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Assessment of microbial communities Encountered in soils with organic and inorganic fertilizers in Afaka area of Kaduna state, Nigeria.

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Abstract
Soil microbial communities are arguably the most diverse communities on earth and soil represents a favorable habitat for micro-organisms. In the present investigation, the extent of the diversity of micro-organisms in organic soil was seen as enormous comparatively in inorganic soil. Soil was collected by simple random sampling directly from Afaka farm, Kaduna State, Nigeria. Samples were processed using a soil dilution plate method. The results showed that the abundance of different microbial groups and total microbial biomass was generally increased in soil with organic fertilizer in comparison to soil with inorganic fertilizer. It was found that the soil with organic manures was rich in bacteria diversity (168 colonies), fungal diversity (25 colonies) and other micro-organisms. Comparatively, in the soil with inorganic fertilizer lower colonies of bacteria (80) and fungi (13) were observed. The total population colonies of micro-organisms obtained in soil with organic fertilizer was 293 while the soil with inorganic being 93. Statistically, there was a significant difference ($P \leq 0.05$) between the microbial populations of the two soil types. The most widely distributed and abundant colony forming taxa were *Penicillium* Spp. *and Aspergillus* Spp (13 colonies) in both soil sample fields. Thus organic fertilizers from this study show a greater microbial diversity when compared with the field sample with inorganic fertilizer. This means more decomposition, biotrans formation, more nitrogen content and higher fertility in the organically fertilized soil samples.

Keywords: bacteria, fungi, inorganically fertilized field, organically fertilized field soil.

1. Introduction
Soil represents a favorable habitat for micro-organisms and is inhibited by a wide range of micro-organisms. Micro-organisms are found in large number in soil usually between one to ten million micro-organisms are present per gram of soil with a dominant number of bacteria and fungi. Soil organisms contribute to important soil function such as food, fiber, energy. They absorb, neutralize and transform compounds which could otherwise become pollutants in the environment. Soil micro-organisms are very important as almost every chemical transformation taking place in soil involves active contribution of these microbes. Soil microbial diversity is influenced by both organic and inorganic matter. Soil organic matter is generally used to represent organic constituents in the soil, excluding undecayed plants and animal tissue, their partial decomposition products and the soil biomass [12]. The soil organic matter provides a favorable habitat for the micro-organisms to grow as compared to inorganic soil. The bacterial diversity present in the soil is greatly influenced by organic matter. It has been consistently reported that soil organic matter favors the growth of bacteria present in the soil. Studies have revealed that bacteria diversity is approximately one hundred times greater than the other microbial diversity [6]. Bacteria are one of the most important components of the soil micro biota and don't occur freely in the soil solutions, but are closely embedded in organic matter even after acting as the dispersing agents [1]. Moreover, they play a major role in organic matter decomposition, biotransformation, biogas production and nitrogen fixation. In particular, they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like potassium, phosphorus, and nitrogen, which are required for plant growth [13].
In most of the aerated and cultivated soil, fungi share a major part of the microbial biomass. Many important plant pathogens and plant growth promoting micro-organisms are fungi.

Fungi are also decomposers in the soil habitat like bacteria; fungi derive nutrients for their growth from organic matter [9]. The rest being Actinomycetes, protozoa, algae and many other which also constitute the microbial diversity of the soil. Microbial biomass in the soil display a positive linear relationship with annual net primary productivity, demonstrating that the growth of micro-organisms and of crops can be controlled and influenced by using organic or inorganic fertilizer [29].

Fertilizer is any organic or inorganic material of natural or synthetic origin (other than liming materials) that is added to a soil to supply one or more plant nutrients essential for growth of plants. Conservative estimates, reports 30 to 50% of crop yields are attributed to natural or synthetic commercial fertilizer. Mined inorganic fertilizers have been used for many centuries, whereas chemically synthesized inorganic fertilizer were only widely developed during the industrial revolution.

Inorganic soil have less microbial diversity which proved that some bacterial or fungi species may be found in inorganic fields, but not able to use probably the microclimate or micro nutrients as can be used by organic microbes. The biggest issue facing the use of chemical fertilizers is ground water contamination. Nitrogen fertilizers break down into nitrates and travel easily into the soil. Because it is water soluble and can remain in ground water for decades, the addition of more nitrogen over the years has accumulative effects. Unfortunately, as unsustainable farming and gardening practices continue, our soil is stripped of it health's and we depend on amendments even greater. This leads to some negative effects of chemical fertilizers. Nitrogen fertilizers break down into nitrate upon application.

Organic fertilizers have been known to improve the biodiversity of soil life and long-term productivity of soil and may prove a large depository for excess carbon-dioxide. Organic nutrients increase the abundance of soil organisms by providing organic matter and micro-nutrients for organisms such as fungal mycorrhiza, (which aids plants in absorbing nutrients), and can drastically reduce external inputs of pesticides, energy and fertilizer, at the cost of decreased yield. Soil fertilized with organic fertilizer contains naturally occurring microbes that turn excess nitrogen into a benign gas, dinitrogen. These microbes are found less frequently and were less active in soil fertilized with chemicals. The more leaching of nitrogen, the greater application is applied, the more microbes and the more poisoning of land

Traditionally, the analysis of soil microbial communities has applied on culturing techniques using a variety of culture media designed to maximize the recovery of different microbial species. This is particularly the case for soil health studies. There are numerous examples where these techniques have revealed a diversity of micro-organisms associated with various soil quality parameters such as disease, oppression and organic matter decomposition [3, 8, 11, 19, 21]. Although there have been recent attempts to device suites of culture media to maximize the recovery of diverse microbial groups from soils [5, 22]. It has been estimated that less than 0.1% of the micro-organisms found in typical agricultural soils are cultural being current media formulations [2, 27]. This is based on comparison between direct microscopic counts of microbes and samples recoverable colony forming units. Microbial characteristics of soils are being evaluated.

Yields are attributed to natural or synthetic commercial fertilizer. Mined inorganic fertilizers have been used for many centuries, whereas chemically synthesized inorganic fertilizer was only widely developed during the industrial revolution. Organic fertilizer includes naturally occurring organic materials, (e.g. chicken liter, manure, worm casting, seaweed, guano, bone meal) or naturally occurring minerals deposited (e.g. saltpeter). Organic fertilizers provided macro or micro plant nutrients and are released as the organic matter decays this may take months or years, organic fertilizer have been known to improve biodiversity and long term productivity of soil [7, 14] and may improve a large depository for excess carbon-dioxide. [15, 16, 25]. Inorganic fertilizer always nearly are readily dissolved and sunless neither added have few other micro and macro plant nutrients nor added any bulk to the soil. Inorganic fertilizers are usually much more concentrated with up to 64% (18-46) of their weights being a given plant nutrients, compared to organic fertilizers that only 0.4% or less of their weight as a given plant nutrient. Combined nitrogen (N), phosphorus (P), and potassium (K) will produce, plants nutrients in easily dissolved form.

1.1 Organic fertilizers

Organic fertilizers have been known to improve biodiversity and long-term productivity of soil, [7, 14] and may prove a large depository for excess carbon-dioxide [16, 15, 25]. Organic nutrients increase the abundance of soil organisms by providing organic matter and micronutrients for organisms such as fungi mycorrhiza, (PIMENTEL, *et al*, 2005). (Which aid plants in absorbing nutrients), and can drastically reduce external inputs of pesticides, energy and fertilizer, at the cost of decreased yield [20]. In general, the nutrients in inorganic fertilizer are both more diluted and also much less readily available to plants. According to the University of California's integrated pest management program, all organic fertilizers are classified as 'slow release' fertilizers, and therefore cannot cause nitrogen to burn [18]. Organic fertilizers from composts and other sources can be quite variable from one batch to the next without batch testing, the amount of applied nutrients cannot be precisely known. Nevertheless, one or more studies have shown they are at least as effective as chemical fertilizers over long periods of use.

1.2 Inorganic fertilizer

Inorganic fertilizers nearly always are readily dissolved and unless neither added have few other micro and macro plant nutrients nor added any 'bulk to the soil'. Nearly all nitrogen that plants use is in the form of (NH_3) or (NO_3) compounds. The usable phosphorus compounds are usually in the form of phosphoric acid (H_3PO_4) and potassium (K) is typically in the form of potassium chloride (KCl). In organic fertilizers, nitrogen, phosphorus and potassium compounds are released from the complex organic compounds as the animal or plant matter decays. In commercial fertilizers, the same required compounds are available in easily dissolved compounds that require no decay-they can be used almost immediately after water is applied. Inorganic fertilizers are usually much more concentrated up to 64% of their weight being a given plant nutrient, compared to organic fertilizers that only provide 0.4% or less of their weights as a given plant nutrient(NPK, 2012). The use of inorganic fertilizers has increased steadily in the last 50 years, rising almost 20-fold to the current rate of 100 milion tones of nitrogen per year [17]. Without commercial fertilizers, it is estimated that about one-third of the food produced now could not be produced (Commercial Fertilizers, 2012). The use of phosphate fertilizers has also increased from 9 million tons per year in 1960 to 40 million per year in 2000.

A maize crop, yielding 6-9 tons of grain per hectare requires 31-50 kg of phosphate fertilizer to be applied, soybeans requires 20-25 kg per hectare. Yara international is the world's largest producer of nitrogen based fertilizers.

1.3 Over fertilization
Over-fertilization of vital nutrients can be as detrimental as under fertilization (Nitrogen fertilization, 2012). 'Fertilizer burn can occur when too much fertilizer is applied, resulting in drying out of the leaves and damage or even death of the plant (Avoiding fertilizer urn, 2012). Fertilizers vary in their tendency to burn roughly in accordance to their salt index ("Understanding salt index, 2012").

1.4 Soil acidification
Also regular use of acidulated fertilizers generally contribute to the accumulation of soil acidity in soil, which progressively increases aluminum availability and hence toxicity. The use of such acidulated fertilizers in the tropical and semi-tropical regions of Indonesia and Malaysia has contributed to soil degradation on a large scale from aluminum toxicity, which can only be cantered by application of limestone or preferably magnesium dolomite, which neutralizes acid soil Ph and also provide essential magnesium. Nitrogen-containing organic and inorganic fertilizers can cause soil acidification when added. This may lead to decreases in nutrients availability which may be offset by liming [26]. Effectively, farmers unknowingly became 100% dependent on 'bought in water soluble, inorganic fertilizer since the sterilization of soil microflora including its mycorrhiza, reduced the availability of other natural and trace minerals within the soil. Fertilizers are broadly divided in to organic fertilizers (composed of organic plant or animal matter), or inorganic or commercial fertilizers. Plants can only absorb their required nutrients if they are present in easily dissolved chemical compounds. Both organic and inorganic fertilizers provide the same needed chemical compounds. Organic fertilizers provided other micro and macro plants nutrients and are released as the organic matter decays this may take months or years. Organic fertilizers nearly always have much lower concentration of plants nutrients and have the usual problem of economic collection, treatment, transportation and distribution. Organic fertilizers include naturally occurring organic materials (e.g. chicken liter, manure, worm casting, seaweed, guano, bone meal), organic fertilizers have been known to improve biodiversity and long-term productivity of soil [7, 14] and may improve a large depository for excess carbon-dioxide [15, 16, 25]. Inorganic fertilizers nearly always is readily dissolved and unless neither added have few other macro and micro plants nutrients nor added any bulk to the soil. Inorganic fertilizers are usually much more concentrated with up to 64% (18-46-0) of their weight being a given plant nutrient, compared to organic fertilizers that only provide 0.4% or less of their weight as a given plant nutrients (NKP ratios 2012).

Combined nitrogen (N) phosphorus (P) and potassium (K) will produce, plant nutrients in easily dissolve form (Potash, 2012).

1.5 Statement of the problem
Inorganic soil has less microbial diversity which proved that some bacterial or fungi species may be found in inorganic fields, but not able to use probably the microclimate or micro nutrients as can be used by organic microbes. The biggest issue facing the use of chemical fertilizers is ground water contamination. Nitrogen fertilizers break down into nitrates and travel easily into the soil. Because it is water soluble and can remain in ground water for decades, the addition of more nitrogen over the years has accumulative effects. Unfortunately, as unsustainable farming and gardening practices continue, our soil is stripped of it health's and we depend on amendments even greater. This leads to some negative effects of chemical fertilizers. Nitrogen fertilizers break down into nitrated upon application. Nitrates are necessary for the plants growth, but an excess will lead into ground water supplies and can contaminates sources miles away. Because nitrogen leaches through the wall more quickly, over application is abundant. These chemical fertilizers have hugely negative effects on plants and aquatic life, as well as human life.

1.6 Justification
Organic fertilizers have been known to improve the biodiversity of soil life and long-term productivity of soil and may prove a large depository for excess carbon-dioxide. Organic nutrients increase the abundance of soil organisms by providing organic matter and micro-nutrients for organisms such as fungal mycorrhiza, (which aids plants in absorbing nutrients), and can drastically reduce external inputs of pesticides, energy and fertilizer, at the cost of decreased yield. Soil fertilized with organic fertilizer contains naturally occurring microbes that turn excess nitrogen into a benign gas, dinitrogen. These microbes are found less frequently and were less active in soil fertilized with chemicals. The more leaching of nitrogen, the greater application is applied, the more microbes and the more poisoning of land and water

The investigate micro-organisms found in farm land using organic and inorganic fertilizer in Afaka area of Kaduna State, Nigeria.
Determine the micro-organism content in the soil as well as Isolation, identification and comparison of micro-organisms associated with organic and inorganic fertilizer.

2. Materials and Methods
2.1 Study area
The study was conducted in Afaka farm, Kaduna. Average temperature of Kaduna is about 23 ^{0}C and the town is located at latitude 10' 25"N to 10' 37"N and longitude 7' 24"E to 7' 30"E. The farm is located in an Igabi Local Government area of the Kaduna metropolis in the settlement of some people around. Afaka farm is about 20 km away from Kaduna city centre and managed by Igabi Local Government Authority.

2.2 Collection of samples
Soil samples were collected by sterile methods from organic and inorganic soils and brought to the laboratory in the air tight polybags.

2.3 Laboratory analysis of samples
2.3. a. Isolation of bacteria
Soil samples were collected by sterile methods from organic and inorganic soils and brought to the laboratory in the air tight polybags. The vertical samples were taken from 5 and 10 cm depths. The samples were processed using a soil dilution plate method. One gram of soil sample were serially dilute with sterile water up to [10-1, 10-2, 10-3, 10-4] and 100 ml of each dilution was added to 20 ml of nutrients agar medium in 90 mm diameter sterile Petri dishes and then enumerated. Simple

separate colonies on the agar plates were selected at random according to standard medium and streaked on the nutrients agar slants and incubated for 24 hrs at ± 30 ^{0}C. Code names were given to each of the isolated plate and stored at ± 40 ^{0}C for characterization and identification by standard methods. Once colonies rose on the media, the subculturing was continued until a pure isolate was obtained. Identification of microbes was done with the help of standard literature. For isolation of bacteria, different media like nutrients agar medium, nutrient broth medium etc. (HI) media were prepared and to differentiate between gram +ive (Positive) and gram –ive (Negative) bacteria gram's staining was done.

2.3. b. Fungal Isolation

The soil borne fungi was isolated and their total population was enumerated by following the method as given below:

First, soil samples were collected from both the organic and inorganic soils, then 3 flasks (250 ml) were taken and 90 ml distilled water was transferred into each flask. Each flask was plugged properly, labeled 1-3 and autoclaved at 151b/inch^2 for 30 minutes. 1gm of soil sample was weighed and transferred into the flask 1 containing 90 ml. it gives the dilution 1:1 i.e 10-1 ml, And then it was shaken for 5minutes gently with a stirrer to get a homogeneous soil suspension. 0.1 ml soil suspension was transferred to dilution into flask 2 containing 90 ml distilled water to get dilution and then mixed gently. Similarly, 1 ml of soil suspension was serially transferred from dilution into flask 3 containing 90 ml water to get the final dilution of and mixed it gently. 1ml of soil suspension was aseptically poured from dilution in different media plate. The plates were gently rotated so as to spread the suspension on medium. The plates were incubated at ± 25 ^{0}C for 4-5 days. Different media from potato agar medium, Marthin's Rose Bengal medium, etc. were prepared for isolation of fungi Lactophenol cotton blue stain was stain was used also called as mounting fluid. The slides were observed under the microscope and fungi were identified by following the mycological literature

3. Results and Discussion

Table 1: Occurrence of fungal colonies in serial dilution in both organic and inorganic soils.

	Organic soil	Inorganic soil
Dilution	**No. of colonies in serial dilution**	**No. of colonies in serial dilution**
10^1	15	7
10^2	7	4
10^3	3	2
10^4	-	-
Total colonies	25	13

Table 2: Percentage of occurrence of various fungus species colonies in examined soil.

Serial No.	Fungi from organic soil	Fungi from organic soil	Fungi from organic soil	Fungi from inorganic soil	Fungi from inorganic soil	Fungi from inorganic soil
	Species	**No. of colonies**	**Occurrence %**	**Species**	**No. of colonies**	**Occurrence %**
1	*Aspergillus ramosa*	3	12	Not found	-	0
2	*A. niger*	5	20		3	23.1
3	*A.candidus*	2	8	-	1	7.7
4	*M. mucedo*	4	16	-	2	15.4
5	*Penicillum rubrum*	5	20	-	4	30.8
6	*P.puberrulum*	3	12	-	1	7.6
7	*Rhizopus oryzae*	3	12	-	2	15.4
Total		25			13	

Table 3: Occurrence of bacteria colonies in serial dilution in both organic and inorganic soils.

	Organic soil	Inorganic soil
Dilution	**No. of colonies in serial dilution**	**No. of colonies in serial dilution**
10^{-1}	84	37
10^{-2}	60	33
10^{-3}	17	9
10^{-4}	7	1
Total	168	80

Table 4: Percentage of occurrence of various bacteria in both organic and inorganic soil

	Bacteria from organic soil			bacteria from inorganic soil		
Serial No.	**Species**	**Col**	**Occurrence %**	**Species**	**Col**	**Occurrence %**
1	*Pseudomonas*	50	41.66		40	50
2	*Pseudomonas*	10	7.69		-	-
3	*Bacillus spp.*	38	18.75		25	31.25
4	*E. coli*	25	14.88		-	-
5	*Streptomyces spp.*	22	13.09		15	18.75
6	*Flavobacterium spp.*	13	7.73		-	-
Total		168			80	

The serial dilution method was followed to determine the microbial diversity of soils. The identification of this isolates resulted in 12 species of microbes, including bacteria (5 species), fungi (7 species). The genera with the greater number species in fungi were *Aspergillus* (3 species), and *Penicillium* (2 species) in the serial dilution plate method. The most widely distributed and abundant colony forming taxa were *Penicillium* (9 colonies), *Aspergillus* 13 colonies) in both soil sample fields. The richest genera in terms of the number of species were *Aspergillus* and *Penicillium.* The six fungi species were isolated and identified from organic and inorganic fields. Two species belonged to genus *Penicillium,* three to *Aspergillus* and one specie belonged to each genus of *Rhizopus, Mucor.* In bacteria the results showed that the number of colonies was found higher in organic field (168 colonies) in comparison to inorganic field (80 colonies). The study also showed that bacteria colonies grown on potato dextrose agar than fungal colonies which proved the earlier research that bacteria produce different kinds of enzymes, which inhibit the other fungi species in soil whether that are useful or pathogenic to the crops. Pseudomonas and Bacillus genera were found in both organic and inorganic fields and were proved higher in species richness among other bacterial species. This agrees with the work of Atlas *et al* (2000) [1]. This data proved that both the bacteria Geneva were able to tolerate adverse microclimate in soil and decompose organic materials and can synthesize inorganic minerals. Moreover, they can sporulate properly and could help in making soil more nutritive with the help of different types of enzymes produced by them. On the other hand, *Streptomyces* Spp., *Flavobacterium Spp.* and *Escherichia coli* were not isolated from inorganic soil samples may be due to their non-occurrence in these types of soil or could not rose in used culture medium. The isolation of various fungal and bacterial species showed that the soil of organic filed is quite rich in microbial flora. This aggress with the work of Bernard *et al.* (2007) who explained that bacterial diversity is approximately 100 times greater than the other microbial diversity. Atlas *et al* also explained that bacterial are one of the most important components of the soil microbiota. A total of 22 colonies of fungi and 168 colonies of bacteria were isolated from organic soil, while a total of 11 colonies of fungi and 80 colonies of bacteria were isolated from inorganic fields. The results showed that the soil was rich in bacterial diversity (248 colonies) comparatively to fungi diversity (33 colonies), however species richness was rich in fungi as eight genera comprising of 8 species were found in both types of soils, while only 3 genera comprising of 8 bacterial species has been found. Species of bacteria and 6 species of fungi were reported which is nourished with chemical fertilizers. Among 8 species of fungi, 6 species were isolated from both organic and inorganic fields while 5 species could not be found in inorganic field. The occurrence of many of the species in genus *Aspergillus* (3) and *Penicillium* (2) are probably due to their capability of producing a diverse range of antibiotics and mycotoxins which protect them from other soil organisms and may also hinder the growth of other fungi species EL Frantroussi *et al*. (2005) also agrees with the importance of the microbes in the soil. He explained that they play a major role in organic matter decomposition, biotransformation, biogas product and decrease in population of microbes in organically fertilized soils may be due to some reasons which include over fertilization, “fertilizer burn” in accordance with salt index (Understanding soil index, 2012). Also regular use of acidulated fertilizers generally contributes to the accumulation of soil acidity in soil, which progressively increases aluminum availability and hence toxicity and death of soil microbes [26]. It is acknowledged now that farmers are becoming 100% dependent on water soluble in organic fertilizers sample soil noted to becoming sterile been devoid of soil natural micro flora and microphage. Thus the soil structure may be destroyed [26]. Comparatively, [7, 14, 16] explained the usefulness of organic fertilizers in a high terms of its improvement of biodiversity, long-term productivity, soil and creating a conducive habitat for micro-organisms. [3, 8, 11, 19, 21] Alverez, *et al*, 2000; Itu and Van Brugger, 2001; Tuld, *et al*, 2000; Boehm, *et al*, 2004; Deleij, *et al*, 2003; Maloney, *et al*, 2007 and workneh, *et al*, 2008) all agreed in their story that revelation of diversity of micro-organisms associated with various soil parameters such as disease suppression and organic matter decomposition were observed.

4. Conclusion

This study proved that organic fertilizers have great capacity to give a good atmosphere for microbial growth comparatively than in inorganic fertilizers because synthetic fertilizers depends on the chemical reactions while due to organic fertilizers, natural physiological activities occur among various microbes. The consequences of the present that the organic farm soils have a great capacity to give space to the microbial survival, which renders a fruitful outcome in the form of good crop production having a great tolerance to atmospheric pathogens and diseases. At the same time, inorganic soil has less microbial diversity which proved that some bacteria or fungi species may be found in inorganic fields, but not able to use properly the microclimate or micronutrients as can be used by organic microbes. *Aspergillus ramosa,* and have been found in ample amounts in organic soils while only a single colony has been found in inorganic soils. Furthermore, the results indicated that common practices of using synthetic fertilizers harm the soil quality in time and consequently low fertility of soil can be observed.

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