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COMPARATIVE ANALYSIS OF HEAVY METAL CONTENT OF WATER HYACINTH (*EICHHORNIA CRASSIPES*) DEPURATED WITH CITRIC ACID AND ETHYLENEDIAMINETETRAACETIC ACID (EDTA)

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ABSTRACT

This study comparatively evaluated the depuration efficiency of citric acid and (EDTA) ethylenediaminetetraacetic acid in reducing heavy metal concentrations in water hyacinth. Fresh plant samples were collected from Ekowe river in Southern Ijaw, Bayelsa State, Nigeria and subjected to chemical depuration using citric acid and EDTA. The samples were cleaned, sorted, dried, milled, sieved and packaged. The water hyacinth sample was divided into three groups: one for citric acid treatment, one for EDTA treatment and a third group serving as the control (untreated). The two water hyacinth samples marked for treatments were subjected to chelator treatment by immersing them in solutions of citric acid and EDTA separately at 5 mM for 24 h. Samples from the 3 groups were subjected to wet-digested and analyzed for zinc (Zn), copper (Cu), cadmium (Cd), nickel (Ni), and lead (Pb) using atomic absorption spectrophotometry. The results of the study showed that the metal concentrations in the samples ranged from 23.33–45.50 mg/kg for Zn, 12.12–43.37 mg/kg for Cu, 0.02–1.15 mg/kg for Cd, 10.25–22.06 mg/kg for Ni, and 0.05–1.26 mg/kg for Pb. The findings of the study showed that chemical depuration significantly reduced the concentrations of all investigated metals, with EDTA achieving higher percentage reductions (48.72–98.26 %) compared to citric acid (36.89–92.06 %). Cadmium and lead levels in the raw samples exceeded FAO/WHO permissible limits but were reduced to safe levels following both treatments. Statistical analysis revealed significant differences ($p < 0.05$) among the raw and treated samples. Overall, EDTA demonstrated superior chelation and metal removal efficiency, while citric acid provided an effective, environmentally friendly alternative. The findings underscore the importance of chemical depuration in minimizing heavy metal risks and support the safe reutilization or disposal of water hyacinth biomass harvested from contaminated aquatic systems.

KEYWORDS: *Water hyacinth, Chemical depuration, Heavy metal contamination, Citric acid, EDTA.*

INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is among the most aggressive aquatic macrophytes globally, flourishing in eutrophic waters and posing significant ecological and management challenges across tropical and subtropical regions (Obasi *et al*., 2023). Owing to its exceptional biomass production and metal uptake capacity, the plant has been widely applied in phytoremediation of contaminated water bodies (Tang *et al*., 2024). However, the same bioaccumulation potential that underpins its remediation efficiency also results in elevated concentrations of toxic heavy metals in harvested biomass, thereby constraining its safe reutilization (Chen *et al*., 2024). Exposure to metals such as cadmium and lead through untreated biomass presents serious risks to human and animal health, necessitating effective post-harvest detoxification strategies (Nwachukwu & Ekpo, 2022).

Chemical depuration, which involves the removal of accumulated contaminants from biological materials, has emerged as a viable approach for mitigating these risks and enabling sustainable biomass valorization (Lee *et al*., 2023). Chelating agents are particularly effective in this context, as they enhance metal mobilization and extraction from plant tissues (Kumar & Bhatia, 2023). Citric acid, a naturally occurring organic chelator, offers an environmentally compatible option due to its biodegradability, low toxicity, and minimal residual effects in treated biomass (Tang *et al*., 2024). Conversely, ethylenediaminetetraacetic acid (EDTA) exhibits high chelation efficiency but is associated with environmental persistence and potential secondary pollution concerns (Lee *et al*., 2023).

Beyond metal removal efficiency, depuration treatments may significantly influence the biochemical composition of water hyacinth, with implications for its nutritional, medicinal, and industrial applications (Adeyemo *et al*., 2023). Despite extensive studies on individual chelators, comparative evaluations of citric acid and EDTA with respect to both depuration efficiency and biochemical integrity of water hyacinth remain limited, highlighting the need for systematic investigation (Chen *et al*., 2024).

Plate 1: Picture of Bunch of water hyacinth plant. Source: Lee *et al*. (2023)

MATERIALS AND METHOD

Sample Pre-processing

The study was conducted at the Biochemistry Laboratory, Department of Science Laboratory Technology, Federal Polytechnic Ekowe, Southern Ijaw, Bayelsa State. Water hyacinth (*Eichhornia crassipes*) was harvested from surface Ekowe river, ensuring young, healthy, and uncontaminated samples. Water hyacinth samples were immediately cleaned with tap water to remove dirt, insects, and surface debris, followed by thorough washing with distilled water. The leaves and stems were separated and blot-dried with absorbent papers to prevent microbial growth. The water hyacinth sample was divided into three groups: one for citric acid treatment, one for EDTA treatment and a third group serving as the control (untreated). The control sample was ground into fine powder using attrition mill and sieved using a mesh sieve of 0.5 mm to ensure uniform particle size, labelled and stored in airtight Ziploc bags.

Depuration Process of Water Hyacinth Flour (WHF)

Water hyacinth flour was prepared according to the method of Chinmah *et al*. (2004) with slight modifications. The two water hyacinth samples marked for treatments were subjected to chelator treatment by immersing them in solutions of citric acid and EDTA separately at 5 mM for 24 h. The depuration, the samples were rinsed with distilled water to remove excess chelators and air-dried to remove surface moisture. The depurated samples were dried in a hot air oven at a temperature between 60 °C and 70 °C for 48 h, depending on the moisture content and tissue thickness. This step ensures the complete removal of moisture, which is essential for preventing microbial degradation and for achieving accurate dry weight-based metal analysis. After drying, the sample was ground into fine powder using attrition mill and sieved using a mesh sieve of 0.5 mm to ensure uniform particle size, which enhances analytical precision during metal quantification and biochemical assays. The water hyacinth flour was labelled and stored in airtight Ziploc bags.

Sample Analysis

Determination of Heavy Metal Content of Water Hyacinth Samples

The method described by Zhang *et al*. (2022) with slight modifications was followed to determine the baseline heavy metal content in both the untreated and treated water hyacinth samples. Fifty milligrams (0.5 g) of each powdered sample was weighed into a 250 mL digestion flask. A mixture of 10 mL of concentrated nitric acid (HNO_3) and 2 mL of perchloric acid ($HClO_4$) were added to each sample. A blank sample was prepared by applying 10 mL of concentrated nitric acid (HNO_3) and 2 mL of perchloric acid ($HClO_4$) into an empty digestion flask. The mixture was heated on a hot plate under a fume hood at 120 °C until the solution became clear, indicating complete digestion (2 h). The digested solution was then allowed to cool, filtered through Whatman No. 1 filter paper, and made up to 50 mL with deionized water in a volumetric flask. The filtrates was analyzed for heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni) and zinc (Zn) using an Atomic Absorption Spectrophotometer (AAS) (Buck Model 210). The characteristic wavelengths of Cd, Cu, Ni, Pb and Zn were first set using the hollow cathode lamp, then digested filtrates samples was aspirated into the flame directly. Concentration was in mg/L (ppm) which was converted to mg/kg by dividing with the volume of sample aspirated. The analysis was conducted in duplicates for each sample.

Determination of percentage (%) Reduction in Heavy Metal Levels of Samples

The % of reduction in heavy metal content of water hyacinth after treatment with citric acid and EDTA was assessed using the methods described by Obasi *et al*. (2023) and Tang *et al*. (2024). Each group consisted of equal weights (50 g) of water hyacinth. From each sample, 0.5 g of the powder was weighed and subjected to wet digestion using a mixture of 10 mL nitric acid (HNO_3) and 2 mL perchloric acid ($HClO_4$) on a hot plate until the digest became clear. After cooling, the digests were filtered and diluted to 50 mL with deionized water in volumetric flasks. Heavy metal concentration (Cd, Cu, Ni, Pb and Zn) were quantified using

Atomic Absorption Spectrophotometry (AAS). The AAS was calibrated using certified standard metal solutions to ensure accuracy. The efficiency of each treatment was calculated by comparing the metal levels in treated samples with those in the untreated control group, using the formula:

% Reduction = $[(C_0 - C_t) / C_0] \times 100$

Where:

C_0 is the initial concentration (control)

C_t is the concentration after treatment.

Method of data Analysis

All experiments were carried out in duplicates to ensure accuracy. The data generated were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Product and Service Solution (SPSS, Version 28) software. Significant means were separated using the Turkey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Table 4.1: Heavy Metals Concentration in Water Hyacinth Samples.

Sample	**Zn (mg/kg)**	**Cu (mg/kg)**	**Cd (mg/kg)**	**Ni (mg/kg)**	**Pb (mg/kg)**
Raw sample	45.50±0.01	43.37±0.11	1.15±0.00	22.06±0.0	1.26±0.03
Sample treated with citric acid	28.71±0.00	18.22±0.02	0.18±0.05	13.09±0.08	0.10±0.00
Sample treated with EDTA	23.33±0.03	12.12±0.06	0.02±0.11	10.25±0.05	0.05±0.01
% Reduction (Citric acid vs Raw)	36.89±0.05	57.98±0.09	84.35±0.02	40.67±0.03	92.06±0.03
% Reduction (EDTA vs Raw)	48.72±0.01	72.05±0.01	98.26±0.00	53.55±0.00	96.03±0.09
WHO/FAO (2020) permissible limit	60	40	0.2	68	0.3

Values are mean ± SD of duplicate. Means within the same column with different letters are significantly ($p<0.05$) different.

Heavy Metals Concentration in Water Hyacinth Samples

The Heavy Metals Concentration in Water Hyacinth Samples is presented in table 4.1 above.

Zinc (Zn)

Zinc concentrations in water hyacinth ranged from 45.50 mg/kg in the raw sample to 23.33 mg/kg in the EDTA-treated sample, confirming the strong zinc bioaccumulation capacity of the plant (Table 4.1). Treatment with citric acid reduced Zn to 28.71 mg/kg (36.89%), whereas EDTA achieved a greater reduction to 23.33 mg/kg (48.72%), reflecting its superior chelation efficiency. All Zn concentrations were below the FAO/WHO (2020) permissible limit of 60 mg/kg, indicating no immediate toxicological risk. Similar patterns of elevated zinc accumulation in untreated water hyacinth have been reported in polluted Nigerian aquatic systems (Chigbo *et al*., 2013), while Alloway (2013) attributed this trend to the high mobility of Zn in water–sediment interfaces.

Copper (Cu)

Copper levels varied significantly across treatments, with the raw sample recording 43.37 mg/kg, compared to 18.22 mg/kg after citric acid treatment (57.98%) and 12.12 mg/kg following EDTA treatment (72.05%). The raw Cu concentration slightly exceeded the FAO/WHO (2020) limit of 40 mg/kg, whereas both treated samples fell within safe thresholds. The superior removal of Cu by EDTA is consistent with its strong affinity for divalent metal ions. Elevated copper accumulation in aquatic macrophytes exposed to anthropogenic discharges has been widely documented (Alloway, 2013; Elbagermi *et al*., 2012).

Cadmium (Cd)

Cadmium concentrations in water hyacinth showed pronounced reduction following chemical depuration, decreasing from 1.15 mg/kg in the raw sample to 0.18 mg/kg with citric acid treatment (84.35%) and 0.02 mg/kg with EDTA treatment (98.26%). The raw Cd concentration exceeded the FAO/WHO (2020) permissible limit of 0.2 mg/kg, indicating significant contamination risk. Both treatments reduced Cd to safe levels, with EDTA demonstrating near-complete removal. These findings align with previous reports highlighting the strong binding affinity of EDTA for cadmium in contaminated plant tissues (Zhuang *et al*., 2009; Jaishankar *et al*., 2014).

Nickel (Ni)

Nickel concentrations ranged from 22.06 mg/kg in the raw sample to 13.09 mg/kg after citric acid treatment (40.67%) and 10.25 mg/kg following EDTA treatment (53.55%). Although all values were below the FAO/WHO (2020) permissible limit of 68 mg/kg, the reductions achieved through depuration are environmentally significant. Similar elevated Ni accumulation in aquatic plants from polluted Nigerian water bodies has been reported by Akan *et al*. (2010), while Alloway (2013) noted that prolonged exposure can induce phytotoxicity and health risks.

Lead (Pb)

Lead concentrations were highest in untreated water hyacinth (1.26 mg/kg), exceeding the FAO/WHO (2020) permissible limit of 0.3 mg/kg. Citric acid treatment reduced Pb to 0.10 mg/kg (92.06%), while EDTA achieved a further reduction to 0.05 mg/kg (96.03%). The exceptionally high removal efficiency, particularly with EDTA, underscores its effectiveness in detoxifying Pb-contaminated biomass. Comparable studies have reported significant lead accumulation in water hyacinth from contaminated rivers, reinforcing its role as a reliable bioindicator of Pb pollution (Sharma & Dubey, 2005; Chigbo *et al*., 2013).

CONCLUSION

This study demonstrated that untreated water hyacinth accumulates high concentrations of heavy metals, confirming its effectiveness as a biomonitor while highlighting the health and environmental risks associated with the direct use of contaminated biomass. Chemical depuration using both citric acid and EDTA significantly reduced the concentrations of Zn, Cu, Cd, Ni, and Pb, indicating their effectiveness in mobilizing and removing bound metals from plant tissues. EDTA consistently achieved higher removal efficiencies than citric acid, particularly for cadmium and lead, with reductions exceeding 95%, due to its strong multidentate chelation properties. Although less aggressive, citric acid successfully reduced all metal concentrations to levels below FAO/WHO permissible limits, demonstrating its suitability as a biodegradable and environmentally friendly alternative. All treated samples met international safety standards, suggesting that depurated water hyacinth biomass can be safely reused for applications such as composting, bioenergy production, or industrial raw materials. Overall, the findings establish chemical depuration—especially EDTA-based treatment—as an effective strategy for detoxifying water hyacinth and converting it from an invasive weed into a safer, value-added resource.

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