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Microbiological Analysis of Top Soil and Rhizosphere Treated with Organic Manure

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Abstract: Microbiological analysis of topsoil and rhizosphere treated with organic manure (Poultry droppings) was carried out. Soil samples were analyzed at three days interval (Day 1, Day 4, Day 7 and Day 10). The total bacterial count recorded for rhizosphere soil treated with poultry droppings (RS:P) had the highest bacterial count ranging between 2.1×10^6 and 5.7×10^6 CFU/g. Top soil treated with poultry droppings had total bacterial count ranging between 1.9×10^6 and 4.9×10^6 CFU/g. The controls (untreated rhizosphere soil) had a total bacterial count ranging between 2.6×10^6 and 4.0×10^6 CFU/g and untreated topsoil had bacterial count ranging between 1.4×10^6 and 2.4×10^6 CFU/g. Total fungal count for top soil treated with poultry droppings ranged between 0.2×10^6 and 0.9×10^6 CFU/g. Total fungal count for rhizosphere soil treated with poultry droppings ranged between 0.2×10^6 and 0.3×10^6 CFU/g. Untreated top soil had total fungal count ranging between 0.1×10^6 and 0.2×10^6 CFU/g while untreated rhizosphere soil had a total fungal count ranging between 0.1×10^6 and 0.2×10^6 CFU/g. Bacterial isolates identified with their percentage frequency of occurrence were Bacillus sp (16.8), *Enterococus* sp (8.4), *Clostridium* sp (4.0), *Staphylococcus* sp (8.0) *Pseudomonas* sp (15.6), *Listeria* sp (12.0), *Micrococcus* sp (14.0), *Serratia* sp (4.8) and *Streptococcus* sp (7.2). Fungal isolates identified with their percentage frequency of occurrence were *Rhizosphere* sp (26.7%), *Penicillium* sp (22.5%), *Aspergillus* sp (21.1%), *Mucor* sp (19.7%) and lastly *Cladosporium* sp (9.8%). Metabolites secreted by the root system act as chemical signal attracting high population of microorganisms. The application of organic manure to the soil enhanced the microbial population of the soil, hence the need to apply organic manure to soil to enhance agricultural sustainably.

Keywords: Soil, Microorganisms, Rhizosphere, Organic Manure

1. Introduction

Soil has been considered as the region on the crust of the earth where biologist and geologist find interest, the platform which provides a habitation for to plant, animals and microorganisms [1]. Soil is compost of microbial life (fungi, bacteria, viruses and protozoa) as well as macroscopic life such as mites, earthworm, nematodes and insects and plant root system. Environmental factors influence the number and type of microorganisms present in the soil. Such factors include; availability of nutrients, moisture content, pH, aeration, temperature etc.

Generally soil is a natural favorable habitat for the growth of microorganisms. Soil fungi may exist in mycorrhizal association with plant roots or may occur as free-living organisms. Microorganisms are found basically at the top 10cm of the soil. Fungi are not commonly found below 30cm soil depth. They are highly populated in aerated and acidic soil [2]. Most fungi grow and carry out metabolic activities under favourable environmental conditions which include; good aeration, good moisture level and presence of degradable substrates [3-4].

Microorganisms play a significant role in the maintaining the environment in its natural state, without microorganisms life would have been miserable. Plants are not able to utilize the needed nutrients without microorganisms breaking the complex

substrates into smaller component for plants to take up [5].

They are highly populated in the soil being that soil is a good habitat for their growth. Organic carbon is needed by most organisms for proliferation; they utilize this from wood, leaves and other organic matter. The rhizosphere also is a source of carbon because plant root system secrets substances needed by microorganisms.

Some bacteria and blue –green algae are microrganisms that can fix nitrogen from air and make it available to plants for utilization. The absence of some specific microbes like mycorrizal fungi can inhibit the growth of some plants and trees. Microorganisms play a significant role in the elimination of challenges associated with pesticides pollution and chemical fertilizers. Microorganisms play a vital role in nature farming and organic agriculture [6, 7]. Their contributions in agricultural sustainability are enormous.

2. Method

2.1. Sample Preparation

A thousand grams of top soil was weighed each and mixed with poultry dung in the following ratios; 1000g of top soil (S) with 100g of poultry dung (TS: P 10:1,) 1000g of top soil with 300g of poultry dung (TS:P 10: 3), 1000g of top soil with 500g of poultry droppings (TS:P 10: 5). Rhizosphere soil was also weighed and mixed with poultry droppings in the following ratios; 1000g of rhizosphere soil with 100g of poultry droppings (RS: P 10:1), 1000g of rhizosphere soil with 300g of poultry droppings (RS:P, 10: 3), 1000g of rhizosphere soil with 500g of poultry droppings (RS:P, 10:5). Top soil and rhizosphere soil not amended with poultry dropping were set up as control. Treated soil samples were thoroughly mixed and put in a perforated polythene bag and labeled properly and each was carried out in duplicate. The bags of samples were kept outside for one week before planting. Maize seeds (four) were planted in each bag for germination studies.

2.2. Microbiological Analysis

Samples (top soil and rhizosphere soil amended with poultry droppings and the controls) were taken at three (3) days interval for microbiological analysis. The emerging visible discrete colonies in the petri dishes were counted and expressed in colony forming units (CFU/g). Cultural, morphological and biochemical characterization of isolates were carried out using the method of [8, 9].

Characterization and identification of fungal isolates was done as pure fungal isolates were characterized and identified based on their morphological and cultural characteristic features as described by.

3. Results

3.1. Total Bacterial Count

Bacterial counts for soil amended with poultry sample TS: P, 100:10 ranged between 1.9×10^6 to 3.4×10^6 CFU/g. while for sample S: P 100:30 total bacterial count ranged between 2.1×10^6 to 3.8×10^6 CFU/g. Total bacterial count was higher for sample S: P 100:50 which ranged between 2.8×10^6 to 4.9×10^6 CFU/g (figure 1).

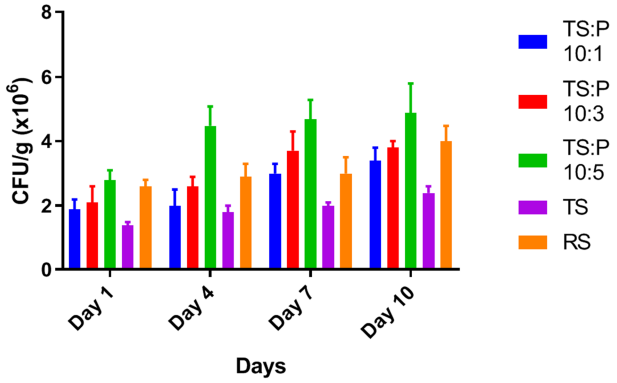

Figure 1. *Total Bacterial Count (TBC) of top soil amended with poultry droppings.*

Total bacterial count of rhizosphere with poultry dung sample RS: P, 100:10 ranged between 2.1×10^6 to 2.8×10^6 CFU/g, while RS: P, 100:30 ranged between 2.4×10^6 to 3.9×10^6 CFU/g. Total bacterial count for RS: P, 100:50 was higher with range between 4.8×10^6 to 5.7×10^6 CFU/g. Total bacterial count for soil without poultry dung (control) ranged between 1.4×10^6 to 2.4×10^6 CFU/g, while total bacterial count for rhizosphere ranged between 2.6×10^6 to 4.0×10^6 CFU/g (figure 2).

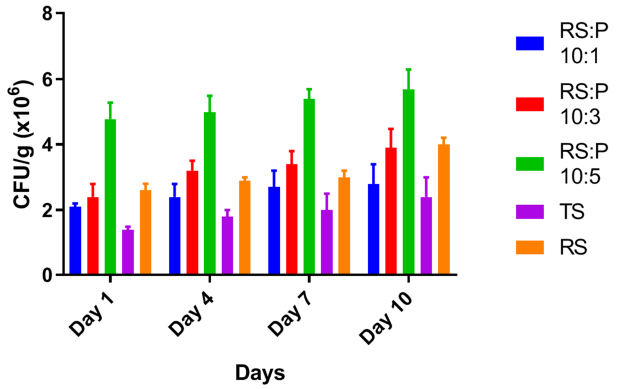

Figure 2. *Total Bacterial Count (TBC) of Rhizosphere soil amended with poultry droppings.*

3.2. Total Fungal Count

Total fungal counts for soil amended with poultry sample S:P, 100:10 and 100:30 ranged between 0.2×10^6 to 0.4×10^6 CFU/g. Total bacterial count was higher for sample S:P 100:50 which ranged between 0.3×10^6 to 0.5×10^6 CFU/g (figure 3).

Total fungal count of rhizosphere with poultry dung sample RS: P, 100:10 ranged between 0.3×10^6 to 0.5×10^6 CFU/g, while RS: P, 100:30 ranged between 0.3×10^6 to 0.6×10^6 CFU/g. Total bacterial count for RS: P, 100:50 was higher with range between 0.4×10^6 to 0.6×10^6 CFU/g. Total bacterial count for soil without poultry dung (control) ranged between 0.1×10^6 to 0.3×10^6 CFU/g, while total bacterial count for rhizosphere ranged between 0.2×10^6 to 0.3×10^6 CFU (figure 4).

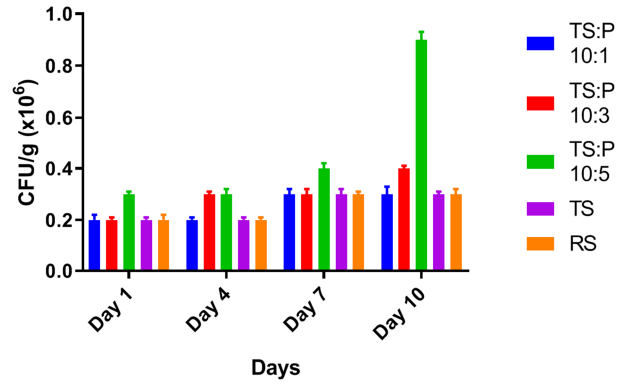

Figure 3. *Total Fungal Count (TFC) of top soil amended with poultry droppings.*

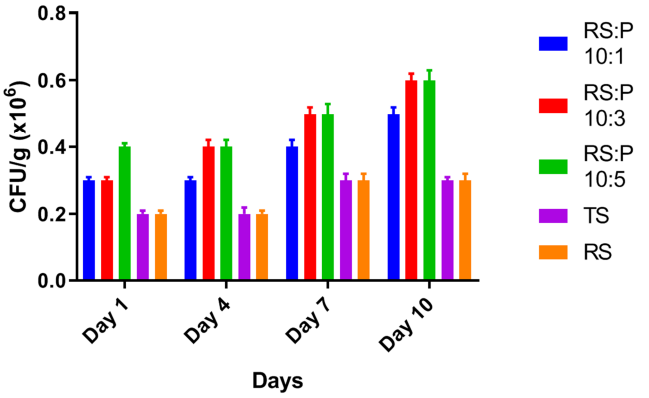

Figure 4. *Total Fungal Count (TFC) of Rhizosphere soil amended with poultry droppings.*

3.3. Characterization and Identification of Bacterial Isolates

The cultural, morphological biochemical characterization of bacterial isolates revealed the following bacterial isolates; *Bacillus* sp, *Enterococcus* sp, *Clostridium* sp, *Staphylococcus aureus*, *Listeria* sp, *Micrococcus* sp, *Serratia* sp, *Streptococcus* sp, *Pseudomonas* sp.

3.4. Characterization and Identification of Fungal Isolates

The cultural characterisitics of fungal isolates revealed the following fungal species; Aspergillus sp, *Rhizopus* sp, *Cladosporium* sp, and *Mucor* sp.

3.5. Percentage Frequency of Occurrence for Bacterial Isolates

The percentage frequency of occurrence for bacterial isolates revealed Bacillus sp the most occurring bacteria (16%) followed by *Pseudomonas* sp (15.6%), with *Micrococcus* sp (13.5%), *Listeria* and *Cladosporium* sp (12%), *Enterococcus* sp and *Staphylococcus* sp (3.6%) as shown in figure 5.

3.6. Percentage Frequency of Occurrence for Fungal Isolates

The percentage frequency of occurrence for fungal isolates revealed *Rhizopus* sp as the most occurring (26.7%) followed by *Penicillium* sp (22.5%), *Aspergillus* sp (21.1%), *Mucor* sp (19.7%) and *Cladosporium* sp (9.8%) as shown in figure 6.

Table 1. *Bacterial succession in soil samples amended with poultry droppings.*

Sample	Days			
	Day 1	**Day 4**	**Day 7**	**Day 10**
TS:P	Bacillus sp	Bacillus sp	Bacillus sp	Bacillus sp
10:1	Clostridium sp	Listeria sp	Clostrdium sp	Enterococcus sp
	Listeria sp	Staphylococcus sp	Pseudomonas sp	Micrococcus sp
TS:P	Listeria sp	Bacillus sp	Staphylococcus sp	Streptococcus sp
10:3	Staphylococcus sp	Clostridium sp	Bacillus sp	Listeria sp
	Pseudomonas sp	Enterococcus sp	Micrococcus sp	Micrococcus sp
TS:P	Clostridium sp	Pseudomonas sp	Micrococcus sp	Enterococcus sp
10:5	Staphylococcus sp	Clostridium sp	Listeria sp	Micrococcus sp
	Enterococcus sp	Bacillus sp	Pseudomonas sp	Listeria sp
RS:P	Staphylococcus sp	Staphylococcus sp	Enterococcus sp	Serratia sp
10:1	Clostridium sp	Micrococcus sp	Pseudomonas sp	Listeria sp
	Listeria sp	Listeria sp	Micrococcus sp	Clostridium sp
RS:P	Bacillus sp	Bacillus sp	Bacillus sp	Serratia sp
10:3	Micrococcus sp	Pseudomonas sp	Listeria sp	Micrococcus sp
	Listeria sp	Micrococcus sp	Micrococcus sp	Pseudomonas sp
RS:P	Pseudomonas sp	Pseudomonas sp	Streptococcus sp	Pseudomonas sp
10:5	Micrococcus sp	Micrococcus sp	Bacillus sp	Streptococcus sp
	Flavobacterium sp	Bacillus sp	Flavobacterium sp	Bacillus sp
TS	*Streptococcus sp*	*Streptococcus sp*	Bacillus sp	Serratia sp
	Micrococcus sp	Clostridium sp	Streptococcus sp	Micrococcus sp
	Pseudomonas sp	Bacillus sp	Pseudomonas sp	Bacillus sp
RS	Staphylococcus sp	Pseudomonas sp	Enterococcus sp	Serratia sp
	Bacillus sp	Micrococcus sp	Pseudomonas sp	Pseudomonas sp
	Pseudomonas sp	Bacillus sp	Micrococcus sp	Bacillus sp

Table 2. *Fungal succession in soil samples amended with poultry droppings.*

Sample	Days			
	Day 1	**Day 4**	**Day 7**	**Day 10**
TS:P	Rhizopus sp	Mucor sp	Mucor sp	Penicillium sp
10:1	Mucor sp	Rhizopus sp	Rhizopus sp	Cladosporium sp
	Penicillium sp			
TS:P	Mucor sp	Penicillium sp	Aspergillus sp	Rhizopus sp
10:3	Penicillium sp	Aspergillus sp	Rhizopus sp	Mucor sp
TS:P	Aspergillus sp	Cladosporium sp	Cladosporium sp	Mucor sp
10:5	Mucor sp	Penicillium sp	Penicillium sp	Aspergillus sp
RS:P	Penicillium sp	Rhizopus sp	Mucor sp	Cladosporium sp
10:1	Rhizopus sp	Aspergillus sp	Penicillium sp	Mucor sp
RS:P	Aspergillus sp	Penicillium sp	Rhizopus sp	Rhizopus sp
10:3	Rhizopus sp	Mucor sp	Penicillium sp	Aspegillus sp
RS:P	Rhizopus sp	Aspergillus sp	Rhizopus sp	Aspergillus sp
10:5	Muccor sp	Penicillium sp	Penicillium sp	Cladosporium sp
TS	*Rhizopus sp*	*Aspergillus sp*	Penicillium sp	Mucor sp
	Aspergillus sp	Mucor sp	Mucor sp	
RS	Aspergillus sp	Penicillium sp	Rhizopus sp	Penicillium sp
	Clasdosporium sp	Aspergillus sp	Cladosprium sp	Rhizopus sp

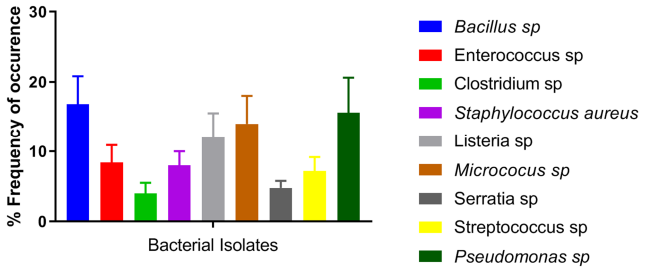

Figure 5. *Percentage frequency of occurrence of bacterial isolates in soils samples.*

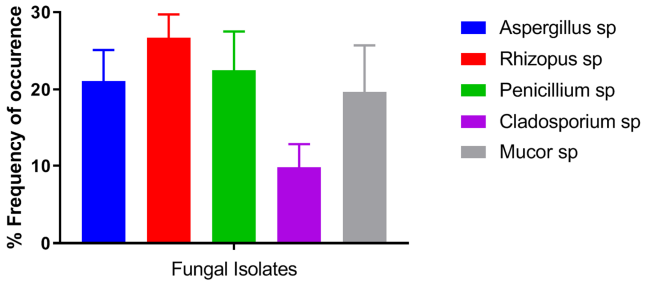

Figure 6. *Percentage frequency of occurrence of fungal isolates in soil samples.*

4. Discussion

The microbiological analysis of soil and rhizosphere treated with poultry manure for an interval of ten days revealed high microbial load in soil and rhizosphere samples treated with high concentration of poultry manure.

Sample S: P 10:5 had a bacterial load ranging between 2.8×10^6 to 4.9×10^6 CFU/g which showed the highest compared to soil samples treated with smaller concentration of poultry manure (figures 1-4). It was observed that bacterial count had a positive correlation with poultry manure added to the soil samples. Rhizosphere treated with high concentration of poultry manure, (RS;, 10:5) revealed high bacterial count compared to samples RS:P, 10:1 and RS:P, 10:3 which had less poultry manure treatment. RS: P, 10:5 recorded total bacterial count ranging between 4.8×10^6 to 5.7×10^6 CFU/g. This is because rhizosphere has been noted to be a competitive environment for diverse range of microorganisms that inhabit it. The metabolites secreted by plant roots act as a chemical signal for bacteria to actively move towards the root surface where they can obtain nutrients, therefore enhancing their growth and population [10]. The total bacterial counts of soil samples were observed to be on the increase till day 10; this is due to the presence of nutrients in the soil samples.

Bacterial species isolated from the soil samples were; *Bacillus* sp, *Enterococcus* sp, *Clostridium* sp, *Staphylococcus* sp, *Listeria* sp, *Micrococcus* sp, *Serratia* sp, *Streptococcus* sp and *Pseudomonas* sp. (table 1). This result is in accordance with the work done by Ogunmonyi et al., [11] on microbiological analysis of top soil selected sites in Obafemi Awolowo University, Nigeria. However, the variations of species differ from one another depending on the sample treatment and composition and also the days of interval. Most of the bacteria isolated in this study have been reported by others [12, 13, 1]. The presence of *Staphylococcus* sp was not a surprise as though this is not a bacterium found in the soil could be associated with poultry manure used in amending the soil samples. Its presence could therefore be as a result of contact of the soil sample with the poultry droppings.

Bacterial succession was observed during the study period. *Bacillus* sp, *Clostridum* sp and *Micrococcus* sp were predominant bacterial species and of high abundance within the study period for soil samples amended with poultry manure. *Psuedomonas* sp, *Micrococcus* sp and *Bacillus* sp were highly abundant bacterial species for rhizosphere soil amended with poultry manure. *Listeria* sp, *Staphylococcus* sp, *Streptococcus* sp and *Listeria* were observed in soil and rhizosphere soil samples ammeneded with poultry manure. This could be associated with the poultry droppings used in amending the soil samples.

The fungal count did not differ from the trend, soil and rhizosphere samples treated with high concentration of poultry manure (TS: P, 10:5 and RS: P, 10:5) showed higher

fungal load compared to samples treated with less poultry manure (figures 3 and 4). The control without poultry manure treatment had far less microbial counts. This result is in agreement with the work of O' Donnell et al., [14] who worked on the effect of organic manure on microbial growth and population of the soil and recorded higher bacterial counts with soils treated with organic manure.

Fungal species isolated and identified were; *Rhizopus* sp, *Aspergillus* sp, *Mucor* sp, *Penicillium* sp and *Cladosporium* sp. The presence of these fungal isolates in the soil samples was not a surprise as there are all soil fungi as reported by Oliveria et al., [15]. Fungal succession was observed during the ten days study period. Predominant fungal isolates were observed to be; *Rhizopus* sp, *Mucor* sp, *Penicillium* sp, *Aspergillus* sp and *Cladosporium* sp.

5. Conclusion

Bacteria and fungi occur in the rhizosphere which is the zone of soil surrounding a plant root where the biology and the chemistry of the soil are influenced by the root system. As plants grow through the soil they mostly release water soluble compound such as amino acid, sugar and organic acids that supplies food for the microorganisms. High level of exudates in the rhizosphere attract plethora of microorganisms to a large extend than elsewhere in the soil. The composition and pattern of root exudates affect microbial activities and the plant productivity depends on the natural conditions of the soil. Microbial population is therefore higher in the rhizosphere than in the soil and poultry manure amended soil harbors more microbial population than soils not treated with poultry manure. The role of microorganisms in soil fertility and plant growth cannot be over emphasized. This work has clearly shown that rhizosphere harbor more microbes than the top soil. Although the significant role of the rhizosphere microbiome in the growth of plant has been generally recognized, there is little knowledge of the roles of this vast community of microbes to the plant. It is there important to understand which microbe is present in the rhizosphere and their respective roles. This work therefore sets the stage for further investigation into the roles of rhizosphere microbiome in soil and plant growth.

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