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ANATOMICAL AND PHYSIOLOGICAL EFFECTS OF CADMIUM IN AQUATIC PLANT *HYDRILLA VERTICILLATA*

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Abstract

The current study was conducted to investigate the anatomical and physiological effects of cadmium in *Hydrilla verticillata* and plant response when exposed to cadmium metals from (January to March 2017). *Hydrilla verticillata* was used to expose to different concentrations (0.5, 1, 3, 6 mg / l) for cadmium cd $^{+2}$ for 15 days laboratory during days (1, 7 and 15). The physiological tests were measured including (Total chlorophyll content, chlorophyll a, b, protein content, catalase enzyme, Superoxide dismutase (SOD)) and anatomical changes showed by preparation tissues slides for Leaves of plant. The highest values of cadmium was recorded during the experimental period in *Hydrilla verticillata* Leaves and highest proportion of removal ratio of the metals was (99.4%) at (3) mg / l during the 15th day. The bioaccumulation factor (BCF) of the Cd^{+2}in the plant Leaves recorded the highest value at 0.5 mg / l during the 15th day.

The current study showed a decrease in protein content during the experiment period, and a gradual increase in the SOD values during the experimental period compared to the control.

The values of the efficiency of catalase enzyme of Cd^{+2} were observed to be the highest during the 7th day and decreased in the 15th day at concentrations 6 mg / l. The anatomical changes of plant showed increased air spaces and small area and mesophyll cells occupied the Leaves center and epidermis cells increased in thickness at 0.5 mg/l of metal, while at 6 mg/l decreased in thickness of Leaves and air space decreased in number and increased in the area and the mesophyll cells has been degradation and epidermis cells increased in thickness. The study concluded that the high concentration of heavy metals was caused by the dissolution of plant cells and plant death.

Key words : Anatomical, Hydrilla, physiological, bioaccumulation, cadmium.

Introduction

Hydrilla is an invasive non-native submerged plant with long slender stems that branch out profusely when they reach water surface. Hydrilla can grow an inch a day and 50% of the standing crop occurs in the top 0.5 m of the water column (Dhir, 2013).

Plants are of great importance to various organisms, including humans. They are necessary to sustain life on earth as source of oxygen necessary for breathing of organisms. In addition to the food factories of other animals (Siracusa and La Rosa, 2006). Aquatic plants are used to treat polluted waste water around the world due to their low cost and ease of maintenance. Aquatic plants play a significant role in protecting many animals from predators and provide available surfaces for adjoining plankton (Warfe and Barmuta, 2006). Because of rapid growth rates, simple growth requirements, bio-accumulation and toxic substances, aquatic plants are used to remove nutrients and nutrients from water and are widely used in wastewater treatment and increasingly in nutrient-rich water remediation (WitWit, 2015).

Studies indicate that many plants have the ability to extract and accumulate heavy metals as a result of having many resistance means that can remove their toxicity, such as the formation of protein-like complexes, as in the case of metallothionines, or the capture of these elements by peptides having low molecular weight known as plant claws (Kachout *et al*., 2010; Aravind *et al*., 2009).

Accumulation of heavy metals in the organism can

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move through the different feeding levels in the food chain and thus have a wider and more serious impact, and disrupting the ecological balance and reducing diversity of life (Canli and Kalay, 1998). Through, a review of the above mentioned, the idea to study and research was conducted to investigate the anatomical and physiological effects of cadmium metals in *Hydrilla verticillata* and plant response when exposed to metals.

Materials and Methods

The study was conducted from January to June 2017 in the Department of Ecology lab. The submersible plant *Hydrilla verticillata* was collected from Euphrates River. Plants were washed several times with tap water then distilled water in order to remove any small invertebrates and algae (Lytle & Smith, 1995). Plants were adapted for day 10 in tap water. After acclimatization, they were exposed to the selected concentration at 0.5, 1, 3, 6 ppm for Cd^{+2} at a time interval of 1, 7, 15 days. Triplicate batch tests were conducted in glass container of dimensions (30*30*30) cm. Chosen heavy metal concentration was added in each container from prepared stock solution. About 100 gm. plant was kept in each container noticeable for the water level. All containers were exposed to light adequately for detention time of 15 days. Every day, tap water was added to maintain the same level in each container. The aquatic plant was exposed separately to the individual metal ion solutions of cadmium ($CdCl_2$). After each time interval the plant was collected and washed with deionized water to remove any metal adhering to its surface. The washed plant samples were carefully dried. They were dried for 48 hrs in an oven at 70ºC. After drying, they were ground and digested according to Orson *et al*. (1992). Chlorophyll, chlorophyll a and b were measured according to Aminot and Rey (2000).

Total protein was measured according to (Pak, 2010). Superoxide dismutase was measured according to (Marklund and Marklund, 1974) and enzyme activity was calculated by equation of Frary *et al*. (2010).

$$\text{SOD activity (units)} = \frac{\text{\% inhibition of pyragallol reduction/50\%} \times \text{reaction volume}}{\text{Total test period}}$$

Catalase enzyme was measured according to Aebi method (1983) and enzyme activity was calculated depending on equation (Frary *et al*., 2010).

Catalase activity (unit /g)= (Δabs\min_x reaction volume)\0.001

The bio concentration factor (BCF) is a useful parameter and it provides the ability index of a plant to accumulate metals with respect to metal concentration in the medium and it was calculated on a dry weight basis (Zayed *et al*., 1998).

BCF = Trace elements concentration in plant tissue ($\mu g.g^{-1}$)/Initial concentration of the element in the external nutrient solution ($mg.l^{-1}$).

The method of Thammathaworn (1996) was used to prepare tissue slide of the plant.

Statistical analysis

All data were subjected to analysis of variance (Two-way ANOVA test) and it was used for further statistical analyses and to determine the significance difference between treatments least significant difference (The value P>0.05) and coefficient of correlation.

Results

The highest values of cd^{+2} concentrations recorded in Leaves of plant was 0.68 mg / l and the lowest values were recorded at the control treatment during the 1 day (0.006) mg / l. The values increased gradually during experiment period and significant differences were recorded below the level of probability ($p > 0.05$) between concentrations and days of experiment except 6 mg / l did not show any significant differences during the 1 and 7 days (fig. 1). The highest values concentration of cd^{+2} (in the ponds of the experiment containing the *H. verticillata*) was (0.51) mg/l during the 1 day at the 6 mg / l and the lowest values was (0.005) at the control treatment during the 15th day the significant differences were recorded below the probability level ($p>0.05$) between concentrations and days of experiment (fig. 2).

The removal ratio highest of cd^{+2} was recorded at 3 mg / l during the 15th day (99.4%) and the lowest recorded at 1 mg / l during the 1 day (table 1).

The accumulation of cd^{+2} in the Leaves recorded highest value at 0.5 mg/l, (82902) during the 15th day and the lowest recorded at 6 mg / l during the 1 day (1194) (table 2). Table 3 showed the variation of the total chlorophyll of *H. verticillata* exposed to cd+2. It increased during the 7 day of all concentrations compared to control and decreased on the 15th day. The highest value was (0.38) mg / g at 3ppm Cd^{+2} during 7th day and the lowest value was (0.052) mg / g at the 6ppm Cd^{+2} during the 15th day. The results of the statistical analysis showed significant differences in the level of probability ($p >0.05$) between days and concentrations during experiment period and the value of LSD = 0.07.

The chlorophyll a decreased gradually during the 7th and 15th days with the exception of the concentration 3

mg/l. It increased during the 15th day compared to the control treatment. There were no differences between the concentrations during the 1 and 15th days, while significant differences were observed during the 7th day (table 3). Chlorophyll b increased during the 7th day and decreased slightly on the 15th day. There were no significant differences between the days of the experiment except the concentration of 6 mg/l and recorded the highest values during the 7th day at 3 mg/l was 0.297 mg /g.

The current study showed a decrease in protein values during the experiment period. Protein values ranged between (2.25-31.25) mg/g to the weight of *H. verticillata* exposed to cd^{+2} (table 4). The results of the statistical analysis showed significant differences in the probability level ($p>0.05$) between some days and concentrations. Gradual increase showed SOD values *of H. verticillata* from day 1 compared to control treatment was between (0.060-0.098) unit/mg. Significant differences were recorded between experimental days and concentrations except the 15th day. No significant differences were observed between the concentrations below the probability level ($P> 0.05$) and the value of LSD = 0.001 (table 4).

The values of the catalase activity range was (0.53-3.72) mg/mg for plant exposed to cd^{+2}. The height of values was observed during the 7th day and decreased on 15th day for the concentrations (3 and 6 mg / l) and the high values compared to the control treatment. Results of statistical analysis were significant differences below the probability level ($p> 0.05$) between days and concentrations (table 4).

Plate 1 shows a cross section of *H. verticillata* Leaves treated with various concentrations of cd^{+2} (0.5,1,3,6) mg/l during the 15th day. It shows increase of the air spaces with small sizes where the mesophyll cells occupied the center of the leaves. Epidermis cells increased in thickness (image B) compared to control treatment (image A) at treatment 0.5 mg/l. Image C shows the treatment 1 mg/l where some walls of air spaces dissembled and occupied the center of leaves, mesophyll cells decreased and occupied edges only, the epidermal cells increased in thickness and multiplied layers. Image (D) showed decrease in leaves thickness in general and air spaces decrease in number of cells with mesophyll cells occupying leaves edges and decreased in area. The epidermal cells increased in thickness and the layers of epidermal cells when treated at 3 mg/l. Image (E) showed a cross section in the plant leaves treated with a concentration of 6 mg /l. The leaves thickness decreased, the air spaces decreased in number and increased in area, and the mesophylls cells were dissociated and epidermis cells increased in thickness.

Discussion

Accumulation in plant

The results show that the content of *H. verticillata* plant increased for the cd^{+2} concentration at (3, 6) mg/ l in plant leaves during 15th day. This could be because the aquatic plants are efficient in removing contaminants, extracting heavy metals and accumulating in their bodies to have resistance means to them, including proteins that bind to elements as metallothionine or low molecular weight peptides called plant claws (Kachout *et al*., 2010). The concentration of heavy metals within plant bodies is controlled by several factors, including pH, presence of other elements and ions, temperature, salinity, light intensity, amount of oxygen, organic volatiles and humic substances (Greger, 1999), or may be to tolerate plant of heavy metals at these concentrations and balance of all enzymatic and molecular antioxidants as well as increase the secretion of cellular metabolites (Wahibi, 2007) and may be the preferred concentrations of plant growth and tolerance. In the study of Srivastava *et al*. (2010) the carrying of aquatic plants to different concentrations of the metals and its continued growth is the result of a balance in the levels of enzymatic and molecular antioxidants such as (Proxidase, proline and total phenols) and the possibility of increasing the secretion of cellular metabolism products such as Cysteine and glutamine. The study agrees with the study (Al-Asadi, 2014) and the study (Tawfiq and Alkbisi, 2014) and the study (Kadhum, 2017).

Bioaccumulation factor values are indicators of plant susceptibility to the accumulation of metals in their tissues (Marbaniang and Chaturvedi, 2014). The results showed the bioaccumulation ratio is the highest accumulation value recorded at 0.5 mg /l during the 15th day of metal and the lowest at high concentration,or the plant's ability to absorb the elements at its preferred concentration is 0.5 mg /l during the experiment period without damage to the plant. Differences in the accumulation of heavy metals within plant tissues may be due to the different cellular mechanisms of aggregation and the biological concentration of necessary and unnecessary elements in plants (Prasad *et al*., 2001).

Chlorophyll

The physiological status of *H. verticillata* study by determination total chlorophyll, chlorophyll a and chlorophyll b. Chlorophyll is the green pigment responsible

for photosynthesis of plants for energy production. This pigment is found within the plant cell in the plastids (Lefsrud and Kopsell, 2005) and is affected by the content of the green pigments of the plant cell with the presence of the necessary elements such as calcium, magnesium and iron responsible for the formation of chlorophyll for photosynthesis process. Iron plays a key role in the formation of chlorophyll through the presence of several enzyme helpers, including Cytochrome Oxidase, Catalase and Peroxidase (Sarvari *et al*., 2008).

The results of the study showed a decrease in total chlorophyll values during the experiment duration when exposed to Cd^{+2} due to the stimulation of ROS (Reactive Oxygen species) for plant stress that directly or indirectly affects photosynthesis (Liu *et al*., 2009; Khataee *et al*., 2012). Dietz *et al*. (1999) the increased of heavy metals concentration stimulates formation of free radicals and some effective forms of oxygen that lead to oxidative stress, or result breakdown of the chlorophyll molecule (Parlak, 2016), or inhibition of enzymes that link with chlorophyll synthesis. The poor processing of magnesium and iron are important in chlorophyll synthesis process (Mishra *et al*., 2006). Heavy metals can interfere with chlorophyll synthesis by directly inhibiting enzymes or causing nutrient deficiencies (Vanassche and Clijsters, 1990) and effect on enzymes that responsible for chlorophyll the cadmium inhibits protochlorophyllate reductase enzyme and manufacturing Amino Levulinic Acid (Stobart *et al*., 1985), or as a result of increased sodium and chloride values that reduce plant pigments (Noori *et al*., 2014).

Protein

The dissolved protein content is an important indicator of the physiological state of plant (Doganlar *et al*., 2010). It has an important role in the metabolism and membrane of the cell as it regulates the processes that overlap the external and internal membrane (Kharat *et al*., 2009). A decrease in the protein content of plant during experimental period is due to plant stress for the formation of ROS (Reactive Oxygen species), which is an oxygen-containing chemical reaction molecule such as Superoxide anion (O_2), hydrogen peroxide H_2O_2 and hydroxyl radical(OH^-), leading to a oxidative stress that produces these compounds as transverse products during metabolism that affect in plant cells and lead to their death as well as the breakdown of protein, fat, and DNA (Smirnoff, 2005). A biotic stress may inhibit the production of certain proteins and stimulate other proteins (Ericson and Alfinito 1984), with trend to general decrease. Mochin and Hosetti (1997) found a reduction in the protein content

Table 1 : Removal ratio for cadmium from *H. verticillata* during experimental period.

15 day	7 day	1 day	Con. ppm	Metal
98.7	98.3	85.3	0.5	Cd^{+2}
98.8	97.7	76	1	
99.4	99.1	89.6	3	
98.8	96.4	89.7	6	

Table 2 : Bioconcentration factor for cadmium in *H. verticillata* Leaves during experimental period.

15 day	7 day	1 day	Con. ppm	Metal
82902	56889	5701	0.5	Cd^{+2}
53100	26471	2222	1	
48333	25775	2192	3	
11333	3426	1194	6	

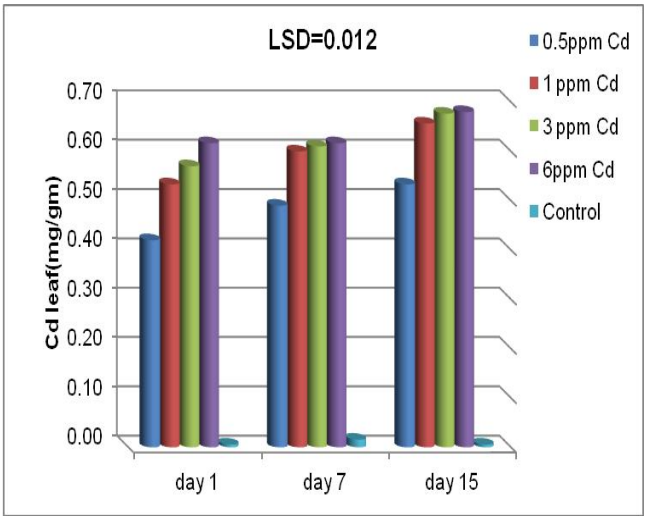

Fig. 1 : Cd^{+2} concentrations in *H. verticillata* Leaves during experiment duration.

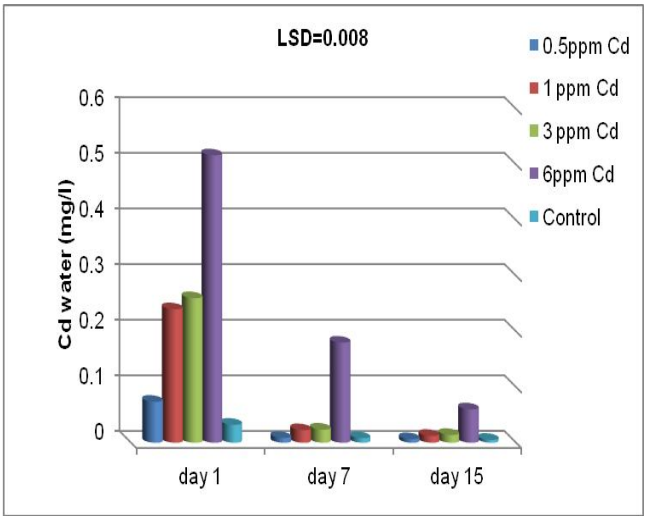

Fig. 2 : Cd^{+2} concentrations in water during experiment duration.

Table 3 : Mean total chlorophyll, chlorophyll a, and b in *Hydrilla verticillata* during exposed period.mg/gm.

Con. of metal		Total chl. mg/gm			Chl. a mg/gm			Chl. b mg/gm		
		1 day	7 day	15 day	1 day	7 day	15 day	1 day	7 day	15 day
Cd	0.5 ppm	5.01	4.76	4.19	1.53	0.99	1.22	3.49	3.78	3.54
	1 ppm	4.98	5.11	4.32	1.57	1.44	1.37	3.41	3.68	3.31
	3 ppm	5.00	6.31	6.17	1.57	1.36	1.98	3.43	4.95	4.66
	6 ppm	4.92	2.67	0.87	1.55	0.59	0.25	3.37	2.08	0.74
	Control	5.03	4.98	4.53	1.52	1.43	1.44	3.53	3.55	3.46
		LSD=0.07			LSD=0.09			LSD=0. 7		

Table 4 : Mean total protein, Superoxide dismutase, and Catalase in *Hydrilla verticillata* during exposed period.

Cd^{+2} conc. ppm	Total protein(mg/gm)			SOD (units/mg)			Catalase(units/mg)		
	1 day	7 day	15 day	1 day	7 day	15 day	1 day	7day	15 day
0.5	11.75	5.75	5.25	0.032	0.070	0.103	0.55	0.57	2.10
1	10.75	5	4.75	0.036	0.073	0.110	1.58	2.23	3.67
3	8.75	3.75	3.25	0.037	0.076	0.111	2.22	3.22	1.58
6	6.25	3.25	2.25	0.042	0.089	0.106	2.68	3.72	2.63
Control	30.75	31.25	29.75	0.038	0.045	0.046	0.54	0.53	0.54
	LSD=0.8			LSD=0.001			LSD=0.11		

Table 5 : Correlation of parameter of *H. verticillata*.

Chlorophyll b	Chlorophyll a	Total Chlorophyll	SOD	Protein	CAT	Cd Plant Leaves	Cd Water	Parameter
							1	Cd water
						1	0.63	Cd plant Leaves
					1	0.86	0.86	CAT
				1	-0.79	-0.99	-0.59	Protein
			1	-0.99	0.87	1.00	0.69	SOD
		1	-0.26	0.19	-0.37	-0.18	-0.73	Total Chlorophyll
	1	0.97	-0.35	0.30	-0.36	-0.26	-0.72	Chlorophyll a
1	0.94	0.99	-0.22	0.14	-0.39	-0.14	-0.73	Chlorophyll b

of *Lemna minor* treated with lead this was attributed to protein degradation due to the increased activity of protease. In the results, we note that there is a positive relationship between the protein content and the amount of chlorophyll and reactive with the value of SOD (table 5).

Catalase enzyme

Catalase is an important, antioxidant and free-radical enzyme (Scandalios *et al*., 1997). It spreads in living organisms and can stimulate the decomposition of hydrogen peroxide into water and oxygen before cellular damage occurs either directly or through the generation of the hydroxyl root (Nicholls *et al*., 2001). It exists in certain organisms in plants such as Peroxisomes and is found in mitochondria, plastids and cytoplasm (Willekens *et al*., 1995).

The increase in enzyme activity during the 7th day and its decrease on the 15th day of the experiment may be due to the plant's susceptibility to possibly stress conditions for the period or may be to the role of cadmium in stimulating the bio-processing of antioxidant enzymes (Aravind and Prasad, 2005).

Superoxide dismutase (SOD)

The SOD is considered the first line of defense against ROS (Alscher et al., 2002). Automatically it splits free radicals and generates H_2O_2 and O_2 (Asada, 1992). It is a basic element in the defense against natural and industrial pollutants, the important function of this enzyme is to restore cell vitality and reduce the speed of destruction. It is used to detect the harmful effects of

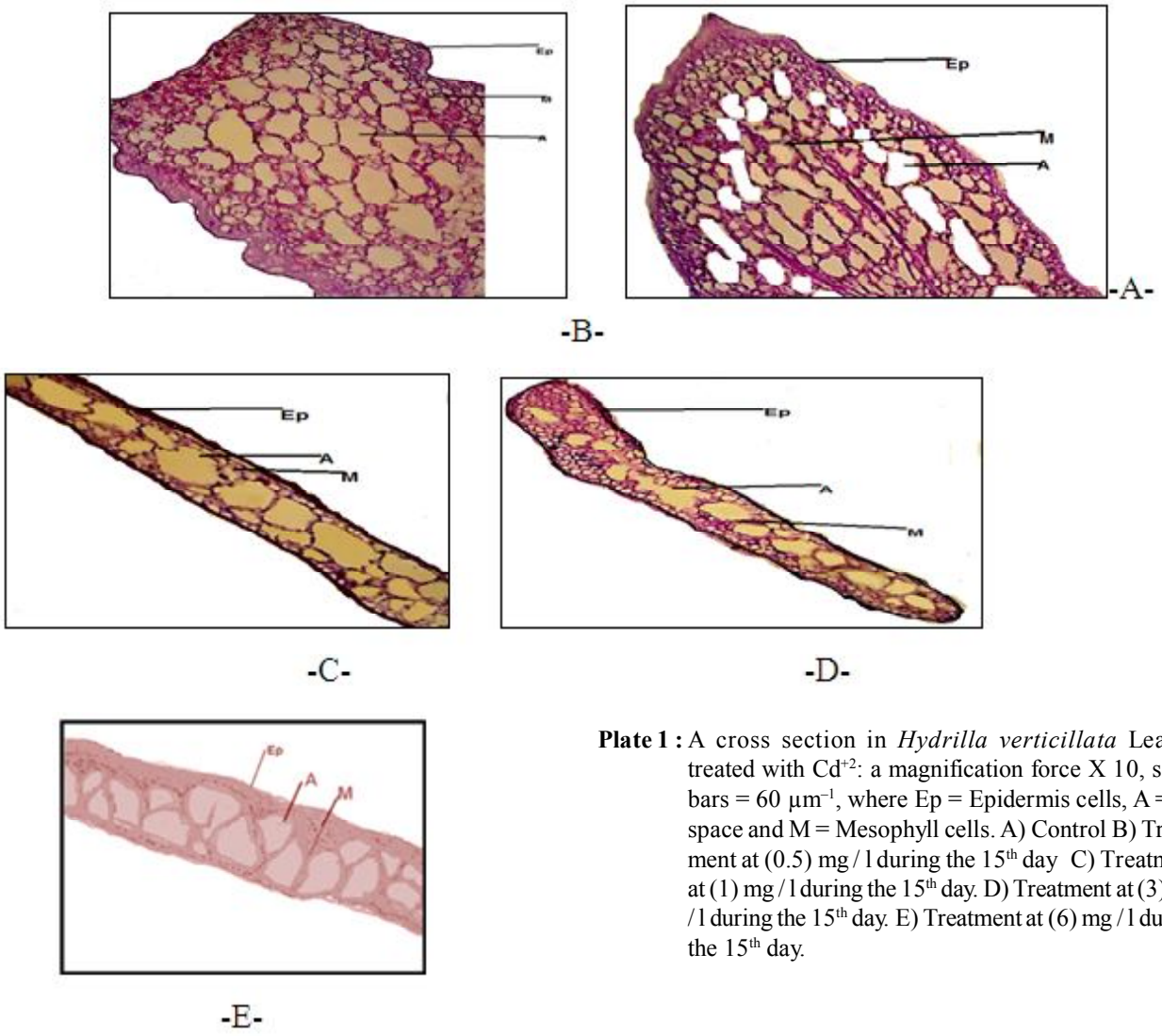

Plate 1 : A cross section in *Hydrilla verticillata* Leaves treated with Cd^{+2}: a magnification force X 10, scale bars = 60 μm^{-1}, where Ep = Epidermis cells, A = air space and M = Mesophyll cells. A) Control B) Treatment at (0.5) mg / l during the 15th day C) Treatment at (1) mg / l during the 15th day. D) Treatment at (3) mg / l during the 15th day. E) Treatment at (6) mg / l during the 15th day.

pollutants in aquatic organisms (Sahan *et al*., 2010). This enzyme is found in green leaves. Its direct effect is to regulate the amount of ROS and its effectiveness in the leaves is higher than its effectiveness in the roots. The high efficiency of the enzyme in the leaves is similar to the leakage of electrons from the chain of transmission of electrons in photosynthesis to the oxygen molecule (Liu *et al*., 2009). The results of current study showed a gradual increase in the SOD values and attributed the reasons for the high effectiveness of the enzyme may be due to the fact that the antioxidants of the enzyme has differed in their effectiveness and the ability of the plant to tolerate stress conditions (Hanfeng *et al*., 2010). Agree the result with Singh *et al*. (2013) The effectiveness of the enzyme has increased in the *H. verticillata* when exposed to different concentrations of Pb and Cd. The low efficiency of the enzyme may be due to the sensitivity of the plant to high concentrations that reduce its effectiveness and thus lead to the collection of ROS in plant tissues and increase the rate of DNA destruction (Ai-jun *et al*., 2007).

Anatomical changes

The anatomical variations in the plant, the thin walls were initially observed during the few concentrations (0.5 and 1) mg /l of Cd while in the high concentrations, the leaves size was reduced in general and the number of cells decreased and degradation. The epidermis is composed of all the plant members of thin-walled cells, which are not calcified by the Cuticle and therefore are non-protective (Yeo *et al*., 1997). The difference in function and structure of epidermis in aquatic plants as compared with that of plants growing in aerial habitat is outstanding. The epidermis is not protective in aquatic plants but absorbs nutrients and gases directly from water. Epidermis on all parts of water hyacinth consists of a single layer of rectangular cells which is characteristically a constant feature of this species. A very thin cuticle and

thin cellulose walls of epidermal cells in a typical hydrophyte assist steady absorption from surrounding water (Mahmood *et al*., 2005). Size variations in epidermal tissues in response to water pollution conditions. Increased thickness of the epidermal cell, as caused by heavy metals, could be associated to adsorption of metals in the cell walls, constituting an alternative pathway for allocation of these ions and stopping their translocation to photosynthetic tissues (Melo *et al*., 2007; Sridhar *et al*., 2005). Exposure to heavy metals leads to a reduction in the thickness of mesophyll cells which agree with (Sandalio *et al*., 2001; AL-Saadi and Qader, 2016). Conditional on anatomical plasticity, some species improve modified leaf tissues that allow better adaptability to different stress conditions (Sridhar *et al*., 2005).

Conclusion

Hydrolysis is highly efficient in cadmium reduction and the concentration used for bio-treatment of elemental accumulation in *H. verticillata* plant which was 0.5 mg / l during the experiment period without damaging plant. The high concentration of Cd^{+2} metals in the study cause plant cell degradation and plant death.

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