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Effect on plant growth and heavy metal accumulation by sunflower

Lucia Helena Garófalo Chaves[1], Maria Alexandra Estrela[1] and Ramara Sena de Souza[1]

[1]Department of Agricultural Engineering, Federal University of Campina Grande, Campina Grande, PB, Brazil

Abstract
The aim of this present study was to determine the effects of heavy metals (Cd, Cu and Zn) on plant growth, tissue concentration and examine their uptake by sunflower in soil. Three experiments (each corresponding to each metal) in a greenhouse were carried out in a complete randomized design, in each experiment, with five treatments and three replications, totaling 15 experimental units. Cadmium was applied at the rate of 0; 10; 20; 30 and 40 mg dm^{-3} of soil; copper and zinc levels were 0; 20; 40; 60 and 80 mg dm^{-3} of soil. Plant tops were harvested from 50 days after sowing. Cadmium and Zn both reduced the plant height of sunflower plant at higher concentration. The higher concentrations of Zn showed a significant decrease in leaf area. The heavy metals have no significant effect on plant dry matter but had significant effect on the accumulation of these elements in the plant parts, stem, leaves and roots.

Keywords: Phytoremediation, Cadmium, Copper, Zinc

INTRODUCTION

Heavy metals, such as zinc (Zn) and copper (Cu) are required in trace amounts by higher plants to complete their life while others, i.e., cadmium (Cd), are non-essential. In extended concentrations, however, all heavy metals are toxic (Riesen and Feller, 2005).

Zinc is an important component of many vital enzymes having catalytic, co-catalytic and structural role as structural stabilizer for proteins, membranes and DNA binding proteins (Zn-fingers). Zn is found to be involved in many cellular functions such as protein metabolism, photosynthetic carbon metabolism and indole acetic acid metabolism, yet its higher concentrations cause toxicity (Sinhal, 2007). One of the first symptoms of Zn deficiency is an inhibition of cell growth and proliferation. Zinc affects growth of shoots and roots and growth symptoms of Zn toxicity in plants, generally, are similar to those of Zn deficiency. One of the primary mechanisms of Zn toxicity may be an increased permeability of root membranes, which will cause nutrients to leak out from the roots (Kabata-Pendias and Pendias,1992).

Copper is one of the essential micronutrients for plant growth. It is involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reactions in mitochondria and chloroplasts (Hansch and Mendel, 2009). However, in excessive quantities copper becomes toxic as it interferes with photosynthetic and respiratory processes, protein synthesis and development of plant organelles (Upadhyay and Panda, 2009). Specifically excess copper can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Bouazizi *et al*., 2010).

Cadmium ranks the highest in terms of damage to plant growth and human health. Even though these metals are not essential for plants but are readily absorbed by the most root systems because of their water solubility. Higher concentrations of essential or nonessential metal ions, i.e. Cd, are deleterious to metal-sensitive enzymes, resulting in growth inhibition and death of the organism.The growth and metabolism of plants are adversely affected by the increasing levels of these metals in the soil environment (John *et al*., 2009), besides this Cd is known to accumulate in different plant parts and enter into the food chain. Therefore, pollution due to heavy metals is significant from nutritional and environmental point of view.

Metals cannot be degraded, and their cleanup requires their immobilization and toxicity reduction or removal. In recent years, scientists have evaluated technologies which include use of live plants for cleaning of polluted areas. Phytoextraction (uptake) is the use of living green plants in order to remove inorganic contaminants, primarily metals, from polluted soils and concentrate them into roots and easily harvestable shoots (Lasat, 2002; Tang *et al*., 2003).

Plants have shown the capacity to withstand relatively high concentration of contaminants without toxic effects. Some of the heavy metals at low doses are essential micronutrients for plants, but in higher doses, may cause metabolic disorder and growth inhabitation for most of the plant species (Sinha *et al*., 2005).

Sunflower (*Helianthus annuus*) is grown as an oilseed crop worldwide in temperate and subtropical climates. Among oilseeds, sunflower generally ranks fifth behind soybeans, rapeseed, cottonseed, and peanuts. Unlike soybean, sunflower is primarily an oil crop, with high protein meal being a by-product. Sunflower is grown on every continent, with nutritionally, sunflower oil has the greater proportion of the unsaturated fatty acids than many other vegetable oils. The objective of this study is to determine the effects of heavy metals on plant growth, tissue concentration and examine

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*Corresponding Author

Lucia Helena Garófalo Chaves
Department of Agricultural Engineering, Federal University of Campina Grande, Campina Grande, PB, Brazil

Tel: +55-31-8333331100 ; Fax:+55-31-8321011307
Email: lhgarofalo@hotmail.com

their uptake by sunflower in soil.

MATERIAL AND METHODS

The study was carried out from September to December 2010 in a semi-controlled greenhouse condition of the Agricultural Engineering Department of the Federal University of Campina Grande, Campina Grande, Paraiba, Brazil. Temperatures ranged from approximately 32°C during the day to 27 °C during the night.

To evaluate the effect of heavy metals cadmium, copper and zinc in the development of sunflower plants (cv Embrapa122/ V-2000) were installed three experiments each corresponding to each metal. Cadmium (Cd) was applied at the rate of 0; 10; 20; 30 and 40 mg dm^{-3} of soil; copper (Cu) and zinc (Zn) levels were 0; 20; 40; 60 and 80 mg dm^{-3} of soil in de form of cadmium, copper and zinc sulphate, respectively. In each experiment the treatments were arranged as a completely randomized design with five treatments and three replications, totaling 15 experimental units.

The soil with pH 6.4 was watered to field capacity and incubated with heavy metal, in the greenhouse for 25 days before sowing. After this period, a basal dose of NPK (100:300:150 mg kg^{-1}) and 2 kg B ha^{-1} (boric acid) were missed in the processed soil and 8.6 Kg of air dried soil was filled in each polyethylene pot. Ten seeds were sown in each pot and after germination 9 seedlings of equal size were retained.

The plants were sampled 50 days after sowing and the various morphometric growth parameters were employed. Then the samples were kept in hot air oven maintained at 70°C for 48 hours. Dry weight of root and shoot was determined. All the dried samples were digested with a mixture of HNO_3:$HClO_4$ acids (10:1) and analyzed for the concentration of Cd, Cu and Zn in digested plant material by atomic absorption spectrophotometer. Using SISVAR-ESAL, the data were subjected to analysis of regression.

RESULTS AND DISCUSSION

In the present investigation Cd and Zn both were found to be phytotoxic in nature and significantly reduced the plant height of sunflower plant at higher concentration (Table 1 and Fig.1).

These results are in close conformity with the findings using Cd and Zn in Vigna radiata (L.) Wilzeck since higher concentration of these elements exerted toxic effects on growth of this plant (Kumari *et al*., 2011). However, increasing Cu level had no significant effect on sunflower plant height. Cadmium is a nonessential element and exert hazardous effects on plant height of plants while Zn is an essential element for plant growth but its excess amount exert toxic effects on plant height. The higher concentration of heavy metals has been reported to retard the cell division and differentiation, reduce their elongation and effect plant growth and development (Soares *et al*., 2001). The depression in height was 25.92% and 18.03% of untreated plants with cadmium and zinc, respectively. Reduction of height of sunflower of 77% due to cadmium was observed by Gopal and Khurana (2011).

Table 1. Analysis of variance for experimental growth traits

	DF	Mean square			
		Shoot height	Leaf area	Dry matter yield	
				Root	Shoot
Treatment	4	168.19*	19.627ns	0.12ns	0.42ns
Error	10	32.92	8.958	0.08	1.30
CV%		8.54	12.46	5.10	17.70
Cadmium level		---------------------------Mean---------------------------			
(mg dm^{-3})		cm $plant^{-1}$	cm^2 $plant^{-1}$	------g $plant^{-1}$------	
0		78.20	708.81	4.67	6.47
10		69.87	655.76	4.43	6.97
20		65.23	661.59	4.70	6.50
30		64.66	440.19	4.73	5.90
40		57.93	473.88	4.27	6.43
Treatment	4	43.43ns	8.067 ns	0.14ns	0.62ns
Error	10	13.21	7.401	0.05	1.11
CV%		4.80	11.58	5.12	16.01
Copper level (mg dm^{-3})		---------------------------Mean--------------------------			
0		78.20	708.81	4.67	6.47
20		80.57	499.57	4.50	7.20
40		76.00	506.42	4.43	6.40
60		72.00	568.37	4.13	6.00
80		71.93	595.42	4.23	6.80
Treatment	4	84.13*	20.672*	0.11ns	0.60ns
Error	10	20.95	5.575	0.09	2.32
CV%		6.59	10.72	6.97	23.14
Zinc level (mg dm^{-3})		---------------------------Mean---------------------------			
0		78.20	708.80	4.67	6.47
20		67.37	499.59	4.33	6.67
40		69.83	407.09	4.27	5.90
60		67.87	397.32	4.70	7.10
80		64.10	579.69	4.50	6.80

ns, * and ** ; Non significant and significant at the 5 and 1 % levels of probability, respectively

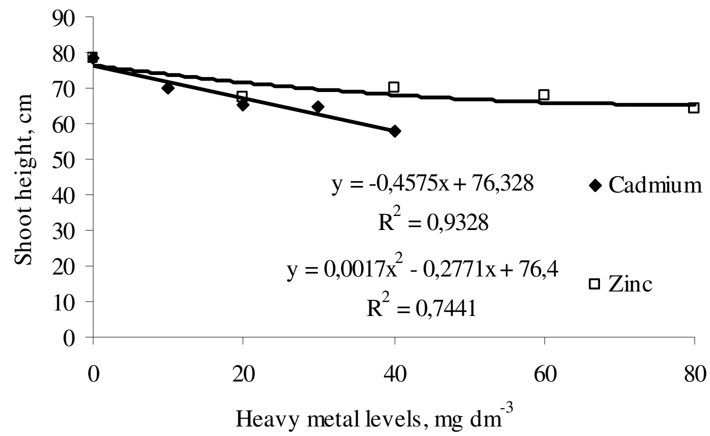


Fig 1. Shoot height as a function of cadmium and zinc levels

Plants treated with Cd and Cu level did not show a significant increase in leaf area, when compared to control, however, the higher concentrations of zinc showed a significant decrease in leaf area (Table 1 and Fig.2). The largest depressions in leaf area were 38%, 29% and 44% of untreated plants with Ca, Cu and Zn, respectively. The inhibitory action of excess zinc in leaf area may be due to reduction in cell division, toxic effect of heavy metal on photosynthesis, respiration and protein synthesis.

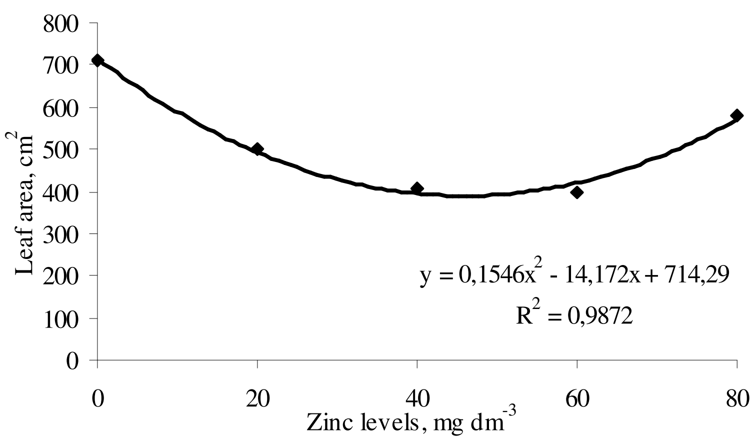


Fig 2. Leaf area as a function of zinc levels

Despite the heavy metals have no significant effect on plant dry matter, the plants treated with cadmium, copper and zinc at low concentrations (10 mg kg^{-1} of Cd and 20 mg kg^{-1} of Cu and Zn) showed a slight increase in dry matter production of shoot, when compared to control. But in higher concentrations, in general, it showed a gradual decline in the dry matter production (Table 1) of shoot and root according to Jadia and Fulekar (2008). Comparing the weight of plant parts was observed that the greatest biomass was the shoots in agreement with the results of Jadia and Fulekar (2008). However these authors researching the heavy metal in sunflower, found smaller biomass than the biomass in this paper.

Reduction of growth of sunflower, Vigna radiata (L.) Wilzeck and black oats due to Cd, Cu and Zn were observed by Gopal and Khurana (2011), Manivasagaperumal *et al*. (2011) and Abranches *et al*. (2009), respectively. The decrease in biomass in excess heavy metal might be due to low protein formation, resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Manivasagaperumal *et al*., 2011).

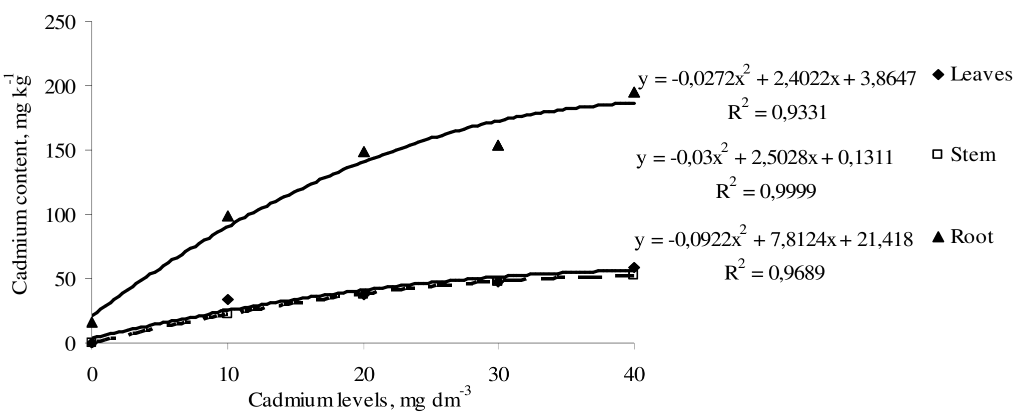
Plants sunflower show high tolerance to heavy metals and therefore, are used in phytoremediation studies (Pilon-Smits, 2005; Schmidt, 2003; Tang *et al*., 2003). Perhaps this justifies the fact that increasing doses of Cd, Cu and Zn have no significant effect on some growth traits.

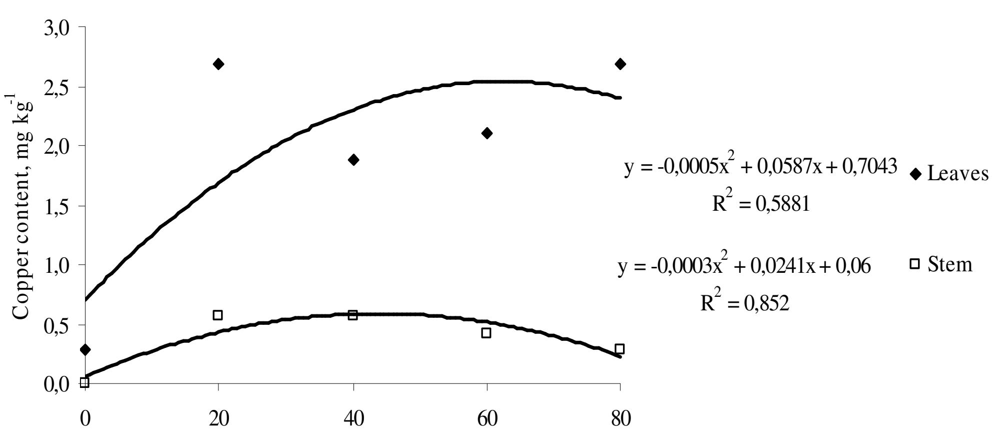
The increasing Cd, Cu and Zn doses had significant effect on the accumulation of these elements in the plant parts, stem, leaves and roots, i.e., the mean uptake of all three metals by sunflower plants increased as the concentration of these metals in the soil increased (Table 2 and Fig.3).

Table 2. Analysis of variance on tissue concentration in different plant parts of sunflower

	DF	Mean square Stem	Leaves	Root
Treatment	4	26.308**	27.626 **	0.139**
Error	10	0.233	0.608	0.0091
CV%		9.66	14.79	9.81
Cadmium level		--------------------Mean, mg kg-1--------------------		
(mg dm-3)		0.00	0.00	15.90
10		22.46	34.06	98.73
20		38.04	37.56	148.63
30		48.07	47.22	153.94
40		52.29	59.18	194.52
Treatment	4	0.293**	0.864**	0.123**
Error	10	0.0024	0.018	0.00046
CV%		9.19	14.67	5.06
Copper level (mg dm-3)		---------------------Mean, mg kg-1-----------------		
0		0.00	0.28	2.81
20		0.56	2.68	29.63
40		0.56	1.88	95.62
60		0.42	2.11	101.17
80		0.28	2.68	174.37
Treatment	4	1038.58**	1713.10**	0.189**
Error	10	5.48	2.94	0.0034
CV%		3.69	2.88	6.31
Zinc level (mg dm-3)		---------------------Mean, mg kg-1--------------------		
0		31.89	18.40	9.43
20		65.85	59.90	95.09
40		64.62	67.32	118.44
60		77.21	75.41	140.24
80		77.31	76.25	136.88

ns, * and ** ; Non significant and significant at the 5 and 1 %levels of probability, respectively





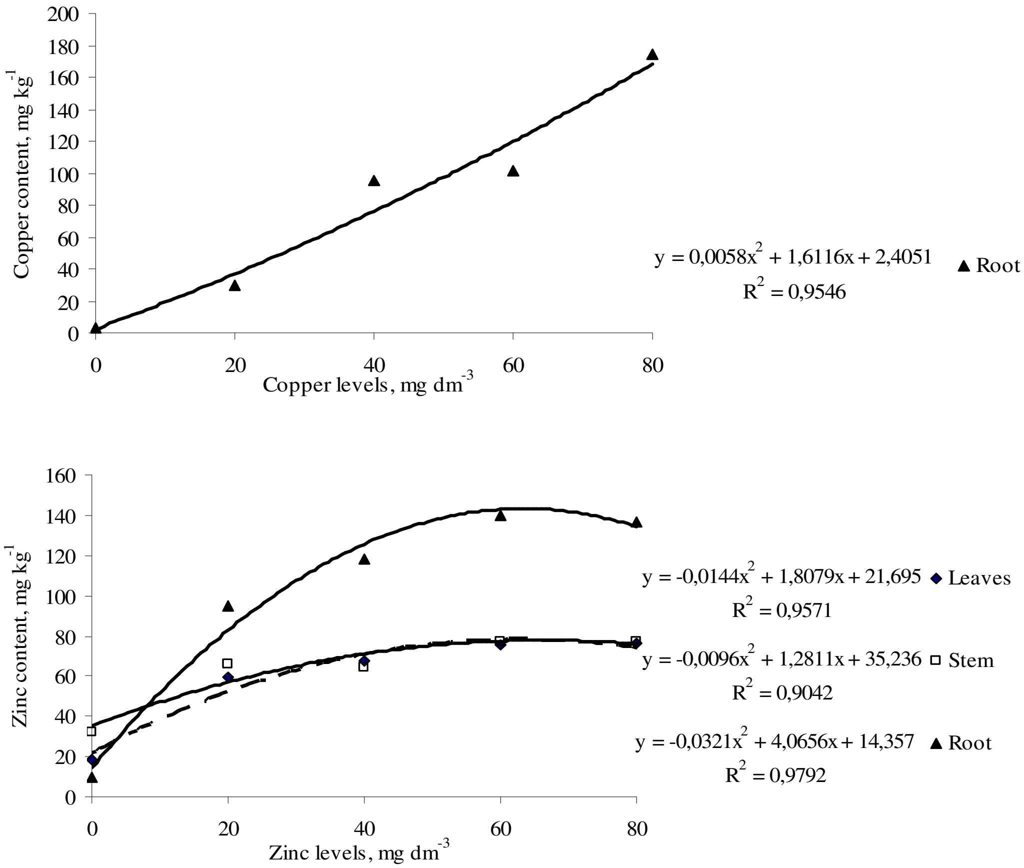


Fig 3. Tissue Cadmium, copper and zinc concentrations in different plant parts of sunflower

In the shoot and root of sunflower the heavy metals were accumulated in the following order: Zn>Cd>Cu and Cd>Zn>Cu, respectively. Among these plant parts, the greater accumulation of these elements was in the roots followed by leaves and stems for Cu; for Cd and Zn the accumulation in the stem and leaves was similar. However, according to Jadia and Fulekar (2008) shoot of sunflower is the major organ of heavy metals accumulation. Nevertheless studies on the heavy metal composition of *Sargassum wightii* showed that the accumulation of Cd was higher in leaves than in stems (Jothinayagi *et al*., 2009) similar to the findings of this study. However, the Cu accumulation was higher in stems, contrary to what was observed.

The Cd concentration in stem and leaves ranged in 0 – 56 mg kg^{-1}, i.e., levels considered toxic, according to Kabatia-Pendias and Pendias (1992) since these authors suggest the value of 5.0 mg kg^{-1} as a value-toxic to plants. According to these results and on the other about roots, the sunflower is considered an accumulator plant since it takes up 10% of the Cd total present in the soil. The Cu concentration in stem and leaves were below critical levels, i.e., below the ranged 20 to 100 mg kg^{-1} but, in root, the Cu concentration corresponded to toxic levels. According to Lasat (2002), a successful decontamination with plants, require plants able to concentrate 1-2% of the excess metal. In this case, these concentrations were obtained only by the roots of sunflower. The Zn concentration in stem and leaves ranged in, more or less, 18-78 mg kg^{-1} and in roots of 9-140 mg kg^{-1}. According to Kabatia-Pendias and Pendias (1992) the values of 100 to 400 mg kg^{-1} are toxic and the plant is accumulator of Zn if the plants take up 1% of the Zn total present in the soil.

CONCLUSION

Thus the present investigation showed that Cd, Cu and Zn at higher concentrations exerted toxic effects on sunflower growth and on the accumulation of these elements in the plant parts, stem, leaves and roots. But, the heavy metals have no significant effect on plant dry matter.

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