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PHYTOEXTRACTION POTENTIAL OF *AMARANTHUS SPINOSUS* L. UNDER NICKEL STRESS IN CO-CULTIVATED CONDITION

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ABSTRACT

Plant used in phytoextraction should accumulate and translocate the specific pollutants especially heavy metals. The aim of work is to assess the tolerance mechanism of *Amaranthus spinosus* L. a selective native hyperaccumulator under nickel stress. Morphometric, biochemical, enzymatic activity, accumulation, translocation and mobility of nickel from soil to root and leaves were studied in co-cultivated hyperaccumulator (*Amaranthu spinosus*) and hypoaccumulator (*Abelmuscus esculentus*) at various levels of nickel. *A. spinosus* accumulated fourfold and fivefold nickel in roots, shoots and leaves, than *A.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 *A. spinosus,* there was no toxicity symptoms and reduction of growth, values of Accumulation factor, translocation factor and mobility of metal. It is well understood that *A. spinosus* showing higher accumulation of nickel, more translocation of nickel from root to shoot and good mobility of nickel was increased from level 1 to level 3, It was revealed that the accumulation of nickel was more in root and shoot of *A.spinosus* than *A.esculentus*. It is inferred from the present study that *A.esculentus* is a hypoaccumulator and is sensitive to nickel and when co-cultivated with *A. spinosus,* shows less of metal toxicity because *A. spinosus* being hyperaccumulator of nickel, accumulate more metal and saves *A. esculentus*.

Key Words: Hyperaccumulator, Nickel Stress, Accumulation Factor, Translocation Factor, Mobility Index

INTRODUCTION

Pollution in the environment especially due to heavy metal contamination in soil, water and air is widespread environmental issue caused by natural and anthropogenic activities. Heavy metal refers to metals and metalloids which produce toxicity and causes adverse effect to the ecosystem (Ali and Khan, 2018), sometimes it involves in the climate change (Ali *et al.* 2019). Agricultural and industrial revolution in the last century have been increasing the level of pollutants in the environment. 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, 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 transformed from one oxidation state or organic complex to another. Therefore, heavy metal pollution poses a great threat to the environment and human health.

Phytoextraction involves the removal of pollutants, the toxic heavy metals and metalloids by the roots of the plants with subsequent transport to aerial plant organs and metabolized to change into non toxic forms (Sidhu *et al*., 2020). Pollutants accumulated in stems and leaves are harvested with accumulating plants removed from the site (Salt *et al.,* 1998). Phytoremediation is the use of plants to treat/clean contaminated sites (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 effects (Bali *et al.* 2020). It is also referred to as green technology and can be applied to both organic and inorganic pollutants present in the soil (solid substrate), water (liquid substrate) or the air (Gratao *et al*., 2005). Phytoremediation takes advantage of the natural ability of plants to extract chemicals from water, soil and air using energy from sunlight. Some of the advantages are that it is less expensive, passive and solar driven, has high public acceptance, retains topsoil and has less secondary waste generation. This technology is being considered as a highly promising technology for the remediation of polluted sites.

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

In the present study, it is aimed to analyse the impact of nickel on the morphometric characters, biochemical, enzymatic features, accumulation factor, translocation factor and mobility index of *A. esculentus,* L. (hypoaccumulator) and *A. spinosus* L. (hyperaccumulator).

MATERIALS AND METHODS

Seeds of *A. esculentus,* L., were procured from the local seed Centre, Sivakasi. *A. spinosus* L. was collected widely from locally fireworks' match industry polluted sites, Sivakasi. *A. esculentus,* L. Var. S7 (Family; Malvaceae) was chosen as experimental plant, whereas the *A. spinosus*L.(Family; *Amaranthus*ceae) was chosen as hyperaccumulator plants for this study. The effect of various concentrations of nickel on the morphometric characters, biochemical, enzymatic features, accumulation factor, translocation factor and mobility index were analyzed on the selected plants on the 45th day after planting (DAP).

EXPERIMENTAL DESIGN

Heavy Metal Stress on *A. esculentus* and *A. spinosus*

The heavy metal 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 metal 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 *A. esculentus,* L. and *A. spinosus* L., were sown uniformly in all the pots for the experimental purpose.

PHYTOREMEDIATION TREATMENT

Co-Cultivation of the Hypoaccumulator and Hyperaccumulator

Optimum number of surface sterilized seeds of both *A. esculentus*, L. (hypo accumulator) and *A. spinosus* L. (hyper accumulator) was sown uniformly in all pots.Appropriate amount of nickel was 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 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 average of five samples was taken from both control and treated sets.The biochemical and enzymatic characters were analysed by adopting 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) were studied. *In vivo* nitrate reductase activity (Jaworski, 1971), peroxidase and catalase (Kar and Mishra, 1976) were assayed.

Accumulation Factor (AF)

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

Accumulation Factor (A) =

$$\frac{Metal\ Concentration\ in\ tissue\ of\ whole\ plant}{Initial\ concentration\ of\ metal\ in\ substrate(soil)}$$

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:

Translocation Factor (TF) =

$$\frac{Metal\ concentration\ in\ stems + leaves}{Metel\ concentration\ in\ roots}$$

However, after co-cultivation the reduction was about 5 % in *Abelmoschus* with *Amaranthus* which was 55 % before co-cultivation. The carotenoid content of *Abelmoschus* has slightly decreased to about 4 % decrease was seen in *Abelmoschus* grown with *Amaranthus* after the application of 10 mM concentration of nickel treatment, whereas the reduction was about at 78 % at 10 mM nickel concentration before co-cultivation. In hyperaccumulators, the carotenoid content also decreased to 20 % in the carotenoids was observed on the *Amaranthus* at 10 mM concentration of nickel 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 has significantly increased the anthocyanin content to about 19% in *Amaranthus* than the control plants. In hypoaccumulator (*Abelmoschus*), anthocyanin content was increased to only 1 % when co-cultivated with *Amaranthus*. Before co-cultivation it was 104 % increase.

The reduction of total soluble sugar content was 16 % on *Amaranthus* nickel treatment at 10 mM concentration.

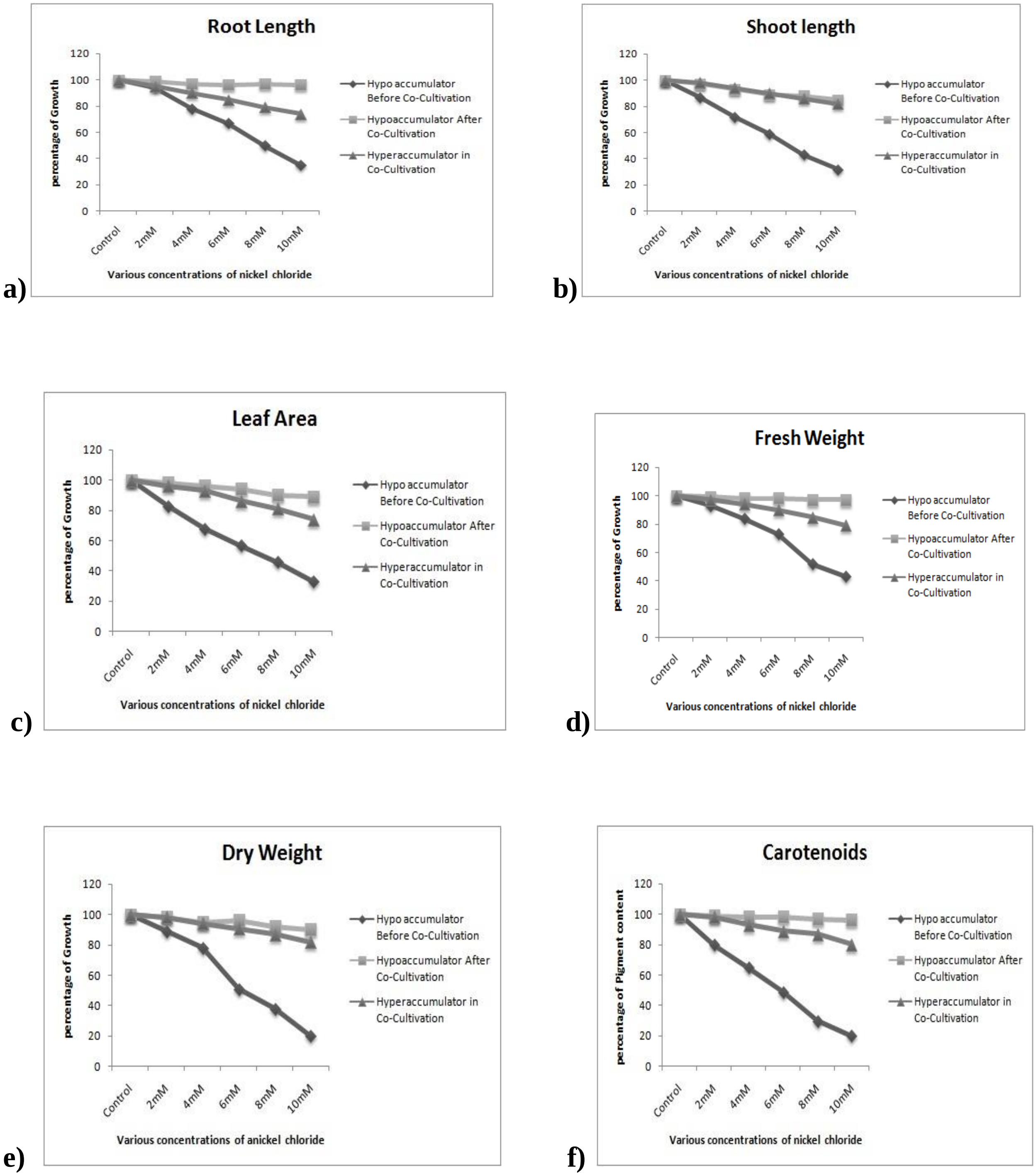

Figure 1: Impact of nickel chloride on the morphometric characteristics (a – d) and pigment (e) of hyperaccumulator (*A. spinosus* L.) and hypoaccumulator (*A. esculentus*, L.)

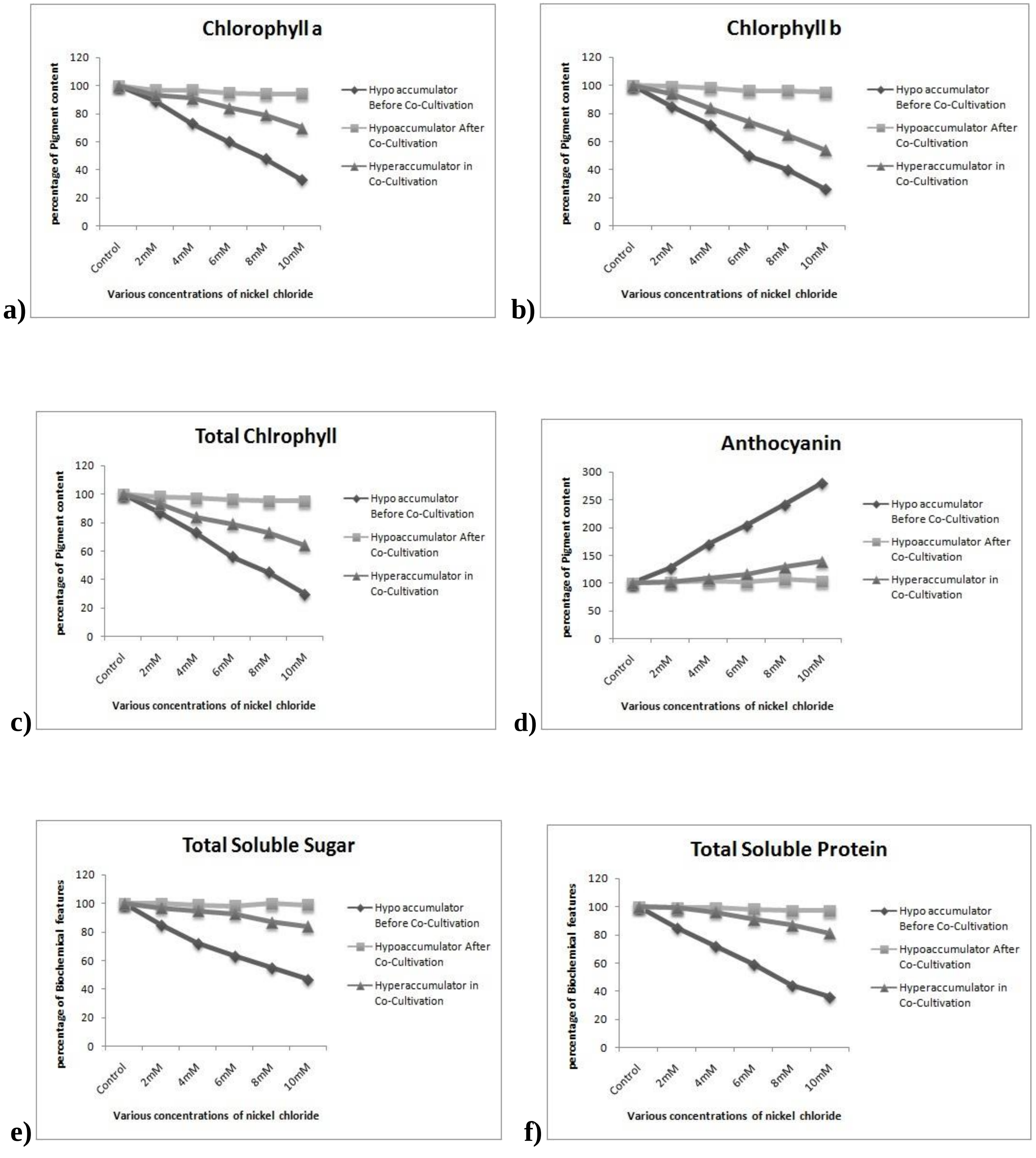

Figure 2: Impact of nickel chloride on the photosynthetic pigment contents (a – d) and biochemical features (e, f) of hyperaccumulator (*A. spinosus* L.) and hypoaccumulator (*A. esculentus*, L.)

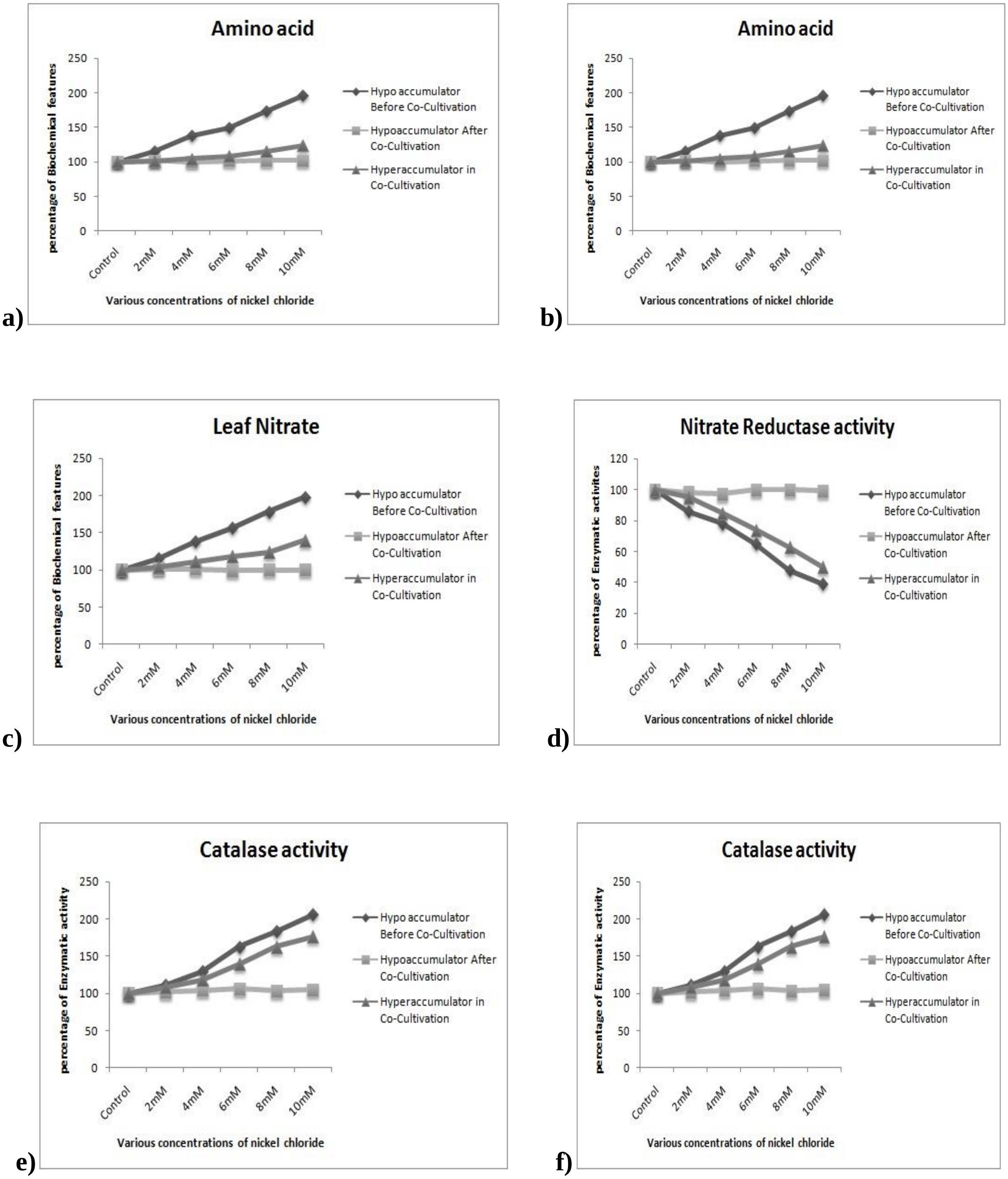

Figure 3: Impact of nickel chloride on the biochemical (a-c) and enzymatic features (d-f) of hyperaccumulator (*A. spinosus* L.) and hypoaccumulator (*A. esculentus*, L.)

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 *Amaranthus*, whereas it was 53% before co-cultivation. In the co-cultivation set, supply of 10 mM concentration of nickel decreased the total soluble protein content of *Amaranthus* 19 % when compared to the control plants. In hypoaccumulator (*Abelmoschus*) the reduction was only 3 % when co-cultivated with *Amaranthus* 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 show that the free aminoacid content of hyperaccumulator, *Amaranthus* where the maximum increase of 24 % at 10 mM nickel treatment than the control plants. Nickel treatment in *Abelmoschus,* the increase was 2 % when co-cultivated with *Amaranthus* but the increase was 96% before co-cultivation. Only 8 % increase of proline content was seen in *Abelmoschus* co-cultivated with *Amaranthus* under the 10 mM nickel treatment. At the same concentration of nickel treatment, it was 147 % more than control before co-cultivation. Nickel treatment in the *Amaranthus* has increased the nitrate level to 40 %, whereas there was no increase in leaf nitrate content when co-cultivated with *Amaranthus.* 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 (Figure 3) that, in vivo nitrate reductase activity in the leaves was significantly inhibited at 10 mM concentration of nickel to about 50 % in *Amaranthus* when compared to the control. In contrary, in the hypoaccumulator *Abelmoschus* when co-cultivated with Amaranthus there was no reduction in nitrate reductase activity. Catalase activity was found to be increased in hyperaccumulators of all the experimental plants than the control. 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 *Amaranthus* under nickel 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 treatment, *Amaranthus* an activity of about 46% more respectively at 6 mM concentration when compared to the control. At the same concentration of nickel, the reduction was about 7 % in hypoaccumulator when co-cultivated with maranthus. 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) were calculated on the effect of nickel on co-cultivately grown A. *esculentus,* L., with A. spinosus, Hk. F. & T. and tabulated in tables 1 and 2.The accumulation factor was significantly increased in hyperaccumulators with the increasing concentrations of nickel. With the increasing concentrations of nickel, the accumulation factor also increased in the hyperaccumulator and more accumulation factor was recorded in *Amaranthus* (1.824) when grown in 10 mM nickel solution. The accumulation factor was not recorded much in the hypoaccumulator, *Abelmoschus.* The seedlings of A. esculentus, L. when co-cultivated with hyperaccumulator *Amaranthus* under the influence of nickel 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 treatment.

In the hyperaccumulators, the translocation factor was increased with the increasing concentrations of nickel. Translocation factor was recorded in *Amaranthus* and when grown in 10 mM nickel solution, it was found to be 1.32. When the hypoaccumulator *Abelmoschus* was co-cultivated with the hyperaccumulator, *Amaranthus* the translocation factor was in the range of 0.765 to 0.711 in nickel treatment.

For Level 1, the mobility index was 0.803 in *Amaranthus* when grown in 10 mM nickel solution. The hypoaccumulator, *Abelmoschus* when co-cultivated with *Amaranthus* did not show the mobility index. For Level 2, in the hyperaccumulators, mobility index was 0.535 in *Amaranthus* when grown in 10 mM nickel solution, *Abelmoschus* when co-cultivated with *Amaranthus* up to 4 mM, the mobility index was below the detectable level for nickeltretment 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 *Amaranthus* under 10 mM nickel treatment. The hypoaccumulator, *Abelmoschus* when co-cultivated with *Amaranthus* up to 4 mM, the mobility index was below detectable level for nickel treatment The *Abelmoschus* when co-cultivated with *Amaranthus*, the mobility index was 0.516 in 10mM nickel.

Table – 1:Impact of nickel chloride concentration in hyperaccumulator (*A. spinosus* L.) and hypoaccumulator (*A. 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.	*Amaranthus spinosus* L.		*Abelmoschus esculentus,* L.	*Amaranthus spinosus* L.
Control	BDL	BDL	BDL	BDL	BDL	BDL
2mM	0.490 ± 0.014	BDL	1.483 ± 0.064	0.125 ± 0.008	BDL	1.103 ± 0.018
4mM	0.301 ± 0.029a*	BDL	1.520 ± 0.072a*	0.121 ± 0.038a*	BDL	1.158 ± 0.093a*
6mM	0.251 ± 0.071a*	0.005 ± 0.026a$^{\#}$	1.586 ± 0.048a*	0.119 ± 0.073a*	BDL	1.196 ± 0.008a*
8mM	0.235 ± 0.026a*	0.004± 0.013a$^{\#}$	1.654 ± 0.013a*	0.112 ± 0.010a*	0.765 ± 0.0021 a$^{\#}$	1.272 ± 0.037a*
10mM	0.213 ± 0.037a*	0.001± 0.061a$^{\#}$	1.824 ± 0.004a*	0.103 ± 0.042a*	0.711 ± 0.034a$^{\#}$	1.327 ± 0.016a*

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

Table –2:Impact of nickel chloride concentration in hyperaccumulator (*A. spinosus* L.) and hypoaccumulator (*A. esculentus,* L.)

Metal Concentration (mM)	Mobility Index (MI)								
	Level 1 (Soil to Root)			Level 2 (Root to Stem)			Level 3 (Stem to Root)		
	Nickel Stress on *A. esculentus,* L.	After Co–Cultivation		Nickel Stress on *A. esculentus,* L.	After Co–Cultivation		Nickel Stress on *A.esculentus,* L.	After Co–Cultivation	
		***A. esculentus,* L.**	*A. spinosus* L.		*A. esculentus,* L.	*A. spinosus* L.		*A. esculentus,* L.	*A. spinosus* L.
Control	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2	0.437 ± 0.068	BDL	0.681 ± 0.074	0.055 ± 0.039	BDL	0.380 ± 0.018	1.630 ± 0.072	BDL	1.378 ± 0.090
4	0.268 ± 0.002a*	BDL	0.705 ± 0.002a*	0.053 ± 0.017 a*	BDL	0.432 ± 0.039a*	1.496 ± 0.015a*	BDL	1.512 ± 0.043a*
6	0.224 ± 0.034a*	0.001 ± 0.055a$^{\#}$	0.704 ± 0.018a*	0.050 ± 0.011a*	BDL	0.436 ± 0.082a*	1.235 ± 0.073a*	BDL	1.656 ± 0.042a*
8	0.212 ± 0.075a*	0.003 ± 0.012a$^{\#}$	0.753 ± 0.069a*	0.050 ± 0.047 a*	0.505 ± 0.012a$^{\#}$	0.528 ± 0.010a*	1.065 ± 0.020 a*	0.585 ± 0.0064a*	1.766 ± 0.043a* b*
10	0.193 ± 0.031a*	0.003 ± 0.078a$^{\#}$	0.803 ± 0.083a*	0.046 ± 0.053a*	0.449 ± 0.034a$^{\#}$	0.535 ± 0.083a*	1.030 ± 0.014a*	0.516 ± 0.0026a*	1.904 ± 0.016a*

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

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 in the case of the hyperaccumulator plants. Results on the co-cultivation of hypoaccumulator *A. esculentus*, L. with hyperaccumulators *A. spinosus* , Hk.F.&T. under various concentrations of nickel are being discussed below.

Heavy metals either retard the growth of the whole plant or plant parts, (Shanker *et al*., 2005). The plant parts, normally the roots which have direct contact with the contaminated soils exhibit rapid and sensitive changes in their growth pattern (Boros-Lajszner *et al*. 2020). Significant effects of number of metals (Cu, Ni, Pb, Cd, Zn, Al, Hg, Cr, As, Fe) on the growth of above-ground plant parts are well documented (Ali *et al.* 2019).

In the present investigation, nickel has caused considerable reduction on the seedling length and leaf area of hyperaccumulator (*Amaranthus)*. 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, which interfered and inhibited the uptake of other essential elements like potassium, calcium, phosphorus and magnesium by the plants (Clarkson, 1985). Sahai *et al*., (1983) reported that, the retardation of plant growth was due to excess quantities of micronutrients and other toxic chemicals.

Reduction of leaf growth is an important visible symptom of heavy metal stress. In many plants, the reduction in leaf area in response to 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. The biomass accumulation represents overall growth of the plants. In the present investigation, the total fresh weight of hyperaccumulator (*Amaranthus*) was gradually reduced with the increase in concentration of metal, but in the hypoaccumulator, no reduction was found and the plants were as same as control plants. This may be due to the removal of metal toxicity by the hyperaccumulator (*Amaranthus*). Similar observation was reported by Lori *et al*.,

(2013) in phytoremoval of *Amaranthus* under nickel stress.

Inhibition of biomass accumulation is directly related to the photosynthetic processes which, in turn, rely upon the pigment level. Considerable reduction in the pigment level was noticed in hyperaccumulator (*Amaranthus*) on the 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 (Lag *et al.*, 2010). Plants exhibit morphological and metabolic changes in response to metal stress that are believed to be adaptive responses. For instance, metal stress not only inhibits growth (Dong *et al.*, 2005), but also brings about changes in various physiological and biochemical characteristics such as water balance, nutrient uptake (Scebba *et al*. 2006) and photosynthetic electron transport around photosystems I and II (Vassilev, *et al.*, 2004). The reduction in growth and biomass due to nickel 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 (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. Similar reduction in pigment level was observed in many plants by various heavy metal treatments (Bauddh and Singh, 2009). Reduction in chlorophyll content paralleled with the reduction in dry weight and net photosynthesis were reported by 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 *A. 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 (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 found to be no change in the hypoaccumulator (*Abelmoschus*) when co-cultivated with *Amaranthus* in nickel treatment. The protective function of plant anthocyanin against the stress condition is fairly clear. 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 (Polit and Krupa, 2006).

There was a considerable reduction in the levels of protein and sugar in the leaves of *Amaranthus* treated with various concentrations of nickel. In contrary, no reduction of sugar and protein contents was observed in the *Abelmoschus* when co-cultivated with the *Amaranthus*. The result coincides with the result of Marchiol *et al*., (2006). As a result of protein degradation, the availability of free amino acids is significantly high in *Amaranthus*. The free amino acid content is increased with increasing concentration of the nickel. 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.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 (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 *Amaranthus* 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 stressed *Amaranthus*, thereby causing a reduction in the nitrate reductase activity. While nickel toxicity was observed in the *Amaranthus*, no such reduction in nitrate reductase activity in the hypoaccumulator *A. 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 *Amaranthus*. 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 enzyme which catalyses the degradation of H_2O_2, which is a natural metabolite toxic to plants. 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 *Amaranthus* with increasing concentrations of nickel. 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 *Amaranthus 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 (Zhelyjazkov *et al*., 2008). In the present investigation, accumulation factor (AF) and translocation factor (TF) values are above one, in *Amaranthus*, suggesting that they are best suited for phytoextraction of nickel toxicity.

The mobility index (MI) of *Amaranthus* 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 lower from roots to stem. Thus, the present results are well corroborated with the observations of Hunter *et al*. (1987a). In contrary, these levels are not noticed in *Abelmoschus*, because the hyperaccumulator plants absorbed the metals and freed the hypoaccumulator*)*. Similar findings were provided by An *et al*., (2004).

Thus, from the above findings it is clear that, the plant *A. spinosus* L. chosen for the study, is acting as hyperaccumulator. This is proved by the results obtained on accumulation factor (AF), translocation factor (TF) and mobility index (MI) studies. Because of the phytoextraction capability of *Amaranthus*, (hypoaccumulator) plant can 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 *A. spinosus* , Hk.F.&T. is best suited for remediating nickel.

Phytoextraction is one of the prominent technologies to remove pollutants from the environment but the co-cultivation of hypoaccumulator with hyperaccumlator is a new one. In this way we can cultivate both plants and maintain the pollution level which is not being affected the hypoaccumulator because the hyperaccumulator absorb toxic (polluted substance) from the soil and save crop plant through this work we assure that in nickel polluted soil, farmers can cultivate the hyperaccumulator (*Amaranthus spinosus*) along with their crop plants.

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