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Phytoremediation of Three Heavy Metals Using Duckweed (Lemna minor)

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Abstract. Today, the world faces the problem of water pollution with heavy metals, which have toxic effects on living organisms, including humans. Treating this water pollution requires using low-cost, environmentally friendly techniques. Therefore, we selected the Lemna minor plant to investigate its capacity to phytoremediate some heavy metals in a laboratory. Three harmful metals for the aquatic environment were chosen: nickel (1, 5, and 10 ppm), cadmium (0.5, 2, and 4 ppm), and lead (0.5, 5, and 10 ppm), in addition to a control group. Removal ratio, relative growth rates (RGR), and bioconcentration factor (BCF) were measured under controlled light and temperature conditions. The highest removal efficiencies by Lemna minor of Cd, Pb, and Ni ranged between 92% (at 0.5 ppm), 99.7% (at 0.5 ppm), and 99.75% (at 1 ppm), respectively. The lower ratio recorded 28.6% in 10 ppm on the 1st day. The bioconcentration factor (BCF) for each metal per unit (kg/L) was recorded as follows: 10,550 for cadmium on the 14th day at 0.5 ppm, 385,352 for nickel at 1 ppm on the 21st day, and 484,382 for lead at 0.5 ppm on the 21st day. The results show significant varying effects of cadmium, nickel, and lead on the removal ratio and relative growth rate of organisms in the closed system. Further analysis is needed to understand the long-term effects and determine the precise mechanisms responsible for these different responses.These results suggest that L. minor is a suitable candidate for the removal of heavy metals from polluted water bodies

Highlights:

1. Heavy metals pollute water, harming organisms.
2. Lemna minor tested for Cd, Pb, Ni phytoremediation.
3. High removal efficiency; suitable for eco-friendly water treatment.

Keywords: BCF, Heavy metals, RGR,. Phytoremediation, Lemna minor.

Introduction

The challenge of heavy metals toxicity in aquatic systems is not peculiar to any one area; it is a global crisis since all living organisms, including human genus beings, are harmed by these metals, where the environment and human health are highly compromised by heavy metal pollution, among which industrial activities, agricultural runoff, and urban waste are always the sources of pollution. Cadmium (Cd), lead (Pb),

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and arsenic (As) are totally harmful metals because of their toxicity nature, persistence in the environment, and ability to be accumulated in living organisms [1,2].

Lemna minor, a floating aquatic plant, also known as duckweed, is getting a lot of interest for its uses in plant remediation, which is an environmentally friendly procedure of removing pollutants from the water and soil [3].

Phytoremediation defined as the use of plants to clean up the polluted environment involves harnessing the plants' natural traits of assimilating, concentrating, and neutralizing contamination. L. minor has been used as a possible species for heavy metal remediation because of its rapid growth, low needs for nutrients, and capability to take up and endure a wide range of heavy metals from polluted water[4]. Some factors, such as exposure time, metal content, and environmental conditions, influenced the capacity of L. minor in removing these metals [5]. It has been shown that plants can absorb heavy metals from water sources highly efficiently[6], as papers have shown a decline of metal concentration after exposure.

Mechanisms of plant uptake, which include possible storage and root uptake in leaf tissues, minimize the impact of toxic metals on the water environment[7] . It is primary to comprehend the unique absorption methods and efficiencies for different heavy metals to maximize the employment of L. minor in phytoremediation techniques. The, study aimed to discuss the efficacy of L. minor phytoremediation of lead, nickel, and cadmium in polluted water environments. The data of results will help create long-term strategies for controlling heavy metal contamination and protecting the ecosystem

Methods

Plant Collect:

This study was conducted from January to November 2024. Where, collected aquatic plants L. minor from Hawizeh Marshe (Um-Al-Naej pond), Latitude °44 29´ 00´´E. They were brought to the laboratory in plastic bags and washed well with river water, then washed several times with tap water and distilled water to clean them from dirt and adhering materials and remove the remains of adhering plankton and small river animals. Ultrasound was used to isolate the algae after washing the plants, and then they were cultivated for 14 days for the plants to adapt to living in tap water containers.

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Reagents:

A stock solution (1000 mg/L) of each metal Cd, Pb, and Ni was prepared in distilled water. An atomic absorption spectrophotometer was used to determine metal concentrations. Sulfuric acid , nitric acid and perchloric acid were used in sample digestion.

Design of experiments:

The study was conducted in the Environmental Laboratory of the Faculty of Science, Kufa University. L. minor plant (30 g) was taken and planted in monocultures in plastic enclosures with dimensions of 30 cm length × 20 cm width × 18 cm height, filled with 8 liters of tap water (storage) per container, and the water level in each container was maintained at the same level throughout the experiment (compensating for the deficiency resulting from evaporation). Before them, the aquatic plants were identified using Iraq-specific references [8].

The experiment of L. minor plant was designed in 30 pots (plastic tank) divided into 9 pots for each metal (Cd ,Ni and Pb) treatments,; in addition to control, three replicates for each element concentration (three treatments exposed to different concentrations of cadmium (0.5, 5, and 10) ppm, three treatments exposed to different concentrations of lead (0.5, 5, and 10) ppm, was a control), and three treatments exposed to different concentrations of nickel (1, 5, and 10) ppm. Table 1. Our experiment was arranged in a completely randomized design, and plant growth parameters (Removal efeciency BCF biomass) were recorded after 1, 7, 14, and 21 days of cultivation with constant light (1500 lux); photoperiod 16/8 light/dark (h); and temperature 28 °C.

Table 1: The experiment design

Metal type	Metal concentration(ppm)	Replicate per concentration
Cd	0.5	3
	2	3
	4	3
Pb	0.5	3
	5	3
	10	3

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Ni	1	3
	5	3
	10	3
Control	C	3

Metals determination:

One hundred ml of water sample was taken and filtered by filter paper of size 0.45 µm (Chm Lab.) and kept in plastic containers until the sample was ready for heavy metal measurement. Plant was taken after drying to digest and 10 ml of the digestion solution consisting of sulfuric acid, nitric acid, and perchloric acid in a ratio of 1:1:3 as indicated by [9]. The flask which was dried on a hot plate was left to pre-dry. After that, five milliliters of the sample were taken, 25 ml of deionized water were added, the sample was filtered through a 0.45 µm filter and the sample was kept in plastic containers until the time of heavy metal assay. Absorption of the samples was determined by the use of an atomic absorption spectrophotometer, where a specific wavelength was used for particular elements [10].

Removal efficiency ratio:

The recovery efficiency was calculated as a percentage, using the general formula below: The percentage (%) of removal efficiency was calculated using the general formula below[11].

Removal efficiency ratio = $((C_i – C_f) / C_i) * 100$.

Where

C_i=initial concentration

C_f= Final concentration

Bioconcentration factors:

The bioconcentration factors (BCF) of each heavy metal (Cd, Ni, and Pb) in L. minor were calculated. The BCF parameter is the ratio of heavy metal concentration in the stems and in the substrate according to the following [12] .

Bioconcentration Factor(BCF)=C_{Plant}/C_{Water}

Relative growth:

The relative growth was estimated over the entire experimental period using the equation described by [13].

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$$Relative\ growth = \frac{FFW}{IFW}$$

Where FFW=Final fresh weight

IFW=initial fresh weight

Statistical analysis:

One way ANOVA is used to compare the means of several treatments at P≥0.05 using the SPSS statistical software (v.26.0). The LSD was used to make comparisons between the means of the treatments.

Result and Discussion

Phytoremediation of heavy metals:

Fig. 1 shows the removal ratios of cadmium metal by the L. minor plant during two weeks of experimentation, where the higher value recorded 92% in 0.5 ppm treatment on the 14th day, while the lower ratio recorded 48.6% in 0.5 ppm on the 1st day. While the removal ratios of nickel metal by the L. minor plant during three weeks of experimentation, where the higher value recorded 99.75% in 1 ppm treatment on the 21st day, while the lower ratio recorded 28.6% in 10 ppm on the 1st day (fig. 2). As for Fig. 3, show the removal ratios of lead metal by the L. minor plant during three weeks of experimentation, where the higher value recorded 99.7% in 0.5 ppm treatment on the 21st day, while the lower ratio recorded 8.5% in 10 ppm on the 1st day. The statistical analysis, with a probability (p >0.05), showed no significant differences between all interactions, of cadmium except time as shown in Table (4) but showed significant differences between all interactions, of nickel and lead tables (5 and 6). This is due to the plant's ability to tolerate high concentrations of heavy metals, as studies have shown that large aquatic plants can survive in a medium containing 3 mg Ni/L or 15 mg Zn. Although the level of heavy metal can affect growth. In another study investigated by [14] about the bioaccumulation and toxic effects of cadmium on Limna plant, the result showed that the plant showed a big ability to accumulate and absorb Cd in the fronds, making it a potential plant for phytoremediation. Our study is in agreement with many studies[15, 16 ,17 ,18] .

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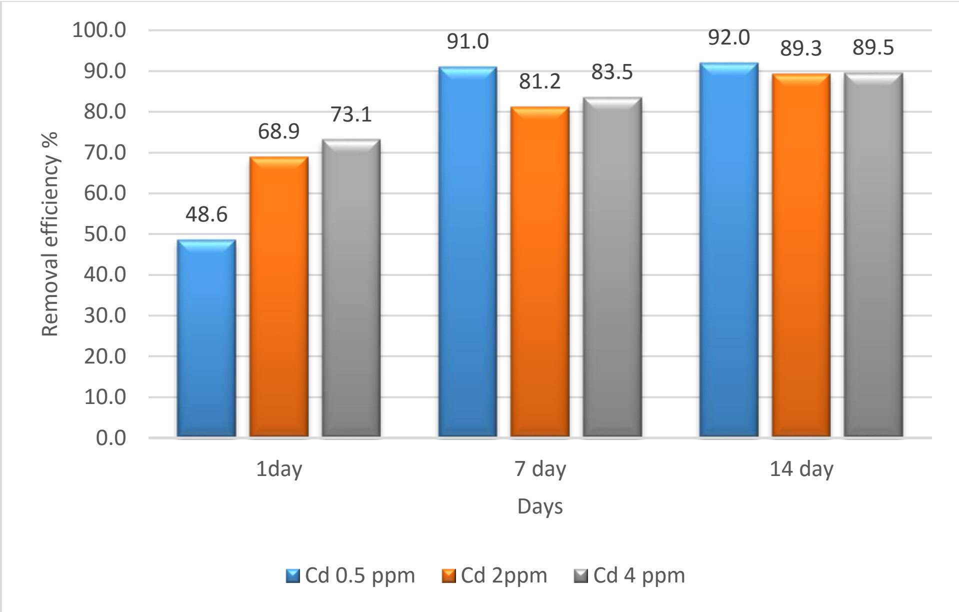

Fig.1: Removal ratio of L. minor for cadmium metal during experment period

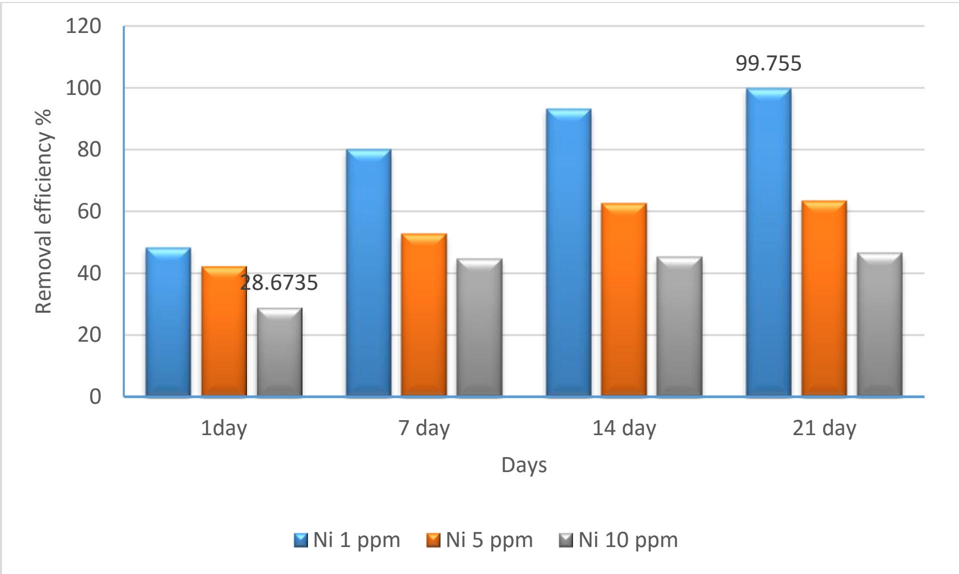

Fig.2: Removal ratio of L. minor for nickel metal during experment period

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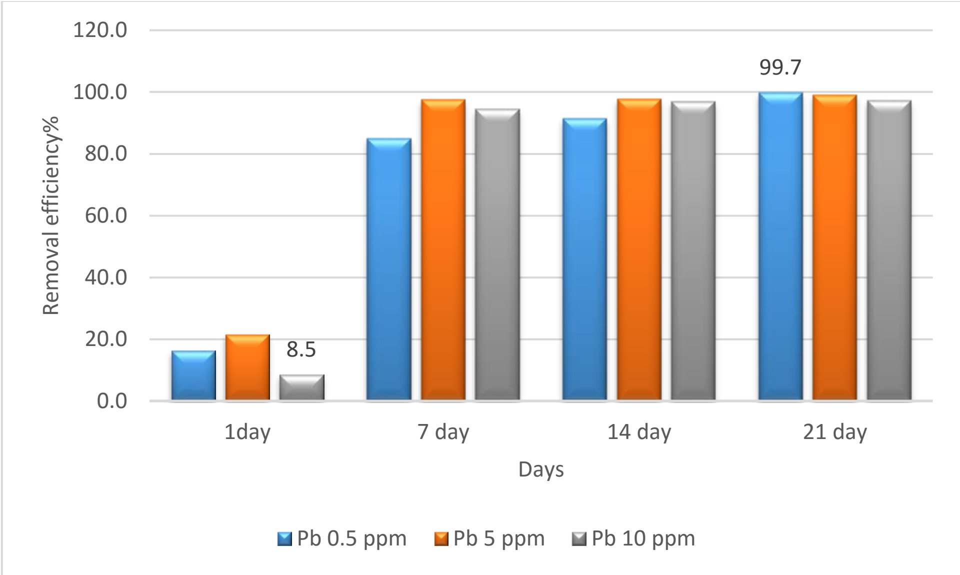

Fig.3: Removal ratio of L. minor for lead(Pb) metal during experment period

BCF:

The lowest biological concentration factor (BCF) for L. minor of cadmium metal was 594 at 2 ppm on the first day, while the highest value recorded was 10,550 at 0.5 ppm on the 14th day (Table 1). The highest value of BCF of nickel was 385,352 at 1 ppm on the 21st day, while the lowest value was 288 at 10 ppm on the 7th day (Table 2). While the highest value of BCF for L. minor of lead, 484.38, was recorded at 0.5 ppm on the 21st day, the lowest value was 479 at 10 ppm on the first day (Table 3).

It could be suggested that plants may be more effective at absorbing cadmium at low concentrations due to L. minor being ability to accumulate the metal at low concentrations (0.5 ppm). This might have to do with the plant's capacity to tolerate low concentrations of metal poisons [19]. High concentrations (2 ppm) result in a decreased capacity of the plant to absorb cadmium in comparison to lower concentrations, although there is a decrease on the first day. This might be because cadmium might initially slow down the plant's absorption of heavy metals, which can have a harmful impact on the plant at the start of the experiment[20] .

The highest BCF of Ni was (385,352) at 1 ppm on the 21st day. This result implies that plants can acquire a large amount of Ni at 1 ppm, particularly in the 21 days. This result might be an indication of the plant's capacity to adapt to Ni due to their seeming to be a noticeable rise in BCF over time [21]. The lowest BCF (288) at 10 ppm on the

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seventh day. After seven days, the higher concentration (10 ppm) of Ni demonstrated a lower ability to accumulate the metal in comparison to Ni at 1 ppm. Maybe this could be explained by the fact that plants may have less capacity to absorb the metal when exposed to high concentrations of Ni. Plants may not be able to absorb Ni effectively at high concentrations, which lowers BCF values [22].

The results showed the highest BCF for Pb was (484,382) at 0.5 ppm on the 21st day. These results is similar to cadmium and nickel, L. minor seems to have a big capacity to absorb lead metal at 0.5 ppm, particularly on day 21. This result is due to that lead may accumulate more efficiently at little concentrations than at higher concentrations. The plant might have been free to create more potent defenses or absorption strategies as a result of the longest period (21 days) [23].. As a lead metal it first generates a poor response. This might have something to increase do with the early harmful effects that plants experience from high lead exposure in their early days. L. minor might not be able to absorb lead at high concentrations at first, according to this response [6] .

Table 1: . Bioconcentration factor value (BCF) (kg/l) of cadmium metal in L. minor during experiment period

Metal concentration (Cd)	Duration time (day)		
	1 day	7 day	14 day
0.5 ppm	1324	8834	10550
2ppm	594	1278	2021
4 ppm	891	2367	1750

Table 2: . Bioconcentration factor value (BCF) (kg/l) of nickel metal in L. minor during experiment period.

Metal concentration (Ni)	Duration time (day)			
	1 day	7 day	14 day	21 day
1 ppm	815	4855	8492	385352
5 ppm	421	452	715	405
10 ppm	7414	288	477	338

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Table 3: . Bioconcentration factor value (BCF) (kg/l) of lead(Pb) metal in L. minor during experiment period.

Metal concentration (Pb)	Duration time (day)			
	1 day	7 day	14 day	21 day
0.5 ppm	1599	6835	19469	484382
5 ppm	928	34134	26238	180173
10 ppm	479	8925	7556	4535

Table 4: ANOVA analysis of removal ratio of cadmium by L.minor

Tests of Between-Subjects Effects

Dependent Variable: Cadmium

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3219.650[a]	8	402.456	2.144	.139	.656
Intercept	114236.423	1	114236.423	608.502	.000	.985
Time	2418.633	2	1209.317	6.442	.018	.589
Con	70.908	2	35.454	.189	.831	.040
Time * Con	730.108	4	182.527	.972	.468	.302
Error	1689.606	9	187.734			
Total	119145.679	18				
Corrected Total	4909.255	17				

a. R Squared = .656 (Adjusted R Squared = .350)

Table5 : ANOVA analysis of removal ratio of nickel by L.minor

Tests of Between-Subjects Effects

Dependent Variable: Nickel

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	10423.413[a]	11	947.583	54.663	.000	.980
Intercept	83416.962	1	83416.962	4812.023	.000	.998
Time	3321.366	3	1107.122	63.866	.000	.941
Con	6268.187	2	3134.094	180.795	.000	.968
Time * Con	833.859	6	138.977	8.017	.001	.800

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Error	208.021	12	17.335
Total	94048.396	24	
Corrected Total	10631.434	23	
a. R Squared = .980 (Adjusted R Squared = .962)			

Table6 : ANOVA analysis of removal ratio of lead by L.minor

Tests of Between-Subjects Effects

Dependent Variable: Lead

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	29339.216[a]	11	2667.201	388.946	.000	.997
Intercept	136655.193	1	136655.193	19927.813	.000	.999
Time	28945.362	3	9648.454	1406.991	.000	.997
Con	149.519	2	74.759	10.902	.002	.645
Time * Con	244.336	6	40.723	5.938	.004	.748
Error	82.290	12	6.858			
Total	166076.699	24				
Corrected Total	29421.506	23				
a. R Squared = .997 (Adjusted R Squared = .995)						

Relative growth rate(RGR):

During the experimental period, the RGR of L.minor plant in the enclosure exposed to varying amounts of cadmium (Cd) decreased in comparison to the control. 0.057 gm was the lowest value in the 2 ppm treatment, while 0.075 gm was the highest in the 0.5 ppm treatment (Fig. 4). With a probability ($p > 0.05$), the statistical analysis revealed no discernible variations across any interactions (Table 7).

In the enclosure exposed to different concentrations of nickel (Ni), the RGR of L.minor plant decreased as compared to the control, with the highest value recorded at 1 ppm (0.073 gm) and the lowest value at 10 ppm (0.064 gm) (Fig. 5). The statistical analysis, with a probability ($p > 0.05$), showed no significant differences between all interactions, as shown in Table 8.

In the enclosure exposed to different concentrations of lead (Pb), the RGR of L.minor plant increased in all treatments compared to the control, with the highest value at 0.5 ppm (0.113 gm) and the lowest value at the control (0.08 gm) (Fig. 6). The

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statistical analysis, with a probability ($p < 0.05$), showed no significant differences between all interactions except between 5 ppm and 10 ppm present significant differences, as shown in Table 9. The results showed a decrease in relative growth rate (RGR) compared to the control group with increasing cadmium concentration. This indicates a toxic effect of cadmium on the organisms in the closed system.

Nickel (Ni): The decrease in RGR with increasing concentration reflects a similar effect of cadmium, although the decrease is less severe.

Lead (Pb): In contrast to cadmium and nickel, the results showed an increase in RGR with all treatments compared to the control group. This may indicate a stimulatory role of lead at low concentrations.

The results of the effect of cadmium and nickel on the relative growth rate highlight the toxic effects of these metals, as they interfere with biological processes such as metabolism, energy production, and nutrient absorption. The gradual decrease in growth rate may indicate an increased accumulation of these metals in the tissues and their impact on general health[24] .

"Lead may have a positive stimulating effect at low concentrations due to a phenomenon called 'hormesis,' where partial exposure stimulates biological processes. Although a decrease in RGR was observed, the differences between the various concentrations were not statistically significant ($p > 0.05$). This could suggest that individual responses vary or that a larger sample size or longer study time are required. Our research supports that of Leblebici & Aksoy [23] . who discovered that L. minor growth rates decreased when Pb concentrations rose.

The varying effects of lead, nickel, and cadmium point to distinct modes of action in living things. This could be due to each metal having a different toxicity or due to organisms being able to tolerate it differently. For example, by attaching itself to proteins and enzymes, cadmium frequently interferes with cellular functions, resulting in oxidative stress and compromised cellular performance[25] .Conversely, nickel can cause disrupted DNA repair processes and carcinogenic consequences [26] . The lead metal is known to replace biominerals like calcium in biological systems, which can have an effect on development and neurological processes, especially in young organisms [27]. In these disparities in toxicity and mechanisms of action, the varying bioavailability and accumulation patterns of each metal within different tissues may also be reflected.

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Additionally, the toxicity concentrations and reactions of certain metals with biological systems can be influenced by particular environmental variables, such as pH and the presence of other pollutants. Comprehending these distinctions is essential to creating cleanup plans that work and safeguarding both human and ecosystem health.

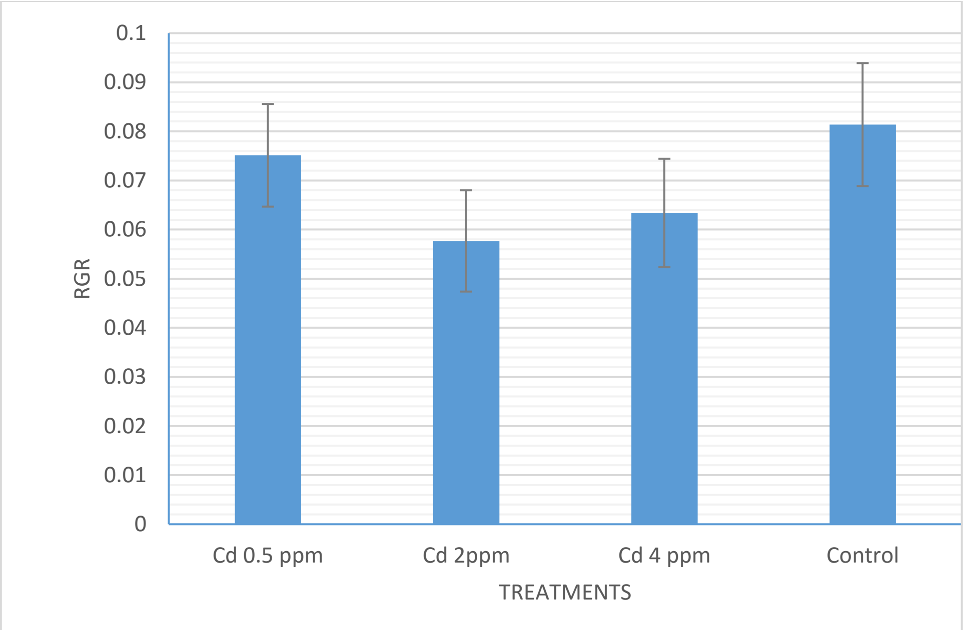

Fig.4: Effect of cadmium(Cd) metal on RGR of L. minor during 2 weeks

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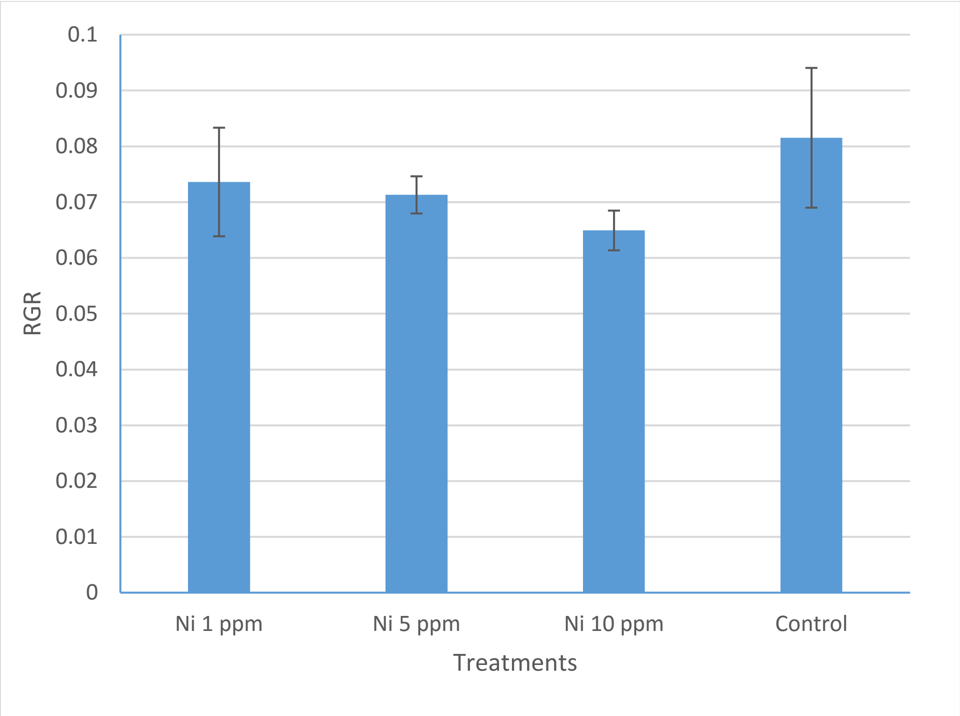

Fig.5: Effect of nickel(Ni) metal on RGR of L. minor during 3 weeks

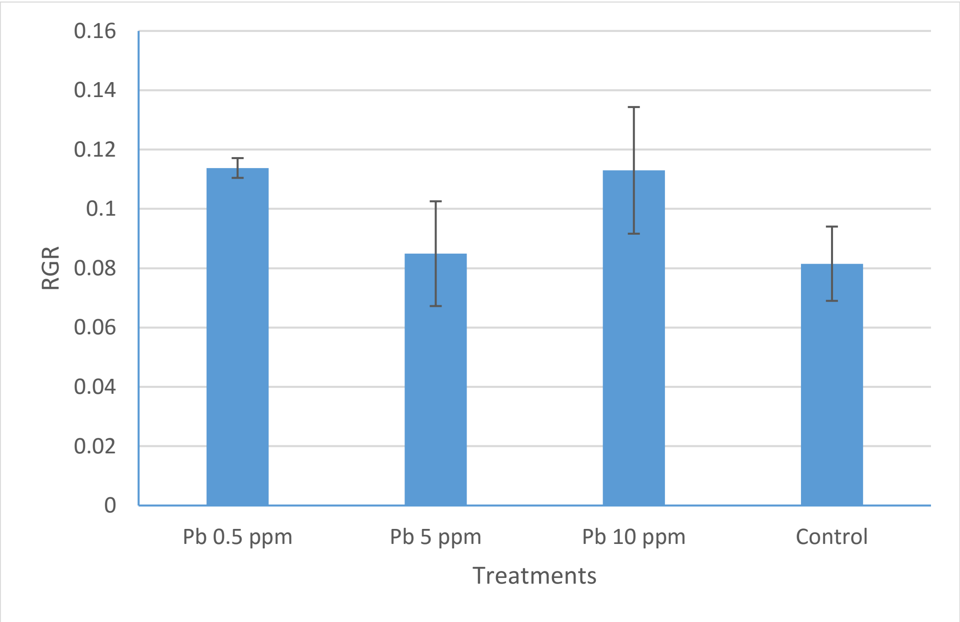

Fig.6: Effect of lead(Pb) metal on RGR of L. minor for during 3 weeks

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Table7 : LSD analysis of cadmium effect on RGR of L.minor

Multiple Comparisons

Dependent Variable: RGR(Cadmium)

	(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	0.5 ppm	2 ppm	.01744	.00865	.091	-.0037-	.0386
		4 ppm	.01172	.00865	.224	-.0095-	.0329
	2ppm	0.5 ppm	-.01744-	.00865	.091	-.0386-	.0037
		4 ppm	-.00571-	.00865	.534	-.0269-	.0155
	4 ppm	0.5 ppm	-.01172-	.00865	.224	-.0329-	.0095
		2 ppm	.00571	.00865	.534	-.0155-	.0269

Table8 : LSD analysis of nickel effect on RGR of L.minor

Multiple Comparisons

Dependent Variable: RGR (Nickel)

	(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	1 ppm	5 ppm	.00231	.00513	.669	-.0102-	.0149
		10 ppm	.00869	.00513	.141	-.0039-	.0212
	5 ppm	1 ppm	-.00231-	.00513	.669	-.0149-	.0102
		10 ppm	.00638	.00513	.260	-.0062-	.0189
	10 ppm	1 ppm	-.00869-	.00513	.141	-.0212-	.0039
		5 ppm	-.00638-	.00513	.260	-.0189-	.0062

Table9 : LSD analysis of lead effect on RGR of L.minor

Multiple Comparisons

Dependent Variable: RGR(Lead)

	(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
LSD	0.5 ppm	5 ppm	-.02708-	.01247	.073	-.0576-	.0034
		10 ppm	.00353	.01247	.787	-.0270-	.0340
	5 ppm	0.5 ppm	.02708	.01247	.073	-.0034-	.0576

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		10 ppm	.03061*	.01247	.049	.0001	.0611
	10 ppm	0.5 ppm	-.00353-	.01247	.787	-.0340-	.0270
		5 ppm	-.03061-*	.01247	.049	-.0611-	-.0001-

*. The mean difference is significant at the 0.05 level.

Conclusion

At low quantities (0.5–1 ppm), L. minor exhibits a greater ability to remove heavy metals such as lead, nickel, and cadmium. High removal rates (up to 99.75% for nickel) confirm its efficiency in phytoremediation. The plant's ability to accumulate metals is affected by concentration and exposure duration, with higher efficiency observed at lower concentrations. Bioaccumulation factors (BCF) are higher at low metal concentrations, decreasing significantly at higher levels due to toxicity. This study suggests L. minor as a viable tool for pollution remediation, especially effective at low concentrations and prolonged exposure periods

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