

# PHYTOREMEDIATION POTENTIAL OF RAPHANUS SATIVUS (L.), BRASSICA JUNCEA (L.) AND TRITICUM AESTIVUM (L.) FOR COPPER CONTAMINATED SOIL

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## ABSTRACT

Phytoremediation is an emerging technology that employs the use of higher plants for the clean up contaminated environment. Phyto extraction, the use of plants to extract toxic metals from contaminated soils, has emerged as a cost-effective, environment-friendly clean up alternative. The present study aimed to find a suitable plants species for use in cleaning up the soil in industrial regions. In this work we were studied crop species, which are cultivated by farmers of North-India. The effects of different concentration of copper were studied in two varieties of wheat (*T. aestivum* L., var. UP 2338 and var. PBW 373), mustard (*Brassica juncea* L.) and radish (*Raphanus sativus* L.) plants. The study included an assessment of heavy metal accumulation in root, shoot and leaf, effect of copper stress on growth parameter (root length, root and shoot dry weight), photosynthetic pigment content, bioaccumulation coefficient (BAC) and the activity of anti-oxidant enzymes. Results demonstrated that plant species were differ significantly in Cu uptake and translocation. Efficient Cu uptake was observed by the roots in all plants. A high metal content in roots, due to localization of ions in the apoplasm. The highest Cu<sup>++</sup> ions accumulated in the roots of radish plant. Root growth was higher in brassicaceae plants (i.e. mustard and radish), as compared to the plants of poaceae family (*T. aestivum*). High concentration of copper (50-100 µM) had a negative effect on growth of all plants. Copper exposure also influenced biochemical and physiological parameters. Administration of excess of copper was followed by an increase of Cu accumulation in leaves, and associated symptoms of toxicity. Typical symptoms of Cu toxicity developed 30 days after the beginning of treatment. Chlorophyll concentration was decreased in response to heavy metal toxicity. Activity of anti-oxidative enzymes e.g. peroxidase and catalase were increased in response to oxidative stress. Atomic absorption spectrophotometer (AAS) was used for analysis of heavy metal in soil and plant samples. Tested plant species were grouped on the basis of their accumulation capability of heavy metal. The results of this research showed that radish and mustard plants of family brassicaceae are hyper accumulator plants that can concentrate heavy metals in their different parts, thus they can be used for remediation of polluted area. Study also showed that potential of metal accumulator plants for extraction of metal from soil occur up to a certain level of concentration, after that when the concentration of metal increased the phyto extraction rate of metal or bioaccumulation coefficient (BAC) were decreased.

Keywords: Phytoremediation, copper, radish, Brassica, wheat, peroxidase, catalase, oxidative stress

## INTRODUCTION

The continuous application of large amounts of fertilizers and other soil amendments to agricultural land has raised concern regarding the possible accumulation of elevated levels of their trace element constituents and potential harm to the environment (Colbourn and Thornton, 1978; Ma and Rao, 1995; Raven and Leoppert, 1997). Furthermore, increasing amounts of urban and industrial wastes ( Haines and Pocock, 1980; Parry *et al.*, 1981; Culbard *et al.*, 1983; Gibson and Farmer, 1983) which may contain significant quantities of heavy metals, are being disposed on the agricultural lands (Raven and Leoppert, 1997). Severe heavy metal contamination in soil may cause a variety of problems, including the reduction of yield and metal toxicity of plant, animals and humans. The decontamination of these soils

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by engineering methods are high costing project ( Baker *et al.*, 1991; Salt *et al.*, 1995). Over the last 15 years there has been an increasing interest in developing a plant based technology to remediate heavy metal contaminated soils ( Chaney, 1983; Cunningham and Berti, 1993; Baker *et al.*, 1994a; Raskin *et al.*, 1994). Phyto extraction is the use of plants to remove heavy metals from contaminated soils. The concept of using plants to clean up contaminated environments is not new. About 300 years ago, plants were proposed for use in the treatment of waste water.

At the end of the 19<sup>th</sup> century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented to accumulate high levels of metals in leaves (Hartman, 1975). The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunomyia (1980) and Chaney (1983), and the first field trial on Zn and Cd phyto extraction was conducted by Baker *et al.* (1991). Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation. Phyto extraction may provide an attractive alternative for the clean up of heavy metal-contaminated soils. The goal of heavy metal phyto extraction is to reduce metal levels in the soil up to the acceptable levels with in a reasonable time frame (Raskin *et al.*, 1994; Nanda-Kumar *et al.*, 1995; Huang *et al.*, 1997). The process of phyto extraction generally requires the translocation of heavy metals to the easily harvestable shoots. A few plant species are able to survive and reproduce on soils heavily contaminated with Zn, Cu, Pb, Cd, Ni, Cr and As. Such species are divided into two main groups. The first group called pseudo metallophytes, that grow on both contaminated and non-contaminated soils, and second group called as absolute metallophytes, that grow only on metal- contaminated and naturally metal rich soil (Baker, 1987). Depending on plant species, metal tolerance may result from two basic strategies: metal exclusion and metal accumulation (Baker, 1981; Baker and Walker, 1990). The exclusion strategy, comprising avoidance of metal uptake and restriction of metal transport to the shoots (De Vos *et al.*, 1991), is usually used by pseudo-metallophytes. The accumulation strategy caused high uptake of metal and storage in vacuoles to prevent metal toxicity. The extreme level of metal tolerance in vascular plants is called hyper-accumulation. Hyper-accumulators are defined as higher plant species whose shoots contain  $\geq 100\text{mg Cd Kg}^{-1}$ ,  $\geq 1000\text{mg Ni, Pb and Cu Kg}^{-1}$  or  $\geq 10,000\text{mg Zn and Mn Kg}^{-1}$  (dry weight) when grown in metal-rich soils (Baker and Brooks, 1989; Baker *et al.* 2000). Crops with both a high metal uptake capacity and a high biomass production are needed to extract metals from soils with in a reasonable time frame (Ebbs and Kochian, 1997). According to Brooks *et al.* (1977), metal hyper accumulation is a rare phenomenon that occurs in some plants called hyper accumulators. However hyper-accumulators are often described as slow growing and low biomass plants (Dushenkov *et al.*, 1995; Nanda-Kumar *et al.*, 1995; Ebbs *et al.*, 1997; Rouhi 1997). The potential of some crop plants from brassicaceae for phyto-remediation has been extensively studied (Baker *et al.*, 1994b; Brown *et al.*, 1995b; Dushenkov *et al.*, 1995; Huang and Cunningham, 1996; Ebbs and Kochian, 1997; Ebbs *et al.*, 1997) and it was demonstrated that some efficient shoot accumulators of the genus brassica contained up to 3.5% on a dry weight basis of heavy metals (Nanda-Kumar *et al.*, 1995).

Heavy metal such as Mn, Cu, Fe, Zn and Ni are essential mineral nutrients for higher plants. Normal concentration of these heavy metals play a crucial role in plant growth and development but generally these heavy metals can caused oxidative stress, eliciting enzymatic and non-enzymatic anti-oxidative reaction responses and lipid per-oxidation in plants. Copper in normal concentration act as co-factors in protein and enzyme conformation. Though it is a component of both photosynthetic (plastocyanin) and respiratory electron chains (cytochrome oxidase), excess copper in the growth environment causes changes in membrane permeability, chromatin structure, protein synthesis, enzymatic activity, photosynthesis and respiratory processes through its phyto-toxic effect, and it also causes lipid per-oxidation and activates senescence. The present research aimed to study the accumulation of copper in roots, shoot and leaves of crop plants belongs to the family of brassicaceae and poaceae. This study also examined the growth performance and physiological response in respect to activity of important enzymes like peroxidase (POD) and catalase (CAT). We identified changes in CAT, POD activity and measured chlorophyll level, to determine the potential efficiency of these crops to remediate the metal from the contaminated soil in which they were cultivated.

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## MATERIALS AND METHODS

### Plant Material and Treatment

To initiate the experiment under controlled condition, air dried soil (2 part sand and 1 part clay soil), artificially polluted by 250 ml/kg CuSO<sub>4</sub> solution of different concentration (i.e. 25µM, 50µM and 100µM). This CuSO<sub>4</sub> solution added twice in two consecutive days. About 2kg of the above treated soil placed into plastic pots (25 cm in diameter and 14 cm in length). In control, plastic pots irrigated by distilled water. Seeds of *Brassica juncea* L., *Raphanus sativus* L. and two varieties of *Triticum aestivum* L. (UP 2338 and PBW 373) were procured from G.B. Pant Agricultural University, Modipuram, Meerut (U.P), India. Seeds were surface sterilized with 0.1% HgCl<sub>2</sub> solution for 1 minutes with frequent shaking, and then were thoroughly washed with distilled water, then were germinated in the dark on filter paper soaked with distilled water. Subsequently, 7 day old seedlings with similar size were selected and transferred in already prepared plastic pots with three replicas for each treatment. Four plants were cultured in each pot. Nutrient solution were added as needed. The composition of nutrient solution were added as needed. The composition of nutrient solution was in mM: K,2.0 (as KNO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and KCl); Ca,0.5 (as Ca(NO<sub>3</sub>)<sub>2</sub>); Mg,0.2 (as MgSO<sub>4</sub>); S,0.2 (as MgSO<sub>4</sub>); NH<sub>4</sub>,0.1 (as NH<sub>4</sub>NO<sub>3</sub>); NO<sub>3</sub>,0.3 (as NH<sub>4</sub>NO<sub>3</sub>, Ca(NO<sub>3</sub>)<sub>2</sub> and KNO<sub>3</sub>); P,0.01 (as KH<sub>2</sub>PO<sub>4</sub>) and in µM: Cl,50 (as KCl); B,12 (as H<sub>3</sub>BO<sub>4</sub>); Mn,2.0 (as MnSO<sub>4</sub>); Zn,0.5 (as ZnSO<sub>4</sub>); Cu,0.2 (as CuSO<sub>4</sub>); Mo,0.1(as Na<sub>2</sub>MoO<sub>4</sub>); Ni,0.1 (as NiSO<sub>4</sub>) and Fe,20 (as Fe-EDTA).The pH of the solution maintained at 4.8-5.0. The experiment was conducted between January to April. Plants were grown in a green house illuminated with natural light, temperature regime of 25/18 °C day/night, 14/10h light/dark period, and a relative humidity of 70/80 %. Soil pH was determined after mixing 1g of soil in 2.5 ml water for about 5 min., allowed ionic exchange to reach equilibrium prior to measuring pH (Peech, 1965).

### Plant harvest and analysis

Plant samples were gently removed from the pots 45 days after sowing (DAS) for the measurement of various growth parameters and biochemical analysis. Shoot and roots were separated and length were measured. Plant shoots and roots were washed with distilled water for 20 minutes and then divided into two bundles of shoot and roots. They were blotted dry on filter paper and dried at 70 °C for 2 days to determine plant dry weight (Bohm, 1979). The biochemical constituents viz., total chlorophyll (a+b) was extracted with 80 % acetone and quantified according to Arnon's (1949). Antioxidant enzymes Catalase (CAT) and Peroxidase (POD) activity were assayed by Chandlee and Scandalios (1984) and Kumar and Khan (1982), respectively. For Catalase (CAT) (EC 1.11.1.6) activity, we homogenized 0.5 g of frozen plant material in a prechilled pestle and mortar with 5 ml of ice cold 50mM sodium phosphate buffer (pH 7.5). The extract was centrifuge at 4 °C for 20 min at 12,500 rpm. The supernatant was used for enzyme assay. The assay mixture contained 2.6 ml of 50mM potassium phosphate buffer (pH 7.0), 400µl of 15 mM H<sub>2</sub>O<sub>2</sub> and 40µl of enzyme extract. The decomposition of H<sub>2</sub>O<sub>2</sub> was followed by a decline in absorption at 240 nm. The assay mixture of Peroxidase (POD) (EC 1.11.1.7) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005M H<sub>2</sub>O<sub>2</sub> and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25 °C and then the reaction was terminated by adding 1 ml of 2.5 N H<sub>2</sub>SO<sub>4</sub>. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H<sub>2</sub>SO<sub>4</sub> at zero time. The time activity was expressed in unit mg<sup>-1</sup> protein. One unit (U) is defined as the change in the absorbance by 0.1 min<sup>-1</sup> mg<sup>-1</sup> protein.

### Determination of Copper contents

Cu content in root, stem and leaf tissues was determined by atomic absorption spectrometry according to De Veries and Tiller (1980). One g oven dried plant samples were ashed in muffle furnace at 450 °C for 2 hours. Transfer this dried powdered material in kjeldahl flask. Add 1 ml of sulphuric acid and 15 ml of double distilled water in kjeldahl flask and incubate it overnight at 80 °C. After this add 5 ml of acid mixture (3:1 nitric acid to perchloric acid) and digested until the nitric acid and perchloric acid were

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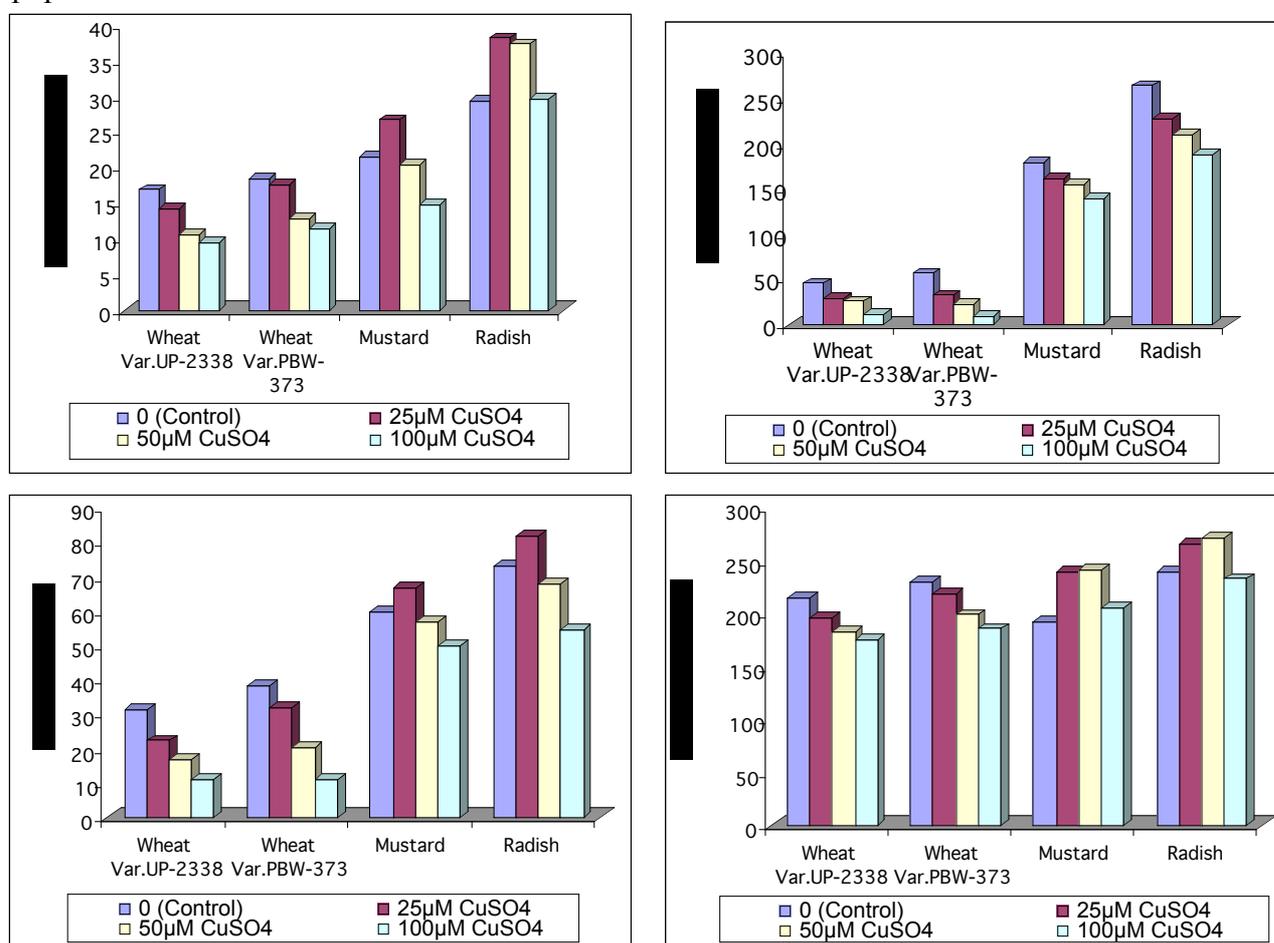
driven off. The digest was cooled, diluted, filtered through Whatman no.42 filter paper, and made up to 50 ml. The solution was directly aspirated to an atomic absorption spectrophotometer (Perkin Elmer 2280).

### The calculation of bioaccumulation coefficient and statistical analysis

The following formula was used for calculation of Bioaccumulation coefficient (BAC) = Element concentration in plant part ( $\mu\text{g metal g dry weight}^{-1}$  of plant part) / Element concentration in soil ( $\mu\text{g metal g dry weight}^{-1}$  of soil) (Bini et al., 1995). The experiments were repeated thrice and the statistical analysis was done using randomized block design.

## RESULTS

The Cu concentration in root, shoot and leaves after exposure to different solution of copper are shown in Table 1. Efficient Cu uptake was observed in the roots of all plants. The highest  $\text{Cu}^{++}$  ions accumulated in the roots of radish plant. The high metal content in roots is due to localization of ions in the apoplasm.

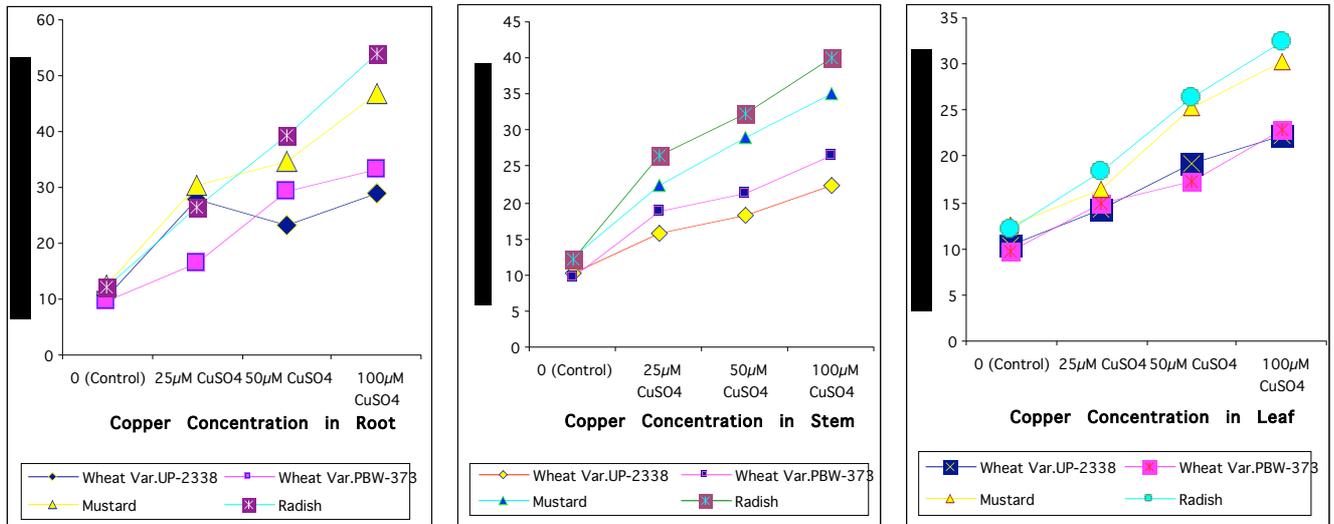


**Figure 1. Shoot and root dry weight (mg/plant), root length (cm/plant) and chlorophyll concentration (mg/g fresh weight) in two varieties of wheat (*Triticum aestivum* L., var. UP 2338 and var. PBW 373), mustard (*Brassica juncea* L.) and radish (*Raphanus sativus* L.) plants in response to elevated levels of copper in the growth medium ( $\mu\text{M}$ ).**

Root growth was significantly reduced in both the wheat varieties (UP 2338 and PBW 373), whereas in both the plants, belongs to brassicaceae family (i.e. mustard and radish) root growth increased by increasing the copper ion concentration up to low level (i.e. 25  $\mu\text{M}$ ). Root length of mustard plant affected more as compared to root length of radish plant under the toxicity of all concentration of copper metal, showed susceptibility to elevated levels of copper metal. Root and shoot dry weight of all four plants had decreased in response to high concentration of  $\text{Cu}^{++}$  ions in the growth medium. The most reduction in shoot and root dry weight in response to Cu metal in growth medium was observed in

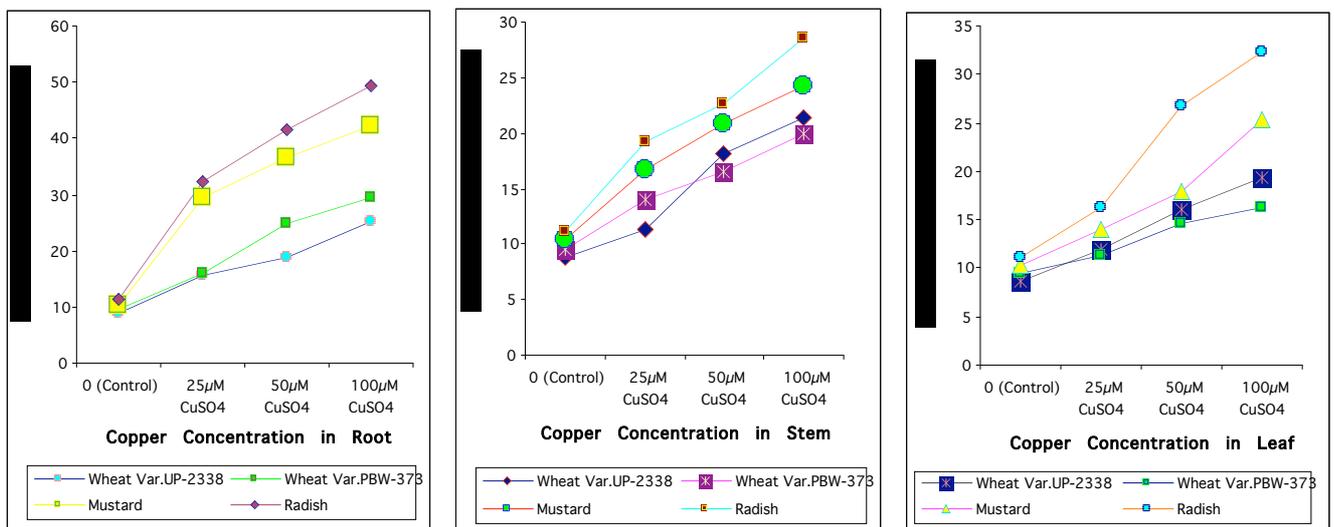
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wheat (var. UP 2338 and var. PBW 373). This means that poaceae plants (i.e. *T. aestivum* var. UP 2338 & var. PBW 373) were the most susceptible species, to the elevated levels of heavy metals in growth medium (Fig. 1).



**Figure 2.** Catalase activity (U/mg protein) in root, stem and leaf of two varieties of wheat (*Triticum aestivum* L., var. UP 2338 and var. PBW 373), mustard (*Brassica juncea* L.) and radish (*Raphanus sativus* L.) plants in response to elevated levels of copper (μM) in the growth medium at 45<sup>th</sup> day.

Copper exposure influenced several biochemical and physiological parameters. Administration of excess amount of copper was followed by an increase of Cu ions and its associated symptoms of toxicity in leaves. Typical symptoms of Cu toxicity developed 30 days after the beginning of treatments. Chlorophyll concentration was decreased in response to heavy metal toxicity. Highest reduction was observed in both the varieties of wheat (var. UP 2338 & var. PBW 373). However, at low and moderate concentration of Cu, amount of chlorophyll increased in both the plants of family brassicaceae (i.e. *B. juncea* & *R. sativus*), which decline by increasing the concentration. Necrotic lesions were seen on the leaves of plants treated with 100μM Cu. Chlorosis symptoms appeared, reflecting a decrease in chlorophyll a and b, confirming that excess copper is damaging to the photosynthetic apparatus (Fig. 1).



**Figure 3.** Peroxidase activity (U/mg protein) in root, stem and leaf of two varieties of wheat (*Triticum aestivum* L., var. UP-2338 and var. PBW-373), mustard (*Brassica juncea* L.) and radish (*Raphanus sativus* L.) plants in Response to elevated levels of copper (μM) in the growth medium at 45<sup>th</sup> day.

CAT activity increases by the increasing the Cu ions concentration in root, shoot and leaf tissues in all the four plants. The highest CAT enzyme activity was observed in root of radish (4.46 fold), where as lowest CAT enzyme activity was observed in leaf tissue of wheat (var. PBW 373) (2.36 fold). The highest CAT enzyme activity was observed after the administration of 100μM Cu in root tissue of radish

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(54.08 ± 9.01 U/mg protein) (Fig. 2). Similarly the highest POD activity, 49.39 U/mg protein was in root tissue of radish after copper treatment. POD enzyme activity is more in roots of radish and mustard plants, if compared with roots of both the varieties of *T. aestivum* (var. UP 2338 & PBW 373). The increase in POD activity in root tissue of brassica and radish was almost same (4.40 and 4.09 fold respectively) and it was 1.5 times more as compared to POD enzyme activity in wheat (Fig. 3).

**Table 1. Cu<sup>++</sup> ion concentration in different plant parts of two varieties of wheat (*Triticum aestivum* L., Var. UP 2338 & PBW 373), Indian mustard (*Brassica juncea* L.) and Radish (*Raphanus sativus* L.); Data are mean ±SD.**

Cu <sup>++</sup> ion Concentration (µg/g dry weight)				
Copper Concentration	Crop Plant	Root	Stem	Leaf
25µM CuSO <sub>4</sub>	Wheat (Var. UP-2338)	165±22.3	144±17.5	102±25.2
	Wheat (Var. PBW-373)	139±26.2	119±12.6	89±12.9
	Mustard	234±19.7	222±20.9	193±12.3
	Radish	268±25.2	213±17.2	190±20.1
50µM CuSO <sub>4</sub>	Wheat Var. UP-2338)	192±27.5	159±17.5	123±12.2
	Wheat (Var. PBW-373)	150±22.5	131±11.8	92±16.2
	Mustard	257±32.2	232±18.9	206±19.2
	Radish	297±20.6	242±26.2	205±32.5
100µM CuSO <sub>4</sub>	Wheat (Var. UP-2338)	196±11.9	149±17.2	128±14.5
	Wheat (Var. PBW-373)	163±24.4	131±31.8	119±15.2
	Mustard	288±26.5	256±22.5	212±22.3
	Radish	339±32.2	289±26.1	232±17.5

**Table 2. Copper ion concentration in artificially polluted soil by three different concentration of CuSO<sub>4</sub> (25µM, 50µM and 100µM) ; Data are mean ±SD.**

Copper Concentration	Cu <sup>++</sup> ion Concentration in soil
25µM	195±12.5
50µM	228±22.7
100µM	259±24.6

**Table 3. Bioaccumulation Coefficient (BAC) of copper metal in different plant parts of two varieties of wheat (*Triticum aestivum* L., Var. UP 2338 & PBW 373), Indian mustard (*Brassica juncea* L.) and Radish (*Raphanus sativus* L.)**

Copper Concentration	Crop Plant	Root	Stem	Leaf
25µM CuSO <sub>4</sub>	Wheat (Var. UP-2338)	0.846	0.736	0.532
	Wheat (Var. PBW-373)	0.712	0.610	0.456
	Mustard	1.24	1.138	0.989
	Radish	1.37	1.092	0.974
50µM CuSO <sub>4</sub>	Wheat Var. UP-2338)	0.842	0.697	0.539
	Wheat (Var. PBW-373)	0.657	0.574	0.403
	Mustard	1.127	1.017	0.903
	Radish	1.302	1.061	0.899
100µM CuSO <sub>4</sub>	Wheat (Var. UP-2338)	0.756	0.575	0.494
	Wheat (Var. PBW-373)	0.629	0.505	0.459
	Mustard	1.111	0.988	0.818
	Radish	1.308	1.115	0.895

The bioaccumulation coefficient/ phytoextraction rate of copper, which was defined as the ratios between µg of metal/g dry weight of shoot, root or leaf and µg of metal/g dry weight of soil (Bini *et al.*, 1995), was calculated in all the four plant species (Table 3). The maximum bioaccumulation coefficient was observed in radish plant in all the solutions of CuSO<sub>4</sub>. In roots, however, a high accumulation rate was observed in all the four plants. It was observed that the potential of all the four plants for extraction of metal from soil occur up to a certain level of concentration, after that when the concentration of metal increased, bioaccumulation rate decreased in all the four plants.

## DISCUSSION

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations etc.) and an atomic number > 20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Zn. Plants grown in metal enriched substrata take up metal ions in varying

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degrees. Uptake is largely influenced by the availability of metals, which is in turn determined by both external (soil associated) and internal (plant associated) factors. In only a limited number of plant species a heritable tolerance or resistance occurs, which enables these plants to grow on metal contaminated soils (Brooks *et al.*, 1977). Soil remediation is needed to eliminate risk to humans or the environment from toxic metals. Several studies dealing with metal hyper accumulating plants, and they have concluded that phyto extraction of metals was a feasible remediation technology for the decontamination of metal polluted soils (Chaney, 1983; Mc Grath *et al.*, 1993; Brown *et al.*, 1994; Brown *et al.*, 1995a, b; Salt *et al.*, 1995). Recent studies looking at the feasibility of phyto extraction, demonstrated that both metal hyper accumulation and good biomass yields are required to make the process efficient (Nanda-Kumar *et al.*, 1995; Blaylock *et al.*, 1997; Huang *et al.*, 1997).

The chemistry of metal interaction with soil matrix is again the important criterion to the phytoremediation concept. Soil pH affects not only metal bioavailability, but also it affects the metal uptake by the roots (Brown *et al.*, 1995a). Sanders (1983) reported that the solubility of heavy metals is generally greater as pH decreases with in the pH range of normal agricultural soils. The high pH values of soils could have accounted for a low transfer of metals from soil to plants. Chaney (1997) reported that soils with high cation exchange capacity (CECs) could absorb large amounts of heavy metals than soil with low CEC. In the relatively short time frame of this experiment, it was observed that, that radish (*Raphanus sativa*) plant produced 10 times more biomass than the other three plants. Accumulation of copper was higher in root tissue of radish and mustard plant, perhaps the result of a tolerance mechanism developed by the plant in order to reduce heavy metal stress. Fernandez and Henriquez (1991) reported that copper tolerant plants prevent copper from reaching stems and leaves by keeping it in their roots. Limitation of transfer of copper to stem and leaves explained copper tolerance in plants (Ozounidou, 1994). In our study, copper treatment caused a decrease in chlorophyll in leaves of moderately and high metal accumulator plants. Van Assche and Clijsters (1990) and Luna *et al.* (1994) reported that copper stopped the formation of chlorophyll and caused destruction of chlorophyll. Chettri *et al.* (1998) reported a decrease in chlorophyll levels *Cladonia rangiformis* after dosing with Cu, Zn and Pb. The decrease of chlorophyll after Cu dosing may be due to blocking of enzymes acting in chlorophyll synthesis or to degradation of chlorophyll. Cell can be protected from reactive oxygen species by the combined action of enzymatic antioxidant systems like catalase (CAT: EC1.11.1.6), peroxidase (POD: EC 1.11.1.7) and non enzymatic antioxidant like ascorbate, glutathione and phenolic compounds. In our experiment peroxidase and catalase enzyme activity was increased under metal stress condition. Previous studies have found a positive relationship between increased POD and CAT enzyme activity and amounts of heavy metals such as Cu, Pb and Zn in plant tissue (Girotti, 1985; Mazhoudi *et al.*, 1997; Mocquot *et al.*, 1996). These enzyme remove superoxide radicals, which are harmful to cell membranes. Peroxidase activity and photosynthetic pigments are sensitive indicators of heavy metal stress and can be used to anticipate events on the organism level (Wu *et al.*, 2003; Mac Farlane and Burchett, 2001.)

Most of the studies on candidate species are mainly based on the interpretation of the analysis of metal concentrations in their plant parts (Nanda-Kumar *et al.*, 1995; Huang and Cunningham, 1996; Huang *et al.*, 1997). Therefore, the content value of metal per plant or organ seems to be a better estimate for heavy metal extraction efficiency in a given species, and reflects the extent of metals which could be removed by an individual plant. Thus on the basis of bioaccumulation coefficient (BAC) analysis plant can be considered in four groups by their capability of heavy metal uptake and sensitivity to high metal pollution (Bini *et al.* 1995):

- Species / plant part that had BAC between 1-10 known high accumulator.
- Species / plant part that had BAC between 0.1-1 known moderately accumulator plants.
- Species / plant part that had BAC between 0.1-0.01 known low accumulator plants.
- Species / plant part that had BAC  $\leq$  0.01 known non accumulator plants

With the above mentioned criterions, it was observed that mustard and radish plant should be considered as high accumulator plants for Cu (Table 3), Where as both the varieties of wheat plants (*T. aestivum*, var. UP 2338 and PBW 373) should be considered as moderately accumulator plants. Study also showed that plants of brassicaceae family accumulated Copper ions mainly in roots and shoots. To this base and with considering the metal accumulation capacity, we suggest that plants of family brassicaceae can be

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used effectively for phytoremediation processing. This is a novel report about their ability to clean up the contaminated soil by the accumulation of Cu element in plant parts. The benefit of this technology is the potential for low cost remediation. This is accordance with finding of Kabata-Pandias (2000), who stated that Chenopodiaceae is one of the family that are good hyper accumulator of heavy metals.

### ACKNOWLEDGEMENTS

We would like to thank Research and Development Division of “Indian Herbs”, Saharanpur (U.P.) and Department of Seed Sciences and Technology, G.B Pant Agricultural University (Western Campus), Meerut (U.P), India for their support in AAS assay of copper and chemical analysis of catalase and peroxidase enzymes. We also like to thank Prof. V.N. Sharma, Director of School of Biotechnology, Meerut Institute of Engineering and Technology, Meerut (U.P) for providing us access to laboratory for this study.

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