastewater by treated *N. officinale* and found that high amount of Ni could be accumulated by *N. officinale*. The high bioaccumulation of Ni in plants may be due to its role as a micronutrient (Assunção et al., 2003). Similar to the results obtained by the earlier studies (Kováčik et al., 2009; Madhava R and Sresty, 2000), in the present study it was found that the uptake of Ni was concentration-dependent. A plant that contains more than 1000 mg/kg of Ni in its tissues is considered to be a Ni-hyperaccumulator.
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Ni. Similarly, Maleva et al. (2009) found that Elodea canadensis accumulated more than 1000 mg/kg of Ni. The roots are thought to be important for element uptake in aquatic plants (Sharma and Gaur, 1995). Nickel can be rapidly taken up by the plant root system (Ali et al., 2005; Choo et al., 2006, Duman et al., 2007). As expected, the roots of  

accumulated a larger amount of Ni than leaves in a concentration and duration-dependent manner. The rate and extent of translocation of metals within plants depended on metal and plant species (Deng et al., 2004). Figure 2 indicates that the Ni accumulated was largely retained in the roots (TF ratio < 1). These results suggest that protective barriers exist to prevent the penetration of Ni from the roots into the leaves (Hozhina et al., 2001).

Yang et al., (1996) stated that high nickel concentrations would cause weak plant growth, leading to depression, metabolic disorders and chlorosis. In this study, low concentrations of nickel caused an increase in biomass. This increase may due to an increase in low molecular weight stress proteins such as antioxidant enzymes (Srivastava et al., 2006). As the concentration of nickel and the duration of exposure increase, the degradation of cell membrane and wall may occur, and as a consequence this may lead to a decline in growth rate and biomass (Kabala et al., 2008).

Protein content may be considered a reliable indicator of oxidative metal stress in plants (Singh et al., 2006; Sinha et al., 2005). In the present study, we observed an increase in protein content in the roots and leaves of  

up to a certain concentration of Ni (5 mg/L). This increase may be due to the increasing activity of some other metal sequestration mechanisms, involved in the detoxification of high Ni doses (Rauser, 1999). However, the protein content decreased at high Ni concentrations (10–25 mg/L). This decrease could be due to the degradation of a number of proteins. Maleva et al. (2009) studied nickel-induced oxidative stress on  

Elodea canadensis, and observed an increase in protein content at up to 10 µmol/L Ni, but if at higher concentrations a decrease was recorded. This phenomenon is in agreement with our results.

Plants are equipped with a defence system to repair the damage created by ROS. Antioxidant enzymes such as SOD, CAT and APX play important roles in this process. It has been reported that antioxidant enzyme activity may increase, decrease or remain unchanged in response to heavy metal exposure (Zhang et al., 2007). In literature, it has been reported that the extent of alteration varied with the type of metal, metal concentration, the enzyme tested, plant species and the specific plant part (Shri et al., 2009; Srivastava et al., 2006; Devi and Prasad, 1998). It was known that Ni treatment induced oxidative stress in plants (Kumar et al., 2007). Sheoran et al., (1990) stated that Ni may inhibit many plant enzymes, including those involved in the Calvin cycle. Our results show that in contrast to CAT, the activity of SOD and APX was more pronounced in leaves than that in roots. Our results show that the difference in the antioxidative enzyme activities of leaves and roots may explain the different tolerance levels of roots and leaves. Moreover, our study indicates that the accumulation of Ni in  

is able to oxidatively stress on  

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may be due to the increasing activity of some other metal

(Fig. 6  Time course of changes in the activity of APX in the roots and leaves of  

Values represent means ± SD (N = 3). Means with the same letter(s) are not significantly different at P < 0.05 according to Duncan’s test.

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over a 7-day period increased as Ni concentration increased in the nutrient solution. However, the activities of the antioxidant enzymes were affected to different extents. All enzymes exhibited similar response curves, with increase of activities at lower concentrations of Ni but decreased activities at higher concentrations. Similar results have also been observed for other metals such as arsenic, copper and chromium (Mishra et al., 2006; Srivastava et al., 2006; Sinha et al., 2005). Reduced activities of SOD, CAT and APX at higher Ni exposures could be the result of enzyme modulation by stress-related effector molecules (Takahashi et al., 1997).

SOD is considered to play a key role in cellular defence mechanisms against ROS. The activity of SOD decreases the risk of OH radical formation which may cause severe damage to membranes, proteins and DNA (Zhang et al., 2007). When the activity of SOD increased significantly in response to exposure to low levels of Ni (up to 5 mg/L), it decreased at high Ni concentrations. Similar results have also been observed for other metals such as arsenic, copper and chromium (Mishra et al., 2006; Srivastava et al., 2006; Sinha et al., 2005). Reduced activities of SOD, CAT and APX at higher Ni exposures could be the result of enzyme modulation by stress-related effector molecules (Takahashi et al., 1997).

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of \( \text{H}_2\text{O}_2 \) (Boominathan and Doran, 2002).

\( \text{H}_2\text{O}_2 \) produced by SOD is toxic and can be converted to \( \text{H}_2\text{O} \) by subsequent reactions. As a result of CAT activity, \( \text{H}_2\text{O}_2 \) breaks down to form water and oxygen (Zhang et al., 2007). Similar to our SOD activity data, the CAT activity increased in response to exposure to up to 5 mg/L Ni. The observed increase in CAT activity is consistent with the observations of Gajewska and Sklodowska, (2007) who studied the effect of nickel on oxidative enzyme activities in wheat leaves. This increase could be due to an increase in the amount of CAT substrate. This result shows that \( \text{N. officinale} \) employs an adaptive mechanism to maintain the level of \( \text{H}_2\text{O}_2 \). However, at high concentrations of Ni (10–25 mg/L), CAT levels decreased. This result suggests that the plant can not cope with increasing \( \text{H}_2\text{O}_2 \) levels.

APX is one of the most important \( \text{H}_2\text{O}_2 \) scavenging enzymes. It has various physiological roles in plant cells and participates in many biochemical reactions. Stimulation of this enzyme can cause alterations in the cell wall, and this in turn may decrease the growth rate. Similar to CAT, this enzyme also breaks down \( \text{H}_2\text{O}_2 \) into water and oxygen. However, the substrate affinity of APX is higher than that of CAT (Siedlecka and Krupa, 2002). The APX activity of both roots and leaves at 1 mg/L Ni was higher than at other Ni concentrations (\( P < 0.05 \)). The increase in APX in response to Ni suggests that APX can affect \( \text{H}_2\text{O}_2 \) detoxification. Kumar et al. (2007) studied the effect of nickel on antioxidative enzymes in maize leaves, and observed that SOD activity first increases, and then decreases with exposure to Ni. This finding is in agreement with our results.

4 Conclusion

Our findings indicate that \( \text{N. officinale} \) can accumulate a significant amount of Ni, and can effectively combat Ni-induced increases in antioxidative stress enzyme concentrations. Thus, \( \text{N. officinale} \) seems suitable for use as a phytoremediator in aquatic ecosystems with moderate Ni pollution.

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References


