Pak. J. Bot., 50(2): 489-493, 2018.

STRUCTURAL AND FUNCTIONAL ASPECTS OF PHOTOSYNTHETIC RESPONSE IN *EICHHORNIA CRASSIPES* (MART.) SOLMS UNDER CADMIUM STRESS

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Abstract

Eichhornia crassipes (Mart.) Solms is a floating hydrophyte that dominates metal-polluted wetlands such as industrial effluents. Keeping in view its capability to hyper-accumulate metal ions, the effect of cadmium stress on structural and functional features of photosynthesis was investigated to assess growth response under metal toxicity. Plants were randomly collected from polluted areas of Sheikhupura-Lahore road and multiplied for about one year at research sites of old Botanic Garden, University of Agriculture, Faisalabad. Plants were thereafter treated with cadmium stress and levels of cadmium were 0 (control), 50, 100 and 150 mg L^{-1} in aqueous solution. *E. crassipes* reduced their biomass, and photosynthetic features like chlorophyll pigments, net CO_2 assimilation rate, sub-stomatal CO_2 concentration and stomatal conductance under cadmium toxicity. Water use efficiency also decreases significantly in response to metal stress, but this species is a floating hydrophyte. Water is frequently available to this species and any decrease in water use efficiency may not affect growth and development. Leaf features i.e. leaf thickness, chlorenchymatous and sclerchymatous area, aerenchymatous area, vascular bundle region (xylem and phloem area) and stomatal area were reduced in response to Cd treatment. Increase in stomatal density on adaxial and abaxial leaf surfaces and bundle sheath cell area under cadmium stress indicate metal tolerance in plants. It is concluded from the present investigations that *E. crassipes* accumulates cadmium and can be utilized for phytoremediation in polluted water.

Key words: Cadmium toxicity, Leaf, Photosynthsesis, Aerenchyma, Phytoremediation.

Introduction

Heavy metals are environmental contaminants and toxic even at their lower concentration. Industrial revolution is the major source of introducing heavy metals on earth (Silva *et al*., 2013). Because microorganisms cannot degrade heavy metals so they are accumulating in environment (Odjegba & Fasidi, 2007).

Cadmium has no biological function in any organism except in marine diatoms and is ranked seventh out of twenty toxins present in this world (Parmar *et al*., 2013). Cadmium is utilized in dye manufacturing (Cd-yellow), Cd-Ni batteries, electroplating, phosphate fertilizer manufacturing and in the formation of plastics (Silva *et al*., 2013). Cadmium is concentrated in body parts of living organisms causing mutation (Fatur *et al*., 2003).

Eichhornia crassipes (Mart.) Solms (water hyacinth) belonging to family Pontederiaceae, is a macrophyte, weedy, monocot, perennial herb and native to South America. Plants of *E. crassipes* have small stem, fibrous floating roots, ranging in length from 30 to 40 cm (Qaisar *et al*., 2005).

E. crassipes eliminates heavy metals from polluted water, to improve the value of water (Das *et al*., 2016). For this reason, *E. crassipes*is is frequently used in phytoremediation i.e. a cheapest technology to remove contaminants from polluted sites by the use of plants (Pilon-Smits & Freeman, 2006).

Metal stresses have severe impact on photosynthetic features, altering the structure of photosynthetic pigments by affecting on their enzymatic functioning (Farnese *et al*., 2014). Heavy metals uptake depends on the specific plant species utilized and nature of heavy metals (Memon *et al*., 2001). Plants used for elimination can concentrate heavy metal 100 to 1000 times more than metal sensitive species (Irfan, 2015). Mechanism of heavy metal uptake in phytoremediationis that metal cations like Cd^{2+}cross the cell membrane barrier by the same transporter used for special cation like Fe^{2+}. By entering of metal ion plants prepare phytochelation (PC) by Phytochelatin (PC) synthase activity immediately, that bound to metal cation and inactivates its functioning, otherwise it may be toxic for enzymatic functioning of plants (Thomine *et al*., 2000; Memon *et al*., 2001).

It is hypothesized that *E. crassipes* concentrate toxic substances like heavy metals in their body tissues and prevents them from spreading to other body parts, so it can be utilized as hyperaccumulator.

Materials and Methods

Experiment was conducted to explore tolerance in *E. crassipes* in polluted water. For this, plants of *E.crassipes* taken from highly polluted sites near Sheikhupura-Lahore road. Plants were grown for one year at research sites of Old Botanic Garden, University of Agriculture, Faisalabad. Young plants were taken and put in separate tubs flooded with water and loam (1:1). These young plants given cadmium stress i.e. 0 (control), 50, 100, and 150 mg $L^{-1}3CdSO_4.6H_2O$, and were collected for various morpho-anatomical and photosynthetic features.

Data regarding photosynthetic characters viz. (stomatal conductance (*gs*), transpiration (*E*) rate and net CO_2 assimilation rate (*A*), were recorded using LCA-4 ADC portable infrared gas analyzer. Photosynthetic characters viz. (carotenoids, chlorophyll a, and b) were estimated following procedure of Arnon (1949).

For anatomical studies, small piece of one cm was cut from mid of leaves. The leaves were kept in (FAA) formalin acetic alcohol, for two days, shifted to acetic alcohol for long term preservation. Permanent slides were prepared by sectioning using serial levels of ethanol for dehydration also using doubled staining procedure. Light microscope (Nikon 104, Japan) fitted with camera was used to take photographs.

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

Results

F-ratio for morphological and photosynthetic parameters from ANOVA is presented in Table 1. All morphological parameters of *E. crassipes* viz., shoot fresh and dry weights, number of leaves/plant, plant height and leaf area reduced in response to cadmium stress.

Photosynthetic parameters viz., net CO_2 assimilation rate was reduced as cadmium level increased. Transpiration rate increased consistently in response to higher doses of cadmium. Sub stomatal CO_2 concentration reduced continually along with increase in cadmium stress. Cadmium toxicity decreased stomatal conductance and also affected on water use efficiency.

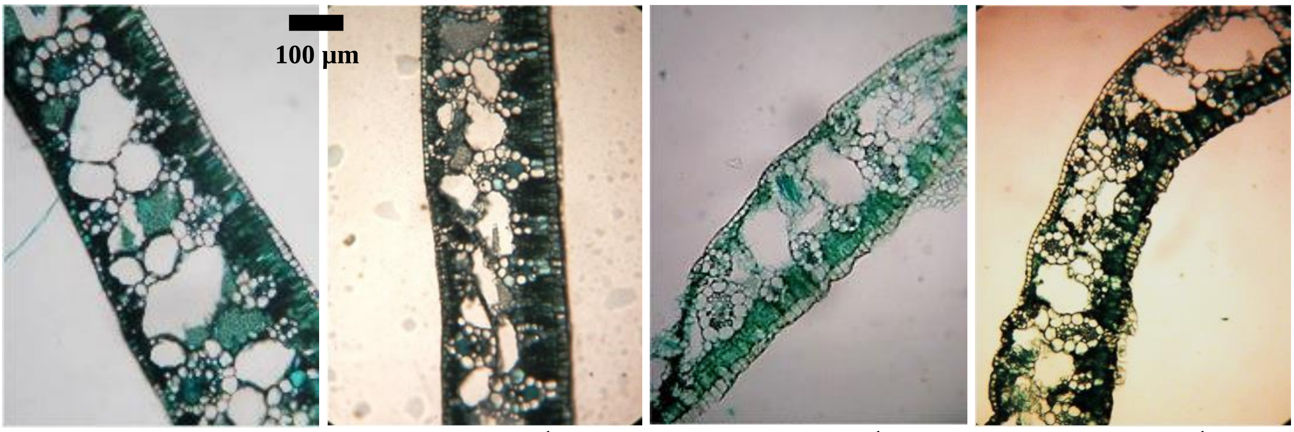
Photosynthetic pigments i.e., Chlorophyll *a* was reduced in response to Cd treatment. Chlorophyll *b* pigment was increased in response to Cd (50 mg L^{-1}) stress, and higher doses of Cd decreased it. Lower dose of Cd (50 mg L^{-1}) had no impact on total chlorophyll and carotenoids but higher doses of Cd decreased it.

Lower level of Cd stress had no impact on upper epidermal cell area, but higher doses of Cadmium (100 and 150 mg L^{-1}) significantly increased its area, but abaxial epidermal cell area was reduced in response to Cd toxicity. Leaf thickness was also reduced by Cd application. Vascular bundle region and aerenchymatous area was significantly affected by metal toxicity. Bundle sheath cell area was increased as Cd levels increased up to 100 mg L^{-1} but higher dose of Cd reduced its area (Fig. 1).

Sclerenchyma area was reduced significantly as Cd levels increased. Chlorenchymatous thickness was decreased by Cd application. Xylem and phloem area was sharply reduced as Cd toxicity increased (Fig. 1). Stomatal number was increased at lower dose of Cd stress but stomatal number was decreased by further increased in Cd concentration while stomatal area consistently decreased in response to Cd stress (Fig. 2).

Table 1. Morpho-physiological and anatomical characteristic of *Eichhornia crassipes* (Mart.) Solms under cadmium stress.

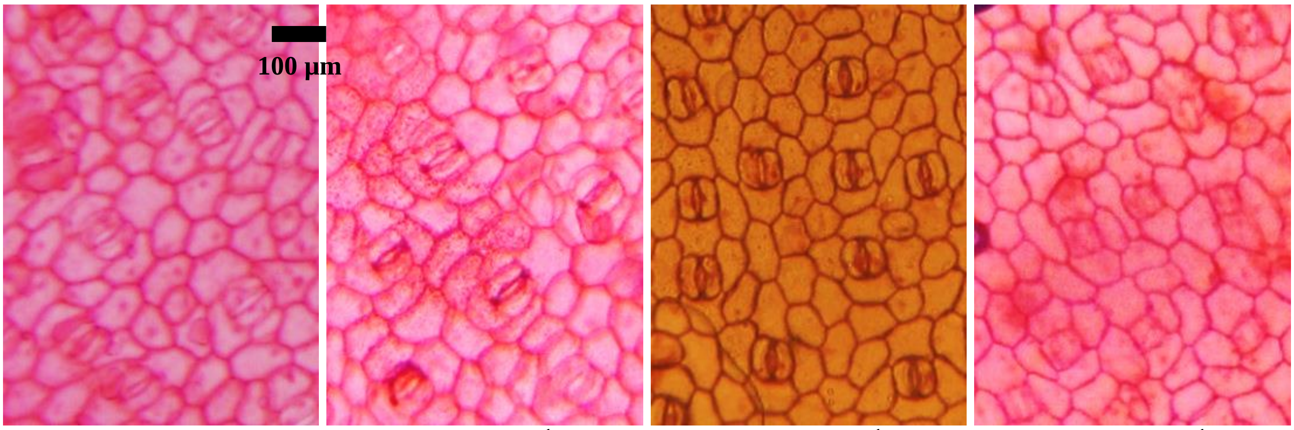
Characteristics	Control (0 mg/L)	50 mg/L	100 mg/L	150 mg/L
Morphological characteristics				
Plant height (cm)	74.6c	71.1c	55.7b	42.5a
Root length (cm)	46.4c	31.9b	28.7b	19.7a
Leaf area (cm^2)	72.8d	42.5b	35.9ab	30.1a
No. of leaves per plant	9.0c	7.0b	6.3b	5.3a
Shoot fresh weight (g $plant^{-1}$)	46.2c	41.6bc	35.2b	23.6a
Shoot dry weight (g $plant^{-1}$)	7.5c	5.4b	4.5ab	3.4a
Metal concentration				
Root Cd content	0.2a	3.6b	9.8c	15.2d
Leaf Cd content	0.2a	0.8b	2.2c	3.2d
Photosynthetic parameters				
Net CO_2 assimilation rate (μmol m^{-2} s^{-1})	10.6d	8.7c	6.3b	4.9a
Transpiration rate (mmol m^{-2} s^{-1})	1.6a	2.5b	2.6b	3.4c
Sub-stomatal CO_2 concentration μmol mol^{-1})	705.6c	444.0b	299.0a	279.3a
Stomatal conductance (mmol m^{-2} s^{-1})	2.4b	1.8ab	1.2a	0.9a
Water use efficiency	6.8d	3.4c	2.3b	1.4a
Photosynthetic pigment				
Chlorophyll *a* (mg g^{-1}f.wt.)	0.44c	0.39bc	0.37b	0.29a
Chlorophyll *b* (mg g^{-1}f.wt.)	0.19b	0.27c	0.19b	0.16a
Carotenoids (mg g^{-1}f.wt.)	0.06c	0.06c	0.05b	0.04a
Leaf anatomical characteristics				
Adaxial epidermal cell area	1220.7a	1203.2a	1569.5b	1796.2c
Abaxial epidermal cell area	767.3c	645.2b	592.9b	418.5a
Leaf thickness	764.3b	516.8a	576.6a	563.0a
Aerenchyma area	78929.3d	52142.8c	41888.6b	24432.1a
Vascular bundle area	33465.6d	13794.3c	12032.9b	7062.8a
Bundle sheath cell area	2441.4b	2563.5c	2528.6c	2179.8a
Sclerenchyma area	17160.1d	12085.3c	5127.1b	2005.5a
Chlorenchyma thickness	212.1b	184.9ab	146.8a	144.1a
Metaxylem area	1220.7c	837.1b	767.3ab	697.5a
Phloem area	10690.2d	4481.8c	3313.4b	2825.1a
Stomatal density	10.0b	12.0c	9.3a	8.6a
Stomatal area	9384.2d	8885.1c	8112.8b	6507.6a



Control **Cd 50 mg L^{-1}** **Cd 100 mg L^{-1}** **Cd 150 mg L^{-1}**

- A significant decrease in leaf thickness under Cd stress
- A consistent decrease in aeranchyma area and palisade thickness along with increase in Cd stress
- A consistent decrease in vascular bundle area along with Cd stress

Fig. 1.Transverse section of leaf of *Eichhornia crassipes* cultivated under different regimes of cadmium stress



Control **Cd 50 mg L^{-1}** **Cd 100 mg L^{-1}** **Cd 150 mg L^{-1}**

- A significant decrease in stomatal area at the highest stress level
- An increase in stomatal density at the highest stress level

Fig. 2. Surface view of epidermis of *Eichhornia crassipes* cultivated under different regimes of cadmium stress (n=6).

Discussion

Heavy metals are affecting terrestrial and water ecosystem by industrial revolution, and have ultimate effect on living organisms and agricultural lands (Ghelich *et al*., 2013). These metal cations when enter in the living organisms, disturb their functions by affecting their cellular or tissue level and cause structural abnormalities (Singh & Sinha, 2004).

Cadmium toxicity has severe effect on all morphological features. Droppingof morphological parameters was also noted in *Hydrilla verticillata* by Singh *et al*., (2012) and in sugarcane by Raza *et al*., 2013. Reduction in growth parameters is due to the progression of reactive oxygen radicles inside the cell that cause necrosis and chlorosis symptoms on the leaves (Mascher *et al*., 2002).It is due to the disturbing of membrane translocation that causes malfunctioning of enzymes and ultimately plant photosynthetic parameters are badly affected (Shaibur *et al*., 2009).

Photosynthesis is decreased by Cd stress because leaf number, leaf area and stomatal area are severely affected by metal toxicity that has direct impact on biomass production (Rai, 2016). Guimarães *et al*., (2012) and Farnese *et al*., (2014) explained that reduction in photosynthetic pigments is mainly due to disruption of carotenoids and chlorophyll by metal stress.

Reduction in photosynthetic pigments is the first symptom of metal toxicity, but stability in chlorophyll structure under stress condition indicates tolerance capacity of plants (Farnese *et al*., 2014; Macfarlane & Burchett, 2001). In the present study in *E. crassipes*, photosynthetic pigments showed stability up to lower doses of Cd stress.

Stomatal conductivity reduced by Cd toxicity has direct impact on gas exchange parameters and on photosynthesis (Agarwal, 2002). Increase in photosynthetic parameters in *E. crassipes* in response to lead stress was reported by Zhou & Han (2005). Increase in photosynthetic parameters directly depends on light and CO_2 capturing capability of plants (Pereira *et al*., 2014), and it also depends on stomatal density, stomatal area and leaf succulence (Zhou & Han, 2005).

Metal accumulator plants exhibit anatomical changes in their parts that protect them from metal toxic effects. These changes include reduction in leaf thickness, vascular regions and parenchymatous cell and enlargement of aerenchyma (Al-Saadi *et al*., 2013). But in this experimental study *E. crassipes* showed dropping of aerenchyma. Al-Saadi *et al*., (2013) demonstrated in *Potamogeton* species, that this sunken hydrophyte was totally dependent on aerenchyma for its existence. But *E. crassipes* is macrophyte that absorbs oxygen openly from fresh air so development of aerenchyma is not crucial for their existence.

Epidermis thickness is beneficial in water conservation, as it reduces evaporation from leaf surfaces. Relationship between leaf turgidity and epidermis thickness in metal toxicity was observed by Gomes *et al*., (2011). Mesophyll tissues help in CO_2 absorption under Cd stress and increase in their mesophyll cells helps plants to carry out their functioning in stress environment and to retain CO_2 concentration inside cell to maintain their photosynthetic level (Pereira *et al*., 2016). Under Cd stress when mesophyll thickness occurs, it helps plants to absorb radiation effectively that are important for photosynthetic process (Pereira *et al*., 2017). Shi & Cai (2009) reported similar results in *Arachis hypogaea*. When plants show stability in leaf anatomical features like mesophyll stability under stress environment, it indicate their metal tolerance (Pereira *et al*., 2014).

Metaxylem is liable for salt and water translocation in vascular tissue, under metal stress reduction in metaxylem also reduced vascular diameter (Ortega *et al*., 2006). When vascular region reduced, it reduces water and solutes transportation that have direct impact on biomass reduction (Ceccoli, 2011).

Stomatal density was important in CO_2 uptake, stomatal density showed no differences under metal polluted environment, while an increase was observed in many cases (Grisi *et al*., 2008; Pereira *et al*., 2014) as also recorded in this experiment.

In conclusion, *E. crassipes* is a metal tolerating plant as it stabilizes different photosynthetic parameters. *E. crassipes* can accumulate high concentration of cadmium and can be used as phytoremediation in metal polluted soil and water. Morphological and physio-anatomical based markers can be used in metal sensitive species to make them metal tolerating plants.

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(Received for publication 25 April 2017)