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Estimation of Barium Toxicity Mitigating Efficacy of *Amaranthus caudatus* L.

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Abstract:

Barium commonly used in the fireworks – cottage industry of this area is the predominant pollutant on growth, biochemical and enzymatic characteristics of *Amaranthus caudatus* L. a widely cultivated crop. This study is aimed at assessing the efficacy of *Amaranthus caudatus* L.in the absorption of barium. The seedlings of *Amanranthus caudatus*L. were treated with various concentration of barium (2mM, 4mM, 6mM, 8mM, 10mM) and its effect on the morphometric, biochemical and enzymatic characters were studied. After ten days of treatment the growth parameters such as leaf area, fresh weight, dry weight, shoot and root length were found decreased than in the control. Biochemical characteristics such as the content of chlorophyll, carotenoid, soluble sugar and protein also decreased with the increase in the concentration of barium. In contrary, the free amino acid, proline, and leaf nitrate increased and the activities of enzymes such as catalase and peroxidase were also found to be increased with the increase in the concentration of barium while the activity of nitrate reductase was found decreased. The result suggest that comparison of the parameters analyzed between the treated and control reveals that barium has seriously affected the *Amaranthus caudatus* L. but at the same time the plant adopts mechanisms such as accumulation of anthocyanin and enhanced activities of antioxidant enzymes to overcome the ill effects of the metal ions. Thus this study is unique in the sense that, this plant *Amaranthus caudatus* L. having been found to be huperaccumulative of metals can be Co-cultivate along with plants susceptible to metal toxicity to overcome the problem of metal toxicity to plants.

Keywords: *Amaranthus caudatus*, Barium, Catalase, Free amino acid, Peroxidase, Proline.

1.0 Introduction:

Environmental pollution is always on the increase due to human activities such as agricultural operations, sewage discharge, energy production, refining, disposal of waste, industrial and vehicular emissions. Heavy metal pollution of soil enhances plant uptake causing accumulation in plant tissues and eventual phytotoxicity and change in plant community (Gimmler *et. al*., 2002). Low concentration of soil heavy metals, regardless of necessary or unnecessary to plants, will not affect the growth of plants in a certain range. But if the concentration is too high, and the content of heavy metals exceeds its tolerance threshold, the plant will be poisoned and it even leads to death (Zhang *et. al*., 1989). Some heavy metal have been found generating reactive oxygen species and involve in altering the metabolism of organisms by replacing enzyme cofactors, inhibiting antioxidative enzymes, causing ionic transport imbalance, damaging DNA and altering protein oxidation (Zhang *et. al*., 1989). The ROS are more toxic, reactive than O_2 and involve in the free radical chain reaction of membrane lipids and protein warranting the involvement of defense mechanisms to reduce the toxic effect of ROS. Panda *et. al*., (2003) have reported Cr induced chlorosis in young leaves of wheat, damaged cells, impaired photosynthesis, altered enzymatic function, stunted growth, and consequently plant death. Similarly, Sangwan *et. al.,* (2014) observed Cr induced decline in growth of cluster bean due to arrest in activities of nitrogenase, nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate dehydrogenase enzymes. Under heavy metal stress due to As the central atom (Mg) of the chlorophyll molecules get dismantled of chlorophyll resulting in

the breakdown of photosynthesis, Yadav *et. al*., (2014). Farrag *et. al*., (2014) noticed that some of the vital physiological parameters of *Amaranthus hybridus* Linn*, Chenopodiumam brosioides* Linn*, Mentha longifolia* Linn and *Typha domingensis* Pers were affected by heavy metal stress. The level of protein in plants under stress has been significantly reduced comparing to their normal counterparts leading to the increase in the activities of catalase, glutathione peroxidase and glutathione reductase.

Recently Muradoglu *et*. *al*., (2015) reported that the excessive Cd reduced chlorophyll contents, increased antioxidant enzyme activities and change plant nutrition concentrations in both roots and leaves. The higher concentration of Cd has negative effect on chlorophyll content and nearly decreased 30% of plant growth in strawberry. The present experiment was undertaken to investigate the changes in growth, biochemical aspects, and enzymatic characteristics of *Amaranthus caudatus* treated with barium and to assess its stress tolerating efficacy. This crop may further be useful in soil reclamation through the process of phytoremediation. In the present day context, our study is very crucial because the soil in our area remains highly polluted due to barium indiscriminately used in fireworks industries which affects the most commonly cultivated *A. caudatus* - the chief crop of vegetable source of the locals. Thus there is an urgent necessity to understand the ill effects of barium on the crop mentioned and the necessary steps to be taken to overcome the problem.

2.0 Materials and Methods:

2.1 Plant Material:

Amaranthus caudatus (Amarantaceae) was selected for present study for its economic importance since it is cultivation offers employment to locals.

2.2 Experimental Design:

In total six earthen pot, each with a capacity of 3 Kg of soil were chosen and named as A, B, C, D, E & F. The pots were filled to 90% of their volume by soil and in each pot 15 seeds were sown. In the meantime the barium chloride solution of the following concentration such as 2 mM, 4 mM, 6 mM, 8 mM, 10 mM were prepared and they kept individually. When the seeds of pot A were treated with water and considered as control, the seeds of the pot B, C, D, E, F were treated with 2 mM, 4 mM, 6 mM, 8 mM, 10 mM solution of barium chloride respectively. This treatment was continued for seven days and on the eighth day the growth parameters such as root length, shoot length, leaf area, fresh weight and dry were analyzed. The various biochemical and enzyme activities were estimated following the methods proposed by those mentioned within the bracket. Chlorophyll and Carotenoids (Wellbum and Lichtenthalar, 1984), Anthocyanin (Mancinelli *et. al*.,(1973)**,** Total soluble sugar (Jayaraman, 1981), Protein content (Lowry *et. al*., 1951), Amino acid content (Jayaraman, 1981), Proline (Bates *et. al*.,1973), Leaf nitrate (Cataldo *et*.*al*.,1978) *in vivo* nitrate reductase activity (Jaworski, 1971), Peroxidase and Catalase activity (Kar and Mishra, 1976).

2.3 Statistics Analysis:

The growth parameters were determined with ten independent replicates. Biochemical characters and enzymatic assay were carried out for five times. The data reported as mean ± SE and within parentheses represent the per cent activity. Statistical analysis (One way ANOVA – Turkey test) was applied using the statistical package, SPSS 16.0.

3.0 Results and Discussion:

3.1Effect of Barium on Plant Growth:

Amaranthus caudatus L. seedlings grown in different concentration of Ba exhibited inhibition in root and shoot length, leaf area, fresh weight, dry weight, with shoot being affected more than root length. After 7 days of treatment, the significantly reduction was 48% and 42% at 10mM in root and shoot length respectively (Figure. 1 & Table 1). The plants do not show any visible toxicity symptoms up to 6 mM Ba treatment. However, Ba treatment at 10 mM concentration showed toxic symptoms like chlorosis and drying edges in seedling. This may be due to heavy metal toxicity and accumulation of Ba content in their leaves. Similarly leaf area, fresh weight and dry weight also significantly decreased to tune of 55%, 75%; 82% respectively at and 10 mM concentration. This is in agreement with the findings of Soleimani *et. al*., (2009) who made similar observation in *Cynodon dactylon* due to Pb. Chlorophyll and carotenoid pigments got reduced significantly in the stressed leaves for their adaptation in $CoCl_2$ stressed condition (Gurusaravanan *et. al*., 2012; Sasmaz *et. al.* 2015; Pant *et. al.,* 2015; Marichali et al. 2016; Das *et. al*., 2016).

The same trend was observed earlier by other workers (Vinod *et. al*., 2012; Tandon and Vikram, 2014). Accumulation of Pb was high in root than in shoot tissues (Malar *et.al*., 2014). Similar changes in the content by various metal treatments were recorded by Muradoglu *et. al*., (2015) with cadmium.

3.2 Effect of Barium on Photosynthetic Pigment Contents:

The effect of different concentration of Ba (2 mM to 10mM) on photosynthetic pigments is depicted in (Figure. 2 and Table. 2) photosynthetic pigments were significantly decreased with increasing level of Ba. The reduction of chlorophyll *a, b*, total chlorophyll and carotenoids was 49%, 63%, 37%, 60% respectively at 10 mM Ba treatment compared to the control. In contrary, the anthocyanin was significantly increased at 10 mM level. Similarly, reduction in the level of photosynthetic pigments, including chl *a*, *b*, total chl and carotenoids after exposure to heavy metals, including Pb has been observed in many plants species (Singh *et al*., 2010; Vinod *et. al*., 2012; Gautam *et. al*., 2015). The findings of the present study agreed with the findings of Bonnet *et. al*., (2000) who reported a net decline in the capacities of photochemical efficiency of photosystem II and in the quantum yield of electron flow throughout PS II in the leaves of rye grass. It has also been reported that alterations in photosynthetic activity and the absorption and disturbance of essential nutrients lead to reduced plant growth. The reduction of chlorophyll was more than overall content. This can be associated with the alterations in pigment composition of photosynthetic approach that possesses lower level of light harvest chlorophyll proteins (LHCPS) (Gill *et. al*., 2012) and similar changes was reported by Gautam *et.al*., (2015) under Zn. Reduction in photosynthetic pigments, such as Chl *a* and *b* has been reported in some earlier studies on *Helianthus annuus* (Akram and Ashraf, 2011). Similar changes in the content after Pb treatments were recorded by Malar *et. al*., (2014) on water hyacinths. According to Chaudhary, (2014) Cadmium alters photosynthetic apparatus including chloroplast structure, photosynthetic pigments, Chl-protein complexes and photosystems resulting in overall decrease in efficiency of carbon assimilation pathway and modify the antioxidant enzymes. Heavy metal inhibits the plant metabolism, but some metals like Cu, Fe, Mn are essential for the photosynthesis. There are number of enzymes found activated by heavy metals and so they are essential for the growth and development of plants and also maintain the optimum metabolism. The deficiency of these metals has direct impact on the process of photosynthesis, but with the increase in concentration of these metals become toxic to plant and affects photosynthesis (Arun *et. al*., 2005). With high concentration of heavy metals, the activities of photosynthetic enzymes degrade which results in the reduction of chlorophyll content (Thapar *et. al*., 2008). In most of the plants Cu is found associated with plastocyanin, an important component of the electron transport chain of PSI in the chloroplast. Copper deficiency reduces PSI electron transport due to decreased formation of plastocyanin (Baszynski *et. al*., 1988). A decrease in PSII is also observed in Cu deficient plants (Henriques, 1989). The biochemical and photochemical reaction centers of photosynthesis are the important sites of inhibition by the action of heavy metals especially by Cu. Similarly, reductions in the level of photosynthetic pigments, including Chl- *a*, *b* and carotenoids, after exposure to heavy metals has been observed in many plant species (Singh *et. al*., 2010; Priya and Balakrishnan, 2013; Pant *et. al*., 2015; Das *et. al*., 2016).

Table 1: Impact of various concentration of barium on the morphometric characteristics of *Amaranthus caudatus* L.

Growth Parameters	Control	2mM	4mM	6mM	8Mm	10mM
Root length (cm)	11.16 ± 0.120 (100)	9.36 ± 0.185 a* (83)	8.61 ± 0.057 a* (77)	7.72 ± .058 a* (69)	6.72 ± 0.123 a* (60)	5.43 ± 0.033 a* (48)
Shoot length(cm)	21.43 ± 0.233 (100)	19.32 ± 0.152 a* (90)	18.52 ± 1.057 a* (86)	16.4 ± 0.578 a* (76)	14.33 ± 0.579 a* (66)	12.46 ±0.033 a* (58)
Leaf Area (cm^2)	8.04 ± 0.005 (100)	7.56 ± 0.008 a* (94)	6.43 ± 0.057 a* (78)	5.8 ± 0.054 a* (71)	4.43 ± 0.012 a* (55)	3.63 ± 0.088 a* (45)
Fresh Weight (gm)	3.24 ± 0.097 (100)	2.19 ± 0.067 a* (65)	1.84 ± 0.031 a* (55)	1.64 ± 0.021 a* (49)	1.48 ± 0.014 a* (41)	0.86 ± 0.008 a* (25)
Dry Weight (gm)	0.43 ± 0.005 (100)	0.31 ± 0.004 a* (72)	0.22 ± 0.008 a* (51)	0.19 ± 0.005 a* (44)	0.14 ± 0.003 a* (33)	0.08 ± 0.002 a* (18)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE.; a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ – Turkey test). $a^{\#}$ - refers to non – significant.

Table 2: Impact of various concentration of barium on the pigments characteristics of *Amaranthus caudatus* L.

Growth Parameters	Control	2mM	4mM	6mM	8mM	10mM
Chlorophyll .a (mg/gLFW)	16.44 ± 0.058 (100)	15.55 ± 0.059 a* (94)	13.56 ± 0.045 a* (83)	12.52 ± 0.067 a* (76)	11.34 ± 0.091 a* (70)	9.56 ± 0.003 a* (51)
Chlorophyll .b (mg/gLFW)	7.58 ± 0.034 (100)	6.51 ± 0.080 a* (85)	5.44 ± 0.003 a* (71)	4.51 ± 0.098 a* (60)	3.55 ± 0.037 a* (47)	2.56 ± 0.023 a* (33)
Total.Chlorophyll (mg/gLFW)	24.37± 0.061 (100)	22.47 ± 0.058 a* (92)	20.49 ± 0.074 a* (84)	19.52 ± 0.064 a* (79)	17.49 ± 0.037 a* (71)	15.48 ± 0.040 a* (63)
Carotenoids (mg/gLFW)	2.40 ± 0.096 (100)	2.005 ± 0.041 a* (83)	1.97 ± 0.013 a* (81)	1.72 ± 0.038 a* (71)	1.15 ± 0.035 a* (47)	1.01 ± 0.002 a* (40)
Anthocyanin (µg/gLFW)	3.62 ± 0.123 (100)	4.44 ± 0.058 a* (122)	5.46 ± 0.061 a* (150)	5.98 ± 0.033 a* (158)	6.40 ± 0.031 a* (176)	8.74 ± 0.034 a* (233)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE.; a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ – Turkey test). $a^{\#}$ - refers to non – significant

Table 3: Impact of various concentration of barium on the biochemical characteristics of *Amaranthus caudatus* L.

Growth Parameters	Control	2mM	4mM	6mM	8mM	10mM
Total Soluble sugar (mg/gLFW)	7.57 ± 0.091 (100)	6.23 ± 0.091 a* (82)	5.25 ± 0.083 a* (70)	4.42 ± 0.065 a* (58)	3.41 ± 0.085 a* (45)	2.41 ± 0.038 a* (31)
Total Soluble Protein (mg/gLFW)	3.23 ± 0.106 (100)	2.48 ± 0.036 a* (77)	2.13 ± 0.011 a* (65)	2.02± 0.006 a* (62)	1.97 ± 0.006 a* (53)	1.26 ± 0.036 a* (39)
Amino acid (µ mole/g LFW)	3.63 ± 0.020 (100)	4.56 ± 0.063 a* (125)	5.35 ± 0.058 a* (147)	6.42 ± 0.104 a* (176)	7.38 ± 0.110 a* (202)	8.59 ± 0.195 a* (236)
Proline (µ mole/g LFW)	2.85 ± 0.036 (100)	3.63 ± 0.073 a* (127)	4.70 ± 0.039 a* (145)	5.60 ± 0.097 a* (164)	6.52 ± 0.712 a* (185)	7.56 ± 0.003 a* (220)
Leaf Nitrate (µg/gLFW)	2.13 ± 2.011 (100)	2.84 ± 0.136 a* (133)	3.46 ± 0.101 a* (152)	4.61 ± 0.124 a* (173)	5.73 ± 0.312 a* (194)	5.77 ± 0.052 a* (219)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE.

a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ – Turkey test). $a^{\#}$ - refers to non – significant

Table 4: Impact of various concentration of barium on the enzyme activity characteristics of *Amaranthus caudatus* L.

Parameters	Control	2mM	4mM	6mM	8mM	10mM
Nitrate Reductase (µ mole/g LFW)	14.62 ± 0.075 (100)	13.54 ± 0.006 a* (92)	12.66 ± 0.118 a* (86)	11.52 ± 0.097 a* (78)	9.47 ± 0.069 a* (64)	7.15 ± 0.038 a* (49)
Catalase activity (µ mole/g LFW)	2.42 ± 0.136 (100)	3.71 ± 0.035 a* (153)	4.52 ± 0.159 a* (186)	5.52 ± 0.132 a* (227)	6.02 ± 0.005 a* (248)	6.52 ± 0.038 a* (269)
Peroxidase activity (µ mole/g LFW)	2.35 ± 0.036 (100)	2.94 ± 0.026 a* (117)	3.32 ± 0.173 a* (131)	4.43 ± 0.092 a* (166)	5.48 ± 0.087 a* (199)	6.16 ± 0.087 a* (233)

Values in parameters indicate percent activity; values are represents means of five observation. Values in parentheses are activity with respect to control. Mean (±) SE.

a - refers to values compared with control in various concentrations of metals, a* - refers to significant ($P \leq 0.05$ – Turkey test). $a^{\#}$ - refers to non – significant

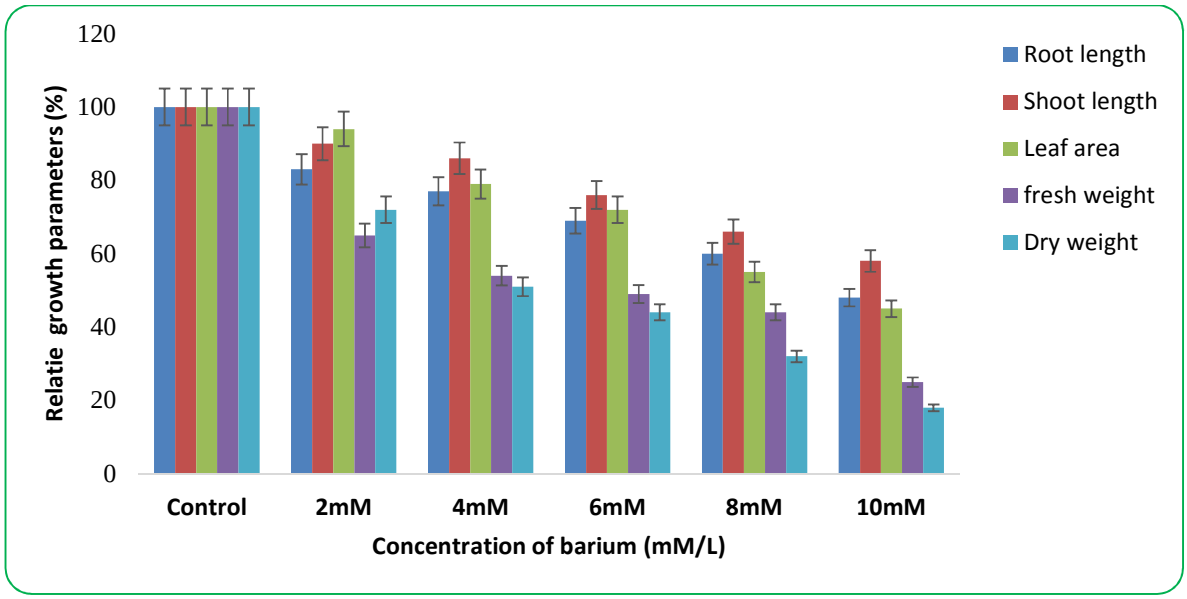

Figure 1: Effect of the various concentration of barium on the morphometric characters of *Amanranthus caudatus* L.

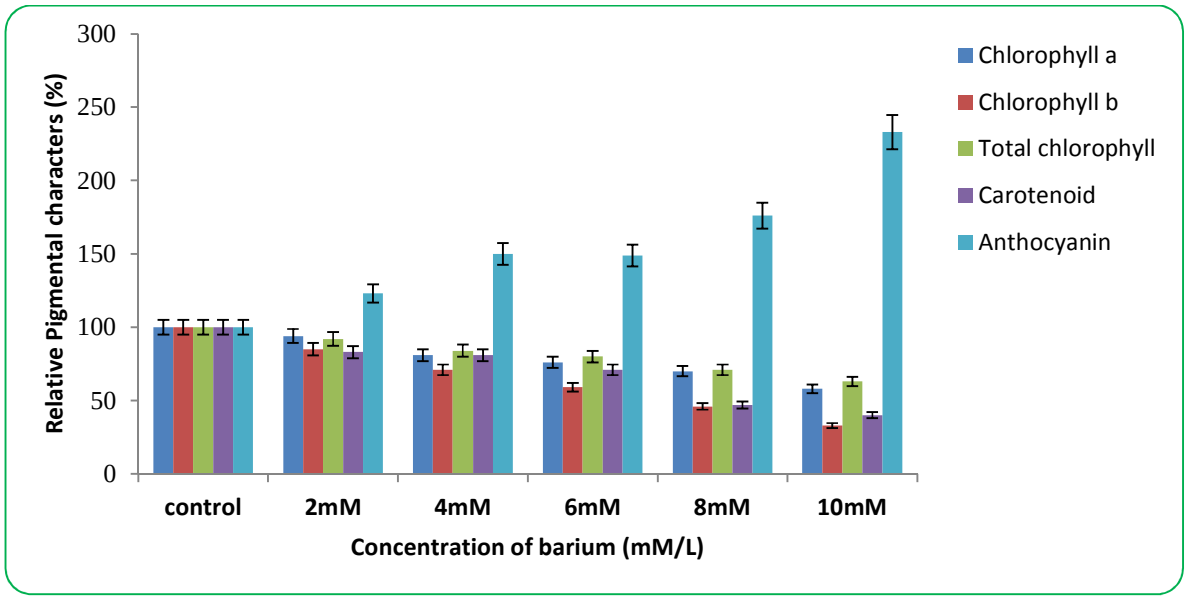

Figure 2: Effect of the various concentration of barium on the photosynthetic pigment contents of *Amanranthus caudatus* L.

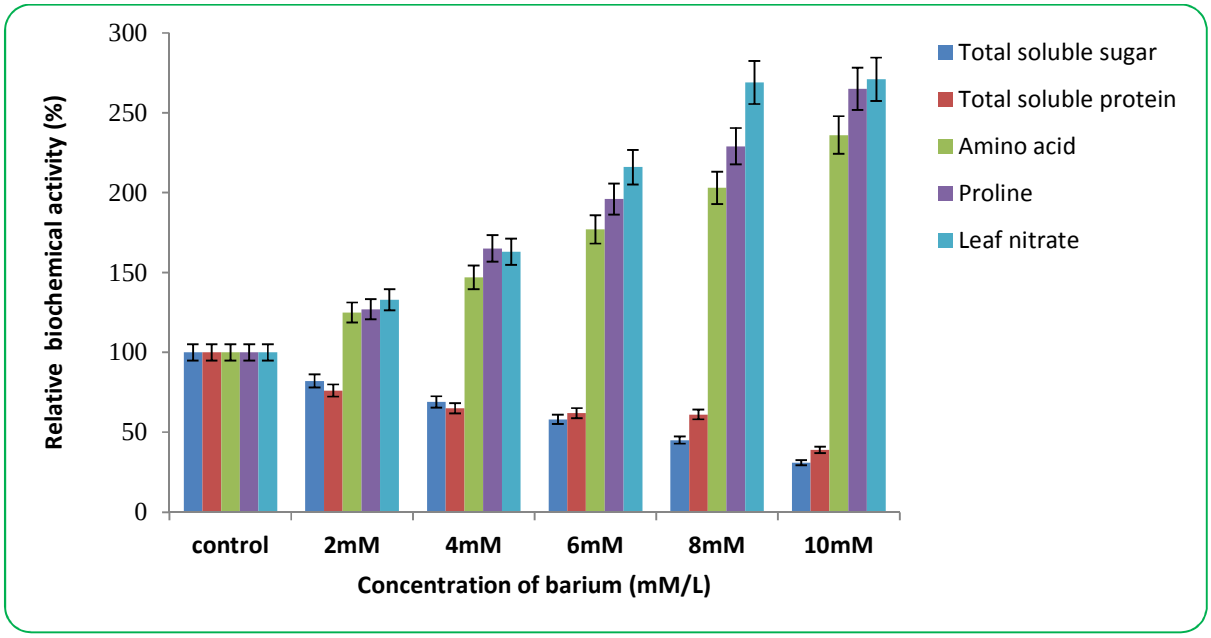

Figure 3: Effect of the various concentration of barium on the biochemical characters of *Amanranthus caudatus* L.

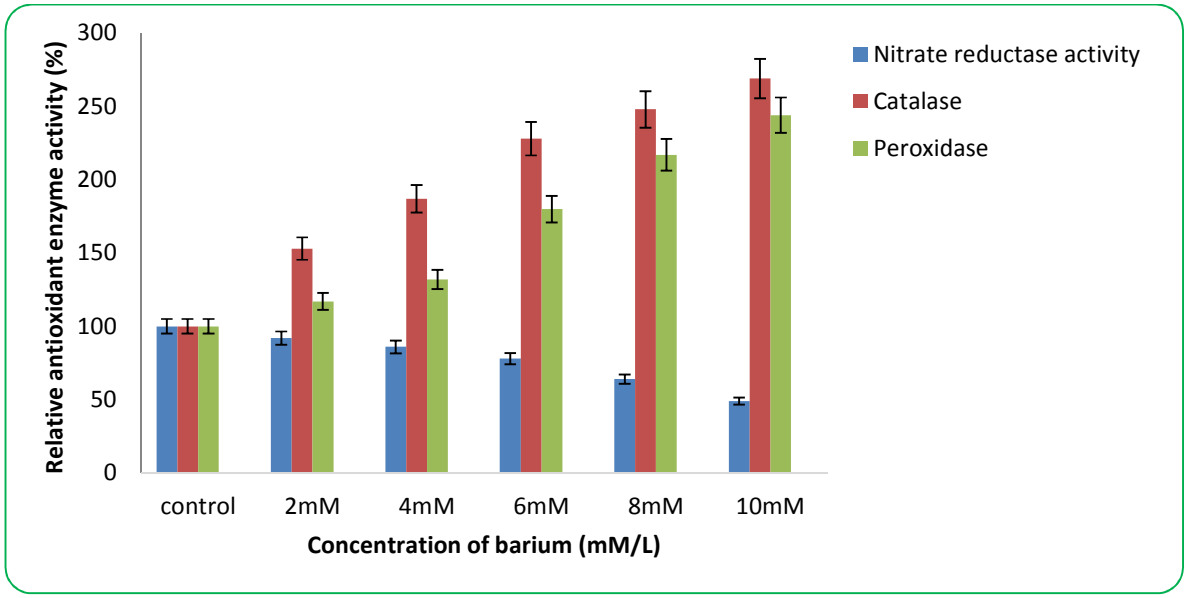

Figure 4: Effect of the various concentration of barium on the enzymatic characters of *Amanranthus caudatus* L.

3.3 Effect of Ba on Biochemical Characters:

3.3.1 Effect of Ba on Total Soluble Sugar:

Heavy metals also modified the total soluble sugar content in *Helainthus annuus* L. leaves (Figure. 3 & Table. 3). At 10 mM Ba concentration significantly decreased the total soluble sugar levels. Our results of decreased total sugar content go along with the findings of Singh *et. al*., (2007) in their work with Cu. Sarma *et. al*., (2009) reported that the reduction of sugar content may be the result of the minimum uptake of phosphate. Vinod *et. al*., (2012) reported that there was significant reduction in total sugar content in *Triticum aestivum* L. under Cu and Zn treatment these findings strongly supported our study. Similar results have been noticed by (Gautam *et.al*., 2015) under Zn treatment on *Spinacea oleracea* L.

3.3.2 Effect of Ba on Protein Contents:

Our results indicate that protein got significantly decrease by Ba treatment at rage of 63% at 10 mM (Figure. 3 & Table. 3). Our results favoured the results of Manivasagaperumal *et. al*., (2011) who found that amino acid and protein content were high at lower concentrations of zinc (10 and 25 mgl-L) further the values decreased with an increase in Zn level. The zinc ions inhibit amino acid and protein formation by binding with the sulfhydryl group of protein and causing deleterious effect on the normal protein form. Reduction protein level in the protein level observed in the study may be attributed to the decrease in protein synthesis or due to the denature of the enzymes involved in the synthesis of protein (Aldesuquy, 1998). The reduction in protein due to Cu and Zn (Vinod *et. al*., 2012) Zn (Gautam *et. al*., 2015) copper, nickel and zinc (Rastgoo *et. al*., 2014) was observed. Bhupendra *et. al*., (2014) also observed the ill effect of arsenic, and chromium on *in vitro* seed germination of black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.) reduction in protein content.

3.3.3 Effect of Ba on Free Amino Acids, Proline and Leaf Nitrate Contents:

As shown in Table (Figure. 3 & Table. 3) the free amino acids, proline and leaf nitrate contents in *Helianthus annuus* L. significantly increase with increasing concentration of Ba. Metal exposure has caused an accumulation of leaf nitrate content with the increase in the concentration of heavy metal than the control. The application of 4mM Ba causes significant increase in the free amino acid and proline contents 139% and 120% respectively. Leaf nitrate content was significantly increased to 119%. As a result of protein degradation during heavy metal condition, the availability of free amino acid is significantly high. It may be due to destruction of protein or due to the biosynthesis of amino acid from the nitrate sources which were not utilized in the protein synthesis (Rastgoo *et. al*., 2014). The results of the present study was confirmed by Ravikumar and Thamizhiniyzn, (2014) who observed that the proline changes in black gram seedlings by increase in Pb. High level of proline, especially in roots, can eliminate hydroxyl radicals, maintain osmoregulation, prevent enzyme destruction, decrease toxicity of heavy metals (Alia and Saradhi, 1991). Similarly increase in leaf nitrate content with increased concentration of cadmium on *Vigna radiata* has been reported earlier (Jayakumar and Ramasubramanian, 2009). These results support our study.

3.3.4 Effect of Ba Exposure on Antioxidant Enzyme Activities:

The result of the present study shows that, *in vivo* NR activity of the leaves was significantly inhibited to 51% at 10mM concentration of Ba. There was a dramatic rise in catalase and peroxidase activity with significant increase in different Ba treatments (Figure. 4 & Table. 4). Catalase activity was found significantly increased in all the experiment plants than the control plants. The increase was about 169% at 6mM. Peroxidase activity was significant increased in all treatments. Plant cells are equipped with a protective system including antioxidant enzymes like catalase and peroxidase which can flush free radicals (Cho and Park, 2000). Reduction in *in vivo* NR activity with increased concentration of cadmium on *Vignaradiata* has been reported earlier (Jayakumar and Ramasubramanian, 2009). Shalini Sharma (2009) in his study on *Brachytheciumpopuleum*, proved that the under the heavy metal stress, catalase and peroxidase enzymes activities were significantly high. In addition, certain changes was observed earlier under cadmium on strawberry (Muradoglu, *et. al*., 2015). Results of previous study by Farrag *et. al*., (2014) on heavy metal contaminated plant support our results. They observed an increase in catalase, peroxidase enzyme activity with increasing heavy metal concentration. Recently Malar *et.al*., (2014) reported that the catalase, peroxidase enzyme activities was increased under high concentration of Pd treatment.

4.0 Conclusions:

The present study provides data on the toxic effect of barium on morphological, biochemical parameters and antioxidant enzymes of *A. caudatus.* Our data suggest that the toxic effects of barium invokes a strong inhibition of root length, shoot length, leaf area, fresh weight, dry weight, photosynthetic pigments (Chlorophyll *a*, *b*, total chlorophyll and carotenoids), protein but a significant enhancement of anthocyanin, proline, free amino acids, leaf nitrate. Likewise the activity of nitrate reductase got significantly reduced while the activity of catalase and peroxidase were increased. Our result suggest that barium in high concentration makes the soil toxic to the plants and results in growth inhibition, structural damage, decline in physiological and biochemical activities of plants. At the same time plants adopt certain damage controlling mechanisms such as increased the activity of antioxidant enzymes and accumulation of anthocyanin as protective measure helping them to overcome the ill effect of the metal stress.

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