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MONITORING OF CADMIUM TOXICITY ON PHYSIOLOGY AND BIOCHEMISTRY OF *Alternanthera philoxeroides*

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ABSTRACT:

The hydroponics experiment was carried out to monitor the cadmium (Cd^{2+}) toxicity on growth, accumulation, antioxidative enzyme responses and total soluble protein content in *Alternanthera philoxeroides* seedlings, treated with different concentrations of Cd (5, 10, 25, 50, 75 mg l^{-1}) for 12 days. Cd concentrations in treated seedlings increased with the higher concentration of Cd. The shoots accumulated 1371.86 mg kg^{-1} Cd dry weight at 75 mg l^{-1} Cd concentration, while the roots accumulated 6801.0 mg kg^{-1} Cd dry weight. *Alternanthera philoxeroides* responded to Cd induced oxidative stress by antioxidative enzymes: catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX). The content of CAT, POX, and APX ratio was gradually increased at the increasing concentration of Cd in both leaves and roots on 12th day. The changes in the activities of photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoides were also observed. The content of total soluble proteins followed the same trends as antioxidative enzymes, gradually increasing in the increasing concentration of Cd. These results suggest that antioxidative defense mechanism play a significant role in detoxification and accumulation of Cd in *A. philoxeroides*.

Keywords: *Alternanthera philoxeroides*, cadmium, antioxidative enzymes, catalase, peroxidase, ascorbate peroxidase.

[I] INTRODUCTION

Cd is highly toxic to humans, animals and plants. Das et al (1997) reported that Cd induced the toxic effects on plants. It enters the environment mainly through industrial and agricultural activities and then is transferred to the food chain. Cd is a priority pollutant not only from the human health perspective, but also from a broader ecosystem view point. In general, it is biologically non-essential, non-biodegradable, persistent type of heavy metal and its compounds are known to have high toxic potentials. Cd uptake can induce the cellular damage [26], Cancer, kidney tubular damage, bone damage, renal dysfunction, glomerular damage and causes of death [28]. Cd is widely used in many industries such as Textile/dye industries, automotive, metal production, electroplating, battery and electric cable manufacturing, mining,

tannery and steel. Textile/dye industries have released most of the Cd effluents into the environment [27]. As the world population increases demands of clothing also increased with the improving sense of fashion and lifestyle thus many textiles are manufactured to meet the growing demands. In India textile production is one of the sources of income. However, this has brought either in a positive way which is an improvement of economy or in a negative way attributed to environmental pollution [12]. The textile industries have been used more than 2000 types of chemicals and over 7000 types of dyes. So the disposal of effluents from these textile industries has become a serious problem. It has been estimated that several industries release effluents at the rate of 1.5 mg d (7 million liters day^{-1}). The release of these effluents without proper treatment into environment are highly toxic and can bioaccumulates in the human body, aquatic life, natural water bodies and also possibly trapped in the soil [27]. So there is a dire need for the removal of Cd heavy metal from the environment. Phytoremediation of heavy metals is a cost effective green technology in which the use of metal accumulating plants to remove toxic metals such as Cd, Cr, Hg and Pb from soil and water [17]. *Alternanthera phlioxeroides* is one of the aquatic macrophytes which are commonly known as alligator weed [14]. It demonstrates very strong reproductive abilities that even small plant fragments are readily established and spread in novel environments. It can grow as either a trailing, terrestrial, herb or as a floating aquatic. Therefore it is also a convenient plant material for ecotoxicoogical investigations [19]. Cardwell et al (2002) and Kamal et al (2004) have also been reported that aquatic macrophytes potential for the simultaneous removal of heavy metals. Qiu et al (2008) reported that Cd has been found to generate free radicals that may damage plant tissues. Depending on its concentration and plant species, Cd can either inhibit or stimulate the activity of several antioxidative enzymes. Reactive oxygen species (ROS) are a main part of free radicals, which can lead to oxidative stress. ROS can react with proteins, pigments and antioxidative enzymes. On the other hand, an antioxidative defense system has been developed in plant cells to fight against toxic free radicals in order to protect themselves from oxidative stress induced by heavy metals. It includes CAT, POX and APX. CAT can convert H_2O_2 to H_2O and molecular oxygen. APX functions in ascorbate/ glutathione cycle, and it uses two molecules of ascorbate to H_2O_2 to water [13]. In our present study increased activity of antioxidative enzymes were reported in *A. philoxeroides* exposed to higher concentration of Cd at 75 mg l^{-1}. Therefore, *A. philoxeroides* seedlings were undertaken to examine the hyper accumulation of Cd heavy metal under hydroponic systems with reference to: (i) growth responses of Cd induced plants; (ii) bioaccumulation of Cd; (iii) changes in content of chlorophyll a, b and carotenoides; (iv) increased activities of CAT, POX and APX; (v) changes in the content of total soluble proteins.

[II] MATERIALS AND METHODS

2.1. Plant material and metal treatment

Alternanthera philoxeroides were collected and the plants were washed several times in tap water to remove the soil particles. Plants having approximately same height and weight were carefully selected and transferred into plastic container filled with full strength Hoagland Nutrient Solution for hydroponic settings [2]. The hydroponic system was set up in the Green House. After 15 days both the root and shoot lengths of hydroponically growing plants were determined and treated with Cd in different concentrations 5, 10, 25, 50, 75 mg l^{-1}, while medium without Cd served as control. The physiological and biochemical indexes were measured from 12 days of culture

2.2. Growth criteria

Both shoot and root lengths were measured before and after Cd treatment. The biomass was estimated by the measurement of shoot and root dry weight. Index of tolerance (IT) and water content of leaves were calculated ((FW-DW)*100 / FW).

2.3. Estimation of Photosynthetic pigments

The spectrophotometric method recommended by Arnon (1949) was used to estimate chlorophyll a, chlorophyll b and carotenoides.

2.4. Analysis of metal accumulation by ICP-AES

The accumulation of cadmium was analyzed by Inductively Coupled Plasma Atomic Emission Spectrometry according to the method of Israr et al., (2006). The digestions of heavy metal treated plants were accomplished using a microwave oven at 105◦C for 15 minutes with 10 ml of concentrated $HN0_3$. Subsequently the sample volume was adjusted to 13ml with double distilled water and all the extracts were analyzed using ICP-AES.

2.5. Determination of CAT activity

CAT activity from the leaves and roots was determined using the method of Aebi (1984).

2.6. Determination of POX activity

POX activity was measured using the method of Castillo et al., (1984).

2.7. Determination of APX activity

APX activity was determined according to the method of Nakano and Asada (1987). **2.8. Determination of protein content**

Total soluble protein content of plant tissue was estimated according to the method of Bradford (1976).

2.9. Statistical analysis

To confirm the validity of data and results, all the data were subjected to an analysis of variance (ANOVA) and determine the significant difference between the treatments. Each result shown in figures is the mean of three replicates and significant difference was statistically evaluated by standard error of mean method.

[III] RESULTS

3.1.Effect of Cd on plant growth parameters

A.Philoxeroides seedlings were exposed to different concentrations (5, 10, 25, 50, 75 mg/l) of Cd for 12 days. Both the shoot and root growth were affected in all the concentrations used in the experiments. Table 1 depicted the effect of Cd on shoot and root length, index of tolerance and relative water content between control and treated plants. Moreover, the shoot and root lengths of *A. philoxeroides* was significantly decreased in the increasing concentration of Cd (Fig 1). The relative water content and the index of tolerance revealed that both shoot and root lengths were also significantly affected with the increasing concentration of cadmium. In addition, the size of the leaves of Cd treated plants was smaller than those in the control plant leaves.

3.2. Effect of Cd on photosynthetic pigments activity

The effect of Cd on photosynthetic pigments viz., of Chl a, Chl b and Car *A. philoxeroides* leaves at different concentrations and different exposure periods shown in Table 2, 3, 4. All photosynthetic pigments increased at lower concentration and reached the highest level (1.54±0.001, 1.55±0.001 and 0.553±0.001 mg g^{-1} FW of 12th day Chl a, Chl b and Car, respectively) at 10mg/l of Cd and then decreased slightly ($p<0.05$) in the higher concentration. A concentration dependent decline of Chl a, Chl b and Car occurred as a consequences of exposure to Cd concentrations of 75mg l^{-1} in 12^{th} day samples.

3.3. Cd accumulation in *A. philoxeroides*

The uptake and accumulation of Cd in *A. philoxeroides* roots and shoots varied with Cd concentration. The highest Cd concentrations in shoots and roots were 1371.86 and 6801.0 mg kg^{-1} DW respectively, when *A. philoxeroides* was treated with 75 mg l^{-1} Cd in the solution. Most of

Cd taken up by *A. philoxeroides* accumulated in the roots and small amounts of Cd were transferred to shoots.

3.4. Effect of Cd on antioxidative enzyme activities in leaves of *A. philoxeroides*

3.4.1. Effect of Cd on CAT activity

The effect of cadmium on CAT activity (U g^{-1} FW) of *A. philoxeorides* leaves at different concentrations and exposure periods were shown in Table 5. The activity of CAT was significantly increased in *A. philoxeorides* seedlings up to 50 mg/l Cd treatments (0.748 U g^{-1}FW) and then decreased in 75 mg/l Cd treatments (0.501 U g^{-1} FW). Also CAT activities differed with increasing concentrations of metals as well as different exposure periods (Fig 5).

3.4.2. Effect of Cd on POX activity

Figure 6 showed the effect of Cd on POX activity of *A. philoxeorides* leaves at different concentrations and exposure periods.. However, a significant increase in the activity of POX (6.487 U g^{-1} FW) was observed at 50 mg l^{-1} Cd treatment. The activity slightly decreased at the concentration of 75 mg l^{-1} Cd; however the activity was appreciably higher with respect to control (Table 6).

3.4.3. Effect of Cd on APX activity

The effects of varying concentration of Cd on APX were depicted in table 7. Cd altered the levels of APX in *A. philoxeroides* leaves after 12 days treatment. Cd at a concentration of 50mg l^{-1} significantly increased (4.618 U g^{-1} FW) APX level with respect to the control. A slight reduction (3.717 U g^{-1} FW) in APX ratio was observed at a concentration of 75 mg l^{-1} (Fig 7).

3.5. Effect of Cd on antioxidative enzyme activities in root tissues of *A. philoxeroides*

Table 8 shows the effect of Cd on CAT, POX, APX activity (U g^{-1} FW) of roots tissues of *A. philoxeorides* at different concentrations after 12 days treatment. The activity of CAT, POX, APX was significantly increased (0.970, 1.036, 1.846 U g^{-1} FW) in the roots of *A. philoxeorides* with increasing (75 mg l^{-1}) Cd treatments (Fig 8). However the antioxidative enzyme activities differed with different concentrations. But the highest increase in APX activity was noticed Cd treated seedlings when compared to other enzyme activities.

Cd (mg/l)	Shoot length (cm)	Root length (cm)	IT values (%)		RWC (%)
			Shoot	Root	
0	26.6±0.08	15.4±0.06	0.0	0.0	66.0
5	20.8±0.10	13.9±0.48	82.8	94.2	65.8
10	19.6±0.12	13.0±0.68	78.0	89.0	65.7
25	19.0±0.08	12.8±0.52	77.6	87.1	64.6
50	18.2±0.17	12.0±0.10	74.6	86.4	63.5
75	16.5±0.29	10.0±0.57	73.9	84.5	63.4

Table: 1. Effect of Cd on shoots and root lengths of *A. philoxeroides*. Data are means ± SE (n=3), significantly different (P<0.05) to control plant**.**

3.6. Effect of Cd on total soluble protein content

The changes of total soluble protein content were depicted in (Fig 9). Accumulation of total soluble protein content in leaves was showed increased trend in 12th day samples but it was decreased in others. However, the significant level of protein accumulation noticed was11.72 and 11.40 mg g^{-1} FW with 50 and 75 mg l^{-1} Cr treatments, respectively (Table 9). This result indicates that the *A.philoxeroides* seedlings were experienced with heavy metal stress at higher Cd concentrations that triggers various proteins as consequence.

Cd (mg/l)	Chlorophyll a (mg g^{-1} FW)			
	3 day	6 day	9 day	12 day
0	1.20±0.01	1.79±0.00	1.34±0.00	1.54±0.00
5	1.20±0.01	1.66±0.00	1.30±0.00	1.41±0.02
10	1.19±0.00	1.59±0.00	1.03±0.01	1.36±0.00
25	1.18±0.00	1.33±0.00	0.99±0.00	1.34±0.03
50	1.09±0.00	1.29±0.00	0.83±0.00	1.13±0.00
75	1.07±0.00	1.00±0.00	0.73±0.00	1.23±0.00

Table 2: Effect of Cd on chlorophyll a of *A. philoxeroides* leaves. Data are means ± SE (n=3), significantly different (p<0.05) to control plants.

Cd (mg/l)	Chlorophyll b (mg g^{-1} FW)			
	3 day	6 day	9 day	12 day
0	1.36±0.00	1.54±0.00	1.39±0.00	1.89±0.01
5	1.34±0.00	1.51±0.00	1.36±0.00	1.58±0.00
10	1.34±0.00	1.40±0.00	1.33±0.00	1.55±0.00
25	1.33±0.02	1.33±0.00	1.29±0.00	1.56±0.00
50	1.33±0.00	1.23±0.00	1.23±0.00	1.54±0.00
75	1.32±0.00	1.10±0.00	1.20±0.00	1.20±0.00

Table 3: Effect of Cd on chlorophyll b of *A. philoxeroides* leaves.

Cd (mg/l	Carotenoides (mg/g FW)			
	3 day	6 day	9 day	12 day
0	0.42±0.00	0.74±0.00	0.45±0.00	0.55±0.00
5	0.41±0.00	0.72±0.00	0.44±0.00	0.52±0.00
10	0.38±0.00	0.62±0.00	0.37±0.00	0.50±0.00
25	0.37±0.00	0.56±0.00	0.35±0.00	0.47±0.00
50	0.37±0.00	0.43±0.00	0.29±0.00	0.42±0.00
75	0.36±0.00	0.34±0.00	0.25±0.00	0.37±0.00

Table 4: Effect of Cd on carotenoides of *A. philoxeroides* leaves. Data are means ± SE (n=3), significantly different ($p<0.05$) to control plants.

Cd (mg/l)	CAT			
	3	6	9	12
0	0.20±0.01	0.20±0.02	0.10±0.03	0.60±0.01
5	0.37±0.05	0.20±0.03	0.16±0.04	0.72±0.01
10	0.44±0.02	0.35±0.01	0.24±0.07	0.71±0.01
25	0.30±0.07	0.41±0.05	0.61±0.02	0.74±0.01
50	0.30±0.00	0.65±0.01	0.48±0.19	0.74±0.02
75	0.51±0.01	0.39±0.07	0.39±0.03	0.50±0.01

Table 5: Effect of Cd on CAT activity (U g^{-1} FW) of *A.philoxeroides* leaves. Data are means ±SE (n=3), significantly different ($P<0.05$) to control plant.

Cd (mg/l)	POX			
	3	6	9	12
0	1.07±0.03	2.64±0.08	3.69±0.02	3.10±0.06
5	0.76±0.11	2.69±0.09	3.81±0.02	3.70±0.04
10	0.75±0.02	2.72±0.05	4.07±0.06	4.23±0.03
25	0.64±0.13	2.68±0.07	4.18±0.02	4.53±0.01
50	0.84±0.19	2.94±0.04	3.86±0.04	4.61±0.02
75	1.40±0.45	2.88±0.03	3.28±0.13	3.71±0.09

Table 6: Effect of Cd on POX activity (U g^{-1} FW) of *A.philoxeroides* leaves. Data are means ± SE (n=3), significantly different ($p<0.05$) to control plants.

Cd mg/l	APX			
	3	6	9	12
0	1.07±0.05	2.64±0.08	3.69±0.02	3.10±0.06
5	0.76±0.16	2.69±0.09	3.81±0.02	3.70±0.05
10	0.75±0.02	2.72±0.05	4.03±0.06	4.23±0.03
25	0.64±0.13	2.68±0.07	4.18±0.02	4.53±0.01
50	0.84±0.19	2.94±0.04	3.86±0.04	4.61±0.02
75	1.40±0.45	2.88±0.03	3.28±0.12	3.71±0.09

Table 7: Effect of Cd on APX activity (U g^{-1} FW) of *A.philoxeroides* leaves. Data are means ± SE (n=3), significantly different ($p<0.05$) to control plants.

Cd (mg/l)	CAT (U g^{-1} FW)	POX (U g^{-1} FW)	APX (U g^{-1} FW)
0	0.64±0.059	0.46±0.050	1.72±0.045
5	0.87±0.005	0.68±0.079	1.48±0.113
10	0.86±0.000	1.01±0.099	1.78±0.012
25	0.91±0.011	1.56±0.042	1.77±0.007
50	0.90±0.045	1.35±0.073	1.74±0.105
75	0.97±0.001	1.03±0.033	1.84±0.021

Table 8: Effect of Cd on antioxidative enzyme activities in root tissues of *A. philoxeroides*. Data are means ±SE (n=3) significantly different ($P<0.05$) to control plants.

Cd mg/l	3rd day	6th day	9th day	12th day
0	8.43±0.21	12.3±0.08	12.1±0.20	10.6±0.16
5	6.74±0.09	4.62±0.59	4.62±0.59	10.2±0.51
10	6.52±0.38	7.18±0.44	7.18±0.44	10.4±0.24
25	6.64±0.44	6.87±0.31	6.87±0.31	10.8±0.19
50	5.51±0.33	8.61±0.08	8.61±0.08	11.7±0.06
75	5.41±0.33	8.36±0.19	8.36±0.19	11.4±0.14

Table 9: Effect of Cd on total soluble protein content (mg g^{-1} FW) of *A. philoxeroides* leaves. Data are means ±SE (n=3), significantly different ($P<0.05$) to control plant.

[IV]DISCUSSION

Cd accumulation in *A. philoxeroides* resulted in considerable physiological and biochemical changes. The obtained results showed that the growth of *A. philoxeroides* seedlings was significantly affected in general but root growth was highly affected than shoot at higher concentrations of Cd (Fig.1). Previous studies also showed that Cd induced physiological

changes in aquatic macrophytes *Lemna trisulca* and *Lemna minor* [21, 30]. Furthermore, IT values and RWC in the seedlings under Cd stress were increased in the lower concentration and it is decreased in higher concentration after 12 days of exposure (Table 1).

A.philoxeroides accumulated significant quantities of Cd in its roots (6801.0 mg kg^{-1} DW) than shoots (1371.86 mg kg^{-1} DW) at 75 mg l^{-1} Cd concentration. The phenomenon of high Cd accumulation in roots is probably because of the absorption of Cd onto negatively charged surface of the root cell wall or sequestering within xylem in the root. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanism. Earlier studies have also been reported that mechanism of Cd mobility and accumulation also occurred in higher plants and aquatic macrophytes [3,5,6,20]. Based on these traits, it is suggested that *A. philoxeroides* seedlings have the ability to accumulate the high level of Cd, since they tolerance to metal toxicity which is crucial characteristic feature for hyper accumulators.

Changes in chlorophyll contents were observed at higher concentrations of Cd treatments. Previous studies have been reported that Cd also interfered in functioning of photosynthetic pigments in higher plants [9,10].

Cd heavy metal accumulation in *A. philoxeroides* seedlings was positively correlated with the induction of antioxidative enzymes such as CAT, POX and APX. In order to repair the damage initiated by ROS, plants have evolved complex antioxidant defense mechanism. It is an important protective mechanism to minimize oxidative damage in polluted environments. In the present study, increased CAT activity in both leaves and roots of *A. philoxeroides* was observed (Fig 5, 8). Where as in the Cd treated *Arabis paniculata*, CAT activity was significantly decreased in roots but it was increased in leaves [32]. The maintenance of high CAT activity in *A. philoxeroides* seedlings Cd stress represents an important feature of metal accumulator tolerance under Cd toxicity. Therefore, it seems that a low concentration of Cd (5 mg/l) (Table 5) in the medium was sufficient to induce alterations in antioxidative enzyme activities which aim to protect plants from heavy metal stress. POX is widely distributed in the plant kingdom and is, one of the principle enzymes involved in the elimination of active oxygen species (AOS). Our results showed that *A. philoxeroides* was able to maintain high levels of POX activity at higher concentrations of Cd (Fig.6, 8). Similar results have also been observed with lead and copper treated plants [23]. Thus increased POX activity might be associated with elevated ROS levels in *A. philoxeroides* seedlings under Cd stress.

APX showed that highest sensitivity reaching maximal activity in *A.philoxeroides* (Fig.7,8). Similar results was also observed in leaves and roots of *Arachis hypogea* seedlings after 10-25 days treatment of (25, 50, 100 µ mol/l) Cd [32]. The increased response of enzymes (CAT, POD, and APX) involved in the activation of ROS to heavy metals greatly depends on the species, plant age and plant growth conditions. Effect of cadmium also induced changes in antioxidative enzyme activities of *Cuscuta reflexa* and *Lemna polyrrhiza* L. [24,31]. These results suggest that Cd triggered antioxidant level in *A.philoxeroides* seedlings responsible for the removal of excessive H_20_2.

Soluble protein content in organisms, is an important indicator of reversible and irreversible changes in metabolism, and is known to respond to a wide variety of stressor such as natural and xenobiotic. In this study the changes in the protein content indicated that the Cd induced oxidative stress appeared obvious in *A.philoxeroides.* Costa and Splitz (1997) also experimented that Cd influence induced changes on protein content of *Lupinus albus*. It is reported that heavy metal stress has been shown to induce

a variety of proteins resulting in an overall increase in protein content [33].

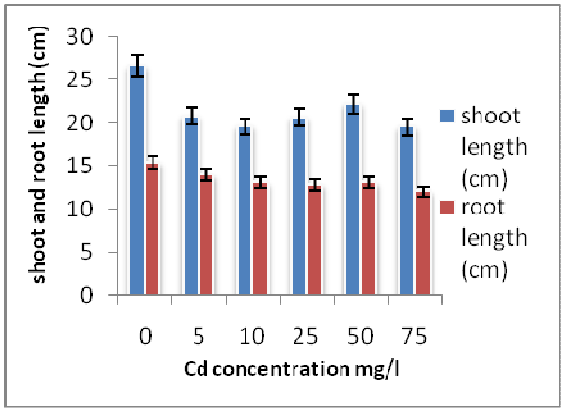

Fig: 1. Effect of Cd on shoots and roots length of *A.philoxeroides*

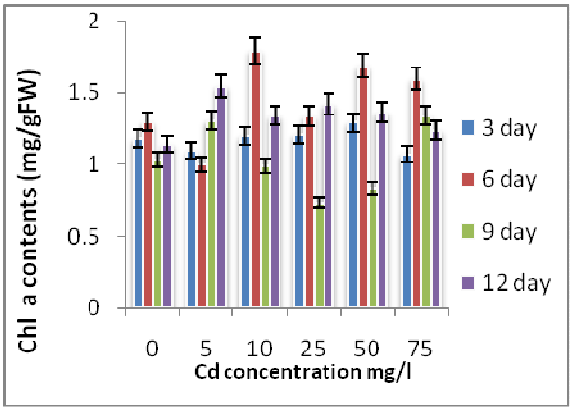

Fig: 2.Effect of Cd on chlorophyll a

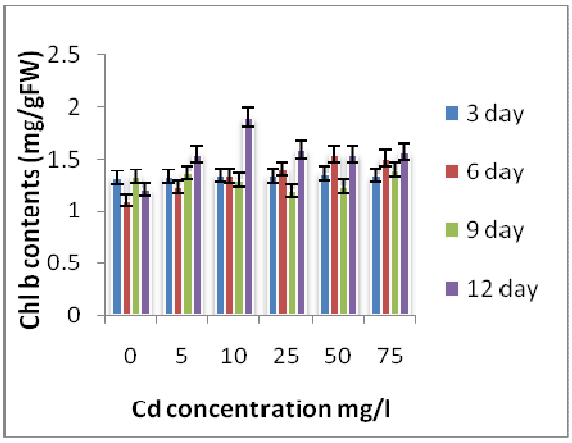

Fig: 3. Effect of Cd on chlorophyll b

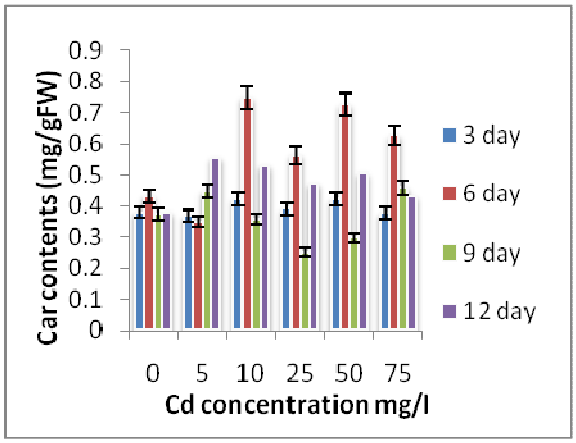

Fig: 4. Effect of Cd on car

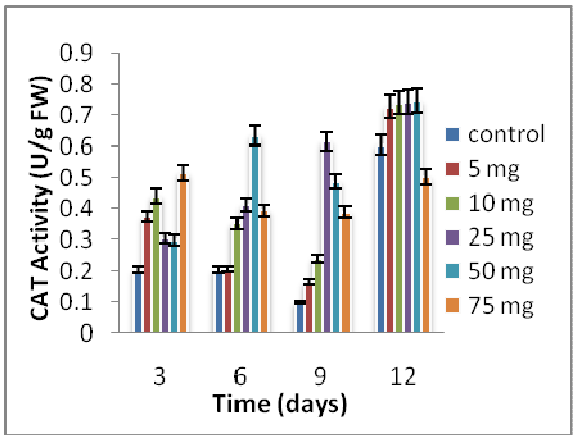

Fig: 5. Effect of Cd on CAT activity

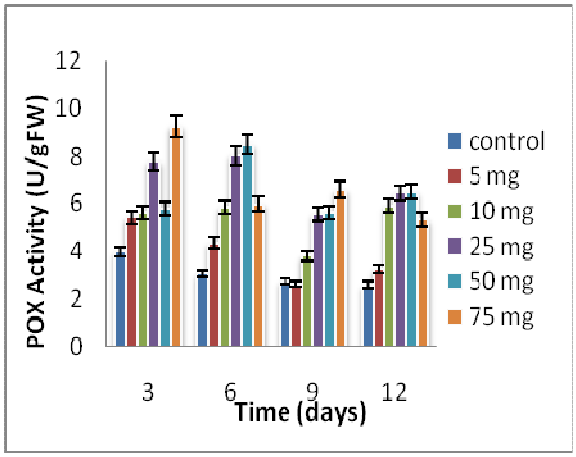

Fig: 6. Effect of Cd on POX activity

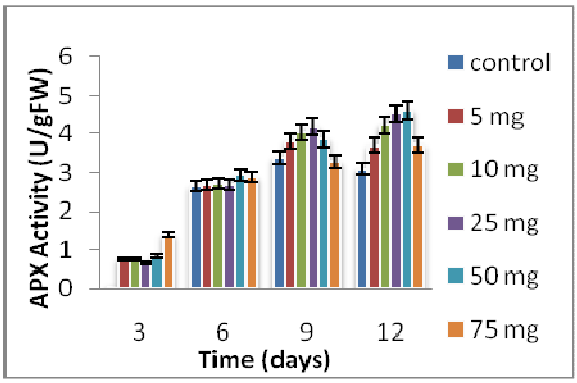

Fig: 7. Effect of Cd on APX activity

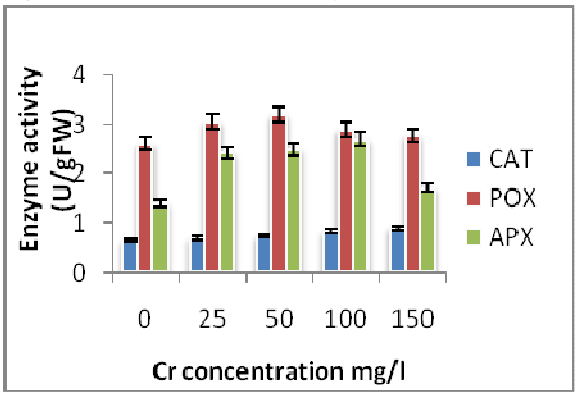

Fig: 8. Effect of Cd on antioxidative enzyme activities in root tissues.

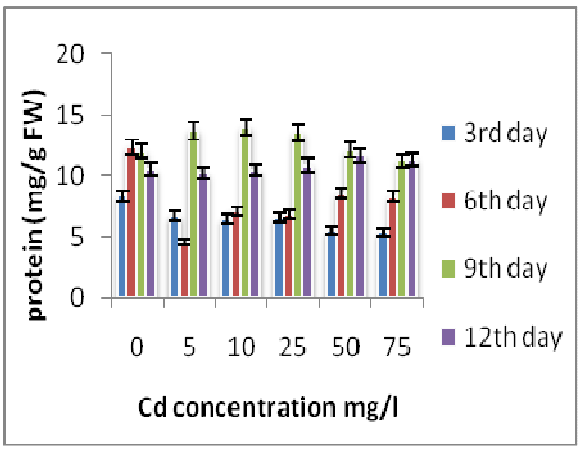

Figure 9: Effect of Cd on total soluble protein content in leaves

[V] CONCLUSION

The results of the present study indicated that *A. philoxeroides* could accumulate high amounts of Cd. Excessive Cd accumulation can be toxic to the plants, hence affecting several physiological and biochemical alterations as evidenced by the changes in the activity of photosynthetic pigments, antioxidative enzymes, and total soluble protein contents suggesting that the possible mechanism involved in *A. philoxeroides* seedlings under Cd metal phytotoxicity. *A. philoxeroides* is a fast growing plant with high biomass production grows on hydroponics solution and has the ability to tolerate (75 mg l^{-1} Cd up to 12 days) high Cd concentrations. In the present study an increase in all the antioxidants and also changes in protein content may be the reason for high level of tolerance exhibited by the *A. philoxeroides* against Cd treatment. Owing to its high tolerance to heavy metals and high biomass production, *A. philoxeroides* has a potential commercial and large scale to remediation and treatment of contaminated sites. Hence *A. philoxeroides* may be considered as hyper accumulator plant for heavy metal phytoremediation including in the future.

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