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The Impact of Organic Amendment on the Anatomical Structure of *Cyperus iria* Linn and *Echinochloa colona* Link Expose to Heavy Metal Pollution

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Abstract: The effect of NPK, orange and plantain peels amendment on anatomical structure of *Cyperus iria* and *Echinochloa colona* in heavy metal polluted soil was investigated in this study. Factorial experiment fitted into Randomized Complete Block Design was adopted. Two kilogram (2kg) of homogenized heavy metal polluted soil were weighed into planting bags and amended with 40g NPK fertilizer, 100g orange peels waste and 100g plantain peel waste in six replications respectively. A double control of unpolluted and polluted soils without amendments in six replications were also set up. Two (2) weeks old seedlings of *Cyperus iria* and *Echinochloa colona* were transplanted from nursery onto the respective experiment blocks. The set up was monitored with constant watering at the rate of 500ml for two months of post treatment. At the end of the experiment, the anatomical structure study of roots and shoots of the study plants grown in control soil and soil amended with NPK 20:20:20 showed distortion in the vacuole bundles and cortex. On the contrary, *Cyperus iria* and *Echinochloa colona grown* in soil amended with orange and plantain peels waste observed to be similar in anatomical structure with *Cyperus iria* and *Echinochloa colona* grown in double control. The result also showed that the vascular bundles of *Cyperus iria* and *Echinochloa colona* grown in NPK amendment treatments and control soil lost their shape in roots and shoots. Therefore, orange and plantain peels amendment were effective in reducing the negative impact of metals on plants anatomical structure. These amendments can be harnessed as chelators in making phytoextraction techniques feasible by controlling wilting of plants during remediation processes.

Keywords: Heavy Metal, Anatomical Structure, Phytoremediation, *Cyperus iria, Echinochloa colona*

1. Introduction

The desire of man to gratify his insatiable wants has led to exploitation of environmental resources, ought to have progressed with environmental sustainability. The environment is basically the effects of all conditions and features which alters the existence of an organisms in its natural habitat. In the growth medium, plants continued existence, adequate growth and reproduction is a function of suitable soil condition (physical and chemical). The continued existence of living organisms (plants and animal) is dependent on their habitat. In other to survive the negative effects of the environment, plants develop tolerant mechanisms to acclimatize to environmental changes such as climate, moisture, temperature, soil condition and essential minerals useful for plant growth. The plant species that cannot adapt to this continuous environmental instability eventually die. [3] accounted that Man is lost in thought in exploiting the environment without taking into consideration the harmful effects of his actions on the environment. Man in all his exploitation actions has been diffident from all his responsibility. According to [15]. Ignoring responsibilities cannot prevent its consequences. Pollution issues are intense in under developed countries of the world than it is in technologically developed countries even with dense populations may be very dense [18]. Metals are ubiquitous since they constitute part of earthcrust formation [14] Human interference contributes immensely in heavy metal occurrence [1]. The harmful effect of heavy metals, their persistence and vulnerability to plants has drawn the attention of researchers [13]. The negative effects of metals on biomolecular processes in plants result in growth decrease [16]. It is reported that metals can only be changed from one state to another [11]. In order to achieve decrease in soil metal concentration, some feasible remediation technologies have been adopted. The solubilization of heavy metal by root exudates increase its availability for plant uptake [5]. Although plants need certain heavy metals for metabolic processes, they can be toxic to plants when in excess. Metals are not biodegradable like hydrocarbons rather they bioaccumulate within plant tissues. Plants have various mechanisms for metal tolerance and to withstand harsh environmental conditions [10]. The tolerance mechanisms of plants against extreme heavy metal toxicity such as reactive oxygen species scavengers production, displacement of ions, preventing heavy metal transport in aerial parts and Storage of metals in non-sensitive parts [8].

Plants developed these strategies to survive in extreme environment. Some of the noxious effects caused by excess heavy metal concentration includes inhibition of cytoplasmic enzymes, damage to cell structures [12].

Heavy metal effects have been reported to morphologically decrease plant growth [20]. The part of plant having direct link with pollutant that is the root exhibit changes in their rooting system in other to adapt and survive such stress [6]. The primary organ that comes in connection with contaminate is the root and it is exposed to toxic effects more than other plant parts [19]. The first pronounced effects of heavy metals on plants are observed in root length inhibition. The morphological and structural effect of heavy metals includes:

1. Decrease in plant root elongation;
2. Decrease in biomass and vessel;
3. Reducing root number;
4. Enhancement in lignification and changes in hypodermis and epidermis structure. [12].

2. Materials and Methods

2.1. Study Area

This research was carried out at the Centre for Ecological Studies, University of Port Harcourt, located in the Niger Delta area of Nigeria on geographical coordinates of Latitude 4.90428°N and Longitude 6.92297°E. The area experiences two distinct seasons - dry and wet seasons. The dry season is from November to March and wet season is from April to October. The annual rainfall is usually at its peaks in July and September. The climate condition of the area is characterized by temperature range of 36°C and 45°C for daily and annual range respectively.

2.2. Sources of Materials and Processing

Land race of sweet orange was acquired from Otutu-Amaumara Ezinihitte Mbaise LGA., Imo State. The sweet orange is popularly known as 'Oroma or Epe' in their native tongue. The ripe plantain was obtained from Kaiama in Kolokuma/Opukuma L.G.A, Bayelsa State. The plantain and orange peels were removed mechanically by hand peeling. The peels (waste) generated from mechanical process was dried and processed into powder form, which was then analyzed to make certain the nutritional value and heavy metals content of the peels (Table 1). The NPK 20:20:20 fertilizer was obtained from Rivers State Agricultural Development Program (ADP) Rumuodomaya, Port Harcourt. A suspected heavy metal polluted soil was acquired from an abandoned metal scrap site at Ikoku Rivers State Port Harcourt on geographical coordinate: Latitude 4.80083°N and Longitude 6.991093°E alongside with uncontaminated soil obtained from a fallow land at University of Port Harcourt, depth 0-20 cm using a spade. The soils collected were analysed to ascertain heavy metal content and other physicochemical properties, this was known as baseline analysis (Table 2). The soils were bulked together, homogenized and transported to the Centre for Ecological Studies University of Port Harcourt on geographical coordinate: Latitude 4.90428°N and Longitude 6.92297°E where the experiment was conducted. The collected soil was mixed thoroughly, dried and sieve through 2 mm mesh to obtain a homogenous soil (fine fraction) composite.

Table 1. *Nutrient and metal of the peels waste used.*

S/N	Parameter	Orange peels waste	Plantain peels waste
1	Phosphorus (mg/kg)	66.51	36.84
2	Sodium (mg/kg)	474.85	137.45
3	Potassium (mg/kg)	66,285	26,743
4	Magnesium (mg/kg)	1208	1614
5	Calcium (mg/kg)	278.70	4,400.10
6	Nitrogen %	0.119	0.196
7	Ash %	11.50	16.40
8	Fe (mg/kg)	767.7	483
9	Zn (mg/kg)	13.05	236.50
10	Pb (mg/kg)	ND	ND
11	Cd (mg/kg)	ND	ND
12	pH	5.56	9.08

ND = Not detected.

2.3. Experimental Design

Factorial experiment fitted into Randomized Complete Block Design (RCBD) was adopted as a design for this experiment. Weighing balance (Setra 480S, USA) calibrated in (kg) was used to weigh two-kilogram (2 kg) of the homogenized soil into twenty-four (30) planting bags. The bags were arranged in 4 sets (A, B, C, and D) alongside with uncontaminated soil designated as batch E.

Table 2. *Physicochemical properties and heavy metal content of polluted and unpolluted soil.*

S/N	Parameter	Unpolluted	Polluted soil
17	pH	5.10	8.43
18	Conductivity (μsCm^{-1})	90	1193
19	Iron (mg/kg)	48.2	4410
20	Zinc (mg/kg)	0.94	107.5
21	Lead (mg/kg)	130	167.3
22	Cadmium (mg/kg)	0.80	15.3

Note: SOM = Soil Organic matter.

2.4. Amendment Treatments

Two kilogram (2kg) of homogenized heavy metal polluted soil were weighed into planting bags and amended with 40g NPK fertilizer, 100g orange peels waste and 100g plantain peel waste in six replications respectively. A double control of unpolluted and polluted soils without amendments in six replications were also set up. Two (2) weeks old seedlings of identical size and vigour of *Cyperus iria* and *Echinochloa colona* were transplanted from nursery onto the respective experiment blocks. The experimental area was shaded with transparent rubber zinc as to control rain water. Watering was done 4 times a week using 500 ml and weeding was done by hand picking when the need arose. The experiment was allowed to stand for 60 days.

2.5. Sampling and Labouratory Analysis

At the 60th day of the experiment, each plot were carefully harvested; the plants shoots were separated from roots by cutting, using a sharp knife. The cut portions of stem from fresh specimens were fixed in formaldehyde, glacial acetic acid and ethanol (FAA) in the ratio of 1 part formaldehyde, 1 part glacial acetic acid and 18 parts of 70% ethanol (v/v). Then they were passed through succession of graded ethanol solutions in the progressive order 30%, 50%, 70%, and 95%. The samples are allowed to stand for 2 hours. The specimens were then transferred to absolute ethanol after passing successively through the different ethanol concentrations this was allowed to stand for 12 hours to enhance dehydration. At the expiration of dehydration the specimen were trimmed, embedded in wax block, hand sectioned, stained with counter stain and mounted on slides, which were viewed under a research microscope and photomicrographs of the sections were taken using digital camera (Osuji, 2003).

3. Result

The addition of amendment influence the anatomical structure of root and shoot as shown in Plates 4.6 and 4. 7. The root and shoot anatomy of *Cyperus iria* and *Echinochloa colona* grown in control soil and soil amended with NPK 20:20:20 of various concentrations 40 g, 80 g and 120 g showed distortion in the vacuole bundles and cortex. On the contrary, *Cyperus iria* and *Echinochloa colona* grown in soil amended with various concentrations of orange and plantain peels waste observed to be similar in anatomical structure with *Cyperus iria* and *Echinochloa colona* grown in double control (unpolluted without amendment). The result also showed that the vascular bundles of *Cyperus iria* and *Echinochloa colona* grown in NPK of various concentration of amendment treatments and control soil lost their shape in roots and shoots.

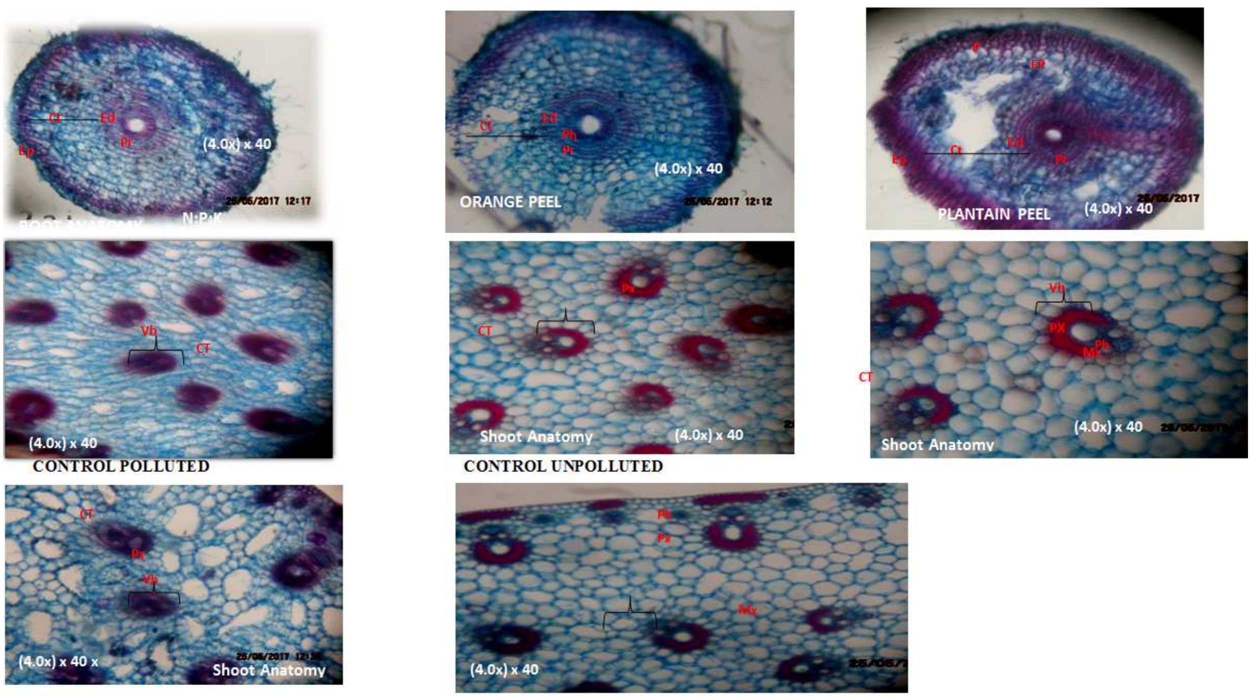

Figure 1. *Anatomical structure of Cyperus iria.*

Figure 1: Photomicrographs (×10) of root and stem anatomy of the genus *Cyperus.* Vb. Vascular tissue, Ct. Cortex, X. Xylem Ph. Phloem, Pr. Pericycle, Ep. Epidermal cell, Ed. Edodermal layer, Mt. metal xylem. Note Xylem cells are stained red and phloem cell are stained blue using counter stain.

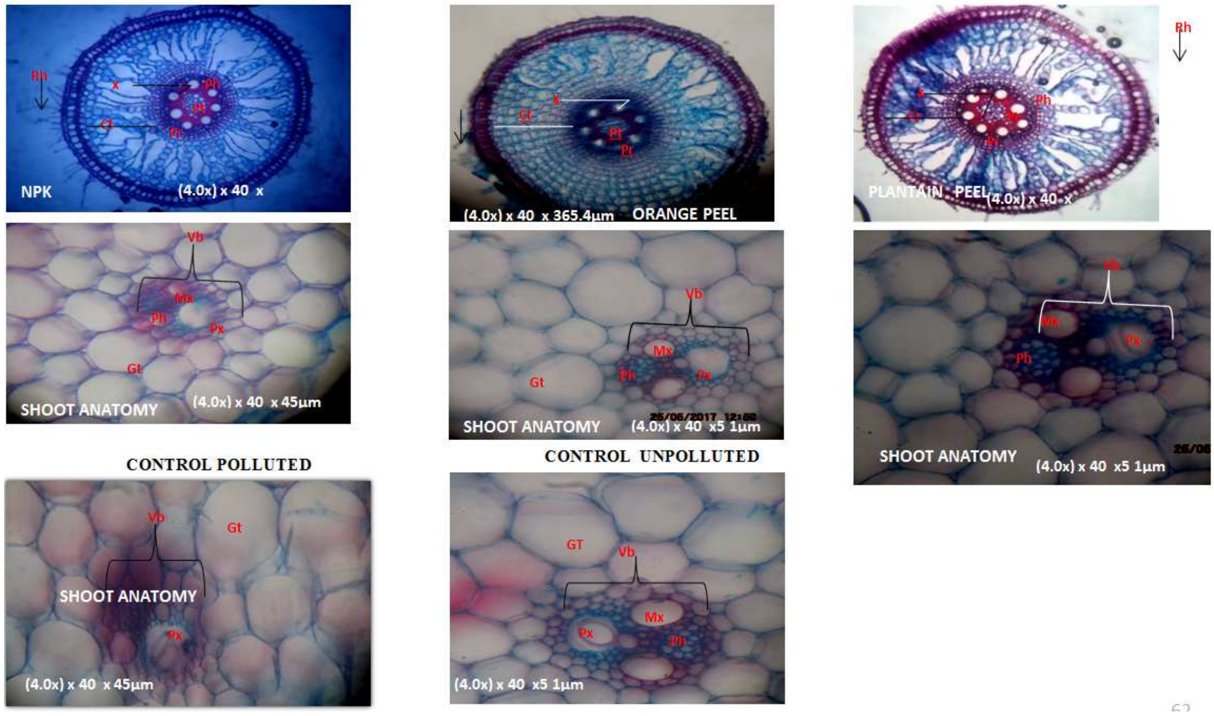

Figure 2. *Anatomical structure of Echinochloa sp.*

Figure 2: Photomicrographs (×10) of root and stem anatomy of the genus *Echinochloa s*p Vb. Vascular tissue, Ct. Cortex, Px. protoxylem Ph. Phloem, Pr. Pericycle, Ep. Epidermal cell, Ed. Edodermal layer, Mt. metal xylem, Pt. Pith, Hd. Hypodermis.

4. Discussion

It is well acknowledged that heavy metal contamination affects the physicochemical characterization and biological properties of soil thus influence negatively the general performance of plants [16; 7]. The effects of heavy metals on plant performance were also detected in *Cyperus iria* and *Echinochloa colona.* The pathway used by plants to accumulate essential mineral is also used in accumulating non-essential mineral that could be detrimental to plant health. [9] report that the pathway used by plants to accumulate essential minerals and metal also gives access to non-metal. As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly. Some of the toxic effects caused by increase in metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress [12]. Additionally, the negative impact heavy metals have on the growth and activities of soil microorganisms may also indirectly affect the growth of plants. This can be achieved by a reduction in the number of beneficial soil microorganisms due to high metal concentration may leading to decrease in organic matter decomposition leading to a decline in soil nutrients. Reactive oxygen species activities useful for plant metabolism may also be hindered due to heavy metal interference with activities of soil microorganisms. The uptake soil nutrients (essential and non-essential) take place through the same mechanism in plant [9]. The anatomical trait observed plant parts may possibly due to the toxicity effects of heavy metals. The effect of heavy metal toxicity on the growth of plants varies according to the particular heavy metal involved in the process. The deterioration in vascular bundles of plant species grown in NPK amendment and control could be pointed to the stress effects. The stress was caused by increase concentration of NPK together-with toxic nature of heavy metals. This agrees with [2] who reported that induced stress owing from heavy metals and NPK fertilizer applied above the recommended doses possibly could have altered the shape and size of vascular bundle. The test plants grown in organic soil amendment showed a related anatomical structure as those in double control. Furthermore, result showed a loss of shape in shoots and roots of *Cyperus iria* and *Echinochloa colona* grown in NPK amendment and control. This variation observed is possible since heavy metals have been known to induced stress in plant biomolecules. This result is in line with [4] who reported that stress induced by heavy metal is capable of damaging plant biomolecules and could also cause a decrease in conducting tissue.

5. Conclusion

The research tested the effects of NPK (20:20:20), powder orange and plantain peels (waste) in addressing the pitfalls in structural impact of heavy metals on plants. The studied plant responded to amendments differently in terms of heavy metals impact on plants plant anatomy. The concept behind using orange peel and plantain peel (waste), was to increase the mineral content of soil, enhance plants growth performance and tolerant ability of the test plants. Conclusively, orange and plantain peel amendments were able to cushion the negative impact of heavy metals on plant anatomical structure.

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