**Phytoremediation** **of Heavy Metals (Cadmium, Lead, Chromium and Copper) from Paint-Based Effluent Using *Azolla microphylla* (Azolla) and *Eichhornia crassipes* (Water Hyacinth)**

**ABSTRACT**

Heavy metals are a major risk to life in general and require urgent attention when found in any environment. Threat is brought to life and the environment at large when toxic wastes (such as heavy metals) are improperly handled and managed. The use of plants, specifically aquatic macrophytes, in the uptake and accumulation of these toxic elements may proffer a more advantageous and economically doable approach. This study was undertaken in the University of Abuja main campus located in Abuja, Nigeria. In this study, two aquatic macrophytes, *Azolla microphylla* and *Eichhornia crassipes* were evaluated for the uptake of four heavy metals (cadmium, chromium, lead and copper) from a paint-based effluent under hydroponic media using two varying dilution factors (50%, TRT 1 and 25%, TRT 2) of the effluent. Pot experiments were carried out under a greenhouse condition at a controlled temperature at the Biological garden, faculty of Science, University of Abuja, Nigeria under maintained temperature. Microsoft Excel were used in the calculation of mean, standard error, and standard deviation and in the plotting of statistical graphs. Statistical Package for the Social Sciences (SPSS) version 25 for Windows was used. One-way Analysis of Variance (ANOVA) and Turkey Post Hoc Test were used to compare means between treatments and other parameters measured. Results obtained revealed significant (P<0.05) removal efficiencies after a 63-day (both acclimatization and treatment) period of treatment for both plants. *Azolla microphylla* reduced cadmium concentration by 90 % and 95%, lead 89% and 78%, chromium 39% and 48% and copper 72% and 99% in both treatments. *Eichhornia crassipes* significantly reduced cadmium by 82% and 48%, lead by 51% and 36%, chromium by 29% and 11% and copper by 76% and 8% in both treatments, respectively. The study concluded that the results obtained from this study suggest the applicability of these two plants grown hydroponically for bioremediation, which promises to be a sustainable and viable option for heavy metal-containing effluents.

**KEYWORDS**: *Azolla microphylla*, *Eichhornia crassipes*, hydroponics, heavy metals, bioremediation.

**INTRODUCTION**

The issue of environmental pollution has become a worldwide and a global problem as an increase in the human population has led to an increase in the establishment of more industries to meet the growing needs of the growing population. Industrial effluent discharges contain many toxic heavy metals and emit a gaseous offensive odor. A pollutant causes alteration to the environment in an undesirable manner and is of different types. They include air, soil, water, noise, and thermal pollution (Kolawole & Iyiola, 2023). Threat is brought to life and the environment at large when toxic wastes (such as heavy metals) are improperly handled and managed (Jaskelevičius and Lynikienė, 2009). The different industries have unique waste composition (organic and inorganic) disposed alongside the wastewater (Mouchet, 1986; Lim *et al.,*2011). Industries produce heavy metal-containing effluents that are highly dangerous to the environment (Ogunfowokan *et al.,*2007). Pollution of surface and groundwater as well as the soil by the heavy metals is brought about as a result of their high solubility (Ahmadkhan, 2001). Aquatic lives, be they marine or freshwater, could be damaged due to heavy metal contamination at different biological magnitudes, thereby resulting in an imbalance in the ecosystem (Zeitoun and Mehana, 2014). Heavy metals are now getting into the sparse water resource availability in Africa (Nriagu, 1992). Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb) and Mercury (Hg) are five heavy metals that exhibit toxicity and are carcinogenic even at very minute concentrations, resulting in harmful effects on humans and on environmental ecology (Leon and Chang, 2020). Heavy metals are an integral part of the environment in which we live since they cannot be broken down or eliminated. Heavy metals are naturally occurring components of the Earth's crust, are persistent environmental pollutants, and have many unfavorable effects on ecosystems (Jomova et al., 2024; Zheng et al., 2024). Epidemiologically, heavy metals have been studied, and they engulf a vast parcel of industrial waste contaminant (Khalifa and Alkhalf, 2018). Pollutants from the industries on the environment, particularly those containing heavy metals and nutrients, are regarded as deadly and toxic to life in general from both zoological and botanical views. Heavy metal has a long biological half-life, they are non-biodegradable, and the pollution that emanates from heavy metals is usually persistent and irreversible (Assayomo et al., 2021). There is a rise in the need for paint globally, and thus, the paint industries have risen to become a point of attraction (Begum *et al.,*2019). Extenders, cellulosic and non-cellulosic thickeners, dyestuffs, organic and inorganic pigments, emulsifying agents, solvents, anti-foaming and coalescing agents are the typical characteristics of water-based paints (Dey *et al.,*2004). The product “paint” is mainly produced as an ornamental gadget or a preservative and, in some cases, for the two purposes concurrently (Virvaghan *et al.,*1991). According to Malakootian *et al.* (2006), the paint industry happens to be among the several industries that have heavy metals included in their waste.

Bioremediation is an aspect of environmental biotechnology that is involved with the use of biological potentials (such as plants) in making environmental contaminants less harmful (Boopathy, 2000). Bioremediation happens to be among the latest technologies that have gained so much interest and attention as it is considered an environmentally friendly and as well as cost-effective approach in dealing with environmental pollution. An adsorbent is regarded as very economical when it is naturally available or a by-product that requires very minimal processing (Bailey *et al.,*1999).

Azolla, *Azolla filiculoides* (*A. filiculoids*) is a loosely floating aquatic fern that is most times, breeded in tropical ecosystems (Akhatr *et al.,*2021). It moves freely with water current and wind circulation, which belongs to the family of salviniaceae (Deval *et al.,*2012; Tayeb *et al.,*2020) and can double its biomass within three to ten days (Hassan et al.,2009). Aquatic macrophytes possess well-formed root systems, which gives them the recognition as superlative bio-accumulators of pollutants (May and Edwards, 2001; Stoltz and Greger, 2002). In the last two decades, plants have been greatly involved in the process of phytotechnologies for the bioremoval of toxicants from the environment, and though the advances are somewhat novel, it has received significant attention (Dhir, 2010). A number of the aquatic plants that have shown to have an affinity for heavy metal removal from aquatic ecosystems are being utilized in the restoration of wastewaters (Abbasi and Rasamani, 1999; Kadlec *et al.,*2000). By virtue of the presence of Azolla in the aquatic ecosystem, it is applied in the bioremediation of wastewater at an economical rate and for an environmentally benign outcome (Deval *et al.,*2012). Zhang *et* *al.* (2008) have suggested the use of high biomass of *Azolla caroliniana* in the renovation of polluted waters. The bioremoval of heavy metals by the macrophyte Azolla has been studied with a successful outcome (Zhao *et al.,*1999; Antunes *et al.,*2021). Research conducted by Xin wei *et al.* (2018) showed that the biomass of Azolla was able to bioabsorb uranium, iron, manganese, copper, zinc, lead and cadmium from the industrial effluent gotten from uranium mills' tailings repository. In addition, *Azolla caroliniana* was able to remove as high as 93% mercury concentration from municipal wastewater after a period of 12 days (Bennicelli *et al.,*2004). In a phytotechnology study carried out by Echiegu *et al.* (2021) using three different aquatic macrophytes, they discovered that *Azolla pinnata* showed the highest percentage reduction of the heavy metal contained in the wastewater. Azolla has been successfully grown under hydroponic conditions in wastewater for heavy metal bioremoval (Deval *et al.,*2012; Echiegu *et al.,*2021).

Water hyacinth, *Eichhornia crassipes* (*E. crassipes*), can be described as a free-floating aquatic macrophyte that belongs to the family of pontedericeae and genus Eichhornia, which has a close correlation with the lily family (Gupta *et al.,*2012; Ali *et al.,*2020). This free-floating invasive macrophyte can be found in all the regions of Asia and also in some other continents and usually forms a heavy covering on the aquatic ecosystem (Cherian and Joseph, 2022). It possesses a very rapid growth rate (doubles biomass in five to fifteen days), is easy to propagate and can successfully contend with other aquatic plants (Nayanathara and Bindu, 2017). Water hyacinth can reproduce both asexually and sexually (Villamagna, 2009), with heights measuring up to 1.5 meters or more (Center *et al.,*2005). According to Ali *et al.* (2020), water hyacinth is the most intrusive and widely distributed tracheophyte worldwide. It has the ability to withstand and grow under diverse environmental conditions, from nutrient variation to temperature (Mahfooz *et al.,*2020). According to Lu (2017), one of the outstanding characteristics of water hyacinth is the bioremoval of heavy metals, e.g., lead, chromium, cadmium and mercury, from contaminated waters. It is very potent in the treatment of industrial wastewater and domestic effluent at an eco-friendly rate (Lin and Li, 2016; Feng *et al.,*2017). Macrophytes are a useful tool when of comes to balancing the ecosystem (Gupta *et al.,*2012), and it is therefore regarded as an effective material for wastewater remediation and removal of pollutants (Cherian and Joseph, 2022). It is greatly recommended for treating industrial effluents as it possesses a high absorption rate of pollutants and can withstand highly contaminated environments (Jafari, 2010). Water hyacinth has been used in the accumulation and phytoremediation of heavy metals (silver, lead, cadmium, chromium, copper and selenium) from polluted wastewaters (Zhu *et al.,*1999). In addition, it was able to reduce the levels of heavy metals in an acid mine drainage and also silver from an industrial effluent within the shortest of time (Pinto *et al.,*1987). Furthermore, it has been able to remediate wastewater containing lead, chromium, cadmium and mercury (Lu, 2017). Water hyacinth is a plant that can be cultured under hydroponics with plant stems above the water level, taking its nutrients straight from the water without soil (Nayanathara and Bindu, 2017). Water hyacinth has been successfully grown hydroponically for wastewater treatment (Echiegu *et al.,*2021).

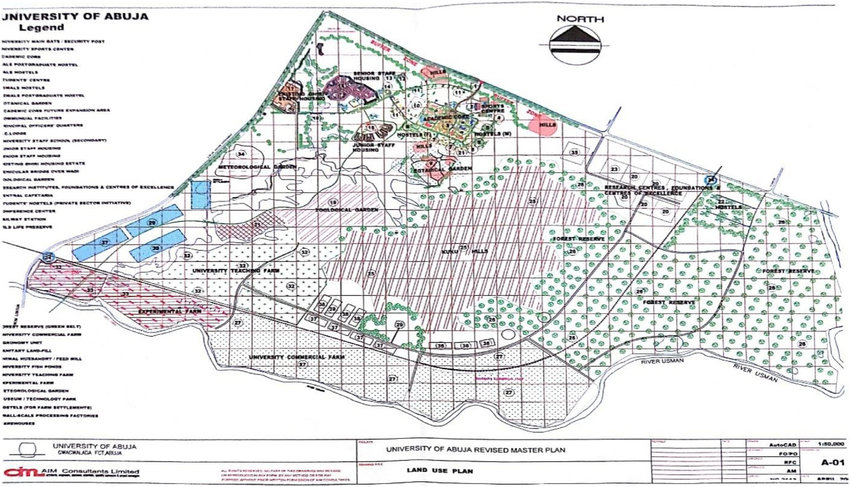
Hydroponics is a system that makes use of nutrients in an aqueous solution to grow plants without soil (Gardea-Torresdey *et al.,*2005). According to Houman *et al.* (2011), hydroponics refers to a soil-less culturing of plants in a controlled domain making use of mineral supplements present in water whereby the roots of the plants are dipped in a nutrient-containing liquid medium. Reports have shown that a good number of terrestrial plants and aquatic macrophytes grown hydroponically have accumulated heavy metals (Ignatius *et al.,*2014; Woraharn *et al.,*2021). Hydroponic systems have been applied in the economical remediation and detoxification of contaminants effectively (Davamani *et al.,*2021) and have been recommended as an alternative technology for the effective treatment of water and wastewater with special reference to the use of both terrestrial and aquatic plants (Moogouei and Chen, 2020).

This study is, therefore, aimed at investigating the bioremediation potentials of two aquatic plants (*Azolla microphylla* and *Eichhornia crassipes*) grown hydroponically in the uptake of cadmium, lead, chromium and copper from a paint-based effluent.

**MATERIALS AND METHODS**

**Sampling Location**

This study was undertaken in the University of Abuja main campus located in Abuja, Nigeria. The university of Abuja which is in Nigeria’s capital city lies between latitude 8o58’ north of the equator and longitude 7o10’ east of the Greenwich meridian. The university is bounded in north by Anagada village, Giri by the west, Kuje is located on the east and south while on the south-west, it is bounded by Gwagwalada (Amarachukwu *et al.,*2020).



**Map 1: Map showing University of Abuja main campus. (Amarachukwu *et al.,*2020)**

**Collection and Preparation of Plants**

The collection of the sample plants *Azolla microphylla* and *Eichhornia crassipes* was done as described by Savitha and Rajan (2018). *Eichhornia crassipes* was obtained from a farm in Jabi Lake, Abuja FCT, Nigeria, while *Azolla microphylla* was obtained from a farm in Onitsha (Strategic Time Fish Farm), Anambara state, Nigeria. The plants were afterwards left to acclimatize and establish in their new environment for some time (Gupta *et al.,*2022). Plants with similar root length and shoot area were selected and after which, they were thoroughly washed under running water and then hydroponically propagated in a nutrient solution (Van Delden *et al.,*2020) for a period of about 14 days before being subjected to the effluent for the bioremoval study. This was to help the test plants acclimatize to the experimental conditions and also to have enough biomass before the experiment (Borker *et al.,*2013; Tablang *et al.,*2021).

**Effluent** **Collection**

The effluent collection followed the pattern described by Nanda *et al.* (2010) and Woldeamanuale and Hassen (2017). The raw paint effluent was obtained from the discharge tank of a paint industry located at Madala, Zuba, Abuja, Nigeria. The effluent sample was collected in newly acquired plastic bottles that were thoroughly washed, cleaned, followed by rinsing with tap water to make it very clean same as all the glasswares (Gupta *et al.,*2022). Preservation of samples was done by storing the samples at 4oC until the time of usage.

**Acid Digestion of the Effluent for Heavy Metal Determination**

The acid digestion of the wastewater sample for the heavy metal determination was carried out according to the method described by Juliani *et al.* (2021) and the United States Environmental Protection Agency (USEPA) (1994). The acid mixture of Nitric acid, HNO3 (heavy metal measurement grade) and hydrogen chloride (Hcl) was added to the effluent sample in a 9:1 ratio, after which it was digested in the laboratory microwave oven. Digestion was then adjusted to a pressure of 800 pounds per square inch (PSI) and set to a temperature of 200oC for a period of 15 minutes. A cellulose acetate membrane filter 0.45 nm was then used to filter the digested sample.

**Determination of Heavy Metal**

The determination of heavy metal contents of the paint-based industrial effluent and the test plants for this study was carried out using the Atomic Absorption Spectroscopy (AAS) Hanna HI Variian AAS SpectraAA 240FS, Italy. A standard solution was run with certified reference material in line with (Zulkafflee *et al.,*2020).

**Experimental Setup**

For this study, the experimental design and the greenhouse maintenance followed the pattern described by Kumar *et al* (2018), Wang *et al* (2018) and Savitha and Rajan (2018), with a slight modification. Acclimatized test plants, *Azolla microphylla* and *Eichhornia crassipes*)was transferred and uniformly spread into plastic pails with a water capacity of 5 litres that contained different dilution factors. Treatment 1 had a 50% dilution rate (i.e. 50% effluent and 50% tap water), and treatment 2 had a 25% dilution rate (25% effluent and 75% tap water). Each of the test plants followed the treatments described above. The pot experiments were carried out under a greenhouse condition at a controlled temperature at the Biological garden, faculty of Science, University of Abuja, Nigeria under maintained temperature. The treatment pails were positioned in such a way that the plants could get adequate sunlight to enhance photosynthesis. The pails were arranged in a completely randomized block design. The effluent was withdrawn on the start and the final day (after 6 weeks) (Echiegu *et al.,*2021) to analyse the heavy metal reduction potential of the study plants *A. microphylla* and *E. crassipes*; this was carried out by comparing the initial heavy metal concentration and the final heavy metal concentration. The acclimatization and the experimental period lasted for a total of 63 days (9 weeks).

**Determination of Removal Efficiency (%) of the Test Plants**

Removal Efficiency was calculated using the following formula (APHA, 2012).

Removal Efficiency (%) = Ci - Ce x 100 …………………….. Equation I

Ci

Where;

Ci = initial concentration of the pollutant.

Ce = final concentration of the pollutant.

**Determination of Relative Growth Rate of the Test Plants**

The relative growth rate was evaluated at the start and at the end of the uptake study, where the harvested plant was weighed for the final weight and compared to the initial fresh weight of the biomass. The length of the leaves and root was also taken with the use of measuring inch tape as described by Kumar *et al.,*(2018) and Gupta *et al.,*(2022).

The formula below was used to calculate the relative growth rate (Aron, 1949).

Relative Growth Rate (RGR) = Ln W2 – Ln W1  ………… Equation II

T2 – T1

Where;

W1 = initial weight of fresh biomass

W2 = final weight of the harvested biomass

T1 and T2 = the duration of the experiment in days.

**Statistical Analysis**

All values described were the means of the replicates. Microsoft Excel were used in the calculation of mean, standard error, and standard deviation and in the plotting of statistical graphs. Statistical Package for the Social Sciences (SPSS) version 25 for Windows was used. Descriptive statistics were employed. Kolmogorov-Smirnov Test was used to check if the data were normally distributed. One-way Analysis of Variance (ANOVA) and Turkey Post Hoc Test were used to compare means between treatments and other parameters measured. Other statistical tools were deployed as well. Significance was used at a 95% confidence interval. Mixed model ANOVA was also used to compare treatments.

**RESULTS AND DISCUSSION**

This study is aimed at investigating the potentiality of two aquatic plants, *Azolla microphylla* and *Eichhornia crassipes,* in the bioremediation of heavy metal-containing paint-based effluent under a hydroponic system. The bioremediation of four heavy metals, lead, cadmium, chromium and copper, were studied.

**Uptake of Lead**

The concentration of lead was analyzed before and after the treatment with the test plants in two different treatments/dilutions (TRT 1, 50% and TRT 2, 25%). From the results obtained in treatment 1 (50% dilution), *A. microphylla* (Azolla) recorded the highest (P<0.05) uptake of lead, which reduced the concentration from 5.34 mg/L to 1.6 mg/L from the effluent at 89%. *Eichhornia crassipes* (Water hyacinth) reduced the concentration of lead significantly (P<0.05), from 5.34 mg/L to the final level of 2.30 mg/L at 51%. In TRT 2, *Azolla microphylla also* recorded the highest (P<0.05) uptake of lead from the effluent sample from 2.9 mg/L to 0.62 mg/L at 78% and *Eichhornia crassipes* (P<0.05) from 2.9 mg/L to 1.81mg/L at 36% (Figure 1).

**Figure 1. Removal efficiency (%) of Lead by the two plants**

**Uptake of Cadmium**

The uptake of cadmium was also carried out by comparing the initial and final concentration of the element after the study. From the results obtained in TRT 1, *Azolla microphylla* significantly reduced cadmium concentration from 7.77 mg/L to 0.16 mg/L at 90% and *Eichhornia crassipes* to 0.33 mg/L at 82%. In TRT 2, *Eichhornia crassipes* recorded the highest (P<0.05) uptake of cadmium from the effluent from the initial concentration of 3.96 mg/L to a final concentration of 0.07 mg/L at resulting to 98% removal while *Azolla microphylla* reduced to 0.24 mg/L at 95% (Figure 2).

**Figure 2. Removal efficiency (%) of cadmium by the two plants**

**Uptake of Chromium**

Chromium was the third heavy metal whose uptake from the effluent was studied. Similar to lead and cadmium, both the initial and final concentrations were recorded. In treatment 1, the highest uptake of chromium from the effluent was observed in the treatment with *Azolla microphylla,* which reduced the concentration significantly (P<0.05) from 0.20 mg/L to 0.15 mg/L with the removal efficiency of 39%, while *Eichhornia crassipes* reduced to a final concentration of 0.18 mg/L at 29%. In treatment 2, the highest uptake was observed in the treatment inoculated with *Azolla microphylla* from the initial concentration of 0.14 mg/L to 0.08 mg/L at 48%. *Eichhornia crassipes,* on the other hand, had 0.12 mg/L against the initial concentration of 0.14 mg/L at 11% (Figure 3).

**Figure 3. Removal efficiency (%) of Chromium by the two plants**

The uptake of copper was investigated. The initial and final concentrations of copper in the water sample were evaluated accordingly. In treatment 1 (TRT 1), *Eichhornia crassipes* had the highest (P<0.05) uptake from 0.53mg/L to 0.13mg/L with a removal efficiency of 76%, while *Azolla microphylla* had the final concentration of 0.15 mg/L at 72%. However, reverse was the case in the second treatment (TRT 2) where *Azolla microphylla* had the highest (P<0.05) uptake from 0.27 mg/L to 0.004 mg/L at 99% and *Eichhornia crassipes* had the final concentration of 0.25 mg/L at 8%, respectively (Figure 4).

**Figure 4. Removal efficiency (%) of Copper by the two plants**

**Relative Growth Rate (RGR)**

The relative growth rate (RGR) is used to measure the growth index of plants or a system. For this study, the RGR was evaluated for both plants. The comparison was drawn between the two treatments and the control. There was a reduced growth rate recorded for *Azolla microphylla* however, *Eichhornia crassipes* showed a slight increase in the relative growth rate after the study in both treatments (Figure 5).

**Figure 5. Relative Growth Rate of the Plants**

**DISCUSSION**

The aim of this study was to check for the heavy metal uptake potential of *Azolla microphylla* and *Eichhornia crassipes* from a heavy metal-containing paint effluent. The plant acclimatization and the heavy metal uptake study lasted for a period of 9 weeks, after which the samples were analyzed for the potential of the different plants in the reduction of the heavy metals present in the effluent. The use of plants for the remediation of heavily polluted systems is beginning to gain much interest due to the many advantages over the conventional waste treatment technologies. A similar observation to the one obtained in this study has been reported in a research carried out under hydroponic condition for the uptake of heavy metals from an electroplating effluent using *Eichhornia. crassipes* (water hyacinth), the removal efficiency recorded was between 90-100% (Savitha and Vel Rajan, 2018). In another hydroponic study carried out for the uptake of heavy metals using different grasses, the data reported showed percentage removal of the heavy metals from the solution at 89% and 97% (Hasssan *et al.,*2020). Photosynthetic plants have shown potency in the absorption hyper-accumulation and can put up with heavy metal toxicity either from the water or soil via their roots.

The relative growth rate (RGR) of the test plants after a 6-week sampling period was evaluated. This gives a clue on the growth of plant within a specified period of time. A slightly notable growth was observed for *Eichhornia crassipes* in both treatments. However, a reduced growth was observed for *Azolla microphylla*. Both increased and reduced biomass yield of plants has been recorded in previous research (Salehi and Shariat, 2024). For the first few days of exposure, the plants seemed to be growing well until some changes in the features began to emerge. Most of the green leaves started to fade and turned yellow, and some eventually turned brownish. A similar observation has also been reported (Phuenghphai *et al.,*2020). In their report, they found that heavy metals at high levels resulted in the death of some plants, which can be linked to the extreme destruction of different metabolisms. Reduced plant growth rate due to the presence of heavy metals has also been reported (Anoliefo *et al.,*2005; Vwioko and Fashemi, 2005; Şentűrk *et al.,*2022).

**CONCLUSION**

There is a need for an efficient remediation of paint-based effluent as most of the heavy metals were found to be above the permissible limits. The data obtained in this study suggests the potentiality of *Azolla microphylla* and *Eichhornia crassipes* grown hydroponically in the uptake of heavy metals (Cadmium, lead, copper and chromium) from multi-metal-containing effluent, considering the toxicity that comes with the presence of heavy metals in the environment.

**COMPETING INTERESTS DISCLAIMER**:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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