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Phytoremediation of cadmium-contaminated water by *Lemna minor*

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ABSTRACT:

The aptitude of *Lemna minor* to remediate cadmium (Cd)-contaminated water has been fairly well established. In light of *L. minor*'s critical function in environmental cleanup, a controlled experiment was conducted to assess its Cd bioaccumulation potential. *Lemna minor* tolerated Cd up to 1.0 ppm for 21 days before exhibiting indications of Cd poisoning, and the fresh and dry weights of *L. minor* declined dramatically. Whereas it dropped considerably with intensifying Cd percentages, the electrical conductivity (EC) of the *L. minor* growth medium reduced considerably (15.7-22.4%) with rising plant-loaded density (PLD). The pH findings of the *L. minor* growth medium and the EC were in conflict. When more Cd was applied, the percentage in the *L. minor* growth culture rose greatly (3.9-29.7%); however, as PLD increased, the percentage of Cd in the *L. minor* fronds declined substantially (48.7-68.4%). The bio-concentration factor for Cd increased (48-68%) with increasing PLD and the Cd concentration tested (36.0-80.0%). Nevertheless, *L. minor* demonstrated promising phytoremediation capacity for the heavy metal Cd studied, with high Cd separation efficiency. Because *L. minor* is an invasive organism that is widely distributed in Egyptian aquatic ecosystems and grows quickly, it is a viable remediation technique for polluted soils and water in progressively degraded environments. Therefore, this study was conducted to test the potential of duckweed (Lemna minor) as a potential remedy to reduce the hazards of lead contamination associated with agricultural operations. It is also easy to handle.

KEYWORDS: Bioaccumulation, Bio concentration factor, Heavy metals, *Lemna minor.*

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1. INTRODUCTION:

A serious concern is that increasing human impacts (such as sewage, urban communities, agriculture, and country communities) on freshwater irrigation and decreasing suitable methods for disposing wastewater is precipitating the diminishment of quality of water and risking ecological integrity **(Proulx et al. 2019; Hader et al. 2020; Khan et al. 2020).** Heavy metals are some of the pollutants that constitute the biggest damage to aquatic life because of their tendency for toxicity, difficulty to biodegrade, and potential to congregate in aquatic animals **(Censi et al. 2006).** Substances classified as heavy metals have a significant atomic weight, a specific gravity greater than 5 grams per milliliter, and a density considerably greater than that of water **(Khan et al. 2020).** Despite they are mainly caused by agricultural and industrial activities, heavy metals can be discharged into the environment by both natural and human processes (Fergusson 1990). Some heavy metals, predominantly Hg, Pb, and Cd, are proven to destroy plants and are not considered micronutrients; on the other hand, certain heavy metals, notably Co, Ni, Mn, Zn, and Cu, are necessary for plant development and are considered micronutrients **(Niess 1999; Gaur and Adholeya 2004; Wani et al. 2017).**

Chemicals, physics, and biology cannot decompose heavy metals into innocuous byproducts; hence they represent a bigger hazard to the environment than organic molecules (Tofighy and Mohammadi 2011). While their chemical makeup can alter through oxidation or reduction, the metals' elemental essence stays constant. These qualities permit them to remain in the ecosystem, resulting in their translocation into the food supply chain and having substantial effects on human wellbeing **(Peralta-Videa et al. 2009; Kabata-Pendias 2011; Zouainia et al. 2016).** Heavy metals, notably Cadmium, can limit root growth, create nuclear aberrations, and cause chromosomal errors (Hemachandra and Pathiratne 2015). Similarly, Cadmium can influence the growth frequencies of aquatic algae **(Magdaleno et al. 2014).** Freshwater plants may uptake and accumulate enormous levels of heavy metals from polluted water, and they can be used to assess water purity and ecological wellbeing **(Cardwell et al. 2002).** To treat these damaged aquatic habitats, swift action is required given the ecological impacts. Among the several methods accessible today is phytoremediation. With widespread public support, it is an affordable method that reduces the amount of time that people, animals, and the environment are exposed to the polluted substrate **(Prasad 2013).** Phytoremediation works best with aquatic plants **(Uqab et al. 2016; Sarma 2011),** because, in comparison to terrestrial plants, they have a higher ability to bio-accumulate harmful metals in enormous quantities **(Pratas et al. 2012).**

In reaction to pollution load, aquatic plants create phytochelatins which are peptides rich in cysteine that adhere to metallic. Phytochelatins assist to eliminate contaminants from the body by interacting with heavy metals to form compounds. In reaction to pollution stress, aquatic plants create phytochelatins, cysteine-rich peptides that bind to metals. Phytochelatins help detoxify contaminants by forming complexes with heavy **metals (Wani et al. 2017).** In Egypt, aquatic plants such as *Ceratophyllum demersum* and *Lemna minor* are employed as heavy metal cleanup technique **(Kumar and Prasad 2004; Ansari et al. 2020).** *L. minor* is an excellent model of a commonly utilized phytoremediation pathway for heavy

metals in aquatic habitats currently **(Rai 2009).** Since it floats, *L. minor* is subjected to contaminants in the air and water (Mohan and Hosetti 1999). *L. minor* has been used in the tertiary treatment of industrial and urban wastewaters **(Cheng et al. 2002; Khan et al. 2020).** *L. minor* is employed to evaluate the phytotoxicity of various phenols, herbicides, and heavy metals **(Vujević et al. 2000).** The Organization for Economic Cooperation and Development and the US Environmental Protection Agency (EPA) have designated it as a biomarker/bioindicator **(OECD 2002; Kiss et al. 2003).** Similarly, the frequency of heavy metal depositing decreases as metal levels rise above particular limits that are detrimental to plants. On the other hand, macrophytes effectively absorb and remove heavy metals from solutions when the concentrations of metals in the water are not very detrimental to them **(Khan et al. 2020).**

Research that investigated *L. minor*'s exposure to heavy metals using deionized water contaminated with cadmium (Cd) (Gounden et al. 2016; Khan **et al. 2020).** If we use pure water that has been enhanced with known amounts of Cadmium, we can assess whether it is over- or under-accumulating. It can also assist us in determining the concentration at which poisoning symptoms manifest, which is not achievable in artificially generated natural water. In general, preparing the medium with clean water provides controlled circumstances to evaluate the additional contaminant's (Cd) impact. Compared to other aquatic macrophytes, *L. minor* is more readily available in Egypt throughout the majority of the year, making it a superior choice for phytoremediation. The aim of this study was to test the effectiveness of duckweed *(Lemna minor)* in eliminating cadmium (Cd) from an artificial solution in a lab setting. The findings of this study will greatly advance our knowledge of the plant's capacity to clean contaminated water and help provide guidelines for potential remedies to reduce the hazards of lead contamination connected with agricultural operations.

2. MATERIALS AND METHODS:

2.1. Site description

The experiment was conducted three times in September and October 2023 at the Soil and Water Department Laboratory, Faculty of Agriculture, Fayoum, Egypt. During the experiment, the laboratory had a daily average temperature of 35 ± 2 °C and a relative humidity of 60.4 ± 3.2%.

2.2. Collection of *L. minor*

The aquatic plant species (e.g., *L. minor*) (Fig. 1) was identified and used in the present study. *L. minor* was provided by the Microbial Research Department at the Soils, Water, and Environment Research Institute, Agricultural Research Center in Giza, Egypt. Zawiat El-Kardsa Village, Fayoum District, Fayoum Governorate, Egypt.

Fig. 1. Illustrates the appearance of the *L. minor*

Healthy *L. minor* plants were gathered in polythene containers and promptly transferred to the laboratory. To guarantee that no contaminants or pollutants remained, the plant samples were carefully cleaned with tap water followed by deionized water. *L. minor* plant was adapted to laboratory settings for 15 days in 30-L plastic containers filled with a half-strength Hoagland-nutrient Arnon's solution, with special care taken to choose plants of equal size and weight (Marin and Oron, 2007). The nutrient solution was changed 7 days later. This step was taken to promote their overall health and performance in the subsequent experiments.

2.3. Culture media

The media was prepared using chemical composition of a half-strength Hoagland-Arnon's nutrient solution as follows: 3.0 mM KNO_3; 2.0 mM $Ca(NO_3)_2$; 0.5 mM $NH_4H_2PO_4$; 1.0 mM $MgSO_4$; 10 µM Fe–EDTA; 1.5 µM H_3BO_3; 0.25 µM $MnSO_4$; 0.1 µM $CuSO_4$; 0.2 µM $ZnSO_4$; and 0.025 µM H_2MOO_4 with pH 7 (Marin and Oron, 2007).

2.4. Preparation of Cd stock solution

Chemicals were obtained from Sigm-Aldrich (3050 Spruce St. Louis, MO 63103 USA). The Cd stock solution was prepared by dissolving 1.3583 g of cadmium nitrate [$Cd(NO_3)_2.4H_2O$] in 0.5 L of deionized distilled water. The stock solution was initially diluted with (Hoagland-Arnon's) medium to achieve the required concentrations of the Cd in the experimental run for Cd (0.00, 0.50, and 1.00 mg L^{-1}) (**Khan et al., 2020**; **Chaudhuri et al., 2014).**

The concentrations of Cd can be calculated using the equation of Rajab and Sami (2017):

$$M_1V_1 = M_2V_2 \quad \ldots\ldots (1)$$

Where: M_1 and V_1 represent the stock concentration (mg L^{-1}) and the volume (mL) of the solution, respectively. Similarly, M_2 and V_2 represent the required concentration (mg L^{-1}) and the volume (mL) of the solution at the required concentration, respectively.

2.5. Experimental Setup

The experiments were carried out in plastic containers having a diameter of

26.0 cm and a depth of 12.0 cm. Each container held 3.0 L of medium (Chen et al., 2015). To cultivate *L. minor*, the medium was supplemented with varying amounts of Cd^{2+} and half-strength modified Hoagland-nutritional Arnon's solution.

The polluted water samples were prepared by dissolving the previously prepared Cd (standard solution) in deionized water at different concentrations. The containers were inoculated with two densities of fresh 10g and 20g of *L. minor*, which was used as a standard inoculum for each treatment. In addition, the control has no cadmium was added. All concentrations of Cd^{2+} were represented by 3 replicates which carried out for the treatments. The inoculated containers were incubated at 35 ± 2°C as an average mean daily temperature, 14-hour light and 10-hour dark for 21 days. The experiments were carried out during September and October 2023. Treatment samples were taken 0, 7, 14, and 21 days after the beginning of the experiments (**Chen et al., 2015**). The solution obtained was measured by U.S. EPA Method 200.7 using the Optima 8300 ICP-OES and prepFAST Auto-Dilution/ Calibration System (EPA, 2001).

A control treatment (a medium free of Cd) was utilized to compare it to other positive therapies. The effect of Cd^{2+} concentrations on fresh and dry weights (El-Shahat, 1997), as well as the accumulation of Ni^{2+} in the studied plants, were assessed based on the dry weight by utilizing US EPA Method 200.7. Using the Optima 8300 ICP-OES with prepFAST Auto-Dilution/Calibration System (EPA, 2001). Figure 2 depicts the duplicate treatments used.

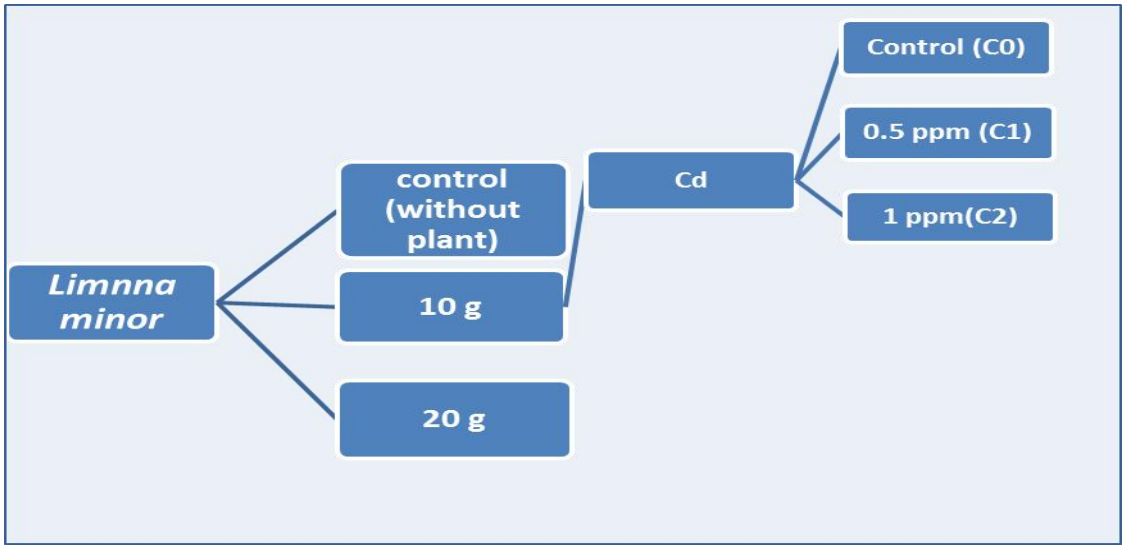

Fig. 2. Schematic sketch of laboratory treatments.

2.6. Determination of physical and chemical parameters in water

The water samples were analyzed according to American public health association standards method for examination of water and waste water (1985).

2.6.1. pHA digital pH meter was used to determine the pH of samples every 7 days for 21 days.

2.6.2. Electrical Conductivity (EC)

Electrical conductivity was determined using an electrical conductivity meter of the samples every 7 days for 21 days.

2.7. Determination of fresh and dry weights of *L. minor*

L. minor plant was harvested then washed with deionized water and placed under shade between two thick layers of blotting tissue papers for approximately 1-2 hours before determining fresh weight. Fresh weight of the plants was measured and expressed as g per container (ElBerashi, 2008). Then, the plants were oven-dried at 70°C until the weights were constant. Weightings were taken after the plant-containing dishes were cooled in a desiccator to room temperature. Dry weight of cells is determined for each replicate by subtracting the obtained weight of dried sample from the glass dish's weight and expressed as g per container.

3.8. Determination of Cd removal efficiency

3.8.1. Digestion of water for heavy metal analysis

A 100 mL sample was taken in an acid washed flask and 5 mL concentrated HNO_3 was added to it. The sample was heated slowly on the hot plate and was evaporated to 20 mL. After cooling, 5 mL concentrated HNO_3 and 10 mL concentrated H_2SO_4 were added to each flask. The sample was again evaporated on the hot plate until dense white fumes of SO_3 appeared. The sample was cooled and diluted to 100 mL with double distilled water. After dilution, the samples were cooled to room temperature, filtered through Whatman No.1 filter paper and were stored in glass vials **(Parnian et al., 2022)**. Sample of digested water was taken to estimate the residual. The Cd is expressed as a percentage of metal removal as given below in Eq. (2) (Von Sperling et al., 2020).

$$\text{Metal removal efficiency} = \frac{C0-Ce}{C0} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where, C0 and Ce are the initial and final metal concentrations in solution (mg L^{-1}), respectively.

3.8.2. Digestion of Aquatic plant for Cd analysis

A 0.2 gram of ground-powder of plant (*L. minor*) oven-dried at 70°C and digested with concentrated sulfuric acid and perchloric acid (3:1, respectively, v/v). The mixture was initially heated at 60 °C for 15 min on a hot plate, then temperature was increased to 120°C and samples were digested. After digestion, the digests were diluted with double distilled water and the volume was made up to 50 mL. To determine the bioconcentration factor of Cd, the following formula was **used (Zayed et al., 1998)** Eq. (3):

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Final metal concentration in biomass}}{\text{Initial metal concentration in biomass}} \ldots\ldots (3)$$

3.9. Statistical Analysis

The examination of the collected data was conducted using a randomized full block design with two parameters [plant loaded density (A) and Cd levels (B), with three repetitions for each parameter. The treatments' averages were contrasted using Snedecor and Cochran's least significant differences (LSD) test (Snedecor and Cochran 1976).

3. RESULTS AND DISCUSSION:

Table 1 shows the effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on the electrical conductivity (EC) of plant media. Regarding PLD, the EC of plant medium

was decreased with increasing PLD. The most effective PLD in decreasing the medium EC was plant loaded density at 20g (PLD_{20}). It decreased EC by 0.0, 16.5, 15.7, and 22.4% at zero time (EC_0), 7, 14, and 21 days after the beginning of the experiment (DAEs), respective For Cd Conc., the EC of plant medium was increased with increasing Cd Conc. The most effective Cd Conc. in increasing the medium EC was cadmium concentration applied at 1.0 ppm ($Cd_{1.0}$). It increased EC by 83.2, 77.7, 72.2, and 72.5% at zero time (EC_0), 7, 14, and 21 DAEs, respectively.

Concerning EC, the EC was generally decreased with increasing DAEs due to the treatment of PLD. The interaction effect of PLD × Cd Conc. treatments was significant (**Table 2**). The most effective interaction treatment that decreased the medium EC was PLD_{20} × Cd Conc.

Table 1. Mean effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on electrical conductivity (EC) of plant medium

Treatment	EC_0 (µS/cm)	EC_7 (µS/cm)	% of EC_0	EC_{14} (µS/cm)	% of EC_0	EC_{21} (µS/cm)	% of EC_0
PLD:	NS	**	-	**	-	**	-
PLD_0 (without	2.54a	2.54a	0.0	2.54 a	0.0	2.54a	0.0
PLD_{10} (10 g)	2.54a	2.27b	- 10.63	2.24 b	-	2.16b	- 14.96
PLD_{20} (20 g)	2.54a	2.12c	- 16. 54	2.14c	-	1.97c	- 22.44
Cd Conc.:	**	**	-	**	-	**	-
$Cd_{0.0}$ (Without Cd)	1.79 c	1.66 c	- 7.26	1.58 c	11.73	1.53 e	14.53
$Cd_{0.5}$ (0.5 ppm)	2.55 b	2.32 b	- 9.02	2.14 b	- 16.1	2.08 b	- 18.4
$Cd_{1.0}$ (1.0 ppm)	3.28 a	2.95 a	- 10.1	2.72 a	- 17.1	2.64 a	- 19.5
PLD × Cd conc.	NS	**	_	**	_	**	_

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$). (**) indicates significant difference at $p \leq 0.01$, and (ns) indicates no significant difference between the treatments.

Table 2. Interaction effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on electrical conductivity (EC) of plant medium

PLD (g)	Cd Conc.	EC_0 (µS/cm)	EC_7 (µS/cm)	% of EC_7	EC_{14} (µS/cm)	% of EC_{14}	EC_7 (µS/cm)	% of EC_{21}
	0.0 ppm	1.79 c	1.79 e	0.0	1.79 e	0.0	1.79 e	0.0
0	0.5 ppm	2.55 b	2.55 c	0.0	2.55 b	0.0	2.55 b	0.0
	1.0 ppm	3.28 a	3.28 a	0.0	3.28 a	0.0	3.28 a	0.0
	0.0 ppm	1.79 c	1.67 f	- 6.70	1.56 f	-12.85	1.45 f	-19.0
10	0.5 ppm	2.55 b	2.28 d	-10.6	1.97 d	-22.7	1.90 e	-25.5
	1.0 ppm	3.28 a	2.87 b	-12.5	2.51 b	-23.5	2.42 c	-26.2
	0.0 ppm	1.79 c	1.53 g	-14.5	1.39 g	22.34	1.34 g	-25.1
20	0.5 ppm	2.55 b	2.12 d	-16.9	1.91 d	-25.1	1.81 e	-29.0
	1.0 ppm	3.28 a	2.70 b	-17.7	2.37 c	-27.7	2.22 d	-32.3

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$). EC = µS/cm

Table 3 presents the effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on pH of the plant medium. Regarding PLD, the pH of the plant medium was increased with increasing PLD. The most effective PLD in increasing the medium pH was PLD_{20}. It increased pH by 0.0, 17.6, 23.1, and 25.6% at zero time, 7, 14, and 21 DAEs, respectively.

For Cd Conc., the pH of the plant medium was decreased with increasing Cd Conc. The most effective Cd Conc. in decreasing the medium pH was cadmium concentration applied at 1.0 ppm ($Cd_{1.0}$). It decreased pH by 24.4, 8.39, 4.67, and 4.59% at zero time (pH_0), 7, 14, and 21 DAEs, respectively.

Concerning pH, the pH was generally increased with increasing DAEs due to the treatment of PLD. The interaction effect of PLD × Cd Conc. treatments was significant (**Table 4**). The most effective interaction treatment that increased the medium pH was PLD_{20} × Cd Conc.

Table 3. Mean effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on pH of plant medium

Treatment	pH_0	% of pH_0	pH_7	% of pH_0	pH_{14}	% of pH_0	pH_{21}	% of pH_0
PLD:	NS		**		**		**	
PLD_0 (without plant)	5.85 a	0.0	5.85 c	0.0	5.85 c	0.0	5.85 b	0.0
PLD_{10} (10 g)	5.85 a	0.0	6.73 b	15.04	7.13 b	21.9	7.21 a	23.2
PLD_{20} (20 g)	5.85 a	0.0	6.87 a	17.6	7.20 a	23.1	7.35 a	25.6
Cd Conc.:	**		**		**		**	
$Cd_{0.0}$ (Without Cd)	6.72 a	0.0	6.79 a	1.04	6.85 a	1.93	6.98 a	3.87
$Cd_{0.5}$ (0.5 ppm)	5.76 b	0.0	6.45 b	12.0	6.80 b	18.1	6.84 b	18.8
$Cd_{1.0}$ (1.0 ppm)	5.08 c	0.0	6.21 c	22.44	6.53 c	28.5	6.59 c	29.7
PLD × Cd conc.	NS		**		**		**	

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$). (**) indicates significant difference at $p \leq 0.01$, and (ns) indicates no significant difference between the treatments.

Table 4. Interaction effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on pH of plant medium

PLD (g)	Cd conc.	pH 0	pH 7	% of pH 0	pH 14	% of pH 0	pH21	% of pH 0
0	0.0 ppm	6.72 a	6.72 c	0.0	6.72 d	0.0	6.72 e	0.0
	0.5 ppm	5.76 b	5.76 d	0.0	5.76 e	0.0	5.76 b	0.0
	1.0 ppm	5.08 c	5.08 e	0.0	5.08 f	0.0	5.08 g	0.0
10	0.0 ppm	6.72 a	6.81 b	1.34	6.88 c	2.38	7.03 d	4.61
	0.5 ppm	5.76 b	6.72 c	16.7	7.26 b	26.04	7.31 b	29.2
	1.0 ppm	5.08 c	6.65 c	30.9	7.25 b	42.7	7.29 b	44.1
20	0.0 ppm	6.72 a	6.84 b	1.76	6.96 c	3.57	7.19 c	6.99
	0.5 ppm	5.76 b	6.87 a	19.3	7.39 a	28.3	7.44 a	30.4
	1.0 ppm	5.08 c	6.92 a	36.2	7.26 b	42.9	7.41 a	45.9

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$).

Table 5 offers the effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on Cd Conc. in plant medium. With regard to PLD, Cd Conc. of the plant medium was decreased with increasing PLD. The most effective PLD in decreasing the medium Cd Conc. was PLD_{20}. It decreased Cd Conc. by 0.0, 47.1, 62.7, and 68.6% at zero time, 7, 14, and 21 DAEs, respectively. For Cd Conc., Cd Conc. of the plant medium was increased with increasing Cd addition to the medium. The most effective Cd Conc. in increasing the medium Cd Conc. was $Cd_{1.0}$.

Concerning Cd Conc., the Cd Conc. was generally decreased with increasing DAEs due to the treatment of PLD. The interaction effect of PLD × Cd Conc. treatments was significant (**Table 6**). The most effective interaction treatment that decreased the medium Cd Conc. was PLD_{20} × Cd Conc.

Table 5. Mean effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on Cd Conc. in plant medium

Treatment	Conc. $_0$	Conc. $_7$	% of Cd_0	Conc. $_{14}$	% of Cd_0	Conc. $_{21}$	% of Cd_0
PLD:	NS	**		**		**	
PLD_0 (without plant)	0.51a	0.51 a	0.0	0.51 a	0.0	0.51 a	0.0
PLD_{10} (10 g)	0.51 a	0.35 b	-30.3	0.29 b	-43.4	0.26 b	-48.7
PLD_{20} (20 g)	0.51 a	0.27 c	-46.1	0.19 c	-63.2	0.16 c	-68.4
Cd Conc.:	NS	**		**		**	
$Cd_{0.0}$ (Without Cd)	0.0	0.0		0.00 c		0.00 c	
$Cd_{0.5}$ (0.5 ppm)	0.51 b	0.39 b	-23.5	0.34 b	-33.3	0.33 b	-35.3
$Cd_{1.0}$ (1.0 ppm)	1.01 a	0.74 a	-26.7	0.64 a	-36.6	0.60 a	-40.6
PLD × Cd conc.	NS	**		**		**	

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$). (**) indicates significant difference at $p \leq 0.01$, and (ns) indicates no significant difference between the treatments.

Table 6. Interaction effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on Cd Conc. in plant medium

LD (g)	Cd conc.	Conc.$_0$	Conc.$_7$	% of Cd_0	Conc.$_{14}$	% of Cd_0	Conc.$_{21}$	% of Cd_0
0	0.0 ppm	0.00c	0.00 g	-	0.00 g	-	0.00 g	
	0.5 ppm	0.51 b	0.51 d	0.0	0.51 c	0.0	0.51 b	0.0
	1.0 ppm	1.01 a	1.01 a	0.0	1.01 a	0.0	1.01 a	0.0
10	0.0 ppm	0.00c	0.00g	0.0	0.00 g	0.0	0.00 g	0.0
	0.5 ppm	0.51 b	0.37 e	-27.1	0.30 e	-41.1	0.28 e	-45.1
	1.0 ppm	1.01 a	0.69 b	-31.7	0.56 b	-44.6	0.50 c	-50.5
20	0.0 ppm	0.00c	0.00g	0.0	0.00 g	0.0	0.00 g	0.0
	0.5 ppm	0.51 b	0.30 f	-41.2	0.21 f	-58.8	0.19 f	-62.7
	1.0 ppm	1.01 a	0.52 c	-48.5	0.35 d	-65.3	0.29 d	-71.3

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$).

Table 7 shows the effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on initial and final Cd Conc. of *L. minor*. For plant loaded density (PLD) of *L. minor*, there are no significant differences in initial Cd Conc. between PLD_{20}, PLD_{10}, and the control. In addition, PLD_{20} collected significant final Cd Conc. compared to PLD_{10}, which, in turn, collected significant final Cd Conc. compared to the control. This result was reflected in BCF which was increased significantly with PLD_{20} compared to PLD_{10}, which, in turn, possessed BCF greater than the control.

For Cd Conc. in plants, there are no significant differences in initial Cd concentrations between $Cd_{1.0}$, $Cd_{0.5}$, and the control ($Cd_{0.0}$). In addition, $Cd_{1.0}$ significantly increased final Cd Conc. compared to $Cd_{0.5}$, which, in turn, increased significantly final Cd Conc. compared to the control. This result was reflected in BCF which was increased significantly with $Cd_{1.0}$ compared to $Cd_{0.5}$, which, in turn, increased significantly BCF compared to the control. The interaction effect of PLD × Cd Conc. treatments was significant (**Table 8**). The most effective interaction treatment that increased BCF was PLD_{20} × Cd Conc.

Table 7. Mean effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on cadmium accumulation by *Lemna minor* at different concentrations of Cd

Treatment	Initial concentration in plants (Ci) (ppm)	Final concentration in plants (Cf) (ppm)	BCF = Cf/Ci
PLD:	NS	**	**
PLD_0 (without plant)	0.000 a	0.000 a	0.00 a
PLD_{10} (10 g)	0.005 a	0.240 b	48.0 b
PLD_{20} (20 g)	0.005 a	0.340 c	68.0 c
Cd Conc.:	NS	**	-
$Cd_{0.0}$ (Without Cd)	0.005 a	0.005 a	1.00 c
$Cd_{0.5}$ (0.5 ppm)	0.005 a	0.180 b	36.0 b
$Cd_{1.0}$ (1.0 ppm)	0.005 a	0.400 c	80.0 a
PLD × Cd conc.	NS	**	**

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$). (**) indicates significant difference at $p \leq 0.01$, and (ns) indicates no significant difference between the treatments.

Table 8. Interaction effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on bioconcentration factor (BCF) in the plant.

LD (g)	Cd conc.	Initial concentration in plants (Ci) (ppm)	Final concentration in plants (Cf) (ppm)	BCF = Cf/Ci
LD_0	0.0 ppm	0.00 a	0.00 a	-
	0.5 ppm	0.00 a	0.00 a	-
	1.0 ppm	0.00 a	0.00 a	-
LD_{10}	0.0 ppm	0.005 a	0.005 a	1.00 e
	0.5 ppm	0.005 a	0.23 d	46 d
	1.0 ppm	0.005 a	0.51 b	102 b
LD_{20}	0.0 ppm	0.005 a	0.005 a	1.0 e
	0.5 ppm	0.005 a	0.32 d	64 c
	1.0 ppm	0.005 a	0.71 e	142 e

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$).

Table 9 shows the effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on fresh and dry weights of *L. minor*. For plant loaded density (PLD) of *L. minor*, fresh and dry weights were significantly increased in PLD_{20} compared to PLD_{10}, which, in turn, increased significantly fresh and dry weights compared to the control. For Cd Conc. in *L. minor*, fresh and dry weights were significantly decreased with $Cd_{1.0}$ compared to $CD_{0.5}$, which, in turn, decreased significantly fresh and dry weights compared to the control. $Cd_{1.0}$ and $CD_{0.5}$ decreased plant fresh weight by 67.9 and 62.3%, respectively, and decreased plant dry weight by 72.8 and 69.6%, respectively, compared to the control.

The interaction effect of PLD × Cd Conc. treatments was significant (Table 10). The most effective interaction treatment that increased fresh and dry weights of *L. minor* plants was PLD_{20} × Cd Conc., followed by PLD_{10} × Cd Conc.

Table 9. Interaction effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on fresh weights and dry weights of plant

Treatment	Fresh weights (g/m^2)	dry weights (g/m^2)
PLD:	**	**
PLD_0 (without plant)	0.00 c	0.00 c
PLD_{10} (10 g)	120.4 b	0.64 b
PLD_{20} (20 g)	173.5 a	0.82 a
Cd Conc.:	NS	**
$Cd_{0.0}$ (Without Cd)	173.1 a	0.92 a
$Cd_{0.5}$ (0.5 ppm)	65.21 b	0.28 b
$Cd_{1.0}$ (1.0 ppm)	55.6 c	0.25 b
PLD × Cd conc.	NS	**

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$). (**) indicates significant difference at $p \leq 0.01$, and (ns) indicates no significant difference between the treatments.

Table 10. Interaction effects of plant loaded density (PLD) and cadmium concentration (Cd Conc.) on fresh weights and dry weights of plant

PLD (g)	Cd conc.	Fresh weights (g/m^2)	dry weights (g/m^2)
PLD_0	0.0 ppm	0.00 f	0.00 d
	0.5 ppm	0.00 f	0.00 e
	1.0 ppm	0.00 f	0.00 e
PLD_{10}	0.0 ppm	216.2 b	1.15 b
	0.5 ppm	77.0 e	0.41 c
	1.0 ppm	67.9 e	0.36 c
PLD_{20}	0.0 ppm	303.1 a	1.61 a
	0.5 ppm	118.7 c	0.45 c
	1.0 ppm	98.7 d	0.39 c

Data are means, different letters after means in each column indicate significant difference at a probability level of 0.05 ($p \leq 0.05$).

Table 11 and **Fig. 3** show the effect of different cadmium (Cd) concentrations on *Lemna minor*. Cd caused visible symptoms indicating damage to *L. minor* at concentrations of 0.5 and 1.0 ppm. The plants were not affected at in the Cd-free growing medium, while mild chlorosis and necrosis were appeared on a few plants at a concentration of 0.5 ppm. The chlorosis was increased along with beginning of dislocation of fronds and marked decreased plant growth.

Table 11. Effect of different cadmium (Cd) concentrations on *Lemna minor*

Concentration (ppm)	Effect
0.0	Plants more or less green in color and in its healthy growth.
0.5	Mild chlorosis and necrosis on a few plants.
1.0	Decreased growth and increased chlorosis with beginning of dislocation of fronds.

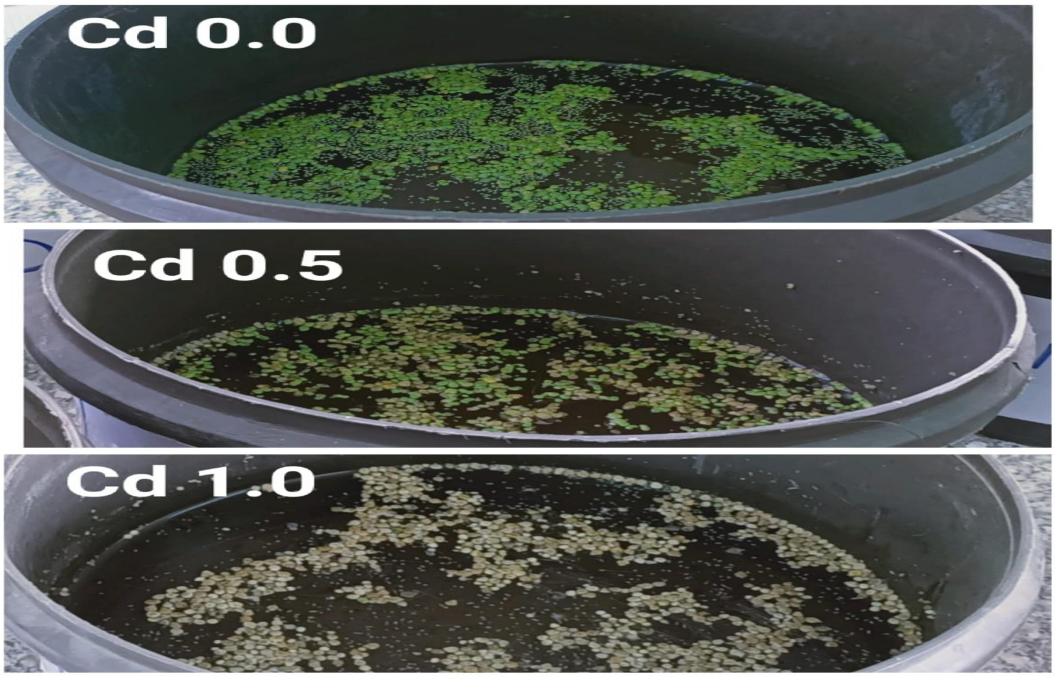

Fig. 3. Effect of different cadmium (Cd) concentrations on *Lemna minor*

Several research have been conducted worldwide on the role of duckweed (*Lemna minor*) in the elimination of heavy metals from wastewater. The purpose of this study was to look into the phytoremediation capability of *L. minor*, which is invading and available all year in Egyptian aquatic systems. This study focuses on *L. minor*'s bioaccumulation of cadmium (Cd) from artificially polluted water.

Because it was a controlled laboratory environment, the temperature measurements were not dramatically higher. This might refer to a laboratory experiment that was conducted under controlled settings.

The electrical conductivity (EC) diminished with rising plant loaded density (PLD), but rose with increasing Cd concentration (Tables 1 and 2). Because of the conductivity, *L. minor* grew well,

especially in diluted pollution, as conductivity decreased, which is a marker of water condition. Its existence is determined by the amount of salt existing in the water. These favorable circumstances are owing to increased photosynthetic activity, which produces O_2 in wastewater and depletes CO_2. Additionally, aerobic oxidation of organic materials in wastewater, and the sedimentation mechanism **(Mahmood et al., 2005; Shah et al., 2014),** and the filtering of suspended particles contribute to the improved elimination of pollutants in planted system treatments (Xiang Shi et al., 2015; Rana and Maiti, 2018). These results agreed with those of Azeez and Sabbar (2012) and Al-Nabhan and Al-Abbawy (2021).

The pH rose with growing plant loaded density (PLD), and declined with raising Cadmium level (Tables 3 and 4). The pH value rose (towards, to some extent, alkalinity) in the *Lemna minor* growth media due to the enhanced photosynthetic activities of the plants by ingesting dissolved CO_2 (Kumar and Deswal 2020). The results showed that the value of pH (6.87–7.35) supports the growth of *Lemna minor*. This result is supported by those of Al-Nabhan and Al-Abbawy (2021).

According to **Chaudhuri et al. (2014),** as the level of Cd in solution elevated, so did the deposition of Cd in *L. minor*. This remains consistent with this investigation as revealed by increasing Cd content in *L. minor* with increasing Cd concentration in plant medium throughout the duration (21 days) of experiments (Tables 5–8).

In relation to the metal content in the surrounding environment, the plant's capacity to collect heavy metal is known as the bioconcentration factor (BCF). The results of this experiment showed that the growth medium's 1.0 ppm level of Cd had the maximum bioconcentration factor (BCF) of Cd (Tables 7 and 8). This outcome supports the findings of Khan **et al (2020).**

When the metal Cd was present in *L. minor* at doses of 0.5 and 1.0 ppm, it induced obvious indications of necrosis and chlorosis (Table 11, Fig. 3). The first toxicity indications of Lemna fronds exposed to Cd were chlorosis, or the frond turning from green to yellow, and frond displacement, or the frond breaking off from colonies. Necrosis developed from these symptoms at 1.0 ppm Cd (Table 11, Fig. 3). After a few days of therapy, 0.5 ppm of cadmium was hazardous and induced damage that was noticeable. Similarly, Khellaf and Zerdaoui (2009) and Khan et al. (2020) found toxicity symptoms on *L. minor* at > 0.4 and 0.5 ppm concentrations of Cd, respectively. Finally, in this study, Cd has been successfully eliminated by more than 70% by adding 20 g of the *L. minor* to 3 liters of water containing Cd at a rate of 1 mg/L.

5. Conclusion:

L. minor is a preferable option for phytoremediation of contaminated waterways because of its widespread distribution, quick growth rate, ease of collecting, and wide tolerance to temperature changes. It also performs better in cleaning up Cd-polluted water. The apparent harmful effects of Cd at 0.5 and 1.0 ppm in *L. minor* are suggestive of possible effects of increasing heavy metal concentrations on the system. The relevance of the current work lies in demonstrating *L. minor*'s efficacy as a phytoremediation for a variety of environmentally significant heavy metals, despite the fact that its uncontrolled growth makes it a nuisance species in water bodies. Even while phytoremediation happens naturally, it will work better if the land is managed, planted, and planned for in a coordinated manner.

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