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COMPARISON OF EDTA-ENHANCED PHYTOEXTRACTION STRATEGIES WITH *NASTURTIUM OFFICINALE* (WATERCRESS) ON AN ARTIFICIALLY ARSENIC CONTAMINATED WATER

DIDEM AYDIN[1] AND OMER FARUK COSKUN[2*]

[1]*Erciyes University, Faculty of Sciences, Department of Biology, Kayseri, Turkey*
[2]*Erciyes University, Institute of Science, Kayseri, Turkey*
[*]*Corresponding author's e-mail: ofcoskun1@hotmail.com*

Abstract

This study was conducted to investigate the effect of EDTA on arsenic uptake, bioaccumulation and growth potential of watercress (*Nasturtium officinale*). Watercress was used as a model plant and was grown in artificially contaminated water. A modified Hoagland's nutrient solution without EDTA was used as the hydroponic medium in this study. The growth media was supported with different As concentration and EDTA. EDTA resulted in more solubilization of arsenite (As (III)) in water. Application of EDTA 15 d prior to harvest increased the amount of arsenite accumulated into watercress with more arsenite accumulated by plants from the media. *Nasturtium officinale* accumulated high arsenite concentration (1353 μgkg^{-1}) in the leaves at As (III) of 600 μgL^{-1} and EDTA of 10^{-4} M after 15 d growth. The application of EDTA had inhibitory effects on the root and leaves dry biomass compared with that in the control. This plant can be used as potential species for chelate-assisted As(III) phytoremediation. However, the use of EDTA in the field should be concerned with their leaching problems.

Introduction

The effects of metal toxicity in plants were indicated by shortening the length of roots and shoots (Ozdener & Kutbay, 2011), limiting of the growth of roots and shoots (Shafi *et al*., 2010), dying of the leaves (Bavi *et al*., 2011). Arsenic is one of the widespread toxic environmental pollutants which have chronic and epidemic effects on humans through water. The phytotoxicity of arsenic depends on its oxidation state. As (III) (Arsenite) and As (V) (arsenate) are inorganic, phytoavailable forms of arsenic and are highly toxic to plants (Mkandawire *et al*., 2004). These inorganic forms are interconvertible, depending on the redox condition of the aquatic ecosystem. As (III) is considered as phytoavailable and the most phytotoxic arsenic species (Mkandawire *et al*., 2004). As (III) is powerful inhibitor of the sulfhydryl groups found in some enzymes and tissue proteins.

Phytoextraction is a technique to remove heavy metals from contaminated soil or water (Achakzai *et al*., 2011). Phytoextraction can be broadly classified as either natural or chemical 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 plant tissue and phytoavailable fraction of metals in rooting medium. In these types of studies, EDTA is still valuable because it allows new results to be compared directly to the broadest segment of the literature. Chelating agents can react with metal ions and influence metal phytotoxicity and phytoextraction (Jean *et al*., 2007; January *et al*., 2008). EDTA is such 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 roots to aboveground 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). Most environmental arsenic problems are the result of mobilization under natural conditions. However, mining activities, combustion of fossil fuels, use of arsenic pesticides, herbicides, and crop desiccants and use of arsenic additives to livestock feed create additional impacts (Mohan *et al*., 2007).

In the present study, *N.officinale* was selected to investigated with the following aims to assess (i) the As(III) tolerance and accumulation ability of *N. officinale,* (ii) the potential of *N.officinale* for EDTA-assisted phytoremediation of As(III) (iii) the influence of As(III) and EDTA supplement on the biomass in *N. officinale.*

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* is used as fodder (Haq *et al*., 2010). The leaves are used as salad and treatment for dyspepsia (Hussain *at al*., 2007). *N. officinale* seedlings were collected in April, 2010 from the Zamantı 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; Saygideger & Dogan, 2005).

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. 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 in this study (Hoagland & Snyder, 1993). Arsenic trioxide (As_2O_3) was used. $Na_2EDTA.2H_2O$ (100% purity) was purchased from Merck. The (As_2O_3) and EDTA were added to the modified Hoagland's nutrient solution to achieve the desired concentrations. For this purpose, growth media supported with (1) 0 μgL^{-1} As(III) (control), (2) 50 μgL^{-1} As(III), (3) 100 μgL^{-1} As(III), (4) 300 μgL^{-1} As(III), (5) 600 μgL^{-1} As(III), (6) 1000 μgL^{-1} As(III), (7) 50 μgL^{-1}

As(III) + 10^{-5} EDTA, (8) 100 μgL^{-1} As(III) + 10^{-5} EDTA, (9) 300 μgL^{-1} As(III) + 10^{-5} EDTA, (10) 600 μgL^{-1} As(III) + 10^{-5} EDTA, (11) 1000 μgL^{-1} As(III) + 10^{-5} EDTA, (12) 50 μgL^{-1} As(III) + 10^{-4} EDTA, (13) 100 μgL^{-1} As(III) + 10^{-4} EDTA, (14) 300 μgL^{-1} As(III) + 10^{-4} EDTA, (15) 600 μgL^{-1} As(III) + 10^{-4} EDTA (16) 1000 μgL^{-1} As(III) + 10^{-4} EDTA. Three 3000 ml plastic cups were used per treatment. Each plastic cup contained 10 seedlings of plants that almost equal morphological characteristics were immersed in modified nutrient solution. The seedlings were incubated in a 16-h light/8-h dark photoperiod at 15°C for 15 d. Distilled water was added every day to maintain water levels. After the 15 d culture period, seedlings were collected, washed with distilled water, and dried with tissues.

The root and leaf parts were dried at 80°C for 2 d, and the weight of dried root and leaf was measured. Dried samples of *N. officinale* were digested with 10 ml of concentrated HNO_3, using a CEM microwave digestion system (Demirezen Yilmaz, 2007). After digestion, the volume of each sample was adjusted to 25 ml using double deionized water. Determinations of the arsenic concentrations in all samples were carried out by ICP-MS. The samples were analyzed in triplicate. The amount of chlorophyll was determined according to the method described by Knudson *et al*., (1977).

Phytoextraction ability: The phytoextraction ability of *N.officinale* plants was assessed using both the bioaccumulation factor (BF) and the translocation factor (TF). The bioaccumulation factor (BF) was calculated as follows (Rahmani & Sternberg, 1999):

$$BF = Conc_{As(III)\ in\ leaves} / Conc_{As\ (III)\ in\ solution}$$

where $Conc_{As\ (III)\ in\ plant}$ is the AS(III) concentration in the plant (μgkg^{-1}) and $Conc_{As\ (III)\ in\ solution}$ is the As(III) concentration in the solution (μgL^{-1});

The translocation factor (TF) gives the leaves/root As (III) concentration and depicts the ability of the species to translocate the metal from roots to shoots or leaves (Ghnaya *et al*., 2007).
TF = As (III) in leaves (μgkg^{-1}) / As (III) + in roots (μgkg^{-1}).

Relative growth rate: The relative growth rate (RGR) based on whole plant dry weight production, was calculated according to Hunt (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 days treatment period, and (t2 – t1) is the duration of this period (Hunt, 1990).

Arsenite absorption efficiency: This parameter was calculated as:

As (III) Abs. Eff = (Q As(III) Pa + Q As(III) R)/ DW Roots average,

where, Q As(III) Pa is the total amount of As(III) accumulated in the leaves at final harvest ($\mu g\ plant^{-1}$) Q As(III) R is the total amount of arsenic accumulated in the roots at final harvest ($\mu g\ plant^{-1}$). DW Roots average is the dry weight logarithmic average of the root system.

Statistical analysis: Correlation and regression analysis were 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 As(III) in *N. officinale*

Arsenite (As(III)) biotoxicity: The effects of As(III) and As(III) plus EDTA treatment on biomass of *N. officinale*, as described by dry weight of plant, were summarized in Fig. 1 (a and b). With the addition of higher EDTA concentration, the dry weight of *N. officinalae* was decreased. When 10^{-4} mM EDTA was supplied on 1000 μgL^{-1} As(III) treated media, the dry weight was reduced by 74% in leaves and 72% in roots of *N. officinale* compared to the plants grown in 1000 μgL^{-1} As(III) treatment alone. Nevertheless, in comparison with controls, the leaves and root dry weight of *N. officinale* increased by 5.5% and 3.7%, respectively, when 300 μgL^{-1} As(III) plus 10^{-4} mM EDTA was supplied. The higher decline of leaves dry weight in this species with the addition of EDTA indicates that leaves are more sensitive than roots to As(III).

The relative growth rate (RGR) given in Fig. 2 also demonstrated that the presence of As(III) in the medium significantly reduced growth activity during treatments. In general, the application of EDTA resulted in the decrease of dry biomass production and RGRs of *N. officinale*. Moreover, visual toxicity symptoms (necrosis on older leaves, not given data) from As(III) or EDTA were observed in plants grown in media supplied with EDTA. Additionally, the significant decrease was observed in the chlorophyll content at application of As(III) without EDTA ($p<0.05$). The most and the least affected groups were those treated with 1000 μgL^{-1} and 50 μgL^{-1} As(III) (Fig. 3).

Arsenate (As(III)) bioaccumulation: In plants cultivated in the presence of As(III) alone or in combination with EDTA, the pollutant mainly accumulated in the leaves. As(III) concentration in the root and leaves parts increased with an increase in aqueous As(III) (Figs. 4a & d). High As(III) concentrations in the root (536 μgkg^{-1}) were observed at solution As(III) concentration of 600 μgL^{-1} (Fig. 4a); however, the As(III) concentrations in the leaves were much higher than those in the root without EDTA (Fig. 4b). Fig. 4c and d also shows that when the solution As(III) concentration was 600 μgL^{-1}, the addition of EDTA enhanced the root and leaves As(III) bioaccumulation in the plant.

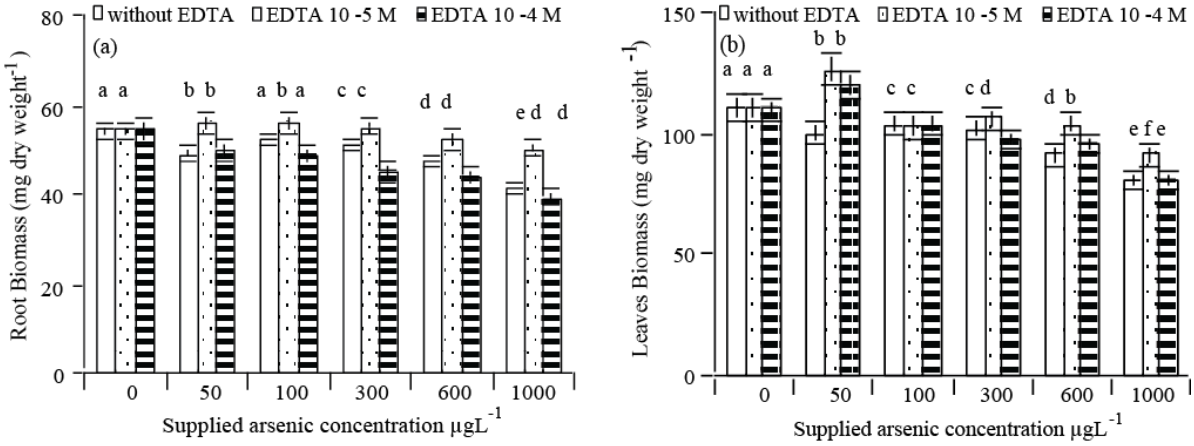

Fig. 1. Effect of As(III) (a) and As+EDTA (b) on dry biomass of roots and leaves in *N.officinale* at different concentrations. Values are means of 3 replicates and vertical bars are standard errors. Means marked with the same letter are not significantly different at $p<0.05$.

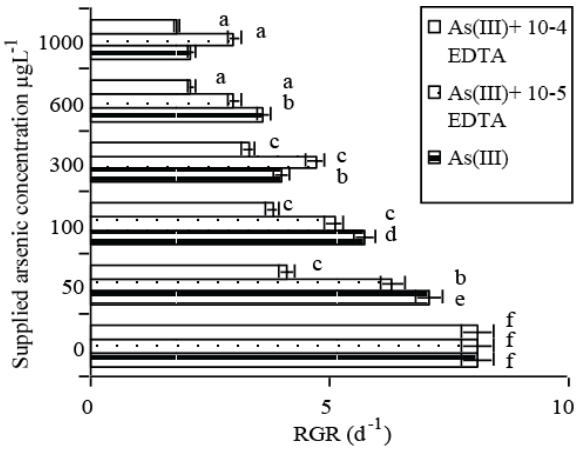

Fig. 2. Changes in the relative growth rate in *N.officinale* plants submitted to different arsenic treatments. Values are means of 3 replicates and vertical bars are standard errors. Means marked with the same letter are not significantly different at $p<0.05$.

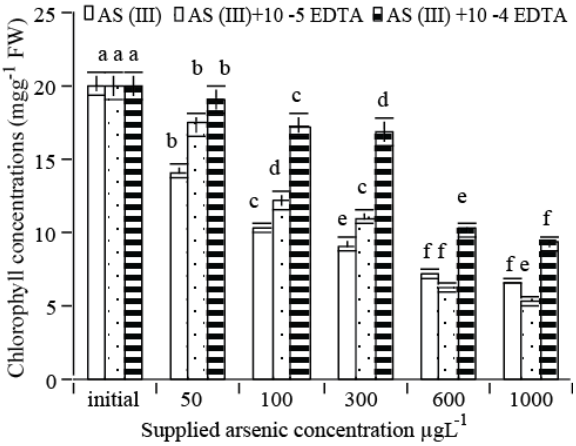

Fig. 3. The total chlorophyll in *N.officinale* under As(III) and As(III)+ EDTA treatment. Values are means of 3 replicates and vertical bars are standard errors. Means marked with the same letter are not significantly different at $p<0.05$.

With the addition of EDTA, the bioaccumulation of As(III) in *N. officinalae* was increased. When 10^{-4} mM EDTA was supplied on 600 μgL^{-1} As(III) treated media, the concentration was increased by % in leaves and % in roots of *N. officinale* compared to the plants grown in 600 μgL^{-1} As(III) treatment alone. Nevertheless, with the addition of higher EDTA concentration (1000 μgL^{-1} As(III)), the bioaccumulation in different parts of this plant was decreased.

BF_{leaves} increased with increased As(III) concentrations with EDTA application (Fig. 5a). Indeed, both the bioaccumulation factor (BF) and translocation factor (TF) used to evaluate the capacity of plants to absorb and to transport metal from the roots to the leaves, were significantly increased by the addition of EDTA (Figs. 5a & b). The present work also showed that the observed increase in the amount of As(III) accumulated by the plants in the presence of EDTA was 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 arsenate by the leaves showed a significant increase when plants were cultivated in the presence of As(III) combined to EDTA as compared to plants exposed to As(III) alone (Fig. 6).

Discussion

In this study it is demonstrated that the presence of As(III) has a significant impact on the relative growth rate. A significant decrease in the dry weight of *N. officinale* was obtained with increase in the concentration of As(III) from 0 to 1000 μgL^{-1} in the absence of EDTA in hydroponic medium. The biomass of this species increased after low level of As(III) treatment with the supplement of EDTA, which is in accordance with studies conducted by John *et al*., (2008). They reported that the fresh weight of *Lemna polyrrhiza* was enhanced with addition of low lead concentration as lead acetate, indicating that lead can stimulate plant growth to some extent. It might be related to the stimulated synthesis of cell wall polysaccharides (Wierzbicka, 1998; Islam *et al*., 2007).

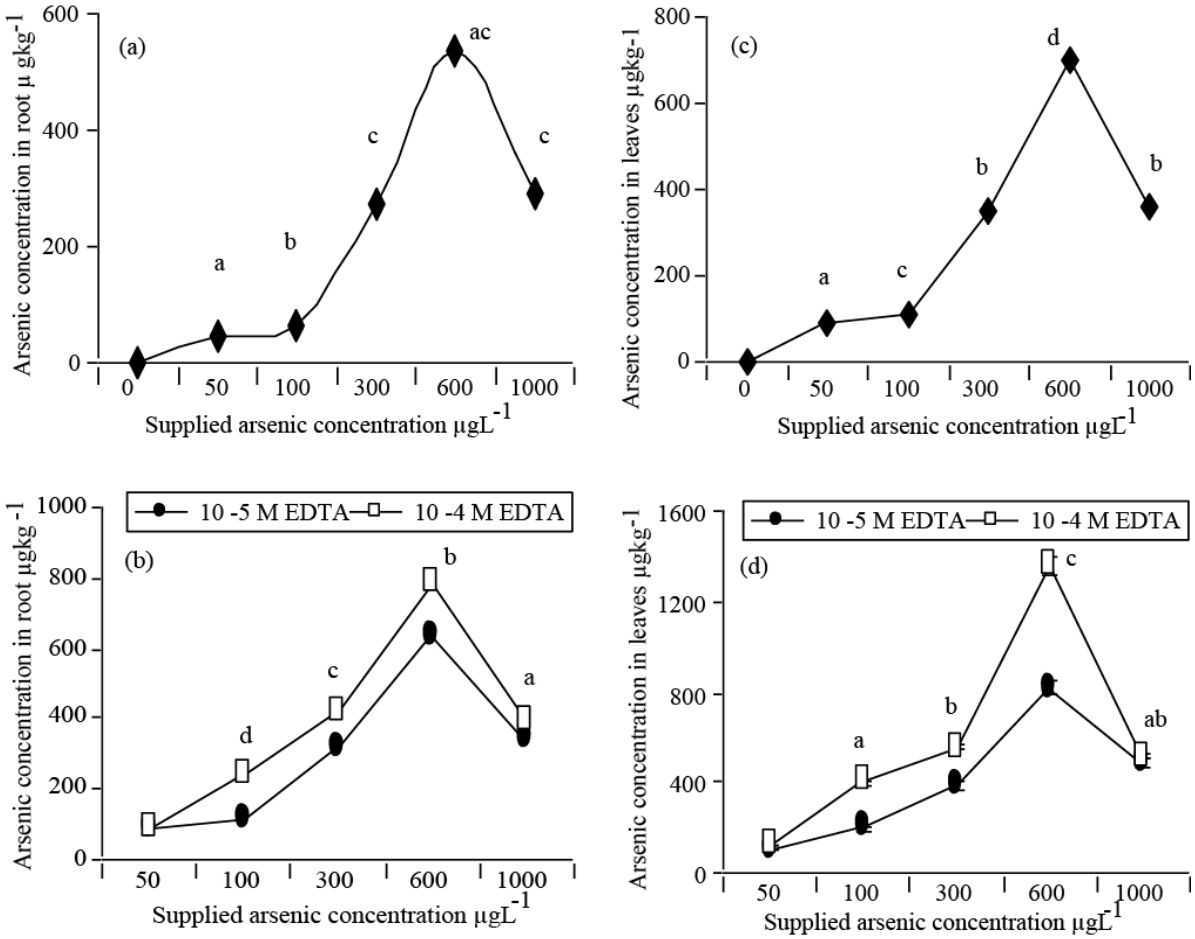

Fig. 4. Arsenic bioaccumulation in root and leaves of *N.officinale* under As(III) (a,c) and As(III)+ EDTA (b,d) treatment. Values are means of 3 replicates and vertical bars are standard errors. Means marked with the same letter are not significantly different at $p<0.05$.

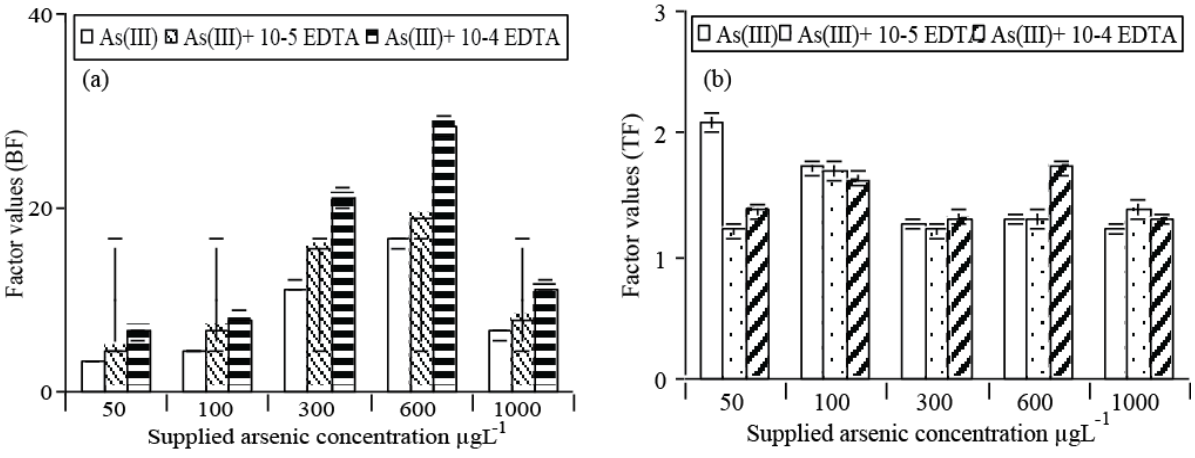

Fig. 5. Bioaccumulation (BF) (a) and Transfer Factor (TF) (b) of As(III) in *N.officinale* grown in As(III) and As(III)- EDTA application after 15 d.

Similarly Bala & Thukral (2008) observed that dry matter yield of *S. polyrrhiza* was decreased as the application rate of chromium (VI) was increased. Additionally, the dry weight of the entire *Miscanthus* plant was 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 decrease in dry biomass in rice seedlings treated with chromium for 24 or 48 h.

In the present study, maximum increase of root and leaves biomass was obtained in the binary combination of 50 μgL^{-1} As(III) and 10^{-4} EDTA in hydroponic medium. However, in the binary combination of 1000 μgL^{-1} As(III) and 10^{-4} EDTA, maximum decrease of 26% in leaves dry weight was observed. The decrease in the biomass production of *N. oficinale* after the application of 10^{-4} M EDTA was due to the combined toxicity of As(III) and EDTA in hydroponic medium. The effects of chelators were dose dependent. The application of chelators alone resulted in the removal of essential metal nutrients from media, leading to deficiencies in the plants (Ruley *et al*., 2006). Grcman *et al*., (2003) found that in all chelate treatments including EDTA, necrotic lesions were observed on cabbage leaves and were more prominent on the older leaves.

It is also demonstrated in this study that the presence of EDTA has a significant impact on the rate of As(III) accumulation in *Nasturtium officinale* (Fig. 4). The earlier reports in literature also supported these findings. According to Chen *et al*., (2010) EDTA-promoted uptake of Cr in *Ipomonea aquatica* was observed. In our data, As(III) uptake was much lower when EDTA was absent even though the dissolved, unchelated heavy metal levels in solution.

With regard to As(III) concentration in *N. officinale*, it was observed to increase with increase in the concentration of As(III) from 0 to 600 μgL^{-1} in hydroponic medium. Our results supported by Mirza *et al*., (2010) who reported an increase in arsenic concentration in *Arundo donax* with increase in arsenic concentration in growth medium. Choo *et al*., (2006) found an increase in the amount of Cr accumulated by water lily with increasing metal concentration in water. Cr (VI) uptake by willows was found to increase linearly with the added Cr(VI) by Yu *et al*., (2007).

In addition to accelerating the rate of As(III) accumulation, the addition of EDTA also had a significant role in stimulating translocation of As(III) from the roots to the leaves. These data are in good agreement with previous results obtained by different authors. For example, according to Meighan *et al*., (2011) EDTA slightly enhanced cadmium translocation to the shoots in mature dwarf sunflowers. Similarly, some researchers suggest that in the absence of additional factors the majority of the heavy metals accumulated by *N. officinale* will remain within the root zone of the plants (Zurayk *et al*., 2001; Saygideger & Dogan, 2005). It is generally believed that metal ion in soluble phase is responsible for transport of metal ion from solid phase to plant root.

In this study, The BF_{leaves} were evaluated. The BF_{leaves}, was extremely high at applied As(III) concentrations of 300-600 μgL^{-1} (Fig. 5a). This suggests that transport of As(III) to leaves occurred fast. 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 (Fig. 5b). In contrast, BF_{leaves} decreased with an increase in As(III) and EDTA concentrations because the formation of As–EDTA may retard the transport of As(III) from root to leaves. Similar results obtained by Chen *et al*., (2010) with chromium-EDTA application. The high BF values also indicate that *N.officinale* is a suitable species for phytoextraction of As(III) from wastewater.

The phytoextraction potential of plant depends not only on metal aboveground organ's concentration but also on biomass production of these organs. The total amount of extracted As(III) is given in Fig. 6 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 As(III) translocation. Indeed, both the translocation factor (TF) and bioaccumulation factor (BF) used to evaluate the capacity of plants to absorb and to transport metal from the roots to the shoots or leaves were significantly changed by the addition of EDTA (Fig. 5). The present work also showed that the observed increase in the amount of As(III) 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 As(III) by the root showed a significant increase when plants were cultivated in the presence of As(III) combined to EDTA as compared to plants exposed to As(III) alone (Fig. 6). In addition, the relationship between the capability of the roots to efficiently absorb As(III) and the ability of the plant to transport As(III) towards the leaves shows a positive correlation. It means, this chelator stimulates the As(III) root-absorption and stimulates of transport from root to leaves in the xylem vessels.

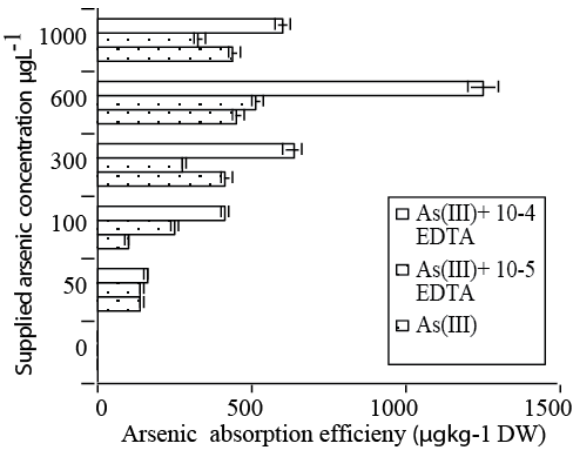

Fig. 6. Arsenic removal efficiency (%) in *N.officinale* plants maintained for 15 days on a solution under As(III) and As(III)+ EDTA treatment. Error bar represents standard deviation.

Several mechanisms have been reported to be responsible for metal uptake of macrophytes, including adsorption, chelation, ionic exchange, precipitation, and intracellular uptake (Maine *et al*., 2004; Sune *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 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 thus finds application to increase the phytoremediation potential of *N. officinale* in As(III) contaminated waters by the use of reducing and chelating compounds.

Conclusions

The results of the present study indicate that *N. officinale* have a tolerance to Arsenic. In the presence of EDTA, As(III) accumulation in leaves was promoted significantly and the highest As(III) concentration was observed in leaves. Therefore, EDTA-assisted As(III) phytoextraction of *N. officinale* could be further enhanced by adjusting application methods. As a result, this species is recommended for their high efficiency to remove arsenic presence of EDTA from waste water.

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