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Growth and bioaccumulation characteristics of watercress (*Nasturtium officinale* R. BR.) exposed to cadmium, cobalt and chromium

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ABSTRACT

The aim of this study is to determine the effects of Cd, Co and Cr on the growth of watercress (*Nasturtium officinale*) and to determine the bioaccumulation properties of these heavy metals by the plant. *N. officinale* individuals were exposed to different concentrations of Cd, Co and Cr for 72 h. Relative growth rates (RGR) and bioconcentration factor (BCF) ratios were calculated for each metal concentration. RGR values of plants exposed to Co slightly increased in lower concentrations, but then decreased again. In contrast, RGR values of plants exposed to Cd and Cr decreased linearly. Significant positive relationships were observed among the concentrations of Cd, Co and Cr in *N. officinale* and in the culture solution. BCF ratios were highest for plants exposed to Co, and lowest for plants exposed to Cr. The most efficient uptake of Cd, Co and Cr occurred at the external solution concentrations at 0.5, 0.5 and 10 mM, respectively.

Keywords: cadmium, cobalt, chromium, growth, bioaccumulation, *Nasturtium officinale*, relative growth rates, bioconcentration factor

1. INTRODUCTION

Heavy metals are serious pollutants of aquatic ecosystems because of their environmental persistence, toxicity and ability to be incorporated into food chains. Aquatic macrophytes may accumulate considerable amounts of heavy metals in their tissues (Deng *et al*., 2004; Duman *et al*., 2007; Hasan *et al*., 2007); therefore, the use of these plants as phytoremediators for the removal of heavy metals from wastewater deserves attention (Mishra and Tripathi, 2008; Kamal *et al*., 2004; Miretzky *et al*., 2004). Metal accumulation by wetland plants is affected by several factors, including the variation in plant species, the growth stage of the plants, elemental absorption, and translocation in plants (Deng *et al*., 2004). Each plant species has different tolerance levels to the different contaminants (Axtell *et al*., 2003; Kamal *et al*., 2004; Sune *et al*., 2007). Growth rate and biomass are important factors for the removal of metal ions (Kara and Zeytunluoglu, 2007). One disadvantage of phytoremediation is that heavy metals at high concentrations may inhibit plant growth and biomass production (Torresdey *et al*., 2005). Plants with high bioconcentration factors (BCF) are considered to be hyperaccumulators (Ghosh and Singh, 2005). Hyperaccumulating plants are those that have a BF >1.0 (Cluis, 2004). There has been an increasing interest in the identification of hyperaccumulators because of their phytoremediation potential (Torresdey *et al*., 2005). A wide range of knowledge about the physiological and biochemical features of plant species is needed in order to identify plants that can remove pollutants from the aquatic environment. The accumulation capacity of aquatic plants is lower than terrestrial plants; nevertheless, they may be excellent hyperaccumulators due to their potentially

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high biomass and their ability to grow in unfavourable conditions (Ghosh and Singh, 2005). Consumption of edible aquatic plants thus increases the risk of heavy metals to enter the food web.

The metal accumulation properties of *N. officinale* have been studied extensively. Zurayk *et al*. (2001a) studied Cr accumulation by *N. officinale* and found that the concentration of Cr increased to 762 μg g^{-1} after being maintained in water containing 1 mg Cr l^{-1} for three weeks. In another study by Zurayk *et al*. (2001b), Cr accumulation in *N. officinale* during the 14 d cultivation period increased as the initial metal concentration (0–8 mg L^{-1}) in solution increased. Other authors that studied metal uptake by *N. officinale* found similar results (Aslan *et al*., 2003; Kara, 2002; Saygideger and Dogan, 2005). Kara (2005) examined the bioaccumulation of Cu, Zn and Ni by *N. officinale* and found that Cu accumulated more effectively that Zn or Ni.

The objectives of this research are to (1) determine the effect of the concentration of Cd, Co and Cr in the growth solution on the amount of metal accumulation in *N. officinale*, (2) compare the bioaccumulation properties of Cd, Co and Cr using BCF and (3) examine the effects of these metals on the relative growth rate of *N. officinale*.

2. MATERIALS AND METHODS

2.1 Sample collection and cultivation

Watercress, *Nasturtium officinale* R. Br., is an aquatic perennial herb growing chiefly in springs or running water. Leaves and stems are partially submerged during growth and this plant is available year-round. This plant is harvested and consumed as a green salad. As a medicinal plant, watercress has been traditionally considered a diuretic, purgative and a tonic (Maranki, 2008).

Seedlings of *N. officinale* were collected in December of 2007 from the Dipsiz Stream in Oymaagac village, Kayseri, Turkey. As sampling area, a narrow field from the spring of the stream was chosen. Submerged samples were selected for sampling. Samples were collected by hand and brought to the laboratory in a bucket. This location has a semi-arid and very cold Mediterranean climate. The average annual mean temperature in the collection 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. Annual mean precipitation is 422.8 kg m^{-2}. The temperature was 2°C at the time of sample collection.

Collected samples were washed in distilled water two times and acclimatised for three days in a climate chamber with a water temperature of 15°C, a relative humidity of 70% and light dark conditions of 16 h light/8 h dark. The plants showing the best condition and having similar size were selected for further experiments.

2.2 Experimental design

Prior to the experiment, glass containers were disinfected by immersing them in 1% (v/v) NaClO for three to five minutes and then rinsed with distilled water three times (Hou *et al*., 2007). The metal compounds used were cobalt nitrate [$Co(NO_3)_2$ $9H_2O$], chromium nitrate [$Cr(NO_3)_3$ $9H_2O$] and cadmium chloride [$CdCl_2$] without purification. Stock solutions of heavy metals were prepared in double distilled water. Stock solutions were then diluted to obtain the appropriate metal concentration. The nutrient solution added to the containers was a Hoagland solution (Eliasson, 1978). The plant samples (about 4 g wet weight) were placed in graded vertical cylinders (400 mL) filled with the Hoagland solution.

Preliminary experiments were conducted to identify the most suitable range of concentrations for *N. officinale* cultivation and the approximate toxicity thresholds for each metal. Plants were exposed to different concentrations of each metal (Cd: 0.5, 1, 1.5, 2, 2.5 and 3 mM; Co: 1, 2, 3, 4, 5 and 6 mM; Cr: 5, 10, 15, 20, 25 and 30 mM) (Zurayk *et al*., 2001b; Kara and Zeytunluoglu, 2007; Gal *et al*., 2007; Srivastav *et al*., 1994). The most important symptoms of metal toxicity are chlorosis and necrosis (Marschner, 1995). Therefore, these symptoms were noted. These experiments lasted 72 h. The volume of evaporated solution was replaced with distilled water daily. *N. officinale* showed visible signs of chlorosis and necrosis when the concentration of Cd was 2 mM, when the concentration of Co was 5 mM and when the concentration of Cr was 20 mM. According to results of preliminary experiments, suitable concentration ranges of Cd, Co and Cr were 0–2, 0–5 and 0–20 mM, respectively. Exposure experiments were then repeated with the metal concentrations within these ranges. Plant samples were harvested and sieved with a plastic griddle. Plants were rinsed with distilled water, drained and then blotted on paper towels for 2 min.

The relative growth rate of *N. officinale* was calculated in each treatment according to Hunt's equation (Tanhan *et al.*, 2007), which is $RGR = [\ln(W_2) - \ln(W_1)]/(t_2 - t_1)$. W_1 and W_2 are the initial and final fresh weights (g), respectively, and $t_2 - t_1$ is the length of the experimental period.

2.3 Sample preparation, chemical analyses and calculations

Plant samples were dried in a drying oven at 70°C for 24 h. Dried samples were pulverised using a mortar and pestle and sieved through 2 mm sieve. Each sample was then digested with 10 mL of pure HNO_3 using a CEM- Marsh 5 (CEM Corporation Mathews, NC, USA) microwave digestion system. The digestion conditions were as follows: maximum power was 1200 W, power was 100%, ramp was 20 min, pressure was 180 psi, temperature was 210°C and hold time was 10 min. After digestion, the volume of each sample was adjusted to 25 mL using double distilled water. The total concentration of each metal was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (Varian-Liberty II, ICP-OES). Stability of the device was evaluated every 10 samples by examining the internal standard. The linearity of the calibration curves was constantly checked. Samples with concentrations above the detection range of the device were diluted to fit within the range of calibration curve. Sample spiking was used to check the accuracy of sample digestion. Spikes of known concentration were added to samples either before or after digestion. 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 on this reference material. Measured values of the reference material were typically $\pm$ 10% of certified values. Accuracy of analytical process could be considered as acceptable. The samples were analysed in triplicate.

Bioconcentration factor (BCF) was calculated as the ratio of metal concentration in the plant ($\mu g\ g^{-1}$ dry weight) to the metal concentration (mM) in the growth medium (Shin *et al.*, 2002).

2.4 Statistical analysis

Data were expressed as means with standard errors (SE). Analysis of variance (ANOVA) was implemented to identify significant differences in the concentration of metals in plants that experienced different initial cultivation conditions ($P \leq 0.05$). All pair-wise mean comparisons were made using the post hoc analyses. Duncan test was used with a degree of significance of 0.05. Correlation analyses were conducted to determine the effect of the initial metal concentration in the solutions on BCF and RGR. All statistical analyses were performed with the software SPSS 15.0.

3. RESULTS

Table 1 shows the mean concentrations of Cd, Co and Cr in *N. officinale* at the end of the 72 h cultivation period. The metal concentrations of control plants were deducted from the concentrations presented in Table 1. Plants from metal loading samples had significantly higher metal concentrations than did plants in the control treatment ($P < 0.05$). Analysis of variance showed a significant difference ($P < 0.05$) between the concentrations of accumulated metals at the end of the experiments and also at all different concentrations as shown in Table 1. Pearson's correlations were used to correlate the initial metal concentrations and the accumulated metal concentration in *N. officinale* for Cd, Co and Cr (Table 2). The accumulation of Cd, Co and Cr within plants depended on their initial concentration in the growth solution.

Table 1 shows that the concentration of metals in the plant during cultivation period increased as the initial metal concentration in solution increased. Also, the highest metal concentration in plants occurred when the initial metal concentration was the highest.

BCF values depended on the metal and the initial concentration of that metal (Figure 1). The highest BCF values were obtained when plants were exposed to 0.5, 0.5 and 10 mM of Cd, Co and Cr, respectively. The highest BCF values of plants exposed to Cd, Co and Cr were 73, 420 and 10.3, respectively. BCF values of Co were higher than those for Cd and Cr.

The highest RGR values were 0.03, 0.03 and 0.08, and these values were found in plants exposed to Cd, Co and Cr concentrations of 0, 3, and 1 mM, respectively (Figure 2). Initial Cd and Cr concentrations in solutions were negatively correlated with RGR ($P < 0.01$). This was, however, not the case for Co. Relative growth rates of plants exposed to Co slightly increased in lower concentrations and then decreased again. Relative growth rates of plants exposed to Cd and Cr, however, decreased linearly.

Table 1 Mean Cd, Co and Cr concentrations of *N. officinale* (μg g^{-1} dry weight) exposed to different concentration of these metals for 72 h. Superscript letters indicate results of significance test (Duncan's test, $P < 0.05$) for each metal, different letters indicate significant differences

		Metal concentration ($n = 6$)
Cd	Control (0 mM)	0.27 ± 0.02^{a}
	0.1	0.8 ± 0.24^{b}
	0.5	37 ± 1.6^{c}
	1	58 ± 1.7^{d}
	1.5	66 ± 2^{e}
	2	78 ± 3^{f}
Co	Control (0 mM)	1.1 ± 0.24^{a}
	0.1	10 ± 1.4^{b}
	0.5	212 ± 2^{c}
	1	270 ± 2^{d}
	3	524 ± 3^{e}
	5	581 ± 9^{f}
Cr	Control (0 mM)	0.6 ± 0.05^{a}
	1	5.4 ± 0.4^{b}
	5	44 ± 4^{c}
	10	103 ± 6^{d}
	15	118 ± 9^{e}
	20	137 ± 13^{f}

4. DISCUSSION

An increase in the accumulation of metals with an increase in the initial metal concentration has been reported in *Eichhornia crassipes* (Chandra and Garg, 1992) and *Azolla filiculoides* (Sela *et al*., 1989). Similarly, Maine *et al*. (2001) found that the higher the initial concentration of Cd, the greater the amount of Cd that the plants removed. Our results are compatible with these literature findings.

The bioconcentration factor (BCF) is an index that indicates the ability of a plant to remove trace elements. Specifically, BCF is the ratio of the amount of the trace element that the plant accumulates to the concentration of that element in the external solution (Zayed *et al*., 1998). The BCF values of *N. officinale* were relatively high for Cd and Co, but relatively low for Cr (Figure 1). Cr is one of the most difficult metals to remove from the water because aquatic macrophytes do not require this element for any physiological purpose (Mishra and Tripathi, 2008). This is consistent with the findings of the present study. The mobility of Cr is low in many plant species because plants have barriers to Cr transport or lack a mechanism to transport Cr from their roots to their shoots (Kleiman and Cogliatti, 1998). *N. officinale* individuals were more efficient at removing Cd and Co from contaminated water.

Heavy metals may cause metabolic disorders and growth inhibition in plants (Hadad *et al*., 2007; Singh *et al*., 2006; Rai *et al*., 1998). Growth inhibition and morphological symptoms, such as yellowing, were especially evident at high concentrations of metals (Gal *et al*., 2008; Lin *et al*., 2007). Chandra and Garg (1992) studied the absorption and toxicity of Cr and Cd in *Limnanthemum cristatum* and found that plants exhibited chlorosis after being exposed to 1 mg Cd L^{-1} for 48 h. Maine *et al*. (2004) studied Cr bioaccumulation in two floating macrophytes (*Salvinia herzogii* and *Pistia stratiotes*); they found a significant negative linear correlation between Cr concentration in the solution (1–6 mg L^{-1}) and relative growth rate of plants. This is in line with our results.

Table 2 Relationships among the added metal in the growth medium and metal concentration in plant

	Added Cd	Added Co	Added Cr
Concentration of metal in plant (μg g^{-1} DW)	0.943*	0.929*	0.956*

* Indicates $P < 0.01$.

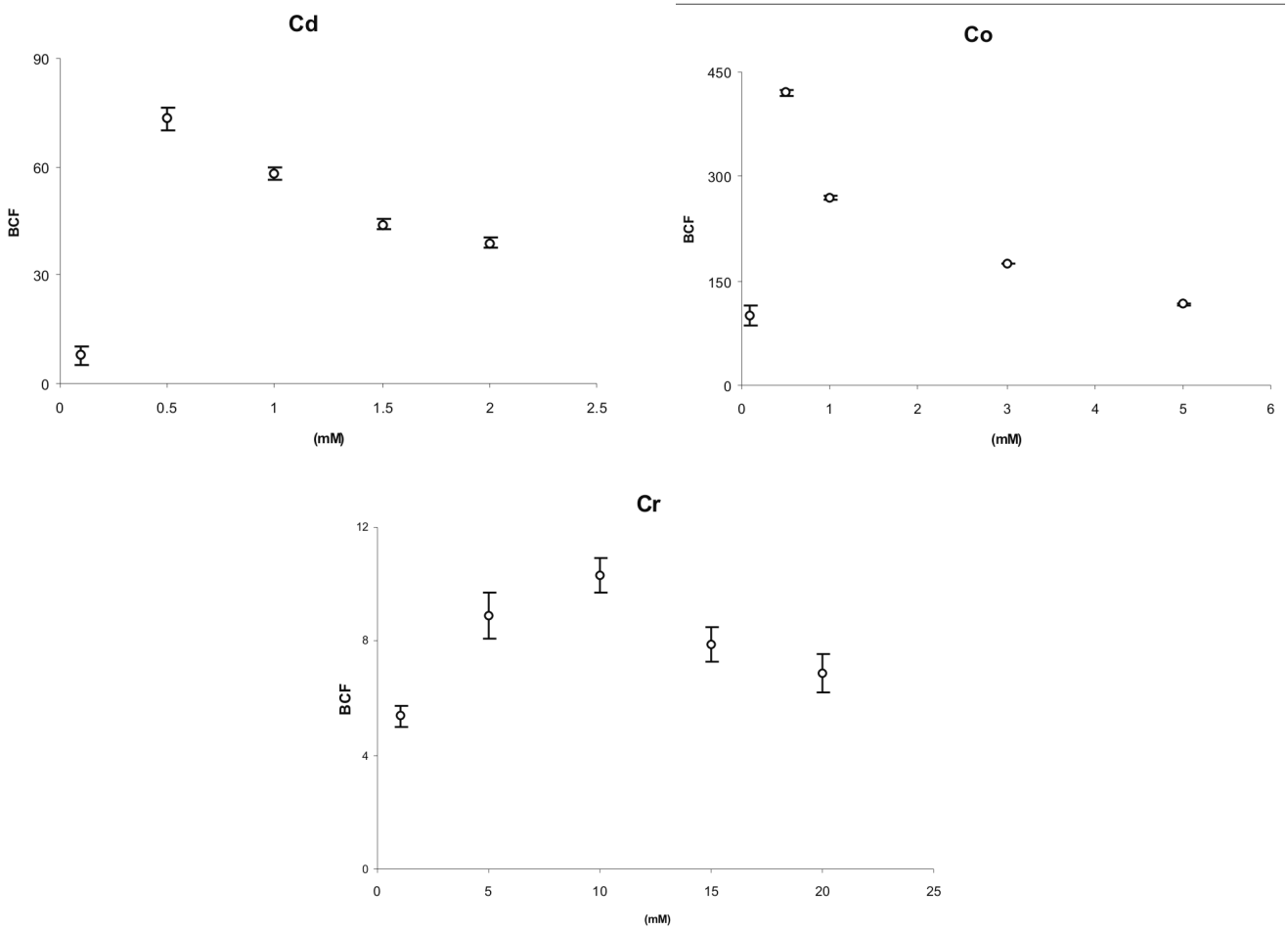

Figure 1 BCF values of *N. officinale* grown in different initial metal concentrations. Error bars represent standard error ($n = 6$).

Although not essential for plant growth, Cd^{2+} ions are readily taken up by roots and transported to the leaves in many plants (Aslan *et al*., 2003; Singh *et al*., 2006; Liu *et al*., 2007). The presence of Cd^{2+} in leaves depresses growth by affecting photosynthesis, chlorophyll fluorescence and nutrient uptake (Mishra and Tripathi, 2008). Cadmium is fairly immobile in plants (Toppi and Gabbrielli, 1999; Singh *et al*., 2006). Ding *et al*. (1994) reported that the concentration of Cd in the roots of *Eichhornia crassipes* was 21.15 μg g^{-1} (wet weight) after plants were grown in water with 2 mg Cd L^{-1} for 24 h. During the present investigation, the highest Cd concentration in plants was 78 μg g^{-1} (Table 1). A hyperaccumulator for Cd would have 100 μg g^{-1} of Cd in its tissue. This is quite high, considering that normal Cd levels in most plants is 0.1 mg kg^{-1} (Brooks, 1998). In the present study it was shown that *N. officinale* was not a hyperaccumulator of Cd. The highest BCF value for Cd was observed in a solution that contained 0.5 mM of Cd (Figure 1). BCF values of Cd decreased as the initial concentration of Cd in the solution increased (Figure 1). The uptake of heavy metals may decrease due to toxicity. Hou *et al*. (2007) indicated that the removal of low levels of Cd from an aqueous solution was efficient for *Lemna minor*. Plant species and varieties vary widely in their tolerance to excess cadmium in the growth medium (Vecchia *et al*., 2005; Singh *et al*., 2006; Toppi *et al*., 2007). Cadmium tolerant plants must be able to either prevent the absorption of excess cadmium or detoxify the cadmium after it is absorbed (Das *et al*., 1997). Vassilev and Yordanov (1997) reviewed the effect of Cd on many plant species and concluded that Cd diminishes RGR by inhibiting the net assimilation rate. In this study, the average relative growth rate was positive when the initial concentration of Cd ranged from 0.1 to 1.5 mM (Figure 2). A similar effect of Cd on relative growth rate has been reported in *Pistia stratiotes* (Maine *et al*., 2001). Although RGR of *N. officinale* in the present study decreased as the initial concentration of Cd increased, there were

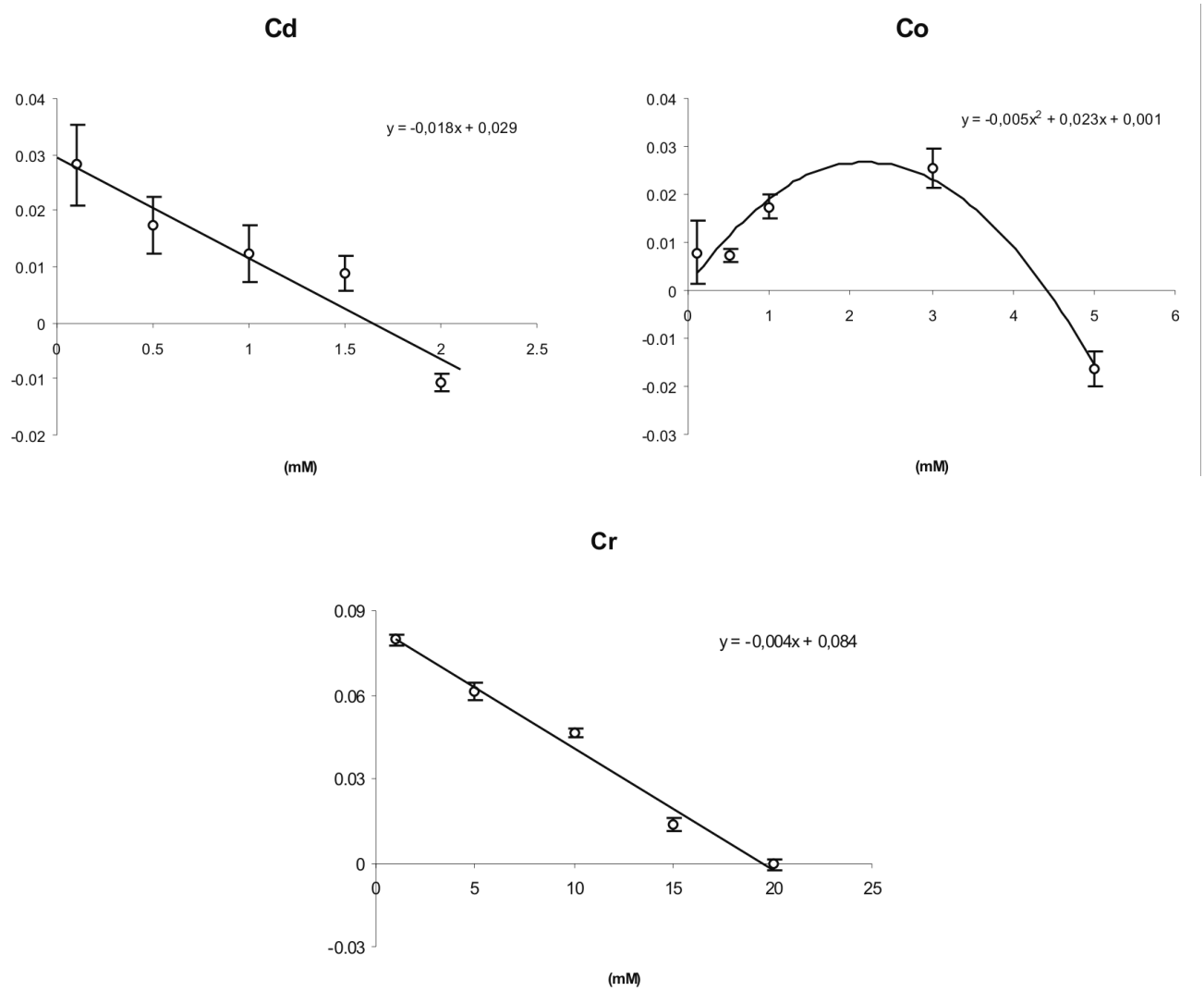

Figure 2 RGR values of *N. officinale* grown in different initial metal concentrations. Error bars represent standard error ($n = 2$).

not any severe macroscopic visual symptoms. Thus, *N. officinale* was apparently well protected against acute Cd stress (Maine *et al*., 2001; Miretzky *et al*., 2004; Toppi *et al*., 2007).

Cobalt is widely used as an alloying ingredient together with nickel, chromium, molybdenum and other elements. Co is essential for animals in trace amounts, as it is an integral component of the vitamin B12 complex (Smith and Carson, 1981). Organisms can bioaccumulate Co at high levels in non-toxic forms (Rainbow, 2002). Severe phytotoxic symptoms, however, can appear for plants that accumulate more than 50–100 μg g^{-1} dry weight of Co from the soil (Chaney, 1983). In this study, the highest Co concentration was 581 μg g^{-1}. A plant that contains more than 1000 μg g^{-1} of Co in its tissues is considered to be a Co-hyperaccumulator (Baker and Brooks, 1989; Malaisse *et al*., 1999). As a result of the present findings, *N. officinale* may not be evaluated as a hyperaccumulator for Co. The BCF values for cobalt ranged from 101 to 420 (Figure 1). The high bioaccumulation of Co in plants may be due to its role as a micronutrient. Szymanowska *et al*. (1999) studied metal bioaccumulation of aquatic plants (*Nymphaea alba*, *Nuphar luteum*, *Ceratophyllum demersum*, *Phragmites communis*, *Typha latifolia* and *Schoenoplectus lacustris*), and they found that these macrophytes had elevated levels of Co. RGR values slightly increased in lower concentrations and then decreased again. According to Figure 2, the relative growth rate was negative when plants were exposed to 5.0 mM of Co. The negative value of RGR indicates metal toxicity (Hasan *et al*., 2007).

Chromium usually occurs in two forms, Cr(III) and Cr(VI). Both of these forms are taken up by plants

(Vajpayee *et al*., 2000). Cr is not involved in plant metabolism (Shanker *et al*., 2005). Its compounds are highly toxic to plants, altering their growth and development (Rai *et al*., 2004). A Cr concentration of 100 μg g^{-1} dry weight is thought to be toxic for most of the higher plants. Plants containing more than 500 mg Cr kg^{-1} in dry weight should be considered hyperaccumulators (Baker *et al*., 1998). In this study, the highest Cr concentration was 137 μg g^{-1}. Therefore, *N. officinale* may not be evaluated as a hyperaccumulator for Cr. Although Cr accumulation by *N. officinale* in the present study depended on the initial concentration of Cr ($P < 0.01$), its highest BCF value occurred at 10 mM of Cr. Lower concentrations (*i.e*. 1–10 mM) facilitated chromium accumulation. Elevated Cr concentrations reduced BCF values. Also, in this study, Cr accumulation by *N. officinale* affected negatively relative growth rate (Figure 2). In a similar experiment, *Vallisneria spiralis* plants had a lower biomass when they were exposed to Cr (Vajpayee *et al*., 2001). The results here indicated that *N. officinale* could tolerate Cr up to a concentration of 20 mM. Earlier workers who studied Cr bioaccumulation stated that aquatic plants could develop strategies to defend themselves against this potential stress (Sinha *et al*., 2005; Vajpayee *et al*., 2000). Observations from the present study support these earlier reports.

Dietary intake may be a major source of the long-term low level accumulation of heavy metals in the bodies of humans. The harmful impact of these metals becomes apparent after several years of exposure (Bahemuka and Mubofu, 1999). According to the recommendation by FAO/WHO (2001), the maximum permissible levels of Cd, Co and Cr in vegetables are 0.3, 50 and 2.3 μg g^{-1}, respectively. It is clear that *N. officinale* can accumulate concentrations of metals that exceed these permissible values. Consumption of aquatic plants that are enriched with toxic metals may cause serious health hazards.

5. CONCLUSIONS

In this study, it was shown that *N. officinale* can grow in water polluted with metals. Moreover, *N. officinale* accumulated appreciable amounts of Cd, Co and Cr. The bioaccumulation of these metals depended on their initial concentrations in the growth solution. In addition, accumulation of metals in *N. officinale* varied from metal to metal. The accumulation of metals increased with an increase in the external metal concentration. Plant growth was negatively affected by Cd and Cr; however, a small amount of Co enhanced the relative growth rate of *N. officinale*. It was concluded that *N. officinale* was not able to bioconcentrate any of the metals to the level usually reported for a hyperaccumulator. The accumulation capacity of *N. officinale* was higher for Cd and Co than it was for Cr. The most efficient uptake of Cd, Co and Cr occurred at external solution concentrations of 0.5, 0.5 and 10 mM, respectively. The consideration of heavy metal bioaccumulation properties of edible aquatic plants is necessary to prevent excessive build-up of these metals in the food chain. *N. officinale* plants gathered from polluted water should not be consumed in order to avoid the accumulation of heavy metals in the body.

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