THE EFFECT OF HEAVY METALS ON THE MORPHOLOGY OF *EICHHORNIA CRASSIPES*-MART. SOLMS. IN PANTEKA STREAM.

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ABSTRACT

Heavy metals are indispensable for normal plant growth and development. This study was conducted to examine heavy metals impact on the morphology of *Eichhornia crassipes*. Morphological parameters of the plant were studied. Water samples were collected from Panteka stream where mechanic and farming activities are carried out. The stream was divided into three points (A, B and C) and pond water, where farming is predominant served as the control site (D). Heavy metals were analyzed during dry and wet seasons for the presence of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn). The result obtained revealed that the highest mean plant height (80.86cm), root length (41.40cm), leaf length (17.68cm), leaf number (21.20), leaf width (14.64cm) and flower number (3) were observed at sampling points A, D, C, B and A respectively. Among the heavy metals analyzed Cd had the highest concentration (0.78mg/kg). Cadmium and Lead were above, while the others were below the standards of FEPA and USEPA. The roots and leaves of *E. crassipes* showed clear signs of abscission and elongated roots at the control sites. The Pearson correlation coefficient between heavy metals at sampling points during the two seasons revealed a significant difference.

keywords: Heavy metals, Morphology, *Eichhornia crassipes* and Abscission.

INTRODUCTION

Heavy metals stress in the plant had in the time past and have continued to severely influence plant productivity, due to the spread of heavy metals in the environment. Heavy metals from industrial, mining, and agricultural activities, increasing soil and water heavy metals concentrations have become an immediate global menace (Thakur *et al.* 2016; Abdu *et al.* 2017). The ever-increasing release of heavy metals into the environment (hydrosphere) has prompted serious focus as a result of their huge bioavailability, bioaccumulation, and biomagnification capability (Fang *et al.* 2017; Binet *et al.* 2018; Song *et al.* 2019). Intake of aquatic organisms is the main route through which heavy metals accumulate in the human body (Fu *et al.* 2019). Examples of heavy metals include; cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and chromium (Cr) have several toxic impacts even at minute concentrations including a series of cancers, anemia, heart attacks, embryotoxicity, neurotoxicity and immune system weakness (Järup, 2003). According to World Health Organization, the maximum permissible limits of these elements have been set to ensure that zero or only the threshold limits of trace levels (part per billion) are allowed in a water resource (Abdullah *et al.* 2019).

Depending on the development conditions, *Eichhornia crassipes* has two unique intermediate morphologies. When water hyacinth mats are less dense, the leaves are roughly round, ovoid, or shaped like a kidney up to 15cm across the width in water with high nutrients and no herbivores. petioles of water hyacinth are one metre long with round leaves which are less than thirty centi-metres with a bulb-like shape when its mats are relatively dense.

However, the seedling's initial leaves are elongated and strap-like, though they may develop into the characteristic spathulate form over time. This demonstrates that both morphologies exhibit distinct photosynthetic behavior (EPPO, 2008). Fruit clusters, leaves, long pendant-like roots, rhizomes, and stolons make up the mature water hyacinth plant. These roots form a dense mat underwater. The average height of water hyacinth is about 40cm, although it can reach a height of 1m. The flowers are 4-7cm in diameter and have 6 to 10 flower leaves. Water hyacinth has tissues (air-filled) in its stems and leaves, which makes it buoyant (Rezania *et al.* 2015).

Heavy metals stress in plants also causes deficiency in water, nutrient absorption, alteration in plasma membrane permeability, and several disorders in biochemical and physiological plant systems, including disturbance in cell division, cell cycle impairment, disorders in mitochondrial respiration, and dysfunctions in the synthesis of photosynthetic pigments (El-Banna *et al.* 2019).

These biochemical and physiological disorders, which are linked to a variety of anatomy and ultrastructure dysfunctions, affect plant cells and tissues. In reed plants, ultrastructural screening revealed a distortion in the epidermis and mesoderm, as well as an inhibition of the radial movement of fluids from roots into peripheral regions and a restriction on the uptake and translocation of nutrients roots and shoots. There are some regular morphological symptoms of heavy metals stress, including chlorosis, withering, dropping leaves, and defects in shoot and root development, along with these biochemical, physiological, and anatomical retrogressions (Baruah *et al.* 2017).

Although, several intensive research has been carried out on wastewater decontamination using *E. crassipes* plants. According to Hussain and Jamil, (1989) the interaction of heavy metals in *E. crassipes* contradicts the terms of biochemical parameters. As shown in the interference of Cd and Mn ions with protein synthesis caused inhibition in nucleic acids content. However, Zn ions gave rise to the opposite effect through increasing nucleic acids content and protein synthesis (Farahat *et al.* 2020). Hence, this present study was carried out to investigate the effect of heavy metals on the morphology of *E. crassipes* growing in two water bodies; stream and pond.

MATERIALS AND METHODS

Study Area

The study area, is the stream flowing through the Panteka mechanic village, located between latitudes 10^0 32’37” and 10^0 33’56” N and longitude 7^0 24’29” and 7^0 25’7” E, at the Northern part of Kaduna, Nigeria. The Panteka stream flows westward, it usually decreases in volume during dry season and *Eichhornia crassipes* grows in the stream. Similarly, Panteka is a mechanic village where all kinds of cars and motorcycle spare parts are sold, and their maintenance is carried out. Alongside this, agricultural activities take place in this area and runoff from the farms and debris washed off from these anthropogenic activities mentioned above enters the stream. The control samples were collected from an enclosed pond, where farming activities are carried out, behind Mal. Abdulrakeem Fish Farm, Farin-Gida Mando Kaduna.The stream was divided into three sampling points, A, B, and C. Sampling point A is 4,218m away from B, and B is 4000m away from C. At sampling point A, the predominant activity done in this area is farming, dumping of car worn-out parts and repairs, while at sampling points B and C, there is more of mechanic repair activities.

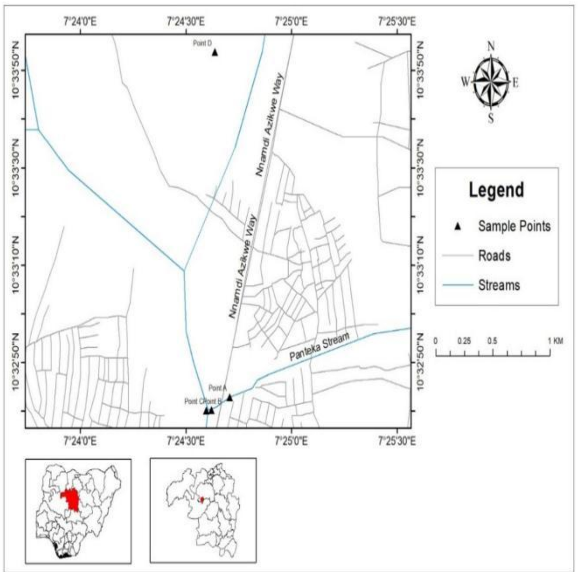

Figure 1: Panteka stream study area map with sampling points, (Source: GIS, KADSU).

Water Sample Collection

Water samples were collected during the dry (April 2020) and wet (August 2020) seasons in sterile plastic containers from the four sampling points. The method of collecting water grab samples as described by Thomas, (2014), was used, and done by holding the uncapped sterile plastic container upside down and submersed it. And, the tip of the container upright and then allowed water to fill the container and was removed from the water, screw-on cap and carefully labeled respectively.

Heavy Metals Analysis of Water

The method described by Wahab and Liasu, (2010) was used to analyze heavy metals in water samples. 50ml of the water samples from each sampling point for each of the seasons were digested with 10ml of HNO_3 on a hot plate. The resulting clear solutions were filtered using Whatman filter papers and reconstituted to 50ml in volumetric flasks with deionized water. 50ml of the filtrates were analyzed for the heavy metals using Atomic Absorption Spectrophotometer (Model: PG-990). The standard solutions of metals to be assayed were prepared and atomic absorption spectrophotometer was allowed to run for 10 minutes before immersing the sipper into the standard metal solutions to calibrate the AAS and acetylene was used as the carrier gas. Flame absorption method at a wavelength of 283.3nm, slit 0.4nm, high voltage of 368V and lamp current of 2.0mA were utilized in the process. The absorbance was read before placing the sipper into the solution of the digested samples and the concentrations were obtained from the AAS.

Plant Material

Plant samples were collected from Panteka's contaminated water as well as control site. Plant samples from the four sampling points were wrapped in labeled polybags for each of the plants and brought to the Biological Sciences Department Laboratory Nigerian Defence Academy, Kaduna.

Plant Sample Processing and Analysis

The plant samples were washed with deionized water to remove unwanted debris Morphological observation was carried out. Followed by herbarium techniques (which include pressing and drying the plant sample using the plant presser, labeling, and storage) and full authentication of the plant. The plant samples were identified and authenticated in the herbarium, Botany laboratory NDA. The specimen with voucher number NDA/BIOH/202030 was kept in the herbarium for reference purposes.

Plant height (cm): The height of the plant was measured with centi-meter rule from the root level to the apex of the plant for each of the plant collected.

***Root length (cm)*:** The length of the root was measured with meter rule from the root level to the tip of the longest root for each of the plant collected.

***Leaf number*:** The leaf number for **e**ach of the plant collected were counted and the mean leaf per plant were determined.

***Leaf width (cm)*:** The leaf width was measured with metre rule from the widest leaf for each of the plant collected.

***Leaf length (cm)*:** The length of leaf was measured with meter rule from the leafstalk to the tip of leaf.

***Number of flowers*:** The flower number for **e**ach of the plant collected were counted and the mean number of flowers per plant were determined (Alvaro *et al.* 2020).

Statistical Analysis

To confirm the variability and validity of the results, the data from the study were subjected to a one-way analysis of variance (ANOVA) and Pearson correlation coefficient (r). All data were expressed as LSD with a probability of $p<0.05$ level of significance using the Statistical Package for Social Sciences (SPSS-version 23.) and Excel version 2016.

RESULTS

Heavy Metals Analysis

Figure 2 and 3 showed heavy metal concentrations at sampling points throughout the dry and wet seasons. During the dry and wet seasons, the maximum concentration of Cadmium (0.79 mg/kg) was recorded at sampling point A, and the lowest concentration of Cadmium (0.054 mg/kg) was recorded at sampling points B and C during the dry season. During the wet season, the maximum concentration of Chromium (0.18 mg/kg) was recorded at sampling point C, and the lowest concentration (0.003 mg/kg) was found during the dry season at sampling point B. The highest concentration of copper was recorded at sampling point C (0.32 mg/kg) and the lowest concentration of copper, (0.215mg/kg) at sampling point C, both were during the wet season.

Furthermore, the concentration of Nickel during the seasons in the different sampling points, the highest concentration of Nickel was recorded at sampling point B, (0.24mg/kg) during the wet season. and the lowest concentration of Nickel, (0.008mg/kg) at sampling point B, during the dry season. The highest concentration of Lead was recorded at sampling point C, (0.39mg/kg) and the lowest concentration of Lead, (0.042mg/kg) at sampling point D, both were during the dry season. However, sampling points A and control showed no trace of Lead during the wet season. The highest concentration of Zinc was recorded at sampling point A, (1.18mg/kg) during the dry season and the lowest concentration of Zinc, (0.052mg/kg) at sampling point B, during the wet season. The control was below the detectible limit, except for Lead and Zinc during the dry season.

In comparison to the Federal Environmental Protection Agency's (FEPA, 1991) and the United States Environmental Protection Agency's (USEPA, 1992) conventional maximum concentration limits, Table 2. Water samples from (sampling points A, B, C, and D) showed that the concentration of cadmium during the dry and wet seasons at the sampling points were all above the limits, except in the control site where it was below the limits in the two seasons. The concentration of chromium in all sampling points during the seasons was below the maximum concentration limit, except for sampling points A and C during the wet season where it showed an increase beyond the maximum concentration limit. Copper concentration at the sampling points was below the maximum concentration limit in all the seasons except for sampling point C during the wet season which showed an increase that was more than the maximum concentration limit. Nickel concentration in all the sampling points was less than the maximum concentration limit in all the seasons, except for sampling points B and C during the wet season where it was beyond the maximum concentration limit. The concentration of Lead in all sampling points was beyond the maximum concentration limit in all the seasons, except in sampling point A where there was no trace of Lead during the wet season. The concentration in the control was below the maximum concentration limit. Zinc concentration at all sampling points was less than the maximum concentration limit, except for sampling points A and B during the dry season where it was beyond the maximum concentration limit.

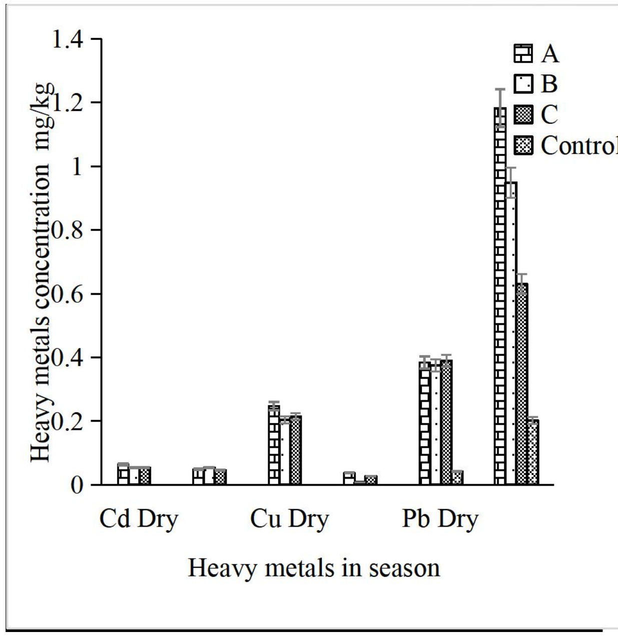

Figure 2: Mean Concentration of Heavy metals at the sampling points during dry seasons.

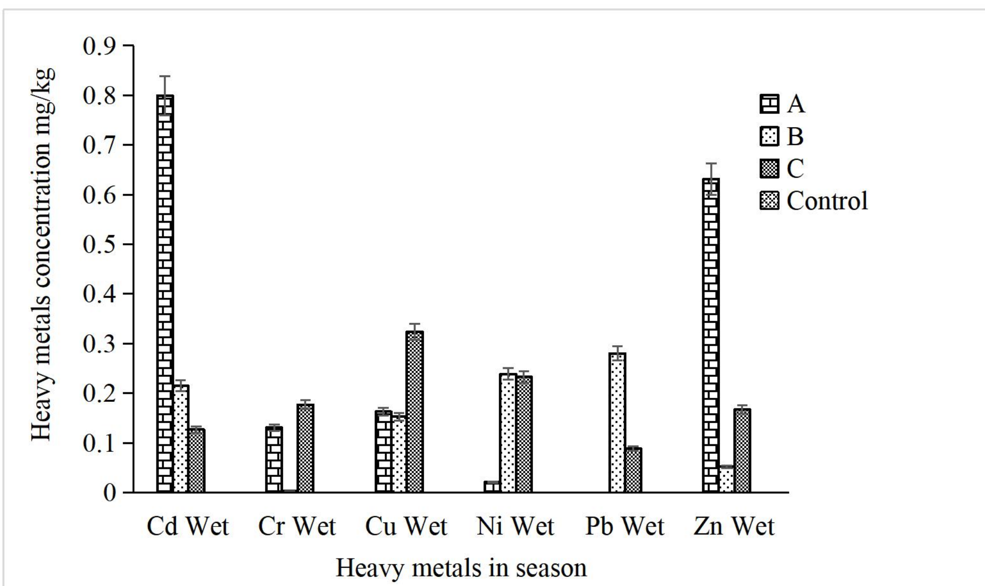

Figure 3: Mean Concentration of Heavy metals at the sampling points during wet seasons.

Morphology of *E. crassipes*

The morphology of *E. crassipes* at the sampling points of Panteka stream and the control are presented in Table 1. The result obtained from all the sampling points showed that the plant height ranged from 60.12 to 80.86cm. The highest mean was recorded at sampling point A with the height of 80.86cm and the lowest was 60.12cm height of the plant at the control. The root length ranged from 17.00 to 41.40cm, with sampling point D (Control) having the highest root length and sampling point B the lowest. The length of the leaf ranged from 7.72 to 17.68cm with sampling point C having the highest length of leaf and sampling point D having the lowest leaf length. The mean number of leaves ranged from 12.40 to 21.20. The highest mean was found at sampling point B, while the lowest mean was recorded at sampling point D. The mean width of the leaf ranged from 8.00 to 14.64cm with sampling point B having the highest mean width and sampling point D having the lowest. The mean number of flowers ranged from 2.40 to 3.00 at only sampling points A and C respectively, other sampling points had no flower.

Correlation Analysis

The Pearson correlation coefficient between heavy metals in water samples at sampling points during the dry and wet seasons revealed a significant difference. All heavy metals showed positive correlations with the two seasons (dry and wet).

Table 1: Morphology of *E. crassipes* at the different sampling points

Sampling points	Plant height	Root length	Leaf number	Leaf width	Leaf length	Flower's number
A	80.86^{a}	21.50^{a}	13.40^{ac}	12.08^{a}	15.08^{a}	2.40^{a}
B	60.12^{a}	17.00^{a}	21.20^{b}	14.64^{b}	15.46^{ab}	0.00^{a}
C	67.33^{a}	25.82^{a}	18.60^{abc}	12.08^{a}	17.68^{b}	3.00^{a}
D	69.20^{a}	41.40^{a}	12.00^{ac}	7.93^{c}	7.72^{c}	0.00^{a}

Down the columns, the means with different superscripts are significantly different ($p< 0.05$ at 95% confidence level.

Table 2: Pearson Correlation Coefficient between Heavy metals during the seasons

Heavy metals	Dry and Wet
Cadmium (mg/kg)	0.784*
Chromium (mg/kg)	0.833*
Copper (mg/kg)	0.833*
Nickel (mg/kg)	0.895*
Lead (mg/kg)	0.457*
Zinc (mg/kg)	0.855*

*Correlation is significant at $p<0.05$ at 95% confidence level.

Table 3: The Maximum Contamination Limit (MCL) standards for Heavy metals (HMs) according to the United States Environmental Protection Agency (USEPA) (Mukesh and Lokendra, 2013) and Federal Environmental Protection Agency (FEPA, 1991).

Heavy Metals	Maximum Contamination Limit (mg/l) USEPA	Maximum Contamination Limit (mg/l) FEPA
Arsenic	0.05	-
Cadmium	0.01	0.01
Chromium	0.05	0.05
Copper	0.25	0.05
Lead	0.006	0.05
Mercury	0.0003	-
Nickel	0.20	0.1-0.2
Zinc	0.80	1.5

DISCUSSION

During the wet season, the concentrations of these heavy metals in water samples from sampling points A, B, C, and D increased. This might be due to the inflow of water from other water sources depositing more of these heavy metals, and the increase in zinc at sampling point A and B, during the dry season might be due to the engine oils, wastes from car parts and other items coated with zinc being washed off into the stream. Among the heavy metals analyzed at Panteka Stream during the wet season, cadmium was observed to have the highest concentration (0.78mg/kg) and this was more than the maximum concentration standards of FEPA (0.01mg/L) and USEPA (0.01mg/L), Table 3.

The high content of these heavy metals might be a result of the release of effluents from car paints, car batteries, and phosphate fertilizers in the stream. The heavy metals concentration conforms with the findings of Ganesh, (2012) who reported that heavy metals were observed from paper dumping sites. Cadmium concentration in the water samples was the highest among the heavy metals studied. This was similar to the reports of Shokrzadeh and Saeedi (2010) who, in their study of heavy metals in sediments collected from the Gorgan coast, reported that zinc had the highest concentration. However, the other heavy metals analyzed were within the recommended limits.

The morphology parameters of *E. crassipes* in the study area revealed that there was no significant difference ($p<0.05$) in plant height and flower number at the various sampling points. The control samples showed a significant difference in root length from the plant growing at the other sampling points. within the various sampling points, there was also a significant difference in the number of leaves, leaf width, and leaf length. *E. crassipes* growing at Panteka stream showed more relative growth of aerial parts (shoots and roots) than those of the control except for the root length.

These results were in agreement with the findings of Ganesh, (2012). Roots in the control samples were observed to be longer than roots at other sampling points. This might be because plants at the control site accumulated a small concentration of Lead, Chromium, Nickel, and Zinc, which may have contributed to the increase in their root length, a phenomenon known as hormesis. Hormesis, defined as the ability of living organisms to adapt to low-level chemical agent exposures, is a common occurrence in nature (Calabrese and Baldwin, 2003). Hormesis is characterized by growth stimulation at low concentrations and inhibition at higher concentrations (Hema *et al.* 2010).

The observed longer root length in the study area might also be owing to an alteration in cell cycles, divisions, and chromosomal abnormalities due to Cadmium as reported by Bernavides *et al.* (2005). Heavy metal stress, according to Ganesh (2012), causes *Eichhornia* to grow abnormally. This might be the first step in the plant's mechanism for accumulating and sequestering heavy metals in various organelles when it is stressed by heavy metals.

The roots and leaves of *Eichhornia crassipes* plants growing at the heavy metal contaminated Panteka stream showed clear signs of abscission. According to Dalcorso *et al.* (2008), heavy metal exposure causes an increase in Abscisic Acid (ABA) and Jasmonates levels in plants. The abscission and browning of stressed roots, root growth, and leaves might be due to this increased ABA level. In addition, Javiera *et al.* (2022), reported that plants exposed to high heavy metal concentrations can cause rigorous effects on its growth and development, such as photosynthesis inhibition, the disruption of cell membrane integrity, root browning, interveinal chlorosis, and, finally, wilting and death (Reichman, 2002; Viehweger, 2014; Ye *et al.* 2015). The production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals through Haber–Weiss and Fenton reactions gave rise to these outcomes mentioned (Thounaojam *et al.* 2012; Viehweger, 2014).

Moreso, the total concentration of these heavy metals in the water, indicated a positive correlation. It connotes that, heavy metals at sampling points increased as these metals increased during the dry and wet seasons and this agrees with the reports of Arifin *et al.* (2012).

CONCLUSION

The results obtained from this study has shown that the morphology of *E. crassipes* growing at Panteka stream may have been affected by heavy metals. There was relative growth in the aerial parts (shoots and roots) than those of the control. But lower growth in the root length. These changes observed might be due to the heavy metal stress. Heavy metals stress could have more effects on the physiology and anatomy of *Eichhornia crassipes* which might be altering some structures and functions resulting in morphological changes.

Assessing other internal compositions could be vital to fully understand the general interaction between these heavy metals and the aforementioned plant. Also, during the seasons, the study area revealed low concentrations of all heavy metals. However, Cadmium was observed to have the highest concentration and Pearson correlation depicts a positive relationship of the heavy metals with seasons at the sampling points. Therefore, a routine check on the freshwater body such as Panteka stream should be conducted regularly and further research is recommended to explore more on this plant under heavy metal stress.

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