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EFFECT OF ZINC TOXICITY ON PLANT PRODUCTIVITY, CHLOROPHYLL AND ZN CONTENTS OF SORGHUM (*SORGHUM BICOLOR*) AND COMMON LAMBSQUARTER (*CHENOPODIUM ALBUM*)

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ABSTRACT: Zinc (Zn) as a heavy metal plays an important role in many biochemical functions of plants. However, the excess amount of zinc is one of the most important growth limiting factors in soils. In the present study, the effects of various concentrations of Zn on biomass, chlorophyll content, and Zn contents of *Sorghum bicolor* and *Chenopodium album* were studied at research field of Urmia University, Urmia, Iran, in 2011. The plants were grown in pots over a 3 month period in soils containing zinc concentration varying between 100.7, 300.7, 500.7, 900.7, 1300.7 and 2100.7 mg_{zn}/kg_{soil}. At the end of growing season, plant height, chlorophyll a, b, and total chlorophyll content, biomass, Zn concentration in the plants and bio-available Zn of the soils were measured. Results indicated that, generally, with increasing Zn concentration in soil, plant height, content of a, b, and total chlorophyll and biomass were decreased significantly ($p \leq 0.05$). With an increase in soil Zn concentration, Zn in Common lambsquarter was increased up to a maximum of 1213 mg/kg (in concentration 2100 mg_{zn}/kg_{soil}). The maximum Zn concentration in sorghum was 2538 mg/kg (in concentration 500 mg_{zn}/kg_{soil}). In addition, there was significant correlation between NH_4NO_3-extractable soil Zn and response of plants to Zn pollution.

Key words: Chenopodium album; phytotoxicity; Sorghum bicolor; Zn; Bio-availability; Biomass.

INTRODUCTION

Zinc (Zn) is one of the important elements of plant growth and development (Bonnet et al., 2000; Misraet al., 2005). Zn plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenases, proteinases, peptidases, and phosphohydrolases (Clarkson and Hanson, 1980; Bowen, 1979). Zinc also plays a vital role in DNA and RNA metabolism, protein biosynthesis, and cytokinesis; it participates in chlorophyll synthesis and protects chlorophyll from decomposition, and it also influences nitrogen assimilation (Alekhina and Kharitonashvilli, 2005). However, high zinc concentrations, like other heavy metals, are toxic for plants (Zhao et al., 2003). Zinc toxicity in crops is far less widespread than Zn deficiency. However, Zn toxicity occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of Zn (Chaney, 1993). Toxicity symptoms usually become visible at [Zn] leaf > 300 $mg_{Zn}\ kg^{-1}$ leaf dry weight (DW), although some crops show toxicity symptoms at [Zn] leaf < 100 $mg_{Zn}\ kg^{-1}$ $leaf_{DW}$ (Chaney, 1993; Marschner, 1995), and toxicity thresholds can be highly variable even within the same species. For example, [Zn] leaf associated with a 50% yield reduction in radish ranged from 36 to 1013 $mg_{Zn}\ kg^{-1}{}_{DW}$ (Davies, 1993). Zn toxicity symptoms include reduced yields and stunted growth, Fe-deficiency-induced chlorosis through reductions in chlorophyll synthesis and chloroplast degradation, and interference with P (and Mg and Mn) uptake (Foy *et al.*, 1978; Chaney, 1993), And disturbance in the intensity of basic physiological processes, i.e., photosynthesis, respiration, and transpiration, and decrease of reproductive performance, too (Ali et al., 2000; Khudsar et al., 2004; Kholodova et al., 2005). Some plant species and genotypes have great tolerance to excessive amounts of Zn. The families Poaceae and Chenopodiaceae are of the most interest, because they include species that are able to grow at high zinc pollution (Likholat et al., 1998; Atabaeva, 2004; Bharagava et al., 2007).

The purpose of this study was to investigate the effect of Zn toxicity on plant growth and chlorophyll content of *Sorghum bicolor* and *Chenopodium album* and also make an estimate of the zinc accumulation in the plants.

MATERIALS AND METHODS

Site description

The experiment was conducted at research field of Urmia University, Urmia, Iran. The regional climate is semiarid-cold, with mean annual precipitation of 335 mm and mean annual temperature of 11°C. The selected soil was from the field of agricultural faculty and soil characteristics were measured according to method of Bauder and Gee (1986).
Table 1 shows some chemical and physical properties of the soil.

Table.1. Some chemical and physical properties of soil.

Clay (%)	Sand (%)	Silt (%)	Texture	pH	CEC	EC* (dSm^{-1})	CCE* (%)	Total Zn(mg/kg)
23	35.75	41.25	loam	7.5	3.45	1.5	15	100.7

*: CEC: Cation Exchange Capacity, EC: Electrical Conductivity, CCE: Calcium Carbonate Equivalent.

The plants were grown in pots over a period of three month in soils containing zinc concentration varying between 100.7, 300.7, 500.7, 900.7, 1300.7 and 2100.7 mg_{Zn}/kg_{soil}.

Determining Growth Parameters

At the end of the experiment, biomass and height of the shoots were measured for each Zn concentration.

Measurement of leaf Chlorophyll (a, b and total) content

Leaf samples were selected randomly from the plants and homogenized in a mortar in acetone. The extract was centrifuged at 5000 g for 5 min. Absorbance of the supernatant was recorded at 663, 645 and 450 nm spectrophotometrically. Chlorophyll (Chl) content was determined following the method of Arnon (1949).

Plant Analysis

Three months after cultivation, plants were harvested and their biomass per pot were recorded and weighed. Prior to analysis of the plant material, they carefully washed with distilled water in order to remove any surface soil or dust deposits, and then oven-dried at 75°C for 72 h, and then milled. Plant samples (2 g) were digested with a mixture of concentrated HNO_3, $HCLO_4$ and H_2SO_4 at a ratio of 40:4:1. The concentration of Zn was determined by flame atomic absorption spectrometry (Shimadzu 6300 AA).

Estimating Relative Yield

In order to assess the phytotoxicity of Zn, yield reductions of the plants were calculated as the relative percentage of dry biomass of a given plant at each treatment (Y_c) to its dry biomass at control treatment (the treatment with no added Zn)(Khodaverdiloo et al., 2011):

$$RY=\left(Y_c / Y_0\right)\times 100$$

Estimating Bio-concentration Factor

To estimate the potential uptake of Zn by the plants, bio-concentration factors of soil Zn by plants were also calculated as follow:

$$\mathrm{BCF}=\frac{total\ Zn\ in\ plant\ dry\ matter\ (mg\ kg^{-1})}{total\ Zn\ in\ soil\ (mg\ kg^{-1})}$$

Where BCF (-) is bio-concentration factor.

Estimating Metal Extraction

For estimating the potential phytoremediation of Zn by the plants, metal extraction of soil Zn by plants were also calculated as follow:

Metal Extraction (mg_{Zn}/pot) = yield (kg/pot) × Zn concentration in plant biomass (mg_{Zn}/kg)

Soil Analysis

Soil extractable Zn, in contaminated soils, were extracted with 0.01 mol l^{-1} $CaCl_2$, 0.1 mol l^{-1} $NaNO_3$, and 1 mol l^{-1} NH_4NO_3 extraction procedures for metal Zn analysis. All the three methods are standardized or undergoing standardization in Europe: 0.01 mol l^{-1} $CaCl_2$ (The Netherlands), 0.1 mol l^{-1} $NaNO_3$ (Switzerland) and 1 mol l^{-1} NH_4NO_3 (Germany). The concentration of Zn was determined by flame atomic absorption spectrometry (Shimadzu 6300 AA).

Statistical analysis

Statistical analysis was performed using SAS Statistical Analysis Software. Duncan multiple rage test ($P \leq 0.05$) was used to establish the significance of the differences among the means.

RESULTS AND DISCUSSION

Growth and Biomass Yield

According to table 2, relative yield of *Chenopodium album* increased with increasing zinc concentration in the soil up to 300.7mg/kg and decreased at higher concentrations, but relative yield of the *Sorghum bicolor* decreased with increasing zinc concentration in the soil .The initial increase could be attributed to the nutritional role of zinc for the plant growth. The decrease of the relative yield at higher concentrations could be due to the toxic effect of Zn that damages plant growth (Atici et al., 2005).

The effect of Zn on plant height and biomass are presented in figures 1 and 2. Both plant height and biomass decreased with growing zinc concentration in the soils. Zinc caused a significant decrease ($p \leq 0.05$) in plant height and biomass. The reduction of biomass by Zn toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis. Excessive amount of Zn may cause decreased uptake of nutrient elements, inhibition of various enzyme activities, and induction of oxidative stress including alterations in enzymes of the antioxidant defense system (Foy et al., 1978; Chaney, 1993). According to the literature, an increase in the zinc content in the soil leads to reduction of the leaf area in some species, which results, in turn, in a decrease in photosynthetic activity (Bonnet et al., 2000; Khudsar et al., 2004; Misra et al., 2005).

Table2. Effect of different concentrations of Zn on relative yield (%) and Zn concentration (mg_{zn}/kg_{plant}) of *Chenopodium album* and *Sorghum bicolor* biomass.

Zn concentration(mg_{Zn}/kg_{Soil})	Relative yield (%)		Zn concentration(mg_{zn}/kg_{plant})	
	Sorghum bicolor	*Chenopodium album*	*Sorghum bicolor*	*Chenopodium album*
100.7	100	100	272.95	86.23
300.7	70.41	109.2	2208.34	462.06
500.7	60.9	89.5	2538.09	666.62
900.7	56.98	85.54	2022.85	1001.36
1300.7	51.94	33.46	1629.3	1067.82
2100.7	41.28	20.08	1714.9	1213.18

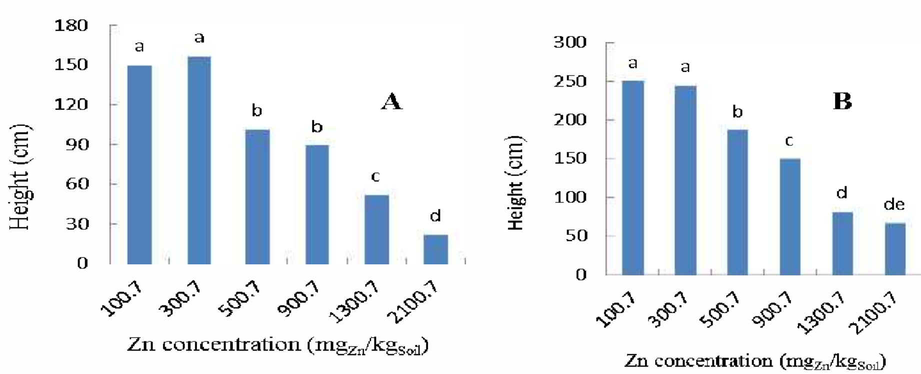

Figure 1. Effect of different concentrations of Zn on plant height of *Chenopodium album* (A) and *Sorghum bicolor* (B).

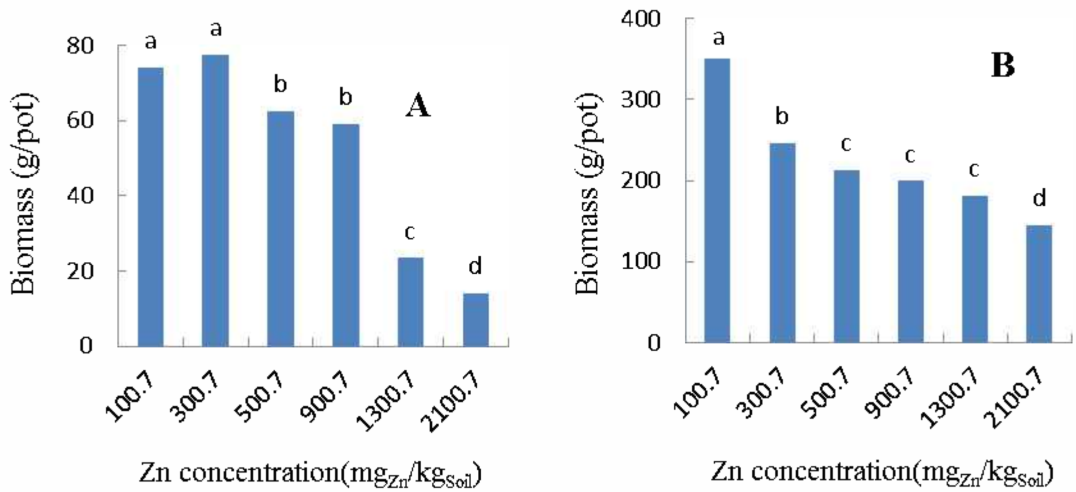

Figure 2. Effect of different concentrations of Zn on biomass of *Chenopodium album* (A) and *Sorghum bicolor* (B).

Chlorophyll Content

The effect of Zn on chlorophyll content is presented in figure3. Levels of Chl a, Chl b and Chl total in control sorghum plants were maximum, but of *Chenpodium album* the maximum amounts were found in concentration 300.7(mg_{zn}/kg_{soil}).

The decline in chlorophyll content in the plants exposed to heavy metals stress is believed to be probably due to (a) inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA-dehydratase) (Padmaja et al., 1990) and protochlorophyllide reductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; and/or (b) impairment of the supply of Mg^{2+} and Fe^{2+} (Kupper et al., 1996). Similar decrease in chlorophyll content under heavy metal stress was reported in sunflower (Zengin and Munzuroglu, 2006) and almond (Elloumi et al., 2007).

Zn Uptake

According to table 2, an increase in soil Zn concentration, increased Zn content in *Chenopodium album* up to a maximum of 1213 mg_{zn}/kg_{plant} (in 2100.7 mg_{zn}/kg_{soil}) and The minimum Zn concentration was 86.23 mg kg^{-1}, which was seen in 100.7 mg_{zn}/kg_{soil}.

Bhahragava *et al* (2008) reported that *Chenopodium spp.* have the ability to accumulate large quantities of heavy metals in the leaf tissue even when they are present in low concentrations in the soil. The concentration of heavy metals in *Chenopodium spp.* is greater than that reported for flax, cotton and hemp; the crops considered suitable for cultivation on metal polluted soils (Yanchev et al., 2000; Angelova et al., 2004).

The maximum and minimum Zn concentrations in sorghum were 2538 mg/kg (in 500.7 mg_{zn}/kg_{soil}) and 272.9 mg/kg (in concentration 100.7 mg_{zn}/kg_{soil}), respectively. Many studies have indicated that certain varieties of high-biomass crops displayed heavy metal tolerance, such as Indian mustard (*Brassica juncea*), oat (*Avena sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*) and ryegrass (*Lolium perenne*) (Shen et al., 2002; Meers et al., 2005; Komarek et al., 2007). *Sorghum bicolor* is a hardy, C_4 grass and it is considered as a great promising energy plant, furthermore, fast-growing and high biomass production. According to our results, Sorghum bicolor could accumulate zinc in high concentrations, in the biomass. Marchiol et al (2007) reported that Zn removal by *S. bicolor* was about 2000 g ha^{-1}.

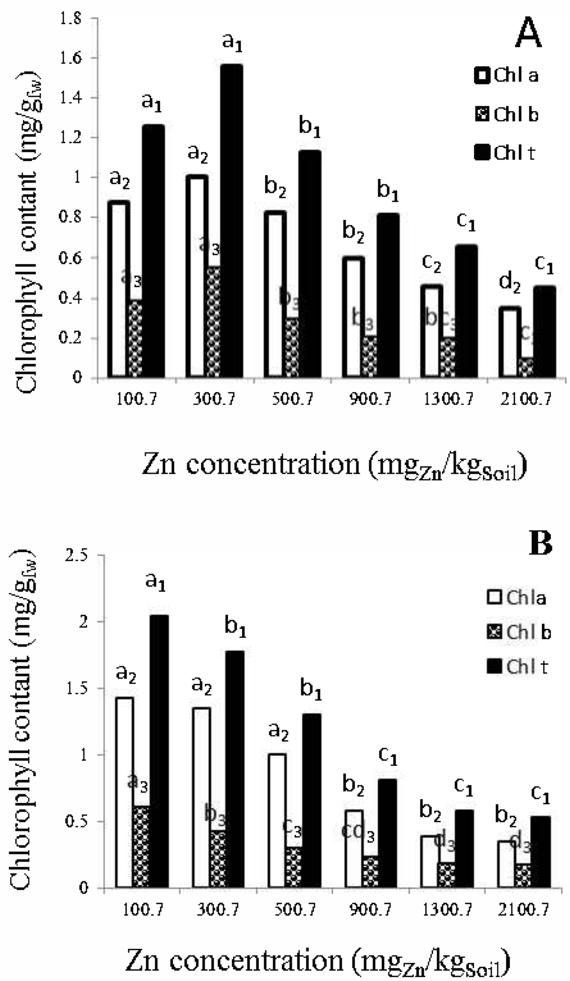

Figure 3. Effect of different concentrations of Zn on leaf chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl t) content of *Chenopodium album* (A) and *Sorghum bicolor* (B). For each property different letters show significant difference ($P \leq 0.05$).Subscripts 1, 2 and 3 for the letters refer to Chl a, Chl b and Chl t, respectively.

Bio-concentration factor

Table 3 shows the mean values of bio-concentration factor (BCF) of the plants for Zn when grown in soils with increasing Zn concentrations. BCF values for sorghum were more than unit in all concentrations except 2100.7 mg_{zn}/kg_{soil}. Maximum BCF in sorghum was 7.3 (in 300.7 mg_{zn}/kg_{soil}). While the BCF values of *Chenopodium album* were more than unit between 300.7 and 900.7 mg_{zn}/kg_{soil} and maximum BCF in *Chenopodium album* was 2.3 (in 500.7 mg_{zn}/kg_{soil}). Both bio-concentration factors (BCF) and translocation factors (TF) can be used to estimate a plant potential for phytoremediation purpose. However, plants with a high bio-concentration factor (BCF, metal concentration ratio of plant biomass to soil) and high translocation factor (TF, metal concentration ratio of plant shoots to roots) have the potential for phytoextraction (Yoon et al., 2006). These results revealed that the plants could be used for remediation of Zn contaminated soils provided that the plants biomass production is increased by some possible means.

Metal Extraction

Table 3 shows the mean values of metal extraction of the plants for Zn when grown on soils with increasing Zn concentrations. Maximum and minimum metal extractions were 90.7 mg_{zn}/pot (in 300.7 mg_{zn}/kg_{soil}) and 16 mg_{zn}/pot (in 100.7 mg_{zn}/kg_{soil}) for *Sorghum bicolor* and 19.7 mg_{zn}/pot (in 900.7 mg_{zn}/kg_{soil}) and 2.2 mg_{zn}/pot (in 100.7 mg_{zn}/kg_{soil}) for *Chenopodium album*, respectively.

Table3. Mean values of bio-concentration factor (BCF) and metal extraction (mg_{zn}/pot) of *Sorghum bicolor* and *Chenopodium album* for Zn when grown in soil with different levels of added Zn.

Zn concentration(mg_{Zn}/kg_{Soil})	BCF (-)		Metal extraction(mg_{zn}/pot)	
	sorghum	*Chenopodium album*	*Sorghum bicolor*	*Chenopodium album*
100.7	2.7	0.9	16	2.2
300.7	7.3	1.5	90.7	12
500.7	5.1	2.3	90	14
900.7	2.2	1.1	67.4	19.7
1300.7	1.3	0.8	50	8.4
2100.7	0.8	0.6	41.4	5.8

Table 4. Extractable zinc concentrations in the soils studied after applying the three mild extraction procedures.

Zn concentration (mg_{Zn}/kg_{Soil})	Extractor		
	NH_4NO_3 (mg_{Zn}/kg_{Soil})	$NaNO_3$(mg_{Zn}/kg_{Soil})	$CaCl_2$(mg_{Zn}/kg_{Soil})
100.7	1.5	0	0
300.7	3.82	0	0.24
500.7	5.63	0	0.15
900.7	13.85	0.7	3.11
1300.7	22.2	1.08	2.35
2100.7	50.7	3.05	4.25

Extraction Efficiency

The Zn extraction efficiency obtained with the three procedures studied has been presented in Table 4. The extractability of Zn obtained with the NH_4NO_3 procedure was higher than that obtained with the $CaCl_2$ and $NaNO_3$ procedures. The lowest extraction efficiency was gained with the $NaNO_3$ procedure. Similar results were reported by Pueyo et al. (2004) but they indicate that for some samples higher extraction efficiencies were obtained with $CaCl_2$ rather than with NH_4NO_3. Extractable soil Zn concentrations with the NH_4NO_3 were closely correlated with total soil Zn concentrations (R^2 = 0.98, Fig.4) as well. Similar results were reported by Langer et al (2009). In addition, there was significant correlation between NH_4NO_3-extractable soil Zn and response of plants to Zn pollution.

The main problem in the use of single extraction methods lies in the lack of uniformity in the different procedures used. In consequence, the results obtained are operationally defined depending on the experimental conditions used (type and concentration of extracting agent, soil mass: volume ratio, shaking time and speed of shaking). This fact makes data comparison difficult and prevents the standardization of these methods. To date, there is no generally accepted method of estimating the bio-availability of heavy metals in soils (Pueyo et al., 2004).

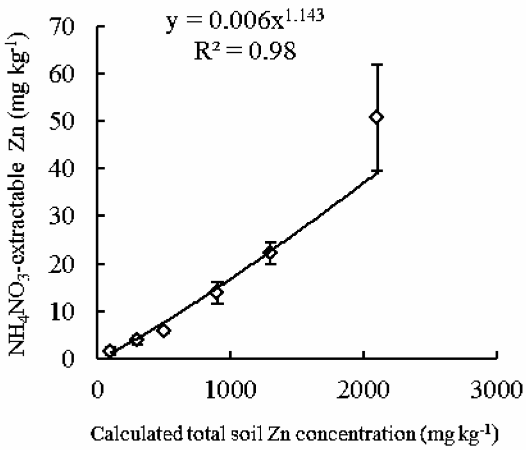

Figure 4. Relationship between NH_4NO_3-extractable and total Zn concentrations in soil.

CONCLUSIONS

Plant genotype is considered as the most important factor affecting the heavy metal uptake by plants. The uptake and accumulation of heavy metals varies greatly with plant species and with cultivars within a species. Results of this study showed that, Common lambsquarter was tolerant at low and medium conentrations (≤900 mg/kg), but sorghum toleranted high concentrastion of zn. In addition, sorghum was more capable in removal of zn from soil as compared to Common lambsquarter, so that sorghum could remediatelow Zn contaminated (≤900 mg/kg) soils. In addition, there was significant correlation between NH_4NO_3-extractable soil Zn and response of plants to Zn pollution.

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