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ORIGINAL ARTICLE

Investigation the effects of different concentration of Copper, Lead and Cadmium of soil on Zinc accumulation inside different organs of *Eucalyptus camaldulensis*

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Phytoremediation is one method which initiated recent decades for amendment and clean up the contaminated soils with organic and mineral compounds. Aim of this research is investigation the effects of different concentration of Copper (Cu), Lead (Pb) and Cadmium (Cd) of soil on Zinc accumulation inside different organs of *Eucalyptus camaldulensis*. In this study 3 treatments of Cadmium (5, 10 and 15 ppm), 3 treatments of copper(5, 10 and 15 ppm) , study 3 treatments of Zinc(1, 3 and 10 ppm), 3 treatments of Lead (5, 100 and 200 ppm), and 3 treatments of combination these elements in 3 level and 6 replication carried out based on Completely Randomized Design for *Eucalyptus camaldulensis*.

Result of this research clearly indicated that concentration and kind of heavy metals existent in soil are affecting on the rate of uptake and accumulation and also location of Zinc accumulation inside different organs of *Eucalyptus camaldulensis*.

Keywords: *Eucalyptus camaldulensis*; copper; Cadmium; Lead; zinc; heavy metals

Introduction

Environmental pollution like soil pollution is one of most important conclusion of disturbance between nature balances. The most important soil contaminants including heavy metals, acid rain and organic materials and amongst them heavy metals are concerned due to unable to decomposition, high toxicity, cumulative effects and carcinogenesis (Mico et al., 2006).

Soil contamination with heavy metals originating vastly from human activities, agriculture and industries (Sharma & Dubey, 2005). Presence of these elements in atmosphere, soil and water even in a very low level could bear in problems for living organisms (Groppa et al., 2007). Due to highly toxicity of metals, the soil contaminated with these elements are faced with environmental problem that required to possible and effective solution (Nascimento & Xing, 2006; Groppa et al., 2007).

There are many ways for removing metal from polluted soil such as soil leaching, chemical application and biological method that imposes high engineering cost also have impacts on biological activities (Khan, 2005; Pulford & Watson; 2003). It seems phytoextraction is most hopeful method and researchers concentrated in a great deal on it (Nascimento & Xing, 2006). In this case phytoremediation is one of the bioremediation methods which presented recent decades for improvement and refining the polluted soil with organic and mineral contaminants. Phytoremediation has preference compared with other chemical and mechanical methods due to low cost and possibility of vast application. Major factor in Phytoremediation is selection of merit plant (Mattina et al., 2003). Researchers these recent years pay attended much on metals accumulation inside plant products, aquatic plants and natural forages. Process of uptake and metal accumulation in different plants depends on metal concentration in soils (Gardeatorresdey et al., 2005).

With regards to increasing needs to forestation it is necessary that complete study on Eucalyptus species and their role to uptake heavy metals to be carried out. In recent decades Eucalyptus plantation rapidly increased and agroforestry attracted public vision. Eucalyptus were prevalent in order vegetation green cover and purification of air in industrial zone like metal melting factories found special position for reducing air and soil pollution. Hence with Eucalyptus plantation which have ability of uptake and accumulation of heavy metal enhance environmental clean up as phytoremediation (Salahi, 2007). Eucalyptus species are preferred with more growth rate and more wood production also uptake and accumulation of elements in soils irrigated with swage and more evapotranspiration compared with low growth rate trees. Different studies were started on ability of heavy metals and other contaminants uptake by tree species. Study on tree establishment and efficiency of eight Eucalyptus species with application of municipal and industrial swage of Yazd city showed that *E. camaldulensis* concern to adaptation, height and diameter growth also water absorption was more successful compared with other species.

Zink toxicity effects on growth and nutrition of *E. maculate* and *E. urophylla* were studied in greenhouse. Critical level of Zink toxicity in *E. maculate* and *E. urophylla* were 835 mg/kg and 697.8 mg/kg respectively (Soares et al., 2001).

Study (Martinez-Sanchez et al., 2012) showed that in arid zone under mining activities Zygophyllum fabago is able to act as Iron accumulator and Arthrocnemum macrostachyum accumulates Iron, Arsenic and Manganese. Also decreasing amount of Zinc is parallel with increasing amount of Cadmium in Maize plants (Naier and Singh, 1989). Research indicated that increasing the level of Cadmium is parallel with decreasing amount of Zinc in tomato (Yildiz, 2005). Decreasing amount of Zinc after increasing amount of Cadmium is probably due to chemical similarity of these 2 elements and their antagonistic relation with each other. Concentration of Iron (Fe), Copper (Cu) and Zinc (Zn) in tea plant increased with increase in Lead (Pb) of soil (Pugh, Dick and Fredeen, 2002).

Hypothesis of recent research is based on this fact that concentration of different heavy metals in soil affecting on specific elemental concentration inside plant organs. Hence goal of this research is investigation the effect of different concentration of Cu, Pb and Cd and composition of these elements in soil and relation of Zn accumulation inside organs of Eucalyptus.

Material and methods

Eucalyptus seedlings obtained from relevant seeds in greenhouse and all seedlings kept in temperature between 18-24 centigrade degree and relative humidity of 60-80% in insulated greenhouse. After 2 months of complete establishment of Eucalyptus seedlings in greenhouse, different treatment heavy metals were applied. In this study 3 treatment of Cadmium (Cd) with concentration of 5, 10 and 15 p.p.m, 3 treatment of Copper (Cu) with concentration of 5, 10 and 20 p.p.m, 3 treatment of Zinc (Zn) with concentration of 1, 3 and 10 p.p.m, 3 treatment of Lead (Pb) with concentration of 5, 100 and 200 p.p.m, and 3 treatment mixture and combination of this elements in 3 levels as Cu (5) Zn (1) Pb (50) Cd (5) p.p.m and Cu (10) Zn (3) Pb (100) Cd (10) p.p.m and Cu (20) Zn (10) Pb (200) Cd (15) p.p.m with 5 replication as Completely Randomized Design for Eucalyptus species were used.

In order to applying these treatments the control seedling consumed 1 liter of distilled water per week while seedling treatment with different concentration of heavy metals provided by weekly irrigation. In this experiment to preparing concentration of heavy metals, elements of Zn, Cd, Cu and Pb provided as $ZnSO_4$, $Cd(NO_3)_2$, $CuSO_4$ and $Pb(NO_3)_2$ and mentioned concentration prepared in different treatment based on ppm. After 13 weeks application of treatment all Eucalyptus plants harvested and divided as leaf, stem and root. All plant samples were dried in oven after 48 hours. All samples changed as powder and after primary preparation concentration of heavy metals inside samples were measured with ICP. Analysis of statistics data carried out with statistic al software of SPSS and LSD Test in level of 95% probability.

Results

Information show that different concentration of Cu, Zn, Cd , Pb and combination treatment in soil have significant effect on Zn uptake into root, stem and leaf of Eucalyptus in level of 5% of probability (Tables 1-4). With increase in Cu concentration of soil until 5 mmol, amount of Zn uptake in root and stem had significant increase but increase in Cu concentration of soil in levels of 10 and 20 mmol, amount of Zn uptake decreased (Table 1). Zn uptake of leaf in *E. camaldulensis* is increased with enhancement of Cu concentration in soil (Table 1). Also variation of Cu of soil leads to variation of Zn uptake in leaf of Eucalyptus so that with increased Pb of soil amount of leaf Pb shows the meaningful increase (Table 5).

Table 1. Zinc (Zn) concentration (ppm) in different treatment of Copper (Cu) inside different organs of *E. camaldulensis*.

Plant components	Control	Cu (5 Mm)	Cu (10 Mm)	Cu (20 Mm)
Leaf	202.30^{b}	195.90^{b}	216.63^{ab}	257.83^{a}
	(12.66)	(19.78)	(12.7)	(35.67)
Stem	136.46^{b}	174.01^{a}	128.93^{b}	139.90^{b}
	(8.35)	(22.92)	(11.81)	(15.95)
Root	202.67^{b}	289.40^{a}	145.00^{c}	232.07^{b}
	(23.71)	(21.63)	(12.94)	(28.29)

Small letters in each row show the significant difference in level of 95% concern to different treatments.

With increase in Cd concentration of soil until 10 mmol, amount of Zn uptake in root increased while increased Zn more than 10 mmol leads to significant decrease in Zn uptake in root of Eucalyptus. Increase in soil Cadmium (Cd) leads to increase amount of Zn uptake in leaf and stem.

Table 2. Zinc (Zn) concentration (ppm) in different treatment of Cadmium (Cd) inside different organs of E.camaldulensis.

Plant components	Control	Cd (5 Mm)	Cd (10 Mm)	Cd (15 Mm)
Leaf	202.30^{b}	273.60^{a}	217.30^{b}	263.03^{a}
	(12.66)	(23.36)	(12.35)	(30.11)
Stem	136.46^{b}	159.44^{b}	183.33^{a}	184.36^{a}
	(8.35)	(18.68)	(12.34)	(6.75)
Root	202.67^{b}	369.73^{a}	376.33^{a}	135.47^{c}

	(23.71)	(20.04)	(11.24)	(6.33)

Small letters in each row show the significant difference in level of 95% concern to different treatments.

With increase in Pb concentration in soil of Eucalyptus seedlings it has not any effect on Zn uptake in stem of these seedlings. While Pb increase leads to increase Pb concentration of root and leaf of Eucalyptus significantly.

Table 3. Zinc (Zn) concentration (ppm) in different treatment of Lead (Pb) inside different organs of *E. camaldulensis*.

Plant components	**Control**	**Pb (5 Mm)**	**Pb (100 Mm)**	**Pb (200 Mm)**
Leaf	202.30^{b}	252.78^{ab}	206.36^{b}	266.40^{a}
	(12.66)	(40.16)	(25.27)	(23.92)
Stem	136.46^{a}	112.00^{a}	136.90^{a}	145.67^{a}
	(8.35)	(28.48)	(22.44)	(31.5)
Root	202.67^{b}	220.13^{b}	489.33^{a}	547.47^{a}
	(23.71)	(40.99)	(50.34)	(40.36)

Small letters in each row show the significant difference in level of 95% concern to different treatments.

Table 4. Zinc (Zn) concentration (ppm) in combination treatment inside different organs of *E. camaldulensis*.

Plant components	**Control**	**Cu(5), Zn(1), Pb(50), Cd(5)**	**Cu(10), Zn(3), Pb(100), Cd(10)**	**Cu(20), Zn(10), Pb(200), Cd(15)**
Leaf	202.30^{c}	402.33^{b}	362.33^{b}	500.90^{a}
	(12.66)	(22.74)	(30.29)	(38.78)
Stem	136.46^{ab}	125.63^{b}	152.05^{ab}	165.87^{a}
	(8.35)	(9.93)	(19.21)	(20.53)
Root	202.67^{b}	230.80^{b}	275.60^{a}	128.57^{c}
	(23.71)	(13.92)	(24.58)	(14.37)

Small letters in each row show the significant difference in level of 95% concern to different treatments.

Table 5. Index of TF relevant to different treatments.

Treatments	**TF**
Cu (5 Mm)	127.82
Cu (10 Mm)	238.32
Cu (20 Mm)	171.38
Zn (1 Mm)	176.68
Zn (3 Mm)	166.98
Zn (10 Mm)	144.74
Pb (5 Mm)	165.71
Pb (100 Mm)	70.15
Pb (200 Mm)	75.27
Cd (5 Mm)	117.12
Cd (10 Mm)	106.46
Cd (15 Mm)	330.25
Cu(5) Zn(1) Pb(50) Cd(5)	228.75
Cu(10) Zn(3) Pb(100) Cd(10)	186.63
Cu(20) Zn(10) Pb(200) Cd(15)	518.6
Control	167.14

Also in combination treatment when amount of Cu, Pb and Cd are increased the concentration of these elements is increased in leaf but show decrease inside root.

TF index or concentration of Zinc inside aerial parts over concentration of Zinc inside root calculated based on following formula:

$$TF=(C_{aeriel\ parts}/C_{roots}) \times 100$$

The least amount of TF is relevant to Pb (100 Mm) and the most amount of TF is relevant to combination treatment of Cu (20) Zn (10) Pb (200) Cd (15).

Conclusion and discussion

Research in recent years indicated that environmental contaminants are enormous and more than former imagination and some of these contaminants persisting in environment for a long time and accumulate in a level that are harmful for human life (Gratao et al., 2005).

Copper and Manganese are required micronutrients for plant growth but in case of high concentration leads to toxicity in plant. For instance if concentration of Copper (Cu) is more than 40 mg/kg dry weight of plant leads to plant toxicity and toxicity in livestock. Hence heavy metal pollution appeared as a serious problem all over the world. One of new method for phytoremediation is Phytoextraction which apply metal accumulator plants to clean up environment containing high amount of heavy metals. These plants are able to accumulate large amount of metals in aerial and harvestable part.

Study on Avicennia marina showed that the highest rate of elements in root and leaf belonged to Zinc (Shete et al., 2007). Mentioned Researchers expressed that Zinc is an essential element hands research by Macfarlane indicate that Avicennia marina has tendency to accumulate essential metals (MacFarlane et al., 2007).

Hyperaccumulation of Zinc (Zn) and lead (Pb) by *Thlaspi praecox* accompanied with Mychohriza fungi were studied in Slovenia by (Vogel-Mikus K, et al 2005) observed accumulation over 14590 mg/kg in aerial parts. The rate of metal in stem/root was 9.6 for Zn and 0.8 for Pb in root (Vogel-Mikus et al., 2005).

Machado (Machado et al., 2002) found the least amount of Zn in leaf. They believed that week transfer of Zn from leaf is due to preservation of Zn in tree.

Based on study of Baycu (Baycu et al., 2006) native species of Populus with regards to rapid growth rate is able to absorb large amount of Nitrogen compounds, Cu, Pb and specially Zn .

From environment and accumulate in leaves. Hence they introduced Populus as the best species to uptake Zn via leaves.

Copper (Cu) accumulation inside stem and rhizome of Nuphar variegatum is higher when the feeding media is sediments while Zn concentration of plant is high when the amount of Zn is high in water (Campbell et al., 1985).

Investigation of short term and long term effect of Iron (Fe) on Cadmium (Cd) uptake in 2 plant species showed that Fe shortage leads to increase in Cadmium uptake in one species so that Cadmium uptake was 3 times when Fe availability is low. With regards to mentioned results treatment with different concentration of Copper (Cu) cause variation of Zinc (Zn) uptake in root, stem and leaves of Eucalyptus (Lombi et al., 2002).

Study of micro and macro elements of mine region of RioTint in Spain regards to rhizosphere soils and different parts of Mediterranean heath (*Erica carnea* L.) show that this plant has ability of growth on different beds containing high Arsenic, Copper, iron and Lead. Study also indicated that Mediterranean heath is able tolerate wide range of Acidity (pH) and toxic elements. High ability of this plant for adaptation to habitat with hard conditions, introduced this plant as a interesting plant for plant establishment and extension of self efficient vegetation cover in Riotinto (Monaci et al., 2011). EPA studied on phytoremediation of groundwater contaminants region of Aberdeen in Meryland city. This region allocated to chemicals and war debris during 1940-47. Chemicals including detergents and industrial solvents and were the main problems of region groundwater therefore Populus alba planted during 1996 in amount of 183. Trees absorbed groundwater contaminants and simultaneously degraded them in rhizosphere. Adjacent groundwater showed that pollution extension toward clean groundwater prevented. Researchers calculated that groundwater contamination to be decreased as much as 85% during 30 years after beginning of project (EPA, 2012).

Soars arranged a study on zinc toxicity on growth and feeding of *Eucalyptus mauclata* and *E. urophylla* to assess effects of additional Zinc on growth and feeding of Eucalyptus inside greenhouse. Critical amount of Zinc was 835 and 697.8 $mg.kg^{-1}$ in *E. maculate* and *E. urophylla* respectively. Study showed that *Eucalyptus maclata* is more resistant to Zinc compared with *E. urophylla* (Soares et al., 2001).

Ghaderian investigated relation between Lead and Zinc mine of Irankuh and its flora. *M. chenopodiifolia* belonged to chrossiferae family is able to grow inside polluted soil with Pb and Zn in Irankuh and has great potential in their leaves fir accumulation of Lead (pb) in natural condition.

Information show that different concentration of Cu, Zn, Cd , Pb and combination treatment in soil have significant effect on Zn uptake into root, stem and leaf of Eucalyptus in level of 5% of probability (Tables 1-4). With increase in Cu concentration of soil until 5 mmol, amount of Zn uptake in root and stem had significant increase but increase in Cu concentration of soil in levels of 10 and 20 mmol, amount of Zn uptake decreased (Table 1). Zn uptake of leaf in *E. camaldulensis* is increased with enhancement of Cu concentration in soil (Table 1). Also variation of Cu of soil leads to variation of Zn uptake in leaf of Eucalyptus so that with increased Pb of soil amount of leaf Pb shows the meaningful increase.

With increase in Cd concentration of soil until 10 mmol, amount of Zn uptake in root increased while increased Zn more than 10 mmol leads to significant decrease in Zn uptake in root of Eucalyptus. Increase in soil Cadmium (Cd) leads to increase amount of Zn uptake in leaf and stem.

With increase in Pb concentration in soil of Eucalyptus seedlings it has not any effect on Zn uptake in stem of these seedlings. While Pb increase leads to increase Pb concentration of root and leaf of Eucalyptus significantly.

Also in combination treatment when amount of Cu, Pb and Cd are increased the concentration of these elements is increased in leaf but show decrease inside root.

The least amount of TF is relevant to Pb) 100 Mm) and the most amount of TF is relevant to combination treatment of Cu (20)

Zn (10) Pb (200) Cd (15).

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