Journal of Experimental Sciences 2011, 2(10): 17-21
ISSN: 2218-1768
www.scholarjournals.org

journal of experimental sciences

www.jexpsciences.com

JES-Life Sciences

Effect of Inoculation with Arbuscular Mycorrhizal Fungi on Green Gram Grown in Soil Containing Heavy Metal Zinc

Shivakumar C.K.[1], Hemavani C.[1], Thippeswamy B.[1*], and Krishnappa. M[2]

[1]*Dept. of P.G. Studies and Research in Microbiology, Kuvempu University, Bio-Science Complex, Jnanasahyadri, Shankaraghatta-577 451, Karnataka, India*
[2]*Dept. of P.G. Studies and Research in Applied Botany, Kuvempu University, Bio-Science Complex, Jnanasahyadri, Shankaraghatta-577 451, Karnataka, India*

Article Info

Article History

Received : 05-07-2011
Revised : 30-09-2011
Accepted : 02-10-2011

*Corresponding Author

Tel : +91-9986917039
Fax : +91-8282256262

Email: thippeswamyb205@gmail.com

©ScholarJournals, SSR

Abstract

Pot culture experiments were conducted to determine the effects of Arbuscular Mycorrhizal Fungi (AMF) on growth and Zn metal uptake by the green gram grown in soil containing 25, 50 and 100 mg kg^{-1} ZnSO4. AMF inoculated and non-AMF inoculated green grams were grown in sterilized substrates containing $ZnSO_4$ and compared its effects on plant growth. Decreased Percentage of AMF colonization was observed with increasing in Zn concentration. Significant increase in root length, shoot length, total biomass, with decreased Zn uptake in shoot region was observed in AMF inoculated plants compared to AMF non-inoculated plants. High Zn accumulation was observed in the root region of mycorrhizal inoculated plant. Over all results indicate that AMF could promote the green gram growth with decreased Zn metal uptake from soil and thus protects the plant from metal toxicity.

Key Words: Arbuscular mycorrhiza; Heavy metal; Accumulation; Green gram

Introduction

Excessive heavy metals contamination in soil have detrimental on ecosystems and pose risks to human health as they enter the food chain via agricultural products (Liang *et al*., 2009). The main source of heavy metal contamination include mining and smelting of metalliferous ores, industrial waste, mineral fertilizers, pesticides, vehicle exhausts and municipal sewage sludge (Qian *et al.,* 2005). When heavy metals particularly Copper, Zinc, Chromium, Lead, Cadmium taken up by plants in higher concentrations, they not only inhibit metabolic process and reduce crop production in plants and also incorporated in to the food chain and potentially cause human liver and brain disorders (Bibi *et al.,* 2006). Technologies presently available for the remediation of metal contaminated soils are expensive, time consuming and may produce secondary waste (Fitz and Wenzel, 2002). Several higher plants developed strategies for heavy metal resistance enabling plants to survive in highly metal contaminated sites (Pilon-Smits, 2005). Zn at low concentration is important as micronutrient but in high concentrations this metal becomes toxic to plants. Plants readily accumulate Zn and excess Zn within tissues, inhibiting plant growth and rendering the crop unfit for human and animal consumption (Jeliazkova and Craker, 2003, Munzuroglu and Geckil, 2002). Heavy metals are toxic at slightly higher levels than those at which they are required. Green gram (*Vigna radiata)* plant was chosen in this study because it is one of the important short duration pulse crop cultivated in India since ancient times and being a drought resistant it can with stand adverse environmental conditions. It is a digestible; protein rich staple food contains about 25% protein, which is almost three times that of cereals, rich in vitamin A, B, niacin and minerals such as potassium, phosphorous and calcium which are necessary for human body. In addition to being an important source of human food and animal feed, green gram also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen through symbiotic association with microbes (Ahmed *et al*., 2006). Many experiments demonstrated that the mutual symbioses established between AMF and more than 80% of the terrestrial plants root, contribute to the heavy metal remediation (Gohre and Paszkowski, 2006). AMF provide a direct physical linkage between the soil and plant roots by their extrametrical mycelia. The objective of this research work was to test the ability of NM (Non Mycorrhizal) and M (Mycorrhizal) inoculated green gram to grow in Zn contaminated soil and to determine if early seedling growth, development and Zn uptake were affected by the presence and absence of AMF. It evaluates how the association of AMF inoculated green gram plants protects from Zn metal toxicity compared to non mycorrhizal inoculated plants.

Materials and Methods

Physicochemical and heavy metals analysis of soil sample:

The soil samples were collected at >10 different locations within the Kuvempu University campus area, which is near and representative of Bhadra wild life. Physicochemical characteristic of soil such as texture, pH, water holding capacity, organic matter (Tan, 1995) and the organic carbon (C) content (Nelson and Sommers, 1982) were determined. For determining heavy metal concentration, the soil sample was dried at 105^0C for 24 h and a portion of the sample (approximately 0.4g) was digested using the nitric acid

(HNO_3)/hydrogen peroxide (H_2O_2) digestion method in a microwave. After digestion, the digested solutions were filtered through filter paper (Whatman No.42) and then diluted with deionized water to a volume of 50 mL in a flask. The heavy metal concentration was determined on a Flame Atomic Absorption Spectrophotometer (FAAS) in the Karnataka State Horticulture Department, Shivamogga (Tseng *et al.,* 2009).

Soil preparation for pot culture:

For pot culture, sand and soil in the ratio 1:1 was taken and sterilized (autoclaved) at 121^{O}c for one hour (sterilized three times with 3 days interval). About 20 kg soil was filled in 17 cm x 15 cm nondraining 18 pots, 9 pots for inoculated AMF and 9 pots for non-inoculated AMF at three different concentration of $ZnSO_4$ (25ppm, 50ppm and 100ppm).

Preparation and inoculation of AM fungal inoculates:

Soil samples were collected from the rhizosphere of *Parthenium hysterophorus*, *Glomus* sp. was inoculated from the soil by wet-sieving and decanting technique (Gerdmann and Nicolson, 1963). Mass multiplication of AMF was done using ragi seeds as host. After 3 months, spores were found in these cultures and the roots of ragi were colonized by AMF.

The mycorrhizal treatments received a suspension of about 400 spores per pot and mixed into the upper 5cm of the soil. The pots were placed in a shaded area and were initially watered with distilled water.

Test Plant:

Seeds of green gram (*Vigna radiata)* were obtained from Agro seed trader, Shimoga Dist. The seeds were surface sterilized with 3% H_2O_2 for 15 min followed by 3 to 4 washing with sterilized distilled water. Ten selected healthy seeds were planted in each pot filled with pasteurized sand consisting of AM fungal spores and AM root segments added as single layer between upper and bottom layer of sand. The pots were placed in a shaded at 25-29^{0}C for 16 h photoperiod and were initially watered with distilled water. Uniform watering in the pots was maintained by keeping them at 60% soil water holding capacity by frequent watering to a constant weight. Surface soil in pots was covered with aluminium foil disks to reduce transpiration.

Treatment with Zn metal:

Three weeks after planting, the pots received 100ml aqueous solutions of 3 levels of ZnSO4.7H2O i.e, 25ppm, 50ppm, 100ppm in triplicate. All test solutions were made in double distilled water (ddH_2O), and ddH_2O was used as a control treatment. The pots were arranged in a block design where a complete set of treatments (3 concentration of ZnSO4.7H2O i.e., AM-fungal propagate free microbes suspension and AM- fungal pot culture) was randomized within each replicate block. The pots were subsequently supplied with 100ml of $ZnSO_4.7H_2O$ solutions at alternate days for 12 weeks.

To determine mycorrhizal root colonization:

Harvested plant roots were washed in tap water, cleaned in 10% KOH for 24 h at room temperature, acidified in 5% lactic acid for 24 h at room temperature and acidified with 0.5N HCl for 2-3 minutes. After staining, the roots were stored in pure lactic acid. Frequency of mycorrhiza in root systems (F %) determined after root samples of both mycorrhizal and nonmycorrhizal plants were stained with 0.05% trypan blue in lactophenol (Engqvist *et al.,* 2006, Nogueira *et al.,* 2004).

To determine shoot length, root length and plant biomass:

After the desired period of time, plants were harvested and their roots were gently washed with distilled water until the complete elimination of the remaining sand. Then, the principle root length, stem length and weight of each plant were recorded after drying at 60^{0}C for 3 days. .

Determination of metal content in plants:

Harvested plants were divided in to different parts with a knife obtaining three samples from each plant, root, stem and leaves. Each biomass section was digested separately to ascertain the metal content in the individual section. Samples were dried overnight at 65^{0}C in an oven with forced air circulation, grounded in a mortar and kept in polyethylene bottles. One g of milled plant tissues was soaked in 20 mL of pure nitric acid for six hours. The mixture was then boiled to 50% of the original volume. 4mL of perchloric acid (70 vol %) was added and the mixture was refluxed for 90 min. The solution was then diluted with distilled water to a total volume of 20 mL and analyzed by FAAS (Cutright *et al.,* 2010). The student t-test was applied to statistically compare the difference between mycorrhizal and nonmycorrhizal treatments ($P < 0.05$).

Results and Discussion

Physicochemical parameters of soil:

Physicochemical and the heavy metals content of the soil are given in the Table 1.

The observed pH, OC, P, K, Cu, Zn and Fe content of the soil is near to permissible limit. High Mn content was observed due to high organic forms in the soil. But high Mn content did not limit the plant growth (Govasmark *et al.,* 2007). Higher the concentration of heavy metals in the soil, greater was the toxic effect observed on plant growth.

Table 1: Physicochemical properties and heavy metal concentration of the test soil used in this study

Parameter	Values
Texture	Sandy clay loam
Type	Alluvial
pH	6.58
Electrical condectivity (Mmhos/cm^2)	0.03
Water holding capacity (cmol kg^{-1})	11.7
Organic matter (g kg^{-1})	1.2
Organic carbon (g kg^{-1})	0.90
Phosphorus (P)	22.0±5.7
Potassium (K)	58.0±8.1
Zinc (Zn) [a]	1.65±0.6 [b]
Copper (Cu)	7.95±2.1
Iron (Fe)	23.01±6.7
Manganese (Mn)	112.7±14.8

[a] All metal concentrations are in mg kg^{-1}
[b] ± Standard deviation
The amount of heavy metal added were higher and equivalent to the concentrations found in the polluted soil near to industrial effluent.

In the present study, the effect of Zn metal on AMF root colonization and growth of green gram in term of shoot length, root length and weight of biomass for each metal treated and control plant (non AMF) was determined.

Mycorrhizal root colonization:

Metals inhibited the extent of mycorrhizal colonization, maximum AMF infection was found in the roots of plants not receiving metal (control) and the least amount of root infection was observed in the roots obtained from the plants receiving highest Zn dose of 100 ppm (Table 2).

Table 2: Percentage colonized by AM fungus in the green gram (*Vigna radiata*) growing in pots receiving $ZnSO_4.7H_2O$ in concentrations of 25, 50 and 100 mg kg^{-1} every 3 weeks for 3 months

Treatments	Values
Control (NM)	0
Zn 25 mg kg^{-1}	10.10
Zn 50 mg kg^{-1}	6.42
Zn 100 mg kg^{-1}	4.23

Values are means of 3 independent experiments. Significant at $P<0.05$

The mean mycorrhizal colonization was affected by Zn application in the absence of AMF. Similar experiment was carried out (Andrade *et al*., 2008) was showed that sunflower plants associated with AMF were less sensitive to cadmium stress than non-associated plants. Strong colonization ability and intensive heavy metal tolerance of the indigenous AMF isolated from multi-metal contaminated soils was observed. Root colonization by AMF in metal polluted fields was relatively high. Hence AMF may play important roles to improve the tolerance of heavy metal for their host plants. The intensive vesicular colonization may be regarded as a part of the survived strategy in the tolerance of heavy metal for the host plants at polluted sites. In this study we isolated *Glomus* sp. from rhizosphere of *Parthenium hysterophorus*, grown in metal contaminated sites. It was found that plant yield was always high when the inoculant used was indigenous to the soil in which the plants were grown (Leyval *et al.,* 1995). Heavy metals were compartmentalized, accumulated and stabilized in the abundant AMF vesicles (Liang *et al.,* 2009). It has also been shown that mycorrhizal dependency differs among crop cultivars and that different AM fungus-plant combinations are functionally diverse. AM fungi are known to enhance phosphorus (P) nutrition of their host plants, particularly in infertile soils and under dry conditions (Quilambo *et al.,* 2010)

Effect of Zn on seed germination, shoot length, root length, and plant biomass:

Zn^{2+} metal is caused strong inhibition of seedling growth at relatively low concentration. The seeds germinated in the presence of high concentrations (100 ppm) of Zn^{2+}, but the subsequent seedling growth was severely inhibited at much lower concentration of metals. Our results suggested that toxicity of Zn^{2+} metals on seed germination dependent on the physiological state of seeds, permeability of metal ions into the embryo (Li *et al.,* 2005).

Seed germination was gradually delayed in the presence of increasing Zn concentration. It may be due to prolong incubation of the seeds that must have resulted in the neutralization of toxic effects of Zn by mechanisms like leaching, chelation, metal binding, accumulation by micraoorganism, paradoxical effect. Lowest doses of Zn improved seed germination and young seedling growth, while highest doses of Zn inhibited germination and decreased growth (Lefevre *et al.,* 2009). Hence physiological strategies employed by the plant to cope with external toxic ions vary according to the specific developmental stage. Toxicity of Zn metals on the growth of green gram was checked. These data indicate that the Zn were toxic to the growth of green gram plants at higher concentrations. Stunted growth with reduction in plant biomass as a result of Zn treatment was observed. The effects of the heavy metals over the shoot growth were different as compared to the effects on root growth. Root and shoot length (Fig.1) of the plant is stimulated at low concentration of Zn and able to grow efficiently upto 100mg kg^{-1} Zn concentration with AMF was evaluated in this study.

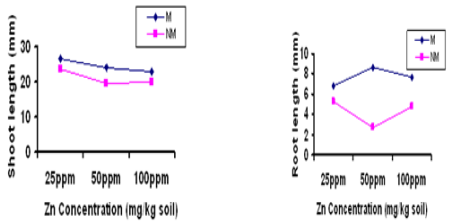

Figure 1: Shoot length and root length of 12 week old green gram plant grown with and without mycorrhizal fungi at 25, 50 and 100ppm of applied Zn. The values of each point is the average of three experiment (P<0.05).

A significant difference ($p<0.05$) was observed in the dry weights of the mycorrhizal and nonmycorrhizal plants. Dry weights of the shoot and root were higher in mycorrhizal plants when compared with the nonmycorrhizal plants (Fig. 2).

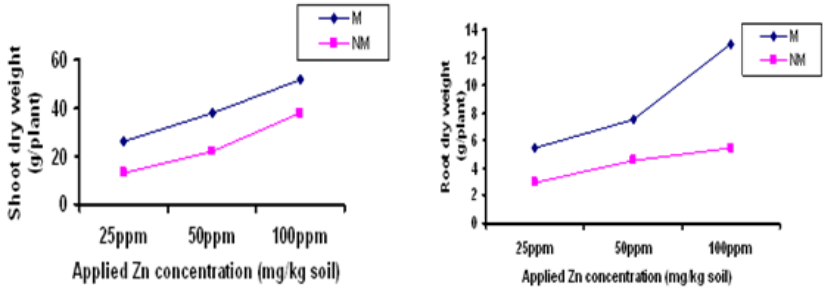

Figure 2: Shoot and root dry weight of 12 week old green gram plant grown with and without mycorrhizal fungi at 25, 50 and 100ppm of applied Zn. The values of each point is the average of three experiment ($P<0.05$).

Dry weight of Zn treated roots was generally lower in mycorrhizal plants when as compared to the nonmycorrhizal plants. The root dry weight of mycorrrhizal plants at Zn level of 25mg kg^{-1} increased sharply but declined at 50mg kg^{-1} and remained low at 100mg kg^{-1}. In the present study, inoculation of AMF did significantly increase the biomasses of roots and shoots than non inoculated groups in the same heavy metal concentration level. A positive mycorrhizal growth response was found in the pot experiment which is agreement with results given by Khan *et al.,* 2000, who reported that mycorrhizae are known to produce growth stimulating substances for plants, thus encouraging for phytoremediation by decontamination of metal polluted soils. It was observed by Shetty *et al.,* 1995 that AM fungi from Zn contaminated soils were more efficient in promoting plant growth when they were grown in soils with high Zn concentrations. Diaz *et al.,* 1996 showed that mycorrhizal plant has greater Zn uptake than non mycorrhizal plant. The biomass of roots and shoots of both inoculated and non-inoculated decreased significantly as the soil metal concentrations increased ($P<0.05$), indicating that the increasing heavy metal might restrain the growth of both AMF inoculated and non-inoculated plants. A wide ranging survey suggests that the excessive heavy metal have detrimental effects on AMF, including a reduction in spore germination, hyphal growth or root colonization (Leyval *et al.,* 1995). Amendment of soil with the heavy metals at concentrations higher than the normal levels resulted in a striking decrease of root length, shoot length and total biomass, due to sensitivity for heavy metals which indicating the alterations in physiology and metabolism of test plants. Loss of biomass (fresh weight) under metal treatment has been reported by many workers. Previous studies have also demonstrated a relatively higher phytotoxicity of Zn. In general, the reduction in dry weight of roots was more severe than the dry weight of shoot following treatment with heavy metals. This is supported by the earlier findings (Athar and Ahmad, 2002) that the influence of relatively higher amounts of Zn, Cu, Pb and Cd in wheat *cv. Vergina* resulted in depressed shoot growth but the most evident symptoms were on the roots. Low levels of phytotoxicity of Zn have been attributed to its insolubility under most soil conditions and it did not affect the plant growth unless the concentrations were very high. The increased leaf area in AM inoculated plants was observed (Quilambo *et al.,* 2010). Hence AM fungi depends on the photosynthetic products of plants and intern it protects the plants from various diseases, increases water holing capacity, helps in the absorption of nutrients from the soil and protects the plants in heavy metal stress conditions.

Accumulation of Zn in root and stem parts of plant:

Lower Zn metal concentration was reported in the shoots of mycorrhizal plants in comparison to non-mycorrhizal plants grown in the soil containing high Zn concentration (Table 3). Majority of the toxic metals were accumulated in the root region instead of transfer from the root to shoot.

Table 3: Concentration of Zn Accumulated by Green gram grown with and without AMF

Treatment	Shoot Zn content (mg $plant^{-1}$)	Root Zn content (mg $plant^{-1}$)	Zn Shoot/Root difference (%)
NM 25ppm	07±2.27	13±3.01	6
NM 50ppm	18±4.96	25±5.28	7
NM 100ppm	38±7.14	53±9.25	15
M 25ppm	08±2.01	15±4.21	7
M 50ppm	14±3.97	34*±6.12	20
M 100PPM	23±4.25	72*±9.24	49

The values are given as means (±S.E.) of three replicates (n=3). *Significant at $P<0.05$.

It is due to the reetention of heavy metal by fungal mycelia through adsorption to the cell wall thereby minimizing metal translocation to the shoot region. This result indicates that the AMF mycelia had a high metal sorption capacity. It

was found that Zn was accumulated to a concentration of over 1- 200 mg kg^{-1} (dry matter) in *G. mosseae* mycelium associated with green gram plants (Liang *et al.,* 2009). Mycorrhiza has been shown to alter the pattern of Zn translocation from root to shoot in the grass *Andropogon gerardii*. The Zn translocation is species-specific and host preference is an important characteristic of the symbiotic association. AMF have potential to promote plant growth or increase heavy metal accumulation.

Conclusion

Detrimental effect of heavy metals can be coping up by isolated *Glomus* mycorrhiza to neutralize Zn toxicity and favors the growth of green gram plants. These data indicated that the green gram plant may be grown in soil in combination with AMF for its effective growth and metal detoxification. Abundant AMF might immobilize Zn to reduce the bioavailability of Zn and other heavy metal toxicity.

References

Ahmed Z.I., Anjum M.S and Rafu C.A. 2006. Effect of *Rhizobium* inoculation on growth and root nodule formation of green gram. *International journal of Agriculture & Biology*. 8:235-237

Andrade S.A.L., Silveira A.P.D., Jorge R.A and Abreu M.F.2008.Cadmium accumulation in sunflower plants influenced by Arbuscular mycorrhiza. *International journal of Phytoremediation*. 10:1-13.

Athar R and Ahmad M. 2002. Heavy metal toxicity: Effect on plant growth and metal uptake by wheat, and on free leaving *Azotobacter*. *Water, Air and Soil Pollution*. 138: 165-180.

Bibi M., Hussain M., Qureshi M.S., Kousar S and Ajaz S. 2006. Heavy metal and nutrient ion accumulation by two black gram cultivars treated with copper and lead. *Journal of Plant Nutritio.* 29:913-920.

Cutright T., Gunda N and Kurt F. 2010. Simultaneous hyperaccumulation of multiple heavy Metals by *Heliantus annuus* grown in a contaminated sandy-loam soil. *International Journal of Phytoremediation*. 12: 6, 562-573.

Diaz G.C., Aguilar A and Honrubia M. 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis crytisoides*. *Plant and Soil*. 180:241-249.

Engqvist L.G., Martensson A., Orlowska E., Turnau K., Belimov A.A., Borisov A.Y and Pearson V.G. 2006. For a successful pea production on polluted soils, inoculation with beneficial microbes requires active interaction between the microbial components and the plant. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*. 56:9-16.

Fitz W.J and Wenzel W.W. 2002. Arsenic transformations in the soil rhizosphere plant system: fundamentals and potential application to phytoremediation. *J Biotechnol*. 99:259-278.

Gerdmann J.W and Nicolson T.H.1963. Spores of mycorrhizal endogone species extracted from soils by Wet- sieving and decanting.*Trans.Br.Mucol.Soc*. 46:235-244.

Gohre V and Paszkowski U.2006.Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation .*Planta*. 223:1115-1122.

Govasmark E., Steen A., Bakken A.K., Strqm T and Hansen S. 2007. Factors affecting the concentration of Zn, Fe and Mn in herbage from organic farms and in relation to dietary requirements of ruminants. *Acta Agaric. Scandinavica Section B-Soil & Plant Science*. 55: 2, 131-142.

Jeliazkova E.A and Craker L.E. 2003. Seed germination of some medicinal and aromatic plants in heavy metal environment. *Journal of Herbs, Spices and Medicinal Plants*. 10:2, 105-112.

Khan A.G., Keuk C., Chaudhry T.M., Khoo C.S and Hayes W.J. 2000. Role of plants, mycorrhizae, and phytochelataros in heavy metal contaminated land remediation. *Chemopshere*. 4:197-207.

Lefevre I., Marchal G., Correal E., Zanuzzi A and Lutts S. 2009. Variation in response to heavy metals during vegetative growth in *Dorycnium pentaphyllum* Scop. *Plant Growth Regul*. 59:1-11.

Leyval C., Singh B.R and Joner E.J. 1995. Occurrence and infectivity of Arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. *Water Air Soil Poll*. 84:203-216.

Li W., Khan M.A., Yamaguchi S and Kamiya Y. 2005. Effect of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. *Plant Growth Regul.* 46:45-50.

Liang C.C., Li T, Xiao Y.P., Liu M.J., Zhang H.B and Zhao Z.W.2009.Effects of inoculation with arbuscular mycorrhizal fungi on maize grown in multi-metal contaminated soils. *Internat. J. Phytoremed*. 11; 692-703.

Munzuroglu O and Geckil H. 2002. Effects of metals on seed germination, root elongation, and coleoptile and hypocotlyls growth in *Triticum aestivum* and *Cucumis sativus*. *Arch Environ Cont Tox*. 43: 203-213.

Nelson D.W and Sommers L.E. 1982. Total carbon, organic carbon, and organic matter. In *Methods of soil analysis, part 2: Chemical and microbiological properties,* ed. A.L. Page, R.H. Miller, and D.R. Keeney. 539-580. Madison, Wisc.

Nogueira M.A., Magalhaes G.C. and Cardoso E.J.B.N. 2004. Manganese Toxicity in Mycorrhizal and Phosphorus-Fertillized Soybean Plants. *J. Plant Nutr*. 27: 1, 141-156.

Pilon-Smits E. 2005. Phytoremediation. *Annu Rev Plant Biol.* 56:15-39.

Qian M., Li X and Shen Z. 2005. Adaptive copper tolerance in *Elsholtzia haichowensis* involves production of Cu-induced thiol peptides. *Plant Growth Regul*. 47:65-73.

Quilambo Q.A., Weissenhorn I., Doddema H., Kuiper P.J.C and Stulen I. 2010. Arbuscular Mycorrhizal Inoculation of Peanut in Low –Fertile Tropical Soil. Host-Fungus Compatibility. *Journal of Plant Nutrition*. 28: 9, 1633-1644.

Shetty K.G., Hertick A.D and Schwab A.P. 1995. Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. *Environmental Pollution*. 88:307-314.

Tan K.H. Soil sampling, preparation and analysis. New York: Marcel Dekker.

Tseng C., Wang J.Y and Yang L. 2009. Accumulation of copper, lead and zinc by in situ plants inoculated with AM fungi in multicontaminated soil. *Communication in soil science and plant analysis*. 40: 21, 3367-3386.