Plant Archives Vol. 25, Supplement 1, 2025 pp. 1138-1150 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.154

ASSESSMENT OF CADMIUM AND COBALT INDUCED RESPONSES IN *THELYPTERIS DENTATA* (FORSSK.) E.P. ST. JOHN: A POTENTIAL HEAVY METAL ACCUMULATOR

Prashan Kumar Chhetri[1,2*], Lagnojita Pal[2], Cyaria Gurung[3] and Jnan Bikash Bhandari[2]
[1]Department of Botany, Gorubathan Government College, Gorubathan, Kalimpong 735231, West Bengal, India.
[2]Pteridology and Palaeobotany Laboratory, Department of Botany, University of North Bengal, Raja Rammohunpur, Darjeeling 734013, West Bengal, India.
[3]Department of Botany, Siliguri College, Siliguri 734001, Darjeeling, West Bengal, India.
*Corresponding author E-mail: prashannbu@gmail.com
(Date of Receiving : 08-2024; Date of Acceptance : 22-10-2024)

ABSTRACT

This hydroponic study investigated the effects of cadmium (Cd) and cobalt (Co) toxicity on *Thelypteris dentata* (Synonym. *Christella dentata* Forssk., Family Thelypteridaceae), a fern species with potential heavy metal accumulation capabilities. Morphological, biochemical, and antioxidative enzyme responses were evaluated at varying concentrations (0, 100, 600, and 1200 ppm) over 5 and 15 days time interval and changes were monitored after 5 and 15 days of exposure time. The concentrations ranging from 100-1200 ppm were chosen considering their environmental relevance and their toxicity thresholds from previous research studies. Short-term exposure (5 days) revealed increased antioxidative enzyme activities (APX, POD, SOD) in Cd and Co-treated plants, except for APX at 1200 ppm concentration of Cd. However, prolonged exposure (15 days) caused significant declines in growth rate, biomass, and relative water content, accompanied by interveinal chlorosis, wilting, and necrosis in Cd-treated plants, leading to the complete death of plants. Cd and Co accumulation increased with increase in concentration in both fronds and roots. The study indicates that *T. dentata* cannot tolerate Cd concentrations ≥100 ppm and Co concentrations >600 ppm. Bioconcentration and translocation factors <1 suggest an exclusion mechanism, with high Cd and Co accumulation in roots. Therefore, it can be concluded that *Thelypteris dentata* exhibits greater tolerance to Co than Cd under long-term heavy metal stress which makes it a potential indicator of Co-contaminated soil.

Keywords **:** Antioxidative enzymes, cadmium, cobalt, relative growth rate, *Thelypteris dentata*

Introduction

Environmental pollution is currently the major global concern for the present population. Heavy metal occupies the second position in terms of danger to human population and may cause many health issues even in very low concentrations (Sytar *et al.*, 2019; Emashogue *et al.,* 2020). The major causes of soil contamination with heavy metals include rapid industrialization, urbanization, and mining activities (Pandey *et al.*, 2022). Heavy metal pollution has become a significant obstacle to higher plant yield and growth in the recent times. Heavy metals commonly found at contaminated sites are arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) (Farooq *et al.*, 2022; Salam *et al.*, 2024). Some of these metals are necessary for the development and growth of living organisms. However, when their concentration surpasses specific thresholds,many heavy metals are extremely hazardous and often could negatively impact in the cellular metabolism by producing reactive oxygen species (ROS) in plants as well as animals (Riyazuddin *et al.*, 2022). Among all the heavy metals, Cadmium (Cd) is a very toxic non-essential heavy metals, which

frequently has a numerous negative impact on plant growth and causes necrosis in some plant species at higher concentrations (Haider *et al.*, 2021; Waheed *et al.*, 2022). Industrial processes, mining, chemical fertilizer, municipal wastes and sewage sludges are the primary man-made sources of Cd in the environment (Bigalke *et al.*, 2017; Al-Khayri *et al*., 2023) and has increased the Cd content in soil from few ppm to even thousands of ppm (Soni *et al.*, 2024). The threshold level of Cd in hyperaccumulator plant species is 100 mg kg^{-1} dry weight (Liu *et al*., 2019).

On the other hand, Cobalt (Co) is an essential heavy metal which is required in trace amount in the growth medium for unaltered growth of many plants (Mahey *et al.*, 2020). However, higher concentrations of cobalt are hazardous and can disrupt plant physiological and biochemical activities (Hu *et al*., 2021). The threshold level of Co in hyperaccumulator plant species is 1000 mg kg^{-1} dry weight (Kikis *et al*., 2024).

Ferns are first vascular land plants, reportedly known for their heavy metal accumulation capacity and stress tolerance activities (Muhamad *et al.*, 2019). Recently, Chinese brake fern (*Pteris vittata* Linn.) and other species of ferns were reported to be the accumulator of arsenic, lead, nickel and cadmium (Yang and He *et al*., 2016; Gupta *et al*., 2022). A previous study (Kumari *et al*., 2013) also showed the feasibility of fern *T. dentata* as a prospective species with heavy metals accumulation which can be employed for the phytoremediation of coal fly ash landfills. However, no researches have been conducted to assess the detailed changes of high cadmium and cobalt stress induced morphological and biochemical changes with antioxidative enzyme activities and its defense mechanism against heavy metal stress in *T. dentata* at different exposure periods under hydroponic systems.

In this study, we investigated the tolerance and bioaccumulation capacity of *T. dentata* when exposed to high concentrations of cadmium (Cd) and cobalt (Co) under hydroponic conditions. We analyzed the effects of separate exposure to each metal on the fern's morphology, biochemistry, and antioxidative enzyme activity over two time periods: 5 days (short-term exposure) and 15 days (long-term exposure) and recorded the data for further analysis.

Materials and Methods

Plant specimen and treatment

Adult healthy ferns with similar leaf and rhizome diameters were initially acclimatized in a 0.2 % Hoagland solution for 14 days under hydroponic system (Hoagland and Arnon, 1950; Bora *et al.,* 2020), with continuous supply of air by an aquarium pump. The pH of the Hoagland solution was maintained at 6.0. After acclimatization, equal number of plants were transferred into hydroponic system supplied with various levels of Cadmium (0, 100, 600 and 1200 ppm) and Cobalt (0, 100, 600 and 1200 ppm) and maintained in green house. Some hyperaccumulator plant species can tolerate and accumulate Cd (>200 mg Cd kg^{-1}) and Co (4000-10000 mg Co kg^{-1}) from contaminated soil (Subašić *et al.,* 2022; Fadzil *et al*., 2024). The pH (6.0±0.5) was maintained of treatment solutions during the entire experiment period using dilute HCl or NaOH. Three independent replicates (n=3) were prepared and maintained in fern house for 5 and 15 days for each heavy metal concentrations. The experiment was carried out in the fern house of the Pteridology and Palaeobotany laboratory, University of North Bengal, West Bengal, where the temperature was maintained between 27 and 32^{o}C and the intermediate photoperiod was 16 hours light/8 hours dark with 80% relative humidity. All the morphological, biochemical and antioxidative enzyme parameters of Cd and Co treated plants were evaluated after 5 and 15 days of exposure period. After 5 and 15 days of exposure period, *T. dentata* plants were harvested and some of the experimental plant replicas were frozen in liquid nitrogen and preserved at - 80^{o}C for biochemical analysis, and others were oven dried for 48 hours at 80^{o}C to evaluate the heavy metal content (Cd and Co) in fronds and roots separately.

Morphological parameters

The methodology and equation provided by (Jafarirad *et al*., 2019) were used to calculate the relative growth rate (RGR). To determine this parameter, at first fresh plant specimens were weighed (W_o) before treating with heavy metal and the weights were recorded. After the appropriate exposure time of 5 and 15 days, the same plants was weighed again (W_t) and weights were recorded. Then RGR was determined by the formula-

$$RGR = (\ln W_o - \ln W_t)/t$$

Plants were divided into fronds and roots for determining plant biomass and relative water contents. Fresh fronds and roots samples were collected and properly washed, and fresh weight (FW) was measured immediately. To calculate the dry biomass (DW), the plant materials were dried in a hot air oven at 80^{o}C for 48 hours. The relative water content (RWC) was calculated by using the formula (Chen *et al.*, 2009; Wiszniewska *et al*., 2019):

$$RWC\ (\%) = (FW-DW)/\ FW \times 100$$

To compute the tolerance index, the ratio of heavy metal treated plant biomass (dry weight) to control plant biomass (dry weight) was determined using the protocol described by (Baker *et al.*, 1994; Wiszniewska *et al.*, 2019).

Pigment analysis

To determine different photosynthetic pigment content, 100% methanol was used to extract photosynthetic pigments from fronds. Chlorophyll and carotenoid content were estimated at 665.2 nm, 652.4 nm, and 470 nm, respectively in UV-VIS spectrophotometer (Lichtenthaler, 1987) and expressed in mg/gm FWT. The following formulas were used to compute pigment concentrations:

Chlorophyll a = $16.72 \times A_{665.2} - 9.16 \times A_{653.4}$ ($\mu g\ mL^{-1}$)

Chlorophyll b = $34.09 \times A_{652.4} - 15.28 \times A_{665.2}$ ($\mu g\ mL^{-1}$)

Total chlorophyll = Chlorophyll (a+b)

Carotenoids = $(1000 \times A_{470} - 1.63 \times \text{Chl a} - 104.96 \times \text{Chl b}) / 221$ ($\mu g\ mL^{-1}$)

where, A is absorbance of the frond extract at wavelengths (665.2, 652.4 and 470 nm).

Extraction and Estimation of osmolytes:

The protocol proposed by (Bates *et al.*, 1973) was applied to estimate the amount of proline concentration with minor changes. First, 5 ml of 3% sulfosalicylic acid solution was mixed with 0.2 g of fronds from control and treated plants and homogenized using a mortar and pestle. The homogenate was centrifuged for 10 minutes at 5000 rpm at 4°C. The supernatant was treated with 1% ninhydrin before partitioning against toluene, and the absorbance of the coloured phase was measured at 520 nm. Proline content was estimated using an L-proline standard curve and expressed in mg / g proline.

For the extraction of total sugar and reducing sugar, the conventional method of (Harborne, 1998) was used. The plant samples (fronds) were extracted with 95% ethanol, andtotal sugar was calculated using Anthrone's reagent (Plummer, 1978). The concentration of reducing sugar was calculated using the Nelson-Somogyi method (Marais *et al*., 1966) and a D-glucose standard curve.

Phenol content

The extraction of phenol was done in 80% ethanol according to the protocol given by (Mahadevan and Sridhar, 1982). Total phenol content was analysed using 20% Na_2CO_3 and Folin-ciocalteu reagent (Bray and Thrope, 1954). The total phenol content was determined using a catechol standard curve.

Antioxidative enzyme activities

Under ice-cold conditions, frond samples (0.2 gm) from each treated and control plants were extracted separately with 2 ml of 50 mM sodium phosphate buffer (pH-6.8) for catalase (CAT) and peroxidase (POD) activity. The ascorbate peroxidase (APX) activity was determined using sodium phosphate buffer (0.05M, pH 7.2) containing 1mM ascorbate and potassium phosphate buffer (100 mM, pH 7.6) was used for superoxide dismutase (SOD) activity. The homogenates were centrifuged at 4°C for 15 minutes at 10,000 rpm. The enzyme activity of the CAT, POD, SOD and APX enzymes were measured in the supernatant. In each sample, the soluble protein content was calculated using Lowry's technique (Lowry *et al.,* 1951) and Bovine serum albumin was used as a standard.

The breakdown of H_2O_2 was measured to assess CAT activity using the methodology by (Maehly and Chance, 1954). In a UV-VIS spectrophotometer, the absorbance decreased at 240 nm from 0 to 3 min. The CAT activity was represented as U g^{-1} protein.

POD activity was measured at 460 nm by monitoring the oxidation of o-dianisidine in the presence of H_2O_2 following the approach by (Chakraborty *et al*., 1993). In a UV-VIS spectrophotometer, the change in absorbance was measured every 30 seconds for up to 3 minutes and expressed as U g^{-1} protein.

The approach of (Asada and Takahashi, 1987) was used to measure APX activity. A UV-VIS spectrophotometer was used to measure ascorbate oxidation as a reduction in absorbance at 290 nm for 3 minutes. The enzyme activity was represented by U g^{-1} protein.

The ability of SOD to prevent nitroblue tetrazolium (NBT) photochemical reduction was assessed using the method of (Dhindsa *et al*., 1981), with some minor modifications. A unit of enzyme activity (EU) was indicated as a 50 % drop in NBT relative to the control set that did not contain any enzyme extract.

Cd and Co accumulation

After 15 days of treatment, *T. dentata* plants were separated into fronds and roots. The plant components were oven dried at 80°C before being pounded into powder with a mortar and pestle. The powdered plant material (1 g) was acid digested with HCl and HNO_3 in a 2:1 ratio. The acid mixture was allowed to evaporate on a hot plate before being mixed with distilled water and filtered. The filtrate was diluted to a

final amount of 50 ml before being analyzed for elemental (Cd and Co) content using Thermo Fischer iCAP 7600, inductively coupled plasma-optical emission spectrometry (ICP-OES). Bioconcentration factor (BCF) and Translocation factor (TF) values were calculated according to the formulas given by (Obinaa and Ebere, 2019; Pietrini *et al*., 2020):

BCF_{fronds}=Metal concentration in fronds (ppm)/ Metal concentration in hydroponic solution (ppm)

BCF_{roots}= Metal concentration in roots (ppm)/ Metal concentration in hydroponic solution (ppm)

TF= Metal concentration in fronds (ppm)/ Metal concentration in roots (ppm)

Statistical analysis

All the observations were recorded with three replications and the statistical analysis was done using one-way ANOVA analysis, followed by post hoc analysis using Duncan's test at $p \leq 0.05$ using IBM SPSS statistics software version 22. The data were represented as mean value ± SE.

Results

Effects of Cd and Co stress in plant growth.

After 5 and 15 days, the RGR of *T. dentata* treated plants at varied concentrations of Cd and Co was evaluated in comparison to control sets (Table 1 & 2). The effect of increasing concentrations of high Cd and Co on *T. dentata* was apparent from their morphological variations. In this context, a significant morphological variation was observed such as interveinal chlorosis, browning of roots and necrotic lesions on the fronds of this plant with increasing high Cd and Cd concentrations as well as exposure duration. After 5 days (short term duration), there was reduction in RGR with increasing Cd concentrations as compared to control set. However, in case of high Co treated plants, there was no significant change in RGR, but in lower concentration (100 ppm) there was an increase of 75% in RGR as compared to control set. After 15 days (long term duration), the RGR of Cd treated plants reduced significantly with respect to control plants. Similarly, all cadmium treated plants after 15 days showed interveinal chlorosis, browning of roots, and necrosis of fronds and ultimately could not survive (Fig. 1 A). Whereas, the reduction in RGR was less significant in Co treated plants (i.e. 100 and 600 ppm), but at highest concentration (i.e. 1200 ppm), there was a significant reduction in RGR compared to that of the control set after 15 days (Fig. 1 B). The effect of Cd and Co on plant biomass, relative water content and tolerance index after 5 and 15 days exposure duration is shown in (Table 1 & 2) respectively. After 5 and 15 days, a noteworthy significant decrease in plant biomass and RWC of *T. dentata* was found with increasing concentration of Cd and Co treatment. After 15 days of exposure to increasing concentrations of Cd and Co, our results showed a significant decline in relative water content (RWC) compared to control groups ($F_{6, 14} = 38.423$, $p = 0.0001$, ANOVA). Notably, the tolerance index decreased substantially, particularly in response to 1200 ppm Cd treatment, which exhibited the highest sensitivity with a tolerance index of 50.5%.

Effects of Cd and Co stress in photosynthetic pigments

After 15 days, all Cd treated plants did not survive; therefore, the pigment composition was not determined. On the contrary, Co treated plants survived at 100 and 600 ppm concentration, except 1200 ppm. Hence, pigment composition of only Cd 100 and Cd 600 ppm treated plants were determined. The result of pigments content is shown in (Table 3 & 4). The chlorophyll a, chlorophyll-b and total chlorophyll content in *T. dentata* increased in the lower Co and Cd concentrations, after 5 days of exposure duration. However, at highest Cd and Co treatment level (1200 ppm) the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content decreased by 23, 28, 25 and 5% respectively with respect to control plants after 5 days of exposure period. On the other hand, in highest Co treatment levels (1200 ppm), chlorophyll b and total chlorophyll content was observed to be 52 and 9% respectively as contrast to control after 5 days. The impact of Cd and Co treatment was noticed in chlorophyll b content. Increasing levels of Co (≥100 ppm) induced increase in the concentration of carotenoids pigments after 5 days of treatment. Since, after 15 days of Cd treatment the plants showed necrosis and browning of leaves and ultimately did not survive. Hence, the pigments composition could not be determined. In case of Co treated plants, after 15 days only two sets of Co treatment levels (100 and 600 ppm) survived. After 15 days of Co treatment (600 ppm), both chlorophyll b and carotenoids decreased by 48 and 41% respectively. In the same Co treatment (600 ppm) chlorophyll a and total chlorophyll content increased by 91 and 1.22% respectively with respect to control after 15 days.

Effect of Cd and Co stress in Biochemical parameters

After 15 days, all Cd treated plants did not survive; therefore, all biochemical parameters were not determined for Cd induced plants. On the other hand, Co treated plants survived at 100 and 600 ppm

concentration, except 1200 ppm. Hence, all biochemical parameters of only Cd 100 and Cd 600 ppm treated plants were determined. The changes in proline content, reducing sugar, total soluble sugar and phenol content of *T. dentata* fronds at different high concentrations of Cd and Co treatment after 5 and 15 days exposure period is shown in (Table 5 & 6).

The proline content increased significantly in both Cd and Co treatments with respect to the control plants with increasing concentration of these metals after 5 days of exposure period ($F_{6,14}=80.785$, $p=0.0001$, ANOVA). During short term exposure (5 days), in Cd and Co treated plants, the proline content ranged between 71% to 228% and 179% to 528% respectively. In Co treated plants, the increase in proline content ranged from 107% to 122% after 15 days. The phenol content also increased significantly ($F_{6, 14}=53.674$, $p=0.0001$, ANOVA) with increase in Cd and Co concentration after 5 days. After 5 days exposure of 600 ppm Cd, plants showed accumulation of reducing sugar which was higher by 133% as compared to control plants. In case of Cd and Co treated plants, the total soluble sugar increased with respect to control plants after 5 days. During long term exposure (15 days), total soluble sugar decreased with increase in Co concentrations (>100ppm).

The result of antioxidative enzyme activity of Cd and Co treated plants after 5 and 15 days of exposure period is shown in (Table 7 & 8). In case of Cd treated plants, the CAT, POD and SOD activity increased with increase in concentration of Cd after 5 days. After 5 days, in case of Co treated plants, POD and SOD activity increased with increased Co concentrations.

The result obtained showed that CAT, POD and SOD activity increased with the increased concentration of Co with respect to control after 15 days. On the other hand, in Co treated plants, there was no such significant change in APX activity after 15 days.

Cd and Co content in fronds and roots

After 15 days, the cadmium (Cd) and cobalt (Co) content in *T. dentata,* fronds and roots were analyzed (Fig. 2 C & D). Results showed a significant increase in Cd and Co accumulation with increasing concentrations (≥100 ppm) in the hydroponic solution, compared to controls. Notably, roots accumulated higher amounts of Cd and Co than fronds.

The bioconcentration factor (BCF) and translocation factor (TF) of Cd and Co are presented in (Fig. 3. E, F & G). At Cd concentrations ≥100 ppm, BCF_{roots} were 0.92, 0.13, and 0.08. However, BCF_{roots} approached 1 at 100 ppm Cd, but plants didn't survive longer exposure times or higher concentrations (≥100 ppm). In contrast, Co-treated plants showed BCF_{roots} of 0.84, 0.22, and 0.18 at increasing concentrations. At 100 ppm Co, BCF_{roots} was near 1, and plants survived.

The translocation factor (TF) for both Cd and Co was <1 after 15 days, indicating Cd and Co accumulation in the root system. Interestingly, Co's TF value was higher than Cd's, suggesting Co phytostabilization near its vicinity, as evidenced by BCF_{roots} approaching 1 at 100 ppm Co.

Discussion

Our experimental observations and results in hydroponic system indicate that growth rate of *T. dentata* decrease significantly when treated with high concentrations of Cd (≥100ppm) above their threshold level of 100 mg/kg DW in shoots of hyperaccumulating plant species. This decrease was more significant for longer exposure time of 15 days which the plants could not tolerate and eventually died due to high toxicity. This decrease in growth rate was associated with reduction in plant biomass with respect to increase in Cd concentration and longer exposure time (Table 2). Cd was found to alter the structural and functional properties of the photosynthetic machinery, resulting in decreased or inhibited photosynthesis and impeding normal plant growth (Parmar *et al.,* 2013). In our study, short term exposure of Cd and Co had no drastic changes in growth rate and plant biomass of *T. dentata* and they could withstand upto 1200 ppm concentration s (Table 1). However, after 15 days of exposure time, Cd treated plants exhibited a sharp decline in tolerance index from 100 to 50.5 % in concentration dependent manner and correlated to negative RGR. In the present study, longer duration of high Cd treatment (15 days) attributed to chlorosis, necrotic lesion and wilting of fronds and ultimately senescence in *T. dentata*. The results of our study coincide with the results for *Salvinia auriculata* - an aquatic fern, treated with cadmium for 10 days (Wolff *et al*., 2012). On the other hand, *T. dentata* showed little chlorosis and browning of fronds at 600 ppm Co treatment, but at highest concentration of (1200 ppm) it could not survive and showed negative RGR.

In this context, excess Co had a negative impact on growth and metabolism of plants, causing leaf necrosis and interveinal chlorosis, as well as suppression of mitosis and chromosomal damage (Wendling *et al.,* 2009; Mahey *et al.,* 2020). This indicates that heavy metal stress or any environmental stress is associated with necrotic lesions and drying of leaves in ferns. However, increasing concentration of Co led to initial increase and subsequent decrease in

the total biomass of *T. dentata.* Similar to our study, (Sun *et al*., 2009) also reported that optimal concentration of Co initiates the growth and development of each plant parts. High cobalt contents in the plant leads to oxidative stress and generate reactive oxygen species (ROS) that can negatively affect normal plant growth and development (Salam *et al.*, 2022). During short term exposure of 5 days, there was no significant change in the relative water content of both Cd and Co treated plants. But after long term exposure of 15 days, substantial decrease in relative water content of *T. dentata* was found with increasing Cd and Co treatment. Heavy metal toxicity can thus affect cell membrane permeability and disrupts the hydraulic conductivity leading to reduction in water content. The decrease in RWC was also reported in *Eruca sativa* L. under Cd and Pb stress by (Yildirim *et al*., 2019). In this context, the decrease in RWC, affected growth rate by interfering with the photosynthetic machinery and negatively impacts the normal plant growth (Zhang *et al.*, 2022).

In response to Cd and Co exposure, *T. dentata's* chlorophyll content showed a brief increase, and then decreased, over the 5-day short-term exposure period, with treated plants exhibiting lower levels than control plants. From this result it can be evident that, the biosynthesis of chlorophyll is inhibited by the accumulation of cadmium and cobalt at higher concentration and increase in exposure period (Table 3 & 4). Similar finding was reported in *Ceratopteris pteridoides* under Cd stress, where chlorophyll content decreased in concentration and exposure time dependent manner (Bora *et al.*, 2021). Previous researchers also discovered that Cd interfered with enzyme activity, altered photosynthetic electron transport and stomatal closure, and decreased intercellular CO_2 concentration (Engineer *et al.*, 2016; Rasafi *et al*., 2020).

Carotenoids are accessory pigment in photosynthetic pathways and works as non-enzymatic antioxidant pigment and protect plants from oxidative damage (Zuluaga *et al*., 2017). In our study, carotenoid content increased with respect to the elevating Cd and Co toxicity period which may be attributed to the defense mechanism of plants under oxidative stress. The high carotenoid content may be supported by its quenching ability of ROS particularly singlet oxygen and to overcome oxidative stress in *Hypoxis hemerocallidea* exposed to Cadmium and Aluminum stress (Okem *et al*., 2024).

Osmolytes play a vital role in plants and help in overcoming oxidative stress and maintaining osmotic balance. Our findings revealed a considerable increase in osmolytes such as soluble sugar and proline content with rising levels of Co stress, indicating a higher level of tolerance via enhancing the plant's inherent defense mechanism under shorter exposure time (Table 5). After a longer exposure period, this increase was noticed only in low dose Co treated plants (100 ppm) (Table 6). After 15 days, the proline level in Co-treated plants was significant higher at the initial concentration (100 ppm), but it decreased at 600 ppm Co treatment. Accumulation of osmolytes in plant leaves is well known to serves as a good indicator of stress tolerance mechanism.

Reducing Carbohydrate and soluble carbohydrate content showed increasing trend with increasing Co stress after 5 days and 15 days. According to Sheel *et al*., (2013), even at high metal concentrations, plants can maintain partial metabolism of soluble, reducing, and non-reducing sugars, potentially serving as a tolerance mechanism against heavy metal stress up to a certain threshold, particularly in response to cobalt (Co) stress, as observed in our study.

After a 5-day exposure period, reducing and total soluble sugar content in *T. dentata* increased at low Cd concentrations but decreased at higher concentrations. Prolonged exposure ultimately led to plant mortality, as all Cd-treated plants succumbed to toxicity. This response mirrors findings by John *et al.*, (2008), who reported similar trends in *Lemna polyrrhiza* L. under cadmium stress.

Our results also revealed an increase in phenol content with increase in Cd and Co treatment especially after longer duration of treatment. The increase in phenol concentration may be attributed to its protective role against toxic heavy metal stress through metal detoxification and scavenging of free radicals (Jańczak-Pieniążek *et al*., 2023). A similar increase in phenol content has been reported in an aquatic fern *Azolla imbricata* when induced to different Cd concentrations and duration of exposure time (Dai *et al*., 2006). Plants cells can protect themselves from oxidative stress through the activity of various antioxidant enzymes like CAT, POD, SOD and APX, which scavenges the ROS and minimizes cellular damage (Du *et al*., 2024). In the present experiment, significant increases in SOD activity in both Cd and Co treated plants, induced to a shorter duration of treatment suggest that *T. dentata* plants has potential to neutralize the increased amount of superoxide radicals.

The result obtained showed that CAT, POD and SOD activity increased with the increased concentration of Co with respect to control after 15

days. On the other hand, in Co treated plants, there was no such significant change in APX activity after 15 days. In this study, the low Co concentrations increased CAT activity, followed by a significant decrease in highest Co concentration (1200 ppm) after 5 days. This demonstrates the inability of *T. dentata,* CAT enzyme to provide adequate protection at higher Cd & Co doses above threshold level (100 mg/kg) and longer exposure period. However, a remarkable increase in CAT activity was noticed in Cd treated plants in short term exposure. This enhanced CAT activity indicates higher defense mechanism of this plant under Cd stress during short term exposure. It has the capability to scavenge the ROS by catalyzing them and provide protection in stress condition (Sinam *et al*., 2012).

Peroxidase enzyme (POD) is a multipurpose enzyme that participates in defensive mechanism and prevents ROS overproduction (Passardi *et al.*, 2005; Das and Roychoudhury, 2014). In this investigation, we found that an increase in POD activity in Cd and Co-treated plants is linked to oxidative reactions caused by an increase in peroxides and free radicals in plant cells as a result of stress. One of the processes taking part in the regulation of Cd and Co concentration in *T. dentata* is the stimulation of antioxidative enzymes such POD and SOD in short term exposure. In our experiment, *T. dentata* showed increasing trend of APX activity with respect to increasing Co stress both after 5 and 15 days. However, at highest Cd treatment (1200 ppm), the APX activity decreased significantly with respect to control after 5 days. (Erdei *et al.,* 2002) also reported increase in APX activity in Cd-treated leaves of Barley plants where up to highest 1mM Cd concentration but its downfall after 3 days of treatment. This indicates the inefficiency of the APX enzyme of *T. dentata* to provide sufficient protection at higher Cd concentrations. The formation of ROS at high quantities disrupts the antioxidant enzyme system, reducing CAT and APX activity in Co and Cd-treated plants after a short period of exposure.

Superoxide dismutase (SOD) is one of the most essential elements of plant defense mechanism and can be used as biomarker of stress in plant body. Our experimental findings showed significant increase in SOD activity with the increasing Co stress after 5 and 15 days and correlated to better management of metal stress. SOD has been shown to effectively eliminate reactive oxygen species (ROS) in plants, protect membrane function and mechanism, and maintain stress resistance (Guo *et al.*, 2020)

After 15 days of exposure, *T. dentata's* roots accumulated significantly more cadmium (Cd) and cobalt (Co) than its fronds (Fig. 2 C & D). To assess metal uptake and accumulation, bioaccumulation factor (BCF) and translocation factor (TF) are employed (Chamba-Eras *et al*., 2022). Notably, Cd rapidly traverses root cortical tissue and is translocated to above-ground tissues, as reported by Wu *et al.,* (2004). In our experiment BCF_{fronds}, BCF_{roots} and TF value for Cd and Co treated plants are shown in (Fig. 3 E, F & G). According to (Bhattacharya and Biswas, 2022), plants with BCF>1 and TF<1 act as phytostabilizers, immobilizing heavy metals in the rhizospheric zone. Conversely, plants with both BCF and TF<1 are classified as excluders, ineffective in metal transfer, and accumulate metals primarily in roots and rhizomes (Usman *et al.,* 2019). In present study, *T. dentata* showed BCF and TF value <1 for Cd and Co treated plants and can be said to be a potent excluder of Cd and Co under 15 days exposure time. Similarly, in response to lead (Pb), *Robinia pseudoacacia* plants also showed BCF and TF values <1, which suggest a potential exclusion mechanism for Pb in this plant and accumulation of high Pb concentrations in its roots (Băbău *et al.*, 2024). The TF of all Cd and Co treated plants were less than one, indicating that *T. dentata* preferentially accumulates Cd and Co in its root system. The maximum TF were reported in the Co containing hydroponic solution, showing that *T. dentata* has a greater potential to translocate Co from roots to fronds than Cd. Based on our present research findings, it can be said that *T. dentata* has a significant accumulation of Cd and Co content in it roots and a poor movement in its fronds; yet, it might be employed as excluder and indicator of Co contaminated soil.

Conclusion

Our findings reveal that exposing *T. dentata* to varied Cd and Co concentrations for 5 and 15 days cause significant changes in growth, pigment content and metabolic markers. Cd had a greater impact on plant growth and development than cobalt over longer exposure duration (15 days). Long-term Cd exposure and high Cd concentrations led to reduction in plant biomass, relative water content, chlorophyll content, photosynthetic inhibition and chlorosis in this plant and ultimately to death. After 15 days, *T. dentata* accumulated more cobalt (Co) than cadmium (Cd), exhibiting reduced toxicity symptoms compared to Cd-treated plants. Although further field trials are necessary, our results indicate that *T. dentata* possesses considerable tolerance to long-term Co stress, characterized by enhanced antioxidative enzyme

activity, carotenoid, proline, and phenol content. Notably, its root system demonstrates potential for excluding and immobilizing Co in soil and can be used in excluding and checking the mobility of Co in the soil.

Table 1: Changes in relative growth rate (RGR), biomass and relative water content (RWC) of *T. dentata* in different concentrations of Cd and Co after 5 days of treatment:

Concentration (ppm)		Biomass (g per plant)			Tolerance index (%)
	RGR	Fresh weight	Dry weight	RWC (%)	
Control	0.004±0.0003^{c}	10.87±0.60^{d}	1.84±0.07^{b}	83.1±0.34^{c}	100.0
Cd 100	0.003±0.0003^{bc}	9.88±0.35^{bcd}	1.71±0.10^{ab}	82.7±0.38^{c}	92.9
Cd 600	0.002±0.0002^{ab}	8.45±0.32^{ab}	1.61±0.10^{ab}	80.9±0.57^{ab}	87.5
Cd 1200	0.002±0.0006^{a}	7.47±0.34^{a}	1.48±0.05^{a}	80.2±0.47^{a}	80.4
Co 100	0.007±0.0003^{d}	10.05±0.60^{cd}	1.72±0.09^{ab}	82.9±0.11^{c}	93.5
Co 600	0.004±0.0006^{c}	9.17±0.61^{bc}	1.63±0.07^{ab}	82.2±0.42^{bc}	88.6
Co 1200	0.003±0.0003^{bc}	8.34±0.44^{ab}	1.51±0.09^{a}	81.9±0.21^{bc}	82.1

Values are the mean±SE (n=3). Different letters in the same column show significantdifferences among different treatments, according to Duncan's post hoc test ($p \leq 0.05$).

Table 2: Changes in relative growth rate (RGR), biomass and relative water content (RWC) of *T. dentata* in different concentrations of Cd and Co after 15 days of treatment:

Concentration (ppm)		BIOMASS (g per plant)			Tolerance index (%)
	RGR	Fresh weight	Dry weight	RWC (%)	
Control	0.009±0.0003e	11.88±0.58e	1.78±0.08d	85.01±0.25d	100.0
Cd 100	-0.010±0.0012c	6.04±0.61c	1.21±0.07bc	79.96±0.82bc	68.0
Cd 600	-0.014±0.0006b	4.38±0.20b	1.02±0.05ab	67.84±1.17a	57.3
Cd 1200	-0.017±0.0006a	2.51±0.33a	0.90±0.04a	64.14±1.83a	50.5
Co 100	0.013±0.0009f	8.11±0.58d	1.33±0.08c	83.54±0.32cd	74.7
Co 600	0.010±0.0012d	6.32±0.52c	1.18±0.05bc	81.05±0.60cd	66.3
Co 1200	-0.015±0.009ab	4.32±0.15b	1.01±0.03ab	76.62±2.36b	56.74

Values are the mean±SE (n=3). Different letters in the same column show significant differences among different treatments, according to Duncan's post hoc test ($p \leq 0.05$).

A negative value indicates a decrease in RGR over the control, while a positive value indicates an increase in RGR over the control.

Table 3: Changes in pigment composition of *T. dentata* in different concentrations of Cd and Co after 5 days of treatment:

Concentration (ppm)	Chlorophyll a (mg g^{-1} FWT)	Chlorophyll b (mg g^{-1} FWT)	Total Chlorophyll (mg g^{-1} FWT)	Carotenoids (mg g^{-1} FWT)
Control	0.404±0.01^{a}	0.469±0.02^{bc}	0.873±0.03^{b}	0.222±0.04^{a}
Cd 100	0.443±0.01^{b}	0.507 ±0.01^{c}	0.950±0.02^{c}	0.268±0.00^{ab}
Cd 600	0.397±0.01^{a}	0.603±0.01^{d}	1.000±0.01^{c}	0.332±0.04^{b}
Cd 1200	0.383±0.01^{a}	0.337±0.00^{a}	0.720±0.01^{a}	0.210±0.02^{a}
Co 100	0.533±0.01^{d}	0.483±0.05^{bc}	1.017±0.05^{c}	0.295±0.03^{ab}
Co 600	0.490±0.02^{c}	0.487±0.01^{bc}	0.977±0.02^{c}	0.348±0.03^{b}
Co 1200	0.393±0.01^{a}	0.423±0.01^{b}	0.817±0.00^{b}	0.207±0.01^{a}

Values are the mean±SE (n=3). Different letters in the same column show significantdifferences among different treatments, according to Duncan's post hoc test ($p \leq 0.05$).

Table 4: Changes in pigment composition of *T. dentata* in different concentrations of Cd andCo after 15 days of treatment:

Concentration (ppm)	Chlorophyll a (mg g^{-1} FWT)	Chlorophyll b(mg g^{-1} FWT)	Total Chlorophyll (mg g^{-1} FWT)	Carotenoids (mg g^{-1} FWT)
Control	0.419 ± 0.004^{a}	0.585 ± 0.02^{b}	1.004 ± 0.019^{a}	0.242 ± 0.014^{b}
Cd 100	NS	NS	NS	NS
Cd 600	NS	NS	NS	NS
Cd 1200	NS	NS	NS	NS
Co 100	0.464 ± 0.004^{c}	0.363 ± 0.01^{a}	0.827 ± 0.01^{b}	0.197 ± 0.006^{a}
Co 600	0.309 ± 0.006^{a}	0.408 ± 0.01^{a}	0.717 ± 0.006^{a}	0.184 ± 0.006^{a}
Co 1200	NS	NS	NS	NS

Values are the mean±SE (n=3). Different letters in the same column show significant differences among different treatments, according to Duncan's post hoc test ($p \leq 0.05$).
NS: Not Survived.

Table 5: Changes in biochemical parameters of *T. dentata* in different concentrations of Cdand Co after 5 days of treatment.

Concentration (ppm)	Proline (mg g^{-1} FWT)	Reducing Sugar (mg g^{-1} FWT)	Total solublesugar (mg g^{-1} FWT)	Phenol (mg g^{-1} FWT)
Control	0.014 ± 0.0020^{a}	1.90 ± 0.04^{b}	2.45 ± 0.050^{b}	0.04 ± 0.001^{a}
Cd 100	0.024 ± 0.002^{b}	3.39 ± 0.005^{cd}	2.47 ± 0.008^{b}	0.06 ± 0.001^{b}
Cd 600	0.038 ± 0.002^{c}	4.44 ± 0.35^{e}	2.77 ± 0.057^{c}	0.07 ± 0.001^{bc}
Cd 1200	0.046 ± 0.004^{c}	1.12 ± 0.003^{a}	2.99 ± 0.064^{d}	0.08 ± 0.002^{cd}
Co 100	0.039 ± 0.004^{c}	3.23 ± 0.16^{c}	3.17 ± 0.022^{e}	0.16 ± 0.006^{e}
Co 600	0.073 ± 0.003^{d}	3.82 ± 0.12^{d}	3.31 ± 0.014^{f}	0.09 ± 0.007^{d}
Co 1200	0.088 ± 0.002^{e}	4.68 ± 0.19^{e}	2.25 ± 0.023^{a}	0.08 ± 0.009^{cd}

Values are the mean±SE (n=3). Different letters in the same column show significant differencesamong different treatments, according to Duncan's post hoc test ($p \leq 0.05$).

Table 6: Changes in biochemical parameters of *T. dentata* in different concentrations of Cdand Co after 15 days of treatment:

Concentration (ppm)	Proline (mg g^{-1} FWT)	Reducing Sugar (mg g^{-1} FWT)	Total soluble sugar (mg g^{-1} FWT)	Phenol (mg g^{-1} FWT)
Control	0.027 ± 0.006^{a}	1.93 ± 0.05^{b}	2.50 ± 0.12^{c}	0.099 ± 0.005^{a}
Cd 100	NS	NS	NS	NS
Cd 600	NS	NS	NS	NS
Cd 1200	NS	NS	NS	NS
Co 100	0.032 ± 0.003^{b}	1.89 ± 0.06^{b}	2.14 ± 0.18^{ab}	0.116 ± 0.003^{b}
Co 600	0.020 ± 0.005^{a}	1.06 ± 0.05^{a}	1.88 ± 0.12^{a}	0.166 ± 0.003^{c}
Co 1200	NS	NS	NS	NS

Values are the mean±SE (n=3). Different letters in the same column show significant differencesamong different treatments, according to Duncan's post hoc test ($p \leq 0.05$).
NS: Not survived

Table 7: Changes in antioxidative enzyme activity in fresh fronds of *T. dentata* in differentconcentrations of Cd and Co after 5 days of treatment:

Treatment (ppm)	CAT (U g^{-1} protein)	POD (U g^{-1} protein)	APX (U g^{-1} protein)	SOD (U g^{-1} protein)
Control	0.019 ± 0.001^{a}	0.016 ± 0.002^{a}	0.027 ± 0.001^{b}	0.019 ± 0.002^{a}
Cd 100	0.024 ± 0.004^{b}	0.046 ± 0.007^{b}	0.038 ± 0.003^{cd}	0.026 ± 0.002^{a}
Cd 600	0.026 ± 0.007^{bc}	0.082 ± 0.006^{d}	0.034 ± 0.003^{bc}	0.042 ± 0.002^{b}
Cd 1200	0.036 ± 0.003^{d}	0.130 ± 0.005^{e}	0.009 ± 0.001^{a}	0.065 ± 0.003^{c}
Co 100	0.025 ± 0.002^{bc}	0.041 ± 0.004^{b}	0.057 ± 0.002^{e}	0.022 ± 0.002^{a}
Co 600	0.029 ± 0.004^{c}	0.066 ± 0.004^{c}	0.042 ± 0.003^{d}	0.039 ± 0.003^{b}
Co 1200	0.016 ± 0.004^{a}	0.156 ± 0.008^{f}	0.033 ± 0.002^{bc}	0.080 ± 0.004^{d}

Values are the mean±SE (n=3). Different letters in the same column show significant differences among different treatments, according to Duncan's post hoc test ($p \leq 0.05$).

Table 8: Changes in antioxidative enzyme activity in the fresh fronds of *T. dentata* indifferent concentrations of Cd and Co after 15 days of treatment:

Treatment (ppm)	CAT (U g^{-1} protein)	POD (U g^{-1} protein)	APX (U g^{-1} protein)	SOD (U g^{-1} protein)
Control	0.015±0.001[a]	0.039±0.005[a]	0.025±0.025[a]	0.022±0.002[a]
Cd 100	NS	NS	NS	NS
Cd 600	NS	NS	NS	NS
Cd 1200	NS	NS	NS	NS
Co 100	0.031±0.004[c]	0.066±0.005[b]	0.034±0.034[a]	0.033±0.007[b]
Co 600	0.019±0.011[b]	0.042±0.004[a]	0.025±0.025[a]	0.045±0.012[c]
Co 1200	NS	NS	NS	NS

Values are the mean±SE (n=3). Different letters in the same column show significant differencesamong different treatments, according to Duncan's post hoc test ($p \leq 0.05$).
NS: Not survived

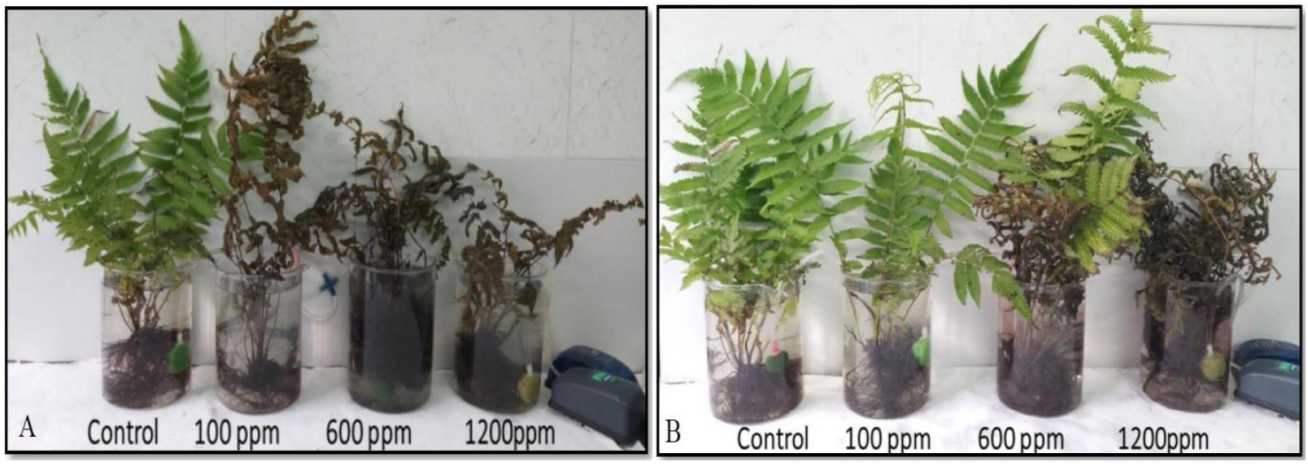

Fig. 1 A, Growth and morphotoxicity symptoms of *T. dentata* (Forssk.) E.P. St. John under different concentrations of Cd stress after 15 days. **B,** Growth and morphotoxicity symptoms of *T. dentata* (Forssk.) E. P. St. John under different concentrations of Co stress after 15 days.

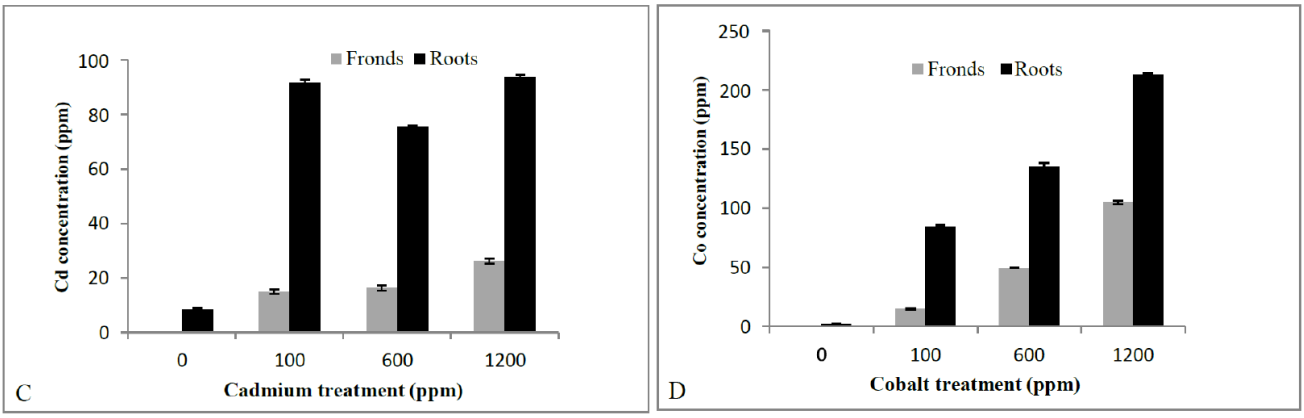

Fig. 2 C, Cd concentration (ppm) in fronds and roots of *T. dentata* subjected to Cd treatments for 15 days. D, Co concentration (ppm) in fronds and roots of *T. dentata* subjected to Co treatments for 15 days. Data are the mean ± SE (expressed as ppm: n=3)

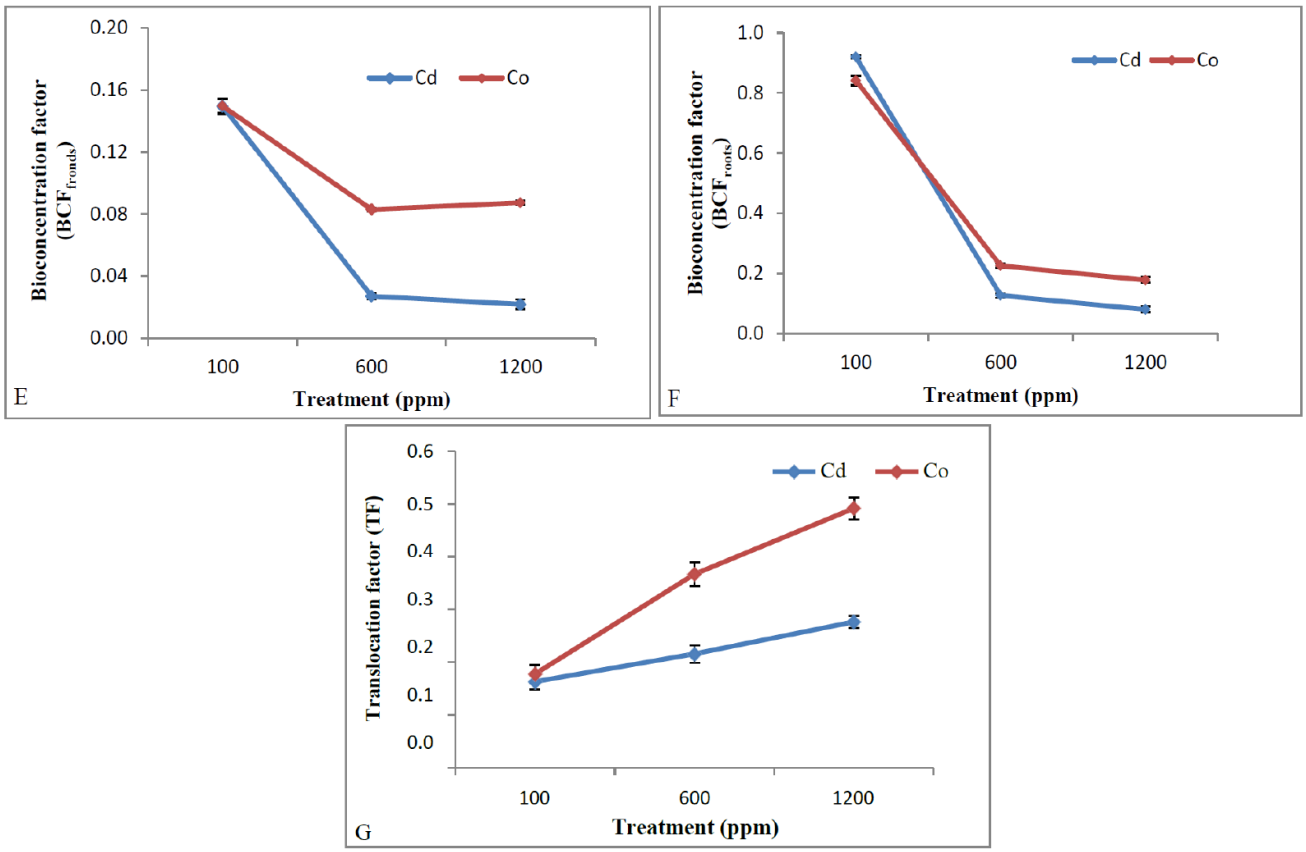

Fig. 3 E, Bioconcentration factor (BCF_{fronds}) of *T. dentata* (fronds) subjected to Cd and Co treatments for 15 days. **F,** Bioconcentration factor (BCF_{roots}) of *T. dentata* (roots) subjected to Cd and Co treatments for 15 days. **G,** Translocation factor (TF) of *T. dentata* subjected to Cd and Co treatments for 15 days. Data are the mean ± SE (n=3).

Abbreviations

APX	Ascorbate peroxidase
BCF	Bioconcentration factor
CAT	Catalase
FWT	Fresh Weight of Tissue
ICP-OES	Inductively coupled plasma-optical emission spectrometry
NS	Not survived
POD	Peroxidase
RGR	Relative growth rate
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase
TI	Tolerance index
TF	Translocation factor
Cd	Cadmium
Co	Cobalt

Acknowledgement

All authors are thankful to the DIST-FIST, University of North Bengal for supplying us thebasic instrumentation facilities. We would also like to acknowledge the HEAD and Staff, SAIF -NEHU, Shillong, for analysing heavy metals in our plant samples by ICP-OES.

References

Al-Khayri, J.M., Banadka, A., Rashmi, R., Nagella, P., Alessa, F.M. and Almaghasia, M.I. (2023). Cadmium toxicity in medicinal plants. An overview of the tolerance strategies, biotechnological and omics approaches to alleviate metal stress. *Front. Plant Sci.,* **13**, 1047410.

Asada, K., Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesis. In, Kyle, D.J., Osmond, C., Arntzen, C.J. (eds.), Photoinhibition, Elsevier, New York, pp. 227-297.

Băbău, A.M.C., Micle, V., Damian, G.E., Sur, L.M. (2024). Lead and copper removal from sterile dumps by phytoremediation with *Robinia pseudoacacia*. Scientific reports, **14**, 9842.

Baker, A.J.M., Reeves, R.D., Hajar, A.S.M. (1994). Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New Phytol.,* **127**, 61-68.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water- stress studies. *Plant Soil*. **39**, 205-207.

Bhattacharya, E., Biswas, S.M. (2022). First report of the hyperaccumulating potential of cadmium and lead by *Cleome rutidosperma* DC. With a brief insight into the

chemical vocabulary of its roots. *Front. Environ. Sci.,* **10**, 830087.

Bigalke, M., Ulrich, A., Rehmus, A., Keller, A. (2017). Accumulation of cadmium and uranium in arable soils in Switzerland. *Environ. Pollut.,* **221**, 85-93.

Bora, M.S., Gogoi, N., Sarma, K.P. (2020).Tolerance mechanism of cadmium in Ceratopteris pteridoides, translocation and subcellular distribution. *Ecotoxicol. Environ. Saf.,* **197**,110599.

Bora, M.S., Sarma, K.P. (2021). Anatomical and ultrastructural alterations in *Ceratopteris pteridoides* under cadmium stress. A mechanism of cadmium tolerance. *Ecotoxicol. Environ. Saf.,* **218**,112285.

Bray, H.G., Thorpe, W. (1954). Analysis of phenolic compounds of interest in metabolism. Methods of biochemical analysis. pp. 27-52.

Chakraborty, U., Chakraborty, B.N., Kapoor, M. (1993). Changes in the levels of peroxidase and phenylalanine ammonia-lyase in *Brassica napus* cultivars showing variable resistance to *Leptosphaeria maculans. Folia Microbiol.,* **38**, 491-496.

Chamba-Eras, I., Griffith, D.M., Kalinhoff, C., Ramírez, J., Gázquez, M.J. (2022). Native hyperaccumulator plants with differential phytoremediation potential in an Artisanal Gold mine of the Ecuadorian Amazon. Plants, **11**, 1186.

Chen, C., Huang, D., Liu, J. (2009). Functions and toxicity of nickel in plants, recent advances and future prospects. *Clean* (Weinh). **37**, 304–313.

Dai, L.P., Xiong, Z.T., Huang, Y., Li, M.J. (2006). Cadmium-induced changes in pigments, total phenolics and phenylalanine ammonia-lyase activity in fronds of *Azolla imbricata. Environ. Toxicol.,* **21**, 505-512.

Das, K., Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci.,* **2**, 53.

Dhindsa, R.S., Matowe, W. (1981). Drought tolerance in two mosses, correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.*, **32**, 79-91.

Du, H., Tan, Lu., Li, S., Wang, Q., Xu, Z., Ryan, P.R., Wu, D., Wang, A. (2024). Effects of cadmium stress on tartary buckwheat seedling. *Plants.* **13**, 1650.

Emashogue, O.A., Kesiye, I.A., Jackson, G., Issac, U. (2020). Assessment of the phytoremediation capabilities of bracken fern (*Pteridium aquilinum*) for the remediation of heavy metals (Pb, Ni and Cd) contaminated water. *Afr. J. Environ. Sci. Technol,*. **14**(10), 336-346.

Engineer, C.B., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordström, M., Azoulay- Shemer, T., Rappel, W.J., Iba, K., Schroeder, J.I. (2016). CO_2 sensing and CO_2 regulation of stomatal conductance, advances and open questions. *Trends in Plant Sci*., **21**(1), 16-30.

Erdei, S., Hegedus, A., Hauptmann, G., Szalai, J., Horvath, G. (2002). Heavy metals induce physiological changes in the antioxidative response system. *Acta Biol. Szeged*., **46**, 89-90.

Fadzil, F.N.M., Mohamad, M.A.N., Repin, R., Harumain, Z.A.S. (2024). Metal uptake and tolerance in hyperaccumulator plants, Advancing phytomining strategies, Rhizosphere, **29**, 2024, 100836.

Farooq, T.H., Rafay, M., Basit, H., Shakoor, A., Shabbir, R., Riaz, M.U., Ali, B., Kumar, U., Qureshi, K.A. and Jaremko, M. (2022). Morpho-physiological growth performance and phytoremediation capabilities of selected xerophytes grass species toward Cr and Pb stress. *Front. Plant Sci*., **13**, 997120.

Guo, H., Jiang, J., Gao, J., Zhang, J., Zeng, L., Cai, M., Zhang, J. (2020). Evaluation of Cadium hyperaccumulation and tolerance potential of *Myriophyllum aquaticum*. *Ecotoxicol. Environ. Saf.,* **195**, 110502.

Gupta, K., Srivastava, S., Saxena, G., Kumar, A. (2022). Application of *Pteris vittata* L. for phytoremediation of arsenic and biomonitoring of the process through cyto-genetic biomarkers of *Trigonella foenum-graecum* L. *Physiol Mol. Biol. Plants*., **28**(1), 91-106.

Haider, F.U., Liqun, C., Coulter, J.A., Cheema, S.A., Wu, J., Zhang, R., Farooq, M. (2021). Cadmium toxicity in plants, Impacts and remediation strategies. *Ecotoxicol. Environ. Saf.,* **211**, 111887.

Harborne, A.J. (1998). Phytochemical methods a guide to modern techniques of plant analysis.

Hoagland, D.R., Arnon, D.I. (1950). The water-culture method for growing plants without soil. *Circular California Agricultural Experiment Station*. **347**, 1-32.

Hu, X., Wei, X., Ling, J. and Chen, J. (2021). Cobalt, An essential micronutrient for plant growth? *Front. Plant Sci.,* **12**, 768523

Jafarirad, S., Hajat Ardehjani, P., Movafeghi, A. (2019). Are the green synthesized nanoparticles safe for environment, A case study of aquatic plant *Azolla filiculoides* as an indicator exposed to magnetite nanoparticles fabricated using microwave hydrothermal treatment and plant extract. *J. Environ. Sci. Health.,* Part A, **54**(6), 516- 527.

Jańczak-Pieniążck, M., Cicheński, J., Michalek, P., Chrzanowski, G. (2023). Effect of heavy metal stress on phenolics compounds accumulation in winter wheat plants. *Molecules*, **28**, 241.

John, R., Ahmad, P., Gadgil, K., Sharma, S. (2008). Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environ.*, **54**(6), 262.

Kikis, C., Thalassinos, G., Antoniadis, V. (2024). Soil Phytomining, Recent developments-A review. *Soil Syst.,* **8**, 8.

Kumari, A., Pandey, V.C., Rai, U.N. (2013). Feasibility of fern *Thelypteris dentata* for revegetation of coal fly ash landfills. *J. Geochem. Explor.,* **128**, 147-152.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids, Pigments of photosynthetic biomembranes. In, Methods in Enzymology. *Academic Press*, pp. 350-382.

Liu, S., Ali, S., Yang, R., Tao, J., Ren, B. (2019) A newly discovered Cd-hyperaccumulator *Lantana camara* L. *J. Hazard Mater.,* **371**,233-242.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*., **193**, 265-275.

Maehly, A.C., Chance, B. (1954). The assay of catalases and peroxidases. *Methods Biochem. Anal.,* **1**, 357-424.

Mahadevan, A., Sridhar, R. (1982). Methods in Physiological Plant Pathology. Sivakami Publication, Madras.

Mahey, S., Kumar, R., Sharma, M., Kumar, V., Bhardwaj, R. (2020). A critical review on toxicity of cobalt and its bioremediation strategies. *SN Appl. Sci.,* **2**, 1279.

Marais, J., De Wit, J.L., Quicke, G.V. (1966). A critical examination of the Nelson-Somogyi method for the

determination of reducing sugars. *Anal. Biochem.,* **15**, 373-381.

Muhamad, I., Aba, L., Kusrini. (2019). The ability of ferns to accumulate heavy metals (Hg, Pb and Cd) in the waters of the Gorontalo River. *Int. J. Appl. Boil*. **3**(1), 36-44.

Obinaa, I.B., Ebere, E.C. (2019). A review, Water pollution by heavy metal and organic pollutants, Brief review of sources, effects and progress on remediation with aquatic plants. *Anal. Methods Environ. Chem. J.,* **2**(3), 5-38.

Okem, A., Stirk, W.A., Fihnie, J.F., Staden, J.V. (2024). Stress-related physiological responses and ultrastructural changes in *Hyponis hemerocallidea* leaves exposed to cadmium and aluminium. *Plant Growth Regul.,* **103**, 721-732.

Pandey, A.K.; Zori´c, L.; Sun, T.; Karanovi´c, D.; Fang, P.; Borišev, M.; Wu, X.; Lukovi´c, J.; Xu, P. (2022). The Anatomical Basis of Heavy Metal Responses in Legumes and Their Impact on Plant-Rhizosphere Interactions. *Plants*, **11**, 2554.

Parmar, P., Kumari, N., Sharma, V. (2013). Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Bot. Stud.,* **54**(45).

Passardi, F., Cosio, C., Penel, C., Dunand, C. (2005). Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep.* **24**, 255-265.

Pietrini, F., Iori, V., Pietrosanti, L., Zacchini, M., Massacci, A. (2020). Evaluation of multiple responses associated with Arsenic tolerance and accumulation in *Pteris vittata* L. Plants exposed to high As concentrations under hydroponics. *Water*, **12**, 3127.

Plummer, D.T. (1978). The practice of column chromatography. In, An Introduction to Practical Biochemistry. McGraw-Hill, New York. pp. 61-66.

Rasafi, T.E., Oukarroum, A., Haddioui, A., Song, H., Kwon, E.E., Bolan, N., Tack, F.M.G., Sebastian, A., Prasad, M.N.V., Rinklebe, J. (2020). Cadmium stress in plants, A critical review of the effects, mechanisms, and tolerance strategies. *Crit. Rev. Environ. Sci. Technol.* **52**(5), 675-726.

Riyazuddin, R., Nisha, N., Ejaz, B., Khan, M.I.R., Kumar, M., Ramteke, P.W., Gupta, R. (2022). A comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules*, 12, 43.

Salam A, Khan AR, Liu L, Yang S, Azhar W, Ulhassan Z, Zeeshan M, Wu J, Fan X, Gan Y. (2022). Seed priming with zinc oxide nanoparticles downplayed ultrastructural damage and improved photosynthetic apparatus in maize under cobalt stress. *J Hazard Mater.* **5**; 423(Pt A), 127021.

Salam, A., Rehman, M., Qi, J., Khan, A.R., Yang, S., Zeeshan, M., Uhhassan, Z., Afridi, M.S., Yang, C., Chen, N. (2024). Cobalt stress induces photosynthetic and ultrastructural distortion by disrupting cellular redox homeostasis in maize. *Environ. Exp. Bot.* **217**, 105562.

Sheel, R., Anand, M., Nisha, K. (2015). Phytoremediation of heavy metals (Zn and Pb) and its toxicity on Azolla filiculoides. Int. J. Sci. Res., **4**(7), 1238-1241.

Sinam, G., Behera, S.K., Mishra, R.K., Sinha, S., Mallick, S., Khare, P.B. (2012). Comparison of two ferns (*Adiantum capillus-veneris* Linn. and *Microsorium punctatum* (Linn.) Copel) for their Cr accumulation potential and antioxidant responses. *Int. J. Phytoremediation*., **14**(7), 629-642.

Soni, S., Jha, A.B., Dubey, R.S., Sharma, P. (2024). Mitigating cadmium accumulation and toxicity in plants, The promising role of nanoparticles. *Sci. Total Environ.,* **20**; 912, 168826.

Subaši´c, M., Šamec, D., Selovi´c, A., Karalija, E. (2022). Phytoremediation of Cadmium Polluted Soils, Current Status and Approaches for Enhancing. *Soil Syst.,* **6**, 3.

Sun, Y., Zhou, Q., Liu, W., An, J., Xu, Z., Wang, L. (2009). Joint effects of arsenic and cadmium on plant growth and metal bioaccumulation, A potential Cd- hyperaccumulator and As-excluder *Bidens pilosa* L. *J. Hazard. Mater.* **165**, 1023- 1028.

Sytar, O., Kumari, P., Yadav, S., Brestic, M., Rastogi, A. (2019). Phytohormone priming regulator for heavy metal stress in plants. *J. Plant Growth Regul.* **38**(2), 739-752.

Usman, K., Al-Ghouti, M.A., Abu-Dieyeh, M.H. (2019). The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse. Scientific reports*, **9**, 5658.

Waheed, A., Haxim, Y., Islam, W., Ahmed, M., Ali, S., Wen, X., Khan, K.A., Ghramh, H.A., Zhang, Z., Zhang, D. (2022). Impact of cadmium stress on growth and physio-biochemical attributes of *Eruca sativa* Mill. *Plants*, **11**, 2981.

Wendling, L.A., Kirby, J.K., Mclaughlin, M.J. (2009). Aging effects on cobalt availability in soils. *Environ. Toxicol. Chem*., **28**, 1609-1617.

Wiszniewska, A., Koźmińska, A., Hanus-Fajerska, E., Dziurka, M., Dziurka, K. (2019). Insight into mechanisms of multiple stresses tolerance in a halophyte *Aster tripolium* subjected to salinity and heavy metal stress. *Ecotoxicol. Environ. Saf.*, **180**,12-22.

Wolff, G., Pereira, G., Cadtro, E., Louzada, J., Coelho, F. (2012). The use of *Salvinia auriculata* as a bioindicator in aquatic ecosystems, biomass and structure dependent on the cadmium concentration. *Braz. J. Biol. Sci.* **72**, 71-77.

Wu, F., Chen, F., Wei, K., Zhang, G. (2004). Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. *Chemosphere*, **57**(6), 447-454.

Yang, G., HE, H. (2016). A review of pteridophyta potential in phytoremediation of heavy metal contaminated environments. *Asian J. Agric. Res.,* **8**(11), 49, 53, 60.

Yildirim, E., Ekinci, M., Ağar, G., Örs, S., Dursun, A., Kul, R., Balci, T. (2019). Impact of cadmium and lead heavy metal stress on plant growth and physiology of Rocket (*Eruca sativa* L.). *KSU J. Agric. Nat.,* **22** (6),843-850.

Zhang, Y., Wu, J., Wang, A. (2022). Comparison of various approaches for estimating leaf water content and stomatal conductance in different plant species using hyperspectral data. *Ecol. Indic.,* **142**, 109278.

Zuluaga, M., Gueguen, V., Pavon-Djavid, G., Letourneur, D. (2017). Carotenoids from microalgae to block oxidative stress. *Bioimpacts,* **7**(1), 1-3.