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Copper and lead trigger distinct responses in two Brazilian mosses

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Abstract Metal contamination, particularly by copper (Cu) and lead (Pb), is a persistent environmental threat, yet the adaptive responses of Neotropical bryophytes to such stressors remain largely unexplored. This study evaluated biomass production, photosynthetic performance, and metal accumulation in two native Brazilian mosses (*Campylopus savannarum* (Müll.Hal.) Mitt. and *Polytrichum juniperinum* Willd. ex Hedw.) exposed to increasing concentrations of Cu and Pb under controlled laboratory conditions. We hypothesized that their contrasting morpho-anatomical traits, especially differences in lamellae structure, photosynthetic surface organization, and the presence of conduction tissues, would lead to distinct physiological strategies of metal tolerance. At the end of 240 days, biomass and maximum quantum efficiency of PSII (*Fv/Fm*) were assessed, and tissue metal concentrations were quantified. The moss *P. juniperinum* exhibited remarkable tolerance, maintaining stable biomass and photosynthetic capacity across all treatments, while *C. savannarum* presented pronounced declines, especially under high Pb exposure. Both species displayed dose-dependent metal accumulation but with distinct patterns: *C. savannarum* accumulated nearly 196-fold more Cu at the highest treatment compared to the control, whereas *P. juniperinum* stored about 44-fold more Pb than the control. These contrasting responses likely reflect species-specific strategies of metal tolerance. These results position Neotropical mosses as valuable bioindicators and potential agents for generating genomic data to improve our understanding of metal tolerance in bryophytes. Future research should investigate tissue-concentration metal partitioning and the molecular mechanisms underlying tolerance in native Brazilian bryophytes.

Keywords Abiotic effects · Bryophytes · Metal contamination · Metal toxicity · Photosynthetic efficiency

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1 Introduction

Soil is widely recognized as a finite and fragile resource, formed over geological time scales (Van Breemen and Buurman 2002). However, it remains highly vulnerable and can be severely degraded by human activities within comparatively short periods (Johnson and Lewis 2007), particularly through agricultural practices (Rashmi et al. 2020; Hu et al. 2021) and industrial processes (Xin et al. 2022). In this context, soil contamination, defined as the accumulation of elements above natural background concentrations that harm organisms (Adriano 2001), has received increasing scientific attention. Recent studies highlight both its far-reaching environmental consequences and the urgent need for effective remediation strategies (Chernysh et al. 2024; Haghighizadeh et al. 2024).

Among soil contaminants, copper (Cu) and lead (Pb) are particularly concerning due to their pronounced toxicity to terrestrial organisms, especially plants (Sağlam et al. 2016; Küpper 2017). Copper, is an essential micronutrient for plants and plays a pivotal role in photosynthesis (Droppa and Horváth 1990; Yruela 2009), by participating in the electron transport chain and facilitating energy flow (Raven et al. 1999; Joliot and Joliot 2006). However, when present in excess, Cu becomes toxic, primarily by inducing oxidative imbalances (Fernandes and Henriques 1991; Mir et al. 2021), reducing water and mineral nutrients uptakes (Shabbir et al. 2020), and impairing photosynthetic process (Barón et al. 1995). In contrast, Pb is a non-essential metal with no biological function in plants (Maestri et al. 2010). Even at low concentrations, Pb can compromise plant health by inducing oxidative imbalance (Pinho and Ladeiro 2012) and disrupting multiple physiological and biochemical pathways, including chlorophyll biosynthesis (Sharma and Dubey 2005; Zulfiqar et al. 2019).

While most plant species are sensitive to the toxic effects of Cu and Pb (e.g., Cunha et al. 2020; Farid et al. 2021; Jin et al. 2021), some have evolved specific physiological and biochemical strategies to avoid or mitigate such toxicity (Emamverdian et al. 2015). Plants may avoid Cu and Pb toxicity by limiting metal uptake through root exudates or by sequestering metals within cell walls (Srivastava and Srivastava 2023; Xu et al. 2024). Additionally, they mitigate toxicity by producing metal-binding peptides such as phytochelatins and metallothioneins, regulating expression of metal transporter genes, and activating antioxidant defense systems to control intracellular metal concentration and minimize oxidative damage (Navabpour et al. 2020; Chen et al. 2022; Gupta et al. 2024; Huang et al. 2024).

Although many studies have examined the effects of Cu and Pb on plants, research has been strongly biased toward commercially important vascular species, such as rice (*Oryza sativa* L.; Rob et al. 2024) and maize (*Zea mays* L.; Abedi et al. 2022). Consequently, plants such as bryophytes remain largely understudied in this context. Bryophytes, including mosses, liverworts, and hornworts, are small plants with a distinct life cycle with the haploid gametophyte as the dominant stage (Shaw and Goffinet 2000; Vanderpoorten and Goffinet 2009; Glime 2017). A unique feature of bryophytes is their ability to act as efficient ion exchangers with their surroundings, making them particularly effective at accumulating metals from the environment (Tyler 1990; Barukial and Hazarika 2022). Curiously, many bryophyte species exhibits remarkable metal tolerance (Salbitani et al. 2023), with certain species growing exclusively in lead-rich soils (e.g., Callaghan and Samson 2023), and others adapted to Cu-rich environments (e.g., Satake et al. 1988; Shaw and Schneider 1995).

Bryophytes, similar to vascular plants, adopt two main strategies to cope with excess metals in the environment: tolerance and avoidance (Stanković et al. 2018). Both the detrimental effects of metals and the mechanisms to mitigate them have been documented in several bryophyte species, including *Physcomitrium patens* (Hedw.) Mitt. (Sassmann et al. 2015; Liu et al. 2020; Schillaci et al. 2023), *Polytrichum commune* L. ex Hedw. (Francis and Petersen 1989; Panda and Choudhury 2005; Šoltés and Gregušková 2013), and *Campylopus schmidii* (Müll. Hal.) A. Jaegr. (Zhang et al. 2022, 2024, 2025). However, knowledge of Neotropical bryophytes and their physiological responses to metal effect remains limited. Brazil, with its rich bryophyte diversity (Costa and Peralta 2015; Brazil Flora Group 2022) and growing soil contamination by Cu and Pb (Paoliello and Capitani 2007; Poggere et al. 2023), presents a valuable yet underexplored setting to investigate metal tolerance mechanisms in Neotropical environments.

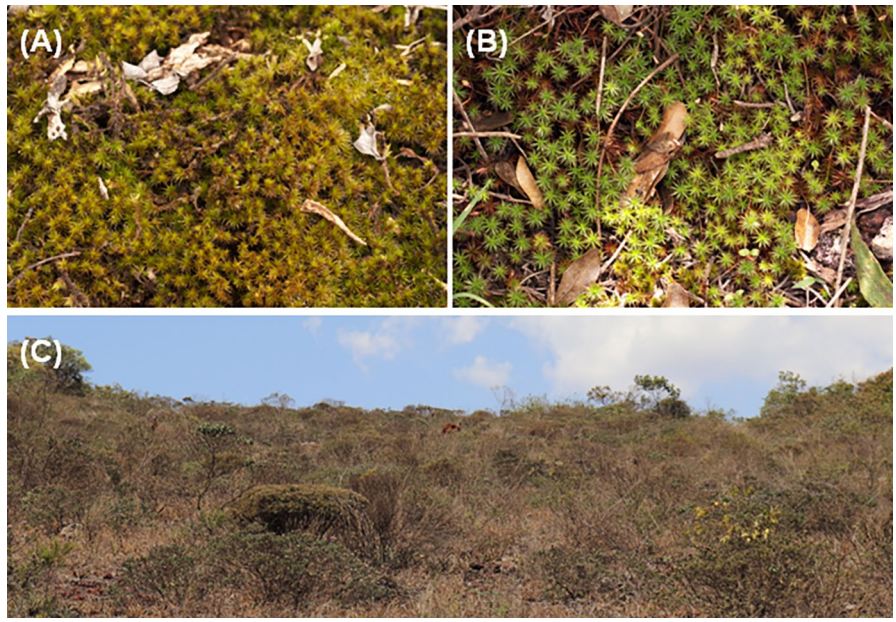


Fig. 1 Overview of the two studied moss species and their collection site. *Campylopus savannarum* (A). *Polytrichum juniperinum* (B). Ironstone outcrop at the Monumento Natural Serra da Calçada, Minas Gerais state, Brazil (C)

Within this framework, our study aimed to investigate the comparative responses of two Brazilian moss species, *Campylopus savannarum* (Müll.Hal.) Mitt. (Dicranaceae) and *Polytrichum juniperinum* Willd. ex Hedw. (Polytrichaceae), to different Cu and Pb treatments. We hypothesized that the contrasting evolutionary histories and morpho-anatomical traits of these species, particularly differences in lamellae architecture, photosynthetic surface organization, and the presence of a differentiated leptome (i.e., phloem-like tissue; Ligrone et al. 2000), would result in distinct physiological strategies to withstand Cu and Pb effects. Consequently, we expected the species to differentially modulate growth (final biomass), photosynthetic performance (maximum quantum yield of PSII, *Fv/Fm*), and metal accumulation under long-term exposure. Understanding how Neotropical bryophytes respond to Cu and Pb provides insights into their physiological tolerance to metal effects, which is relevant both for evaluating their potential vulnerability in impacted environments and for elucidating the evolution of metal-tolerance mechanisms in early land plants.

2 Material and methods

2.1 Target species description and sampling procedures

The moss species selected for this study were *Campylopus savannarum* and *Polytrichum juniperinum*, chosen for their distinct phylogenetic lineages and relatively large gametophytes, which facilitate handling and analysis. Both species occur across all Brazilian biomes, with *C. savannarum* being pantropical and *P. juniperinum* having a broad global distribution (Costa and Pôrto 2022). *Campylopus savannarum* forms large tufts ranging from light green to yellowish (Fig. 1A), whereas *P. juniperinum* forms medium to large tufts with colors ranging from dark green to reddish-brown (Fig. 1B). Both species possess leaf structures that enhance photosynthetic surface area, with abaxial costellae in *C. savannarum* and adaxial lamellae in *P. juniperinum* (Proctor 1984; Frahm 1991; Peralta and Yano 2010). Additionally, the stems of both species possess water-conducting tissues, consisting of a central strand of hydroids (Frahm 1991; Ligrone et al. 2000). However, only *P. juniperinum* presents a differentiated leptome (i.e., a phloem-like tissue; Ligrone et al. 2000), whereas *C. savannarum* lacks this specialized conducting tissue (Frahm 1991).

Five samples of each species (10 in total, ~ 10 cm^2 each) were collected on 12 April 2019 from distinct patches at least 2 m apart on ironstone outcrops of the Monumento Natural Serra da Calçada, Minas Gerais state, Brazil (Fig. 1C; 20° 05′ 43″ S, 43° 58′ 59″ W). Common in southeastern Brazil, ironstone outcrops are characterized by iron-rich soils, unique vegetation, and a high abundance of bryophytes (e.g., Peñaloza-Bojacá et al. 2018; Oliveira et al. 2021; Teixeira et al. 2024). These ecosystems host metal-tolerant plant species adapted to harsh environmental conditions but are highly threatened by open-pit mining and remain poorly studied despite their remarkable biodiversity (Jacobi et al. 2007; Jacobi and Carmo 2008).

Sampling was carried out using a spatula, with specimens carefully numbered and stored in hermetically sealed zip-lock plastic bags. In the laboratory, a portion of each sample was refrigerated at 10 °C until the experimental setup. The remaining material was used for taxonomic identification following Frahm (1991) and Peralta and Yano (2010), with all specimens examined under both stereomicroscope and optical microscope. Subsequently, they were deposited in the BHCB Herbarium, Universidade Federal de Minas Gerais (voucher numbers 204045 to 204,052, 204,061, and 204,062).

2.2 Pre-experimental handling of moss samples

A minimum of 96 gametophyte shoots from each collected sample was randomly selected, totaling 480 shoots per species, thereby constituting a composite sample for each species. The shoots were cleaned in an ultrasonic bath for two cycles of 480 s, apically trimmed to approximately 1 cm in length, and placed, moistened with deionized water, into two sealed 500 mL plastic containers, each containing only one species (*C. savannarum* or *P. juniperinum*). The mosses were acclimated in a growth chamber for one week under controlled conditions: average temperature of 21 °C, 12-h photoperiod, and light intensity of approximately 24 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Following a two-week acclimation period, the mosses were arranged for the experimental setup.

2.3 Soil characterization

The soil used in this study was commercially sourced and chemically characterized prior to the experiment. Samples were sent to the Laboratório de Análises de Solo at the Universidade Federal de Lavras (Minas Gerais, Brazil), where chemical analyses were performed following the protocols described by Claessen et al. (1997) and Santos et al. (2018). Mean values from two subsamples were as follow: pH = 6.45; potassium (K) = 87.67 mg dm^{-3}; phosphorus (P) = 9.01 mg dm^{-3}; sodium (Na) = 3.50 mg dm^{-3}; calcium (Ca) = 1.67 $cmol_c\ dm^{-3}$; magnesium (Mg) = 0.80 $cmol_c\ dm^{-3}$; aluminum (Al) = 0.10 $cmol_c\ dm^{-3}$; potential acidity ($H^+ + Al^{3+}$) = 1.90 $cmol_c\ dm^{-3}$; sum of bases = 2.70 $cmol_c\ dm^{-3}$; effective cation exchange capacity = 2.80 $cmol_c\ dm^{-3}$; total cation exchange capacity = 4.60 $cmol_c\ dm^{-3}$; base saturation (V%) = 58.76%; aluminum saturation = 3.58%; organic matter = 15 g kg^{-1}; remaining phosphorus (P-rem) = 52.10 mg L^{-1}; zinc (Zn) = 0.80 mg dm^{-3};iron (Fe) = 21.50 mg dm^{-3}; manganese (Mn) = 4.30 mg dm^{-3}; boron (B) = 0.10 mg dm^{-3}.

2.4 Cu and Pb treatments

Based on Santos et al. (2014), who established guideline values for soil and groundwater contaminants in Brazil, solutions of Cu (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$) and Pb (II) chloride ($PbCl_2$) were prepared (Table 1). The concentrations correspond to

Table 1 Concentrations of copper (Cu) and lead (Pb) used in the experiment, showing nominal treatment concentration, corresponding solution concentrations, and estimated metal availability in soil (mg dm^{-3})

Metal	Concentration	Solution (mg L^{-1})	Availability (mg dm^{-3})
Copper (Cu)	1	35	28.9
	2	60	54.61
	3	120	116.49
	4	180	166.57
Lead (Pb)	1	17	14.34
	2	72	53.50
	3	144	113.33
	4	216	162.98

the standard value (concentration 1, Cu = 35 mg L^{-1}, Pb = 17 mg L^{-1}), the threshold value (concentration 2, Cu = 60 mg L^{-1}, Pb = 72 mg L^{-1}), and two- and three-fold the threshold value (concentrations 3 and 4, Cu = 120 and 180 mg L^{-1}; Pb = 144 and 216 mg L^{-1}, respectively).

For soil contamination, each soil unit (~0.2 dm^3), properly labeled by treatment and replicate, received a single application of 40 mL of the respective metal solution. Samples were thoroughly homogenized and incubated in sealed plastic bags at room temperature for 15 days. Control soils (concentration 0) received 40 mL of autoclaved distilled water. After this stabilization period, subsamples from each replicate were analyzed at the Laboratório de Análises de Solo of the Universidade Federal de Lavras (Minas Gerais, Brazil) to determine Cu and Pb availability, following Claessen et al. (1997) and Santos et al. (2018). These analyses confirmed that the prepared Cu and Pb solutions, once mixed into the soil, yielded concentrations very close to the intended values (Table 1). In addition, background concentrations in the control soil were quantified as 0.86 mg dm^{-3} for Cu and 2.26 mg dm^{-3} for Pb, both within the soil quality standards established by Santos et al. (2014).

2.5 Experimental setup

The experiment was conducted using a completely randomized design (2 × 2 × 4 × 48), comprising of two moss species (*C. savannarum* and *P. juniperinum*), two metals (Cu and Pb), four concentrations (1, 2, 3, and 4, as detailed in Table 1), and 48 replicates per treatment. Notably, for each species, the control treatment was the same across both metal types, meaning that the same 48 control plants of each species served as reference for both Cu and Pb treatments. In total, the experiment involved 864 plants. Sterile 24-well culture plates were used, with each well (3.5 mL) filled with soil treatments up to one-third of its volume. A single gametophyte was then inserted upright into the soil of each well.

The plates were placed in 5.5 L trays containing moist vermiculite at the bottom and sealed with plastic film to minimize evaporation and prevent contamination. All plants were maintained for 240 days under controlled growth conditions, with an average temperature of 21 °C, a 12-h photoperiod, and a light intensity of approximately 74 µmol m^{-2} s^{-1}. Substrate moisture in the culture plates was maintained throughout the experimental period by regular supplementation with distilled water. Vermiculite was placed at the base of the trays to help sustain adequate humidity around the plates.

2.6 Biomass and photosynthesis of mosses under metal exposure

We used final biomass as a proxy for plant growth and physiological tolerance under metal exposure, assessing it at the end of the 240-day cultivation period. For each treatment, biomass was determined using three replicates of 16 gametophytes each, as weighing individual plants was not feasible due to their small size. This approach ensured consistent biomass estimation across treatments. Samples were cleaned, air-dried for 24 h, and weighed on an analytical balance (0.0001 g precision).

Photosynthetic efficiency was estimated by measuring chlorophyll fluorescence (*Fv/Fm*), an established proxy for physiological tolerance and photochemical integrity under metal treatments, representing the maximum quantum efficiency of Photosystem II (PSII; Maxwell and Johnson 2000). The *Fv/Fm* ratio is calculated as (*Fm* – *Fo*)/*Fm*, where *Fo* is the minimal fluorescence after dark adaptation, and *Fm* is the maximal fluorescence following a saturating light pulse (Maxwell and Johnson 2000). Lower values of *Fv/Fm* indicate damage to the photosynthetic apparatus, while values ranging from 0.70 to 0.83 are typical for healthy, non-stressed bryophytes (Tuba et al. 2010). Measurements were carried out according to Tuba et al. (2010) by taking two time points: day 0 (before metal exposure, to establish baseline values), and at 240 days post-inoculation. Plants were dark-adapted for 24 h prior to measurement using a Mini-PAM fluorometer (Walz, Germany). The fiber-optic probe was positioned over each well, preferably centered on the gametophyte, and a saturating light pulse of 3,000 µmol m^{-2} s^{-1} was applied for 0.8 s, after which the *Fv/Fm* value was recorded.

2.7 Metal accumulation in plants

Mosses samples were analyzed for internal metal concentrations (Terra Análises para Agropecuária, Goiás, Brazil). Due to the minimum sample mass

required for accurate analysis (0.5 g), all plants from the same treatment were combined, resulting in one pooled sample of 48 gametophytes per treatment analyzed collectively for each species. Metal concentration, including Cu and Pb, was determined by atomic absorption spectrophotometry (AAS) following standard protocols for nitric-perchloric acid digestion of plant material, according to the methodology described by Malavolta et al. (1997). To confirm that experimentally used gametophytes did not contain pre-existing metal contamination, a separate pool of 48 gametophytes per species was collected directly from the field. Only the apices (~ 1 cm) of these field samples were used, matching the portion of gametophytes exposed in the cultivation setup.

2.8 Data analysis

To evaluate the effects of bryophyte species and metal concentration 240 days after inoculation, separate Generalized Linear Models (GLMs) were fitted for each combination of response variable and metal. Biomass and maximum quantum efficiency of PSII (*Fv/Fm*) were analyzed as response variables, while the predictors included bryophyte species (*C. savannarum* and *P. juniperinum*) and metal concentration (control and four concentrations for Cu; four concentrations for Pb). Separate models were used for Cu and Pb to allow species-specific responses to each metal to be assessed independently. To determine the most appropriate error distribution, three GLM families were tested: Gaussian (identity), Gamma (log), and quasi-Gamma (log with variance proportional to the squared mean). Model fit was assessed using residual deviance per degree of freedom and AIC (for Gaussian and Gamma). Based on these criteria, Gamma was selected for Cu biomass, quasi-Gamma for Pb biomass, and Gaussian for *Fv/Fm* for both metals. Type III analysis of deviance (*car* package; Fox and Weisberg 2018) was performed to test main effects of species and concentration, as well as their interaction. Pairwise comparisons of estimated marginal means were conducted using the *emmeans* package (Lenth 2024) with Sidak adjustment. Additionally, metal accumulation between species at the two highest concentrations (3 and 4) for each metal was assessed using Pearson's Chi-squared tests with Yates' continuity correction; these analyses are descriptive only due to pooled samples and are intended to illustrate contrasting accumulation patterns without implying statistical inference. All analyses were conducted in R 4.0.2 (R Core Team 2024).

3 Results

3.1 Biomass production

After 240 days of cultivation, most *C. savannarum* shoots remained green across all treatments, showing growth but limited branching (Fig. 2A, B), while *P. juniperinum* plants, also predominantly green regardless of treatment, invested in producing new etiolated branches (Fig. 2C, D). The GLM results indicated that metal concentration had a significant effect on biomass for both Cu and Pb (Table 2), while the overall biomass did not differ significantly between species, and the interaction between species and concentration was not significant (Table 2). Pairwise comparisons revealed that, for *C. savannarum*, biomass in the control treatment was significantly higher than at Cu concentration 3 and Pb concentrations 3 and 4 (Fig. 3). In contrast, *P. juniperinum* showed no significant differences in biomass among concentrations for either metal (Fig. 3), indicating that while species exhibit similar overall biomass patterns, their responses to increasing metal treatments may differ under specific concentrations. Although the general GLM did not detect a species vs. concentration interaction, post hoc tests indicated that at Cu concentration 3, biomass differed between species, with *C. savannarum* showing lower values than *P. juniperinum*.

3.2 Photosynthetic responses

At 240 days post-inoculation, photosynthetic efficiency (*Fv/Fm*) was significantly influenced by metal concentration and by the interaction between moss species and concentration, while moss species alone had no significant effect (Table 3). At the highest Cu concentrations, *C. savannarum* and *P. juniperinum* differed in *Fv/Fm*, with higher values observed in *P. juniperinum* (Fig. 4). However, comparisons among Cu concentrations within each species revealed no significant differences relative to the control (Fig. 4), indicating that *Fv/Fm* was largely unaffected by Cu exposure in both species. In contrast, Pb exposure

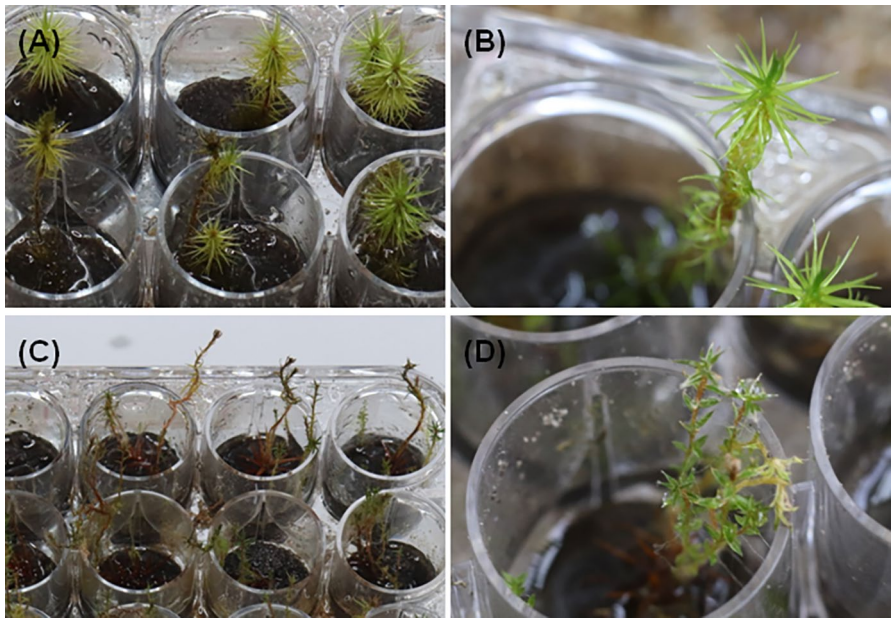


Fig. 2 Moss gametophytes responses after 240 days of cultivation. *Campylopus savannarum* gametophytes growing under Cu concentration 1 (A). Detail showing predominantly apical growth with limited branching (B). *Polytrichum juniperinum* gametophytes under Cu concentration 2 (C). Close-up of the numerous etiolated branches produced by *P. juniperinum* at the end of the experiment (D)

Table 2 - Type III Analysis of Deviance from Generalized Linear Models (GLMs) assessing the effects of bryophyte species, metal concentration, and their interaction on biomass for each metal

Copper (Cu)			
Effect	LR Chisq	Df	Pr(> Chisq)
Species	0.6523	1	0.4193
Concentration	10.3686	4	**0.0347**
Species × Concentration	2.7117	4	0.6072
Lead (Pb)			
Effect	LR Chisq	Df	Pr(> Chisq)
Species	1.8919	1	0.1690
Concentration	26.2977	4	**> 0.0001**
Species × Concentration	0.3863	4	0.9836

Likelihood ratio chi-square statistic (LR Chisq), degrees of freedom (df), and p-values are presented. Statistically significant effects ($p < 0.05$) are indicated in bold. Model fit was evaluated using residual deviance divided by residual degrees of freedom (deviance/df) and Akaike Information Criterion (AIC, where applicable). For copper (Cu), a Gamma distribution with log link was used (deviance = 2.93, df_residual = 20, deviance/df = 0.15, AIC = 313), and for lead (Pb), a quasi-Gamma model with log link and variance proportional to the squared mean was used (deviance = 0.55, df_residual = 20, deviance/df = 0.027)

led to more pronounced differences between species across all concentrations except the control (Fig. 4), with *C. savannarum* consistently showing lower *Fv/Fm* than *P. juniperinum*. When comparing Pb concentrations within species, concentrations 3 and 4 significantly differed from the control in *C. savannarum* (Fig. 4). Interestingly, *P. juniperinum* exhibited significantly higher *Fv/Fm* at concentration 2 compared to the control (Fig. 4), suggesting a non-linear response to Pb exposure.

For reference, before any exposure to metals, the mean *Fv/Fm* value calculated from all 432 gametophytes of each species used in the experiment was $0.77 \pm 0.06$for *C. savannarum* and $0.69 \pm 0.11$for *P. juniperinum*. These baseline values represent the photosynthetic efficiency of healthy plants of each species

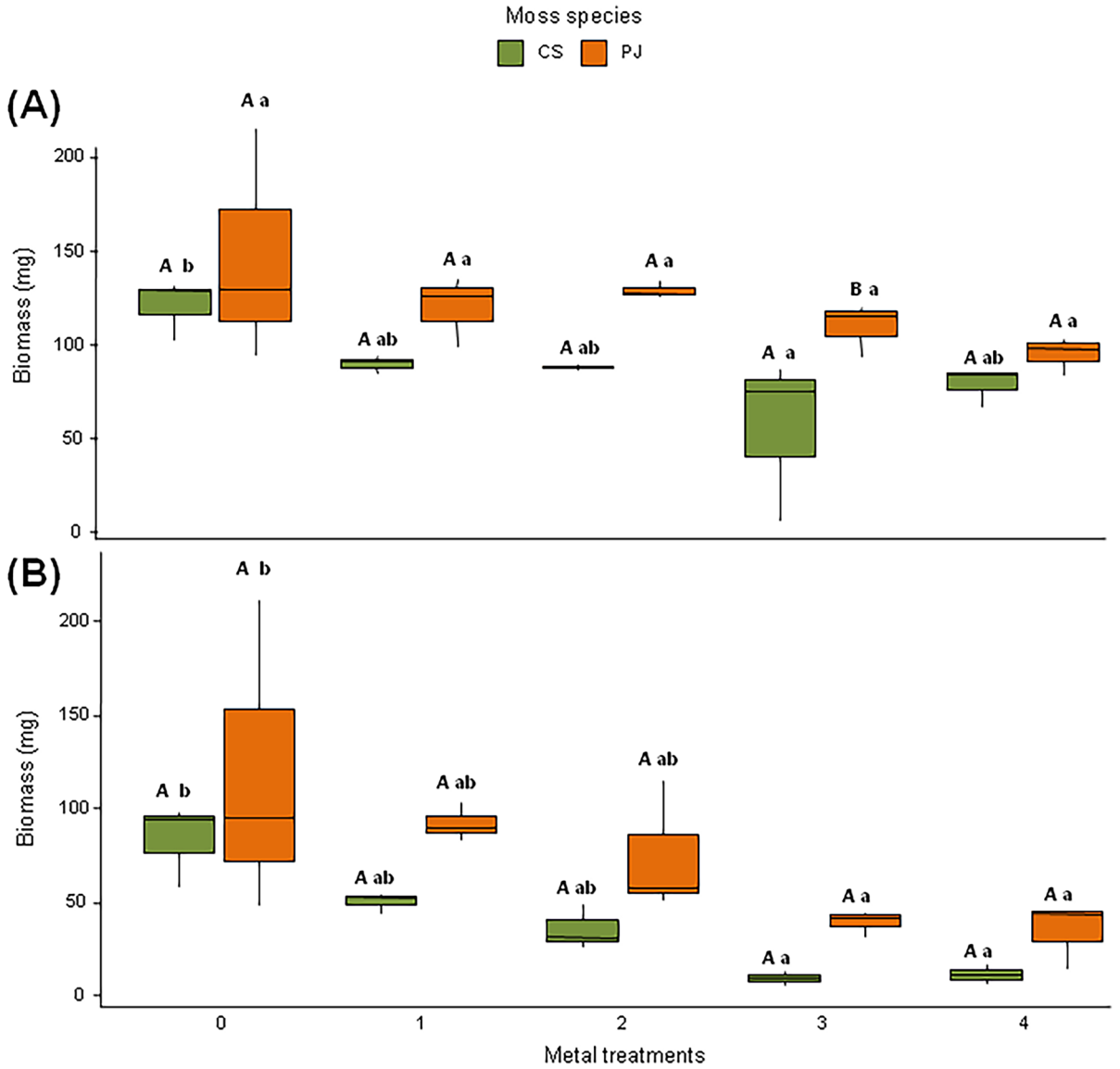


Fig. 3 Boxplots of biomass (mg) for each bryophyte species across metal concentration treatments. Copper treatments, where concentrations 1 = 28.9 mg dm^{-3}; 2 = 54.6 mg dm^{-3}; 3 = 116.5 mg dm^{-3}; 4 = 166.6 mg dm^{-3} (A). Lead treatments, where concentrations 1 = 14.3 mg dm^{-3}; 2 = 53.5 mg dm^{-3}; 3 = 113.3 mg dm^{-3}; 4 = 163.0 mg dm^{-3} (B). Boxes show the median (central line) and interquartile range (IQR; 25–75%), with whiskers extending to 1.5 × IQR. Lowercase letters indicate significant differences among concentrations within the same species, while uppercase letters indicate significant differences between species at the same concentration (GLM followed by Sidak-adjusted pairwise comparisons, α = 0.05). CS = *Campylopus savannarum*; PJ = *Polytrichum juniperinum*

under the initial cultivation conditions. After 240 days of growth under the established treatments, the mean *Fv/Fm* values for the 48 gametophytes per treatment deviated from these baselines (Table 4). Even the control values decreased, reaching 0.65 ± 0.08 For *C. savannarum* and 0.67 ± 0.09 for *P. juniperinum*. However, some treatments showed higher values than the baseline, particularly for *P. juniperinum* at high Cu concentrations and at Pb concentration 2, which recorded the highest mean *Fv/Fm* (Table 4). Conversely, the lowest *Fv/Fm* was observed in *C. savannarum* at Pb concentration 3 (Table 4).

Table 3 - Type III Analysis of Deviance from Generalized Linear Models (GLMs) assessing the effects of bryophyte species, metal concentration, and their interaction on photosynthetic efficiency (*Fv/Fm*)

Copper (Cu)			
Effect	LR Chisq	Df	Pr(> Chisq)
Species	1.867	1	0.172
Concentration	14.830	4	**0.005**
Species × Concentration	11.239	4	**0.024**
Lead (Pb)			
Effect	LR Chisq	Df	Pr(> Chisq)
Species	1.126	1	0.289
Concentration	59.074	4	**> 0.001**
Species × Concentration	32.031	4	**> 0.001**

Likelihood ratio chi-square statistic (LR Chisq), degrees of freedom (df), and p-values are presented. Statistically significant effects ($p < 0.05$) are indicated in bold. Model fit for each GLM with Gaussian distribution with identity link is summarized as residual deviance divided by residual degrees of freedom (deviance/df) and Akaike Information Criterion (AIC). For Cu: deviance/df = 0.009, AIC = −887.1. For Pb: deviance/df = 0.015, AIC = −644.3

3.3 Metal accumulation patterns

Samples of *C. savannarum* collected directly from the natural environment and those from the control group exhibited low Cu concentrations (Fig. 5). Cu accumulation increased markedly in treated samples, ranging from 69 mg kg^{-1} at concentration 1 to 980 mg kg^{-1} at concentration 4, corresponding to a 196-fold increase relative to the control. A similar pattern was observed for *P. juniperinum*: plants with no exposure to Cu had low metal concentrations, while treated samples showed a substantial increase, from 60 mg kg^{-1} at concentration 1 to 800 mg kg^{-1} at concentration 4, representing a 113-fold increase compared to the control (Fig. 5). Overall, both species displayed a clear dose-dependent increase in Cu accumulation, with *C. savannarum* reaching slightly higher maximum values than *P. juniperinum*.

On the other hand, regarding Pb accumulation, *P. juniperinum* showed higher accumulation compared to *C. savannarum* under equivalent treatment conditions. In *P. juniperinum*, Pb concentrations increased from 30 mg kg^{-1} at the lowest treatment concentration to 490 mg kg^{-1} at the highest, representing a 44-fold increase relative to the control, while control and natural samples exhibited low concentrations of 11 mg kg^{-1} and 13 mg kg^{-1}, respectively (Fig. 5). For *C. savannarum*, Pb concentrations ranged from 40 mg kg^{-1} (concentration 1) to 330 mg kg^{-1} (concentration 4), corresponding to a 24-fold increase compared to the control, with control and natural groups showing 14 mg kg^{-1} and 10 mg kg^{-1}. Despite both species responding positively to increasing Pb exposure, *P. juniperinum* consistently accumulated greater amounts across the treatment gradient.

The species-specific accumulation patterns were further supported by Pearson's Chi-squared tests. At concentration 3, the test was highly significant ($X^2 = 55.91$, $df = 1$, $P < 0.001$), indicating that the two mosses responded differently: *C. savannarum* accumulated more Cu, whereas *P. juniperinum* accumulated more Pb. This divergence became even more pronounced at concentration 4, where the Chi-squared test again revealed a highly significant difference ($X^2 = 48.68$, $df = 1$, $P < 0.001$), reinforcing the contrasting accumulation strategies of the two species.

4 Discussion

4.1 Species-specific physiological strategies underlying Cu and Pb responses

Our study revealed species-specific strategies in response to Cu and Pb exposure. *C. savannarum* exhibited high Cu accumulation despite reductions in biomass and photosynthetic efficiency (*Fv/Fm*) at elevated metal concentrations, particularly under Pb,

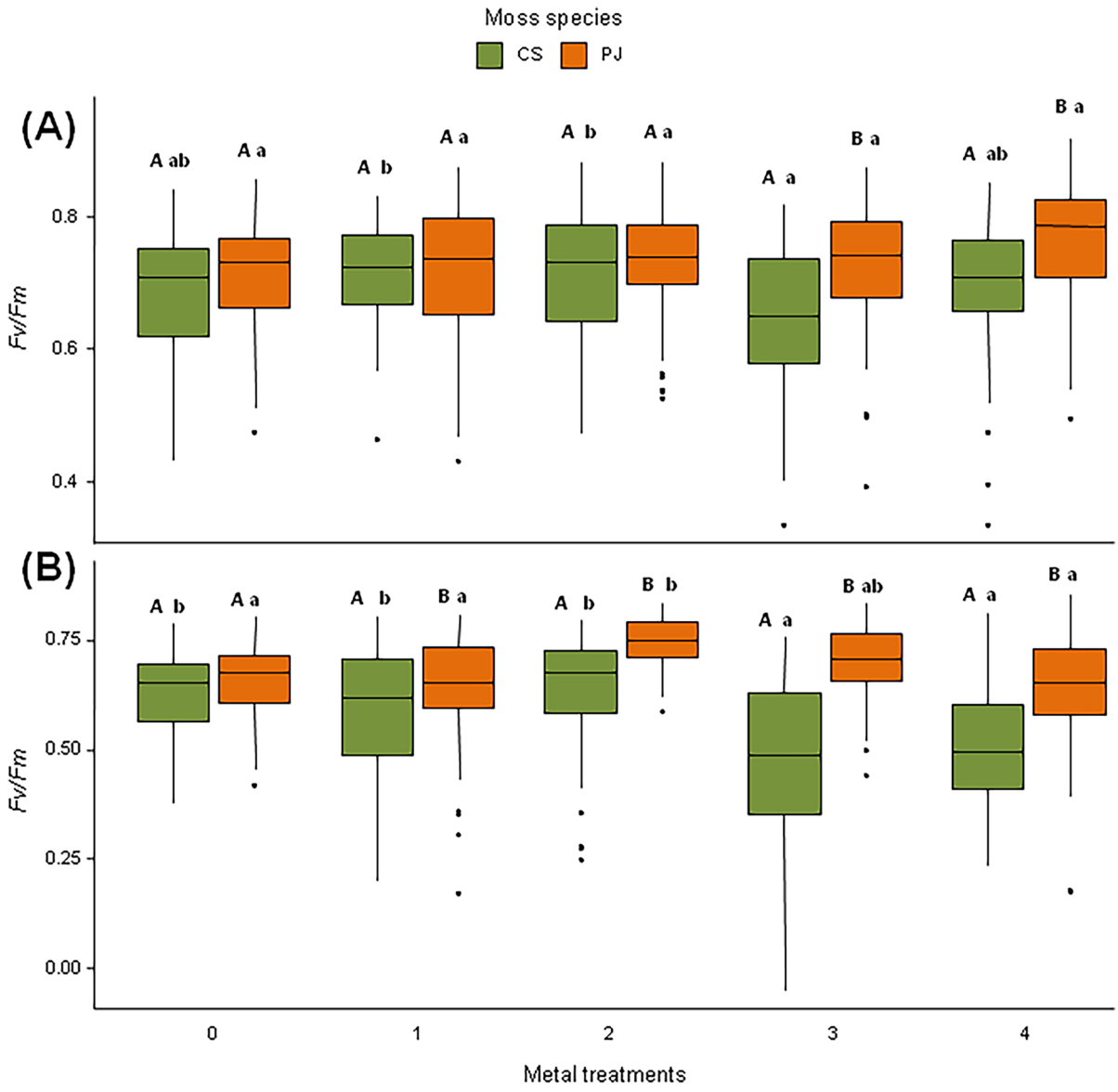


Fig. 4 Boxplots showing photosynthetic efficiency (*Fv/Fm*) for each species across different metal concentrations, separated by metal type. Copper treatments, where concentrations 1 = 28.9 mg dm^{-3}; 2 = 54.6 mg dm^{-3}; 3 = 116.5 mg dm^{-3}; 4 = 166.6 mg dm^{-3} (A). Lead treatments, where concentrations 1 = 14.3 mg dm^{-3}; 2 = 53.5 mg dm^{-3}; 3 = 113.3 mg dm^{-3}; 4 = 163.0 mg dm^{-3} (B). Boxes show the median (central line) and interquartile range (IQR; 25–75%), with whiskers extending to 1.5 × IQR. Lowercase letters indicate significant differences among concentrations within the same species, while uppercase letters indicate significant differences between species at the same concentration (GLM followed by Sidak-adjusted pairwise comparisons, $\alpha = 0.05$). CS = *Campylopus savannarum*; PJ = *Polytrichumjuniperinum*

indicating a trade-off between metal uptake and physiological performance. In contrast, *P. juniperinum* maintained stable growth and photosynthetic function while accumulating more Pb, suggesting effective tolerance mechanisms. These contrasting patterns align with observations in related mosses, where metal-specific accumulation and differential sensitivity reflect adaptations to local metal conditions (Francis and Petersen 1989; Panda and Choudhury 2005; Šoltés and Gregušková 2013; Zhang et al. 2024, 2025).

Table 4 Mean *Fv/Fm* values (± standard deviation; SD) for each bryophyte species under each treatment, including the control and the initial time point

Species	Treatment (mg dm^{-3})	*Fv/Fm* mean (± SD)
Campylopus savannarum	Initial	0.77 ± 0.06
	Control	0.65 ± 0.08
	Concentration 1-Cu (28.9)	0.67 ± 0.10
	Concentration 2-Cu (54.61)	0.67 ± 0.11
	Concentration 3-Cu (116.49)	0.61 ± 0.11
	Concentration 4-Cu (166.57)	0.65 ± 0.10
	Concentration 1-Pb (14.34)	0.61 ± 0.17
	Concentration 2-Pb (53.50)	0.65 ± 0.17
	Concentration 3-Pb (113.33)	0.50 ± 0.16
	Concentration 4-Pb (162.98)	0.54 ± 0.12
Polytrichum juniperinum	Initial	0.69 ± 0.11
	Control	0.67 ± 0.09
	Concentration 1-Cu (28.9)	0.68 ± 0.09
	Concentration 2-Cu (54.61)	0.69 ± 0.09
	Concentration 3-Cu (116.49)	0.68 ± 0.09
	Concentration 4-Cu (166.57)	0.72 ± 0.09
	Concentration 1-Pb (14.34)	0.66 ± 0.12
	Concentration 2-Pb (53.50)	0.76 ± 0.11
	Concentration 3-Pb (113.33)	0.71 ± 0.11
	Concentration 4-Pb (162.98)	0.66 ± 0.11

The "Initial" values were calculated from all 432 gametophytes per species before any exposure to metals, representing the baseline photosynthetic efficiency of healthy plants under the initial cultivation conditions. All other values correspond to measurements taken after 240 days of growth under the respective treatments, based on 48 gametophytes per treatment

Our findings are consistent with the mechanistic basis we hypothesized for species-specific tolerance. *P. juniperinum* possesses well-developed lamellae that increase the effective photosynthetic surface and reduce CO_2-diffusion constraints, traits known to support higher PSII stability under adverse environmental conditions (Proctor 2005). By enabling efficient use of high irradiance without excessive reliance on non-photochemical quenching, these lamellae may limit ROS overproduction when metal toxicity constrains photosynthetic electron transport. In addition, the presence of a central strand and a hydroid-based conduction system (Ligrone et al. 2000) likely facilitates more effective internal translocation or compartmentalization of absorbed metals, contributing to the stable *Fv/Fm* values and biomass observed even under elevated Cu and Pb. In contrast, *C. savannarum*, with simpler lamellae and reduced internal conduction tissues (Frahm 1991), likely has more limited photoprotective capacity and internal sequestration pathways, making it more susceptible to Pb-induced photoinhibition and growth suppression. These anatomical differences provide a plausible mechanistic explanation for the contrasting physiological responses observed between the species.

4.2 Differential metal tolerance

In our experiment, the physiological and growth responses of *C. savannarum* differed depending on the metal evaluated. The relatively moderate responses observed under Cu are consistent with observations in other *Campylopus* species, such as *C. schmidii*, which exhibits reduced sensitivity to Cu compared with other metals (Zhang et al. 2024). While Zhang et al. (2024) applied Cu at concentrations of 10–50 µM $CuSO_4$ (approximately 0.64–3.2 mg Cu dm^{-3}), our study used higher Cu treatments (13.05 to 67.05 mg Cu dm^{-3} from $CuCl_2 \cdot 2H_2O$), yet *C. savannarum* maintained relatively stable physiological performance across most concentrations. Moreover, *C. schmidii* responds to cadmium (Cd) with transcriptional changes in gene

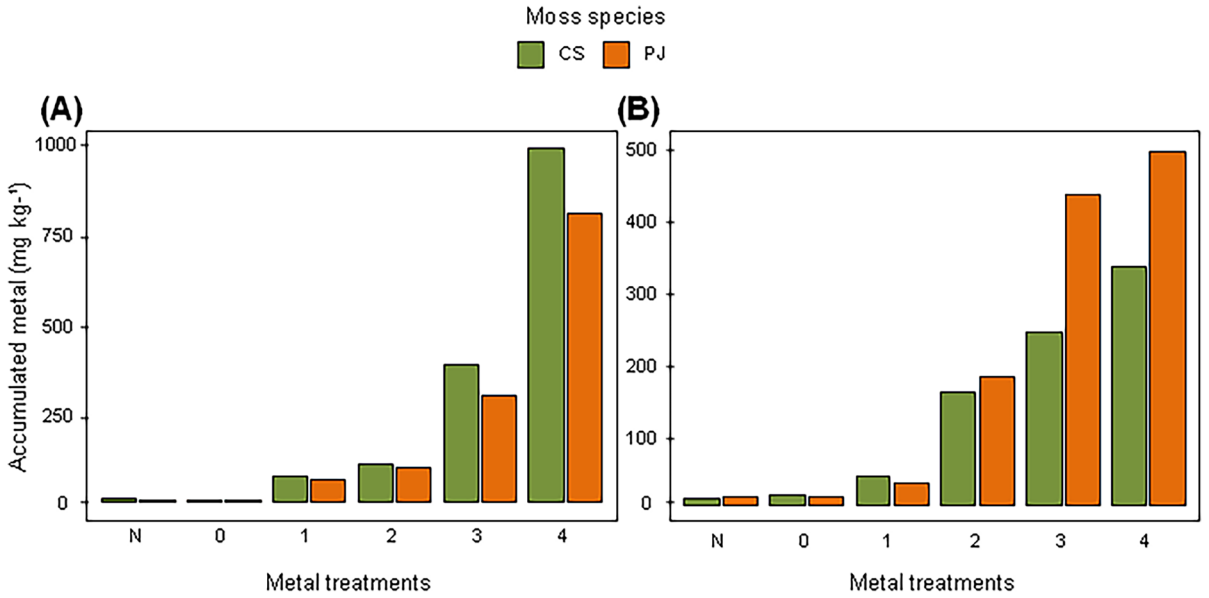


Fig. 5 Copper (Cu) and lead (Pb) accumulation in *Campylopus savannarum* and *Polytrichum juniperinum* across treatments. Copper treatments, where concentrations 1 = 28.9 mg dm^{-3}; 2 = 54.6 mg dm^{-3}; 3 = 116.5 mg dm^{-3}; 4 = 166.6 mg dm^{-3} (A). Lead treatments, where concentrations 1 = 14.3 mg dm^{-3}; 2 = 53.5 mg dm^{-3}; 3 = 113.3 mg dm^{-3}; 4 = 163.0 mg dm^{-3} (B). Bars represent metal concentrations (mg kg^{-1}) measured in plants collected directly from the natural environment (N), the control, and four increasing treatment concentrations (1–4) for each metal. For each species and treatment, values correspond to one pooled sample composed of 48 individuals analyzed collectively

linked to cell structure, signaling, and metabolism, suggesting molecular strategies to mitigate with metal toxicity (Zhang et al. 2025). Similar mechanisms may occur in *C. savannarum*, although they were not directly assessed here.

For instance, certain metals have markedly deleterious effects on *Campylopus* species. Chromium (Cr) exposure significantly reduces photosynthesis in *C. schmidii* (Huang et al. 2025), mirroring the Pb-induced decline in photosynthetic efficiency observed in *C. savannarum*. Although some *Campylopus* species such as *Campylopus lamellatus* Mont. and *C. schmidii* predominantly accumulate metals in their cell walls (Oliveira et al. 2025; Zhang et al. 2024), suggesting a genus-wide sequestration mechanism that may aid in metal avoidance, this does not appear to be the case for *C. savannarum*. The pronounced decline in *Fv/Fm* and biomass under Pb exposure indicates that lead exerts cellular toxicity, likely by generating oxidative imbalance and disrupting chlorophyll biosynthesis (Pinho and Ladeiro 2012; Sharma and Dubey 2005; Zulfiqar et al. 2019). These observations indicate metal-specific response patterns within the experimental context rather than a direct quantitative comparison of Cu versus Pb tolerance.

In contrast, *P. juniperinum* maintained relatively stable growth and photosynthetic performance under the Cu and Pb concentrations tested in this study, which contrasts with responses reported for some other *Polytrichum* species under different experimental conditions. In fact, Panda and Choudhury (2005) reported that *P. commune*, when exposed to Cu concentrations of 0–64 mg Cu dm^{-3} (as $CuSO_4$), experienced reduced nitrate reductase activity, decreased chlorophyll concentration, and increased lipid peroxidation after only 24–48 h, despite dose-dependent metal accumulation. However, direct comparisons between these studies should be made with caution. Methodological differences, such as the use of direct gametophyte inoculation on soil versus exposure in liquid medium, and the range of endpoints assessed, may contribute to the observed discrepancies in metal tolerance between studies. Considering that other *Polytrichum* species accumulate metals symplastically (e.g., aluminum; Oliveira et al. 2025), internal sequestration mechanisms may play a role

in tolerance within this genus, although this was not directly tested here.

4.3 Metal accumulation capacity and implications for tolerance mechanisms

In our study, *C. savannarum* and *P. juniperinum* accumulated up to 980 mg kg^{-1} and 800 mg kg^{-1} of Cu, and 330 mg kg^{-1} and 490 mg kg^{-1} of Pb, respectively—values below the widely accepted hyperaccumulation threshold for bryophytes (< 1,000 mg kg^{-1} of dry mass; Reeves and Baker 2000; Rascio and Navari-Izzo 2011). Nevertheless, future studies with more refined analytical approaches to quantify metal concentrations in individual gametophytes (< 0.001 mg) may provide clearer evidence as to whether these species can be considered hyperaccumulators. In fact, true hyperaccumulation in bryophytes is rare but has been reported in *Scopelophila cataractae*, which can reach up to 3% Cu in dry mass (Satake et al. 1988) and is recognized as a genuine Cu hyperaccumulator (Rascio and Navari-Izzo 2011). Thus, the metal accumulation observed in both species points to a potential morphophysiological capacity for metal detoxification and sequestration, possibly involving the synthesis of metal-chelating compounds, regulation of metal transport pathways, and upregulation of antioxidant defense responses (Navabpour et al. 2020; Chen et al. 2022; Gupta et al. 2024; Huang et al. 2024).

High metal concentrations can trigger overproduction of reactive oxygen species (ROS), causing oxidative imbalance, lipid peroxidation, protein degradation, and DNA damage (Prasad 2004; Dazy et al. 2009). The apparently stable growth and photosynthetic efficiency observed in *P. juniperinum* under elevated Cu and Pb concentrations are descriptive and suggest that this species may deploy effective antioxidant defenses, similar to those reported in *Fontinalis antipyretica* Hedw., *Pleurochaete squarrosa* (Brid.) Lindb., and *Timmiella barbuloides* (Brid.) Moenk under metal exposure (Dazy et al. 2009; Aydoğan et al. 2017), even though these mechanisms were not directly measured in our study.

4.4 Future directions

Across bryophytes, responses to toxic metals are generally species-specific, with tolerance commonly linked to cell wall or vacuolar sequestration and the activation of antioxidant defenses (Panda and Choudhury 2005; Dazy et al. 2009; Zhang et al. 2024; Oliveira et al. 2025). In many cases, such tolerance reflects genetic adaptations rather than plasticity, with polluted habitats imposing strong selective pressures that favor resistant ecotypes (Meharg 1994). Examples such as *Funaria hygrometrica* Hedw., *Ceratodon purpureus* (Hedw.) Brid., and *Pseudoscleropodium purum* (Hedw.) M. Fleish. illustrate how evolutionary history shapes physiological performance under adverse environmental conditions, as some populations have evolved ecotypes fully adapted to high metal concentrations (Jules and Shaw 1994; Basile et al. 2001; Boquete et al. 2016).

On the other hand, many plant species exhibit epigenetic mechanisms (i.e., reversible modifications in gene expression without changes in the DNA sequence) that enable them to adjust to environmental conditions and transmit these adaptive responses across generations (Fasani et al. 2023). In the case of *C. savannarum* and *P. juniperinum*, which originated from non-contaminated soils, their observed tolerance to Cu and Pb may reflect constitutive morphophysiological traits or also possibly rapid plastic responses or epigenetic adjustments.

5 Conclusions

This study demonstrates species-specific physiological strategies of metal tolerance in Neotropical mosses. *Polytrichum juniperinum* effectively maintained growth and photosynthetic efficiency under both Cu and Pb treatments, while *Campylopus savannarum* was more sensitive, particularly to Pb. Descriptive observations of metal accumulation indicate contrasting patterns: *C. savannarum* accumulated more Cu, while *P. juniperinum* accumulated more Pb. These findings highlight their ecological relevance and potential application in restoration, while future studies should investigate metal partitioning within tissues and the underlying molecular tolerance mechanisms.

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Author contributions Mateus Fernandes Oliveira led the conceptualization, methodology, validation, formal analysis, investigation, data curation, and preparation of the original draft. Tiago Vilas-Boas, Felipe Della-Torre, and Najla Meireles-Medeiros contributed to methodology, investigation, data curation, and manuscript review and editing. Raphael Ricon de Oliveira, Lucas Anjos de Souza, and Adaíses Simone Maciel-Silva contributed to conceptualization and methodology, with Adaíses Simone Maciel-Silva also responsible for validation, supervision, and project administration. Funding acquisition was carried out by Adaíses Simone Maciel-Silva and Raphael Ricon de Oliveira. Marcel Giovanni Costa França provided resources and contributed to manuscript review and editing. All authors read and approved the final version of the manuscript.

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Data Availability All data generated or analyzed during this study are included in this published article. Voucher specimens are deposited in the BHCB Herbarium (Universidade Federal de Minas Gerais, Brazil) and are available for loan upon request. Additional information can be obtained from the authors upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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