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Seedling response of three *Eucalyptus* species to copper and zinc toxic concentrations

M.H. Assareh, A. Shariat* and A. Ghamari-Zare

Research Institute of Forests and Rangelands, P.O.Box 13185-116, Tehran, Iran.
** Corresponding author's E-mail: shariat_ana@yahoo.com*

ABSTRACT

Human activities have continuously increased the level of heavy metal ions circulating in the environment. It is important to understand the tolerance of plant species to high concentrations of heavy metals. Very little is known on the response of *Eucalyptus* species to Zn and Cu toxic concentrations *Eucalyptus camaldulensis*, *E. microtheca*, and *E. occidentalis* seeds were grown in silt and irrigated by nutrient solution. Forty two days old seedlings were exposed to ten Zn and Cu treatments for ten months.

The tolerance order of the species to toxic concentrations of Cu was *Eucalyptus camaldulensis*> *E. occidentalis*> *E. microtheca* and the tolerance order of the species to toxic concentrations of Zn was *E. occidentalis*> *E. microtheca*> *E. camaldulensis*. Tissue concentration increased as Zn and Cu concentration increased for the species. Root tissue concentrations regards to Zn and Cu concentrations were higher than shoot tissue concentration at all solution. These results provided the first quantified metals in *Eucalyptus* species and the second explored relationships between concentrations of metals in nutrient solution and plant organs.

Keywords: Heavy metals, *Eucalyptus camaldulensis*, *E. microtheca*, *E. occidentalis*, Toxic concentration.

INTRODUCTION

There are a large number of sites world wide polluted with trace elements as a result of human activities. The mining industry has produced a significant legacy of polluted and degraded soils (Careillo- Gonzalez and Gonzalez-Chvez, 2006). Approximately 400 metal hyperaccumulators have been identified so far (Baker *et al.,* 1994). Hyper-accumulator plants are grown on soils high in metals to determine their potential for management of polluted soils and especially for metal extraction (Shallari *et al.*, 1998). The concentration of heavy metals in above ground part of the hyperaccumulator plants is 10-500 times more than that of other plants (Shen and liu, 1998). Utilization of the remarkable potential of green plants to accumulate elements and compounds from the environment and to perform biochemical transformation is becoming a new frontier of plant biology (Yanqun *et al*., 2004). However, the distribution, ecology, and phytochemistry of the *Eucalyptus* species in the pollutant area are poorly understood. *Eucalyptus* species grow faster than many native tree species In Iran. They are not invasive, having been planted in Khuzestan province in south part of the country for more than 50 years (Djavanchir & Mossadegh, 1973). *Eucalyptus camaldulensis,, E. microtheca, E. occidentalis* were selected for this study because of their important attributes such as tolerance to a wide range of soil types, and pH. In particular, they are fast growing trees so that when they are planted on suitable sites and managed properly, they have high potential for producing commercial products such as pulpwood, mulch, and energy wood, and even for remediation of some environmental problems (Abo-Hassan, *et al.,* 1988 and Djavanchir & Mossadegh, 1973). Patterns of heavy metal accumulation in plants, regarding the type of soil, biological peculiarities of plants, the elements nature and concentration, are discussed by others (Basta and Gradwohl, 2000; Golovatyj, 2000; Lukin *et al*., 2000, 2001; Keller *et al*., 2002., Grytsyuk, *et al*., 2006).

This paper examines the tolerance of three *Eucalyptus* species with a wide distribution in the world (especially *E. camaldulensis*) and may be used in rehabilitation activities on Zn and Cu toxicity in solution culture. Our objectives were:

a) To quantify metal concentration in the plant species.
b) To explore relationships between concentrations of the metals in nutrient solution and plants to evaluate the potentials for the associated risks of elevated pollutant uptake.

MATERIALS AND METHODS

Three *Eucalyptus* species were grown in well-controlled experiments to measure their heavy metals uptake. Seeds of *Eucalyptus camaldulensis*, *E. microtheca*, and *E. occidentali* were germinated in sterilized pot filled by silt in a controlled temperature glasshouse (20° C day/15°C night). When seedlings grown to the two-leaf stage the nutrient solution was used for irrigation. Nutrient solution was prepared based on a method described by Heidari Sharif Abad (1994). After 10 weeks, when the seedling were grown to 15 centimeters, sufficient $ZnSO_4.7H_2O$ and $CuSO_4.2H_2O$ were added to the nutrient solution drums to give initial ten treatments. Zn concentrations were 1, 3 and 10 mM Cu concentrations were 5, 10 and 20 mM Mixture of the two elements were also used in three levels a) Zn(1mM), Cu(5mM); b) Zn(3mM), Cu(10mM); c) Zn(10mM), Cu(20mM).

Table 1. Cu and Zn treatments.

T	Treatment	T	Treatment
1	Cu (5 mM)	6	Zn (10 mM)
2	Cu (10 mM)	7	Cu(5),Zn(1)
3	Cu (20 mM)	8	Cu(10),Zn(3)
4	Zn (1 mM)	9	Cu(20),Zn(10)
5	Zn (3 mM)	10	Cu(0),Zn(0)

Harvesting and chemical analysis

Plants were harvested ten months after being exposed to Zn and Cu treatments. Plants were rinsed four times in deionized water. Roots, stems and leaves were separated. The plant parts were placed in paper bags and dehydrated at 70°C for 48 hours then digested in %65 nitric acid, %37 hydrochloric acid and %30 hydrogen peroxide (Westerma, 1990). Heavy metal analysis was performed using individually coupled plasma-optical emission spectrometry (ICP-OES Integra XL,GBC, Australia). Detection limit for ICP-OES is $6\mu gL^{-1}$ for Zn and $10\mu gL^{-1}$ for Cu.

Statistical analysis:

Factorial analysis of variance was performed on the data groups based on completely randomized experiment using SPSS 13 and CurveExpert 1.3 for estimating relationship between concentration of the metals in soil and the plant parts. The main factors were species of *Eucalyptus* (three levels), Organs (three levels) and metal treatments (ten levels).

Effects of the metal treatments on leaves, roots and stems on the three *Eucalyptus* species were analyzed by ANOVA (SPSS13) followed by Duncan multiple range tests. The data were normalized by Tukey transformation where necessary. Tukey transformation was used in this study because this method improved linearity of regression better than the other methods of transformation. This method improved normality of distribution and stabilized approximately the variance. Experimental data were analyzed by CurveExpert 1.3 for estimation of relationship between the metals concentrations in the nutrient solution and the plant species.

Enrichment coefficient equator

Enrichment coefficient was described as heavy metal element concentration in plant above ground part, which can be used to evaluate accumulating capacity of plant to the heavy metal.

RESULTES

Analysis of variance revealed significant differences between the main effects, treatments, organs and species and all their interactions ($p<0.01$) for the studied attributes (Zn and Cu concentrations). It would indicate that there are significant differences between stems, leaves and roots absorbing Zn and Cu. This also indicates different tolerance to metals for the studied species. Significant interaction of organs and treatments revealed different effects of the treatments on stems, leaves and roots. The interaction of organs and species was significant, this would indicate that the changes in Cu and Zn contents of the organs are not constant in the three *Eucalyptus* species. Triple interaction effect (Interaction between the organs, treatments and the species) was significant (Table 2). However, significant interactions indicated that not only the studied characteristics differ between the species and organs within the species but also the rate of the changes are not constant between the factors levels.

Mean classification

As a result of Duncan multiple range test, leaf, stem and root Cu contents were significantly different so that roots Cu content was 17 times more than that of leaves. There wasn't any significant differences between Zn contents in roots and leaves (Table 3).

Duncan analysis classified Cu treatments to 8 groups, but Zn absorption was classified to 7 groups (Table4). *E. microtheca* and *E. occidentalis* were classified in one group based on Cu absorption they significantly differed with *E. camaldulensis*. The species were classified in three groups based on Zn absorption (Table 5).

Different trends on distribution of Zn and Cu concentration and similar trends in the distribution of cooper were observed between species of *E. microtheca* and *E.camaldulensis,* but *E.camaldulensis* showed greater concentration of cooper.

The order of Cu enrichment coefficient was *E. microtheca*>*E. camaldulensis*> *E. occidentalis*. The order of Zn enrichment coefficient was *E. occidentalis*> *E. microtheca*>*E. camaldulensis*. It can be concluded that *E. microtheca* and *E. camaldulensis* had more ability to accumulate Cu than *E. occidentalis*. *E. occidentalis* had more ability to accumulate Zn than the other two species (Table 6).

Comparing the accumulation and translocation of the studied metals within the plants showed that the metals uptakes by roots are higher than those by stems and leaves (figure 1). Zn and Cu uptakes by the leaves and stems of the three *Eucalyptus* species are lower than those of roots. For instance Cu concentration in roots of *E. camaldulensis* in third treatment with Cu: 20 mgl^{-1} was about 2500 mgkg^{-1} but Cu concentration in Leaves and stems were 80 and 180 mgkg^{-1} dry mass respectively. The highest Cu uptake was observed in *E. camaldulensis* and the lowest Cu uptake was observed on *E. microtheca*. Leaves Zn concentrations were generally higher than those of stems (figure1). For instance Zn concentration in roots of *E. camaldulensis* in the sixth treatment level (Zn=10 mgl^{-1}) was 520 mgkg^{-1} in roots. Leaves and stems Cu concentration were 510 and 300 mgkg^{-1} dry mass respectively. The highest Zn uptake was observed in *E. occidentalis* and the lowest Zn uptake was observed in *E.* camaldulensis.

Quadratic relationship between Cu concentration (mg kg-1 dry weight) in the three Eucalyptus species and soil which is presented in figure 2 and the following equation.

Y= 3.705+7.624X-0.14X2,

in which Y is Cu concentration in plant and X is Cu concentration in nutrient solution. Linear relationship between Zn concentrations (mg kg-1 dry weight) in the three *Eucalyptus* species. Nutrient solution relationship is presented in figure 3 and the corresponding equation is as follows:

Y=230.98+2.58X,

in which Y is Zn concentration in plant and X is Zn concentration in nutrient solution.

Table 2. Mean squares of heavy metals concentration in the three *Eucalyptus* species

Sources of variation	d.f.	Cu (mg kg^{-1} D.W.)	Zn (mg kg^{-1} D.W.)
Organ	2	13035405.6**	693699.9**
Treatment	9	1583917.8**	174116.3**
Species	2	25895.2**	29096.6**
Organ*treatment	18	1232990.8**	32930.2**
Organ*species	4	29117.2**	38091.6**
Treatment*species	18	79989.5**	17291.4**
Organ*treatment*species	36	87081.6**	12097**
Error	180	2713.4	600.1

**: p<0.01

Table 3: Duncan multiple range test of organs for Cu and Zn.

Organ	Cu (mg kg^{-1} D.W.)	Zn (mg kg^{-1} D.W.)
Leaf	41.7± 2.3 a	324.7± 12.7 b
Stem	75.8± 5.4 b	169.3± 4.3 a
Root	717.3± 71.9 c	317.8± 14.9 b

Means±SE, n=3, means in a column followed by a different letter are significantly different (P<0.05) according to Duncan test.

Table 4. Duncan multiple range test of Cu and Zn treatments.

Treatment	Cu (mg kg^{-1} D.W.)	Zn (mg kg^{-1} D.W.)
5 mM Cu	170.0± 33.0 c	221.7± 10.8 c
10 mM Cu	361.2± 75.8 e	183.6± 10.9 a
20 mM Cu	699.9± 175.5 h	257.2± 17.8 d
1 mM Zn	77.8± 15.3 b	325.3± 27.0 e
3 mM Zn	38.2± 5.6 a	359.6± 31.1 f
10 mM Zn	60.8± 10.0 ab	433.9± 30.4 g
5 Cu, 1 Zn (mM)	238.9± 61.4 d	199.8± 16.6 b
10 Cu, 3 Zn (mM)	463.9± 111.6 f	257.6± 16.3 d
20 Cu, 10 Zn (mM)	604.1± 147.2 g	268.9± 23.3 d
0, 0 (mM)	67.8± 17.8 b	198.8± 9.2 b

Means±SE, n=3, means in a column followed by a different letter are significantly different (P<0.05) according to Duncan test.

Table 5. Duncan multiple range test of *Eucalyptus* species for Cu and Zn treatments.

Species	Cu (mg kg^{-1} D.W.)	Zn (mg kg^{-1} D.W.)
E.camaldulensis	297.8± 60.2 b	253.0± 12.7 a
E.microtheca	268.0± 43.9 a	270.0± 13.8 b
E.occidentalis	268.9± 53.8 a	288.9± 14.7 c

Means±SE, n=3, means in a column followed by a different letter are significantly different (P<0.05) according to Duncan test.

Table 6. Enrichment coefficient of three *Eucalyptus* species for Cu and Zn.

Species	Cu (mg kg^{-1} D.W.)	Zn (mg kg^{-1} D.W.)
E.camaldulensis	5.0± 0.5b	128.5± 28.6a
E.microtheca	5.2± 0.5b	139.7± 32.8b
E.occidentalis	4.4± 0.5a	155.2± 40.3c

Means±SE, n=3, means in a column followed by a different letter are significantly different (P<0.05) according to Duncan test.

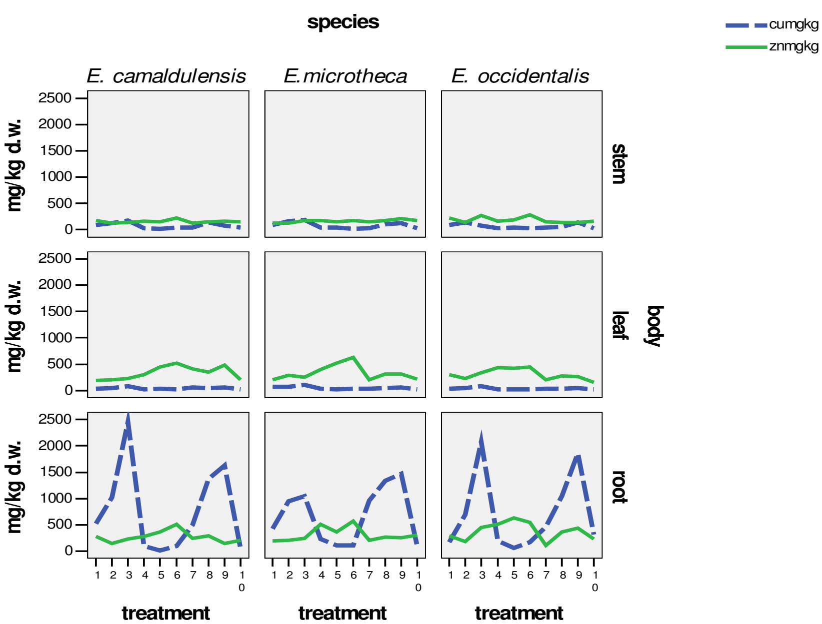

Fig 1. Effects of 10 heavy metals treatments on three *Eucalyptus* species on different parts of trees.

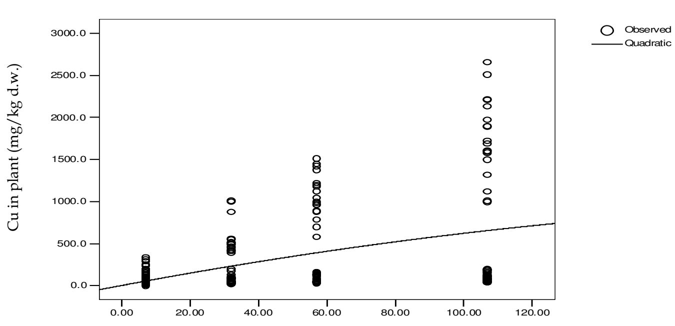

Fig 2. Quadratic relationship between Cu concentration (mg kg-1 dry weight) in three Eucalyptus species and nutrient solution, Y= 3.705+7.624X-0.14X2.

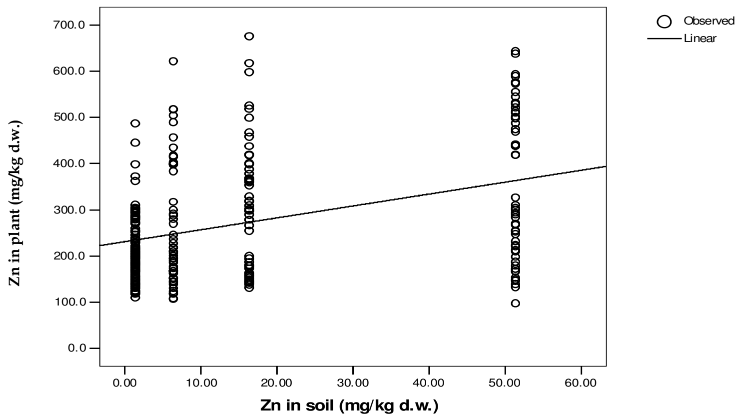

Fig 3. Linear relationship between Zn concentration (mg kg-1 dry weight) in three Eucalyptus species and nutrient solution, y=230.98+2.58X

DISCUSSION

Heavy metals are released in the environment by power generating stations, heating systems, waste incinerators, metal working industries, and many other sources. Their accumulation in soils can become dangerous to all kinds of organisms, including plants (Gichner, *et al.*, 2006). In this paper, four distinctly different treatments were used: a slightly contaminated solution, elevated level of Zn, Cu and high level of the elements. Between the studied species, *E. camaldulensis* indicated the highest Cu concentration (297.8 mg/kg) and *E. occidentalis* indicated the highest Zn concentration (288.9 mg/kg). Aten and Gupta (1996) observed good correlations between shoot Zn and Cu content in *Lactuca sativa* and *lolium perenne* and extractable soil concentrations using diluted salt solutions. Numerous studies reported that, heavy metals compartmenttalization occurs predominantly in actively growing tissues (Pulford and Watson, 2003). Riddle-Black (1994) observed foliage concentrations to be greater than stem concentrations for four *willow* species grown on sludge amended soil. Most plants can not transfere metals to the shoot system even if metal concentrations in the soil-slag mixtures were high (Carrillo-Gonzalez and Gonzalez-Chavez, 2006). Freitas *et al*., (2004) found a wide range of plant families growing on trace element contaminated soils. The authors reported that non endemic tree species, such as: *Eucalyptus* sp., *Casuarina cunninghamiana* and *Schinus moll* were able to grow on the residues, but they do not produce a leafy crown and the trunks showed clear signs and poor growth. These plants had tiny leaves, which show chlorosis, necrotic brown spots and dried tips. In some cases they died after few years (5 to 10 years) exposure to the slag.

Bioaccumulation of Cu in environmental settings occurs when this element is biologically available (Bettiol and Camargo, 2000). In general, vegetation growth in soils with high Cu contents reflects the Cu levels in plant tissues, varying according to the physiological requirements of each species (WHO, 2001). It is estimated that in unpolluted soils, Cu concentration in vegetable tissues ranges between 6-25 mg/kg. However, in Cu polluted soils the concentration of this element in vegetable tissues may reach to 80 mg/kg (WHO, 2001). The existent Cu concentrations in roots and stems of *E. camaldulensis* were above 2000 and 180 mg/kg respectively (figure 1). When Cu levels are higher than 150 mg/kg, agricultural species may show adverse effects. An experimental study, where young sugar cane plants grew in solutions containing levels of Cu between 50 and 500µM concluded that solution with concentration over 100µM of Cu inhibited the development rate in 48% (Sereno, 2004).

Heavy metals accumulation in crops as a function of their concentration in soil is verified by rather high values of correlation coefficient R^2(R^2 >0.70). High correlation between heavy metal content in soil and in plant parts was found for all plant species. Plants species, type of soil and physicochemical properties of heavy metals determine the most important parameters of this dependence (Grytsyuk *et al.*, 2006). Zn uptake to the leaves by *Eucalyptus* roots was not significantly different, but Cu uptake

betw-
een the organs was highly different so that the Cu contents in roots was 17 times more than that in leaves.

The results of this study suggest a possible interest in monitoring the zones under direct influence of zinc and copper activities, for controlling of surrounding pollutant area. In order to avoid a potential future increase of toxic levels of metals, it would also be important to perform an exhaustive evaluation of application of *Eucalyptus camaldulensis* on industrial residues. The results of this study also indicate that *Eucalyptus* is able to grow in areas where some metals in soils are accumulated. The interaction between metals, fertilizers and herbicides could be also a subject of further studies on *Eucalyptus* species.

REFERENCES

Abo-Hassan, A., Kandeel, S.A. and Kherallah, I.E. (1988) *New eucalyt species introduction in the Saudi Arabia central zone.* In: Proceedings of the international forestry conference, Australian Bicentenary, vol.5. Australian Forest Development Institute. Alburry, Australia. pp. 1-16.

Aten, C.F. and Gupta, S.K. (1996) On heavy metals in soil: rationalization of extraction by dilute salt solutions, comparison of the extracted concentration with uptake by ryegrass and lettuce, and the possible influence of pyrophosphate on plant uptake. *Sci. Total Environ.* **178,** 45-53.

Baker, A.J.M., Reeves, R.D. and Hajar, A.S.M. (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (*Brassicaceae*). *New Phytol.* **127,** 61–68.

Basta, N. and Gradwohl, R. (2000) Estimation of Cd, Pb and Zn bioavalibility in smelter contaminated soils by a sequential extraction procedure. *J Soil Contam.* **9**(2), 149-164.

Bettiol, W. and Camargo, O.A. (2000) Impacto ambiental do uso agricola do lodo de esgoto. Jagauriuna. EMBRAPA.

Carrillo-Gonzalez, R. and Gonzalez-Chavez, M.C.A. (2006) Metal accumulation in wild plants surrinding mining wastes, *Environmental Pollution.* **144,** 84-92.

Djavanchir, K. and Mossadegh, A. (1973) *Eucalyptus*. Tehran University Press. 435p.

Freitas, H.M., Prasad, M.N.V. and Pratas, J. (2004) Plant community tolerance to trace elements growing on the degraded soil of Sao Domingos mine in the south east of Portugal: environmental implications. Environ. *Int.* **30,** 65-72.

Gichner, T., Patkova, Z., Szakova, J. and Demnerova, K. (2006) Toxicity and DNA damage in tobacco and potato plants growing on soil polluted with heavy metals. *Ecotoxicology and Environmental safety.* **65,** 420-426.

Golovatyi,, S.Y. (2002). Heavy metals in agroecosystems. *Minsk, Russia*. pp. 92-119.

Grytsyuk, N., Arapis, G., Perepelyatnikova, L, Ivanova, T. and Vynogradska, V. (2006) Heavy metals effects on forage crops yields and estimation of elements accumulation in plants as affected by soil. *Science of the Total Environment.* **354,** 224-231.

Heidari Sharif Abad, H. (1994) Variation in the sensivity of nodulation and nitrogen fixation to nitrate in annual *Medicago* species. *A thesis for the degree of doctor of philosophy. Waite Agricultural Research Institute, Glen Osmond, South Australia.*

Keller, A., Abbaspour, K.C. and Schulin, R. (2002) Assessment of uncertainty and risk in modeling regional heavy-metal accumulation in agricultural soils. *Enviro Qual.* **31,** 175-187.

Lukin, S.V. Soldat, I.Y. and Pendjurin, Y.V. (2001) Regulatities of zinc accumulation in agricultural crops. *Agrochimija.* **4,** 46-49.

Lukin, S.V., Javtushenko, V.Y. and Soldat, I.Y. (2000) Cadmium accumulation in agricultural crops in dependence on the level of soil contamination. *Agrochimija.* **2,** 73-77.

Pulford, I.D. and Watson, C. (2003) Phytoremediation of heavy metal-contaminated land by trees- a review. *Environ. Int.* **29,** 529-540.

Riddle-Black, D. (1994) Heavy metal uptake by fast growing willow species. In: Aronsson, P., Perttu, K. (Eds.). Willow vegetation filters for municipal wastewaters and sediments: A biological purifycation system. Proceeding of a study tour, Conference and workshop in Sweden. Swedish University of Agricultural Science, Section of short rotation Forestry, Rapport 50, June 5-10, pp.133-144.

Sereno, M.L. (2004) Avaliacao da tolerancia de Cana-de-acucar (Saccharum spp.) a metais. Expressao dos genes de metalot-ioneina. *Teses. Universidade de Sao Paulo-Escola Superior de Agricultura "Luiz de Queiroz"*. 94 pp.

Shallari, S., Schwartz, C., Hasko, A. and Morel, J.L. (1998) Heavy metals in soils and plants of serpentine and industrial sites of Albania. *Sci. Total Environ.* **209,** 133-142.

Shen, Z.G. and Liu, Y.L. (1998) progress in the study on the plants that hypera-ccumulate heavy metal. *Plant Physiol Commun.* **34,** 133-139.

Westerma, R.E.L. (1990) Soil testing and plant analysis., SSSA. Madison wisconsin, USA.

WHO (World Health Organization), (2001) Copper. Environmental health criteiavol. 200. Geneva: International Programme on Chemical Safety.

Yanqun, Z., Yuan, L., Schvartz, C., Langlade, L.and Fan, L. (2004) Accumulation of Pb, Cd, Cu and Zn in plants and hypera-ccumulator choice in lanping lead-zinc mine area. *china, Environment International.* **30,** 567-576.

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