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PHYTOREMEDIATION POTENTIAL OF BRASSICA JUNCEA IN NICKEL CONTAMINATED SOIL

KEYWORD: Hyperacumulator, Nickel Stress, Accumulation Factor, Translocation Factor, Mobility Index

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ABSTRACT

The aim of this research was to assess the growth performance, biochemical, enzymatic activity, accumulation, translocation and mobility of nickel chloride form soil to root and leaves were studied in co-cultivated hyperaccumulator (Brassica juncea) and hypoaccumulator (Abelmuscus esculentus) at various levels of nickel . B.juncea accumulated fourfold and fivefold nickel in roots, shoots and leaves, respectively than Abelmuscus esculentus L. A.esculentus seedlings when cultivated alone were seen sensitive to nickel 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. This is well understand that Brassica juncea showing higher accumulation of nickel, more translocation of nickel from root to shoot and good mobility of nickel was increased form level 1 to level 3, It was revealed that the accumulation of nickel was more in root and shoot of B.juncea than A.esculentus. It is inferred from the present study that A.esculentus is a hypoaccumulator and is sensitive to nickel chloride. When co-cultivated with Brassica juncea showing less of metal toxicity because Brassica juncea being hyperaccumulator of nickel chloride, accumulate more metal and save Abelmuscus esculentus.

INTRODUCTION

Due to global industrialization and the increase in human population in the twentieth century, heavy metal contamination of soil, water and air has posed various uncompromising and fatal effects on humans and the stability of the ecosystem. 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 organisation 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 (Sidhu, 2016; Kumar *et al.,* 2019). 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.

Nickel (Ni) is an essential element that can be toxic and possibly carcinogenic in high concentrations. Ni is ubiquitously distributed in nature. It is found in different concentrations in all soil types of diverse climatic regions (Srivastava *et al*., 2005). Naturally derived soils from serpentine rocks are rich in Ni, but due to various industrial and anthropogenic activities such as mining, refining of Ni ores, burning of fossil fuels and residual oil and sewage sludge, other areas have also become prone to Ni contamination (Sengar *et al*., 2008). The normal range of Ni in soil is 2 to 750 ppm, with a critical soil concentration at 100 ppm (Gardea - Torresdey *et al*., 2005). Heavy metals accumulated in soil can affect flora, fauna and human livings in the vicinity of contaminated sites. The most of nickel is used to make stainless steel as a productive and ornamental coating for less corrosion. Nickel alloys are used in making coins and heat exchange items like valves. Nickel is combined with many other elements, including chlorine, sulfur and oxygen. Nickel compounds are used in plating, coloring ceramics making some batteries and as chemical reaction catalysts for dyes, molds, cast propellers and valve seats. The problem of nickel toxicity acquires a series concern because of agriculture use of sewage sludge that is usually rich in nickel (Juste and Mench, 1992) and the industrial use of nickel in the production of Ni – Cd batteries which lead to discharge of nickel effluents.

Phytoremediation is the use of plants to treat/clean contaminated sites (Schnoor *et al*., 1995; Salt *et al*., 1998; Meagher, 2000; Dietz and Schnoor, 2001; Newman and Reynolds, 2004; Suresh and Ravishankar, 2004; Pilon-Smits and Freeman, 2006; Lal and Srivastava, 2010) and it can be defined as the use of green plants to remove pollutants from the environment or to render them harmless (Berti and Cunningham, 2000; Salt *et al*., 1994). 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 (Gratao *et al*., 2005; Salt *et al*., 1998). 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 (Salt *et al*., 1995.). This technology is being considered as a highly promising technology for the remediation of polluted sites (Lukatkin *et al.,* 2020).

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 (Reeves and Baker, 2000; Prasad and

Freitas 2003), 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 (Kamnev and Van Der Lelie, 2000).

In the present study, it is aimed to analyse the impact of nickel 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. & T.

MATERIALS AND METHODS

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

Experimental Design

I) Heavy Metals Stress On *Abelmoschus, Brassica*

The heavy metals nickel 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. & 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 analysed on the 35th day after planting (DAP).

Phytoremediation Treatment

II) Co-cultivation Of The Hypoaccumulator And Hyperaccumulator

Optimum number of surface sterilized seeds of both *Abelmoschus esculentus,* L. (hypoaccumulator) and *Brassica juncea,* Hk. F. & T. (hyperaccumulator) were sown uniformly in all pots.

Appropriate amount of nickel chloride were given separately for the experimental plants with different concentration as 2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v) in five replicates. Morphometric, biochemical, enzymatic parameters and metal concentration in plants such as accumulation factor, translocation factor and mobility index were analysed on the 35th day after planting (DAP).

MORPHOMETRIC PARAMETERS

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

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

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 (Ghosh and Singh, 2005; Yoon *et al.*, 2006):

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

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 (Mellem *et al.,* 2009). It is represented by the ratio:

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

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*etal*(2009)

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

RESULTS

The results on the effect of nickel chloride on the morphometric characters of co-cultivated hypoaccumulator *Abelmoschus esculentus,* L. and hyperaccumulators *Brassica juncea,* Hk. F. & 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 10mM concentration of nickel chloride. 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.&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 nickel 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 nickel chloride 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 hyperaccumulator *Brassica juncea,* Hk.F.&T. with the increasing concentrations of nickel chloride. Nickel chloride has reduced the 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 nickel chloride 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 nickel treatment. The dry weight was analysed 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 nickel chloride 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 nickel chloride on the pigment contents of co-cultivated hypoaccumulator *Abelmoschus* *esculentus,* L. and hyperaccumulators *Brassica juncea,* Hk. F. & 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 nickel chloride treatment, whereas the reduction was about at 78 % at 10 mM nickel chloride 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 nickel chloride 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 nickel chloride 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.

The reduction of total soluble sugar content was 16 % on *Brassica* nickel chloride treatment at 10 mM concentration. At the same concentration of nickel 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 mM concentration of nickel chloride 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 nickel 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 aminoacid content. The results of the study shows that the free aminoacid content of hyperaccumulator, *Brassica* where the maximum increase of 24 % at 10 mM nickel chloride treatment than the control plants. Nickel chloride 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 nickel chloride treatment. At the same concentration of nickel treatment, it was 147 % more than control before co-cultivation. Nickel chloride 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.

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 nickel chloride to about 50 % in *Brassica* when compared to the control. In contrary, the hypoaccumulator *Abelmoschus* when co-cultivated with *Brassica* no reduction in nitrte reductase activity under 10 mM nickel 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 nickel chloride 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 nickel chloride treatment, *Brassica* an activity of about 46% more respectively at 6 mM concentration when compared to the control. At the same concentration of nickel chloride, 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 mobiliyt in the plant tissue, the Accumulation Factor (AF), Translocation Factor (TF) and Mobility Index (MI) was calculated on the effect of nickel chloride on co-cultivately grown *Abelmoschus esculentus,* L., with *Brassica juncea,* Hk.F.&T. and tabulated in tables 8 and 9.

The accumulation factor was significantly increased in hyperaccumulators with the increasing concentrations of nickel chloride. With the increasing concentrations of nickel chloride, the accumulation factor also increased in the hyperaccumulator and more accumulation factor was recorded in *Brassica* (1.824) when grown in 10 mM nickel chloride 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 nickel chloride 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 nickel chloride treatment. In the hyperaccumulators, the translocation factor was increased with the increasing concentrations of nickel chloride. Translocation factor was recorded in *Brassica* and when grown in 10 mM nickel chloride 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 nickel chloride 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 nickel chloride 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 nickel chloride solution, *Abelmoschus* when co-cultivated with *Brassica* up to 4 mM, the mobility index was below the detectable level for nickel tretment 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 nickel chloride treatment. The hypoaccumulator, *Abelmoschus* when co-cultivated with *Brassica* up to 4 mM, the mobility index was below detectable level for nickel treatment The *Abelmoschus* when co-cultivated with *Brassica*, the mobility index was 0.516 in 10mM nickel chloride.

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 analysed in this article.

Results on the co-cultivation of hypoaccumulator *Abelmoschus esculentus,* L. with hyperaccumulators *Brassica juncea,* Hk.F.&T. under various concentrations of nickel chloride are being discussed below.

Heavy metals either retard the growth of the whole plant or plant parts (Shafiq and Iqbal, 2005; Shanker *et al.*, 2005). The plant parts normally the roots which have direct contact with the contaminated soils exhibit rapid and sensitive changes in their growth pattern (Baker and Walker, 1989). 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 (Wong and Bradshaw 1982).

In the present investigation, nickel chloride 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 nickel chloride, which interfered and inhibited the uptake of other essential elements like potassium, calcium, phosphorus and magnesium by the plants (Clarkson, 1985). Sahai *et al.*, (1983) and Dolar *et al.*, (1972) 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 nickel treatment was also related to accumulation of nickel in leaves, where the size of the leaf was also decreased (Panday and Sharma, 2002).

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 (Arduini *et al.*, 1996). 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.*, (2005) 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 nickel treatment, which was not in the hypoaccumulator (*Abelmoschus*). Heavy metal stress reduces nutrient and water uptake, impairs photosynthesis and inhibits growth of the plants (Chaudhary and Singh, 2000; Jihen *et al.*, 2010; Lag *et al.*, 2010).

Plants exhibit morphological and metabolic changes in response to metal stress that are believed to be adaptive responses (Singh and Sinha, 2004, Ma *et al.,* 2019). For instance, metal stress not only inhibits growth (Lunackova *et al.*, 2003, Dong *et al.*, 2005), but also brings about changes in various physiological and biochemical characteristics such as water balance, nutrient uptake (Vassilev *et al.*, 1997, Scebba *et al.* 2006) and photosynthetic electron transport around photosystems I and II (Skorzynska-Polit and Baszynski, 1995, Vassilev, *et al.* 2004). The reduction in growth and biomass due to nickel chloride 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 (Bharti and Singh, 1994; Di Toppi and Gabbrielli, 1999; Chaundari and Singh, 2000; Sharma *et al.*, 2010).

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 by Ewais (1997). Similar reduction in pigment level was observed in many plants by various heavy metal treatments (Padmaja *et al.*, 1990; Gajewska *et al.*, 2006; Bauddh and Singh, 2009, Zhou *etal.* 2020).

Reduction in the chlorophyll content paralleled with the reduction in dry weight and the net photosynthesis were reported (Kumar *et al.*, 2007). In this study, there was a reduction in root length and chlorophyll content associated with the reduction in dry matter in hyperaccumulator, which did not occur in hypoaccumulator (*Abelmoschus*). It may be due to the hyperaccumulator accumulating all the toxicity, so the *Abelmoschus esculentus,* L. is free from metals toxicity. In heavy metal treated plants, the reduction in chlorophyll content could be due to a block in the chlorophyll biosynthetic pathway or induction of chlorophyll degradation by chloropyllase (Kupper *et al.*, 1996; Kupper *et al.*, 1998; Kupper *et al.*, 2002; Dong *et al.,* 2005). 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 nickel treatment. The protective function of plant anthocyanin against the stress condition is fairly clear (Moroni *et al.*, 1991) 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 (Yu, 2005; Mishra and Agarwal, 2006; Polit and Krupa, 2006).

There was a considerable reduction in the levels of protein and sugar in the leaves of *Brassica* treated with various concentrations of nickel chloride. 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.*, (2006).

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 nickel chloride. It may be due to the destruction of protein or increase in the biosynthesis of amino acids from the nitrate source, which were not utilised in the protein synthesis (Schmoger *et al.,* 2000). The degradation of protein may lead to an increase in free amino acid content. It is an adaptive mechanism employed by the plant cell to overcome post stress metabolism (Singh and Vijayakumar, 1974).

Proline accumulation 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 quarternary ammonium compounds such as glycine, betaine and proline are considered as osmotic adjustments in higher plants during water stress (Acevedo *et al.*, 1979; Boyer and Meyer, 1979). Rout and Shaw (1998) analysed the possibility of proline accumulation as a consequence of impaired protein synthesis.

Under stress, inhibition of growth of cells, leaves and the whole plant is accompanied by an accumulation of nitrate in plant tissue particularly in leaves (Sinha and Nicholas, 1981). The leaf nitrate content was analysed and found to be more in *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 (2009) and industrial effluent on *Abelmoschus esculentus* by Jeyarathi and Ramasubramanian (2002).

Nitrate Reductase (NR) enzyme is one of the cytoplasmic substrate inducible enzymes. The NR activity was found to be decreased in 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 (Hanser and Hitz, 1982). Thus, it is possible to assume that, a mechanism similar to this might have operated in the nickel chloride stressed *Brassica* thereby causing a reduction in the nitrate reductase activity. While nickel 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 (Balakumar *et al*., 1993). Hence, in the present study, activities of enzyme like catalase and peroxidase were analysed. 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 nickel 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 analysed 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 (Guo *et al.* 2019; Zeng *et al*. 2020). Nashikkar and Chakrabarti (1994) 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 nickel chloride. 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*., (2001). 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* (Raskin *et al*., 1994).

More or less similar results have been reported in the accumulation pattern of heavy metals in *Bidens tripartita* (Zheljazkov *et al*., 2008). Those authors suggested that accumulation potential of plants towards heavy metal depends on the availability of the metals in the soil/ growth media as well as on the plant genotype. But in the present study, 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 (Yoon *et al*., 2006; Zhelyjazkov *et al*., 2008). 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 nickel 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*. (1987a, 1987b, 1987c). 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*., (2002); An *et al*., (2004). Thus, from the above findings it is clear that, the plant *Brassica juncea,* Hk.F.&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.&T. is best suited for remediating nickel.

CONCLUSION

The co-cultivated experiment shows that the metal concentration factors of plants such as accumulation factor, translocation factor and mobility index were below detectable level (BDL) for the concentration of nickel in hypoaccumulator (*Abelmoschus*) plant when co-cultivated with hyperaccumulator (*Brassica*). But in the hyperaccumulator plant, the accumulation factor, translocation factor and mobility index gradually increased with increase in the concentration of metals. Due to phytoextraction properties of the hyperaccumulator it was observed that, after the co-cultivation with the hyperaccumulators in heavy metal nickel, the experimental plant, *Abelmoschus esculentus*, L. experienced less stress. Thus, from the above findings, it is clear that the plant *Brassica juncea*, Hk.F.&T. chosen for this study, are acting as hyperaccumulators. 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* (hyperaccumulator), the experimental plant, *Abelmoschus esculentus,* L. (hypoaccumulator) could grow well in metal stressed environment when it is co-cultivated. It is suggested that *Brassica juncea*, Hk.F.&T. is best suited for remediating nickel.

Table – 1 Impact Of Nickel Chloride On The Morphometric Characteristics Of Hyperaccumulator (*brassica Juncea,* Hk.f.&t.) And Hypoaccumulator (*abelmoschus Esculentus,* L.)

Metal Concentration	Root Length (cm)			Shoot Length (cm)			Leaf Area (cm^2)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,*	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&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)
2mM	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)

4mM	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)
6mM	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)
8mM	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)
10mM	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 ($P \leq 0.05$ – Turkey test). a# – refers to non-significant.

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

Metal Concentration	Fresh Weight (gm.)			Dry Weight (gm.)		
	Nickel Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea*, Hk.F.&T.		*Abelmoschus esculentus*, L.	*Brassica juncea*, Hk.F.&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)
2mM	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)
4mM	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)
6mM	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)
8mM	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)
10mM	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 ($P \leq 0.05$ – Turkey test). a# – refers to non-significant.

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

Metal Concentration	Chlorophyll .a (mg/gLFW)			Chlorophyll .b (mg/gLFW)			TotaL. Chlorophyll (mg/gLFW)		
	Nickel Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus*, L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea*, Hk.F.&T.		*Abelmoschus esculentus*, L.	*Brassica juncea*, Hk.F.&T.		*Abelmoschus esculentus*, L.	*Brassica juncea*, Hk.F.&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)
2mM	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)
4mM	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*
6mM	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)
8mM	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)
10mM	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 ($P \leq 0.05$ – Turkey test). a# – refers to non-significant.

Table – 4 Impact of nickel chloride on the pigments of hyperaccumulator (Brassica juncea, Hk.F.&T.) and hypoaccumulator (Abelmoschus esculentus, L.)

Metal Concentration	Carotenoids (mg/gLFW)			Anthocyanin (μg /gLFW)			Total Soluble Sugar (mg/gLFW)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&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)
2mM	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)
4mM	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)
6mM	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)
8mM	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)
10mM	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 activitywithrespecttocontrol.Mean±S

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

Table – 5 Impact Of Nickel Chloride On The Biochemical Features Of Hyperaccumulator (brassica Juncea, Hk.f.&t.) And Hypoaccumulator (abelmoschus Esculentus, L.)

Metal Concentration	Total Soluble Protein(mg/gLFW)			Amino acid (μ mole/g LFW)			Proline (μ mole/g LFW)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&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)
2mM	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)
4mM	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)
6mM	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)
8mM	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)
10mM	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 – Turkey test). a# – refers to non-significant.

Table – 6 Impact of nickel chloride on the biochemical and enzymatic features of hyperaccumulator (Brassica juncea, Hk.F.&T.) and hypoaccumulator (Abelmoschus esculentus, L.)

Metal Concentration	Leaf Nitrate (μ mole/g LFW)			Nitrate Reductase activity (μ mole/g LFW)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&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)
2mM	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)
4mM	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)

6mM	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)
8mM	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)
10mM	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 ($P \leq 0.05$ – Turkey test). a# – refers to non-significant.

Table – 7 Impact of nickel chloride on the enzymatic features of hyperaccumulator (Brassica juncea, Hk.F.&T.) and hypoaccumulator (Abelmoschus esculentus, L.)

Metal Concentration	Catalase activity (µ mole/g LFW)			Peroxidase activity (µ mole/g LFW)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&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)
2mM	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)
4mM	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)
6mM	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)
8mM	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)
10mM	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 ($P \leq 0.05$ – Turkey test). a# – refers to non-significant.

Table – 8 Impact of nickel chloride concentration in hyperaccumulator (Brassica juncea, Hk.F.&T.) and hypoaccumulator (Abelmoschus esculentus, L.)

Metal Concentration	Accumulation Factor (AF)			Translocation Factor (TF)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&T.
Control	BDL	BDL	BDL	BDL	BDL	BDL
2mM	0.490 ± 0.0014	BDL	1.483 ± 0.0064	0.125 ± 0.0008	BDL	1.103 ± 0.0018
4mM	0.301 ± 0.0029a*	BDL	1.520 ± 0.0072a*	0.121 ± 0.0038a*	BDL	1.158 ± 0.0093a*
6mM	0.251 ± 0.0071a*	0.005 ± 0.0026a$^{\#}$	1.586 ± 0.0048a*	0.119 ± 0.0073a*	BDL	1.196 ± 0.0008a*
8mM	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*
10mM	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 ($P \leq 0.05$ – Turkey 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 nickel chloride concentration in hyperaccumulator (Brassica juncea, Hk.F.&T.) and hypoaccumulator (Abelmoschus esculentus, L.)

Metal Concentration	Mobility Index (MI)								
	Level 1 (Soil to Root)			Level 2 (Root to Stem)			Level 3 (Stem to Root)		
	Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation		Nickel Stress on *Abelmoschus esculentus,* L.	After Co–Cultivation	
		Abelmoschus esculentus, L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&T.		*Abelmoschus esculentus,* L.	*Brassica juncea,* Hk.F.&T.
Control	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2mM	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
4mM	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*
6mM	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*
8mM	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*
10mM	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 ($P \leq 0.05$ – Turkey 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

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