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Effects of EDTA on Cr^{+3} Uptake, Accumulation, and Biomass in *Nasturtium officinale* (Watercress)

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Abstract
A pot study was used to examine the effects of ethylenediaminetetraacetic acid (EDTA) on the growth potential, uptake, and mobilization of Cr by Watercress (*Nasturtium officinale*) in contaminated water at different concentration levels of the chelating agent EDTA (0, 10^{-5}, and 10^{-4} M) and four concentration levels of Cr (0, 1, 3, and 10 mgL^{-1}). The EDTA resulted in more solubilization of Cr in water. The application of EDTA 15 d prior to harvest increased the amount of Cr accumulated in watercress with more Cr accumulated by the plants from the media. *Nasturtium officinale* accumulated high Cr concentration (317 $mgkg^{-1}$) in the root at a concentration of 10 mgL^{-1} Cr^{3+} and 10^{4-} M EDTA after 15 d growth. The application of EDTA had inhibitory effects on the root and shoot dry biomass compared with that in the control. This plant can be used as potential species for chelate-assisted Cr phytoremediation.
Keywords: Biomass, Chromium, EDTA, *Nasturtium officinale*, Phytoexraction

***Nasturtium officinale*'de (Su Teresi) Cr^{+3} Alımı, Birikimi ve Biyokütlesi Üzerinde EDTA'nın Etkisi**
Özet
Bu çalışmada Cr'un dört farklı seviyesi (0, 1, 3 ve 10 mgL^{-1}) ve farklı seviyelerdeki EDTA (0, 10^{-5} ve 10^{-4} M) ile kontamine olmuş suda, Su Teresi (*Nasturtium officinale*) kullanılarak bitki üzerinde Cr'un büyüme potansiyeli, alınımı ve taşınmasında EDTA' nın etkisi incelenmiştir. EDTA sudaki Cr'un daha fazla çözünmesini sağlamıştır. Hasattan 15 gün önceki uygulamada bitkiler tarafından daha fazla Cr biriktirilmesiyle, su teresindeki Cr miktarı artmıştır. 15 günlük büyüme sonunda 10^{4-}M EDTA ve 10 mgL^{-1} Cr^{3+} uygulanan *Nasturtium officinale* köklerinde yüksek Cr konsantrasyonu (317 $mgkg^{-1}$) hesaplanmıştır. EDTA'nın, kontrolle karşılaştırıldığında bitkinin kök ve gövde kuru ağırlıklarında inhibe etki yaptığı gözlemlenmiştir. Bu bitkiler Cr için arıtmada bitkilerin kullanılmasında potansiyel bir tür olarak kullanılabilir.
Anahtar Kelimeler: Bitkisel Arıtma, Biyokütle, Krom, EDTA, *Nasturtium officinale*.

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INTRODUCTION

Phytoextraction is a green technology that uses plants to remove inorganic contaminants, primarily heavy metals, from soils and waters. Phytoextraction can be broadly classified as either natural or chemically assisted. The natural phytoextraction strategy utilizes metal hyperaccumulating plant species (Kumar et al. 1995). The success of phytoextraction, either natural or chemically assisted, is largely determined by plant biomass, metal concentration in the plant tissue, and the phytoavailable fraction of metals in the rooting medium.

In these types of studies EDTA is still valuable because it allows new results to be compared directly to the broadest segments of literature. Chelating agents can react with metal ions and influence metal phytotoxicity and phytoextraction (Jean et al. 2007, January et al. 2008). EDTA is a chelating agent often used to enhance metal uptake by plants in field and pot experiments (Wu et al. 2004, Luo et al. 2006). Also, chelating agents can translocate the metal from the roots to the above ground parts of plants (Li et al. 2009). The presence of EDTA alters the metal speciation and metal phytotoxicity (Huang et al. 2008, Wang et al. 2008).

The impact of heavy metals is manifested on the environment (Akinci and Caliskan 2010, Demirayak et al. 2011). Environmental pollution by toxic metals has increased since the industrial revolution, thereby causing ecological problems (Huseyinova et al. 2009).

Wastewater from many industries, including leather tanning, electroplating, finishing,

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metallurgical, refractory, and dyeing along with other industries utilizing pigments, may contain high levels of chromium ions and potentially also chelating agents and chloride (Fu and Viraraghavan 2001, Shanker et al. 2005). The most stable states of chromium in the environment are the trivalent and hexavalent species; however, Cr^{6+} is rapidly reduced to Cr^{3+} in the presence of organic matters and in reducing environments (Zayed and Terry 2003). A high concentration of chromium is highly toxic to plants (Chatterjee and Chatterjee 2000, Shanker at al. 2005).

In this study, *N. officinale* was selected to evaluate its potential to phytoextract Cr^{3+} in an aqueous environment. Influences of the Cr^{3+} speciation on Cr phytoremediation by *N. officinale* were also investigated. The influences of Cr speciation on phytotoxicity and phytoextraction of *N. officinale* were investigated in the presence of EDTA.

MATERIAL AND METHODS

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. *N. officinale* seedlings were collected in April, 2010 from the Zamanti Stream in Kayseri, Turkey. *Nasturtium officinale* are one of the target species that continue to hold excellent promise for phytoextraction (Zurayk et al. 2001, Aslan et al. 2003).

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 a photoperiod of 16 hr/8 hr (light/dark). The plants that were in the best condition were selected for subsequent experiments. Seedlings of similar size were selected for the following hydroponic experiments.

Hydroponic Experiment

A modified Hoagland's nutrient solution without EDTA was used as the hydroponic medium, because the 2 x 10^{-5} M Na–EDTA in the Hoagland's nutrient solution (Hoagland and Snyder 1993, Xiong et al. 2002) would interfere with the Cr^{3+} speciation in this study and an extra pure grade of $CrCl_3.6H_2O$ was used. The $Na_2EDTA.2H_2O$ (100% purity) was purchased from Merck. The $CrCl_3$ and EDTA were added to the modified Hoagland's nutrient solution to achieve the desired concentrations. For this purpose, the growth media was supported with (1) 0 ppm Cr^{3+} (control), (2) 1 ppm Cr^{3+}, (3) 3 ppm Cr^{3+}, (4) 10 ppm Cr^{3+}, (5) 1 ppm Cr^{3+} + 10^{-4} EDTA, (6) 3 ppm Cr^{3+}+ 10^{-4} M EDTA, (7) 10 ppm Cr^{3+}+ 10^{-4} M EDTA, (8) 1 ppm Cr^{3+}+ 10^{-5} M EDTA, (9) 3 ppm Cr^{3+}+ 10^{-5} M EDTA, and (10) 10 ppm Cr^{3+}+ 10^{-5} M EDTA. Three 3000 ml plastic cups were used per treatment. Each plastic cup contained 10 seedlings in 1800 mL of modified Hoagland solution. The seedlings were incubated in a 16-h light/8-h dark photoperiod at 15°C for 15 d. Distilled water was added each day to maintain water levels. After the 15 d culture period, the seedlings were collected, washed with distilled water, and dried with tissues.

Then, the root and shoot parts were dried at 80°C for 2 d, and the dried root weight and shoot weight were measured. The dried samples of *N. officinale* were then digested with 10 mL of concentrated HNO_3, using a CEM microwave digestion system (Demirezen Yılmaz 2007). After digestion, the volume of each sample was adjusted to 25 mL using double deionized water. Determinations of the heavy metal concentrations in all samples were carried out by Inductively Coupled Plasma Optical Emission Spectrometry (Varian). The samples were analyzed in triplicate.

The amount of chlorophyll was determined according to the method described by Knudson et al. (1977).

Bioconcentration Factor

The bioconcentration factor (BCF) was calculated as follows (Rahmani and Sternberg 1999):

$BCF_{plant} = Conc_{Cr\ in\ plant} = Conc_{Cr\ in\ solution}$

Where $Conc_{Cr\ in\ plant}$ is the Cr concentration in the plant ($mgkg^{-1}$)

and $Conc_{Cr\ in\ solution}$ is the Cr concentration in the solution (mgL^{-1});

Relative Growth Rate

The relative growth rate (RGR), based on whole plant dry weight production, was calculated according to Hunt et al. 1990 as RGR = (Ln (W2) – Ln (W1))/ (t2 – t1), where W is the dry matter at the beginning (W1) and the end (W2) of the 15 day treatment period, and (t2 – t1) is the duration of this period.

Chromium Absorption Efficiency

This parameter was calculated as:

Cr^{3+} Abs. Eff= (Q Cr^{3+} Pa + Q Cr^{3+} R)/ DW Root average, where

Q Cr^{3+} Pa is the total amount of chromium accumulated in the shoots at final harvest (µg $plant^{-1}$) Q Cr^{3+} R is the total amount of chromium accumulated in the roots at final harvest (µg $plant^{-1}$). DW root average is the dry weight logarithmic average of the root system.

Translocation Factor

The translocation factor (TF) gives the shoot and root chromium concentration and depicts the ability of the species to translocate the metal from the roots to the shoots (Ghnaya et al. 2007).

TF= Cr^{3+} in shoots (mgkg-1) / Cr^{3+} + in roots (mg kg^{-1}).

Statistical Analysis

A correlation and regression analysis was performed. ANOVA was performed using SPSS-11 to identify significant differences in parameters in the different treatments. Differences were considered significant for $p<0.05$.

RESULTS

Biotoxicity and Bioaccumulation of Cr in *N. officinale*

Chromium Biotoxicity

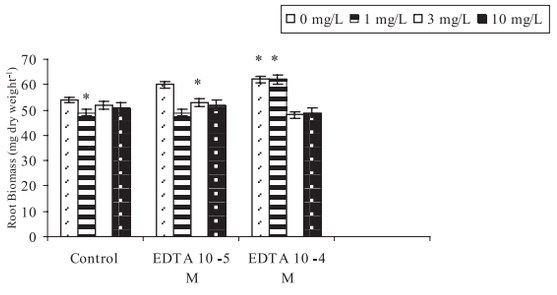
The effects of the Cr^{3+} and Cr^{3+} plus EDTA treatment on the biomass of *N. officinale*, as described by the dry weight of the plant, are summarized in Figures 1a and 1b. Figure 1 indicates that neither the applied EDTA nor the Cr^{3+} concentrations had any obvious effect on the root and shoot biomass in the Cr-EDTA mixture.

A significant decrease was observed in the chlorophyll content with the application of Cr without EDTA ($p < 0.05$). The most and the least affected groups were those treated with 10 and 1 mgL^{-1} Cr. There was approximately a 1.5 fold differences between the control and 10 mgL^{-1} Cr application with EDTA (Figure 2). No significant difference was observed between the control and 10 mgL^{-1} Cr with 10^{-4} M EDTA levels.

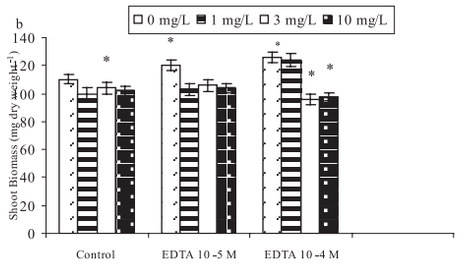
The relative growth rate (RGR) given in Fig. 3 also shows that the presence of Cr^{3+} in the medium significantly reduced the growth activity during treatments. On the other hand, EDTA in the absence of Cr^{3+} had no impact on the RGR (Fig. 3).

Chromium Bioaccumulation

In plants cultivated in the presence of Cr^{+3} alone or in combination with EDTA, the pollutant mainly accumulated in the roots. Figures 4a and 4b indicate that Cr in the roots and shoots increased with an increase in aqueous Cr^{3+}. High Cr concentrations



Root (a)



Shoot (b)

Fig. 1. Root (a) and shoot (b) biomass of *N. officinale* with Cr^{3+} and Cr^{3+} + EDTA treatment. Values represent mean±SE (n=3). Means marked with ⋆ are significantly different at $p < 0.05$.

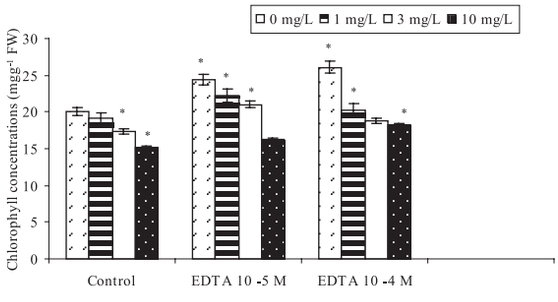


Fig. 2. The total chlorophyll in *N. officinale* with Cr^{3+} and Cr^{3+} + EDTA treatment. Error bar represents standard deviation. Means marked with the same letter are not significantly different at $p < 0.05$.

in the root (317 $mgkg^{-1}$) were observed in a Cr^{3+}solution concentration of 10 mgL^{-1} (Figure 4 a); however, the Cr concentrations in the shoot were much lower than those in the root without EDTA (Figure 4 b). Figure 4 a and b also shows that when the solution Cr^{3+} concentration was 10 mgL^{-1}, the addition of EDTA enhanced the root and shoot Cr bioaccumulation but, decreased the Cr concentrations in the shoot.

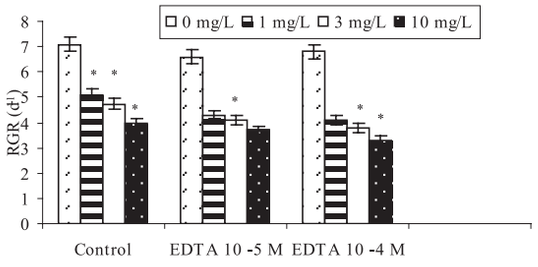


Fig. 3. Changes in the relative growth rate in *N. officinale* plants submitted to different treatments. Means marked with the same letter are not significantly different at $p < 0.05$.

Table 1. Bioconcentration Factor (BCF) of Cr^{3+} in *N. officinale* grown in a Cr- EDTA application after 15 d.

Cr (mgL^{-1})			
EDTA (M)			
BCF_{root} (1 g)	0	10^{-5}	10^{-4}
1	0.2±0.001	1.6±0.001	1.28±0.09
3	0.2±0.002	2.1±0.01	1.58±0.02
10	0.7±0.003	2.7±0.1	3.36±0.05
EDTA (M)			
Cr (mgL^{-1})			
BCF_{shoot} (1 g)	0	10^{-5}	10^{-4}
1	0.1±0.001	0.56±0.001	0.01±0.003
3	0.7±0.007	0.74±0.03	0.02±0.004
10	0.2±0.004	1.14±0.002	0.03±0.004

Table 2. Transfer Factor (TF) of Cr^{3+} in N.officinale grown in a Cr- EDTA application after 15d.

Cr (mgL^{-1})			
	EDTA (M)		
TF	0	10^{-5}	10^{-4}
1	0.6±0.001	0.27±0.004	0.17±0.009
3	0.28±0.001	1.16±0.002	0.22±0.008
10	0.53±0.008	0.42±0.02	0.14±0.001

Table 1 indicates that both BCF_{root} and BCF_{shoot} increased with an increased Cr^{3+} concentrations with EDTA application. The BCF_{root} also increased with increased EDTA concentrations (Table 1). It is probable that the formation of Cr–EDTA enhanced the mass transfer of Cr^{3+} ions to the root surface.

Indeed, both the bioconcentration factor (BCF) and translocation factor (TF) used to evaluate the capacity of plants to absorb and to transport metal from the roots to the shoots, were significantly increased by the addition of EDTA (Tables 1 and 2). The range of the bioconcentration factor (BCF) for the root is 0.2 - 3.36 (1 g) and for shoot 0.01 - 1.14 (1 g). So, the minimum values of BCF implied that the spread in values compared different EDTA concentrations. The results also showed that the transfer factor (TF) values were significantly increased by the addition of EDTA. For example,

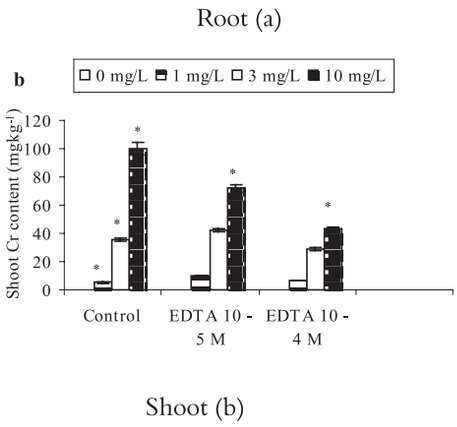


Fig. 4. Cr^{3+} bioaccumulation in root (a) and shoot (b) of *N. officinale* with Cr^{3+} and Cr^{3+}+ EDTA treatment. Error bar represents standard deviation. Means marked with the same letter are not significantly different at $p < 0.05$.

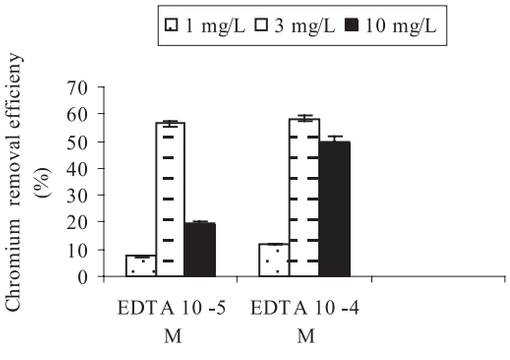


Fig. 5. Chromium removal efficiency (%) in *N. officinale* plants maintained for 15 days in a solution with Cr^{3+} and Cr^{3+}+ EDTA treatment. Error bar represents standard deviation. Means marked with the same letter are not significantly different at $p < 0.05$

the range of TF is 0.14-0.53 with the addition of EDTA. As a result, the present work also shows that the observed increase in the amount of Cr^{3+} accumulated by the plants in the presence of EDTA is the consequence of an increased absorption of this metal in the presence of a chelating agent. Hence, the calculation of the efficiency of the absorption of chromium by the root showed a significant increase when the plants were cultivated in the presence of Cr^{3+} combined with EDTA as compared to plants exposed to Cr^{3+} alone (Figure 5).

DISCUSSION

This study demonstrated that the presence of Cr^{3+} had a significant impact on the relative growth rate. A significant decrease in the dry weight of *N.*

officinale was obtained with an increase in the concentration of chromium from 0 to 10 mgL^{-1} in the absence of EDTA in the hydroponic medium. Similarly Bala and Thukral (2008) observed that the dry matter yield of *S. polyrrhiza* decreased as the application rate of chromium (VI) was increased. Additionally, the dry weight of the entire Miscanthus plant decreased by 17% with 50 mg L^{-1} Cr, by 37% with 100 mg L^{-1} Cr, and by approximately 59% with the two highest chromium concentrations (Arduini et al. 2006). Panda (2007) also reported a decrease in the dry biomass in rice seedlings treated with chromium for 24 and 48 h.

In the present study, the maximum increase of root and shoot biomass was obtained in the binary combination of 1 mgL^{-1} Cr^{3+} and 10^{-4} EDTA in the hydroponic medium. However, in the binary combination of 10 mgL-1 Cr^{3+} and 10^{-4} EDTA, a maximum decrease of 31.3% in shoot dry weight was observed. The decrease in the biomass production of *N. officinale* after the application of 10^{-4} M EDTA was due to the combined toxicity of Cr and EDTA in the hydroponic medium. The effects of chelators were dose and time dependent. The application of chelators alone resulted in the removal of essential metal nutrients from the media, leading to deficiencies in the plants (Ruley et al. 2006). Grcman et al. (2003), discovered that in all chelate treatments, including EDTA, necrotic lesions were observed on cabbage leaves and were more prominent on the older leaves.

This study is also demonstrated that the presence of EDTA had a significant impact on the rate of chromium accumulation in *Nasturtium officinale* (Figure 4). Earlier reports in literature also supported the study. According to Chen et al. 2010, the EDTA-promoted uptake of Cr in *Ipomonea aquatica*. In our data, the chromium uptake was much lower when EDTA was absent even with the dissolved, unchelated chromium levels in the solution

With regard to chromium concentration in *N. officinale*, it was observed to increase with an increase in the concentration of chromium from 0 to 10 mgL^{-1} in the hydroponic medium. Our results were supported by Dirilgen, 1998 who reported an increase in chromium concentration in *L. minor* fronds with an increase in the chromium concentration in the growth medium. Choo et al., 2006 found an increase in the amount of Cr accumulated by the water lily with an increasing metal concentration in the water. Cr (VI) uptake by willows was found to increase linearly with the addition of Cr (VI) (Yu et al. 2007).

Several studies have reported the Cr bioaccumulation by macrophytes in the root being higher than that in the shoot (Zayed and Terry 2003, Manine at al. 2004, Shanker at al. 2005, Paiva et al. 2009). The reason for the poor translocation of Cr from root to shoot is that plants have no specific transport system for Cr^{3+}; plant Cr uptake occurs only through passive transport (apolastic transport) (Zayed and Terry 2003). The enhanced root Cr concentration after the addition of EDTA could be explained by the increase in Cr^{3+} solubility in the solution, which enhanced the mass transfer of Cr^{3+} to the plant root surface. The shoot Cr concentration decrease brought about by the addition of EDTA is possibly due to the large molecular weight of the Cr–EDTA complex which impedes its transport through the roots to the shoots. The inhibition of EDTA on Cd and Cr bioaccumulation in the shoot of *Ipomoea aquatica* was also observed by other researchers (Wang et al. 2008, Chen at al. 2010). Paiva et al. 2009 reported that the root Cr concentrations of *Eichhornia crassipes* was 417 and 1258 $mgkg^{-1}$ at a Cr^{3+} concentrations of 1 and 10 mM, respectively, after 2 d of incubation.

In addition to accelerating the rate of chromium accumulation, the addition of EDTA also had a significant role in inhibiting translocation of chromium from the roots to the shoots. This data is in good agreement with previous results obtained by different authors, and suggest that in the absence of additional factors the majority of the chromium accumulated by *N. officinale* will remain within the root zone of the plants (Zurayk et al. 2001, Saygıdeğer and Doğan 2005). However, these results are in contrast to a recent report by Meighan et al. (2011), who saw that adding EDTA slightly enhanced the cadmium translocation to the shoots in mature dwarf sunflowers. It is generally believed that the metal ion in the soluble phase is responsible for the transport of the metal ion from the solid phase to the plant root.

In this study, The BCF_{root} and BCF_{shoot} were evaluated. Table 1 indicates that the BCF_{root} was extremely high at applied Cr^{3+} concentrations of 1–10 mgL^{-1}. This suggests that the transport of Cr^{3+} to the root surface occurred quickly. However, it has

also been observed that too much EDTA can result in a reduced biomass that overwhelms the advantages of increased translocation and leads to a decrease in the total amount of target metal extracted (Table 1). It is probable that the formation of Cr–EDTA enhanced the mass transfer of Cr^{3+} ions to the root surface. In contrast, the BCF_{shoot} decreased with an increase in EDTA concentrations because the formation of Cr–EDTA may retard the transport of Cr from the root to the shoot (Chen at al 2010).

The high BCF values also indicate that *N. officinale* is a suitable species for phytoextraction of Cr^{3+} from wastewater.

The phytoextraction potential of plants depends not only on metal shoot concentration but also on shoot biomass production. The total amount of extracted Cr^{3+} is given in Figure 4 and was clearly enhanced by the addition of EDTA. This result is not related to an increase in biomass production in the presence of EDTA, but to the stimulatory effect of EDTA on Cr^{3+} translocation. Indeed, both the translocation factor (TF) and bioconcentration factor (BCF) used to evaluate the capacity of plants to absorb and to transport metal from the roots to the shoots, was significantly changed by the addition of EDTA (Table 1). The present work also showed that the observed increase in the amount of Cr^{3+} accumulated by the plants in the presence of EDTA is the consequence of an increased absorption of this metal in the presence of chelating agent. Hence, the calculation of the efficiency of the absorption of chromium by the root showed a significant increase when plants were cultivated in the presence of Cr^{3+} combined to EDTA as compared to plants exposed to Cr^{3+} alone (Fig. 5). In addition, the relationship between the capability of the roots to efficiently absorb Cr^{3+} and the ability of the plant to transport Cr^{3+} towards the shoots shows a negative correlation. It means, this chelator stimulates the Cr^{3+} root-absorption, however, inhibits of transport from root to shoot in the xylem vessels.

Several mechanisms have been reported to be responsible for metal uptake of macrophytes, including adsorption, chelation, ionic exchange, precipitation, and intracellular uptake (Maine at al. 2004, Suñe et al. 2007). It is important to note that the plant, chelator source, and level will make a difference in metal uptake. The macrophytic plants will directly affect the available biomass for storage and translocation as well as resistance and susceptibility to toxicity. The chelator type and source, in conjunction with the medium, will impact bioavailability. It may also affect the selectivity by forming chelatore metal complexes that alters the uptake rate (Turgut et al. 2004).

As a result, the study found an application to increase the phytoremediation potential of *N. officinale* in Cr^{3+}contaminated waters by the use of reducing and chelating compounds.

CONCLUSIONS

It may be concluded from the present study that the concentration of Cr^{3+} for the growth of *N. officinale* is 3 and 10 mgL^{-1} in the hydroponic media and that the dry weight of *N. officinale* plants decreases with increase in the concentration of chromium in the media. Chromium concentration in the plants increases with an increase in the concentration of chromium in the media. When *N. officinale* was introduced to chromium without EDTA, they proved to be poor phytoremediators. As a result, this species is recommended for their high efficiency to remove chromium in the presence of EDTA from waste water.

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