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Water hyssop as bioaccumulator for remediation of Lead, Nickel and Iron

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Abstract

Phytoremediation is eco-friendly and cost-effective technology compared to traditional treatments. It uses plants and associated microbes to remove organic and inorganic pollutants from soil, water, and sediment. This study aims to evaluate the ability of water hyssop *Bacopa monnieri* L. to remediate lead (Pb), nickel (Ni), and iron (Fe) and to illustrate some physiological and biochemical changes that occur in the plant. The experiments were done in the laboratory for forty days. The parameters measured were metal residual in water, removal efficiency, bioconcentration factor (BCF), fresh and dry weight, relative growth rate (RGR), total chlorophyll, protein, and proline content. The results showed that the metal residual in water declined in all treatments with different values. The reduction was higher in low concentrations than in high concentrations. Calculating the removal efficiency, the highest value was 80% at 1 mg/L of Fe, and the lowest was 16.875% at 8 mg/L of Ni. The result revealed that the highest BCF value was at 1 mg/L of Fe and the lowest value was at 8 mg/L of Ni. The result showed that the highest reduction in fresh weight, RGR, total chlorophyll, and protein was respectively at 8 mg/L of Ni and the Fe boosted the plant's growth. The results also indicate that the proline content increased as Ni and Pb concentrations increased compared with the control. The conclusion of this study indicates that the plant can survive and tolerate selected metal concentrations, making it a good candidate for use in phytoremediation.

Keywords: heavy metal, phytoremediation, toxicity, bio concentration factor (BCF), biochemical response.

Introduction

Water is now a necessity around the world because of its environmental importance. Water has been the most important human requirement for survival. Because of the destruction of natural water supplies, the major environmental impact of water contamination and insufficiency is developing, as is its limited availability (Carolin *et al*., 2017). The discharge of polluted wastewater has been increasing during the past decades because of many human activities like urbanization, industrialization, and agricultural activity (Renuka *et al*., 2013; Gonçalves *et al*., 2017; Al- Nabhan, 2022). Heavy metal is a term used to describe an element with a higher density and is harmful even at low concentrations. It is defined as an element with an atomic weight of between 63.5 and 200.6 and a density greater than 5.0 g/cm^3 (Srivastava *et al*., 2008; Al-Nabhan and Al-abbawy, 2021). Heavy metal pollution is a major environmental impact due to its availability, accumulation, non-biodegradability, and persistence in the environment for a long time (Peligro *et al*., 2016). Heavy metal concentrations in the environment have increased because of anthropogenic activities such as burning fossil fuels, the discharge of municipal wastes, using fertilizers and pesticides, and so on. This elevation in heavy metal concentration is a major cause of concern for both humans and the environment (Wani *et al*., 2017). Many traditional techniques are prohibitively expensive, time-consuming, and ineffective. Phytoremediation is a unique concept and multidisciplinary approach that can potentially treat polluted systems using plants and associated microbes to remove different types of pollutants (Uqab *et al*., 2016). Aquatic plants are of key significance because, compared to terrestrial plants, they can bioaccumulate harmful metals and nutrients in huge quantities (Pratas *et al*., 2012). Furthermore, these macrophytes have been proven to absorb contaminants at varied rates and efficiencies depending on their biochemical makeup, species, abundance, and habitat. Plants can remove metal pollution from both water and soil. Heavy metals are known to be removed by aquatic plants of all varieties, whether free-floating, submerged, or emergent (Kinnersley, 1993). *Bacopa monnieri* L. is a medicinal herb that belongs to the *Scrophulariaceae* family. It can be found in ponds, wetlands, and marshes in sunny and shady regions. It can tolerate high temperatures but not drought. According to its description, it is an annual plant with white flowers that are not aromatic. It can grow to 10–30 cm by forming roots at the nodes. It has numerous medical applications, including antioxidants, increased brain activity, lower blood pressure, and others (Srivastava *et al*., 2012). Dogan, 2019 studied the ability of *Bacopa monnieri* to accumulate lead, cadmium, and chromium and the effects of these metals on the plant's chlorophyll and protein. The results showed that the plant could accumulate metals with different values. The results also indicated that the chlorophyll and protein of the plant decreased. It was Cd > Cr > Pb, respectively. This study aims to investigate the ability of *B. monnieri* L. to remove and accumulate Pb, Ni, and Fe and to study some of the toxicological effects of these metals on the plant.

Materials and Methods

Plant collection and acclimatization

The aquatic plant *Bacopa monnieri* L. was collected from Al-Hammar Marshes in labeled plastic bags after being washed several times with a water marsh to remove solids. The plant is transferred to the laboratory. In the lab, the plant was washed with tap water and put in a plastic aquarium filled with tap water for one month of acclimatization and growth.

Metal concentration and aquarium prepared.

The standard salt of Lead acetate (CH_3COOPb) and iron sulfate ($FeSO_4 . 6H_2O$) was used to prepare Pb (II) and Fe (II). Otherwise, the stock solution of 1000 mg/L of Ni was used to prepare the desired concentration of Ni. The concentration was (1, 4, and 8) mg/L. plastic aquarium capacity 1.6 L filled with 1 L of desired concentrations as triplicate for each metal treatment and three aquariums without metal concentrations used as control.

Preliminary test

According to the literature review and practical experiments, the plant was exposed to different strengths of Hoagland nutrient solution to choose the desired concentration for the plant to supply the nutrients to the end of the experiment. The plant was exposed to (1, 3, 5, 10) % of Hoagland strength solution. The preliminary tests indicated that the suitable strength for the plant to the end of the experiment was 1%.

Experimental setup

The experiments were carried out in the laboratory. Each metal concentration has a triplicate aquarium. It is filled with 1 L of desired concentrations (1, 4, 8) mg/L. 15 g (about 7-8 individual plants approximately the same in size and height) in each aquarium of healthy plants were selected after acclimatization. It was washed several times with tap and distilled water to remove solid particles. Depending on the preliminary test, the suitable strength of the Hoagland nutrient solution was 1% added for each aquarium. The control treatments contained plant growth in Hoagland nutrient solution only as control. The selected parameters in the plant were measured before and after the experiments. Otherwise, the metal residual in water and removal efficiency calculated measured at the end of experiments.

Parameters measuring

Metal residual in water and removal efficiency

The metal residual in water is measured as the method mentioned in APHA, (2005). The concentrations are expressed in mg/L. The removal efficiency was calculated as an equation in Khan *et al*. (2009)

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

Metal concentration in plant and Bioconcentration factor (BCF)

Plant metal concentrations were measured using the method described in ROPME, 1982. The concentration was measured using atomic absorption spectroscopy. The concentration expressed in mg/g. The BCF was calculated as described in Abdalla (2012)

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

Fresh, dry weight, and relative growth rate (RGR)

The fresh weight was measured at the end of the experiments. The plant is taken out from each aquarium and washed several times with tap and distilled water to remove debris. After that, the Sartorius (four digits) balance was used to record the final fresh weight in g. The RGR was calculated using the equation described in Xiaomei *et al*. (2004).

$$\text{Relative growth rate} = \frac{final\ fresh\ weight\ (gram)}{intial\ fresh\ weight\ (gram)}$$

Total chlorophyll content

The Total chlorophyll content was measured as the procedure mentioned in Arnon (1949). About 0.2 g of fresh weight was extracted from 20 ml of 80% Acetone. The clear solution was separated using centrifuged at 5000 rpm for ten minutes. The spectrophotometric was used to measure the Total chlorophyll at the optical density of 645 and 663. The concentrations are expressed in µg/g.

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

OD: optical density at 645 and 663 nm

Protein ratio

The protein ratio was found as the method described in Page *et al.* (1982). It represented calculating the total Nitrogen and multiplying by 6.25.

Proline content (mg/g)

The proline content was measured using the method mentioned in Trollant and Lindsley (1955). The proline content is expressed in mg/g.

Statistical analysis

The data analysis using SPSS (Statistical Package for Social Science) version 23. Descriptive Statistical applied to analyze the data. One-way ANOVA was also applied at a probability of 0.05 ($p \leq 0.05$) for metal concentrations and control.

Results

Metal residual in water and removal efficiency

The result indicated that the metal residual in water declined in all treatments at the end of experiments, with a significant difference of 0.05 ($p \leq 0.05$) between metal treatment and control. The reduction was greater in low concentrations than in high concentrations. The higher reduction was 0.2 mg/L at 1 mg/L of Fe, and the lowest reduction was 6.65 mg/L at 8 mg/L of Ni. Table 1

The result revealed that the highest removal efficiency was 80% at 1 mg/L of Fe, and the lowest was 16.975 % at 8 mg/L of Ni (Table1).

Table 1: The average metal residual in water and removal efficiency at the end of experiments for Pb, Ni, and Fe

Metal	Concentrations (mg/L)	Metal residual in the water (mg/L)	Removal efficiency %
Pb	1	0.224 ± 0.001 a	77.6 ± 0.1 a
	4	1.57 ± 0.06 b	60.75 ± 1.50 b
	8	3.54 ± 0.23 c	55.75 ± 2.87 bc
Ni	1	0.432 ± 0.023 a	56.8 ± 2.30 a
	4	2.72 ± 0.377 b	32 ± 9.425 b
	8	6.65 ± 0.216 c	16.875 ± 2.70 c
Fe	1	0.2 ± 0.01 a	80 ± 1.00 a
	4	1.146 ± 0.066 b	71.35 ± 1.65 b
	8	2.43 ± 0.011c	69.625 ± 0.186 bc

The difference letter between rows for each metal refer to significant difference 0.05 ($p \leq 0.05$)

BCF

The BCF value depends on concentration in plant. The result showed that the BCF was highest in 2 mg/L of Fe and lowest in 8 mg/L of Ni (Figure 1).

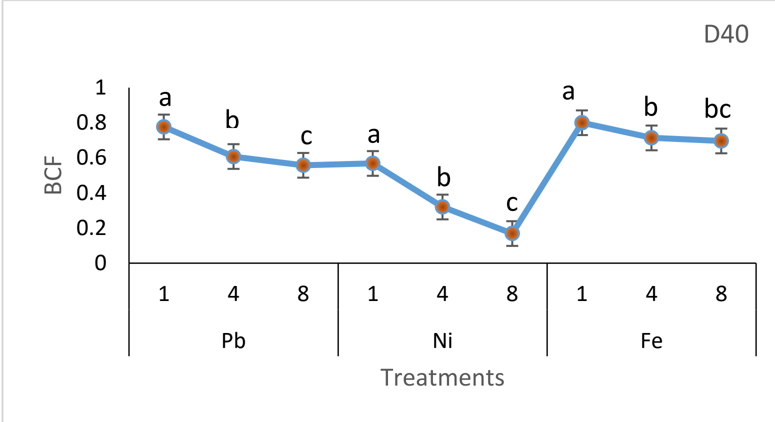

Figure1: the average BCF of Pb, Ni and Fe at the end of experiments

The difference letter refers to a significant difference of 0.05 ($p \leq 0.05$)

Fresh weight and relative growth rate

Except for iron, all selected metal concentrations showed morphological toxicity signals. In contrast to Fe, which stimulated plant growth, excess Pb and Ni inhibited plant development and altered some metabolic parameters.

The initial fresh weight of the plant was 15±0.5g. The results indicated that the fresh weight was reduced in all treatments of Pb and Ni and increased in all treatments of Fe. The statistical analysis ANOVA indicated a significant difference of 0.05 ($p \leq 0.05$) between treatments (Table 2).

Table 2: The average change in fresh weight and RGR ± SD at the end of experiments for all treatments and control

treatments	Concentrations (mg/L)	Fresh weight (g)	Relative growth rate RGR
Control	0	17.005 ± 0.495 a	1.093 ± 0.001 a
Pb	1	13.965 ± 0.099 b	0.893 ± 0.001 b
	4	11.786 ± 0.220 c	0.760 ± 0.001 c
	8	10.987 ± 0.222 d	0.579 ± 0.0035 d
Control	0	17.005 ± 0.495 a	1.093 ± 0.001 a
Ni	1	10.786 ± 0.276 b	0.702 ± 0.005 b
	4	7.112 ± 0.267 c	0.467 ± 0.001 c
	8	4.765 ± 0.155 a	0.306 ± 0.041 d
Control	0	17.005 ± 0.495 a	1.093 ± 0.001 a
Fe	1	18.124 ± 0.116 b	1.161± 0.001 b
	4	17.057 ± 0.027 c	1.102 ± 0.001 c
	8	15.987 ± 4.409 a	1.035 ± 0.001 a

The difference letter refers to a significant difference of 0.05 ($p \leq 0.05$)

Total chlorophyll content

The average initial concentration of total chlorophyll was 8.034 µg/g. At the end of experiments after forty days, the results showed a reduction in total chlorophyll content for all concentrations of Pb and Ni; otherwise, it increased in all concentrations of Fe. The higher reduction was Ni> Pb. The lowest reduction was 4.9902 µg/g at 8 mg/L of Ni. The statistical analysis using ANOVA one-way test indicated that significant difference at a probability of 0.05 ($p \leq 0.05$). Figure2.

The total chlorophyll concentration gradually reduced as Ni and Pb concentrations increased. The value was 8.164, 8.472 and 8.596 µg/g at 1, 4 and 8 mg/L of Iron, respectively. A concentration of 8 mg/L of Ni was discovered to be its lowest total chlorophyll level, while the control showed 8.014 µg/g.

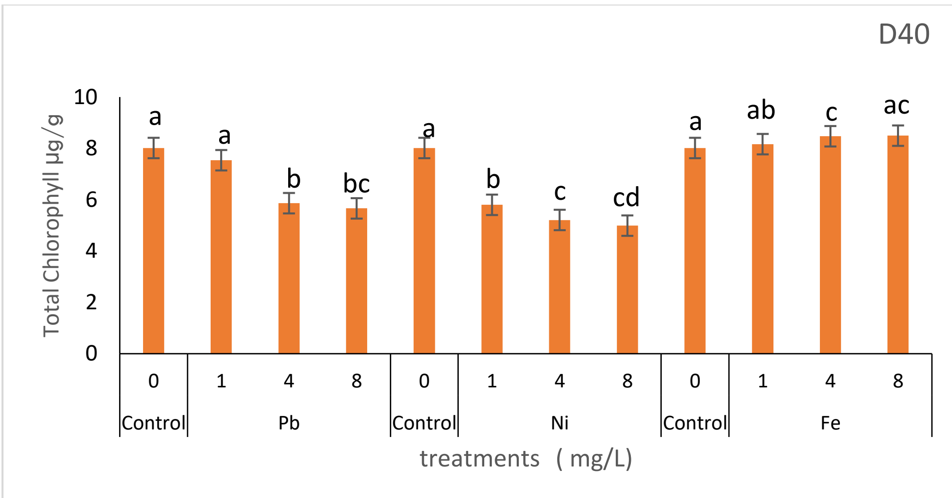

Figure 2: the average changing of Total chlorophyll at the end of experiments for Pb, Ni, Fe and control.

The difference letter refers to a significant difference of 0.05 ($p \leq 0.05$)

Protein %

The average initial concentration of protein was 40.653 %. The results indicated that the protein content decreased in all treatments of Ni and Pb. The highest reduction was 25.332% at 8 mg/L of Ni, and at all treatments of Fe, the plant showed an increase in protein content, as shown in figure 3. The statistical analysis indicated a significant difference between treatments at 0.05 ($p \leq 0.05$).

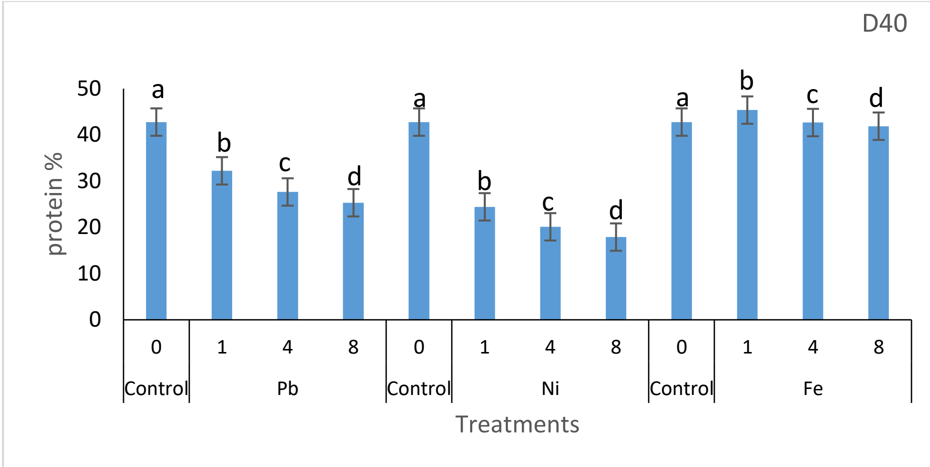

Figure 3: the average protein % at the end of experiments for Pb, Ni, Fe, and Control

The difference letter refers to a significant difference of 0.05 (p≤0.05)

Proline content (mg/g)

The initial concentration of proline in the plant was 32.545 mg/g. At the end of the experiments, the result indicated that the proline content increased in all treatments with different values. The highest increase was 220 mg/g at 8 mg/L of Ni, and the lowest value was 34.533 mg/g in the control treatment. Figure 4.

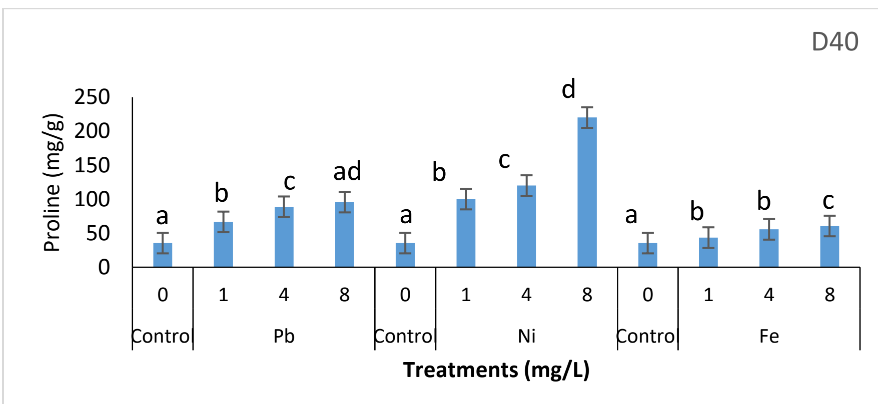

Figure 4: The average concentration of Proline at the end of experiments for Pb, Ni, Fe and control treatment.

The difference letter refers to a significant difference of 0.05 (p≤0.05)

Discussion

Aquatic plants normally experience morphological and physiological changes after metal accumulation, some of which increase the plants' tolerance (Gratão *et al*., 2005).

Depending on the presence of the plant in the aquatic ecosystem as a biofilter, the metal residual in water decreased in all treatments at the end of experiments with different values. The plant can use the rhizofilteration mechanism to absorb selected metals from an aqueous solution. These results agreed with Mahdi and Al-Abbawy (2019).

The removal efficiency of metals varied from aqueous solution and BCF value inside plant tissue depending on the plant's ability to tolerate selected heavy metal stress. This result agreed with Azeez and Sabbar (2012); Iha and Bianchini (2015); Azeez (2021).

The physiological and morphological parameters were examined after forty days of exposure to Ni, Pb, and Fe. The result indicated that plant samples with a green color and necrosis indications were gathered with different values depending on the metal. The plant is affected by Ni and Pb with different values. At the same time, Fe causes positive plant growth because the plant can use Fe as a micronutrient for growth. This result agreed with (Malar *et al*., 2016; Al-Nabhan, 2022).

Reduced plant biomass is also a crucial indicator of a plant's physiological reaction (Muratova *et al*., 2015). According to this study, iron (Fe) had a beneficial effect on plant growth at all Fe concentrations, whereas fresh weight and relative growth characteristics of *Bacopa monnieri* L. gradually declined as nickel and lead concentrations increased. The average plant biomass in shoots and roots has decreased significantly during heavy metal stress in several plant species (Toler *et al.,* 2005; Muratova *et al*., 2015). Fresh weight and relative growth are inhibited by the reduction carried out due to the restriction of metabolic activity, which also impacts the passage of water and nutrients through the plasma membrane because they alter its structure and function. This result agreed with Pandian *et al*., 2020.

Three mechanisms may cause the effects of heavy metals on plants' levels of photosynthetic pigments. First, heavy metals may build up abnormally quickly in plant chloroplasts, causing oxidative stress, which would cause damage like peroxidation of chloroplast membranes (Puertas *et al*. 2004). They have the potential to directly harm the structure and functionality of the chloroplast by attaching to the -SH group of the enzyme. They may also slow down the entire process of chlorophyll biosynthesis by focusing on Mg^{2+} and Fe^{2+}. Second, the antagonistic actions of heavy metal ions, which hinder the uptake and transportation of additional metal elements like manganese and iron, diminish the plants' capacity to generate color (Das *et al*. 1997). Third, exposure to heavy metals may stimulate pigment enzymes, accelerating the breakdown of pigment (Wenhua *et al*. 2007). When the reactions produced by *Bacopa monnieri* under the metal stress of Ni and Pb are examined, it is evident that Ni has a more consistent and significant effect than Pb on the physiological processes of plants. It is well known that iron treatment increases plants' chlorophyll and protein content, enabling them to utilize and tolerate higher iron concentrations (Samreen *et al.,* 2017). At the same time, other researchers described the reduction in chlorophyll content as the plant cell membranes commonly thought to be the principal sites of metal injury. Lipid peroxidation can be generated by activated oxygen species (ROS). When the amount of ROS produced exceeds the plant's ability to quench it, peroxidation of chloroplast membranes occurs, resulting in further pigment loss (Baszynski *et al*., 1988). On the other hand, lipoxygenase action is usually attributed to membrane disruption. Excess Ni and Pb also caused oxidative stress in chloroplasts (Prasad and Strzalka, 1999). Metal ions block electron flow in photosystem II (PS II), creating exciting chlorophyll. This result agreed with Jayasri, and Suthindhiran, (2017).

Total soluble protein levels on *Bacopa monnieri* L. plants significantly reduced as Ni and Pb treatment concentrations increased. As demonstrated in the current study, numerous studies have linked exposure to heavy metals to a decrease in the soluble protein content as a response to heavy metal stress (Tauqeer *et al*., 2016; Ali *et al*., 2012). The protein concentration was frequently higher even under extreme stress, indicating that the plants could tolerate a particular level of heavy metal stress. Otherwise, it's interesting to note that plants exposed to Fe concentrations saw a rapid increase in protein content accompanied by an increase in the activity of antioxidant enzymes, especially in plant shoots. These protein content changes in response to abiotic stress may be used as potential indications to identify the main mechanism behind plants' stress responses (Tran and Popova, 2013). This result agreed with Polińska *et al*., 2022.

Higher plants accumulate huge levels of proline as a stress indicator in response to environmental stresses (Ashraf *et al*., 2012). Protein defense, osmoregulation, and protection against oxidative damage depend on proline. Cellular membranes are also fixed as a response (Slama *et al*., 2015). Several plant species under the stress of heavy metals have shown proline accumulation (Gajewska *et al*. 2006). Proline's increased concentration in this study also showed that it has the antioxidant capacity to detoxify the accumulation of heavy metals in treated plants. This result agreed with John *et al*., 2009.

Conclusions

In conclusion, *Bacopa monnieri* L. demonstrated promising potential as a biosorbent for removing Ni, Pb, and Fe from polluted waters. The presence of these heavy metals causes significant morphological and physiological changes in plants, thereby inhibiting their growth and development. Notably, Ni exhibited greater toxicity than Pb in relation to increasing concentrations and was compared to the control treatment with Fe. Treatment with Ni and Pb resulted in a higher proline content than that of Fe. These findings highlight the efficacy of *B. monnieri* in removing heavy metals and its importance in mitigating their adverse effects on aquatic ecosystems.

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نبات Water hyssop كمراكم حيوي لمعالجة الرصاص، النيكل والحديد

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المستخلص

تعد المعالجة النباتية تقنية صديقة للبيئة، واقل تكلفةً من تكاليف المعالجات التقليدية الأخرى. تعد المعالجة النباتية طريقة تستعمل فيها النباتات والاحياء المجهرية المرافقة لإزالة أنواع مختلفة من الملوثات العضوية واللاعضوية من الترب والمياه العادمة بأنواعها والرواسب. تهدف الدراسة الحالية الى تقييم قدرة نبات *Bacopa monnieri* L. على معالجة وإزالة عنصر النيكل والرصاص والحديد من المياه الملوثة ومعرفة بعض التغيرات البيوكيميائية التي تحدث على النبات. أجريت التجارب في المختبر لمدة أربعون يوما. قيس في التجربة التركيز المتبقي بالماء وكفاءة الازالة ومعامل التركيز الحيوي والوزن الطري والجاف ومعدل النمو النسبي كما تم تقدير الكلوروفيل الكلي والبروتين والبرولين. أظهرت النتائج ان المعدن المتبقي في الماء انخفض في جميع التراكيز وبنسب مختلفة. اذ كان الانخفاض الأكبر في التراكيز القليلة. اظهرت كفاءة الازالة ان الكفاءة الأعلى وصلت 80% عند التركيز 1 ملغم/ لتر من الحديد بينما كانت الأقل 16.875 % عند التركيز 8 ملغم/ لتر من النيكل. كما بينت النتائج ان معامل التركيز الحيوي كان الأعلى عند التركيز 1 ملغم/ لتر من الحديد والاقل عند 8 ملغم/ لتر من النيكل. كما بينت النتائج ان التأثر الأكبر للنبات في الوزن الطري والنمو النسبي والمحتوى البروتيني والكلوروفيلي كان بتأثير عنصر النيكل عند التركيز 8 ملغم/ لتر. بينما اظهر عنصر الحديد زيادة نمو النبات في جميع التراكيز. واظهرت النتائج ان المحتوى البروليني ازداد بزيادة تركيز عنصري الرصاص والنيكل. نستنتج من الدراسة ان النبات تحمل تراكيز العناصر المختارة في الدراسة وبالتالي إمكانية اعتباره مرشح فعال لإزالة المعادن الثقيلة من المياه الملوثة.

الكلمات المفتاحية: المعادن الثقيلة، المعالجة النباتية، السمية، معامل التركيز الحيوي، الاستجابة البايوكيميائية