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Full Length Article

Contribution of Structural and Functional Traits in Turgor Maintenance of *Pistia stratiotes* under Cadmium Toxicity

Aneela Zahoor[1], Farooq Ahmad[1], Mansoor Hameed[1*] and Shahzad Maqsood Ahmad Basra[2]
[1]*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan*
[2]*Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan*
[*]For correspondence: hameedmansoor@yahoo.com

Abstract

Plants of *Pistia stratiotes* L. were collected from industrial effluents along Sheikhupura-Lahore road in order to examine morpho-anatomical and physiological mechanism for turgor maintenance to cope metal toxicity. Plants were subjected to cadmium stress ($3CdSO_4.6H_2O$) and levels for Cd^{2+}, were 0, 50, 100 and 150 mg L^{-1} in aqueous medium. Growth of the *P. stratiotes* L. in terms of plant height and biomass production was reduced under cadmium stress. *P. stratiotes* accumulated proline, total proteins and total free amino acids in higher amount under cadmium stress. Metals increased uptake of both Ca^{2+} and K^+ in roots but higher doses of cadmium disturbed ionic balance. Root epidermal cell area increased as the stress level increased but higher level of cadmium showed reduction in this parameter. Cortical region thickness and cortical cell area increased significantly under cadmium stress, and this can be related to accumulation of organic and inorganic substances in storage parenchyma. A significant increase was also recorded in aerenchymatous area and phloem area that ensures efficient gaseous exchange and translocation of photosynthates, hence successful survival under polluted environments. Leaf anatomical characteristics like abaxial or adaxial epidermal cell area were relatively more sensitive to Cd stress showing a significant reduction at higher Cd levels. Trichome number increased at all levels of stress enabling *P. stratiotes* to resist water loss through leaf surface, indicating its high degree of tolerance. *P. stratiotes* accumulated Cd mainly in roots, and therefore it is concluded that this species is metal accumulator and can be used for phytoremediation of Cd-polluted soils and water.

Keywords: Cations; Organic osmotica; Cadmium toxicity; Trichomes; Storage parenchyma; Phytoremediation

Introduction

Heavy metal contamination is disturbing aquatic ecosystem due to humans and industrial activities (Meagher, 2000). The greatest rise in heavy metal contamination has become a serious problem over the earth (Parmar *et al*., 2013). The capacity of macrophytes to absorb pollutant from their surrounding areas is very important that have many implications (Hadad *et al*., 2006).

Heavy metals i.e., cadmium (Cd) is highly lethal to living organisms (Deckert, 2005). Cd absorbent plants can accumulate greater than 100 μg g^{-1} dry weight of metal (Das *et al*., 2016), so they increase their biomass, reproduce more efficiently, and have bio-concentration factor (BCF) and translocation factor (TF) values greater than one (Garbisu and Alkorta, 2001).

Pistia stratiotes (water lettuce) belongs to family Araceae and used in phytoremediation. It floats on the water upper surface and has hanging roots emerging from floating leaves (Dipu *et al*., 2011). *P. stratiotes* shows better nutrient absorption competency with increasing reproduction rate (Gupta *et al*., 2012). The phytoremediation by macrophytes may be possible due to the occurrence of specific enzymes, stress proteins and phytochelatins. The concentration of metals at highest levels causes retardation of biomass, biological and chemical activities and also production of enzymes containing sulfhydryl (-SH) group (Rolli *et al*., 2013).

The ions are present in the vacuoles and the organic osmotica in the cytoplasm of cell. Organic osmotica protect sub-cellular structures and reduce oxidative damage initiated in response to oxidative radicals in different abiotic stress conditions. Accumulation of osmolytes in plant cells has been associated with greater stress tolerance by protecting from free radicals and defensive enzymes (Szabados *et al*., 2011).

In response to stress hyper accumulator plants develop various mechanisms that enable them to survive under stress condition. Plants produce different types of organic osmotica e.g., amino acids, sugar and proline that act as a signaling molecules, metal chelators and protect plant cells from oxidative molecules. Overproduction of organic osmotica combats with oxidative molecules and balance their range within cell that protects plant from ion

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leakage and stabilizes membrane that maintain cell turgidity (Hayat *et al*., 2012).

Plants develop different anatomical changes that protect them against metal stress like variation in dermal and conducting tissues and distribution of ions and nutrients also vary between different plants parts that act as survival mechanism (Gomes *et al*., 2012). Plant root is the main organ that protects all other parts from metal ions and act as barrier for plant (Gupta and Chakrabarti, 2013). Thickness in leaf structure, vascular and cortical cells and increase in trichome number and bulliform cells and reduction in stomatal area and density are the fundamental features that protect hyperaccumulator plants from stress toxicity (Mukhtar *et al*., 2013).

It is hypothesized that *P. stratiotes* can absorb and accumulate metal in their body parts and prevent their surroundings from toxic metals and can be used as hyperaccumulator for metals.

Materials and Methods

Pistia stratiotes L. was collected from road side water near Sheikhupura-Lahore area that was heavily affected by industrial effluents. The *P. stratiotes* was first established in Faisalabad environment for twelve months in old botanical garden University of Agriculture, Faisalabad. Medium for the Cd application was sand and loam (1:1) mixed in equivalent quantity and flooded with water. In the experiment, four cadmium levels were applied after their establishment for two month, viz., 0 (control with no Cd treatment), 50, 100, and 150 mg L^{-1} $3CdSO_4.6H_2O$. The plants were taken carefully and washed completely with distilled water for numerous morpho-anatomical and physiological studies. Morphological parameters, plant size, root length, leaf area, number of leaves per plant, fresh and dry biomass, ionic contents and organic osmotica, as well as anatomical parameters including the occurrence and extent of dermal, parenchymatous, mechanical and conducting tissue were assessed.

For recording dry weight, plants were dried for 10 days until a constant weight was achieved.

Ionic Content of Root and Leaf

Plant samples were oven-dried at 65˚C for analysis of inorganic ions. The ground dried samples were analyzed for K^+ and Ca^{2+} after H_2SO_4 digestion (Wolf, 1982) using a flame photometer (Jenway, PFP-7). The Cd concentration in leaf and root (mg g^{-1} d. wt.) were determined in the acid digests of leaves and roots by Atomic Absorption Spectrophotometer (AAnalyst-300, Perkin Elmer, Germany).

Osmolytes Measurement

Proline determination: Free proline was assessed following method of Bates *et al*. (1973).

Total soluble proteins: Total soluble proteins were assessed using the method of Lowry *et al*. (1951).

Total soluble sugars: Total soluble sugars were estimated following the method of Yemm and Willis (1954).

Total free amino acids: Total free amino acids were determined following the method of Moor and Stein (1948).

Anatomical studies of T.S. of root, leaf and leaf epidermis were carried out by free-hand sectioning. Pieces of tissue (1 cm) were cut from the base of the main root and from the leaf of the same plants that were used for other morphological and physiological characteristics. The material was fixed in formalin acetic alcohol (FAA) solution (5% formalin, 10% acetic acid, 50% ethanol, 35% distilled water by volume) for about 48 h. A double staining technique was used (Ruzin, 1999) with safranin for lignified tissues (secondary cell walls such as metaxylem and sclerenchyma) and fast green for subrinized tissue (primary cell walls). Anatomical measurements were made with an ocular micrometer at eyepiece resolution 10X and objective lens 20X, and pictures taken with a Carl–Zeiss camera-equipped microscope.

Statistical Analysis

The experiment was planned in (CRD) completely randomized design. Microsoft Excel software and Minitab statistical software was used for statistical analysis and analysis of variance (ANOVA) and LSD for comparison of mean values.

Results

Morphological Characteristics

Plant height, root length, leaf area, root fresh weight, shoot fresh and dry weight and number of leaves per plant of *P. stratiotes* were severely affected by cadmium stress. Root dry weight increased up to 100 mg L^{-1} Cd level, but it decreased at higher levels (Table 1).

Ionic Content

In *P. stratiotes*, lower doses of Cd (50 and 100 mg L^{-1}) showed increase in root K^+ content, but root Ca^{2+} boosted up consistently in response to higher doses of Cd stress. Cadmium showed an increase in shoot K^+ at lower concentrations (50 mg L^{-1}), but root Ca^{2+} increased consistently at (50 and 100 mg L^{-1}) doses of cadmium stress. Root and leaf Cd^{2+} content progressively increased as doses of cadmium increased (Table 1).

Organic Osmotica

Proline and protein content increased significantly as the cadmium level increased. Cadmium doses at lower concentration (50 and 100 mg L^{-1}) have no significant effects on total soluble sugars but sugar contents increased significantly at higher doses of cadmium stress (150 mg L^{-1})

Table 1: Morpho-physiological characteristic of *P. stratiotes* under cadmium stress

Characteristics	Control (0 mg/L)	50 mg/L	100 mg/L	150 mg/L
Morphological characteristics				
Plant height (cm)	42.01c	35.43b	33.67ab	29.10a
Root length (cm)	29.33c	24.67b	21.67ab	18.33a
Leaf area (cm^2)	100.09c	77.86b	55.02ab	43.67a
No. of leaves per plant	15.67b	13.63a	13.32a	12.34a
Shoot fresh weight (g $plant^{-1}$)	44.42c	38.77bc	31.60b	21.21a
Root fresh weight (g $plant^{-1}$)	10.60c	9.50bc	8.90b	5.70a
Shoot dry weight (g $plant^{-1}$)	8.02c	6.19b	5.33ab	4.72a
Root dry weight (g $plant^{-1}$)	1.68a	2.64b	1.81a	1.13a
Ionic content				
Root K^+ (mg g^{-1} d. wt.)	8.43a	14.50b	12.50ab	7.33a
Root Ca^{2+} (mg g^{-1} d. wt.)	12.53a	18.54b	21.33bc	23.45c
Leaf K^+ (mg g^{-1} d. wt.)	9.50b	14.23c	9.50b	6.50a
Leaf Ca^{2+} (mg g^{-1} d. wt.)	24.01a	27.52b	25.23b	22.50a
Metal content				
Root Cd^{2+} (mg g^{-1} d. wt.)	0.16a	2.02b	7.05c	9.03d
Leaf Cd^{2+} (mg g^{-1} d. wt.)	0.16a	0.67ab	1.85b	2.13c
Organic osmotica				
Proline (mg g^{-1} f. wt.)	51.43a	60.32ab	89.67b	128.33c
Total soluble proteins (mg g^{-1} f. wt.)	37.23a	48.67ab	55.33b	64.67c
Total soluble sugar (mg g^{-1} f. wt.)	40.33a	37.33a	44.33a	65.32b
Total free amino acids (mg g^{-1} f. wt.)	1128.21a	1217.07ab	1327.43b	1730.33c

(Table 1). A significant and gradual amplification in total free amino acids was observed in *P. stratiotes*.

Root Anatomical Characteristics

Cadmium stress has non-significant effect on root area at (50 mg L^{-1}) and root area reduced significantly at higher doses of cadmium stress. Epidermal cell area increased significantly at lower Cd levels up to (100 mg L^{-1}) but reduced at the highest cadmium level. Cortical cell area and aerenchymatous area increased significantly in response to increase in cadmium stress (Table 2 and Fig. 1).

Pith area and pith cell area increased significantly at lower levels of Cd stress (50 mg L^{-1}) but a significant reduction occurs as the stress level increased. Endodermal area reduced significantly as cadmium doses increased. Vascular bundles i.e., metaxylem area, xylem area and phloem area also enlarged significantly in response to increase in cadmium level up to (100 mg L^{-1}) but as the cadmium doses further increased reduction in vascular bundles area was observed (Table 2 and Fig. 1).

Leaf Anatomical Characteristics

Leaf anatomical characteristics i.e., adaxial and abaxial epidermal cell area, aerenchymatous area, vascular bundle area, leaf and lamina thickness were more affected as cadmium level increased. These parameters reduced significantly in response to cadmium stress. In response to stress *P. stratiotes* increase their number of trichomes. Length of trichomes enlarged as the stress level rose up to

Table 2: Anatomical characteristics of *P. stratiotes* under cadmium stress

Characteristics	Control	50 mg/L	100 mg/L	150 mg/L
Root anatomical characteristics				
Root area (mm^2)	2.52c	2.50c	2.45b	2.23a
Epidermal cell area (μm^2)	576.90ab	978.98b	978.98b	419.56a
Cortex thickness (µm)	626.36a	667.21b	697.17c	713.51c
Cortical cell area (μm^2)	2867.02a	3360.05b	3867.02c	4492.83d
Aerenchyma area (μm^2)	30558.28a	33582.64b	38198.23c	41536.89d
Pith area (μm^2)	34107.14c	43040.33d	29929.31b	24614.45a
Pith cell area (μm^2)	576.90b	1048.91c	576.90b	367.11a
Endodermal cell area (μm^2)	1485.95d	1398.54c	1098.54b	699.27a
Metaxylem area (μm^2)	3933.41b	4213.14c	4842.47d	3076.80a
Xylem area (μm^2)	75748.88b	108544.83d	82410.22c	71028.78a
Phloem area (μm^2)	10751.34b	15157.15c	20698.52d	5996.27a
Leaf anatomical characteristics				
Adaxial epidermal cell area (μm^2)	816.68c	952.79d	680.57b	340.28a
Abaxial epidermal cell area (μm^2)	3470.91c	4423.71d	3062.57b	2381.92a
Leaf thickness (µm)	3309.97c	3895.67d	2778.01b	1262.73a
Aerenchyma area (μm^2)	118555.52c	120801.42d	86228.35b	43692.66a
Vascular bundle area (μm^2)	63837.56c	55126.25b	56487.42b	47912.22a
Lamina thickness (µm)	558.82b	553.45b	521.21ab	488.97a
Trichome number	1612.23a	2041.86c	2041.86c	1988.13b
Trichome length (µm)	671.67ab	698.53b	693.16b	666.29a
Subsidiary cell area (μm^2)	128015.24b	213631.32d	162112.32c	101949.51a

(100 mg L^{-1}) but further increased in cadmium doses reduced trichomes length (Table 2 and Fig. 2). Subsidiary cell area increased up to (100 mg L^{-1}) but reduced at higher cadmium stress (150 mg L^{-1}) (Table 2 and Fig. 3).

Discussion

A very serious environmental problem today, is the addition of heavy metals (HMs) to the aquatic as well as terrestrial areas that is contaminating them severely (Ghelich and Zarinkamar, 2013). Metals induce structural modifications (Bosabalidis and Panou-Filotheou, 2004), at molecular or tissue level (Singh and Sinha, 2004) by the formation of reactive oxygen species (ROS) (Gill, 2014), that may disturb morphological, biochemical and physiological functioning (Emamverdian *et al*., 2015). In response, plants possesses different mechanisms to deactivate heavy metal toxicity, i.e., production of metal binding chelates (Yadav, 2010), and retain these complexes in their vacuoles (McGrath and Zhao, 2003) away from metabolically active sites (Hassan and Aarts, 2011).

In the present investigations all morphological parameters in *P. stratiotes* L. were adversely affected by cadmium stress that was also observed by (Bosabalidis and Panou-Filotheou, 2004; Sharma and Dubey, 2005; Vollenweider *et al*., 2006; Heidari and Sarani, 2011). Higher doses of Cd cause root abnormalities due to which movement of salts to above ground plants parts is restricted (Irfan, 2015). Reduction in growth parameters was also observed in *Hydrilla verticillata* by Singh *et al*. (2012) and in sugarcane by Raza *et al*. (2013). Root parameters indicated little resistance to higher treatment

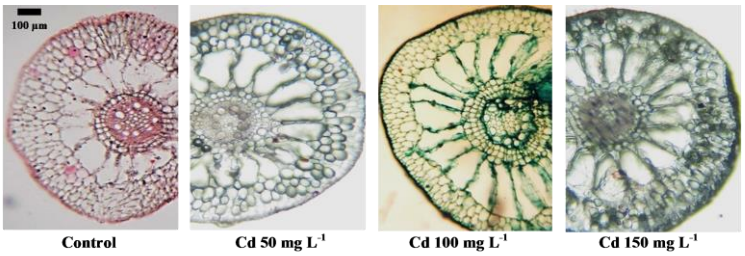

Fig. 1: Transverse section of root of *Pistia stratiotes* cultivated under different regimes of cadmium stress
A consistent decrease in root cross-sectional area along with increase in Cd stress
A significant decrease in peripheral parenchyma at the highest Cd level
Deformation of inner cortical region and vascular tissue at the highest Cd level

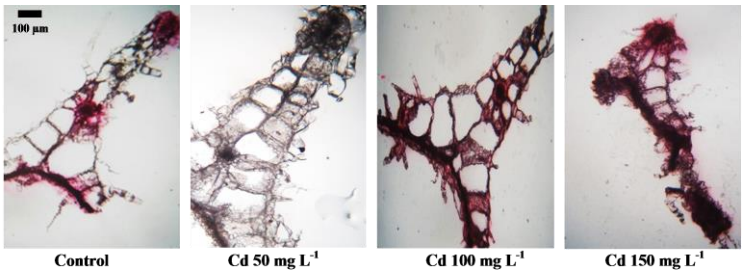

Fig. 2: Transverse section of leaf of *P. stratiotes* cultivated under different regimes of cadmium stress
A significant increase in leaf thickness at 50 mg L^{-1} level, but thereafter a consistent decrease along with increase in stress
Disintegration of leaf tissue at the highest Cd level

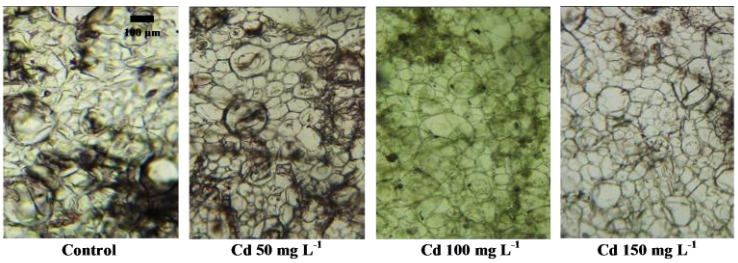

Fig. 3: Surface view of epidermis of *P. stratiotes* cultivated under different regimes of cadmium stress (n=6)
A consistent decrease in stomatal cavity and subsidiary cell area along with increasing stress levels
A significant decrease in stomatal density at the highest stress level

of Cd that showed hyper accumulator response to Cd treatment that neutralizes protein disintegration, lipid oxidation and damaging of photosynthetic pigments (Päivöke and Simola, 2001). Resistance in root has also been studied by Bosabalidis and Panou-Filotheou (2004), Vollenweider *et al.* (2006), Soares *et al.* (2011) in response to heavy metal because of enhancement in vascular tissue proportion.

Growth parameters are reduced by the production of reactive oxygen species at cell wall or inside cell that cause necrosis and chlorosis in above ground plant parts (Mascher *et al.*, 2002). It will also reduce membrane permeability, enzymetic activity and photosynthetic functioning, and in a result translocation of micronutrients is affected (Shaibur *et al.*, 2009).

Organic osmolytes are produced in response to osmotic stress conditions like metal toxicity (Hassanein *et al.*, 2012). Metals disturb nitrogenous compounds of plants so it will disturb the amino acid accumulation (Less and Galili, 2008). Proline synthesis is significant in maintaining turgidity in plants in response to stress conditions like metal toxicity (Hayat *et al.*, 2012). It helps in detoxification of toxic metal ions and increases the tolerance capacity of plants (Emamverdian *et al.*, 2015).

Cations like Ca^{2+} and K^{+} were badly affected by cadmium stress. Cadmium raised uptake of both cations (Ca^{2+} and K^{+}) in shoot and roots up to moderate stress levels. But in sensitive plants, heavy metals caused lipids peroxidation in response to free radicle formation, leading to membrane disruption that caused K^{+} leakage (Farnese *et al.*,

2014).

Root area, root xylem and metaxylem area, endodermal cell area, root pith and pith cell area increased in response to cadmium treatment, as reported by Silva *et al*. (2013) in *P. stratiotes*. Epidermal cells have thickest outer walls that are a barrier to toxic cations like metal ions that disturb metabolically active sites of plant cell (Gostin, 2009). Exodermis and endodermis cells area poplastic barrier that hamper movement of toxic ions inside cell (Lux *et al*., 2004). These tissues block the movement of toxic cation and protect softer tissues from dehydration (Silva *et al*., 2013).

Vascular bundle area increased under moderate cadmium stresses. Increase in vascular region facilitates the movement of water, minerals and photosynthates in plants (Pereira *et al*., 2014). *P. stratiotes* increased absorption of water and minerals from roots to leaves, that caused more efficient growth of plant that can compete stressful environment. Increase in phloem regions in response to metal toxicity indicated tolerance capacity of plants as it increased transport of photoassimilate from leaf to roots, and in result more efficient growth of roots occurs. This tolerance mechanism was also observed by Periera *et al.* (2014) in *Eichhornia crassipes* under lead toxicity.

Metal toxicity caused reduction in root length but root size increased in diameter (Bosabalidis and Panou-Filotheou, 2004). In the present investigations, root size, root fresh and dry weight reduced in response to cadmium treatment, because stress hinders cell division that reduced root growth and development, as reported by Bosabalidis and Panou-Filotheou, (2004), Vollenweider *et al*. (2006), Heidari and Sarani (2011). These characters are relating to sensitive plants but *P. stratiotes* is metal accumulator species that responded differently.

Many anatomical changes takes place in plants in response to metal toxicity, in which leaf area and vascular area of plants reduced but aerenchyma increased in number (Al-Saadi *et al*., 2013). But in this experiment aerenchyma formation reduced at the highest cadmium level, the reason for reduction in aerenchyma formation is that *P. stratiotes* is a floating plant and it takes oxygen directly from the surrounding environment therefore aerenchyma formation is not essential for their survival while Al-Saadi *et al.* (2013) performed his experiment on *Potamogeton* species that is a submerged aquatic plant and completely depends on aerenchyma development for its survival.

In *P. stratiotes*, leaf abaxial and adaxial surface was more responded to cadmium stress. Leaf abaxial and adaxial epidermal surfaces become thick, that protect plant from desiccation. In metal affected environment a positive relationship between leaf epidermis thickness and turgidity in leaf blade was observed by Gomes *et al.* (2011).

Mesophyll tissues increased under Cd treatment that capture more CO_2 and in result increased photosynthetic rates (Pereira *et al*., 2016), same results were also observed in *Arachis hypogaea* by Shi and Cai (2009). Consistency in leaf anatomical characteristics like retention of mesophyll tissues under metal treated environment indicates hyper accumulator features of plant (Pereira *et al*., 2014). Metaxylem vessels are responsible for water and mineral distribution within vascular area and under metal toxicity when vascular bundle area is affected it also reduced metaxylem vessels area (Ortega *et al*., 2006) so it directly influence plant growth characteristics (Ceccoli *et al*., 2011).

C4 plants have bundle sheath cells that increase their photon capture capacity (Chen *et al*., 2009). Metal toxicity has no significant effect on stomatal density in most experiments however it is positively correlated to CO_2 capture. Stomatal density and number are also responsible for transpiration control (Pereira *et al*., 2014). Pereira *et al.* (2014) and Grisi *et al.* (2008) observed that in more tolerant plants there is an increase in stomatal density under stress conditions.

Conclusion

It is concluded that *P. stratiotes* can tolerate high concentration of cadmium. Anatomical modifications like high proportion of storage parenchyma (cortex), thick epidermis and increased amount of phloem can be related to high degree of tolerance to metal toxicity in *P. stratiotes*, Another advantage is the development of large aerenchymatous cavities under Cd stress, and this can modification not only important for gaseous exchange but also for dumping off toxic metals like Cd. It is metal accumulator and can be used for phytoremediation and metal affected soil. Morpho-anatomical and physiological based markers can be incorporated in other metal sensitive plants to increase tolerance level.

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