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Research article

Bioaccumulation of Zinc and Cadmium by two aquatic plants: *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L. under nursery conditions

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Abstract: This study investigated the bioaccumulation abilities of *Eichhornia crassipes* and *Pistia stratiotes* for zinc and cadmium under nursery conditions. The plants were grown in tap water medium spiked with varying concentrations of 5 mg l^{-1}, 10 mg l^{-1} and 20 mg l^{-1} of zinc and cadmium salts while the control experiments was not spiked with any of the metallic salts. The biomass of each plant species was harvested after six weeks and separated into leaves, stems and roots and the metal concentrations in their tissues were determined according to various treatments. It was observed that both the fresh weight and dry weight decreased serially with the increase in the concentration of the metals. In *Pistia stratiotes*, the Bioconcentration factor (BCF) was higher for cadmium than zinc; on the other hand, the BCF was higher for zinc than cadmium in *Eichhornia crassipes*. Significant differences occurred between the control and the treatment ($P \leq 0.05$) in the tissues of both plant species for zinc and cadmium contents which shows that the plants were able to bioaccumulate the metals. Comparing the two species based on the total amount of metals accumulated by the plants, *Pistia stratiotes* showed better bioaccumulation values for both cadmium and zinc than *Eichhornia crassipes*. Overall, the study revealed that the two aquatic macrophytes are potential bioaccumulators of cadmium and zinc and thus may be considered as candidate species for the phytoremediation of water bodies contaminated with heavy metals.

Keywords: *Eichhornia crassipes* - *Pistia stratiotes* - Zinc - Cadmium - Bioaccumulation.

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INTRODUCTION

Aquatic bodies are essential compartments of ecosystems, serving as sources of water supply to humans and as habitats to numerous aquatic plants and animals. The quality assessment carried out has shown that many aquatic bodies in developing countries are heavily polluted due to industrial and sewage discharge (Jonnalagadda & Mhere 2001). Cadmium does not have any known useful function in higher organism Cadmium has been reported to exert deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic activities (ATSDR 1999, Lippmann 2000, Risso-de-faverney *et al.* 2001). Cadmium accumulates in animals, especially in the kidney, liver, and reproductive organs. Elevated levels of cadmium in humans can cause kidney damage, and low levels of cadmium in the diet are linked to renal dysfunction (Järup *et al.* 2000). Cadmium poisoning is known worldwide and is one of the metals under scrutiny by the United States Environmental Protection Agency (USEPA) (Hogue 2004) and contamination from it occurs in more than eight percent (8%) of hazardous waste sites in the world (Yeung & Hsu 2005).

Generally, zinc is an essential element that acts as a plant nutrient (Shier 1994, Welch 1995); however, becomes toxic at higher concentrations. When it is assimilated early by plants, it can be extremely phytotoxic. Inhibition of growth is a common phenomenon of zinc toxicity (Collins 1981). Genotoxicity of zinc in micro

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and macro flora has been elucidated by various researchers (Sharma *et al*. 1985, Subhadra & Panda 1994). There is proof of copper deficiency at low intakes of 100–300 mg Zn/d and this has been suggested to impinge with the usage of copper and iron or adversely affect levels of cholesterol in organisms (Fosmire 1990). According to Fosmire (1990) zinc toxicity has also been linked to alterations of blood lipoprotein levels. In general, they are not biodegradable and therefore, they bioaccumulate in fish, oyster, mussels, sediments and other components of aquatic ecosystems that have been reported from all over the world (Majid *et al.* 2014).

The issue of heavy metal bioaccumulation of aquatic organisms also requires constant inspection and surveillance due to the biomagnifying ability of toxic metals in the human food chain (Das & Kaviraj 2000, Laxi 2005, Jayakumar & Paul 2006). Removal of toxic heavy metals from polluted waters is essential from the standpoint of environmental pollution control (Yuan *et al.* 2001). A number of toxins, including toxic heavy metals (Cadmium and Zinc), have been reported to be widespread in waterways, lakes and reservoirs are harmful to aquatic ecosystems (Olsson 1998). The phytoremediation of heavy metals from water bodies is a cost effect technology (Chaney *et al.* 1997, El-Gendy 2006). The aquatic plants in metal-polluted water bodies act as biological filters and biomonitors of environmental metal levels (Sujatha *et al.* 2001, Liao & Change 2004).

The concepts and ideas of the phytoremediation systems are to clean up polluted water, including the detection and use of productive aquatic plants, the absorption of dissolved nutrients and metals by growing plants, and the recycling and the beneficial use of plant biomass provided by the remediation method (Lu 2009). About four hundred (400) plant species have been identified as metal hyperaccumulators (Prasad & Freitas 2003, Ndimele *et al.* 2014); among these are *Eichhornia crassipes* (Mart) Solms and *Pistia stratiotes* L. used in this study. *Eichhornia crassipes* (Mart) Solms generally referred to as water hyacinth is known to be the most obnoxious aquatic plant in the world. It has a prolific growth rate and according to Thyagarajan (1984), it is a mat-forming plant which can multiply rapidly and can double its population within 12–14 days under favourable environmental conditions; thus posing problems of navigation, nutrient depletion and health issues to aquatic life (Uka & Chukwuka 2008).

Pistia stratiotes widely referred to as water lettuce or water cabbage is a free floating aquatic plant of streams, lakes and ponds. *P. stratiotes* belongs to Araceae family (Quattrocchi 2000). As a floating weed, it forms dense mats on the surface of water bodies, modifying the microclimate of the aquatic flora and fauna underneath and thus adversely affects the water ecosystem and hinders water flow, fishing, swimming, boating, water sports and navigation (Attionu 1976, Bruner 1982, Sharma 1984, Holm *et al*. 1991, Khan *et al*. 2014). It is therefore imperative to explore the potential of these noxious weeds for environmentally beneficial purposes (such as phytoremediation) and thus form the major objective for this study.

MATERIALS AND METHODS

Study area

The research was carried out in the Department of Botany, University of Ibadan, Ibadan-Nigeria. This area lies between latitude 3° 53'47.1" E and longitude 7° 26' 35.1" N and altitude of 215 m above sea level. The area has a mean daily temperature of 24.6°C and average annual rainfall above 1300 mm (Akin-Oriola 2003).

Plant collection and authentication

Water hyacinth plants used for this study were collected from the Awba Dam, University of Ibadan, Ibadan-Nigeria while the water lettuce was collected from Eleyele River, Ibadan, Nigeria. Samples of both plants were identified and authenticated as water hyacinth (Voucher No.: UIH-22491) and water lettuce (Voucher No.: UIH-22492) at the University Herbarium Ibadan (UIH) of the Department of Botany, University of Ibadan, Ibadan-Nigeria.

Phytoremediation study

The plants were washed with clean water to eliminate dirt and other attached organisms or their eggs and were placed in clean tap water for a week for acclimatization purposes. Solutions of heavy metals were prepared for each metal by dissolving salts of the metal in tap water. Solutions corresponding to 5 mg l^{-1}, 10 mg l^{-1} and 20 mg l^{-1} of cadmium and zinc were prepared by dissolving cadmium sulphate ($Cd.SO_4.H_2O$) and zinc chloride ($ZnCl_2$) respectively in water. These varying concentrations of cadmium and zinc solutions were added to 29 l of water to make up 30 l of tap water solution in each plastic bowl used for the study. Six plants each were cultured per plastic bowl and left to grow for six weeks before harvest.

Experimental design

The experiments were conducted in a complete randomized design with three replications per treatment group.

Biomass determination

The plants were harvested according to treatments after six weeks and the fresh weights were measured and recorded. They were then oven-dried at 90°C for 48 hours and the biomass readings were also taken for each of the plant samples.

Metal analysis

One gram of biomass matter of the plant samples was weighed into 50 ml beakers as described by Yusuf *et al*. (2002). Ten ml mixture of analytical grade acids: HNO_3:H_2SO_4:$HClO_4$ in the ratio of 1:1:1 was then added to the beakers containing the samples and were covered with watch glasses and left overnight. The digestion was carried out at a temperature of 90°C until 4 ml of the mixture was left in the beaker. Then, an additional 10ml of the acid mixture was added. This mixture was allowed to evaporate to a volume of about 4 ml; which after cooling the solution was filtered to remove small quantities of waxy solids and made up to a final volume of 50ml with distilled water. Metal concentrations were then determined using Atomic Absorption Spectrophotometer (Model A Analyst Spectrophotometer 700).

Water samples were also collected individually from each bowl according to various treatments and their metal concentrations determined by Atomic Absorption Spectrophotometer (Model A Analyst Spectrophotometer 700)

Bioconcentration factor (BCF)

The bioconcentration factor was calculated as the ratio of the heavy metal concentration in the harvested entire plant tissues biomass in relation to the concentration of the element in the external environment (*i.e.* tap water medium) (Vesely *et al*. 2002, Rivelli *et al*. 2012).

$$\text{BCF} = \frac{\text{Metals in plant tissues}}{\text{Metals in surrounding medium}}$$

Translocation factor (TF)

The translocation factor was calculated as the ratio of the heavy metal concentration in shoots to roots (Subhashini & Swamy 2013).

$$\text{TF} = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in roots}}$$

Statistical analysis

The data generated from the analysis were subjected to One Way analysis of variance (ANOVA) using the Statistical Analysis System (SAS) Software Version 9.1 to determine any significant differences between the treatments and also compared to the control. Means were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

RESULTS AND DISCUSSION

The bioaccumulation potential of *Eichhornia crassipes* and *Pistia stratiotes* in different concentrations of zinc and cadmium occurring in their respective growth medium was investigated in this study. Tables 1 and 2 show that both fresh and dry weights of *Eichhornia crassipes* and *Pistia stratiotes* were affected by various concentrations of cadmium and zinc in different but varying ways. For cadmium, fresh and dry weights of both plants decreased serially with the increase in the concentration of the metal and this observation is similar to the report of Selvam & Wong (2009) who observed a decline in biomass production of *Brassica napus* L. when grown in cadmium contaminated soils. Borker *et al.* (2013) also reported that cadmium chloride caused a decrease in biomass of *Eichhornia crassipes* in cadmium polluted water. The decrease in biomass reported in this study may be due to the leaf chlorosis noticed in the plants which affected their primary production and growth probably resulting from the inhibition of root Fe (III) reductase induced by cadmium which led to Fe (II) deficiency, and reduced photosynthesis as reported by Alcantara *et al*. (1994) on the effects of heavy metals on both induction and function of root in *Cucumis sativus* L. because of an irreversible inhibition exerted by cadmium on the proton pump responsible for the process (Aidid & Okamoto 1992).

Exposure of both test plants to zinc showed significant differences in biomass production of *Eichhornia crassipes* and *Pistia stratiotes* as the concentration of the metal increased. The plants showed a slight increase in both fresh weight and dry weight at 5 mg l^{-1} compared to control but at higher concentrations, there were decrease in weight of the two plants which is similar to the findings reported by Okunowo & Ogunkanmi (2010) that high zinc concentration led to a decrease in biomass of *Eichhornia crassipes.* Decrease in biomass is one of the symptoms of zinc phytotoxicity reported by many researchers; others include stunting of growth, browning of stems and chlorosis due to inhibition of iron translocation from roots to tops, inactivation of enzymes and displacement of essential enzymes from functional sites (Ambler *et al*. 1970, Barcelo & Poschenneder 1990, Ben *et al.* 2000, Liu *et al*. 2005, Rivelli *et al*. 2012).

Table 1. Effects of different concentrations of Zinc and Cadmium on the fresh and dry weights of *Eichhornia crassipes* (Mart.) Solms.

Concentrations (mg l^{-1})	Fresh Weights of plants (g)	Dry Weights of plants (g)
Zinc		
0	126.02±10.94^{b}	5.75± 0.24^{b}
5	151.03±5.66^{a}	6.41±0.39^{a}
10	118.44±8.24^{b}	3.99±0.77^{d}
20	82.40±5.49^{c}	4.21±0.93^{c}
Cadmium		
0	128.09±16.50^{a}	6.17±0.42^{a}
5	98.24±7.43^{b}	5.53±0.13^{b}
10	89.54±3.22^{b}	4.72±0.30^{c}
20	74.11±4.92^{b}	4.45±1.00^{c}

Note: Each value is a mean of three observations; values with the same superscripts in the same column are not significantly ($p \leq 0.05$) different.

Apart from the reduction in biomass and chlorosis of leaves previously mentioned, another interesting effect of the heavy metals was in the case of the flowering of *Eichhornia crassipes*. The first set of plants to flower were those grown at 5 mg l^{-1} of zinc, followed by the control and then those at 10 mg l^{-1} and 20 mg l^{-1}. For the cadmium polluted treatments, *Eichhornia crassipes* produced flowers at 5 mg l^{-1} and 10 mg l^{-1} but those grown at 20 mg l^{-1} produced no flowers although the flowers produced by the lower concentrations of cadmium withered after one week.

Table 2. Effects of different concentrations of Cadmium and Zinc (mg l^{-1}) on the fresh weights and dry weights (g) of *Pistia stratiotes* L.

Heavy metal	Fresh Weights of plants (g)	Dry Weights of plants (g)
Zinc		
0	67.21±2.59^{b}	2.48±0.27^{c}
5	74.93±3.41^{a}	3.29±0.33^{a}
10	56.39±4.80^{c}	2.94±1.15^{a}
20	45.28±3.70^{d}	1.46±0.27^{d}
Cadmium		
0	68.01±3.73^{a}	2.56±0.55^{a}
5	62.02±4.35^{a}	2.50±0.54^{a}
10	45.13±3.70^{b}	1.48±0.20^{b}
20	39.36±1.31^{c}	1.24±0.33^{b}

Note: Each value is a mean ± standard error (n=3). Values with the same superscripts in the same column are not significantly different ($p \leq 0.05$).

Table 3 showed that the amount of metals bioaccumulated by *Eichhornia crassipes* grown in polluted water was higher than the amount of metals in *E. crassipes* in the control which suggests that the plants were able to bioaccumulate the heavy metals, although the rates of uptake varied for the plant parts and concentrations. The plants bioaccumulated higher amounts of zinc (2.55 ± 0.45) occurring at 10 mgl^{-1} than cadmium. The highest amount of cadmium (2.34 ± 0.84) was bioaccumulated at 20 mg l^{-1}. The concentration of the two metals in the plants decreased from roots > stems > leaves. This is in line with Vesely *et al*. (2012) who reported that metal bioaccumulation in water hyacinth increased in the order of leaves < stems < roots.

The bioaccumulation of zinc and cadmium by *Pistia stratiotes* is as shown in table 4. The bioaccumulation rates varied among the plant parts and concentrations of the heavy metals. The highest amount of zinc (4.91 ± 0.50) uptake was recorded at 20 mg l^{-1} in the roots while that of cadmium was (7.10 ± 0.41) also at 20 mg l^{-1} in the roots. These uptake values also showed that *P. stratiotes* accumulated cadmium better than zinc and the metals uptake also decreased in the plant parts from root > leaves. The residual concentration of both metals were minimum (0.025 and 0.013) for zinc and cadmium respectively in the control, and maximum (0.87 ± 0.14 and 2.37 ± 0.56) for zinc and cadmium at 20 mg l^{-1} (Table 5).

The observations of tables 3 and 4 showed that *Pistia stratiotes* had better bioaccumulating ability for both cadmium and zinc than *Eichhornia crassipes*. This is similar to the findings of Goswami *et al.* (2009), where

Pistia stratiotes was able to accumulate 9.05 mg g^{-1} of cadmium while *Eichhornia crassipes* accumulated only 6.29 mg g^{-1} of the same cadmium when both plants were grown in cadmium contaminated water of the same concentration.

Table 3. Heavy metals absorbed by *Eichhornia crassipes* (Mart.) Solms.

Concentration (mg l^{-1})	Leaves (mg g^{-1})	Stem(mg/g)	Roots (mg g^{-1})
Zinc			
0	0.03±0.00^{c}	0.04±0.00^{d}	0.07±0.01^{c}
5	0.07±0.02^{b}	0.23±0.07^{c}	0.96± 0.06^{b}
10	0.20±0.03^{a}	0.57±0.06^{b}	2.55±0.45^{a}
20	0.23±0.05^{a}	0.71±0.22^{a}	2.28±0.55^{a}
Cadmium			
0	0.01±0.01^{b}	0.01±0.00^{b}	0.01±0.00^{c}
5	0.10 ±0.03^{a}	0.13±0.05^{a}	1.39±0.48^{b}
10	0.10 ±0.03^{a}	0.25±0.05^{a}	2.11±0.76^{a}
20	0.12 ±0.02^{a}	0.24±0.11^{a}	2.34±0.84^{a}

Note: Each value is a mean ±standard error (n=3). Values with the same superscripts in the same column are not significantly different ($p \leq 0.05$).

Table 4. Heavy metals absorbed by *Pistia stratiotes* L.

Concentration (mg l^{-1})	Leaves	Roots	Total
Zinc			
0	0.09±0.01^{d}	0.07±0.01^{d}	0.16
5	0.64±0.26^{c}	1.88±0.90^{c}	2.51
10	1.25±0.59^{b}	4.57±1.02^{a}	5.82
20	1.87±0.55^{a}	4.91±0.50^{a}	6.79
Cadmium			
0	0.01±0.01^{c}	0.02±0.00^{d}	0.03
5	0.97±0.37^{b}	2.83±1.16^{c}	3.80
10	0.66±0.26^{b}	3.15±0.31^{b}	3.81
20	1.95±0.12^{a}	7.10±0.41^{a}	9.05

Note: Each value is a mean ±standard error (n=3). Values with the same superscripts in the same column are not significantly different ($p \leq 0.05$).

Table 5. Residual Pollutants left in water after harvesting the plants.

Concentration (mg l^{-1})	*Eichhornia crassipes*	*Pistia stratiotes*
Zinc		
0	0.03±0.01	0.03±0.01
5	0.12±0.02	0.12±0.02
10	0.17±0.05	0.32±0.12
20	0.29±0.10	0.87±0.14
Cadmium		
0	0.01±0.00	0.01±0.01
5	0.37±0.12	0.17±0.02
10	0.98±0.22	0.19±0.02
20	1.85±0.53	2.37±0.56

Note: Each value is a mean ± standard error (n=3). Values with the same superscripts in the same column are not significantly different ($p \leq 0.05$).

Table 6. Bioconcentration Factors of *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L.

Concentration (mg l^{-1})	*Eichhornia crassipes*	*Pistia stratiotes*
Cadmium		
5	4.44	22.33
10	2.51	20.38
20	1.46	3.82
Zinc		
5	10.50	12.63
10	19.56	18.18
20	11.09	7.53

From table 6, it can be seen that *Eichhornia crassipes* had higher BCF values for zinc than cadmium, with the highest for zinc being 19.56 mg g^{-1} at 10 mg l^{-1} and that of cadmium being 4.44 mg g^{-1} at 5 mg l^{-1} which shows that *E. crassipes* demonstrates a higher affinity for zinc than cadmium. This is in line with the findings of

Kamel (2013) who reported that *E. crassipes* found on El- Temsah Lake in Egypt had BCF value of 1172.80 ppm for zinc and 2.35 ppm for cadmium.

When BCFs were calculated for *Pistia stratiotes*, it was observed that *P. stratiotes* had higher values for cadmium than zinc, with the highest for cadmium being 22.33 at 5 mg l^{-1} and that of zinc being 18.18 at 10 mg l^{-1} which shows that *P. stratiotes* although has a high affinity for both metals since the BCFs are greater than 2, it tends to have a higher affinity for cadmium than zinc. Comparing the two plants, *Pistia stratiotes* had higher BCF for cadmium than *Eichhornia crassipes* while *Eicchornia crassipes* had higher values than *Pistia stratiotes* for zinc only at 10 mg l^{-1} and 20 mg l^{-1}. Bioconcentration Factor tends to decrease with increasing concentration of both metals except for zinc at 10 mg l^{-1}. According to Zhu *et al*. (1999), Abd-Elmoniem (2003) and Borker *et al*. (2013), the ratio between the plant metal concentration and the metal concentration in the growth media expresses the bioconcentration factor which reflects the affinity of aquatic macrophytes to a specific heavy metal. Liu *et al*. (2005) stated that the concentration of heavy metals in any hydrophyte is dependent on the concentration of the surrounding medium while Ndimele *et al*. (2014) opined that BCF values greater than 2 are regarded as high which means that the plants have high affinity to the metals while those lower than two are considered low.

Table 7. Translocation factors of zinc and cadmium in the leaves and stems of water hyacinth.

Concentration (mg l^{-1})	Leaves	Stem	Total
Zinc			
5	0.07	0.24	0.31
10	0.08	0.22	0.30
20	0.10	0.31	0.41
Cadmium			
5	0.07	0.09	0.17
10	0.05	0.12	0.17
20	0.05	0.10	0.15

Table 8. Translocation factors of metals in leaves of *Pistia stratiotes* L.

Concentration (mg l^{-1})	Leaves
Zinc	
5	0.34
10	0.27
20	0.38
Cadmium	
5	0.34
10	0.21
20	0.27

For a plant to be considered to have good translocation of metal, its translocation factor has to be greater than 1 (Ndimele *et al*. 2014). The evaluation of the translocation factors (TF) of both *Eicchornia crassipes* and *Pistia stratiotes* for zinc and cadmium are shown in tables 7 and 8. These showed that the two plants have poor translocation abilities for zinc and cadmium since all the translocation factors had values less than 1 and this might be due to some physiological barriers against metal transport to aerial parts of the plants under study (Liu *et al*. 2005). The TF value for zinc was 0.41 while that of cadmium was 0.15 at 20 mg l^{-1} in *Eicchornia crassipes*. In *Pistia stratiotes* however, the highest for zinc was 0.38 at 20 mg l^{-1} while that of cadmium was 0.34 and 5 mg l^{-1}.

In summary, two aquatic plants *Eicchornia crassipes* and *Pistia stratiotes* were tested for the removal of zinc and cadmium from metal-polluted water. These macrophytes proved highly effective for the uptake of zinc and cadmium at different concentrations studied. These plants were able to remove cadmium and zinc to a great extent, but *Pistia stratiotes* was observed to show better accumulation potential for both zinc and cadmium than *Eicchornia crassipes*. Overall, this study showed that the two aquatic macrophytes are good candidates for the bioaccumulation of cadmium and zinc in heavy metal polluted water bodies and thus can be used for the treatment of such water systems.

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