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Uptake of Heavy Metals by Water Hyacinth, *Eichhornia crassipes* (Mart)

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ABSTRACT: Water hyacinth, *Eichhornia crassipes* was used to conduct a 15-day study on the uptake of heavy metals Lead (Pb); Cadmium (Cd) and Copper (Cu). 5 plants were in each case emersed in each of graded metal salt solutions (5, 10 and 15mg/l) over a 15 – day period. Metals in plant and water samples were analyzed to determine their metal concentrations by means of an atomic absorption spectrometer (AAS) in separate experiments. Results show that by day 3 of the study period, more than 90% of the metal ions in solution were taken up by the plant. In the 5mg/l treatment, metal uptake increased from day 3 to day 12 as follows: Pb, from 0.263 to 0.344mg/kg; Cd, 0.315 to 0.357mg/kg and Cu, 0.191 to 0.287mg/kg while metal ions decreased in the original solution as follows: Pb, from 0.017 to 0.009mg/l; Cd, 0.169 to 0.122mg/l and Cu, 0.430 to 0.190mg/l. In the 10mg/l treatment, increase in metal ions uptake from day 3 to day 12 were as follows: Pb, from 0.201 to 0.282µm/kg Cd, 0.275 to 0.478 mg/kg and Cu, 0.337 to 0.384 mg/kg. Metal ions decrease in the original solution were as follows: Pb, from 0.021 to 0.012mg/l; Cd, 0.404 to 0.302mg/l and Cu, 0.598 to 0.328mg/l. In the 15mg/l treatment, metals uptake increase were: Pb from 0.237 to 0.283mg/kg; Cd, 0.330 to 0.488mg/kg and Cu, 0.359 to 0.538mg/kg while metal values decrease in the original solution were: Pb, 0.008mg/l, Cd, 0.416mg/l and Cu, 0.348mg/l from day 3 to day 9. Plants in the 15mg/l treatment did not survive beyond day 9 of the study period. Significant differences ($F = 2.3479$, $P < 0.05$) were recorded in the concentrations of metals taken up by plants in the 5-plant scheme. Pb had the highest mean bioaccumulation quotient and had the highest uptake. The concentrations of metals left in solution were below the limits recommended by the Federal Ministry of Environment (FMENV) and the World Health Organization (WHO) for portable drinking water.

Keywords: Heavy Metals; Water hyacinths; *Eichhornia crassipes*.

Introduction

Over the years, imbalance has been created in the earth's ecosystem by the numerous activities of man who discharge toxic substances including heavy metals into the aquatic environment. According to Meadows *et al.*, (1992) pollution of the aquatic and terrestrial environments including other types of environmental degradation in any community or society are due to the combined effects of population increase, urbanization, affluence and technological developments. Apart from natural sources, anthropogenic sources are the principal source of pollutants arising from municipal wastes, refuse heaps, agricultural practices and industrial waste water (Smith, 1985).

Among the pollutants, heavy metals such as lead, cadmium, copper, nickel, chromium and mercury are retained in the soil, water bodies and waterbeds. Some of these metals (zinc, copper, iron) are essential to the life processes of organisms in trace concentrations unlike the non-essential ones (cadmium, mercury and arsenic) which are not needed by organisms because of their toxic effects (Connell and Miller, 1984).

The concentrations of these metals have increased tremendously in recent times as a result of human activities. Fish, aquatic plants and other aquatic organisms take up these metals, which are passed along the food chain to other organisms including humans. While selenium, mercury and arsenic can be methylated and degraded by bacteria and then volatilized into the surrounding atmospheric environment (Losi and Frankenberger, 1997) most heavy metals cannot be degraded. However, certain bacteria, fungi and algae can concentrate toxic inorganics including heavy metals but unfortunately, these microorganisms are too small to be harvested and used to bioremediate polluted waters.

Based on this fact, the use of plants remain the best option to phytoremediate polluted waters. Inorganic metals cannot be mineralized but can be physically removed from polluted waters or converted to biologically inert form, implying biomass or contaminant volatilization removal (Cunningham and Ow, 1996). According to Environmental Protection Agency (EPA, 1998) phytoremediation is more environmental friendly, safe and cost effective technique for bioremediating polluted waters compared to other conventional methods. Some plants have been effectively used to remediate polluted waters. They include the Indian mustard, *Brassica juncea*, the cat tail, *Typha latifolia*, the salt grass, *Distichlis spicata*, and the wild Azolla, *Azolla caroliniana* (Pilon-Smits *et al.*, 1999). The water hyacinth has also been used to remediate water polluted with selenium (Pilon-Smits *et al.*, 1999) and clean up waters polluted with heavy metals, hydrocarbons, organic wastes, organo-chlorides and explosives because of its large biomass and fast growth rate (Matagi *et al.*, 1998).

In the Nigeria aquatic environment, water hyacinth is commonly known because of its nuisance value. Its harmful effects on fishing activities and water transport have been reported by several research workers including Holm *et al.* (1977). There is dearth of information on the use of water hyacinth in remediating polluted waters in Nigeria. The aim of this study was to determine the effectiveness of the use of water hyacinth as a phytoremediating agent for water polluted by selected heavy metals (lead, cadmium and copper).

Materials and Methods

Study Samples and Test Salts

The water hyacinth used for this study were harvested from a concrete nursery water storage tank in the Department of Botany, University of Benin. They have average length of 6cm, leaf width of 7.2cm, root length of 26cm and an average wet weight of 21.10g. The heavy metals (lead, cadmium and copper) used for the study were sourced from analar grade salts of Lead sulphate (PbSO_4), Cadmium sulphate ($\text{CdSO}_4 \cdot 2\text{H}_2\text{O}$) and Copper sulphate (CuSO_4).

Experimental Units and Procedures

The experimental units were made up of 12, 15litre plastic bowls, each with a depth of 16cm, a diameter of 39cm and containing 7L of water. The experimental set-up consisted of 5 plants which were each emersed in graded solutions of 5mg/l, 10mg/l and 15mg/l concentrations of metal salts with controls and replicates. Stock solutions of metal salts were prepared and were serially diluted to give 5mg/l, 10mg/l and 15mg/l treatments. The experimental plants, *Eichhornia crassipes*, were picked randomly and placed one at a time into each unit until the required number of plants per unit was attained. The set-up was allowed to stand for 15days. Water samples were taken at three-day intervals from each treatment and stirred to resuspend all precipitated metal salts after which they were stored at -4°C prior to analysis.

Digestion and Analysis

Plant samples were collected from each experimental unit at three-day intervals and allowed to drip-dry. They were oven dried at a temperature of 80°C to constant weight and milled separately into powder by means of a hammer dry mill. For the digestion process, 0.5g of dried ground plant sample was in each case accurately weighed into 100ml Erlenmeyer flask, 0.5ml nitric acid and 1ml sulphuric acid were added. Digestion was performed on a hot plate at 45°C for 6hours under a hood until solutions were clear (Van Loon, 1980). Digests were allowed to cool and made up to 50ml with distilled water and stored prior to analysis.

Water samples were digested with preconcentrated nitric acid according to the method of Parker (1972). Digests were analyzed by aspiration into the flames of an atomic absorption spectrophotometer (UNICAN 929) for heavy metal determination. Values obtained were expressed in mg/l. Digested plant samples were analyzed for their heavy metals concentration as per water and the values obtained were expressed in mg/kg dry wt. Digested samples were analyzed thrice. The standard solutions for instrument calibration were prepared by dissolving 1000mg analar grade metal salts in 1L of distilled water. Standard and controls were run with each set of experiments. Analysis of variance (ANOVA) was used in all cases for mean metals comparisons, at 5% probability level. Means were separated using the Duncan Multiple Range Test.

Bioaccumulation Quotient (BAQ)

The Bioaccumulation Quotient is the ratio between the accumulated concentration of heavy metals in the water hyacinth and its dissolved concentration in the various solutions of metal salts.

$$\text{BAQ} = \frac{\text{Metal concentration in plant (mg/kg)}}{\text{Metal concentration in the water (mg/l)}}$$

The actual concentration of each metal in a given sample was calculated using the formula:

$$\text{Actual concentration of metal in sample} = \text{PPMR} \times \text{Dilution factor (Olaifa et al., 2004)}$$

Where PPMR = AAS reading of digest

$$\text{Dilution factor} = \frac{\text{Volume of digest used}}{\text{Weight of sample digested}}$$

The physico-chemical parameters of the water samples determined were conductivity and pH. Conductivity of water samples was determined by means of a conductivity meter, while pH was determined by means of a Javey pH meter.

Results

Results of uptake of lead (Pb), cadmium (Cd) and Copper (Cu) by water hyacinth, *E. crassipes* and a accompanying parameters (water pH and conductivity) are presented in Table 1. The mean bioaccumulation Quotient (BAQ) values for metal ions are shown in Table 2 while ANOVA for the 5 plant scheme is presented in Table 3.

Analysis of plant and water samples in the 5mg/l, 10mg/l and 15mg/l units under 5-plant scheme showed an increase in mean metal concentrations as follows: In the 5mg/l treatment, the original concentration of metals increased from day 3 to day 12 as follows: Pb from 0.263 to 0.344mg/kg; Cd, 0.315 to 0.357mg/kg and Cu, 0.191 to 0.287mg/kg. Results show that Cd uptake by the plants had the highest mean value (0.357mg/kg) while Cu uptake had the lowest mean value (0.287mg/kg). In the 10mg/l treatment, metal concentrations increased from day 3 to day 12 as follows: Pb, from 0.201 to 0.282mg/kg; Cd, 0.275 to 0.478mg/kg and Cu, 0.337 to 0.384mg/kg. Again, Cd uptake by water hyacinth had the highest mean value (0.478mg/kg) while Pb had the lowest value (0.282mg/kg) of uptake.

Metal concentrations also increased from day 3 to day 9 in the 15mg/l plant treatment with the following values: Pb, 0.237 to 0.283mg/kg; Cd, 0.330 to 0.488mg/kg and Cu, 0.359 to 0.538mg/kg. Pb had the lowest value (0.283mg/kg) of metal uptake by the plants. Plants in the 15mg/l treatment did not survive beyond day 9 due to observed yellowing of the leaves (chlorosis). Significant differences ($F = 2.3479$, $P < 0.05$) were recorded in the concentrations of metals taken up by plants in the 5-plant scheme of 5mg/l, 10mg/l and 15mg/l treatments. The concentrations of metals in the solutions (5mg/l, 10mg/l and 15mg/l) decreased due to metal uptake by the plants. The original concentration of metals in the 5mg/l treatment by day 3 decreased as follows: Pb, from 0.017 to 0.009mg/l; Cd, 0.169 to 0.122mg/l and Cu, 0.430 to 0.190mg/l by day 12 of the study period. Significant differences were recorded between metal concentrations in the 5mg/l solutions, ($F = 3.493$, $P < 0.05$), Table 3. While water pH increased from 5.7 to 6.4 (tending to alkaline), water conductivity decreased from 377 to 215 μScm^{-1} (Table 1). The original metal concentrations

in the 10mg/l solution decreased as follows: from day 3 to day 12: Pb, from 0.021 to 0.012mg/l; Cd, 0.404 to 0.302mg/l and Cu, 0.598 to 0.328mg/l. In the 15mg/l solutions of metal salts, the decrease in values were: Pb, 0.008mg/l; Cd, 0.416mg/l and Cu, 0.348mg/l. In the 10mg/l treatment, water pH increased from 5.4 by day 3 to 6.1 by day 12. Conductivity value decreased from 382 μScm^{-1} by day 3 to 254 μScm^{-1} by day 12.

Table 1: Mean pH, Conductivity and Concentrations of lead, cadmium and copper in plant and water samples in a 5-Plant Treatment Scheme

Concentrations (mg/l)	Time (days)	pH	Conductivity (μScm^{-1})	Metal Concentrations					
				Plant (mg/kg)			Water (mg/l)		
	Initial Concentrations	–	–	Pb	Cd	Cu	Pb	Cd	Cu
				–	–	–	5.0	5.0	5.0
5mg/l	Day 3	5.7	377	0.263	0.315	0.191	0.017	0.169	0.430
	Day 6	5.8	328	0.305	0.337	0.275	0.014	0.149	0.621
	Day 9	5.9	267	0.342	0.354	0.270	0.010	0.140	0.502
	Day 12	6.4	215	0.344	0.357	0.287	0.009	0.122	0.191
	Initial conc.	–	–	–	–	–	10.0	10.0	10.0
10mg/l	Day 3	5.4	382	0.201	0.275	0.337	0.021	0.404	0.598
	Day 6	5.7	301	0.215	0.310	0.368	0.018	0.378	0.552
	Day 9	5.8	262	0.225	0.454	0.372	0.017	0.336	0.374
	Day 12	6.1	254	0.282	0.478	0.384	0.012	0.302	0.328
	Initial conc.	–	–	–	–	–	15.0	15.0	15.0
15mg/l	Day 3	6.0	256	0.237	0.330	0.359	0.013	0.488	0.508
	Day 6	6.0	248	0.269	0.418	0.430	0.012	0.480	0.419
	Day 9	6.3	204	0.283	0.488	0.538	0.008	0.416	0.348
	Day 12	–	–	–	–	–	–	–	–

The mean bioaccumulation of metal ions in the 5-plant treatment scheme show that Pb had the highest mean bioaccumulation quotients (38.22 and 23.50) in the 5 and 10mg/l treatments respectively by day 12, and 35.38 in the 15mg/l treatment by day 9. (Table 2). Cu had the lowest mean bioaccumulation quotients (1.50 and 1.17) in the 5 and 10mg/l treatments respectively by day 12.

Table 2: Mean Bioaccumulation Quotient of Heavy metal ions in water hyacinth of a 5-plant Treatment Scheme.

Concentration	Time (days)	Bioaccumulation Quotient		
		Pb	Cd	Cu
5mg/l	Day 3	15.47	1.86	0.44
	Day 6	21.79	2.26	0.44
	Day 9	34.20	2.53	0.54
	Day 12	38.22	2.93	1.50
10mg/l	Day 3	9.57	0.68	0.56
	Day 6	11.94	0.82	0.67
	Day 9	13.24	1.35	1.00
	Day 12	23.50	1.58	1.17
15mg/l	Day 3	18.23	0.68	0.71
	Day 6	22.24	0.87	1.03
	Day 9	35.38	1.17	1.55
	Day 12	–	–	–

Table 3-ANOVA: Two Factor Without Replication- 5 Plant Scheme

<i>SUMMARY</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
5mg/l	3	0.811	0.2703	0.0047
	3	0.971	0.3237	0.0018
	3	0.988	0.3293	0.0014
	3	0.540	0.1800	0.0033
10mg/l	3	0.827	0.2757	0.0037
	3	0.893	0.2977	0.0006
	3	1.089	0.3630	0.0092
	3	1.144	0.3813	0.0096
15mg/l	3	1.014	0.3380	0.0085
	3	1.129	0.3763	0.0086
	3	1.317	0.4390	0.0187
	3	1.317	0.4390	0.0187
Pb	11	2.956	0.2687	0.0024
Cd	11	3.712	0.3375	0.0165
Cu	11	4.055	0.3686	0.0048

ANOVA							
Source of variation	of	SS	df	MS	F	P-value	F crit.
Rows		0.1429	10	0.0143	3.0511	0.016178	2.3479 sig.
Columns		0.0575	2	0.0287	6.1369	0.008352	3.4928 sig.
Error		0.0937	20	0.0047			
Total		0.2941	32				

Discussion

Water hyacinth, *Eichhornia crassipes*, is known in Nigeria for its nuisance value. It was however, reported to have been used to clean up water polluted by heavy metals, hydrocarbons, organic wastes, organochlorides and explosives (Matagi *et al.*, 1998). The plant's biomass and fast growth rate have combined to make it a good accumulator of pollutants including heavy metals. These characteristics according to Matagi *et al.*, (1998), are prerequisites for the choice of plants to phytoremediate polluted waters. According to Alabaster and Lloyd (1982), the uptake and absorption of metals by living organisms depend on the chemical properties and the prevailing water chemistry of the water in which the organism is found. The period of exposure also plays an important role as it determines the level of metal uptake within a given time frame.

In the 5mg/l unit, the concentration of heavy metals (Pb, Cd and Cu) taken up by water hyacinth increased with the number of days from day 3 to day 12. Results show that the plants took up a higher concentration of Cd (0.357mg/kg) while Cu uptake yielded the lowest value (0.287mg/kg). In the 10mg/l treatment, Cd uptake yielded the highest value (0.478mg/kg) while Pb uptake yielded the lowest value (0.282mg/kg). Cu uptake in the 15mg/l treatment yielded the highest value (0.538mg/kg) while Pb uptake yielded the lowest value (0.283mg/kg).

Uptake of metals by plants in the various treatments (5, 10 and 15mg/l) appear to follow similar trend and showed increase of metal uptake from day 3 to day 12 except in the 15mg/l treatment where plants survived only to day 9, attributable to the higher concentration of the treatment. Though the yellowing of leaves (chlorosis) was observed by day 3 in all three (5, 10 and 15mg/l) treatments, it was more pronounced in plants in the 15mg/l scheme. Significant differences ($P < 0.05$) were recorded in the concentrations of metals taken up by the plants among the different treatments. This may not be unconnected with the low plant population associated with the 5-plant treatment. Increase in metal uptake is dependent on the bioavailability of metal ions and the pH of the medium (Oguzie, 1999).

The regulation of metal ions in the aquatic environment was observed to be disturbed by elevated H^+ ions concentration (Tessier *et al.*, 1989) and Gerhardt (1992). According to Sverdrup and Warvunge (1990), metal precipitation and subsequent discharge onto substrate materials in the medium occurred in waters with high pH where substrate-bound rather than substrate-free materials existed. In the present study, water pH increased from 5.7 from day 3 to 6.4 by day 12 (tending to alkaline condition), while water conductivity (a measure of total ions in the solution) decreased from 317 by day 3 to $215\mu S m^{-1}$ by day 12. This situation resulted to decrease of bioavailable metal ions in solution for uptake by the plants. However, metal uptake by the plants increased from day 3 to day 12 in the various treatments (5, 10 and 15mg/l) while the concentrations of metal ions in solutions decreased due to metal ions uptake by plants.

The predominance of free metal ions in natural waters under various pH conditions showed that at low pH (increasing acidity), H^+ and metal ions compete for binding sites in the biota within the aquatic environment (Gerhardt, 1992). The effects of pH in the coprecipitation of metals with various metal hydroxides resulting in low metals bioavailability in the water column and subsequent low uptake was reported by Gerhardt (1992). However, results from this study showed that over a specific time frame, the plants in the 15mg/l treatment recorded a higher rate of metal ions uptake than plants in the 5 and 10mg/l treatments. This was explained by the number of plant deaths recorded in this medium compared to similar values for the 5 and 10mg/l treatments. By day 9, all the plants in the 15mg/l treatment had withered and died. This suggested that the plants in the 15mg/l treatment absorbed more metal ions over time compared to plants in the 5 and 10mg/l treatments. In the test solutions, it was observed that more than 95% of the initial metal concentrations was taken up by the plants within the first three days of the study. This observation corroborated previous reports by Ivo *et al.*, (1999) who used dry ground biomass of water hyacinth in a similar study. He reported that 75% of the 6.3mg/kg Cu introduced into the solution were taken up within 30minutes. The large biomass of the plant made up of numerous roots, large leaves and stalk might suggest the enhanced metal ions uptake in a very short time. According to Brix (1993) the plant's root system often help to increase the reach of the plant and provided a large surface area for metal uptake.

In the present study, bioaccumulation which is the net accumulation of contaminant heavy metals from the medium by the tissues and organs of the plant showed that the plants in the various treatments (5, 10 and 15mg/l) bioaccumulated the different metal ions to varying degrees. When the rate of uptake exceeded the rate of elimination, there was a gradual build up and this adversely affected the plants as was observed

in this study. The plants in the various treatments suffered chlorosis especially these in the 15mg/l treatment which later died due to the high concentration of metal ions in the solution. In this study, Pb had the highest mean bioaccumulation quotients (38.22 and 23.50 by day 12 and 35.38 by day 9) in the 5, 10 and 15mg/l treatments respectively. This implied that water hyacinth has a higher preference to take up Pb than either Cd or Cu from polluted waters. Data on water samples showed that 21.10g of water hyacinth, *E. crassipes* successfully reduced the concentration of lead (Pb) in all three (3) treatments (5, 10 and 15mg/l) by about 95% within the first three days of this study which is within the World Health Organization (WHO, 1989) maximum acceptable limit (0.05mg/l) for lead in drinking water. This implies that water hyacinth could effectively be used as a phytoremediating agent for industrial effluents in Nigeria. The plant could also be used to phytoremediate Cd and Cu in polluted industrial effluents because their reduction levels in metal salt solutions fall below the limits (0.05 and 0.1mg/l) respectively recommended by the Federal Ministry of Environment (FMENV, 1991) and the World Health Organization (WHO, 1989) for portable drinking water. Industrial effluents are emphasised because anthropogenic sources were implicated as the major source of inland water pollution in Nigeria.

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