Acta Physiologiae Plantarum (2024) 46:74
https://doi.org/10.1007/s11738-024-03700-5

ORIGINAL ARTICLE

Adaptive responses of *Alternanthera tenella* Colla. to cadmium stress through physiology, elemental allocation and morpho-anatomical modifications

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Received: 2 October 2023 / Revised: 4 June 2024 / Accepted: 31 July 2024 / Published online: 16 August 2024

Abstract

Industrialization has accelerated the rate of heavy metal discharge into the environment and among trace metals, cadmium (Cd) gains attention due to its relative mobility from soil to plant and potential toxicity to humans. Phytoremediation is a plant-based, cost-effective approach to remediate the contaminated soil and water, and an attempt has been made in the present study to explore the potential of an invasive plant *Alternanthera tenella* for Cd removal. The physiological and morpho-anatomical modifications of plant tissues including the elemental allocation pattern and bioaccumulation potential were studied in response to 170 µM of $Cd(NO_3)_2$. Cd negatively affects the growth parameters, biomass, and photosynthetic efficacy of the plant. Cd treatment influenced the distribution of macro and microelements in the plant and the structural moieties in the biomolecules on the interaction of metal ions. Anatomical modifications included the alterations in the diameter and thickness of cell walls, especially xylem walls, the presence of cell structural distortions and blockage, and fully opened stomata with thick guard cells and depositions. Metabolites like proline, flavonoids, phenol, and malondialdehyde marked a significant increase in stress tolerance. Despite having a relatively low transfer factor (TF), *A. tenella* exhibits high values of biological concentration factor (BCF) and biological accumulation factor (BAF), suggesting its suitability for phytostabilization of Cd-contaminated environments.

Keywords *Alternanthera tenella* · Cadmium · Phytoextraction · Elemental distribution · FTIR · SEM · Bioaccumulation · Phytoremediation

Introduction

The rapid urban development along with industrial and population growth over the past few decades has accelerated the concentration of environmental pollutants including heavy metals (HMs) like mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), etc. that is fatal at even low doses due to their carcinogenicity and ability to cause genomic instability and epigenetic alterations (Balali-Mood et al. 2021). Among metals, Cd is a highly toxic and widespread industrial and environmental pollutant that has been labelled as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC 1993), a Group 2a carcinogen by the Environmental Protection Agency (EPA), and a 1B carcinogen by the European Chemical Agency (IPCS 1992; ATSDR 2012) carcinogen classification systems. Cd is released into the environment from mining, copper and nickel smelting, use of synthetic phosphate fertilizers, recycling of electronic wastes, fossil fuel combustion, and leachate generated from landfill sites, compost, and sludges (Genchi et al. 2020). Cd^{2+} and Cd-chelates are the two forms of Cd in the soil solution (Abedi and Mojiri 2020). Cd is highly mobile and assimilable, hence it enters the roots as both inorganic and organic complexes and translocates to the shoot in ionic form (Kubier et al. 2019; Dong et al. 2019). Chlorosis, stunted growth, and plant necrosis are the visible symptoms of Cd in plants (Xu et al. 2020). Cd has deleterious effects on the plant's physiological processes like photosynthesis, seedling germination, enzyme activities,

Communicated by S. Srivastava.

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stomatal conductance, transpiration, uptake, and transport of essential minerals (Nazar et al. 2012; Zhang et al. 2020). Cd can easily enter the human body as it replaces Calcium (Ca); the mass Cd contamination of food and water had caused the Itai-Itai disease in Japan and the patients suffered from kidney failure, painful degenerative bone disease, and gastrointestinal and lung disorders (Nishijo et al. 2017). Therefore, developing efficient ways to eliminate Cd from the environment is essential.

Phytoremediation, particularly employing hyperaccumulator plants, presents a promising and sustainable approach for heavy metal (HM) decontamination, offering cost-effective and eco-friendly solutions. Invasive plants possess specific attributes advantageous for establishing in contaminated areas and enhancing metal absorption, including rapid growth, extensive root systems, and robust symbiotic microbial associations in roots, making them suitable candidates for remediating metal-polluted soils, especially in riparian zones near industries (Wang et al. 2020; El-Bakatoushi and Elframawy 2016). While various weeds like *Praxelis clematidea*, *Bidens pilosa*, *Chromolaena odorata* and *Pennisetum purpureum* have been utilized for Cd phytoremediation (Wei et al.2018; Yang et al. 2020), research on the potential and mechanisms of invasive plants for remediating heavy metal-contaminated sites remains limited. *Alternanthera tenella* Colla. (Amaranthaceae), a common invasive alien plant in India, shows promise due to its ability to thrive in industrially polluted areas. Previous studies have highlighted the response of *A. tenella* to HM stress, with evidence suggesting its capacity to accumulate Cd under controlled conditions (Rodrigues et al. 2017; Chinmayee et al. 2014; Firdous et al. 2023). Further research on this species could confirm its potential application as a phytoremediator for heavy metal decontamination. This study aims to assess the efficacy of *A. tenella* for phytoremediating Cd-contaminated sites, while also investigating the morpho-anatomical and physiological changes associated with Cd stress.

Materials and methods

Plant material and Cd treatment

Healthy stem cuttings of *A. tenella*, each measuring 25–30 cm in length, were carefully selected for the experiment. These cuttings were maintained under controlled conditions with a relative humidity of $60 \pm 5\%$ and a temperature of 30 ± 2 °C to optimize plant growth throughout the study. Initially, the stem cuttings were dipped in distilled water for a week to induce rooting. Later, the rooted plantlets were subjected to a standardized concentration of $Cd(NO_3)_2$ (170 µM) for a duration of 21 days using hydroponic culture techniques. The hydroponic setup consisted of glass test tubes sized 25×150 mm, filled with half-strength Hoagland's solution (50 mL). Plants were subjected to 21-day treatments with various concentrations of $Cd(NO_3)_2$ ranging from 0 to 200 µM, prepared from a 1 M stock solution, with five replicates for each concentration. The range of concentrations was gradually narrowed down, initially with a difference of 50 µM and later with a difference of 10 µM between concentrations. Following screening, it was established that exposure to 170 µM $Cd(NO_3)_2$ induced substantial stress in the plants, leading to approximately 50% growth inhibition, thus selected for further studies (data given in Table S1). For studying the physio-chemical effects of cadmium stress on *A. tenella*, samples were harvested at different time intervals (0, 7, 14, and 21 days) after Cd stress treatment. Five biological replicates were collected for each treatment to ensure reliability and consistency in the results. Hereupon, cadmium stress (Cd) will be used to refer $Cd(NO_3)_2$ treatments.

Plant growth

The whole plants were harvested at specific intervals of Cd exposure, washed with distilled water and blotted dry. The length of roots and shoots and leaf area were measured respectively with a graduated scale and a graph paper, respectively. The dry weight of whole plants was recorded and the tolerance index percentage of the plantlets was calculated (Turner 1994). The plant parts (root, stem, and leaf) were separated after weighing and stored for further analysis. The remaining samples were oven dried at 100 °C for 1 h, then at 60 °C till a constant mass is achieved to calculate dry weight of the plant samples.

Bioaccumulation of cadmium

The fresh plant samples harvested (root, stem, and leaves) on 21 d for Cd content determination were dried in at 100 °C for 1 h and then at 60 °C until a constant dry weight was achieved. The dried samples were then ground and finely powdered. 200 mg from each sample were left in a mixed solution of nitric and hydrochloric acids ($4HNO_3$: 1HCl) in closed PTFE vessels, followed by digestion in a microwave oven for two hours at 200 °C. Estimation of Cd present in the *A. tenella* digest was carried out using an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) (Perkin Elmer Avio 200, USA). The Cd accumulation and rate in plant and distribution proportion of Cd in roots were calculated using formulas given by Fang et al. (2017). The phytoremediation potential of the *A. tenella* against Cd stress was calculated using different indices like biological accumulation coefficient (BAC), translocation factor (TF), and bioconcentration factor (BCF) using the formulas of Yanqun et al. (2005) and Yoon et al. (2006).

Anatomical parameters

The root, stem, and leaf samples of *A. tenella*, both from the control and Cd treatments, were sectioned after 21 days and immersed in a solution containing 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) overnight at room temperature for fixation. After fixation, the specimens underwent two rinses with double-distilled water and were dehydrated using an ascending alcohol series. The dehydrated samples were affixed onto aluminium stubs using double-sided adhesive conducting carbon tape. These prepared specimens were then coated with gold and photographed using the photographic attachment of a scanning electron microscope (SEM, Jeol 6390LA) operating at 20 kV.

Element analysis in tissues

The dehydrated samples of root, stem, and leaf from *A. tenella* were subjected to examination using a high-resolution SEM (Jeol 6390LA) at a magnification of × 300,000 to analyse their elemental distribution patterns. Employing an Inca analyser EDX spectrophotometer and following the methodology outlined by Cocozza et al. (2008), a quantitative compositional analysis of the elements was conducted. Utilizing energy dispersive X-ray microanalysis (EDXMA), three micro spots were analyzed in the root, stem, and leaves of both control and metal-treated plantlets, labelled as spectrum 1, 2, and 3 (Sarath et al. 2022).

Photosynthetic efficiency

Photosynthetic pigments. The chlorophyll and carotenoid contents of the pigments in the leaf samples were estimated according to the method of Arnon (1949).

Metabolites

The metabolites such as total proteins, soluble sugars, total phenolics, flavonoids, proline and malondialdehyde content in roots, shoots, and leaves of plant samples were estimated according to the protocol of Lowry et al. (1951), Dubois et al. (1956), Bray and Thorpe (1954), El-far and Taie (2009), Bates et al. (1973), and Li et al. (2010) respectively. The D-glucose, bovine serine albumin, quercetin, L-proline and tannic acid were used as standards for the estimation of total sugar, total proteins, proline and total phenolics respectively.

Fourier transform infrared (FTIR) analysis

The dried powder obtained from leaf, stem, and root samples was mixed with dried, water-free KBr at a ratio of 1:150 mg (sample: KBr). The mixture was then subjected to a hydraulic pressure of 10 tons to form KBr discs suitable for infrared (IR) analysis. These discs were positioned in the path of the instrument beam of a Fourier-transform infrared (FT-IR) spectrometer (JASCO 4100, Shanghai, China) to measure the solid-state spectrum. The IR analysis was conducted within the range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1} (Sarath et al. 2022).

Statistical analysis

The results of the study were statistically examined using one-way ANOVA. All significant treatment effects were determined using Tukey's HSD test at $p < 0.05$. Data are average recordings from three independent experiments, eachssss with five replicates (i.e., $n = 15$). The data represent mean ± standard error (SE).

Results

Plant growth

Throughout the treatment period, all plant growth indices displayed a consistent decrease under Cd stress. While control roots exhibited a two-fold increase in growth, the root length of Cd-treated plantlets notably declined from 11% at 7d to 41% at 21d. Similarly, the shoot length of *A. tenella* plantlets subjected to Cd stress showed a significant reduction, particularly evident in the later stages of stress, with a 22.3% decrease observed at 21 days compared to the control plants. Cd treatment also led to inhibited growth of newly emerged leaves in *A. tenella*, with a substantial 48% reduction in leaf area observed at 21 days, contrasting with significant increases in leaf area in control plants. The tolerance index of Cd-treated plantlets gradually declined from 11% at 7d to 41% at 21d, indicating diminishing tolerance over the stress period (Table 1). The leaf relative water content and biomass of the plantlets also reflected a similar pattern.

Significant reductions in shoot biomass were observed specifically on 21d of the Cd treatment period, with a decrease of 16% compared to control plantlets. Similarly, root biomass exhibited a consistent decrease under Cd stress compared to control plants, although no significant differences were found among treated plants at this specific interval ($p < 0.05$). Furthermore, Cd treatment led to a reduction in the RWC of leaves in *A. tenella* compared to control plants. The maximum decrease in RWC occurred on 21d, with a reduction of 19% (Table 2).

Bioaccumulation of cadmium

A. tenella plantlets were exposed to 170 μM of Cd for 21d. Roots showed a greater concentration of Cd compared to the aerial parts of plant, which indicates that the majority of the Cd taken up by the plantlets was retained in the roots and only a small portion was transported to the stem and leaves. On 21d, the roots of treated *A. tenella* had a Cd content of 317.25 $mgkg^{-1}$ DW, while the stem and leaves of plants had Cd concentrations of 87.462 $mgkg^{-1}$ DW and 72.825 $mgkg^{-1}$ DW, respectively (Table 3). The plant showed a total Cd accumulation ($Cd_{root} + Cd_{shoot}$) of 5925.608 $mgkg^{-1}$ DW on 21d, with the Cd distribution proportion of root as 0.57. The BCF value for *A. tenella* under Cd treatment was determined to be 8.39 based on the ratio of the metal concentration in treated plant roots to that of the medium. The BAC value calculates the ratio of the metal content in the shoot (including the stem and leaves) to the medium. The BAC value of Cd treated *A. tenella* was equally high, at 16.6. The BTC value, which gives an estimate of the metal concentration translocated from the root to the aboveground part of the plant, was discovered to be 0.505 for *A. tenella*, making it suitable for phytostabilization of Cd.

Anatomical parameters

Cd stress-induced significant structural changes especially in the vascular regions and inner pith were observed on the

Table 1 Root length (cm), Shoot length (cm), leaf area (cm^2), and tolerance index (%) of *A. tenella* subjected under control and Cd-treated conditions at different days

Day	Root length (cm)		Shoot length (cm)		Leaf area (cm^2)		Tolerance index (%)
	Control	Cd	Control	Cd	Control	Cd	Cd
0	6.4 ± 0.28^{a}	6.4 ± 0.28^{a}	39.94 ± 0.52^{a}	39.94 ± 0.52^{a}	1.82 ± 0.13^{a}	1.78 ± 0.08^{a}	100^{a}
7	8.4 ± 0.28^{ab}	7.44 ± 0.15^{a}	42.66 ± 0.53^{ab}	40.78 ± 0.26^{a}	2.7 ± 0.21^{ab}	1.9 ± 0.07^{ac}	88.74 ± 1.31^{b}
14	10.8 ± 0.21^{b}	7.62 ± 0.17^{ab}	49.38 ± 0.44^{b}	41.44 ± 0.37^{b}	3.06 ± 0.16^{b}	1.92 ± 0.07^{b}	70.61 ± 1.65^{c}
21	13.26 ± 0.32^{b}	7.78 ± 0.24^{b}	53.42 ± 0.38^{b}	41.48 ± 0.38^{b}	3.7 ± 0.2^{c}	1.92 ± 0.07^{c}	58.77 ± 2.04^{d}

The data are presented with the mean ± SE of five replicates. Values with different letters are significantly different ($p < 0.05$)

Table 2 Shoot and root dry biomass, leaf RWC of *A. tenella* under control and Cd-treated conditions at different days

Day	Biomass ($mgkg^{-1}$)				Leaf RWC	
	Control		Cd		Control	Cd treated
	Shoot	Root	Shoot	Root		
0	29.13 ± 0.55^{a}	7.37 ± 0.2^{a}	29.03 ± 0.54^{a}	7.93 ± 0.56^{a}	87 ± 0.89^{a}	87 ± 0.71^{a}
7	32.09 ± 0.52^{ab}	9.02 ± 0.21^{bc}	30.48 ± 0.42^{a}	9.03 ± 0.25^{a}	88 ± 1.38^{bcd}	83 ± 0.71^{bcd}
14	35.49 ± 0.32^{b}	11.65 ± 0.43^{c}	31.35 ± 0.38^{b}	10.04 ± 0.22^{b}	92 ± 1.0^{c}	75 ± 1.09^{c}
21	37.94 ± 0.12^{b}	14.08 ± 0.2^{c}	31.8 ± 0.3^{b}	10.57 ± 0.15^{b}	95 ± 0.71^{c}	77 ± 0.71^{cd}

The data are presented with the mean ± SE of five replicates. Values with different letters are significantly different ($p < 0.05$)

Table 3 Cadmium concentration and accumulation in various parts of control and Cd-treated *A. tenella* on 21d

Sample	Dry weight, DW ($mgkg^{-1}$)		Cd concentration, [Cd] ($mgkg^{-1}$)		Cd accumulation = [Cd]*DW ($mgkg^{-1}$ DW)	
	Control	Treated	Control	Treated	Control	Treated
Leaf	18.66 ± 0.21	14.28 ± 0.18	ND	72.825	ND	1039.941
Stem	19.28 ± 0.17	17.52 ± 0.2	ND	87.462	ND	1532.334
Root	14.08 ± 0.2	10.57 ± 0.15	ND	317.25	ND	3353.333

Values are the means ± SE of five replicates

ND not detected

scanning electron micrographs of the root, stem and leaves of the Cd treated *A. tenella* plantlets on 21d.

Root. Cd treatment resulted in a reduction in the root diameter compared to the control group. Notably, Cd exposure led to significant structural alterations in the root's vascular system (Fig. 1). The thickness of the xylem wall and the diameter of xylem vessels exhibited marked changes in Cd-treated plants. Specifically, the thickness of the xylem wall in roots increased (3.22 ± 0.32) compared to control plants (3.06 ± 1.18), and a similar trend was observed for xylem vessel diameter (Table 4). The cellular morphology and structure were notably distorted in Cd-treated plant roots, with a reduction in parenchymatous pith. Moreover, the presence of dense granules, likely containing Cd, was observed within cortical cells and vascular elements (Fig. 1D).

Stem. Cd treatment resulted in minimal structural changes in the stems of *A. tenella* compared to the control group, with the exception of cortical cells, which exhibited significant distortion and occlusions (Fig. 2D, Fig. 3). Cd exposure induced an increase in the thickness of the xylem wall and a reduction in the diameter of the xylem. Specifically, Cd-treated plantlets showed a 29% increase in the thickness of the xylem wall and a 32% reduction in xylem diameter compared to control samples (Table 4) (Fig. 3).

Table 4 Xylem diameter (μm) and thickness of xylem wall (μm) in various parts of control and Cd-treated *A. tenella*

Sample	Diameter(μm)		Thickness(μm)	
	Control	Cadmium	Control	Cadmium
Root	7.72 ± 1.66^{a}	9.97 ± 1.04^{c}	1.15 ± 0.11^{a}	3.22 ± 0.32^{a}
Stem	42.24 ± 2.91^{ab}	28.58 ± 2.16^{b}	6.04 ± 0.48^{ab}	7.82 ± 0.46^{b}
Leaf	19.29 ± 1.13^{bc}	14.82 ± 1.27^{c}	2.62 ± 0.45^{b}	4.11 ± 0.26^{b}

The data are presented with the mean ± SE of five replicates. Values with different letters are significantly different ($p < 0.05$)

Leaf. In leaf samples of plants subjected to Cd stress, there was an increase in both the diameter of the xylem and the thickness of the xylem wall. The thickness of the xylem wall in leaves significantly increased in Cd-treated plants (4.11 ± 0.26) compared to control plants (2.62 ± 0.45), whereas the diameter of the xylem vessels decreased from 19.29 ± 1.13 in control leaves to 14.82 ± 1.27 in leaves of Cd-treated plants, marking a reduction of 23% (Table 4). However, no significant changes in the shape and structure of leaf cells were observed between Cd-treated plants and controls. Occlusions were observed in the tracheary elements of the stele in leaves of Cd-treated plants, which are likely attributed to Cd deposition (Fig. 4B). *A. tenella* typically possesses non-glandular trichomes and diacytic shaped stomata scattered over the lamina of leaf surfaces (see Fig. 5A).

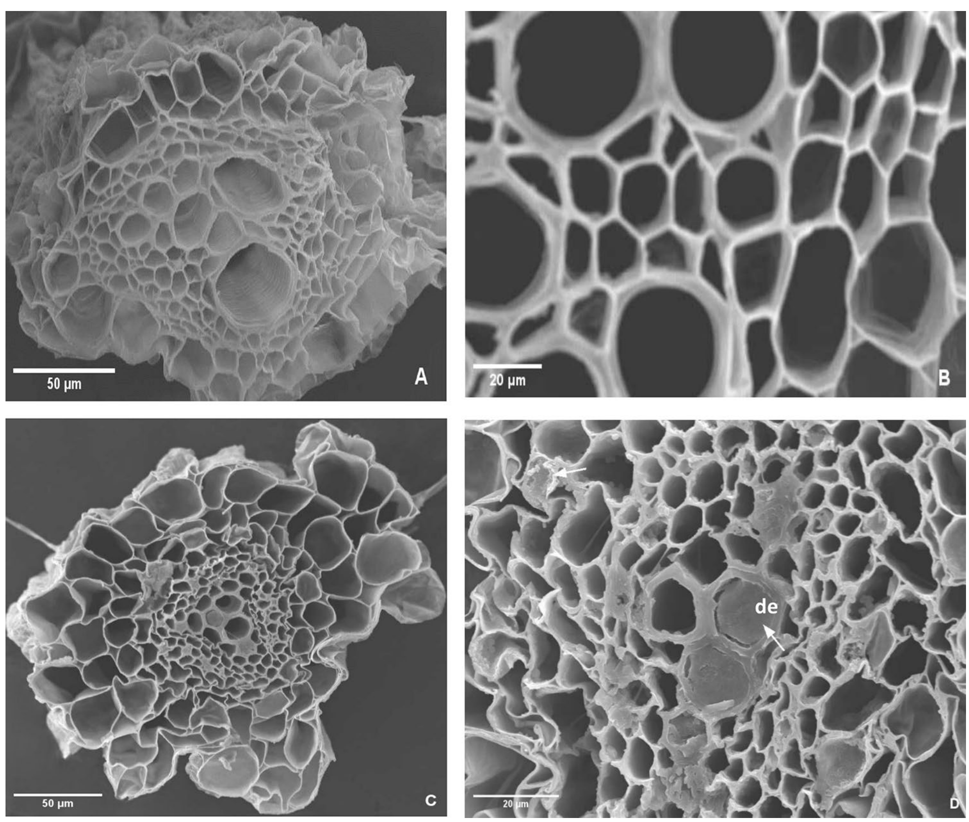

Fig. 1 SEM images of *A. tenella* root under control and Cd stress. **A**, **C** Cross section of control and treated root; **B**, **D** Enlarged view of vascular region of control and treated roots (de – deposition)

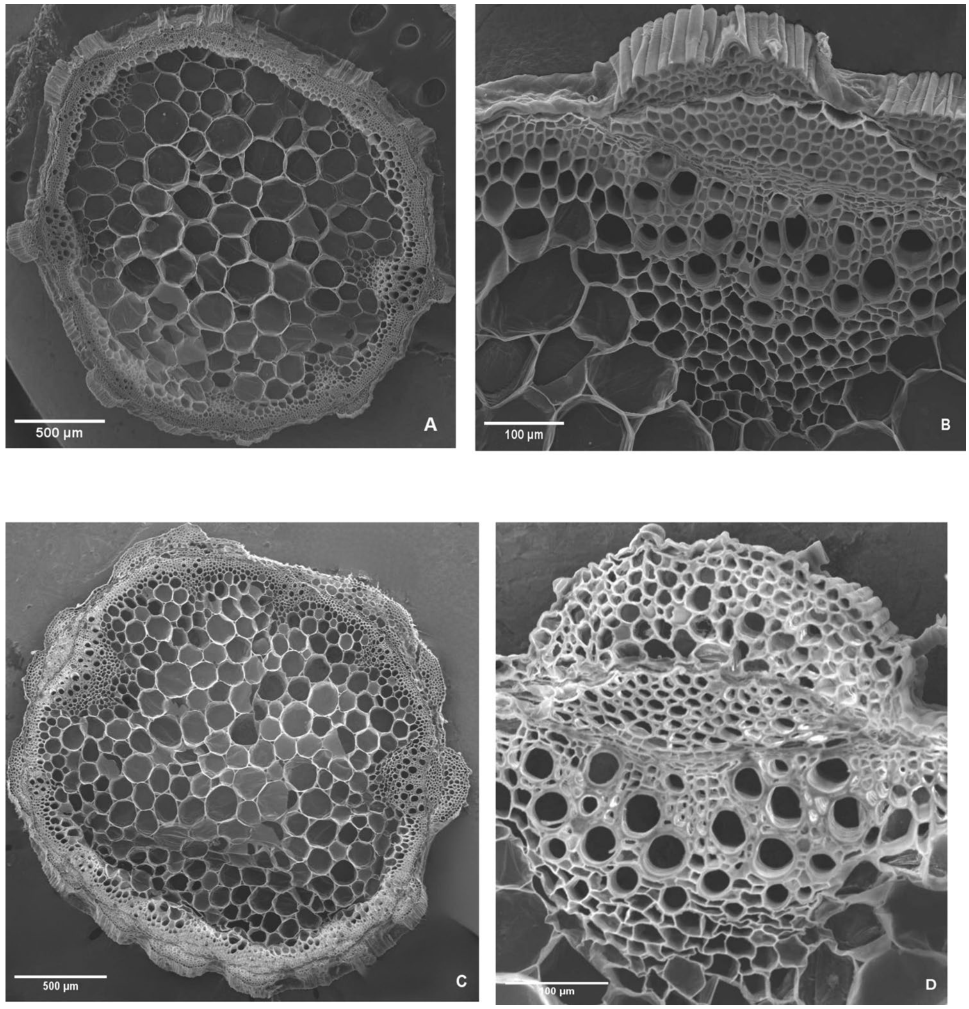

Fig. 2 SEM images of *A. tenella* stem under control and Cd stress. **A**, **C** Cross section of control and treated stem; **B**, **D** Enlarged view of vascular region of control and treated stem

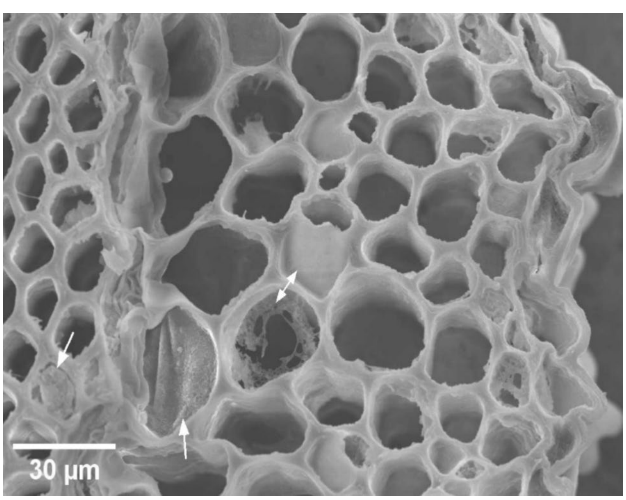

Fig. 3 SEM image of the cortical cells of Cd-treated *A. tenella* stem

A reduction in the number of trichomes was observed in the leaves of Cd-treated plants compared to controls. The number of stomata was higher on the abaxial surface of the leaf. Stomata observed in both control and Cd-treated leaf samples appeared open and fully matured, with well-defined borders and guard cells. However, in Cd-treated *A. tenella* leaves, some stomata appeared partially opened, while others were widely opened with thick guard cells and deposits, presumably Cd (Fig. 5D). The presence of mass depositions in the stomata suggests the possibility for Cd transpiration, making the plant potentially suitable for phytovolatilization.

Element analysis in tissues

EDXMA analysis on the three different regions of the plant parts (root, stem and leaf) in control and Cd treated plants showed the significant changes caused by Cd in the elemental distribution pattern within the plant tissues. The regions selected for the analysis were marked as spectrum 1, spectrum 2, and spectrum 3, respectively, for the outer

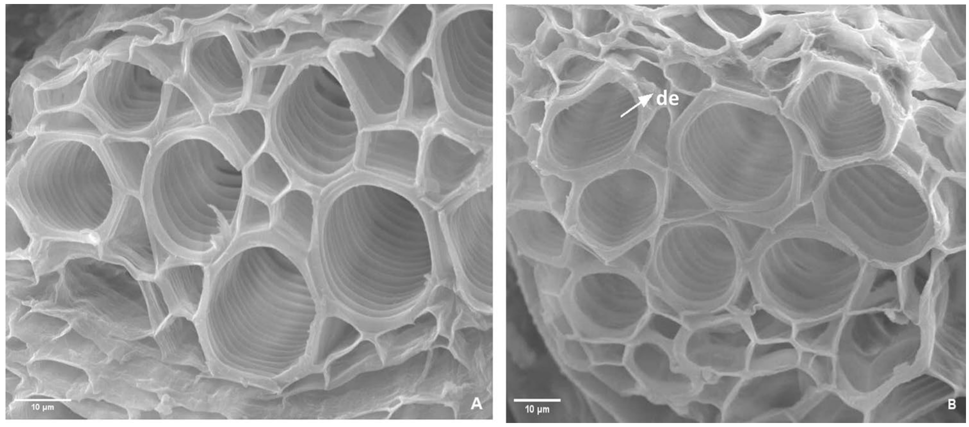

Fig. 4 SEM images showing enlarged view of xylem walls of control (**A**) and Cd-treated (**B**) *A. tenella* leaf (de- deposition)

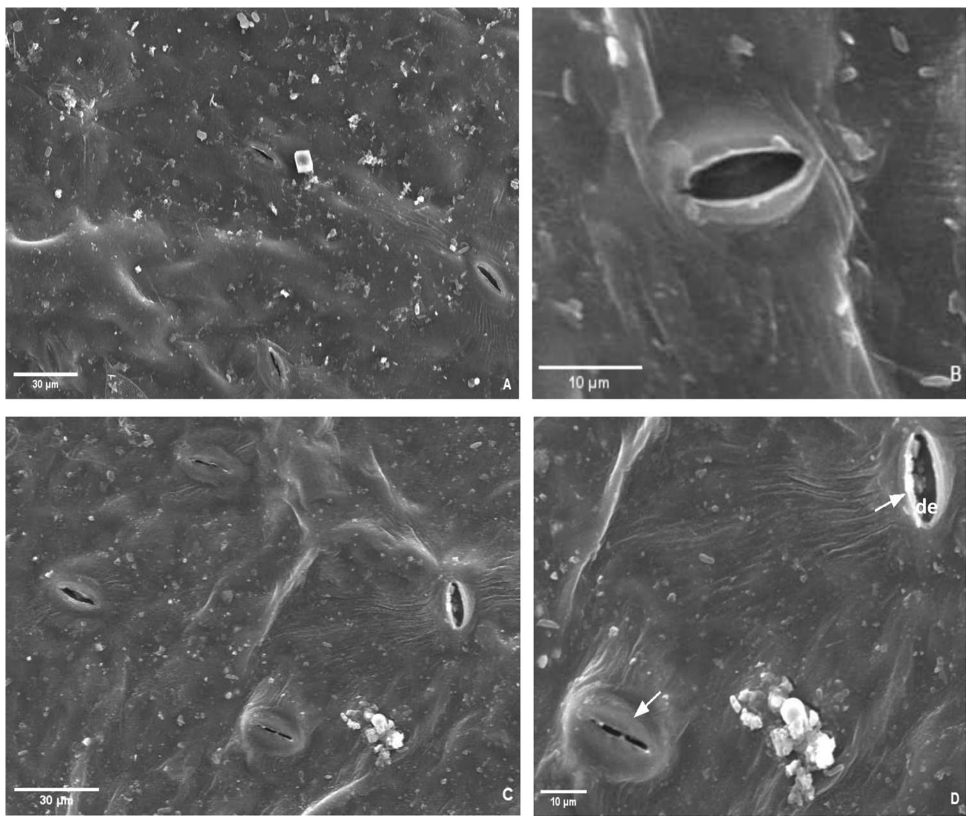

Fig. 5 SEM images of *A. tenella* leaf under control and Cd stress. **A**, **C** Surface view of control and treated leaf epidermis; **B**, **D** Enlarged view of stomata of control and treated roots (de – deposition)

region (epidermis and cortex), a middle region (endodermis and stele) and the inner pith cells.

Root. Carbon (C), Oxygen (O), Potassium (K), Phosphorus (P), and Sulphur (S) were evenly distributed across all regions of both control and Cd-treated roots. However, Cd treatment led to a reduction in C content in the outer and middle regions of the roots, while the inner region remained unaffected. O distribution decreased in the middle and inner regions of Cd-treated plants but increased by 18% in the outer region. Cd treatment significantly enhanced the levels of K and P in the middle region of roots by 94% each. S content decreased in the outer region of Cd-treated roots but showed slight increases in other regions. Iron (Fe) concentration increased in the inner regions of both samples upon Cd treatment. Magnesium (Mg), Aluminium (Al), and Silicon (Si) were present in the outer region of control roots but were undetectable in any regions of Cd-treated roots. Conversely, Copper (Cu) and Sodium (Na) were only detected in the roots of Cd-treated plants, showing a reverse trend (Table 5).

Stem. The number of elements in the stem of control and treated samples was fewer compared to the leaf and root.

Table 5 EDX spectral data of element concentrations (% weight) in outer region (spectrum 1), middle region (spectrum 2), and inner region (spectrum 3) in different parts of control and Cd-treated *A. tenella*

Element	ROOT						STEM						LEAF					
	Control			Cd treated			Control			Cd treated			Control			Cd treated		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
C	63.69	56.04	57.49	55.36	35.48	57.57	53.37	52.96	51.67	56.56	59.22	53.06	56.5	53.42	51.44	59.24	54.91	60.59
K	0.24	1.08	0.9	1.41	19.15	0.61	–	–	–	0.18	–	–	1.24	3.19	2.39	0.4	0.61	0.9
O	33.96	41.75	40.2	41.85	34.52	39.89	42.72	42.66	46.78	41.65	39.89	46.42	41.46	40.18	44.06	39.57	42.8	32.38
Ca	0.31	0.36	0.28	–	–	–	–	–	0.39	0.47	0.46	0.27	0.28	–	–	0.29	0.2	0.77
Cu	–	–	–	0.53	0.98	–	2.58	2.76	1.15	0.87	–	–	–	0.45	0.58	0.51	0.84	2.3
P	0.32	0.5	0.39	0.63	9.11	0.44	–	–	–	–	–	–	–	0.88	0.56	–	0.28	–
Fe	–	–	0.55	–	–	1.13	–	–	–	–	–	–	–	–	–	–	–	1.17
S	0.47	0.26	0.18	0.22	0.35	0.36	–	–	–	0.3	0.43	0.25	–	–	–	–	0.19	0.19
Al	0.31	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	–	–	–
Si	0.49	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16	0.38
Mg	0.22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Na	–	–	–	–	0.42	–	–	–	–	–	–	–	0.39	0.41	0.53	–	–	0.49
Zn	–	–	–	–	–	–	1.33	1.62	–	–	–	–	–	0.8	–	–	–	–
Cl	–	–	–	–	–	–	–	–	–	–	–	–	0.13	0.67	0.3	–	–	0.83

The C content increased in all regions of roots exposed to Cd. The distribution of O decreased in the middle region of Cd-treated roots, with the other two regions showing less significant changes. K was only present in the outer region of treated roots. Ca was solely detected in the inner region of control roots. Cd induced the distribution of Ca in all root regions but decreased in the inner region. The pattern was reversed for Cu. The C content was high in all three regions of control roots, whereas it was only detected in the outer region of Cd-treated plants with a reduced concentration. Zn and S were exclusively present in the roots of control and Cd-treated plants, respectively (Table 5).

Leaf. C, O and K were the macro elements distributed throughout the leaf tissues of both samples (control and Cd-treated). The C content increased in all three regions, with the maximum peak in the inner region by 15%. The distribution of O varied significantly in the control and Cd-treated leaf. While the middle region of the Cd-treated leaf showed a slight decrease in O content, the inner and outer regions exhibited reductions by 4% and 26.5%, respectively, compared to the control. Cd treatment led to a reduction in K content in all regions. Calcium (Ca), which was present only in the outer region of control plants, was found to be distributed in all leaf regions exposed to Cd. Cu content increased upon Cd treatment, while levels of P, Na, Zinc (Zn), and Chlorine (Cl) decreased S, Si, and Fe were present only in the Cd-treated roots (Table 5).

Photosynthetic efficiency

Photosynthetic pigments. Control plants showed an increase in total chlorophyll content throughout the treatment period, whereas Cd-treated plants exhibited a decline compared to control plantlets. A 39% reduction in chlorophyll content was observed on day 21 in *A. tenella* after Cd treatment. The carotenoid contents of both control and treated samples followed a similar trend (Fig. 6).

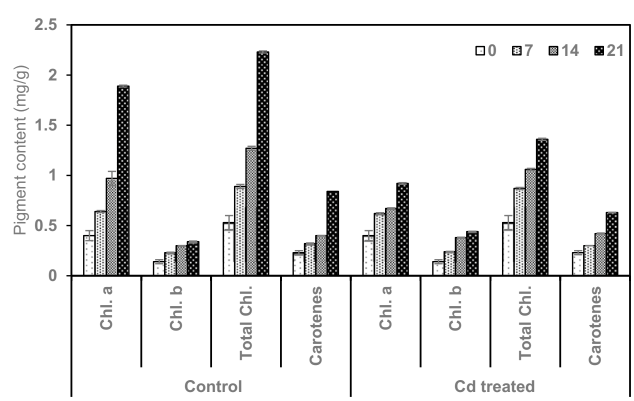

Fig. 6 Graph showing comparison of chlorophyll (in mg/g FW) and carotene content (in mg/g FW) in *A. tenella* under Cd stress at different time intervals. The data are presented with the mean ± SE of five replicates

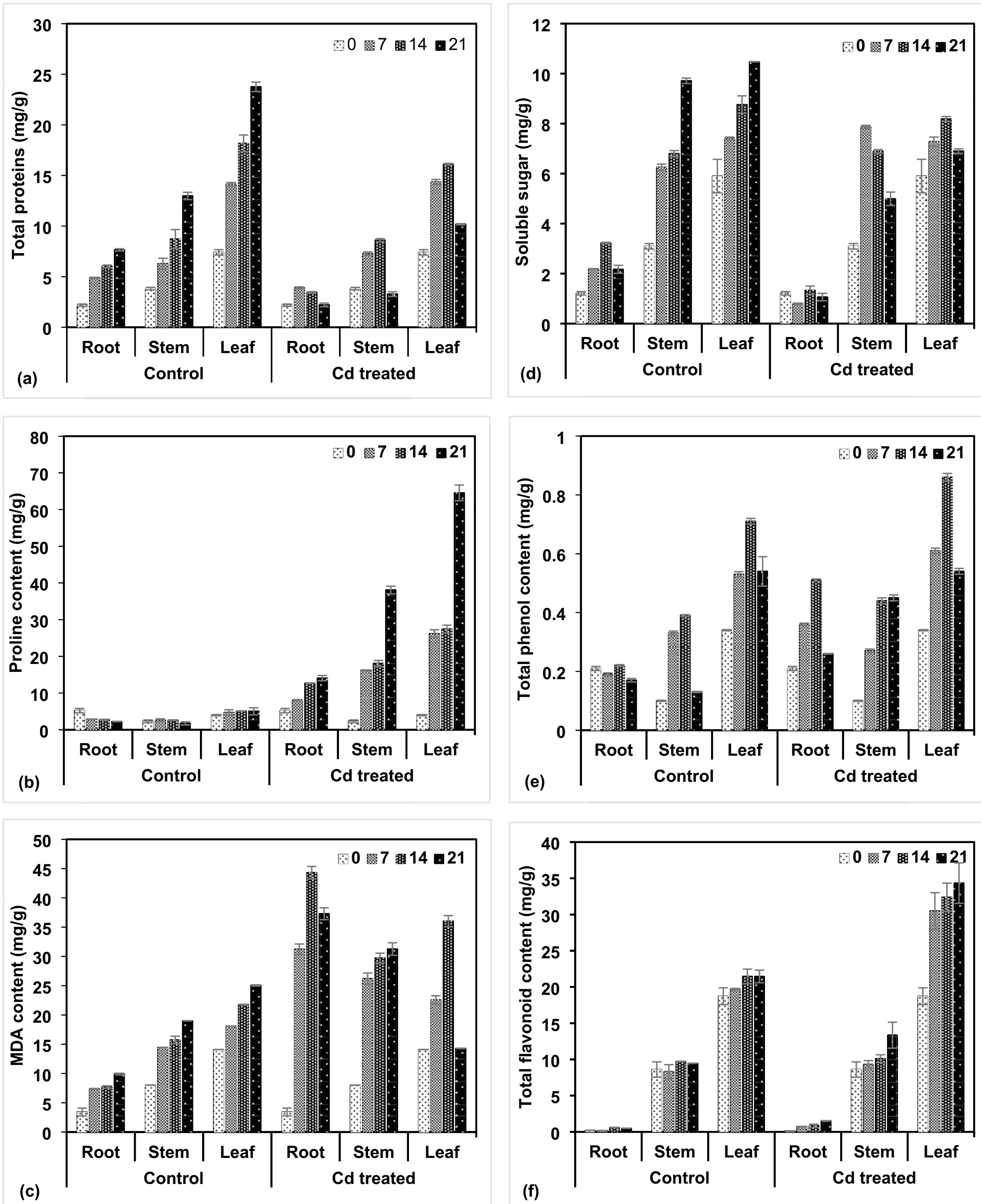

Fig. 7 Total protein (**a**), proline (**b**), malondialdehyde (**c**), soluble sugar (**d**), phenolics (**e**), and flavonoid (**f**) content in various parts of *A. tenella* subjected to Cd stress compared to control. The data are presented with the mean ± SE of five replicates

Metabolites

Protein content. The root, stem, and leaf tissues of the control plantlets exhibited a significant increase in total protein content. On 21d, the total protein content in the roots, stems, and leaves of control plants increased over the treated samples by 42.69%, 1.26%, and 11.3%, respectively. Following Cd treatment, there was an initial increase followed by a subsequent reduction in the total protein content found in the root, stem, and leaf samples (Fig. 7a).

Proline. In comparison to control samples, the proline content significantly increased in all plant tissues exposed to Cd over the treatment period. The proline level in root and stem samples of control plants decreased. In Cd-treated plants, the proline content in the stem and leaf showed a rapid increase within the initial week itself, reaching 95.45% and 92.2% higher levels, respectively, than in control plants by 21d. However, in roots, Cd induced a gradual production of proline compared to control plants, leading to nearly a sevenfold increase in proline content by 21d (Fig. 7b).

Malondialdehyde. Lipid peroxidation induced by metal stress can be quantified by measuring the amount of malondialdehyde (MDA) synthesized in plant tissues. The MDA content increased in all plant parts of both control and treated samples over the stress period. However, the increase in MDA content was significantly higher in tissues of Cd-treated plants compared to control (Fig. 7c).

Soluble sugar. The root tissues of *A. tenella* contained less soluble sugar than the stem and leaves of both control and Cd-treated plants. In control samples, particularly the stem and leaves, there was an increase in soluble sugar content over the treatment period from 0 to 21d, while Cd-treated plants exhibited a gradual decline in soluble sugar content after an initial increase during the early phase (7d). All tissues of Cd-treated *A. tenella* demonstrated a significant decrease in the accumulation of soluble sugar compared to control samples, with the maximum reduction occurring on 21d of stress (Fig. 7d).

Total phenols. The *A. tenella* plant possesses a lower total phenol concentration, with leaf tissues exhibiting a higher phenolic content than other plant tissues. Cd treatment triggered the production of phenolics in the plant. There was no significant variation in the total phenol content of control samples, but in Cd-treated *A. tenella*, the total phenolic content in roots, stems, and leaves increased slightly compared to control plants, with the maximum concentration observed on 14d (Fig. 7e).

Flavonoids. A significant enhancement in flavonoid content was observed in plants subjected to Cd treatment compared to control samples. While a gradual increase in flavonoid levels was recorded in the stems and leaves of control samples, the concentration was comparatively lower compared to that of Cd-treated plants. On 21d, the total flavonoid content in the roots, stems, and leaves of treated plants increased over control plants by 66.8%, 28%, and 37%, respectively (Fig. 7f).

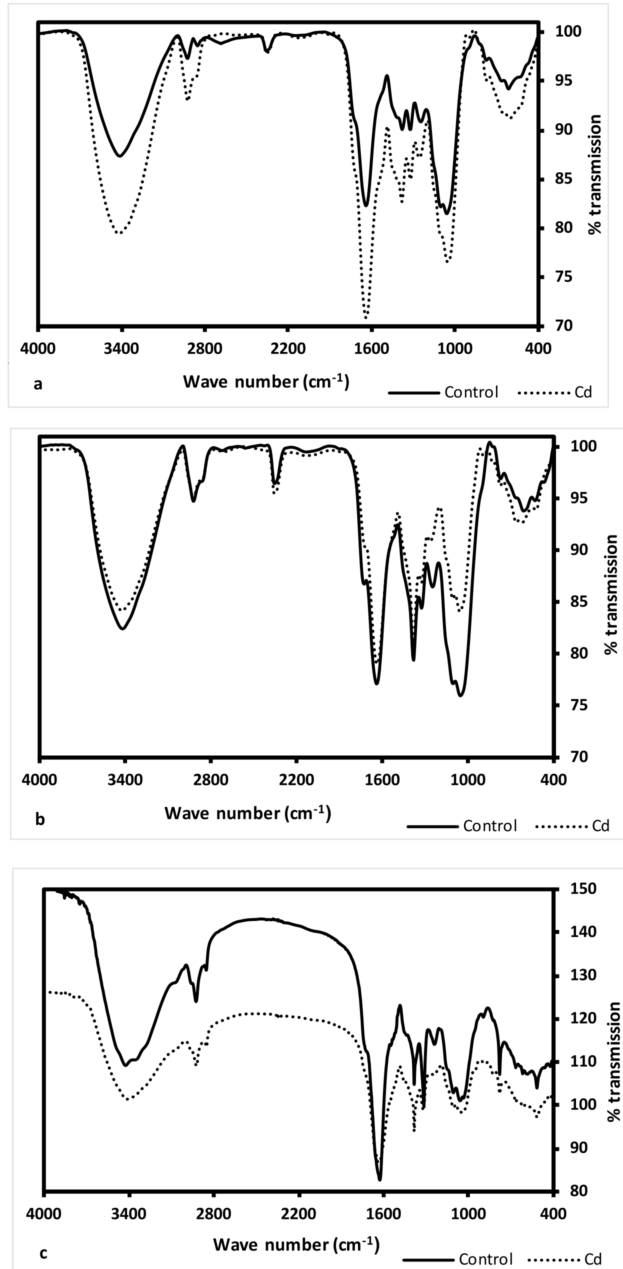

Fig. 8 FTIR spectra of root (**a**), stem (**b**), and leaf (**c**) of *A. tenella* subjected to Cd stress compared to control

Fourier transform infrared (FTIR) analysis

Analyzing the infrared light absorption of biomolecules enables the identification of functional group interactions with transition metals in biological samples, such as phenols, aldehydes, and carbohydrates. In Cd-treated plants, shifts in peak positions were observed across various infrared regions, indicating alterations in the chemical composition and structural properties of biomolecules (Fig. 8a–c). Notable changes were observed in the 3200–3500 cm^{-1}

region, corresponding to N–H groups of primary amines (protein) and O–H groups (alcohols, phenols, carboxylic acids) of lignin, cellulose, saponin, and tannin (Bora and Sarma 2021). Furthermore, alterations were noted in the 2600–2000 cm^{-1} range, characteristic of symmetric stretching vibrations of groups like O = C = O and N = C = N, as well as in the 1600–1670 cm^{-1} range, representing symmetric stretching of C = C. The 1400–1000 cm^{-1} region, characterized by groups S = O, C–O, C–N, and C–F, also exhibited notable alterations. Additionally, changes were observed in the 900–1100 cm^{-1} region, characterized by symmetric stretching of > P⁼O of phosphodiesters, and in the 400–800 cm^{-1} region, attributed to –C–OH stretching vibrations and C–S stretching of disulfide. These shifts collectively indicate significant modifications in the molecular structure and composition of proteins, nucleic acids, phospholipids, and other cellular components in response to Cd stress.

Cd stress induced significant phenotypic and physiological alterations in *A. tenella* plantlets. Notable reductions in the elongation of root and shoot, leaf expansion and overall biomass accumulation in Cd-treated plantlets indicates the impaired growth dynamics under Cd toxicity. Anatomical modifications such as thickening of xylem walls, reduction in xylem diameter, and evident distortion in root and stem structure underscore the impact of Cd stress on plant morphology. Elemental profiling revealed altered distribution patterns of macro and micro-elements within the plant tissues, reflecting disturbances in nutrient uptake and allocation. Biochemical assays indicated reduced chlorophyll content and simultaneous accumulation of stress-related metabolites like proline and malondialdehyde. Moreover, differential Cd accumulation patterns were observed, with roots exhibiting higher metal uptake compared to stems and leaves, suggesting a potential role for phytostabilization mechanisms. These findings highlight the comprehensive effects of Cd stress on *A. tenella*, impacting growth, anatomy, physiology, and biochemistry.

Discussion

Excess HMs in environment induce stress on plants since they have detrimental effects and affects the overall growth and development. Cd is a highly toxic metal that directly influence the morphology, metabolism, anatomy, and physiological activities in plants. *A. tenella* is a profusely growing invasive plant species in the industrial riparian zones. Therefore, evaluating the alterations in the metabolic processes and structure induced by Cd stress, and the ability of *A. tenella* to tolerate Cd become crucial.

Plant growth

The onset of HM toxicity in plants is often marked by visible morphological alterations such as leaf chlorosis, root and shoot growth retardation, wilting, low biomass accumulation, and necrosis. In *A. tenella*, exposure to Cd stress negatively impacted plant growth, leading to reduced root and shoot length, leaf area, and biomass. The toxicity of Cd to plant structure and photosynthetic organs likely contributed to the decrease in net growth of plant height and biomass (Zhao et al. 2021). Inhibition of root length elongation and biomass is an early and prominent effect of Cd toxicity, stemming from decreased mitotic activity in root meristems caused by chromosome aberrations and depolymerization of microtubules in the cell cytoskeleton under Cd stress (Subašic et al. 2022; Shanying et al. 2017). The retardation in plant growth may also result from limited uptake of essential minerals and reduction in the production of photo assimilates (Wang et al. 2008). The negative effects of Cd toxicity are more evident in aerial parts of the plant than in roots, making leaf relative water content (RWC) a potential indicator of the plant's ability to tolerate stress (Waheed et al. 2022). The leaf RWC of *A. tenella* was found to be decreased compared to control plants. The decline in water uptake due to interaction of Cd^{2+} with aquaporin proteins may contribute to the reduction in RWC of treated plants (Shackira and Puthur 2019). *A. tenella* exhibited a low tolerance index value after Cd treatment, indicating that high Cd concentration interferes with cell division and severely hinders root growth. The defence mechanism of a plant incurs a reduction in growth and reproduction (Karasov et al. 2017), suggesting that the decreased growth rate and biomass may serve as a coping mechanism for *A. tenella* to mitigate the deleterious effects of Cd^{2+}. Reduction in growth parameters due to Cd stress have been reported in several plants like *Eruca sativa*, *Erigeron annuus*, and *Vicia faba*, (Waheed et al. 2022; Zhang et al. 2021; Piršelová et al. 2021).

Bioaccumulation of cadmium

In our study, the concentration of Cd was observed to be highest in the roots of Cd treated *A. tenella* (317.25 $mgkg^{-1}$ DW) followed by 87.462 $mgkg^{-1}$ DW in the stem and 72.825 $mgkg^{-1}$ DW in leaves. Typically, Cd concentration in plant parts follows the pattern: roots > stem > leaves (Ahmadpour et al. 2015). Roots are the primary organs to come into contact with Cd in the growth medium. Due to its high mobility, Cd is readily absorbed by roots and transported to shoots through the xylem. However, to mitigate damage to plant tissues and the photosynthetic apparatus caused by Cd, plants often restrict Cd transport to shoots and accumulate it in root cell walls and vacuoles (Hanikenne et al. 2011). The higher Cd concentration in roots compared to aerial parts of the plant is attributed to low translocation indices and Cd accumulation in cell walls and vacuoles (Zhang et al. 2022; Pereira et al. 2018). This serves as a defence mechanism to protect above-ground tissues and enables plants to tolerate Cd toxicity. The elevated Cd accumulation in root tissues suggests *A. tenella*'s potential for phytostabilization of Cd. Similar observations have been made in *Acacia mangium*, *Jatropha curcas*, and *Manihot esculenta* (Taeprayoon et al. 2022). BCF and TF values can be used to assess the ability of plants to translocate heavy metals from soil to plant parts and from root to shoot respectively (Siyar et al. 2022). Plants with BCF values greater than 1 and low TF and bioaccumulation coefficient (BAC) levels indicate phytostabilization potential (González-Chávez and Carrillo-González 2013). In our study, *A. tenella* exhibited a BCFroot value > 1 but TF < 1, supporting its ability for Cd phytostabilization. BAC measures metal tolerance and accumulation in total plant biomass. The higher BAC of Cd-exposed *A. tenella* indicates its tolerance and Cd accumulation capacity, especially in roots. Given its prolific growth, invasive nature, high BCF, and BAC values, along with limited economic utility, *A. tenella* emerges as a promising candidate for Cd-contaminated site phytoremediation.

Anatomical parameters

Significant anatomical modifications were observed in various parts of *A. tenella* in response to Cd stress. SEM images revealed distortion of cell structures, thickening of cell walls, and occlusions in vascular elements and parenchyma cells as major changes due to Cd uptake and accumulation. Distinct tissues such as the epidermis, cortex, vascular region, and pith observed in control roots and shoots became distorted after Cd treatment. Roots and stems exhibited more damage compared to leaves, showing severe deformation of cell structures and the presence of deposits within tissues, consistent with previous studies (Bora and Sarma 2021; Liza et al. 2020). Cd deposition was clearly observed in root tissues of Cd-treated *A. tenella*, indicating predominant Cd retention in the roots. This is similar to the results in *A. ilicifolius*, suggesting the complexation of Cd with phytochelatin in vacuoles to impede vertical Cd transport via xylem (Shackira and Puthur 2019). Limited Cd translocation to aerial parts is attributed to apoplastic Cd fractions in roots and binding to cell walls, serving as a defence mechanism against Cd toxicity. According to previous reports, various polysaccharides like pectin, cellulose, and proteins of the cell wall are responsible for Cd binding to negatively charged sites (Chandrasekhar and Ray 2019; Bora and Sarma 2021). The findings from our FTIR analysis further corroborate the binding of Cd to polysaccharides and proteins, evidenced by the shifting of their respective peak positions in the Cd-treated biomass of *A. tenella*. The thickening and deformation of cell walls act as barriers against HM transport by resisting radial water flow (Pandey et al. 2022). Changes in xylem structure, including depositions and alterations in vessel characteristics, further hinder HM entry and transport to the plant shoot (Yadav et al. 2021). Cd-induced anatomical changes, such as reduced xylem tracheid diameter, can be attributed to lignin deposition in the cell wall, consistent with previous literature (Bora and Sarma 2021). Up-regulation of lignin biosynthesis and lignifying enzymes such as peroxidases under Cd stress suggests a defence response and mechanical adaptation (Rui et al. 2016; Liu et al. 2018). Anatomical changes in roots under Cd stress reduce water and mineral uptake, inhibiting plant growth (Pérez Chaca et al. 2014); hence validating the results of the morphological and EDX studies. In the stem, xylem elements and surrounding tissues are primarily affected sites for HM entry via the vascular system (Pandey et al. 2022). The reduction in stem diameter, number and size of xylem vessels, increased cell wall thickness, number of trichomes and sclerenchyma above the phloem have been reported as an act of defence against Cd stress in plants by several authors (Yadav et al. 2021; Liza et al. 2020; Shackira and Puthur 2019). Limited metal translocation to leaves is a defensive strategy to safeguard the photosynthetic system, with even small doses of HM causing severe anatomical changes, such as reduced cell and vessel size, affecting pigment synthesis and stomatal parameters (Pandey et al. 2022). Most stomata in Cd-treated *A. tenella* leaves appeared closed, a common response to metal exposure (Batool et al. 2015). Stomatal density, size, and partial/full closure decrease under Cd stress. Increased Ca^{2+} levels in roots or abscisic acid accumulation in guard cells induce leaf turgor reduction and stomatal closure, limiting gas exchange (Rucinska-Sobkowiak 2016; Sha et al. 2019). Surprisingly, some stomata were widely opened with thick guard cells and visible depositions, possibly indicating Cd transpiration, making the plant suitable for phytovolatilization (Yan et al. 2020).

Element analysis in tissues

In soil, Cd typically exists as Cd^{2+} ions and Cd-chelates. Its uptake in plants primarily occurs through interaction with absorption sites, often associated with other nutrient minerals such as Ca^{2+} channels found in the guard cells of plasma membranes (Haider et al. 2021). Utilizing the apoplastic pathway, regulated by membrane potential, Cd enters the roots and is subsequently translocated either in its ionic form through transporters or via the ascent of sap into the stelar region of shoots, depending on its concentration (Dong et al. 2019; Kuriakose and Prasad 2008). However, this transportation process, along with the detoxification and storage of Cd within plant cells, triggers anatomical and physiological changes that ultimately hinder the uptake of water and essential nutrients by the roots (Perez Chaca et al. 2014). Studies by Nazar et al. (2012) have demonstrated a decrease in the concentration of essential minerals such as Mg, Ca, and K in various plant species including *Cucumis sativa*, *Lycopersicon esculentum*, *Lactuca sativa*, and *Zea mays*, attributed to Cd exposure. Though the mineral nutrients are essential for plant growth and development, they also get allocated to stress tolerance (including HM stress) in plants under adverse environmental conditions (Jalloh et al. 2009).

Treatment with Cd resulted in a reduction of C content in the roots but a significant increase in the stems and leaves of *A. tenella* compared to control plants. C, a crucial macronutrient, constitutes about 50% of a plant cell's dry weight and is integral to various plant biomolecules such as carbohydrates, proteins, and nucleic acids. The observed increase in soluble sugar content in Cd-treated *A. tenella* leaves suggests a defence mechanism against Cd stress rather than a promotion of plant growth, as sugar translocation from leaves to roots is hindered by Cd, resulting in lower C content in roots (Li et al. 2020). Furthermore, Cd toxicity induces full or partial stomatal closure, inhibiting carbon fixation and reducing photosynthetic activity upon continuous exposure to Cd (Haider et al. 2021). Cd stress induced variations in the distribution of O in all three spectra studied in roots, stems, and leaves. Elevated oxygen levels can enhance plant growth and development by facilitating nutrient uptake and cellular respiration. K levels were significantly higher in the roots of Cd-treated *A. tenella* but slightly decreased in leaves. K^+ ions play various roles in plants, including enhancing root-system proliferation, upregulating antioxidant enzyme activity, maintaining osmoregulation, and activating enzymes involved in metabolic pathways such as nitrogen (N) and C metabolism, sugar transport, photosynthesis, and protein synthesis (Xu et al. 2020; Jia et al. 2008). The increase in K assimilation in roots can be an act of tolerance imparted against Cd stress in the plant. K^+ ions also regulate the opening and closing of stomata (Hasanuzzaman et al. 2018); hence a decrease in K content in the leaves of Cd treated plants can be the reason for closed stomata and reduced photosynthetic activity observed in our study. Changes in the translocation of K and other nutrients to the shoot have been linked to alterations in the vascular system, including reductions in the numbers and diameters of xylem elements (Ouzounidou et al. 1995). P content increased in the roots of Cd-treated plants compared to controls, possibly due to enhanced ATP synthesis from increased mitochondrial activity and antioxidant enzymes (Sarath et al. 2022). Increasing P concentration has been reported to reduce Cd mobility in plants, leading to its accumulation in roots rather than aerial parts (Ma et al. 2022; Jia et al. 2024), which is consistent with the bioaccumulation results obtained using ICP-AES in our study. Interestingly, Ca was detected in control roots but not in Cd-treated plants, and vice versa for stem and leaf samples. Ca, known for its crucial role in signalling pathways, is readily absorbed by roots, translocates vertically, and induces stomatal closure upon reaching the leaves (Schroeder et al. 2001). Studies indicate that Ca can alleviate Cd toxicity by neutralizing negatively charged plasma membrane surfaces, and potentially reducing the influx of Cd (Sarwar et al. 2010).

Among several micronutrients, Cu and Na were present only in the roots of Cd-treated plants, while Mg, Al, Si, and S were present only in control roots. Cu and Zn were present only in control stems, while S was present in Cd-treated stems. Cl, Al, Zn, and Na were present only in control leaves, while Fe was observed only in Cd treated leaves. Cu content increased in Cd-treated leaves compared to controls. The increased S content in *A. tenella* shoots may be attributed to increased levels of phytochelatins or glutathione, which play a role in heavy metal stress tolerance (Gielen et al. 2017). The lower Zn content in the shoots of Cd treated plants compared to controls may be attributed to P-Zn interactions, where Zn deficiencies in leaves enhance P uptake rates by roots and its translocation to the shoots (Gomes et al. 2013). Fe has been shown to alleviate Cd-induced oxidative stress in plants, as it serves as an essential cofactor for antioxidant enzymes like catalase and ascorbate peroxidase, thus maintaining high Fe levels in the roots during peak Cd concentrations can enhance the activity of these enzymes, serving as a crucial defence mechanism against ROS generated by Cd stress (Wang et al. 2013; Sharma et al. 2004). Thus, SEM–EDX data indicate that the effect of Cd on the distribution and content of both macro and microelements in *A. tenella* varies depending on the plant parts.

Photosynthetic efficiency

Photosynthesis is an important physiological process in plants which is severely affected during heavy metal stress. Studies have shown that Cd toxicity impairs the photosynthetic system inhibiting the fixation of carbon and the photosynthesis (Srivastava et al. 2021; Ahanger et al. 2020). Consistent with these findings, our results show a notable decrease in chlorophyll and carotenoid content in *A. tenella*. This decrease correlates with a reduction in plant dry weight, as photosynthesis contributes to biomass production (Li et al. 2020). Reduction in chlorophyll content due to Cd stress has been observed in various plant species such as *Broussonetia papyrifera* and *Erigeron annuus* (Zhang et al. 2020, 2021). The decline in chlorophyll content may be attributed to Cd replacing Mg in chlorophyll molecules or to increased chlorophyllase activity (Shackira and Puthur 2019). Cd-induced degradation of photosynthetic pigments like Chlorophyll a and b reduces the net photosynthetic rate, correlating with dry biomass (Zhao et al. 2021), which is consistent with our findings in *A. tenella*. Carotenoid levels in *A. tenella* showed a similar trend to chlorophyll under Cd stress. The decrease in carotenoid content induced by Cd may result from the inhibition of enzymes in pigment biosynthesis or from overproduction of reactive oxygen species (ROS) affecting carotenoid metabolic pathways (Qian et al. 2009; Mishra et al. 2006).

Metabolites

The total protein content of plant parts of *A. tenella* after Cd treatment showed a decline on 21d after a gradual increase in the initial phase. The initial rise may be attributed to the synthesis of stress proteins such as heat shock proteins (HSPs), phytochelatins, metallothioneins, or antioxidants like glutathione (GSH), which are known to protect plants from metal toxicity (Verma and Dubey 2003; Mishra et al. 2006). Metal stress induces an increase in proteins associated with transcriptional and translational control, antioxidant pathways, biosynthetic metabolism, and molecular chaperones, impacting proteins linked to food metabolism (Jain et al. 2018). Metal accumulation in plants triggers the release of phytohormones such as ethylene and jasmonic acid, as observed in *Phaseolus coccineus* (Pell et al. 1997; Maksymiec et al. 2005). Cd toxicity also stimulates the production of signalling proteins like MAPK, which in turn activate genes responsible for metal transport and degradation (Jonak et al. 2004). An increase in total protein content under Cd stress has been reported in various plant species (Popova et al. 2012; Lee et al. 2010; Semane et al. 2010). However, prolonged exposure to HMs can lead to protein fragmentation and degradation, affecting the structural and functional properties of proteins, thus exerting a detrimental effect on protein composition during extended growth periods (John et al. 2009). Decreased protein synthesis in lettuce is attributed to increased protein degradation and reduced Rubisco activity (Monteiro et al. 2009).

The soluble sugar content in Cd treated *A. tenella* recorded an increase initially followed a decrease after 14d. The initial rise in soluble sugar content may be attributed to increased carbon assimilation, serving as a defence response of the plant against osmotic and oxidative stress induced by Cd. Soluble sugars play a crucial role in ROS scavenging, maintaining osmotic balance in cells, and stabilizing membranous structures under stress conditions (Wang et al. 2021). Our findings regarding soluble sugars align with observations made in mung bean, Kentucky bluegrass and maize (Anwar et al. 2021; Wang et al. 2020; Li et al. 2020). The leaves of Cd-treated *A. tenella* exhibited higher levels of soluble sugars compared to the roots. This disparity may be due to impaired sugar translocation by Cd, leading to reduced transport of sugars from synthesizing sites (leaves) to sink tissues (roots) (Jha and Dubey 2004; Mishra and Dubey 2013). While sugars are primarily utilized for plant growth, they also play a crucial role in maintaining osmotic balance under severe osmotic stress conditions (Li et al. 2020). The decrease in soluble sugar content in *A. tenella* during later stages of stress could be attributed to reduced photosynthesis resulting from damage to the photosynthetic system and a decrease in leaf area (Armendariz et al. 2016; Zhao et al. 2021).

Cd-treated *A. tenella* exhibited significantly higher contents of proline, phenols, flavonoids, and malondialdehyde (MDA), consistent with earlier reports (Ahmad et al. 2015; Hadi et al. 2016). Increased proline accumulation serves as a defence mechanism against environmental stresses, including HM toxicity (Clemens 2006). Proline synthesis is upregulated in leaves under stress conditions, resulting in higher concentrations compared to roots. Proline acts as an effective osmoprotectant and antioxidant defence molecule, playing multiple roles during metal stress such as free radical scavenging, protein stabilization, metal chelation, and protection of membrane integrity (Hosseinifard et al. 2022; Spormann et al. 2023). Increased proline levels in response to Cd stress have been reported in *Medicago truncatula* and *Cucurbita pepo* (Garcia de la Torre et al. 2022; Labidi et al. 2021). Elevated levels of phenolic compounds represent another defence mechanism of plants against heavy metal stress. Plants exposed to heavy metal stress biosynthesize phenols and flavonoids for ROS scavenging and detoxifying hydrogen peroxide (H_2O_2) (Sakihama et al. 2002). Phenolic compounds protect plants from oxidative stress induced by metals and induce anatomical modifications such as cell wall thickening and formation of physical barriers (Michalak 2006). Flavonoids enhance metal chelation, reducing the level of hydroxyl ions (OH^-) in plant cells (Mira et al. 2002). The higher content of phenols and flavonoids in *A. tenella* is consistent with previous studies (Dobrikova et al. 2021; Jańczak-Pieniążek et al. 2022). Oxidative stress induced by Cd modifies proteins and carbohydrates, fluidity and permeability of membranes, and inactivates/damages nucleic acids (Malkowski et al. 2019). Excess production of ROS in response to heavy metal stress leads to lipid peroxidation, increasing MDA content. Therefore, MDA concentration serves as a reliable indicator of physiological stress in plants. Our study showed an increase in MDA content in all parts of *A. tenella*, consistent with findings in Cd-treated Citrus and strawberry (Giannakoula et al. 2021; Muradoglu et al. 2015).

Fourier transform infrared (FTIR) analysis

FTIR profiling of *A. tenella* biomass serves as a tool for identifying potential plant metabolites involved in Cd binding. Through analysis of the infrared spectra, it becomes possible to discern metal-induced alterations in the structure of biomolecules within the samples. Comparison of FTIR profiles between control and Cd-treated biomass revealed interactions between the metal and various functional groups such as O–H, N–H, C–H, C≡C, C = O, P = O, –C–OH, and C–S present in different metabolites. This result is in conformity with previous studies (Bora and Sarma 2021; Su et al. 2017). The peaks and their corresponding shifts due to metal interaction in the plant parts of *A. tenella* that was found to be consistent in all tissues were studied in detail. After exposing to Cd for 21d, the absorption peak at 3412 cm^{-1} for root control (RoC) representing the hydroxyl group (–OH) corresponding to phenols and alcohols shifted to a higher frequency region (3419 cm^{-1}) in treated roots (RoT). In stem, absorption peak at 3419 cm^{-1} for control (SoC) was shifted to 3423 cm^{-1} for stem treated (SoT). The interaction of Cd^{2+} ions with the aliphatic primary amine group for metal oxygen binding produces these shifts. RoC showed a band at 1641 cm^{-1} shifting to 1639 cm^{-1} for RoT, from 1640 cm^{-1} for SoC to 1635 cm^{-1} for SoT, 1627 cm^{-1} for LoC to 1638 cm^{-1} region for LoT. These shifts were due to the cationic interaction of Cd with carbonyl groups (ketones/aldehydes). There were decrease in bands intensity from RoC at 1060 to 1052 cm^{-1} for RoT, SoC from 1054 to 1057 cm^{-1} for SoT, LoC from 1060 at 1051 cm^{-1} LoT. Strong metal binding with C–C, C–O, and C–O–P causes these shifts (Sheng et al. 2004). The band shifts correspond to the symmetric bending vibration of C–H and –COOH mainly from hemicellulose, cellulose and pectin on the cell wall on interaction with metal ions (Sruthi and Puthur 2019). A major shift in the absorption peak was recorded from 2145 cm^{-1} for RoC to 2125 cm^{-1} for RoT which characterizes the N = C = N (carbodiimide) stretch, which is important for the preparation of many functional groups including carboxylic acids. According to Panda et al. (2007), mostly amino sugars mediate the interactions between plant biomass and the transition metals. The cations H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, and Fe^+ can be found at these sites. So, the presence of metals like Cd may lead to metal substitution (Schneider et al. 2001). Hence, in *A. tenella*, Cd interact with plant biomass through carbonyl and amine groups. Kumar et al. (2017) and Panda et al. (2007) have suggested that the modifications to the structural moieties of lignin, cellulose, and proteins by the interaction of their functional groups and transition metals have an impact on the plant growth, development, photosynthetic, and antioxidant activities. Finally, FTIR results showed a consistent decrease in band intensity of RoT as compared to SoT that was comparable with the ICP-AES result, which showed that *A. tenella* adsorbs more Cd in the root with subsequent translocation to the stem and leaf. Wang et al. (2009) have reported the ten-fold higher concentration of Cd in plant roots than the aboveground tissues that indicates the role of roots as a barrier in Cd translocation, and this efficacy of the roots is attributed to the functional groups available in the cell wall like hydroxyl, carboxyl groups that interact and form complexes with the heavy metals (Shah et al. 2019; Nishizano et al. 1987).

Conclusion

Morphological, anatomical, and physiological modifications were induced on treatment with Cd in *A. tenella* which enable the plant to withstand metal toxicity. Growth retardation and the changes in distribution pattern of macro and micro elements indicates the reallocation of resources to stress tolerance for survival under adverse environmental conditions. The fully opened stomata with thick guard cells and depositions, the production of metabolites like stress proteins, proline and phenols, increase in the thickness of xylem elements and occlusions in the tracheary elements of stele clearly demonstrated the mechanisms of *A. tenella* to cope with Cd stress. The availability of functional groups like carbonyl and amine groups to interact with the Cd^{2+} ions modify the biomolecules, thereby enhancing metal absorption and tolerance. The accumulation of Cd in the root cells limiting its translocation to aboveground tissues reveals the phytostabilization potential of the plant. *A. tenella* is a suitable candidate for the mitigation of Cd from the contaminated soil or water and even for the revegetation of such sites.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11738-024-03700-5.

Acknowledgements K.A. Firdous acknowledge the CSIR-SRF fellowship (CSIR-SRF Grant No. 08/592(0007)/2020-EMR-I) from Council of Scientific & Industrial Research (CSIR), New Delhi. The authors acknowledge the technical assistance provided by Sophisticated test and Instrumentation Centre, Cochin university of science and technology, Ernakulam, Kerala for the SEM-EDX analysis.

Author contributions KAF and MSR conceived the idea and designed the experiments. KAF and KPN collected and analysed the data with assistance from MSR. KAF led writing with input from MSR. KAF, MSR and PJV reviewed and edited further details of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Science and Engineering Research Board (SERB) India, under DST-SERB SURE grant project no. SUR/2022/003505.

Availability of data and materials Data will be made available upon request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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