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Absorption and enrichment characteristics of aquatic plants under cobalt stress

Yonghong Zheng[a,b,c], Fangling Chen[a], Zhiguo Zhang[a,*], Weiqing Cai[a], Chengnan Ma[a], Yongqiang Deng[a], Chao Fang[a], Zhilin Zhang[a]

[a] School of Earth and Environment, Anhui University of Science and Technology, Huainan, Anhui 232001 China
[b] Huainan Mining (Group) Co., Ltd., China, Huainan 232001 China
[c] National Engineering Laboratory for Protection of Colliery Eco-Environment, Huainan 232001 China

*Corresponding author, e-mail: zzgaust@aust.edu.cn

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ABSTRACT: The 6 different genera *Canna indica L.*, *Eichhornia crassipes*, *Nelumbo nucifera*, *Iris pseudacorus L.*, *Ceratophyllum demersum L.*, and *Myriophyllum spicatum L.* were selected as experimental plants. All genera could remove cobalt (Co) from water, and *E. crassipes* showed the highest removal efficiency of 94.86%. *C. demersum L.* showed the strongest enrichment ability with a maximum comprehensive enrichment coefficient of 4214.08. For different aquatic plant species, the best harvest time should be considered, and different harvesting methods should be adopted to avoid secondary pollution caused by the decomposition of aquatic plants. In combination with the landscape effect of aquatic plants, the floating plants, *E. crassipes* and *C. demersum L.*, can be considered potential species for the remediation of Co-polluted water body.

KEYWORDS: aquatic plants, absorption model, cobalt (Co), enrichment coefficient, transfer coefficient

INTRODUCTION

With the rapid development of China's economy and the continuous improvement of people's living standards, the emission of various pollutants has greatly exceeded the self-purification capacity of ecosystems [1, 2]. Currently, water pollution is one of the most pressing environmental issues, as large amounts of industrial wastewater containing toxic compounds and nutrients are discharged into water body, potentially leading to the accumulation of heavy metals and eutrophication [3, 4]. Moreover, due to the well-documented impacts of heavy metals to human health, a significant amount of research has been conducted on methods of removing heavy metals from drinking water sources [5] as well as municipal wastewater, industrial wastewater, and other water sources [6]. Cobalt (Co) pollution is mainly the result of nuclear weapon test waste, atomic energy industrial waste, mineral exploitation, hospital sewage discharge, and laboratory sewage disposal [7]. Most of the compounds in such sewage are difficult to degrade, accumulate and migrate easily, and are highly toxic. As they cannot be degraded naturally, they pose a risk to aquatic organisms and human health [8].

The heavy metal Co is a trace element important for plant growth and a crucial part of vitamin B_{12}. At appropriate doses, it promotes plant growth [9]. However, soluble cobalt salt can adversely interfere with cell division, induce chromosome aberration in plants, and make plants toxic [10]. Along with all living organisms, humans need varying amounts of heavy metals. In humans, Co plays an important role in regulating blood pressure, maintaining normal thyroid function, and decreasing small-cell anemia [11, 12]. However, excessive Co intake has several negative impacts on human health [13, 14]; for example, it can cause gastrointestinal dysfunction, deafness, goitre, heart damage, myocardial ischemia, and other problems [15]. In recent years, the dispersion and complexity of polluted water bodies have been high, and the treatment did not achieve the desired effect due to disadvantages such as large investments and high costs [16]. To date, few papers have surveyed the use of low-cost materials for the removal of heavy metals from water [17]. However, several studies have shown that phytoremediation is an economically and environmentally friendly pollution control for nutrients, heavy metals, petroleum hydrocarbons, or radionuclides [18, 19].

In this study, we used the aquatic plant species, *C. indica L.*, *E. crassipes*, *N. nucifera*, *I. pseudacorus L.*, *C. demersum L.*, and *M. spicatum L.*, for Co removal from heavy metal-polluted water with the aim to identify the most suitable absorption model and to provide a scientific basis for increased sewage treatment.

MATERIALS AND METHODS

Test materials

Experimental plants

The 6 different genera, *C. indica L.*, *E. crassipes*, *N. nucifera*, *I. pseudacorus L.*, *C. demersum L.*, and *M. spicatum L.*, were selected as experimental plants. Aquatic plants were collected from the Aquatic Botanical Garden (117E and 3237N) located in Huainan, Anhui Province, China.

Table 1 The physicochemical properties of the prepared water.

Parameter†	Concentration		
	High	Medium	Low
Co	10 mg/ml	5 mg/ml	1 mg/ml
TP	5 mg/l	5 mg/l	5 mg/l
TN	20 mg/l	20 mg/l	20 mg/l

† TP = total phosphorus; TN = total nitrogen.

Experimental plants

According to previous studies, standard cobalt solutions were added to prepare high (10 mg/ml), medium (5 mg/ml), and low (1 mg/ml) concentration Co-polluted wastewater [20]. At the same time, a total of 60 ml potassium nitrate (5 g/l) and 75 ml potassium dihydrogen phosphate (1 g/l) were added to give final concentrations of 20 mg/ml and 5 mg/ml, respectively, into 15 l of experimental water to give final concentrations of 20 mg/ml and 5 mg/ml, respectively. Potassium nitrate and potassium dihydrogen phosphate can provide the nutrient elements, potassium and phosphorus, needed by aquatic plants; the physicochemical properties of the prepared water are shown in Table 1.

Experimental design and sample pre-treatment

Experimental design

The experiment was performed in the laboratory. Each plastic bucket (36 cm diameter, 32 cm height) was filled with 15 l of test water. The roots of aquatic plant seedlings were repeatedly washed with tap water, and then rinsed with deionized water repeatedly. The plants were acclimated with ultrapure water for 1 week. The aquatic plants were transplanted into each bucket when their growth characteristics reached the values as shown in Table 2. Each treatment was repeated 3 times. The 6 plants were numbered A1-*C. demersum L.*, A2-*M. spicatum L.*, A3-*C. indica L.*, A4-*I. pseudacorus L.*, A5-*N. nucifera*, and A6-*E. crassipes*, and K stands for blank: no aquatic plants. The aquatic plant culture experiment lasted for 70 d (June 7–August 30, 2020). During this period, the photoperiod was 10 h each day. The water temperature range was 19.2–30.8 °C, and the pH range was 6.7–8.1. During the experiment, 20 ml of water samples were collected from each treated bucket (with plants) at regular 14-day intervals to determine the heavy metal content. After 70 d of seedling transplantation, all plants were harvested.

Sample pre-treatment

The plant samples were repeatedly rinsed with tap water, then deionized water for 2–3 times, placed in an oven at 105 °C for 30 min, and dried when the oven temperature drops to 70 °C to constant weight. The plant samples were crushed through a 60-mesh nylon sieve and packed in a plastic bag for later use. Plant sample of 0.5000 g was weighed into a digestion tube; 6 ml of HNO_3, 4 ml of $HClO_4$, and 4 ml of H_2O_2 were added to the digestion tube in sequence and let stand for 12 h before digestion. After the sample is digested completely, it was filtered and kept sealed for later use.

Twenty ml of water sample was taken into the digestion tube; 6 ml of HNO_3 and 4 ml of H_2SO_4 were added to the tube in sequence and let stand for 12 h for digestion. After the sample is digested completely, it was filtered, sealed, and stored for later use. The contents of P and K elements in the plants were determined by digestion with concentrated H_2SO_4-H_2O_2. The method is described in detail elsewhere [21, 22].

Determination method and data analysis

Determination method

Total phosphorus content in water and plants was determined by ammonium molybdate spectrophotometry (HJ 671-2013, China). The contents of Co and K in water and plants were determined using an atomic absorption spectrophotometer (PE AA800, USA).

Calculated parameters for Co transfer and enrichment ability of plants

As roots, stems, and leaves of submerged plants cannot easily be distinguished, the transfer coefficient of submerged plants was not considered [23]. To determine the transfer coefficient (TF), the enrichment coefficient (BCF), and the comprehensive enrichment factor (BCF*), we used the following equations:

$$TF_{\text{Floating/Emerged}} = \frac{C_{\text{above water surface}}}{C_{\text{below water surface}}}, \tag{1}$$

$$BCF = \frac{C_{\text{plant}}}{C_{\text{water}}}, \tag{2}$$

$$BCF* = \frac{BCF_{\text{root}} + BCF_{\text{stem}} + BCF_{\text{leaf}}}{3}. \tag{3}$$

Here, $C_{\text{above water surface}}$, $C_{\text{below water surface}}$, C_{plant}, and C_{water} are the Cobalt content (mg/kg) in the plant parts above and below water surface as well as in the total plant and in the water, respectively. The BCF_{root}, BCF_{stem} and BCF_{leaf} are the enrichment coefficients of plant roots, stems, and leaves, respectively. The transfer coefficient and comprehensive enrichment coefficient were used to evaluate the ability of plants to transfer and enrich heavy metals.

Statistical analysis

For data analysis and absorption curve simulation, we used SPSS 25.0. The software Origin 2018 was used to draw the graphs.

Table 2 Experimental design of aquatic plants.

Category	Plant code and name	No. of plant columns	Plant length (cm)	Fresh weight (g)
Blank control group†	Blank sample 1; Blank sample 2; Blank sample 3	–	–	–
Aquatic plant group	A1: *Ceratophyllum demersum L.*	19	5 ± 1	39.79
	A2: *Myriophyllum spicatum L.*	19	5 ± 1	26.99
	A3: *Canna indical L.*	3	30 ± 2	52.22
	A4: *Iris pseudacorus L.*	3	28 ± 2	56.18
	A5: *Nelumbo nucifera*	6	6 ± 1	77.90
	A6: *Eichhornia crassipes*	6	7 ± 2	98.13

† Blank samples 1, 2, 3 are at high, medium, and low concentrations of cobalt, respectively.

Table 3 Correlation analysis of metal contents in aquatic plants with potassium and phosphorus.

Co in different parts	Correlation coefficient	
	Potassium	Phosphorus
Root	−0.120	−0.290
Stem	−0.028	−0.257
Leaf	−0.391	−0.129

Quality control

All reagents were of analytical grade. To generate the standard curve, we used heavy metal reference material (GSB 04-1766, China Shanghai forthright biological technology Co., Ltd., China); for quality control, we used plant reference material (GBW10011(GSB-2), China Wuhan Zhongchang National Research Standard Technology Co., Ltd., China). The error of the standard sample determination results was within the allowable range.

RESULTS AND DISCUSSION

Changes in the biomass of aquatic plants

Throughout the experiment, the water pH remained neutral. Changes in the biomass of *I. pseudacorus L.*, *N. nucifera*, and *E. crassipes* at low Co concentrations were higher than those in the other plant species (Fig. 1a). The biomass changes in A4, A5, and A6 were greater than the average biomass changes of all plants (28.55 g). The average growth rate of biomass in each concentration group was as follows: low concentration (24%) > medium concentration (19%) > high concentration (18%). Based on our results, all species could adapt to the synthetic sewage and tolerate Co pollution.

Effects of Co stress on nutrient levels in aquatic plants

Different aquatic plants have different growth characteristics. We observed changes in phosphorus (P) and potassium (K) levels in all 6 plant species under Co stress. The changes in P after 70 d of cultivation are shown in Fig. 1b. With increasing Co stress, the plant P levels decreased gradually (except for A1); the average P content in each concentration group was as follows: low concentration (70%) > medium concentration (55%) > high concentration (32%). At a low Co concentration (1 mg/l), the P levels in plants were highest, following the order: A5 (17.45 mg/l) > A6 (10.36 mg/l) > A3 (10.28 mg/l) > A1 (7.29 mg/l) > A2 (4.21 mg/l) > A4 (3.85 mg/l). Therefore, under the same concentration, Co stress had a greater effect on A2, *M. spicatum L.* and A4, *I. pseudacorus L.*

The changes in the K levels of the 6 aquatic plant species under different Co concentrations are shown in Fig. 1c. With increasing Co stress, the plant K levels decreased gradually. The average K content in each concentration group was as follows: low concentration (72%) > medium concentration (61%) > high concentration (40%). At the low Co concentration, the levels of plant K were highest, following the order: A3 (877.29 mg/l) > A5 (871.28 mg/l) > A4 (665.48 mg/l) > A6 (294.317 mg/l) > A2 (158.741 mg/l) > A1 (92.173 mg/l). Therefore, under the same concentration, Co stress had a greater influence on *C. demersum L.* and *M. spicatum L.* Cobalt poisoning mainly results in a disturbed nutrient balance; our findings agree with previous studies [24]. Generally, at low Co levels, P and K adsorption by aquatic plants is low. Also, *C. indica L.*, *N. nucifera*, and *E. crassipes* species showed better tolerance to Co pollution.

Correlation analysis of nutrient levels is shown in Table 3. Co stress was negatively correlated with the contents of P and K in plants ($n = 64$), indicating that high Co concentrations affect plant growth. In addition, Co stress in leaves was negatively correlated with K levels ($p < 0.01$, $n = 64$); there was a significant negative correlation between stress and plant root P level ($p < 0.05$, $n = 64$). In this sense, under Co stress, the influence of plant leaves on K was more significant, and the impact of plant roots on P levels was higher.

Absorption of Co by aquatic plants

Over time, the Co concentration in each experimental group was lower than that in the blank, indicating that aquatic plants removed Co from the water. Over a period of 28 d and with high Co concentrations, *E. crassipes* (64.97%) and *N. nucifera* (57.49%) showed a higher removal efficiency than the other species. From 28 to 42 d, the removal efficiency of *C. indica L.*

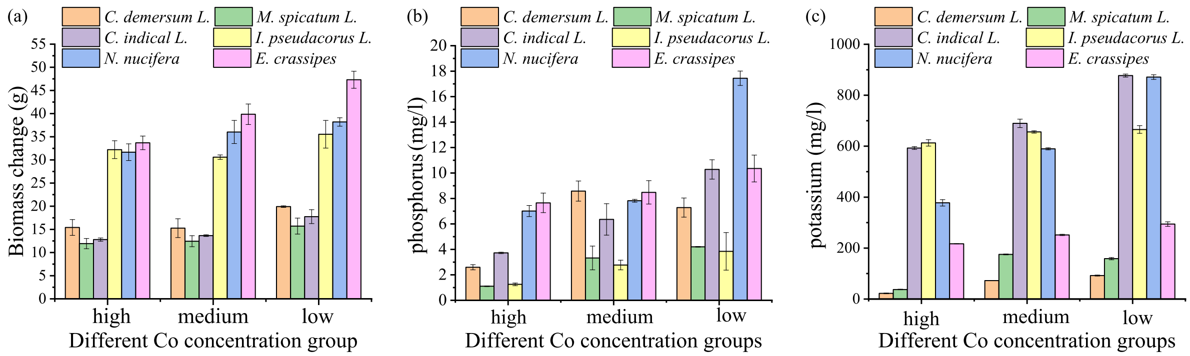

Fig. 1 Plants treated with different levels of Co. Changes in biomass of aquatic plants (0–70 d) (a); changes in concentration of phosphorus (b) and potassium (c) elements in aquatic plants.

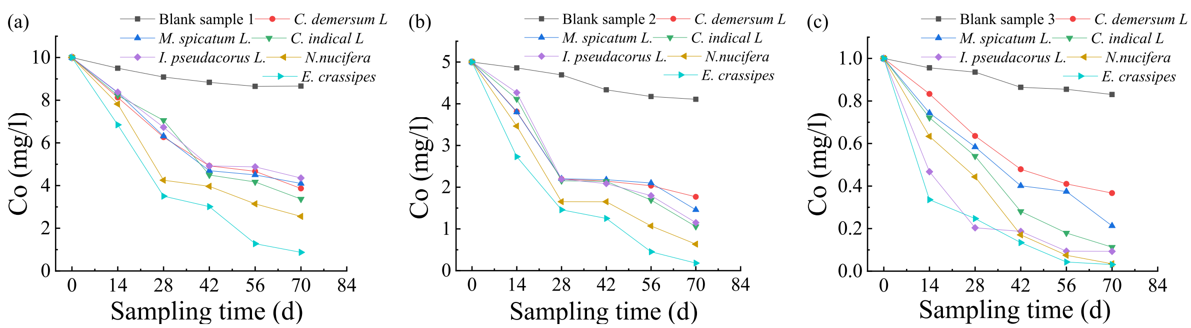

Fig. 2 Absorption of Co by aquatic plants treated with different levels of Co at high concentration (a), medium concentration (b), and low concentration (c) for 70-day exposure.

decreased, and that of *C. indica L.* increased. The removal efficiency of *C. indica L.* was 55.05% and higher than those of *E. crassipes*, *C. demersum L.*, and *I. pseudacorus L.* From 42 to 56 d, the removal efficiencies of *C. indica L.*, *I. pseudacorus L.*, *M. spicatum L.*, and *C. demersum L.* decreased, whereas those of *E. crassipes* and *N. nucifera* increased. At 70 d, the total removal efficiency followed the order: *E. crassipes* (91.29%), *N. nucifera* (74.45%), *C. indica L.* (66.34%), *C. demersum L.* (61.36%), *M. spicatum L.* (59.01%), and *I. pseudacorus L.* (56.46%). The aquatic plant with the highest removal efficiency was *E. crassipes* (Fig. 2a).

Under medium Co concentration, in the first 28 d, the maximum removal efficiency was observed in *E. crassipes* (70.83%). From 28 to 42 d, the removal efficiencies of all species decreased significantly while higher removal efficiencies were observed from 42 to 56 d. At 70 d, the total removal efficiency followed the order: *E. crassipes* (96.40%) > *N. nucifera* (87.30%) > *C. indica L.* (78.85%) > *I. pseudacorus L.* (76.99%) > *C. demersum L.* (70.82%) > *M. spicatum L.* (64.62%) (Fig. 2b).

Under low Co concentration, the highest removal efficiency of the 6 aquatic plants over 14 d was observed with *E. crassipes* (66.43%). From 14 to 28 d, the removal efficiency of *E. crassipes* decreased significantly, and that of *I. pseudacorus L.* exceeded that of *E. crassipes* by 79.60%. From 28 to 42 d, the removal efficiency of *I. pseudacorus L.* was significantly lower than that of the previous period, and the removal efficiencies of *E. crassipes* and *N. nucifera* were higher than that of *I. pseudacorus L.* At 70 d, the total removal efficiency followed the order: *E. crassipes* (96.89%) > *N. nucifera* (96.54%) > *I. pseudacorus L.* (90.69%) > *C. indica L.* (88.74%) > *M. spicatum L.* (78.70%) > *C. demersum L.* (63.30%) (Fig. 2c).

Table 4 Three functional equations and models of *E. crassipes* at different Co concentrations.

Concentration	Equation†	R^2	F	Sig
High	$Y = 10.24\,e^{-0.035x}$	0.972	141.006	0.000
Medium	$Y = 5.49\,e^{-0.046x}$	0.955	105.438	0.001
Low	$Y = 1.21\,e^{-0.059x}$	0.946	108.742	0.000

† Y is Co concentration in water (mg/l), x is the number of days of hydroponics.

A model of Co uptake by aquatic plants

Establishment and prediction of Co uptake by aquatic plants

The regression linear analysis was carried out by SPSS 25.0 software to screen out the optimal function equa-

Table 5 Measured and predicted values of Co concentration at high, medium, and low concentrations.

Day	High concentration			Medium concentration			Low concentration		
(d)	Measured value	Simulated predicted value	Error	Measured value	Simulated predicted value	Error	Measured value	Simulated predicted value	Error
0	9.4983	9.5223	0.25%	4.8603	4.7593	−2.08%	0.9559	0.9439	−1.26%
14	6.8481	6.8177	0.44%	2.7318	2.7648	1.21%	0.3357	0.4837	4.41%
28	3.5035	3.4203	2.37%	1.4583	1.4915	2.28%	0.2472	0.3006	1.38%
42	3.0120	3.1036	3.04%	1.2510	1.2626	0.93%	0.1341	0.1747	3.43%
56	1.2757	1.2703	−0.42%	0.4516	0.4656	3.10%	0.0431	0.0267	−14.85%
70	0.8709	0.8766	0.65%	0.1802	0.1631	−11.15%	0.0311	0.0171	−12.86%

tion. The Co absorption curves of the 6 aquatic plants were in line with the exponential function model, and the Co removal efficiency of *E. crassipes* was generally good (Table 4). Therefore, different function models of Co absorption by *E. crassipes* were established, and the best model was selected. Based on the results, 3 models with different functional equations are obtained, at high, medium, and low concentrations. The Co absorption models for *E. crassipes* were all in accordance with the exponential function type ($p < 0.005$).

By establishing the function model, a more objective prediction of the Co concentration in water can be obtained. Through data analysis, the most suitable harvest time can be predicted, ensuring the maximum Co absorption by aquatic plants and preventing secondary pollution.

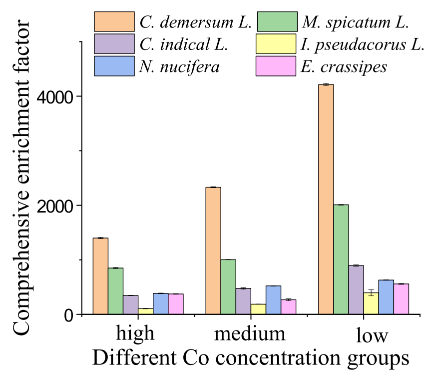

Fig. 3 Comprehensive enrichment coefficients of plants treated with different levels of Co.

Absorption model test

Comparative analysis of the predicted and the measured values can reflect the prediction effect of the model (Table 5). The differences between the simulated and the measured values were small with error values from 0.25–10% for 15 values and above 10% for 3 values. The results show that the function model can reliably predict the Co concentration of aquatic plants.

Enrichment and transfer characteristics

Enrichment capacity of Co in aquatic plants

The comprehensive enrichment coefficient is the average value of the enrichment coefficient of a certain heavy metal in each part of the plant body and can reflect the whole plant's ability to accumulate a certain heavy metal.

Data analysis was conducted for the submerged plant species, *C. demersum L.* and *M. spicatum L.* The maximum comprehensive enrichment coefficients of *C. demersum L.* and *M. spicatum L.* reached 4214.08 and 2009.27, respectively. Analysis of other plants led to the same conclusion; at low concentration, Co enrichment by aquatic plants was highest, indicating a higher accumulation capacity at low Co concentration. This also suggests that at higher Co concentrations, the capacity to accumulate Co is inhibited most likely because of the toxic effects of Co. In addition, at higher concentrations and lower Co stress periods, this inhibition effect increases.

The average comprehensive enrichment coefficients of the different treatment groups were A1 (2648.33), A2 (1287.43), and A3 (563.03). Under different Co concentrations, the average comprehensive enrichment coefficients of *C. demersum L.* and *M. spicatum L.*, respectively, were as follows: high concentration (756.24 and 450.13), medium concentration (1097.89 and 642.77), and low concentration (1680.01 and 963.71). As the concentration of Co decreased, most of the comprehensive enrichment coefficients of submerged plants in each group showed an increasing trend (Fig. 3). Overall, the enrichment ability of *C. demersum L.* was the strongest while that of *I. pseudacorus L.* was the weakest compared with the other 5 aquatic plants. At decreasing Co levels in the water, the comprehensive enrichment coefficients of aquatic plants in each group increased continuously, which further confirmed that high Co levels inhibit the growth of aquatic plants [25].

Ability of the aquatic plants to transport Co

The transfer coefficient is the ratio of heavy metal content in the plant parts above water surface and below water surface. The transfer coefficients of different types of aquatic plants were different. The transport capacity of plants for heavy metals is generally assessed using this coefficient. The transfer coefficient of *C. indica L.* at A3-high was greater than 1, whereas those of *I. pseudacorus L.* were all below 1. More than half of the transfer coefficients of *N. nucifera* were greater than 1. In general, *E. crassipes* has the highest Co transfer capacity and *I. pseudacorus L.* the lowest one. In this sense, the roots of this species should be harvested in the later growth stage.

CONCLUSION

Under Co stress, with decreasing Co levels, the removal efficiency of aquatic plants in each group increased continuously, and the cut-off point of stress was at a medium concentration (5 mg/l). The exponential function model established can objectively predict the Co concentrations in water treated with *E. crassipes*. Different types of aquatic plants showed different enrichment abilities for Co. *C. demersum L.* showed the strongest enrichment ability with a maximum comprehensive enrichment coefficient of 4214.08. Different types of aquatic plants differed in their ability to transfer Co. The transfer coefficient of *E. crassipes* was above 1, and that of *I. pseudacorus L.* was below 1. For different aquatic plants, different harvesting methods should be used in the later growth stage. Only the above water surface part of *E. crassipes* should be harvested, whereas for *I. pseudacorus L.*, the entire plant should be harvested. Among the aquatic plants, *E. crassipes* and *C. demersum L.* showed the highest Co removal capacity, making them suitable species in the remediation of polluted water bodies.

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