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High efficiency phytoextraction of barium using *Amaranthus viridis* l.

Fitoextracción de bario de alta eficiencia utilizando *Amaranthus viridis* l.

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ABSTRACT

Heavy metal pollutants in the environment are emerging global concern. Barium is one of the heavy metal abundantly used in the manufacture of firecrackers and match industries. This work is aim to eradicate barium from these industrial sites; the new-flanged phytoextraction technology is used to mitigate the metal pollution through hyperaccumulators. Plant used in phytoextraction should accumulate and translocate specific pollutants especially heavy metals. This work aims to assess the tolerance mechanism of *Amaranthus viridis* L. a selective native hyperaccumulator under barium chloride stress. Morphometric, biochemical, enzymatic activity, accumulation, translocation and mobility of barium form soil to root and leaves were studied in co-cultivated hyperaccumulator (*Amaranthus viridis*) and hypoaccumulator (*Abelmuscus esculentus*) at various concentration levels of barium. *Amaranthus viridis* accumulated fourfold to fivefold barium in roots, shoots and leaves than *Abelmuscus esculentus*L. This is well understand that *Amaranthus viridis* showing higher accumulation of barium, more translocation of barium from root to shoot and good mobility. The mobility of barium was increased form level 1 to level 3. It was revealed that the accumulation of barium was more in root and shoot of *Amaranthus viridis*. It is

inferred from the present study that *A.esculentus* is a hypoaccumulator and is sensitive to barium. When co-cultivated with *Amaranthus viridis* showing less of metal toxicity because *Amaranthus viridis* being hyperaccumulator of barium, accumulate more metal and save *Abelmuscus esculentus*. It is strongly suggest that the hyperaccumulator *Amaranthus viridis* L. should grown in the barium polluted sites and make the environment sans heavy metal pollution.
Keywords: Phytoextraction, barium pollutant, accumulation factor, mobility index, metal pollution.

RESUMEN

Los contaminantes de metales pesados en el medio ambiente son una preocupación global emergente. El bario es uno de los metales pesados más utilizados en la fabricación de petardos e industrias de cerillas. Este trabajo tiene como objetivo erradicar el bario de estos sitios industriales; la nueva tecnología de fitoextracción con bridas se utiliza para mitigar la contaminación por metales a través de hiperacumuladores. La planta utilizada en la fitoextracción debe acumular y trasladar contaminantes específicos, especialmente metales pesados. Este trabajo tiene como objetivo evaluar el mecanismo de tolerancia de *Amaranthus viridis* L. un hiperacumulador nativo selectivo bajo estrés por cloruro de bario. La actividad morfométrica, bioquímica, enzimática, la acumulación, la translocación y la movilidad del bario desde el suelo hasta la raíz y las hojas se estudiaron en cultivos hiperacumuladores (*Amaranthus viridis*) e hipoacumuladores (*Abelmuscus esculentus*) cocultivados a varios niveles de concentración de bario. *Amaranthus viridis* acumuló cuatro o cinco veces más bario en raíces, brotes y hojas que *Abelmuscus esculentus* L. Esto es bien entendido que *Amaranthus viridis* muestra una mayor acumulación de bario, más translocación de bario de la raíz al brote y buena movilidad. La movilidad del bario aumentó del nivel 1 al nivel 3. Se reveló que la acumulación de bario era mayor en la raíz y el brote de *Amaranthus viridis*. Se infiere del presente estudio que *A. esculentus* es un hipoacumulador y es sensible al bario. Cuando se co-cultiva con *Amaranthus viridis* muestra menos toxicidad por metales debido a que *Amaranthus viridis* es hiperacumulador de bario, acumula más metal y salva a *Abelmuscus esculentus*. Se recomienda encarecidamente que el hiperacumulador *Amaranthus viridis* L. crezca en los sitios contaminados con bario y haga que el medio ambiente esté libre de contaminación por metales pesados.
Palabras clave: Fitoextracción, contaminante de bario, factor de acumulación, índice de movilidad, contaminación por metales.

INTRODUCTION

Barium is an important metal element which is abundantly used in the manufacturing of firecrackers and match industries in and around Sivakasi, Virudhunagar District, Tamilnadu, India. Barium mainly in used in the preparation of sparkling crackers for various colouring effect, after manufacturing remaining scrapheap are dump in the waste land around these industries. The concentration of barium increased in the soil is pose major problem to the plants; it directly affects the plant growth and their metabolic activity, finally leads to the devastation of agriculture in the industrial area. These heavy metals are dangerous because of their non-biodegradable form and a capacity to accumulate in the living organisms (Marchand *et al.,* 2016). Many techniques developed so for to eliminate this metal pollution from the environment but the new innovative technique phytoremediation has been attracted many scientist due to its cost efficiency.

Pollution in the environment especially heavy metal contamination in soil, water and air is a widespread environmental issues caused by natural and anthropogenic activities (Kumar *et al.* 2019;Ali *et al.* 2020). Heavy metal refers to metals and metalloids which produce toxicity and adverse effect to ecosystem (Ali and Khan 2018) and are sometimes it involves in the climate change also (Ali *et al.* 2019). Among different toxins, increasing levels of salts, heavy metal, pesticides and other chemicals are posing a threat to agricultural as well as natural ecosystems of the world. Industrial and urban wastes, in particular the uncontrolled disposal of waste and the application of various substances to agricultural soils, have resulted in the contamination of our ecosystem. The heavy metal pollution is due to emission, effluents, and solid discharge from industries, vehicle exhaustion, smelting and mining, and also as soluble salts (natural and artificial). The use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture land, and excessive use of fertilizers. Each source of contamination has its own damaging effects on plants, animals, and ultimately on human health. Heavy metals of soil and water are of serious concern to the environment due to their non-degradable state. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another. Therefore, heavy metal pollution poses a great threat to the environment and human health.

Phytoextraction involves the removal of pollutants, the toxic heavy metals and metalloids, by the roots of the plants with subsequent transport to aerial plant organs and metabolized to change into non-toxic forms (Sidhu*et al*., 2020). Phytoremediation is the use of plants to treat/clean contaminated sites (Suresh and Ravishankar, 2004; Lal and Srivastava, 2010) and it can be defined as the use of green plants to remove pollutants from the environment or to render

them harmless (Berti and Cunningham, 2000; Bali*et al.* 2020). It is also referred to as green technology and can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Grataoe*t al*., 2005;). Phytoremediation takes advantage of the natural ability of plants to extract chemicals from water, soil and air using energy from sunlight. It is less expensive, passive and solar driven, has high public acceptance, retains topsoil, and has less secondary waste generation. This technology is being considered as a highly promising technology for the remediation of polluted sites.

The effectiveness of a Hyperaccumulation is dependent on the selection of the appropriate plant. Plants native to the target area should be considered since they are adapted to the local climate, insects and diseases (Lia*et al*. 2020). Any plant used as a phytoremediator must be able to tolerate high concentrations of the toxic substances of interest, in addition to any other pollutants found at the particular site, as candidate site for phytoremediation usually have multiple contaminants (Peer *et al.,* 2003). *Amaranthus* is commonly grown in the pollution site. It is used for accumulation of cadmium, zinc and iron (Jonnalagdda *et al.* 2006) and this is ability to bioconcentrate various heavy metals in leaves. *Amaranthus* is generally used to remediate lead and heavy metal contaminated soil (Anoliefo *et al.* 2008).

In the present study, it is aimed to analyse the impact of barium on the morphometric characters, biochemical, enzymatic features, of *Abelmoschus esculentus,* L*.* as hypoaccumulator. Also studied the effect of co-cultivation of *Amaranthus viridis* L. as hyperaccumulator along with *Abelmoschus escuentus* in barium treatment.

MATERIAL AND METHODS

Seeds of *Abelmoschus esculentus,* L*.* and *Amaranthus viridis* L. were procured from local seed centre, Sivakasi. *Abelmoschus esculentus,* L.Var. S7 (Family; Malvaceae) was chosen as experimental plant, whereas the *Amaranthus viridis* L.(Family; *Amaranthusceae*) was chosen as hyperaccumulator plants for this study. The effect of various concentrations of barium on the morphometric characters, biochemical, enzymatic features, accumulation factor, translocation factor and mobility index were analyzed on the selected plants.

I) Heavy metals stress on *Abelmoschus*: The heavy metals barium was treated separately in the experimental plants with different concentrations *viz.,* 2 mM, 4 mM, 6mM, 8 mM and 10 mM (w/v) in five replicates. The aqueous solutions of heavy metals were applied to the soil after the development of first leaves in the seedlings. Then the plants were watered with the respective concentration of metals on every alternate day. A set of plants without heavy metal treatment was maintained as control.

II) Co-cultivation of the hypoaccumulator and hyperaccumulator: Optimum number of surface sterilized seeds of both *Abelmoschus esculentus,* L. (hypoaccumulator) and *Amaranthus viridis* L.(hyperaccumulator) were sown uniformly in all pots. Appropriate amount of barium were given separately for the experimental plants with different concentration as 2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v) in five replicates. Morphometric, biochemical, enzymatic parameters and metal concentration in plants such as accumulation factor, translocation factor and mobility index were analysed on the 45th day after planting (DAP).

For all the morphometric characteristics, root length, shoot length, leaf area, fresh weight and dry weight were analysed, the seedlings numbering ten have been taken from both experimental and control sets and the results indicate the average of ten seedlings along with their standard error.

For all the biochemical analysis, the result indicates the average of five samples taken from both control and treated sets.

The biochemical characters and enzymatic charters were analysed by the following methods. Chlorophyll and carotenoids (Wellburn, and Lichtenthaler, 1984), anthocyanin (Swain and Hills, 1959), total soluble sugar and amino acid (Jayaraman,1981), Protein content (Lowry *et al.,* 1951), leaf nitrate (Cataldo*et al.,* 1978). *In vivo* nitrate reductase activity (Jaworski, 1971), peroxidase and catalase (Kar and Mishra, 1976).

The Accumulation Factor (AF) was considered to determine the quantity of heavy metals absorbed by the plant from soil. This is an index of the plant to accumulate a particular metal with respect to its concentration in the soil and is calculated using the formula (Ghosh and Singh, 2005; Yoon *et al*., 2006):

$$\text{Accumulation Factor (AF)} = \frac{Metal\ Concentration\ in\ tissue\ of\ \ whole\ \ plant}{Initial\ \ concentration\ of\ \ metal\ in\ substrate\ (soil)}$$

To evaluate the potential of plant species for phytoextraction, the Translocation Factor (TF) was considered. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Mellem *et al.,*2009). It is represented by the ratio:

$$\text{Translocation Factor (TF)} = \frac{Metal\ concentration\ in\ stems + leaves}{Metel\ concentration\ \ in\ roots}$$

Mobility Index (MI) was considered to determine the biomobility and transport of heavy metals in different plant parts. The whole experiment was divided into three

categories: Level 1 (Soil – Roots), Level 2 (Roots – Stems) and Level 3 (Stems – leaves). It was calculated by the methods of Kumar *et al*. (2009)

$$\text{Mobility Index (MI)} = \frac{Concentration\,of\ metal\,in\,the\,receiving\ level}{Concentration\,of\ metal\,in\,the\,source\,level}$$

RESULTS AND DISCUSSION

The results on the effect of barium on the morphometric, biochemical and enzymatic characters *Abelmoschus esculentus,* L.has been presented in Table 1. The same plant after co-cultivation with hyperaccumulator *Amaranthus viridis* L., the barium effect on the both the pants have been presented in the Figure 1, 2 and 3 and discussed below:

Barium treated *Abelmoschus esculentus,*L. shows the reduction of all morphometric, biochemical characters with increasing concentration of metal, however savaging enzyme anthocycnic, proline, catalase and peroxidases showed increased with increasing metal concentration. Heavy metals either retard the growth of the whole plant or plant parts (Shafiq and Iqbal, 2005; Shanker*et al.,* 2005). The plant parts normally the roots which have direct contact with the contaminated soils exhibit rapid and sensitive changes in their growth pattern (Boros-Lajszner*et al*. 2020). Significant effects of number of metals (Cu, Ni, Pb, Cd, Zn, Al, Hg, Cr, As, Fe) on the growth of above-ground plant parts is well documented (Ali *et al*. 2019).

The co-cultivation of hypoaccumulator *Abelmoschus esculentus,* L. with hyperaccumulators *Amaranthus viridis* L. under various concentrations of barium are being discussed below. In the present investigation of co-cultivated *Abelmoschus* with *Amaranthus*, barium has caused considerable reduction on the seedling length (Fig. 1a,b) and leaf area (Fig.1c) of hyperaccumulators *Amaranthus*. However, not much reduction in the hypoaccumulator *Abelmoschus* was recorded when compared with plant treated without co-cultivation. Inhibition of the root and shoot lengths at higher concentration of the metals is due to the high levels of toxicity present in barium, which interfered and inhibited the uptake of other essential elements like potassium, calcium, phosphorus and magnesium by the plants (Clarkson, 1985). Sahai *et al*., (1983) reported that, the retardation of plant growth was due to excess quantities of micronutrients and other toxic chemicals. Reduction of leaf growth is an important visible symptom of heavy metal stress. In many plants, the reduction in leaf area in response to barium treatment was also related to accumulation of barium in leaves, where the size of the leaf was also decreased (Panday and Sharma, 2002).

Table 1: Impact of various concentration of arsenic chloride on the morphometric, pigment, biochemical and enzymatic characteristics of *Abelmoschus esculentus*, L.

Parameters	Control	2mM	4mM	6mM	8mM	10mM

Root Length (cm)	29.7 ± 0.92 (100)	27.03 ± 0.67 a* (91)	21.68 ± 0.51 a* (73)	17.82 ± 0.72 a* (60)	12.47 ± 0.32 a* (42)	8.91 ± 0.41 a* (30)
Shoot Length (cm)	25.4 ± 0.43 (100)	20.32 ± 0.26 a* (80)	17.02 ± 0.48 a* (67)	12.7 ± 0.23 a* (50)	8.38 ± 0.25 a* (33)	5.59 ± 0.85 a* (22)
Leaf Area (cm^2)	12.54 ± 0.52 (100)	9.31 ± 0.12 a* (74)	7.04 ± 0.57 a* (56)	5.16 ± 0.15 a* (41)	3.52 ± 0.45 a* (28)	2.10 ± 0.31 a* (17)
Fresh Weight (gm)	16.09 ± 0.17 (100)	13.82 ± 0.63 a* (86)	12.0 ± 0.51 a* (75)	9.13 ± 0.24 a* (58)	6.82 ± 0.17 a* (42)	5.01 ± 0.26 a* (31)
Dry Weight (gm)	10.15 ± 0.37 (100)	8.17 ± 0.16 a* (83)	7.35 ± 0.34 a* (72)	4.70 ± 0.48 a* (46)	2.51 ± 0.63 a* (25)	1.34 ± 0.77 a* (13)
Chlorophyll .a (mg/gLFW)	5.76 ± 0.19 (100)	4.87 ± 0.50 a# (85)	4.04 ± 0.35 a* (70)	3.16 ± 0.42 a* (55)	2.27 ± 0.18 a* (39)	1.49 ± 0.43 a* (26)
Chlorophyll .b (mg/gLFW)	4.13 ± 0.91 (100)	3.26 ± 0.41 a* (79)	2.53 ± 0.38 a* (61)	1.78 ± 0.70 a* (43)	1.15 ± 0.84 a* (28)	0.841 ± 0.15 a* (20)
TotaL.Chlorophyll (mg/gLFW)	9.89 ± 0.77 (100)	8.03 ± 0.08 a* (81)	6.57 ± 0.26 a* (66)	4.94 ± 0.67 a* (50)	3.42 ± 0.12 a* (35)	2.233 ± 0.38 a* (24)
Carotenoids (mg/gLFW)	3.78 ± 0.23 (100)	2.96 ± 0.74 a* (78)	2.01 ± 0.17 a* (53)	1.43 ± 0.11 a* (38)	0.96 ± 0.88 a* (25)	0.53 ± 0.53 a* (14)
Anthocyanin (µg /gLFW)	1.65 ± 0.83 (100)	2.14 ± 0.87 a* (130)	2.99 ± 0.28 a* (181)	3.68 ± 0.82 a* (223)	4.32 ± 0.35 a* (262)	4.93 ± 0.91 a* (299)
Total Soluble Sugar (mg/gLFW)	7.63 ± 0.14 (100)	6.14 ± 0.76 a* (80)	5.03 ± 0.25 a* (66)	4.27 ± 0.86 a* (56)	3.79 ± 0.18 a* (50)	2.81 ± 0.15 a* (37)
Total Soluble Protein(mg/gLFW)	4.76 ± 0.41 (100)	3.88 ± 0.28 a* (82)	3.19 ± 0.82 a* (67)	2.54 ± 0.57 a* (53)	1.93 ± 0.37 a* (41)	1.17 ± 0.89 a* (25)
Amino acid (µ mole/g LFW)	3.57 ± 0.30 (100)	4.39 ± 0.67 a* (123)	5.28 ± 0.69 a* (148)	5.94 ± 0.44 a* (166)	6.48 ± 0.79 a* (181)	7.13 ± 0.19 a* (200)
Proline (µ mole/g LFW)	1.968 ± 0.38 (100)	2.54 ± 0.21 a* (129)	3.18 ± 0.47 a* (161)	3.87 ± 0.25 a* (196)	4.36 ± 0.51 a* (185)	5.08 ± 0.19 a* (258)
Leaf Nitrate (µ mole/g LFW)	3.52 ± 0.30 (100)	4.29 ± 0.13 a* (122)	4.96 ± 0.19 a* (141)	5.73 ± 0.15 a* (163)	6.52 ± 0.21 a* (185)	7.14 ± 0.29 a* (203)
Nitrate Reductase activity (µ mole/g LFW)	8.03 ± 0.78 (100)	6.52 ± 0.64 a* (81)	5.97 ± 0.16 a* (74)	4.69 ± 0.69 a* (58)	3.58 ± 0.33 a* (45)	2.83 ± 0.12 a* (35)
Catalase activity (µ mole/g LFW)	2.67 ± 0.47 (100)	3.16 ± 0.68 a# (118)	3.92 ± 0.12 a* (147)	4.63 ± 0.08 a* (173)	5.28 ± 0.26 a* (198)	5.96 ± 0.57 a* (223)
Peroxidase activity (µ mole/g LFW)	1.63 ± 0.20 (100)	2.38 ± 0.71 a* (146)	2.94 ± 0.38 a* (180)	3.62 ± 0.51 a* (222)	4.51 ± 0.49 a* (277)	7.97 ± 0.16 a* (305)

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE

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

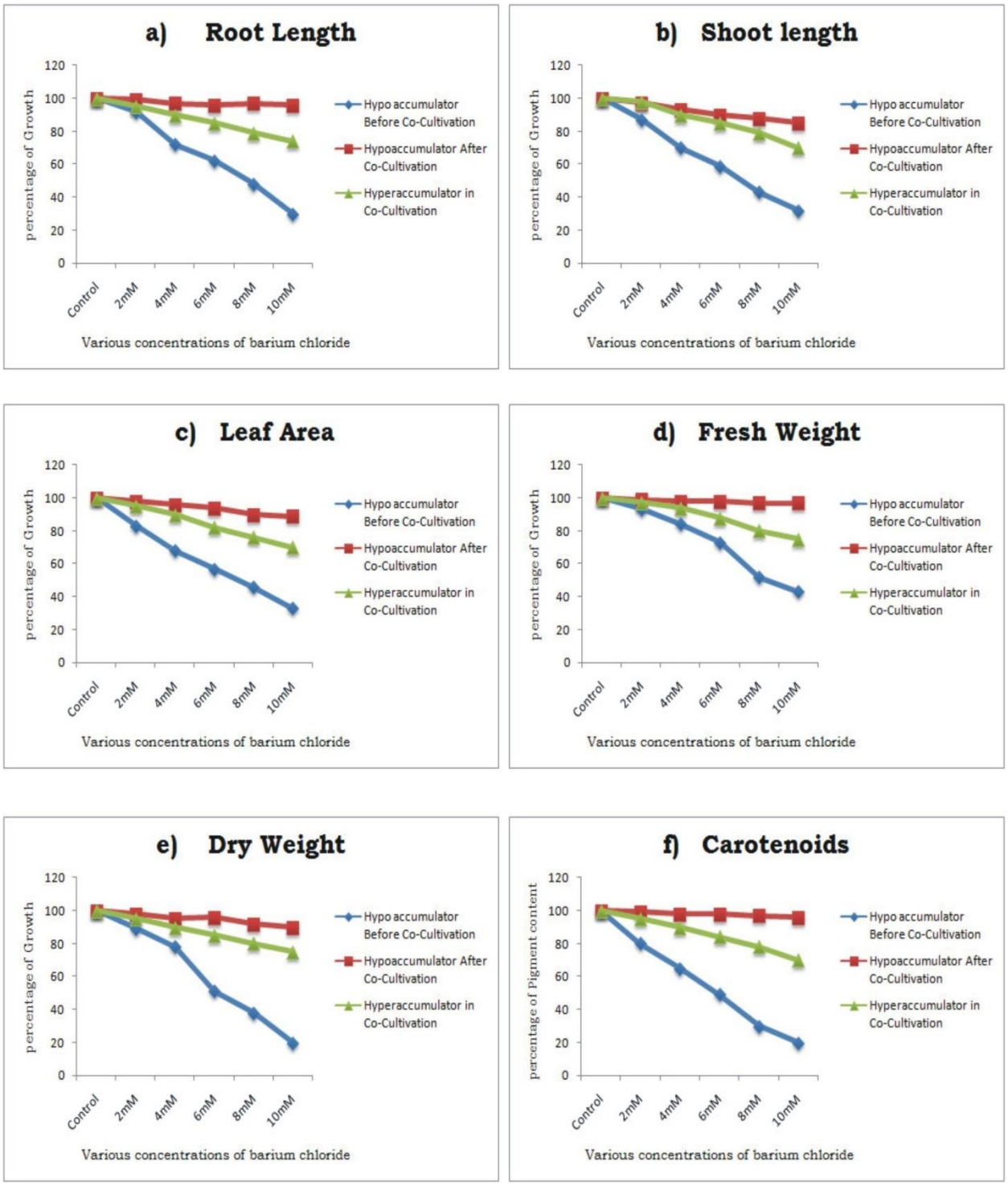

Figure 1: Impact of barium chloride on the morphometric characteristics (a d) and pigment (e) of hyperaccumulator (*Amaranthus viridis* L.) and hypoaccumulator (*Abelmoschus esculentus*, L.)

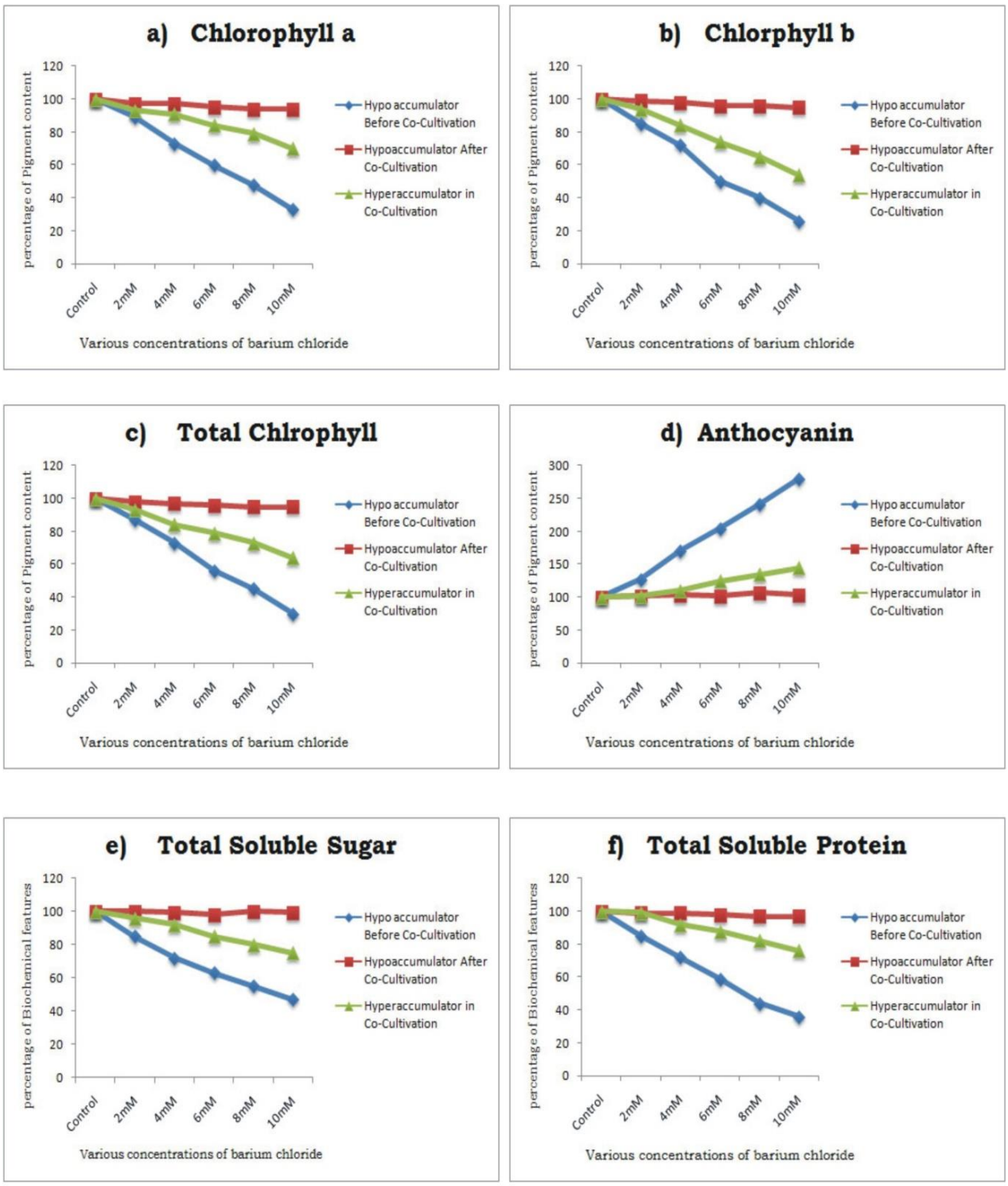

Figure 2: Impact of barium chloride on photosynthetic pigment contents (a - d) and biochemical features (e, f) of hyperaccumulator (*Amaranthus viridis* L.) and hypoaccumulator (*Abelmoschus esculentus*, L.)

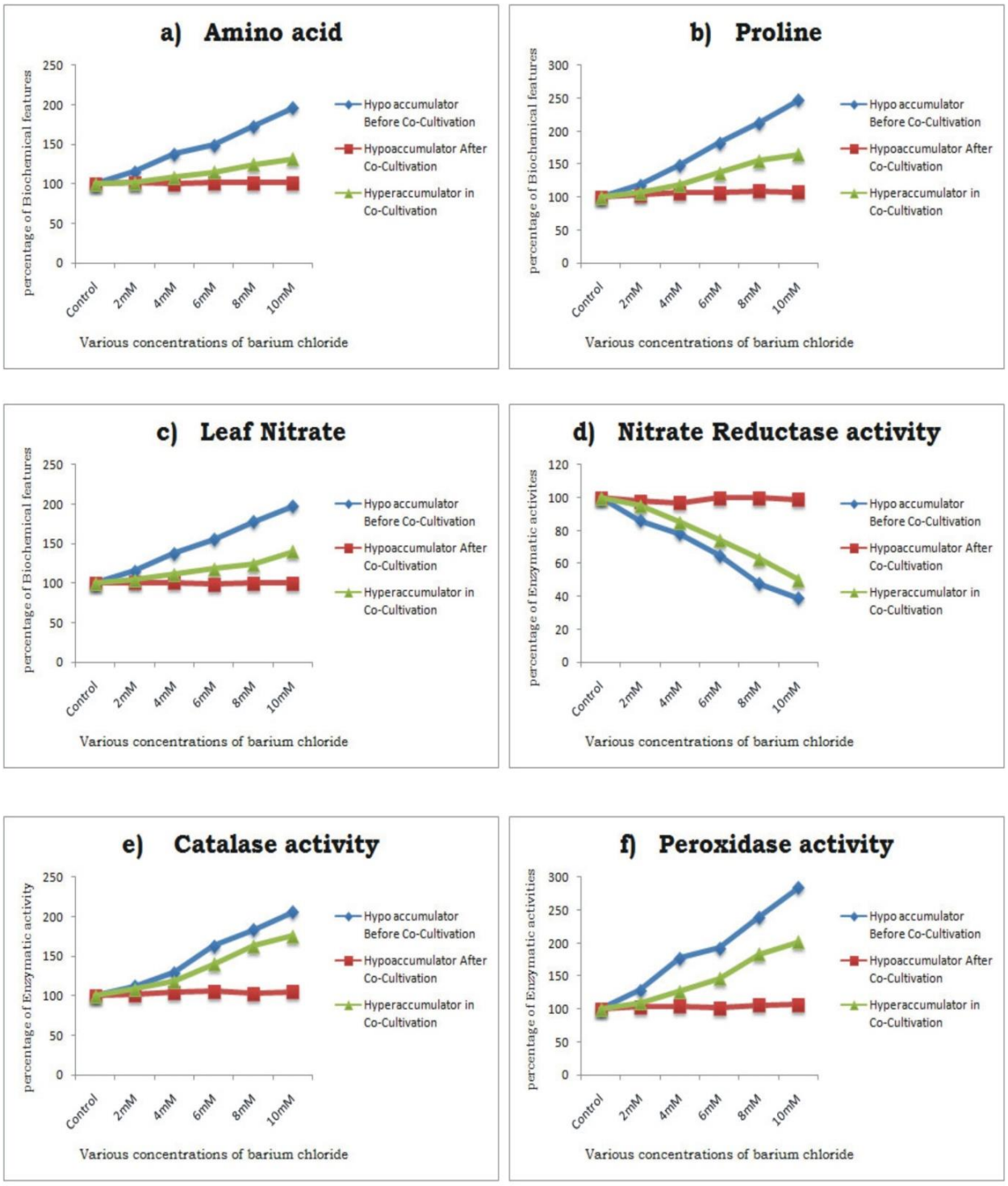

Figure 3: Impact of barium chloride on biochemical (a - c) and enzymatic features (c - f) of hyperaccumulator (*Amaranthus viridis* L.) and hypoaccumulator (*Abelmoschus esculentus*, L.)

The observed inhibition of shoot and root growth and leaf area is the main cause for the decrease in fresh weight and dry weight of seedlings (Fig. 1d, e). In plants, uptake of metals occurs primarily through the roots, so roots are the primary site for regulating the accumulation of metals (Arduini*et al*., 1996). The biomass accumulation represents overall growth of the plants. In the present investigation, the total fresh weight of hyperaccumulator *(Amaranthus*) was gradually reduced with the increase in concentration of metal, but in the hypoaccumulator, no reduction was found and the plants were as like as control plants when co-cultivated. This may be due to the removal of metal toxicity by the hyperaccumulator (*Amaranthus*). Similar observation was reported by Iori *et al*., (2013) in phytoremoval of *Amaranthus* under nickel stress.

Inhibition of biomass accumulation is directly related to the photosynthetic processes which, in turn, rely upon the pigment level. Considerable reduction in the pigment level was noticed in hyperaccumulator (*Amaranthus*) on the barium treatment, which was not observed in the hypoaccumulator (*Abelmoschus*) after co-cultivation. Heavy metal stress reduces nutrient and water uptake, impairs photosynthesis and inhibits growth of the plants (Jihen *et al*., 2010; Lag *et al*., 2010).

In co-cultivation the chlorophyll content (Fig.2 a-c), which is an indicator of the photosynthetic efficiency of the plant, showed a marked reduction in all the treatments in the hyperaccumulator plant but not in hypoaccumulator plant. In plants increasing concentrations of heavy metal and its toxic effects on the plant chlorophyll content was reported by Ewais (1997). Similar reduction in pigment level was observed in many plants by various heavy metal treatments (Bauddh and Singh, 2009).Reduction in the chlorophyll content paralleled with the reduction in dry weight and the net photosynthesis were reported (Kumar *et al*., 2007). In this study, there was a reduction in root length and chlorophyll content associated with the reduction in dry matter in hyperaccumulator, which did not occur in hypoaccumulator (*Abelmoschus*) in co-cultivation. It may be due to the hyperaccumulator accumulating all the toxicity, so the *Abelmoschus esculentus*, L. is free from metals toxicity. In heavy metal treated plants, the reduction in chlorophyll content could be due to a block in the chlorophyll biosynthetic pathway or induction of chlorophyll degradation by chloropyllase (Kupper*et al*., 2002; Dong *et al*., 2005). In the present study, similar declining trend was observed in the carotenoid content (Fig. 1f) in hyperaccumulator which was not seen in hypoaccumulator after co-cultivation.

The anthocyanin content (Fig. 2d) was, however, found increasing in the hyperaccumulator, whereas there was no change found in the hypoaccumulator (*Abelmoschus*) when co-cultivated with *Amaranthus* in barium treatment. The protective

function of plant anthocyanin against the stress condition is fairly clear (Moroni*et al.,* 1991; Genge*t al.* 2020) The anthocyanin accumulated in the leaves exposed to heavy metal or pollutants could act as scavengers, before the metal or pollutants reaches the sensitive targets such as chloroplast and other organelle (Mishra and Agarwal, 2006; Polit and Krupa, 2006). There was a considerable reduction in the levels of sugar and protein (Fig. 2e, f) in the leaves of *Amaranthus* treated with various concentrations of barium. In contrary, no reduction of sugar and protein contents was observed in the *Abelmoschus* when co-cultivated with the *Amaranthus*. The result coincides with the result of Marchiol *et al*., (2006).

As a result of protein degradation, the availability of free amino acids is significantly high in *Amaranthus* (Fig 3a). The free amino acid content is increased with increasing concentration of the barium, these increasing trend was observed in *Abelmoschus* in co-cultivation. It may be due to the destruction of protein or increase in the biosynthesis of amino acids from the nitrate source, which were not utilized in the protein synthesis (Schmoger*et al*., 2000). The degradation of protein may lead to an increase in free amino acid content. It is an adaptive mechanism employed by the plant cell to overcome post stress metabolism (Singh and Vijayakumar, 1974).

Proline accumulation (Fig. 3b) was observed in *Amaranthus* with increasing concentration of metal stress indicating highly stressed nature by metal, however in *Abelmoschus,* there is no much accumulation indicating the plants are relieved from stress after co-cultivation. Proline is considered to be a protective mechanism for the plants to preserve water, which is necessary to tide over any internal water deficit. Accumulation of amino acids, organic anions and quaternary ammonium compounds such as glycine, betaine and proline are considered as osmotic adjustments in higher plants during water stress (Boyer and Meyer, 1979). Rout and Shaw (1998) analysed the possibility of proline accumulation as a consequence of impaired protein synthesis.

Under stress, inhibition of growth of cells, leaves and the whole plant is accompanied by an accumulation of nitrate in plant tissue particularly in leaves (Sinha and Nicholas, 1981). The leaf nitrate content was analysed and found to be more in *Amaranthus*, than in the *Ablemoschus* plants (Fig. 3c) in co-cultivation. In *Ablemoschus*all the treatments the leaf nitrate content was more or less similar to the control plant. Indeed, the accumulation of leaf nitrate content was found to be paralleled with the reduction in nitrate reductase (NR) activity (Fig. 3d). Similar increase in leaf nitrate content and reduction in *in vivo* nitrate reductaseactivities with increase in concentration of cadmium treatment on *Vigna radiata* was observed by Jayakumar and Ramasubramanian (2009). Similar type of observations with the

increase in the concentration of industrial effluent on *Abelmoschus esculentus*was also observed by Jeyarathi and Ramasubramanian (2002).

Nitrate Reductase (NR) enzyme is one of the cytoplasmic substrate inducible enzymes. The NR activity was found to be decreased in *Amaranthus* (Fig.3d). In metal stressed plants, lowering of nitrate reductase activity reflects a decreased rate of enzyme synthesis or an increased rate of enzyme degradation (Hanser and Hitz, 1982). Thus, it is possible to assume that, a mechanism similar to this might have operated in the barium stressed *Amaranthus* thereby causing a reduction in the nitrate reductase activity. While barium toxicity on nitrate reductase activity was observed in the *Amaranthus*, no such reduction was observed during co-cultivationin of the hypoaccumulator *Abelmoschu sesculentus*, L.

Physiological stress manifests itself in metabolic disturbance and oxidative injury by producing reactive oxygen species. Resistance to any stress is exhibited by the antioxidant capacity or increased level of one or more antioxidants which can prevent stress damage (Balakumar*et al.*, 1993). Hence, in the present study, activities of enzyme like catalase and peroxidase were analysed (Fig. 3e, f). Peroxidase is an enzyme which utilizes hydrogen peroxide as a substrate and it also oxidizes a wide range of hydrogen donors such as phenolic substances, cytochrome-c-oxidase. The peroxidase activity was observed with the increasing concentrations of the barium in the *Amaranthus*. The increased peroxidase activity might have caused a major impact on the chlorophyll degradation but there is no such increased peroxidase activity was seen in *Abelmoschus* when co-cultivated with *Amaranthus*.

Catalase is another anti-oxidant scavenging enzyme. It is also analysed in the present study and found to be increased with the increasing concentrations of barium. Catalase is a special type of peroxidative enzymes which catalyses the degradation of H_2O_2, which is a natural metabolite toxic to plants. Nashikkar and Chakrabarti (1994) reported that increasing concentrations of sodium chloride has caused enhanced catalase activity. However, in *Abelmoschus* plants during co-cultivation both the catalase and peroxidase activities were found to be on par with control plant indicating stress relieved nature.

The accumulation factor and translocation factor of both metals show a gradual increase in the *Amaranthus* with increasing concentrations of barium (Table 2). But in the *Abelmoschus*, the accumulation factor (AF) and translocation factor (TF) were very less even in 4mM concentration of metal treatment after co-cultivation. Both factors were recorded below the detectable level which coincides with the findings of Ma *et al*., (2001). It is also reported that comparatively low TF values of chromium and high TF values of mercury in *Amaranthus*, reveal that very low and high translocation of these metals indicating the translocation potential *Amaranthus diffusa* (Raskin *et al*., 1994).

Table 2: Impact of arsenic chloride concentration in hyperaccumulator (*Amaranthus viridis* L.) and hypoaccumulator (*Abelmoschus esculentus,* L.)

Metal Concentration	Accumulation Factor (AF)			Translocation Factor (TF)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.		After Co–Cultivation
		Abelmoschus esculentus, L.	*Amaranthus viridis* L.		*Abelmoschus esculentus,* L.	*Amaranthus viridis* L.
Control	BDL	BDL	BDL	BDL	BDL	BDL
2mM	0.490 ± 0.001	BDL	1.303 ± 0.007	0.125 ± 0.001	BDL	1.044 ± 0.0042
4mM	0.301 ± 0.002 a*	0.005 ± 0.007 a#	1.303 ± 0.002 a#	0.121 ± 0.003 a*	0.175 ± 0.0071a*	1.097 ± 0.001 a*
6mM	0.251 ± 0.007 a*	0.003 ± 0.009 a#	1.149 ± 0.007 a*	0.119 ± 0.007 a*	0.098 ± 0.0082a*	1.172 ± 0.001 a*
8mM	0.235 ± 0.002 a*	0.003 ± 0.008 a#	1.465 ± 0.009 a*	0.112 ± 0.001 a*	0.087± 0.0018a*	1.181 ± 0.001 a*
10mM	0.213 ± 0.003 a*	0.001 ± 0.001 a#	1.559 ± 0.002 a*	0.103 ± 0.004 a*	0.036± 0.0054a*	1.253 ± 0.002 a*

Values are an average of three observations. Mean ± SE, a – refers to value compared with control in various concentrations of metals, a* – refers to significant (P ≤ 0.05 – Tukey test). a# – refers to non-significant.

BDL – Below Detectable Level, S – R: Soil to Root, R – S: Root to Stem, S – L: Stem to Leaf

More or less similar results have been reported in the accumulation pattern of heavy metals in *Bidenstri partita* (Zheljazkov *et al*., 2008). Those authors suggested that accumulation potential of plants towards heavy metal depends on the availability of the metals in the soil/ growth media as well as on the plant genotype. But in the present study, after co-cultivaton, the accumulation factor and translocation factor were less in the hypoaccumulator (*Abelmoschus*)was observed. This may be due to the hyperaccumulator accumulating more metals and leave hypoaccumulator free from metal toxicity.

If the accumulation factor (AF) and translocation factor (TF) values are above one, the plant is suitable for phytoremediation (Yoon *et al*., 2006; Zhelyjazkov*et al*., 2008). In the present investigation, accumulation factor (AF) and translocation factor (TF) values are above one, in *Amaranthus*, suggesting that they are the best suited for phytoextraction of barium toxicity.

The mobility index (MI) (Table 3) of *Amaranthus* is higher than one for Level 3, the mobility index was more than 0.7 and 0.4 respectively for Levels 1 and 2, indicating the moderate rate of mobility of metals form soil to roots, higher mobility rate in stem to leaves, and low from roots to stem. Thus, the present results are well corroborated with the observations of Hunter *et al*. (1987a, 1987b, 1987c). In contrary, in the hypoaccumulator *Abelmoschus* these levels are not traceable, because the hyperaccumulator plants absorbed

the metals whichf reed the hypoaccumulator *Abelmoschus*. Similar findings were provided by Yusuf *et al*., (2002) and An *et al.,* (2004).

Table 3: Impact of arsenic chloride concentration in hyperaccumulator (*Amaranthus viridis* L.) and hypoaccumulator (*Abelmoschus esculentus,* L.)

Metal Concentration	Mobility Index (MI)								
	Level 1 (Soil to Root)			Level 2 (Root to Stem)			Level 3 (Stem to Root)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation *Abelmoschus esculentus,* L.	After Co–Cultivation *Amaranthus viridis* L.	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation *Abelmoschus esculentus,* L.	After Co–Cultivation *Amaranthus viridis* L.	Arsenic Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation *Abelmoschus esculentus,* L.	After Co–Cultivation *Amaranthus viridis* L.
Control	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2mM	0.437 ± 0.006	BDL	0.681 ± 0.007	0.055 ± 0.003	BDL	0.380 ± 0.001	1.630 ± 0.007	BDL	1.378 ± 0.009
4mM	0.268 ± 0.000 a*	BDL	0.705 ± 0.000 a*	0.053 ± 0.001 a*	BDL	0.432 ± 0.003 a*	1.496 ± 0.001 a*	BDL	1.512 ± 0.004 a*
6mM	0.224 ± 0.003 a*	0.001 ± 0.005 a#	0.704 ± 0.001 a*	0.050 ± 0.001 a*	BDL	0.436 ± 0.008 a*	1.235 ± 0.007 a*	BDL	1.656 ± 0.004 a*
8mM	0.212 ± 0.007 a*	0.003 ± 0.001 a#	0.753 ± 0.006 a*	0.050 ± 0.004 a*	0.505 ± 0.001 a#	0.528 ± 0.001 a*	1.065 ± 0.002 a*	0.585 ± 0.006 a*	1.766 ± 0.004 a*b*
10mM	0.193 ± 0.003 a*	0.003 ± 0.007 a#	0.803 ± 0.008 a*	0.046 ± 0.005 a*	0.449 ± 0.003 a#	0.535 ± 0.008 a*	1.030 ± 0.001 a*	0.516 ± 0.002 a*	1.904 ± 0.001 a*

Values are an average of three observations. Mean ± SE, a – refers to value compared with control in various concentrations of metals, a* – refers to significant (P ≤ 0.05 – Tukey test). a# – refers to non-significant.

BDL – Below Detectable Level, S – R: Soil to Root, R – S: Root to Stem, S – L: Stem to Leaf

Thus, from the above findings it is clear that, the plant *Amaranthus viridis* L. chosen for the study, are acting as hyperaccumulator. This is proved by the results obtained on accumulation factor (AF), translocation factor (TF) and mobility index (MI) studies. Because of the phytoextraction capability of *Amaranthus* (hyperaccumulator), *Abelmoschus,* the hypoaccumulator plant, could grow well in metal stressed environment when it is co-cultivated. Based on the result obtained on accumulation factor (AF), translocation factor (TF) and mobility index (MI), it is suggested that *Amaranthus viridis* L. is best suited for remediating barium contaminated soil.

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