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Research Article

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Microbial diversity under different levels of organic and inorganic soil treatment

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Abstract

This research investigated microbial diversity in heavy metal-contaminated soil subjected to different levels of organic and inorganic amendments, using standard analytical methods. The trial treatments were as follows: A1: NPK (40g/2kg); A2: NPK (80g/2kg); A3: NPK (120g/2kg); B1: Orange peel (100g/2kg); B2: Orange peel (200g/2kg); B3: Orange peel (300g/2kg); C1: Plantain peel (100g/2kg); C2: Plantain peel (200g/2kg); C3: Plantain peel (300g/2kg). Blocks D and E, which received no amendments, served as the control and double control, respectively Results indicated that the highest increase in total heterotrophic bacterial count (THBC) was observed in *Cyperus iria* grown soil, with values of 26 x 10^4 and 48.0 x 10^4 at 60 and 120 days, respectively. Similarly, *Echinochloa colona* showed THBC values of 27.6 x 10^4 and 50.0 x 10^4 in 300g amended soil at the same intervals. The lowest THBC was recorded in the control soil at 60 days, while the NPK treatments of 80g and 120g showed the smallest decrease at 120 days. The probable fungi isolates included *Aspergillus niger*, *Penicillium sp.*, *Rhizopus*, *Tritirachium sp.*, and *Cladosporium sp*., with higher occurrences in both organic and inorganic treated soils. The highest increase in fungi occurrence was found in the 300g plantain peel amended soil. Overall, this research suggests that adding amendments significantly aids in the microbial restoration of polluted soil. Specifically, the 300g plantain peel (organic waste) amendment proved to be the most effective and suitable for enhancing microbial populations in heavy metal-contaminated soil, highlighting its potential as a valuable agent in soil restoration efforts.

Keywords: Microorganisms, soil, nutrient, enhancer, organic and inorganic

Introduction

Soil's complex ecosystem and the intricate interactions between its diverse inhabitants play a fundamental role in supporting plant life. Understanding these relationships and processes is crucial for sustainable agriculture and ecosystem management. Plant physiologists observe that soil is a complex and dynamic ecosystem that supports a vast array of organisms, including mammals, bacteria, fungi, and protists, while also serving as an effective nutrient supply for plants (Hayat *et al*., 2010). The diversity and interactions within this ecosystem are critical for maintaining soil health and fertility, which directly impact plant growth and productivity. The soil is categorically a home to various species of organisms, each contributing to the ecosystem's functionality: Small mammals like moles and rodents play a role in soil aeration and mixing, which enhances water infiltration and root penetration. Soil microorganisms are crucial also for decomposing organic matter and nutrient cycling. They convert organic compounds into forms that plants can absorb, such as ammonium and nitrate from nitrogen-fixing bacteria (Fierer 2017).Mycorrhizal fungi form symbiotic relationships with plant roots, extending their reach and improving water and nutrient uptake. Saprophytic fungi decompose complex organic materials, releasing nutrients back into the soil.Soil microbes play a vital role in plant growth and optimum performance by manipulating hormonal signaling pathways. Lopez-Bucio *et al*., (2007) describe how certain soil bacteria produce phytohormones or modulate the plant's hormonal balance, leading to enhanced root growth, increased resistance to stress, and overall improved plant health. Microbes also enhance plant productivity by suppressing the growth of plant pathogens through various mechanisms. Wang *et al*., (2021a), highlight the role of beneficial microbes in producing antimicrobial compounds, outcompeting pathogens for resources, and inducing plant defense responses, thereby protecting plants from diseases. Harmful pathogens, capable of decreasing optimal performance of plants are certain species of microorganisms (Huet *et al.*, 2023).Macro and micronutrients, such as sulfur, phosphorus, and nitrogen, are essential for plant growth but often exist in forms that are not readily accessible to plants. These nutrients bind to organic molecules in the soil, and their availability depends on microbial activity(Jacoby *et al*., 2017).Microbes oxidize sulfur compounds, converting them into sulfate; Phosphorus is often bound to soil particles or organic matter. Mycorrhizal fungi release enzymes that solubilize phosphorus a form which Nitrogen-fixing bacteria convert atmospheric nitrogen into ammonium and nitrate these nutrient are present in form which plants can uptake and utilize for growth. (Jacoby *et al*., 2017).In the Niger Delta region, microbial populations and diversity are particularly influenced by anthropogenic activities such as heavy metal contamination and crude oil pollution. These pollutants disrupt the delicate balance of the soil microbiome, leading to reduced microbial diversity and altered community structures. The impact of these anthropogenic activities extends to the physical properties of the soil, including increased erosion and changes in soil organic matter inputs, which further affect microbial populations (Crecchio *et al*., 2018).Xu *et al*. (2018) highlighted that the removal of vegetation due to human activities can have profound effects on the soil microclimate and microbial habitat. Vegetation loss leads to a decline in fungal decomposers and mycorrhizae, which are essential for nutrient cycling and plant health. The reduction in these key microbial groups compromises soil structure and function, ultimately affecting plant growth and ecosystem stability.

Aim of this research.

The study aim to provide insights into the mechanisms of soil restoration and the potential for microbial communities to recover and thrive in contaminated soils. By understanding the specific needs and interactions of soil microorganisms, strategies can be developed to enhance microbial diversity and activity, thereby promoting soil health and sustainable plant

growth. The study of soil microbial populations and their interactions is vital for the restoration of degraded soils.

Materials and Methods

Study area

This research was conducted at the Center for Ecological Studies, University of Port Harcourt. The center is located in the Niger Delta region of Nigeria, at geographical coordinate's 4.90428° N latitude and 6.92297° E longitude. The area experiences two distinct seasons: the dry season from November to March, and the wet season from April to October. Annual rainfall peaks in July and September (Uko and Tamunobereton-Ari, 2013). The climate is characterized by daily temperatures ranging from 36°C to 45°C throughout the year.

Sources of Soil enhancers and processing

A land race of sweet orange, locally known as 'Oroma' or 'Epe' in the native language, was acquired from Otutu-Amaumara Ezinihitte Mbaise LGA in Imo State. Ripe plantains, referred to as 'Beribe', were sourced from Kaiama in Kolokuma/Opukuma L.G.A, Bayelsa State. The peels of both the sweet orange and plantain were removed mechanically by hand peeling. These peels were then dried and processed into powder form. The resulting powder was analyzed to determine its nutritional value and heavy metal content (see Table 3.1). Additionally, NPK 20:20:20 fertilizer was obtained from the Rivers State Agricultural Development Program (ADP) in Rumuodomaya, Port Harcourt.

Table 1: Nutrient value of amendments

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	pH	5.56	9.08

Experimental Design

A factorial experiment designed within a Randomized Complete Block Design (RCBD) framework was employed for this study. Suspected heavy metal-polluted soil was acquired from an abandoned metal scrap site in Ikoku, Rivers State, Port Harcourt, at geographical coordinate's 4.80083° N latitude and 6.991093° E longitude. Additionally, uncontaminated soil was collected from fallow land at the University of Port Harcourt at a depth of 0-20 cm using a spade. Both soils were analyzed to determine their heavy metal content and other physicochemical properties, referred to as baseline analysis. The collected soils were bulked together, homogenized, and transported to the Centre for Ecological Studies at the University of Port Harcourt, located at 4.90428° N latitude and 6.92297° E longitude, where the experiment was conducted. The soils were thoroughly mixed, dried, and sieved through a 2 mm mesh to obtain a homogenous soil (fine fraction) composite. Using a calibrated weighing balance (Setra 480S, USA), two kilograms (2 kg) of the homogenized soil were weighed into planting bags. The bags were arranged into four batches (A, B, C, and D) along with uncontaminated soil designated as

batch E. Batch A was further subdivided into three subplots labeled A1, A2, and A3. The same subdivision process was applied to batches B and C, resulting in subplots B1, B2, and B3, and C1, C2, and C3, respectively, with 12 replications for each subplot.

Table 2. Physicochemical properties of the growth medium substrate (soil)

S/N	**Parameter**	**Unpolluted**	**Polluted soil**
1	Moisture (%)	45	43
2	Bulk density (%)	1.5	1.7
3	Particle density (%)	5.8	5.1
4	Porosity	0.35	0.3
5	SOM (%)	12	24
6	Sand (%)	95.6	93.6
7	Silt (%)	0.10	0.7
8	Clay (%)	4.3	5.7
9	Chloride (mg/kg)	213	3687
10	Sulphate (mg/kg)	28.4	269
11	Nitrate (mg/kg)	71.9	138
12	Phosphorus (mg/kg)	1.35	0.82
13	Calcium (mg/kg)	110	120
14	Magnesium (mg/kg)	258	280
15	Potassium (mg/kg)	43	68
16	pH	5.10	8.43
17	Conductivity (μsCm^{-1})	90	1193
18	Lead (mg/kg)	130	167.3
20	SOM	32	12
21	THBC	1.21×10^5	1.0×10^5
22	TFC	4.5×10^4	2.1×10^4
23	HUBC (CFU/g)	1.01×10^5	1.1×10^5
24	HUFC	3.1×10^4	2.3×10^4

Treatment application

Using a calibrated weighing balance (Setra 480S, USA), amendment treatments were added as follows:

Treatment A1: NPK (40g/2kg)
Treatment A2: NPK (80g/2kg)
Treatment A3: NPK (120g/2kg)
Treatment B1: Orange peel (100g/2kg)
Treatment B2: Orange peel (200g/2kg)
Treatment B3: Orange peel (300g/2kg)
Treatment C1: Plantain peel (100g/2kg)
Treatment C2: Plantain peel (200g/2kg)
-Treatment C3:Plantain peel (300g/2kg)

No amendments were added to blocks D and E, which served as the control and double control, respectively, with 0g amendment. After two weeks of post-amendment treatment, the amendments along with the control and double control were divided into two groups. Two seedlings each of the test (phytoremediation) plants, raised in the nursery from seeds for three weeks and properly identified at the University of Port Harcourt Herbarium as *Cyperus iria* Linn and (L.) Link, were planted in each group. Thus, *Cyperus iria*was planted in one group of 132 bags, and *Echinochloa colona*in the other group of 132 bags, as shown in Table 3.3. The planted seedlings were of identical size and vigor. The experimental area was shaded with transparent rubber zinc to control rainwater. Watering was

done four times a week using 500 ml of water, and weeding was performed by hand as needed. The experiment was monitored at 60-day intervals at termination soil sample collection was done as describe:

Sample Collection

The soil samples from each amendment treatment were carefully collected directly from the soil atmosphere. These samples were then transported to the Microbiology Environmental Laboratory at the University of Port Harcourt for analysis.

Media Preparation

Nutrient agar (NA) is a widely used medium for the cultivation of bacteria. The preparation of nutrient agar was done precisely according to manufacturer standard to ensure effective growth of a wide range of non-fastidious microorganisms. Below is an elaboration on the preparation process, ensuring adherence to the manufacturer's standards.

Preparation Steps

Weighing the Nutrient Agar Powder was done in response with the manufacturer's instructions on the quantity of nutrient agar powder required per liter of distilled water. Typically, this is about 23 grams per liter. The appropriate amount of nutrient agar powder required was achieved using an accurate weighing balance.The require amount of distilled water was measured into a conical flask or Erlenmeyer flask with gradual addition the nutrient agar powder to the flask while stirring continuously to ensure the powder dissolves completely.

Sterilization

All glass wares such as Petri dishes, test tubes, conical flask etc, were sterilized by autoclaving at 121 ^{0}C at 15 pounds per square inch (psi) for 15 minutes.

Diluents

Physiological saline was prepared by suspending 0.85g of NaCl into 250 ml conical flask (sterile) containing 1000 ml of distilled water. About nine milliliters (9 ml) from the stock was dispensed into test tubes, plugged with cotton wool, and sterilized by autoclaving at 121 ^{0}C for 15 psi at 15 minutes and allowed to cool.

Total heterotrophic bacterial count (THBC)

For this analysis, nutrient agar medium was used. The medium was prepared according to the manufacturer's standard by weighing and dissolving 28 grams of nutrient agar powder in 1 liter of distilled water. The solution was then sterilized by autoclaving at 121°C for 15 minutes and allowed to cool for about 45 minutes. It was subsequently poured into sterile Petri dishes and left to solidify. Excess moisture was removed from the solidified agar by drying it in a hot air oven set at 60°C.

To carry out a serial dilution, 1 gram of the soil sample was weighed and added to 9 mL of sterile diluent (normal saline). An aliquot of 0.1 mL of this diluted sample was aseptically inoculated onto the surface of duplicate agar plates using a sterile pipette. The inoculum was spread uniformly using a flame-sterilized glass rod. The plates were then incubated at 37°C for 24 hours. After incubation, colony forming units (CFU/g) were calculated based on the number of colonies observed.

Cultivation, Characterization and Identification of Fungal Isolate

This procedure according to a standard method was adopted. A 1:10 ratio of sample to diluent was prepared, and the mixture was shaken thoroughly before being serially diluted to 10^4. A 0.1 mL aliquot of this diluted sample was then plated on potato dextrose agar (PDA) supplemented with 1.0% lactic acid. The spread plate technique was used for cultivation. The plates were incubated for seven days, after which the fungal isolates were characterized.

Fungal Macroscopy

This procedure aimed to determine the colonial characteristics of the isolates on potato dextrose agar (PDA) plates. The isolates were aseptically inoculated onto freshly prepared PDA plates and incubated at 28°C for 3 to 5 days. After incubation, various properties such as the presence of aerial and substrate mycelia, as well as their different shapes, forms, and diameters, were observed.

Fungal Microscopy

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 which was further 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.

Determination of Frequency of Occurrence of Fungi

A standard method was employed. The number of times each fungi occurred divided by the total number of fungi per plate.

Results and Discussion

Total Heterotrophic Bacteria Count (THBC) for Soil with Test Plants

Tables 3 and 4 present the total heterotrophic bacteria count (THC) for soil with *Cyperus iria*. The addition of amendments significantly increased the bacterial load over time. The highest microbial titre was recorded at 26 x 10^4 and 48.0 x 10^4 cfu/ml for the 300 g plantain peel soil amendment at 60 and 120 days, respectively. In contrast, the amended soil with 40 g NPK (20:20:20) and the control soil showed the least decrease in microbial titre at 60 and 120 days, respectively. Significantly, the lowest microbial load was recorded at 6.0x10^4 and 7.0x 10^4 cfu/ml for the control (polluted remediated soil) at 60 and 120 days in *Echinochloa colona* remediated soil. The highest microbial titre observed in the 300 g plantain peel soil amendment at both 60 and 120 days was significant within and between amendments at p=0.05.This data underscores the efficacy of organic amendments, particularly plantain peels, in enhancing microbial activity and soil health, demonstrating a substantial increase in bacterial populations over time compared to other amendments and control soils.

The variation in the total heterotrophic bacteria count (THC) across different soil amendments can be attributed to the diverse impacts of organic and inorganic amendments on microbial activity and soil health. Organic amendments such as plantain peels provide rich sources of nutrients and organic matter, which promote the growth and activity of soil microbes. In contrast, inorganic amendments like NPK fertilizers offer essential nutrients but may not support microbial activity to the same extent due to the lack of organic matter.The highest microbial titre, recorded for the 300 g plantain peel soil amendment at 60 and 120 days respectively, can be attributed to the rich organic content of plantain peels. Plantain peels are a valuable source of organic matter, containing a variety of nutrients essential for microbial growth. These nutrients include carbohydrates, proteins, and lipids, which serve as energy sources and building blocks for microbial cells.Plantain peels are high in carbohydrates, which are a primary energy source for heterotrophic bacteria. The presence of sugars and starches in the peels provides easily accessible carbon that microbes can metabolize, leading to rapid growth and reproduction. Carbohydrates also stimulate microbial activity by enhancing the production of extracellular enzymes that degrade complex organic compounds (Awasthi *et al*., 2020).The protein content in plantain peels supplies essential amino

acids that microbes require for synthesizing new proteins and other cellular components. Proteins and amino acids are vital for cell structure, enzyme function, and metabolic processes. The availability of these nitrogen-rich compounds can significantly boost microbial biomass and activity (Gupta *et al.,* 2019).Lipids in plantain peels provide long-term energy reserves and structural components for microbial cell membranes. The degradation of lipids by soil microbes releases fatty acids, which are used in various metabolic pathways. This contributes to sustained microbial activity and growth over extended periods (Meena *et al.,* 2020).In addition to macronutrients, plantain peels contain trace elements and micronutrients such as potassium, magnesium, and calcium. These elements are crucial for enzymatic reactions and maintaining microbial cell stability. Their presence in the soil amendment ensures that microbes have a balanced nutrient supply, supporting optimal growth conditions (Bardgett *et al*., 2013).The addition of plantain peels improves soil structure by increasing organic matter content. This enhances soil aeration, water retention, and the formation of soil aggregates. Improved soil structure creates a favorable microenvironment for microbes, promoting their colonization and activity in the soil (Van der Heijden *et al*., 2008). Similar result was also reported by Gupta *et al*., (2019) that organic matter from plantain peels provides a steady supply of carbon and other nutrients, which are essential for microbial growth and proliferation. The amended soil with 40 g NPK (20:20:20) recorded the least decrease in microbial titre at 60 and 120 days. NPK fertilizers provide essential nutrients (nitrogen, phosphorus, and potassium) that support plant growth but do not supply organic matter, which is critical for sustaining microbial populations. While these nutrients can enhance the growth of some microbial groups, they may not support the diverse microbial community that thrives on organic matter (Bardgett *et al*., 2013).The control soil recorded the lowest microbial load at 60 and 120 days, respectively. This variation observed is appreciable since the low rate of microbial activity can be linked to the lack of added nutrients and organic matter in the control soil.

Polluted soils often have compromised microbial communities due to the presence of contaminants and lack of essential nutrients (Van der Heijden *et al*., 2008).

Table 3: Total Heterotrophic Bacteria Count (THC) for soil with *Cyperus iria*

SAMPLE	TOTAL VIABLE COUNT		TOTAL VIABLE COUNT
TREATMENTS	60 DAYS	TREATMENTS	120 DAYS
N_{40}	$6.2X10^4$	N_{40}	$8.7X10^4$
N_{80}	$6.4X10^4$	N_{80}	$7.9X10^4$
N_{120}	$7.1X10^4$	N_{120}	$7.9X10^4$
O_{100}	$17.8X10^4$	O_{100}	$40.1X10^4$
O_{200}	$17.3X10^4$	O_{200}	$36.4X10^4$
O_{300}	$17.3X10^4$	O_{300}	$42.4X10^4$
P_{100}	$20.8X10^4$	P_{100}	$42.2X10^4$
P_{200}	$20.8X10^4$	P_{200}	$44.7X10^4$
P_{300}	$26.2X10^4$	P_{300}	$48.0X10^4$
CP	$6.0X10^4$	CP	$8.2X10^4$
UP	$13.3X10^4$	UP	$18.0X10^4$

Table 4: Total Heterotrophic Bacteria Count (THC) for soil with *Echinochloa colona*

SAMPLE	TOTAL VIABLE COUNT		TOTAL VIABLE COUNT
TREATMENTS	60 DAYS	TREATMENTS	120 DAYS
N_{40}	$6.1X10^4$	N_{40}	$7.7X10^4$
N_{80}	$5.8\ X10^4$	N_{80}	$8.9X10^4$
N_{120}	$4.9X10^4$	N_{120}	$6.9X10^4$
O_{100}	$14.8X10^4$	O_{100}	$35.1X10^4$
O_{200}	$15.3X10^4$	O_{200}	$30.4X10^4$
O_{300}	$17.3X10^4$	O_{300}	$41.4X10^4$
P_{100}	$20.8X10^4$	P_{100}	$40.2X10^4$
P_{200}	$22.8X10^4$	P_{200}	$43.7X10^4$
P_{300}	$26.2X10^4$	P_{300}	$50.0X10^4$
CP	$6.0X10^4$	CP	$7.0X10^4$
UP	$15.3X10^4$	UP	$19.0X10^4$

Effects of amendment of frequency of occurrence of isolated fungus

Figures 1a, 1b, 2a, and 2b show the frequency of occurrence of isolated fungi in percentage. The highest occurrence of *Aspergillus niger* in soil rhizosphere grown with *Cyperus iria* at 60 days was recorded for the double control, while the lowest occurrence of *Aspergillus sp*. was observed in the control soil. The highest increase in the occurrence of *Penicillium sp*. was found in 200 g orange peel amended soil, with the least decrease observed in the control. The highest increase in the occurrence of *Rhizopus sp., Tritirachium sp.*, and *Cladosporium sp*. was recorded for the 100 g plantain peel soil amendment, while the least decrease was documented in control soil for *Rhizopus sp*. and *Cladosporium sp*., and the least decrease in *Tritirachium sp*. was observed in the 40 g NPK soil amendment, as shown in Figure 1a. At 120 days, the highest occurrence of *Aspergillus niger* in soil rhizosphere grown with *Cyperus iria* was recorded for the 300 g plantain peels soil amendment, while the lowest occurrence of *Aspergillus sp*. was observed in the 80 g and 120 g NPK soil amendments, which was insignificant. The highest increase in the occurrence of *Penicillium sp., Rhizopus sp*., *Tritirachium sp*., and *Cladosporium sp*. was documented for the 100 g plantain peel soil amendment, while the least decrease was documented in control soil for *Penicillium sp*., *Rhizopus sp.*, and *Cladosporium sp*., and the least decrease in *Tritirachium* sp. was observed in the 40 g NPK treated soil, as shown in Figure 1b.

An increase in the occurrence of *Aspergillus niger, Penicillium sp*., and *Cladosporium sp*. in soil rhizosphere grown with *Echinochloa colona*at 60 days was recorded for the 300 g plantain peels soil amendment, while the least increase was recorded for the 40 g NPK soil amendment, control (0 g) polluted remediated soil, and 120 g NPK amendment respectively for *Aspergillus niger, Penicillium sp*., and *Cladosporium sp*. The highest occurrence of *Rhizopus sp.* and *Tritirachium sp* was documented for the 100 g plantain peels soil treatment, while the least occurrence was found in the 120 g NPK soil amendment, as shown in Figure 2a.

In Figure 2b, the highest occurrence of *Aspergillus niger* in soil rhizosphere grown with *Echinochloa colona* at 60 days was recorded for the 200 g plantain peels soil amendment, while the 120 g NPK treated soil showed a decrease. An increase in the occurrence of *Penicillium sp*. and

Cladosporium sp. was recorded for the 100 g plantain peels soil amendment, while the least decrease was found in control soil and the 120 g NPK soil amendment, respectively, for *Penicillium sp* and *Cladosporium sp* . The highest occurrence of *Rhizopus sp* and *Tritirachium sp*. was documented for the 100 g plantain peels soil amendment, while the least occurrence was found in the 120 g and 40 g NPK soil amendments, respectively, for *Rhizopus sp* and Tritirachium *sp.*, as shown in Figure 2b.

The observed variations in the frequency of occurrence of isolated fungi across different soil treatments can be attributed to the varying effects of soil amendments on fungal growth and activity. Soil amendments, such as plantain peels, orange peels, and NPK fertilizers, introduce different nutrients and organic matter into the soil, which can significantly influence the microbial community structure and function.The highest occurrence of *Aspergillus niger, Penicillium* sp., *Rhizopus* sp., *Tritirachium* sp., and *Cladosporium* sp. was often recorded for soils amended with 100 g and 300 g of plantain peels. This can be attributed to the high organic content and nutrient availability in plantain peels (Table 1), which provide an enriched environment for fungal growth. Organic amendments like plantain peels can enhance soil fertility, improve soil structure, and increase microbial biomass and activity. This finding corroborated with Gupta *et al*., (2019); Meena *et al*., (2020) who reported that high organic content and nutrient availability in plantain peels create an enriched environment conducive to fungal growth. Organic amendments such as plantain peels play a crucial role in enhancing soil fertility by supplying essential nutrients and organic matter that promote microbial activity. These amendments improve soil structure, which enhances water retention and aeration, creating favorable conditions for fungi to thrive. Additionally, the introduction of organic matter from plantain peels can increase microbial biomass and activity, further supporting the proliferation ofbeneficial fungi. This synergistic effect of improved soil properties and nutrient availability underscores the effectiveness of organic amendments in fostering a healthy and diverse soil microbial community.The highest increase in the occurrence of *Penicillium* sp. in 200 g orange peel amended soil is understandable because orange peels are rich in essential nutrients and organic compounds, making them a valuable soil amendment for promoting the growth of specific fungal species. These peels contain sugars, vitamins, and minerals that serve as excellent substrates for fungi, providing the energy and nutrients necessary for their growth and reproduction. The organic acids and aromatic compounds present in orange peels can also create a more favorable soil pH and enhance microbial activity. This nutrient-rich environment not only supports the proliferation of fungi but also contributes to the overall health and diversity of the soil microbial community. By incorporating orange peels into the soil, it is possible to enhance biological activity and improve soil health, leading to more robust and resilient ecosystems. This agreeswith Iqbal*et al*., (2015), who reported that orange peels contain essential nutrients and organic compounds that can promote the growth of specific fungal species. The lowest occurrences of *Aspergillus* sp., *Penicillium* sp., and *Cladosporium* sp. were often observed in soils treated with 40 g and 120 g of NPK fertilizers. While NPK fertilizers supply essential nutrients like nitrogen, phosphorus, and potassium, they may not provide the same level of organic matter as plant-based amendments, potentially limiting the growth of organic matter-dependent fungi (Bardgett *et al.*, 2013).

The use of some plants such as *Cyperus iria* and *Echinochloa colona* also influences fungal occurrence. Certain plant species can alter soil properties, root exudation patterns, and microbial community dynamics, which can affect fungal populations (Wenzel, 2009).At 60 and 120 days, the highest occurrences of *Aspergillus niger* were observed in soils grown with *Cyperus iria* and amended with plantain peels. The interaction between plant roots and organic amendments can create favorable microenvironments for fungi by enhancing root exudates and organic matter content (Hrynkiewicz and Baum, 2012).Similarly, soils grown with *Echinochloa colona* and amended with plantain peels exhibited increased

occurrences of fungi. The plantain peel amendments likely provided additional organic substrates that supported fungal growth, while the plants' root systems contributed to creating suitable niches for fungi (Wang *et al*., 2017).

The variations in fungal occurrence between control soils and amended soils highlight the influence of natural soil conditions and the impact of amendments. Polluted soils often exhibit compromised microbial communities due to the presence of contaminants and a lack of essential nutrients. Contaminants such as heavy metals, hydrocarbons, and industrial chemicals can be toxic to soil microorganisms, inhibiting their growth and activity. These pollutants can disrupt microbial cell structures, interfere with metabolic processes, and even lead to cell death, resulting in reduced microbial diversity and biomass. This finding also agrees with Van der Heijden *et al.*, (2008) who reported that control soils typically exhibited lower fungal occurrences, emphasizing the importance of adding organic matter and nutrients in promoting microbial activities in a polluted soil

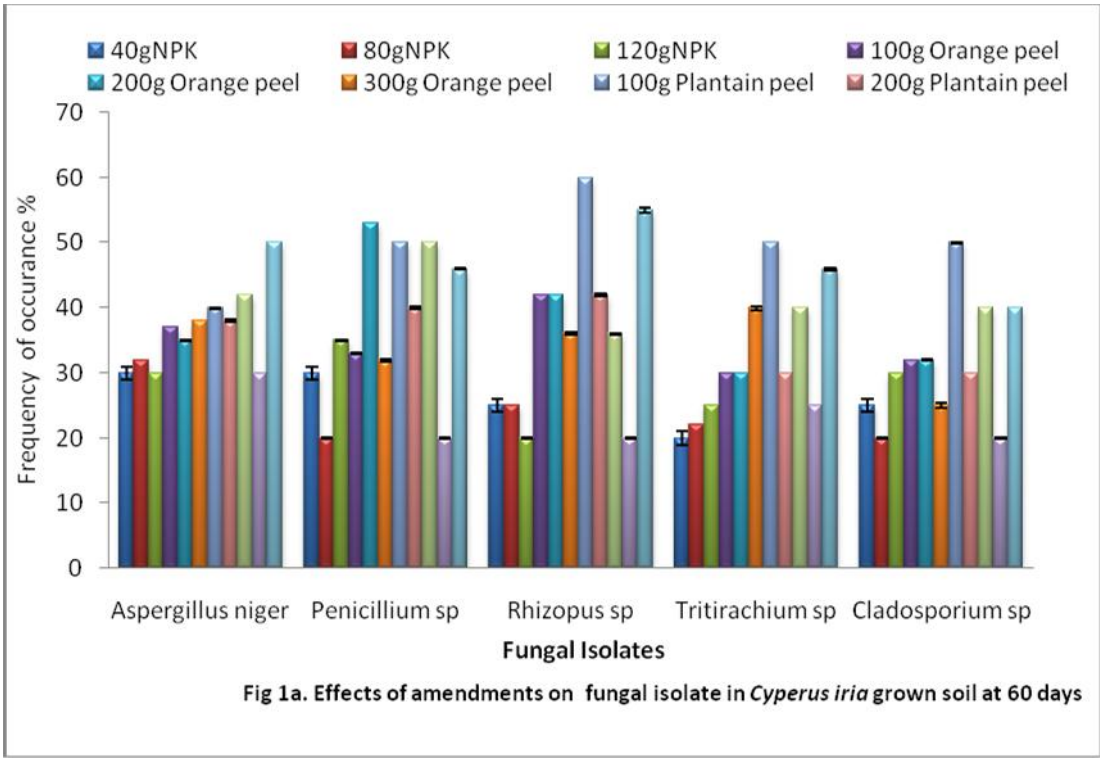


Fig 1a. Effects of amendments on fungal isolate in *Cyperus iria* grown soil at 60 days

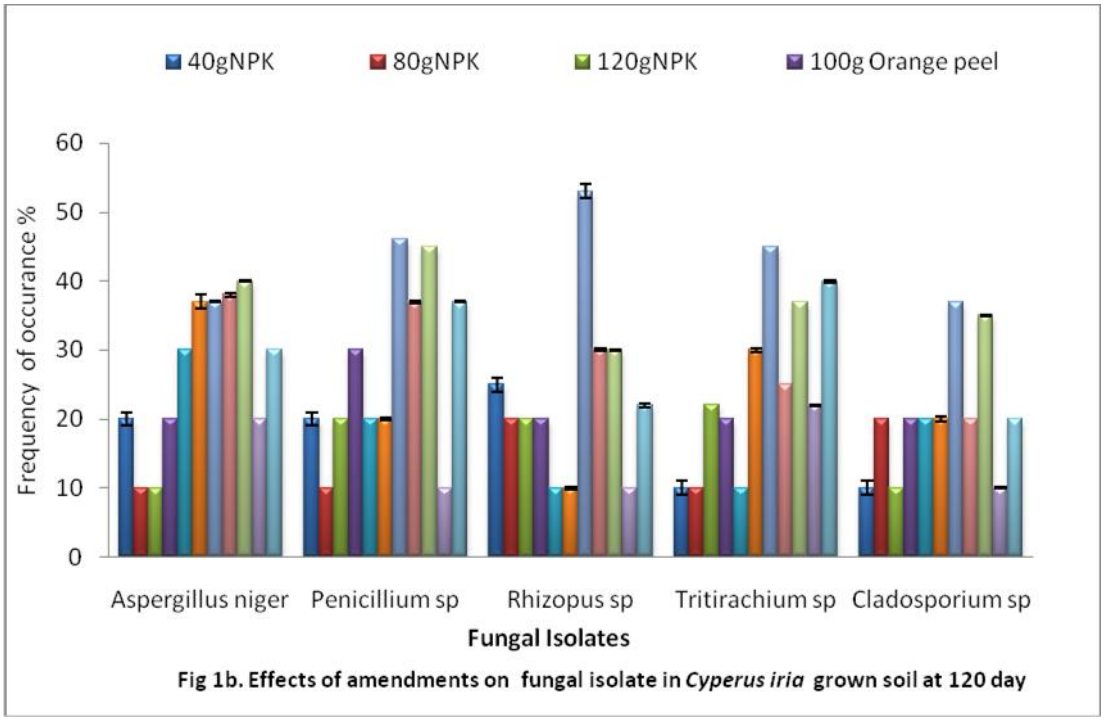


Fig 1b. Effects of amendments on fungal isolate in *Cyperus iria* grown soil at 120 day

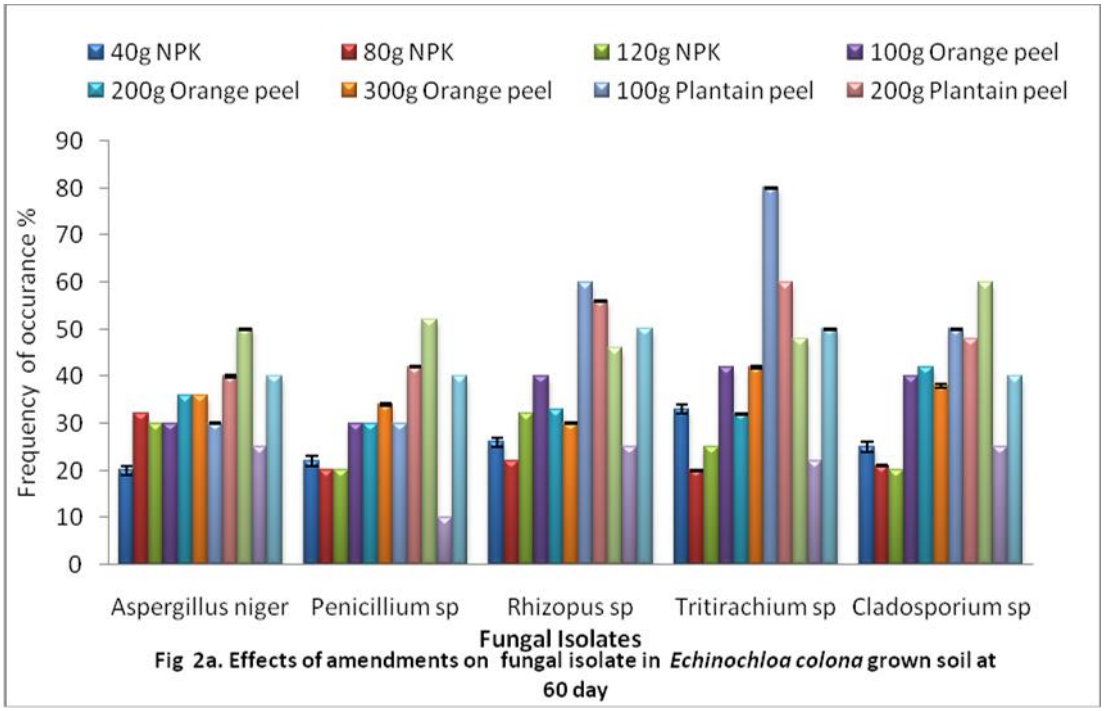


Fig 2a. Effects of amendments on fungal isolate in *Echinochloa colona* grown soil at 60 day

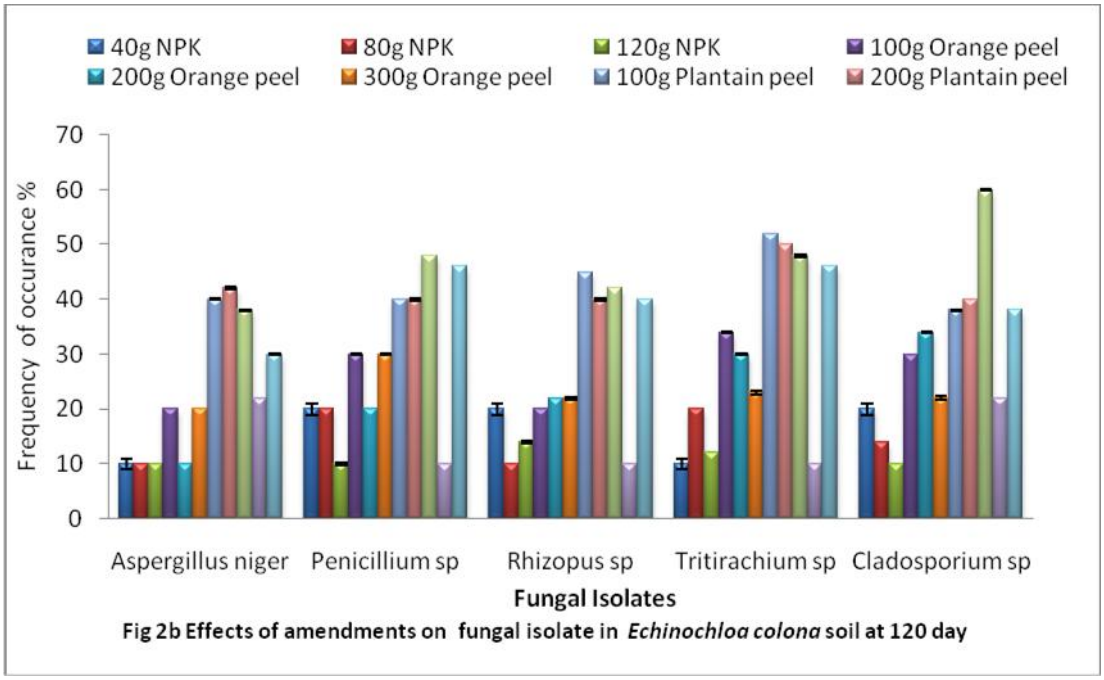
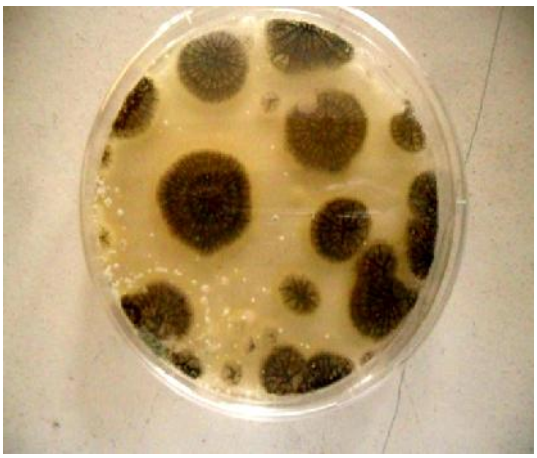


Fig 2b Effects of amendments on fungal isolate in *Echinochloa colona* soil at 120 day



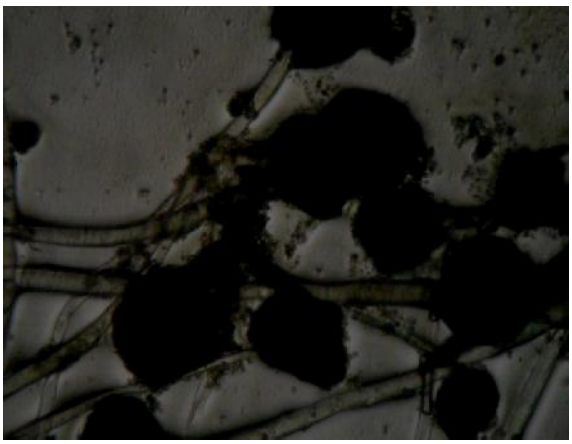
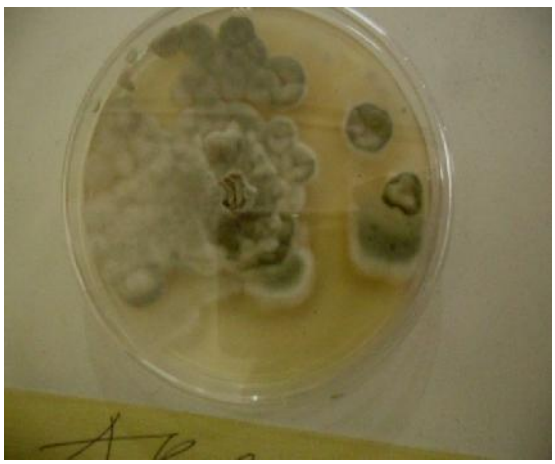


Plate 1: Macroscopic and Microscopic view of *Aspergillus niger*



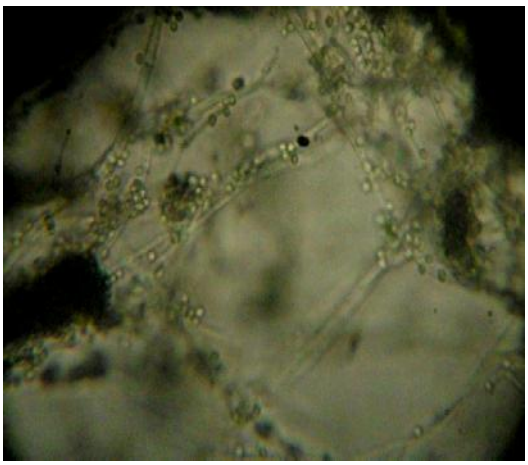


Plate 2. Macroscopic and Microscopic view of *Penicillium* sp

Plate 3: Macroscopic and Microscopic view of *Rhizopus* sp

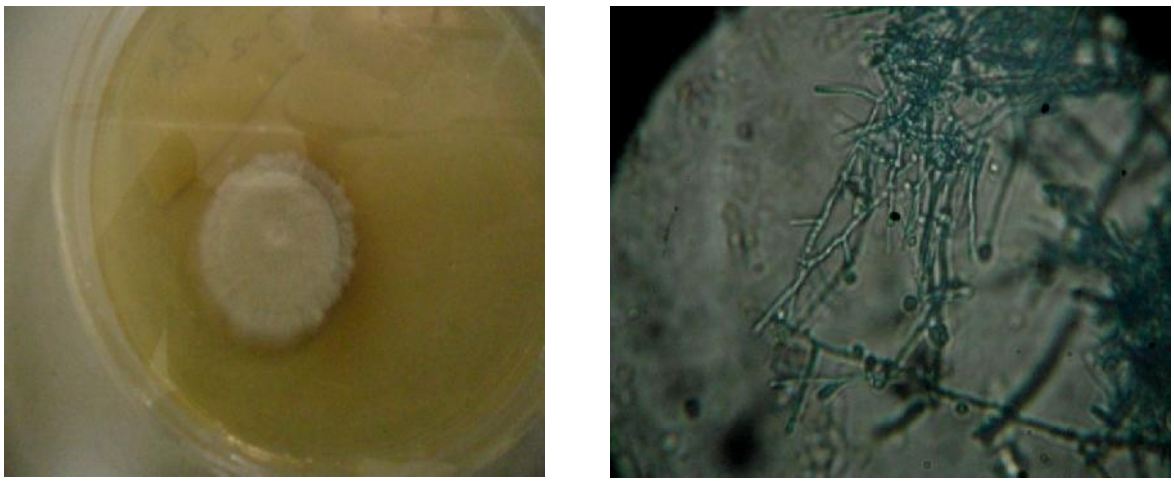


Plate 4: Macroscopic and Microscopic view of *Tritirachium* sp

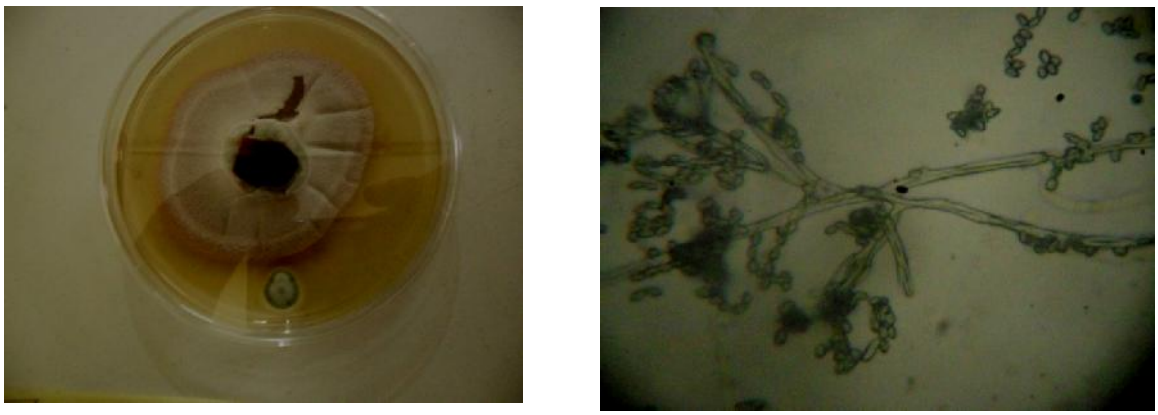


Plate 5: Macroscopic and Microscopic view of *Cladosporium* sp

Conclusion

The decrease in Total Heterotrophic Bacteria Count and the frequency of occurrence of isolated fungi across different soil amendments underscores the crucial role of organic matter in supporting microbial activity. Organic amendments such as plantain and orange peels provide essential nutrients and organic matter that foster microbial growth, while NPK fertilizers may have a limited effect on fungi that depend on organic substrates. The control soils, with minimal nutrient addition, show the lowest microbial activity, highlighting the importance of amendments in enhancing soil microbial health. In conclusion, the high microbial titre observed in the 300 g plantain peel soil amendment at 60 and 120 days can be attributed to the rich organic content of plantain peels. The combination of carbohydrates, proteins, lipids, trace elements, and the resulting improvement in soil structure creates an optimal environment for microbial growth and activity. This demonstrates the effectiveness of plantain peels as an organic amendment in promoting soil microbial health and enhancing soil fertility.

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