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Accumulation and Removal of Selected Heavy Elements by *Ceratophyllum demersum* L. from Aqueous Solutions

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Abstract:

This study investigates the phytoremediation potential of the submerged aquatic plant *Ceratophyllum demersum* L. for the removal and accumulation of selected elements, boron (B), barium (Ba), strontium (Sr), and iron (Fe) from aqueous solutions under controlled laboratory conditions. A 30-day experiment was conducted using five initial concentrations (1, 5, 20, 40, and 80 mg/L) with control treatments. Results showed a continuous decline in residual metal concentrations over time, with removal efficiencies reaching 97% for the Fe, 83.6% for Sr, 61.7% for B, and 29.5% for Ba. Metal accumulation in plant tissues increased with concentration, with a maximum of 22.32 mg/g recorded for barium. The bioconcentration factor (BCF) was highest for Fe at low concentrations (up to 0.97). Physiological responses indicated stress effects. Including reduced growth rate (0.54-0.69) and chlorophyll content, alongside increased proline levels. Despite these effects, the plant maintained high tolerance. These findings demonstrate that *C. demersum* is an effective, low-cost phytoremediation plant for multi-element contaminated water.

Keywords: coontail, phytoremediation, heavy elements, bioconcentration factor, aquatic macrophytes.

Introduction:

The contamination of aquatic environments by heavy metals has emerged as a critical global environmental issue due to their persistence, toxicity, and non-biodegradable nature. Unlike organic pollutants, heavy metals cannot be chemically or biologically degraded and tend to accumulate in water bodies, sediments, and biota, posing long-term ecological and human health risks (Tchounwou *et al*., 2012; Ali *et al*., 2013). The intensification of industrial activities, agricultural practices, urban expansion, and energy production has significantly increased the discharge of these elements into freshwater systems, particularly in developing regions where wastewater treatment infrastructure remains inadequate (Ali & Sajad, 2013; Briffa *et al*., 2020). As a consequence, heavy metals can enter aquatic food chains through bioaccumulation and biomagnification processes, ultimately threatening ecosystem stability and public health (Lee *et al*., 2007; Reza & Singh, 2010).

Conventional technologies for heavy metal removal, including chemical precipitation, ion exchange, membrane filtration, and electrochemical treatments, are widely used but often incur high operational costs, require intensive energy, and generate secondary pollutants that require further treatment (Fu & Wang, 2011; Barakat, 2011). These limitations have stimulated growing interest in sustainable and environmentally benign alternatives, among which phytoremediation has gained considerable attention. Phytoremediation is a plant-based technology that harnesses plants' natural capacity to absorb, accumulate, and detoxify pollutants from contaminated environments. It offers several advantages, including cost-effectiveness, low energy demand, minimal maintenance, and reduced secondary waste generation, making it particularly suitable for large-scale and low-resource applications (Al-Nabhan & Al-Abbawy, 2021). Among aquatic macrophytes, submerged species are of particular importance due to their continuous contact with the water column and their enhanced capacity for direct uptake of dissolved contaminants. *Ceratophyllum demersum* L., a rootless submerged aquatic plant widely distributed in freshwater ecosystems, has been identified as a promising candidate for phytoremediation. Its high growth rate, substantial biomass production, and ability to accumulate a broad spectrum of pollutants, including heavy metals, contribute to its effectiveness in wastewater treatment systems (Duman *et al*., 2010; Bonanno & Giudice, 2010). Moreover, the absence of a root system allows the plant to absorb contaminants directly through its entire surface area, thereby increasing its remediation efficiency in aqueous environments. Despite extensive research on the removal of conventional heavy metals such as cadmium, lead, and nickel using *C. demersum*, limited attention has been given to its performance in multi-metal systems involving elements such as boron, barium, and strontium. These elements are increasingly detected in industrial effluents and energy-related discharges, yet their behavior in phytoremediation systems remains insufficiently characterized. Boron, although essential in trace amounts, can become toxic at elevated concentrations, with typical freshwater levels below 0.1 mg/L, but concentrations can increase significantly in polluted environments (Howe, 1998). Barium, a relatively abundant element in the Earth’s crust, can accumulate in environmental compartments due to anthropogenic activities, particularly industrial processes (Kunesh, 1978). Strontium, an alkaline earth metal with chemical properties similar to calcium and barium, is widely distributed in natural systems and may pose ecological risks at elevated concentrations (Aziz *et al*., 2017). Iron, while essential for plant metabolism, can disrupt physiological processes when present in excessive amounts, despite its generally low solubility in natural waters (Taylor, 1964; Wang & Dou, 1998). Furthermore, the interactions among metal accumulation, plant growth dynamics, and biochemical responses across varying concentration gradients remain poorly understood. In particular, the balance between phytoremediation efficiency and physiological stress responses represents a critical aspect in evaluating the practical applicability of aquatic plants.

Therefore, the present study aims to investigate the capacity of *Ceratophyllum* *demersum* to remove and accumulate selected heavy elements (B, Ba, Sr, and Fe) from aqueous solutions under controlled laboratory conditions. In addition, the study examines the associated physiological and biochemical responses, including growth performance, chlorophyll content, protein levels, and proline accumulation, to elucidate the mechanisms underlying metal tolerance and stress adaptation. The outcomes of this research are expected to contribute to the development of efficient, low-cost, and sustainable phytoremediation strategies for the treatment of multi-metal contaminated aquatic environments.

1. Materials and Methods:

2.1 Experimental Design

A controlled laboratory experiment was conducted to evaluate the phytoremediation potential of *Ceratophyllum demersum* for selected heavy metals, namely boron (B), barium (Ba), strontium (Sr), and iron (Fe). The experiment was designed as a batch system under controlled environmental conditions over 30 days. Five concentrations of each metal were prepared: 1, 5, 20, 40, and 80 mg/L, along with a control treatment with no added metals. Each treatment was performed in triplicate to ensure reproducibility and statistical reliability.

2.2 Plant Material Collection and Preparation

Healthy specimens of *Ceratophyllum demersum* L. were collected from freshwater environments in the Shatt Al-Arab River. The plants were thoroughly rinsed with tap water followed by distilled water to remove adhered particles, epiphytes, and contaminants. Prior to experimentation, plants were acclimatized under laboratory conditions for a defined period to minimize physiological stress and ensure uniformity in growth conditions.

2.3 Preparation of Metal Solutions

Stock solutions of Boric acid (H_3BO_3), Barium chloride (BaCl2 · 2H2O), Strontium chloride (SrCl2 · 6H2O), and Ferric Chloride ($FeCl_3$) were prepared using analytical-grade reagents. Working concentrations (1–80 mg/L) were prepared by serial dilution in distilled water. Each experimental unit consisted of a defined volume of metal solution into which a known biomass of *C. demersum* was introduced (Abbas et *al*., 2014).

2.4 Physicochemical Measurements

2.4.1 pH and Electrical Conductivity (EC)

The pH and electrical conductivity of the aqueous solutions were measured using calibrated digital meters at the beginning and end of the experimental period.

2.4.2 Residual Metal Concentration

Water samples were collected periodically and subjected to digestion using standard procedures prior to analysis. The residual concentrations of metals in solution were determined using appropriate analytical techniques following APHA standard methods.

2.5 Removal Efficiency Calculation

The removal efficiency (%) of each metal was calculated according to the following equation (Kumar & Deswal, 2020):

$$\text{Removal Efficiency (\%)}=\frac{Intial\ Concentration\left(\frac{mg}{L}\right)-final\ Concentration\left(\frac{mg}{L}\right)}{Intial\ concentration\ \left(\frac{mg}{L}\right)}\times 100$$

2.6 Plant Analysis

2.6.1 Fresh and Dry Biomass

Fresh biomass was determined by gently rinsing plant samples with distilled water to remove adhered particles, blotting excess moisture with filter paper, and weighing approximately 25 g of plant material. Dry biomass was measured after oven-drying equivalent samples at 70 °C for 48 h until constant weight.

2.6.2 Relative Growth Rate (RGR)

Plant growth was assessed using relative growth rate, calculated according to Xiaomei *et al.* (2004):

$$\text{RGR}=\frac{\text{final fresh weight (gram)}}{\text{,initial fresh weight (gram)}}$$

2.6.3 Tolerance Index (TI)

The tolerance index was calculated following Wilkins (1978):

$$\text{TI(\%)}=\frac{\text{dry weight of the plant exposed to the element}}{\text{dry weight of the plant in the control tank without concentration}}*100$$

2.6.4 Determination of Metal Concentration in Plant Tissue

At the end of the experiment, plant samples were harvested, washed, dried, and digested. The concentration of accumulated metals in plant tissues was determined using standard analytical techniques, the concentration was in mg/gram.

2.6.5 Bioconcentration Factor (BCF)

The bioconcentration factor was determined according to Abdallah (2012):

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

2.7 Biochemical Analyses

2.7.1 Chlorophyll, Proline, and Protein Content

Total chlorophyll content was determined spectrophotometrically after extraction using standard solvent methods (Oron *et al*.,1988). Proline levels were measured as an indicator of physiological stress response using established biochemical protocols. Total protein content was determined to evaluate metabolic alterations under heavy metal exposure.

2.8 Statistical Analysis

Statistical comparisons between sampling sites were conducted using an independent samples t-test at a significance level of $p \leq 0.05$. Data analysis was performed using GenStat software (version 12) to evaluate differences among means (Al-Asadi, 2019).

2. Results and Discussion:

3.1 Metal Accumulation in Plant Tissues

The results demonstrated a clear concentration-dependent increase in metal accumulation within the tissues of *Ceratophyllum demersum*. As shown in Table 1, the concentrations of boron (B), barium (Ba), strontium (Sr), and iron (Fe) in plant tissues increased progressively with increasing external exposure levels. The highest accumulation was observed for barium, reaching 22.32 mg/g at 80 mg/L, indicating a strong affinity of *C. demersum* for this element. This pattern suggests that *C. demersum* exhibits efficient phytoaccumulation capacity, likely mediated by both intracellular sequestration and surface adsorption. Similar trends have been reported for submerged macrophytes, in which metal uptake is driven by concentration gradients and by binding to cell wall functional groups such as carboxyl and hydroxyl groups.

Table 1: The average concentration of metal in *C. demersum* at the end of the experiments using

Concentration mg/L	Concentration of the metals in *C. demersum* (mg/g)			
	B	Ba	Sr	Fe
Control	0.13	0.56	0.51	-
1	0.62	0.31	0.83	0.97
5	1.41	1.50	1.26	0.79
20	5.53	5.99	5.14	2.24
40	7.53	11.64	7.08	4.37
80	11.25	22.32	8.4	6.70

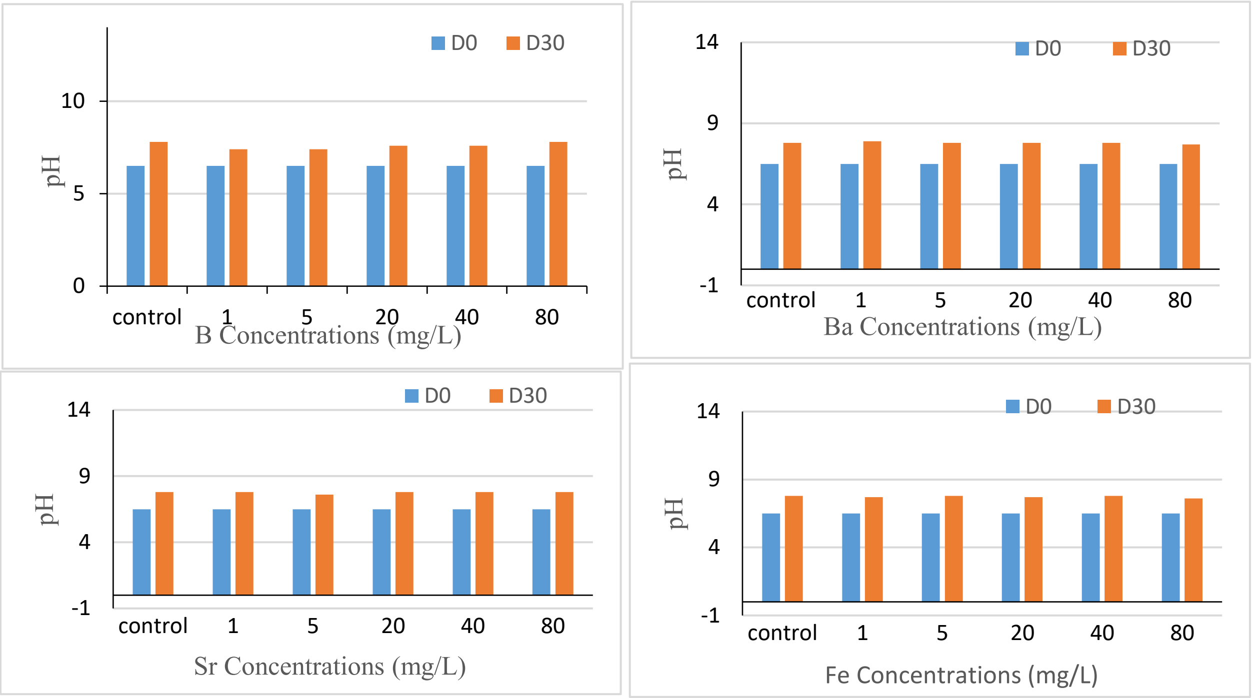

Figure 1: Changes in pH of the aqueous medium at the initial and final stages of the experiment under exposure to selected heavy metals using *Ceratophyllum demersum*

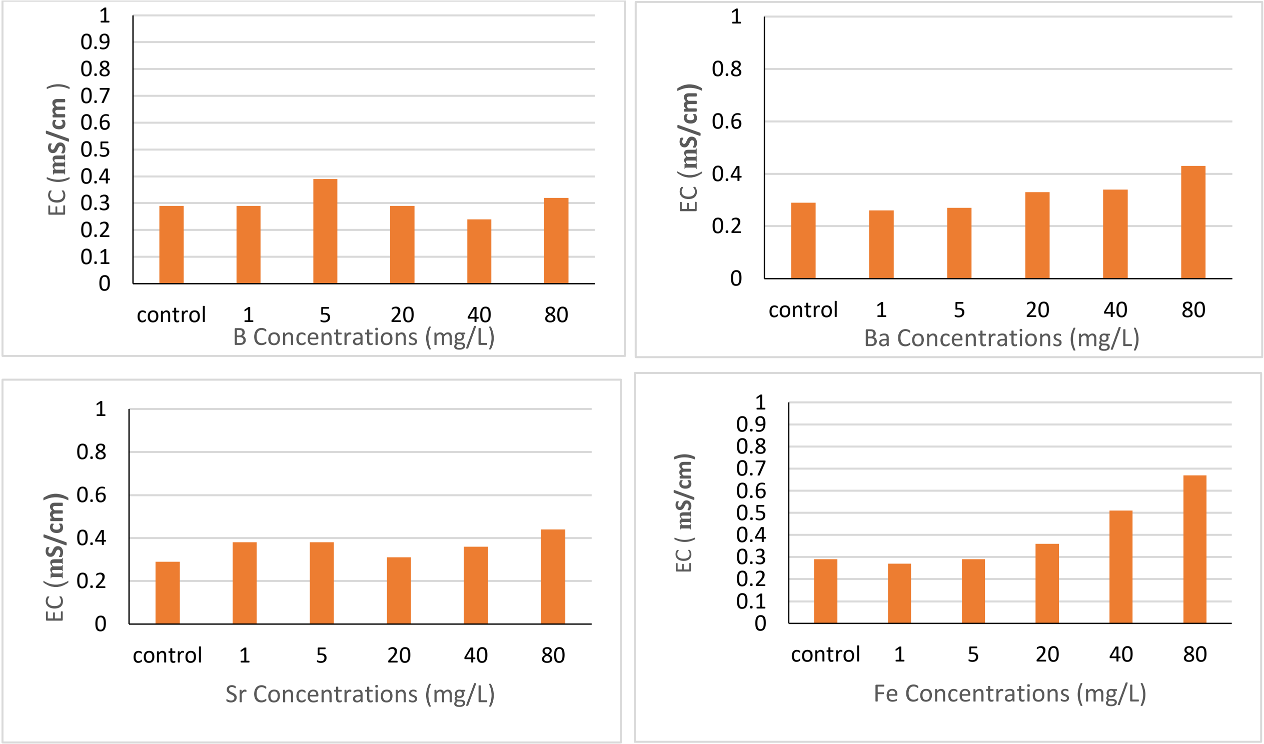

Figure 2: Variation in electrical conductivity (EC) of the aqueous medium at the beginning and end of the experimental period under exposure to selected heavy metals using *Ceratophyllum demersum*

3.2 Growth Response and Biomass Variation

The toxicological impact of heavy metals on plant growth was evident through reductions in fresh biomass, relative growth rate (RGR), and tolerance index (TI). As presented in Table 2, fresh weight declined significantly with increasing metal concentrations, with the most pronounced reduction observed at 80 mg/L. Among the tested metals, barium exhibited the strongest inhibitory effect on biomass, followed by strontium and boron. This decline in biomass can be attributed to disruption of cellular metabolism, inhibition of nutrient uptake, and impairment of enzymatic activity under metal-stress conditions. Despite these inhibitory effects, *C. demersum* maintained relatively high tolerance index values across treatments, particularly at lower concentrations. This indicates the presence of adaptive mechanisms that enable the plant to withstand moderate levels of heavy metal stress.

3.3 Bioconcentration Factor (BCF)

The bioconcentration factor values varied depending on both metal type and concentration. Higher BCF values were generally observed at lower concentrations, particularly for iron, indicating greater uptake efficiency under low contamination conditions. At higher concentrations, BCF values declined, suggesting potential saturation of uptake sites or physiological limitations due to toxicity. This inverse relationship between concentration and BCF is consistent with previously reported phytoremediation studies and reflects a transition from active uptake to stress-dominated conditions.

Table 2: The average toxicological effect of selected metals on *C. demersum* at the end of the experiment.

Con. mg/L	Fresh Weight(g)				Relative growth (RGR)%				Tolerance Index Rate (TIR) %				BCF			
	B	Ba	Sr	Fe	B	Ba	Sr	Fe	B	Ba	Sr	Fe	B	Ba	Sr	Fe
1	25.21	25.21	25.21	25.21	0.69	0.82	0.75	0.82	77.4	92.6	89.3	96.6	0.620	0.317	0.836	0.97
5	20.45	18.72	20.42	17.38	0.66	0.75	0.71	0.76	73.7	90.2	88.7	96.3	0.282	0.3	0.252	0.158
20	19.05	17.8	18.33	16.45	0.57	0.66	0.67	0.74	68.7	75.8	88.3	86.4	0.276	0.299	0.25	0.112
40	18.54	16.8	16.39	14.46	0.56	0.59	0.57	0.67	65.06	75.6	83.8	76.7	0.193	0.291	0.177	0.109
80	16.59	14.31	14.84	14.07	0.54	0.50	0.51	0.58	60.7	73.3	68.1	76.3	0.140	0.279	0.105	0.083

3.4 Chlorophyll Content and Photosynthetic Activity

A significant reduction in total chlorophyll content was observed as metal concentrations increased (Table 3). Compared to control treatments (8.46 µg/g), chlorophyll levels declined progressively, reaching minimum values at higher concentrations. This reduction indicates impairment of the photosynthetic apparatus, which may result from inhibition of chlorophyll biosynthesis, degradation of chloroplast structure, and disruption of enzymatic pathways involved in pigment formation. Heavy metals are known to interfere with protein synthesis and bind strongly to sulfhydryl groups of enzymes, thereby inhibiting key metabolic processes required for chlorophyll production (Cenkci *et al*., 2010; Parmar *et al*., 2013; Elloumi *et al*., 2014).

3.5 Protein Content

Protein content decreased with increasing metal concentrations. The control treatment showed the highest protein level (33.3%), while significant reductions were recorded under metal exposure. This decline can be explained by inhibition of protein synthesis pathways, structural damage to ribosomes, and enhanced protein degradation under oxidative stress Heavy metals are known to form complexes with proteins, altering their structure and function, and ultimately affecting cellular stability (Wu *et al*., 2010).

Table3: Integrated toxicological responses of *C. demersum* under different heavy metal concentrations

Concentration mg/L	Chlorophyll				Protein%			
	B	Ba	Sr	Fe	B	Ba	Sr	Fe
Control	8.46	8.46	8.46	8.46	33.3	33.3	33.3	33.3
1	5.21	6.93	6.41	7.49	31.4	30.4	30.7	18.7
5	3.92	5.89	5.93	7.31	28	28.9	27.4	28.5
20	3.81	4.96	4.99	6.31	24.7	24.4	22.3	26.5
40	3.46	3.69	3.03	5.03	22.6	18.9	15.2	23.5
80	3.38	3.41	2.58	4.05	16.4	14.6	12.8	19.7

3.6 Proline Accumulation and Stress Response

Proline accumulation increased markedly with increasing metal concentrations, reaching a maximum value of 305 µg/g under iron exposure at 80 mg/L. This accumulation reflects a typical plant response to abiotic stress. Proline plays a critical role in osmotic regulation, protein stabilization, and membrane stabilization. The observed increase in proline content suggests activation of defense mechanisms against heavy metal-induced oxidative stress.

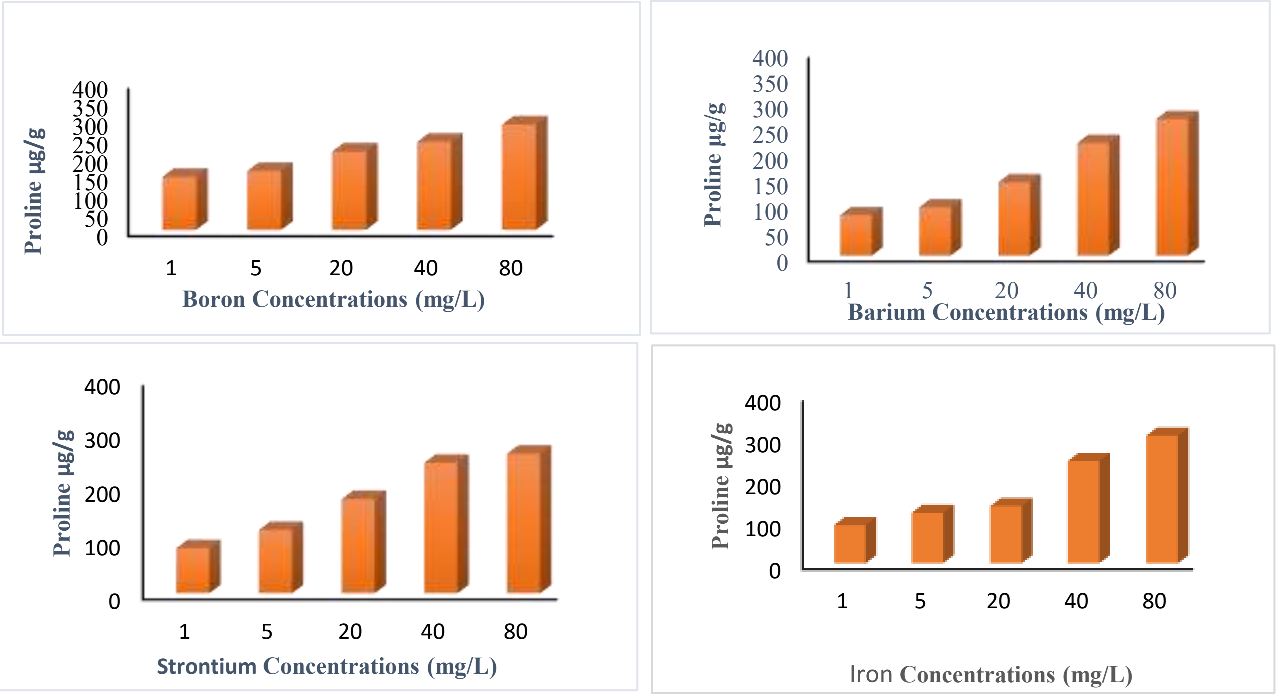

Figure: The levels of proline in Boron concentrations at the end of experiments using *C. demersum*

Conclusion:

Ceratophyllum demersum demonstrated high efficiency in removing and accumulating multiple elements from aqueous solutions, particularly iron and strontium. Despite exposure to elevated concentrations, the plant maintained significant tolerance and adaptive capacity. The results confirm that *C. demersum* is a promising candidate for phytoremediation of contaminated aquatic environments due to its high removal efficiency, strong capacity for accumulation, and physiological resilience.

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تراكم وإزالة العناصر الثقيلة المختارة بواسطة النبات المائي الغاطس *Ceratophyllum demersum L.* من محاليل مائية

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المستخلص

تتناول هذه الدراسة تقييم القدرة المعالجة النباتية للنبات المائي الغاطس *Ceratophyllum demersum L.* في إزالة وتراكم مجموعة من العناصر المختارة، وهي البورون(B) ، الباريوم(Ba) ، السترونشيوم(Sr) ، والحديد (Fe) من المحاليل المائية تحت ظروف مختبرية مضبوطة .تم إجراء تجربة لمدة 30 يوماً باستخدام خمسة تراكيز ابتدائية (1، 5، 20، 40، و80 ملغم/لتر) مع وجود معاملات سيطرة. أظهرت النتائج انخفاضاً مستمراً في تراكيز المعادن المتبقية مع مرور الوقت، حيث بلغت كفاءة الإزالة 97% للحديد، و83.6% للسترونشيوم، و61.7% للبورون، و29.5% للباريوم .كما ازداد تراكم المعادن في أنسجة النبات مع زيادة التركيز، حيث تم تسجيل أعلى قيمة للباريوم بلغت 22.32 ملغم/غم. وكان عامل التركيز الحيوي (BCF) الأعلى للحديد عند التراكيز المنخفضة، إذ وصل إلى 0.97 .أظهرت الاستجابات الفسيولوجية وجود تأثيرات إجهادية، تمثلت في انخفاض معدل النمو (0.54–0.69) ومحتوى الكلوروفيل، إلى جانب زيادة مستويات البرولين. وعلى الرغم من هذه التأثيرات، حافظ النبات على درجة تحمل عالية .تشير هذه النتائج إلى أن *C. demersum* يُعد نباتاً فعالاً ومنخفض الكلفة لمعالجة المياه الملوثة بعدة عناصر باستخدام تقنيات المعالجة النباتية.