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Research article

Cadmium accumulation by *Phragmites australis* and *Iris pseudacorus* from stormwater in floating treatment wetlands microcosms: Insights into plant tolerance and utility for phytoremediation

Muhammad Mohsin [a,b,*], Nicole Nawrot [b], Ewa Wojciechowska [b,**], Suvi Kuittinen [a], Katarzyna Szczepańska [c], Grażyna Dembska [c], Ari Pappinen [a]

[a] *School of Forest Sciences, University of Eastern Finland, Yliopistokatu 7, P.O. Box 111, 80100, Joensuu, Finland*
[b] *Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Narutowicza 11/12, 80-233, Gdansk, Poland*
[c] *Department of Environmental Protection, Gdynia Maritime University Maritime Institute, Gdynia, Poland*

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ABSTRACT

Environmentally sustainable remediation is needed to protect freshwater resources which are deteriorating due to severe industrial, mining, and agricultural activities. Treatment by floating wetlands could be a sustainable solution to remediate water bodies. The study aimed to examine the effects of Cd on *Phragmites australis* and *Iris pseudacorus* growth (height, biomass, root length and chlorophyll contents), anatomy, Cd accumulation in their biomass and their ability to remove Cd, N and P. Seedlings of both plants were grown in a greenhouse for 50 days in artificially prepared stormwater amended with Cd, N, and P. The treatments were: control (Cd _0), Cd_1, Cd_2, and Cd_4 mg L^{-1}. N and P contents were 4 mg L^{-1} and 1.8 mg L^{-1}, respectively. In the case of *P. australis*, the maximum plant height, root length, and total dry biomass production was increased in medium dose (Cd_2) treatment while the chlorophyll index (CCI) increased in high dose (Cd_4) treatment as compared to all treatments. For *I. pseudacorus*, the maximum plant height and total dry biomass production, root length and CCI values were improved in low dose (Cd_1) and high dose (Cd_4) treatments, respectively among all treatments. Results showed that *P. australis* accumulated 10.94–1821.59 μg · $(0.05\ m^2)^{-1}$ in roots and 2.45–334.65 μg · $(0.05\ m^2)^{-1}$ in shoots under Cd_0, Cd_1 and Cd_4 treatments. *I. pseudacorus* accumulated the highest Cd in roots up to 5.84–4900 μg · $(0.05\ m^2)^{-1}$ and 3.40–609 μg · $(0.05\ m^2)^{-1}$ in shoots under Cd_0, Cd_1 and Cd_4 treatments. The translocation factor was observed as <1 and the bioconcentration factor >1 for both species, which indicates their phytostabilization potential. Results demonstrate that *P. australis* and *I. pseudacorus* are suitable for use in floating wetlands to remediate contaminated sites.

1. Introduction

Over the last century, urban stormwater pollution has affected the world's surface water by 30–50% and become a predominant reason for river ecosystem degradation and urban water quality deterioration (Wang et al., 2022). Stormwater runoff consists of nutrients, coliforms, heavy metals, toxic chemicals (e.g., polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls, organochlorines) and visually apparent components such as debris, litter, and sediment. These stormwater contaminants can present risks to animals, plants, and humans as well as technical and aesthetic problems (Malaviya and Singh, 2012). Potentially toxic elements (PTEs) (like Pb, Zn, Cu and Cd) as well as nutrients are commonly reported as typical contaminants present in stormwater runoff (Liang et al., 2013). In urban areas, the runoff carries several contaminants, the type and loads of which are connected to catchment characteristics, land use, rainfall intensity and duration, air pollution etc. Due to dry deposition some atmospheric contaminants, including heavy metals, settle on streets and urban lands and are thereafter washed off with stormwater runoff (Al Masum et al., 2022; Malaviya and Singh, 2012). Urban stormwater runoff poses a threat to the global marine environment (Wang et al., 2021). Pollutants washed off urban surfaces, for example, can have a negative impact on

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* Corresponding author. School of Forest Sciences, University of Eastern Finland, Yliopistokatu 7, P.O. Box 111, 80100, Joensuu, Finland.;

** Corresponding author.

E-mail addresses: muham@uef.fi (M. Mohsin), esien@pg.edu.pl (E. Wojciechowska).

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the quality of urban water (Liu et al., 2016). Therefore, mitigating urban stormwater pollution is essential in order to achieve sustainable development in society.

About 97% of the Baltic Sea area is eutrophicated (high N and P loads), and individual load of these nutrients in seawater are N (78%) and P (95%). According to Wojciechowska et al. (2019) and Zima (2019), the most important dispersed sources of these nutrients include agriculture (45% of the N load and 45% of the P load) and municipal sewage (12% of the N load and 20% of the P load). For example, the semi-closed parts of the Baltic, like the Gulf of Finland and the Gulf of Gdansk as well as many inland lakes are suffering from eutrophication, which is a well-known and persistent problem. Moreover, heavy metals are also recognized as the major pollutants in aquatic ecosystems (Antoniadis et al., 2019). Among them, Cd plays a significant role in soil and water pollution and poses major risks to human health. Several anthropogenic sources including the burning of fossil fuels, mining operations, sewage sludge, and the use of phosphate fertilizers are responsible for Cd supplementations in the ecosystem (Li et al., 2016; Liu et al., 2020). These contaminants can pose risk to drinking water supplies recreational areas, and agricultural production systems (Liang et al., 2013). To protect the final receivers of wastewater and stormwater, nutrient and heavy metal run-off must be reduced more efficiently, particularly in the agriculture and industrial sectors (Finnish Environment Institute, 2019). Therefore, an economically, and environmentally friendly approach needs to be developed to remediate the contaminated water to protect inland waterways globally.

Phytoremediation provides an environmentally sustainable, publicly demandable, and cost-efficient solution to remediate polluted soils and water as well as ensure the circular use of metals to secure supply (Mohsin et al., 2022). In this context, wetland technology has received significant attention as an alternative solution to conventional wastewater treatment systems due to lower energy consumption, minimum maintenance requirement, and moderate capital cost (Shahid et al., 2019). Floating treatment wetland (FTW) is a nature-based method used to remediate different wastewaters. In this method, naturally emergent macrophytes develop their root systems hydroponically to eliminate metals from water/wastewater (Barco et al., 2021; Colares et al., 2021). Moreover, FTW has been applicable in reducing nutrient loads originating from municipal, industrial, agricultural, and stormwater runoff (White, 2021). FTW has been also acknowledged as a landscape improver, habitat for birds and fishes as well as a barrier for the littoral zone (Afzal et al., 2019). At the same time, this technology is easy to apply to pre-existing water bodies, like retention tanks for stormwater in urban areas.

Macrophytes are aquatic plants that have proven to be crucial in the removal of heavy metals from wastewater (Kurniawan et al., 2021). *Phragmites australis* (common reed) has been reported widely to remediate Cu, Ni, Zn, Cr, Cd, and Pb (Ghassemzadeh et al., 2008; Nawrot et al., 2021b; Rahman and Hasegawa, 2011). *P. australis* is a common species on almost all continents. Its habitat includes climate zones ranging from cool to tropical and arid. For all European countries, this species is recognized as native (Packer et al., 2017). It has been planted extensively in constructed wetlands for the remediation of wastewater and effluents (Anning et al., 2013). In urban areas, it is applied to create green zones, due to increasing awareness of sustainable techniques (Nawrot et al., 2021a). *Phragmites* species have also been highlighted as a potential feedstock for bioenergy production, in terms of vigorous biomass production on degraded or marginal wastelands (Young et al., 2011). Moreover, *Iris pseudacorus* (yellow flag) is indigenous to Europe, western Asia, and northwest Africa, and has shown potential to remediate different wastewaters (Yousefi and Mohseni-Bandpei, 2010) and improve the quality of inland waters. *I. pseudacorus* is an ornamental plant that can be grown for both urban landscaping (parks, forests, and vertical gardens) and wastewater remediation (Kwon et al., 2021).

However, the literature contains fewer research findings about these species with a particular emphasis on nutrient uptake and translocation in FTW technology (Garcia Chance et al., 2019). Furthermore, the increasing demand for plant biomass, to be utilized as a substitute for fossil sources offers immense potential to generate low-cost revenue streams for low-income communities, as well as initiate a new, scalable, and more environmentally sustainable source of protein and fiber (Rodriguez-Dominguez et al., 2022). Moreover, nutrient-enriched plant biomass could be used in biorefineries in a circular economy approach to produce valuable biobased products and fertilizers to enhance bioeconomy development (Mohsin et al., 2021).

In this context, the study aimed to investigate a) the effects of Cd on *P. australis* and *I. pseudacorus* height growth, root length, biomass production, and chlorophyll content index, b) the effect of Cd dose on nutrients removal ability of FTWs with *P. australis* and *I. pseudacorus*, c) Cd accumulation in *P. australis* and *I. pseudacorus*, and d) the impact of Cd on *P. australis* and *I. pseudacorus* tissue development. The study results offer new insights on Cd uptake and tolerance by two plant species widespread throughout the temperate climate zone, which have potential to be applied for phytoremediation of water bodies using FTW.

2. Materials and methods

2.1. Plant culture and phytoremediation experimental design

New cuttings of two native macrophytes in Poland (*P. australis* and *I. pseudacorus)* were ordered from a water nursery dealing with aquatic and hydrophilous plants cultivation. During transport, cuttings were protected against damage to the stems and roots and access to water. The species selection was established based on their specific features such as high tolerance towards PTEs (especially documented for *P. australis*, but also *Iris* species such as *Iris versicolor*), fast growth, and the ability for PTEs uptake. Cuttings were washed thoroughly with tap water to remove all visible soil particles which were stored in a box pallet and then finally washed with distilled and redistilled water according to the procedure described by Shamshad et al. (2015). New cuttings of *P. australis* and *I. pseudacorus* were transplanted (in the number of three cuttings in each reactor) and subjected to acclimatization in plastic containers (15 L, 40 cm height, 35 cm diameter) with tap water in a greenhouse within approx. 2 months (April and May 2021). The islands were created using a plastic 0.05 m^2 floating mat and the achieved coverage ratio was 50%. Floating mats were formed from plastic baskets with PE foam ensuring buoyancy. After the acclimatization period, eight reactors presented in Fig. 1 were loaded with 10 L of synthetic wastewaters containing nitrogen and phosphorus (the initial ratio of total nitrogen to total phosphorus–N_{tot}:P_{tot} was established as 4:1; in real concentration 7.5 mg/L of N_{tot} and 1.8 mg/L of P_{tot} as P-PO_4^{3-} were prepared). To six reactors (II. – IV.) additional doses of Cd were added (in the initial concentrations II. 1 mg/L, III. 2 mg/L, and IV. 4 mg/L). The stock solution was prepared as a mixture of ammonium nitrate NH_4NO_3, potassium dihydrogen phosphate KH_2PO_4, and Cd trace standard (10,000 μg/mL ICP, Tusnovic Standard).

The experiment lasted for 50 days (June 2nd and July 22nd, 2021) when liquid samples were collected five times (at the beginning of the experiment, after 10, 20, 35, and 50 days). At the beginning and the end of the experiment, plant tissues were collected for analyses of Cd concentrations. The experiment was carried out in a completely randomized design to simulate the natural environment, with natural light and temperature conditions representative of Gdansk, Poland (temperate climate). A transparent plexiglass cover was placed over the experimental reactors to keep rainwater out. The experiment was conducted under a roof in a greenhouse from the beginning of June 2021. The temperature in the greenhouse with open windows and doors was 3–6° higher than the outside temperature. The archival data on the temperature outside and in the greenhouse are presented in Fig. S1 (meteoblue.com.pl).

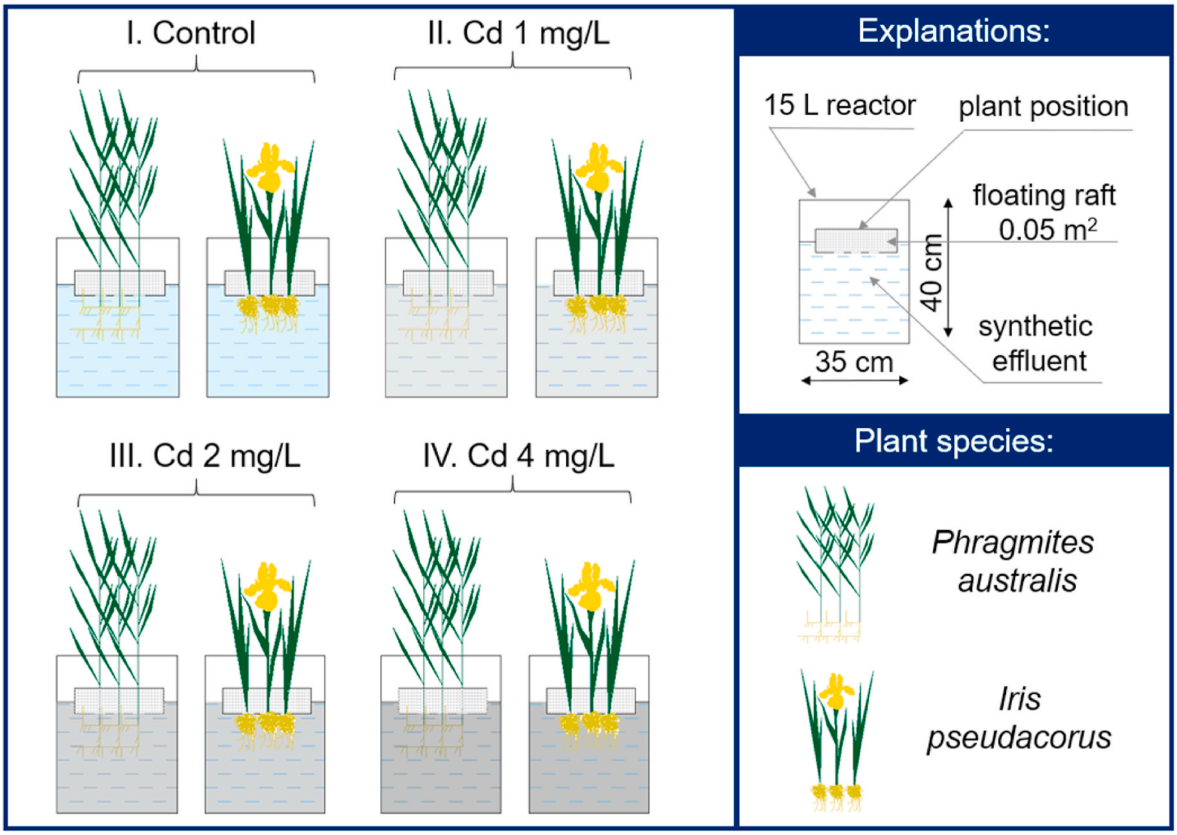

Fig. 1. The testing stand consisted of 8 reactors with *P. australis* and I. pseudacorus placed in floating rafts under different doses of Cd: I. Control, II. Cd_1 (1 mg/L of Cd), III. Cd_2 (2 mg/L of Cd), and IV. Cd_4 (4 mg/L of Cd).

2.2. Chemical analysis

2.2.1. Plants

Cd accumulation in the *P. australis* and *I. pseudacorus* were assessed in min and max Cd treatments 1 mg L^{-1} and 4 mg L^{-1}, respectively. The collected biomass (shoots and roots) samples of *P. australis* and *I. pseudacorus* for chemical analyses were preliminarily cleaned with distilled and redistilled water (Milli-Q Ultrapure Water System, Merck) and then separated into roots and rhizomes (below water parts – BWP), as well as stems and leaves (above water parts – AWP), was performed. Split BWP and AWP parts were placed in Petri dishes and dried at 40 °C to a constant weight. Then, the plant parts were weighed and ground in a mill (Mill Mix 20, Domel). The dried biomass harvested from each reactor was given in relation to the area of the artificial island (0.05 m^2). The accumulation of Cd was established in reference to concentration [mg/kg d. w.] and to harvested dried biomass [g].

Sample digestion was performed in a Multiwave 3000 Microwave Digestion System (Anton Paar) using nitric acid (65.5%, Emplura, Merck), hydrochloric acid (36%, Suprapur, Merck), and hydrogen peroxide solution (30.9%, for ultrarace analysis, Merck). Preparation of blanks and samples of certified reference material (CRM, LGC7162, Strawberry Leaves, LGC Standards) were subjected to the same digestion procedure as *P. australis* and *I. pseudacorus* samples. After the digestion procedure, the solutions were transferred quantitively to 25 mL volumetric flasks and brought up to full volume with deionized water. Determination of Cd content in prepared samples was performed via the ICP–OES technique using spectrometer Agilent 5800 VDV. Analysis accuracy was determined by CRM and the obtained results from four independently prepared CRM samples were compared with certified values. All results fell within the uncertainty interval of the manufacturer. Accuracy was expressed as a percentage recovery and the mean value of this parameter was 99.4%.

2.2.2. Liquid samples

During the growth period, liquid samples were analysed for conductivity (μS/cm), pH, temperature (°C), redox (mV), and dissolved oxygen (%) from the reactors planted with *P. australis* and *I. pseudacorus* using the multi-parameter meter (model HQ4300, Hach Lange). These measurements are depicted in the supplementary materials (S1 and S2). N_{tot} and P_{tot} concentrations were analysed with a DR3900 (Hach Lange) spectrophotometer using cuvette tests: LCK138 (according to EN ISO 11905–1 Persulphate mineralization) and LCK349 (according to EN ISO 6878 Phosphomolybdenum blue method). Liquid samples for Cd analyses were collected using a pure syringe, filtered with Millipore filters (0.45 μm), and stored in PP tubes with a nitric acid HNO_3 65% (Suprapur, Merck) addition (100 μL of HNO3 per 10 mL of the sample). The PP tubes were kept in cool conditions (8 °C). The Cd content in the liquid samples was determined via the ICP-OES method using an Agilent 5800 VDV spectrometer. Blank samples were measured according to the same procedure. To determine analysis accuracy, an LGC6027 certified reference material (LGC Standards) was used. The mean recovery value was 99.8%.

2.3. Plant development assessment

2.3.1. Light microscopy procedure

Semi-thin (0.5–1 mm) sections of plant tissues were cut with a razor blade and placed in a watch glass in redistilled water (Milli-Q Ultrapure Water System, Merck). The cross-sections were soaked for 2–3 min in water and then transferred to the staining solution and immerse for 1 min. Toluidine blue was used to differentiate tissue structures (Parker et al., 1982). After staining, sections were rinsed in redistilled water, placed on a clean glass slide with a drop of water, and covered with a coverslip. Light microscope (Biolar, PZO Poland) at powers up 5x, 10× magnification of lens and 10× magnification of eyepiece was used to observe roots, stems, and leaves cross-sections.

2.3.2. *Chlorophyll content index (CCI)*

A chlorophyll content meter device (CCM-200 Plus, Opti-Sciences) was used to calculate the relative chlorophyll content. It has a measurement diameter of 9.5 mm, and it is calculated using an index value of the chlorophyll content (CCI), giving a ratio of optical absorbance at 653 nm and 931 nm. The meter provides non-destructive measurements of plants during their growth and cultivation. One sample consisted of a leaf apex of 0.7 cm^2. Measurements were taken for each replicate of each treatment following the methodology proposed by Ling et al. (2011) and Higbie et al. (2010).

2.3.3. *Plant tolerance index and phytoextraction efficiency*

The tolerance index for mean dry biomass, root length, and stem height were calculated to evaluate the plants growth capability under metal stress according to the following equations (1)–(3) (Patra et al., 2020).

$$Dry\ Biomass\ Tolerance\ Index\ (DBTI) = \frac{Dry\ biomass\ of\ treated\ plant\ [g]}{Dry\ biomass\ of\ control\ plant\ [g]} \bullet 100\ [\%] \tag{1}$$

$$Stem\ Length\ Tolerance\ Index\ (SLTI) = \frac{Stem\ length\ of\ treated\ plant\ [cm]}{Stem\ length\ of\ control\ plant\ [cm]} \bullet 100\ [\%] \tag{2}$$

$$Root\ Length\ Tolerance\ Index\ (RLTI) = \frac{Root\ length\ of\ treated\ plant\ [cm]}{Root\ length\ of\ control\ plant\ [cm]} \bullet 100\ [\%] \tag{3}$$

Phytoextraction efficiency such as translocation factor was calculated by dividing the Cd concentration in shoots by the Cd concentration in roots (equation (4)) and bioconcentration factor was determined by dividing the Cd concentration in roots by the Cd concentration in hydroponic solution (equation (5)) as followed by Mohsin et al. (2022).

$$Translocation\ Factor = \frac{Cd\ concentration\ in\ ABW[mg/kg\ d.w.]}{Cd\ concentration\ in\ BWP} \tag{4}$$

$$Bioconcentration\ Factor = \frac{Cd\ concentration\ in\ BWP[mg/kg\ d.w.]}{Cd\ concentration\ in\ hydroponic\ solution\ [mg/L]} \tag{5}$$

2.4. *Statistical analysis*

Analysis of variance (ANOVA) was run using SPSS software (IBM version 27, NY, USA) to examine the effects of Cd treatments on studied plant species growth parameters (height, biomass production, root length and chlorophyll content index) and Cd accumulation at a significance level $p < 0.05$ followed by the Tukey (HSD) test. The results are presented as the means ± standard error (SE) of three replicates.

3. Results

3.1. *Growth parameters assessment and tolerance indices*

Growth parameters of *P. australis* and *I. pseudacorus* including mean growth height, root length, and CCI are shown in Table 1. *P. australis* showed no significant difference for growth height in control, Cd_2 and Cd_4 treatments excluding Cd_1 which exhibited a minor decline in growth height while for BWP the maximum root length growth was observed in Cd_2 treatment followed by control, Cd_1 and Cd_4. For *I. pseudacorus,* the greatest plant height and root length were noted in Cd_1 and Cd_4 treatments, respectively. The lowest height and root length were observed in the control. The lowest CCI value was observed in the control for *P. australis* and *I. pseudacorus* (0.5 and 0.93, respectively). However, both species presented a linear increase in CCI value under Cd treatments as compared to the control.

Table 1

Effects of Cd on *P. australis* and *I. pseudacorus* mean height (cm), root length (cm) and CCI values for 50 days (mean ± SE, n = 3). Small different alphabetical letters indicate a significant difference between treatments for each species separately.

Species	Treatment	Plant height [cm] AWP	Root length [cm] BWP	CCI value [-]
P. australis	Control	56.29 ± 3.26^{ab}	9.40 ± 1.01^{a}	0.50 ± 0.04^{a}
	Cd_1	49.40 ± 1.47^{a}	7.30 ± 0.48^{b}	1.40 ± 0.12^{b}
	Cd_2	57 ± 1.30^{ab}	9.70 ± 1.11^{a}	2.30 ± 0.20^{c}
	Cd_4	55.80 ± 1.11^{b}	6.90 ± 1.46^{b}	3.30 ± 0.29^{d}
I. pseudacorus	Control	37.20 ± 1.74^{a}	9.30 ± 0.94^{a}	0.93 ± 0.08^{a}
	Cd_1	56.40 ± 2.01^{b}	13.94 ± 2.46^{a}	1.90 ± 0.17^{b}
	Cd_2	51.80 ± 1.66^{c}	11.62 ± 2.91^{a}	2.80 ± 0.25^{c}
	Cd_4	55.80 ± 4.51^{bc}	14.06 ± 4.22^{a}	3.72 ± 0.34^{d}

AWP: above water parts; BWP: below water parts.

In the case of dry biomass production, Fig. 2A illustrates that *P. australis* produced the highest total dry biomass (6.80 g) in the medium-dose Cd treatment (Cd_2), which demonstrated a 43% increase compared to the control. The lowest total dry biomass (3.05 g) was noticed in the high dose Cd treatment (Cd_4) which was comparatively 36% lower than the control. However, a significant difference was observed among all treatments. For *I. pseudacorus*, the highest total dry biomass (6.13 g) was noticed in low Cd dose treatment (Cd_1) and, when compared with the control, the biomass was about 55% more (Fig. 2B).

Furthermore, both species have shown a remarkable propensity towards DBTI, SLTI, and RLTI (Table 2). Under Cd load, *P. australis* presented the highest DBTI and RLTI in medium dose Cd treatment (Cd_2) and RLTI in low dose Cd treatment (Cd_1). In the case of *I. pseudacorus*, the highest DBTI and SLTI were noticed in low dose Cd treatment (Cd_1) and RLTI in high dose Cd treatment (Cd_4).

3.2. *Nutrients removal from hydroponic solutions with different Cd doses*

N_{tot} removal by *P. australis* and *I. pseudacorus* under Cd loads within days (0, 10, 20, 35, and 50) intervals are independently shown in Fig. 3. Fig. 3A indicates that the highest N_{tot} removal (81%) was observed in the reactor with *P. australis* on day 35 in the control as compared to the initial N_{tot} dose (7.5 mg/L). Furthermore, N_{tot} removal in different treatments in 10–50 days were 57–81% (Control), 37–48% (Cd_2), 21–33% (Cd_1), and 20–24% (Cd_4) compared to their initial N_{tot} doses. In the case of reactors with *I. pseudacorus*, the highest N_{tot} removal (80%) was observed on day 50 in the low dose Cd treatment (Cd_1) as compared to the initial N_{tot} dose (7.5 mg/L). Overall, an expressive N_{tot} removal was noticed on day 50 in the medium and high dose Cd treatments such as 76% (Cd_2) and 59% (Cd_4) excluding the control, which showed the highest removal (73%) on day 20 concerning their N_{tot} initial doses (Fig. 3B).

Moreover, P_{tot} removal by *P. australis* and *I. pseudacorus* under Cd load with days (0, 10, 20, 35, and 50) intervals is depicted in Fig. 4. *P. australis* has shown an almost similar P_{tot} removal trend in low and medium Cd dose treatments (Cd_1 and Cd_2) in 10–35 days except for control and high dose Cd treatment (Cd_4). Nevertheless, the P_{tot} removal was distinct on day 50 in all the treatments. The control (54%)

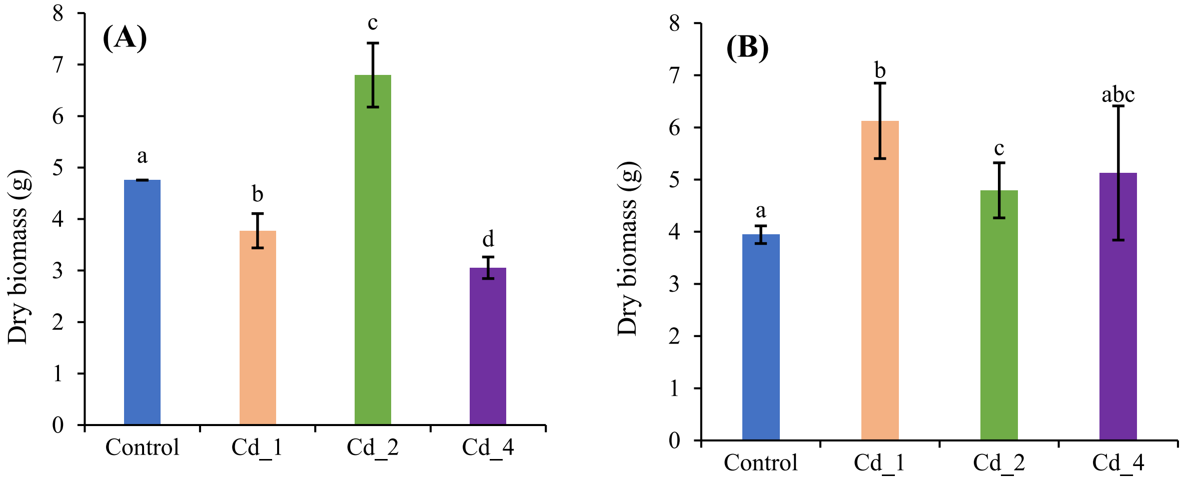

Fig. 2. Total mean dry biomass production (sum of shoots + roots) by *P. australis* (A) and *I. pseudacorus* (B) grown under different doses of Cd (Mean ± SE, n = 3) for 50 days. Small different letters indicate a significant difference between Cd treatments at $p < 0.05$.

Table 2
Effects of Cd treatments on *P. australis* and *I. pseudacorus* tolerance indexes for 50 days.

Species	Treatment	DBTI [%]	SLTI [%]	RLTI [%]
P. australis	Cd_1	79	**106**	78
	Cd_2	**143**	101	**103**
	Cd_4	64	99	73
I. pseudacorus	Cd_1	**155**	**152**	150
	Cd_2	122	139	125
	Cd_4	130	150	**151**

DBTI - dry biomass tolerance index; SLTI - stem length tolerance index; RLTI - root length tolerance index.
Bold – values indicate the highest tolerance indices.

> Cd_4 (27%) > Cd_1 (18%) > Cd_2 (10%) once compared with the initial P_{tot} doses (Fig. 4A). Since *P. australis* and *I. pseudacorus* also demonstrated a quite similar P_{tot} removal pattern in all treatments on day 50 under Cd load, for example, control (60%) > Cd_4 (32%) > Cd_2 (7%) > Cd_1 (−1%) when compared with the initial P_{tot} dose (Fig. 4B).

3.3. Cd uptake

Cd removal by *P. australis* and *I. pseudacorus* over 50 days period is depicted in Fig. 5AB. *P. australis* have exhibited the Cd removal from low to high dose Cd treatments up to 86% (Cd_1), 72% (Cd_2) and 76% (Cd_4) based on their respective initial doses (Fig. 5A). In the case of *I. pseudacorus*, Cd removal under a different Cd dose treatment was observed as 62% (Cd_1), 56% (Cd_2) and 69% (Cd_4) as compared to their initial doses (Fig. 5B). However, the results indicate that both species were capable to uptake the highest Cd concentration under low dose Cd treatment on day 50.

3.4. Cadmium accumulation, translocation factor (TF) and bioconcentration factor (BF)

Fig. 6A shows that Cd accumulation in *P. australis* AWP and BWP varied from 2.45 to 334.65 μg · $(0.05\ m^2)^{-1}$ and 10.94–1821.59 μg · $(0.05\ m^2)^{-1}$ in all treatments, respectively. The greatest accumulation was observed in BWP than AWP and particularly Cd accumulation in AWP and BWP was considerably increased with an increase of Cd dose. For example, compared to the control, an incredible increase of 3658% and 13,559% Cd accumulation in AWP was noticed in the low (Cd_1) and high dose (Cd_4) Cd treatments. Meanwhile, up to 263% proliferation of Cd accumulation in AWP was found in high dose treatment than low dose treatment. For BWP, the largest Cd accumulation was found in high dose Cd treatment revealing a magnificent increase of 16,550% and 35% compared to control and low dose Cd treatment, respectively.

Moreover, in the *I. pseudacorus*, the Cd accumulation in AWP and

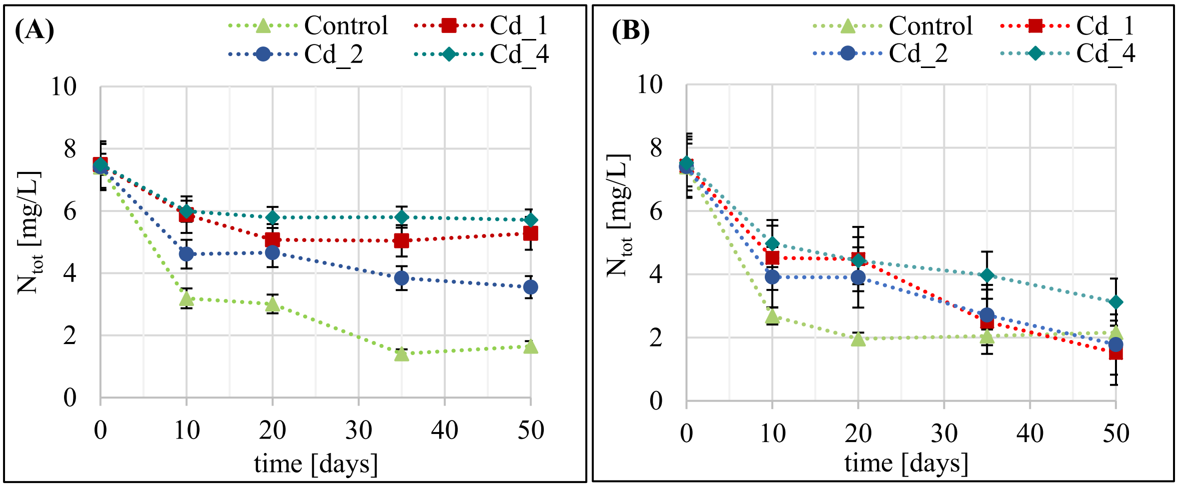

Fig. 3. Total nitrogen (N_{tot}) concentration fluctuation during 50 days of the experiment under FTW planted by **(A)** *P. australis* and **(B)** *I. pseudacorus*.

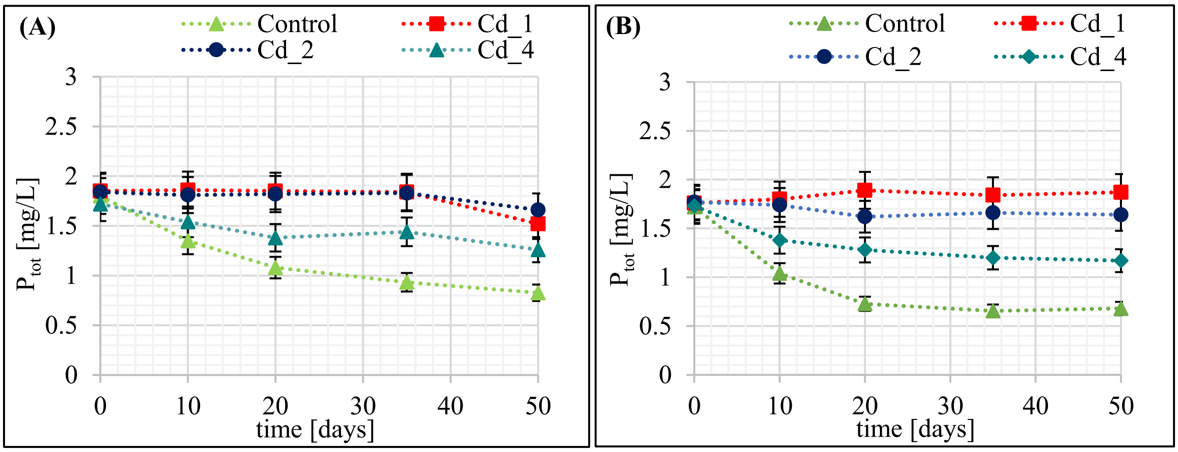

Fig. 4. Total phosphorus (P_{tot}) concentration fluctuation during 50 days of the experiment under FTW planted by **(A)** *P. australis* and **(B)** *I. pseudacorus*.

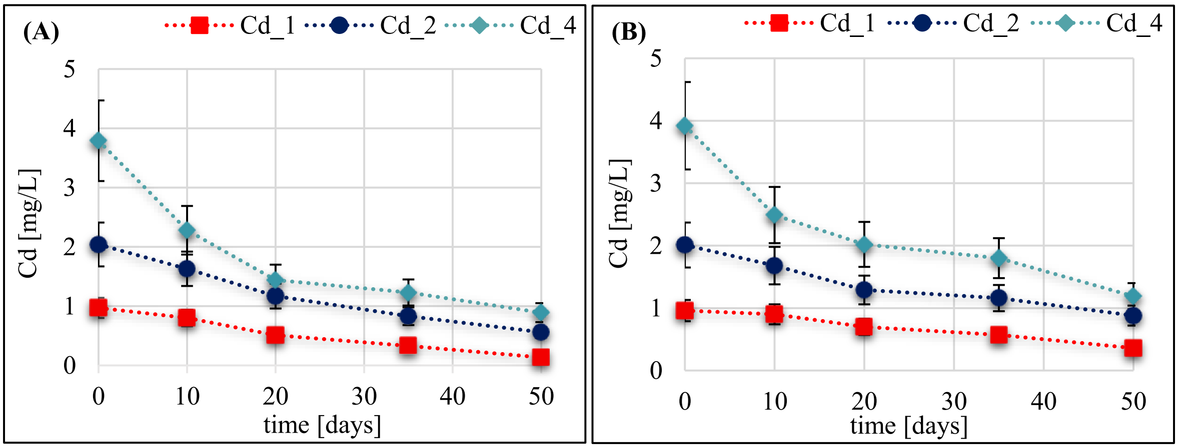

Fig. 5. Cd concentration fluctuation during 50 days of the experiment under FTW planted by **(A)** *P. australis* and **(B)** *I. pseudacorus*.

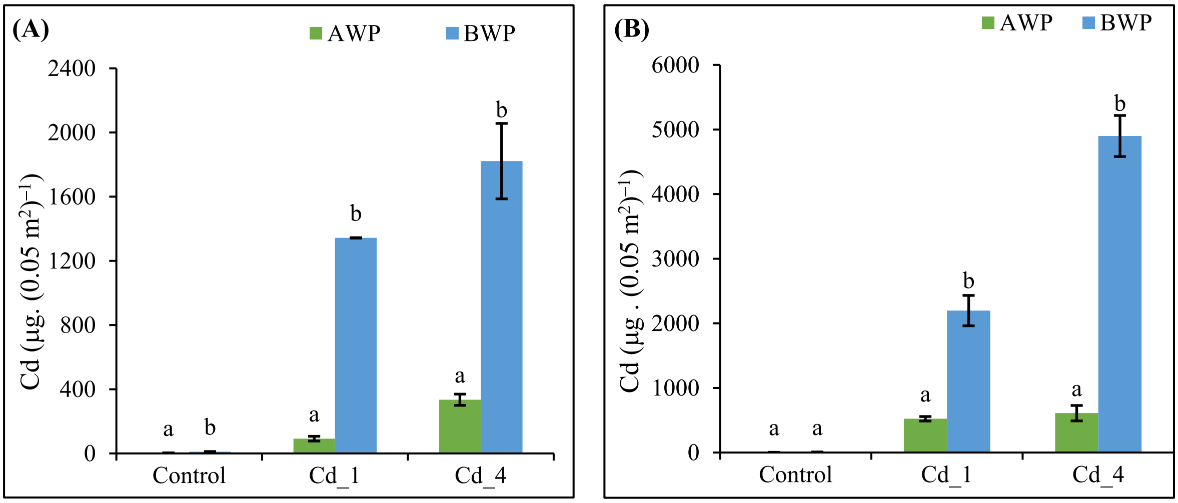

Fig. 6. Cadmium accumulation by **(A)** *P. australis* and **(B)** *I. pseudacorus* in AWP (above water parts) and BWP (below water parts) in hydroponics for 50 days. (Mean ± SE, n = 3). The small letters (a, b) indicate a significant difference between AWP and BWP in each treatment at $p < 0.05$.

BWP correspondingly ranged between 3.40 and 609 µg · $(0.05\ m^2)^{-1}$ and 5.84–4900 µg · $(0.05\ m^2)^{-1}$ in all treatments (Fig. 6B). Like *P. australis*, the *I. pseudacorus* has also demonstrated a similar trend for Cd accumulation as the highest accumulation found in BWP than AWP. The results indicate that Cd accumulation in both AWP and BWP was improved with an increase in Cd dose. When compared with the control, a massive increase of 15,252% and 17,811% of Cd accumulation in AWP was revealed in the low and high dose Cd treatment. However, an increase of 16% Cd accumulation in AWP was noticed in high-dose Cd treatment than low dose Cd treatment. Furthermore, the highest Cd accumulation in BWP was detected in high dose Cd treatment which represents a remarkable increase of 83,804% and 123% compared to control and low dose Cd treatment, respectively.

Fig. 7 demonstrated that *P. australis* had the highest TF (0.18) in high dose Cd treatment (Cd_4) but the lowest TF (0.07) was noticed in low Cd dose treatment (Cd_1). *I. pseudacorus* presented the highest TF (0.24) in low dose Cd treatment (Cd_1) whereas the lowest TF (0.12) was observed in high dose Cd treatment (Cd_4). Generally, TF indicates low translocation for both species. Furthermore, the highest translocations in roots (BF) for both species (1.34 and 2.20) were noticed in low Cd dose treatment (Cd_1) as compared to high Cd dose treatment (Cd_4). Noticeably, *I. pseudacorus* also indicated BF > 1 (1.22) in high dose Cd treatment (Cd_4).

3.5. Anatomical-morphological response of *P. australis* and *I. pseudacorus*

Representative light microscopy images of the plants are shown in Fig. 8 to identify the root structure obtained in the control, Cd_1, and Cd_4 treatments and to establish comparisons between them. The examination of a transverse section of roots shows the external layer of the epidermis (Ep), internal layer of endodermis (En), central cylinder (stele, S), and cortex (C). In *P. australis* roots, large intercellular channels – aerenchyma (Aer) are visible (Fig. 8a), whereas, in *I. pseudacorus* Cd_4 treatment, root hairs (RH) and lateral root (LR) are visible (Fig. 8b). Vessel elements arrangement in S *P. australis* is regular. Ep and En cells of *P. australis* are closely packed without disturbances in all treatments. Aer spaces tend to be two times smaller with Cd_4 treatment in comparison to control. In the case of *I. pseudacorus,* the cortical parenchyma (C) between Ep and En in Cd_4 treatment presented some increase in cell size (25 layers) in comparison to the control (11 layers). The C width in Cd_2 treatment is two times larger (600 μm) compared to the control and Cd_4 treatment. Generally, light-optical observations did not reveal any significant differences in the structures of the central cylinder; the size is of comparable size (150 μm) in the control and Cd treated reactors.

Light micrographs of *P. australis* stem (Fig. 9) in the control and Cd_1 treatment show proper development of Ep, En, vallecular canals (Vc), and vascular bundle (Vb). Pith cells are closely and regularly packed. The Vb are arranged in a ring that lies alternately with the Vc of the cortex (C). Central canal (Cc) is properly developed in both control and Cd_1, respectively. It is formed due to the rapid elongation of the internodal region. In the Cd_1 and Cd_4 treatments, multiple periderms are observed. Moreover, in Cd_4 the pith cavity (Cc) is being formed or indicates a malformation. The Vb and pith cells show irregular formation in comparison to the control.

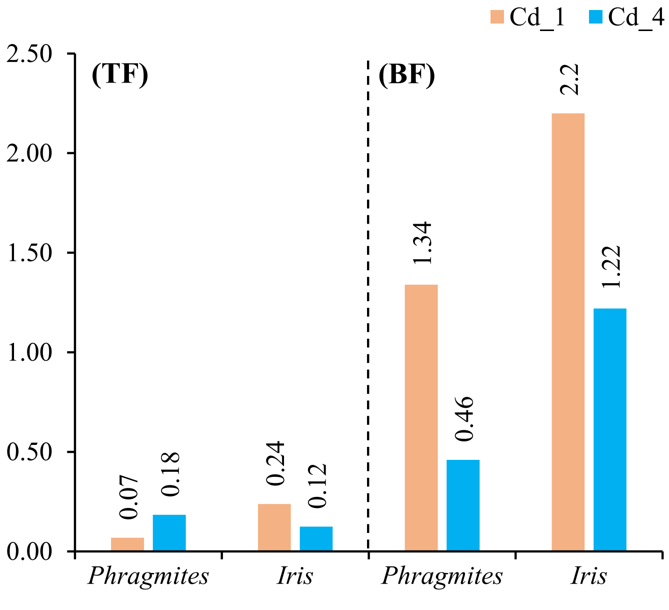

Fig. 7. Translocation factor (TF) and bioconcentration factor (BF) by *P. australis* and *I. pseudacorus* under low and high Cd dose treatments (Cd_1 and Cd_4).

Fig. 10 shows the transverse section of *P. australis* where new shoots in Cd_1 and Cd_4 treatments were formed at the end of the experiment. In the Cd_2 treatment, Ep, En, Vc, Vb, and Cc were already formed in a regular way. In the case of Cd_4 treatment, Ep and En consisted of 1–2 cellular layers, and Vc and Cc were not fully developed.

4. Discussion

4.1. Effects of Cd on *P. australis* and *I. pseudacorus* growth parameters

P. australis and *I. pseudacorus* displayed 100% survival with no toxicity symptoms such as withering, necrosis or chlorosis according to visual observations throughout the growth period, which shows that the elements (N, P and Cd) level in the hydroponic solution was tolerated by *P. australis* and *I. pseudacorus*. The results revealed that Cd doses did not cause an undesirable impact on the *P. australis* and *I. pseudacorus* height growth, root length, biomass production and CCI values as compared with the control (Fig. 4).

Growth parameters, such as biomass as well as shoot and root growth, were used to evaluate metal toxicity in plants and especially root growth was particularly sensitive to metal toxicity (Hakmaoui et al., 2007). Stress tolerance indices (DBTI, SLTI and RLTI) are important parameters to grasp the impact of Cd stress on morphological features of the plant (Mohajel Kazemi et al., 2020). The TI, based on root growth, signifies the effects of Cd toxicity as the results show that both *P. australis* and *I. pseudacorus* could be described as Cd tolerant plants with medium (2 mg/L) and high (4 mg/L) Cd dose treatment, respectively (Table 2). An increase in growth parameters under Cd application might be due to the inducement of cell proliferation, efficient photosystem−II, electron transport rate by Cd or high nutrient uptake (Mohsin et al., 2022). N and P addition might improve plant antioxidant defense ability and the stress to adversity by showing increased level of free proline and carotenoid, which increases the plant's resistance to heavy metal (Tang et al., 2021). Hence, we contemplate this reason in line with our study as N and P jointly promoted the *P. australis* and *I. pseudacorus* growth under Cd exposure.

To establish the possible stress response of *P. australis* and *I. pseudacorus* on additional Cd doses, CCI as a reflection of the photosynthesis process was measured. Chlorophyll, as a photosynthetic pigment, is critical in the conversion of solar energy to chemical energy (Yang et al., 2020). Generally, the CCI decrease when plants are exposed to highly toxic compounds. According to the literature, chlorophyll content and related CCI is a well-known and acceptable indicator of the presence of stressors or contaminants, and to some extent it can be useful for predicting plant toxicity (Spinedi et al., 2019). Under this study, the increase in CCI with increasing Cd dosages in treatments demonstrated the strong tolerance of *P. australis* and *I. pseudacorus* to Cd, which may be attributed to the root system, which has a great capacity to adsorb and retain metal and to reduce the toxicity of metals to the leaves. Yang et al. (2020) revealed similar data for *Davidia involucrata* in relation to Pb and Cd, which explain this effect owing to phytochelatin chelation to trace metals, hence lowering toxicity. An adverse effect was reported by (Spinedi et al., 2019) who identify the CCI decrease with increasing anthracene concentrations in *Marchantia polymorpha*. Based on the CCI data, it may be concluded that both species are adaptable to Cd presence.

4.2. Impact of Cd on nutrient removal in FTW microcosms

It may be claimed that additional Cd loads disrupted the *P. australis* uptake and nitrification-denitrification processes which result in the

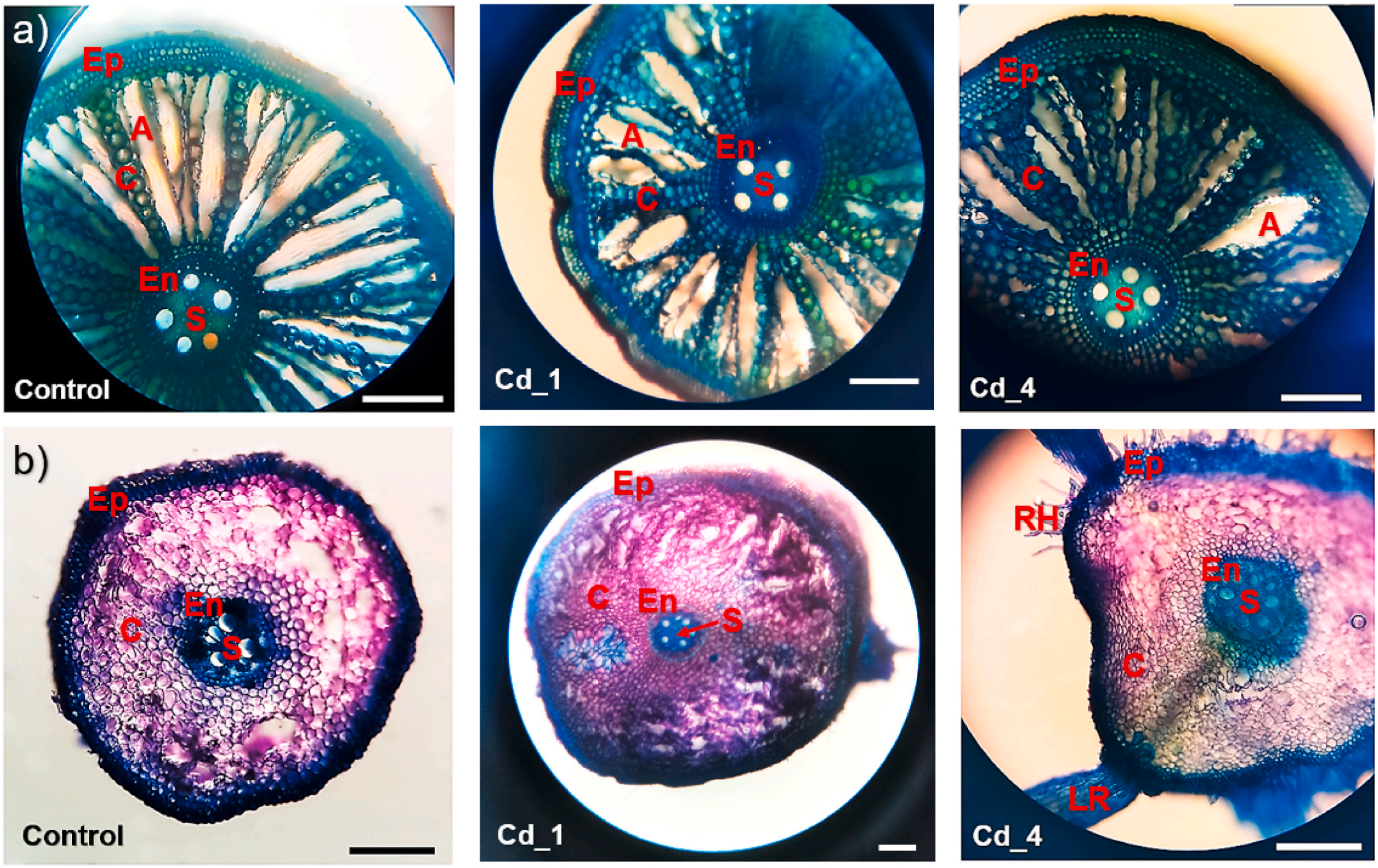

Fig. 8. Light micrographs showing the transverse section of a) *P. australis* and b) *I. pseudacorus* roots of control, Cd_1 (1 mg/L), and Cd_4 (4 mg/L) treated plants sampled on the last day of metal treatment and stained with toluidine blue (The scale bar is 150 μm); Ep – epidermis; En – endodermis; LR – lateral root; RH – root hairs; S – stele (central cylinder); C – cortex; A – aerenchym

least N_{tot} reduction as compared to *I. pseudacorus*. According to Tran and Petrova (2013), Cd can reduce the enzymatic activity of nitrate reductase in shoots and minimize nitrate absorption and its transport from roots to aboveground parts of dicotyledonous species. Moreover, competition between essential nutrients and Cd inhibits the transportation of macro and micronutrients in plants (Haider et al., 2021).

In the case of P_{tot} highest concentration decrease was noticed at day 50 under medium Cd loads (Cd_2) and low Cd loads (Cd_1) respectively for *P. australis* and *I. pseudacorus* (Fig. 4AB). Generally, it is well recognized that Cd and P interact antagonistically (Kabata-Pendias and Pendias, 1999), which is in line with obtained results which pointed out that additional Cd loads (Cd_1, Cd_2, and Cd_4) disrupted the removal efficiency of P_{tot}. The antagonistic interaction of Cd, according to Kabata-Pendias, (2001), can reduce P uptake by plants by up to 40% of the control values. The results agree with Awad et al. (2022) who reported the potentiality of native wetlands plants (*Baume a rubiginosa* and *P. australis*) for removing N and P from synthetic stormwater and domestic wastewater in constructed wetlands. The bioactive compounds released from the macrophytes roots influence the physicochemical properties of water which may enhance the sedimentation and sorption processes in FTW (Headley and Tanner, 2012). We observed the highest removal of Cd at day 50 for *P. australis* 86% and *I. pseudacorus* 69% when exposed to low dose Cd treatment (Cd_1) and high dose treatment (Cd_4), respectively (Fig. 5AB). Gill et al. (2017) have revealed the removal efficiency of *P. australis* and *Typha latifolia* for different metals for example Cd (5%), Cu (31%), Pb (31%) and Zn (86%) once treating stormwater runoff for nine years in a constructed wetland. The considerable removal of N and P in this study could be due to the large surface area of plant roots that provides a habitat for microorganisms (biofilm) and accelerate nutrient removal through phytodepuration and confiscate the suspended particles within the water column (Awad et al., 2022).

4.3. Cd accumulation, translocation, and effects on *P. australis* and *I. pseudacorus* tissues

Cd is a non-essential element; however, it can be easily uptaken by root and leaf systems. Agriculture and industry development cause the culmination of high Cd concentrations in agricultural soils (Chen et al., 2016), which can be easily leached to nearby watersheds during rain events and, at the same time, due to the high mobility of Cd. Deposition of Cd in plants, polluted soils, sediments, and waters poses a severe problem for animals and humans, due to its toxicity effect on multiple organs and high affinity to accumulation in the kidneys, etc. What is more, Cd in minerals replaces Ca because of similar chemical behaviors, identical charge, and ionical radius alike (Kubier et al., 2019). In the case of plants, the first sign of Cd depletion is chlorosis and shunted growth (Jali et al., 2016), while higher toxicity even inhibits plant growth and results in necrosis (Haider et al., 2021). Cd toxicity decreases the chlorophyll content, and photosynthetic activity, and inhibits carbon fixation (Dobrikova et al., 2021). According to Kabata-Pendias and Pendias, (1999) plants able to accumulate more than 100 ppm of Cd are hyperaccumulators. In this context, both species qualify for this purpose (both AWP and BWP have Cd levels above 100 ppm). Generally, hyperaccumulators that store unexpectedly high amounts of metals in aerial parts (leaves and stems) usually develop a lower biomass, due to the high energy consumption of mechanisms needed to adapt to unfavorable conditions. Such effect was observed in Cd_4 for *P. australis*, where the biomass could be lower due to negative Cd impact. Generally, the metal accumulation in plants is contingent on

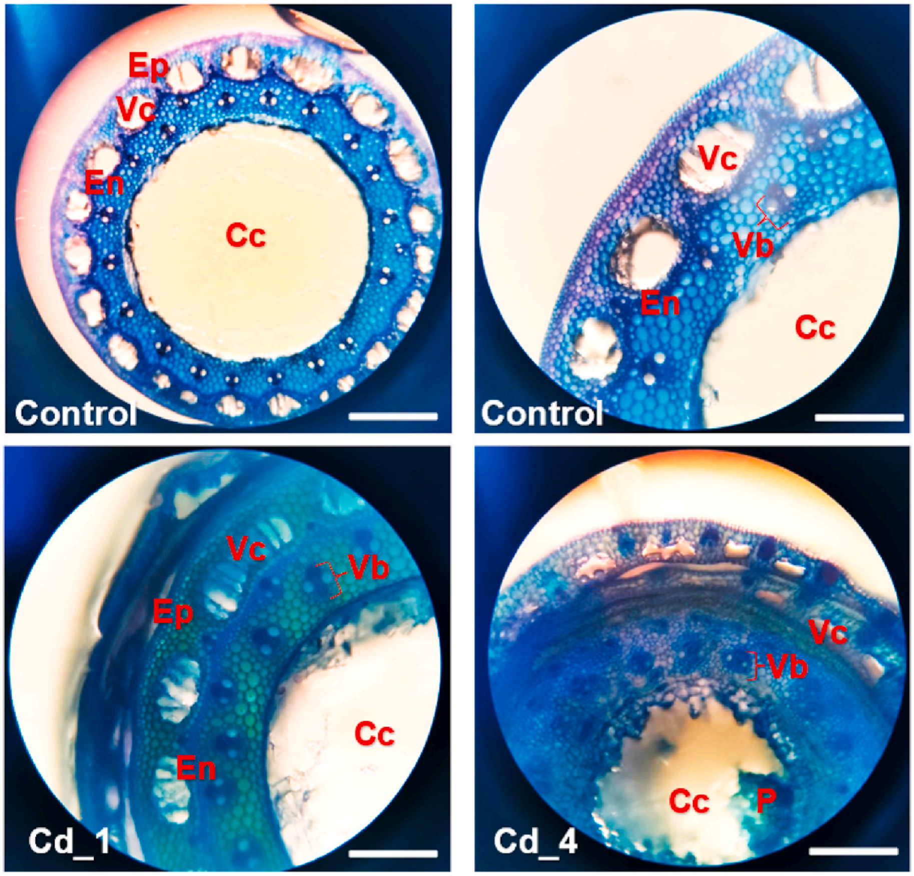

Fig. 9. Light micrographs showing a transverse section of *P. australis* stem of control (scale bar 100 μm and 50 μm, respectively), Cd_1 (1 mg/L) (scale bar 50 μm), and Cd_4 (4 mg/L) (scale bar 100 μm) treated plants sampled on the last day of metal treatment and stained with toluidine blue; Ep – epidermis; En – endodermis; Cc – central canal; Vc – vallecular canal; Vb – vascular bundle; P – pith. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

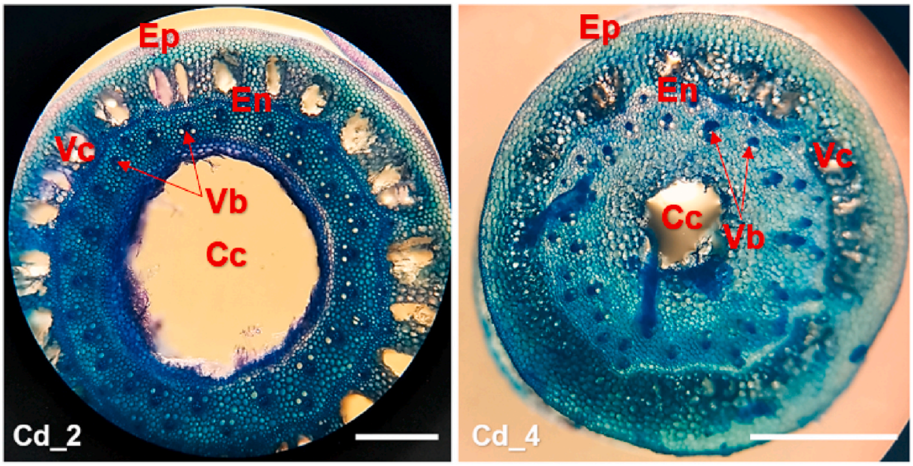

Fig. 10. Light micrographs showing a transverse section of *P. australis* new shoots of Cd_2 (2 mg/L) and Cd_4 (4 mg/L) treated plants sampled on the last day of metal treatment and stained with toluidine blue (The scale bar is 200 μm and 100 μm, respectively)*;* Ep – epidermis; En – endodermis; Cc – central canal; Vc – vallecular canal; Vb – vascular bundle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

numerous factors for example plant species abilities, pH level, chemical form and amount of metal, the presence of multiple elements causing synergistic or antagonistic interactions, redox potential and conductivity of soils and waters (Drzewiecka et al., 2012; Mohsin et al., 2022).

In the study, the highest Cd accumulation was observed in *P. australis* and *I. pseudacorus* roots which are in line with Al-Homaidan et al. (2020) and Nawrot et al. (2021a) who reported the highest element concentrations in *P. australis* roots. Gao et al. (2015) also reported the highest Cd accumulation in BWP of *Iris sibirica* in constructed wetlands. Other studies, by Vymazal et al. (2007) and Bonanno (2011) state that heavy metal concentrations in *Phragmites* roots were observed higher than in shoots. Moreover, the present results concerning the highest Cd accumulation in BWP are also in agreement with the former reports on aquatic plants (Ae and Fasidi, 2007; Zhang et al., 2010). Instead, Zhang et al. (2012) reported the maximum accumulation of Cd in wetland plant *Juncus subsecundus* shoots compared with roots once grown in Cd contaminated soils under glasshouse. Research unveils that P availability under Cd exposure could enhance polysaccharide production and cell wall affinity for Cd as well as promote xylem development via cell division stimulation, which may eventually improve Cd accumulation

(Liu et al., 2022). Regarding Cd, the phytotoxic levels in roots and the low plant/root movement may indicate that roots are integrally tolerant to Cd and act as filters to prevent toxic distribution in the plant. Concentrations of heavy metals in wetland plants are commonly higher in the roots and rhizomes as compared to stems, leaves, and flowers (Galletti et al., 2010; Vymazal, 2016; Vymazal et al., 2009) since the roots are primary sites for heavy metal uptake and the further translocation to shoots may be restricted (Vymazal, 2016). Another probable reason of Cd immobilization in nutrient solution and/or inside roots could be due to the formation of Cd–P complexes in plants vacuoles and cell walls, which can reduce Cd uptake in plants (Rizwan et al., 2017). Like our findings, Silva et al. (2016) detected high Cd concentration in roots compared to shoots of different forage grasses including *Panicum maximum* Jacq. Cv. Aruana and cv. Tanzânia; *Brachiaria brizantha* cv. Xaraés and cv. Marandu; and *Brachiaria decumbens* cv. Basilisk. This poor metal transport from plant roots to shoots could be due to negative charges present on the root surface capable of binding the metal cations (Zhivotovsky et al., 2011). Yang et al. (2020) described that N could enhance the Cd bio-available content and exchange capacity, Cd accumulation and uptake in plants, while also improving the tolerance of plants to Cd by regulating cell wall isolation and oxidative resistance, and producing more phytochelatins (PCs), nitric oxide (NO) and glutathione (GSH).

The presented result indicates that Cd accumulated by plants was largely retained in roots (Fig. 5AB), as shown in Fig. 6 (TF $<$ 1 and BF $>$ 1). Nouri et al. (2011) stated that plants with a high BF value and low TF value could be suitable for phytostabilization if both BF and TF values are greater than one could be used for phytoextraction. In the present study, *P. australis* and *I. pseudacorus* BF values for Cd are $>$1 and TF values $<$ 1, in this case, both plants could be used for phytostabilization of Cd. At the same time, *I. pseudacorus* presented a better predisposition to Cd phytostabilization then *P. australis* due to the highest BF in low (Cd_1) and high (Cd_4) Cd loads treatments (2.2 and 1.22, respectively). According to Gao et al. (2015), the TF value should not be considered an important criterion regarding the phytoremediation of water because AWP can be harvested easily. Based on the present results, it can be concluded that the BWP are mainly responsible for Cd phytoextraction. The studied wetland plants have shown the ability to reduce metal translocation from roots to shoots which makes them suitable for ecological restoration such as phytostabilization (Gomes et al., 2014). Furthermore, the limited Cd translocation to AWP may indicate effective protection mechanisms against metal translocation to the most important plant organs in the context of photosynthesis.

Changes in tissue levels were detected in the roots of *P. australis* and *I. pseudacorus* growing in Cd-contaminated media. Light-optical measurements revealed that Cd toxicity reduces the root size of the air cavities significantly for *P. australis* (Fig. 8a) and increases the size of cells in cortical parenchyma (C) between Ep and En for *I. pseudacorus* in Cd_4 treatments. Despite these changes, both species exhibit the emergence of resistance mechanisms that reflect the interaction of parts and organs in the whole plant system. *I. pseudacorus* roots developed RH and LR, while *P. australis* grew new shoots in Cd_2 and Cd_4 treatments. Minkina et al. (2019) observed similar anatomical changes in the leaves and roots of *Typha australis* when grown in soil contaminated with PTEs. According to Minkina et al. (2018), PTEs in *P. australis* favored the disruption of the orderly arrangement of cortical cells and a considerable reduction in air-bearing spaces. However, such structural changes in the roots, which have direct contact with the dissolved substances, eventually enable macrophyte plants to adapt to adverse environmental conditions. PTEs accumulates and is distributed throughout plant tissues as a result of pollution, which also alters the morphological and anatomical level (Minkina et al., 2021).

5. Conclusions

P. australis and *I. pseudacorus* were not exposed to metal toxicity for example necrosis, chlorosis or withering under multi-metal environment based on visual observations. Results indicate that the studied plant species demonstrated productive responses for all growth parameters (growth height, biomass production, root length, DBTI, SLTI, RLTI and CC1) under varied Cd doses.

The overall fluctuations in Cd removal were about 76–86% for *P. australis* and 56–69% for *I. pseudacorus* in a 0−50-day period. The largest Cd accumulation in *P. australis* and *I. pseudacorus* was observed in roots (BWP) compared with shoots (AWP) during high dose (Cd_4) treatment. Both species which displayed the TF at $<$1 and BF $>$ 1 shows their potential to immobilize the metals in BWP as phytostabilizers. Anatomical observations revealed that Cd toxicity reduced the root size of the air cavities significantly for *P. australis*, while also increasing the size of cells in cortical parenchyma (C) between Ep and En for *I. pseudacorus* in Cd_4 treatments. Nevertheless, both species exhibited the emergence of resistance mechanisms that reflect the interaction of parts and organs in the whole plant system. The findings indicate that *P. australis* and *I. pseudacorus* could be utilized for the remediation of Cd enriched water bodies. The experimental period was noticeably short; therefore, N, P and Cd removal as well as Cd accumulation in the studied plant roots and translocation to the shoots are relatively complex processes and still require more clarification in the long run. Further research should focus on explanation of the role of FTW components (plants, microbiome, and their interactions) under meso and full-scale treatments.

Credit author statement

Muhammad Mohsin: Investigation, Formal analysis, Writing – original draft preparation. Nicole Nawrot: Investigation, Formal analysis, Writing – original draft preparation and Funding acquisition. Katarzyna Szczepańska: Sample analysis. Grażyna Dembska: Sample analysis. Suvi Kuittinen: Writing – review & editing. Ewa Wojciechowska & Ari Pappinen: Supervision; Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.117339.

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