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Research Article

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# Assessment of heavy metal uptake ability of *Echinochloa colona* in orange peel amended soil.

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## Abstract

This research work focuses on the assessment of heavy metal uptake of *Echinochloa colona* in orange peel amended soil.This research was carried out at the Centre for Ecological Studies, University of Port Harcourt. This centre is located in the Niger Delta area of Nigeria on geographical coordinates: Latitude 4.90428$^o$N and Longitude 6.92297$^o$E. Weighing balance (Setra 480S, USA) was use to weigh two (2) kilograms of the homogenized soil into planting bags of height 18cm, diameter 14cm and with a surface area of 0.0985m$^2$. The planting bags were arranged in 4 blocks (1,2,3,4) and the unpolluted soil was filled into block 5 with 12 replications each. 100g, 200g and 300g of orange peel waste was added to block 1,2 and 3 while block 4 and 5 was without orange peel waste amendment. Equal height and weight of *Echinochloa colona* from nursery was transplanted into all blocks. *Echinochloa colona* and its corresponding soil samples were analysed at two-month interval for Cd and Pb content using anatomical absorption spectrophotometry (AAS). Pb accumulation was higher in 100g 200g, 300g and orange peel amended soil. While Cd accumulation was highest in 100g orange peel waste. All concentration of amendment added were effective in enhancing the availability, mobility and uptake of Cd and Pb from below ground level to the harvestable tissue with TF > 1.Orange peel waste functioned efficiently in enhancing metal availability into solution phase. The study therefore recommends all levels of orange peel waste as an amendment in addressing metal accumulation problems.

**Keywords:** *Echinochloa colona*, heavy metal, Orange peel waste, Cd and Pb.

## 1. Introduction

Environment is considered as the comprehensive influencer of all biotic factors such as organisms, food and its interactions together with abiotic factors which include soil, air, water, climate etc on individual species, population or community affecting its growth and survival. Living organisms in response to changes in their micro, macro, physical and biotic environment is known as adaptation. The final appearance of everything present outside and organisms is known as the environment. The concept of environment has its origin before first human ancestors. The observation of the environment was carried by Babylonia and Egyptians basically for planting of crops and religions reasons. From creation, plants were design to grow in their natural habitats playing a specific role in nutrient circulation in the environment. The concepts of weeds (as an unwanted plants) came with man's perturbation of the stable natural environment which has resulted to successional shift. Man's eagerness in satisfying his needs (which are insatiable)has modified the environment to meet his agricultural, industrialization and aesthetic needs. Man's disturbance of the natural vegetation has resulted in the addition or increase of certain harmful materials into the environments. The contamination of the natural vegetation with pollutants resulting from human activities such as crude oil, pesticides and heavy metals which have much corollary on the environment appear to be a multidimensional problem. For example, some heavy metals such as Cd, Pb and Zn are not only detrimental effects on natural vegetation but also pose a threat to human’s health (Gill *et al*., 2000). Cd toxicity has been found to reduction the growth and development of plants by influencing their net photosynthetic rate and $CO_2$ concentration. Although, some of these metals are essential to plants as a trace element in the enhancement of its performance but detrimental at high concentrations. High concentrations of heavy metal have been found to interfere with basic plant physiological and biochemical processes like respiration, respiration and regeneration of plants cells which can lead to death of plants when inhibited. Metal toxicity problem is not pronounced in natural soils with its original vegetation. Resident plants with time accumulate particular heavy metal in the environment. These metals become toxic to plants in excess (Nagajyoti*et al*., 2010). However, the most available heavy metals are those in solution phase. These heavy metals in solution phase are in soluble components that are solubilized by plant root exudates (Blaylock and Huang, 2000). Plants need certain heavy metals for metabolic function, but these metals become noxious to plant at certain threshold (Garbisu and Alkorta, 2001). The uptake soil nutrients (essential and non-essential) takes place through the same mechanism in plant (Djingova and Kuleff, 2000). Metals are not biodegreadable like hydrocarbons, they bioaccumulate within plant tissue. Plants have various mechanisms to tolerant and withstand harsh environmental conditions (Garbisu and Alkorta, 2001).

## The tolerance mechanisms of plant in extreme heavy metal toxicity include:

1. Reactive Oxygen Species scavenger’s production
2. Displacement of ions
3. Preventing heavy metal transport in aerial parts (Clemens, 2006).
4. Storage of metals in non-sensitive parts

The mercurial and multidimensional problem of heavy metal today is due to its major characteristic of not been biodegradable which makes them dangerous and toxic, they can only bioaccumulate and biomagnified along food chain. The toxic nature of heavy metals and the associated environmental risk has made it mandatory to apply some remedial measures in reducing its concentration and toxicity in the natural vegetation. Numerous techniques have been adapted for the cleaning of heavy metal polluted site which rate from conventional to non- conventional methods. The conventional remedial method is always based on civil engineering method. This method depend heavy on expensive sophisticated equipment for operation and its application is not environmentally friendly since it disrupts the natural vegetation such as plants, animals and microorganisms. A certain eco-friendly approach has been proven to be a viable, inexpensive and easy to use method in the remediation of polluted environment. This easy to use method is known as bioremediation which involve the use of living things, with plant it is known as phytoremediation. Phytoremediation involves the growing of higher plants for the *in-situ* reduction of contaminant in soil or water. It uses approaches such as Phyto stabilization, phytodegradation, phytovolatilization, rhizofiltration and phytoextraction to decontaminate polluted environment. Phytoextraction has been known to be the most effective and affordable technological solution used to remove metal pollutants from contaminated soil without affecting soil texture and structure. (Salt and Kramer, 1997; Ghosh and Singh 2005).

## Ecology of *Echinochloacolona*

This plant species is commonly known as jungle-rice. It is an erect annual weed rooting at the nodes, and can be reproduce from seed. With 60cm high the stem is a greenish round and densely tufted at its base. The leaves are linear about 30 cm long and 7mm wide. The inflorescence is a raceme showing various colour with ascending branches that bear closely crowded ovate spikelets.

**Habit:**

It is a common weed of rice farm, this plant species grows in a wide range of soil moisture conditions, ranging from swampy or hydromorphic soils to dry land.

This study aims at investigating the ability of *Echinochloa colona* heavy metal accumulating ability in orange peel amended soil. This choice of the species is based on its abundance and availability in the study area. This result will add to the existing knowledge on plants and the type of amendment that can enhance heavy metal uptake.

## 2. Materials and Methods

This research was carried out at the Centre for Ecological Studies, University of Port Harcourt. This centre is located in the Niger Delta area of Nigeria on geographical coordinates: Latitude 4.90428$^o$N and Longitude 6.92297$^o$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 at its peaks in July and September (Uko and Tamunobereton-Ari, 2013). The climate condition of the area is characterized by temperature range of 36$^o$Cand 45$^o$C for daily and annual range respectively.Soil collected from an abandoned metal scraps dumpsite at Ikoku metals scrap site which is located at the heart of Port Harcourt in Rivers State, Nigeria. The soil sample of depth 0-20cm was excavated with a spade after baseline analysis confirmed high concentration of Pb and Cd present in the excavated soil. The excavated soil was transported to the experimental site.An experimental design that was adopted is a Completely Randomized Design (CRD). After a proper mixing of the excavated soil, it was the dried and sieve through a 2mm wire mesh as to obtain a homogenous soil composition. Weighing balance (Setra 480S, USA) was use to weigh two kilograms of the homogenized soil into 60 planting bags of height 18cm, diameter 14cm and with a surface area of 0.0985m$^2$. The planting bags were arranged in 4 blocks of 12 replications each. The unpolluted soil was filled into block 5 with similar replications.

### 2.1 Sources of material and processing

Land race of sweet orange was acquired from Otutu-Amaumara Ezinihitte Mbaise LGA., Imo State. The sweet orange is popularly known as 'Oromaor Epe' in their native tongue. The 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 ascertain the nutritional value and heavy metals content of the peels (Table 3.1).

Table 3.1: Nutrient and metal of the peels waste used

| S/N | Parameter | Orange peels waste |
|---|---|---|
| 1 | Phosphorus (mg/kg) | 66.51 |
| 2 | Sodium (mg/kg) | 474.85 |
| 3 | Potassium (mg/kg) | 66,285 |
| 4 | Magnesium (mg/kg) | 1208 |
| 5 | Calcium (mg/kg) | 278.70 |
| 6 | Nitrogen % | 0.119 |
| 7 | Ash % | 11.50 |
| 8 | Fe (mg/kg) | 767.7 |
| 9 | Zn (mg/kg) | 13.05 |
| 10 | Pb (mg/kg) | ND |
| 11 | Cd (mg/kg) | ND |
| 12 | pH | 5.56 |

ND = Not detected

Then 100g,200g, 300g of analyzedorange peels in powder form was added intoeach of the block 1,2,3 respectively, while block 4 and 5 acted as control and double control (polluted and unpolluted without amendment), respectively. The treatment was mixed thoroughly in the planting bags to enhance harmonization. The bags were allowed to stand for 3 weeks before seedings of *Echinochloa colona* was transplanted from nursery into block 1,2,3,4 and 5.

## 2.2 Amendment Treatment

The treatment of organic waste was applied as shown:

Block 1 contaminated soil + 100g powder orange peel +*Echinochloa colona*

Block 2 contaminated soil + 200g powder orange peel +*Echinochloa colona*

Block 3 contaminated soil + 300g powder orange peel +*Echinochloa colona*

Block 4 contaminated soil + 0g powder orange peel +*Echinochloa colona*

Block 5uncontaminated soil + 0g powder orange peel +*Echinochloa colona*

Plants species used where carefully selected after ensuring they are of the same size, vigour and age. In other to control some extraneous environmental factors, transparent roofing sheet was done to control natural rain. Watering (50cl/planting bag) was done twice daily with distilled water. The experiment was monitored for 4 months but analyses were done at two-month interval. At every two-month interval, the soil from all the blocks and its replicate were collected, and air-dried for 3 days. *Echinochloa colona* plant species from each block was carefully harvested by uprooting them from the bags. The plants were separated into roots and shoots which were washed to get rid of all adhering soil and air dried. The composite soil samples were air-dried using a bulb for 1 month to constant weights. Two grams of air-dried soil were accurately weighed and passed through (2 mm sieve) and then transferred to 250 ml conical flask. A measured volume of well- mixed hyperchloric acid, nitric acid and sulphuric acid were added into flask containing soil sample in the ratio of 1:2:2 in the fume hood. Heat at about (15-20 mins) in the hot plate till a white fume is observed, stop digestion and cool, 20 ml of distilled water was added after cooling. This was boiled to bring the metal into solution. After cooling filter using whatmann 42 filter paper in a 100 ml volumetric flask and make to mark with distilled water, then transfer to 100 ml plastic can for Atomic Absorption Spectrophotometer Analysis (PG Instrument AA 500 Spectrophotometer).

Plant roots and shoots samples was dried to constant weight. Each dried sample was ground to fine powder using a wearing blender (Model type A 10 Janke and Kunkel GBH a Co. KG). Two grams of each sample was used for analysis. These samples were digested and analysed adopting the same procedure to those for soil.

The plant translocation factor, accumulation factor and bioaccumulation factor were also calculated.

Translocation Factor (TF) is given as the ratio of concentration of metal in the shoot to that in the roots. It was evaluated using the formula according to Cui *et al*. 2007 as follows:

$$TFCd = \frac{c(Cd)shoot}{c(Cd)root} \quad \text{and} \quad TFPb = \frac{c(Pb)shoot}{c(Pb)root}$$

Bioaccumulation factor (BF) was calculated with the formula according to Baker *et al*. (1987) as follows:

$$BFCd = \frac{c(Cd)\ plant}{c(Cd)soil} \quad \text{and} \quad BFPb = \frac{c(Pb)\ plant}{c(Pb)\ soil}$$

### Data Analysis.

The data generated were subjected to statistical analysis of variance (ANOVA) using Statistical Analysis System (SAS,2002). Further validity of differences among treatment means was estimated using the Least Significance Difference (LSD)method. The Cuand Pb transport from soil to plant and from roots to shoots were evaluated using the translocation factor and accumulation factor respectively. The percentage reduction of metals was calculated by the total concentration of elements by the initial status of elements presents in soil.

## 3. Results

Figure 1 and 2 showed the effect of orange peel treatment on the accumulation of Pb in the root and shoot of *Echinochloa colona*. There was a significant decrease in the accumulation of Pb in shoot at 2 months. The highest increase in the accumulation rate of Pb in shoot of *Echinochloa colona* was found in 200g orange peel amendments at 2 months with double control showing the least decrease. 100g and 200g orange peel amendment showed a slight decrease in Pb accumulation rate as compared with 200g orange peel amendment. There was a significant increase in the accumulation of Pb in shoot of *Echinochloa colona* across treatment at month 4. The highest increase was recorded for 100g and 300g orange peel amendment whiledouble control showing the least decrease. There was a significant decrease in the Pb accumulation at month 2 and 4 as shown in Figure 2. The height increase in the presence of Pb in root of *Echinochloa colona* plant species was recorded for control soil at month 2 and 4 while the least was found in 300g orange peel amended soil at month 2 and double control at month 4.

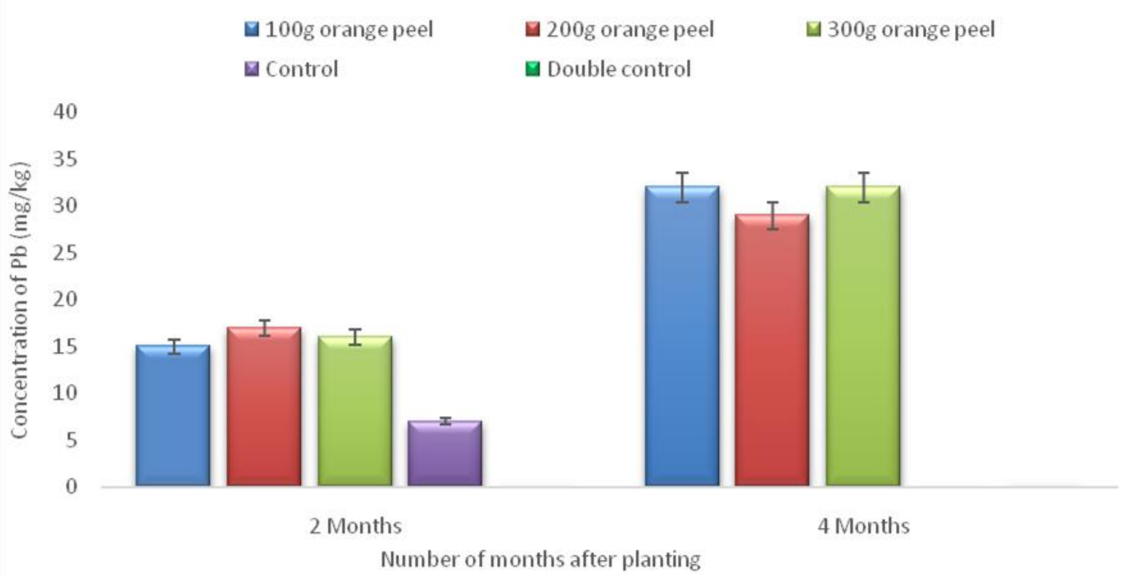


Figure 1. Effects of treatent in the concentration of Pb in *Echinochloa colona* shoot

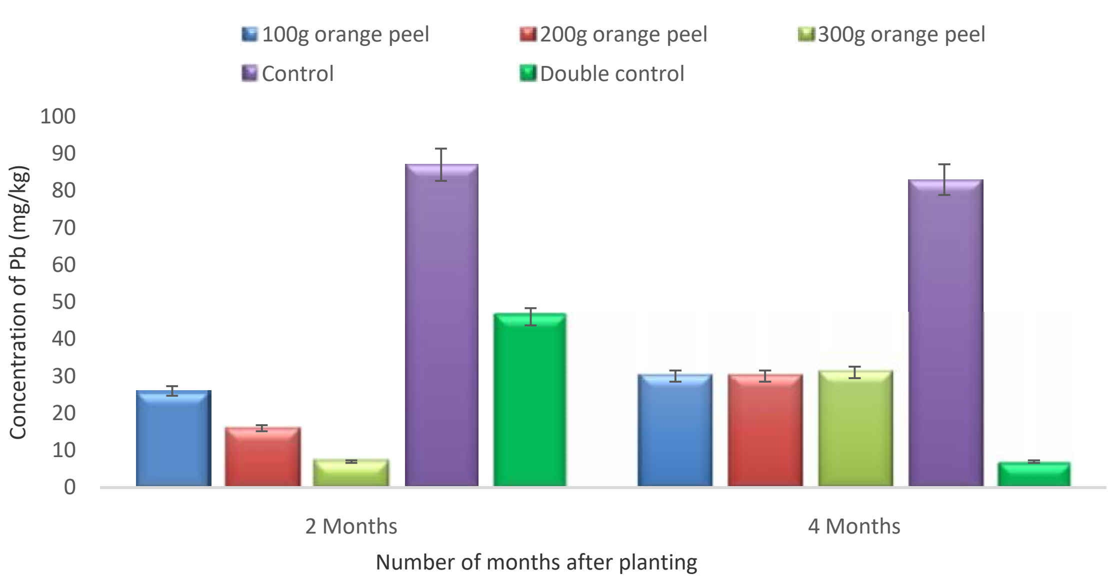


Figure 2. Effects of treatent in the concentration of Pb in *Echinochloa colona* root

Figure 3 and 4 showed the effect of orange peel treatment on the accumulation of Cd in the shoot and shoot of *Echinochloa colona.* There was a significant decrease in the accumulation of Cd in shoot at 2 months. The highest increase in the accumulation rate of Cd in shoot of *Echinochloa colona* was found in 100g orange peel amendments at 2 months with double control showing the least decrease. There was a significant increase in the accumulation of Cd in shoot of *Echinochloa colona* across treatment at month 4. The highest increase was recorded for 300g orange peel amendment while double control showing the least decrease. There was a significant decrease in the Pb accumulation at month 2 as shown in Figure 2. The height increase in the presence of Pb in root of *Echinochloa colona* plant species was recorded for 200g and 300g orange peel amended soil at month 2 while the least was found in double control. It was found that an increase in the Cd concentration was recorded for 100g orange peel amended soil while control and double control showed least decrease.

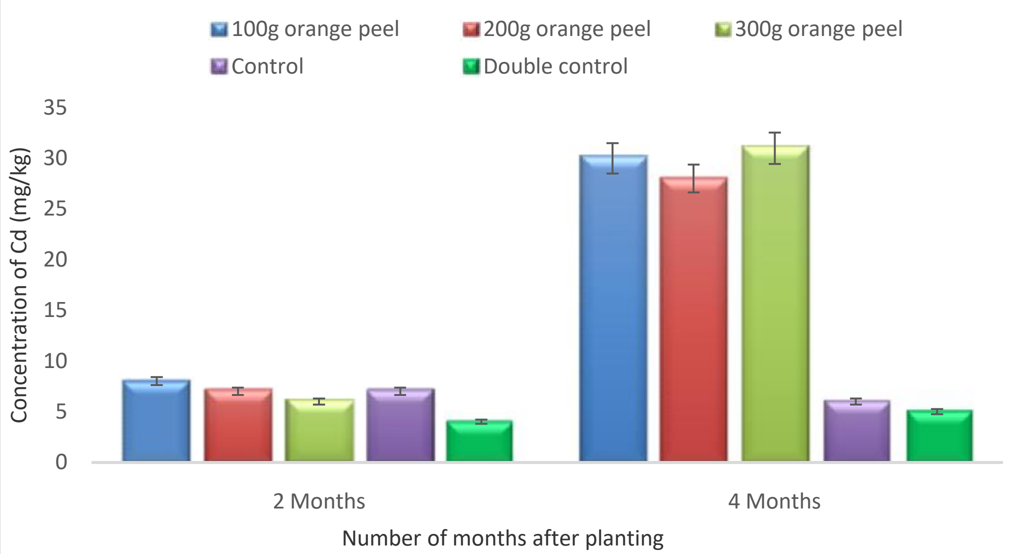


Figure 3. Effects of treatent in the concentration of Cd in *Echinochloa colona* shoot

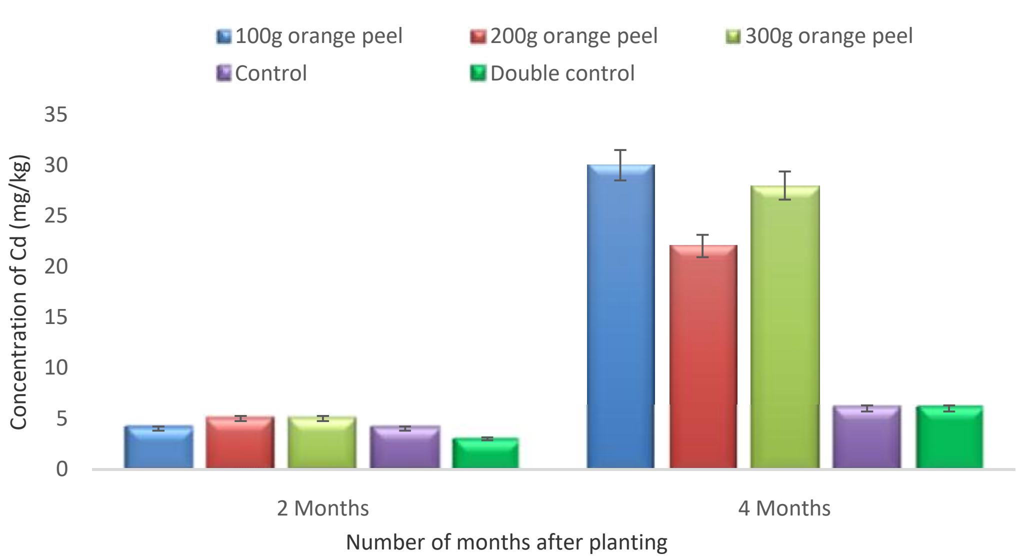


Figure 4. Effects of treatent in the concentration of Cd in *Echinochloa colona* root

**Table 2. The concentration of Pb in soil phytoextracted with *Echinochloa colona* DPR tolerant limit**

| | Baseline | | | | | |
|---|---|---|---|---|---|---|
| **Number of accumulation month** | **polluted** | **unpolluted** | **100g** | **200g** | **300g** | **DPR T.L** |
| **2 months** | 130.5± 2[a] | 16.73±2[a] | 59.4±0.7[a] | 84.3±1[a] | 122±1[a] | 85 |
| **4 months** | 130.5± 2[a] | 16.73±2[a] | 98.1±0.1[b] | 80±0.1[a] | 68±1[b] | 0.8 |

**Table 3. The concentration of Cd in soil phytoextracted with *Echinochloa colona* in comparison with DPR tolerant limit**

| | Baseline | | | | | |
|---|---|---|---|---|---|---|
| **Number of accumulation month** | **polluted** | **unpolluted** | **100g** | **200g** | **300g** | **DPR T.L** |
| **2 months** | 0.8±0.1[b] | 15.3±1[a] | 0.08±0.002[c] | 0.07±0.001[c] | 0.05±0.003[e] | 85 |
| **4 months** | 0.8±0.1[b] | 15.3±1[a] | 0.01±0.0001[g] | 0.03±0.002[e] | 0.02±0.004[f] | 0.8 |

**Table 4. Translocation factor of Pb in *Echinohloa colona***

| | 2 Months | 4 Months |
|---|---|---|
| | TF | TF |
| Orange 100g | 0.58 | 1.08 |
| Orange 200g | 1.07 | 1.06 |
| Orange 300g | 2.13 | 1.04 |
| Polluted | 0.008 | 0.002 |
| Unpolluted | 0.002 | 0.02 |

**Table 5. Translocation factor of Cd in *Echinohloa colona***

| | 2 Months | 4 Months |
|---|---|---|
| | TF | TF |
| Orange 100g | 1.70 | 1.01 |
| Orange 200g | 1.39 | 1.25 |
| Orange 300g | 1.13 | 1.06 |
| Polluted | 0.89 | 0.8 |
| Unpolluted | 0.72 | 0.93 |

## 4. Discussion

This study showed that plants stands a better chance in addressing issues of increasing heavy metal contamination which has become frequent. The bioavailability of metal and its uptake is governed by numerous factors. The most important factor happens to be the total metal concentration, organic meter, redox conditions and pH, while the uptake mechanism is based on metal concentration, its availability and plant associated factors (internal factor). Result showed that orange peel waste as an organic amendment influences the bioavailability ofheavy metals (Pb and Cd) and also its extraction by plants. The variation found between the phytoextraction and accumulation of the studied metal in response to plants uptake can be attribution to the organic amendment used which may have increased the organic matter of the soil. This finding is in agreement with the report of McGrath et al (1992) that organic matter content of a soil an important factor influencing availability of metals. The variation in heavy metal accumulation of *Echinohloa colona* grown in 200g, 300g for Pb and 100g for Cd in orange peel waste amended soil, could be attributed to the inherent ability of plants to response to perturbations emanating from the environment. This finding also corroborated with the report of Salt *at al*. (1995) who had reported that the uptake and accumulation of metals by plants and its parts was plant species dependent. The concept of translocation factor is a visible factor that explains the ability of plant or the effectiveness of amendment in phytoremediation. Translocation factors tells if a given plant can be classified as hyperaccumulator or not. It makes known the effectiveness of plants and its corresponding amendment's in facilitating the movement of metals from root to shoot.TF >1 indicates that metals are no longer concentrated in the soil/ root arena but in the harvestable parts.It was observed9 that orange peel waste at various levels increased the availability and accumulation of Cd and Pb since the translocation value >1 was recorded.This result agrees with the findings of Clement *et al.* (2002) who stated that plant species posses the capability to mobilize metals from soil in its solution phase and translocate it to its harvestable part.

## 5. Conclusion

Results obtained showed the effectiveness of orange peel waste in enhancing metal availability into solution phase. This can will help address the problems of environmental pollution emanating from addition of soil amendments such as EDTA to enhance the mobility of heavy metal into solution phase. The study therefore recommends all levels of orange peel waste can enhance the efficacy of plant species in metal accumulation. Plants has the tendency of becoming a hyperaccumulator when planted in an ideal environment.

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