INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 6, p. 68-75, 2013

RESEARCH PAPER OPEN ACCESS

Study on the accumulation of copper from soil by shoots and roots of some selective plant species

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Key words: Phytoremediation, copper, wheat, Poaceae, pollution, hyper accumulator.

doi: http://dx.doi.org/10.12692/ijb/3.6.68-75 Article published on June 22, 2013

Abstract

Phytoextraction, 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 Cu from copper contaminated soil. Soil is artificially polluted by different concentration of Cu and the effects were studied in four verities of plant *Triticumaestivum* L (corn),*Abelmoschusesculentus* L, *Basella alba* L, and *Solanumlycopersicum* L (vegetables) from different family Poaceae, Malvaceae, Basellaceae and Solanaceae respectively those usually abundantly grow in Bangladesh. Atomic absorption spectrophotometer (AAS) was used for analysis of heavy metal in soil and plant samples.Results demonstrated that plant species were differing significantly in Cu uptake. Wheat plants (*Triticumaestivum* L) were more tolerant to the conditions tested with the highest dry matter production and no visual toxicity symptoms. The efficiency of Cu uptake from soil by *Triticumaestivum* L was observed by roots (803.14 mg kg^{-1}) and shoots (418.43 mg kg^{-1}). *Triticumaestivum* L totally accumulated 1221.57 mg kg^{-1} in dry weight and demonstrated as a hyper accumulator, other plants were not accumulated copper as higher as wheat. Thus *Triticumaestivum* L can be used for remediation of Cu polluted area.

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Introduction

Environmental pollution through heavy metals is one of the major serious concerns all over the world at present time. Heavy metals may come into the environment especially into air, soil and water from various human activities such as, industrial production, waste disposal, construction work, utilization of chemical fertilizers and fuel consumption(Chen*et al.*, 2005).These heavy metalscause a variety of problems, including the reduction of yield and metal toxicity of plant, animals and humans.

Copper is one of the most abundant trace metal and micronutrient of great importance in agricultural production and occurs as Cu^{+} and as Cu^{2+} (Luo and Christie, 1998). Copper in soil occurs almost exclusively in the divalent form, which shows isomorphism with Zn^{2+}, Mg^{2+} and Fe^{2+}. In normal concentration copper acts as cofactor in protein and enzyme conformation both in plants as well as animals. Cu is also required as a cofactor of Cu-Zn superoxide dismutase(Brown *et al.*,1994); and a vital component of both photosynthetic (plastocyanin) and respiratory electron chains (cytochrome oxidase). Though normal concentration of this heavy metal play a crucial role in plant growth and development but excess copper in the growth environment causes changes in membrane permeability, chromatin structure, protein synthesis, photosynthesis and respiratory processes through its phyto toxic effect, caused oxidative stress, eliciting enzymatic and non enzymaticantioxidative reaction responses, and lipid per oxidation in plants and activates senescence. Copper is also essential to human life and is required for various biological processes, but like all heavy metals, is potentially toxic as well (Hifsa*et al.*, 2010). The average abundance of copper in the earth's crust is recorded as 24 to 55 ppm (Deepa *et al.*, 2006). The acceptable limit for human consumption of Cu is 10 ppm (Nair *et al.*,1997). When Cu exceeds its safe level concentration, it causes hypertension, sporadic fever, uremias, coma, etc. The presence of copper ions cause serious toxicological concern, it is usually known to deposit in brain, skin, liver, pancreas and myocardium (Davis, 2000).

The decontamination of these pollutants from environment by engineering methods is high costing project (Salt *et al.*, 1998). Currently attempts are made to remediate the environmental heavy metals with conventional remediation technologies such as solidification and stabilization, soil flushing, electro kinetics, chemical reduction and oxidation, soil washing, low temperature thermal desorption, incineration, vitrification, pneumatic fracturing, excavation and retrieval, and landfill disposal (Jeanna, 2000). However, these are expensive and destructive. Over the last 15 years there has been an increasing interest in developing a plant based technology (phytoremediation) to remediate heavy metal contaminated soils (Cunningham and Berti, 1993; Raskin*et al.*, 1994). The phytoremediation method was simple, efficient, cost effective and environmental friendly (Schnoor and McCutcheon, 2003) as compared to traditional engineering practices that rely on intensive soil manipulation.The potential of some crop plants from brassicaceae for phytoremediation has been extensively studied (Dushenkov*et al.*,1995; Huang and Cunningham, 1997).

To reduce the limit of copper from our cultivated land and environment by phytoremediation the green, succulent, hairy root in stem and leaf, bunch of root nature plant are selected from different family of crop plant such as Poaceae, Malvaceae, Basellaceae and Solanaceae.The aim of this study was to investigate the accumulation of copper in the shoots and roots of *Triticumaestivum*L and whole plant of *Abelmoschusesculentus* L, *Basellaalba*L, *Solanumlycopersicum* L and to compare the efficiency of copper removal of the four species. Throughout the investigation we examined these plants because they have desirable characteristics such as high shoot biomass, metals tolerance, short life cycle and handling ease.Among all species *Triticumaestivum*L totally accumulated 1221.57 mg kg^{-1} in dry weight and demonstrated as a hyper accumulator than other species.*Triticumaestivum*L might be cultivated in large scale for removal of copper from contaminated agricultural lands.

Materials and methods

Soil sampling

Surface soils were collected from the garden of Soil Resource Development Institute (SRDI) zonal research centre muraridah, Jhenaidah district in Bangladesh. The soils were dried in natural air, clean to remove grass and dirt through 2 mm iron sieve and were then grinded.

Preparation of copper sulphate solution

Copper (II) sulphate pentahydrate were used for the preparation of different concentration of Cu solution as the experiment desire i.e. 25 µM, 50 µM, 75 µM, 100 µM.

Plant material and treatment

To initiate the experiment under controlled condition, air dried soil, artificially polluted by 250 ml/kg CuSO4 solution of different concentration (i.e. 25 µM, 50 µM, 75 µM and 100 µM). This CuSO4 solution added twice after 4 days interval. About 8 kg soils were collected and filled into 16 plastic pots using water bottle 25 cm in diameter and 14 cm in length. During experiment, plastic pots irrigated by distilled water. Seeds of experimented plant was collected from local market (BADC, kushtiazonal office).Seeds were surface sterilized with 0.1% $HgCl_2$ solution for 1 minutes with frequent shaking, and were then thoroughly washed with distilled water, were then germinated in the dark on filter paper soaked with distilled water. Subsequently, 5-7 days old seedlings with similar size were selected and transferred into prepared plastic pots with four replicas for each treatment. 8-12 plants were cultured in each pot. The experiment was conducted between October to November, 2010. Plants were grown in normal temperature and light.

Plant harvest and laboratory analysis

Plant samples were gently removed from the pots 18 days after sowing for the measurement of copper accumulation or extraction, then washed with distilled water for 20 minutes to remove of adhere soils. Shoot and roots of *Triticumaestivum*L were separated and *Abelmoschusesculentus* L,*Basellaalba*L, *Solanumlycopersicum* L whole plants were collected. They were blotted dry on filter paper and oven dried at high temperature for 2-3 minutes. Then plant samples were digested with 65% HNO_3, mortared separately and incubated for 48 hours. Plant samples were heated over water bath in a fume cupboard for 4 hours. After that plant samples were diluted to 100 ml each by distilled water and were filtered (Whatman No. 40). The plant sample solutions were preserved by 100 ml volumetric flask in freeze temperature until use. The resultant extracts for Cu content were carried out using Atomic Absorption Spectrophometer (AAS).

The chemical and physical properties of the soils were determined prior to planting. pH of soil sample was measured electrochemically using a glass electrode pH meter at a soil: water ratio of 1: 2.5 as described in Imamul and Alam (2005). The organic carbon of the soil was determined volumetrically by wet oxidation method of Walkley and Black as described in Imamuland Alam (2005). The phosphorus content of the soil was determined by Molybdophosphoric Blue Color Method in Sulfuric Acid System. Calcium and Magnesium were determined by EDTA (Ethylenediamine Tetra Acetic Acid) Titration. Potassium was determined by Flame photometry Method. The Sulfer content of the soil sample was determined by turbidity method. The total nitrogen content of the soil sample was determined by Kjeldahl's method. Heavy metal content (Cu, Mn, Fe, Zn, B) of the soil sample was determined by AAs.

Statistical analysis

Analysis of Variance (ANOVA) using SPSS version 20 was performed. The statistical significance was set at the $P<0.05$ confidence level. Test of significance of the means was by the Least Significance Difference (LSD).

Results

Different components in soil

Soil sample were measured by using various methods to find out the amounts of different components that are essential for plant growth (Table 1).

Table 1. Nutrient elements and heavy metal content of soil before experiment

Parameters	Value	Comments
pH	8.0	Slight base
Organic Carbon	0.47%	Very low
Ca	12.38 ml-equiv./100gm	Very high
Mg	1.75 ml-equiv./100gm	High
K	0.13 ml-equiv./100gm	Low
Total nitrogen (N)	0.02 %	Very low
P	10.46 µg/g	Low
S	10.85 µg/g	Low
B	0.30 µg/g	Low
Zn	0.45 µg/g	Very low
Fe	1.02 µg/g	Very low
Mn	2.57 µg/g	Normal
Cu	1.17 µg/g	Very high

Copper accumulation by shoots and roots of Triticumaestivum L

The Cu accumulation in shoots and roots of *Triticumaestivum* L cultivars varied depending on the different copper concentrations used (Table 2).Copper ions were accumulated highly in the roots, and low amounts of Cu were transferred to the shoots. The Cu content in roots and shoots increased significantly ($p<0.05$) with increasing Cu concentration. The total copper accumulation levels increased with the increasing concentration of copper (Table 2).

Table 2. Effect of copper accumulation on shoots and roots of*Triticumaestivum* L

Concentration (µM)	Copper accumulation (mg kg^{-1} dry weight)		
	Shoots	Roots	Total
25	180.71	197.55	378.26
50	177.73	152.15	329.88
75	322.53	604.88	927.41
100	418.43	803.14	1221.57

The clustered column chart shows that *Triticumaestivum*L accumulate maximum 1221.57 mg kg^{-1} (in dry weight) copper, that is in the parameter of hyper accumulation. So it was found that *Triticumaestivum*L is a copper hyper accumulator plant. By the way, when the concentration of metal increased, the accumulation rate increased (Fig. 1).

Table 3. Effect of copper accumulation of (Whole plants) *Triticumaestivum* L, *Abelmoschusesculentus* (L.),*Basellaalba* L. and *Solanumlycopersicum* L.

Concentration (µM)	Copper accumulation (mg kg^{-1} dry weight)			
	Triticumaestivum L	*Abelmoschusesculentus* L	*Basella alba* L	*Solanumlycopersicum*L.
25	378.26	256.54	190.60	38.65
50	329.88	500.25	577.24	55.27
75	927.41	307.07	639.71	67.50
100	1221.57	396.68	748.22	254.98

Copper accumulation by Abelmoschusesculentus L ,Basella alba L, Solanumlycopersicum L and Triticumaestivum L.

Abelmoschus esculentus L accumulated highest 500.25 mg kg^{-1} (dry weight) copper in 50 µM copper polluted pot. On the other case, there is an upward increase of copper accumulation (Figure 2 and Table 3).*Basellaalba*Laccumulated highest amount of copper 748.22 mg kg^{-1} in 100µM copper polluted soil and lowest in 25 µM polluted soil. Among different plant sample *Solanumlycopersicum*Ldetermined as lowest copper accumulator. The highest accumulation was 254.98 mg kg^{-1} at 100 µM solution polluted soil (Fig. 2 and Table 3).

Moreover, *Triticumaestivum*L. accumulated highest amount of copper from contaminated soil than other tested plants. It can now define as a hyper accumulator plant. *Basellaalba*accumulated 2nd highest amount of copper than other plants (Figure 2 and Table 3). Cu accumulation by different experimented plant was found in the order of *Triticumaestivum*>*Basellaalba*>*Abelmoschusesculentus*>*Solanumlycopersicum*(Table 3 and Figure 2).

Discussion

In our work, *Triticumaestivum*L totally accumulated 1221.57 mg kg^{-1} in dry weight and demonstrated as a hyper accumulator than other species. In general, the normal concentration of copper in plant tissues is approximately 5-25 mg/kg. Plant copper concentrations are controlled within a remarkably narrow range and plant copper concentrations above 100 mg/kg are rare even in the presence in high soil copper. The variation in copper accumulation may be related to soil pH, soil moisture, the season of the year, individual genotypic variability and varying degrees of soil contamination (Reeves *et al.*, 2000).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; Brown *et al.*, 1994; Brown *et al.*, 1995; 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 *et al.*, 1995; Blaylock *et al.*, 1997; Huang *et al.*, 1997). If an individual plant accumulates Cu above 1000 mgkg^{-1} plant dry weight this is considered as hyperaccumulator (Baker and Brooks, 1989).Hyperaccumulator plants tend to grow relatively slowly, which limits their usefulness for phytoremediation (Chaney, 1983). Nevertheless, their growth rate may be improved through selective breeding and the transfer of metal Hyperaccumulation genes to high biomass; fast growing species may also help to circumvent the problem (Chaney *et al.*, 2000). In the relatively short time frame of this experiment, it was observed that wheat (*Triticumaestivum*L.) plant produced more biomass than the other three plants. On the basis of biomass plant species accumulate heavy metals and large amount of biomass of plants accumulate higher amount of heavy metals.Accumulation of copper was higher in root tissue of wheat 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. The growth of plant species was different in each composition. It was observed that *Triticumaestivum*L. grew well in the experimental pots and there was no toxicity showed. Some Chlorosis in stems and leaves were found in the *Abelmoschusesculentus*(L.) Moench and *Basellaalba* L. within 15 days during experiment.

Pterisvitatta has been shown to accumulate 14,500 mg kg^{-1} arsenic without showing symptoms of toxicity (Ma *et al.*, 2001). The accumulation of Cu was 1,110 mg kg-1 dw of water hyacinth plant species. This amount was the lowest level as compared to *Spinaciaoleracea*plant species (Gupta *et al.*, 1994). The research shows that copper accumulation of *Triticumaestivum*L. 1221.57 mg kg^{-1} (dry weight) is 5 fold greater than *Solanumlycopersicum* L. and 2 to 3 fold than *Abelmoschusesculentus*(L.) Moench. On the other hand, *Basellaalba* L. accumulate 3 fold greater copper than *Solanumlycopersicum* L. The plant *Triticumaestivum*L. demonstrated as a hyperaccumulator. Whereas *Basellaalba* L. should be considered as a moderately accumulator plants. The study also demonstrated that plants of Poaceae family accumulates copper mainly in roots and shoots. To this base and with considering the metal accumulation capacity, we suggest that plants of family Poaceae can be used effectively for phytoremediation processing.

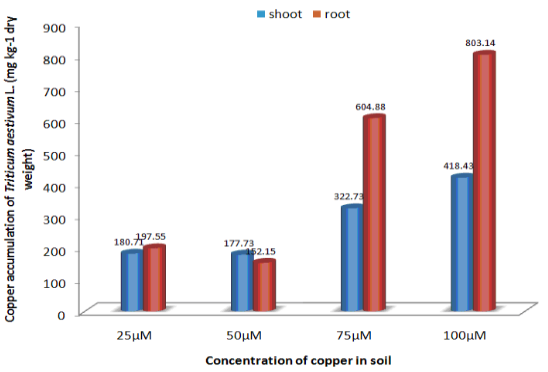

Fig. 1. Comparison between cooper accumulation by shoot and root of *Triticumaestivum* L.

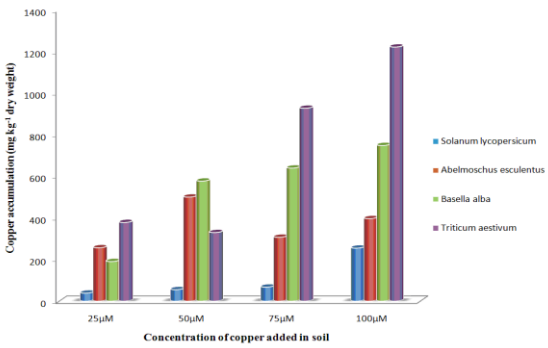

Fig. 2. Comparison of copper accumulation among different experimental plants.

Conclusion

*Triticumaestivum*L totally accumulated 1221.57 mg kg^{-1} copperin dry weight by shoots and roots.But the copper concentration in shoots of *Triticumaestivum*L. should not exceed 1,000 mg kg^{-1} dry weight. Therefore, *Triticumaestivum*L. could be regarded as a hyperaccumulator for copper remediation in contaminated soils. The copper concentration of *Basellaalba*L.was low (748.22 mg kg^{-1}). However, there was no sign of copper toxicity in this plant when testing in moderately contaminated soil. Thus, they could be classified as a copper tolerant species. For *Basellaalba*L., chelates, organic acids and certain chemical compounds should be applied to increase the solubility of copper in the soil solution and copper accumulation in the plants.

Acknowledgements

The authors would like to thank to Bangladesh atomic energy commission for supporting the laboratory work. We would also like to thank the department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh for cooperation of the project.

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