Microbiological Analysis of Top Soil and Rhizosphere Treated with Organic Manure

Aniefon Alphonsus Ibuot*, Iniobong Ime James, Mayen Godwin Ben, Christiana Utibe Etuk, Agnes Monday Jones, Emmanuel Anthony Umoren, Elizabeth Lazarus Akpan, Esther Ndarake Akpan

Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Nigeria

Email address:

anialphonsus@yahoo.com (A. A. Ibuot)

*Corresponding author

To cite this article:

Aniefon Alphonsus Ibuot, Iniobong Ime James, Mayen Godwin Ben, Christiana Utibe Etuk, Agnes Monday Jones, Emmanuel Anthony Umoren, Elizabeth Lazarus Akpan, Esther Ndarake Akpan. Microbiological Analysis of Top Soil and Rhizosphere Treated with Organic Manure. *Advances in Bioscience and Bioengineering*. Vol. 7, No. 2, 2019, pp. 27-31. doi: 10.11648/j.abb.20190702.13

Received: June 6, 2019; Accepted: July 12, 2019; Published: July 26, 2019

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), *Enterococcus* 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 secretes substances needed by microorganisms.

Some bacteria and blue –green algae are microorganisms that can fix nitrogen from air and make it available to plants for utilization. The absence of some specific microbes like mycorrhizal 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 characteristics 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 10:1	<i>Bacillus</i> sp	<i>Bacillus</i> sp	<i>Bacillus</i> sp	<i>Bacillus</i> sp	
	<i>Clostridium</i> sp	<i>Listeria</i> sp	<i>Clostridium</i> sp	<i>Enterococcus</i> sp	
	<i>Listeria</i> sp	<i>Staphylococcus</i> sp	<i>Pseudomonas</i> sp	<i>Micrococcus</i> sp	
TS:P 10:3	<i>Listeria</i> sp	<i>Bacillus</i> sp	<i>Staphylococcus</i> sp	<i>Streptococcus</i> sp	
	<i>Staphylococcus</i> sp	<i>Clostridium</i> sp	<i>Bacillus</i> sp	<i>Listeria</i> sp	
	<i>Pseudomonas</i> sp	<i>Enterococcus</i> sp	<i>Micrococcus</i> sp	<i>Micrococcus</i> sp	
TS:P 10:5	<i>Clostridium</i> sp	<i>Pseudomonas</i> sp	<i>Micrococcus</i> sp	<i>Enterococcus</i> sp	
	<i>Staphylococcus</i> sp	<i>Clostridium</i> sp	<i>Listeria</i> sp	<i>Micrococcus</i> sp	
	<i>Enterococcus</i> sp	<i>Bacillus</i> sp	<i>Pseudomonas</i> sp	<i>Listeria</i> sp	
RS:P 10:1	<i>Staphylococcus</i> sp	<i>Staphylococcus</i> sp	<i>Enterococcus</i> sp	<i>Serratia</i> sp	
	<i>Clostridium</i> sp	<i>Micrococcus</i> sp	<i>Pseudomonas</i> sp	<i>Listeria</i> sp	
	<i>Listeria</i> sp	<i>Listeria</i> sp	<i>Micrococcus</i> sp	<i>Clostridium</i> sp	
RS:P 10:3	<i>Bacillus</i> sp	<i>Bacillus</i> sp	<i>Bacillus</i> sp	<i>Serratia</i> sp	
	<i>Micrococcus</i> sp	<i>Pseudomonas</i> sp	<i>Listeria</i> sp	<i>Micrococcus</i> sp	
	<i>Listeria</i> sp	<i>Micrococcus</i> sp	<i>Micrococcus</i> sp	<i>Pseudomonas</i> sp	
RS:P 10:5	<i>Pseudomonas</i> sp	<i>Pseudomonas</i> sp	<i>Streptococcus</i> sp	<i>Pseudomonas</i> sp	
	<i>Micrococcus</i> sp	<i>Micrococcus</i> sp	<i>Bacillus</i> sp	<i>Streptococcus</i> sp	
	<i>Flavobacterium</i> sp	<i>Bacillus</i> sp	<i>Flavobacterium</i> sp	<i>Bacillus</i> sp	
TS	<i>Streptococcus</i> sp	<i>Streptococcus</i> sp	<i>Bacillus</i> sp	<i>Serratia</i> sp	
	<i>Micrococcus</i> sp	<i>Clostridium</i> sp	<i>Streptococcus</i> sp	<i>Micrococcus</i> sp	
	<i>Pseudomonas</i> sp	<i>Bacillus</i> sp	<i>Pseudomonas</i> sp	<i>Bacillus</i> sp	
RS	<i>Staphylococcus</i> sp	<i>Pseudomonas</i> sp	<i>Enterococcus</i> sp	<i>Serratia</i> sp	
	<i>Bacillus</i> sp	<i>Micrococcus</i> sp	<i>Pseudomonas</i> sp	<i>Pseudomonas</i> sp	
	<i>Pseudomonas</i> sp	<i>Bacillus</i> sp	<i>Micrococcus</i> sp	<i>Bacillus</i> 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	Aspergillus sp
RS:P	Rhizopus sp	Aspergillus sp	Rhizopus sp	Aspergillus sp
10:5	Mucor 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
	Cladosporium sp	Aspergillus sp	Cladosporium 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, *Clostridium* sp and *Micrococcus* sp were predominant bacterial species and of high abundance within the study period for soil samples amended with poultry manure. *Pseudomonas* 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 amended 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

fungus 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 extent 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.

References

- [1] Pelczar, M. J., Chan, E., Krieg, N. R. (2001). Microbiology: Concept and Application. International Edition McGraw- Hill USA Pp 281.
- [2] Domsch, K. H., Gawas, W., Anderson, T. H. (2003). *Compendium of soil fungi*. London Academic Press 859-860.
- [3] Ferando HC, Amanda V, Wright JS (1994). Tropical forest litter decomposition under seasonal drought nutrient release, fungi and bacteria. *Oikos*. (70): 183-190.
- [4] Miyanoto T, Igaraslic T, Takahashi K (2002). Lignin-degradation ability of litter decomposing basidiomycetes from picea forest of Hokkaida *Myco. sci.* (41): 105-110.
- [5] Ibut, A. A. and Bajhaiya, A (2013). Biodegradation of crude oil sludge using municipal solid waste as bulking agent. *Asian Journal of Biological Sciences* 6 (4) 207-213.
- [6] Higga, T. (2001). Effect Microorganism: A biotechnology for mankind U.S. Department of Agriculture USA (16).
- [7] Parr, J., Hornick, S. And Kaufmman, D. (2004) Use of microbial inoculants and organic fertilizers in Agricultural production Published by the food and fertilizer technology center Tapei 91 (32).
- [8] Cheesbrough, M. (2006). *District Laboratory practice in tropical countries* (part 2) Cambridge University Press. Hon Kong. Pp 64-70.
- [9] Bergey (1989). Bergey's manual of systematic bacteriology Sterley JT (Ed). Vol. 3 Williams and Eilkins, Baltimore. p. 450.
- [10] Nihorimbere, V., Ogena, M., Thonart, P. (2016). Beneficial effect of the Rhizosphere microbial community for plant growth and health. *BASE* 15: 2.
- [11] Dundas, E., Paul, O., John, H. (2002). *Mashavira is dated the Jains* London ISBN 0-415.
- [12] Amir, H., Pineau R., (1998). Influence of plant and cropping on microbiological characteristics of some new Caledonian ultamatic soils. *Aust. J. Soil Res.* 36, 3: 457-470.
- [13] Okoh, L. A., Badejo, M. A., Nathaniel, I. T., Tian G. (1999). Studies on the bacteria, fungi, and springtails (Collembola) of an agroforestry arboretum in Nigeria. *Pedobio* 43: 18-27. Olivier, R., Bacchin, P., Robertiello, A., Oddo, N., Degen, L., And Tonolo, A., 2006. Microbial degradation of soil spills enhanced by a slow release fertilizer. *App. Environ. Microbiol.* 31: 629-634.
- [14] O' Donnell AG, Seasman M, Macrae A, Waite I, Davies JT (2001). Plants and Fertilizers as drivers of change in microbial community structure and function in soil. *Plant Soil* (232): 135-45.
- [15] Olivier, R., Bacchin, P., Robertiello, A., Oddo, N., Degen, L., And Tonolo, A., 2006. Microbial degradation of soil spills enhanced by a slow release fertilizer. *App. Environ. Microbiol.* 31: 629-634.