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RESEARCH ARTICLE

Comparative Study of Two organic amendments for their biostimulating potential in Heavy Metal Polluted Soil

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This research was carried out in collaboration among all authors. Both Authors read and approved the final manuscript

ABSTRACT
Decrease in soil nutrient resulting from heavy metal pollution is one of the major challenges inhibiting phytoextraction processes. This research work compared the effectiveness of two biostimulants in a metal polluted soil. Randomized Complete Block Design (RCBD) was adopted, 2kg weight of polluted soil in bags were arranged into two groups of 4 sets (T1, T2, T3, and T4) with reference soil designated as T5. The Groups1and 2 were treated with different grams of orange peel (waste) and yam peels (waste) respectively, while T4 and T5 with 0g of orange peels waste stand as control and double control and the test plants were transplanted from nursery to all bags and monitored for 8weeks. Highest accumulation of Cd and Pb was in 300g of orange treatment as 23 mg/kg and 224 mg/kg respectively while with yam of similar concentration also increased Cd uptake as 21 mg/kg and 138 mg/kg for Pb. The accumulation factor > 1 for Pb and Cd in orange peel treated soil were in order: Pb: T1 (3.4) > T2 (2.32) > T3 (1.21) Cd: T1& T3 (17.0) > T2 (16.1) and accumulation factor > 1 for Pb and Cd in yam peel treated soil were in order: Pb: T1 (1.8) > T2 (1.26) > T3 (1.02) Cd: T3 (15.0) > T2 (14.2) > T3 (8.0) > T4 (6.03) > T5 (1.12). Biostimulants addition of various concentrations enhanced soil nutrient level, heavy metal availability and mobility. Since the accumulation factor were > 1 in all biostimulated soil, it confirms the effectiveness of orange and yam peel waste as a good bio stimulating agent in Pb and Cd phytoextraction processes. Therefore, the use of orange and yam peels waste as biostimulants should be harnessed for the remediation of Cd and Pb impacted soil.

Key Words: Biostimulants, heavy metal, Phytoextraction, *Phyllanthus amarus*

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1.1. INTRODUCTION

In the realm of modern agricultural demands, the quest of an efficient and sustainable crop production method has been increasingly paramount and of great necessity (Hazrat *et al*., 2019). Increase in population growth in the world required a deliberate attempt to enhance agricultural activities and other industrial processes in other to sustain the growing population with minimal environmental impact. An estimate of 95% of total human activities are done on the environment and as such environmental stability is hampered after such activities especially in developing countries of the world where environmental issues are yet to take-over the daily news headlines (Hazrat *et al*., 2019). Human activities such as crude oil exploitation and their uses, associated with effluent discharges and indiscriminate waste disposal as well as, bush burning deposit certain harmful materials into the environment (Giller *et al*. 1998). The frequency of this environmental perturbation is overwhelming and is now beyond the carry capacity of the environment, most of these pollutant released are recalcitrant in the environment, particularly of inorganic component such as heavy metals which pose threat to ecosystem structure (Amadi *et al*., 2022). Heavy metals especially Pb and Cd have been reported to have not only detrimental effects on ecosystem functioning but also pose a potential health risk (*Giller et al.*, 1998). Heavy metal inhibit optimal plant performance with its effect shown in morphological reduction in root and shoot length; it also has a greater impact on plant physiological and biochemical processes such as photosynthesis, respiration and degradation of main cell organelles, thus can led to death due to increasing reactive oxygen species (ROS) (stress triggers) (Amadi *et al* 2023; Schwartz *et al* 2003). It is thus, imperative to apply some remedial measures to reduce or remove heavy metals toxicity levels. Man is in daily search for an ecofriendly methods that will help decrease the impact of heavy metals on plants and also useful in restoration of soil physicochemical properties hence the quest for biostimulants (Amadi *et al*. 2017). Biostimulants have emerged as a promising solution in decreasing heavy metal impact on the ecosystem in light of its' efficacy in soil nutrient restoration, promote plant growth, improve crop resilience and optimize general plant performance (Amadi *et al*. 2017). Biostimulants are biodegradable materials capable of stimulating natural processes within the plant for optimal performance by enhancing plant growth, nutrient uptake, stress tolerance (Amadi *et al* 2023). Organic biostimulated have been reported to contain essential nutrient and microbial population than inorganic (chemical biostimulants) (Amadi *et al*., 2018). These organic biostimulants are readily available and obtained from plant extracts, orange, plantain peels waste, plant and animal waste (poultry and cow dung). This study attempts to investigate the efficacy of two organic biostimulants in optimization of plant general performance in heavy metal polluted soil. Results obtained will broaden the knowledge on the role of biostimulants in phytoextraction mechanisms.

2.0. MATERIALS AND METHODS

2.1 *Experimental Design & Treatment Application*

This study was carried out at the Ecological Unit, Rivers State University. It is located in the Niger Delta area of Nigeria. Randomized Complete Block Design (RCBD) was used for the experiment. Heavy metal polluted soil sample was obtained from an abandoned metal scrap site at 0-20 cm depth using a spade and the reference soil was obtain at a fallow farm land at Rivers State University. The total soil was mixed thoroughly, dried and sieved through a 2 mm wire mesh to obtain a homogenous 'fine fraction' of soil composites. A baseline analysis was carried out. Two (2) kilograms of homogenous soil composite was weighed into polythene bags using a weighing balance (Setra 480S, USA).The bags were arranged into two groups of 4 sets (T1, T2, T3, and T4) along with reference soil designated as T5. Landraces of sweet orange and yam acquired from Otutu-Amaumara Imo State and Taraba State respectively were removed mechanically by hand peeling. Their peel (waste) were dried and processed into powder form and a given measurement was used as treatment amendments. Group1: contains 300g, 200g and 100g of orange peel (waste) for set T1, T2, T3 respectively, while T4 and T5 with 0g of orange peels waste stand as polluted control and unpolluted control (reference soil). This amendment application process was also repeated for Group 2 using yam peel waste. Two seedlings (identical size and vigour) of the test (phytoremediation) plant which was raised in the nursery and properly identified at the University of Port Harcourt Herbarium as *Phyllanthus amarus* was planted in each sets belonging to group 1 and 2. The experimental site was devoid of natural rain by shedding. Watering was done three times a week. The quantity of water used for watering the planting bags was 50 cl per bag. Weeding was done when the need arose.

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Table1: Nutrient and heavy metal composition and concentration of the biostimulants.

S/N	Parameter	Orange peel	Yam peel
1	Phosphorus (mg/kg)	66.51	58.5
2	Nitrate (mg/kg)	64	45
3	Potassium (mg/kg)	145	345
4	Magnesium (mg/kg)	12.05	11
5	Calcium (mg/kg)	279	8
6	Pb (mg/kg)	Nil	Nil
7	Cd (mg/kg)	Nil	Nil
8	pH	5.56	5.3
9	Conductivity	9.2	8.6

Table 2: Chemical content of soil samples

S/N	Physicochemical Ppt	Normal soil	Metal soil
1	pH	5.2	8.49
2	Conductivity (µsCm^{-1})	65	1187
5	Lead (mg/kg)	138.6	234.6
6	Cadmium (mg/kg)	1.0	19.01
7	Nitrate (mg/kg)	75.6	145
8	Phosphorus (mg/kg)	1.0	0.12
9	Calcium (mg/kg)	110	120
10	Magnesium (mg/kg)	210	232
11	Potassium (mg/kg)	65	23

2.2 *Determination of Parameters*

The Pb and Cd content in soil and plant material, as well as soil nutrients' contents were analyzed after 8 weeks of cultivation. The plants were carefully harvested from the bags from each treatment dipped into a bucket full of water to remove soil particles. All collected samples were labelled in accordance with treatment application and were taken to the laboratory to test for concentrations of metals and physicochemical properties (pH, conductivity, potassium, nitrate, magnesium, calcium and soil organic matter) in plant and soil. Plant sample was first rinsed with distilled water and oven dried at 100°C for 48 hours. The dried plant sample was ground to fine powder before digestion and analyzed for Pb and Cd concentration using BUCK scientific 200A model of Atomic Absorption Spectrophotometer (AAS). The soil was also dried and sieved through a 2mm sieve to remove coarse soil particles before analysis. The determination of soil nutrient and heavy metals was by Spectropotometer (AAS) (BUCK scientific 200A model) after digestion after digestion on a hot plate for 20 minutes with 1:2:2 ratio of perchloric, nitric and sulphuric acids (API-RP-45, 2005).

The soil phosphate determination was by UV visible spectrophotometer after adding 40 ml of Olsen's extracting solution to 2 grams of soil which was then filtered using Whatman paper with 4 ml of B-reagent 10 minutes (APHA, 2004).

Soil nitrate was determined using UV visible spectrophotometer after adding 20 ml sodium acetate to 5 grams which was then wobbled for one (1) minute and allowed to stand for 5 minutes (ASTM, 2004).

Soil sulphate was determined using UV visible spectrophotometer by adding 250 ml of KH_2PO_4, and shaking for 30 minutes and allowed to stand for 5 minutes (APHA, 2004). Soil chloride was titrated with $AgNO_3$ solution (APHA, 2004).

The soil pH and conductivity were determined electronically using a glass electrode pH metre and conductivity metre (HANNA HI Series), respectively.

Soil organic matter (SOM) determination was by oven dry method. The oven dried soil with crucibles were transferred into a muffle furnace after weighing for 3 hours at the temperature of 400°C , Then, the crucible was removed from the furnace and weighed again, that is (burned soil + crucible) - crucible to obtain weight of burned soil alone. Based on these data, the following parameters were calculated:

Inorganic soil fraction percentage = (Burned soil/dry soil) x 100%.

Organic soil fraction percentage = 100% - soil fraction percentage.

Accumulator factor (AF) was calculated with the formula according to Baker (1981) as:

$$AF = \frac{c(Pb)plant}{c(Pb)soil}$$

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2.3 *Data Analysis*

The data generated were subjected to statistical analysis of variance (ANOVA) using Statistical Analysis System (2002). Further validity of differences among treatment means was estimated using Least Significance Difference (LSD) method

3.0. RESULTS AND DISCUSSION

Table 3: Showed the rate of cadmium accumulation in biomass of *Phyllanthus amarus* grown in the test soils. The rate of cadmium uptake by plant varies with treatment and concentrations applied. There was significant difference in Cd accumulation rate between and within treatments at (p=0.05). Highest increment in cd accumulation was found in plant biomass grown in 300g and 200g of soil amended with orange peels and yam waste respectively (T1). While the least in cadmium uptake was recorded for plant species grown in 0g treated (orange and yam peels waste) polluted control soil designated as (T4). However, heavy metal uptake mechanisms were also noticed with Pb accumulation in orange and yam peel treated soil (Table 4). The highest in Pb accumulation was recorded for plant grown in 300g amended soil with orange and yam peels. Least decrease in Pb accumulation was control untreated soil. There was significant differences between and within treatments at (P=0.05).

Result showed that treatment application was able to optimize plant tolerance ability and increase in heavy metal availability in solution phase in readiness for uptake. This findings is line since increase in organic matter content influence metal availability and absorption mechanisms. This assertion agrees with McGrath *et al*.(1992) who worked on the efficacy of organic matter soil to heavy metal availability. This result also agrees with findings of Ho *et al*., (2008) who reported that biostimulants are capable of promoting biomass yield results in bioaccumulation of heavy metals in individual plants. It was concluded that organic matter is an important soil factor influencing heavy metal availability in solution phase. The uptake of metals in study plant was higher in orange peel treated soil of various concentrations than yam peel treated soil. This observation could be attributed to the concentration of orange peel organic biostimulants added. Orange peels contains high level of citric acid often reported as low molecular weight organic acid which is able to acidify the rhizosphere hence triggers heavy metal mobility and availability. The least decrease in heavy metal availability and uptake in plants could be as a result of the toxic nature of the test soils decrease in organic matter level and essential nutrient required to enhance plant growth. This assertion corroborated with the report of Amadi *et al*. (2023), explained how the impact of pollution on soil physicochemical properties (depletion of soil nutrient, soil water content and decrease in microbial activities) and its inhibiting techniques on optimal plant performance (morphological reduction in root and shoot length, physiological and biochemical processes) can be improved with biostimulants addition.

Table 3: Cd accumulation in plant biomass grown in different concentration of biostimulants

S/N	Treatments	T1	T2	T3	T4	T5
1	Orange peels waste	23±0.1^{a}	16.0±0.06^{b}	17.0±0.04^{b}	5.10±0.08^{bc}	1.0±0.001^{c}
2	Yam peel waste	21±0.01^{a}	14.2± 0.01^{b}	15.0±0.04^{b}	6.03±0.01^{c}	1.12±0.001^{d}

*accumulation unit is in mg/kg

Table 4: Pb accumulation in plant biomass grown in different concentration of biostimulants

S/N	Treatments	T1	T2	T3	T4	T5
1	Orange peels waste	224±5.0^{a}	221±5^{a}	115.2±1.0^{b}	65±0.5^{c}	6±0.001^{d}
2	Yam peel waste	138±2.0^{a}	120±2.0^{b}	98.1±0.1^{c}	92.01±0.1^{c}	6.5±0.02^{d}

*accumulation unit is in mg/kg

The addition of various concentrations of amendments improved soil fertility. There was an improvement in nutrient concentration in amended soil compared to control soil. There was a significant difference in the effects of amendments on soil nutrient quantity between and within treatments at (p=0.05). The variation in soil chemical properties in orange peels amended soil is presented as: SOM: >T2<T4; P:>T3<T4; K:>T3<T4; N:>T2<T4; pH:>T4<T3; Cond: >T4<T5; Pb:>T4<T5; Cd:>T4<T5. Strong negative correlation was recorded between SOM, K, against Cd while strong positive correlation was recorded between pH and EC against Pb and Cd

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	SOM	P	K	N	PH	EC	PB	CD
SOM	1	0.048352	0.2308	0.021393	0.25906	0.29865	0.57088	0.25586
P	0.88106***	1	0.14469	0.22528	0.40259	0.51119*	0.8650***	0.43145
K	0.65443**	0.74953***	1	0.47565	0.055941	0.10724	0.2566	0.081101
N	0.9313***	0.66018**	0.42499	1	0.3226	0.31646	0.51453	0.28807
PH	-0.62554**	-0.48953	-0.8687***	-0.56345*	1	0.001038	0.028437	0.000431
EC	-0.58645*	-0.39439	-0.7959***	-0.56931*	0.99091***	1	0.011234	0.000778
PB	-0.34394	-0.10616	-0.62803**	-0.39154	0.91682***	0.9553***	1	0.023762
CD	-0.62877**	-0.46374	-0.8312***	-0.59675*	0.99494***	0.9925***	0.9262***	1

*****Correlation is significant at 0.01 and level *Correction is significant at 0.05 level**

The variation in soil chemical properties in yam peels amended soil is presented as: SOM: >T2<T4; P:>T1<T4; K:>T3<T4; N:>T1<T4; pH:>T4<T3; Cond: >T4<T5; Pb:>T4<T5; Cd:>T4<T5.

	SOM	P	K	N	PH	EC	PB	CD
SOM	1	0.11183	0.49285	0.23467	0.23288	0.37925	0.61406**	0.25967
P	0.78997***	1	0.51679	0.030614	0.22692	0.51573	0.66521**	0.21191
K	0.41012	0.3896	1	0.20941	0.016233	0.0032543	0.0011734	0.016396
N	0.65042**	0.9129***	0.67695**	1	0.06578	0.25616	0.30961	0.054286
PH	-0.6522**	-0.6584**	-0.9429***	-0.85355***	1	0.019777	0.044221	0.00025961
EC	-0.5106	-0.3905	-0.9805***	-0.62846**	0.93482***	1	0.0055892	0.022574
PB	-0.3080	-0.2661	-0.9901***	-0.57587	0.88802***	0.97204***	1	0.043658
CD	-0.6249**	-0.6742**	-0.9425***	-0.87139***	0.9964***	0.92877***	0.88898***	1

*****Correlation is significant at 0.01 and level *Correction is significant at 0.05 level**

Strong negative correlation was recorded between SOM, K, P, and N against Cd, this was also recorded for K against Pb while strong positive correlation was recorded between pH and EC against Pb and Cd.

Soil physicochemical properties were optimized with nutrient amendments as compared with control. This increase in nutrient levels could be attributed to their concentration in the peels used as biostimulants. The decomposition of peels waste in organic matter released nutrients in the peels and decrease in pH level enhanced it availability in solution phase pH is one of the factors which influence the bioavailability and transport of heavy metals in the soil. Sauve *et al*. (1997) observed a connection between soil pH and metal speciation, solubility and adsorption. Metal uptake has been reported to increase due to decreasing pH (Brown *et al.* 1994; Amadi *et al*. 2018). As soil pH decreases, metals compete with the extra H+ for positions on the exchange sites thereby making the metals to be soluble and available as free metal ions in the soil solution.

Table 5: Effects of orange peel waste biostimulants on soil physicochemical properties

Tn	SOM %	Phosphorus Mg/kg	Potassium Mg/kg	Nitrate Mg/kg	pH	Cond	Pb Mg/kg	Cd Mg/kg
T1	32±0.1^{b}	6.2±0.02^{b}	67.3±1.0^{b}	65±3.0^{b}	5.6±0.01^{b}	231±5.2^{b}	92.5±3.0b	0.03±0.001^{a}
T2	46±0.1^{a}	6.6±0.01^{b}	55.7±2.1c	102±6^{a}	5.61±0.1^{b}	143±9^{bc}	86.0±0.2bc	0.04±0.001^{a}
T3	34±0.01^{b}	7.3±0.03^{a}	82.3±0.4^{d}	55±3.0^{c}	5.3±0.01^{b}	143±9.7b^{c}	88.1±0.2bc	0.04±0.001^{a}
T4	12±0.001^{d}	0.3±0.001^{c}	20±0.33^{c}	30±0.2^{d}	8.4±0.03^{a}	1181±10^{a}	200.3±3a	1±0.002^{a}
T5	16±0.02^{c}	1.1±0.001^{c}	48.5±2.0^{a}	45±0.1c^{d}	5.5±0.01^{b}	61±0.03d	34±0.002d	0.02±0.0001^{a}

Table 6: Effects of yam peel waste biostimulants on soil physicochemical properties

Tn	SOM %	Phosphorus Mg/kg	Potassium Mg/kg	Nitrate Mg/kg	pH	Cond	Pb Mg/kg	Cd Mg/kg
Ti	21±0.02^{b}	7.2±0.03a	71.1±4.0a	62±3.02a	5.6±0.02b	512±10b	100.2±7b	0.02±0.003a
T2	32±3.0^{a}	7.1±0.01a	71±4.0a	55±4.0b	5.6±0.02b	289.2±8c	95.0±4b	0.02±0.001a
T3	30±3.0a	6.5±0.003ab	72.1±0.04a	54±3.4b	5.3±0.002b	287±8c	95.5±4b	0.01±0.001a
T4	12±0.1c	0.3±0.002d	52±2.0b	30±3.3cd	8.4±0.01a	1181±19a	200.3±12a	1±0.001a
T5	16±0.4c	1.1±0.001c	78.5±4.0a	45±4.3c	5.5±0.002b	61±0.2d	34±0.23c	0.02±0.001a

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Accumulation factor (AF) is a measure of the ability of plants to bio accumulate metals from soil to its biomass. In table 7 & 8 showed that the highest increment in Cd and Pb AF rate was recorded for plants in treated soil of various concentrations. The accumulation factor > 1 for Pb and Cd in orange peel treated soil were in order: Pb: T1 (3.4) > T2 (2.32) > T3 (1.21) Cd: T1& T3 (17.0) > T2 (16.1) and accumulation factor > 1 for Pb and Cd in yam peel treated soil were in order: Pb: T1 (1.8) > T2 (1.26) > T3 (1.02) Cd: T3 (15.0) > T2 (14.2) > T3 (8.0) > T4 (6.03) > T5 (1.12). The highest increment in the concentration of AF could be attributed to the peels used. The pH concentration in the peel is slightly acidic and this may have increased the acidity of the soil hence increase the availability and mobility of metals in plants. A strong positive correlation of r-0.880 and r-0.996 for Pb and Cd respectively showed the influence of pH in the availability and mobility of nutrient. This finding agrees with McBride *et al* (1997); Khoshgoflarmanesh and Kalbasi (2002) who conducted an experiment on the effects of biostimulants on the availability of nutrient and it was concluded that decrease in soil pH can influence the availability and mobility of soil nutrient. Results also showed that the availability of soil nutrients varies among treatment and concentrations applied.

Table 7: Accumulation factor of plant in Pb biostimulated soils.

Tn	Orange amended soil	Yam amended soil
T1	3.4	1.8
T2	2.326316	1.263158
T3	1.206283	1.027225
T4	0.324513	0.459361
T5	0.176471	0.191176

Table 8: Accumulation factor of plant in Cd biostimulated soils.

Tn	Orange amended soil	Yam amended soil
T1	17	8
T2	16.09	14.02
T3	17.0	15.0
T4	5.01	6.03
T5	1.02	1.12

CONCLUSION

The results obtained from this experimental research shows that application of different concentrations of biostimulants improved soil nutrient quantity and quality and it also influenced heavy metal availability and mobility. Since the accumulation factor were > 1 in all biostimulated soil indicates the effectiveness of orange and yam peel waste as a good bio stimulating agent in Pb and Cd phytoextraction processes. Therefore, between the two bio-stimulators orange peel waste of various concentrations triggered the highest increment in soil metal availability and mobility in plants hence its potential should be harnessed for the remediation of Cd and Pb impacted soil.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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