Trends Journal of Sciences Research, 2019, Volume 4, Issue 3, 111-120
http://www.tjsr.org/journal/index.php/tjsr
DOI: 10.31586/Chemistry.0403.04

Responses of Hydroponically Grown Sorghum (*Sorghum bicolor L.M*) to Zinc (Zn) Stress

Hamza Badamasi[1,*], Muhammad Saminu Dagari[2]

[1]Department of Chemistry, Federal University Dutse, Nigeria.
[2]Department of Chemistry, Federal University Gashua, Nigeria.
*Corresponding author: Hamza Badamasi (hamza.badamasi@fud.edu.ng)

Research Article

Open Access

How to cite this article: Badamasi, H., & Dagari, M. (2019). Responses of Hydroponically Grown Sorghum (Sorghum bicolor L.M) to Zinc (Zn) Stress. *Trends Journal Of Sciences Research, 4*(3), 111-120.

Received: May 11, 2019

Accepted: June 22, 2019

Published: June 24, 2019

Abstract Abiotic stress especially due heavy metals is one of the major environmental problems that threatens food security and pose greater risks to human health worldwide. In this research, greenhouse hydroponic experiments were carried out to study the morphological and biochemical responses of *Sorghum bicolor L.M* to different Zinc (Zn) levels. Two-week-old seedlings transplanted in hydroponic solutions were treated with different doses of Zn in the concentration ranges of 5, 25, 50, 100 and 200 mg/L supplied as $ZnSO_4$. $5H_2O$. After 21 day of culture, the plants were harvested, blotted to dryness and separated into roots and shoots. The root and shoot lengths, dry weights and non-enzymatic biochemical parameters such as proline, Chlorophyll a, b, Carotenoids (pigments) were determined. The results indicate that Zn applications significantly (P<0.05) depressed the lengths of root and shoot, dry weights and pigment contents compared to untreated plants (control). The effects were more pronounced with increased Zn dosage. The accumulation of the metal and proline contents in treated plants however, increase gradually with increasing Zn concentrations (P<0.05). The changes in these parameters had resulted in toxicity symptoms and overall growth retardation especially at elevated concentrations and the estimated critical toxicity thresholds in both solution and tissue concentrations suggest that *sorghum bicolor L.M* should not be grown beyond Zn concentration of above 3.2 mg/L.

Keywords: *Critical toxicity; threshold; Greenhouse; Hydroponic; Proline; Sorghum bicolor L.M; Zn*

1. Introduction

Abiotic stress and its effects on plants is a topic that is receiving worldwide attention because of its potential impacts on crop quality and productivity [1,2]. Among various stresses encountered by plants, heavy metal stress is one of the major environmental problems that threatens food security and pose greater risk to human health worldwide [3,4,5,6]. It has been documented that more than 70 % of the yield of important crops may be lost to various abiotic stresses [7,8]. Zinc (Zn) is an essential heavy metal that is needed as a micronutrient for optimal growth and development of plants [9]. It acts as a plant nutrient and plays an important role in many metabolic processes [10]. However, at higher levels it becomes highly phytotoxic to plants, causing the inhibition of growth and even death of plants [11,12]. Excess Zn in plant's cell can generate reactive oxygen species (ROS). This lead to induction of oxidative damage to important biomolecules such as lipids, pigments and nucleic acids; which is detrimental to plant growth and development [13,14,15]. Visual symptoms of Zn toxicity are reported to be wilting and chlorosis in young leave which progresses to older leaves, appearance brown colouration in leaves and stunted growth [16,17].

Sorghum bicolor L.M belongs to the family *poacea* and *rapogeneae* and subtribe *sorghastrae*. It is the fifth most important cereal crop in the world and represents Africa's main contribution to the world food supply due to its wide use as food,

animal feed, alcoholic beverages and biofuel [18,19]. Zn deficiency is uncommon in *Sorghum bicolor L.M* plants due to its ability to release phytosiderophore compounds from roots to overcome low levels of Zn [20,21]. It has been reported that concentrations of Zn found in contaminated soils usually exceed to those required as nutrients and may cause phytotoxicity [22]. For this reason, this study was designed with the aim of examining the impacts of Zn fertilizations on the growth, morphological and biochemical changes in *Sorghum bicolor L.M* and to establish threshold for Zn toxicity using hydroponic culture protocol as a model to soil solutions.

2. Material and Methods

2.1. Plant Material and Experimental Design

The seeds of *Sorghum bicolor L.M* were obtained from the Institute for Tropical Agriculture (IITA), Kano state, Nigeria. They were surface sterilized with 1% (v/v) sodium hypochlorite for 20 minutes and washed several times with distilled water to eliminate pest contamination [51]. The seeds were sown in the Agronomy experiment farm, Bayero University, Kano, Nigeria. After growing for 2 week, seedlings of similar sizes and vigor were transplanted to a modified Hoagland hydroponic solution in 1-L plastic vessels (three plants per vessels) containing the following compositions: NH_4NO_3 1.5 mM, $CaCl_2$ 1.00 mM, $MgSO_4$ 1.60 mM, K_2SO_4 1.00 mM, KH_2PO_4 0.30 mM H_3BO_3 2.0 µM, $MnSO_4$ 5.0 µM, $ZnSO_4$ 0.5 µM, $CuSO_4$ 0.2 µM and $(NH4)_6Mo_7O_{24}$ 0.05 µM. Iron was supplied as Fe-EDTA at 0.1 mM. The nutrient solutions were not aerated and replaced every week. The plants were allowed to grow for 10 day in hydroponic culture in order to adapt to a new hydroponic environment which served as control (CR). Zn was added as $ZnSO_4.5H_2O$ in the concentrations of 5, 25, 50, 100 and 200 mg/L. The plants were cultured for 21 day in treated solution under controlled conditions with a day/night temperature of 30 ±3 ℃ /25±3 ℃, and a relative humidity of 75 ±3% in a greenhouse. The pH of the nutrient solution was maintained at 5.3 and adjusted using NaOH or HCl. After treatments, the plants were harvested, separated into roots and shoots, soaked in 20 mM Na_2EDTA for 15 minutes to remove the metals attached to the roots surface and then washed with tap water followed by deionized water [23].

2.2. Measurement of Morphological Parameters

Root and shoot lengths (cm) of fresh plants were recorded using calibrate ruler. The plants were oven dried at 65 ℃ to a constant weight and dry weights of root and shoot were determined using weighing balance (Shimadzu, AP124Y). Another set of fresh plants was used for proline and pigments estimations.

2.3. Estimating of Toxicity Threshold

Toxicity threshold was established in terms of percentage relative yield at 10% dry weight reduction using the following relation [17]:

$$RY = \frac{Y_t}{Y_c} \times 100\% \quad (1)$$

Y_t = the relative percentage of dry biomass of a given plant at each treatment
Y_c = the relative percentage of dry biomass of a control.
Toxicity thresholds were calculated from the data using second order polynomial growth curve.

2.4. Determination of Zn^{2+} Concentration in Plants

The dried samples of plants were ground, weighed and made to ashes in a muffle furnace at 450 ℃ for 4 h. The ashes were dissolved in 0.10 M HNO_3 and then filtered through Whatman No. 42 filter paper. The filtrate was transferred to a 50 cm^3 volumetric flask. Zn content was analyzed using Atomic Absorption Spectrophotometer (Angstrom, AA320N). Zn concentrations in the samples were obtained from a calibration curve prepared from the standard solution. Blanks were also analyzed using similar protocol.

2.5. Estimation of Proline

The amount of proline was measured according to the method of Bates et al., [24]. 0.5 g of a fresh leaf was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman No. 2 filter paper. 2 mL of the filtrate was then mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid and heated at 100 ℃ for 60 min. The reaction was terminated in an ice bath and 4 mL of toluene was added to the mixture and contents of tubes were stirred for 20 s. The absorbance of the pink-red upper phase was recorded at 520 nm against toluene blank using UV-visible spectrophotometer (Hitachi, U-2900/U-2910 double beam). The concentration of proline was determined from a standard curve using L-proline as standard and calculated on a fresh weight basis using the relation:

$$\text{Proline}\left(\frac{\mu g}{g}\right)\text{FW} = \frac{\mu g/\text{ml proline} \times \text{vol. of toluene} \times \text{vol. of salicylic acid}}{\text{weight of plant} \times \text{molecular weight of proline}} \quad (2)$$

2.6. Pigment Analysis

The estimation of pigments in both control and treated plants were carried out according to the method of described by Arnon [25]: Two grams of fresh leaf tissue of each sample were homogenized using 80% acetone. The homogenate was centrifuged for 10 minutes and a supernatant was collected. The residue was again extracted and the supernatant was pooled together. The extraction process was repeated until the residue became colorless. The volume of the combined supernatant was noted. The absorbance of the solution was measured at 645 nm, 663 nm and 470 nm for both chlorophyll a, b and carotenoids respectively using UV-visible spectrophotometer (Hitachi, U-2900/U-2910 double beam). The amount chlorophyll a, b (Chl a Chl b) and carotenoids (Car) were calculated according to Lichtenthaler and Wellburn [26] using the relations:

$$\textbf{Chl a}\left(mg\ g-1\right) = 12.7 \times \left(A663\right) - 2.69 \times \left(A645\right) \times V\ /\ 1000 \times W \quad (3)$$

$$\textbf{Chl b}\left(mg\ g-1\right) = 22.9 \times \left(A645\right) - 4.68 \times \left(A663\right) \times V\ /\ 1000 \times W \quad (4)$$

$$\textbf{Car}\left(mg\ g-1\right) = 1000\ A470 - 1.90\ Chl\ a - 63.14\ Chl\ b\ /\ 214 \quad (5)$$

2.7. Statistical Analysis

A randomized complete block experimental design was used with each treatment replicated three times. All data were expressed as mean of three replicates and treated using Excel 2013 program (Microsoft office window 10). The data were also subjected to One-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test at P = .05. Zn toxicity thresholds at 10% DW reduction (PT10) were determined using second order polynomial regression growth curve at $\alpha = 0.05$.

3. Results and Discussion

3.1. Effects of Zn Level on Root and Shoot Lengths

The results of this study indicate that Zn application had adversely effected the morphology of *Sorghum bicolor L.M* by gradually decreasing the length of roots and shoots. The parameters were significantly reduced (P=.05) at all Zn concentrations relative to control plants (Figure 1a). The maximum decreased were 1.60 and 3.20 cm for roots and shoots respectively at 200 mg/L Zn level while the minimum were 6.70 and 13.0 cm respectively at 5 mg/L. Yellowing of the main root and lateral roots were also apparent during the period of exposure. At the end of the culture, majority of roots exposed to Zn concentrations beyond 50 mg/L were found to be stunted or death. Similar observations were reported by Sharma and Sharma [27] and Balashouri [28]. Cherif et al., [29] had studied the effects of Zn on the growth and antioxidative systems in tomato (*Solanum lycopersicum*) plants. They observed that High levels of Zn had exhibited severe phytotoxic effects and retarded the growth and development of plants by interfering with some important metabolic activities. Barcelό and

Poschenrieder, [30], Sagardoy et al., [31] and Li et al., [32] had also reported that higher Zn concentrations were found to reduce the growth of the main root and affect cell division and elongation in various plants.

3.2. Effects of Zn Level on Dry Weights (Biomass)

The roots and shoots dry weight followed a similar pattern of lengths of root and shoot; decreasing gradually (P =.05) with increasing Zn treatments (Figure 1b). The maximum decrease of 135.00 mg was observed at 200 mg/L and a minimum of 934.00 mg was recorded at 5 mg/L Zn level for roots. However, for shoots, the maximum reduction was 245.00 mg at 200 mg/L and the minimum was 2108.00 mg at 5 mg/L. Our results were in good agreement with Hasan et al., [17] who observed a similar pattern in *Sorghum Bicolor L. (Moench)* and *Chenopodium Album* when exposed to Zn stress. Manivasagaperuma et al., [33] also observed a similar reduction of dry matter yield in *Vignaradiata L* by application of excess Zn concentrations. According to Tripathy and Moharty [34], the decreased in dry weight might be due interference of Zn with other essential metal such as Manganese (Mn) and Iron (Fe).

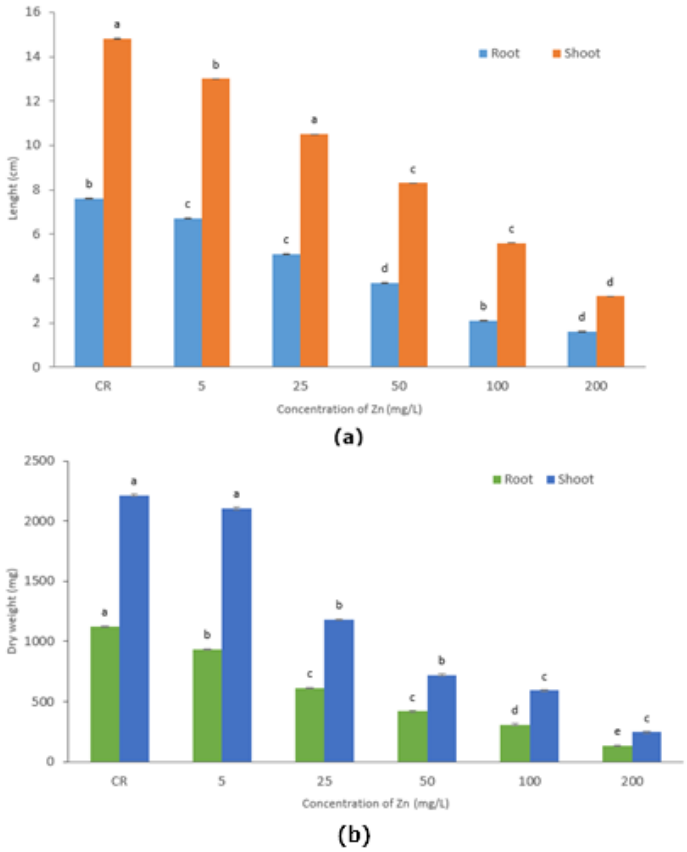

Figure 1. Effects of Zn Levels on **(a)** Fresh root and shoot lengths **(b)** Dry weight (biomass) of *sorghum bicolor L.M* plants after 21 day of exposure. *The vertical bars represent standard error of mean (n = 3). Different letters above the bars indicate significant differences at P<0.05 as determined by Fisher's LSD test.*

3.3. Estimation of Critical Toxicity Threshold Values

Dry weight yield decrease has generally been accepted as the standard measure for comparisons of plant's toxicity [16]. Critical toxicity threshold level is defined as the tissue concentration resulting in a 10% reduction in growth or yield in terms of dry weight of a particular plant [16,35]. The critical concentration of Zn in the hydroponic solution associated with 10% dry weight reduction of *Sorghum bicolor LM* was estimated to be 3.20 mg/L (Figure 2a). This is indicates that *sorghum bicolor L.M* should not be grown beyond Zn concentrations of above 3.2 mg/L. Zn-induced physical injury in the plants such as chlorosis (yellowing of leaves), appearance of brown spot on leaves and drying of older leaves were observed above 5 mg/L Zn level. This have vindicated the above claim concerning the estimated critical threshold value. For estimation of critical tissue concentration, second order polynomial regression growth curve was used at $\alpha = 0.05$. The critical tissue

toxicity level of Zn in the shoot was estimated to be 158.90 mg/kg (Figure 2b). It is also comparable to the values of 173.1 mg/Zn kg determined by Long et al., [3] for Chinese cabbage, 143 mg/kg for *Acacia auriculaeformis* [35], 200 mg/kg for Alfalfa [36] and 217.7 mg/kg for maize [37].

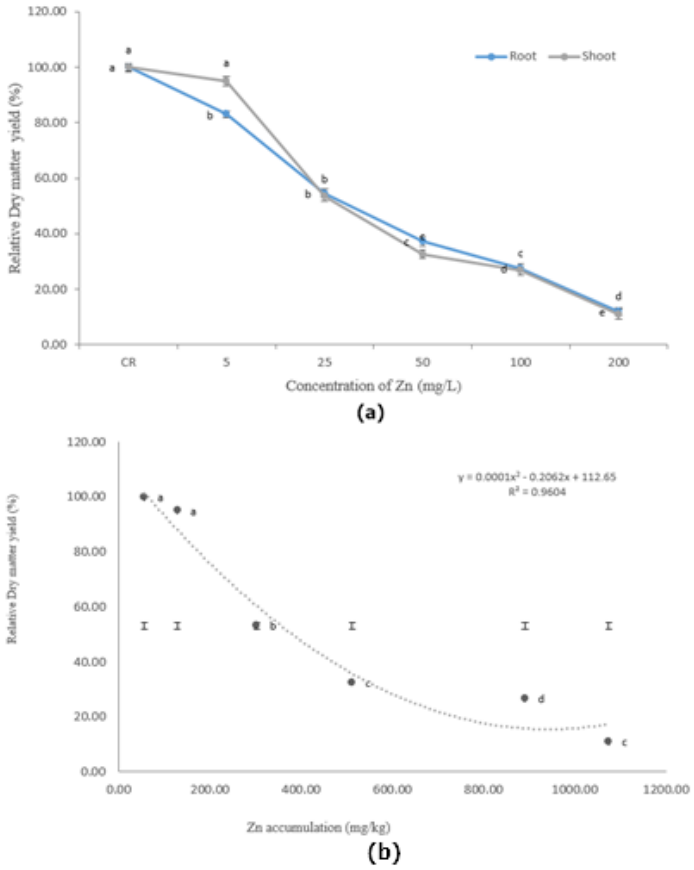

Figure 2. (a) Relationships Between Solution Zn Concentrations and Relative Dry Matter Yield and **(b)** between Plant Tissue Zn Concentrations and Relative Dry Yield of *Sorghum Bicolor L.M* exposed to 21 day of Zn Treatments. *Different letters above the bars indicate significant differences at P<0.05 as determined by Fisher's LSD test.*

3.4. Effects of Zn Levels on Zn Uptake

The results presented in Figure 3a show the mean Zn contents of *Sorghum bicolor LM* exposed to different doses of Zn. Both shoot and root Zn concentrations increased significantly ($P < 0.05$) with increasing Zn levels, with a maximum uptake of 2711.853 followed by 1470.744 mg/kg at 200 and 100 mg/L Zn levels respectively. The minimum uptake was recorded at control followed by 329.093 mg/kg at 5 mg/L for the shoot. In the case of roots, the maximum uptake was observed at 1074.217 (13 fold) followed by 890.66 mg/kg at 200 and 100 mg/L Zn treatments. While the minimum uptake was recorded at control treatments followed by 12.14 mg/kg at 5 mg/L Zn level. Davis and Parker [38] and Soudek et al., [39] also made a similar observation that uptake of Zn increased with increased application of Zn in peanut plants. The roots were affected more strongly by Zn treatments than shoots [38]. These results confirmed other studies that the Zn uptakes were higher in the roots than the shoots. The reason for this may likely be that the roots are organs in direct contact with Zn or because of metal tolerance mechanisms by the plants [40,41]

3.5. Effects of Zn level on the Accumulation of Proline

One of the strategies of plant's tolerance to Zn stress and toxicity is the generation and accumulation of proline [42,43]. Proline is believed to protect the plant's cells against oxidative damage caused by Zn-induced toxicity [41,44]. *Sorghum bicolor L.M* exposed to Zn stress exhibited a significant ($P = .05$) elevation in proline levels. Proline accumulation increased progressively with increasing Zn dosage (Figure 3b). The accumulations were more apparent in the leaves than root and shoots. The highest accumulation was obtained at 200 mg/L Zn level (3.657 µg/g) while the lowest (0.157 µg/g) was recorded

at control treatments for the leaves. In the case of roots, the maximum accumulation was found at highest Zn level (3.091 µg/g) and the minimum was recorded at control. The proline contents for shoots were lowest in comparison with that of the leaves and roots with maximum accumulation at 200 mg/L (2.35 µg/g) and minimum at control (0.08 µg/g). This is in good agreement with work carried out by Saradhi, and Saradhi [45] who observed that heavy metal stresses greatly the increased amount of proline. It has also been reported by numerous authors that proline acts as a metal chelator and the production of proline in response to Zn stress is dosage dependent [46,47]. The variation in proline accumulation may be due to differences in metabolic activities in different tissues [48]. Proline is reported to act as an antioxidant and have ROS scavenging activity thereby protecting cells against heavy metal-induced injury [49,50]. Based on this study however, accumulation of proline could not protect the studied plants from Zn-induced toxicity especially at higher Zn concentrations. This probably because the excess Zn had overpowered protective role of proline.

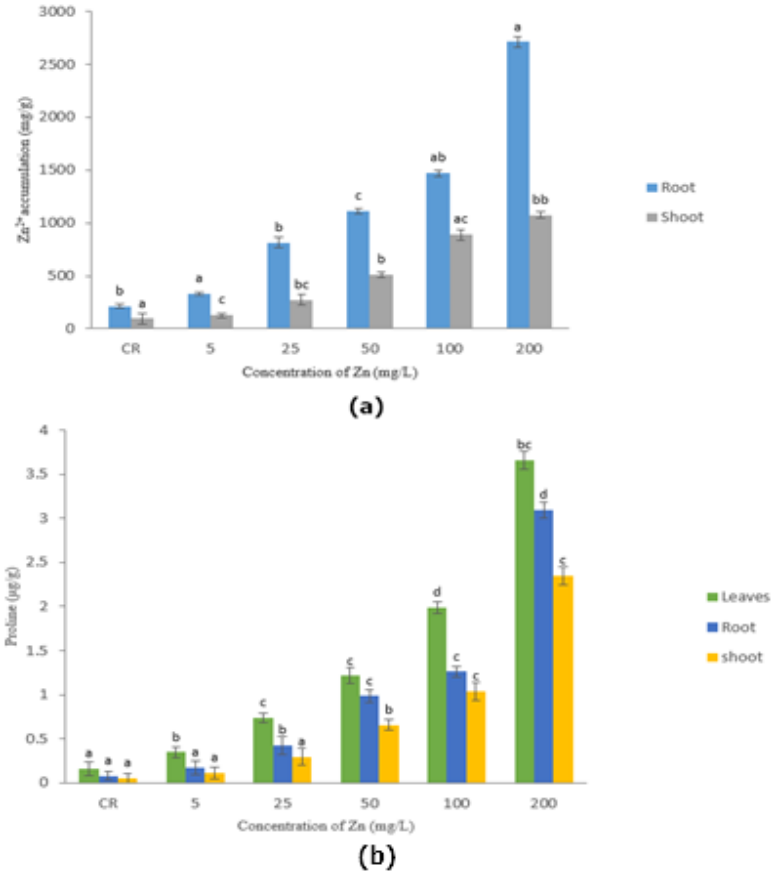

Figure 3. Effects of Zn Levels on **(a)** Zn uptake **(b)** accumulation of proline in sorghum bicolor L.M plants after 21 day of exposure. The vertical bars represent standard error of mean (n = 3). Different letters above the bars indicate significant differences at P<0.05 as determined by Fisher's LSD test.

3.6. Effects of Zn Level on Pigments

To assess the effects Zn toxicity on *sorghum bicolor L.M* plants pigments (Chl a, Chlb and Car.) contents were determined. Elevated levels of Zn in plants showed a remarkable decrease in pigments contents (P =.05) that causes remarkable reduction of photosynthesis rate [51]. The changes in pigment content are linked to visual symptoms of plants illness and photosynthetic production [52]. In this study, addition of Zn to hydroponic solution had resulted in severe depletion of pigment contents by inhibiting the Chl a, Chl b and Car (Figure 4) formation. The strongest inhibitory effect was recorded at the highest Zn dosage (200 mg/L). The pigments reduction followed the trend Car > Chl b > Chl a. Excess Zn has been reported to promote the decrease in pigments contents of *Triticumaestivum* seedlings [47]. The Zn-induced reduction in pigment contents is found to be associated with overproduction of reactive species oxygen (ROS), which is resulted from induction of oxidative stress [53]. The destruction of photosynthetic pigments by metal could be due to replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecules by Zn^{2+} [28,52,54]. It might also be due to impairment of the electron transport chain and inhibition of important enzymes associated with chlorophyll biosynthesis [52,55,56].

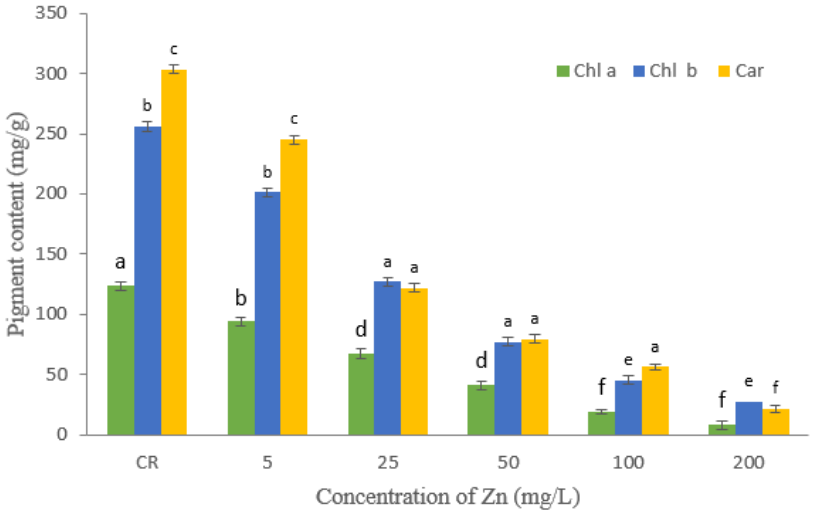

Figure 4. Effects of Zn Levels on Pigment Contents of Sorghum bicolor L.M leaves after 21 day of exposure. The vertical bars represent standard error of mean (n = 3). Different letters above the bars indicate significant differences at $P < 0.05$ as determined by Fisher's LSD test.

4. Conclusion

It can be concluded based on the results of the study that Zn uptake and toxicity in *sorghum bicolor L.M* were concentration dependent. Excess Zn reduced plant growth and development by depressing root and shoot lengths, biomass and the leaves showed chlorosis symptoms and signs of damage as judged by reduced pigment contents. Proline accumulations were markedly increased with increased Zn dosage. The critical toxicity threshold had shown that *sorghum bicolor L.M* should not be grown beyond Zn concentration of above 3.2 mg/L.

Competing interests

We declare that no competing of interest exists regarding this paper.

References

[1] Paul EV, Manu A, Surekha KA, Jianhua Z, Jian-Kang Z. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant J. 2006:45:523-539.

[2] Qin F, Shinozaki K, Yamaguchi-Shinozaki K. Achievements and Challenges in Understanding Plant Abiotic Stress Responses and Tolerance. Plant & Cell Physiol. 2011:52:1569–1582. DOI: https://doi.org/10.1093/pcp/pcr106.

[3] Long XX, Yang XE, Ni WZ, Ye ZQ, He ZL, Calvert DV, Stoffella JP. Assessing Zinc Thresholds for Phytotoxicity and Potential Dietary Toxicity in Selected Vegetable Crops. Comm. in Soil Sci. & Plant Analysis. 2003:34:1421-1434. DOI: https://doi.org/10.1081/CSS-120020454.

[4] Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters. 2010:8:199–216.

[5] Fahad S, Bajwa, Nazir U, Anjum AS, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Shah S, ZI, Muhammad, Alharby, Wu C, Wang D, Huang J. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. Front. Plant Sci. 2017:8:1147. DOI:https://dx.doi.org/10.3389%2Ffpls.2017.01147.

[6] Lekshmi S, Jayadev A. Effect of Heavy Metal Stress (Cadmium) on Morphological Physiological Activity and Anatomy of Cow Pea Plant (*Vigna Uncuigulata).* Int. J Research in Applied Sci. & Eng. Tech. 2017:281-290.

[7] Boyer JS. Plant Productivity and Environment. Science, 218, 1982:443-448. DOI: https://doi.org/10.1126/science.218.4571.443.

[8] Acquaah G. Principles of plant genetics and breeding; Blackwell, Oxford, UK. 2007: pp. 569.

[9] Misra A, Srivastava AK, Srivastava NK, Khan A. Zn-acquisition and its role in growth, photosynthesis, photosynthetic pigments, and biochemical changes in essential monoterpene oil(s) of Pelargonium graveolens. Photosynthetica. 2005:43:153-155.

[10] Hafeez B, Khanif M, Saleem M. Role of zinc in plant nutrition: a review. Am. J. Exp. Agric. 2013:374–391.DOI: 10.9734/AJEA/2013/2746.

[11] Hossain MA, Hasanuzzaman M, Fujita M. Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiol. & Molecular Biol. Plants. 2010:16:259–72.

[12] Zhao FJ, Lombi E, McGrath SP. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspicaerulescens*. Plant Soil. 2003:249:37-43.

[13] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003:91:179–194.

[14] Sharma P, Dubey RS. Involvement of Oxidative Stress and Role of Antioxidative Defense System in Growing Rice Seedlings Exposed to Toxic Concentrations of Aluminum. Plant Cell Reports. 2007:26:2027-2038.DOI: http://dx.doi.org/10.1007/s00299-007-0416-6.

[15] Gao JM, Xiao Q, Ding LP, Chen MJ, Yin L, Li J Z, Zhou SY, He GY. Differential responses of lipid peroxidation and antioxidants in *Alternanther aphiloxeroides* and *Oryza sativa* subjected to drought stress. Plant Growth Regul. 2008:56:89–95.

[16] Reichman SM. The responses of plants to metal toxicity: A review focusing on copper, manganese and zinc. Australian Minerals & Energy Environment Foundation. 2002. Accessed on November 21, 2011. Available: http://www.plantstress.com/Articles/toxicity-i/Metal-toxicity.pdf.

[17] Hasan MH, Reza A, Habib K. Effect of Zinc Toxicity on Plant Productivity, Chlorophyll and Zn Contents of Sorghum (*Sorghum Bicolor*) and Common Lambsquarter (*Chenopodium Album*). Int. J. of Agric. Research and Review. 2012:2:247-254.

[18] DeVries, J. Securing the Harvest. Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller Foundation, New York, USA, 2001; pp. 224.

[19] Xin Z, Wang ML, Barkley NA, Burow G, Franks C, Pederson G. Applying genotyping and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biology. 2008:8:103**.**

[20] Cakmak I, Gulut KY, Marschner H, Graham RD. Effect of zinc and iron deficiency on phytosiderophore release in wheat genotypes differing in zinc efficiency. J. Plant Nutr. 1994:17:1–17.

[21] Hopkins BG, Whitney DA, Lamond RE, Jolly VD. Phytosiderophore release by Sorghum, wheat, and corn under zinc deficiency. J Plant Nutri. 2008: 22623-2637. DOI: https://doi.org/10.1080/01904169809365593**.**

[22] Yadav SK. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African J Bot. 2010:76:167-179. DOI: https://doi.org/10.1016/j.sajb.2009.10.007.

[23] Ahmad M, Seyed GM, Henk S. The effect of EDDS and citrate on the uptake of lead in hydroponically grown *Matthiolaflavida***.** Chemosphere. 2013: 93:986–989.

[24] Bates LS, Waldren RP, Teare ID. Rapid Determination of Free Proline for Water-Stress Studies. Plant & Soil. 1973:39:205 – 207.

[25] Arnon DI. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in *Beta Vulgaris*. Plant Physiol. 1949:24:1–15.

[26] Lichtenthaler HK, Wellburn AR. Determination of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents. Biochemical Society Trans. 1983:11, 591-603.

[27] Sharma DC, Sharma CP. Chromium Uptake and its Effects on Growth and biological Yield of Wheat. Cereal Research Comm. 1993:21:317-322.

[28] Balashouri P. Effect of zinc on germination, growth and pigment content and phytomass of *Vignaradiata* and *Sorghum bicolour*. J Ecobio. 1995:7:109–114.

[29] Cherif J, Mediouni C, Ammar WB, Jemal F. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solanum lycopersicum*) J. Environ. Sci. 2011:23:837-844.

[30] Barceló J. Poschenrieder, C. Plant water relations are affected by heavy metals: a review. J. Plant Nutr. 1990:13:1–37.

[31] Sagardoy R, Morales F, Lopez-Millan AF, Abadia A, Abadia J. Effects of zinc toxicity on sugar beet (*Beta vulgaris L.*) plants grown in hydroponics. Plant Biol. 2008*:* 11: 339-350.

[32] Li TQ, Yang XE, Lu LL, Islam E, He ZL. Effects of zinc and cadmium interactions on root morphology and metal translocation in a hyperaccumulating species under hydroponic conditions. J. Hazard Mater. 2009:169:734–741. DOI: 10.1016/j.jhazmat.2009.04.004.

[33] Manivasagaperuma R, Vijayarengan P, Balamurugan S, Thiyagarajan G. Effect of zinc on growth, dry matter yield and nutrient content of *Vigna Radiata (L.)* Wilczek. Int. J. Recent Scientific Research. 2012:3:687-692.

[34] Tripathy BC, Mohanty P. Zinc-inhibited electron transport of photosynthesis in isolated barley chloroplasts. Plant Physiol. 1980*:*66:1174-1178.

[35] Zhang ZQ, Wong MH, Nie XP, Lan CY. Effects of zinc (zinc sulfate) on rhizobia-earleaf acacia (*Acacia auriculaeformis*) symbiotic association. Bioresource Tech. 1998:64:97-104.

[36] Stanisławska-Glubiak E, Korzeniowska J. Criteria for Assessing the Toxicity of Zinc to Plants. IUNGPIB. 2005:107:12.

[37] Baran A. Assessment of *Zea mays* Sensitivity to Toxic Content of Zinc in Soil. Pol. J. Environ. Stud. 2013:22: 77-83.

[38] Davis JG, Parker MB. Zinc toxicity symptom development and portioning of biomass and zinc in peanut plants. J. Plant Nutr. 1993:16:2353-2369.

[39] Soudek P, Petrova S, Vankova R, Song J, Vanek T. Accumulation of heavy metals using Sorghum sp., Chemosphere. 2014:15-24.

[40] Anamika S, Eaparn S, Fulekar M. Phytoremediation of Cd, Pb and Zn by *Brassica Juncea L.* Czern and Cross J. Applied Biosci. 2009:13:726-736.

[41] Mourato MP, Moreira IN, Leitão, I, Pinto FR, Sales JR, Martins LL. Effect of Heavy Metals in Plants of the Genus *Brassica*. Int. J. Mol. Sci. 2015:16*:*17975-17998. DOI: 10.3390/ijms160817975.

[42] Choudhary M, Jetley UK, Khan MA, Zutshi S, Fatma T. Effect of heavy metal stress on proline, malondialdehyde, and superoxidedismutase activity in the cyanobacterium Spirulina platensis-S5. Ecotoxicol. Environ. Safety. 2007:66:204–209.

[43] Szabados L, Savouré, A. Proline: A multifunctional amino acid. Trends in Plant Sci. 2009*:*15:89-97.

[44] Bac˘kor M, Fahselt D, Wu CT. Free proline content ispositively correlated with copper tolerance of the lichen *photobiont Trebouxiaerici (Chlorophyta).* Plant Sci. 2004:167: 151–157.

[45] Saradhi A, Saradhi PP. Proline accumulation under heavy metal stress. J Plant Physiol. 1991:138: 554-558.

[46] Radic ´S, Babic ´M, Skobic ´D, Roje V, Pevalek-Kozlina. B. Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor L*. Ecotoxicol. Environ. Saf. 2010:73:336-342. DOI:10.1016/j.ecoenv.2009.10.014.

[47] Bassi R, Sharma SS. Proline accumulation in wheat seedlings exposed to zinc and copper. Phytochemistry. 1993:33:1339-1342.

[48] Handique GK, Handique AK. Proline accumulation in lemongrass (*Cymbopogon flexuosus Stapf.*) due to heavy metal stress. J Environ Biol. 2009:30:299-302.

[49] Matysik J, Alia BB, Mohanty P. Molecular mechanism of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 2002*:*82:525–532.

[50] Mishra S, Dubey R.S. Inhibition of ribonuclease and protease activities in arsenic exposed rice seedlings: Role of proline as enzyme protectant. J. Plant Physiol. 2006:163:927–936.

[51] Leila R, Abbas A. Biochemical responses of Gouan (*Aeluropuslittoralis*) to heavy metals stress. Australian J. Crop Sci. 2011:5:375-383.

[52] Küpper H, Küpper F, Spiller M. In situ detection of heavy metal substitution chlorophylls in water plants. Photosyn Res. 1998:58:123-133.

[53] Madhava RKV, Sresty TVS. Antioxidative parameters in the seedlings of pigeon pea (*Cajanuscajan (L.) Millspaugh*) in response to Zn and Ni stresses Plant Sci. 2000: 157:113-128.

[54] Van Assche F, Clijsters H. Effects of metals on enzyme activity in plants. Plant Cell Environ. 1990:13:195–206.

[55] Sandalio LM, Dalurzo HC, Go ́mez M, Romero-Puertas M.C, Delrı´o, L.A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot. 2001:52:2115–2126.

[56] Fikriye KZ, Omer M. Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in Bean (*Phaseolus vulgaris*) seedlings. Acta Biologica Cracoviensia. 2005:47:157-164.