Chapter 1
Competitive Bioaccumulation by *Ceratophyllum demersum* L.

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Abstract Heavy metals produced during anthropic activities and as a consequence of geological processes affect biological systems via bioaccumulation. Bioaccumulation is a process within phytoremediation. Specific classes of elements are bioaccumulated relative to other element classes and their concentrations. The aim of this chapter is to detail an investigation of the interaction and simultaneous absorption of two heavy metals by the aquatic plant *Ceratophyllum demersum* L.

Plants were cultivated for 8 days in nutrient solutions enriched with incrementally increasing concentrations of cadmium (Cd) and nickel (Ni). To examine the preferred/competitive absorption of these metals by plants, Ni and Cd concentrations were measured in initial, final growth solutions and in the plant matter. The highest absorption percentages were observed in treatments containing Cd 4 mg l^{-1} and Ni 1 mg l^{-1}, being 86.5% and 79.0%, respectively.

Increase of the heavy metals concentration correlates with plant absorption of the pollutants but was limited by toxicity effects. The performance of *C. demersum* was sufficient for simultaneous removal of Cd and Ni. Competitive bioaccumulation via

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J. N. Furze et al. (eds.), *Earth Systems Protection and Sustainability*,
https://doi.org/10.1007/978-3-030-98584-4_1

phytoremediation and its effective absorption dynamic offers great hope for remediation of aquatic and semi-aquatic ecosystems as well as restorative potential for land subjected to industrial activity or intensive agriculture.

Keywords *Ceratophyllum demersum* L. · Competitive bioaccumulation · Phytoremediation · Cadmium (Cd) · Nickel (Ni)

1.1 Introduction

Heavy metals are elements with a specific gravity of 5.0 $g.cm^{-3}$ or more and an atomic weight ranging from 63.5 to 200.6 $g.g^{-1}.mol^{-1}$ (Srivastava and Majumder 2008). Among these metals nickel (Ni) and cadmium (Cd) are released as divalent cations into the environment through human activities including metal finishing, automotive, electroplating, battery production, tannery, electric cable production, mining, steel, and textile industries. Different concentrations of metals consequently accumulate in wastewaters through runoff. Unless constrained by high pH values, these cations are highly mobile and bioavailable (Bonfranceschi et al. 2009). Heavy metals are toxic to the aquatic ecosystem and flow freely to water bodies, providing direct risks and danger to human and ecosystem health. In water bodies, several metals exist at toxic levels, resulting in undesirable living conditions and severe trophic consequences (Saygıdeğer and Doğan 2004). Heavy metals accumulate in organisms in tolerance levels (Sawidis et al. 2001; Demirezen et al. 2007).

The residual and persistent presence of Cd poses great environmental concerns due to its high toxicity to animals and humans. Cd is readily bioaccumulated by plants in levels that are not harmful to them though are toxic to the animals consuming those plants. In turn, humans are more sensitive to Cd toxicity, due to the metals' cumulative concentration and concentrated accumulation in internal organs following consumption of Cd-contaminated foods (Tudoreanu and Phillips 2004; Kirkham 2006). Toxic levels of Cd decrease water uptake, photosynthetic processes, and nutrient uptake in plants (Wojcik and Tukiendorf 2004; Mohanpuria et al. 2007).

Ni is essential for plant and ecosystem survival (Chorom et al. 2012). In plants it is required in small but critical concentrations. However, Ni causes serious kidney, lung, stomach, and skin problems in humans and animals and is a known carcinogen (Fu and Wang 2011). Ni toxicity in plants disrupts membrane functionality, effects ionic imbalance (of K^+) in the cytoplasm, and alters internal water relations (Pandey and Sharma 2002).

Phytoremediation is the use of plants to remediate contaminated environments. The biological method remediates air, soil, and aquatic environments (Pivetz 2001). Phytoremediation is promising, low cost, and environmentally sustainable and may be used in the remediation of contaminated water containing trace elements (Chorom et al. 2012; Parnian et al. 2016).

Plants have two physically differentiated means by which they remediate contaminants, foliar surfaces and root systems, enabling versatility depending on which area has contact with the contamination (Demirezen et al. 2007; Sawidis et al. 2001). The phytoremediation active surface area is the plant's organ/part that is in direct contact with pollutants and plays a major role in the remediation process (Dhir 2013). In aquatic medium phytoremediation primary interfaces are commonly the roots or shoots (Parnian and Furze 2021; Thomas et al. 2016; Upadhyay et al. 2014). The higher active surface area of a plant effects the remediation performance of it, and is further supported by additional absorption uptake interfaces; accompanied by activities of suitable microorganisms (Pivetz 2001). Phytoremediation encompasses various mechanisms, the most successful being phytostabilization, rhizodegradation, rhizofiltration, phytodegradation, phytoextraction, phytoaccumulation, and phytovolatilization (Jeevantham et al. 2019).

Phytoremediation technology has been applied in the cleanup of aquatic environments including groundwater (Martino et al. 2019), seawater (Bastos et al. 2019), drainage (Parnian et al. 2017), and wastewater (Panja et al. 2020). There are numerous (originally aquatic) plants with a capacity for aquatic environment remediation (Ansari et al. 2020), some of which have migrated to terrestrial areas (Darajeh et al. 2019). All plants have restorative functions in ecosystems. Furthermore, phytoremediation approaches seek to make use of the most effective plants as candidates for different settings of environmental cleanup. The choice of species used in phytoremediation of contaminated environments is dependent on the following criteria: a high ability to absorb contaminants, a high biomass production, and a high ability of transferring contaminants from absorbing surfaces to "removable" parts. Optimistically, endemic or noninvasive plant species should be used, having a capacity for rapid adaptation to local climatic conditions, thus reducing economical costs and environmental impacts of the remediation.

Ceratophyllum demersum L. normally grows with its stem buried in sandy or silty substrates and is prone to dislodgement (Global Invasive Species Database 2022). It is a submerged, rootless, free-floating plant which achieves stable, cosmopolitan distribution (Szabó et al. 2022). It belongs to the order Nymphaeales, family Ceratophyllaceae (hornworts). Its growth proliferates in shallow, muddy, and quiescent water bodies at low light intensities (Aravind and Prasad 2005). *C. demersum* is known for its use in heavy metal remediation, including that of Cd (Polechońska and Klink 2021), Pb (Mahmoud et al. 2018), and Ni (Polechońska et al. 2018).

C. demersum remediates aquatic mediums through the following mechanisms:

(i) Phytoextraction: The uptake of a contaminant from contaminated environments by an absorbing organ/part of a plant and its translocation into harvestable/removable organs/parts. *C. demersum* does not possess a relatively large active root, though uptakes elements with its entire body. The physiology of the species enables its use in highly effective bioaccumulation of contaminants, and may be removed using biological control and mechanical methods following remediation (Jacobs and Best 1990; Engel 1990; Verma and Charudattan 1993; Wells et al. 2003). Further, although *C. demersum* is considered highly invasive, invasion of ecosystems can be moderated ecologically; complementary, selection effects amongst biodiversity and similar functional groups being present where it is introduced have been seen to be effective (Petruzella et al. 2018). *C. demersum* is effective in heavy metal removal (Parnian et al. 2016).

(ii) Rhizodegradation: A special kind of surface degradation, contaminant degradation occurs in the contaminated environment, commonly in the rhizospheric areas. In most submerged aquatic plants, the entire surface of the plant's body is considered as a special surface; microorganisms are also involved in the degradation process. *C. demersum* effectively reduces chemical oxygen demand (COD) of water bodies (Luo et al. 2019).

(iii) Rhizofiltration or phytofiltration: Contaminant absorption from polluted environments. In the process contaminants are adhered onto the plant active area and then filtered through the plant. The technique is used in hydroponic growth of plants, though *C. demersum* commonly remediates waters of suspended solids, thereby reducing water turbidity (Beheary et al. 2019).

(iv) Phytodegradation: The process of contaminant degradation by plants as a result of the plant metabolism. Nitrate removal and remediation of contaminated water bodies using *C. demersum* exemplify the degradation process (Xu et al. 2019).

Hydrophyte uptake and accumulation of elements occur through the foliar surface and/or the root system (Shi et al. 2021). Moreover, hydrophytes' uptake of metals is dependent on biotic and abiotic factors such as temperature, pH, salinity, and the presence of dissolved ions in water (Ebrahimbabaie et al. 2020). In many regions, metals appear in combination together. It has become clear that toxic trace elements affect seed germination and plant growth, though the relationships affecting heavy metal accumulation in plants remain poorly understood. In this study, *C. demersum* is the chosen species as it accumulates high concentrations of trace elements (Khan et al. 2009). *C. demersum* has a high capacity for vegetative propagation and biomass production even under modest nutritional conditions. It is useful as an oxygenator and has found use in closed equilibrated biological aquatic systems (CEBAS). *C. demersum* can be a biofilter for heavy metals.

The objective of this chapter is to show the competitive bioaccumulation of heavy metals (Cd and Ni) by the free-floating hydrophyte *C. demersum* L.

1.2 Climatic Conditions of the Competitive Bioaccumulation Experimental Site

The experimental study was conducted between November 2009 and February 2010 at the College of Agriculture, Shahid Chamran University (SCU), in the city of Ahvaz in Iran (31° 18′ 12.2″ N, 48° 39′ 29.6″ E). Climatic conditions incuded a mean daily temperature of 24 ± 5 ° C, mean daily relative humidity of 55 ± 25%, daily maximum global radiation between 650 and 1100 Wm^{-2}, and direct radiation between 400 and 1050 Wm^{-2}, according to the university meteorological center (SCU Meteorological Data Collection Center 2010).

1.3 Maintenance of Stock Cultures of *Ceratophyllum demersum* L.

Hornwort cedar moss (*C. demersum* L.) is commonly available in Ahvaz County, Iran. Cultures of *C. demersum* L. were obtained from an irrigation dyke of Shahid Chamran University shown in Fig. 1.1. Five kilograms of plants were collected by hand during an irrigation cycle as shown in Fig. 1.2. Irrigation water properties are given in Table 1.1. Plants were placed outdoors in 30 L plastic containers shown in Fig. 1.3, filled with half-strength Hoagland-Arnon nutrient solution (Marin and Oron 2007); the composition used is given in Table 1.2.

Plants were cultivated for 4 weeks prior to the beginning of the experiment. The nutrient solution was stabilized at a pH equal to 7.0 and was replenished every 3 days. Preliminary tests for pH were performed to determine the optimum pH range for plant growth and metal accumulation.

1.4 Experimental Setup and Heavy Metal Analysis

Eight-day batch experiments were conducted to evaluate Cd and Ni removal by *C. demersum*. In this study, nickel nitrate ($Ni(NO_3)2{\cdot}6H_2O$) and cadmium chloride ($CdCl_2.4H_2O$) (Merck) were used in treatments. The experiments were performed

Fig. 1.1 *C. demersum* L. collection from irrigation dyke of Shahid Chamran University, Ahvaz, Khuzestan Province, Iran, 2010

Fig. 1.2 Dry irrigation dyke, Shahid Chamran University, Ahvaz, Khuzestan Province, Iran, 2010

Table 1.1 Irrigation water properties, Shahid Chamran University, Ahvaz, Khuzestan Province, Iran, 2010

Water properties	Turbidity	EC	pH	HCO_3^-	TDS	NO_3^-	COD	BOD	DO	TSS
Unit	NTU	dSm^{-1}	–	$mEql^{-1}$	$mg\ l^{-1}$					
Concentration	5	2.69	7.7	3.12	1722	7.58	13.2	3.58	6.4	60

EC electrical conductivity, HCO_3^- bicarbonate, *TDS* total dissolvable solids, NO_3^- nitrate, *COD* chemical oxygen demand, *BOD* biological oxygen demand, *DO* dissolved oxygen, *TSS* total suspended solids, *NTU* nephelometric turbidity units, dSm^{-1} deciSiemens per meter, $mEql^{-1}$ milliequivalents of solute per liter of solution, $mg\ l^{-1}$ milligrams per liter

outdoors in 3 L plastic receptacles with a surface area of 432 cm^2. A total of 16 different treatments were tested in half-strength Hoagland-Arnon nutrient solution. The selected Cd and Ni concentrations are shown in Table 1.3.

Table 1.3 shows the increasing concentrations of heavy metals investigated in the batch experiments.

Three replicates were made for each treatment; three receptacles without *C. demersum* were set as controls. Each replicate was filled with 2.5 L of half-strength Hoagland-Arnon hydroponic nutrient solution contaminated with a combination of Cd and Ni as shown in Fig. 1.4.

The solutions were adjusted to pH 7.0 with 0.01 M NaOH and 0.01 M HCl (Parneyan et al. 2011). Every day during cultivation, the solution volume was maintained with deionized water. Preliminary tests for Ni and Cd concentrations

Fig. 1.3 *C. demersum* L. collected and cultivated outdoors in half-strength modified Hoagland-Arnon nutrient solution, Shahid Chamran University, Ahvaz, Khuzestan Province, Iran, 2010

Table 1.2 The composition of half-strength modified Hoagland-Arnon nutrient solution

Chemical name	Chemical formula	Concentration	Unit
Potassium nitrate	KNO_3	3	mM
Calcium nitrate	$Ca(NO_3)_2$	2	mM
Ammonium dihydrogen phosphate	$NH_4H_2PO_4$	0.5	mM
Magnesium sulfate	$MgSO_4$	1	mM
Ferric EDTA	Fe–EDTA	10	μM
Boric acid	H_3BO_3	1.5	μM
Manganese(II) sulfate	$MnSO_4$	0.25	μM
Copper(II) sulfate	$CuSO_4$	0.1	μM
Zinc sulfate	$ZnSO_4$	0.2	μM
Molybdic acid	H_2MoO_4	0.025	μM

Table 1.3 Cd and Ni concentrations in treatments

Ni:Cd (mg l^{-1})			
0.00:0.00	0.00:1.00	0.00:2.00	0.00:4.00
1.00:0.00	1.00:1.00	1.00:2.00	1.00:4.00
2.00:0.00	2.00:1.00	2.00:2.00	2.00:4.00
4.00:0.00	4.00:1.00	4.00:2.00	4.00:4.00

were performed to determine the appropriate sensitivity range of *C. demersum* (Parnian et al. 2016; Chorom et al. 2012). Twenty grams fresh weight (FW) of plants were added to each treatment replicate set. Treatment and control vessels were randomly arranged.

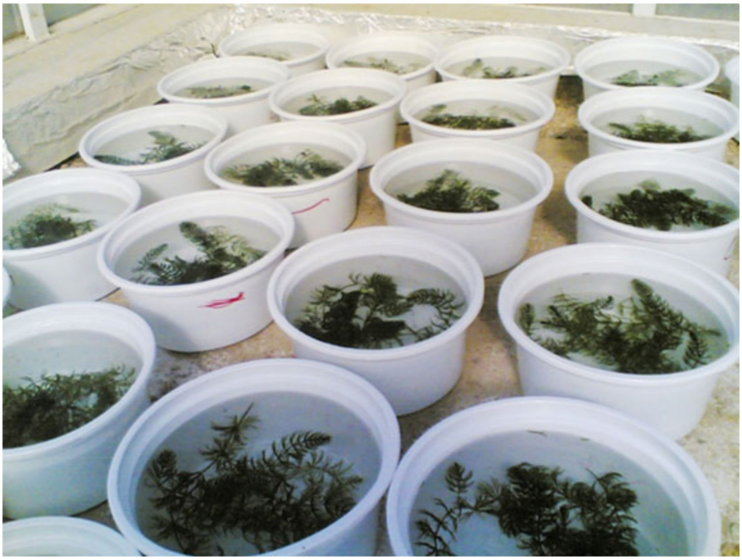

Fig. 1.4 *C. demersum* cultivated in contaminated hydroponic nutrient solution, Shahid Chamran University, Ahvaz, Khuzestan Province, Iran

1.5 Biomass Production Measurements

Plant biomass production, Pr (g FW day^{-1}), was calculated as follows:

$$\mathrm{Pr} = (\mathrm{FW2} - \mathrm{FW1})/\Delta t \tag{1.1}$$

where FW_1 and FW_2 were plant fresh weight (g) at time 1 and time 2, respectively, and Δt is the difference between time 1 and time 2 (Parnian et al. 2016).

1.6 Quantification of Metals Removed from Nutrient Solutions by Plants Active Uptake

Removal of the metals from the nutrient solutions was determined by measuring the concentration of metal left in the medium. The water sampling period commenced at day 0, with 2 ml samples being taken daily to ascertain the heavy metal concentration. Heavy metal presence and concentrations in water samples were determined by atomic absorption spectrophotometry according to standard methods for examination of water and wastewater (APHA 2005). Removal of metals was calculated using the following equation:

$$R(\%) = [(C_0 - C_t)/C_0] \times 100 \tag{1.2}$$

where C_0 and C_t represent the residual concentration of metal at the beginning of the experiment and at time t, respectively (Khellaf and Zerdaoui 2009).

1.7 Statistical Analysis

One-way ANOVA was performed to identify significant differences in metal concentrations in the different treatments. Differences were considered as significant at $p < 0.05$. Statistical analyses were performed with SPSS 16 and Excel 2010.

1.8 Cd and Ni Removal from Water

Table 1.4 shows the percentage of Ni removal for different initial concentrations by *C. demersum* in competition with different initial concentrations of Cd.

Under the conditions of this study, the highest removal efficiencies of Ni by *C. demersum* occurred in all of the treatments with an initial concentration of Ni equal to 1 mg l^{-1} as shown in Table 1.4. Overall, the Ni removal values by *C. demersum* increased with an increase of Cd initial concentration with the following three exceptions of Ni initial concentration and Cd initial concentration: 2 mg l^{-1}, 4 mg l^{-1}; 4 mg l^{-1}, 2 mg l^{-1}; and 4 mg l^{-1}, 4 mg l^{-1}. In these three treatments, *C. demersum* showed a low capacity of Ni removal. *C. demersum* growth was slowed when heavy metal concentrations were high; the Ni removal was thus impaired by heavy metal toxicity (Parnian et al. 2016). The increasing effect of Cd on the Ni removal efficiency of *C. demersum* is caused by decreasing cellular membrane selective permeability, causing plants to lose their controlled uptake ability (Marschner and Marschner 2012; Chen et al. 2015). Previous studies have shown that the submerged aquatic plant *C. demersum* can be successfully used for heavy metal (Pb, Zn, and Cu) removal under dilute metal concentrations (Keskinkan et al. 2004). Other macrophytes (*Spirodela polyrhiza*, *Pistia stratiotes*, and *Eichhornia crassipes*) demonstrated Ni and Cd removal percentages over 45% and more than 50%, respectively, in a 15-day period (Mishra and Tripathi 2008).

Table 1.4 *C. demersum* Ni removal efficiency for different initial concentrations of Ni and Cd

	Cd = 0 mg l^{-1}	Cd = 1 mg l^{-1}	Cd = 2 mg l^{-1}	Cd = 4 mg l^{-1}
Ni = 0 mg l^{-1}	0%^{a}	0%^{a}	0%^{a}	0%^{a}
Ni = 1 mg l^{-1}	77%^{a}	67%^{c}	71%^{b}	79%^{a}
Ni = 2 mg l^{-1}	52%^{b}	60%^{a}	62%^{a}	32%^{c}
Ni = 4 mg l^{-1}	50%^{b}	58%^{a}	30%^{d}	35%^{c}

Different letters in the same row indicate a significant difference at $p < 0.05$, $n = 3$

Table 1.5 *C. demersum* Cd removal efficiency for different initial concentrations of Ni and Cd

	Ni = 0 mg l^{-1}	Ni = 1 mg l^{-1}	Ni = 2 mg l^{-1}	Ni = 4 mg l^{-1}
Cd = 0 mg l^{-1}	0%^{a}	0%^{a}	0%^{a}	0%^{a}
Cd = 1 mg l^{-1}	74%^{b}	77%$^{a, b}$	38%^{c}	81%^{a}
Cd = 2 mg l^{-1}	84%^{a}	79%^{b}	82%$^{a, b}$	29%^{c}
Cd = 4 mg l^{-1}	35%^{c}	86%^{a}	48%^{b}	37%^{c}

Different letters in the same row indicate a significant difference at $p < 0.05$, $n = 3$

Table 1.6 Heavy metal effect on *C. demersum* biomass production

	Ni = 0 mg l^{-1}	Ni = 1 mg l^{-1}	Ni = 2 mg l^{-1}	Ni = 4 mg l^{-1}
Cd = 0 mg l^{-1}	2.30 ± 0.13	1.63 ± 0.08	1.27 ± 0.14	1.02 ± 0.05
Cd = 1 mg l^{-1}	1.84 ± 0.06	1.62 ± 0.10	1.15 ± 0.12	1.03 ± 0.11
Cd = 2 mg l^{-1}	1.49 ± 0.19	1.45 ± 0.13	1.01 ± 0.14	0.80 ± 0.09
Cd = 4 mg l^{-1}	1.32 ± 0.22	1.29 ± 0.16	0.83 ± 0.19	0.78 ± 0.10

Table 1.5 shows *C. demersum* percentage of Cd removal for different initial concentrations in competition with different initial concentrations of Ni ions.

Results showed that the highest removal efficiency of Ni by *C. demersum* (86%) occurred in the treatment with initial concentration of Ni 1 mg l^{-1} and Cd 1 mg l^{-1}. Overall the Cd removal values by *C. demersum* increased with increasing of metals' initial concentration. It has been proven that heavy metals can cause dysfunctions in cell plasma membranes (Devi and Prasad 1999); the latter is caused by a nickel distraction effect in ion transfer, affecting the plasma membrane of cells. In higher concentrations of Cd, this phenomenon effectively demolishes cellular membrane selective permeability (Marschner and Marschner 2012). Decreased Cd removal efficiency in high concentrations of heavy metals especially in 2 mg l^{-1} Ni, 4 mg l^{-1} Cd; 4 mg l^{-1} Ni, 2 mg l^{-1} Cd; and 4 mg l^{-1} Ni, 4 mg l^{-1} Cd initial concentrations results due to the decrease in plant biomass production as seen in Tables 1.5 and 1.6. These results are consistent with trace element removal efficiencies in different plants (Parnian et al. 2016; Chorom et al. 2012; Marin and Oron 2007).

In general, the competitive bioaccumulation of heavy metals (Ni and Cd) by *C. demersum* seen in Tables 1.4 and 1.5 shows critical metal concentrations of Ni equal to 4 mg l^{-1} and Cd equal to 4 mg l^{-1}; these concentrations of the metals mutually increased the removal efficiency of plants. At high concentrations, plant growth is reduced by the toxicity of the metal, although the metal removal efficiency may increase in certain conditions (Marschner and Marschner 2012; Parnian et al. 2016).

In highly contaminated mediums, heavy metals have been seen to demolish the cell membrane with the consequence of the cytoplasm being freely exposed to the outside environment (Wang et al. 2010). Active sites for metal adsorption will increase in such circumstance by inner sites being engaged, including proteins and the inner sides of cell membranes in addition to the outer cell membranes (Ruta et al.

2017). It may be surmised that a higher metal adsorption occurs after cell membrane defection. Exposing both the inner and outer sides of cell membranes to contaminated aqueous medium results in the defected plants adsorbing more heavy metals. In the current study, this explains *C. demersum*'s higher Cd removal efficiency when compared to 1 mg l^{-1} Ni:4 mg l^{-1} Cd and 0 mg l^{-1} Ni:4 mg l^{-1} Cd and when compared to 4 mg l^{-1} Ni:1 mg l^{-1} Cd and 2 mg l^{-1} Ni:1 mg l^{-1} Cd.

1.9 Heavy Metal Effect on Plant Biomass Production

Accumulation of heavy metal in hydrophytes produces significant growth responses (Thomas et al. 2016). Nonessential and highly toxic heavy metal Cd causes serious defects in *C. demersum* (Parnian et al. 2016). Trace concentrations of Ni are essential for plants, whereas high doses of Ni effect significant damage in *C. demersum* (Chorom et al. 2012). The biomass production results of the current study are shown in Table 1.6. Mean biomass production for *C. demersum* in the cultivation period was between 2.30 ± 0.13 and 0.78 ± 0.10 g FW day^{-1} in contaminated aquatic mediums. The mean of all treatments biomass production was in the order of Cd treated > Ni treated mediums indicating that Ni was more toxic to *C. demersum* than Cd in the experimental conditions of the current study. The latter is in agreement with published data where the same effective Ni toxicity was shown (Parnian et al. 2016). The crucial concentration of an element is one that effects its maximum absorption; at higher levels, plants show obvious physiological or morphological defects including leaf/root irregularities and additional internal dysfunction, which disrupts uptake of water/elements. The crucial concentrations for both heavy metals were 4 mg l^{-1} or less; in lower concentrations of metals, plants showed greater removal efficiency seen in Tables 1.4 and 1.5 and biomass production as in Table 1.6.

The different concentrations measured in Table 1.6 are expressed as: average ± standard deviation, $n = 3$. *C. demersum* biomass production was calculated according to equation (1.1).

In the three treatments (2 mg l^{-1} Ni, 4 mg l^{-1} Cd), (4 mg l^{-1} Ni, 2 mg l^{-1} Cd), and (4 mg l^{-1} Ni, 4 mg l^{-1} Cd), *C. demersum* showed an extreme reduction in biomass production and plant growth among these initial concentrations of contamination; the resultant change in plant morphology is illustrated in Fig. 1.5. Plant biomass production affects metal removal by reducing metal absorbance mass (Parnian et al. 2017). Hydrophytes may be selected for aquatic medium restoration based on their toxicity characteristics and removal efficiencies (Rezania et al. 2016). The tolerance of *C. demersum* to Cd and Ni indicates that this plant is effective for Cd and Ni remediation from contaminated water.

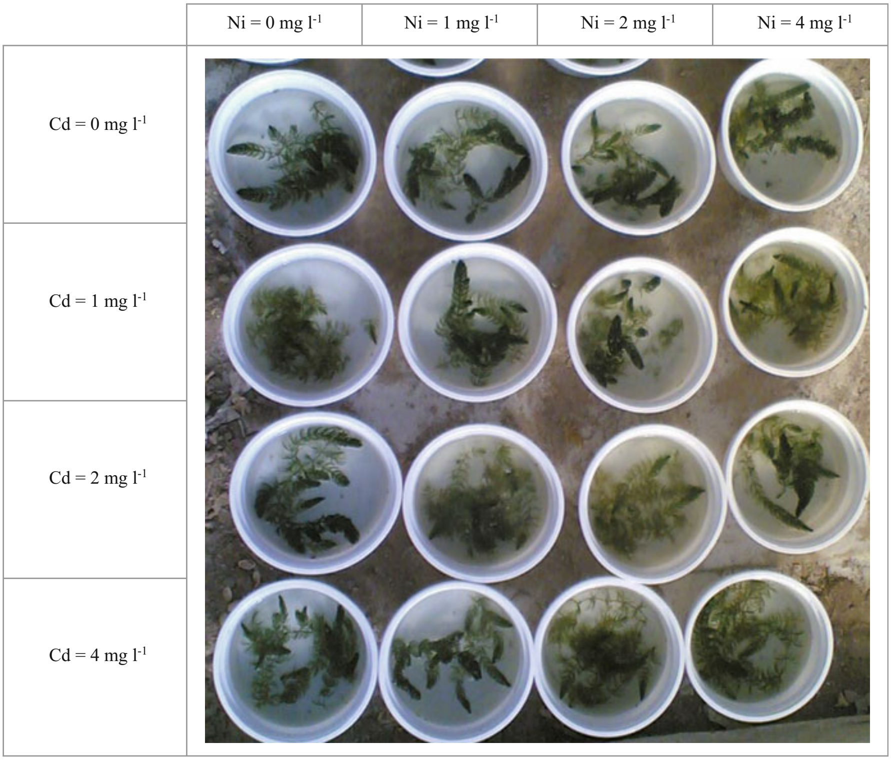

Fig. 1.5 Heavy metal effect on *C. demersum*. Day 5 of the experiment (Shahid Chamran University, Ahvaz, Khuzestan Province, Iran)

1.10 Conclusion

Heavy metal pollution poses a great risk to the environment. This pollution is present in aquatic areas including urban wastewater, drainage water, and industrial wastewater. The solution to this critical environmental problem is the use of phytotechniques.

In the current chapter, we demonstrated that the copious hydrophyte *C. demersum* of Khuzestan and Iran is very effective for phytoremediation of Ni and Cd from nutrient medium. Further the crucial metal concentration of both Ni and Cd was identified (4 mg l^{-1}) for each metal. *C. demersum* effectively mutually removed both heavy metal elements before this concentration. Concentrations greater than the crucial value impair removal efficiency.

C. demersum can be utilized for the large-scale treatment of heavy metals from contaminated effluent including steal industrial wastewater, mine and mine waste drainages, and battery factory wastewater. Further work is required with this plant in pilot studies ranging to full-scale constructed wetland with the recommended aim of mixed-metal polluted water phytoremediation. *C. demersum* may upgrade waste stabilization lagoons in industrial zones. *C. demersum* is considered a cosmopolitan plant; integrating an ecological mosaic model involving additional species will assist in resolving complex chemical pollution, and with consideration of combinations of the correct species ultimately benefit biodiversity enrichment across the Earth.

Acknowledgments This chapter was a part of the research program "Phytoremediation of heavy metals (Ni and Cd) using two aquatic macrophytes (free-floating and floating-leaved) from steel industrial wastewater and synthetic medium", and was financially supported by the vice president for research and technology from Shahid Chamran University in Iran.

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