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RESEARCH ARTICLE

PHYTOEXTRACTION OF SOME HEAVY METALS BY OKRA (*Abelmoschus esculentus*) AND ROSSELLE (*Hibiscus sabdariffa* L.) IN DIFFERENT POLLUTED SOILS

***Hammad Khalifeh Hammad Aldal'in**

Department of Medical Support, Al-Balqa Applied University, Al-Karak University College, Al-Karak, Jordan

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ABSTRACT

The potential of Okra and Rosselle for phytoremediation of Fe, Mn, Cu and Zn was investigated. A pot experiment using three types of soil with different properties and concentration of examined metals was conducted. The pots were filed with 3 kg of garden soil. The plants were grown for a period of 40 days. The results showed that Okra and Rosselle are hyperaccumulators for Fe, where the highest translocation factor values were 312 and 119 respectively. The high content of organic matter in polluted soil increased the growth parameters of both crops, however, crops performed better as metal extractors in the moderately polluted soil.

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INTRODUCTION

Human activities are the main reasons for soil pollution and release of contaminants and heavy metals into environment. Heavy metals are generally considered as those metals and semi metals with potential human or environmental toxicity and defined as metals with a density higher than 5 g/cm^3 (Weast, 1984). Seventeen heavy metals are available and have importance for living cells and ecosystem, among these metals are Fe and Mn as micro-nutrients and Cu and Zn as toxic elements with low or higher importance as trace elements (Nies, 1999). Heavy metals make significant contribution to environmental pollution as a result of anthropogenic activities such as mining, energy- and fuel production, power transmission, intensive agricultural practices, sludge and industrial effluent dumping and military operations (Pilon-Smits, 2005). Metals such as cadmium, cobalt, chromium, copper, lead, manganese, mercury, nickel, selenium and zinc were considered as the major environmental pollutants and their toxic effects on plants has already been established (Cseh, 2002; Fodor, 2002). A common characteristic feature of heavy metals in general regardless of whether they are biologically essential or not is that they may exert toxic effects to plants in low concentrations compared to macro nutrients and these metals are considered to be cytotoxic and mutagenic although a few of them are essential for metabolic processes (Kabata-Pendias and Pendias, 2001).

****Corresponding author: Hammad Khalifeh Hammad Aldal'in***
Department of Medical Support, Al-Balqa Applied University, Al-Karak University College, Al-Karak, Jordan.

Availability and toxicity of heavy metals in the environment, responses and adaptive strategies of plants to metal toxicity and phytoremediation technology etc. have been extensively been explained and were reviewed by several authors (Migocka and Klobus, 2007). Elevated concentrations of both essential and non-essential heavy metals in the soil and water can lead to toxicity symptoms and growth inhibition in most plants (Jindal, and Kaur, 2000; Hall, 2002). Absorption, translocation and accumulation of heavy metal ions of Fe, Mn, Cu and Zn reduce qualitative and quantitative productivity of the plant species and cause serious health hazards through the food chain to other life forms. In this study, the removal of micro-nutrients including Fe, Mn, Cu and Zn by Rosselle and Okra plants from contaminated soil was examined in pot experiments.

MATERIALS AND METHODS

Soil sampling and preparation

Homogeneous soil samples were collected from contaminated and non-contaminated sites at depth of 0-30 cm using hand spade. All the samples were air dried for five days, pulverized, passed through 2mmsieve and preserved for initial analysis and use in experimental pots

Pot culture experiment

The experiment was arranged in randomized complete block design (RCBD) in three replicates for Okra and Rosselle and three types of polluted soil plus non cultivated pots to estimate the leaching of metals by irrigation water. Plastic pots were used in the experiment. Three Kg of air-dried soil were weighed and transferred into a plastic pot 20 cm in diameter. Five seeds of each particular crop were planted at a depth of two cm in the soil in each pot. One week after the germination, the seedlings were thinned to two plants for each crop. No fertilizer added, irrigation was performed equally for all experimental pots, weeds were controlled manually by hand. Plants were harvested after 40 days of germination for heavy metals analysis.

Plant analysis

Samples preparation, digestion and determination of heavy metals

After 40 days of the germination, the soil of each pot was broken up and whole plants were gently removed from the pots. Harvested plants were washed thoroughly with running tap water, then deionized water to remove adhered soil particles. Roots and shoots were separated, oven-dried at 72 C for 48 h to a constant weight.

Wet digestion for plant materials

Oven dry samples of shoots and roots were ground and passed through 1.0 mm sieve and digested with a mixture of HNO_3/H_2O_2. The temperature was maintained at 120˚C for 2 h during digestion of 1.0 g of plant sample with 16 mL of 3:1 HNO_3/H_2O_2 mixtures on the hot plate. After cooling, the volume was completed to 50 mL by adding distilled water. The solution was filtered through filter paper 45 µ. Metal contents of the final solution were determined by ICP-AES.

Soil analysis

Ethylenediaminetetraacetic acid-EDTA solution at concentration of 0.05 M was used for determining the extractable metals in the soil samples before and after the experiment. Ten gram of soil of each sample and 20 ml of EDTA solution were added to a conical flask 50 cm3. The mixture was shaken using end to end shaker at 220 rpm for one hour. All suspensions were passed through filter paper 45 µ before analysis at ICP-AES.

Determination of Soil pH

50 ml of distilled water was added to 50 g of air dried soil and allowed to equilibrate for 30 minutes. The pH of the solution was measured using a calibrated pocket pH meter. Calibration of the pH meter was done using two buffer solutions with pH’s 4 and 7.

Soil salinity determination

For each soil sample, salinity was determined from electrical conductivity (EC) measurements in soil/water suspensions at soil: water ratios (1:1 w/v). Electrical conductivity (EC) of soil/water suspensions was measured by using distilled water in soil saturated paste extract. After mixing, soil saturated pastes were left to stand for overnight (25 C) before filtration. Soil/water suspensions were shaken manually and then filtered through Whatman filter paper No 42. EC extracts were measured by using a calibrated conductivity meter.

Organic Matter (OM)

A weighed amount of oven-dried soil sample (105ºC; 24 h) was placed in a high-form porcelain crucible and set in a muffle furnace (± 5ºC precision) for combustion at 550ºC for 5 hours (Goldin, 1987). The organic matter content was determined through the mass difference in relation to the original soil sample.

Calculation of TF and BCF

Translocation and bio-concentration factors were calculated for the investigated crops to compare their suitability for phytoremediation. The two factors were calculated according to (Kabata- Pendias and Pendias, 2001; Fitz and Wenzel, 2002) as follows:

Calculation of translocation factor (TF)

TF = ((Metal) shoot /(Metal)Root)…………………………(1)

BCF = ((Metal) root) / ((Metal) soil)………………………. (2)

RESULTS AND DISCUSSION

Soil analysis

The initial concentration of all examined metals in the three soils is exceeding the permissible limit as shown on table (1). The content of organic matter in these soils is an indication of the organic waste dumping and pollution. Fe, Mn, Cu and Zn are micro-nutrients essential for normal plant growth, but only a small amount of these metals are required for the normal growth.

Translocation factor (TF)

Translocation factor is defined as the ability of plants to transfer accumulated metals from the roots to the shoots and measured by dividing the concentration of metal in the shoot by that in the roots (Cui *et al*., 2007). All plants have TF and BCF more than one (>1) for specific metal have the potentiality to be used for phytoextraction for that metal.

Table 1. Initial soil properties of study areas

Level of soil pollution	pH	EC/ppm	O.M (%)	Metals conc. (ppm)				Soil texture
				Fe	Mn	Zn	Cu	
High	6.8	742.4	13.78	164.75	12.35	65.25	46.63	Loamy sand
Moderate	7.7	1113.6	4.37	24.05	12.00	25.40	10.94	Loamy sand
Slight	8.2	41.6	2.04	18.50	16.00	24.20	3.31	Sand
Permissible limit				5*	n/a	1.5*	0.5*	

*= limits described by Soltanpour, 1985.

As it is shown on Table (2), the translocation factor is different for the soils and metals. Okra and rossele showed very high extraction for Fe in all polluted soils. The extraction of Mn by okra is high in moderate and slight polluted soil and low in high polluted soil. Rossele showed the opposite where the extraction Mn was higher in the high and moderate polluted soil and week in the slight soil. The extraction of Cu and Zn by okra was very week, the content was below the detection level. On the other hand the extraction of Zn and Cu by rosselle was observed in high and moderate polluted soil. From the results it is clearly that metal uptake by plants can be affected by several factors including metal concentrations in soils, soil pH, occurrence of other metals and cationic exchangeable capacity of the soil , organic matter content, plant species , and plant age. However, the concentration of the metal is dominant factor as reported by Adriano, 1986. Plant can be classified as hyper accumulator for Cu, Zn and Mn when the concentration of the metal is 100 mg/Kg for Cu and 10.000 mg/kg for Zn and Mn and there is no evident of toxicity symptoms (Baker and Brooks, 1989). According to the results of this study, both plants are not hyperaccumulator for Mn, Cu and Zn.

The reason of better growth of these crops in the polluted soil could be mainly attributed to the high content of the organic matter and nutrients which exceeding the permissible limit.

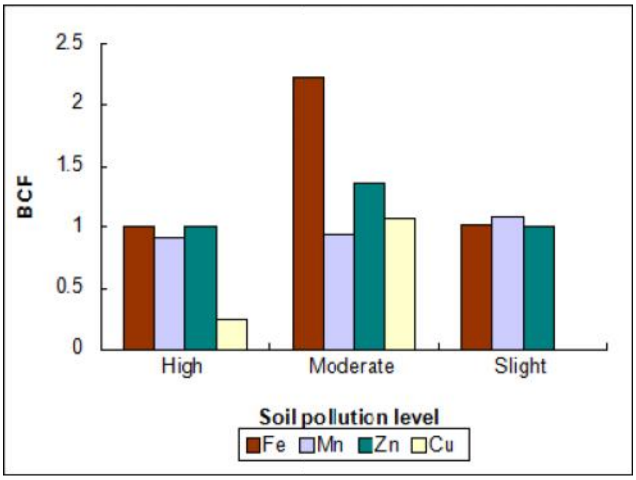

Figure 1. Bioconcentration factor for Okra

Table 2. Translocation factor for Okra and Rosselle

Metals	Crop					
	Okra			Rosselle		
	Soil pollution level			Soil pollution level		
	High	Moderate	Slight	High	Moderate	Slight
Fe	36.07	73.15	312.99	25.75	119.03	85.06
Mn	0.38	1.33	3.65	70.00	4.3	0
Zn	0.00	0	0	76.00	0.79	0
Cu	0.00	0	0	0.00	2.37	0

Table 3. Effect of soil pollution on the growth parameters of Okra and Rosselle

Crop	Soil pollution level	Shoot fresh weight /plant /g	Shoot dry weight /plant /g	Root fresh weight/plant/g	Root dry weight/plant/g	Plant height/cm	Root length/cm
Okra	High	7.34 ± 0.7	1.05 ± 0.2	1.32 ± 0.4	0.21 ± 0.1	40.5 ± 3.5	39.0 ± 17
	Moderate	6.02 ± 0.5	1.24 ± 0.0	1.44 ± 0.1	0.29 ± 0.0	36.0± 2.8	42.5 ± 16
	Slight	1.65 ± 0.1	0.34 ± 0.0	1.57 ± 0.3	0.17 ± 0.0	22.0 ± 1.4	20.5 ± 4
Rosselle	High	5.01 ± 0.6	0.70 ± 0.1	0.87 ± 0.1	0.16 ± 0.0	30.0 ± 1.4	30.5 ± 0.7
	Moderate	4.00 ± 0.3	0.80± 0.0	2.10 ± 0.1	0.29± 0.0	23.0± 1.4	34.5± 3.5
	Slight	1.40 ± 0.1	0.27 ± 0.0	1.51 ± 0.1	0.19± 0.0	18.0 ± 2.8	25.4 ± 2.1

Bioconcentration factor (BCF)

Figure 1 and 2 show the bioconcentration factor (BCF) or the ratio of metals concentration in plants roots to concentration of the metals in soil. The root BCF factor of each crop is different based on the concentration of metal. Okra and rosselle showed the highest BCF factor for Fe in the moderate polluted soil, where the values were 2.23 and 2.21. The concentration of Cu was not detected in the roots of both crops cultivated in the slight polluted soil.

Plant growth parameters

The growth parameters of Okra and Rosselle were measured at the end of experiment. Plant height from the soil level to the top, root length, shoot and root fresh and dry weight on two plants selected from each replicate and for each treatment. All the values of growth parameters for okra and rosselle were higher in the high and moderately soil than the slightly polluted, however, some symptoms of toxicity were observed.

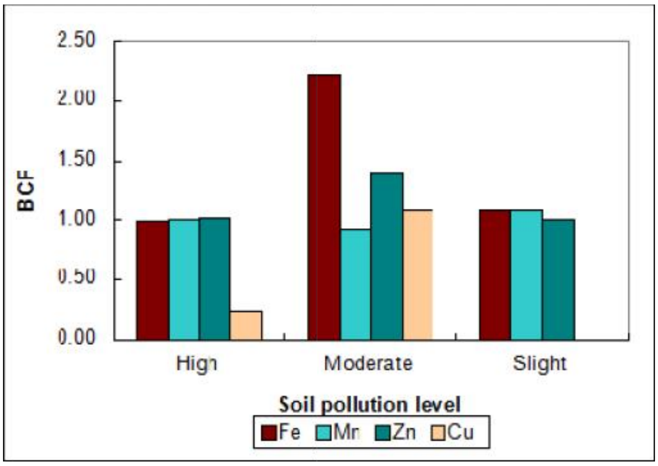

Figure 2. Bioconcentration factor for Rosselle

Figure 3. Variation on plant height due to organic matter content and toxicity stress

Figure 4. Toxicity symptoms of Fe on Rosselle leaves

Conclusion

This study examined the relative phytoextraction capacity of Okra and Rosselle plants for Fe, Mn, Cu and Zn in different contaminated soils. The results showed that both plants are suitable for extraction of Fe. High content of organic matter improved the growth parameters for both plants, however, the extraction of examined metals was decreased. Okra and Rosselle are hyper-accumulator of Fe, however, their practical use for soil remediation is difficult due to their poor root growth.

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