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BIOCHEMICAL AND ENZYMATIC VARIATIONS IN THE MOSS *LEUCODON SCIUROIDES* UNDER ZINC-INDUCED STRESS

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Abstract: Anthropogenic air pollution poses a significant threat to ecosystems and human health, particularly through the release of non-biodegradable heavy metals that accumulate in the environment. *Leucodon sciuroides*, a terrestrial bryophyte widely distributed in the Souk Ahras region, is well-adapted to harsh environmental conditions and exhibits a high capacity for metal accumulation, rendering it an ideal bioindicator for environmental assessment. In the present study, the oxidative stress response of *L. sciuroides* was evaluated following hydroponic exposure to varying gradients of zinc (Zn) concentrations (0.005, 0.05, 10, and 20 mg/ml) for 2, 4, 6, and 8 days. A range of physiological, biochemical, and enzymatic parameters, including chlorophyll pigments, total proteins, proline, reduced glutathione (GSH), and antioxidant enzymes (catalase CAT; ascorbate peroxidase APX), were analyzed to assess the impact of Zn-induced stress. The results showed differences among Zn treatments. Low Zn concentrations stimulated chlorophyll (a, b, and a+b) and protein contents, indicating a micronutrient effect, whereas high Zn concentrations led to marked reductions, reflecting Zn toxicity. Proline accumulation increased in a dose- and time-dependent manner, indicating oxidative stress. Antioxidant responses, including GSH content and the activities of the enzymes CAT and APX, varied according to Zn concentrations and exposure duration. The highest values were observed at intermediate Zn concentrations and during early to intermediate exposure periods, whereas lower values were recorded during prolonged exposures. Statistical analysis revealed that zinc concentration, exposure duration, and their interaction significantly affected photosynthetic pigments, protein and proline contents, as well as antioxidant defenses (APX, CAT, and GSH), indicating a physiological response strongly dependent on both concentration and time under Zn-induced stress ($p < 0.05$). These findings highlight the strong antioxidant capacity and stress tolerance of *L. sciuroides* and confirm its relevance as a sensitive bioindicator for monitoring zinc pollution in terrestrial ecosystems.

Keywords: bryophytes, *Leucodon sciuroides*, heavy metals, souk Ahras, oxidative stress.

INTRODUCTION

Environmental pollution and contamination, though distinct, often overlap as contaminants frequently function as pollutants with adverse ecological effects. Pollution involves undesirable alterations in the physical, chemical, or biological properties of air, water, and soil, negatively affecting living organisms, including plants and animals. Such changes may involve chemical substances or forms of energy, such as noise, heat, or light (Wong et al., 2012).

Air pollution resulting from urbanization, industrialization, rapid expansion of motorized transport, and population growth represents one of the most severe threats to the environment and human health worldwide, particularly in urban areas. Atmospheric heavy metals, such as chromium (Cr), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb), constitute a critical subset of these pollutants (Li et al., 2017). Monitoring and managing air pollution are well-established practices that remain essential for environmental protection (Ram et al., 2015).

Various biological matrices, including soil crusts (Wojtuń et al., 2013) and lichens (Paoli et al., 2015), have been employed in biomonitoring to assess air quality and atmospheric metal levels. In recent years, the integration of biomonitoring with chemical analyses has gained broad research and applied significance (Brignole et al., 2018).

Heavy metal contamination poses increasing environmental challenges in developing countries, driven by both natural geologic processes and intensified anthropogenic activities, leading to elevated metal concentrations detrimental to ecosystems (Duffus, 2002). Traffic emissions in agricultural areas can further introduce heavy metal pollution, contaminating crops and posing health risks to local residents via inhalation of airborne dust or dermal contact (Tchounwou et al., 2012).

The biological impacts of heavy metals have been extensively studied in both animals and plants, revealing effects ranging from oxidative stress to genotoxicity. Metal-tolerant plants have evolved diverse strategies to cope with toxic metal concentrations, as metal ions can interact with DNA and nuclear proteins, causing genotoxicity and conformational changes. Hyperaccumulator plants can thrive in heavily contaminated sites, accumulating metals at levels exceeding established thresholds (Shrivastava et al., 2019).

Industrialization and urbanization, fueled by rapid global population growth, have escalated air pollution to a critical environmental concern. This situation is

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exacerbated by dust and particulate matter (PM), particularly fine particles (PM2.5 and PM10), emitted through both natural (dust storms, soil erosion, volcanic activity, rock weathering) and anthropogenic processes (industrial operations and transportation) (Soleimani et al., 2018).

Bryophytes, a diverse group of terrestrial non-vascular plants, rank second only to angiosperms in species richness. Despite limited research due to their modest biomass, slow growth, and low economic value, bryophytes exhibit remarkable adaptations, including unique secondary metabolites, tolerance to drought, salinity, and toxic elements, as well as distinctive biochemical pathways (Sabovljević et al., 2016).

Bryophytes play a crucial role in aquatic and adjacent terrestrial ecosystems, especially where anthropogenic activities elevate heavy metal levels. These plants absorb and accumulate metals via surface uptake from substrates such as soil and rock. Many species possess rhizoids fine, filamentous root-like structures that enhance cation exchange, making bryophytes ideal for monitoring heavy metal pollution in soil and water (Chen et al., 2019). For instance, the aquatic moss *L. riparium* (Hedw.) Warnst. exhibits high tolerance and accumulation of cadmium (Cd), serving as a reliable bioindicator across diverse habitats and pollution sources (Maresca et al., 2022).

Due to the absence of vascular tissues, bryophytes rely on cellular responses to survive in polluted environments. These traits allow them to colonize metal-contaminated sites and accumulate substantial metal quantities without apparent negative effects (Stanković et al., 2018). Consequently, mosses and lichens are commonly used to detect atmospheric metal deposition in terrestrial ecosystems, particularly in large-scale surveys, owing to their morphological structures, accumulation mechanisms, and ecophysiology (Harmens et al., 2007).

Plants employ diverse strategies to tolerate and mitigate heavy metal toxicity (Hossain et al., 2012). Chelation is a primary mechanism extensively documented in bryophytes (Fasani et al., 2022). Heavy metals induce oxidative stress by promoting reactive oxygen species (ROS) formation, including superoxide anions (O_2^-), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2), which damage cellular membranes and DNA, potentially leading to cell death (Hossain et al., 2012).

Unlike organic pollutants, heavy metals such as zinc (Zn) are non-biodegradable and persist in the environment. Although Zn is an essential micronutrient for plant growth, elevated concentrations become toxic, inhibiting growth and disrupting physiological processes (Panda et al., 2003), including lipid peroxidation (Ribarov & Benov, 1981). Plants have evolved enzymatic (e.g., catalase [CAT], peroxidases) and non-enzymatic (e.g., glutathione, ascorbate) antioxidant systems to counteract ROS toxicity, which are upregulated under both biotic and abiotic stresses (Foyer et al., 1994). Cr, Cu, and Zn trigger ROS production in cells, with Cu showing the highest catalytic activity in Fenton reactions, followed by Cr and Zn (Panda et al., 2003). Although the effects of Cr (VI) remain poorly understood, ROS formation from these metals disrupts antioxidant metabolism, enhancing the activity of enzymes such as CAT, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) (Choudhury & Panda, 2004).

Mosses, characterized by rapid growth and reproduction, efficiently accumulate metals in urban and industrial areas, making them effective biomonitors for atmospheric heavy metal deposition (Stanković et al., 2018; Ucarli et al., 2020). Bryophytes have been widely employed to investigate metal accumulation mechanisms and uptake kinetics from metal-enriched environments (Brown & Beckett, 1985). Due to their lack of a localized root system and protective cuticle, bryophytes, alongside vascular plant leaves, serve as reliable bioindicators for heavy metal contamination from diverse sources (Kosior et al., 2015). For example, Lequy et al., (2022) demonstrated that *Grimmia pulvinata* collected from 77 and 51 cemeteries around Paris and Lyon effectively reflected fine-scale urban air quality through passive biomonitoring.

MATERIALS AND METHODS

Chemical material

The chemical reagent used in this study was zinc (Zn), a bluish-white metal, slightly malleable, and capable of being polished to a fine sheen. Zinc of analytical grade was used for all experimental treatments.

Fig. 1. The bryophyte species *L. sciuroides*.

Biological material

A bryophyte survey was conducted in the Talal agglomeration (Ain Seynour), which enabled the selection of *L. sciuroides* as the study species. Multiple thalli of this species were collected from tree bark at various sites under standardized conditions, with samples taken at a height of 1.50–2.00 m above the ground (Figure 1). Sampling was carried out in March 2024. All experimental procedures were performed at the Pedagogical Laboratory of Mohamed Cherif Messaadia University.

Study area

The sampling site was selected in the Ain Seynour region, located northwest of Souk-Ahras and southeast of Guelma (Figure 2). The site is bordered to the north by the Mechroha daïra (7 km), to the east by the Ouled Driss daïra (14 km), to the west by the Hennancha commune, and to the south by Souk-Ahras province (13 km). The geographical coordinates of the site are 36°19′32″ N and 7°54′38″ E (decimal latitude: 36.319773).

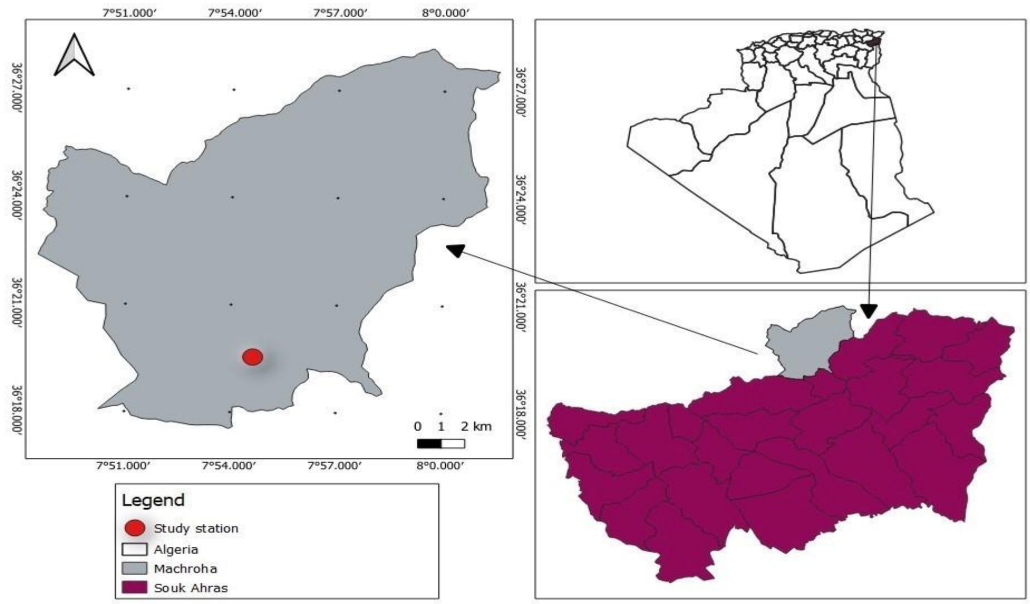

Fig. 2. Geographical location of the “Ain Seynour” sampling site.

Treatment application

Zinc (Zn) was used for the treatment of *L. sciuroides* thalli.

Preparation of zinc solutions: Zinc was dissolved in distilled water to prepare solutions at concentrations of 0.005, 0.05, 10, and 20 mg L^{-1}.

Treatment procedure: Approximately 2 g of moss thalli were immersed in 200 mL of the prepared Zn solutions. Samples were exposed for durations of 2, 4, 6, and 8 days under controlled laboratory conditions.

Assays

Chlorophyll content: Chlorophyll pigments were extracted and quantified following the method of Holden (1975), which involves maceration of plant material in acetone.

Proline content: Proline levels were determined according to Monneveux and Nemmar (1986).

Total protein content: Total protein content was determined using the Bradford method (1976), with bovine serum albumin (BSA) as a standard.

Antioxidant enzyme activities: Catalase (CAT) activity was assessed following Cakmak and Horst (1991), while ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1987).

Glutathione (GSH) content: GSH levels were quantified using the method described by Weckberker and Cory (1988).

Statistical analysis

Data were analyzed using SPSS software (version 25). Results are presented as mean ± standard error (mean ± SE). The effects of zinc concentration, exposure duration, and their interaction were assessed using two-way analysis of variance (two-way ANOVA). Additionally, a one-way ANOVA was applied for each exposure duration to compare the effects of different zinc concentrations. Statistical significance was set at $p < 0.05$.

RESULTS

Chlorophyll pigment contents

Chlorophyll a

High Zn concentrations (10 and 20 mg/ml) significantly reduced chlorophyll *a* levels compared with the control, with the most pronounced declines observed at 4 and 8 days, indicating toxic effects (Figure 3). Conversely, low Zn concentrations (0.05 and 0.005 mg/ml) maintained chlorophyll *a* content close to control levels and even exhibited a stimulatory effect at day 6, suggesting a hormetic response.

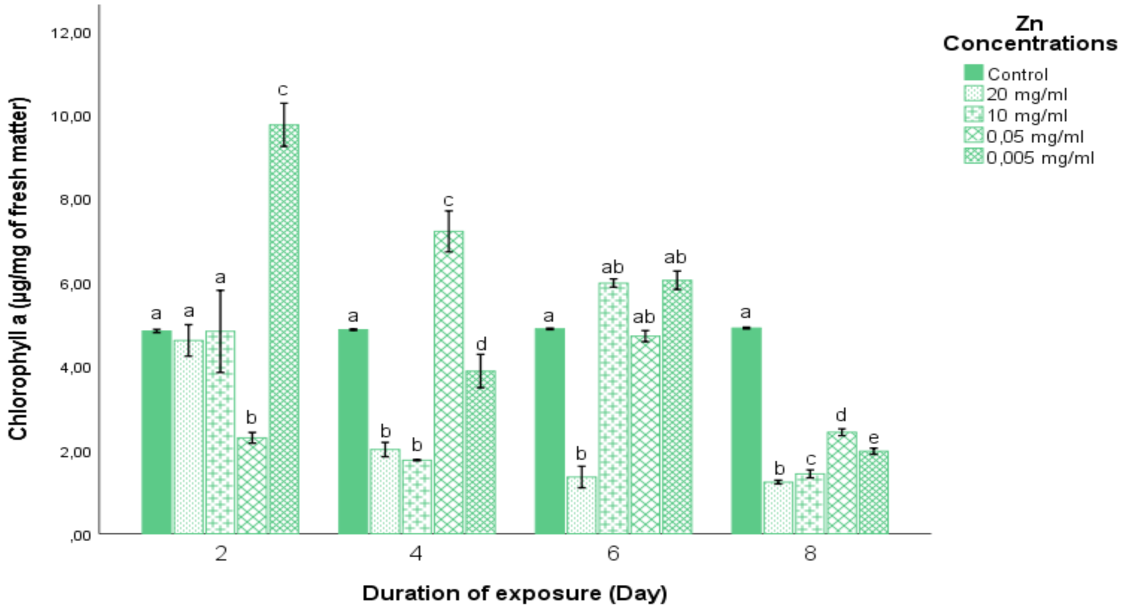

Fig. 3. Effect of zinc (Zn) concentrations on chlorophyll a content (μg/mg fresh matter) in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

Chlorophyll *a* content was significantly affected by Zn exposure at all time points ($p < 0.05$). The marked decreases at higher concentrations suggest impairment of photosystem II, whereas some treatments showed transient increases, indicating a stress-induced acclimation response. Overall, chlorophyll *a* levels decreased with increasing Zn concentration and prolonged exposure, highlighting the dual role of Zn as an essential micronutrient at low doses and a toxic element at higher concentrations.

Chlorophyll b

A significant decrease in chlorophyll *b* content was observed at high Zn concentrations (10 and 20 mg/ml) compared with the control across most exposure periods, with the most pronounced reductions occurring at 4 and 8 days, indicating clear Zn toxicity (Figure 4). In contrast, low Zn concentrations (0.05 and 0.005 mg/ml) maintained chlorophyll *b* levels close to the control and even induced a significant increase at early (2 days) and intermediate (4–6 days) exposure times, suggesting a hormetic response.

Chlorophyll *b* levels were significantly affected by Zn treatment ($p < 0.05$). The decreases observed at higher concentrations likely reflect disruption of the light-harvesting complexes, whereas the occasional transient increases at lower doses suggest a possible acclimation mechanism. Overall, chlorophyll *b* content declined with increasing Zn concentration and prolonged exposure duration, highlighting the dual role of Zn as an essential micronutrient at low doses and a toxic factor at higher concentrations.

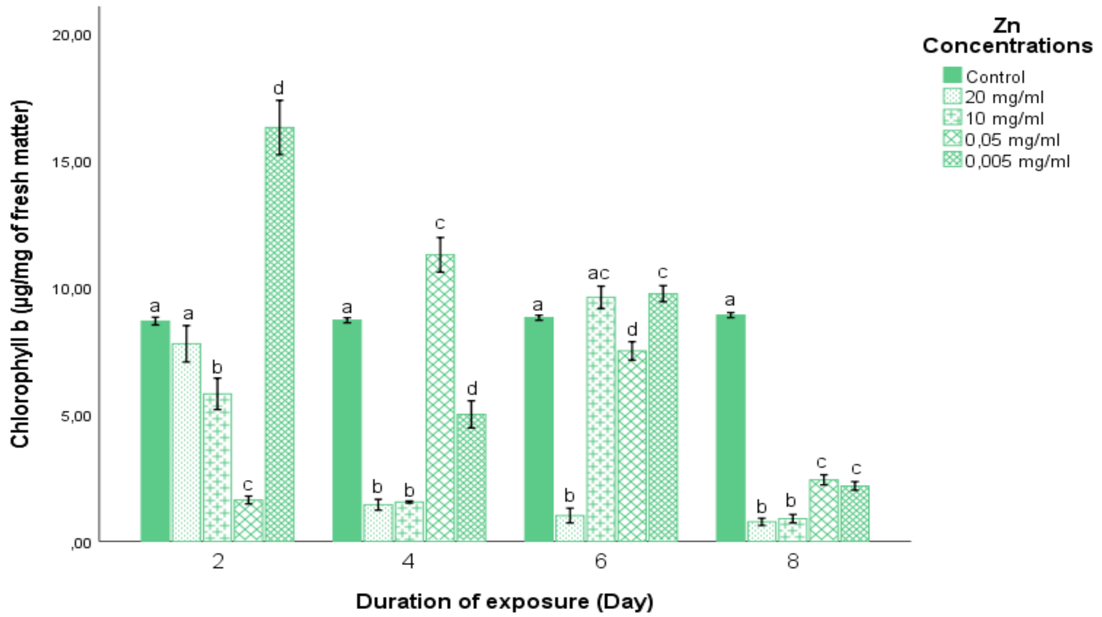

Fig. 4. Effect of zinc (Zn) concentrations on chlorophyll b content (μg/mg fresh matter) in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

Chlorophyll a+b

Total chlorophyll (a + b) content remained relatively stable in the control treatment throughout the experimental period. In contrast, exposure to high Zn concentrations (10 and 20 mg/ml) resulted in a significant reduction in total chlorophyll content at all exposure times compared with the control, with the most pronounced decreases observed at 4 and 8 days, indicating severe Zn-induced stress and impairment of photosynthetic pigment synthesis (Figure 5).

Conversely, low Zn concentrations (0.05 and 0.005 mg/ml) significantly increased chlorophyll (a + b) content, particularly at early (2 days) and intermediate (4–6 days) exposure periods, with the highest pigment accumulation recorded at 0.005 mg/ml after 2 days of exposure. However, a slight decline was observed at day 8, suggesting that prolonged exposure may attenuate this stimulatory effect.

Total chlorophyll (a + b) content was significantly affected by Zn exposure ($p < 0.05$). The overall reductions at higher concentrations indicate photosynthetic impairment, whereas the transient increases observed at lower doses suggest adaptive responses to Zn-induced stress. Overall, chlorophyll (a + b) content exhibited a clear concentration- and time-dependent response to Zn, confirming its dual role as an essential micronutrient at low doses and a toxic factor at elevated concentrations.

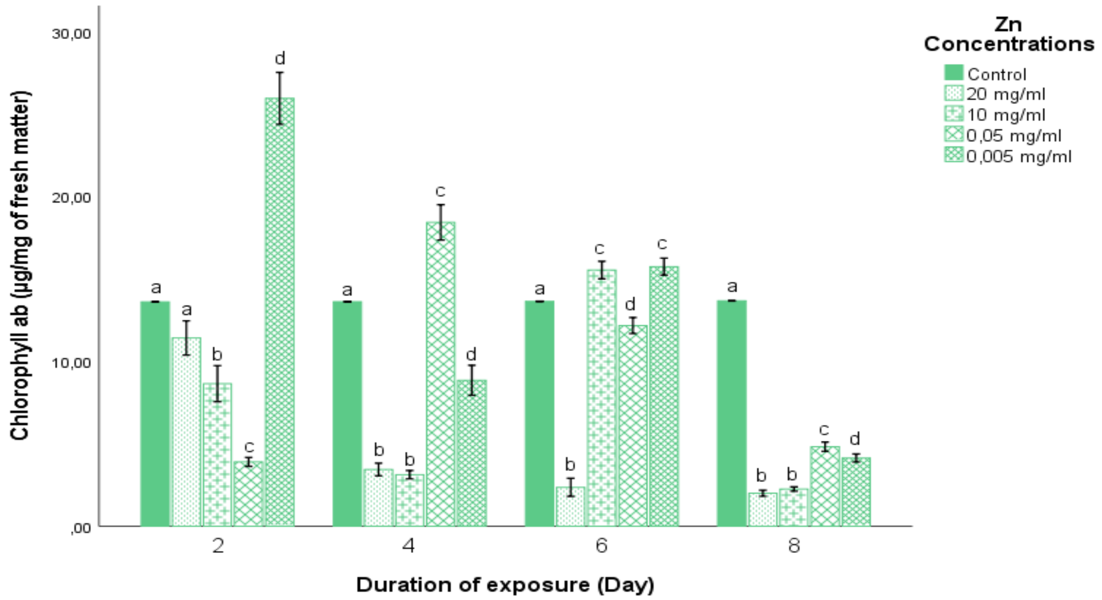

Fig. 5. Effect of zinc (Zn) concentrations on total chlorophyll (a + b) content (µg/mg fresh matter) in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

Total protein content

Protein content in the control treatment remained relatively stable throughout the experimental period. Exposure to low Zn concentrations (0.05 and 0.005 mg/ml) resulted in a significant increase in protein content at early (2 days) and intermediate (4–6 days) exposure times, with the highest values recorded at 0.005 mg/ml, particularly after 6 days, indicating a stimulatory effect at low doses (Figure 6).

In contrast, high Zn concentrations (10 and 20 mg/ml) caused a significant reduction in protein content compared with low-dose treatments and, in some cases, relative to the control, especially at prolonged exposure times. The most pronounced decreases were observed at 4 and 6 days, suggesting that excessive Zn interferes with protein synthesis or promotes protein degradation. By day 8, protein levels across treatments showed slight convergence, possibly reflecting physiological adjustment or stress-induced metabolic constraints.

Overall, total protein content exhibited a clear concentration- and time-dependent response to Zn exposure, reinforcing the dual role of Zn as an essential micronutrient that enhances metabolic activity at low concentrations and as a toxic stressor that disrupts protein metabolism at elevated levels. Protein content was significantly affected by Zn treatment ($p < 0.001$), confirming metabolic adjustments under Zn-induced stress.

Proline

Proline content in plant tissues showed a significant and consistent response to Zn treatments across all exposure durations (2, 4, 6, and 8 days). Compared with the control, all Zn treated samples exhibited a marked increase in proline accumulation, reflecting the activation of stress-induced physiological responses. Proline levels increased in a concentration-dependent manner, with higher Zn doses inducing greater accumulation. After 2 days of exposure, the highest proline content was recorded at 20 mg/ml Zn, followed by 10 mg/ml, while lower concentrations produced moderate but significant increases (Figure 7).

A similar pattern was observed after 4 days, although proline levels were slightly reduced compared with those at day 2, suggesting partial acclimation to Zn stress. Maximum proline accumulation occurred at day 6, particularly under the highest Zn concentration, indicating intensified stress conditions and enhanced osmoprotective and antioxidant responses. By day 8, proline levels declined slightly but remained significantly higher than the control across all treatments.

Proline accumulation was significantly affected by Zn treatment ($p < 0.001$), with values consistently higher than in controls at most concentrations and exposure times, indicating a strong osmoprotective response to heavy metal stress. Overall, these findings demonstrate that Zn stress induces proline biosynthesis in a dose- and time-dependent manner, supporting its role as an important biochemical marker of stress tolerance in plants.

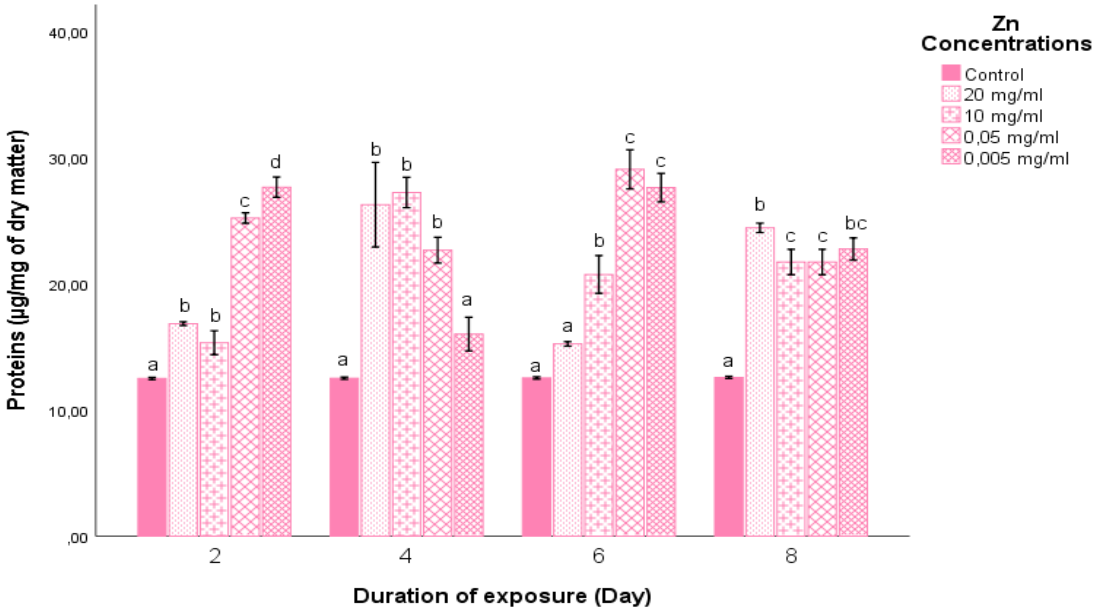

Fig. 6. Effect of zinc (Zn) concentrations on total protein content (µg/mg dry matter) in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

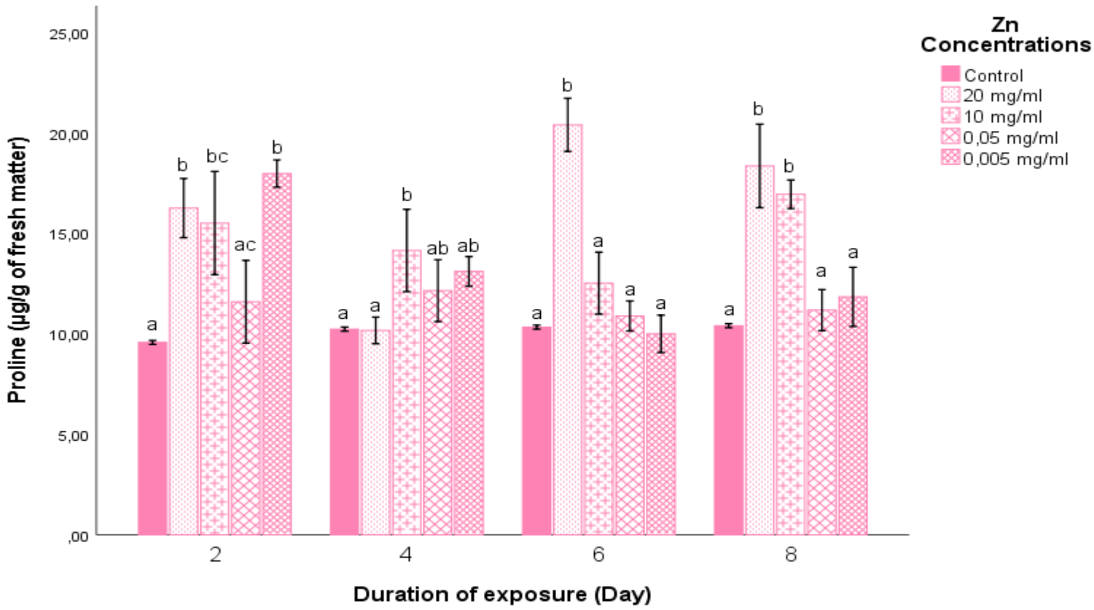

Fig. 7. Effect of zinc (Zn) concentrations on proline content (µg/mg fresh matter) in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

Enzymatic Biomarker Activity

The activities of catalase (CAT) and ascorbate peroxidase (APX), key enzymes involved in oxidative stress management, varied significantly in response to Zn exposure (Figures 8 and 9).

APX activity

APX activity exhibited significant variations across Zn concentrations and exposure durations. Compared with the control, all Zn treatments induced a marked increase in APX activity, particularly during the early and mid-exposure periods. After 2 days, APX activity was strongly stimulated at all Zn concentrations, indicating a rapid antioxidant response to Zn induced oxidative stress (Figure 8). By day 4, APX activity remained elevated, with moderate Zn concentrations inducing higher activity than the lowest dose. The highest APX activity was observed after 6 days at intermediate Zn concentrations, suggesting optimal enzyme activation under moderate stress conditions.

In contrast, prolonged exposure (8 days) led to a general decline in APX activity across all treatments. This reduction may reflect enzyme inhibition or oxidative damage resulting from sustained Zn stress. Nevertheless, APX activity in Zn treated samples remained significantly higher than in controls ($p < 0.05$–0.001), confirming the activation of this enzymatic antioxidant defense. Overall, these results demonstrate that Zn triggers an adaptive antioxidant response, with APX playing a crucial role in mitigating oxidative stress, particularly during short- to medium term exposures.

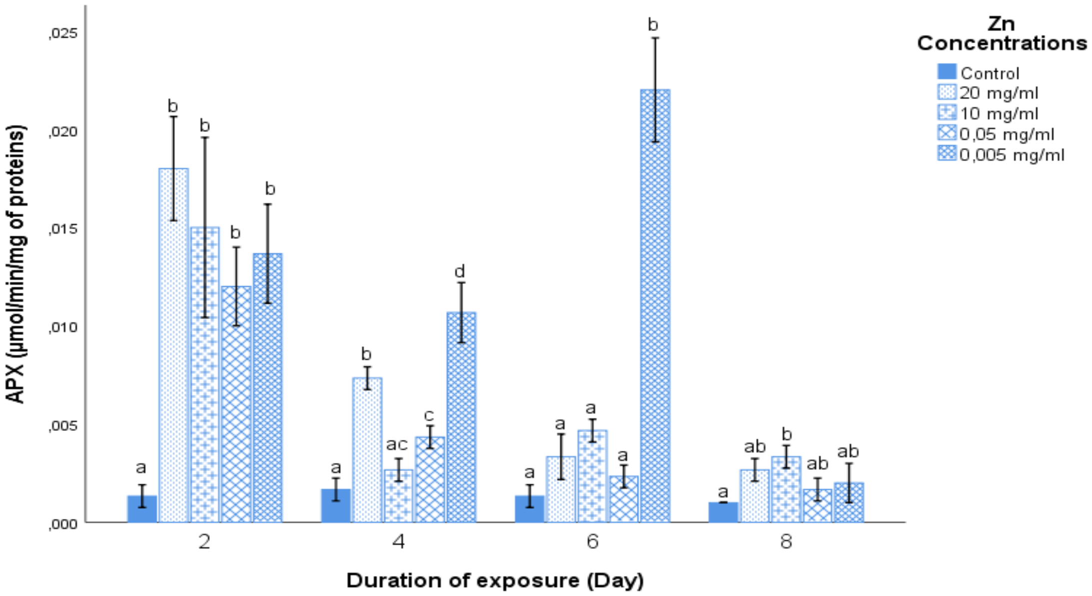

Fig. 8. Effect of zinc (Zn) on ascorbate peroxidase (APX) activity in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

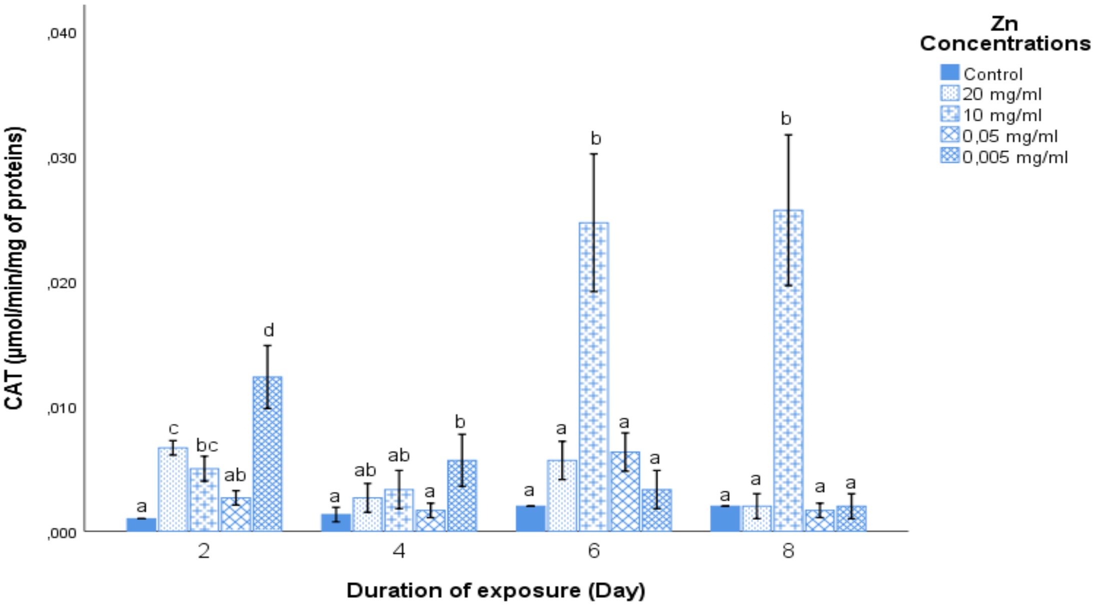

Fig. 9. Effect of zinc (Zn) on catalase (CAT) activity in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

CAT activity

CAT activity was significantly influenced by both Zn concentration (0.005, 0.05, 10, and 20 mg/ml) and exposure duration. Compared with the control, all Zn treated samples showed a clear induction of CAT activity, indicating activation of antioxidant defenses. After 2 days of exposure, CAT activity increased significantly at 0.05 and 10 mg/ml, while the lowest concentration (0.005 mg/ml) induced only a slight response (Figure 9).

By day 4, CAT activity further increased, with 10 and 20 mg/ml exhibiting higher enzyme levels than the lower concentrations. The highest CAT activity was recorded after 6 days at 0.05 and 10 mg/ml, suggesting optimal enzyme activation under moderate Zn stress. At 20 mg/ml, CAT activity increased but remained lower than at intermediate concentrations.

After 8 days, CAT activity declined or stabilized across all treatments, approaching control values. Nevertheless, CAT activity remained significantly higher in Zn exposed plants compared to controls ($p < 0.05–0.001$), reflecting its role in scavenging H_2O_2 and protecting cells from oxidative damage. This decrease may be attributed to enzyme inhibition or oxidative damage caused by prolonged Zn exposure.

Overall, CAT activity exhibited a clear dose- and time-dependent response, with intermediate Zn concentrations (0.05–10 mg/ml) eliciting the strongest antioxidant response.

Levels of non-enzymatic biomarkers

GSH activity

Zinc (Zn) exposure significantly affected reduced glutathione (GSH) content in *L. sciuroides* (Figure 10), indicating the activation of antioxidant defense mechanisms in response to metal-induced oxidative stress. Higher Zn concentrations (10 and 20 mg/mL) induced the strongest GSH accumulation, particularly after 2 and 4 days of exposure, reflecting an early stress response. In contrast, prolonged exposure (6 and 8 days) led to a gradual decline in GSH content, suggesting either exhaustion of the antioxidant system or physiological acclimation. Lower Zn concentrations (0.005 and 0.05 mg/mL) caused a moderate but relatively stable increase in GSH throughout the exposure period. Statistically significant differences, indicated by different letters, confirm the combined effect of Zn concentration and exposure duration (ANOVA test). GSH levels were significantly higher in treated samples compared with controls ($p < 0.05–0.001$), highlighting its role in detoxification and maintenance of redox balance under Zn stress.

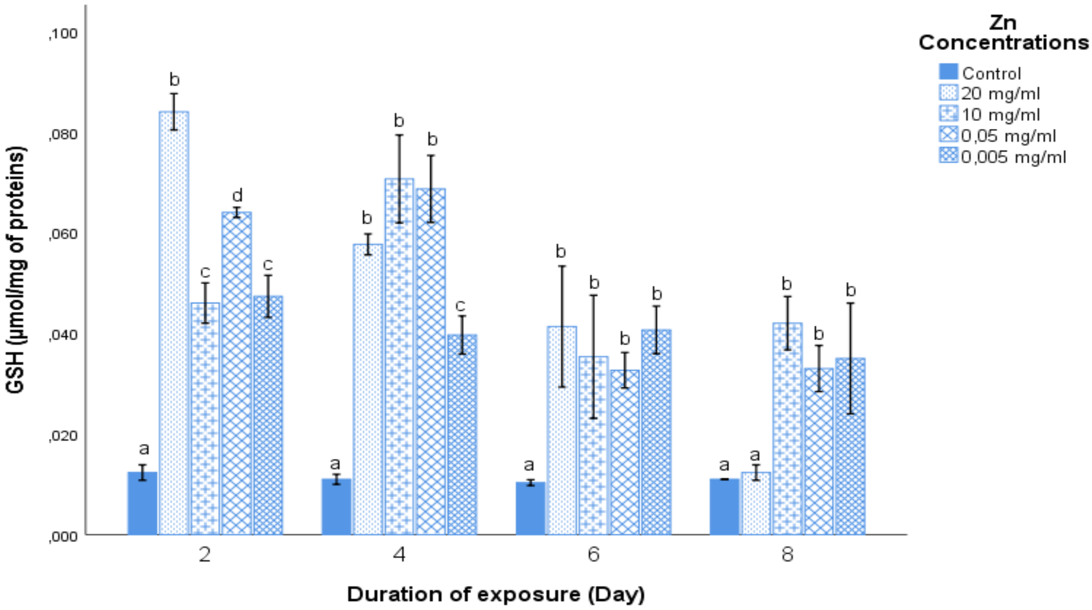

Fig. 10. Effect of zinc (Zn) on glutathione (GSH) activity in *L. sciuroides* after 2, 4, 6, and 8 days of exposure (ANOVA test).

DISCUSSION

The present results show that *L. sciuroides* responds to zinc exposure in a concentration- and time-dependent manner. The decline in chlorophyll content at high Zn concentrations aligns with previous studies reporting that heavy metals impair chlorophyll biosynthesis and promote pigment degradation (Panda et al., 2003). Although Zn is considered less harmful than Cr and Cu in terms of chlorophyll degradation, excessive Zn levels can still disrupt photosynthetic processes.

Furthermore, the limited variation in chlorophyll content observed across stressed mosses in previous studies supports our observation that chlorophyll responses may be less sensitive than antioxidant markers, highlighting the importance of using multiple biochemical parameters. Overall, these results emphasize the need for controlled, species-specific studies to validate mosses as biomonitors and suggest that *L. sciuroides*, with its robust antioxidant response, could be a reliable candidate for zinc pollution assessment, provided that standardized calibration is established for field conditions (Stanković et al., 2021).

The increase in proline content and the modulation of antioxidant enzymes (CAT, APX) and GSH indicate the induction of oxidative stress and activation of defense mechanisms. Higher antioxidant activity at intermediate Zn concentrations suggests an adaptive response, whereas prolonged exposure may lead to partial inhibition of the defense system. Consistent with earlier reports Bargagli (1998), bryophytes act as efficient metal accumulators due to their high surface-to-volume ratio and effective uptake mechanisms. Therefore, the physiological and biochemical changes observed confirm the metal-accumulating capacity and bioindicator potential of *L. sciuroides* under Zn stress.

This study provides new insights into the zinc tolerance mechanisms of the terrestrial moss *L. sciuroides* and supports previous findings on heavy metal responses in bryophytes. Zinc exerted a dual effect, acting as a micronutrient at low concentrations by stimulating chlorophyll and protein synthesis, while inducing toxicity at higher concentrations, as reflected by reductions in photosynthetic pigments and protein content, consistent with the general role of heavy metals in plant physiology (Basile et al., 2012; Esposito et al., 2018; Lentini et al., 2018).

The dose and time dependent accumulation of proline indicates enhanced oxidative stress and the activation of protective mechanisms. Moreover, the modulation of antioxidant defenses particularly the increase in reduced glutathione (GSH) and the activities of catalase (CAT) and ascorbate peroxidase (APX) at intermediate zinc concentrations and during early exposure periods highlights the involvement of an efficient antioxidant system in stress mitigation.

These responses are consistent with biochemical evidence reported for the aquatic moss *L. riparium*, in which heavy metal tolerance is associated with thiol-based detoxification mechanisms involving GSH and related peptides (Bellini et al., 2020). Together, these results suggest that *L. sciuroides* relies on conserved antioxidant and thiol-mediated pathways to counteract zinc-induced oxidative stress, supporting its relevance as a sensitive bioindicator of metal pollution.

The responses of *L. sciuroides* to zinc exposure align with previously reported findings on heavy metal tolerance in mosses. The stimulation of chlorophyll and protein contents at low Zn concentrations reflects effective micronutrient homeostasis, as reported by Basile et al., (2008), whereas the inhibitory effects observed at higher concentrations indicate Zn toxicity. Furthermore, the accumulation of proline and the modulation of GSH, CAT, and APX activities demonstrate the activation of antioxidant defenses in response to oxidative stress, in agreement with the findings of Rau et al., (2007).

Although these results support the potential of *L. sciuroides* as a biomonitor species, the presence of multiple coexisting metals in natural environments may complicate the interpretation of metal-specific biological responses. Most previous studies have focused on the effects of individual heavy metals (Rau et al., 2007), whereas in natural environments, mosses are typically exposed to multiple metals simultaneously. This co-exposure makes the interpretation of metal-specific effects challenging.

In addition, other environmental variables may further modulate these responses. Therefore, although the present study provides a promising methodological framework, further calibration and standardization are required to reliably link pollution levels with biological responses in potential plant bioremediators and to disentangle the contributions of individual metals to the observed effects.

The responses of *L. sciuroides* to zinc exposure observed in the present study are consistent with reports on heavy metal induced oxidative stress in *Fontinalis antipyretica*. In both species, heavy metal exposure disrupted cellular redox homeostasis, as evidenced by significant changes in antioxidant defenses and stress related metabolites. The modulation of CAT and APX activities, along with the increase in reduced glutathione (GSH) and proline in *L. sciuroides*, reflects the activation of antioxidant mechanisms similar to those described for *F. antipyretica*, where APX responded broadly to different metals while other enzymes were induced depending on the contaminant (Dazy et al., 2009).

The oxidative stress responses observed in *L. sciuroides* under zinc exposure are broadly consistent with those reported for the aquatic bryophyte *F. antipyretica* exposed to multiple heavy metals. In both species, heavy metal stress triggered increased lipid peroxidation and activation of antioxidant defenses, indicating disruption of cellular redox balance. Similar to *F. antipyretica*, where APX responded across different metals and CAT activity was induced depending on the contaminant, *L. sciuroides* showed significant modulation of APX and CAT activities along with increased GSH and proline levels, suggesting activation of conserved antioxidant pathways (Dazy et al., 2009).

Moreover, the decline in antioxidant responses and the stronger stress effects observed at higher Zn concentrations and prolonged exposure in *L. sciuroides* support the conclusion that defense systems can become overwhelmed under excessive metal stress, as also observed in *F. antipyretica* at the highest contaminant levels. Overall, these similarities reinforce the view that bryophytes employ comparable biochemical strategies to cope with heavy metal pollution, supporting the potential of *L. sciuroides* as a reliable bioindicator for environmental metal contamination (Dazy et al., 2009).

The zinc-induced responses of *L. sciuroides* observed in this study align with the idea that moss reactions to heavy metal stress are both species and metal specific, as demonstrated in controlled experiments with the acrocarpous *Atrichum undulatum* and pleurocarpous *Hypnum cupressiforme*. Similar to these findings, *L. sciuroides* exhibited clear concentration- and time dependent effects, with low Zn levels stimulating photosynthetic pigments and protein content, while higher concentrations caused toxicity and reduced physiological performance (Maevskaya et al., 2001; Stanković et al., 2018).

The results obtained for *L. sciuroides* under zinc exposure are also consistent with the general pattern of species and metal specific responses observed in other bryophytes, such as *Pleurochaete squarrosa* and *Timmiella barbuloides*. Similar to these mosses, *L. sciuroides* showed a clear threshold between micronutrient and toxic Zn concentrations, with low Zn stimulating chlorophyll and protein synthesis and high Zn causing marked reductions in growth related parameters (Aydoğan et al., 2017).

This pattern mirrors the differential metal accumulation and tolerance reported for *P. squarrosa* and *T. barbuloides*, where non-Fenton metals (Ni, Pb) were accumulated to higher levels with limited toxicity, whereas the redox-active metal Cu caused strong oxidative damage, reduced dry weight, and decreased chlorophyll a/b ratio. In *L. sciuroides*, the dose and time dependent increase in proline, along with modulation of GSH, CAT, and APX, indicates activation of antioxidant defenses to mitigate Zn induced oxidative stress, similar to the elevated antioxidant responses observed in *T. barbuloides* under Cu stress (Aydoğan et al., 2017).

However, as observed with Cu toxicity, prolonged exposure to high Zn concentrations eventually overwhelmed the antioxidant system in *L. sciuroides*, leading to reduced defense enzyme activity and potential cellular damage. These parallels suggest that bryophytes share conserved mechanisms of metal tolerance, particularly the ability to maintain membrane integrity and regulate antioxidant metabolism, while exhibiting species specific sensitivity to different metals. Collectively, the present findings reinforce the value of *L. sciuroides* as a bioindicator of zinc pollution and highlight the importance of using multiple biomarkers such as dry weight, chlorophyll ratios, antioxidant status, and lipid peroxidation to accurately assess heavy metal toxicity in mosses (Aydoğan et al., 2017).

The metabolic responses of *L. sciuroides* to zinc stress resemble those reported for *L. riparium*, where heavy metal exposure induces specific metabolic changes and phytochelatin synthesis. In both species, antioxidant defenses including GSH, CAT, APX, and proline increase under moderate metal stress, suggesting activation of thiol-based detoxification pathways. Although phytochelatins were not measured in *L. sciuroides*, the observed rise in GSH supports the likelihood of similar detoxification mechanisms. Given that phytochelatin induction has been documented in only a few bryophytes, including *L. riparium* and *L. cruciata*, further investigation in *L. sciuroides* would help clarify whether phytochelatins can serve as specific biomarkers of metal exposure in this species (Maresca et al., 2020, 2022).

The objective of this study was to analyze the effects of zinc induced oxidative stress on *L. sciuroides* by examining various biochemical parameters, including enzymatic and non-enzymatic biomarkers. The results indicate that zinc exerts a dose- and time dependent toxic effect, as evidenced by the modulation of metabolites and the activities of key antioxidant enzymes, including catalase (CAT), ascorbate peroxidase (APX), and reduced glutathione (GSH).

Exposure to low Zn concentrations (0.005 and 0.05 mg/mL) stimulated chlorophyll and protein synthesis, suggesting a beneficial micronutrient effect, whereas high Zn concentrations (10 and 20 mg/mL) caused significant reductions in these parameters, reflecting oxidative stress and impaired photosynthetic and metabolic processes. Both enzymatic (CAT, APX) and non-enzymatic (GSH) biomarkers showed clear activation in response to Zn, highlighting the strong antioxidant capacity of *L. sciuroides* and its ability to mount protective defenses against metal induced oxidative stress.

To maintain moss hydration throughout the experimental period, a specific in situ exposure system was developed. Monitoring vitality parameters, such as chlorophyll content, metabolites, and antioxidant biomarker activities, confirmed the effectiveness of this system for *L. sciuroides*.

Although the existence of specific detoxification mechanisms, such as cytochrome P450-mediated pathways, cannot be confirmed or excluded, further studies are needed to explore their role in heavy metal metabolism in bryophytes. Assessing photosynthetic activity could also provide complementary insight into the physiological impact of Zn exposure. Interestingly, the relatively modest variation in peroxidase activities observed in our experiments may reflect the high tolerance of *L. sciuroides* to metal stress, as more sensitive species typically exhibit stronger enzymatic responses.

Oxidative stress biomarkers in mosses present certain limitations for metal toxicity studies, as their natural variability affected by intrinsic factors such as water content and age may interfere with xenobiotic effects. These fluctuations can reflect both Zn-induced disturbances and inherent stress-resistance mechanisms, highlighting the remarkable ability of mosses to cope with environmental stressors, including metal pollution.

This resilience makes mosses particularly valuable and suitable models for studying metal accumulation and tolerance in highly contaminated environments. Future research could explore higher Zn concentrations and long-term exposure to better understand the dynamics of antioxidant biomarker activation, providing deeper insight into the mechanisms of heavy metal tolerance in *L. sciuroides*.

AUTHORS CONTRIBUTIONS

All authors contributed to the design and data acquisition on which this article is based. Hana Bouzahouane processed and analyzed the data using the team's tools and guidance. Khaoula Menaiaia drafted the initial manuscript. Malek Belkhir, Chaima Bouamrane, Amina Ouahab, and Sameh Rouainia participated in the collection of experimental data, the interpretation of results, and the critical revision of the manuscript. All authors discussed the results and contributed to revising and finalizing the final version.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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