Biochemistry & Physiology: Open Access

Selvaraj et al., Biochem Physiol 2015, 4:3
DOI: 10.4172/2168-9652.1000172

Research Article Open Access

Phytoextraction: Using Brassica as a Hyper Accumulator

Selvaraj K[1]*, Sevugaperumal R[2] and Ramasubramanian V[2]

[1]Department of Botany, Sri S Ramasamy Naidu Memorial College (Autonomous), Sattur – 626 203, Tamil Nadu, India
[2]Department of Botany, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi – 626 124 Virudhunagar District, Tamil Nadu, India

Abstract

Phytoextraction is a cost-effective method that could be an alternative to remediate polluted sites. Growth performance, biochemical, enzymatic activity, accumulation, translocation and mobility of arsenic form soil to root and leaves were studied in co-cultivated hyper accumulator (*Brassica juncea*) and hypo accumulator (*Abelmuscus esculentus*) at various levels of arsenic. *B.juncea* accumulated fourfold and fivefold arsenic in roots, shoots and leaves, respectively than *Abelmuscus esculentus* L. *A. esculentus* seedlings when cultivated alone were seen sensitive to arsenic with decrease growth, poor values of accumulation factor, translocation factor and mobility of metal. But the same plant when co-cultivated with *Brassica juncea* there is no toxicity symptoms and reduction of growth, values of Accumulation factor, translocation factor and mobility of metal. This is well understand that *Brassica juncea* showing higher accumulation of nickel, more translocation of arsenic from root to shoot and good mobility of arsenic was increased form level 1 to level 3, It was revealed that the accumulation of arsenic was more in root and shoot of *B. juncea* than *A. esculentus*. It is inferred from the present study that *A. esculentus* is a hypo accumulator and is sensitive to arsenic. When co-cultivated with *Brassica juncea* showing less of metal toxicity because *Brassica juncea* being hyper accumulator of arsenic, accumulate more metal and save *Abelmuscus esculentus*.

Keywords: Hyperacumulator; Arsenic stress; Accumulation factor; Translocation factor; Mobility index

Introduction

The agricultural and industrial revolutions in the last few decades have resulted in increased concentration of toxins in our environment that are the major causes of toxicity in plants and animals. 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. Human activities have dramatically been changing the composition and organization of the soil on earth. 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 includes point sources such as emission, effluents, and solid discharge from industries, vehicle exhaustion, smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), 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.

Phytoremediation is the use of plants to treat/clean contaminated sites [1-7] and it can be defined as the use of green plants to remove pollutants from the environment or to render them harmless [8,9]. 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 [2,10]. Phytoremediation takes advantage of the natural ability of plants to extract chemicals from water, soil and air using energy from sunlight. It's some of the advantages are that it is less expensive, is passive and solar driven, has high public acceptance, retains topsoil, and has less secondary waste generation. In this respect, plants can be compared to solar driven pumps capable of extracting and concentrating certain elements from their environment [11]. This technology is being considered as a highly promising technology for the remediation of polluted sites.

The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time. Many families of vascular plants have been identified as metal hyperaccumulator [12,13], and many of them belongs to Brassicaceae and Amaranthaceae. These hyperaccumulator are metal selective, having slow growth rate, produce small amounts of biomass and can be used in their natural habitats only [14].

In the present study, it is aimed to analyze the impact of arsenic on the morphometric characters, biochemical, enzymatic features, accumulation factor, translocation factor and mobility index of *Abelmoschus esculentus,* L. (hypoaccumulator) and hyperaccumulator *Brassica juncea,* Hk. F. and T.

Materials and Methods

Seeds of *Abelmoschus esculentus* L., and *Brassica juncea,* Hk. F. and T. were procured from local seed center, Sivakasi. *Abelmoschus esculentus* L. Var. S7 (Family; Malvaceae) was chosen as experimental plant, whereas the *Brassica juncea,* Hk. F. and T. (Family; Brassicaceae) was chosen as hyperaccumulator plants for this study. The effect of various concentrations of arsenic on the morphometric characters, biochemical, enzymatic features, accumulation factor, translocation factor and mobility index were analyzed on the selected plants.

Experimental design

***Corresponding author:** Selvaraj K, Department of Botany, Sri S Ramasamy Naidu Memorial College (Autonomous), Sattur – 626 203, Tamilnadu, India, Tel: +91 9789240653; E-mail: kselvarajphd@gmail.com

Received June 26, 2015; **Accepted** July 15, 2015; **Published** July 22, 2015

Citation: Selvaraj K, Sevugaperumal R, Ramasubramanian V (2015) Phytoextraction: Using Brassica as a Hyper Accumulator. Biochem Physiol 4: 172. doi: 10.4172/2168-9652.1000172

Copyright: © 2015 Selvaraj K, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Heavy metals stress on *Abelmoschus, Brassica*: The heavy metals arsenic was treated separately in the experimental plants with different concentrations *viz.,* 2 mM, 4 mM, 6 mM, 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 days. A set of plants without heavy metal treatment was maintained as control.

Ten surface sterilized seeds of *Abelmoschus esculentus* L., and *Brassica juncea,* Hk. F. and T. were sown uniformly in all the pots for the experimental purpose. Morphometric, biochemical, enzymatic parameters and metal concentration in plants such as accumulation, translocation factor and mobility index were analyzed on the 35th day after planting (DAP).

Phytoremediation treatment

Co-cultivation of the hypoaccumulator and hyperaccumulator: Optimum number of surface sterilized seeds of *Abelmoschus esculentus* L. (hypo accumulator) and *Brassica juncea,* Hk. F. and T. (hyperaccumulator) were sown uniformly in all pots. Appropriate amount of arsenic 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 analyzed on the 35th day after planting (DAP).

Morphometric parameters

For all the morphometric characteristics, root length, shoot length, leaf area, fresh weight and dry weight were analyzed, 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.

Biochemical and enzymatic features

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 analyzed by the following methods. Chlorophyll and carotenoids [15], anthocyanin [16], total soluble sugar and amino acid [17], Protein content [18], leaf nitrate [19]. *In vivo* nitrate reductase activity [20], peroxidase and catalase [21].

Accumulation Factor (AF)

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 [22,23]:

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

Translocation Factor (TF)

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 [24]. It is represented by the ratio:

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

Mobility Index (MI)

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. [25].

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

Results

The results on the effect of arsenic on the morphometric characters of co-cultivated hypoaccumulator *Abelmoschus esculentus* L. and hyper accumulators *Brassica juncea,* Hk. F. and T. have been presented in the Tables 1and 2.

The reduction in root length of hyperaccumulators was found to be 26% in *Brassica* at 10 mM concentration of arsenic. However, at the same concentration the reduction in *Abelmoschus* was only 4% after co-cultivation, and 65% before co-cultivation. Shoot length has also followed a similar declining trend, in the hyper accumulator *Brassica juncea,* Hk. F. and T. the reduction was about 18% compared to the control plants; In contrary, the *Abelmoschus* showed only 15% reduction when co-cultivated with *Brassica*. Before co-cultivation, the *Abelmoschus* showed a reduction of 68% in arsenic treatment. The increasing concentration of metal application has caused significant reduction in the leaf area of hyperaccumulators and was about 26% (*Brassica*) under 10 mM concentration of arsenic treatment. At the same concentration, the reduction in *Abelmoschus* was only 11% after co-cultivation, which was 67% before co-cultivation. The fresh weight was also reduced in the hyper accumulator *Brassica juncea,* Hk. F. and T. with the increasing concentrations of arsenic. Arsenic has reduced the

Metal Concentration	Root Length (cm)			Shoot Length (cm)			Leaf Area (cm^2)		
	Arsenic Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea*, Hk. F. and T.		*Abelmoschus esculentus*, L.	*Brassica juncea*, Hk. F. and T.		*Abelmoschus esculentus*, L.	*Brassica juncea*, Hk. F. and T.
Control	29.7 ± 0.921 (100)	29.9 ± 0.357 (100)	20.80 ± 0.465 (100)	25.4 ± 0.437 (100)	26.1 ± 0.173 (100)	25.0 ± 0.197 (100)	12.54 ± 0.524 (100)	13.2 ± 0.306 (100)	15.1 ± 0.519 (100)
2 mM	27.92 ± 0.817 a˙ (94)	29.60 ± 0.164 a# (99)	19.76 ± 0.195 a˙ (95)	22.1 ± 0.150 a˙ (87)	25.32 ± 0.197 a# (97)	24.5 ± 0.413 a˙ (98)	10.45 ± 0.793 a˙ (83)	12.9 ± 0.520 a# (98)	15.02 ± 0.387 a˙ (96)
4 mM	23.17 ± 0.911 a˙ (78)	29.0 ± 0.289 a# (97)	18.72 ± 0.373 a˙ (90)	18.29 ± 0.245 a˙ (72)	24.27 ± 0.194 a# (93)	23.5 ± 0.419 a˙ (94)	8.52 ± 0.263 a˙ (68)	12.7 ± 0.192 a# (96)	14.66 ± 0.128 a˙ (93)
6 mM	19.90 ± 0.676 a˙ (67)	28.70 ± 0.157 a# (96)	17.68 ± 0.176 a˙ (85)	14.99 ± 0.193 a˙ (59)	23.23 ± 0.314 a˙ (89)	22.5 ± 0.571 a˙ (90)	7.17 ± 0.753 a˙ (57)	12.4 ± 0.164 a˙ (94)	13.53 ± 0.184 a˙ (86)
8 mM	14.85 ± 0.737 a˙ (50)	29.01 ± 0.176 a# (97)	16.43 ± 0.452 a˙ (79)	10.92 ± 0.546 a˙ (43)	22.97 ± 0.715 a˙ (88)	21.5 ± 0.326 a˙ (86)	5.84 ± 0.291 a˙ (46)	11.9 ± 0.157 a˙ (90)	12.74 ± 0.371 a˙ (81)
10 mM	10.40 ± 0.809 a˙ (35)	28.70 ± 0.159 a# (96)	15.39 ± 0.291 a˙ (74)	8.13 ± 0.437 a˙ (32)	22.19 ± 0.362 a˙ (85)	20.5 ± 0.425 a˙ (82)	4.13 ± 0.564 a˙ (33)	11.6 ± 0.613 a˙ (89)	11.68 ± 0.129 a˙ (74)

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 ≤ 0.05 – Tukey test).

Table 1: Impact of arsenic chloride on the morphometric characteristics of hyper accumulator (*Brassica juncea,* Hk. F. and T.) and hypoaccumulator (*Abelmoschus esculentus* L.).

Metal Concentration	Fresh Weight (gm.)			Dry Weight (gm.)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	16.09 ± 0.179 (100)	16.17 ± 0.419 (100)	19.87 ± 0.357 (100)	10.15 ± 0.371 (100)	10.37 ± 0.163 (100)	14.07 ± 0.174 (100)
2 mM	14.91 ± 0.947 a* (93)	16.03 ± 0.715 a# (99)	19.42 ± 0.419 a# (97)	9.04 ± 0.134 a* (89)	10.14 ± 0.756 a# (98)	13.82 ± 0.543 a* (98)
4 mM	13.47 ± 0.731 a* (84)	15.92 ± 0.452 a# (98)	18.71 ± 0.164 a* (94)	7.92 ±0.316 a* (78)	9.84 ± 0.867 a# (95)	13.16 ± 0.294 a* (94)
6 mM	11.70 ± 0.398 a* (73)	15.90 ± 0.194 a# (98)	17.82 ± 0.518 a* (90)	5.14 ± 0.675 a* (51)	9.91 ± 0.512 a# (96)	12.87 ± 0.359 a* (91)
8 mM	8.36 ± 0.671 a* (52)	15.73 ± 0.456 a# (97)	16.98 ± 0.473 a*(85)	3.83 ± 0.219 a* (38)	9.52 ± 0.149 a# (92)	12.24 ± 0.783 a* (87)
10 mM	6.98 ± 0.738 a* (43)	15.69 ± 0.129 a# (97)	15.73 ± 0.431 a* (79)	2.07 ± 0.519 a* (20)	9.37 ± 0.542 a* (90)	11.53 ± 0.648 a* (82)

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 ≤ 0.05 – Tukey test). a# – refers to non-significant.

Table 2: Impact of arsenic chloride on the biomass of hyperaccumulator (*Brassica juncea,* Hk. F. and T.) and hypoaccumulator (*Abelmoschus esculentus,* L.).

fresh weight up to 79% in *Brassica* than the control plants. There was no reduction in fresh weight in *Abelmoschus* when co-cultivated with *Brassica* (hyperaccumulators), under the 10 mM arsenic treatment, the *Abelmoschus* showed only 3% reduction when co-cultivated with *Brassica* when *Abelmoschus* alone grown, the reduction was 57% under the same concentration of arsenic treatment. The dry weight was analyzed in the control and heavy metal treated plants of co-cultivated hypoaccumulator and hyperaccumulator. The reduction was about 82% in *Brassica* under 10mM concentration of arsenic treatment. Whereas, *Abelmoschus* when co-cultivated with *Brassica* showed 10% reduction However, the reduction was about 80% when *Abelmoschus* was cultivated individually. The results on the effect of arsenic on the pigment contents of co-cultivated hypoaccumulator *Abelmoschus esculentus,* L. and hyper accumulators *Brassica juncea,* Hk. F. and T. have been presented in the tables 3 and 4.

In the hyperaccumulators, the reduction in total chlorophyll content was about 27% in *Brassica* compared to the control plants. However, after co-cultivation the reduction was about 5% in *Abelmoschus* with *Brassica* which was 55% before co-cultivation. The carotenoid content of *Abelmoschus* has slightly decreased to about 4% decrease were seen in *Abelmoschus* grown with *Brassica* after the application of 10 mM concentration of arsenic treatment, whereas the reduction was about at 78% at 10 mM arsenic concentration before co-cultivation. In hyperaccumulators, the carotenoid content also decreased to 20% reduction in the carotenoids was observed on the *Brassica* at 10 mM concentration of arsenic treatment than the control plants. In contrary to the photosynthetic pigments, the anthocyanin content was increased with the increasing concentrations in both the metals when co-cultivated with hyperaccumulators. But in hypoaccumulator, anthocyanin content was not increased in all the concentrations and it was more are less equal to the control plant. In hyperaccumulator plants, the application of 6 mM concentration of arsenic has significantly increased the anthocyanin content to about 19% in *Brassica* than the control plants. In hypoaccumulator (*Abelmoschus*), anthocyanin content was increased to only 1% when co-cultivated with *Brassica.* Before co-cultivation it was 104% increase (Table 5).

The reduction of total soluble sugar content was 16% on *Brassica* arsenic treatment at 10 mM concentration. At the same concentration of arsenic treatment, in the hypoaccumulator (*Abelmoschus*) in all concentrations total soluble sugar content was more or less similar to control plants when co-cultivated with *Brassica*, whereas it was 53% before co-cultivation. In the co-cultivation set, supply of 10

Metal Concentration	Chlorophyll .a (mg/gLFW)			Chlorophyll .b (mg/gLFW)			TotaL. Chlorophyll (mg/gLFW)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	5.76 ± 0.197 (100)	6.14 ± 0.362 (100)	9.76 ± 0.097 (100)	4.13 ± 0.914 (100)	4.42 ± 0.568 (100)	7.31 ± 0.473 (100)	9.89 ± 0.771 (100)	10.56 ± 0.761 (100)	17.07 ± 0.128 (100)
2 mM	5.10 ± 0.108 a* (89)	5.98 ± 0.419 a# (97)	9.12 ± 0.165 a* (93)	3.52 ± 0.793 a* (85)	4.37 ± 0.317 a# (99)	6.84 ± 0.136 a* (94)	8.62 ± 0.314 a* (87)	10.35 ± 0.516 a# (98)	15.96 ± 0.139 a* (93)
4 mM	4.23 ± 0.461 a* (73)	5.95 ± 0.716 a# (97)	8.86 ± 0.119 a* (91)	2.99 ± 0.147 a* (72)	4.33 ± 0.479 a# (98)	6.12 ± 0.307 a* (84)	7.22 ± 0.658 a* (73)	10.28 ± 0.815 a# (97)	15.0 ± 0.213 a*
6 mM	3.49 ± 0.640 a* (60)	5.86 ± 0.134 a# (95)	8.16 ± 0.306 a* (84)	2.08 ± 0.186 a* (50)	4.26 ± 0.294 a# (96)	5.38 ± 0.096 a* (74)	5.57 ± 0.025 a* (56)	10.12 ± 0.143 a# (96)	13.54 ± 0.518 a* (79)
8 mM	2.78 ± 0.517 a* (48)	5.80 ± 0.617 a* (94)	7.73 ± 0.177 a* (79)	1.65 ± 0.492 a* (40)	4.27 ± 0.915 a* (96)	4.72 ± 0.149 a* (65)	4.43 ± 0.158 a* (45)	10.07 ± 0.205 a# (95)	12.45 ± 0.375 a* (73)
10 mM	1.98 ± 0.376 a* (33)	5.77 ± 0.237 a* (94)	6.87 ± 0.253 a* (70)	1.07 ± 0.315 a* (26)	4.21 ± 0.518 a* (95)	3.911 ± 0.465 a* (54)	2.99 ± 0.213 a* (30)	9.98 ± 0.314 a* (95)	10.84 ± 0.197 a* (64)

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 ≤ 0.05 – Tukey test). a# – refers to non-significant.

Table 3: Impact of arsenic chloride on the photosynthetic pigment contents of hyperaccumulator (*Brassica juncea,* Hk.F. and T.) and hypoaccumulator (*Abelmoschus esculentus,* L.).

Metal Concentration	Carotenoids (mg/gLFW)			Anthocyanin (µg /gLFW)			Total Soluble Sugar (mg/gLFW)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	3.78 ± 0.236 (100)	3.84 ± 0.173 (100)	6.75 ± 0.093 (100)	1.65 ± 0.832 (100)	1.58 ± 0.276 (100)	2.67 ± 0.086 (100)	7.63 ± 0.147 (100)	7.61 ± 0.326 (100)	12.38 ± 0.367 (100)
2 mM	3.04 ± 0.197 a* (80)	3.80 ± 0.419 a# (99)	6.62 ± 0.086 a# (98)	2.09 ± 0.334 a* (127)	1.60 ± 0.241 a# (101)	2.72 ± 0.384 a* (102)	6.51 ± 0.313 a* (85)	7.59 ± 0.257 a# (100)	12.04 ± 0.283 a# (97)
4 mM	2.47 ± 0.360 a* (65)	3.76 ± 0.237 a# (98)	6.31 ± 0.098 a* (93)	2.81 ± 0.151 a* (170)	1.62 ± 0.378 a# (103)	2.91 ± 0.399 a* (109)	5.47 ± 0.173 a* (72)	7.56 ± 0.721 a# (99)	11.80 ± 0.176 a* (95)
6 mM	1.86 ± 0.314 a* (49)	3.77 ± 0.581 a# (98)	6.04 ± 0.136 a* (89)	3.36 ± 0.249 a* (204)	1.59 ± 0.352 a# (101)	3.17 ± 0.674 a* (119)	4.83 ± 0.842 a* (63)	7.49 ± 0.342 a# (98)	11.46 ± 0.354 a* (93)
8 mM	1.12 ± 0.527 a* (30)	3.73 ± 0.729 a# (97)	5.87 ± 0.142 a* (87)	3.99 ± 0.167 a* (241)	1.64 ± 0.247 a* (107)	3.45 ± 0.413 a* (129)	4.16 ± 0.760 a* (55)	7.58 ± 0.346 a# (100)	10.97 ± 0.602 a* (87)
10 mM	0.849 ± 0.674 a* (22)	3.70 ± 0.365 a# (96)	5.39 ± 0.479 a* (80)	4.63 ± 0.184 a* (280)	1.63 ± 0.187 a# (103)	3.72 ± 0.638 a* (139)	3.56 ± 0.221 a* (47)	7.53 ± 0.148 a# (99)	10.45 ± 0.567 a* (84)

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 ≤ 0.05 – Tukey test). a# – refers to non-significant.

Table 4: Impact of arsenic chloride on the pigments of hyper accumulator (*Brassica juncea,* Hk. F. and T.) and hypo accumulator (*Abelmoschus esculentus,* L.).

mM concentration of arsenic decreased the total soluble protein content of *Brassica* 19% when compared to the control plants. In hypoaccumulator (*Abelmoschus*) the reduction was only 3% when co-cultivated with *Brassica* under 10 mM arsenic treatment. At the same concentration, it was about 64% before co-cultivation. A reduction in soluble protein level eventually leads to an increase in free amino acid content. The results of the study show that the free amino acid content of hyperaccumulator, *Brassica* where the maximum increase of 24% at 10 mM arsenic treatment than the control plants. Arsenic treatment in *Abelmoschus,* the increase was 2% when co-cultivated with *Brassica* but the increase was 96% before co-cultivation. Only 8% increase of proline content was seen in *Abelmoschus* co-cultivated with *Brassica* under the 10 mM arsenic treatment. At the same concentration of arsenic treatment, it was 147% more than control before co-cultivation. Arsenic treatment in the *Brassica* has increased the nitrate level to 40%, whereas, no increase in leaf nitrate content when co-cultivated with *Brassica*. In all concentrations, the leaf nitrate content was about equal to control plant, whereas it was 98% before co-cultivation (Table 6).

The results of the present study shows (Table 7) that, *in vivo* nitrate reductase activity of the leaves was significantly inhibited at 10 mM concentration of arsenic to about 50% in *Brassica* when compared to the control. In contrary, the hypoaccumulator *Abelmoschus* when co-cultivated with *Brassica,* no reduction in nitrate reductase activity under 10 mM arsenic treatments. Catalase activity was found to be increased in hyperaccumulators of all the experimental plants than the control. The increase was respectively, about 76% when compared to the control plants. In *Abelmoschus,* there was only 5% increase when co-cultivated with *Brassica* under arsenic treatment, which was 206% when grown alone. Peroxidase is another antioxidant enzyme that also showed an increasing trend as catalase in hyperaccumulators and in hypoaccumulator it showed on par activity with control. In arsenic treatment, *Brassica* an activity of about 46% more respectively at 6 mM concentration when compared to the control. At the same concentration of arsenic, the reduction was about 7% in hypoaccumulator when co-cultivated with *Brassica*. This was 284% when grown alone.

Heavy metal concentrations

To evaluate the heavy metal accumulation, translocation and mobility in the plant tissue, the Accumulation Factor (AF), Translocation Factor (TF) and Mobility Index (MI) was calculated on

Metal Concentration	Total Soluble Protein(mg/gLFW)			Amino acid (µ mole/g LFW)			Proline (µ mole/g LFW)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	4.76 ± 0.412 (100)	4.79 ± 0.168 (100)	7.61 ± 0.275 (100)	3.57 ± 0.301 (100)	3.63 ± 0.079 (100)	6.57 ± 0.450 (100)	1.968 ± 0.386 (100)	1.984 ± 0.116 (100)	3.84 ± 0.176 (100)
2 mM	4.05 ± 0.216 a* (85)	4.73 ± 0.214 a# (99)	7.53 ± 0.318 a# (99)	4.13 ± 0.379 a* (115)	3.69 ± 0.428 a# (102)	6.69 ± 0.428 a* (102)	2.325 ± 0.228 a* (118)	2.047 ± 0.173 a# (103)	4.12 ± 0.215 a* (107)
4 mM	3.41 ± 0.237 a* (72)	4.75 ± 0.346 a# (99)	7.34 ± 0.425 a* (96)	4.96 ± 0.657 a* (138)	3.64 ± 0.754 a# (100)	6.88 ± 0.534 a* (105)	2.941 ± 0.206 a* (149)	2.125 ± 0.234 a# (107)	4.57 ± 0.161 a* (119)
6 mM	2.83 ± 0.677 a* (59)	4.69 ± 0.872 a# (98)	6.91 ± 0.638 a* (91)	5.34 ± 0.138 a* (149)	3.67 ± 0.082 a# (101)	7.19 ± 0.251 a* (109)	3.579 0.382 a* (182)	2.113 ± 0.315 a# (107)	5.25 ± 0.755 a* (137)
8 mM	2.10 ± 0.136 a* (44)	4.64 ± 0.311 a# (97)	6.65 ± 0.346 a*(87)	6.19 ± 0.463 a* (173)	3.72 ± 0.486 a# (102)	7.53 ± 0.682 a* (115)	4.184 ± 0.472 a* (213)	2.167 ± 0.324 a# (109)	5.98 ± 0.183 a* (156)
10 mM	1.72 ± 0.254 a* (36)	4.66 ± 0.267 a# (97)	6.18 ± 0.212 a* (81)	6.98 ± 0.249 a* (196)	3.70 ± 0.512 a# (102)	8.14 ± 0.743 a* (124)	4.866 ± 0.637 a* (247)	2.148 ± 0.167 a* (108)	6.32 ± 0.198 a* (165)

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 ≤ 0.05 – Tukey test). a# – refers to non-significant.

Table 5: Impact of arsenic chloride on the biochemical features of hyper accumulator (*Brassica juncea,* Hk. F. and T.) and hypoaccumulator (*Abelmoschus esculentus,* L.)

Metal Concentration	Leaf Nitrate (µ mole/g LFW)			Nitrate Reductase activity (µ mole/g LFW)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	3.52 ± 0.308 (100)	3.55 ± 0.273 (100)	7.57 ± 0.085 (100)	8.03 ± 0.781 (100)	8.14 ± 0.126 (100)	12.53 ± 0.364 (100)
2 mM	4.06 ± 0.432 a* (115)	3.59 ± 0.126 a# (101)	7.84 ± 0.093 a* (104)	6.87 ± 0.160 a* (86)	8.00 ± 0.634 a# (98)	11.86 ± 0.803 a* (95)
4 mM	4.84 ± 0.467 a* (138)	3.58 ± 0.264 a# (101)	8.39 ± 0.148 a* (111)	6.24 ± 0.284 a* (78)	7.93 ± 0.518 a# (97)	10.62 ± 0.516 a* (85)
6 mM	5.49 ± 0.510 a* (156)	3.51 ± 0.325 a# (99)	8.96 ± 0.102 a* (118)	5.21 ± 0.418 a* (65)	8.12 ± 0.193 a# (100)	9.27 ± 0.234 a* (74)
8 mM	6.27 ± 0.521 a* (178)	3.54 ± 0.314 a# (100)	9.42 ± 0.386 a* (124)	3.879 ± 0.367 a* (48)	8.16 ± 0.509 a# (100)	7.84 ± 0.732 a* (63)
10 mM	6.98 ± 0.549 a* (198)	3.56 ± 0.431 a# (100)	10.61 ± 0.257 a* (140)	3.132 ± 0.319 a* (39)	8.09 ± 0.341 a# (99)	6.31 ± 0.747 a* (50)

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 ≤ 0.05 – Tukey test). a# – refers to non-significant.

Table 6: Impact of arsenic chloride on the biochemical and enzymatic features of hyper accumulator (*Brassica juncea,* Hk. F. and T.) and hypo accumulator (*Abelmoschus esculentus,* L.).

Metal Concentration	Catalase activity (µ mole/g LFW)			Peroxidase activity (µ mole/g LFW)		
	Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	2.67 ± 0.472 (100)	2.54 ± 0.376 (100)	5.48 ± 0.433 (100)	1.63 ± 0.207 (100)	1.56 ± 0.087 (100)	3.60 ± 0.231 (100)
2 mM	2.99 ± 0.587 a* (112)	2.59 ± 0.147 a# (102)	5.97 ± 0.670 a* (109)	2.08 ± 0.324 a* (128)	1.61 ± 0.096 a# (103)	3.94 ± 0.436 a* (109)
4 mM	3.48 ± 0.542 (130)	2.63 ± 0.139 a# (104)	6.49 ± 0.481 a* (118)	2.88 ± 0.469 a* (177)	1.63 ± 0.125 a# (104)	4.59 ± 0.485 a* (127)
6 mM	4.35 ± 0.419 a* (163)	2.68 ± 0.272 a# (106)	7.65 ± 0.143 a* (140)	3.14 ± 0.479 a* (193)	1.59 ± 0.149 a# (102)	5.27 ± 0.354 a* (146)
8 mM	4.92 ± 0.205 a* (184)	2.61 ± 0.897 a# (103)	8.94 ± 0.376 a* (163)	3.92 ± 0.273 a* (240)	1.66 ± 0.182 a# (106)	6.63 ± 0.417 a* (184)
10 mM	5.49 ± 0.059 a* (206)	2.66 ± 0.643 a# (105)	9.62 ± 0.265 a* (176)	4.63 ± 0.167 a* (284)	1.67 ± 0.195 a# (107)	7.28 ± 0.163 a* (202)

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 ≤ 0.05 – Tukey test). a# – refers to non-significant.

Table 7: Impact of arsenic chloride on the enzymatic features of hyper accumulator (*Brassica juncea,* Hk.F. and T.) 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.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	BDL	BDL	BDL	BDL	BDL	BDL
2 mM	0.490 ± 0.0014	BDL	1.483 ± 0.0064	0.125 ± 0.0008	BDL	1.103 ± 0.0018
4 mM	0.301 ± 0.0029a*	BDL	1.520 ± 0.0072a*	0.121 ± 0.0038a*	BDL	1.158 ± 0.0093a*
6 mM	0.251 ± 0.0071a*	0.005 ± 0.0026a#	1.586 ± 0.0048a*	0.119 ± 0.0073a*	BDL	1.196 ± 0.0008a*
8 mM	0.235 ± 0.0026a*	0.004± 0.0013a#	1.654 ± 0.0013a*	0.112 ± 0.0010a*	0.765 ± 0.0021 a#	1.272 ± 0.0037a*
10 mM	0.213 ± 0.0037a*	0.001± 0.0061a#	1.824 ± 0.0004a*	0.103 ± 0.0042a*	0.711 ± 0.0034a#	1.327 ± 0.0016a*

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

Table 8: Impact of arsenic chloride concentration in hyper accumulator (*Brassica juncea,* Hk. F. and T.) and hypo accumulator (*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		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Arsenic Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk. F. and T.
Control	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2 mM	0.437 ± 0.0068	BDL	0.681 ± 0.0074	0.055 ± 0.0039	BDL	0.380 ± 0.0018	1.630 ± 0.0072	BDL	1.378 ± 0.0090
4 mM	0.268 ± 0.0002a*	BDL	0.705 ± 0.0002a*	0.053 ± 0.0017 a*	BDL	0.432 ± 0.0039a*	1.496 ± 0.0015a*	BDL	1.512 ± 0.0043a*
6 mM	0.224 ± 0.0034a*	0.001 ± 0.0055a#	0.704 ± 0.0018a*	0.050 ± 0.0011a*	BDL	0.436 ± 0.0082a*	1.235 ± 0.0073a*	BDL	1.656 ± 0.0042a*
8 mM	0.212 ± 0.0075a*	0.003 ± 0.0012a#	0.753 ± 0.0069a*	0.050 ± 0.0047 a*	0.505 ± 0.0012a#	0.528 ± 0.0010a*	1.065 ± 0.0020 a*	0.585 ± 0.0064a*	1.766 ± 0.0043a* b*
10 mM	0.193 ± 0.0031a*	0.003 ± 0.0078a#	0.803 ± 0.0083a*	0.046 ± 0.0053a*	0.449 ± 0.0034a#	0.535 ± 0.0083a*	1.030 ± 0.0014a*	0.516 ± 0.0026a*	1.904 ± 0.0016a*

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

Table 9: Impact of arsenic chloride concentration in hyper accumulator (*Brassica juncea,* Hk. F. and T.) and hypoaccumulator (*Abelmoschus esculentus,* L.).

the effect of arsenic on co-cultivately grown *Abelmoschus esculentus* L., with *Brassica juncea,* Hk. F. and T. and tabulated in tables 8 and 9.

The accumulation factor was significantly increased in hyperaccumulators with the increasing concentrations of arsenic. With the increasing concentrations of arsenic, the accumulation factor also increased in the hyperaccumulator and more accumulation factor was recorded in *Brassica* (1.824) when grown in 10 mM arsenic solution. The accumulation factor was not recorded much in the hypoaccumulator, *Abelmoschus.* The seedlings of *Abelmoschus esculentus,* L. when co-cultivated with hyperaccumulator *Brassica* under the influence of arsenic up to 4 mM the accumulation factor was below detectable level (BDL) and 6 mM to 10 mM it was ranging from 0.015 to 0.003 in arsenic treatment. In the hyperaccumulators, the translocation factor was increased with the increasing concentrations of arsenic. Translocation factor was recorded in *Brassica* and when grown in 10 mM arsenic solution. It was found to be 1.32. When the hypoaccumulator *Abelmoschus* was co-cultivated with the hyperaccumulator, *Brassica* the translocation factor was in the range of 0.765 to 0.711 in arsenic treatment.

The mobility index was divided into three parts; Level 1- Soil to Root; Level 2- Root to Stem and Level 3- Stem to Leaf. For Level 1, the mobility index was 0.803 in *Brassica* when grown in 10 mM arsenic solution. The hypoaccumulator, *Abelmoschus* when co-cultivated with *Brassica* did not show the mobility index. For Level 2, in the hyperaccumulators, mobility index was 0.535 in *Brassica* when grown in 10 mM arsenic solution, *Abelmoschus* when co-cultivated with *Brassica* up to 4 mM, the mobility index was below the detectable level for arsenic treatment and in 6 mM to 10 mM concentration, the mobility index was ranging from 0.073 to 0.058. For Level 3, the mobility index was 1.904 in *Brassica* under 10 mM arsenic treatment. The hypoaccumulator, *Abelmoschus* when co-cultivated with *Brassica* up to 4 mM, the mobility index was below detectable level for arsenic treatment The *Abelmoschus* when co-cultivated with *Brassica*, the mobility index was 0.516 in 10 mM arsenic.

Discussion

Phytoextraction is a soil remediation technology that makes use of the plants to extract metals from contaminated soils. When using non-hyperaccumulators as phytoextractors, one of the greatest factors limiting the success of this technology is the solubility of metals in the soil solution. Since plants can only accumulate metals in the labile fraction of the soil, the success of phytoextraction would be restricted by the unavailability of soil metals. Generally, at high contaminant concentrations in soil or water, plants are able to metabolize these harmful elements. However, some plants can survive and even grow well when they accumulate high concentration of toxic elements, as is the case of the hyperaccumulator plants. So, the co-cultivation of hypoaccumulator with hyperaccumulator has been analyzed in this chapter.

Results on the co-cultivation of hypoaccumulator *Abelmoschus esculentus,* L. with hyper accumulators *Brassica juncea,* Hk. F. and T. under various concentrations of arsenic are being discussed below.

Heavy metals either retard the growth of the whole plant or plant parts [26,27]. The plant parts normally the roots which have direct contact with the contaminated soils exhibit rapid and sensitive changes in their growth pattern. 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 [28].

In the present investigation, arsenic has caused considerable

reduction on the seedling length and leaf area of hyperaccumulators *Brassica*. However, not much reduction in the hypoaccumulator *Abelmoschus* was recorded when compared with plant treated with metal alone. Inhibition of the root and shoot lengths at higher concentration of the metals is due to the high levels of toxicity present in arsenic, which interfered and inhibited the uptake of other essential elements like potassium, calcium, phosphorus and magnesium by the plants [29]. Sahai et al. [30] and Dolar et al. [31] 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 arsenic treatment was also related to accumulation of arsenic in leaves, where the size of the leaf was also decreased [32].

The observed pronounced inhibition of shoot and root growth and leaf area is the main cause for the decrease in fresh weight and dry weight of seedlings. In plants, uptake of metals occurs primarily through the roots, so roots are the primary site for regulating the accumulation of metals [33]. The biomass accumulation represents overall growth of the plants. In the present investigation, the total fresh weight of hyperaccumulator (*Brassica*) 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. This may be due to the removal of metal toxicity by the hyperaccumulator (*Brasssica*). Similar observation was reported by Quartacci et al. [34] in phytoextraction of cadmium by the Indian mustard.

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 (*Brassica*) on the arsenic treatment, which was not in the hypoaccumulator (*Abelmoschus*). Heavy metal stress reduces nutrient and water uptake, impairs photosynthesis and inhibits growth of the plants [35-37].

Plants exhibit morphological and metabolic changes in response to metal stress that are believed to be adaptive responses [38]. For instance, metal stress not only inhibits growth [39,40], but also brings about changes in various physiological and biochemical characteristics such as water balance, nutrient uptake [41,42] and photosynthetic electron transport around photosystems I and II [43-45]. The reduction in growth and biomass due to arsenic stress may result in many biochemical, physiological and molecular changes in the plants. Heavy metal stress in plants has been reflected as stunted growth, leaf chlorosis and alteration in the activity of key enzymes of various metabolic pathways [35,46-48].

The chlorophyll content, 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. Similar reduction in pigment level was observed in many plants by various heavy metal treatments [49-51].

Reduction in the chlorophyll content paralleled with the reduction in dry weight and the net photosynthesis were reported. 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*). 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 [40,52-54]. In the present study, similar declining trend was observed in the carotenoid content in hyperaccumulator.

The anthocyanin content was, however, found increasing in the hyperaccumulator, whereas there was no change found in the hypoaccumulator (*Abelmoschus*) when co-cultivated with *Brassica* in arsenic treatment. The protective function of plant anthocyanin against the stress condition is fairly clear [55]. The anthocyanin accumulated in the leaves exposed to heavy metal or pollutants could act as scavengers, before it reaches the sensitive targets such as chloroplast and other organelle [56-58].

There was a considerable reduction in the levels of protein and sugar in the leaves of *Brassica* treated with various concentrations of arsenic. In contrary, no reduction of sugar and protein contents was observed in the *Abelmoschus* when co-cultivated with the *Brassica*. The result coincides with the result of Marchiol et al. [59].

As a result of protein degradation, the availability of free amino acids is significantly high in *Brassica*. The free amino acid content is increased with increasing concentration of the arsenic. 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 [60]. 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 [61].

Proline accumulation 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 [62,63]. Rout and Shaw [64] analyzed 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 [65]. The leaf nitrate content was analyzed and found to be more in *Brasssica*, than in the *Ablemoschus* plants. In 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. Similar increase in leaf nitrate content, reduction in *in vivo* nitrate reductase activities with increase in concentration of cadmium treatment on *Vigna radiata* was observed by Jayakumar and Ramasubramanian [66] and industrial effluent on *Abelmoschus esculentus* by Jeyarathi and Ramasubramanian [67].

Nitrate Reductase (NR) enzyme is one of the cytoplasmic substrate inducible enzymes. The NR activity was found to be decreased in both the *Brassica* in both metal treatments. In metal stressed plants, lowering of nitrate reductase activity reflects a decreased rate of enzyme synthesis or an increased rate of enzyme degradation [68]. Thus, it is possible to assume that, a mechanism similar to this might have operated in the arsenic stressed *Brassica* thereby causing a reduction in the nitrate reductase activity. While arsenic toxicity was observed in the *Brassica*, no such reduction in nitrate reductase activity in the hypoaccumulator *Abelmoschus esculentus* L. was observed.

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 [69]. Hence, in the present study, activities of enzyme like catalase and peroxidase were analyzed. 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 to be increased with the increasing concentrations of the arsenic in the *Brassica*. The increased peroxidase activity caused a major impact on the chlorophyll degradation.

Catalase is another anti-oxidant scavenging enzyme. It is also analyzed in the present study and found to be increased with the increasing concentrations of nickel. 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 [70] reported that increasing concentrations of sodium chloride has caused enhanced catalase activity. However, in *Abelmoschus* plants, both the catalase and peroxidase activities were found to be on par with control pant indicating stress relived nature.

The accumulation factor and translocation factor of both metals show a gradual increase in the *Brassica* with increasing concentrations of arsenic. But in the *Abelmoschus*, the accumulation factor (AF) and translocation factor (TF) were very less even in 4mM concentration of metal treatment. Both factors were recorded below the detectable level which coincides with the findings of Ma et al. [71]. Comparatively low TF values of chromium and high TF values of mercury reveal very low and high translocation of these metals indicating the translocation potential *Brassica diffusa*.

More or less similar results have been reported in the accumulation pattern of heavy metals in *Bidens tripartita* [72]. 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, the accumulation factor and translocation factor were less in the hypoaccumulator (*Abelmoschus*). 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 [23,72]. In the present investigation, accumulation factor (AF) and translocation factor (TF) values are above one, in *Brassica*, suggesting that they are best suited for phytoextraction of arsenic toxicity.

The mobility index (MI) of *Brassica* is higher than one for Level 3, the mobility index was more than 0.6 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. [73-75]. In contrary, in the hypoaccumulator *Abelmoschus* these levels are not noticed, because the hyperaccumulator plants absorbed the metals freed the hypoaccumulator *Abelmoschus*. Similar findings were provided by Yusuf et al. [76] and An et al. [77].

Thus, from the above findings it is clear that, the plant *Brassica juncea,* Hk. F. and T. 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 *Brassica*, (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 *Brassica juncea,* Hk. F. and T. is best suited for remediating nickel.

References

1. Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29: 318A-23A.
2. Salt DE, Smith RD, Raskin I (1998) PHYTOREMEDIATION. Annu Rev Plant Physiol Plant Mol Biol 49: 643-668.
3. Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3: 153-162.
4. Dietz A, Schnoor JL (2001) Advances in phytoremediation. Environ. Health Persp 109: 163 – 168.
5. Newman LA, Reynolds CM (2004) Phytodegradation of organic compounds. Curr Opin Biotechnol 15: 225-230.
6. Suresh B, Ravishankar GA (2004) Phytoremediation--a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24: 97-124.
7. Pilon-Smits EAH, Freeman JL (2006) Environmental cleanup using plants: Biotechnological advances and ecological considerations. Fron. Ecol. Environ. 4: 203 – 210
8. Berti W, Cunningham SD (2000) Phytostabilization of metals. In: Phytoremediation of toxic metals: using plants to clean-up the environment. Eds. Raskin I, EnsleY B. D. New York, John Wiley and Sons, PP. 176 – 189.
9. Salt DE, Kumar PBAN, Dushenkov S, Raskin I (1994) Phytoremediation: A New Technology for the Environmental Cleanup of Toxic Metals. International Symposium Research on Conservation and Environmental Technology for Metallic Industry. Toronto, Canada.
10. Gratao PL, Prasad MNV, Cardoso PF, Lea PJ, Azevedo RA, et al. (2005) Phytoremediaion: Green technology for the clean-up of toxic metals in the environment. Braz J Plant Physiol 17: 53 – 64.
11. Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, et al. (1995) Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology (N Y) 13: 468-474.
12. Reeves RD, Baker AJM (2000) Metal accumulating plants. In: Phytoremediation of toxic metals: Using plants to clean-up the environment. Eds. Raskin I and Ensley BD New York, John Wiley and Sons: 193 – 230.
13. Prasad MNV, Freitas H (2003) Metal hyper accumulation in plants - Biodiversity prospecting for phytoremediation technology. Electronic J Biotechnol 6: 275 – 321.
14. Kamnev AA, van der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20: 239-258.
15. Wellburn AR, Lichtenthaler H (1984) Formulae and program to determine total carotenoids and chlorophyll a and b of leaf extracts in different solvents. In: Advances in photosynthesis Research Eds. Sybesma, Martinus Nijhoof Co., The Hague: 9 – 12.
16. Swain T, Hills WE (1959) The phenolic constituents of *Prunus domestica* L. The quantitative analysis of phenolic constitution. J Sci Food Agric 10: 63-68.
17. Jayaraman J (1981) Laboratory manual in Biochemistry, Willey-Eastern company Limited, Madras: 1– 65.
18. Lowry OH, Rosenbury NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193: 265– 275.
19. Cataldo DA, Haroon M, Schroader LE, Younger VC (1978) Rapid colorimetric determination of Nitrate in plant by nitrate salicylic acid. Commun Soil Sci Plant Anal 6: 71 – 80.
20. Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. Biochem Biophys Res Commun 43: 1274-1279.
21. Kar M, Mishra D (1976) Catalase, Peroxidase, and Polyphenoloxidase Activities during Rice Leaf Senescence. Plant Physiol 57: 315-319.
22. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its by-products. App Ecol Environ Res 3: 1 – 18.
23. Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368: 456-464.

24. Mellem JJ, Baijnath H, Odhav B (2009) Translocation and accumulation of Cr, Hg, As, Pb, Cu and Ni by *Amaranthus dubius* (Amaranthaceae) from contaminated sites. J Environ Sci Health A Tox Hazard Subst Environ Eng 44: 568-575.

25. Kumar JIN, Soni H, Kumar RN, Bhatt I (2009) Hyper accumulation and mobility of heavy metals in vegetable crops in India. J Agricul and Environ 10: 29 – 38.

26. Shaq M, Iqbal MZ (2006) Tolerance of Peltophorum pterocarpum D. C. Baker Ex K. Heyne. seedlings to lead and cadmium treatment. J New Seeds 7: 83 – 94.

27. Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31: 739-753

28. Wong MH, Bradshaw AD (1982) A comparison of the toxicity of heavy metals, using root elongation of rye grass, Lolium perenne. New Phytol 91: 255 – 261.

29. Clarkson DT (1985) Factors affecting mineral nutrient acquisition by plants, Annu. Rev. Plant Physiol 36 77-115.

30. Sahai R, Jabeen S, Saxena PK (1983) Effect of distillery waste on seed germination, seedling growth and pigment content of rice. Ind J Ecol 10: 7 – 10.

31. Dolar SG, Boyle JR, Keeney DR (1972) Paper mill sludge disposal on soils: e?ects on the yields and mineral nutrition of oats. J Environ Qual 1: 405 – 409.

32. Panday N, Sharma CP (2002) Effect of heavy metals Co2+, Ni2+ and Cd2+ on growth and metabolism of Cabbage. Plant Sci 63: 753 – 758.

33. Arduini I, Godbold DL, Onnis A (1995) Influence of copper on root growth and morphology of *Pinus pinea* L. and *Pinus pinaster* Ait. seedlings. Tree Physiol 15: 411-415.

34. Quartacci MF, Baker AJ, Navari-Izzo F (2005) Nitrilotriacetate- and citric acid-assisted phytoextraction of cadmium by Indian mustard (*Brassica juncea* (L.) Czernj, Brassicaceae). Chemosphere 59: 1249-1255.

35. Chaudhary A, Singh RB (2000) Cadmium induced change in diamine oxidase activity and polyamine label in Vigna radiata Wilczek. J Plant Physiol 156: 704 – 710.

36. Jihen el H, Fatima H, Nouha A, Baati T, Imed M, et al. (2010) Cadmium retention increase: A probable key mechanism of the protective effect of zinc on cadmium-induced toxicity in the kidney. Toxicol Lett 196: 104-109.

37. Låg M, Rodionov D, Ovrevik J, Bakke O, Schwarze PE, et al. (2010) Cadmium-induced inflammatory responses in cells relevant for lung toxicity: Expression and release of cytokines in fibroblasts, epithelial cells and macrophages. Toxicol Lett 193: 252-260.

38. Singh S, Sinha S (2004) Scanning electron microscopic studies and growth response of the plants of *Helianthus annuus* L. grown on tannery sludge amended soil. Environ Int 30: 389-395.

39. Lunácková L, Masarovicová E, Král'ová K, Stresko V (2003) Response of fast growing woody plants from family Salicaceae to cadmium treatment. Bull Environ Contam Toxicol 70: 576-585.

40. Dong J, Wu FB, Zhang GP (2005) Effect of cadmium on growth and photosynthesis of tomato seedlings. J Zhejiang Univ Sci B 6: 974-980.

41. Vassilev A, Yordanov I, Tsonev T (1997) Effects of Cd2+ on the physiological state and photosynthetic activity of young barley plants. Photosynthetica 34: 293 – 302.

42. Scebba F, Arduini I, Ercoli L, Sebastiani L (2006) Cadmium effects on growth and antioxidant enzymes activities in Miscanthus sinensis. Biol Plant 50: 688 – 692.

43. Siedlecka A, Baszynski T (1993) Inhibition of electron Now around photosystem I in chloroplasts of Cd-treated maize plants is due to Cd-induced iron deficiency.

44. Skorzynska-Polit E, Baszynski T (1995) Photochemical activity of primary leaves in cadmium stressed Phaseolus coccineus depends on their growth stages. Acta. Soc. Bot. Pol. 64: 273 – 279. Plant Physiol 87: 199 – 202.

45. Vassilev A, Lidon F, Scotti P, Da Graca M, Yordanov I, et al. (2004) Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants. Biol Plant 48: 153 – 156.

46. Bharti N, Singh RP (1994) Antagonistic effect of NaCl to different heavy metal toxicity regarding in vivo nitrate reductase activity and organic nitrogen contents of roots and leaves of *Sesamum indicum* L. cv PB – 1. Phytochemistry. 35: 1157 – 1161.

47. Di Toppi LS, Gabrilli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41: 105 – 130.

48. Sharma A, Sainger M, Dwivedi S, Srivastava S, Tripathi RD, et al. (2010) Genotype variation in *Brassica juncea* L. Czern. Cultivars and growth, nitrate assimilation, antioxidants response and phytoremediation potential using cadmium stress. J Environ Biol 31: 773 – 780.

49. Padmaja K, Prasad DDK, Prasad ARK (1990) Inhibition of chlorophyll synthesis in *Phaseolus valgaris* seedlings by cadmium acetate. Photosynthetica 24: 399 – 405.

50. Gajeswska E, SlabaM, Andrezejewska R, Sklodowska M (2006) Arsenic induced inhibition of wheat root growth is related to H2O2 production, but not to lipid peroxidation. Plant Growth Regul 49: 95 – 103.

51. Bauddh K, Singh RP (2009) Genotypic differences in arsenic toxicity in Indian mustard (*Brassica juncea* L.). Pollut Res 28: 699 – 704.

52. Kupper H, Kupper F, Spiller M (1998) In situ detection of heavy metal substituted chlorophylls in water plants. Photosynth Res 58:123 – 133.

53. Kupper H, Kupper F, Spiller M (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. J Exp Bot 47: 259 – 266.

54. Kupper H, Setlik I, Spiller M, Kupper FC, Prasil O, et al. (2002) Heavy metal-induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. J Phycol 38: 429 – 441.

55. Moroni JS, Briggs KG, Taylor GJ (1991) Chlorophyll content and leaf elongation rate in wheat seedlings as a measure of manganese tolerance. Plant Soil 136: 1–9.

56. Yu W (2005) Impact of anthocyanin from Malva sylvestris on plasma lipids and free radicals. J Forest Res 16: 228 – 232.

57. Mishra S, Agarwal SB (2006) Interactive effects between supplemental ultraviolet–B radiation and heavy metals on the growth and biochemical characteristics of *Spinacia oleracea* L. Braz J Plant Physiol 18: 742 – 748.

58. SkÃ³rzyÅ, ska-Polit E, Krupa Z (2006) Lipid peroxidation in cadmium-treated *Phaseolus coccineus* plants. Arch Environ Contam Toxicol 50: 482-487.

59. Marchiol L, Assolari S, Fellet G, Zerbi G (2006) Germination and seedling growth of Indian mustard exposed to cadmium and chromium. Ital J Agron Riv Agron 1: 45 – 49.

60. Schmoger ME, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122: 793 – 801.

61. Singh DS, Vijayakumar KP (1974) Carry out the effects of Salinity on yield and quality of wheat seed. Seed Res 21: 13 – 18.

62. Acevedo E, Fereres E, Hsiao TC, Henderson DW (1979) Diurnal growth trends, water potential, and osmotic adjustment of maize and sorghum leaves in the field. Plant Physiol 64: 476-480.

63. Boyer JS, Meyer RF (1979) Antagonistic effect of NaCl to different heavy metal toxicity regarding in vivo nitrate reductase activity and organic nitrogen contents of roots and leaves of *Sesamum indicum* L. cv PB – 1. Rains DW, Valentine RC, Hollaender A (Eds), Plenum, New York.

64. Rout N, Shaw B (1998) Salinity tolerance in aquatic macrophytes: Probable role of proline, the enzymes involved in its synthesis and C4 type of metabolism. Plant Sci 136:121 – 130.

65. Sinha SK, DJD Nicholas (1981) In The physiology and biochemistry of drought resistance in plants. Eds. Paleg LG, Aspinall D. Academic Press, London: 145 – 169.

66. Jayakumar S, Ramasubramanian V (2009) Bioremoval of chromium using seaweeds as biosorbents. J Basic and App Biol 3(3 and 4): 121 – 128.

67. Jeyarathi KP, Ramasubramanian V (2002) Analysis of sugar mill Effluents and its impact on the growth and biochemical characteristics of *Abelmoschus esculentus* (L.) Mediakus. In: Recent Trends in Biotechnol. Eds. Harikumar, Grobios Publication, Jodhpur. 245 – 253.

68. HanserAD, Hitz WD (1982) Enzymes and metals. Ann Rev Plant Physiol 33: 163 – 203.

69. Balakumar T, Hanibabu Vincent V, Kailash Paliwal (1993) Interaction of UV Radiation (280-315 nm) with water stress in crop plants. Physiol Plant 87: 217 – 222.

70. Nashikkar VJ, Chakrabarthi T (1994) Catalase and peroxidase activities in plants and indicator of heavy metal toxicity. Ind J Exp Biol 32: 520 – 521.

71. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, et al. (2001) A fern that hyper accumulates arsenic. Nature 409: 579

72. Zheljazkov VD, Cantrell CL, Tekwani B, Khan SI (2008) Content, composition, and bioactivity of the essential oils of three basil genotypes as a function of harvesting. J Agric Food Chem 56: 380-385.

73. Hunter BA, Johnson MS, Thompson DJ (1989b) Ecotoxicology in copper and cadmium in a contaminated grassland ecosystem. II. Invertebrates. J Appl Ecol 24: 589– 599.

74. Hunter BA, Johnson MS, Thompson DJ (1987c) Ecotoxicology in copper and cadmium in a contaminated grassland ecosystem. II. Small mammals. J Appl Ecol 24: 601 – 614.

75. Hunter BA, Johnson MS, Thompson DJ (1987a) Ecotoxicology in copper and cadmium in a contaminated grassland ecosystem. I soil vegetation contamination. J Appl Ecol 24: 573 – 586.

76. Yusuf AA, Arowolo TA, Bamgbose O (2003) Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. Food Chem Toxicol 41: 375-378.

77. An YJ, Kim YM, Kwon TI, Jeong SW (2004) Combined effect of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. Sci Total Environ 326: 85-93.