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The ability of *Ceratophyllum demersum* L. to remediate polluted water with Chromium, Cadmium, and Cobalt

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Abstract

The aquatic plant is an essential biofilter to remove different pollutants from the watershed. This study focuses on the ability of *Ceratophyllum demersum* L. to remediate cadmium (Cd), Chromium (Cr), and Cobalt (Co) from aqueous solution and also examines some toxicological effects of metals on this plant species. *Ceratophyllum demersum* L. was exposed to 3 mg/L of selected metals in single and combination experiments, and a plant without metals was set as a control. The experiments were done in a laboratory for ten days. The parameters were measured at the beginning and end of the experiments. It included metals in the plant, bioconcentration factor (BCF), fresh and dry weight, relative growth rate (RGR), total chlorophyll, protein, and proline content, and tolerance index rate. The metal residual in the water and the removal efficiency were measured at the end of the experiments. The results revealed that the removal efficiency of metals in single metal experiments was higher than in combined metal experiments. It was Co > Cr > Cd. The results also improved that the reduction of fresh and dry weight, relative growth rate, total chlorophyll, and protein content was highest in combinations of three metals; it was 2.23 g, 0.309 g, 0.218, 2.714 µg/g, and 18.32%, respectively. The proline content was increased as a response to heavy metals. In combinations of three metal experiments, it was 355.76 mg/g. The conclusion indicates that *Ceratophyllum demersum* was a good candidate to remediate polluted water with a low concentration of heavy metals.

Key words: Removal efficiency, Heavy metal, Toxicological effects, Biochemical, Phytoremediation

Introduction

The aquatic environment has become unhealthy for many organisms due to the different pollutants released. Heavy metals are one of the dangerous pollutants in the aquatic environment and have become a severe challenge. Lead, mercury, cadmium, nickel, chromium, copper, and other dangerous heavy metals are among them. The critical problem with heavy metals is that, unlike organic pollutants, they are non-biodegradable, persistent in nature, and bio-accumulated in various environmental components, mainly living beings. (Zhuang *et al*., 2014; Mahdi, 2018). Heavy metals are discharging into the environment mostly via natural and anthropogenic sources such as industrial effluents, fuel production, mining, smelting operations, agricultural chemicals, small-scale industries, and electronic trash disposal, among others (Chaudhuri *et al.*, 2014; Mahdi and Al-abbawy, 2019). Ion exchange, reverse osmosis, precipitation, adsorption, electro-coagulation, and other traditional metal removal procedures are expensive; they require excavating, design by specialist people, energy, and many units to remove metals from polluted water (Rahman & Hasegawa, 2011). Phytoremediation is an eco-friendly technology that uses living green plants to remove toxins from soil and water in situ. Because each species has its own genetic, morphological, physiological, and anatomical properties, the efficiency of phytoremediation differs significantly between species. (Wang *et al*., 2008; Narayan, 2011; Mahdi, 2018). The submerged aquatic plant (*Ceratophyllum demersum* L.) of the Ceratophyllacae botanical family is considered an excellent plant for removing various organic and inorganic contaminants. *Ceratophyllum demeresum* L. is a rootless plant with 1-2.5 cm and 20–100 cm long leaves. It prefers shallow water and marshes with a slow current of water. It also prefers moderate to high nitrogen levels as well as low salinity. (Chorom *et al*., 2012; DiTomaso and Kyser, 2013). Afaj *et al.* (2017) studied the toxicological effect of lead on *C. demersum* on morphological growth and chlorophyll content in a laboratory experiment for fifteen days. His results indicated that the reduction of growth and chlorophyll content occurred with increasing concentrations. Chen *et al*. (2015) studied the bioaccumulation, BCF, protein, fresh weight, and growth of *C. demersum* L. exposed to different lead concentrations. The result indicated that the reduction of selected parameters increased with increasing concentration. This study aims to evaluate the ability of *C. demersum L.* to accumulate Cd, Cr, and Co in single and combination metals experiments and to evaluate the changes in some biochemical parameters.

Materials and methods

Plant collection and acclimatization

The *Ceratophyllum demersum* was collected from the Shatt Al-Arab River in a labeled plastic bag and transferred to the laboratory. The plant was washed several times with tap water to remove debris. The plastic aquarium, with a capacity of 20 liters, is filled with tap water for one-week acclimatization.

Preliminary test

According to the literature review and practical experiments, the plant was exposed to different concentrations of selected metals (1, 5, 10, and 20) mg/L. The preliminary tests indicated that the plant could not tolerate more than 3mg/L of Cd and Cr to the end of experiments at 5 mg/L and above. Therefore, the selected concentration was less than 5 mg/L. It was 3 mg/L.

Heavy metal preparation and experiment design

The standard salt of $CdSO_4.5H_2O$, K_2CrO_4, and $CoSO_4.5H_2O$ were used to prepare 3 mg/L of selected metals Cd (II), Cr (VI), and Co (II) respectively. Triplicate treatments were used for each single and combination experiment as named below. Three aquariums without metals are set as a control. Plastic aquariums with a 1.6-liter capacity filled with 1 L of selected concentration were used. 10±0.5 g of healthy plant matter was put in each aquarium after acclimatization and washing with tap and distilled water. 3% of Hoagland nutrient solution was added to apply a sufficient amount of nutrients during the experiments. The selected parameters were measured before and after the experiments.

T1: Control treatment (plant grow with distilled water and 3 % Hoagland nutrient solution
T2: Cr concentration only with plant and 3% nutrient solution
T3: Cd concentration only with plant and 3% nutrient solution
T4: Co concentration only with plant and 3% nutrient solution
T5: Cd + Cr concentration with plant and 3% nutrient solution
T6: Co + Cr concentration with plant and 3% nutrient solution
T7: Cd + Co concentration with plant and 3% nutrient solution
T8: Cr + Cd +Co concentration with plant and 3% nutrient solution

Metal residual in the water

According to APHA (2005), the concentration of metal residual in water was measured. A 100 mL sample of water was obtained. After adding 5 mL of concentrated nitric acid to the sample, it was heated on a hot plate to ensure that it was entirely digested (near dryness) Then, another 5 ml of concentrated nitric acid was added and returned to the hot plate near drying). After cooling, it was placed in a volumetric flask and filled with distilled water. The samples were analyzed using a flame atomic absorption spectroscopy (FAAS), with the results expressed in mg/L.

Removal efficiency (RE)%

The removal efficiency was determined using the Khan *et al*. (2009) equation.

$$\textbf{Removal efficiency } \% = \frac{C1 - C2}{C1} \times 100$$

C1 : intial metal concentration in water in mg/L
C2 : Final metal concentration in water in mg/L

Bio-concentration factor (BCF)

The BCF was calculated using the equation mentioned in Abdalla (2012)

$$\text{BCF} = \frac{metal\ concentrations\ in\ plant\ tissue \frac{mg}{gram}}{intilal\ metal\ concentration\ in\ water\ (\frac{mg}{L})}$$

Fresh, dry weight and relative growth rate

After washing the plant with tap and distilled water and putting them on filter paper to eliminate excess water, the fresh weight was calculated by weighing it with a 4-digit balance and recording the weight in grams. The plant was dried until it reached a constant weight in an oven at 70–80 °C, and the dry weight was recorded in grams. The relative growth rate was calculated using the algorithm provided by Xiaomei *et al*. (2004).

$$\textbf{Relative growth rate } = \frac{final\ fresh\ weight\ (g)}{intial\ fresh\ weight\ (g)}$$

Total Chlorophyll

The chlorophyll content was calculated using the Arnon method (1949). It involves extracting the chlorophyll with 0.2 g of fresh weight in 20 ml of 80 % acetone, centrifuging for 5 minutes at 5000 rpm to remove any residual particles, and measuring the absorbance of the extracted solution with a spectrophotometer at 645 and 663 nm to calculate the total chlorophyll content in µg/g.

$$\textbf{Total chlorophyll (µg/g)} = (12.7 * \text{OD } 663) + (16.8 * \text{OD } 645)$$

OD: optical density at 663 and 645 nm

Protein ratio

According to Page *et al.* (1982), the protein content of the plant was calculated as a percentage by multiplying the total nitrogen content by a factor of 6.25.

Proline content

Proline content was determined using the method described by Trollant and Lindsley (1955). 0.2 g of dry, powdered plant was mixed with 5 ml of 95 % ethanol for an hour before being centrifuged for five minutes at 5000 rpm. The translucent portion of the extract was evaporated until it was nearly dry. Two mL- distilled water was added to the mixture. Using spectroscopy at 520 nm, the proline standard curve using the concentrations (10, 20, 30, 40, 50, 60, 100, and 500) mg/L for measured the proline content of a 1 mL extract solution. It was a drawing of a standard curve to calculate the proline content in plant sample in mg/g.

Tolerance index Rate (TIR)%

The tolerance index rate is calculated according to the equation mentioned in Wilkins (1978).

$$\text{TIR } \% = \frac{dry\ weight\ of\ the\ plant\ in\ metal\ treatment}{dry\ weight\ of\ the\ plant\ in\ control\ treatment} \times 100$$

Statistical analysis

SPSS program version 23 was used to analyze the data. The result was expressed as descriptive statistics, and ANOVA at a significant level of 0.05 ($p \leq 0.05$) was used between treatments and control.

Result and discussion

Metal residual in water and removal efficiency

The metal residual in water deceased at the end of experiments in all treatments with different values with a significant difference between them. The highest reduction was in T4, and the lowest was in T8. Calculating the removal efficiency, the highest in T4 and the lowest was in T8, with a significant difference at 0.05 ($p \leq 0.05$) between each metal in single and combined metal experiments, as shown in Table1.

BCF

Metals in contaminated water and substrates accumulate in aquatic plants (Rai *et al*., 1995). In the current study, the heavy metal accumulation capacity of the plant for various metals was presented in figure 1. The plant accumulated a large amount of Co from the aqueous solutions compared to the other metals. Single metal experiments showed more accumulated metal than combined metal experiments.

Table 1: The metal remaining in the water and the removal efficiency of the selected metal treatment at the end of the experiments.

Treatments	Metal	The metal residual in the water (mg/L)	Removal efficiency %
T2	**Cr**	0.883 ± 0.027 *a	70.55 ± 0.90 *A
T3	**Cd**	1.595 ± 0.028 **a	46.815 ± 0.945 **A
T4	**Co**	0.503 ± 0.076 ***a	85.885 ± 0.125 ***A
T5	**Cd**	1.87 ± 0.120 **b	37.667 ± 4.00 **B
	Cr	1.45 ± 0.09 *b	51.667 ± 3.00 *B
T6	**Co**	1.120 ± 0.088 ***b	62.655 ± 2.95 ***B
	Cr	1.425 ± 0.025 *c	52.5 ± 0.83*C
T7	**Cd**	1.985 ± 0.025 **c	32.83 ± 0.84 **C
	Co	1.5 ± 0.01 ***c	50 ± 0.333 ***C
T8	**Cr**	1.922 ± 0.015 *d	35.933 ± 0.500 *D
	Cd	2.216 ± 0.026 **d	26.111 ± 3.421**D
	Co	1.699 ± 0.024 ***d	43.344 ± 0.80 ***D

*a, *b, *c, *d: The different letters for each column refers to a significant difference at 0.05 ($p \leq 0.05$) between Cr in single and combination experiments for remaining Cr metal in water

**a, **b, **c, **d: The difference letter for each column refers to a significant difference at 0.05 ($p \leq 0.05$) between Cd in single and combination experiments for remaining Cd metal in water

***a, ***b, ***c, ***d: The difference letter for each column refer to a significant difference at 0.05 ($p \leq 0.05$) between Co in single and combination experiments for remaining Co metal in water

*A, *B, *C, *D: The different letters refer to a significant difference at 0.05 ($p \leq 0.05$) between Cr in single and combination experiments for removal efficiency

**A, **B, **C, **D: The difference letter refer to a significant difference at 0.05 ($p \leq 0.05$) between Cd in single and combination experiments for removal efficiency

***A, ***B, ***C, ***D: The difference letter refers to a significant difference at 0.05 ($p \leq 0.05$) between Co in single and combination experiments for removal efficiency

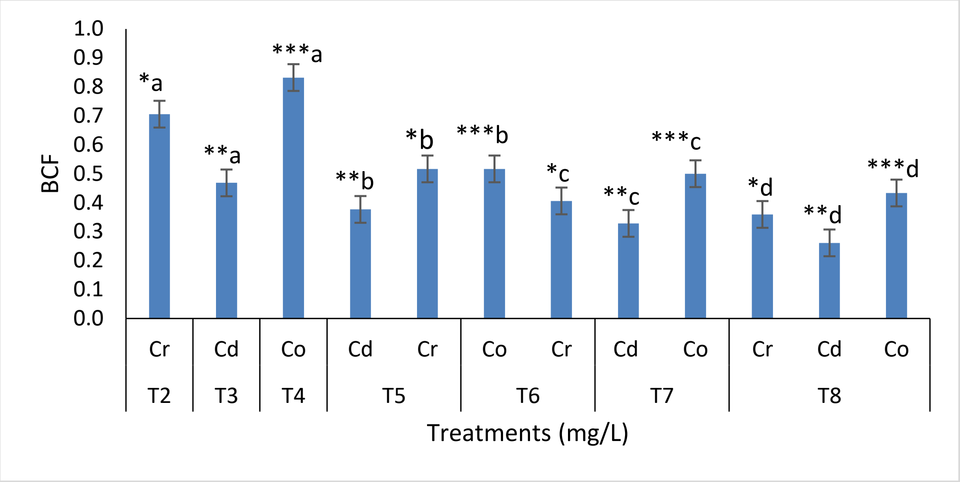

Figure 1: The average BCF in the plant at the end of experiments

*a, *b, *c, *d: The different letters refer to a significant difference at 0.05 ($p \leq 0.05$) between Cr in single and combination experiments for BCF of Cr in plant
**a, **b, **c, **d: The difference letter refers to a significant difference at 0.05 ($p \leq 0.05$) between Cd in single and combination experiments for BCF of Cd in plant
***a, ***b, ***c, ***d: The difference letter refers to a significant difference at 0.05 ($p \leq 0.05$) between Co in single and combination experiments for BCF of Co in plant

Fresh, Dry and Relative Growth rate

The results showed in figures (2-4) that the stress of selected heavy metals in the experiment affected the average fresh weight, dry weight, and relative growth rate of Ceratophyllum demersum L. under the same concentrations and laboratory conditions. The plant's more significant effect was mixing metals than single metals. The reduction was T8>T5>T7>T6>T3>T2>T4 Compared to the increase in fresh weight in T1.

Total Chlorophyll and Protein

The average initial concentration of total chlorophyll, protein, and proline was 7.915 µg/g, 35.076 %, and 30.593 mg/g, respectively. The result improved that the heavy metal stress affected the total chlorophyll content in all treatments compared with increased in control. At the end of the experiments, the reduction was more in mixed metals than in single metal experiments. The reduction in T8 reached 2.714 µg/g compared with 8.938 µg/g in T1. Table 2

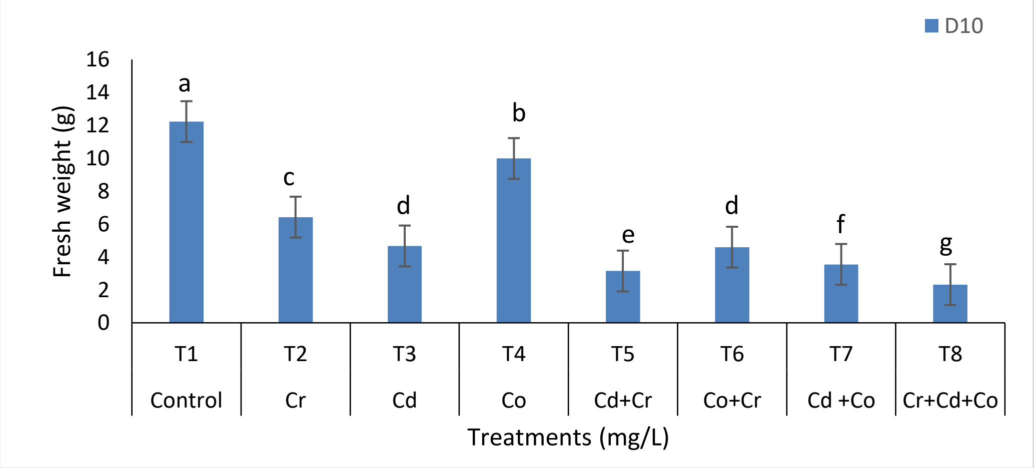

Figure 2: The average changes in fresh weight at the end of experiments

The difference letter refers to the significant difference between treatments at the end of experiments at 0.05 ($p \leq 0.05$)

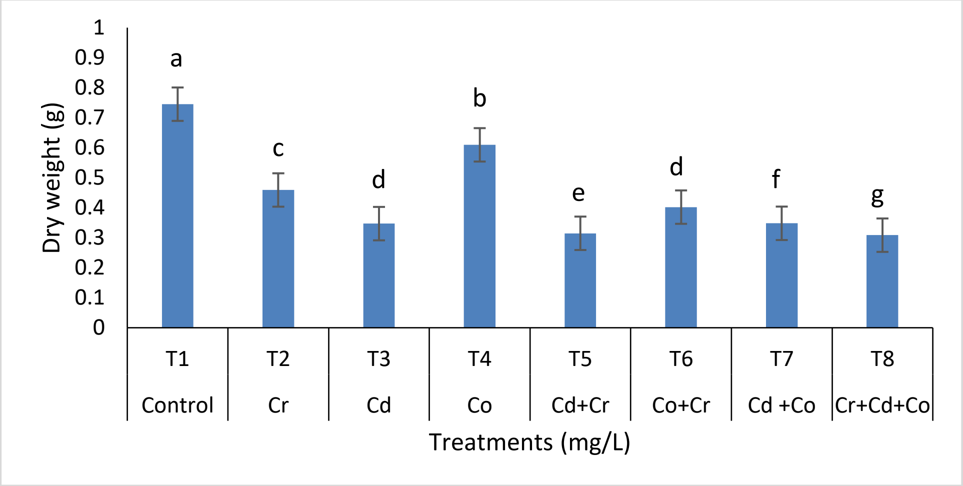

Figure 3: The average change in dry weight at the end of the experiments

The difference letter refers to the significant difference between treatments at the end of experiments at 0.05 ($p \leq 0.05$)

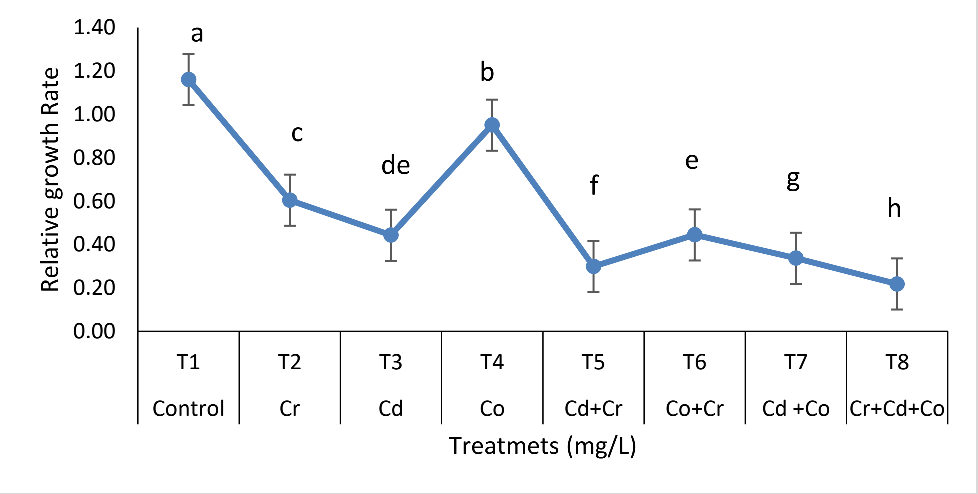

Figure 4: The average change in relative growth rate at the end of experiments

The difference letter refers to the significant difference between treatments at the end of experiments at 0.05 ($p \leq 0.05$)

Table 2: The average change in Total chlorophyll, protein, and proline after ten days of exposure

Treatments	Total chlorophyll (µg/g)	Protein %	Proline (mg/g)
T1	8.938 ± 0.06 a	38.6 ± 0.05 a	31.940 ± 0.82 g
T2	4.165 ± 0.078 c	25.49 ± 0.16 c	148.044 ± 2.501 f
T3	3.818 ± 0.059 de	22.7325 ± 0.032 d	184.2885 ± 1.523 e
T4	7.7385 ± 0.084 b	36.825 ± 0.065 b	41.705 ± 1.055 h
T5	3.194 ± 0.03 f	20.605 ± 0.045 f	289.262 ± 1.392 c
T6	3.8325 ± 0.154 d	22.265 ± 0.21 e	218.26 ± 2.390 d
T7	3.26 ± 0.051 g	28.37 ± 0.50 g	253.33 ± 2.430 b
T8	2.7145 ± 0.063 h	17.335 ± 0.315 h	378.207 ± 2.447 a

The different letter for each column refers to a significant difference at 0.05 ($p \leq 0.05$) for the parameter calculate

Tolerance Index Rate (TIR)

At the end of the experiments, the ability of plants to tolerate selected metals were varied with a significant difference of 0.05 ($p \leq 0.05$) between treatments. The highest Tir was 71.598 % for Cobalt (T4) in single metal experiments and 35.740 % for T6 in combined metals experiments, as shown in figure 5.

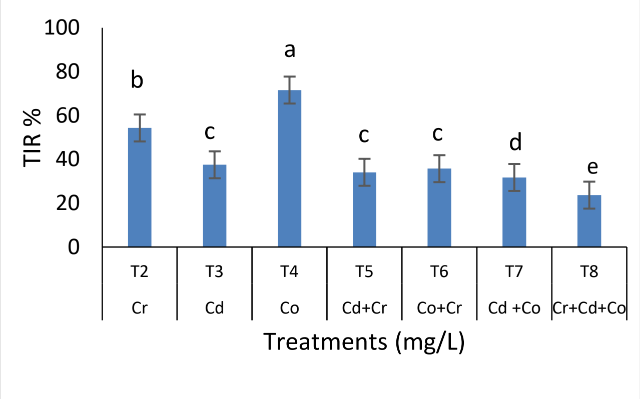

Figure 5: The average tolerance rate in all-metal treatments at the end of experiments

The different letter for each column refers to a significant difference at 0.05 ($p \leq 0.05$) for treatments.

Discussion

The metal residual in water decreased in all treatments. The reduction was due to the ability of plants to absorb heavy metals from an aqueous solution depending on the rhizofilteration mechanism. The plant can absorb metal from its root or whole body part. This result agreed with Phukan *et al*. (2015)

Plant biomass reduction is the main sign of physiological response in plants and is considered the most crucial agricultural index of heavy metals tolerance (Muratova *et al*., 2015). The inhibition of fresh and dry weight in combination experiments was more than in single metal experiments. The reduction may be attributed to the toxic metals bioaccumulation has been related to harmful impacts on critical metabolic activity and plant development. The decrease in mitotic index seen in the case of Cd and Cr was more than Co exposure may be linked to growth inhibition (Vecchia *et al*., 2005). The effect on the components of the plasma membrane and restricting the passage of fluids across it by altering its structure and function may be responsible for the decrease in fresh and dry weight and relative growth. Heavy metal exposure reduces plant growth by interfering with the photosynthesis process and inhibiting the transfer of metabolic components between cells (Mam, 2002). The reduction may be due to an increase in the production of abscisic acid, which closes the stomata and reduces the accumulation of potassium, which is vital in the plant's metabolic processes (Jouyban, 2012). This result agreed with Piotrowska (2010)

The reduction in total chlorophyll contents could be attributed to free radical-induced oxidation or reduction in chlorophyll pigment production, chloroplast breakdown, and chlorophyllase enzyme activity (Kato & Shimizu, 1985; Gill *et al*., 2012). HMs may restrict chlorophyll biosynthesis by reducing the intake of essential minerals for photosynthetic pigment production, such as magnesium, potassium, calcium, and iron (Piotrowska *et al*., 2009). The decreasing photosynthetic pigment may be due to increased ROS generation (Sharma *et al*., 2012). This result agreed with Malar *et al*. (2016)

Proteins are one of the most crucial components of each plant cell, as heavy metal stress causes a decrease in protein production and accumulation. Different antioxidant enzymes and other enzymes involved in GSH and PC production and some heat shock proteins make up stress protein synthesis. However, there was a considerable reduction in protein content at higher metal concentrations, which could be owing to metal-induced protein oxidation mediated by H_2O_2 and enhanced proteolytic activity. Proteolytic activity and protein degradation have been suggested as oxidative stress indicators Singh & Tewari (2003). This result agreed with Mahdi and Al-Abbawy (2019).

In higher plants, proline acts as a stress marker, accumulating enormous amounts in response to environmental stressors (Ashraf *et al*., 2012). Proline is vital for protein protection, osmoregulation, the prevention of oxidative damage, and the stabilization of cellular membranes (Slama *et al*., 2015). Proline accumulation has been found in HMs-stressed plants of diverse species (Gajewska *et al*., 2006). This study's increased proline level also indicated its antioxidant capacity in detoxifying HMs buildup in treated plants. This result agreed with Pandian *et al*. (2020).

The tolerance index rate is an indicator of the ability of the plant to tolerate environmental stressors based on the dry weight. In these experiments, the plant tolerates Co more than other metals in single and mixed metal experiments. The reason for that may be due to the essentiality of Co for plant metabolism activity. In contrast, the reduction of tolerance index rate in other treatments was due to the reduction in fresh and dry weight in the experiments compared with control. This result agreed with Umebese and Motajo (2008)

Conclusion

The aquatic plant Ceratophyllum demersum was effective as a biosorbent for removing Cd and Cr and Co from a nutrient medium in this study. Metal accumulation results in significant biochemical changes. The findings suggest that heavy metals have an inhibiting impact on plant growth and development. Cd was more harmful than Cr compared to Co and control treatments. The rate of degradation of chlorophyll and proteins was different from their value. Treatment with Cd and Pb resulted in higher proline content than treatment with Co.

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قابلية نبات الشمبلان *Ceratophyllum demersum* L. في معالجة المياه الملوثة بعناصر الكروم والكادميوم والكوبلت

ايناس عوني مهدي النبهان

قسم علم البيئة، كلية العلوم، جامعة البصرة

المستخلص

تعد النباتات المائية مرشحات لإزالة الملوثات من البيئة المائية. هدفت الدراسة الحالية لتقييم قدرة نبات الشمبلان على معالجة المياه الملوثة بالكادميوم والكروم والكوبلت من محاليلها المائية ودراسة بعض التأثيرات السمية لهذه العناصرعلى النبات. اذ عُرض نبات الشمبلان لتركيز 3 ملغم/ لتر من العناصر المختارة في الدراسة في تجارب مفردة وخلط العناصرمع بعض ووضع احواض سيطرة بدون معادن ثقيلة. أجريت التجربة مختبريا لمدة 10 أيام. قيست صفات النبات في بداية ونهاية التجربة وشملت الوزن الطري والجاف ومعدل النمو النسبي والتراكم الكلوروفيل الكلي والبروتين والبرولين ومعدل التحمل النسبي . اما الصفات التي قيست في نهاية التحربة في الماء تمثلت بتركيز العنصر المتبقي في الماء وحساب كفاءة الازالة. أظهرت النتائج ان كفاءة إزالة العناصر في التجارب المفردة كانت اعلى من تجارب خلط العناصر. اذا كانت Cd< Cr<Co . كما بينت التجارب انخفاض الوزن الطري والجاف ومعدل النمو النسبي ومحتوى الكلوروفيل الكلي والبروتين وكان الانحفاض الاكبرعند خلط المعادن الثلاثة معا. اذ بلغ 2.23 غرام و 0.309 غرام و0.218 و 2.714 مايكروغرام / غرام و 18.32 % على التوالي. كما اثبتت التجارب ارتفاع المحتوى البروليني في كل التجارب مقارنة مع احواض السيطرة اذ بلغ اعلى ارتفاع عند خلط المعادن الثقيلة الثلاثة معا ووصل الى 355.76 ملغم/ غرام. استنتجت التجارب ان نبات الشمبلان مرشح فعال لمعالجة المياه الملوثة بالتراكيز القليلة للمعادن الثقيلة.

الكلمات المفتاحية: - كفاءة الازالة، المعادن الثقيلة، التأثيرات السمية، التأثيرات البايوكيميائية، المعالجة النباتية