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 ~~triplicate~~ three levels ~~(0,150, 300, 450 ppm)~~ of zinc concentration as zinc sulphate heptahydrate(0,150, 300, 450 ppm), laid in ~~Completely~~ completely ~~Randomized~~ randomized ~~Design~~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 ($ZnSO_4.7H_2O$) 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 carefully 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 (P≤~~=~~0.05) between treatments in zinc accumulation. Results further showed that zinc was accumulated in the leaves more than the roots. Concentrations of zinc in plant tissues of both vegetables followed a similar order of leaves > roots > stem indicating their efficiency in zinc ~~translocating~~translocation ~~zinc~~ from roots to leaves. ~~Translocation factors were generally greater than unity.~~

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

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

1. INTRODUCTION

Zinc (Zn) 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~~ Zn 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.

Just like other heavy metals, ~~zinc~~ Zn 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].

Amaranthus and Corchorus spp*.* are fast-growing humid tropical vegetables, usually grown for their leaves, as well as fiber in the case of *Corchoru*.~~*s*~~. *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~~ Zn phytoextraction. This study was aimed to determine and compare the phytoextraction potentials of *C. olitorius* and *A. caudatus.*

2.0 MATERIALS AND METHODS

2.1 Experimental site

The experiment was conducted using plastic pots placed inside a housing facility for growing phyto-planktons at the National Institute for Freshwater Fisheries Technology (Latitude 09^o 53'N and long 04^o 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~~ Two kg each was weighed into 24 experimental pots, and laid in a ~~Completely~~ completely ~~Randomized~~ randomized ~~Design~~design.

A. caudatus and *C. olitorius* seeds were raised in a nursery bed in the orchard. After 16 days, seedlings were transplanted ~~to~~ in 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 ($ZnSO_4.7H_2O$) at the rate of 0, 150, 300, 450 ppm in triplicate for each vegetable. The maximum permissible limit of total ~~zinc~~ Zn in soil is 300 ppm [15] however total ~~zinc~~ Zn concentrations in contaminated farm lands in Nigeria could reach up to 1575 ppm [16].

Data were collected for plant height and number of leaves at 7 days interval for 3 weeks. Visible symptoms of toxicity were clear at 3rd week after transplanting (WAT). Plants were then carefully 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 (TF) was calculated as a ratio of ~~zinc~~ Zn concentration in shoot to ~~zinc~~ Zn concentration in root.

Comment [A. A.1]: Please indicate the method of Zn analysis to determination translocation factor

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^{-1}, 5.94 mg kg^{-1} and 0.73 g kg^{-1}, while calcium dominated the exchange site with a mean value of 7.70 cmol kg^{-1}. Extractable zinc concentration was 0.67 mg kg^{-1}. Sims and Johnson [17] reported the critical zinc range for most crops as 0.5 – 2.0 mg kg^{-1} (DTPA) and 0.5 – 3.0 mg kg-1 (Melich-1). Zinc is usually deficient in soils with high sand and low organic carbon contents [18].

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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^{-1}	0.67	Digestion with 3:1 HCl:HNO$_3$, AAS

Sodium	cmol kg^{-1}	0.53	Ammonium acetate extraction
Potassium	cmol kg^{-1}	0.30	??
Magnesium	cmol kg^{-1}	1.62	??
Calcium	cmol kg^{-1}	7.70	??
Organic carbon	g kg^{-1}	2.00	Wet oxidation (modified) [19]
Total Nitrogen	g kg^{-1}	0.73	Micro Kjeldahl [20]
Available Phosphorus	mg kg^{-1}	5.94	Bray-1 [21]
Sand	g kg^{-1}	874.8	Hydrometer method (1:1 soil to water)
Silt	g kg^{-1}	76.4	
Clay	g kg^{-1}	48.8	

3.2 Effects of ~~zinc~~ Zn concentration on growth and yield of vegetables

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

Peak plant height values followed a similar trend at ~~zinc~~ Zn 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 3rd WAT for *C. olitorius*. Toxicity effects were visually observed in both vegetables at 300 and 450 ppm (Plates 1 and 2). Plant species differ in ~~zinc~~ Zn 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

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

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

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

Kloke [15] estimated maximum of 300 ppm ~~zinc~~ ZN concentration for most crops; however for *A. caudatus* and *C. olitorius* growth and yield reduced at 300 ppm. This means that similar soil type having ~~zinc~~ Zn concentration of 300 ppm could be toxic for these vegetables.

At ~~zinc~~ 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 ~~zinc~~ Zn levels and therefore they are not hyperaccumulators [8].

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

	Amaranthus caudatus				*Corchorus olitorius*			
Treatment	**Root**	**Stem**	**Leaves**	**Total**	**Root**	**Stem**	**Leaves**	**Total**
Fresh matter yield (g)								
0 ppm	5.21^{a}	9.52^{a}	6.72^{a}	21.45	0.50^{a}	1.46^{a}	2.56^{a}	4.52
150 ppm	2.07^{a}	7.84^{ab}	6.88^{a}	16.79	0.53^{a}	1.88^{a}	2.84^{a}	5.25
300 ppm	3.39^{a}	6.29^{ab}	5.55^{a}	15.23	0.53^{a}	1.43^{a}	2.32^{a}	4.28
450 ppm	1.37^{a}	4.84^{a}	4.25^{a}	10.46	0.64^{a}	1.19^{a}	2.23^{a}	4.06
Dry matter yield (g)								
0 ppm	0.92^{a}	1.65^{a}	1.56^{a}	4.13	0.29^{a}	0.33^{a}	0.80^{a}	1.42
150 ppm	0.70^{a}	1.67^{a}	1.70^{a}	4.07	0.34^{a}	0.43^{a}	0.79^{a}	1.56
300 ppm	0.70^{a}	1.14^{a}	1.34^{a}	3.18	0.23^{a}	0.31^{a}	0.68^{a}	1.14
450 ppm	0.43^{a}	0.87^{a}	1.12^{a}	2.42	0.24^{a}	0.25^{a}	0.66^{a}	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~~ Zn concentrations in vegetables (Table 4). As seen in results above, vegetables grown in 450 ppm ~~zinc~~ Zn concentration had the lowest growth and yield; resultant of the highest total concentration in tissues (735.57 mg kg^{-1} for *A. caudatus* and 640 mg kg^{-1} for *C. olitorius*). Compared to other studies [23, 24, 25] ~~zinc~~ Zn concentration in plant tissues treated with ~~zinc~~ Zn salt were low (21.18 – 735.57 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 ~~zinc~~ Zn plant tissue concentration of 300 – 1000 mg kg^{-1}. Leaf ~~zinc~~ Zn concentrations of ≥ 300 mg kg^{-1} led to the vegetables yield reductions.

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

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

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

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

Translocation of ~~zinc~~ Zn from root and shoot ranged between 1.47 and 1.62 for *A. caudatus* while in *C. olitorius* it was between 1.58 and 1.84 (Table 4). TF is used to estimate plants' potential for phytoextraction. In the present study TF was above unity indicating the efficiency in translocating ~~zinc~~ Zn from roots to the shoot. Contrary to the findings of Mavengahama et al. [25] leaves concentrated more ~~zinc~~ Zn than roots indicating good potential for phytoremediation [28, 29]. Contrasting results in studies may be attributed to differences in soil type. TF 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 ~~zinc~~ Zn uptake. Generally, *C. olitorius* consistently had higher TF than *A. 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. *C. olitorius* had higher translocation factor than *A. caudatus* indicating better phytoextraction potential.

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