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Assessing Heavy Metal Toxicity: Lead and Cadmium Uptake and their Influence on Chlorophyll Dynamics of *Taxiphyllum taxirameum* (Mitt.) M. Fleisch

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Abstract
This study evaluates the comparative phytotoxic effects of lead (Pb) and cadmium (Cd) on the chlorophyll dynamics and metal accumulation in the moss *Taxiphyllum taxirameum*. Experimental exposure revealed that Cd induces acute toxicity, resulting in rapid chlorophyll depletion and complete pigment degradation at concentrations ≥200 ppm within 45 days. In contrast, Pb exposure led to a slower but cumulative reduction in chlorophyll, reaching total loss at 350–500 ppm by 60 days. Metal accumulation showed a clear concentration- and time-dependent pattern, with Cd levels rising to 581.6 ppm and Pb to 701.8 ppm over 75 days, indicating a greater retention capacity for Pb in moss tissue. Statistical analyses, including Pearson's correlation and principal component analysis, demonstrated strong negative relationships between metal uptake and chlorophyll content, with metal concentration emerging as the dominant driver of pigment loss (variance explained: Pb = 72.2%, Cd = 72.8%), while exposure duration played a significant secondary role (~20% variance in PC2). Notably, chlorophyll loss followed a non-linear, threshold-driven pattern, highlighting the interaction between metal burden, exposure time, and physiological stress in the organism. Together, these findings provide new insights into the mechanisms of heavy metal toxicity in mosses and reinforce the value of *T. taxirameum* as a biomonitor for environmental contamination by Pb and Cd.

Keywords: Taxiphyllum taxirameum; Heavy metal toxicity; metal bioaccumulation; Chlorophyll degradation; Metal uptake; PCA; Biomonitoring

Introduction

Heavy metal pollution poses a significant threat to environmental health and human well-being worldwide. The World Health Organization (WHO) has identified several chemicals of major public health concern, including lead (Pb) and cadmium (Cd), which substantially contribute to global morbidity and mortality (WHO, 2021). In 2019 alone, over two million deaths were attributed to chemical exposures, with nearly half resulting from lead-induced cardiovascular diseases. Their environmental persistence and bio accumulative nature raise serious ecological and toxicological concerns (Mitra et al., 2022). Mosses are widely recognized as effective biomonitors for monitoring atmospheric heavy metal pollution due to their simple structure, widespread distribution, and high capability to bioconcentrate metals beyond physiological needs (Macedo-Miranda et al., 2016; Tessier and Boisvert, 1999). The cell walls of mosses contain abundant exchangeable cations, facilitating passive uptake of metal ions and reflecting ambient environmental metal loads (Kosior et al., 2020; Grodzińska and Szarek-Łukaszewska, 2001). This characteristic enables mosses to serve as sensitive monitors of spatial and temporal variations in metal pollution, particularly in urban and industrial areas (Chen et al., 2010; Mao et al., 2022). Heavy metal toxicity affects multiple physiological and biochemical processes in plants, including protein synthesis, respiration,

photosynthesis, and membrane integrity across diverse taxa (Hart and Scaife, 1977; Azeez and Banerjee, 1986; Tyler, 1990). In mosses, exposure to metals such as copper, zinc, lead, and cadmium has been shown to reduce chlorophyll content, disrupt photosynthetic efficiency, and alter species composition (Shakya et al., 2008; Phaenark et al., 2024; Nanda et al., 2024).

Taxiphyllum taxirameum (Mitt.) M. Fleisch is notable for its efficient bioconcentration of heavy metals and suitability as a biomonitor for air pollution (Chen et al., 2010; Mao et al., 2022). Changes in chlorophyll content serves as critical indicator of heavy metal-induced stress, given chlorophyll's essential role in photosynthesis and plant vitality (Tanaka and Ito, 2025). Despite the ecological significance of *T. taxirameum*, differential impacts of Pb and Cd on its chlorophyll dynamics and metal accumulation patterns remain insufficiently explored. This study aims to quantify the accumulation of Pb and Cd in *Taxiphyllum taxirameum*, and evaluate their respective effects on chlorophyll (chl a), chlorophyll (chl b), and total chlorophyll (total chl) content over time. Understanding these responses will enhance insights into heavy metal phytotoxicity mechanisms and reinforce the use of mosses in ecological biomonitoring and pollution assessment.

Materials and methods

Sampling of Moss *Taxiphyllum taxirameum*

Samples of *T. taxirameum* were collected from unpolluted sites on the forested foothill of Summerhill, approximately 6 km from Shimla, with geographical coordinates of 31˚07'13.3"N, 77˚08'25.3"E. Sampling was conducted during the rainy season (August–September), taking care to minimize material loss and contamination. Moss specimens were collected using a spatula and carefully stored in polythene bags for transportation.

Preparation of Plant Material

In the laboratory, fresh samples were placed on plastic trays and cleaned using plastic tweezers to remove soil particles, insects, and non-target gametophytes. Only green and light green-brown moss plants were transferred to petri-plates and maintained at room temperature.

Preparation of Metal Solutions and Treatment

Separate aqueous stock solutions of Cd and Pb (1000 ppm) were prepared by dissolving 2.036 g $CdCl_2.2\ H_2O$ and 1.6g of lead nitrate Pb $(NO3)2$ (analytical grade, Merck) in 1 Litre of double deionized water. Solutions were diluted to working concentrations ranging from 10 to 500 ppm. Moss samples were sprayed with these solutions at specified intervals in triplicate. Control samples were sprayed with distilled water. Samples were harvested at 15-day intervals up to 75 days for chlorophyll quantification.

Determination of Chlorophyll Content

The chlorophyll estimation was measured spectrophotometrically following Pavlicevic et al. (2023). Treated moss clump were rinsed with deionized water and blotted dry. Approximately 100 mg fresh tissue was homogenized in 5 mL of 80% acetone in cold, dark environment with addition of magnesium carbonate to neutralize acids. The final volume of filtrate was made to 10 mL with 80% acetone. The homogenate was filtered to 10 mL with 80% acetone and centrifuged at 5000 rpm for 10 minutes at 4°C (SIGMA 3-30K centrifuge, Germany). Absorbance of the supernatant was measured at 663 nm and 645 nm using a double-beam spectrophotometer (Thermo Fischer, Evolution 201, USA), using 80% acetone as blank.

Analysis of Heavy Metal Accumulation

Samples after 45 and 75 days of treatment were thoroughly washed with tap water and deionized water, dried at 50˚ in a hot air oven (Memmert) to constant weight, and ground in liquid nitrogen. About 0.1 g of powered sample was digested in Teflon vessels with 3ml of 69% HNO_3 (Fluka 84385) and 1 mL of 30% H_2O_2 (Carlo Erba 412072) using Milestone Ethos UP microwave digestion system. Digestion consisted of two 20-minute steps at 180°C for complete organic matter degradation. Metal concentrations were determined using ICP-MS (Agilent 7850), calibrated with IMS-102 multi-element standards.

Data Analyses

Net Enrichment

Net enrichment (NE) was determined as the difference between the metal concentration in moss at the end and at the beginning of the exposure period, following the approach described by Ares et al. (2012) and García-Seoane et al. (2023).

Statistical analysis

Normality of the data was assessed using Shapiro Wilk test. Two-way ANOVA with multiple but equal number of observations per cell was used to evaluate the effects of metal concentration (10–500 ppm) and exposure time (15, 30, 45, 60, and 75 days) on the chlorophyll parameters (chl a, chl b, and total chl), with an equal number of replicates per group. Significant differences between treatment groups were determined using Tukey's Honest Significant Difference (HSD) post-hoc test. Separate two-way ANOVAs were also conducted to examine the effects of metal concentration and exposure time (45 and 75 days) on uptake of Pb and Cd by moss samples. Post-hoc comparisons were performed using Tukey's HSD test to identify significant pairwise differences between specific treatment groups. In all statistical analyses, a p value less than 0.05 was considered statistically significant. Pearson correlation was applied to assess linear relationships between heavy metal concentrations, exposure duration, metal uptake, and chlorophyll content in moss. PCA was used to identify main factors influencing chlorophyll degradation under metal stress.

Results and discussion

This study investigated the effects of Pb and Cd exposure (10 – 500ppm) on chl a, chl b, and total chl content in *T. taxirameum* over a 75days period. Concentration and time dependent trends were analysed to determine the phytotoxic thresholds of Pb and Cd. Metal uptake was quantified on days 45 and 75 to assess the accumulation potential of the moss and to correlate Heavy metal accumulation and chlorophyll degradation.

Table 1 Chlorophyll a, Chlorophyll b and Total Chlorophyll content (mg/g) when treated with different concentrations of Lead.

Chlorophyll a content mg/g (Mean ± SEM; n = 3)									
Time	Control	10 ppm	30 ppm	60 ppm	100 ppm	150 ppm	200 ppm	350 ppm	500 ppm
0	14.75 ± 0.049^{aA}	14.75 ± 0.049^{aB}	14.75 ± 0.049^{aC}	14.75 ± 0.049^{aD}	14.75 ± 0.049^{aE}	14.75 ± 0.049^{aF}	14.75 ± 0.049^{aG}	14.75 ± 0.049^{aH}	14.75 ± 0.049^{aH}
15	13.57 ± 0.015^{cA}	10.06 ± 0.02^{cB}	10.63 ± 0.033^{cC}	8.71 ± 0.015^{cD}	8.66 ± 0.006^{cE}	8.52 ± 0.03^{cF}	7.5 ± 0.023^{cG}	4.74 ± 0.02^{cH}	3.52 ± 0.014^{cH}
30	13.63 ± 0.015^{bA}	12.08 ± 0.02^{bB}	11.47 ± 0.02^{bC}	11.32 ± 0.013^{bD}	9.68 ± 0.014^{bE}	8.71 ± 0.006^{bF}	7.47 ± 0.02^{bG}	2.72 ± 0.012^{bH}	2.16 ± 0.017^{bH}
45	11.43 ± 0.023^{dA}	9.44 ± 0.02^{dB}	9.2 ± 0.02^{dC}	8.62 ± 0.02^{dD}	8.08 ± 0.023^{dE}	7.79 ± 0.014^{dF}	7.55 ± 0.023^{dG}	1.34 ± 0.012^{dH}	0.76 ± 0.003^{dH}
60	11.26 ± 0.012^{eA}	9.31 ± 0.016^{eB}	9.12 ± 0.023^{eC}	8.57 ± 0.02^{eD}	8.17 ± 0.005^{eE}	7.56 ± 0.01^{eF}	6.99 ± 0.02^{eG}	0	0
75	10.96 ± 0.029^{fA}	9 ± 0.015^{fB}	8.58 ± 0.015^{fC}	8.11 ± 0.003^{fD}	7.73 ± 0.02^{fE}	7.41 ± 0.026^{fF}	5.74 ± 0.026^{fG}	0	0
Chlorophyll b content mg/g (Mean ± SEM; n = 3)									
Time	Control	10 ppm	30 ppm	60 ppm	100 ppm	150 ppm	200 ppm	350 ppm	500 ppm
0	10.96 ± 0.096^{aA}	10.96 ± 0.096^{aB}	10.96 ± 0.096^{aC}	10.96 ± 0.096^{aD}	10.96 ± 0.096^{aE}	10.96 ± 0.096^{aF}	10.96 ± 0.096^{aG}	10.96 ± 0.096^{aH}	10.96 ± 0.096^{aI}
15	8.01 ± 0.057^{cA}	7.01 ± 0.02^{cB}	5.48 ± 0.033^{cC}	3.44 ± 0.015^{cD}	3.14 ± 0.006^{cE}	3.03 ± 0.034^{cF}	3.32 ± 0.023^{cG}	3.98 ± 0.02^{cH}	2.04 ± 0.014^{cI}
30	9.22 ± 0.044^{bA}	6.13 ± 0.032^{bB}	6.05 ± 0.02^{bC}	5.60 ± 0.013^{bD}	5.29 ± 0.014^{bE}	4.76 ± 0.006^{bF}	4.42 ± 0.02^{bG}	1.64 ± 0.012^{bH}	1.33 ± 0.0145^{bI}
45	7.28 ± 0.047^{dA}	4.5 ± 0.02^{dB}	4.34 ± 0.02^{dC}	4.10 ± 0.025^{dD}	3.90 ± 0.023^{dE}	3.58 ± 0.014^{dF}	3.93 ± 0.023^{dG}	1.32 ± 0.012^{dH}	0.44 ± 0.003^{dI}
60	7.24 ± 0.055^{eA}	4.40 ± 0.016^{eB}	4.38 ± 0.023^{eC}	4.00 ± 0.02^{eD}	3.57 ± 0.005^{eE}	3.32 ± 0.01^{eF}	2.9 ± 0.02^{eG}	0	0
75	6.92 ± 0.04^{fA}	3.96 ± 0.016^{fB}	3.65 ± 0.015^{fC}	3.44 ± 0.003^{fD}	2.64 ± 0.028^{fE}	2.5 ± 0.026^{fF}	2.50 ± 0.044^{fG}	0	0
Total Chlorophyll content mg/g (Mean ± SEM; n = 3)									
Time	Control	10 ppm	30 ppm	60 ppm	100 ppm	150 ppm	200 ppm	350 ppm	500 ppm
0	25.71 ± 0.144^{aA}	25.71 ± 0.144^{aB}	25.71 ± 0.144^{aC}	25.71 ± 0.144^{aD}	25.71 ± 0.144^{aE}	25.71 ± 0.144^{aF}	25.71 ± 0.144^{aG}	25.71 ± 0.144^{aH}	25.71 ± 0.144^{aI}
15	21.58 ± 0.072^{cA}	17.16 ± 0.044^{cB}	16.11 ± 0.035^{cC}	12.15 ± 0.037^{cD}	11.81 ± 0.038^{cE}	11.55 ± 0.023^{cF}	10.83 ± 0.023^{cG}	8.73 ± 0.031^{cH}	5.56 ± 0.055^{cI}
30	22.85 ± 0.057^{bA}	18.22 ± 0.052^{bB}	17.52 ± 0.062^{bC}	16.93 ± 0.033^{bD}	14.97 ± 0.040^{bE}	13.48 ± 0.061^{bF}	11.89 ± 0.057^{bG}	4.36 ± 0.046^{bH}	3.50 ± 0.040^{bI}
45	18.72 ± 0.061^{dA}	13.94 ± 0.042^{dB}	13.54 ± 0.040^{dC}	12.72 ± 0.029^{dD}	11.98 ± 0.043^{dE}	11.38 ± 0.020^{dF}	10.95 ± 0.008^{dG}	2.67 ± 0.14^{dH}	1.21 ± 0.036^{dI}
60	18.50 ± 0.063^{eA}	13.71 ± 0.058^{eB}	13.51 ± 0.050^{eC}	12.57 ± 0.086^{eD}	11.74 ± 0.086^{eE}	10.88 ± 0.049^{eF}	9.89 ± 0.052^{eG}	0	0
75	17.89 ± 0.069^{fA}	12.96 ± 0.069^{fB}	12.23 ± 0.040^{fC}	11.55 ± 0.046^{fD}	10.38 ± 0.073^{fE}	9.91 ± 0.05^{fF}	8.24 ± 0.056^{fG}	0	0

^{a,b,c,d,e,f} within columns, value with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

^{A,B,C,D,E,F,G,H,I} within rows, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

Effects of Lead on Chlorophyll Content

Pb exposure caused a significant concentration- and time-dependent decline in chl a, chl b and total chl content in *T. taxirameum* (Table 1). Statistical analysis revealed significant main effects of both Pb concentration ($p < 0.05$, $\eta^2 = 0.999–1.000$) and exposure time ($p < 0.05$, $\eta^2 = 0.993–0.999$), with significant interaction effects ($p < 0.05$, $\eta^2 = 0.988–0.998$), emphasizing the joint influence of these variables on pigment degradation. Regression analysis (Fig. 1) indicated a progressive decline in pigment levels, with chl a decreasing by 38.98% at 10 ppm and complete pigment loss occurring at 350–500 ppm after 60 days. Notably, chl b exhibited greater sensitivity to Pb, showing a loss of up to 95.9% loss at 500 ppm within 45 days. These findings are consistent with prior reports of Pb induced chronic phytotoxicity in mosses (Shakya et al., 2008; Dogan et al., 2018).

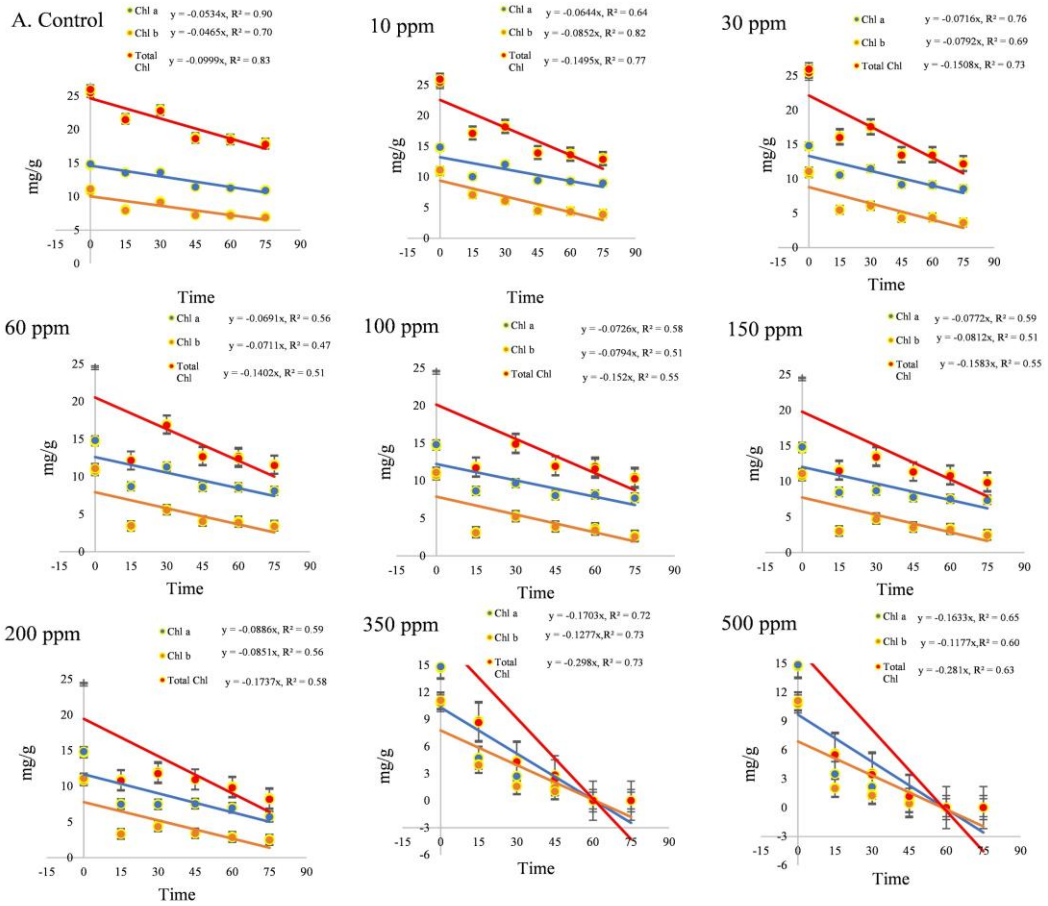

Fig. 1. Time dependent regression of Chlorophyll a Chlorophyll b and Total Chlorophyll content under varying Lead concentrations.

Effects of Cadmium on Chlorophyll Content

Cd exposure produced more acute phytotoxicity than Pb, causing marked declines in chlorophyll content (Table 2). Two-way ANOVA confirmed highly significant effects of both concentration and time ($p < 0.05$, $\eta^2 = 1.000–0.999$) and interact significantly ($p < 0.05$, $\eta^2 = 0.998–0.994$). Regression curves (Fig. 2) demonstrated a marked drop in chl a and chl b at lower concentrations (10–60 ppm), culminating in total pigment loss at concentrations ≥ 200 ppm by 45 days. As with Pb, chl b experienced a faster rate of decline than chl a, highlighting this pigment heightened sensitivity to metal stress (Muradoglu et al., 2015). The pattern of acute toxicity is supported by related studies in mosses and aquatic plants (Huihui et al., 2020; Dogan et al., 2018).

Comparative Effects of Lead and Cadmium on Chlorophyll a, Chlorophyll b, and Total Chlorophyll

The comparative effects of Pb and Cd on chl a, chl b, and total chl content in *T. taxirameum* demonstrate distinct patterns of phytotoxicity, highlighting the differential impact of these metals on photosynthetic pigments. Exposure to Cd resulted in sharp and immediate reduction in chl a, particularly at concentrations of 100 ppm and above, with levels declining by 92.95% by day 75 and complete depletion observed at concentrations from 200 to 500 ppm (Fig. 3A). In contrast, Pb induced a more gradual decline, with significant reductions manifesting only beyond 200 ppm; complete chlorophyll loss was observed at 350–500 ppm by day 60 (Fig. 3B). These results indicate that Cd exerts a much more acute toxic effect on *T. taxirameum* compared to Pb, consistent with

previous reports of greater moss sensitivity to Cd stress than Pb exposure (Dogan et al., 2018; Rau et al., 2007; Phaenark et al., 2022; Huihui et al., 2020). After 75 days of exposure, moss treated with 200 ppm of Pb retained 5.74 mg/g chl a, whereas under Cd stress, a comparable amount of chl a was found only at 30 ppm (6.6 mg/g), demonstrating that *T. taxirameum* tolerates higher Pb concentrations more effectively than Cd (Dogan et al., 2018; Phaenark et al., 2022). Chl b displayed even greater susceptibility, especially to Cd: concentrations dropped sharply to 0.65 mg/g at 200 ppm and 0.11 mg/g at 500 ppm by day 15, with complete depletion occurring above 350 ppm by day 45 (Fig. 3C). In contrast, Pb-exposed moss maintained higher chl b levels with a more gradual decline (3.32 mg/g at 200 ppm and 2.04 mg/g at 500 ppm by day 15) and measurable chl b (2.50 mg/g) at 200 ppm by day 75 (Fig. 3D).

Table 2. Chlorophyll a, Chlorophyll b and Total Chlorophyll content (mg/g) when treated with different concentrations of Cadmium

Chl a content mg/g (Mean ± SEM; n = 3) when treated with different conc. (ppm) of Cadmium									
Time	Control	10 ppm	30 ppm	60 ppm	100 ppm	150 ppm	200 ppm	350 ppm	500 ppm
0	14.75 ± 0.049^{aA}	14.75 ± 0.049^{aB}	14.75 ± 0.049^{aC}	14.75 ± 0.049^{aD}	14.75 ± 0.049^{aE}	14.75 ± 0.049^{aF}	14.75 ± 0.049^{aG}	14.75 ± 0.04^{aH}	14.75 ± 0.049^{aH}
15	13.57 ± 0.015^{cA}	8.95 ± 0.034^{cB}	7.64 ± 0.029^{cC}	6.1 ± 0.032^{cD}	2.4 ± 0.011^{cE}	1.79 ± 0.026^{cF}	1.03 ± 0.021^{cG}	0.57 ± 0.008^{cH}	0.48 ± 0.017^{cH}
30	13.63 ± 0.015^{bA}	10.16 ± 0.027^{bB}	8.08 ± 0.059^{bC}	6.55 ± 0.023^{bD}	3.32 ± 0.085^{bE}	0.96 ± 0.011^{bF}	0.53 ± 0.017^{bG}	0.35 ± 0.014^{bH}	0.38 ± 0.014^{bH}
45	11.43 ± 0.023^{dA}	8.65 ± 0.041^{dB}	8.1 ± 0.026^{dC}	6.46 ± 0.028^{dD}	2.35 ± 0.014^{dE}	0.9 ± 0.012^{dF}	0.6 ± 0.020^{dG}	0	0
60	11.26 ± 0.012^{eA}	8.34 ± 0.020^{eB}	6.91 ± 0.008^{eC}	4.31 ± 0.028^{eD}	1.88 ± 0.015^{eE}	0.81 ± 0.017^{eF}	0.37 ± 0.021^{eG}	0	0
75	10.96 ± 0.029^{fA}	8.09 ± 0.026^{fB}	6.6 ± 0.014^{fC}	4.29 ± 0.014^{fD}	1.04 ± 0.015^{fE}	0.63 ± 0.024^{fF}	0	0	0
Chl b content mg/g (Mean ± SEM; n = 3) when treated with different conc. (ppm) of Cadmium									
Time	Control	10 ppm	30 ppm	60 ppm	100 ppm	150 ppm	200 ppm	350 ppm	500 ppm
0	10.96 ± 0.096^{aA}	10.96 ± 0.096^{aB}	10.96 ± 0.096^{aC}	10.96 ± 0.096^{aC}	10.96 ± 0.096^{aD}	10.96 ± 0.096^{aE}	10.96 ± 0.096^{aF}	10.96 ± 0.096^{aG}	10.96 ± 0.096^{aG}
15	8.01 ± 0.057^{cA}	4.65 ± 0.018^{cB}	4.87 ± 0.035^{cC}	4.53 ± 0.021^{cC}	1.78 ± 0.040^{cD}	1.17 ± 0.036^{cE}	0.65 ± 0.020^{cF}	0.20 ± 0.017^{cG}	0.11 ± 0.012^{cG}
30	9.22 ± 0.044^{bA}	4.86 ± 0.018^{bB}	4.66 ± 0.02^{bC}	4.03 ± 0.014^{bC}	2.79 ± 0.058^{bD}	0.87 ± 0.026^{bE}	0.7 ± 0.035^{bF}	0.17 ± 0.0^{bG}	0.12 ± 0.014^{bG}
45	7.28 ± 0.047^{dA}	3.96 ± 0.055^{dB}	3.50 ± 0.053^{dC}	3.7 ± 0.079^{dC}	1.57 ± 0.04^{dD}	0.59 ± 0.015^{dE}	0.38 ± 0.027^{dF}	0	0
60	7.24 ± 0.055^{eA}	3.47 ± 0.072^{eB}	2.90 ± 0.072^{eC}	3.50 ± 0.062^{eC}	1.41 ± 0.033^{eD}	0.53 ± 0.023^{eE}	0.11 ± 0.015^{eF}	0	0
75	6.92 ± 0.04^{fA}	3.42 ± 0.053^{fB}	2.73 ± 0.028^{fC}	2.93 ± 0.024^{fC}	1.75 ± 0.015^{fD}	0.21 ± 0.008^{fE}	0	0	0
Total Chl content mg/g (Mean ± SEM; n = 3) when treated with different conc. (ppm) of Cadmium									
Time	Control	10 ppm	30 ppm	60 ppm	100 ppm	150 ppm	200 ppm	350 ppm	500 ppm
0	25.71 ± 0.144^{aA}	25.71 ± 0.144^{aB}	25.71 ± 0.144^{aC}	25.71 ± 0.144^{aD}	25.71 ± 0.144^{aE}	25.71 ± 0.144^{aF}	25.71 ± 0.144^{aG}	25.71 ± 0.144^{aH}	25.71 ± 0.144^{aH}
15	21.58 ± 0.072^{cA}	13.60 ± 0.052^{cB}	12.51 ± 0.063^{cC}	10.64 ± 0.049^{cD}	4.18 ± 0.052^{cE}	2.96 ± 0.061^{cF}	1.69 ± 0.041^{cG}	0.78 ± 0.026^{cH}	0.62 ± 0.011^{cH}
30	22.85 ± 0.057^{bA}	15.03 ± 0.040^{bB}	12.73 ± 0.083^{bC}	10.58 ± 0.067^{bD}	6.11 ± 0.13^{bE}	1.83 ± 0.037^{bF}	1.24 ± 0.05^{bG}	0.60 ± 0.034^{bH}	0.50 ± 0.029^{bH}
45	18.72 ± 0.061^{dA}	12.61 ± 0.095^{dB}	11.61 ± 0.079^{dC}	10.16 ± 0.092^{dD}	3.93 ± 0.055^{dE}	1.49 ± 0.026^{dF}	0.99 ± 0.047^{dG}	0	0
60	18.50 ± 0.063^{eA}	11.81 ± 0.092^{eB}	9.82 ± 0.080^{eC}	7.82 ± 0.072^{eD}	3.29 ± 0.049^{eE}	1.34 ± 0.039^{eF}	0.50 ± 0.047^{eG}	0	0
75	17.89 ± 0.069^{fA}	11.51 ± 0.063^{fB}	9.33 ± 0.042^{fC}	7.22 ± 0.046^{fD}	2.79 ± 0.028^{fE}	0.85 ± 0.032^{fF}	0	0	0

^{a,b,c,d,e,f} within columns, value with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

^{A,B,C,D,E,F,G,H,I} within rows, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

This differential pattern illustrates the acute toxicity of Cd compared to the slower, chronic effects of Pb (Dogan et al., 2018; Huihui et al., 2020; Muradoglu et al., 2015). Total chl content mirrored these trends. Cd-treated moss exhibited a rapid decline to 4.18 mg/g at 100 ppm by day 15, compared with 11.81 mg/g in Pb-treated samples (Fig. 3E). By day 45, total chl in Cd-exposed moss had dropped to to 3.93 ± 0.055 mg/g, while Pb-exposed samples retained higher levels (11.98 ± 0.043 mg/g). Complete pigment loss in Cd treatment occurred at concentrations ≥200 ppm by day 60-75, whereas Pb-treated moss retained substantial pigment even at high concentrations after 75 days (8.24 mg/g; Fig. 3F). These findings emphasize the species- and metal-specific nature of chlorophyll degradation induced by heavy metal stress (Michel et al., 2024; Sheng et al., 2022).

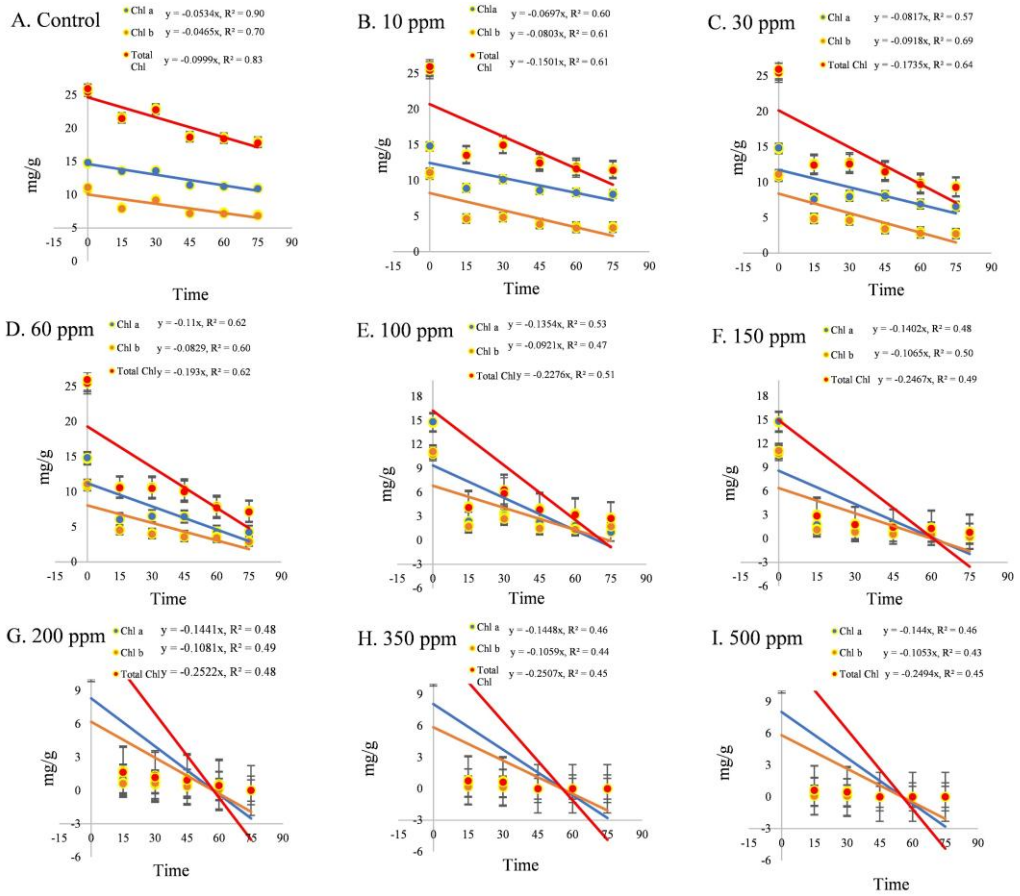

Fig. 2. Time dependent regression of Chlorophyll a Chlorophyll b and Total Chlorophyll content under varying Cadmium concentrations.

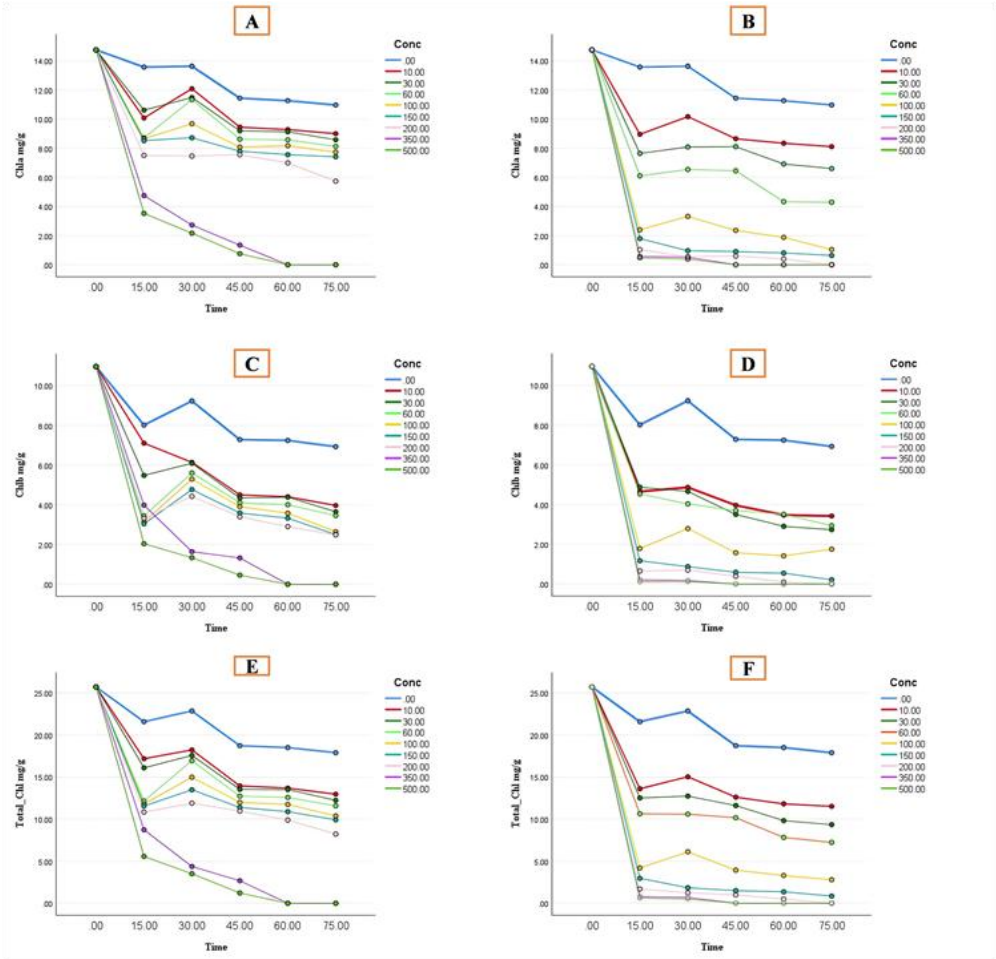

Fig. 3. Comparative Effects of Lead and Cadmium on Chlorophyll a, Chlorophyll b, and Total Chlorophyll content.

The pronounced sensitivity of chl b compared to chl a and total chl under both Pb and Cd stress further highlights the preferential vulnerability of accessory pigments to metal toxicity, which is consistent with prior reports in aquatic and terrestrial plants (Muradoglu et al., 2015; Dogan et al., 2018). The stronger negative impact of Cd on chlorophyll parameters, as compared to Pb, may result from Cd' greater chemical reactivity and its disruption of chlorophyll biosynthesis, potentially by substituting for Mg^{2+} at the core of chlorophyll molecules and increasing oxidative stress (Tanaka and Ito, 2025; Huihui et al., 2020; Shakya et al., 2008).

Statistical Relationships Between Metal Stress and Chlorophyll Dynamics

Pearson Correlation Analysis

Pearson correlation analysis (Fig. 4) revealed significant inverse relationships between metal (Cd, Pb) concentrations, exposure duration, and chlorophyll content. Variables associated with Pb exhibited stronger negative correlations with both metal concentration (chl a : $r = -0.653$; chl b: $r = -0.436$; total chl: $r = -0.568$; $p < 0.01$) and exposure time (chl a: $r = -0.551$; chl b: $r = -0.665$; total chl: $r = -0.611$; $p < 0.01$) compared to those associated with Cd concentration (chl a: $r = -0.487$; chl b: $r = -.599$; total chl: $r = -0.453$; $p < 0.01$) and exposure time (chl a : $r = -0.529$; chl b: $r = -0.599$; total chl: $r = -0.562$; $p < 0.01$). These findings indicate that Pb exerts a more potent and persistent influence on chlorophyll depletion over time compared to Cd. Additionally, strong positive correlations ($r \geq 0.933$ for Pb treatments and $r \geq 0.974$ for Cd treatments) among chl a, chl b, and total chl suggest these pigments decrease concurrently under metal toxicity related to photosynthetic pigment loss (Sheng et al., 2022; Zafar-Ul-Hye et al., 2020).

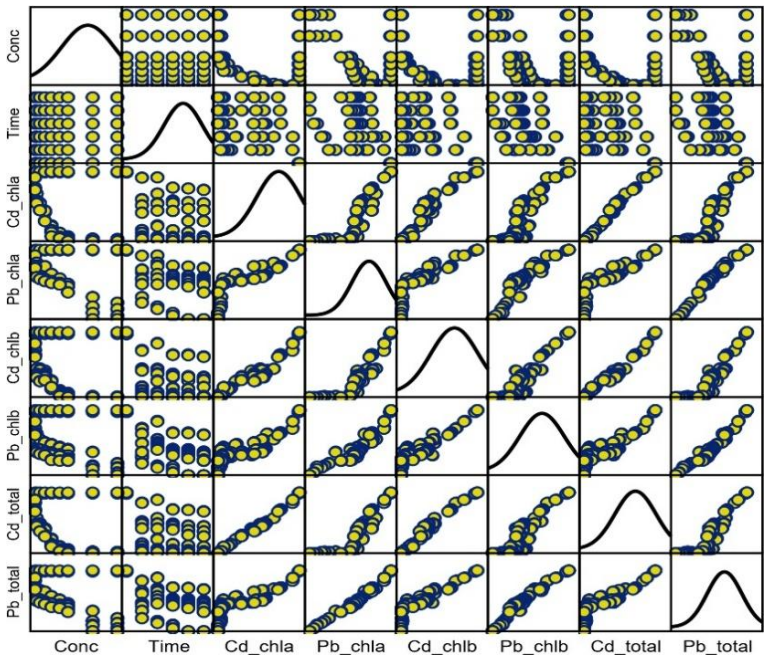

Fig. 4. Pearson Correlation matrix showing relationships between heavy metal concentrations, time, and chlorophyll content in moss treated with Lead and Cadmium.

Principal Component Analysis

Principal Component Analysis (PCA) was performed to identify the dominant factors controlling chlorophyll degradation in *T. taxirameum* under heavy metal stress. For Pb treatment (Fig. 5), the first two principal components (PCs) explained 92.41% of total variance, with PC1 accounting for 72.25% and PC2 for 20.16%. PC1 showed a strong negative loading for Pb concentration (-0.49231), indicating a dose-dependent inhibitory effect on chlorophyll content. Correspondingly, chl a, chl b, and total chl had strong positive loadings on PC1 (0.51655, 0.45919, and 0.51484, respectively), reflecting significant pigment reduction with increasing Pb levels. PC2 was mainly associated with exposure time (loading of 0.96691), highlighting the secondary but important role of prolonged exposure in exacerbating chlorophyll loss. This separation of groups along PC1 emphasizes a threshold-driven chlorophyll degradation pattern in response to Pb, beyond which pigment reduction becomes critical. PCA of Cd-treated samples (Fig. 6) similarly accounted for 92.76% of the variance (PC1, 72.75%; PC2, 20.01%). PC1 exhibited strong positive loadings for chl a (0.515), chl b (0.513), and total chl (0.518), while Cd concentration had a significant negative loading (-0.443), confirming its predominant role in dose-dependent pigment suppression. PC2 was strongly

correlated with exposure duration (0.984), indicating that extended Cd exposure further intensifies chlorophyll degradation. The distinct clustering of high-concentration Cd treatments apart from controls reveals a pronounced threshold effect, consistent with acute Cd phytotoxicity. These findings align with previous PCA-based studies (Shahid et al., 2019), which showed that Cd stress causes greater chlorophyll reduction in older than younger leaves, with older tissues reflecting prolonged toxic exposure. Comparable clustering patterns have been observed among species, where higher pollutant accumulation coincides with greater physiological alterations, whereas low-uptake species display minimal effects on chlorophyll and related traits (De Agostini et al., 2022).

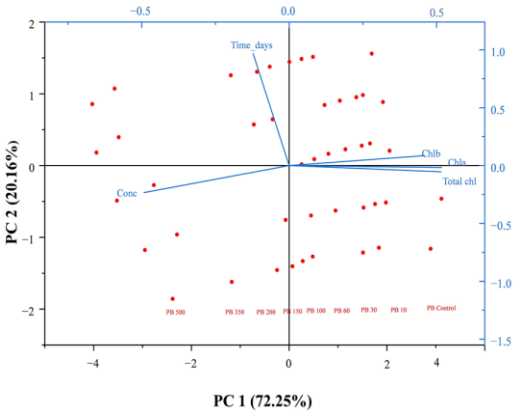

Fig. 5. Biplot represents Principal Component Analysis of the relationship between Lead concentrations, treatment duration and chlorophyll content (Chlorophyll a, Chlorophyll b, and Total Chlorophyll).

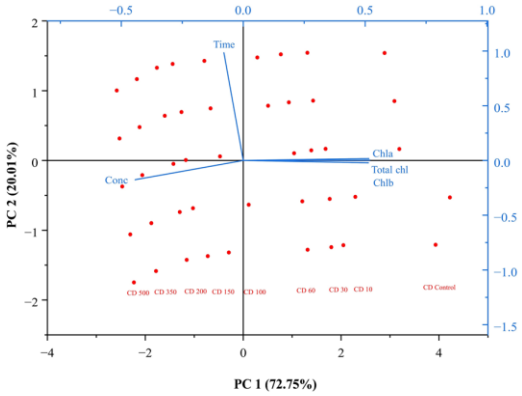

Fig. 6. Biplot represents Principal Component Analysis of the relationship between Cadmium concentrations, treatment duration and chlorophyll content (Chlorophyll a, Chlorophyll b, and Total Chlorophyll).

Metal Uptake

Bioaccumulation experiments revealed a clear, concentration- and time-dependent increase in Cd and Pb accumulated within the tissues of *T. taxirameum* across all exposure treatments (Table 3). Metal concentrations in treated moss samples were statistically significant ($p < 0.05$), with Pb exhibiting a higher overall accumulation than Cd, as evidenced by maximum values of 701.81 ppm for Pb and 581.58 ppm for Cd at 500 ppm exposure after 75 days. These findings corroborate previous studies indicating a high retention capacity for certain heavy metals in moss species, including *Taxiphyllum*, supporting their effectiveness as bio-monitors of environmental pollution (Macedo-Miranda et al., 2024; Ares et al., 2018; Chen et al., 2010). Net enrichment—defined as the difference between metal concentration at the end and start of exposure—increased with both higher external metal concentration and prolonged duration, reflecting cumulative, passive uptake mechanisms (Ares et al., 2012; García-Seoane et al., 2023).

This trend was observed for both metals: as dosage increased from 10 to 500 ppm, net enrichment for Cd ranged from 3.012 to 581.56 ppm, while Pb ranged from 6.82 to 700.52 ppm. Comparative studies with *Barbula consanguinea* and *Hyophila involuta* showed similar high adsorption rates for Pb (93% and 94% respectively), and slightly lower values for Cd (87% and 94%) (Phaenark et al., 2024). This enhanced accumulation reflects the physiological characteristics of mosses, particularly their lack of protective cuticle and abundance of cation-exchange sites in the cell wall, which favours passive metal ion adsorption. (Chaudhuri & Roy, 2024; Macedo-Miranda et al., 2016). The high cation-exchange capacity of *Taxiphyllum* measured at approximately 0.65 mmol/g for Cd further enhances its capacity to outcompete other mosses such as *Sphagnum* for metal binding (Mahapatra et al., 2019). Field studies confirm that *T. taxirameum*, when exposed using moss bags, reliably reflects spatial and temporal variability in atmospheric deposition across both urban and industrial environments (Mao et al., 2022).

Relationships Between Heavy Metals concentrations, Exposure Time, Metal Uptake and Chlorophyll Levels

Pearson Correlation Analysis

Pearson correlation analysis was conducted to elucidate the relationships between heavy metal concentrations (Cd and Pb), exposure duration, metal uptake, and chlorophyll content (chl a, chl b, and total chl) in *T. taxirameum*. The analysis yielded strong, statistically significant correlations ($p <$

0.05), illuminating physiological effects of metals on moss pigment dynamics. The correlation matrix (Fig. 7) showed a strong positive correlation between applied Cd and Pb concentrations and their respective uptake (r = 0.918), suggesting a concentration-dependent adsorption pattern (Phaenark et al., 2024; Tőzsér et al., 2023; Muradoglu et al., 2015). Significant negative correlations were observed between metal uptake and chlorophyll content, indicating that increased bioconcentration inhibits photosynthetic pigment levels. Specifically, Cd uptake negatively correlated with chl *a* (r = -0.617), chl *b* (r = -0.605), and total chl (r = -0.618), while Pb uptake exhibited even stronger negative correlations with chl *a* (r = -0.904), chl *b* (r = -0.813), and total chl (r = -0.886). These findings are consistent with published studies on *Taxiphyllum barbieri* and *Vesicularia montagnei*, which report strong negative correlations between heavy metal accumulation and chlorophyll levels (Jose and Joseph, 2022). Similar trends have been observed for Zn and Cd hyperaccumulation in *Vesicularia montagnei*, altering chlorophyll (a:b) ratios and reducing total content in a concentration-dependent manner (Taeprayoon et al., 2024).

Table 3. Metal bio concentration (ppm ± SEM), and net enrichments of moss bioassays.

Sample	Metal concentration (ppm)			Net enrichment	
Cd	0day	45day	75day	45 day	75day
Control	0.019 ± 0.001^{aA}	0.027 ± 0.001^{aB}	0.03 ± 0.001^{aC}	0.007 ± 0.001	0.011 ± 0.001
10 ppm	0.019 ± 0.001^{aA}	3.03 ± 0.009^{aB}	5.18 ± 0.04^{aC}	3.012 ± 0.009	5.16 ± 0.048
30 ppm	0.019 ± 0.001^{bA}	8.17 ± 0.035^{bB}	14.57 ± 0.13^{bC}	8.155 ± 0.03	14.55 ± 0.13
60 ppm	0.019 ± 0.001^{cA}	16.40 ± 0.11^{cB}	29.12 ± 0.25^{cC}	16.38 ± 0.11	29.1 ± 0.25
100 ppm	0.019 ± 0.001^{dA}	30.56 ± 0.21^{dB}	57.23 ± 0.11^{dC}	30.54 ± 0.21	57.21 ± 0.11
150 ppm	0.019 ± 0.001^{eA}	58.83 ± 0.56^{eB}	109.36 ± 1.03^{eC}	58.81 ± 0.56	109.34 ± 1.03
200 ppm	0.019 ± 0.001^{fA}	69.87 ± 0.82^{fB}	133.75 ± 1.06^{fC}	69.85 ± 0.82	133.73 ± 1.06
50 ppm	0.019 ± 0.001^{gA}	80.89 ± 1.78^{gB}	349.63 ± 3.29^{gC}	180.87 ± 1.78	349.61 ± 3.29
500 ppm	0.019 ± 0.001^{hA}	310.16 ± 2.17^{hB}	581.58 ± 5.46^{hC}	310.14 ± 2.17	581.56 ± 5.46
Pb	0day	45day	75day	45day	75day
Control	1.29 ± 0.033^{aA}	1.36 ± 0.010^{aB}	1.37 ± 0.012^{aC}	0.067 ± 0.010	0.076 ± 0.012
10ppm	1.29 ± 0.033^{bA}	8.12 ± 0.05^{bB}	15.19 ± 0.012^{bC}	6.82 ± 0.05	13.89 ± 0.10
30ppm	1.29 ± 0.033^{cA}	14.10 ± 0.06^{cB}	32.51 ± 0.10^{cC}	12.81 ± 0.06	31.22 ± 0.15
60ppm	1.29 ± 0.033^{dA}	30.82 ± 0.22^{dB}	66.77 ± 0.26^{dC}	29.53 ± 0.22	65.48 ± 0.26
100ppm	1.29 ± 0.033^{eA}	55.24 ± 0.35^{eB}	119.57 ± 0.66^{eC}	53.95 ± 0.35	118.28 ± 0.66
150ppm	1.29 ± 0.033^{fA}	79.39 ± 0.55^{fB}	154.54 ± 0.93^{fC}	78.10 ± 0.55	153.25 ± 0.93
200ppm	1.29 ± 0.033^{gA}	112.89 ± 1.23^{gB}	212.51 ± 1.49^{gC}	111.60 ± 1.23	211.22 ± 1.49
350ppm	1.29 ± 0.033^{hA}	244.60 ± 3.01^{hB}	450.55 ± 2.54^{hC}	243.30 ± 3.01	449.26 ± 2.54
500ppm	1.29 ± 0.033^{iA}	361.16 ± 4.53^{iB}	701.81 ± 5.40^{iC}	359.87 ± 4.53	700.52 ± 5.40

^{a,b,c,d,e,f,g,h,i} within columns, value with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

^{A,B,C,} within rows, values with different superscript letters are significantly different ($p < 0.05$) according to Tukey's test.

PCA analysis

PCA was carried out to identify the key factors influencing chlorophyll content and metal uptake in *T. taxirameum* under Cd and Pb exposure. The first two principal components (PC1 and PC2) accounted for 86.94% of the total variance, with PC1 explaining 69.36% and PC2 contributing 17.58% (Fig. 8). PC1 exhibited strong negative loadings for both metal concentration (-0.4428) and metal uptake (-0.4118), indicating that higher concentrations and accumulation of metals in moss tissues were closely associated with reduced chlorophyll content. Conversely, chl a (0.4661), chl b (0.4322), and total chl (0.4680) loaded positively on PC1, affirming the tight coupling between pigment decline and metal exposure. These results accord with established mechanisms whereby Cd and Pb can substitute for magnesium in the chlorophyll molecule, disrupting biosynthesis and

accelerating degradation, ultimately impairing photosynthetic capacity (Shakya et al., 2008; Tanaka and Ito, 2024). PC2 was strongly associated with exposure duration (loading 0.9421), reflecting the cumulative impact of metal exposure and consequent progressive pigment decline over time. The positive correlation between exposure time and metal accumulation reinforces the notion that prolonged exposure exacerbates metal bioaccumulation, which intensifies chlorophyll loss, a phenomenon substantiated by multiple studies (swislowski et al., 2021). Notably, chl b showed a distinct negative loading on PC2 (-0.2004), suggesting higher vulnerability to temporal degradation relative to chl a, underscoring differential pigment sensitivities under chronic metals stress.

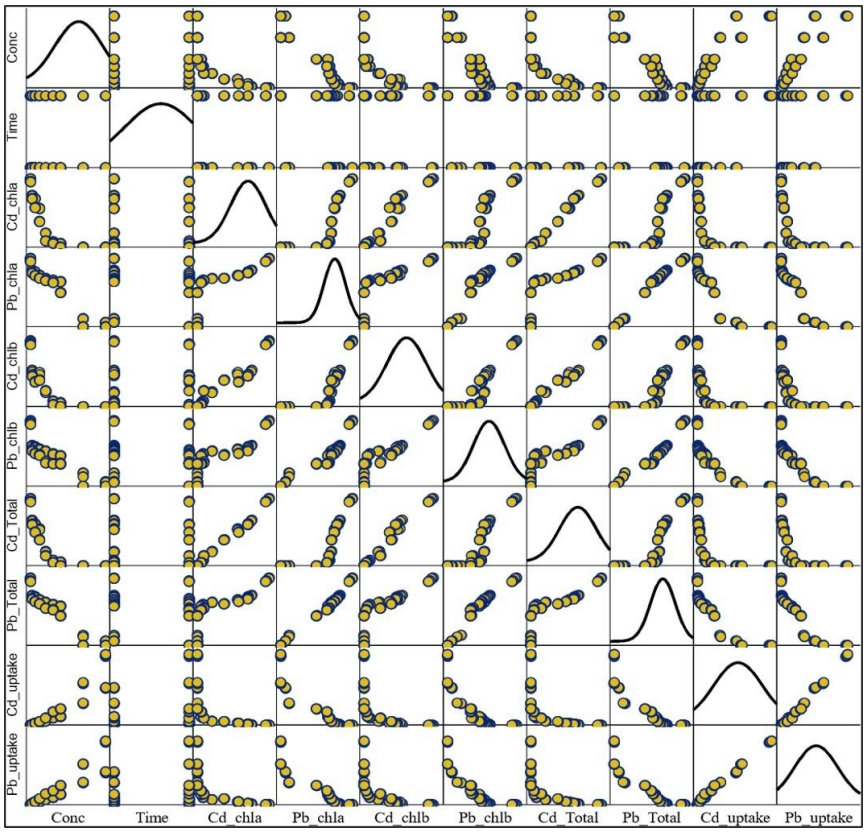

Fig. 7. Pearson correlation matrix showing the relationship between heavy metals (Lead and Cadmium) concentrations, exposure time, their uptake, and chlorophyll content (Chlorophyll a, Chlorophyll b, and Total Chlorophyll) in moss after 45 and 75 days of treatment.

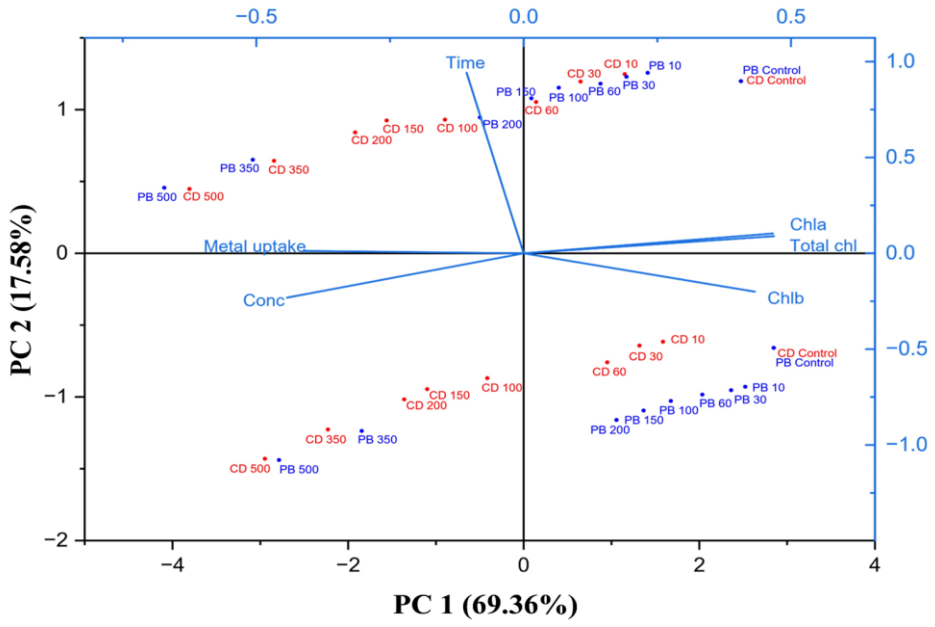

Fig. 8. Biplot represents Principal Component Analysis of the relationship between Cadmium and Lead concentrations, metal uptake, chlorophyll content (Chlorophyll a, Chlorophyll b, and Total Chlorophyll), and treatment duration.

Conclusion

This investigation provides clear evidence of the distinct phytotoxic impacts of Pb and Cd on chlorophyll dynamics in *T. taxirameum*, thereby offering robust insight into heavy metal toxicity mechanisms. Both metals significantly accelerate chlorophyll degradation, driven primarily by metal concentration and exposure duration, as confirmed by robust statistical analyses (two-way ANOVA, regression, PCA). Cadmium demonstrates acute toxicity with rapid chlorophyll loss, achieving complete degradation at concentrations ≥200 ppm by 45 days, whereas lead causes a slower, chronic decline, reaching total chlorophyll loss at 350–500 ppm by 60 days. Principal Component Analysis confirmed metal concentration as the dominant factor influencing chlorophyll degradation and metal uptake, with exposure time as a secondary but significant contributor. The strong negative correlations between metal accumulation and chlorophyll content underscore the physiological stress imposed by metal uptake. Although cadmium is overall more toxic, lead uptake exhibits a more pronounced and linear association with pigment depletion, possibly due to differences in detoxification strategies within the physiology of moss. These findings not only advance understanding of moss-based biomonitoring but also underscore the environmental significance of *T. taxirameum* as a sentinel species for heavy metal contamination. Future research may further clarify the species-specific mechanisms of heavy metal tolerance and extend biomonitoring applications across varied ecosystems.

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UT, AR, MS, DM and NT conceived the concept, wrote and approved the manuscript.

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Competing interest
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