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CADMIUM REMOVAL BY AQUATIC MACROPHYTE (*NASTURTIUM OFFICINALE)* AND POTENTIAL FOR OIL PRODUCTION FROM THE BIOMASS

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ABSTRACT

Toxic metal pollution of water bodies is a major environmental problem and has accelerated drastically since the beginning of industrial revolution. Aquatic plants are known to accumulate metals and other toxic elements from contaminated water. This study was carried out to investigate short term uptake of cadmium (Cd) by *Nasturtium officinale* and Cd effects on chlorophyll contents and oil production. Plants were exposed to 1, 5 and 10 mg L^{-1} of Cd in hydroponics and sampling was done after 3, 6, 9 and 12 days to check accumulation and toxicity patterns. A control treatment (without Cd) was also included in the experiment to compare the results. The plants treated with 10 mg L^{-1} of Cd at day 12 had the highest concentration in shoots (35.7 mg kg^{-1} Cd) and roots (18.8 mg kg^{-1}). The chlorophyll contents decreased from 0.25 to 0.82 mg g^{-1} fresh weight at levels of Cd from control to highest level of Cd at day 12, respectively. Oil contents dropped from 258±11.5 to 218±3.5 mL kg^{-1} of fresh biomass over an exposure period of 12 days at 10 mg L^{-1} of Cd. Keeping in view the bioaccumulation capacity and oil production potential, this technique appears as environmentally and economically sustainable.

Keywords: Cadmium, chlorophyll, phytoremediation, wastewater.

INTRODUCTION

Land and water are valuable natural resources capable of ensuring the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to utmost exploitation and sternly polluted because of anthropogenic activities (Arshad *et al.,* 2008; Shahid *et al.,* 2014). There is a worldwide concern regarding water pollution that is due to the random and unplanned disposal of untreated domestic sewage and industrial effluents into

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water bodies (Mashiatullah *et al.,* 2005). However, wastewater is considered as one of the most important resources that can become productive or useful if treated and managed well to be reused. It is well known that heavy metals are the most dangerous elements or substances in the environment due to their high level of stability and toxicity to the biota (Alkorta *et al.,* 2004). A metal in concentration more than its permissible limits like Cd is an environmental toxin. It is utilized in manufacturing batteries, electronics components, automobile radiators, and in photography. It is also a component of petrol, tires, lubricating oils, phosphatic fertilizers and diesel fuel (Naja and Volesky, 2009). Once released to the environment, it persists and do not degrade like organics through chemical oxidation or by microbial activity. Therefore, Cd contamination appears to be a significant problem due to its long-term persistence and toxic nature in water systems, humans and animal life. Cadmium exerts most of their toxicity by destroying important proteins by stealing off sulphur from them, many of which are enzymes, hormones, or cell receptors. It is also regarded as a potential human pancreatic carcinogen (Brian *et al.,* 2012).

Cadmium toxicity causes inhibition and abnormalities of general growth in many plant species. After long-term exposure of Cd, roots become mucilaginous, browning, and decomposing while reduction of shoot and root elongation, rolling of leaves, and chlorosis can occur as well. Cd was found to inhibit lateral root formation while the main root became brown, rigid, and twisted (Rascio and Navari-Izzo, 2011). The core reason reported is disordered division and abnormal enlargement of epidermal and cortical cell layers in the apical region. The changes in the leaf include low contents of chlorophyll and alterations in chloroplast ultrastructure, which caused chlorosis, as well as restricted activity of photosynthesis (Miyadate *et al.,* 2011).

Several studies tested the ability of both terrestrial and aquatic plants to remediate toxic contaminants such as zinc (Zn), cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), nickel (Ni), etc. (Zurayk *et al.*, 2001; Marchiol *et al.*, 2004; Kara, 2005; Arshad *et al*., 2008; Shahid *et al*., 2014). Some aquatic plants are reported to accumulate considerable levels of Cd from contaminated water, like *Nasturtium officinale* (watercress) upon an exposure of 14 days to Cd metal (Aslan *et al.*, 2003). It is a perennial member of the Brassicaceae family and naturally grows in abundance near springs and open-running waterways in Europe, America and Asia. It has asexual propagation and every part of plant is in contact with the water to produce adventitious roots and develop a complete plant (Jafari and Hassandokht, 2012). The present study was therefore intended to assess Cd accumulation potential of *Nasturtium officinale,* toxic effects on chlorophyll contents and bio-oil production from the biomass over a period of three weeks. Previously, only up to 5 mg L^{-1} Cd concentration was considered (Aslan *et al*., 2003) while 10 mg L^{-1} of Cd was the highest selected level in present study. Moreover, possibility of oil production in combination with remediation was not considered earlier. Based upon the work done, it can be suggested that rhizofiltration technique is economically sustainable where the metal free oil produced can help to offset the expenditures for the treatment of wastewater.

MATERIALS AND METHODS

Plant material

One week old *Nasturtium officinale* plants with approximately the uniform size and weight were collected from National Agriculture Research Centre, Islamabad, Pakistan. The plants were washed thoroughly with tap water, followed by de-ionized water prior to the experiment onset. All the plants were grown in experimental tanks filled with 6L of distilled water along with Hoagland solution in hydroponics setup. Cadmium sulphate ($CdSO_4$) salt was dissolved in distilled water to get the desired contamination level of 1, 5 and 10 mg Cd L^{-1}. A control treatment was also included where the plants were grown in Hoagland solution only. All the experimental sets were maintained in triplicate. Test plants (roots and shoots) were harvested after 3, 6, 9 and 12 days of exposure.

Cadmium analysis of shoots and roots

Separated root and shoot samples were oven dried at 60 °C to remove all the moisture. The oven dried samples were weighed and then ground to powder. For digestion, 0.5 and 1.0 g of roots and shoots were weighed, respectively and the samples were digested for about 45 minutes with HNO_3–$HClO_4$ in 2:1 ratio (v/v) using hotplate and diluted to 100 mL with de-ionized water. Samples were filtered prior to analysis. The digested plant samples were analyzed for heavy metals by means of Atomic Absorption Spectrophotometry (PHOENIX-986) with air/ acetylene burner. Quality check samples/blanks/standards purchased from PG Instruments, Ltd, UK (Cd Standards) were also processed during the analysis to ensure the correctness of procedures.

Chlorophyll estimation

Fresh leaves weighing 2g were plucked during morning for chlorophyll analysis. The method reported by Kavulicova *et al.* (2012) was adopted for chlorophyll estimation. Leaves were crushed thoroughly with 80% acetone in order to extract the chlorophyll content and then decanted the supernatant through the filter paper (Whatman # 41) into 100 mL volumetric flask. Five milliliter of supernatant solution was transferred into a 100 mL volumetric flask and volume was made up to the mark with 80 percent acetone solution. Absorbance of the leaf tissue extract at 663 and 645 nm was measured by spectrophotometer (UV-VIS Spectronic, GENESYS-5). Calculations for finding chlorophyll concentrations were performed by following formula (Arnon, 1949).

$$C\ (mg^{-1}) = (20.2 \times A645 + 8.02 \times A663) \times 50/1000 \times 100/5 \times 1/2$$

Oil Extraction

For oil extraction, similar procedure as above mentioned was repeated but samples were taken at the intervals of 6 and 12 days. The samples were oven dried at 60 °C and were ground to powder form. The extracts were obtained in a

Soxhlet apparatus for 5 hours at the boiling points of the solvent (n-hexane 69 °C). The ratio of solvent to sample was 10:1 (Saxena *et al.,* 2011). In order to separate oil from n-hexane, the sample was run into rotary evaporator at 70 °C and 30 rpm. Then in order to separate pigments from oil, 0.2 g of bleaching activated earth powder was added to bind pigments and centrifuged at 6000 rpm for 10 minutes.

Statistical Analysis

This study was performed in three replicates and outcomes are shown as the mean ± SD (Standard Deviation). Results were analyzed using ANOVA ($P<0.05$) with data analysis tools in Excel and LSD test was performed to determine the effect of different treatment means.

RESULTS AND DISCUSSION

Plant biomass and Cd uptake by *Nasturtium officinale*

Results related to dry biomass (DW) are presented in Figure 1. In control treatment, there was no significant difference for biomass among 3, 6 and 9 days values. However, it was significantly ($P<0.05$) increased for 12 day culture. The maximum biomass was recorded in control, i.e. 1.72 g DW while the minimum value was 1.08 g DW at day 12 with 10 mg L^{-1} of Cd treatment. In general, as the exposure time and concentration increased, dry biomass decreased significantly. Presence of Cd metal in hydroponics could lead to inhibition of growth hormones which regulate biomass production (Schellingen *et al.*, 2014). As reported by Arshad *et al.* (2012), balanced availability of auxins and cytokinins are required for adequate plant growth.

Cadmium uptake by plant from water occurs either passively with the mass flow of water into the roots, or through active transport that crosses the plasma membrane of root epidermal cells. Plants can potentially accumulate certain metal ions under normal growing conditions (Kim *et al*., 2003). Results of this study showed that test plant apparently exhibited a high capability of concentrating Cd from water. This trend is depicted in the metal accumulation patterns for *Nasturtium officinale* (Figure 2) from different concentrations of Cd in solutions. Cd concentration in plant roots varied from 0.92 to 18.84 mg kg^{-1} (Figure 2a). The values increased as the exposure time increased ($P<0.05$). Exposure level had a significant effect ($P<0.05$) and the highest values were recorded at 10 mg L^{-1} of Cd in solution. Concentrations of Cd in shoots are presented in Figure 2b. The values ranged from 1.84 to 35.48 mg kg^{-1}. With increasing exposure time and concentration in solution, shoot levels of Cd were significantly increased ($P<0.05$). The trends were similar as for the roots.

Results of Cd uptake per plant are presented in Figure 3. The values ranged from 2.5 to 33.8 µg per plant over the period of 12 days. There was gradual increase in Cd uptake with increase in exposure time as well as the concentration in solution. The continuous increase in Cd contents is encouraging for rhizofiltration purposes as the plants still show capacity for further accumulation of the metal. Kara (2005) found *Nasturtium officinale* as very enduring to low dose (1 mg L^{-1}) and longer term (3 days) exposure to certain heavy metals. Since the model plant has short life cycle, there could be some problems for long term accumulation studies with watercress. However, in soil medium, long term field studies is a promising option as it has been reported using Pelargonium species for heavy metal accumulation (Shahid *et al.,* 2012).

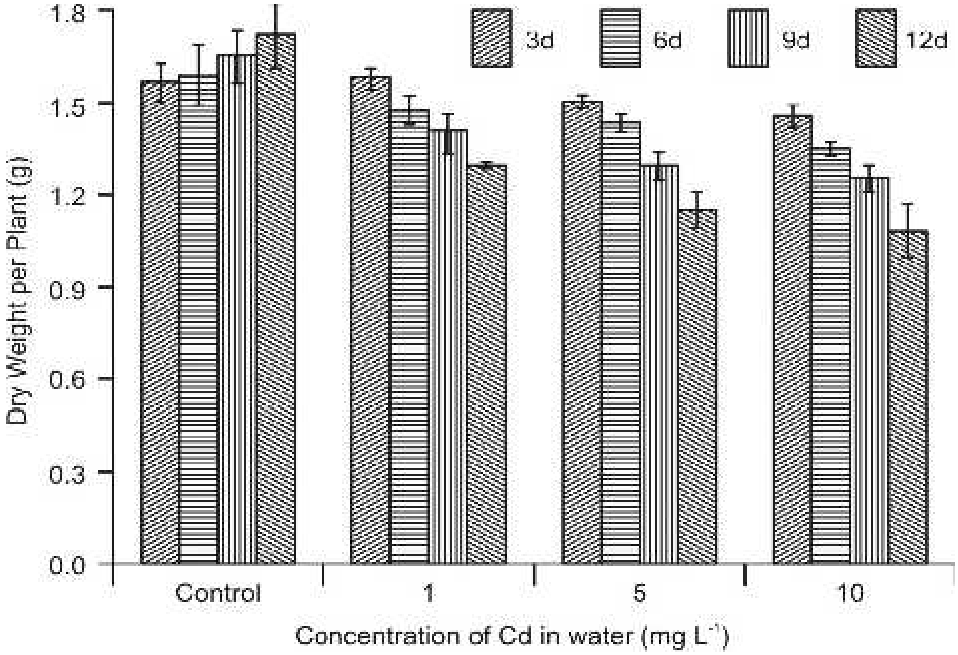

Figure 1. Biomass of *Nasturtium officinale* over a period of 12 days in response to exposure to different levels of Cd.

Bioconcentration and translocation factors (BCF and TF)

The plant's ability to uptake/remove metals from water can be estimated by BCF. It is defined as the ratio of metal concentration in roots and external environment i.e. soil or water (Ghosh and Singh, 2005). Furthermore, the plant's ability to translocate metal from roots to shoots is measured by calculating TF, which is the mobilization ratio of metal from roots to shoots (Marchiol *et al*., 2004). The process of phytoextraction generally entails the translocation of heavy metal to the easily harvestable plant parts, i.e. shoots. Plants exhibiting TF and particularly BCF values greater than one are regarded as metal accumulators. By comparing BCF and TF, plants ability can be determined for uptake of metals from water. Metal tolerant plants have a tendency to restrict the transfer of metal to easily harvestable part and consequently have much less accumulation in their biomass (Fitz and Wenzel, 2002).

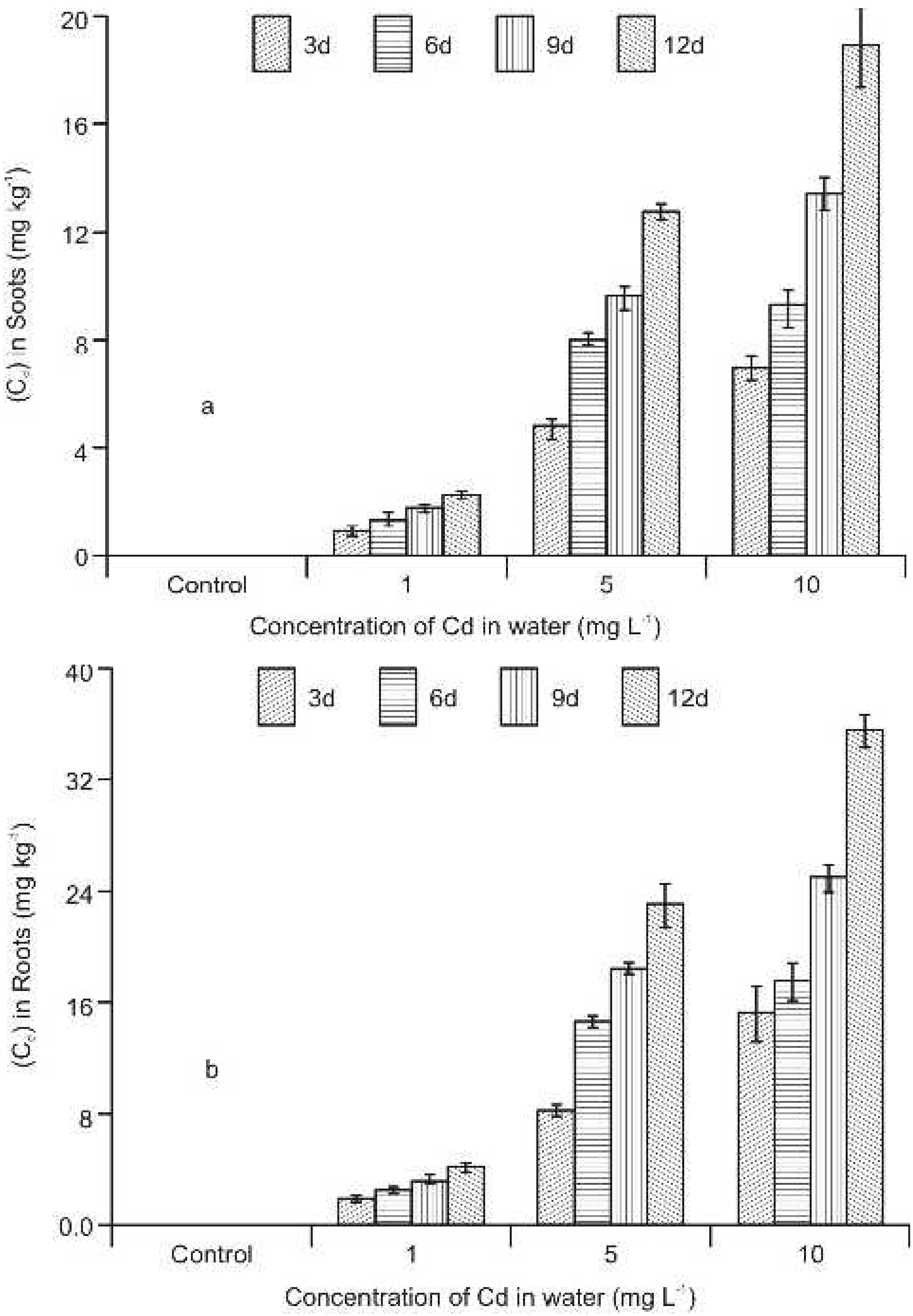

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Figure 2. Cd concentration in plant parts of *Nasturtium officinale*. a) in roots, b) in shoots

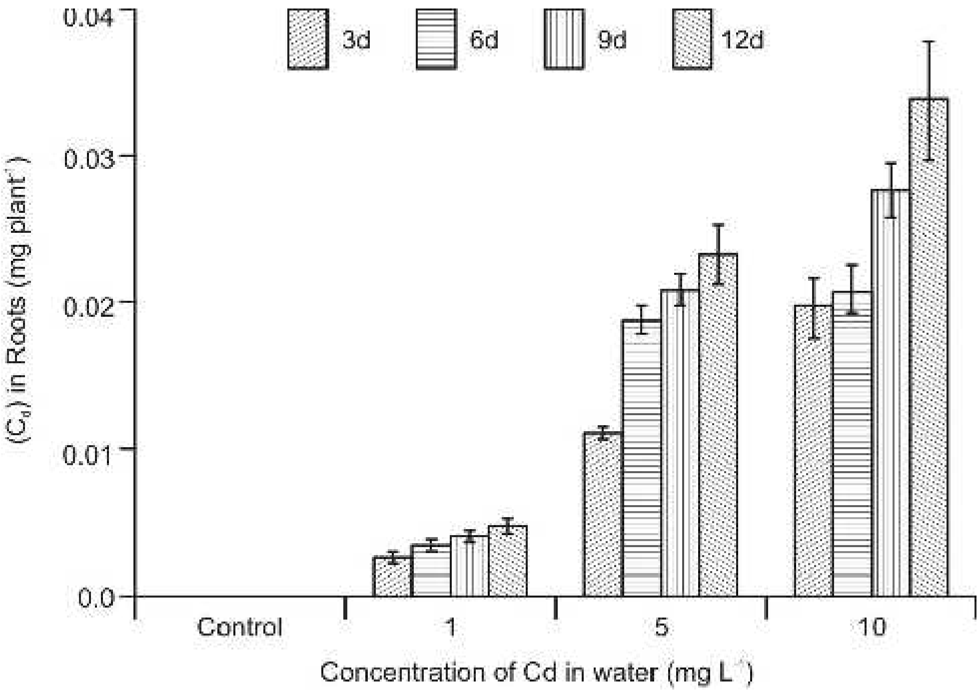

Figure 3. Cd uptake by *Nasturtium officinale* over a period of 12 days in response to exposure to different levels of Cd.

The BCF values of *Nasturtium officinale* varied from 2.7 to 19.4 (Figure 4a). These values were the highest for all the levels at 12 days exposure while the lowest values were recorded at 3 days exposure. This data is promising that even after exposure of 12 days, accumulation was going on. Based on the experimental data, it is clear that the test species grown in higher concentration of Cd with longer exposure exhibits BCF values greater than 2. Therefore, it can be concluded that *Nasturtium officinale* is very efficient in accumulating Cd in plant roots.

The translocation factor values are presented in Figure 4b. The results showed more accumulation in shoots as compared to the roots. The plants treated with 10 mg L^{-1} of Cd at day 12 accumulated the highest concentration in shoots (35.7 mg Cd kg^{-1}). In the roots, the highest mean concentration of Cd in plants grown in 10 mg Cd L^{-1} solutions was 18.8 mg kg^{-1}. The TF values ranged from 1.7 to 2.2. Statistically, there was no significant difference among different treatments and exposure periods. Moreover, all the values of TF were greater than one which indicates higher accumulation in shoot component. Since, the translocation factor helps in determining the mobilization of metal from roots to shoots; it can be very useful parameter in selection of metal accumulator or tolerant plant species (Xiong, 1998) with availability of more space for storage in shoots.

Effects of Cd on chlorophyll and oil contents

Results (Figure 5) indicate that *Nasturtium officinale* expressed the symptoms of Cd toxicity leading to the decrease in total chlorophyll content at higher levels (5 and 10 mg $L^{-1)}$ of Cd. On the contrary, Cd ions positively affect the physiological parameters of *Nasturtium officinale* when low metal concentrations (1 mg L^{-1}) were added. Slight increase in chlorophyll content was recorded from 1.75 to 1.04 mg L^{-1} after 3 days of exposure to 1 mg Cd L^{-1}. This could happen probably due to possible replacement of Mg ion in chlorophyll by Cd ions. Kavulicova *et al.* (2012) also reported similar pattern of chlorophyll increase when toxic effects of low metal concentrations of copper (Cu), cadmium (Cd) and zinc (Zn) were studied on *Linum usitatissimum*. The maximum decrease of the chlorophyll content was observed at the higher Cd exposure (10 mg L^{-1}) and at day 12, chlorophyll contents dropped from 2.25 to 0.82 mg g^{-1} FW. Furthermore, plant leaves treated with higher concentrations of Cd (5 and 10 mg L^{-1}) were observed small, curled and discolored whereas a slight stimulation in plant organs (leaves and stem) have been recorded at the lower Cd concentrations. Aslan *et al.* (2003) reported a significant reduction in chlorophyll concentrations in *Nasturtium officinale* leaves after 14 days of exposure to 1.0 and 5.0 mg L^{-1} Cd. The toxic Cd concentration can inhibit plant growth or cause death by changing the lipid organization of membranes, affecting photosynthesis, or inhibiting CO_2 fixing enzyme (Tran and Popva, 2013).

Zurayk *et al.* (2001) collected and studied twelve Mediterranean hydro-phyte species from Lebanon. These were investigated for their potential as bio-indicator species for heavy metal pollution. They were inoculated in nutrient cultures enriched with 1 mg L^{-1} chromium (Cr), nickel (Ni) and cadmium (Cd). *Nasturtium officinale* was included in the list of investigated plant species. The growth rate of watercress remained high during the experimental period, indicating that the plants were little affected by the presence of the metal at the different concentrations. Results showed that *Nasturtium officinale* has a potential to act as bioindicator species for Cr, Cd and especially for Ni.

Results showing oil contents in fresh biomass are presented in Figure 6. By analyzing the data, it was observed that amount of oil extracted from *Nasturtium officinale* remains almost same with varying treatment concentrations of Cd (Figure 6) at day 6. However, there was slight decline in the amount of oil at 12 days exposure to 5 and 10 mg L^{-1} with statistically significant differences among the levels of Cd. The qualitative analysis of oil extracted from *Nasturtium officinale* was carried out by XRF in order to assess the presence of heavy metals particularly, Cd. The XRF analyses demonstrated the absence of Cd and presence of calcium, potassium, iron, zinc and titanium in all oil samples (Data not shown). The plant can therefore be utilized for bio-oil production and its use may be safe. However, toxicity assessments are recommended before any further use.

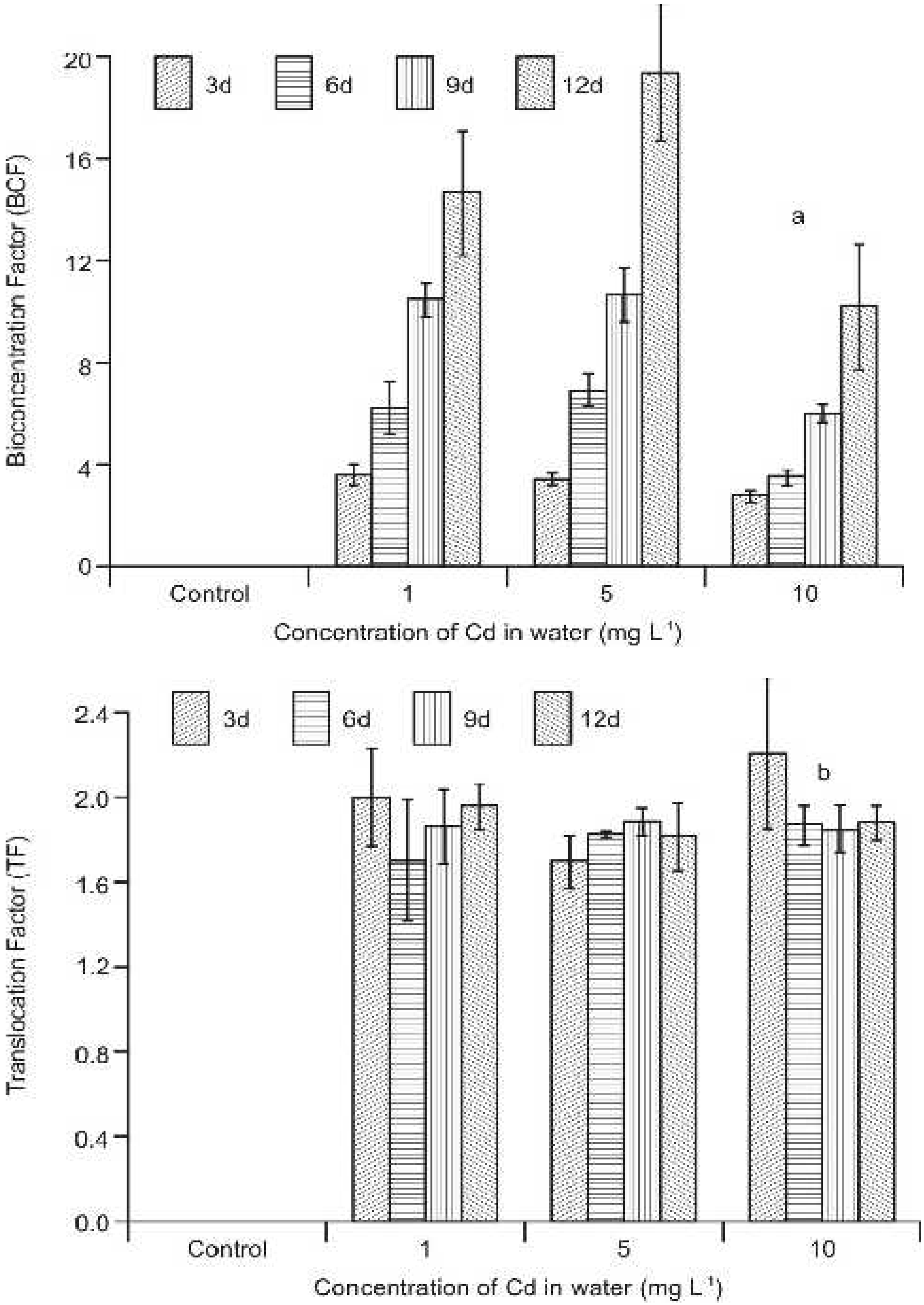

Figure 4. Cd accumulation patterns of *Nasturtium officinale.* a) Bioconcentration factor (BCF) and, b) Translocation factor (TF)

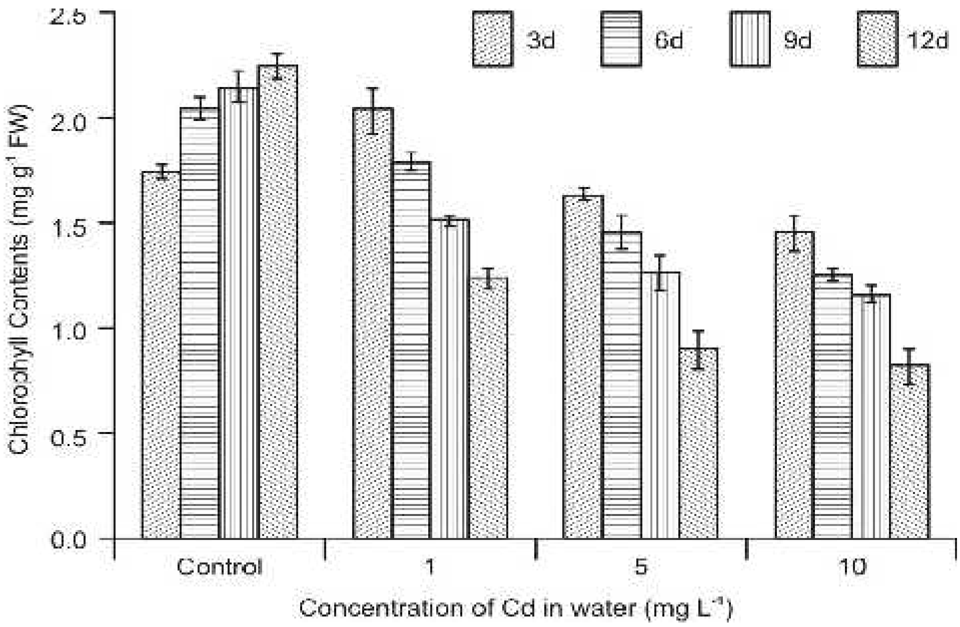

Figure 5. Chlorophyll contents in *Nasturtium officinale* plants over a period of 12 days in response to different levels of Cd in hydroponics

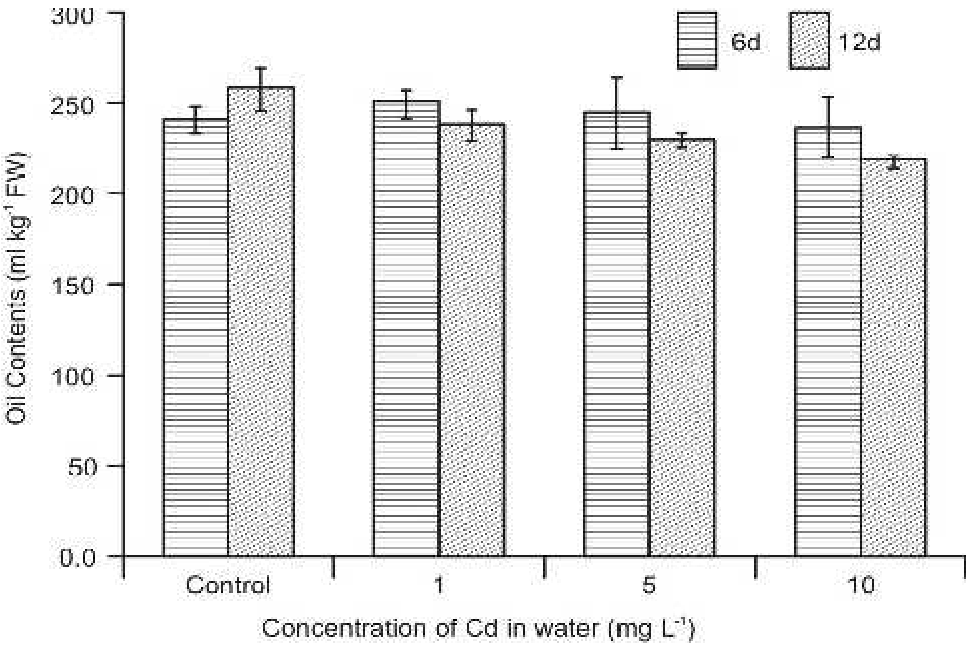

Figure 6. Oil contents of *Nasturtium officinale* extracted at 6th and 12th days of exposure to different levels of Cd

CONCLUSION

The present investigation revealed that *Nasturtium officinale* could be a suitable plant for rhizofiltration of Cd. The overall Cd uptake by the plant was dependent upon the concentration of Cd and the duration of exposure. Both BCF and TF values were higher than one, indicating that *Nasturtium officinale* is potentially useful for remediation of Cd contaminated waters. Moreover, metal free production of oil can be helpful to compensate the expenditure for application of this technology.

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