

Ecotoxicological Evaluation of *Azolla pinnata* and *Lemna minor* for Biomonitoring Cd and Pb Contamination in Aquatic Environments

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Abstract— Heavy metal pollution in aquatic environments has led to devastating long-term effects in nature. Therefore, immediate action is required to develop ecotoxicological monitoring strategies to combat this environmental issue. This study evaluated the response of two aquatic plants, *Azolla pinnata* (Mosquito fern) and *Lemna minor* (Duckweed), to a concentration series (0.1–10.0 mg L⁻¹) of two heavy metals, Cadmium (Cd) and Lead (Pb), over a 3-day and 7-day microcosm exposure. A multiparameter approach was used where vegetative growth (frond number, wet weight, root length and plant width), biochemical changes related to oxidative stress (glutathione (GSH), peroxidase (POD), superoxide dismutase (T-SOD) activity), as well as chlorophyll a content were measured. Both plants showed an increase in growth at lower metal concentrations (up to 1 mg L⁻¹), but growth decreased dose-dependently with Cd being more toxic than Pb ($p < 0.05$), shown by severe necrosis and browning in plants. Results of the biochemical tests revealed more complex defense mechanisms at play. Elevated POD activity (112–119 U mg⁻¹) and T-SOD activity (2.4–14.24 U mg⁻¹) at higher concentrations of Cd exposure, coupled with a significant decrease in chlorophyll a (8.92 to 3.93 units, $p < 0.05$), indicating targeting of the photosynthetic apparatus, were some of the trends observed. Both plants demonstrated consistent responses to heavy metal exposure within relevant environmental ranges, indicating their suitability to be used as ethical plant models for ecotoxicity testing and environmental monitoring.

I. INTRODUCTION

Heavy metal contamination is a persistent global threat to aquatic ecosystems. Unlike organic pollutants, metals such as cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), and arsenic (As) resist biological degradation, accumulating in water, sediments, and biological tissues. Their mobility, and toxicity make them critical contaminants for ecotoxicological monitoring and ecological risk assessment [1,2]. The biological consequences of heavy metal contamination extend from molecular and biochemical disturbances to impaired growth, reproduction, and ecosystem structure.

Consequently, biological responses complement conventional chemical measurement. While chemical analysis quantifies contaminant concentrations, biological monitoring indicates whether contaminants are biologically available and capable of producing measurable effects in living organisms [3]. Biomonitoring complements environmental assessment through measurable structural, physiological, biochemical or molecular responses. Ideal bioindicator organisms are sensitive, available, easy to maintain, and reproducible. Aquatic organisms have traditionally included fish, amphibians, algae, and invertebrates; however, plant-based systems have increasingly received attention because of their ecological relevance and contaminant accumulation [3,4].

Among aquatic plants, *Lemna* spp. (duckweed) is a primary model for toxicity assessment. These rapidly growing, free-floating freshwater plants have a simple morphology and vegetative reproduction. Their small size, short generation time, high growth rate, and ease of cultivation under controlled conditions make them suitable for laboratory-based toxicity testing [5]. *Lemna* species accumulate heavy metals, and toxic exposure produces measurable changes in frond number, biomass, frond area, root development, and photosynthetic characteristics [5,6]. These properties support using duckweed for pollution detection. The suitability of *Lemna* for standardized ecotoxicological assessment is further demonstrated by the OECD *Lemna* growth inhibition test. While frond number is a principal measurement variable, other parameters such as area and biomass provide additional information on toxicity [7]. Such standardized endpoints enable comparative assessment and sublethal effects detection.

Another promising aquatic plant is *Azolla* spp. (mosquito fern), a small, fast-growing floating fern with high biomass production. Its extensive surface contact with water and capacity to accumulate inorganic contaminants make it relevant to ecotoxicity studies. Several *Azolla* species accumulate metals including Cd, Pb, Cr, Ni, supporting their role in biomonitoring [8–10]. Furthermore, metal exposure induces visible morphological and biochemical changes, offering reliable endpoints for assessing environmental stress.

The value of *Azolla* and *Lemna* extends beyond metal accumulation, as heavy metal exposure triggers cellular defense mechanisms associated with oxidative stress. Enzymes such as superoxide dismutase (SOD) and peroxidases (POD), alongside non-enzymatic antioxidants such as glutathione (GSH), protect cells from oxidative damage [11]. Consequently, changes in antioxidant activity provide sensitive early-warning indicators before severe morphological damage occurs. Other endpoints, including chlorophyll content, lipid peroxidation, DNA damage, and growth inhibition, can provide complementary physiological and cellular insights. Using multiple endpoints enables biological responses to be evaluated across different organizational levels rather than relying solely on visible toxicity.

Additionally, *Lemna* and *Azolla* provide insights into both exposure and biological effect. Metal accumulation indicates contaminant presence and bioavailability, whereas physiological shifts – such as growth, pigmentation, and antioxidant responses, reflect toxicity. This combination is crucial as total metal concentration alone does not necessarily represent the fraction of a contaminant that is capable of causing ecological effects [12]. Integrating chemical data with plant-based biological responses strengthens environmental assessments and risk interpretation. Aquatic plants also offer practical biomonitoring advantages. Compared to animal-based bioassays, plant systems are easily maintained, require minimal space and resources, and allow repeated measurements. Consequently, they support rapid screening of contaminated waters as a cost-effective complement to instrumental chemical analysis.

Despite these advantages, the responses of aquatic plants to heavy metals vary with plant species, metal type and concentration, exposure duration, environmental conditions and interactions among contaminants. Although individual effects of Cd and Pb have been documented, solely relying on these findings may risk an incomplete assessment, and direct comparative studies evaluating morphological, biochemical, and photosynthetic endpoints across both *Azolla* and *Lemna* under microcosm conditions remain scarce. Accordingly, this study examines vegetative parameters, antioxidant responses, and chlorophyll a content in both plants following Cd and Pb exposure to provide a more comprehensive understanding of their suitability as biomonitoring organisms

II. METHODOLOGY

A. Test Plant Selection

Wild samples of *Azolla pinnata* and *Lemna minor* collected from a natural water body were maintained in two separate glass tanks filled with dechlorinated tap water and kept outdoors under natural diurnal sunlight. Ambient temperatures during the experimental period ranged from 22°C to 32°C. From these cultures, plants with uniform morphology, specifically *Azolla* with 12-

13 fronds and *Lemna* with 3 fronds, were selected for the exposure assays.

B. Preparation of Heavy Metal Solutions

Analytical grade cadmium chloride hydrate ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$; molecular weight 201.32 g mol⁻¹) and lead nitrate ($\text{Pb}(\text{NO}_3)_2$; molecular weight 331.21 g mol⁻¹) were dissolved in dechlorinated tap water to prepare individual 1000 mg L⁻¹ (ppm) stock solutions. Working solutions of 0.1, 1.0 and 10.0 mg L⁻¹ were prepared by a serial dilution of the respective stocks into a final volume of 2L. This 0.1-10.0 mg L⁻¹ range was selected to span environmentally relevant contamination levels up to severe industrial pollution thresholds.

C. Individual Metal Exposure

The responses of *Azolla* sp. and *Lemna* sp. to Cd and Pb were investigated under controlled conditions. For each concentration, 500 mL of the respective exposure solution was added to clean glass troughs. *Azolla* plants were introduced at a density of 20 plants per experimental unit. Separate exposure groups were established using both root-intact and rootless plants. Corresponding controls used 500 mL of dechlorinated water without added heavy metals. The same procedure was followed for *Lemna* sp. Three replicate units were established per concentration. Troughs were maintained under natural light and ventilation for 3 and 7 days without additional treatments (Table 1).

TABLE I. SUMMARY OF THE EXPERIMENTAL TREATMENT GROUPS FOR *AZOLLA PINNATA* AND *LEMNA MINOR*

Metal Type	Concentration (mg L ⁻¹)	Exposure Durations	Plant Condition/Replication
Control	0.0	3 & 7 days	RI & RL; triplicated
Cd	0.1, 1.0, 10	3 & 7 days	RI & RL; triplicated
Pb	0.1, 1.0, 10	3 & 7 days	RI & RL; triplicated

Cd-Cadmium, Pb-Lead, RI-Root-intact, RL-Rootless

D. Assessment Following Exposure

After each exposure period, five plants per replicate, that is 15 plants per treatment concentration across the three replicate units, were randomly selected for biological assessment, while remaining tissue was harvested for biochemical analyses.

III. RESULTS

A. Effects of Cd and Pb Exposure on *Azolla*

Exposure to Cd and Pb produced concentration-dependent changes in the vegetative characteristics of *Azolla* sp. across the tested concentration range of 0.1–10.0 mg L⁻¹ (ppm; Figure 1). The experimental results were obtained for both 3-day and 7-day exposure periods, with the more pronounced responses generally evident following the longer exposure period.

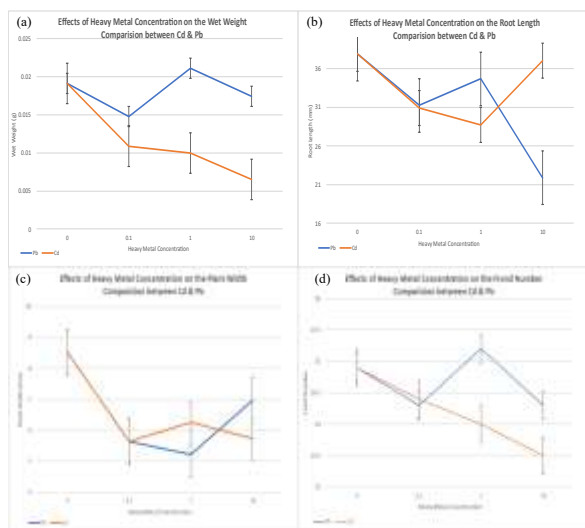


Figure 1. Comparison between single-metal exposure of Cd and Pb on the vegetative parameters of *Azolla* (a) Wet weight (b) Root Length (c) Plant Width (d) Frond Number

Cd exposure resulted in a progressive reduction in several vegetative parameters of *Azolla* as the exposure concentration increased. The reduction in wet biomass was particularly evident. The number of fronds also generally declined with increasing Cd concentration, with the strongest effects occurring at the highest concentration tested (10.0 mg L⁻¹). Root-intact plants generally exhibited more pronounced responses than rootless plants. Changes in root length were less consistent than those observed for biomass and frond production.

The response of *Azolla* to Pb differed from that observed under Cd exposure. Frond production remained relatively stable, and in some treatments demonstrated a slight increase, at concentrations up to 1.0 mg L⁻¹. A clear reduction in frond number became evident at 10.0 mg L⁻¹ Pb. Wet biomass similarly remained comparatively stable at lower Pb concentrations, with a slight increase at 1.0 mg L⁻¹ followed by a marked reduction at 10.0 mg L⁻¹. Root length also showed a generally decreasing trend with increasing Pb concentration, although some recovery was observed at 1.0 mg L⁻¹. Plant width generally decreased with increasing Pb concentration, while a slight increase was observed at the lowest concentration.

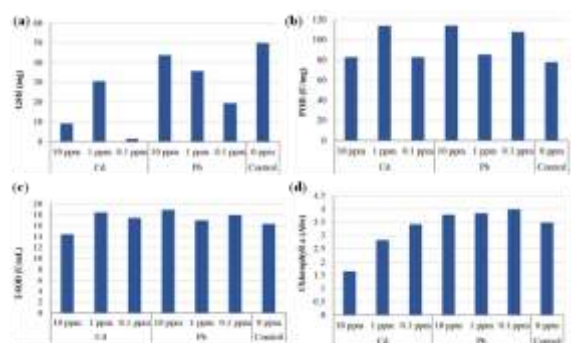


Figure 2. Alterations of Biochemical parameters (a) GSH-Glutathione levels (b) POD- Peroxidase levels (c) T-SOD-Superoxide Dismutase levels and (d) Chlorophyll a levels of *Azolla* upon exposure to Cd and Pb (0.1-10 mg L⁻¹) for 7 days

To assess physiological responses GSH content, POD, T-SOD, and chlorophyll a were assayed in *Azolla*. Control GSH content was 50 mg. Cd exposure significantly reduced GSH to 9 mg at 0.1 mg L⁻¹ (Kruskal-Wallis, $p < 0.05$, Figure 2(a)), whereas levels were higher at 1.0 and 10.0 mg L⁻¹. Similarly, Pb exposure yielded the lowest GSH at 0.1 mg L⁻¹ (20 mg), compared to 36 mg (1.0 mg L⁻¹) and 43 mg (10.0 mg L⁻¹). Both metals—particularly at low doses—reduced endogenous GSH, indicating cellular stress, with Cd showing a more pronounced reduction than Pb. Control POD activity (75 U mg⁻¹) increased gradually under both Cd and Pb treatments, peaking at 1.0 mg L⁻¹ (110 U mg⁻¹), though this trend was not significant (Kruskal-Wallis, $p > 0.05$, Figure 2(b)). Overall, metal exposure induced higher POD activity than the control, signaling an oxidative stress response. Control T-SOD activity was approximately 16 U mg⁻¹ (Figure 2(c)), remaining relatively stable across treatments (Kruskal-Wallis, $p > 0.05$). The highest level (~ 18 U mg⁻¹) occurred at 0.1 mg L⁻¹ Cd, with slightly elevated levels at lower Cd concentrations versus the control. Chlorophyll a absorbance (Figure 2(d)) declined significantly at 10.0 mg L⁻¹ Cd (Kruskal-Wallis, $p < 0.05$). Conversely, Pb treatment showed no considerable effect, maintaining levels comparable to or slightly exceeding the control.

B. Effects of Cd and Pb Exposure on Lemna

Similar to *Azolla*, *Lemna* sp. showed measurable responses following exposure to Cd and Pb at 0.1, 1.0 and 10.0 mg L⁻¹ (Figure 3).

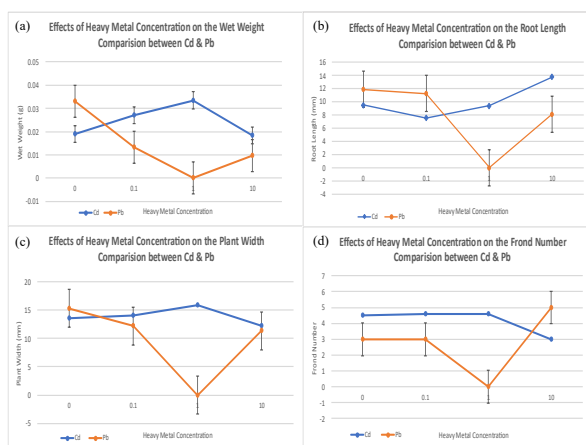


Figure 3. Comparison between single-metal exposure of Cd and Pb on the vegetative parameters of *Lemna* (a) Wet weight (b) Root Length (c) Plant Width (d) Frond Number

Cd produced a pronounced inhibitory effect on *Lemna* growth. Frond number declined sharply with increasing Cd exposure, and the reduction was evident even at the lower tested concentrations. The number of fronds remained relatively low at higher Cd concentrations.

Root-related responses differed between the exposure groups. In root-intact plants, root length exhibited an increasing trend at some Cd concentrations, followed by a marked decline at 10.0 mg L⁻¹. In rootless plants, no substantial root regeneration was observed during the exposure period. The effects on plant width also became increasingly evident with increasing metal concentration, with the strongest reduction occurring at the highest exposure level.

Pb exposure produced a comparatively less severe response in *Lemna* at the lower concentrations. Frond number showed a slight increase from the control condition to 1.0 mg L⁻¹ and remained relatively stable at this concentration. However, a clear decline in frond production was observed at 10.0 mg L⁻¹ Pb.

The responses of wet biomass, root length and plant width similarly varied according to Pb concentration. The overall pattern indicated greater effects at 10.0 mg L⁻¹, whereas lower concentrations produced comparatively limited changes. Thus, the response of *Lemna* to Pb differed from its response to Cd, particularly at the lower and intermediate concentrations

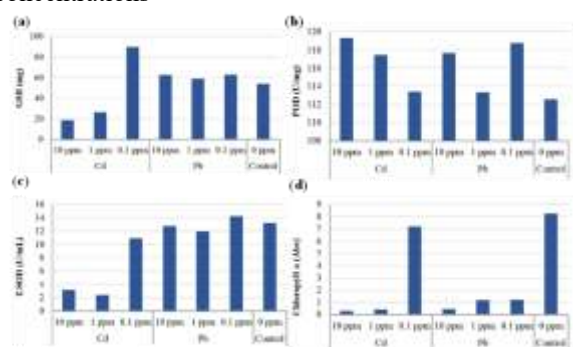


Figure 4. Alterations of Biochemical parameters (a) GSH- Glutathione levels (b) POD- Peroxidase levels (c) T-SOD- Superoxide Dismutase levels and (d) Chlorophyll a levels of *Lemna* upon exposure to Cd and Pb (0.1-10 mg L⁻¹) for seven days

To assess physiological responses GSH content, POD, T-SOD, and chlorophyll a were assayed in *Lemna*. Control GSH (55 mg) declined to 20 mg at 10.0 mg L⁻¹ Cd, marking the highest toxicity (Kruskal-Wallis, $p < 0.05$, Figure 4(a)). Low Cd (0.1 mg L⁻¹) increased GSH above control levels, whereas high Cd (10.0 mg L⁻¹) reduced it. Conversely, Pb exposure maintained GSH above 55 mg across all concentrations. Control POD activity (110 U mg⁻¹) increased to 118 – 119 U mg⁻¹ at 10.0 mg L⁻¹ for both Cd and Pb (Figure 4(b)), though Pb peaked highest at 0.1 mg L⁻¹. Control T-SOD (14 U mg⁻¹) changed significantly under Cd exposure but remained stable under Pb (Figure 4(c)). Specifically, Cd at 10.0 mg L⁻¹ caused a significant decline to 3 U mg⁻¹ (Kruskal-Wallis, $p < 0.05$), whereas Pb treatments stayed between 10 and 13 U mg⁻¹. Both Cd and Pb exposure significantly reduced chlorophyll a content (Figure 4(d)). High Cd severely decreased levels, while Pb maintained reduced levels relative to the control across all concentrations.

IV. DISCUSSION & CONCLUSION

To fulfill the primary research objective of evaluating *Azolla pinnata* and *Lemna minor* as effective biomonitoring organisms, this study shows that both plants are sensitive and reliable bioindicators for heavy metal contamination in water, providing measurable physiological and morphological endpoints for environmental assessment. Pb exposure induced less severe morphological and biochemical disruption, with certain lower concentrations corresponding to a slight increase in growth parameters, relative to Cd, which was far more toxic and hindered growth severely.

A. Comparative Responses of *Azolla* and *Lemna* to Cd and Pb

The present study demonstrates clear differences in the responses of *Azolla* sp. and *Lemna* sp. to Cd and Pb, with Cd generally producing more pronounced adverse effects than Pb. The effects were evident at both morphological and biochemical levels and became more apparent with increasing exposure concentration and duration. These observations are consistent with the established toxicity of Cd in plants, where disruption of nutrient acquisition, oxidative balance, photosynthesis and cellular metabolism can occur even at relatively low concentrations [1,2,13].

The pronounced sensitivity to Cd observed in both aquatic plants was reflected by reductions in frond production, wet biomass and plant width. In *Azolla*, the decline in wet weight was particularly evident with increasing Cd concentration, whereas *Lemna* showed a marked reduction in frond number even at relatively low Cd exposure. Such responses are important from a biomonitoring perspective because changes in growth are integrated responses to several underlying physiological disturbances. Duckweed growth, in particular, has previously been demonstrated to provide a sensitive endpoint for assessing contaminant toxicity, and the *Lemna* root-regrowth test has been validated as a standardized approach for toxicity assessment [11,12]. The present observations therefore support the use of vegetative characteristics as relatively simple and readily measurable indicators of heavy-metal stress for monitoring applications.

The comparatively lower toxicity of Pb observed in the present study is also consistent with the different physiological behaviour of Pb in plants. Pb is generally poorly mobile within plant tissues and can become associated with cell walls and extracellular binding sites, although sufficiently high exposure can still disrupt membrane integrity, nutrient balance, photosynthesis and growth [3,14]. In the present experiment, Pb exposure produced relatively stable or slightly increased values for some growth parameters at lower concentrations, followed by clear inhibition at 10.0 mg L⁻¹. This contrast with Cd suggests that the two metals should not necessarily be treated as having equivalent toxicity when aquatic plants are used as biomonitoring organisms.

The slight stimulation of some growth parameters at lower Pb concentrations may represent a low-dose stimulatory or hormetic response. Hormesis describes a biphasic response in which low levels of a stressor may stimulate particular biological processes whereas higher concentrations become inhibitory [4]. Similar low-dose stimulation has been reported in plants exposed to environmental stressors, although the biological mechanisms responsible can vary among species and experimental conditions. Therefore, the slight increases observed at lower Pb concentrations should be interpreted cautiously without asserting definitive nutritional benefits.

B. Morphological Responses as Indicators of Heavy Metal Contamination

The different responses of frond number, wet weight, root length and plant width demonstrate the value of using multiple morphological endpoints rather than relying on a single growth parameter. Frond number provides a particularly useful measure because both *Azolla* and *Lemna* reproduce rapidly, allowing changes in growth to become visible over relatively short exposure periods. The strong inhibition of frond development under Cd exposure in *Lemna* indicates that reproductive or vegetative propagation may be particularly sensitive to Cd stress.

The reduction in plant width observed at higher concentrations in both species is also ecologically relevant because plant surface area is closely associated with photosynthetic activity and biomass production. A reduction in vegetative growth may therefore represent the cumulative effect of impaired metabolism rather than an isolated morphological response. Previous studies have similarly demonstrated that heavy metals can alter growth, morphology and physiological performance in duckweed and other aquatic macrophytes [5,11].

Root responses provided additional evidence of species- and metal-specific toxicity. When comparing species systematically, *Azolla* generally produced a reduction in root length with increasing Cd/Pb concentration. *Lemna* exhibited a distinct biphasic pattern in root length, increasing under lower to intermediate Cd exposure before declining sharply at 10.0 mg L⁻¹. These differences emphasize that root growth cannot always be interpreted as a simple linear indicator of exposure. Instead, root responses may reflect a balance between metal uptake, stress-induced growth modification and the capacity of the plant to restrict metal translocation.

The comparison between root-intact and rootless plants is particularly informative. The generally stronger responses observed in root-intact plants indicate that roots contribute substantially to the interaction between the plant and dissolved metals. Roots can act as sites of metal adsorption, uptake and sequestration, while also representing an important barrier to movement of metals into other plant tissues. This interpretation agrees with the recognized role of plant roots in metal immobilization and detoxification [6]. At the same time, responses in rootless plants indicate that metal interaction with above-ground tissues should not be

disregarded. Consequently, whole plants may provide a more comprehensive measure of environmental exposure because both roots and aerial tissues contribute to contaminant interception.

C. Heavy Metal-Induced Oxidative Stress and Antioxidant Responses

The biochemical responses provide evidence that the morphological changes observed in the present study were accompanied by alterations in cellular stress responses. Heavy metals can promote oxidative stress through several mechanisms, including disruption of electron transport, interference with antioxidant systems and indirect stimulation of reactive oxygen species (ROS) production [2,7,13]. Plants consequently activate both enzymatic and non-enzymatic antioxidant mechanisms to maintain cellular redox balance.

When evaluating species differences systematically, antioxidant and biochemical pathways diverged between *Azolla* and *Lemna*. GSH showed substantial differences between species and metals. In *Azolla*, Cd and Pb exposure generally reduced GSH, particularly under stronger metal stress. In *Lemna*, however, low Cd exposure resulted in an increase in GSH followed by a substantial reduction at higher Cd concentrations. This biphasic response is biologically plausible because an initial increase in GSH may represent activation of protective mechanisms, whereas sustained or excessive oxidative stress can exceed the capacity for GSH synthesis and regeneration. GSH is important not only as a direct antioxidant but also in the sequestration and detoxification of potentially toxic metals. The importance of glutathione and related antioxidant pathways in plant responses to heavy-metal stress has been widely recognized [2,7]. The differences between *Azolla* and *Lemna* indicate that GSH may be a useful biomarker of metal stress, but its interpretation should be based on concentration and species. A reduction in GSH may indicate depletion under severe oxidative stress, whereas an increase may indicate an activated defence response. Thus, GSH measurements are potentially more informative when interpreted together with enzymatic antioxidant activities and growth responses.

POD activity generally increased following exposure to both Cd and Pb in the present study. The pronounced increase in POD, particularly at intermediate or higher exposure concentrations, suggests activation of enzymatic defence mechanisms. Peroxidases participate in the removal of hydrogen peroxide and are therefore important components of plant protection against oxidative injury. Similar increases in antioxidant enzyme activity have been documented in aquatic plants exposed to heavy metals, including *Lemna* [15]. The observed POD response therefore provides biochemical evidence supporting the interpretation that heavy-metal exposure generated oxidative stress before or alongside visible growth impairment.

In contrast, T-SOD responses were less uniform. *Azolla* maintained relatively stable SOD activity across much of the exposure range, whereas *Lemna* exhibited

a marked reduction in SOD activity under Cd exposure, particularly at higher concentrations. SOD constitutes one of the primary enzymatic barriers against oxidative stress by converting superoxide radicals to hydrogen peroxide. Consequently, suppression of SOD at high Cd concentrations may indicate that the antioxidant system was being impaired or overwhelmed rather than simply activated. The contrasting responses of POD and SOD demonstrate that antioxidant enzymes do not necessarily respond synchronously to metal exposure and that species-specific differences in antioxidant capacity are important when selecting biomarkers [7,15].

The combined interpretation of GSH, POD and T-SOD therefore provide stronger evidence of metal-induced oxidative stress than any individual biomarker. In particular, the combination of reduced GSH, increased POD and decreased SOD at higher Cd exposure in *Lemna* suggests a transition from an adaptive antioxidant response at lower exposure to physiological stress at higher exposure.

D. Effects on Chlorophyll and Photosynthetic Performance

The decline in chlorophyll a provides an important link between biochemical stress and the observed reduction in plant growth. Chlorophyll is particularly vulnerable to heavy-metal-induced physiological disturbances because metal exposure can interfere with pigment biosynthesis, chloroplast structure and photosynthetic electron transport [8,16]. In the present study, Cd produced a pronounced reduction in chlorophyll a in both *Azolla* and *Lemna*, with the strongest effects occurring at higher exposure concentrations.

The greater effect of Cd on chlorophyll compared with Pb is consistent with the overall pattern of Cd toxicity observed for the vegetative parameters. Previous research has demonstrated that Cd can interfere with photosynthesis both directly and indirectly, including effects on chloroplast function and enzymes associated with carbon fixation [8,16]. Similar effects have been reported for duckweed exposed to heavy metals, supporting the usefulness of chlorophyll content as a physiological indicator of contamination [15].

Although Pb produced less severe effects than Cd, chlorophyll a was nevertheless reduced relative to controls in *Lemna*. This indicates that the apparently greater tolerance of Pb at the morphological level does not necessarily indicate the absence of physiological stress. Such a distinction is important for biomonitoring because biochemical or physiological endpoints may respond before severe morphological deterioration becomes evident

This study investigated the ecotoxicological responses of *Azolla pinnata* and *Lemna minor* exposed to Cd and Pb across a 0.1–10.0 mg L⁻¹ concentration gradient. The main finding is that both species demonstrate distinct, concentration-dependent toxicity profiles, with Cd causing severe growth inhibition and

chlorophyll a degradation compared to the more moderate responses triggered by Pb. Regarding critical endpoint patterns, growth inhibition and reduced chlorophyll a served as robust macroscopic indicators, while coordinated shifts in antioxidant enzymes (POD, T-SOD) and glutathione (GSH) revealed underlying oxidative stress and species-specific defense thresholds. By directly comparing these integrated multi-level endpoints, this study contributes a validated baseline framework for aquatic ecotoxicity testing and biomonitoring. Ultimately, future work must focus on field-based validation under environmentally realistic conditions—accounting for variables like pH and metal speciation—to confirm the quantitative reliability of these plant models for real-world environmental monitoring.

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