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IMPACTS OF CITRIC ACID ON THE PHYTOEXTRACTION OF ZINC (Zn) USING SORGHUM (*Sorghum bicolor (L.)* Moench) PLANTS

(Kesan Asid Sitrik Terhadap Pengekstrakan Fito Zink (Zn) Mengunakan Tumbuhan Sorghum (*Sorghum bicolor (L.)* Moench))

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Abstract

Greenhouse hydroponic experiments were carried out to examine the impacts of citric acid on Zn uptake and phytoextraction potentials of sorghum (S*orghum bicolor (L.)* Moench). Two-week-old seedlings transplanted in hydroponic solutions were treated with different doses of Zn in the concentration range of 5, 25, 50, 100, and 200 mg/L alone or in combination with 10 mM citric acid. After 21 days of culture, the plants were harvested, separated into roots and shoots, and then dried. Fresh and dry weights were measured using Sartorius balance, Zn uptakes in the roots and shoots were determined by atomic absorption spectrometry. Translocation factor (TF) was determined by dividing Zn concentrations in roots by Zn concentration in the shoots, bioconcentration factor (BCF) was determined as a ratio of Zn concentration in the roots to Zn concentration in the hydroponic solution. Proline, pigments, protein, and ascorbate content were measured spectrophotometrically using acid ninhydrin, acetone, Lowry assay, and dinitrophenyl hydrazine methods respectively. The results indicate that Zn uptake, fresh and dry weights, TF, BCF, proline, and ascorbate contents were concentration dependent with a more significant increase ($p < 0.05$) after the application of citric acid. Pigments and protein contents were, however severely decreased with increasing Zn concentrations and appreciated gradually with the addition of citric acid. Thus, citric acid efficiently increased phytoextractability of Zn and reduced Zn-induced toxicity; *Sorghum bicolor LM* was non-hyperaccumulator of Zn but may be used for phytoremediation of Zn contaminated environments with assistance of citric acid.

Keywords: citric acid, hydroponic, phytoextraction, *Sorghum bicolor (L)* Moench, Zn

Abstrak

Kajian hidroponik rumah hijau telah dijalankan untuk menkaji kesan asid sitrik terhadap pengambilan Zn dan potensi pengekstrakan fito bagi sorghum (S*orghum bicolor (L.)* Moench). Pembenihan selama 2 minggu di dalam larutan hidroponik telah dirawat mengunakan dos Zn yang berbeza di dalam julat kepekatan 5, 25, 50, 100, dan 200 mg/L tunggal atau gabungan bersama 10 mM asid sitrik. Selepas 21 hari dikultur, tumhuban dituai, diasingkan kepada bahagian pucuk dan akar, dan kemudian dikeringkan. Berat asal dan kering telah diukur mengunakan penimbang Sartorius, manakala pengambilan Zn pada bahagian akar

dan pucuk telah ditentukan mengunakan spektrometri serapan atom. Faktor translokasi (TF) telah ditentukan dengan membahagi kepekatan Zn di dalam akar dengan kepekatan Zn pada bahagian pucuk, manakala factor biokepekatan (BCF) telah ditentukan dengan nisbah kepekatan Zn di dalam akar kepada kepekatan Zn di dalam larutan hidroponik. Prolin, pigmen, protein, dan kandungan askorbat telah diukur mengunakan spektrofoto masing-masing mengunakan kaedah asid ninhydrin, aseton, ujian Lowry, dan dinitrofenil hidrazin. Keputusan menunjukan pengambilan Zn, berat asal dan kering, TF, BCF, prolin, dan kandungan askorbat mempunyai kepekatan berbeza dengan peningkatan signifikan ($p < 0.05$) selepas aplikasi asid sitrik. Kandungan pigmen dan protein walaubagaimana pun merosot dengan peningkatan kepekatan Zn dan meningkat dengan penambahan asid sitrik. Maka, asid sitrik berkesan meningkatkan kebolehupayaan fito bagi Zn dan mengurangkan ransangan ketoksikan Zn; *Sorghum bicolor LM* tak berfungsi penumpukan hiper bagi Zn tetapi boleh digunakan bagi pemulihan fito bagi persekitaran yang tercemar dengan Zn dengan bantuan asid sitrik.

Kata kunci: asid sitrik, hidroponik, pengesktrakan fito, *Sorghum bicolor (L)* Moench, Zn

Introduction

Pollution through heavy metals is an issue of worldwide concern due to their negative effects on public and environmental health [1]. Zinc (Zn) is a heavy metal essential for optimal growth and development; however, at elevated concentrations, it is highly toxic to plants and animals leading to impaired growth [2]. Contamination of soils by Zn had become one of the major environmental problems due to frequent Zn related anthropogenic activities such as mining, fertilization, etc. [3]. Higher Zn levels often lead to the production of reactive oxygen species (ROS) resulting in the stimulation of oxidative damage to plants [4]. This will result in stunted growth, reduced chlorophyll, and overall illness [5].

Phytoextraction is a cost-effective and green method of de-contamination for an environment polluted by heavy metals; it involves the use of plants to remove heavy metals from the soil, water, or any other substrate by extracting the metals from the roots and translocating them to the above-ground portions [6, 7]. Phytoextraction is a method of choice and substituted the traditional physicochemical methods because of their high cost and adverse effects on the environment [6]. Many researches had proven the effectiveness of chelating agents, such as citric acids in enhancing and assisting the phytoextraction of metals in plants [8]. Citric acid is a low molecular weight organic acid that belongs to the carboxylic acid group. It is weakly acid and usually found in citrus and in the vacuoles of many plants [9]. Besides it cost-effectiveness, citric acid is reported to have a low affinity to group I and II metals; this unique feature makes it to easily complex Zn forming strong and stable Zn-citrate complex [10]. Citric acid also increases the solubility and availability of Zn for plant uptake [10, 11].

Sorghum bicolor (L.) Moench belongs to the family poacea, rapogeneae and subtribe sorghastrae. It has been ranked fourth in importance among the cereal crops [12]. The crop is environmentally friendly as it is resistant to drought, water-efficient, and requires little or no fertilizer. It is an excellent material for heavy metal stress studies especially Zn because of its higher biomass production, vascular nature, heavy metal tolerance as well as the accumulation of a large quantity of Zn in shoot [13]. The widespread Zn contamination in the environment as a result frequent use of Zn related anthropogenic activities request for an urgent cost-effective and environmentally friendly response [2]. For this reason, this work is was aimed to examine the potential use of *Sorghum bicolor (L.)* Moench for phytoextraction of Zn and to evaluate the impact of citric acid on the *Sorghum bicolor (L.)* Moench growth, Zn uptake, and accumulation.

Materials and Methods

Plant material

Two hundred (200) seeds of *Sorghum bicolor (L.)* Moench were obtained from the Institute of Tropical Agriculture (IITA), Tarauni station, Kano state. The variety is an early maturing one, which has been adopted by many farmers of West Africa [13].

Soil sampling and analysis

Six different sampling points were used to bring together five soil samples as recommended by Patterson

[14]. Parameters namely: total Zn content, soil texture, pH, total organic matter, CEC were determined using atomic absorption spectrometer, hydrometer, pH meter, Walkley and Black wet oxidation and ammonium acetate methods respectively [15, 16].

Field experiment

The experiment was conducted at the Faculty of Agronomy Experimental Farm, Bayero University. The seeds of *Sorghum bicolor (L.)* Moench were raised on the farm for 2 weeks. Seedlings of equal height and strength were selected for control and hydroponic culture experiments.

Hydroponic experiment

The experiment took place in a greenhouse of the department of plant biology's botanical garden, Bayero University, Kano. After 2 weeks of harvest, uniform seedlings were cautiously removed from the soil and transferred immediately to a 1L good plastic containers containing the hydroponic solution of various treatment. Three seedlings were chosen per treatment with one seedling in each container. The hydroponic solution was prepared based on a modified Hoagland solution as described by Taiz and Zeiger [17].

Three treatments were designed in a completely randomized manner which composed of:

i. A hydroponic solution only (control)
ii. Hydroponic solution + Zn supplied as $ZnSO_4 .5H_2O$ at 5, 25, 100 and 200 mg/L.
iii. Hydroponic solution + Zn supplied as $ZnSO_4 .5H_2O$ at 5, 25, 100, and 200 mg/L + 10 mM citric acid added as citrate ($C_6H_5Na_3O_7$).

Measurement of growth parameters

After 6 weeks of treatment, the plants were harvested; cleaned with tap water, and then distilled water. The plants were cut and divided into shoots and roots. Fresh weight was immediately recorded using Sartorius balance (ENTRIS64-1S). The plants were subsequently dried and weight. The roots and shoots were placed in different dark polyethylene bags for further analysis.

Determination of Zn concentration

The dried samples were ground, weighed based on availability, and burned in a furnace at 450 °C for 4 hours. The ash obtained was dissolved in 0.10M HNO_3 and then filtered into a 50 cm^3 volumetric flask. Zn contents in the roots and shoots were analysed using atomic absorption Spectrometer. Zn concentration in the samples was obtained from a calibration curve prepared from the Zn stock solution. Blank determinations were carried out in the same way.

Determination of Translocation factor (TF) and Bioconcentration Factor (BCF)

TF and BCF were estimated using the following relations [18]:

$$TF = \frac{\text{Zn Conc. in the Shoot}}{\text{Zn Conc. in the Root}} \quad (1)$$

$$BCF = \frac{\text{Zn Conc. in the Root}}{\text{Zn Conc. in the Hydroponic Solution}} \quad (2)$$

Determination of proline Contents

The proline contents were measured based on the method described by Bates et al. [19]. A proline standard curve was used to estimate the concentrations of proline and computed using Equation 3.

Estimation of pigment contents

The estimation of pigments content in control and treated plants were carried as per the method of Arnon [20]. The amount Chl a, Chl b and CR were calculated using Equations 4 - 6 [21].

Protein estimation

Protein contents were based on the method of Lowry et al. [22]. An amount 1 g of treated and control plants were milled with 5 ml of $K_2(SO_4)_3$ buffer and centrifuged at 12000 for 15 minutes. About 0.5 ml of the supernatant were mixed with 1 ml of complex-developing reagent (solution A: 2% Na_2CO_3 in distilled water, solution B: 1% $CuSO_4 \cdot 5H_2O$ in distilled water and solution C: 2 % sodium potassium tartrate in distilled water) and then cooled for 15 minutes. A 0.1 ml of diluted Folin reagent was added, cooled for 1 hour and the absorbance was read at 550 nm using a spectrophotometer. The unknown concentration of

protein was determined by plotting a standard curve of absorbance as a function of initial protein concentration.

Analysis of ascorbic acid

Ascorbic acid estimation was accomplished using the method described by Mukherjee and Choudhari [23]. An amount 2 g of control and treated plants were ground in 6% trichloroacetic acid and the extract was filtered and centrifuged at 1000 rpm for 20 minutes and the filtered was made up to 15 mL with trichloroacetic acid. Five mL of extract was mixed with 3 ml of 2% dinitrophenyl hydrazine followed by an addition of 10% thiourea. The mixture was boiled for 20 minutes in a water bath. A 5 mL of 80% H_2SO_4 was added to the mixture in an ice bath. The absorbance was recorded at 530 nm using a spectrophotometer. The concentration of ascorbic acid was calculated from a standard curve plotted with a known concentration of ascorbic acid.

Statistical analysis

The data were analysed in triplets and expressed as mean and standard deviation. The mean of all treatments was subjected to a One-way analysis of variance (ANOVA) using IBM SPSS Statistics 23 software and mean differences were performed using the Tukey test. All graphs were plotted using Microsoft® Excel 2013.

$$\text{Proline}\left(\frac{\mu g}{g}\right) FW = \frac{\text{Proline}\left(\frac{\mu g}{ml}\right) \times \text{Vol. of toluene} \times \text{Vol. of salicylic acid}}{\text{Weight of plant} \times \text{Molecular weight of proline}} \quad (3)$$

$$\text{Chl a (mg g}^{-1}) = \frac{12.7 \times (A663) - 2.69 \times (A645) X\ V}{1000 \times W} \quad (4)$$

$$\text{Chl b (mg g}^{-1}) = \frac{22.9 \times (A645) - 4.68 \times (A663)\ X\ V}{1000 \times W} \quad (5)$$

$$\text{CR (mg g}^{-1}) = \frac{1000A470 \times 1.90\text{Chl a} - 63.14\text{Chl b}}{214} \quad (6)$$

Results and Discussion

The suitability of the *Sorghum bicolor (L.)* Moench optimal growth and development was evaluated by analysing the soil parameters in the studied farm soil (Table 1). The pH value of 7.4±0.133 was in the range (4.0-8.5) suitable for *Sorghum bicolor (L.)* Moench cultivation. The pH value obtained is indicating that Zn was not readily available in soluble form in the soil. The background Zn concentration in the soil was 15.698±0.34 mg/kg. This was within the range of 10-300 mg/kg of uncontaminated soil [24]. Cation exchange capacity (CEC) of 40.12 cmolc/kg±0.10 is demonstrating that Zn is readily be adsorbed by soil and thus leaching will be minimized. High organic matter content (27.49±0.38 g/kg) is signifying the fertility of the soil.

Effects of Zn and citric acid on fresh weight (FW) and dry weight (DW)

A dose-dependent decrease in FW and DW of both roots and shoots was observed after the application of Zn (Table 2). There were maximum reductions of 60% and 46% in FW of root and shoot respectively after 200 mg/L Zn exposure compared to control. The minimum reductions were 25% and 4% at 5 mg/L Zn level respectively. Several authors had reported that higher reduction of FW in the roots and shoots at elevated concentrations might be due to the interference with photosystem alteration which subsequently resulted in stunted growth and chlorosis [24]. A 58% and 71% decrease in root and shoot DW were respectively recorded at maximum Zn dosage. At a minimum dose, 10 % and 18% decline in root and shoot DW were observed in that order. Hasan et al. [25] detected the same pattern in *Sorghum bicolor (L.)* Moench and *Chenopodium album* when treated with Zn.

Manivasagaperuma et al. [26] also witnessed related decline after the application of high Zn concentrations. The reduction is more obvious in roots. This might be because roots are the first tissue in contact with Zn and perhaps the tolerance of *Sorghum bicolor (L.)* Moench to Zn stress [27]. The reduction in weight is reported to be associated with a deficiency of other micronutrients [28]. However, the addition of 10 mM citric acid significantly ($p < 0.05$) improved FW and DW of both root and shoots. There were 11% and 10 % increase in root and shoot FW at maximum Zn dosage. At a minimum, 18% and 2% improvement in root and shoot FW were noted respectively compared to control treatments. Root and shoot DW also followed the same pattern. Since a fixed amount of citric was used across all concentration levels, a citric acid-induced increment is Zn concentration-dependent.

Table 1. Properties of soil

Parameter	**Value**
Texture (%)	Clay (%) 0.46 ±0.07
	Sand (%) 86.50±0.81
	Silt (%) 5.15±0.62^{a}
pH (%)	7.4±0.13^{a}
Organic matter(g/kg)	27.49±0.38^{b}
CEC ($cmol_c$/kg)	40.12±0.10^{d}
Background Zn Conc. (mg/kg)	11.698±0.28^{c}

The result is expressed as mean ± SD. Different letters above denote significant different at P < 0.05 according Tukey test

Table 2. Effects of Zn and citric acid on roots and shoots FW and DW

Treatments	**Parameters**			
	Root FW (mg)	**Shoot FW (mg)**	**Root DW (mg)**	**Shoot DW (mg)**
Control	57.54 ± 0.32^{cd}	122.54 ± 0.62^{c}	15.6 ± 0.21^{a}	38.14 ±0.13^{da}
Zn 5	42.83 ± 0.43^{a}	117.19 ± 0.43^{ab}	14.1 ± 0.41^{b}	31.22 ± 0.23^{c}
Zn 25	33.21 ± 0.51^{b}	97.73 ± 0.51^{d}	11.65 ± 0.51^{d}	17.10 ± 0.11^{b}
Zn 100	27.89 ± 0.32^{a}	72.19 ± 0.32^{a}	8.12 ± 0.13^{c}	14.76 ± 0.32^{a}
Zn200	22.77 ± 0.63^{cb}	65.95 ± 0.63^{a}	6.5 ± 0.12^{ab}	11.11 ± 0.13^{d}
Zn 5 + 10 mM CA	53.70 ± 0.70^{c}	120.39 ± 0.70^{c}	14.76 ± 0.24^{c}	22.76 ± 0.20^{c}
Zn 25 + 10 mM CA	37.23 ± 0.43^{ab}	92.09 ± 0.43^{bd}	13.71 ± 0.13^{d}	18.71 ± 0.32^{a}
Zn 100 + 10 mM CA	31.93 ± 0.53^{b}	81.11 ± 0.53^{b}	12.03 ± 0.32^{b}	17.98± 0.25^{b}
Zn 200 + 10 mM CA	29.45 ± 0.61^{d}	78.15 ± 0.61^{d}	11.81 ± 0.41^{a}	15.05 ± 0.21^{d}

The result is expressed as mean ± SD. Different letters above denote significant different at P < 0.05 according Tukey test

Effects of Zn and citric acid on Zn uptake

The uptake of Zn in the roots and shoots of *Sorghum bicolor (L.)* moench on the addition of different levels of Zn (5, 25, 100, and 200 mg/L) and 10 mM of citric acid are shown in Figure 1.

The addition of Zn to hydroponics enhanced the metal uptake considerably in both roots and shoots ($p < 0.05$). The uptakes increased with increasing Zn levels and roots accumulated more Zn than the shoots. Davis and Parker [29] made similar observations that uptake of Zn increased with increasing application of Zn in peanut plants. The maximum uptakes at 200 mg/L Zn were 2711.83±0.27 and 1068.21±0.31 mg/kg for the root and shoot respectively. The minimum uptakes for root and shoot were 329.09±0.23 and 79.14±0.21 mg/kg. This is in line with the work reported by literature [30, 31]. The addition of citric increased the uptake further. At a minimum Zn level, there was 16% and 13% increase in root and shoot Zn accumulation respectively. At maximum Zn level, the root and shoot uptakes were accelerated to 40% and 69% respectively. Turgut et al. [32] studied the impact of citric acid on metals uptake and accumulation in *Helianthus annus*. Their results indicated that citric acid had considerably increased the metal bioavailability and enhanced the metal uptake in the plant. This study followed the same pattern with the above study. Tandy *et al.* [33] also found that citric acid enhanced the solubility of Zn in the soils and thus increase their bioavailability for plant uptake.

Effects of Zn and citric acid on TF and BCF

TF and BCF were respectively used to estimate the hyperaccumulating and phytoremediation potential of *Sorghum bicolor (L.)* Moench TF increased significantly ($p < 0.05$) from 0.059±0.09 in control to 0.414±0.06 when the concentration of Zn reached a maximum of 200 mg/L (Figure 2). The TF was less than one (TF<1) in all treatments indicating the non-hyperaccumulation capacity of *sorghum bicolor (L.)* Moench TF appreciated further with increased Zn application. The increase might be due to the accumulation of more Zn in the shoots with increasing Zn concentrations [29]. BCF however, decreased gradually with increasing Zn concentration (Figure 3). The decrease may be due to the resistance of metal transfer from roots to the above parts [34]. There was a significant increase ($p < 0.05$) when citric acid was added. The highest increase was observed at 200 mg/L + 10 mM concentrations and the lowest at control. Han *et al.* [35] reported a similar pattern that BCF of Cd significantly reduced with increasing metal concentrations levels. BCF was greater than one in all treatments. For viable phytoextraction, the BCF ought to be greater than one [36, 37]. This is suggesting that *Sorghum bicolor (L.)* Moench is a potential candidate for phytoextraction of Zn contaminated environment.

Effects of Zn and citric acid on proline and ascorbic acid contents

Proline and ascorbic acid are dominant antioxidants that play an important role in the plant's defence mechanism against heavy metals stress. *Sorghum bicolor (L.)* Moench under Zn stress shows a significant upsurge in proline levels. Proline which is considered as a protective mechanism against heavy metal stress increased considerably ($p < 0.05$) from 0.23± 0.01 µg/g in control to a maximum of 3.66±0.02 µg/g at 200 mg/L Zn application (Figure 4). The elevated levels of proline suggest induction of osmotic stress under Zn stress [37]. Addition of citric acid generally lower proline value especially at lower Zn levels. The lowering of proline values may be due to the reaction of Zn with citric acid; forming stable Zn-citrate complex that reduces Zn stress [38]. Proline accumulation in plants assists in preserving the integrity of proteins cytoplasm, functions as carbon and nitrogen sources, and prevents denaturation of enzymes [37]. Kishor et al. [39] proposed that enhanced proline accumulation in plants could be owing to the protection of feedback inhibition of all biosynthetic enzymes by sequestrating proline out of its direction. Bohnet and Jersen [40] stated that proline protects plants from stress by removing the toxic reactive oxygen species and safeguarding of membrane and protein. Ascorbic acid plays an essential role in chlorophyll formation and functions as a substrate for the enzyme, the ascorbate peroxidase. A rise in ascorbic acid is signaling heavy metal stress and consequently the toxicity in plants [41]. The findings of this study indicate a significant elevation (50%) of ascorbic acid at

maximum Zn level; while at minimum Zn concentration, there was a 10% increment. Kandziora-Ciupa et al. [41] had documented that ascorbic acid content was positively correlated with the Zn level. Olkhovych et al. [42] also made similar observations. Hydroponic experiments conducted by Dolatabadian and Jouneghani [43] reveals that ascorbic acid detoxified the damaging effects of reactive oxygen species (ROS). However, after the addition of citric acid, a significant ($p < 0.05$) decrease in ascorbic acid was noted. The study conducted by Chen et al. [44] revealed that citric acid is capable of reducing the toxicity of heavy metals.

Effects of Zn and citric acid on pigments and protein contents

Table 3 shows the changes in the pigments and protein contents with respect to an increase in the concentration of Zn and 10 mM citric acid.

The changes in pigments are associated with visual symptoms of plant disorder and photosynthesis [45]. The amounts Chl a, Chl b, and Car were severely affected by Zn treatments, especially at higher Zn dosages. The addition of citric acid however, increased significantly ($p < 0.05$) the pigment contents compared to free Zn treatments. The reduction of pigments in plants with increasing Zn concentration might be due to the exchange of central Mg^{2+} in chlorophyll by Zn^{2+} [46]. Fikrye and Omer [47] reported that the generation of reactive oxygen species which later caused oxidative damage might lead to reduction in pigment levels. Formation Zn-citrate complex after citric acid addition led to decreased pigment levels and reduced Zn toxicity [48]. Zn application had significantly ($p < 0.05$) affected the protein contents. 36%, 57%, 65%, and 78% decline in protein contents were observed when 5, 25, 100, and 200 mg/L Zn concentration levels were respectively applied. This is in line with the investigation performed by Alia et al. [49]. Previous research indicated that the decline in protein is linked to a decrease in pigment contents and impairment of nitrogen absorption [50]. However, there was considerable improvement in the value of protein when 10 mM Citric acid was applied. There were only 2%, 9%, 21%, and 42% decrease in protein level at 5, 25, 100, and 200 mg/L Zn levels respectively compared to when Zn was used alone.

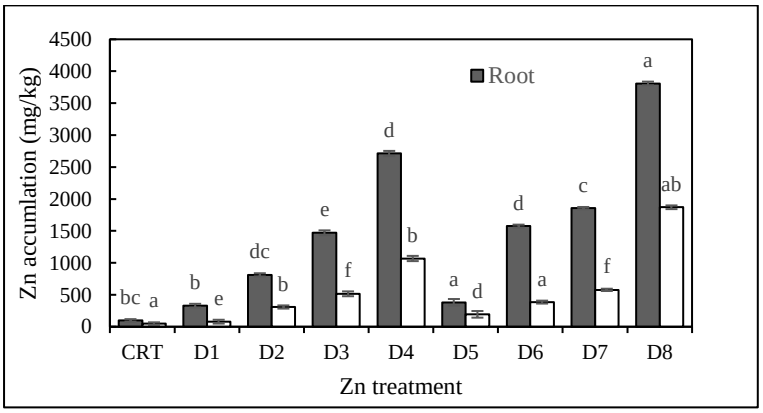

Figure 1. Effect of added Zn and 10 mM citric acid on Zn uptake in the roots and shoots of *sorghum bicolor L.M*. **CRT** = Control, **D**1 = Zn 5**, D2** = Zn 25, **D3** = Zn 100, **D4** = Zn 200, **D5** = Zn 5 + 10 mM CA, **D6** = Zn 25 + 10 mM CA, **D7** = Zn 100 + 10 mM CA, **D8** = Zn 200 + 10 mM CA

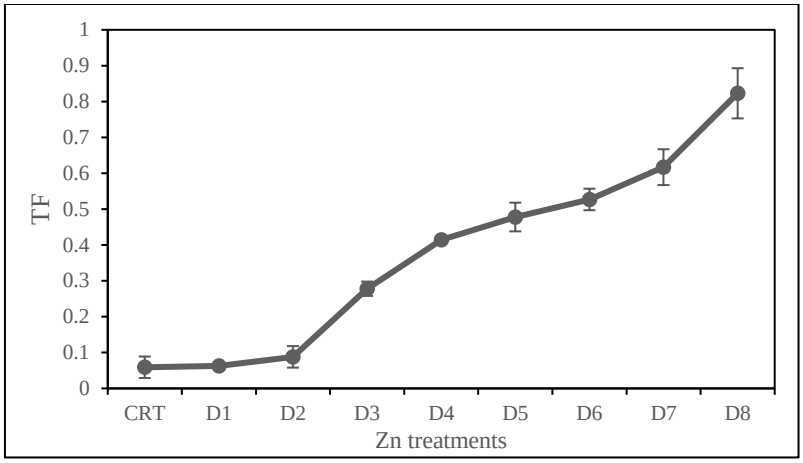

Figure 2. Effects of Zn and 10 mM citric acid on TF. **CRT** = Control, **D**1 = Zn 5**, D2** = Zn 25, **D3** = Zn 100, **D4** = Zn 200, **D5** = Zn 5 + 10 mM CA, **D6** = Zn 25 + 10 mM CA, **D7** = Zn 100 + 10 mM CA, **D8** = Zn 200 + 10 mM CA

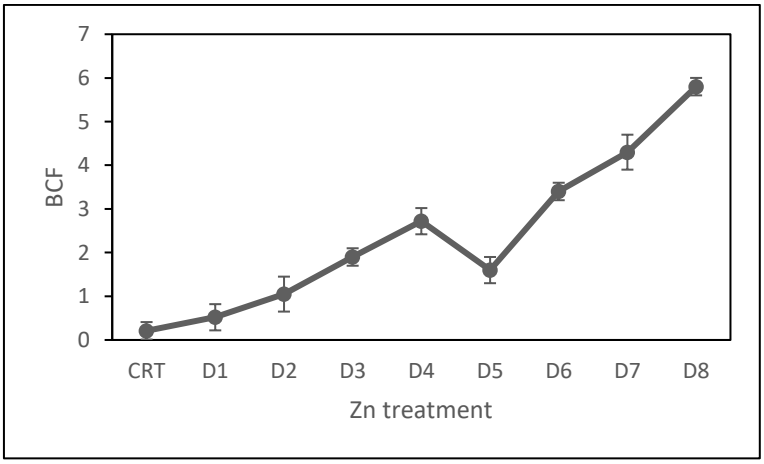

Figure 3. Effects of Zn and 10 mM citric acid on BCF. **CRT** = Control, **D**1 = Zn 5**, D2** = Zn 25, **D3** = Zn 100, **D4** = Zn 200, **D5** = Zn 5 + 10 mM CA, **D6** = Zn 25 + 10 mM CA, **D7** = Zn 100 + 10 mM CA, **D8** = Zn 200 + 10 mM CA

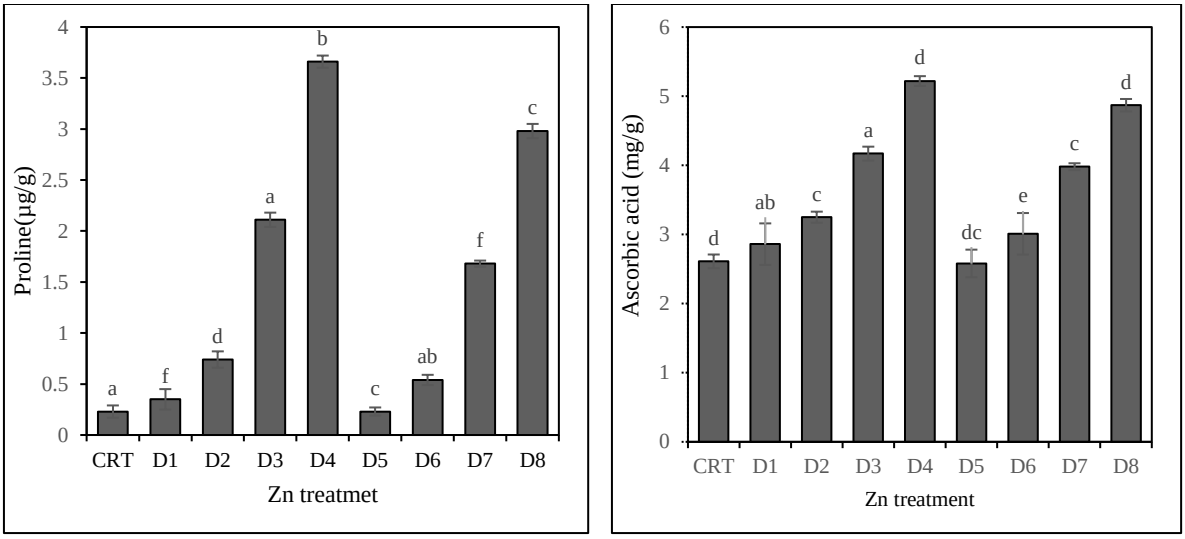

Figure 4. Effects of added Zn levels and citric acid on accumulation of proline and ascorbic acid contents. **CRT** = Control, **D**1 = Zn 5**, D2** = Zn 25, **D3** = Zn 100, **D4** = Zn 200, **D5** = Zn 5 + 10 mM CA, **D6** = Zn 25 + 10 mM CA, **D7** = Zn 100 + 10 mM CA, **D8** = Zn 200 + 10 mM CA

Table 3. Effects of Zn and citric acid on pigment and protein content

Treatments	**Parameters**			
	Chl a (mg/g)	**Chl b (mg/g)**	**CR (mg/g)**	**Protein (mg/g)**
Control	79.39 ± 0.45^{a}	126.86 ± 0.71^{c}	330.05 ± 0.31^{a}	6.05 ± 0.11^{da}
Zn 5	76.28 ± 0.32^{ab}	121.21 ± 0.44^{b}	325.78 ± 0.45^{bc}	3.78 ± 0.21^{c}
Zn 25	70.31 ± 0.51^{c}	17.77 ± 0.31^{b}	316.14 ± 0.85^{a}	2.62 ± 0.15^{b}
Zn 100	63.98 ± 0.32^{a}	102.52 ± 0.35^{a}	210.79 ± 0.72^{c}	2.11 ± 0.12^{a}
Zn200	48.36 ± 0.77^{d}	87.57 ± 0.42^{a}	156.71 ± 0.92^{c}	1.34 ± 0.13^{d}
Zn 5 + 10 mM CA	72.59 ± 0.62^{a}	115.61 ± 0.60^{ab}	320.17 ± 0.76^{b}	5.91 ± 0.30^{c}
Zn 25 + 10 mM CA	69.03 ± 0.45^{dc}	94.07 ± 0.23^{cd}	300.14 ± 0.65^{a}	5.52 ± 0.32^{a}
Zn 100 + 10 mM CA	51.23 ± 0.53^{b}	86.12 ± 0.46^{b}	240.13 ± 0.42^{d}	4.76 ± 0.35^{b}
Zn 200 + 10 mM CA	49.87 ± 0.74^{c}	74.81 ± 0.33^{a}	187.67 ± 0.45^{a}	3.53 ± 0.21^{d}

The result is expressed as mean ± SD. Different letters above denote significant different at $P < 0.05$ according to Tukey test

Conclusion

Results of this study indicated that addition Zn to different hydroponic treatments significantly affect the various parameters such as pigments, proline, protein contents of *Sorghum bicolor (L.)* Moench by inducing the toxicity symptoms. The effects were more pronounced with increasing Zn level. However, the addition of citric acid significantly alleviated these effects. The TF was less than one (TF<1) in all treatments but the BCF was greater than one in all treatment (BCF > 1). This is suggesting that citric acid efficiently increased phytoextractability of Zn and

Sorghum bicolor (L.) Moench was not hyperaccumulator of Zn but it may successfully be used in the phytoextraction of Zn contaminated environment with the assistance of citric acid. This study provides recommendation for future use of *Sorghum bicolor (L.)* Moench and its related species for large scale phytoremediation of Zn and other metals contaminated areas in a cheap and environmentally friendly techniques.

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References

1. Blaylock, M. J. and Huang, J. W. (2000). Phytoremediation of toxic metals using plants to clean up the environment. John Wiley & Sons Inc., New York: pp. 53-70.
2. Alloway, B. J. (2004). Zinc in soils and crop nutrition. IZA Publications, International Zinc Association, Brussels: pp. 135-150.
3. Chaney, R. L. (1993). Plant uptake of inorganic waste constituents. Park Ridge, Noyes Data Corporation, New Jersey, USA: pp. 50-76.
4. Sharma. P. and Dubey, R. S. (2007). Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. *Plant Cell Reports*, 26(1): 2027-2038.
5. Lo'pez-Mosquera, M.E., Moiro'n, C. and Carral, E. (2000). Use of dairy-industry sludge as fertilizer for grasslands in northwest Spain: heavy metal levels in the soil and plants. *Resources, Conservation and Recycling*, 30(1): 95-109.
6. Islam, E., Yang, X. E., He, Z. L. and Mahmood, Q. (2007). Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *Journal of Zhejiang University of Science B,* 20(1): 1-13.
7. Achakzai, A. K. K., Bazai, Z. A. and Kayani, S. A. (2011). Accumulation of heavy metals by lettuce (*Lactuca sativa L.)* irrigated with different levels of wastewater of Quetta city. *Pakistan Journal of Botany*, 43: 2953-2960.
8. Zhao, Z., Xi, M., Liang, G., Liu, X., Bai, Z. and Huang, Y. (2010). Effects of IDSA, EDDS and EDTA on heavy metals accumulation in hydroponically grown maize (*Zea mays, L.*). *Journal of Hazardous Materials*, 181(2): 455-459.
9. Gunawardana, B., Singhal, N. and Johnson, A. (2010). Amendments and their combined application for enhanced copper, cadmium, lead uptake by *Lolium perenne*. *Plant and Soil*, 329 (3):283-294.
10. Bassi, R., Prasher, S. O. and Simpson, B. K (2000). Extraction of metals from a contaminated sandy soil using citric acid. *Environmental Progress*, 19(4): 275-282.
11. Irannajad, M., Meshkini, M. and Azadmehr, A. R. (2013). Leaching of zinc from low grade oxide ore using citric acid. *Physicochemical problems of Mineral Processing*. 49(5):547-555.
12. Aba, D. A., Marley, P. S. and Maigida, D. N. (2008). Cereals crops of Nigeria; principles production and utilization. Ade-commercial press, Zaria, Nigeria: pp. 40-53.
13. Obilana, A. T. (1981). International Symposium on Sorghum grain quality, ICRISAT, Patancheru: pp.40-45.
14. Patterson, J. J. and Olson, J. J. (1983). Effects of heavy metals on radicle growth of selected woody species germinated on filter paper, mineral and organic soil substrates. *Canadian Journal of Forest Research*, 13 (1): 233-238.
15. IITA (1979). Laboratory manual on basic soil and plant analyses, 3rd edition, Longman London, UK: pp. 3-12.
16. Juo, A. S. B. (1988). Selected methods for soil and plant analysis, Manual Series No.1 International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria: pp. 10-30.
17. Taiz, L. and Zeiger, E. (2002). Plant physiology, Sinauer Associates, (eds.). Sunderland, U.S.A: pp. 67-86.
18. Shrikrishna, R. S. and Singh. S. (2012). Effect of zinc on yield, nutrient uptake and quality of Indian mustard. *Journal of Indian Society of soil Science*, 40(3): 321-325.

19. Bates, L. S., Waldren, R. P. and Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39(1):205-207.
20. Arnon, D. I. (1949). Copper enzymes in isolated chloroplast, polyphenoloxidase in *Beta vulgaris*. *Plant Physiolology*, 24(5): 1-15.
21. Lichtenthaler, H. and Wellburn, A. (1983). Determination of total carotenoids and chlorophylls a and b of leaf extract in different solvent. *Biochemical Society Transaction*, 11(1): 591-592.
22. Lowry, O.H., Rosembrough, N. J., Farr, A. L and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chem*istry, 193(1): 267-275.
23. Mukherjee, S. P. and Choudhari, M. A. (1983). Implication of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen perioxide in *Vigna* seedlings. *Physiology Plant Journal*, 58: 166-170.
24. Hafeez, B., Khanif, Y. M. and Saleem, M. (2013). Role of zinc in plant nutrition-A review. *American Journal of Experimental Agriculture*, 3(2):374-391.
25. Hasan, M. H., Reza, A. and Habib K. (20120. Effect of zinc toxicity on plant productivity, chlorophyll and Zn contents of Sorghum (*Sorghum bicolor*) and common lambsquarter (*Chenopodium album*). *International Journal of Agricultural Research and Review*, 2(1): 247-254.
26. Manivasagaperuma, R., Vijayarengan, P., Balamurugan, S. and Thiyagarajan G. (2012). Effect of zinc on growth, dry matter yield and nutrient content of *Vigna Radiata (L.) Wilczek*. *International Journal of Recent Scientific Research*, 3(3):687-692.
27. Anamika, S., Eaparn, S. and Fulekar, M. (2009). Phytoremediation of Cd, Pb and Zn by *Brassica juncea L*, Czern. *Cross Journal of Applied Biosciences*, 13(1): 726-736.
28. Wang, C., Zhang, S. H., Wang, P. F., Hou, J., Zhang, W. J., Li, W. and Lin, Z. P. (2009). The effect of excess Zn on mineral nutrition and ant oxidative response in rapeseed seedlings. *Chemosphere*, 75:1468-1476.
29. Davis, J. G. and Parker, M. B. (1993). Zinc toxicity symptom development and partitioning of biomass and Zn in peanut plants. *Journal of plant Nutrition*, 16(2): 2353-2369.
30. Wong, M. H., Li, W. C. and Zhang, Y. C. (2009). Effect of bacteria on enhanced metal uptake of the Cd/Zn hyperaccumulating plant *Sedum alferedii*. *Journal of Experimental Botany*, 58(1): 4173-4182.
31. Carl, E. S., Heather, D. T., Timothy, W. C. and Williams, M. R. (2013). Zn availability in hydroponics culture influences Glucosinolate concentration in *Brassica rapa. Horticultural Science*, 39: 84-86.
32. Turgut, C., Pepe, M. K. and Culright, T. J. (2008). Effects of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annus*. *Environmental Pollution*, 131:147-154.
33. Tandy, S., Schulin, R. and Norwaek (2008). Uptake of metals and Chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration. *Environmental Science and Technology*, 40(1): 2753-2758.
34. Justin, V., Majid, N., Islam, M. M. and Abdu A, (2011). Assessment of heavy metal uptake and translocation in *Acacia mangium* for phytoremediation of cadmium contaminated soil. *Journal of Food Agriculture and Environ*ment, 9(2): 588-592.
35. Han, F., Shan, X. Q., Zhang, S. Z., Wen, B. and Owens, B. (2011). Enhanced cadmium accumulation in maize roots: the impact of organic acids. *Plant and Soil*, 289: 355-368.
36. Ma, L.Q., Komar, K. M., Zhang, T. U. C., Caiv, W. and Kenmelley E. D. (2012). A fern that hyperacumulates arsenic. *Nature*, 11(1): 409-579.
37. Sharma, S. S., Schat, H. and Vooijs, R. (1998). In vitro alleviation of heavy metal-induced enzyme inhibition by proline. *Phytochemistry*, 49:1531-1535.
38. Siripornadulsil, S., Traina, S., Verma, D. P. S. and Sayre, R. T. (2014). Molecular mechanisms of proline–mediated tolerance to heavy metals in transgenic microalgae, *Plant Cell*, 14:2837-2947.

39. Kishor, P. B. K, Sangam, S., Amrutha, R. N., Laxmip, S. N., Naidu, K. R., Rao, K. R. S. S., Rao, S., Reddy, K. S., Theriappan, P. and Screenivasule, N. (1995). Regulation of proline synthesis, degradation, uptake and transport in higher plants; its implication in plant growth and abiotic stress tolerance. *Current Science*, 88: 424-438.
40. Bohnet, H. J. and Jersen, R. J. (1993). Strategies for engineering water–stress tolerance in plants. *Trends in Biotechnology*, 14(1): 89-97.
41. Kandziora-Ciupa, M., Nadgórska-Soch, A., Barczyk, G. and Ciepa, R. (2017). Bioaccumulation of heavy metals and ecophysiological responses to heavy metal stress in selected populations of *Vaccinium myrtillus L*. and *Vaccinium vitis-idaea L*. *Ecotoxicology*, 26(1): 966-980.
42. Olkhovych, O., Volkogon. M., Taran. N., Batsmanova, L. and Kravchenko, I. (2016). The effect of copper and zinc nanoparticles on the growth parameters, contents of ascorbic acid, and qualitative composition of amino acids and acylcarnitines in *Pistia stratiotes L. (Araceae)*. *Nanoscale Research Letters*, 11:218-228.
43. Dolatabadian, A. and Jouneghani, S. (2009). Impact of exogenous ascorbic acid on antioxidant activity and some physiological traits of common bean subjected to salinity stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 37(2): 165-172.
44. Chen, Y. X., Lin, Q., Luo, Y. M., He, Y. J., Zhen, S., Yu, Y. L., Tian, W. and Ming G. M. (2003). The role of citric acid on the phytoremediation of heavy metal contaminated soil. *Chemosphere*, 50(1): 807-811.
45. Küpper, H. F. and Spiller, M. (1996). Environmental relevance of heavy metal substituted chlorophyll using the example of water plant. *Journal of Experimental Bio*logy, 47:259-266.
46. Balashouri, P. (2009). Effects of zinc on germination, growth, pigment content and phytomass of *Vignia Radiata* and sorghum. *Journal of Ecobiology*, 7:109-114.
47. Fikriye, K. Z. and Omer, M. (2008). Effects of some heavy metals on concentration of chlorophyll, proline and antioxidant chemicals in beans (*Phaseoley Vulgarus. L*) seedlings. *Biological Craco-Viensia.* 47:157-164.
48. Doncheva, S. and Stoyanova, Z. (2010). The effect of Zinc supply and succinate treatment on plant growth and mineral uptake in pea plant. *Brazilian Journal of Plant Physio*logy, 14(1):1871-1880.
49. Alia, N., Sarda, K., Said, M., Salma, K., Sadia, A., Sadaf, S., Toqeer, A. and Miklas, S. (2015). Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. *International Journal of Environmental Resources and Public Health*, 12(2):74-84.
50. Lin, R. Z., Wang, X. R., Luo, Y., Du, W. C., Guo, H. Y., Yin, D. Q. (2007). Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). *Chemosphere*, 69(2): 89-98.