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Full Length Research Paper

Development of a phytoremediation system for metals-contaminated soil

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The present study is dealing with the development of a phytoremediation system such as using the herbal plant ryegrass, *Lolium multiflorum* as excluder plant to investigate its ability to exclude the two metals, copper and zinc from contaminated soil. The plant was grown in different levels of metals-contaminated soil. The translocation of metals in the plant was compared considering root to shoot transport and redistribution of metals in the root and shoot system. The trace metal contents from root and shoot parts were determined using atomic absorption spectrometer. The results showed that the percent of copper transferred to ryegrass plant was 96.01%, while the remained percentage in soil was 3.99% following 60 days of treatment. On the other hand, the percent of zinc transferred to ryegrass plant was 84.51%, while the remained percentage in soil was 15.49% following the same time interval. The soil-plant transfer index in root and shoot system of ryegrass was found to be 0.60 and 0.37, respectively. However these values in case of zinc were 0.51 and 0.32, respectively. These findings indicated that ryegrass is a promising excluder plant and able to exclude either copper or zinc from soil.

Keywords: Phytoremediation, metals, copper, zinc, ryegrass plant, excluder

INTRODUCTION

Heavy metals are considered one of the most serious environmental problems as contaminants of food supply due to their highly persistence in the environment and their potential impact on human and animal health. They are not biodegradable, have long biological half-lives and have the potential to accumulate in different organs causing significant implications for human health (Jarup, 2003). Copper and zinc are essential elements for important biochemical and physiological functions and are carefully regulated by physiological mechanisms in most organisms (ATSDR, 1994; Bremner and Beattie, 1995). However, they are regarded as potential hazards that can endanger both animal and human health. Contamination with copper and zinc can come from electroplating industry, smelting and refining, mining and bio-solids (Liu et al 2005) using pesticides and fertilizers (McGrath et al 2001). Heavy metals are toxic to plants and most of plants cannot survive on polluted soil (Wong, 2003). Many methods have been carried out to develop technologies for soil remediation such as physical-chemical methods and immobilization of metals (Rulkens et al, 1995). The use of plants for contaminated soil remediation named as phytoremediation has gained increasing attention as an emerging effective and inexpensive technology (Macek et al, 2000, Susarla et al, 2002 and Xia et al, 2003). Also, it has a minimal impact on the environment (McKinlay and Kasperek, 1999). There are different categories of phytoremediation, including phytostabilization, phytodegradation, phytovolatilization, phytoextraction and phytofiltration depending on the mechanisms of remediation (Garbisu and Alkorta, 2001 and Greipsson, 2011). Phytoremediation of heavy metal polluted soils was investigated by many investigators (Chen et al, 2004; Wei et al, 2005; Page et at, 2006; Olowoyo et al, 2012; Badr et al, 2012,; Luo et al, 2012). Lone et al, 2008 reviewed the phytoremediation of heavy metal polluted soils and explained that the mechanisms of metal uptake, accumulation, exclusion, translocation and osmoregulation vary with each plant species and determine its specific role in phytoremediation. The aim of our study is to investigate the uptake and transloca-

Table 1: Standard conditions used in determination of copper and zinc and their detection limits using Atomic Absorption Spectrometer.

Element	Wavelength	Slit width (nm)	Fuel	Support	Fuel flow L/min	Detection limit (ppm)
Copper	Copper	324.7	0.5	Acetylene	Air	1.0
Zinc	Zinc	213.9	1.0	Acetylene	Air	1.0

ion of the heavy metals, copper and zinc in the roots and shoots of the herbal plants ryegrass, *Lolium multiflorum* and determine its capability for cleaning polluted soil as a cheaper technology.

MATERIALS AND METHODS

Experimental method

Soil for the experiment was taken from the farm of the College of Agriculture, Alexandria University, Egypt. The soil samples of a depth of 30 cm were taken, air dried, sieved to remove stones, roots and other plant materials and stored at 4 °C prior to use. The pH value and organic matter content in soil were determined and found to be 1.52% and 6.5, respectively. Plastic pots (1L) were used for the experiment. Soil (250 g) was added for each pot. De-ionized water was subsequently added to the soil to maintain the soils at 80% of field capacity. No fertilizer was added into the pot soils. All the treatments were three replicated. Five seeds of *Lolium multiflorum* were planted each pot. The pots were daily irrigated with deionized water and the plant left to grow under greenhouse conditions where the temperature kept at 20°C and daily light period of 13 hrs. Metal salts of copper carbonate and zinc carbonate at different concentrations of 0, 100, 200, 400 and 600 µg/g soil were spiked into the pot soil. Meanwhile, the treatment without external heavy metal addition was regarded as the control. Plants were harvested after 60 days of the beginning of the experiment. Plants were washed carefully to remove dust and fine soil particles and divided into root and shoot parts. Roots and shoots were left to dry at room temperature. The samples were dried at 105 °C in an oven for 24 hrs. Dried samples were ground to a powder. Five grams of each sample were placed in crucible and few drops of concentrated nitric acid were added to the solid as an ashing aid. Dry-ashing process was carried out in a muffle furnace at 600°C. The ash was rinsed with 1M nitric acid and filtered.

Spectroscopic analysis

Samples were subsequently analyzed for heavy metal contents, as dry weight basis, using an atomic absorption spectrometer (AAS). Measurements were made using the hollow cathode lamps for Cu and Zn at the proper wavelength and the slit width were adjusted and other AAS conditions employed in these determinations are summarized in Table 1.

The flame type used for all elements was air-acetylene. Working solutions were prepared by dilution just before the use of standard solutions for atomic absorption spectroscopy (1000 ppm). For the determination, two solutions were prepared for each sample and three separate readings were made for each solution. The means of these figures were used to calculate the concentrations.

Quality assurance

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Samples were generally carefully handled to avoid contamination. Glassware was properly cleaned and the reagents were of analytical grade. Double distilled deionized water was used throughout the study. Reagents blank determinations were used to correct the instrument readings.

Statistical Analysis

Values of heavy metal content are means of three replicates. Differences in heavy metal content in root and shoot systems were tested for statistical significance using ANOVA. A probability of 0.05 or less was considered significant.

RESULTS AND DISSCUSSION

As a biological process, all plants have a natural ability to uptake elements from soil and translocate them between roots and shoot systems. Heavy metals can also be taken up by roots and transported in the different parts of the plant (Ximenez-Embun et al, 2002). The data obtained showed that the herbal plant ryegrass, *Lolium multiflorum* able to uptake and translocate either copper or zinc. The amount of metal uptake was directly proportional with the contamination level. The amounts of copper in whole plant were found to be 89.20, 193.4, 422.91 and 588.78µg/g at the contamination levels of

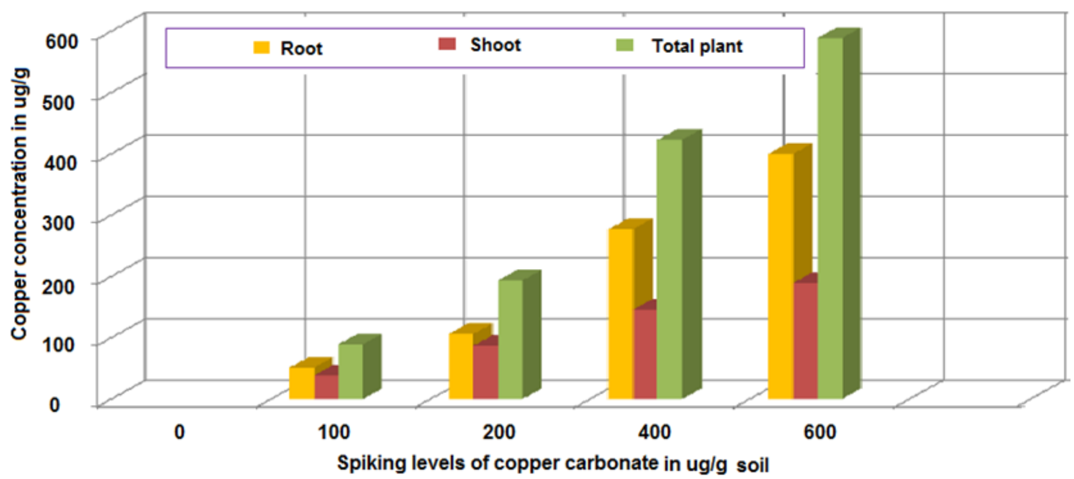

Figure 1: Copper concentration in different parts of the herbal plant ryegrass, *Lolium multiflorum* 60day following spiking of different levels of copper carbonate

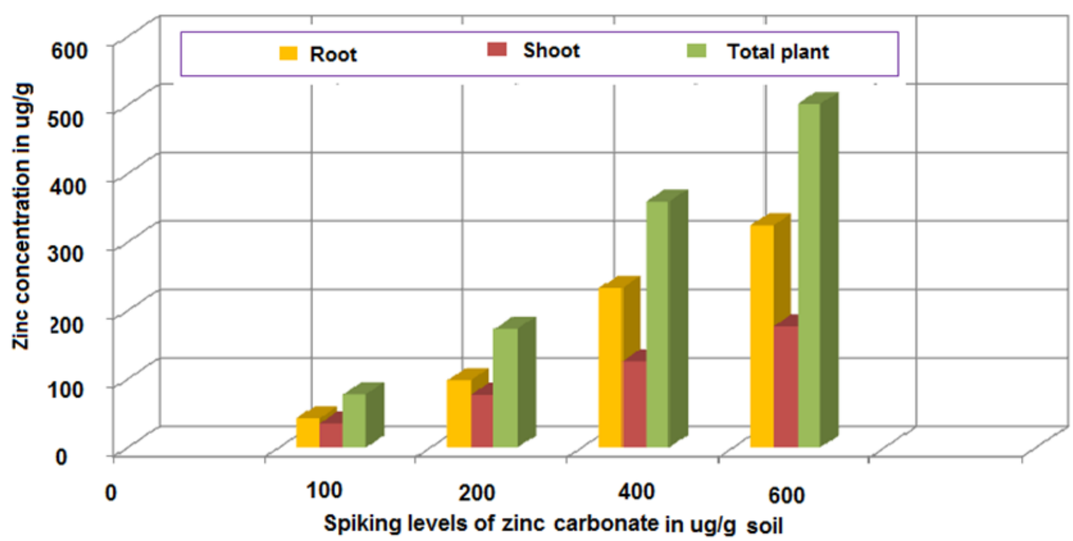

Figure 2: Zinc concentration in different parts of the herbal plant ryegrass, *Lolium multiflorum* 60day following spiking of different levels of zinc carbonate.

100, 200, 400 and 600µg/g, respectively, 60 days following application (Figure 1). In case of zinc, these amounts were 77.87, 173.52, 359.07 and 501.70µg/g at the contamination levels of 100, 200, 400 and 600µg/g, respectively (Figure 2). The average amount of copper uptake was higher than that found in case of zinc where they were 323.57 and 278.04 µg/g for copper and zinc, respectively.

The translocation of copper and zinc in ryegrass was compared considering root to shoot transport and redistribution of metals in the root and shoot system. The trace

metal contents from root and shoot parts were determined. The results also, indicated that the amount of copper found in roots were 51.01, 106.33, 277.14 and 399.67µg/g at the contamination levels of 100, 200, 400 and 600µg/g, respectively, with an average of 208.54µg/g. However, the amounts found in shoots were 38.19, 87.10, 145.77 and 189.11µg/g at the contamination levels of 100, 200, 400 and 600µg/g, respectively, with an average of 115.04µg/g. The results also indicated that the amounts of copper in roots were higher than that found in shoots. Our findings are in agreement with that found by Wei et al (2005). They found that the amount of copper and zinc in roots were higher than that found in shoots in seven weed plants at the spiking levels of 400 µg/g for copper and 1000 µg/g for zinc. The percent of copper transferred to ryegrass plant was 96.01%, while the remained percentage in soil was 3.99% following 60 days of treatment (Figure 3). On

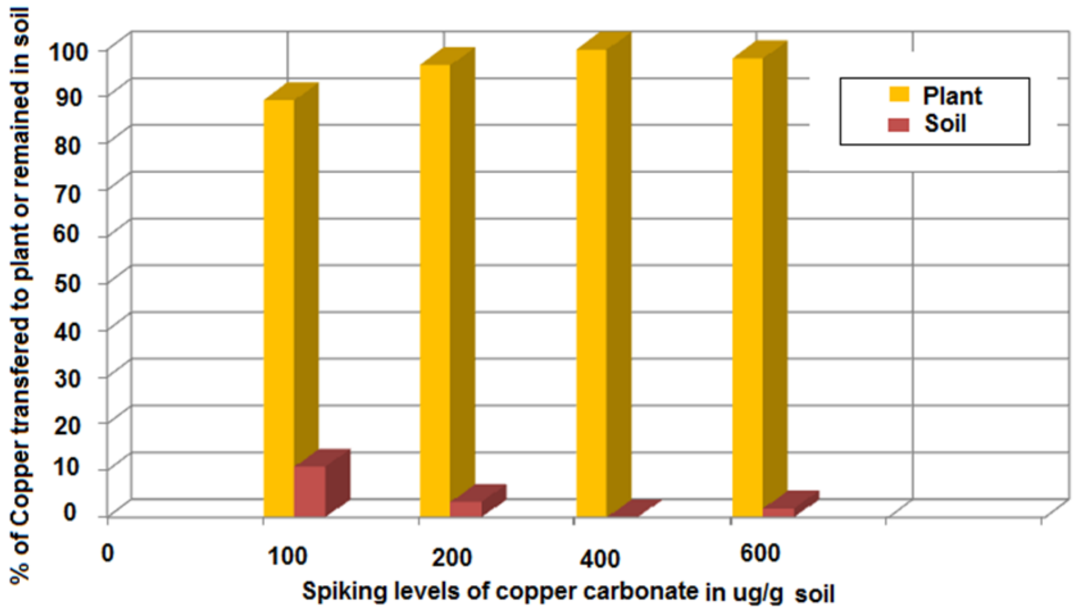

Figure 3: Percent of copper of transferred to ryegrass plant or remained in soil following 60 days of treatment.

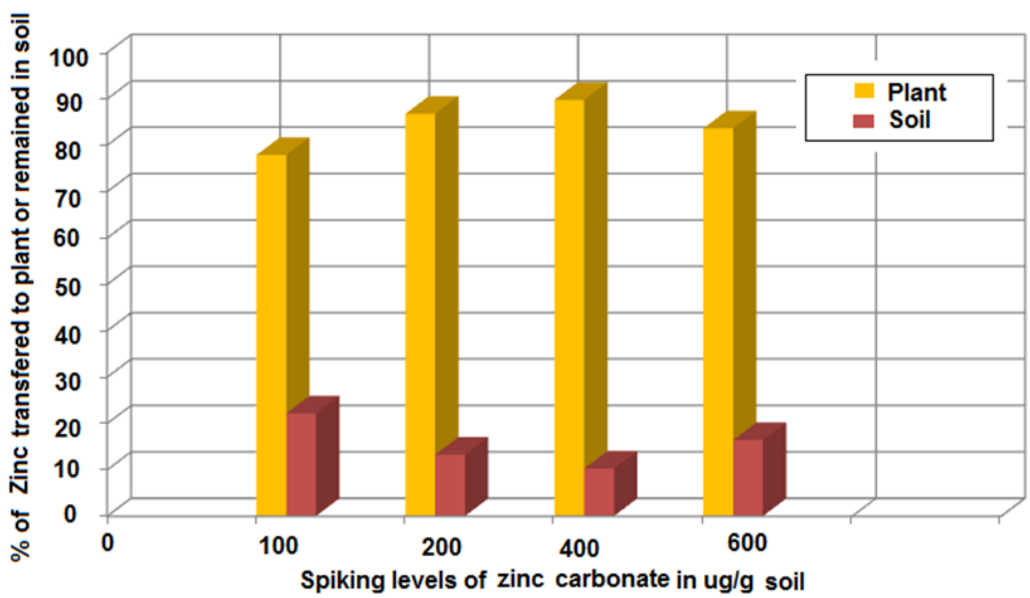

Figure 4: Percent of Zinc transferred to ryegrass plant or remained in soil following 60 days of treatment.

the other hand, the percent of zinc transferred to ryegrass plant was 84.51%, while the remained percentage in soil was 15.49% following 60 days of treatment (Figure 4).

The soil-plant transfer index in root and shoot system of ryegrass was found to be 0.6 and 0.37, respectively (Figure 5). However these values in case of zinc were 0.51 and 0.32, respectively (Figure 6). The transfer factor was calculated as the concentration of heavy metal in plant parts to the concentration present in the soil according to Olowoyo et al (2010). They stated that transfer factor ratios <1 indicated that plants exclude the element from soil (excluder). Shtangeeva et al (2001) concluded that the cultivation of plants produced important variations in soil chemistry because the organic compounds released from plant roots can partially dissolve tightly bound forms of heavy metals, resulting in increase of element uptake. Our findings indicated that the herbal plant ryegrass is copper and zinc excluder plant where it is able to exclude either copper or zinc from soil. Such excluder plants can normally survive in heavy metals-contaminated soil

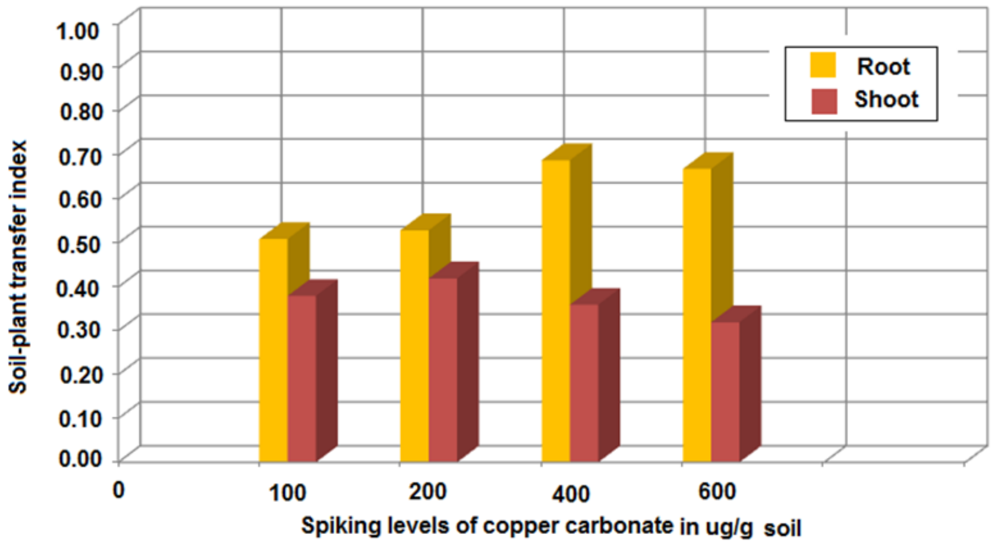

Figure 5: transfer factor for copper concentration in different parts of plant

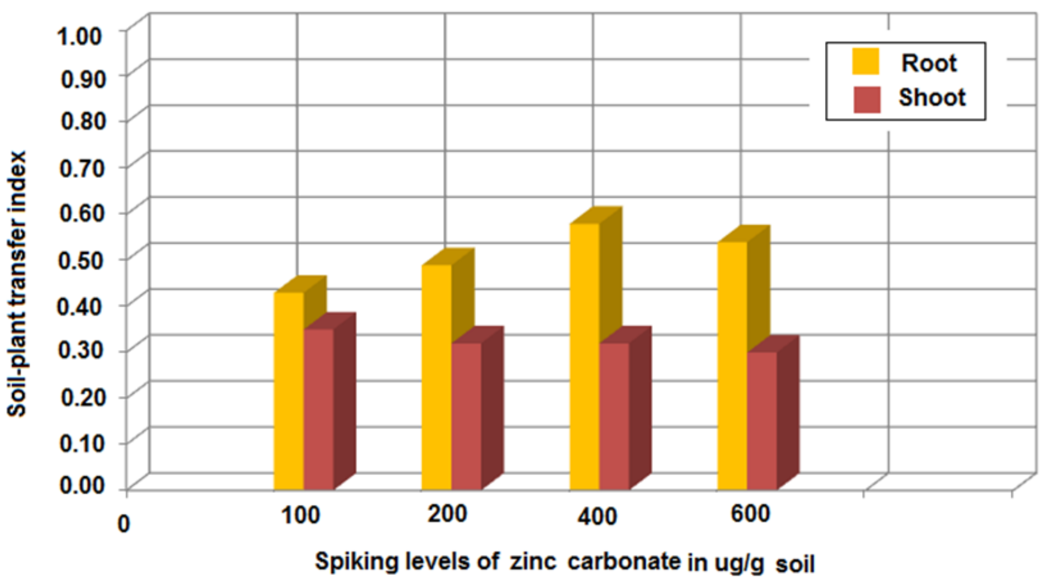

Figure 6: transfer factor for Zinc concentration in different parts of plant

where low heavy metal concentrations occurred in shoots compare with roots. The contamination of heavy metals is of great concern due to its potential impact on human and animal health. Therefore, cheaper and effective technologies are needed to protect our natural resources and biological lives. Phytoremediation is a promising technology for remediating contaminated soils.

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