Phytoextraction: Using Brassica as a Hyper Accumulator

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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 (Abelmoscus esculentus) at various levels of arsenic. B. juncea accumulated fourfold and fivefold arsenic in roots, shoots and leaves, respectively than 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 Abelmoscus esculentus.

Keywords: Hyperaccumulator; 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 belong 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

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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 day. 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 charts 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:

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

**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 hyperaccumulator *Abelmoschus esculentus* L. and hyper accumulators *Brassica juncea*, Hk. F. and T. have been presented in the Tables 1 and 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 hyperaccumulator *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

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

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Root Length (cm)</th>
<th>Shoot Length (cm)</th>
<th>Leaf Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).


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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 stress. The dry weight was analyzed in the control and heavy metal treated plants of co-cultivated hypooaccumulator and hyperaccumulator. The reduction was about 82% in *Brassica* under 10mM concentration of arsenic stress. Whereas, *Abelmoschus* when with co-cultivated 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 hyperaccumulator *Abelmoschus esculentus*, L. and hyper accumulators *Brassica juncea*, 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 esculentus* 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 hypooaccumulator, anthocyanin content was not increased in all the concentrations and it was more 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 hypooaccumulator (*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 hypooaccumulator (*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

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

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Fresh Weight (gm.)</th>
<th>Dry Weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.09 ± 0.179 (100)</td>
<td>10.15 ± 0.371 (100)</td>
</tr>
<tr>
<td>2 mM</td>
<td>14.91 ± 0.9474 (93)</td>
<td>10.37 ± 0.163 (100)</td>
</tr>
<tr>
<td>4 mM</td>
<td>13.47 ± 0.7314 (64)</td>
<td>14.07 ± 0.174 (100)</td>
</tr>
<tr>
<td>6 mM</td>
<td>11.70 ± 0.3988 (73)</td>
<td>6.62 ± 0.086 (100)</td>
</tr>
<tr>
<td>8 mM</td>
<td>8.36 ± 0.6714 (52)</td>
<td>17.07 ± 0.128 (100)</td>
</tr>
<tr>
<td>10 mM</td>
<td>6.98 ± 0.7385 (43)</td>
<td>15.73 ± 0.431 (79)</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parenthese 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 biomass of hyperaccumulator (*Brassica juncea*, F. and T.) and hypooaccumulator (*Abelmoschus esculentus*, L.)

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Chlorophyll a (mg/gFW)</th>
<th>Chlorophyll b (mg/gFW)</th>
<th>Total Chlorophyll (mg/gFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.76 ± 0.197 (100)</td>
<td>8.78 ± 0.007 (100)</td>
<td>2.70 ± 0.159 (20)</td>
</tr>
<tr>
<td>2 mM</td>
<td>5.10 ± 0.168 (89)</td>
<td>9.02 ± 0.137 (90)</td>
<td>6.82 ± 0.314 (87)</td>
</tr>
<tr>
<td>4 mM</td>
<td>4.23 ± 0.461 (73)</td>
<td>6.95 ± 0.016 (70)</td>
<td>7.22 ± 0.684 (73)</td>
</tr>
<tr>
<td>6 mM</td>
<td>3.49 ± 0.640 (86)</td>
<td>8.86 ± 0.016 (55)</td>
<td>5.57 ± 0.025 (56)</td>
</tr>
<tr>
<td>8 mM</td>
<td>2.78 ± 0.517 (48)</td>
<td>8.80 ± 0.016 (54)</td>
<td>10.12 ± 0.143 (46)</td>
</tr>
<tr>
<td>10 mM</td>
<td>1.98 ± 0.376 (33)</td>
<td>7.07 ± 0.273 (43)</td>
<td>8.90 ± 0.466 (54)</td>
</tr>
</tbody>
</table>

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 hyperaccumulator (*Brassica juncea*, F. and T.) and hypooaccumulator (*Abelmoschus esculentus*, L.)

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Carotenoids (mg/gFW)</th>
<th>Anthocyanin (µg/gFW)</th>
<th>Total Soluble Sugar (mg/gFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.78 ± 0.236 (100)</td>
<td>7.67 ± 0.093 (100)</td>
<td>7.63 ± 0.147 (100)</td>
</tr>
<tr>
<td>2 mM</td>
<td>3.04 ± 0.197 (90)</td>
<td>6.62 ± 0.086 (98)</td>
<td>6.51 ± 0.313 (85)</td>
</tr>
<tr>
<td>4 mM</td>
<td>2.47 ± 0.360 (85)</td>
<td>6.31 ± 0.096 (93)</td>
<td>5.47 ± 0.173 (72)</td>
</tr>
<tr>
<td>6 mM</td>
<td>1.86 ± 0.314 (49)</td>
<td>6.04 ± 0.136 (89)</td>
<td>4.83 ± 0.042 (63)</td>
</tr>
<tr>
<td>8 mM</td>
<td>1.12 ± 0.527 (36)</td>
<td>5.87 ± 0.147 (87)</td>
<td>4.16 ± 0.260 (55)</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.849 ± 0.674 (22)</td>
<td>5.10 ± 0.365 (96)</td>
<td>7.53 ± 0.148 (96)</td>
</tr>
</tbody>
</table>

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.
mM concentration of arsenic decreased the total soluble protein content of *Brassica* 19% when compared to the control plants. In hyperaccumulator (*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 *Abelmoschus*. 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 hyperaccumulator *Abelmoschus* when co-cultivated with *Brassica*, no reduction in nitrate reductase activity under 10 mM arsenic treatments. Cathalase 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 cathale in hyper accumulators and in hyperaccumulator 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 hyperaccumulator 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

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

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Total Soluble Protein (mg/LFW)</th>
<th>Amino acid (µ mole/LFW)</th>
<th>Proline (µ mole/LFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arsenic Stress on <em>Abelmoschus</em> esculentus, L.</td>
<td>Arsenic Stress on <em>Brassica</em> juncea, Hk. F. and T.</td>
<td>Arsenic Stress on <em>Brassica</em> juncea, Hk. F. and T.</td>
</tr>
<tr>
<td></td>
<td>After Co–Cultivation</td>
<td>After Co–Cultivation</td>
<td>After Co–Cultivation</td>
</tr>
<tr>
<td>Control</td>
<td>4.76 ± 0.412 (100)</td>
<td>7.61 ± 0.275 (100)</td>
<td>3.67 ± 0.096 (100)</td>
</tr>
<tr>
<td>2 mM</td>
<td>4.05 ± 0.216 (80)</td>
<td>7.63 ± 0.316 (100)</td>
<td>4.92 ± 0.168 (100)</td>
</tr>
<tr>
<td>4 mM</td>
<td>3.41 ± 0.237 (72)</td>
<td>7.94 ± 0.425 (100)</td>
<td>6.31 ± 0.747 (100)</td>
</tr>
<tr>
<td>6 mM</td>
<td>2.83 ± 0.677 (56)</td>
<td>8.60 ± 0.672 (100)</td>
<td>3.52 ± 0.308 (100)</td>
</tr>
<tr>
<td>8 mM</td>
<td>2.10 ± 0.136 (44)</td>
<td>8.64 ± 0.311 (97)</td>
<td>5.49 ± 0.510 (156)</td>
</tr>
<tr>
<td>10 mM</td>
<td>1.72 ± 0.254 (36)</td>
<td>8.66 ± 0.267 (97)</td>
<td>6.27 ± 0.521 (178)</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percent 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.)

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Catalase activity (µ mole/LFW)</th>
<th>Peroxidase activity (µ mole/LFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arsenic Stress on <em>Abelmoschus</em> esculentus, L.</td>
<td>Arsenic Stress on <em>Brassica</em> juncea, Hk. F. and T.</td>
</tr>
<tr>
<td></td>
<td>After Co–Cultivation</td>
<td>After Co–Cultivation</td>
</tr>
<tr>
<td>Control</td>
<td>2.67 ± 0.472 (100)</td>
<td>5.48 ± 0.433 (100)</td>
</tr>
<tr>
<td>2 mM</td>
<td>2.99 ± 0.587 a (112)</td>
<td>5.97 ± 0.670 a (109)</td>
</tr>
<tr>
<td>4 mM</td>
<td>3.48 ± 0.542 (130)</td>
<td>6.49 ± 0.481 a (118)</td>
</tr>
<tr>
<td>6 mM</td>
<td>4.35 ± 0.419 a (163)</td>
<td>7.65 ± 0.143 a (140)</td>
</tr>
<tr>
<td>8 mM</td>
<td>4.92 ± 0.205 a (184)</td>
<td>8.94 ± 0.376 a (163)</td>
</tr>
<tr>
<td>10 mM</td>
<td>5.49 ± 0.059 a (206)</td>
<td>9.62 ± 0.265 a (176)</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percent 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 8: Impact of arsenic chloride concentration in hyper accumulator (Brassica juncea, Hf. F. and T.) and hypo accumulator (Abelmoschus esculentus, L.).

<table>
<thead>
<tr>
<th>Metal Concentration</th>
<th>Accumulation Factor (AF)</th>
<th>Translocation Factor (TF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mM</td>
<td>0.490 ± 0.0014</td>
<td>1.654 ± 0.0013a</td>
</tr>
<tr>
<td>4 mM</td>
<td>0.301 ± 0.0029a</td>
<td>0.585 ± 0.0064a</td>
</tr>
<tr>
<td>6 mM</td>
<td>0.251 ± 0.0071a</td>
<td>0.535 ± 0.0083a</td>
</tr>
<tr>
<td>8 mM</td>
<td>0.235 ± 0.0026a</td>
<td>0.193 ± 0.0031a</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.213 ± 0.0037a</td>
<td>0.050 ± 0.0047a</td>
</tr>
</tbody>
</table>

The accumulation factor was significantly increased in hyperaccumulators with the increasing concentrations of arsenic. The translocation factor was also increased in the hyperaccumulator and 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 translocation 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 below detectable level for arsenic treatment. 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 availability 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, Hf. 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 (Brassica). 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 chloroplylase [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 Marchioli 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 Brassica, than in the Abelmoschus 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$_2$O$_2$, 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 relieved 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 low 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 difussa*.

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


