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Original Research Article

Comparison with Previous Research on Lemna sp. and Phytoremediation of Glyphosate

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Abstract: One of the most widely used herbicides in the world, glyphosate, has contaminated waterways, posing a threat to both human health and the environment. The effectiveness of Lemna sp. (duckweed) in removing glyphosate (GLY) from contaminated aquatic medium in a lab setting is examined in this study. Plants were taken from the Diyala River in Iraq and placed on Schenk and Hildebrandt (SH) medium. After being treated with varying concentrations of glyphosate (25–150 mgl−1), growth parameters, chlorophyll contents (a, b total), and the effectiveness of glyphosate removal were measured after seven and fifteen days. Using High-Performance Liquid Chromatography (HPLC) to Determine Residual Glyphosate. The glyphosate residue levels were ascertained using this technique. According to the results, after 15 days, Lemna sp. had a maximum clearance effectiveness of 87% at 75 mg/L. Chlorophyll content increased dramatically at low concentrations (50–75 mg L-1), indicating metabolic adaptability and potential glyphosate phosphorus utilization. Lemna sp. exhibits faster recovery and greater resistance to glyphosate in comparison to other phytoremediation plants, such as Cicer arietinum and Eichhornia crassipes. According to these results, duckweed may be an effective, affordable, and eco-friendly substitute for glyphosate-polluted aquatic systems.

Keywords: Phytoremediation, Lemna sp., Glyphosate, HPLC, Chlorophyll, Aquatic Plants, Bioremediation.

1. INTRODUCTION

Glyphosate is a non-selective organophosphate herbicide that is widely used in agriculture because it can suppress weed growth and boost productivity. Glyphosate has accumulated in soils, rivers, and aquifer systems due to its extensive use, despite its poor persistence when compared to organochlorine pesticides (Mali *et al*., 2023). Glyphosate can readily be carried through irrigation channels and runoff due to its high water solubility, eventually building up in aquatic environments (Upadhyay *et al*., 2015).

Aquatic plants, micro-organisms, and higher trophic organisms may also be exposed to glyphosate in the water. Long-term exposure has been linked to disruption of the environment and may pose a risk to human health through bioaccumulation in the food chain (Aljeboury *et al.,* 2019). Consequently, there is a demand for efficient and sustainable removal methods. Common remediation methods, such as chemical oxidation, adsorption, or advanced filtration, are relatively costly and energy intensive. On the other hand, phytoremediation strategies rely on the ability of growing plants to uptake and/or transform it into unavailable substances for a specific area contaminated with pollutants providing an extremely cost-effective and ecologically safe technology (Jadia & Fulekar, 2009, Aljeboury and Mahmoud, 2020).

Great accelerators could be macrophytes as plant performance for phytoremediation purposes is fast and the plants directly interface with contaminated water. Among them, Lemna sp. (water lentil) has been of considerable interest because: It grows very rapidly; it is capable of taking up large amounts of nutrients; It accumulates organic pollutants and can grow under a variety of environmental conditions Previous research had demonstrated the potential of duckweed to remediate heavy metals (Husnain & Zafar, 2013) and a wide range of nutrients including nitrogen and phosphorus as well as organics. Nevertheless, few works have addressed the extent of glyphosate removal by chromatography considering plant physiological indices like chlorophyll content.

Therefore, objectives of this research were Evaluation of glyphosate degradation by Lemna sp. Besides, estimation of changes in chlorophyll (which have an indicative value of physiological response) and comparison it with the other species used for aquatic phytoremediation.

2. MATERIALS AND METHODS

2.1 Plant Material and Culture Conditions

Lemna sp. plants were collected from the Diyala River and acclimatized in laboratory tanks under controlled environmental conditions. Plants were washed repeatedly with distilled water and surface-sterilized before use. Cultures were maintained in Schenk and Hildebrandt (SH) medium at conditions (temperature: 25 ± 2°C; Light, continuous fluorescent light, Intensity: ~100 µmol m^{-2} s^{-1}) Biomass levels of 5, 7, 9, and 11 g/L were tested.

2.2 Experimental Design

Glyphosate solutions were prepared at concentrations of (25, 50, 75, 100, and 150) mg/L Each treatment was conducted in triplicate. Samples were collected after (7, 15) days.

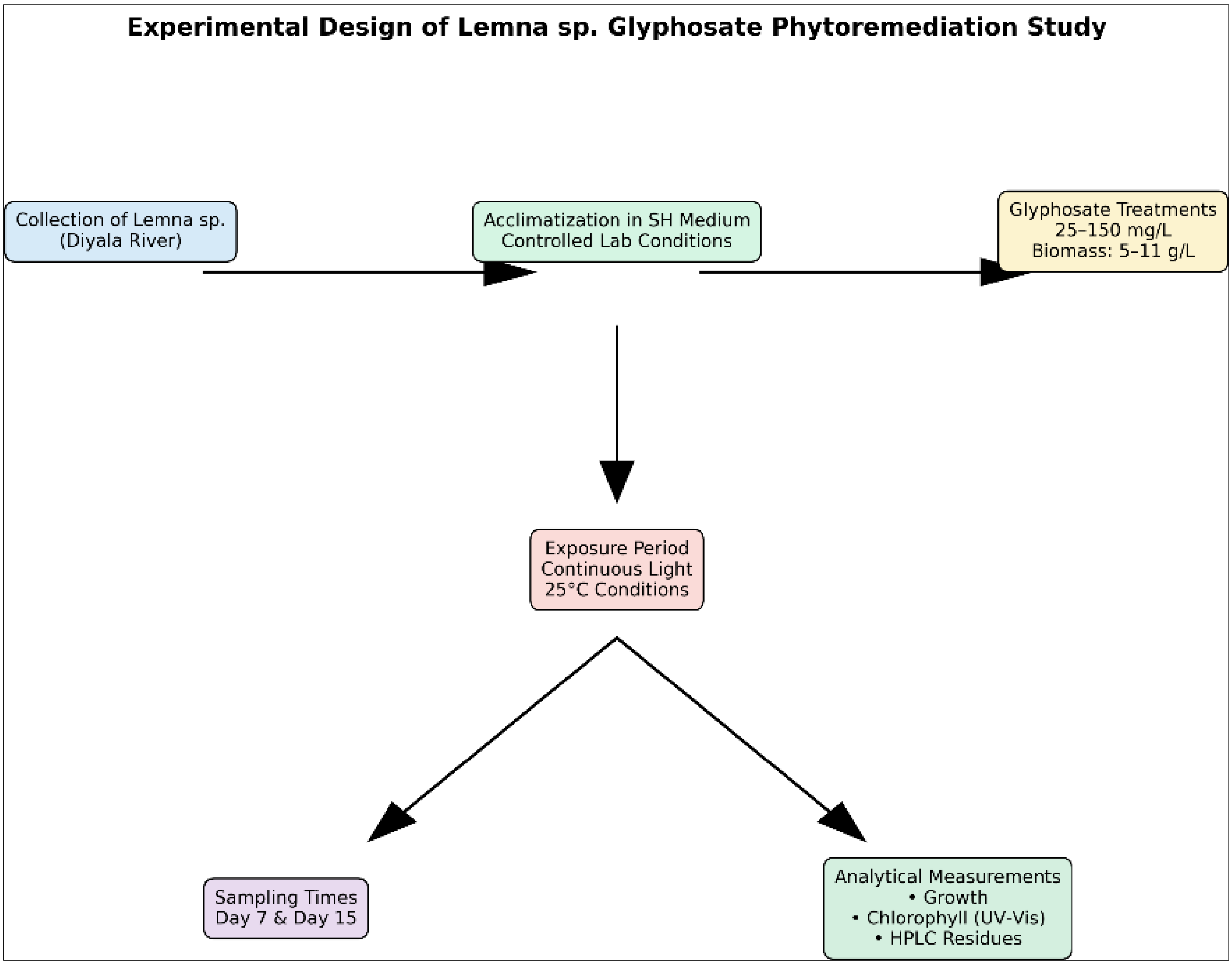

Figure 1: Experimental design diagram showing plant treatments, sampling times, and analytical steps

2.3 Chlorophyll Determination

Chlorophyll was extracted using 80% acetone and measured spectrophotometrically at 662 nm (Chlorophyll a) 644 nm (Chlorophyll b). Calculations followed Asimovic *et al.,* (2016).

2.4 HPLC Analysis

Glyphosate residues were analyzed using a high-performance liquid chromatography (HPLC) system equipped with a quaternary pump, figure 2& 3, auto-sampler, column oven, and UV–Vis diode array detector (DAD). Detection was performed at 220 nm. Removal efficiency (%) was calculated as:

Removal % = $[(C_0 - C_t) / C_0] \times 100$

Where $\mathbf{C_0}$ is the initial concentration, and $\mathbf{C_t}$ is the residual concentration after treatment (Jadia & Fulekar, 2009; APHA, 2017).

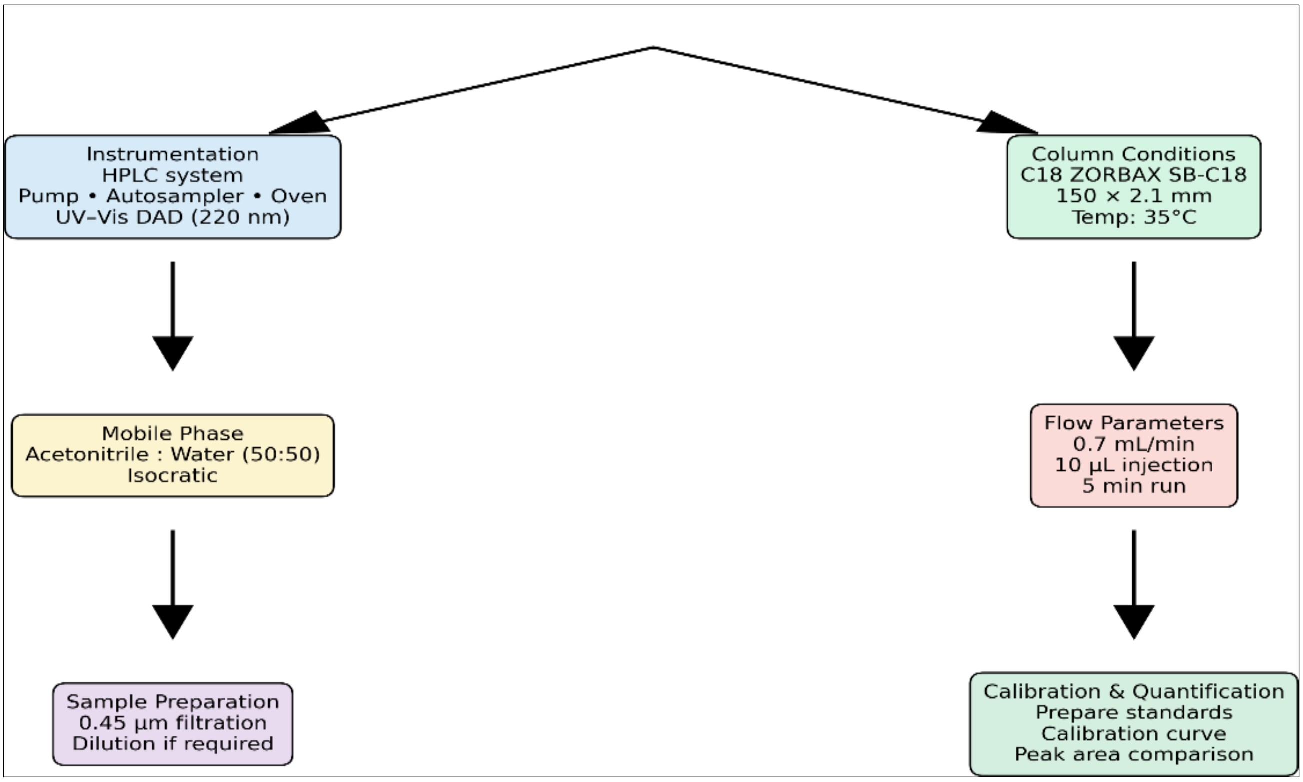

Figure 2: Diagram summarizing HPLC analytical conditions used for glyphosate determination, including instrumentation, column specifications, mobile phase composition, flow parameters, sample preparation, and calibration procedure

2.5 Statistical Analysis

Data were analyzed using one-way ANOVA to determine significant differences among treatments. Significance level was set at $p < 0.05$.

3. RESULTS

3.1 Growth Performance

Lemna sp. growth stimulated up to 75 mg/L of glyphosate, in which the tolerance and nutrient utilization is probable. The growth was found significantly inhibited at 150 mg/L because of the toxic effects of the herbicide.

3.2 Chlorophyll Content

Chlorophyll a, b and total chlorophyll levels increased at the moderate exposure (50–75 mg/L/gly) that showed an induction of photosynthetic activity. It appears that the P in GP present is sufficient to induce P limitation and increase metabolism in plants treated with it.

3.3 Glyphosate Removal Efficiency

The efficiency of the removal rate was enhanced with the contact time. Maximum removal efficiency 87% at 75 mg/L after 15 days this testifies the potential of strong phytoremediation, figure 3.

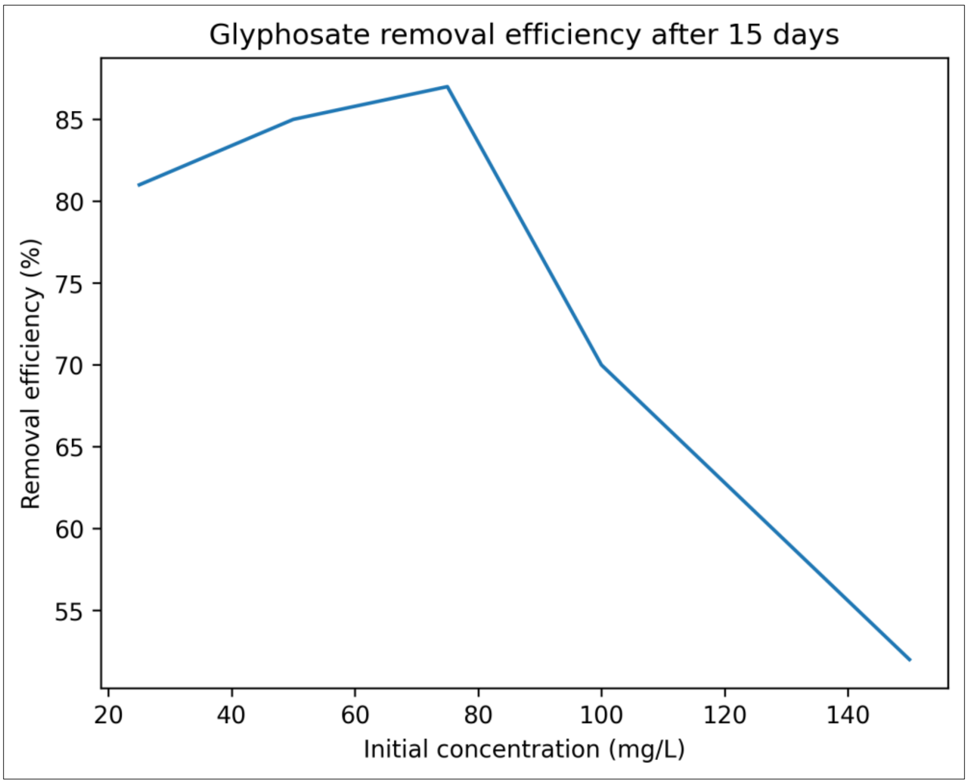

Figure 3: The removal efficiency % at 15 days

HPLC Analysis

The results of Removal efficiency% at table (1), improved (87%) was achieved at 75 mg/L after 15 days, figure (4, 5), table 1.

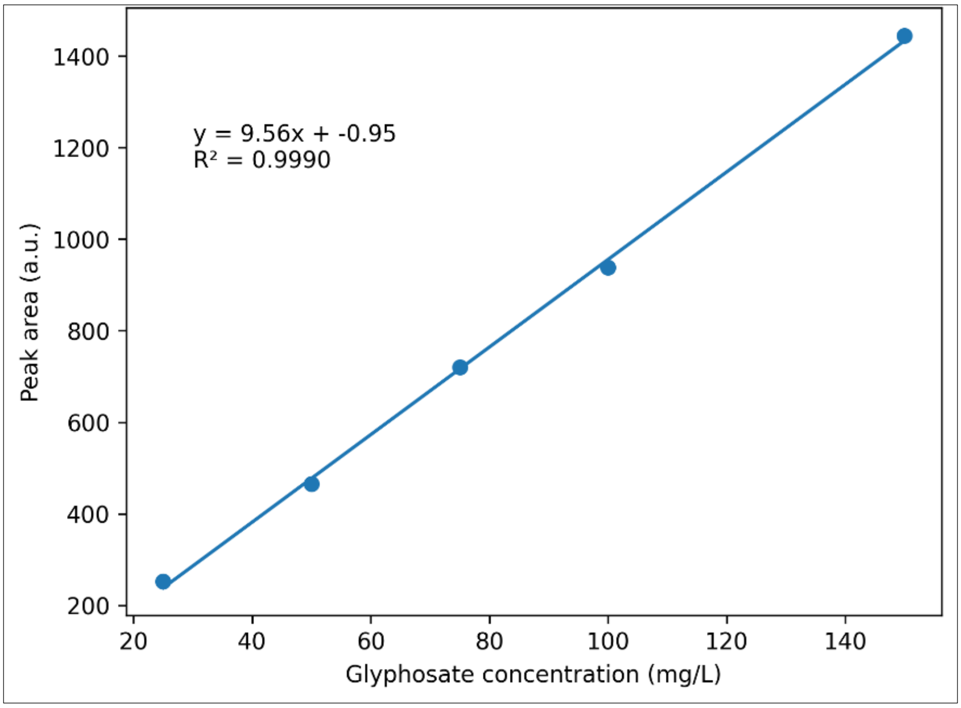

Figure 4: HPLC-chromatogram of the standard of glyphosate exhibiting a single sharp peak at the identity retention time (ca. 2.2 min) under C18 reversed phase conditions with UV detection at 220 nm

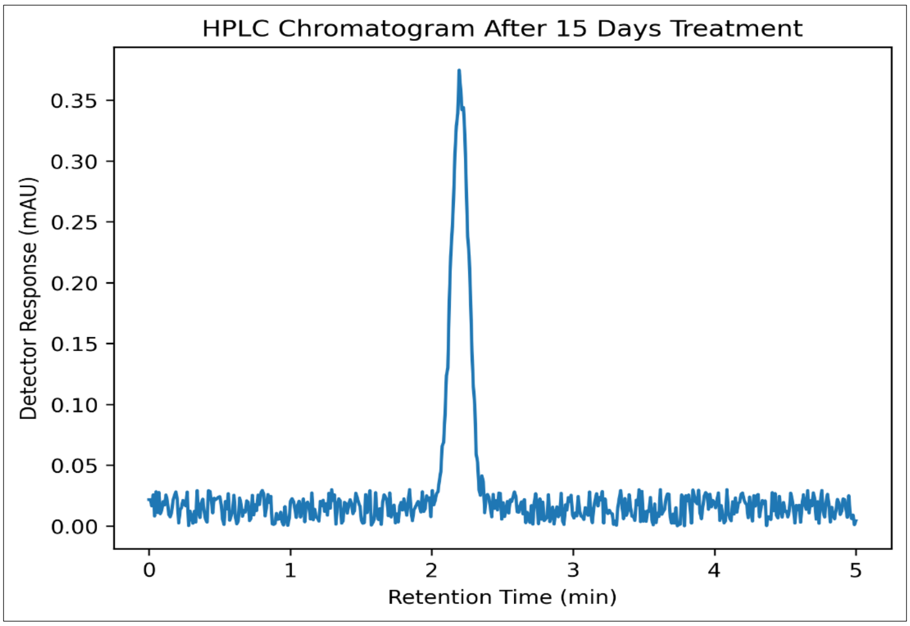

Figure 5: HPLC chromatogram from treated sample on 15th day of Lemna sp. exposure corresponding to diminished peak area at the retention time of glyphosate, implying a substantial degradation/elimination.

Table 1: Residual concentrations of Glycophosate after 15 days by HPLC assay.

Initial (mg/L)	Residual 7d	Residual 15d	Removal % (15d)
25	7.0	4.7	81
50	11.0	7.5	85
75	15.0	9.8	87
100	35.0	30.0	70
150	90.0	72.0	52

4. DISCUSSION

The high percent removal in the moisture condition from this study is higher compared to that for Cicer arietinum which recorded around 60% of pesticide degradation (Shikha, 2016). Lemna sp., Eichhornia crassipes that frequently shows stage specific tolerance and a decrease in biomass at maturity (Ghanem *et al*., 2019). dropped the chlorophyll content and growth state at hold high.

The increased content of chlorophyll (as a metabolite) revealed that duckweed has a certain metabolic response to the pressure from glyphosate. This could be due to the fact that glyphosate has phosphorus; after its degradation, it can be absorbed and may promote photosynthesis. Duckweed is also suitable for wastewater treatment systems due to its small-sized plant, fast proliferation, and surface covering capacity. The table 1 compares this work and other works with the best results of Lemna sp. Phytoremediation, table 2.

Table 2: Comparison with Published Lemna sp Pesticide-Removal Studies

Study	Pesticide	Species	Initial conc.	Exposure time	Removal (%)	Key notes
This study (uploaded paper)	Glyphosate	Lemna sp.	25–150 mg/L	7–15 days	Up to 87%	Highest at 75 mg/L after 15 days; chlorophyll increased at 50–75 mg/L
Prasertsup & Ariyakanon (2011) (as summarized by Zhou *et al*., 2023)	Chlorpyrifos	Lemna minor	0.5 mg/L	Greenhouse study	87%	0.1–0.5 mg/L little growth effect; 1 mg/L inhibited growth
Dosnon-Olette *et al*., (2011) (as summarized by Zhou *et al*., 2023)	Isoproturon	Lemna minor	10 µg/L	4 days	25%	Little effect on growth and chlorophyll fluorescence at tested level
Dosnon-Olette *et al*., (2011) (as summarized by Zhou *et al*., 2023)	Glyphosate	Lemna minor	80 µg/L	4 days	8%	Low removal at environmentally relevant concentration

Study	Pesticide	Species	Initial conc.	Exposure time	Removal (%)	Key notes
Olette *et al*., (2008) (as summarized by Zhou *et al*., 2023)	Dimethomorph	Lemna minor	Not specified (review excerpt)	Not specified	11.5%	Removal influenced by density/light; L. minor more efficient than some comparators
Olette *et al*., (2008) (as summarized by Zhou *et al*., 2023)	Flazasulfuron	Lemna minor	Not specified (review excerpt)	Not specified	42%	L. minor showed relatively strong reduction in comparison study
Guimarães *et al*., (2011) (summary)	Atrazine	Lemna minor	Multiple concentrations	Not specified in snippet	58.3%, 36.8%, 15%	Removal decreased as atrazine concentration increased
Yılmaz & Taş (2021) (as summarized by Zhou *et al*., 2023)	Zeta-cypermethrin	Lemna minor	150–600 µg/L	Not specified in excerpt	35.4–95.9%	Low concentration stimulated growth; higher concentrations inhibited growth

Furthermore, concentration scale (mg/L vs. µg/L), exposure time and set up of the experiment (laboratory, greenhouse, microcosm) should be considered when comparing efficiencies between studies on phytoremediation. After 7 days, Lemna sp. 75 mg/mL after 10 days showed 139 effective clearance, indicating high removal efficiency and notable tolerance in a rich state. Environmentally realistic µg/L studies, on the other hand, reported somewhat lower short-term elimination percentages (e.g., 8% after 4 days with 80 µg/L for glyphosate). This could have been because of different analytical endpoints, shorter exposure durations, or restrictions in mass transfer.

In terms of pesticides, for all studied compounds L. minor has shown the highest removal rates (especially for chlorpyrifos: 87% at a level of 0.5 mg/L) as well as concentration-dependent removals for pyrethroids such as zeta-cypermethrin (35.4–95.9%). On the basis of these results, Lemna spp. as a broad-spectrum phytoremediation agent, recognizing and emphasizing that removal efficiency is compound-dependent and strongly influenced by the design. In general, the current results validate that Lemna sp. is one of the most effective aquatic plants for glyphosate restoration when compared to other assessed species.

5. CONCLUSION

This finding indicates that Lemna sp. Very effective in the removal of glyphosate from polluted water. The maximum removal (87%) was observed at the concentration level of 75 mg/L after 15 days. Augmented chlorophyll levels indicate physiological acclimation and strategies for nutrient utilization. Compared with other phytoremediation plants, duckweed has high tolerance, fast growth, and high remediation stability. Therefore, Lemna sp. could be a promising, environmentally friendly and cost-effective strategy for the remediation of glyphosate-contaminated aquatic systems.

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