

## Original Research Article

### ZINC ACCUMULATION IN *AMARANTHUS CAUDATUS* AND *CORCHORUS OLITORIUS*: RELEVANCE FOR PHYTOEXTRACTION

#### ABSTRACT

Aim: In a pot experiment the accumulation of Zinc in *Amaranthus caudatus* and *Corchorus olitorius* from contaminated soil and its relevance for phytoextraction were studied.

Study design: Plants were exposed to different levels (0,150, 300, 450 ppm) of Zinc concentration as Zinc sulfate heptahydrate, laid in Completely Randomized Design.

Place and duration of study: The study lasted for a total of 37 days in a housing facility used for phytoplankton culture at National Institute for Freshwater Fisheries Technology New-Bussa Nigeria.

Materials and Methods: Seeds were raised in a nursery for 16 days, and transplanted to pots containing each 2 kg of air-dried and sieved soil. Zinc was artificially applied to soil as sulphate ( $ZnSO_4 \cdot 7H_2O$ ) at the rate of 0, 150, 300, 450 ppm for each vegetable. They were watered at 60-70 % field capacity and growth parameters measured every 7 days. Plants were uprooted after 3 weeks of transplanting, washed, and weighed before and after oven drying. They were analyzed for zinc concentration.

Results: Results of this study showed decreases in growth and yield at 300 and 450 ppm Zinc concentrations. Plant height for both vegetables increased at 150 ppm however these increases were significantly different ( $P=0.05$ ) with control for *Corchorus* only. There were also significant differences

**Comment [MSOffice1]:**  
Correct: significantly different means that ( $P \leq 0.05$ ) not ( $P = 0.05$ )

( $P=0.05$ ) between treatments in Zn accumulation. Results further showed that Zn was accumulated in the leaves more than the roots. Concentrations of Zn in plant tissues of both vegetables followed a similar order of leaves > roots > stem indicating their efficiency in translocating Zn from roots to leaves. Translocation factors were generally greater than unity.

**Comment [MSOffice2]:** The same as previous comment

**Comment [MSOffice3]:** Replace "unity" by "1"

Conclusion: Despite their lack of hyperaccumulation properties, both vegetables exhibited good potential for phytoextraction. The higher translocation factor of *Corchorus olitorius* indicated better phytoextraction potential.

Keywords: Zinc contamination, *Amaranthus caudatus*, *Corchorus olitorius*, phytoextraction

**Comment [MSOffice4]:** use words that you did not use in the title

## 1. INTRODUCTION

Zinc is one of the essential micronutrients needed for plant growth and development. It occurs naturally in the environment but may reach toxic levels in soils due to human activities. In addition to industrial sources, zinc could be introduced in the agro ecosystem through uses as pesticides, fertilizers and irrigation with wastewater [1, 2, 3, 4, 5, 6]. At levels exceeding crop limit, it becomes a great environmental concern as it is non-biodegradable.

**Comment [MSOffice5]:** Add these references:  
•Mohammed Mesnoui, Enrique Mateos-Naranjo, Jesús Alberto Pérez-Romero, José María Barcia-Piedras, Brahim Lotmani, Susana Redondo-Gómez: *Combined effect of Cr-toxicity and temperature rise on physiological and biochemical responses of Atriplex halimus L.* Plant Physiology and Biochemistry 08/2018; 132., DOI:10.1016/j.plaphy.2018.08.025  
•Mohammed Mesnoui, Enrique Mateos-Naranjo, José María Barcia-Piedras, Jesús Alberto Pérez-Romero, Brahim Lotmani, Susana Redondo-Gómez: *Physiological and biochemical mechanisms preventing Cd-toxicity in the hyperaccumulator Atriplex halimus L.* Plant Physiology and Biochemistry 09/2016; 106:30-38., DOI:10.1016/j.plaphy.2016.04.041  
•Stress abiotiques sur Atriplex halimus L : Effet des métaux lourds et caractérisation des biomarqueurs et bioindicateurs. Thèse, Mohammed MESNOUA (2016). Université Abdelhamid Ben Badis-Mostaganem.

Just like other heavy metals zinc pollution destroys biodiversity making soils unsuitable for plant development. But several plants are known to thrive in polluted soils due to peculiar physiological characteristics and as such have been identified as phytoremediators. These plants are able to tolerate relatively high levels of heavy metal stress and in the process absorb pollutants from soil. This process is known as phytoextraction and is considered efficient and cheap; as low as \$ 0.05 per cubic meter of land [7]. Some common criteria for plant selection for phytoextraction as listed by Sarma [8] include: ability to uptake, translocate and accumulate pollutants, tolerance to extreme weather conditions, rapid growth rate, luxurious biomass, deep root system, and nativity to the soil type being remediated. These factors are also dependent on plant species, soil type and type of heavy metal [9, 10, 11].

**Comment [MSOffice6]:** Rewrite this sentence

*Amaranthus* and *Corchorus* spp. are fast-growing humid tropical vegetables, usually grown for their leaves, as well as fiber in the case of *Corchorus*. *Amaranthus* is annual plant while *Corchorus* is annual to short term perennial plant. Both vegetables are fast growing and can be harvested several times in a growing season. They are sometimes referred to as wild vegetables [12, 13, 14] because they are able to grow in the wild without cultivation, and this shows their hardy nature to survive in harsh weather conditions.

Earlier studies have shown accumulation of significant amount of heavy metals by *Corchorus* and *Amaranthus* species in contaminated soils, however few studies have been done on *Corchorus olitorius* and *Amaranthus caudatus* with respect to zinc phytoextraction. This study was aimed to determine and compare the phytoextraction potentials of *Corchorus olitorius* and *Amaranthus caudatus*.

## **2.0 MATERIALS AND METHODS**

### **2.1 Experimental site**

The experiment was conducted using plastic pots placed inside a housing facility for growing phytoplanktons at the National Institute for Freshwater Fisheries Technology (Latitude 09° 53'N and long 04° 31'E), New-Bussa Nigeria.

### **2.2 Pot experiment**

Bulk soil sample was collected from the orchard at Federal College of Freshwater Fisheries Technology New-Bussa, Nigeria. Soil sample was air-dried, ground and passed through 2mm sieve. It was mixed to ensure homogeneity. 2 kg each was weighed into 24 experimental pots, and laid in a Completely Randomized Design.

*Amaranthus caudatus* and *Corchorus olitorius* seeds were raised in a nursery bed in the orchard. After 16 days, seedlings were transplanted to pots at 3 seedlings per pot. Uniform watering with deionized water at 60 - 70% field capacity (by weight) was done as required for all treatments.

Zinc was artificially applied to soil as sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) at the rate of 0, 150, 300, 450 ppm for each vegetable. The maximum permissible limit of total zinc in soil is 300 ppm [15] however total Zn concentrations in contaminated farm lands in Nigeria could reach up to 1575 ppm [16].

**Comment [MSOffice7]:** Why you didn't use this concentration in your experiment

Data were collected for plant height and number of leaves at 7 days interval for 3 weeks. Visible symptoms of toxicity were clear at 3<sup>rd</sup> week after transplanting (WAT). Plants were then uprooted and washed thoroughly but gently with deionized water. Fresh biomass was weighed while dry matter weight was taken after oven drying to constant weight at 70°C.

Translocation factor was calculated as a ratio of Zn concentration in shoot to Zn concentration in root.

### 2.3 Data Analysis

Significant treatment effects were tested using Analysis of Variance (SPSS vs 13) evaluated at  $P \leq 0.05$ . Means were separated using Duncan Multiple Range Test (DMRT).

## 3.0 RESULTS AND DISCUSSION

### 3.1 Soil physical and chemical properties

Physical and chemical properties of soil used in the study are shown in Table 1. The soil was slightly acidic with pH of 6.3, and texture was sandy loam. Organic carbon, available phosphorus and total nitrogen were low with values of 2.00 g kg<sup>-1</sup>, 5.94 mg kg<sup>-1</sup> and 0.73 g kg<sup>-1</sup>, while calcium dominated the exchange site with a mean value of 7.70 cmol kg<sup>-1</sup>. Extractable zinc concentration was 0.67 mg kg<sup>-1</sup>. Sims and Johnson [17] reported the critical zinc range for most crops as 0.5 – 2.0 mg kg<sup>-1</sup> (DTPA) and 0.5 – 3.0 mg kg<sup>-1</sup> (Melich-1). Zinc is usually deficient in soils with high sand and low organic carbon contents [18].

**Table 1: Chemical and physical properties of soil used for the study**

Parameters	Unit	Values	Method
pH	-	6.3	pH electrode meter
Extractable Zinc	mg kg <sup>-1</sup>	0.67	Digestion with 3:1 HCl:HNO <sub>3</sub> , AAS
Sodium	cmol kg <sup>-1</sup>	0.53	Ammonium acetate extraction
Potassium	cmol kg <sup>-1</sup>	0.30	
Magnesium	cmol kg <sup>-1</sup>	1.62	
Calcium	cmol kg <sup>-1</sup>	7.70	
Organic carbon	g kg <sup>-1</sup>	2.00	Wet oxidation (modified) [19]
Total Nitrogen	g kg <sup>-1</sup>	0.73	Micro Kjeldahl [20]
Available Phosphorus	mg kg <sup>-1</sup>	5.94	Bray-1 [21]
Sand	g kg <sup>-1</sup>	874.8	Hydrometer method (1:1 soil to water)
Silt	g kg <sup>-1</sup>	76.4	
Clay	g kg <sup>-1</sup>	48.8	

### 3.2 Effects of Zinc concentration on growth and yield of vegetables

Zinc concentrations had significant effect on plant heights of both vegetables in the 2<sup>nd</sup> and 3<sup>rd</sup> week after transplanting (WAT) (Table 2). However, number of leaves showed significant difference at 3<sup>rd</sup> WAT for *Amaranthus caudatus* only.

Peak plant height values followed a similar trend at zinc concentration of 150 ppm for both vegetables, after which it decreased gradually with the least plant height at 450 ppm. Moreover, the highest plant heights at 150 ppm were not significantly different from the control except at 3<sup>rd</sup> WAT for *Corchorus olitorius*. Toxicity effects were visually observed in both vegetables at 300 and 450 ppm (Plates 1 and 2). Plant species differ in zinc uptake, threshold toxicity and time of exposure stress [22]. Results indicated similar response of both vegetables to time of exposure stress and threshold toxicity.



Plate 1: Effect of Zinc concentration on growth of *Amaranthus caudatus*

A = 0 ppm B = 150 ppm C = 300 ppm D = 450 ppm



Plate 2: Effect of Zinc concentration on growth of *Corchorus olitorius*

A = 0 ppm B = 150 ppm C = 300 ppm D = 450 ppm

**Table 2: Plant height and number of leaves of vegetables as influenced by zinc concentrations**

Treatment	<i>Amaranthus caudatus</i>						<i>Corchorus olitorius</i>					
	Plant height (cm)			Number of leaves			Plant height (cm)			Number of leaves		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
	<b>Week After Transplanting</b>											
<b>0 ppm</b>	15.86 <sup>a</sup>	22.71 <sup>ab</sup>	24.63 <sup>a</sup>	8.44 <sup>a</sup>	11.11 <sup>a</sup>	11.56 <sup>ab</sup>	8.19 <sup>a</sup>	10.26 <sup>ab</sup>	13.80 <sup>b</sup>	6.44 <sup>a</sup>	9.11 <sup>a</sup>	16.33 <sup>a</sup>
<b>150 ppm</b>	17.59 <sup>a</sup>	23.56 <sup>a</sup>	25.43 <sup>a</sup>	8.00 <sup>a</sup>	11.12 <sup>a</sup>	13.11 <sup>a</sup>	7.11 <sup>a</sup>	12.54 <sup>a</sup>	17.49 <sup>a</sup>	6.67 <sup>a</sup>	9.13 <sup>a</sup>	19.63 <sup>a</sup>
<b>300 ppm</b>	16.38 <sup>a</sup>	20.33 <sup>bc</sup>	22.60 <sup>ab</sup>	7.78 <sup>a</sup>	9.78 <sup>a</sup>	13.71 <sup>a</sup>	7.72 <sup>a</sup>	11.74 <sup>a</sup>	16.34 <sup>ab</sup>	6.89 <sup>a</sup>	8.33 <sup>a</sup>	15.78 <sup>a</sup>
<b>450 ppm</b>	16.59 <sup>a</sup>	19.57 <sup>c</sup>	19.87 <sup>b</sup>	8.56 <sup>a</sup>	10.00 <sup>a</sup>	9.89 <sup>b</sup>	5.05 <sup>a</sup>	7.79 <sup>b</sup>	10.22 <sup>c</sup>	6.00 <sup>a</sup>	7.89 <sup>a</sup>	15.11 <sup>a</sup>

Means followed by different alphabets in the same column are significantly different (DMRT P=0.05)

Despite significant differences in plant heights at 2<sup>nd</sup> and 3<sup>rd</sup> WAT, both vegetables showed no significance in their fresh and dry matter yields (Table 3). However, *Amaranthus* had the highest fresh and dry yields of 21.45 and 4.13 g at 0 ppm while *Corchorus* had highest values of 5.25 and 1.56 g of fresh and dry yield at 150 ppm. This suggests that *Corchorus olitorius* may be a better accumulator than *Amaranthus caudatus*. Generally, yields were consistently lowest at 450 ppm.



Kloke [15] estimated maximum of 300 ppm Zn concentration for most crops; however for Amaranthus and Corchorus growth and yield reduced at 300 ppm. This means that similar soil type having zinc concentration of 300 ppm could be toxic for these vegetables.

At Zn concentrations of 300 and 450 ppm plants showed symptoms of toxicity. There were necrotic patches on leaves and evidence of stunted growth. This shows their intolerance to high Zn levels and therefore they are not hyperaccumulators [8].

**Table 3: Effects of zinc concentrations on fresh and dry matter yields of vegetables**

	<i>Amaranthus caudatus</i>				<i>Corchorus olitorius</i>			
Treatment	Root	Stem	Leaves	Total	Root	Stem	Leaves	Total
<b>Fresh matter yield (g)</b>								
0 ppm	5.21 <sup>a</sup>	9.52 <sup>a</sup>	6.72 <sup>a</sup>	21.45	0.50 <sup>a</sup>	1.46 <sup>a</sup>	2.56 <sup>a</sup>	4.52
150 ppm	2.07 <sup>a</sup>	7.84 <sup>ab</sup>	6.88 <sup>a</sup>	16.79	0.53 <sup>a</sup>	1.88 <sup>a</sup>	2.84 <sup>a</sup>	5.25
300 ppm	3.39 <sup>a</sup>	6.29 <sup>ab</sup>	5.55 <sup>a</sup>	15.23	0.53 <sup>a</sup>	1.43 <sup>a</sup>	2.32 <sup>a</sup>	4.28
450 ppm	1.37 <sup>a</sup>	4.84 <sup>a</sup>	4.25 <sup>a</sup>	10.46	0.64 <sup>a</sup>	1.19 <sup>a</sup>	2.23 <sup>a</sup>	4.06
<b>Dry matter yield (g)</b>								
0 ppm	0.92 <sup>a</sup>	1.65 <sup>a</sup>	1.56 <sup>a</sup>	4.13	0.29 <sup>a</sup>	0.33 <sup>a</sup>	0.80 <sup>a</sup>	1.42
150 ppm	0.70 <sup>a</sup>	1.67 <sup>a</sup>	1.70 <sup>a</sup>	4.07	0.34 <sup>a</sup>	0.43 <sup>a</sup>	0.79 <sup>a</sup>	1.56
300 ppm	0.70 <sup>a</sup>	1.14 <sup>a</sup>	1.34 <sup>a</sup>	3.18	0.23 <sup>a</sup>	0.31 <sup>a</sup>	0.68 <sup>a</sup>	1.14
450 ppm	0.43 <sup>a</sup>	0.87 <sup>a</sup>	1.12 <sup>a</sup>	2.42	0.24 <sup>a</sup>	0.25 <sup>a</sup>	0.66 <sup>a</sup>	1.15

Means followed by different alphabets in the same column of each section are significantly different (DMRT P=0.05)

### 3.3 Zinc accumulation and translocation factors in Vegetables

There were significant increases ( $P = 0.05$ ) in Zinc concentrations in vegetables (Table 4). As seen in results above, vegetables grown in 450 ppm zinc concentration had the lowest growth and yield; resultant of the highest total concentration in tissues ( $735.57 \text{ mg kg}^{-1}$  for Amaranthus and  $640 \text{ mg kg}^{-1}$  for Corchorus). Compared to other studies [23, 24, 25] Zn concentration in plant tissues treated with Zn salt were low ( $21.18 - 735.57 \text{ mg kg}^{-1}$ ). This may be as a result of little time of exposure (21 days). However, Hafeez et al. [22] stated that yield may be reduced at Zn plant tissue concentration of  $300 - 1000 \text{ mg kg}^{-1}$ . Leaf Zn concentrations of  $\geq 300 \text{ mg kg}^{-1}$  led to the vegetables yield reductions.

Baker and Brooks [26] had classified zinc hyperaccumulators as plants that can absorb  $>10,000 \text{ mg kg}^{-1}$  of zinc in the dry matter. On this basis, present study shows that neither Amaranthus nor Corchorus are hyperaccumulators. This finding corroborates that of Nazir et al. [27].

**Comment [MSOffice8]:** This because you used a low concentrations f Zn. If you used à Zn concentrations (up to 5000ppm) you probably can find another results, and you can also know the tolerance limit of yours plants

**Table 4: Zinc concentrations ( $\text{mg kg}^{-1}$ ) and Translocation factors (TF) in vegetables**

	<i>Amaranthus caudatus</i>					<i>Corchorus olitorius</i>				
	Root	Stem	Leaves	Total	TF	Root	Stem	Leaves	Total	TF
<b>0 ppm</b>	50.71 <sup>c</sup>	25.35 <sup>c</sup>	54.01 <sup>c</sup>	130.07	1.57	45.56 <sup>c</sup>	21.18 <sup>c</sup>	52.06 <sup>c</sup>	118.80	1.61
<b>150 ppm</b>	147.38 <sup>b</sup>	56.56 <sup>b</sup>	167.68 <sup>b</sup>	371.62	1.52	125.21 <sup>b</sup>	50.31 <sup>b</sup>	148.07 <sup>b</sup>	323.59	1.58
<b>300 ppm</b>	229.20 <sup>ab</sup>	65.72 <sup>ab</sup>	270.61 <sup>b</sup>	565.53	1.47	177.37 <sup>b</sup>	61.56 <sup>ab</sup>	265.29 <sup>ab</sup>	504.22	1.84
<b>450 ppm</b>	280.55 <sup>a</sup>	73.54 <sup>a</sup>	381.48 <sup>a</sup>	735.57	1.62	238.07 <sup>a</sup>	70.66 <sup>a</sup>	331.55 <sup>a</sup>	640.28	1.69

Means followed by different alphabets in the same column are significantly different (DMRT  $P=0.05$ )

Translocation of zinc from root and shoot ranged between 1.47 and 1.62 for Amaranthus while in Corchorus it was between 1.58 and 1.84 (Table 4). Translocation factor is used to estimate plants' potential for phytoextraction. In the present study TF was above unity indicating the efficiency in translocating Zn from roots to the shoot. Contrary to the findings of Mavengahama et al. [25] leaves

**Comment [MSOffice9]:** Replace "unity" by "1"

concentrated more Zn than roots indicating good potential for phytoremediation [28, 29]. Contrasting results in studies may be attributed to differences in soil type. Translocation factor may be influenced by adsorption capacity of soils [30, 31]. The more metals are adsorbed to colloidal surfaces the less release for uptake. Moreover, other factors such as high concentrations of other heavy metals [32] could also influence Zn uptake. Generally, *Corchorus olitorius* consistently had higher TF than *Amaranthus caudatus*.

#### 4.0 Conclusion

The two vegetables investigated had similar responses to zinc concentrations. Higher zinc concentrations had negative effects on vegetables. Based on shoot biomass and accumulated zinc concentrations in plant tissues, both vegetables are not hyper accumulators however they exhibited good potential for phytoextraction. *Corchorus olitorius* had higher translocation factor than *Amaranthus caudatus* indicating better phytoextraction potential.

#### REFERENCES

- [1] Singh KP, Mohan D, Sinha S, Dalwani R. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health agricultural, and environmental quality in the wastewater disposal area. *Chemosphere* 2004; 55: 227-255.
- [2] He ZL, Yang XE, Stoffella PJ. Trace elements in agroecosystems and impacts on the environment. *J. Trace Elem. Med. Bio.* 2005; 19(2-3):125-140.
- [3] Gobarah ME, Mohamed MH, Tawfik MM. Effect of phosphorus fertilizer and foliar spraying with zinc on growth, yield and quality of groundnut under reclaimed sandy soils. *J. Appl. Sci. Res.* 2006; 2: 491– 496.

- [4] Phattarakul N, Rerkasem B, Li LJ, Wu LH, Zou CQ, Ram H, Sohu VS, Kang BS, Surek H, Kalayci M, Yazici A, Zhang FS, Cakmak I. Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant Soil*. 2012; 361:131–141.
- [5] Barua D, Saikia M. Agronomic biofortification in rice varieties through zinc fertilization under aerobic condition. *Indian J. Agric. Res.* 2018; 52(1): 89-92.
- [6] Zaman Q, Aslam Z, Yaseen M, Ihsan MZ, Khaliq A, Fahad S, Bashir S, Ramzani PMA, Naeem M. Zinc biofortification in rice: leveraging agriculture to moderate hidden hunger in developing countries. *Arch. Agron. Soil Sci.* 2018; 64(2):147-161.
- [7] Cunningham SD, Shann JR, Crowley D, Anderson TA. In: Krueger EL, Anderson TA, Coats JP (eds.). *Phytoremediation of Soil and Water Contaminants*. American Chemical Society, Washington, DC; 1997.
- [8] Sarma H. Metal Hyperaccumulation in Plants: A Review Focusing on Phytoremediation Technology. *J. Environ. Sci. Technol.* 2011; 4: 118-138.
- [9] Barman SC, Kisku GC, Salve PR, Misra D, Sahu RK, Ramteke PW, Bhargava SK. Assessment of industrial effluent and its impact on soil and plants. *J. Environ. Biol.* 2001; 22: 251-256.
- [10] Spinoza-Quinones FR, Zacarkim CE, Palacio SM, Obregon CL, Zenatti DC, *et al.* Removal of heavy metal from polluted river water using aquatic macrophytes *Salvinia* sp. *Braz. J. Physiol.* 2005; 35: 744-746.
- [11] Otte ML, Haarsma MS, Broekman RA, Rozema J. Relation between heavy metal concentrations in salt marsh plants and soil. *Environ. Pollut.* 1993; 82: 13-22.
- [12] Bruni R, Medici A, Guerrini A, Scalia S, Poli F, Muzzoli M, Sacchetti G. Wild *Amaranthus caudatus* Seed Oil, a Nutraceutical Resource from Ecuadorian Flora. *J. Agric. Food Chem.* 2001; 49: 5455–5460.

- [13] Balemie K, Kebebew F. Ethnobotanical study of wild edible plants in Derashe and Kucha Districts, S Ethiopia. *J. Ethnobiol. Ethnomed.* 2006; 2: 53. (doi: 10.1186/ 1746-4269-2-53).
- [14] Ndlovu J, Afolayan AJ. Nutritional analysis of South African wild vegetable *Corchorus olitorius* L. *Asian J. Plant Sci.* 2008; 7(6): 615-618.
- [15] Kloke A. Guidelines for tolerable total contents of some elements in cultivated soil. *Messages of VDLUFA.* 1980; 1-3: 9-11.
- [16] Aloh OG, Obasi NA, Chukwu KE, Agu AN. Effects of lead-zinc mining activities on water and soil quality in Ameka mining area of Ezza South, Ebonyi State, Nigeria. *Int. Res. J. Nat. Appl. Sci.* 2016; 3(7): 194-231.
- [17] Sims JT, Johnson GV. Micronutrient soil tests. In: Mortvedt J, Cox F, Shuman L, Welch R. (eds). *Micronutrients in Agriculture.* Soil Science Society of America, Madison, Wisconsin, pp. 427-476; 1991.
- [18] Alloway BJ. *Zinc in Soils and Crop Nutrition* Second edition, published by IZA and IFA Brussels, Belgium and Paris, France; 2008.
- [19] Nelson DW, Sommers LE. Total Carbon, Organic Carbon and Organic Matter. In: Page AL, Miller RH, Keeny DR. (Eds.) *Methods of Soil Analysis, Part-2, 2nd Edition, Agronomy Monograph No. 9,* ASA and SSSA, Madison, 539-579; 1982.
- [20] Jackson ML. *Soil chemical analysis.* Englewood cliffs, NY: Prentice Hall; 1962.
- [21] American Public Health Association (APHA). *19th Edition of Standard Methods for the Examination of Water and Wastewater,* American Public Health Association, 800 I Street, NW, Washington, DC 20001-3710; 1995.

- [22] Hafeez B, Khanif YM, Saleem M. Role of Zinc in Plant Nutrition- A Review. Am. J. Exp. Agric. 2013; 3(2): 374-391.
- [23] Santos, GCG, Rodella, AA, Abreu, CA, Coscione, AR. Vegetable species for phytoextraction of boron, copper, lead, manganese and zinc from contaminated soil. Scientia Agricola 2010; 67(6), 713-719.
- [24] Malik N, Chamon A, Mondol M, Elahi S, Faiz S. Effects of different levels of zinc on growth and yield of red amaranth ( *Amaranthus* sp. ) and rice ( *Oryza sativa* , Variety-BR49). J. Bangladesh Assoc. Young Res. 2011; 1(1): 79-91.
- [25] Mavengahama S, de Clercq WP, McLachlan, M. Trace element composition of two wild vegetables in response to soil-applied micronutrients. South African Journal of Science, 2014; 110(9-10): 1-5.
- [26] Baker AJM, Brooks RR. Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. Biorecovery. 1989; 1; 81-126.
- [27] Nazir A, Malik R, Ajaib M, Khan N, Siddiqui M. Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. Pak. J. Bot. 2011; 43: 1925-1933.
- [28] Ebbs SD, Kochian LV. Phytoextraction of zinc by Oat (*Avena sativa*), Barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). Environ. Sci. Technol. 1998; 32: 802-806.
- [29] Reeves RD, Baker AJM. Metal-accumulating plants. In: Raskin I, Ensley BD (Eds.) Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. John Wiley & Sons, Inc., New York, pp. 193-229; 2000.
- [30] Elliott HA, Liberati MR, Huang CP. Competitive Adsorption of Heavy Metals by Soils. J. Environ. Qual. 1986; 15:214-219.
- [31] Basta NT, Pantone DJ, Tabatabai MA. Path Analysis of Heavy Metal Adsorption by Soil. Agron. J. 1993; 85:1054-1057.

[32] Xue SG, Chen YX, Reeves, RD, Alan JM, Baker Q, Denise L, Fernando R. Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Environ. Pollut.* 2004; 131: 393-399.

UNDER PEER REVIEW