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Research Article

Bioremediation of Cadmium and Nickel from a Saline Aquatic Environment Using *Ceratophyllum demersum*

Amir Parnian*[a], Mostafa Chorom [b], Neemat Jaafarzadeh [c], Hadi Pirasteh Anosheh [a], Munir Ozturk*[d], Dilek Unal [e], Dilek Demirezen Yilmaz [f] and Volkan Altay [g]

[a] National Salinity Research Center, Agricultural Research, Education and Extension Organization, Yazd 8917917451, Iran.
[b] Faculty of Agriculture, Department of Soil Science, Shahid Chamran University, Ahvaz, Iran.
[c] Environmental Technologies Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran and Department of Environmental Health Engineering, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[d] Botany Department, Science Faculty, Ege University, Izmir 35040, Turkey.
[e] Department of Molecular Biology and Genetics, Faculty of Science and Arts, Bilecik Seyh Edebali University, Bilecik 11600, Turkey.
[f] Biology Department, Faculty of Science and Arts, Erciyes University, Kayseri 38039, Turkey.
[g] Biology Department, Faculty of Science and Arts, Hatay Mustafa Kemal University, Hatay 31060, Turkey.

*Author for correspondence; e-mail: amir.parnian86@gmail.com, munirozturk@gmail.com

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ABSTRACT

In this study, effect of salinity on growth was examined together with cadmium (Cd) and nickel (Ni) accumulation capacity of coontail a free-floating hydrophyte (*Ceratophyllum demersum* L.) under controlled conditions. Different saline waters (EC = 2.8, 5.5 and 9.5 dS m^{-1}) were made with the base of farm drainage water (EC = 13.2 dS m^{-1}). A total of four concentrations of $Ni(NO_3)_2$ and $CdCl_2$ (0, 1, 2, 4 mg L^{-1}) were added to these. The results showed that there was a decrease in the growth rate as the water salinity level increased. The biomass production was also inhibited with the increase in salinity. At 9.5 dS m^{-1} salinity level growth rate was the lowest (0.81 ± 0.05 g FW d^{-1}) among the treatments. This decrease was accelerated by metal contaminations. High salinity levels decreased the removal of nickel. Highest removal was recorded as R= 86% (Ni = 1 mg L^{-1}, EC = 5.5 dS m^{-1}). The lowest cadmium removal was observed as R = 35% (Cd = 4 mg L^{-1}, EC = 2.8 dS m^{-1}) in the lowest salinity medium. In general, phytoremediation efficiency of coontail decreased with enhancing nickel concentrations, but increased when cadmium concentration increased. An increase in the salinity levels of water lead towards a parallel increase in the removal efficiency of coontail. It was concluded that this floating hydrophyte has a good potential for phytoremediation of cadmium and nickel from a saline aquatic environment.

Keywords: coontail, salinity, cadmium, nickel, phytoremediation

1. INTRODUCTION

Several toxic elements are contaminating our environment [1-3]. The aquatic ecosystems get an equal share from such contaminations, often creating unacceptable conditions for the biota living in there [4-6]. Submerged macrophytes distributed in our freshwater ecosystems are highly important for maintaining water quality as well as ecological functions of such ecosystems. There is a global progressive loss of submerged aquatic vegetation due to climate change, eutrophication and land reclamation [7]. Heavy metal pollution originating from different sources is also an important environmental problem in such ecosystems [8-12]. Different kinds of industries release heavy metals like cadmium (Cd) and nickel (Ni) in wastewaters in various concentrations, which prove highly toxic for human health [13]. After entering our aquatic ecosystems, bio-accumulation of heavy metals in different organisms varies in the food chain and different organisms show different tolerance levels [14].

The nickel and cadmium are released as divalent cations into the environment from different industrial sources, showing high mobility and bioavailability except at high pH values [15]. There is a great concern about cadmium contamination in the environment, because of its high toxicity to animals and humans. Any accumulation in plants may not show any toxicity however, their consumption by the other living beings may in turn show toxicity in the consuming organisms. In fact, cadmium contamination affects humans more than animals, because we are at the top of food chain [16]. Generally, high levels of cadmium in plants lead towards a reduction in photosynthesis, water uptake, and to some extent nutrient uptake as well [13, 17, 18]. The nickel is an essential trace element for our ecosystems in general [19]. However, If its critical levels exceed, it may lead to serious health disorders such as; lung and kidney problems in addition to the gastrointestinal disturbances, pulmonary fibrosis and skin dermatitis, also known as human carcinogen [20-22]. It has been reported that, Ni^{2+}-treated plants suffer from membrane functionality and ion balance in the cytoplasm, particularly K^+, the most mobile ion across the plant cell membrane gets affected and ends up with changes in water balance [23].

The metal bioaccumulation depends upon such biotic and abiotic factors as; temperature, pH, salinity, alkalinity, water stress and dissolved ions in water [14, 24]. However, salinity has a notable affect on germination and growth. Uptill now the relationship between heavy metal accumulation in salinity-stressed plants has not been investigated to a large extent [14].

Hydrophytes are known as good heavy metal accumulators, therefore accepted as highly valuable for phytoremediation [25, 26]. The aquatic plant, coontail (*Ceratophyllum demersum* L.), from the family Ceratophyllaceae is a cosmopolitan, submerged, rootless, free floating perennial hydrophyte. It is evaluated as an oxygenator in closed aquatic systems, showing very high vegetative propagation and biomass production even under modest nutritional conditions [27, 28]. This plant can serve as a good biofilter for heavy metals, such as cadmium [29-31] and nickel [32, 33]. Our aim in this study was to determine the effects of salinity on growth and accumulation capacity of cadmium and nickel in *C. demersum*.

2. MATERIALS AND METHODS

2.1 Experimental Studies

The experiment was conducted under the following experimental conditions: mean daily temperature of 24 ºC, mean daily relative humidity of 55%, daily maximum global radiation ranging between 650-1100 W m^{-2} and direct radiation ranging between 400-1050 W m^{-2}.

C. demersum plants were collected from the irrigation dike and they were grown outdoors in 30-liter plastic containers filled with half-strength Hoagland's nutrient solution [34]. The plants grow better and metal accumulation for 4 weeks prior to the start of experiment. The nutrient solution with pH 7.0 was changed every 3 days, and preliminary tests for pH were carried out to determine the best pH range at which the test plants grow and metal accumulation can occur [35].

Eight-day batch experiments were carried out to evaluate the removal of cadmium and nickel by the plants from saline waters. The nickel nitrate [$Ni(NO_3)_2 \bullet 6H_2O$], cadmium chloride ($CdCl_2.4H_2O$), distilled water and agricultural drainage water (EC = 13.2 dS m^{-1}) were used without further purification for adjusting the treatments. Preliminary tests for nickel and cadmium concentrations, as well as salinity ranges were performed to determine the appropriate sensitivity range for the main experiment. The plants were cultivated in 36 receptacles containing 2.5 liter solutions with three different saline water levels (2.8, 5.5 and 9 dS m^{-1}, prepared by adding agricultural drainage water to the distilled water). These were contaminated by four different cadmium and nickel concentrations (0.00, 1.00, 2.00 and 4.00 mg L^{-1}). In all three replicates were set, three further receptacles without *C. demersum* were used as controls for check of adding contaminants.

During cultivation period, pH of the solutions was adjusted at 7.0 by using 0.01 M NaOH and 0.01 M HCl every day. The evaporated volume of water was replaced with distilled water [36]. After daily water sampling starting from the 8th day, the experiment was terminated. The plants were taken out, washed three times with tap water, fresh and dry weights (24 h at 70 °C) were recorded.

Plant biomass production Pr (g FW d^{-1}) was calculated as follows [37]:

$$Pr = (FW2 - FW1)/\Delta t, \qquad (1)$$

Where FW1 and FW2 were fresh weights (g) at time 1 and time 2, respectively, and Δt the difference between time 1 and time 2 (days).

2.2 Heavy Metal Measurements

The sampled waters were analysed by using "Atomic Absorption Spectrophotometer" (Perkin Elmer 3100), following the standard methods for the analysis of water and wastewater [38]. Metal removal was determined by quantifying the concentration of the leftover in the medium after incubation with plants. 2-ml water samples were taken daily and the removal of metals was then calculated using the following equation [39]:

$$R\ (\%) = [(C0 - Ct)/\ C0] \times 100 \qquad (2)$$

where C0 and Ct represent the residual concentration of metal at the beginning of the experiment and at time t, respectively.

2.3 Statistical Analyses

Data was subjected to analysis of variance (ANOVA) and the means were separated using Duncan`s multiple range test at 5% probability level. Pearson's correlation was also performed using statistical programme. Statistical analyses was performed with the software SPSS 16 and MS-Excel 2013.

3. RESULTS AND DISCUSSION

Aquatic plants could be used for aquatic environment rehabilitation, based on their removal efficiencies and endurance to the toxicity levels [40]. Many aquatic plants have been used in remediation studies [29, 35, 37, 41]. Earlier findings have reported that *C. demersum* has a good capacity for removing heavy metals [29]. Keeping this in view, we also investigated the remediation capacity of *C. demersum* using the heavy metals nickel and cadmium, but under different salinity conditions. The results showed that the highest removal efficiency of nickel was at treatments with Ni = 1 mg L^{-1} initial concentration, under different salinities used (Table 1). The nickel removal percentages in our test plant increased with an increase in salinity and decreased with an increase in initial nickel concentration. The treatments with the salinity of 9.5 dS m^{-1} have shown (Table 1) a lower nickel removal efficiency than with salinity of 5.5 dS m^{-1}. *C. demersum* is affected by the water salinity [42], the trend observed here might be since we see lower biomass production in the plant, due to the high salinity effect on growth (Table 2). When heavy

Table 1. Nickel (Ni) and Cadmium (Cd) removal efficiency (%) by *Ceratophyllum demersum* under different water salinity levels and Ni and Cd concentrations.

Water salinity (dS/m)	**Ni concentration (mg/L)**			**Mean**
	1	**2**	**4**	
2.8	77.0^{aB}	52.0^{bA}	50.0^{bB}	59.7^{C}
5.5	86.0^{aA}	54.5^{cA}	58.3^{bA}	66.3^{A}
9.5	79.0^{aB}	46.0^{cB}	53.0^{bB}	61.8^{B}
Mean	80.7^{a}	50.8^{b}	53.8^{c}	-
Water salinity (dS/m)	**Cd concentrations (mg/L)**			**Mean**
	1	**2**	**4**	
2.8	74.0^{bB}	84.0^{aB}	35.0^{cC}	64.3^{C}
5.5	83.0^{bA}	94.5^{aA}	94.3^{aA}	90.6^{A}
9.5	71.0^{cB}	97.0^{aA}	87.5^{bB}	85.2^{B}
Mean	76.0^{b}	91.8^{a}	72.3^{b}	-

Note: Different lowercase letters in each row and uppercase letters in each column indicate a significant difference at $P < 0.05$.

Table 2. Biomass production (gFW/d) of *Ceratophyllum demersum* under different water salinity levels and nickel (Ni) and cadmium (Cd) concentrations.

Water salinity (dS/m)	**Ni concentrations (mg/L)**				**Mean**
	0	**1**	**2**	**4**	
2.8	2.30^{aA}	1.63^{bA}	1.27^{cA}	1.02^{dA}	1.56
5.5	1.38^{aB}	1.00^{bB}	0.62^{cB}	0.46^{dB}	0.87
9.5	0.81^{aC}	0.52^{bC}	0.50^{bC}	0.35^{cC}	0.22
Mean	1.50^{a}	1.05^{b}	0.80^{c}	0.61^{d}	-
Water salinity (dS/m)	**Cd concentrations (mg/L)**				**Mean**
	0	**1**	**2**	**4**	
2.8	2.30^{aA}	1.84^{bA}	1.49^{cA}	1.32^{cA}	1.74^{A}
5.5	1.38^{aB}	0.80^{bB}	0.48^{cB}	0.45^{cB}	0.78^{B}
9.5	1.01^{aC}	0.46^{bC}	0.38^{cC}	0.15^{dC}	0.50^{C}
Mean	1.56^{a}	1.03^{b}	0.78^{c}	0.64^{d}	-

Note: Different lowercase letters in each row and uppercase letters in each column indicate a significant difference at $P < 0.05$.

Table 3. Pearson's correlation tests the Ni and Cd removing rate and biomass results under different salinity range.

Ni	**1**			**2**			**4**		
	Removing	**Biomass**	**Salinity**	**Removing**	**Biomass**	**Salinity**	**Removing**	**Biomass**	**Salinity**
Salinity	0.1535	−0.9776*	-	−0.5558*	−0.8616*	-	0.1854	−0.8691*	-
Removing	-	−0.3486	0.1535	-	−0.3040	−0.5558*	-	−0.3342	0.1854
Biomass	−0.3486	-	−0.9776*	−0.3040	-	−0.8616*	−0.3342	-	−0.8691*
Cd	**1**			**2**			**4**		
	Removing	**Biomass**	**Salinity**	**Removing**	**Biomass**	**Salinity**	**Removing**	**Biomass**	**Salinity**
Salinity	−0.269	−0.9216*	-	−0.9013*	−0.8318*	-	0.7403*	−0.9066*	-
Removing	-	−0.0966	−0.269	-	−0.9694*	0.9013*	-	−0.9164*	0.7403*
Biomass	−0.0966	-	−0.9216*	−0.9694*	-	−0.8318*	−0.9164*	-	−0.9066*

Note: asterisks denote statistically significant correlation coefficients ($P < 0.05$).

metal concentrations are higher, nickel removal is impaired due to heavy metal toxicity in *C. demersum*, which might be because of the cell membrane dysfunction caused by nickel toxicity [23]. The decrease in heavy metal removal efficiency has also been reported earlier [37].

In the present study, Pearson's correlation results indicated that the rate of salinity was not significantly correlated with nickel removal efficiency (Table 3). However, the biomass production showed strongly negative correlation with salinity rate in aquatic system (Table 3).

Both nickel and cadmium have shown toxic effects on *C. demersum* [31, 43]. The percentages of cadmium removal by *C. demersum* for different initial salinity levels in the aquatic medium are presented in table 1. The results show that the highest removal efficiency of cadmium by *C. demersum* (94%) has been observed in the treatment with initial concentration of cadmium as 2 mg L^{-1} and EC = 9.5 dS m^{-1}. Except for the lowest growth medium salinity EC = 2.8 dS m^{-1}, the *C. demersum* removal efficiency increased with increase in the initial concentration of cadmium. The results in an earlier report have demonstrated that heavy metals may result in malfunctions in cell plasma membrane [44], and this could be the reason for such a of cadmium removal trend. Under toxic cadmium concentration levels, cell plasma membrane disorder is also involved in the irregular cellular membrane selective permeability [45]. The cadmium removal decreases under higher concentrations of salinity at EC = 2.8 dS m^{-1} (Table 1). The reason might be that plant biomass production also decreases. In Table 2, we observe this data and similar results for a reduction in the removal percentages have been stressed by other workers [37, 46].

The accumulation in hydrophytes are mostly detrimental if the heavy metal levels are toxic, since they decrease the plant biomass production [47]. As a highly toxic non-essential heavy metal Cadmium causes negative responses after uptake in *C. demersum*. The same was the case with nickel, which causes some functional damages in high doses in *C. demersum* [37]. The biomass production results for nickel and cadmium contaminated waters are presented in table 2. Mean biomass productions for *C. demersum* in the cultivation period was between 2.30 ± 0.13 (under no heavy metal contamination and lowest EC) and 0.78 ± 0.10 g FW d^{-1} (for 2 mg L^{-1} cadmium contamination and salinity of 5.5 dS m^{-1}) in contaminated aquatic system (Table 1). The data presented in the table 2 reveal that reduction in the plant biomass is a normal reaction to salinity in *C. demersum*, as pointed out by other researches [42, 48, 49]. Nickel also ends up with some negative effects on this plant [50]. The data presented in Table 2 shows that a reduction in growth performance follows an increase in the salinity. Similar findings have been reported for other trace elements [48]. It has been emphasized that the ability of *C. demersum* towards an accumulation of some metals decreases with an increase in the salinity in its growth medium and toxicity also goes down but the plant biomass production goes up.

Our results show that cadmium in lower salinity (EC = 2.8 dS m^{-1}) levels generally has lower toxicity in the case of *C. demersum* (Table 2) and these findings fully agree with those of Parnian et al. [37]. However, the plant biomass production in saline waters (EC = 5.5 and 9.5 dS m^{-1}) was higher for nickel contaminated waters than cadmium. This could be due to the fact that cadmium shows higher bioaccumulation potential under higher salinity than nickel in the case of *C. demersum* (Table 1). The date presented in Table 2 also points out that with an increase in the salinity and cadmium concentration in the aquatic systems, biomass production of *C. demersum* rapidly decreases, because of the combined detrimental effects of salinity and cadmium. Similar reports have been published for some higher plants [51-57]. Pearson's correlation results support these findings fully (Table 3).

The salinity level effect of water appear to be related to biomass production and an efficient removal capacity of *C. demersum* (Table 3). The rate of cadmium remediation is strongly positively correlated with salinity, depending on its concentration (Table 3). The biomass production rate shows strong negative correlation as observed with salinity level of our aquatic system. A negative correlation in the biomass level of *C. demersum* has been recorded with the remediation efficiency of *C. demersum* (Table 3). This demonstrates that the tolerance of *C. demersum* to cadmium and nickel under different salinity level varies. The plant did not grow well under saline conditions, however, it could be useful for cadmium and nickel remediation from contaminated waters.

4. CONCLUSIONS

The cadmium and nickel accumulation capacity and the growth rate of *C. demersum* are significantly affected by the salinity. Plants growing under high salinity conditions produce a significantly lower biomass as compared to the lower salinity levels. The highest removal efficiency for the heavy metals has been recorded to lie around 86% and 97% respectively, as obtained in Ni = 1 mg L^{-1}, EC = 5.5 dS m^{-1} and Cd = 2 mg L^{-1}, EC = 9.5 dS m^{-1}. Our results revealed that any increase in the heavy metal concentration becomes a detrimental factor in cadmium removal, but for nickel the same factor affects the removal percentage due to different effects of nickel and cadmium on *C. demersum*. The effects are visible in the growth performance and the functions of uptake by the cells. We found that nickel is less detrimental in the plant used, but a higher removal efficiency for cadmium is seen under similar condition. *C. demersum* seems to be an appropriate candidate for cadmium and nickel remediation from normal contaminated waters.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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