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Biostimulating effect of plantain peels (waste) on soil chemical properties, microbial loads and growth of *Cyperus iria*(Linn)in a heavy metal polluted soil

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Abstract

This study investigated the effects of plantain peel (waste) as a biostimulant on soil chemical properties, microbial population and growth of *Cyperus iria* in a heavy metal polluted soil. The research was conducted at the Center for Ecological Studies, University of Port Harcourt. Two (2) kilograms of homogenous heavy metal soil was weighed into planting bags arranged in 4 treatments (T1, T2, T3, and T4) along with non-polluted soil (T5) of 6 replications each. Pulverized plantain peels of 100g, 200g and 300g were added as bio-stimulant into T1, T2, T3 respectively, while T4 and T5 with 0g bio-stimulant stand as controlI (polluted) and control II (non-polluted). Seedlings of *Cyperus iria* were transplanted into all treatments. At 60Day after planting (DAP), soil chemical properties, microbial population and plant growth morphology of the test plant were assessed. Results showed an increase in soil chemical properties (Ca^{2+}, P, K, and N), and soil organic matter and plant morphological characters in bio-stimulated soil as compared to the control I and control II. Increase in plant height at 60 DAP were as follows; 34.3 < 50.3 <51 < 58.7 < 62.2 for control I (T4), T1, T5 (control II),T2and T3 respectively.The probable fungal isolates showed that *Aspergillus sp* was abundance in T3 while *Penicillium sp*, *Rhizopus sp*, *Tritirachium sp* and *Cladosporium sp* were highest in occurrence in T1. This indicated that plantain peels addition as a bio-stimulant is effective in restoring soil nutrient deficits, enhancing microbial growth and promote the growth of *Cyperus iria* in heavy metal polluted soil.

Keywords: soil nutrient, heavy metal, microbial activity, plant morphology, biostimulation, fungal isolates

1. Introduction

Centuries ago, human interference on the environment was insignificant. The environment as a self-sufficient system was highly stable (nature was dominant). Uncontrolled increase in human population and industrialization have resulted in pollution of the environment (Ramesh *et al.*, 2013; Babagana *et al.*, 2015). Pollution problems are felt locally as well as globally. Presently, significant efforts have been put forward by environmental scientists, NGO's, government at all levels and others in minimizing the noxious side-effect of pollution on human health by decontaminating the chief components of the environment (Ramesh *et al.*, 2013).In reality, fertilization of soil appears to be effective in the restoration of soil nutrients which has helped in improving plant growth. However, regular application of chemical fertilizer will increase its concentration in environment and with time it causes soil toxicity (Khan *et al.* 2018). The toxicity nature of excessive use of fertilizer contaminates surface and groundwater. In addition, some chemicals present in fertilizer (such as urea, phosphate etc.) pose a risk on human health through accumulation in food chain. The use of various arrays of chemical fertilizer can also influenced negatively on soil nutrients balance and subsequent uptake by plant. Soil physical, chemical and biological composition are disrupted adversely (Korschens *et al*. 2013; Wang *et al*. 2020). Excessive use of nitrogen fertilizers impaired the optimal growth of microbial abundance and some essential biogeochemical cycle (Bell *et al* .2015; Zhang, 2020). Fertility of a soil is categorized on the bases of microbial population and the stability of other relevant soil characteristics. On this premise, addition of biostimulants is seen as the most effective and efficient ways of improving soil composition (Randeep, 2021). Biostimulation is an aspect of bioremediation techniques which involves the addition of biodegradable substance to soil which help improve plant nutrient stability and soil components (Du Jardin, 2012; Randeep, 2021). The use of biostimulants to facilitate soil potentiality appear to be more effective in improving soil stability at any concentration. Most biostimulants improve plants optimal performance especially in a polluted soil with depleted nutrients. Plant-based benefit of biostimulant is also found to increase shoot growth, high biomass production, high crop yield, adequate translocation of nutrient, increase in soil microbial activities and enhance plant disease resistance (Abdel-Raouf *et al*.2012; Halpern *et al*. 2015).Some biostimulants added to soil pose a health risk to human especially those compost obtained from a waste dump sites with increase in heavy metal content.

Since pollution pose a risk to crop production. There is need to proper design measures to ameliorate the negative effect of pollution on soil as to boast crop production (Osuji *etal*. 2005). One of such measures is the use of stimulant. Severally studies on the effect of stimulant on polluted soil have been done such as the use of inorganic chelates (eg EDTA). No much work has been done on the use of local, cheap and organic materials such as plantain peel (waste). Hence this study aimed at investigating the biostimulating effect of waste plantain peels on the soil chemical, microbial load and morphological properties of *Cyperus iris* in a heavy metal polluted soil.

2. Materials and Methods

Study Location: The research was carried out at the Center for Ecological Studies, University of Port Harcourt, located at Latitude 4.90428^{o}N and Longitude 6.92297^{o}E. Two (2) distinct seasons - dry and wet seasons are experienced. 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).

2.1 Biostimulant

Land race of ripe plantain was obtained from Kaiama in Kolokuma/Opukuma L.G.A, Bayelsa State which is referred as 'Beribe' in their local dialect. The plantain was removed mechanically by hand peeling. The peels (waste) generated was dried and processed into powder which was further analyzed to ascertain its nutrient and heavy metals content (Table 1).

Table 1: Nutrient and metal contents of the peels waste

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

2.2 Heavy Metal Polluted Soil

A suspected heavy metal polluted soil was acquired from an abandoned metal scrap site at Ikoku Port Harcourt, Rivers State on geographical coordinate: Latitude 4.80083^{o}N and Longitude 6.991093^{o}E alongside with non-polluted soil obtained from a fallow land with history of heavy pollution at University of Port Harcourt was also collected. The soils were collected at depth of 0-20 cm using soil auger. The soils collected were analyzed to ascertain heavy metal content and other chemical properties, (Table 2). The soils were bulked together and homogenized.

Table 2. Chemical properties of the studied soil

S/N	**Parameter**	**Unpolluted**	**Polluted soil**
1	SOM (%)	24	12
2	Chloride (mg/kg)	213	3687
3	Sulphate (mg/kg)	28.4	269
4	Nitrate (mg/kg)	71.9	13.8
5	Phosphorus (mg/kg)	1.35	0.82
6	Sodium (mg/kg)	120	132
7	Calcium (mg/kg)	110	120
8	Magnesium (mg/kg)	258	280
9	Potassium (mg/kg)	68	43
10	pH	5.10	8.43
11	Lead (mg/kg)	130	167.3
12	Cadmium (mg/kg)	0.80	15.3

2.3 Experimental Design

A Completely Randomized Design (CRD) was adopted for this experiment, the collected soil was thoroughly mixed, dried and sieve using 2 mm wire mesh to obtain a homogenous fine soil composite. A calibrated weighing balance (Setra 480S, USA) was used to weigh two (2) kilogram of the fine soil composite into 30 planting bags of height, diameter and surface area of 18cm, 14 cm and $0.095m^2$ respectively. The planting bags with soil content were set in four (5) treatments (1, 2, 3 and 4) for polluted soil and treatment 5 contains non-polluted soils. Each treatment was replicated 6 times.

Addition of biostimulant Plantain peels

The bio-stimulant was added as follows:
T1: polluted soil + 100 grams plantain peels waste
T2: polluted soil + 200 grams plantain peels waste
T3: polluted soil + 300 grams plantain peels waste
T4: polluted soil + 0 grams plantain peels waste (control 1)
T5: non-polluted + 0 grams plantain peels waste (control II)

After the addition of biostimulant (amendments), the treatments were allowed to stand for 3 weeks before two seedlings of *Cyperus iria* of similar height and vigor were transplanted from the nursery into various treatments. The experiment was kept in a shedding environment with transparent roofing sheets to help control rainfall. Watering was done using 50cl/bag twice daily and the experiment was monitored for 60 days.

Determination of soil chemical, microbial and plant growth properties.

At termination, the soil from the treatments and their replicates were collected for chemical properties, microbial loads and selected plants morphological parameters.

Soil Parameter assessment. The determination of soil organic matter was done accordingly by calculation method using the formula cited by Osuji *et al*. (2005). Soil cadmium (Cd), lead (Pb), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), nitrate (N), and sulphate (S)contents were determined using atomic absorption spectrometer (AAS) through the digestion method. Soil Phosphate was determined by ascorbic and oxidation methods. The pH value of the soil was determined using Equip-tronics (Model EQ-610) pH meter. Soil chloride (Cl) was determined by electrometric method as described by Arao and Akira (1931).

Microbial Load Determination. The medium nutrient agar was used for the analysis of total heterotrophic bacteria count (THBC). In preparation of nutrient agar, 28g of the powder was added in 1 L of distilled water and characterized by autoclaving at 121 ^oC for 15 minutes and allow to stand for about 45 minutes. It was poured into sterilize Petri dishes and allow to solidify and excess moisture was eradicated from agar by drying in hot air oven set at 60 ^oC. Then 1g of the soil sample was weighed into 9 mL sterile diluent (normal saline), to carry out serial dilution. Exactly 0.l ml aliquote of inoculum was aseptically inoculated on duplicated agar surface using a sterile pipette and flame-sterilized glass –rod was used to spread the inoculum uniformly and incubate at 37 ^oC for 24 hours followed by colony to obtain colony forming unit (CFU/g) calculation.

Cultivation, Characterization and Identification of Fungal Isolate. A ratio of 1:10 of sample to diluents was prepared. The mixture was shaken and then serially diluted to 10^{-4}. Exactly 0.1 ml was plated on potatoes dextrose agar (PDA) impregnated with 1.0 % lactic acid. The spread plate technique was employed in the cultivation. The plates were incubated for seven (7) days then theprobable fungal isolates were characterized.

Microscopically, features like cell shape, type of hypha, presence of spores and spore arrangement were also observed. The cells were first stained prior to microscopic examination. Yeast-like fungal isolates were emulsified on clean, grease-free slides with a loopful of water smeared and allowed to dry before fixing. The smears were stained with crystal violet and after 1 min of dryness, the stain was gently washed off using 70 % alcohol. The smear was then gently rinsed with water and allowed to dry. The slide was then examined under oil immersion objective. The isolates were aseptically cut and placed on a clean slide, flooded cotton blue lacto phenol dye and mounted under a cover slip and viewed with the x 40 objective lens. The number of times each fungi occurred divided by the total number of fungi per plate.

Plant Morphological characters. Shoot length (cm) was measured using a centimeter rule which was placed from the stem at intercept with the soil surface to the terminal shoot apex. This shoot measurement was done weekly. Fresh weight was obtained by measuring the plant immediately after harvest using a digital weighing balance (Ohaus, HSC4010). The dry weight was also determined by loss on drying method. Root growth was determined at the end of the experimentusing a spadeto carefully extract 6 replicates of the root samples from each treatment which was then washed, and the length of each replicate was measured using a centimeter rule with 1 mm of precision.

Data Analysis

The data generated were subjected to statistical analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2002) to test the significant of bio-stimulant on soil characteristics and plant growth.Least Significant Difference (LSD) were used to analyze the data obtained.

3. Results

Effects of Bio-stimulant of different concentrations on soil Chemical Composition

The addition of bio-stimulantsignificantly decreased pH level of heavymetal polluted soil as compared to the control and double control. Addition of different concentrations of bio-stimulant showed an effect on the concentration of other soil chemical properties such as soil cadmium (Cd), lead (Pb), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), nitrate (N), sulphate (S), sodium (Na), chloride (Cl), SOM and phosphate (P) (Table 3). There were increase in Ca^{2+},P,K^+, sulphate, Na and nitrate. A Significant decrease in the concentration of soil Pb, Cd, Cl and Mg^{2+} were found in bio-stimulating soil of various concentrations compared to control. The highest increase in SOM was recorded for 200g bio-stimulant addition.

Morphological characteristics of test plant at 60 DAP

Results showed a remarkable effects of biostimulant on some selected plant morphological characters such as shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root length(Table 4) and shoot length (Table 5) of *Cyperus iria*. It was observed that the aforementioned morphological parameters of the studied plant (*Cyperus iria*) grown in bio-stimulated soil showed increase (p=0.05)with the highest at T3 (300g biostimulant) while the lowest root length in the control I (polluted soil without bio-stimulation).

Effect of biostimulant onTHBC and probable fungal isolates

Biostimulant application increased the populations of soil microorganisms. Biostimulants of different concentration enhanced soil microbial population (Table 6). The highest population of total heterotrophic bacteria count (THBC) was recorded in treatment 3(soil with 300g bio-stimulant), while Treatments 1 & 2 (100 and 200g bio-stimulant respectively) also showed a marked increase in THBC as compared to control Iand control II. There was abundance of *Aspergillus sp* Treatment 3 while *Penicillium sp*, *Rhizopus sp*, T*ritirachium sp* and *Cladosporium sp* have highest in occurrence in treatment 1 as compared to the controls.

Table 3: Effect of Biostimulant treatment of different concentrations on soil chemical properties(mg/kg) at 60 DAP

Treatments	Mg^{2+}	Ca^{2+}	P	K^{+}	S	N	SOM	Pb	Cd	Na	pH	Cl
T1	216 ± 0.33^{b}	200 ± 0.79^{c}	8.99 ± 0.02^{b}	43.3 ± 0.33^{b}	84.15 ± 0.02^{d}	56.0 ± 0.57^{a}	13.2 ± 0.001^{d}	98.4 ± 1.9^{c}	0.05 ± 0.001^{c}	189 ± 0.3^{b}	6.34 ± 0.01^{b}	212.63 ± 0.66^{b}
T2	100 ± 0.33^{b}	210 ± 1.45^{b}	8.92 ± 0.02^{b}	56.66 ± 1.33^{a}	121.65 ± 0.5^{b}	55.01 ± 0.5^{1ab}	36 ± 0.01^{a}	106 ± 0.055^{b}	0.18 ± 0.001^{c}	175 ± 2.0^{a}	6.21 ± 0.01^{b}	163.13 ± 0.03^{c}
T3	$150\pm.57^{c}$	300 ± 1.33^{a}	10.09 ± 0.01^{a}	67.66 ± 0.33^{b}	144.2 ± 0.53^{a}	56.4 ± 0.53^{a}	30.33 ± 0.2^{b}	129 ± 0.16^{b}	0.21 ± 0.003^{b}	200 ± 2.0^{a}	6.29 ± 0.01^{b}	141.52 ± 0.18^{c}
T4 (control I)	280 ± 2.0^{a}	120 ± 1.0^{d}	0.82 ± 0.21^{d}	43 ± 0.54^{c}	132 ± 1.02^{b}	13.2 ± 0.01^{c}	12 ± 0.17^{d}	167.3 ± 1.20^{a}	15.3 ± 0.023^{a}	132 ± 2.0^{ab}	8.43 ± 0.21^{a}	3687 ± 1.2^{a}
T5(controlII)	258 ± 2.01^{a}	110 ± 1.0^{d}	1.35 ± 0.31^{c}	68 ± 0.61^{ab}	120 ± 1.12^{c}	71.9 ± 1.0^{bc}	24 ± 0.7^{c}	13.0 ± 0.02^{d}	0.80 ± 0.001^{c}	120 ± 2.5^{b}	5.10 ± 0.001^{bc}	213 ± 0.01^{bc}

Table 4: Effect of Biostimulant treatment of different concentrations on the Plant Morphological characteristicsof *Cyperus iria* at the 60 DAP.

Treatments	Root length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
T1	7.01^{b}	26.6^{b}	13.57^{c}	9^{c}	2.92^{b}
T2	7.22^{b}	29.7^{b}	11.23^{c}	12^{b}	5.14^{a}
T3	10.5^{a}	48.6^{a}	30.73^{a}	16^{a}	6.98^{a}
T4 (Control I)	4.81^{c}	17.5^{c}	9.65c^{d}	2^{d}	1.41^{c}
T5 (Control II)	10.1^{ab}	30.3^{ab}	19.01^{b}	16^{a}	1.72^{c}

Table 5. Effects of bio-stimulant of different concentrations on the height of *Cyperus iria* from 1-8 weeks

Nos of Weeks	T5 (Control I) (cm)	T4 (control I) (cm)	T1 (cm)	T2 (cm)	T3 (cm)
Week 1	6	2.0	11.0	9.8	10.0
Week 2	9.2	5.0	13.8	14.0	13.5
Week 3	9.8	5.2	14.0	14.2	14.0
Week 4	9.8	5.22	14.2	14.3	14.32
Week 5	10	6.1	15.2	16.0	16.7
Week 6	19	10.0	28.9	29.23	32.0
Week 7	28	11.0	42.0	48.0	50.0
Week 8	51	34.3	50.3	58.66	62.2

Table 6. Effects of bio-stimulant treatment of different concentrations on total heterotrophic bacteria count (THBC) and fungal isolates

Biostimulant	THC	Aspergillus niger	Penicillium sp	Rhizopus sp	Tritirachium sp	Cladosporium sp
T1	$20.8X10^4$	40	50	60	50	50
T2	$20.8X10^4$	38	40	42	30	30
T3	$26.2X10^4$	42	50	36	40	40
T4 control I	$6.0X10^4$	30	20	20	25	20
T5 control 11	$13.3X10^4$	50	46	55	46	40

4. Discussion

The benefits of soil for agricultural purposes cannot be neglected. Increase in agricultural products and environmental restoration directly depend on the stability of soil. The quality of a soil is easily observed on the health of any vegetation through biomass production, shoot and root length. Soil plays a crucial role in supporting plant life and microbial activities since it holds the essential nutrients needed for their survival and optimal productivity(Voroney *et al*., 2015).Result showed that soil chemical properties such as Ca^{2+}, P, K, N and soil organic matter were higher in bio-stimulated soil as compared to the control. This variation in chemical propertiesis crucial since decrease in levels of the selected chemical properties were found in control I and II. The added bio-stimulant may have influence the soil chemistry thereby increasing its chemical properties since the potential nutrients in the organic bio-stimulant are made available for the soil during decomposition. This finding also corroborated with the investigation of (Sootahar *et al.* 2019) who reported that the application of organic bio-stimulants is capable of improving soil fertility significantly thereby maintaining nutrient balance; Amadi *et al*. (2018)also explained that the application of organic biodegradable waste like plantain peels has the capacity of improving soil fertility and the available nutrient will be utilized by plants for proper growth and productivity.

Result revealed that the addition of organic bio-stimulant increased the soil organic matter content (SOM). Highest increase in soil organic matter was found in soil with 200g bio-stimulant (T2). This could be attributed to the nutrient quality of the amendment used since the increase SOM level is not proportional with the concentration of bio-stimulant added. This suggests that microbial degradation process is not directly linked with the concentration or quantity of amendment added but on the quality of the essential nutrient required by microbes for metabolic processes. Bago (2000), have also recognized that microbial metabolic processes is not a function of the concentration of biodegradable amendment added and its metabolic activity help to increase soil nutrient through waste degradation process. This results also correspond with Wang *et al.*(2014) who observed a remarkable increase in soil organic matter with addition of organic bio-stimulant. This findings disagrees with the report of Sootahar *et al.*(2019) who reported that the organic particles present in soil is responsible in promoting the concentration of organic matter through the formation of fulvic acids.

Result also revealed that addition of organic bio-stimulant of various concentration led to a significant increase in microbial activity. This increase in soil microbial activity could be as a result of the prebiotic and probiotic molecules obtained from the fermentation process of humic acids and bacterial strain respectively. This finding also corresponds with Vassileva *et al.*(2020) who reported that diversity found in microorganisms and soil microbial health is directly linked with the activity of prebiotic and probiotic molecules. Increase in the frequency of occurrence of total heterotrophic bacterial count and fungal isolate; (*Penicillium sp*, *Rhizopus sp*, T*ritirachium sp* and *Cladosporium sp*) was more in bio-stimulated soil. This observation could be attributed to the nutrient quality of the peels added might have created an optimal environment for microorganisms to strive through production of the needed nutrients which is needed to enhance microbial diversity (species richness and evenness) and microbial health. This finding also agrees with Yuan *et al*. (2017), who informed that changes in soil microbial community is usually reported in organic bio-stimulated soil.

Plant optimal performance is characterized by increase in soil nutrient and microbial metabolism and favoring greater root growth. Result showed that the addition of organic bio-stimulant of different concentrations were able to increase in morphological characters (shoot length, root length, shoot fresh and dry weight, root fresh and dry weight). This is an indication that the added organic bio-stimulant is a good source of nutrient that will help compliment the depleted soil nutrient. This increase in soil nutrient quality is achieved by the presence of microorganisms through decomposition process of the bio-stimulant. This available nutrient positively influence the production of more root hairs and lateral root system. This findings is in agreement with the assertion of Ibrahim (2013), who reported that the shoot length and biomass of plants found growing in a favorable soil condition are influenced positively by nutrient availability. Amadi *et al.*(2023) also testified that organic bio-stimulant contains sundry of proteins decomposed during the fermentation process which was humidified into amino acids. The humidified amino acids have the capacity to induce increase in bacterial life in the soil which can directly influence plant growth and performance by stimulating its root activity. This result corroborates with (Barone *et al*. 2018) who also reported that quite a few studies have shown that bio-stimulating of plants with humic substances is capable of inducing proliferation of lateral roots and root hairs. This assertion was also in agreement with Barone *et al*. (2018), who reported an improvement in root growth primarily due to application of bio-stimulant which became relevant in helping plants access soil nutrients and water at different depths.

5. Conclusion

This research tested the potentiality of organic bio-stimulant (plantain peel waste) in improving polluted soil quality. Results from the study revealed that the application of plantain peel (waste) significantly improved soil chemical (fertility) properties leading to increase in microbial activity and hence resulting in optimal performance and growth (morphological character) of *Cyperus iria* in a heavy metal polluted soil. Thus the study has shown that different concentrations of organic amendment can enhance soil stability and growth performance of plants.

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