



Comparative rhizosphere microbiological and physicochemical properties of *Arachis Hypogaeae* (Groundnut) and *Hibiscus Esculentus* (Okro)

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ABSTRACT

The rhizosphere microbiological and physicochemical properties of *Hibiscus esculentus* (okro) and *Arachis hypogaeae* (groundnut) were examined for eight weeks. Samples from rhizosphere and non-rhizosphere soil (control) were collected and analyzed using standard microbiological and physicochemical methods. The mean total aerobic bacterial count (cfu/g) of rhizosphere and non-rhizosphere soil for weeks 4, 6 and 8 ranged from 8×10^9 - 2×10^{10} and 4×10^9 - 3×10^{10} in rhizosphere of okro and groundnut respectively while non-rhizosphere soil had count of 3×10^8 cfu/g. The mean total anaerobic bacteria count (cfu/g) of rhizosphere and non-rhizosphere soil for weeks 4, 6 and 8 ranged from 1×10^9 - 1×10^{10} and 4×10^8 - 2×10^{10} in rhizosphere of okro and groundnut respectively while non-rhizosphere soil had a count of 2×10^8 cfu/g. The mean total fungal count (cfu/g) of rhizosphere and non-rhizosphere soil for weeks 4, 6 and 8 ranges from 1×10^7 and 2×10^7 - 3×10^7 in rhizosphere of okro and groundnut respectively while the non-rhizosphere soil had a count 4×10^6 cfu/g and the mean total actinomycetes count (cfu/g) of rhizosphere and non-rhizosphere soil for weeks 4, 6 and 8 ranged from 6×10^9 - 8×10^9 and 1×10^9 - 7×10^{10} in rhizosphere of okro and groundnut respectively while the non-rhizosphere soil a count of had 2×10^8 cfu/g. Microorganisms isolated from rhizosphere soils were species of *Bacillus*, *Micrococcus*, *Streptococcus*, *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium*, *Fusarium*, *Actinomyces*, *Streptomyces* and *Nocardia* while the microorganisms isolated from the non-rhizosphere soil were species of *Bacillus*, *Micrococcus*, *Aspergillus*, *Mucor* and *Actinomyces*. The results of the physicochemical analysis revealed that there were significant differences ($P < 0.05$) in the mean electrical conductivity, potassium, phosphorus, nitrogen, organic carbon (%), organic matter (%), moisture (%) and sulphur between weeks 4, 6 and 8 but there was no significant difference ($P > 0.05$) in their mean pH and sodium. There was also a significant difference ($P < 0.05$) between the microbiological and physicochemical properties of rhizosphere compared with non-rhizosphere soil. The result of this study revealed the presence of several microorganisms in the rhizosphere of okro and groundnut that can serve economic importance.

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KEYWORDS

Rhizosphere;
Microbiological;
Physicochemical;
Hibiscus esculentus;
Arachis hypogaeae.

INTRODUCTION

Rhizosphere is the portion of soil that is influenced by the root of plant where there is interaction between the microbiological and physicochemical properties of the soil^[3,5]. Exudates, from the plant are released into the soil via the root which serves as nutrient to different microorganisms thereby controlling the populations, types and association of microorganisms found in the root of different plants^[28].

The microbiological and physicochemical properties vary for different plants. *Arachis hypogaea* (groundnut) is a leguminous plant widely grown in Nigeria and is used in production of oil and also serve as food (en.wikipedia.org/wiki/groundnut). Okro is a vegetable plant. Its pods contain a gummy substance that thickens and it is mucilaginous. It is valued for its edible green seed pod. It is common in Nigeria and used in making soup.

The aims and objectives of this research are;

1. To enumerate, isolate and identify microorganisms present in the rhizospheres of groundnut, okro and the non-rhizosphere.
2. To determine the physicochemical properties of rhizosphere soil of groundnut, okro and non-rhizosphere soil.

MATERIAL AND METHODS

Site location and land preparation

The site for cultivation of the groundnut, spinach and okro was located at 15m behind Chapel of Grace building in Federal University of Technology Minna, Bosso Campus, Niger State, Minna is located in the northern Guinea savannah zone with wet season from May to October and dry season from November to April. The field was ploughed, ridged at 50cm apart and irrigated. The seeds were planted in the month of March at 2.5cm depth, 3.0cm apart and were irrigated daily.

Collection of sample

Rhizosphere soil samples were collected by carefully uprooting each plant and shaking the soil adhering to the roots into a corresponding sterile labelled polyethylene bags, the non-rhizosphere soil (without roots)

was also collected into sterile polythene bag from the same site at 4th, 6th and 8th weeks age of the plants and were transported to the microbiology laboratory of Federal University of Technology, Minna for microbiological analysis following the method described by Oyeyiola^[3].

Media and sterilization

The media used were Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) for isolation of bacteria and fungi respectively. To isolate actinomycetes, a formulated medium containing: 1L water, 15g agar, 10g glycerin, 1g sodium asparaginate, 1g glucose, 1.5g $\text{NH}_4\text{H}_2\text{PO}_4$, 0.2g MgSO_4 , 0.1g CaCl_2 , 0.1g KCl , 0.1g nystatin and trace FeCl_3 was used. The media were sterilized using autoclave at 121°C for 15 minutes following the method described by Ogbulie *et al.*^[18].

Enumeration and isolation of microorganisms from rhizosphere and non-rhizosphere soil

Ten fold serial dilutions were carried out on one gram of each soil samples. One millilitre (1ml) of 10^{-8} diluent was used for the isolation of bacteria and actinomycete while 1ml of the 10^{-6} serial dilution was used for isolation of fungi. One millilitre (1ml) of each suspension was inoculated aseptically by pour plate method in two replicates following the method described by Ogbulie *et al.*^[18]. SDA plates were then incubated at $28 \pm 1^\circ\text{C}$ for 48-72 hours for fungi growth, NA plates was incubated in the incubator at 37°C for 24 hours both aerobically and anaerobically using anaerobic jar and actinomycetes was incubated at 30°C for 3-5 days. After incubation, colonies which developed were counted using a colony counter and expressed as colony forming unit per gram (cfu/g). Colonies differing in size, shape and colors were sub cultured using streak plate method to obtain a pure isolate. Pure culture was maintained in agar slant for further characterization and identification^[3].

Characterization and identification of bacterial isolates

Bacteria isolates were characterized using colonial morphology, cultural characteristics and biochemical tests which include Gram staining reactions, production of catalase, coagulase, indole, utilization of citrate, fermentation of sugars, urease test, starch hydrolysis and

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voges proskauer test as described by Ogbulie et al.^[18]. The isolates were identified by comparing their characteristics with those of known taxa.

Characterization and identification of fungal isolates

The mould isolates were characterized based on the color of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spores, sporangio-phore and conidiophores, and the characteristics of spore head.

A small portion of the mycelia growth was carefully picked with the aid of a pair of sterile inoculating needles and placed in a drop of lactophenol cotton blue on a microscope slide and covered with a cover slip. The slide was examined under the microscope, first with (x10) and then with (x40) objective lens for morphological examination as described by Ogbulie et al.^[18]. The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Domsch and Gams^[4].

Characterization and identification of actinomycetes

Identification of actinomycetes was done carried out using Gram's staining reaction, catalase test, urease test and cultural characteristics^[4]. The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Ochei and Kohalka, (2007).

Physicochemical analysis of soil samples

The soil samples were analysed for their pH, conductivity, moisture, organic carbon, organic matter, phosphorous, total nitrogen, sulphide, sodium (Na) and potassium (K) using the methods described by International Institute for Tropical Agriculture, IITA (1979).

Statistical analysis of data

The data obtained from this study was subjected to statistical analysis using one way analysis of variance (ANOVA) and Pearson correlation with MINITAB 14 package.

RESULT

Total Aerobic Bacterial Count (cfu/g) of rhizosphere

and non rhizosphere soils for weeks 4, 6 and 8.

TABLE 1 shows the mean total aerobic bacterial count (cfu/g) of rhizosphere and non rhizosphere soil for weeks 4, 6 and 8. In week 4, the highest aerobic bacterial count (8×10^9 cfu/g) was obtained in rhizosphere soil of okro. In week 6 the highest aerobic bacterial count (2×10^{10} cfu/g) was obtained in rhizosphere soil of okro and in week 8 the highest aerobic bacterial count (3×10^{10} cfu/g) was obtained in rhizosphere soil of groundnut. The highest mean total aerobic bacterial count (1×10^{10} cfu/g) was obtained in weeks 6 and 8 while the mean total aerobic bacterial count in week 4 was 2×10^9 cfu/g.

TABLE 1 : Total aerobic bacteria count (cfu/g) of rhizosphere and non rhizosphere soil for week4, 6 and 8.

Samples	Week 4	Week 6	Week 8
Rhizosphere of Okro (cfu/g)	$8 \times 10^{9a} \pm 6 \times 10^7$	$2 \times 10^{10a} \pm 3 \times 10^8$	$3 \times 10^{9a} \pm 6 \times 10^7$
Rhizosphere of Groundnut (cfu/g)	$4 \times 10^{9b} \pm 3 \times 10^8$	$1 \times 10^{10b} \pm 3 \times 10^8$	$3 \times 10^{10b} \pm 3 \times 10^7$
Non Rhizosphere (cfu/g)	$3 \times 10^{8c} \pm 3 \times 10^7$	$3 \times 10^{8c} \pm 3 \times 10^7$	$3 \times 10^{8c} \pm 3 \times 10^7$
Mean (cfu/g)	$2 \times 10^{9a} \pm 1 \times 10^8$	$1 \times 10^{10b} \pm 6 \times 10^5$	$1 \times 10^{10b} \pm 6 \times 10^6$

NB: Values are means of three replicates; \pm = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two superscripts do no differ significantly ($P > 0.05$) from the two values.

TABLE 2 : Total anaerobic bacteria count (cfu/g) of rhizosphere and non rhizosphere soil for week4, 6 and 8.

Samples	Week 4	Week 6	Week 8
Rhizosphere of Okro (cfu/g)	$2 \times 10^{9a} \pm 6 \times 10^7$	$1 \times 10^{10a} \pm 3 \times 10^8$	$1 \times 10^{9a} \pm 6 \times 10^7$
Rhizosphere of Groundnut (cfu/g)	$4 \times 10^{8b} \pm 6 \times 10^6$	$1 \times 10^{10a} \pm 6 \times 10^7$	$2 \times 10^{10b} \pm 6 \times 10^7$
Non Rhizosphere (cfu/g)	$2 \times 10^{8c} \pm 6 \times 10^6$	$2 \times 10^{8b} \pm 6 \times 10^6$	$2 \times 10^{8c} \pm 6 \times 10^6$
Mean (cfu/g)	$7 \times 10^{8a} \pm 6 \times 10^6$	$8 \times 10^{9b} \pm 6 \times 10^6$	$5 \times 10^{9c} \pm 6 \times 10^6$

NB: Values are means of three replicates; \pm = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two superscripts do no differ significantly ($P > 0.05$) from the two values.

Total anaerobic bacterial count (cfu/g) of rhizosphere and non rhizosphere soils for weeks 4, 6 and 8.

TABLE 2 shows the total anaerobic bacterial count (cfu/g) of rhizosphere and non rhizosphere soil for weeks 4, 6 and 8. In week 4, the highest anaerobic bacterial count (2×10^9 cfu/g) was obtained in rhizosphere soil of okro. In week 6 the anaerobic bacterial count (1×10^{10} cfu/g) obtained was the same in rhizosphere of

okro and groundnut while in week 8 the highest anaerobic bacterial count (2×10^{10} cfu/g) was obtained in rhizosphere soil of groundnut. There was significant difference ($P < 0.05$) between the mean total anaerobic bacterial count obtained in weeks 4, 6 and 8.

Total fungal count (cfu/g) of rhizosphere and non rhizosphere soils for week 4, 6 and 8

TABLE 3 shows the total fungal count (cfu/g) of rhizosphere and non rhizosphere soil for weeks 4, 6 and 8. The highest fungal count (2×10^7 cfu/g), (2×10^7 cfu/g) and (3×10^7 cfu/g) in week 4, 6 and 8 respectively were obtained in rhizosphere of groundnut.

TABLE 3 : Total fungal count (cfu/g) of rhizosphere and non rhizosphere soil for week 4, 6 and 8.

Samples	Week 4	Week 6	Week 8
Rhizosphere Okro (cfu/g)	$1 \times 10^{7a} \pm 3 \times 10^5$	$1 \times 10^{7a} \pm 4 \times 10^5$	$1 \times 10^{7a} \pm 3 \times 10^5$
Rhizosphere of Groundnut (cfu/g)	$2 \times 10^{7b} \pm 6 \times 10^5$	$2 \times 10^{7b} \pm 3 \times 10^5$	$3 \times 10^{7b} \pm 3 \times 10^5$
Non-Rhizosphere (cfu/g)	$4 \times 10^{6c} \pm 6 \times 10^4$	$4 \times 10^{6c} \pm 6 \times 10^4$	$4 \times 10^{6c} \pm 6 \times 10^4$
Mean (cfu/g)	$1 \times 10^{7a} \pm 6 \times 10^4$	$1 \times 10^{7a} \pm 6 \times 10^4$	$1 \times 10^{7a} \pm 6 \times 10^5$

NB: Values are means of three replicates; \pm = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two superscripts do no differ significantly ($P > 0.05$) from the two values.

TABLE 4 : Total actinomycetes count (cfu/g) of rhizosphere and non rhizosphere soil for week 4, 6 and 8.

Samples	Week 4	Week 6	Week 8
Rhizosphere Okro (cfu/g)	$6 \times 10^{9a} \pm 6 \times 10^5$	$8 \times 10^{9a} \pm 6 \times 10^7$	$6 \times 10^{9a} \pm 3 \times 10^7$
Rhizosphere of Groundnut (cfu/g)	$2 \times 10^{9b} \pm 3 \times 10^5$	$1 \times 10^{9b} \pm 3 \times 10^6$	$7 \times 10^{9b} \pm 6 \times 10^7$
Non-Rhizosphere (cfu/g)	$2 \times 10^{8c} \pm 6 \times 10^4$	$2 \times 10^{8c} \pm 6 \times 10^4$	$2 \times 10^{8c} \pm 6 \times 10^4$
Mean (cfu/g)	$3 \times 10^{8a} \pm 6 \times 10^4$	$5 \times 10^{9b} \pm 6 \times 10^6$	$7 \times 10^{9c} \pm 6 \times 10^6$

NB: Values are means of three replicates; \pm = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two superscripts do no differ significantly ($P > 0.05$) from the two values.

Total actinomycetes count (cfu/g) of rhizosphere and non rhizosphere soils for week 4, 6 and 8

TABLE 4 shows the total actinomycetes count (cfu/g) of rhizosphere and non rhizosphere soil for weeks 4, 6 and 8. The highest total actinomycetes count (6×10^9 cfu/g) and (8×10^9 cfu/g) was obtained in rhizosphere soil of okro in week 4 and 6 respectively and in week 8 the highest total actinomycetes count (7×10^9 cfu/

g) was obtained in rhizosphere soil of groundnut. The highest mean total actinomycetes count (cfu/g) (7×10^9 cfu/g) was obtained in weeks 8.

Microorganisms isolated from rhizosphere and non rhizosphere soils for week 4

TABLE 5 shows the microorganisms isolated from rhizosphere and non rhizosphere soil for week 4. Bacteria isolated from rhizosphere of okro and groundnut were species of *Bacillus*, *Micrococcus*, *Streptococcus* sp. (not found in rhizosphere of okro) and *Staphylococcus* sp. Fungi isolated from rhizosphere soils of okro and groundnut were species of *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium* and *Fusarium* with exception of *Alternaria* in groundnut. Species of *Actinomyces*, *Nocardia* and *Streptomyces* were also isolated from rhizosphere of okro and groundnut but *Bacillus megaterium*, *Micrococcus luteus*, *Alternaria* sp., *Aspergillus flavus*, *Penicillium* sp., *Actinomyces* sp. and *Nocardia* sp. were not isolated from the non rhizosphere soil.

Microorganisms isolated from rhizosphere and non rhizosphere soil for week 6

TABLE 6 shows the microorganisms isolated from rhizosphere and non rhizosphere soil for week 6. Bacteria isolated from rhizosphere of okro and groundnut were species of *Bacillus*, *Micrococcus*, and *Staphylococcus* with addition of *Streptococcus* in rhizosphere soil of groundnut. Fungi isolated from rhizosphere of okro and groundnut were species of *Aspergillus*, *Mucor*, *Penicillium* and *Fusarium*. Species of *Actinomyces*, *Nocardia* and *Streptomyces* were also isolated from rhizosphere soils of okro and groundnut but *Bacillus megaterium*, *Bacillus mycoides*, *Micrococcus luteus*, *Streptococcus* sp., *Aspergillus flavus*, *Penicillium* sp., *Actinomyces* sp. and *Nocardia* sp. were not isolated from the non rhizosphere soil.

Microorganisms isolated from rhizosphere and non rhizosphere soil for week 8

TABLE 7 shows the microorganisms isolated from rhizosphere and non rhizosphere soil for week 8. Bacteria isolated from rhizosphere of okro and groundnut were species of *Bacillus*, *Micrococcus* and *Staphylococcus*. with addition of *Streptococcus* sp. in rhizosphere of groundnut. Fungi isolated from rhizosphere of okro and

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groundnut were species of *Aspergillus*, *Mucor*, *Penicillium* and *Fusarium* with addition of *Alternaria* in okro. Species of *Actinomyces*, *Nocardia* and *Streptomyces* were also isolated from rhizosphere soils of okro

and groundnut but *Bacillus megaterium*, *Micrococcus luteus*, *Alternaria* sp., *Aspergillus flavus*, *Penicillium* sp. *Actinomyces* sp. and *Nocardia* sp. were not found in the non rhizosphere soil.

TABLE 5 : Microorganisms isolated from rhizosphere and non rhizosphere soil for week 4.

Isolate	Week 4 Rhizosphere of okro	Rhizosphere of Groundnut	Non Rhizosphere
Bacteria	<i>Bacillus cereus</i>	<i>Bacillus cereus</i> <i>B. megaterium</i>	<i>Bacillus subtilis</i> <i>Micrococcus</i> sp.
	<i>B. subtilis</i> <i>Micrococcus luteus</i>	<i>Micrococcus</i> sp. <i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>
	<i>Micrococcus</i> sp. <i>Staphylococcus aureus</i>	<i>aureus</i> <i>Streptococcus</i> sp.	
Fungi	<i>Alternaria</i> sp. <i>Aspergillus niger</i>	<i>Aspergillus niger</i> <i>Fusarium</i> sp.	<i>Aspergillus fumigatus</i> <i>A. niger</i>
	<i>Fusarium</i> sp. <i>Mucor</i> sp. <i>Penicillium</i> sp.	<i>Mucor</i> sp. <i>Penicillium</i> sp.	<i>Mucor</i> sp.
Actinomycetes	<i>Actinomyces</i> sp. <i>Nocardia</i> sp.	<i>Actinomyces</i> sp. <i>Nocardia</i> sp.	<i>Streptomyces</i> sp.
	<i>Streptomyces</i> sp.	<i>Streptomyces</i> sp.	

TABLE 6 : Microorganisms isolated from rhizosphere and non rhizosphere soil for week 6.

Isolate	Week 6 Rhizosphere of okro	Rhizosphere of Groundnut	Non Rhizosphere
Bacteria	<i>Bacillus cereus</i> <i>B. subtilis</i> <i>B. megaterium</i>	<i>Bacillus subtilis</i> <i>B. megaterium</i> <i>B. mycooides</i>	<i>B. subtilis</i>
	<i>B. mycooides</i> <i>Micrococcus luteus</i>	<i>Micrococcus</i> sp. <i>M. luteus</i>	<i>Micrococcus</i> sp.
	<i>Micrococcus</i> sp. <i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>
Fungi	<i>Aspergillus flavus</i> <i>Alternaria</i> sp. <i>A. fumigatus</i>	<i>Aspergillus flavus</i> <i>A. fumigatus</i> <i>A. niger</i>	<i>Aspergillus fumigatus</i>
	<i>A. niger</i> <i>Fusarium</i> sp. <i>Mucor</i> sp.	<i>Fusarium</i> sp. <i>Mucor</i> sp.	<i>A. niger</i> <i>Mucor</i> sp.
Actinomycetes	<i>Penicillium</i> sp.	<i>Penicillium</i> sp.	
	<i>Actinomyces</i> sp. <i>Nocardia</i> sp.	<i>Actinomyces</i> sp. <i>Nocardia</i> sp.	<i>Streptomyces</i> sp.
	<i>Streptomyces</i> sp.	<i>Streptomyces</i> sp.	

TABLE 7 : Microorganisms isolated from rhizosphere and non rhizosphere soil for week 8.

Isolate	Week 8 Rhizosphere of okro	Rhizosphere of Groundnut	Non Rhizosphere
Bacteria	<i>Bacillus cereus</i> <i>B. subtilis</i>	<i>Bacillus subtilis</i> <i>B. mycooides</i>	<i>B. subtilis</i> <i>Micrococcus</i> sp.
	<i>Micrococcus luteus</i> <i>Micrococcus</i> sp.	<i>Micrococcus luteus</i> <i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i> <i>aci</i>
Fungi	<i>Staphylococcus aureus</i>	<i>Streptococcus</i> sp.	
	<i>Aspergillus flavus</i> <i>Alternaria</i> sp. <i>A. niger</i>	<i>Aspergillus niger</i> <i>Fusarium</i> sp.	<i>Aspergillus fumigatus</i> <i>A. niger</i>
Actinomycetes	<i>Fusarium</i> sp. <i>Mucor</i> sp.	<i>Mucor</i> sp. <i>Penicillium</i> sp.	<i>Mucor</i> sp.
	<i>Penicillium</i> sp.		
Actinomycetes	<i>Actinomyces</i> sp. <i>Nocardia</i> sp.	<i>Actinomyces</i> sp. <i>Nocardia</i> sp.	<i>Streptomyces</i> sp.
	<i>Streptomyces</i> sp.	<i>Streptomyces</i> sp.	

Physicochemical properties of the rhizosphere and non rhizosphere soils for week 4

TABLE 8 shows the physicochemical properties of rhizosphere and non rhizosphere for week 4. There was significant difference ($P < 0.05$) in pH, sodium, potassium, phosphorus, nitrogen, organic carbon (%), organic matter (%) and sulphur between rhizosphere of okro, groundnut and the non rhizosphere soil. There was no significant difference ($P > 0.05$) in electrical conductivity between rhizosphere soil of groundnut and the non rhizosphere soil. The non rhizosphere soil has the highest moisture content of 0.9%.

Physicochemical properties of the rhizosphere and non-rhizosphere soils for week 6

TABLE 9 shows the physicochemical properties of rhizosphere and non rhizosphere for week 6. There was significant difference ($P < 0.05$) in Conductivity, sodium, phosphorus, organic carbon (%) and sulphur between rhizosphere of okro, groundnut and the non rhizosphere soil. There was no significant difference ($P > 0.05$) in pH and potassium between rhizosphere soils of spinach, okro, and groundnut. The non rhizosphere soil has the highest moisture and sulphur content of 0.9% and 69mg/l while the rhizosphere soil of okro has the highest organic matter of 4.3%.

Physicochemical properties of the rhizosphere and non rhizosphere soil for Week 8

TABLE 10 shows the physicochemical properties

of rhizosphere and non rhizosphere for week 8. There was significant difference ($P < 0.05$) in pH, electrical conductivity, sodium, potassium, phosphorus, organic carbon (%) and sulphur between rhizosphere soils of spinach, groundnut and the non rhizosphere soil. There was no significant difference ($P > 0.05$) in moisture between rhizosphere soil of spinach and the non rhizosphere soil.

TABLE 8 : Physicochemical properties of the rhizosphere and non-rhizosphere soil for Week 4.

Parameter	Week 4 Rhizosphere of Okro	Rhizosphere of Groundnut	Non-Rhizosphere
pH	7.680 ^a ±0.017	7.620 ^b ±0.006	7.520 ^c ±0.049
Conductivity (mS/m)	191.30 ^a ±4.372	97.00 ^b ±0.577	102.00 ^c ±1.155
Sodium (mg/l)	12.00 ^a ±0.057	10.70 ^b ±0.115	10.20 ^b ±0.057
Potassium (mg/l)	12.40 ^a ±0.115	10.20 ^b ±0.115	9.50 ^c ±0.115
Phosphorus (mg/l)	5.067 ^a ±0.088	3.300 ^b ±0.115	6.100 ^c ±0.173
Nitrogen (%)	0.210 ^a ±0.006	0.180 ^b ±0.012	0.260 ^a ±0.006
Organic carbon (%)	2.530 ^a ±0.006	1.950 ^b ±0.006	1.590 ^c ±0.006
Organic matter (%)	4.400 ^a ±0.115	3.400 ^b ±0.058	2.800 ^c ±0.058
Moisture (%)	0.400 ^a ±0.115	0.200 ^b ±0.012	0.900 ^c ±0.058
Sulphur (mg/l)	49.600 ^a ±0.115	67.700 ^b ±0.115	69.800 ^c ±0.058

NB: Values are means of three replicates; ± = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two or three superscripts do no differ significantly ($P > 0.05$) from the two or three values.

TABLE 9 : Physicochemical properties of the rhizosphere and non-rhizosphere soil for week 6.

Parameter	Week 6 Rhizosphere of Okro	Rhizosphere of Groundnut	Non Rhizosphere
pH	7.620 ^a ±0.006	7.580 ^{ab} ±0.026	7.370 ^c ±0.006
Conductivity (mS/m)	174.0 ^a ±0.577	108.0 ^b ±1.732	102.0 ^{bc} ±1.154
Sodium (mg/l)	12.00 ^a ±0.115	11.50 ^b ±0.580	10.20 ^c ±0.580
Potassium (mg/l)	12.00 ^a ±0.058	11.70 ^{ab} ±0.058	9.50 ^c ±0.115
Phosphorus (mg/l)	14.20 ^a ±0.058	7.700 ^b ±0.058	6.100 ^c ±0.173
Nitrogen (%)	0.240 ^a ±0.012	0.350 ^b ±0.006	0.260 ^{ac} ±0.006
Organic carbon (%)	2.490 ^a ±0.012	1.820 ^b ±0.017	1.590 ^c ±0.006
Organic matter (%)	4.300 ^a ±0.058	3.100 ^b ±0.115	2.800 ^c ±0.058
Moisture (%)	0.500 ^a ±0.058	0.300 ^b ±0.058	0.900 ^c ±0.058
Sulphur (mg/l)	54.200 ^a ±0.058	65.800 ^b ±0.058	69.800 ^c ±0.058

NB: Values are means of three replicates; ± = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two or three superscripts do no differ significantly ($P > 0.05$) from the two or three values.

TABLE 10 : Physicochemical properties of the rhizosphere and non rhizosphere soil for week 8.

Parameter	Week 8 Rhizosphere of Okro	Rhizosphere of Groundnut	Non Rhizosphere
pH	6.720 ^a ±0.006	7.060 ^b ±0.017	7.370 ^c ±0.006
Conductivity (mS/m)	125.00 ^a ±1.155	108.0 ^b ±1.155	90.0 ^c ±1.155
Sodium (mg/l)	7.50 ^a ±0.580	11.50 ^b ±0.580	6.80 ^c ±0.580
Potassium (mg/l)	10.80 ^a ±0.115	9.90 ^b ±0.058	9.50 ^c ±0.115
Phosphorus (mg/l)	14.20 ^a ±0.058	7.700 ^b ±0.058	6.100 ^c ±0.173
Nitrogen	0.900 ^a ±0.006	0.800 ^{ab} ±0.012	0.260 ^c ±0.006
Organic carbon (%)	2.790 ^a ±0.006	1.390 ^b ±0.006	1.590 ^c ±0.006
Organic matter (%)	4.800 ^a ±0.115	2.400 ^b ±0.058	2.800 ^{bc} ±0.058
Moisture (%)	0.700 ^a ±0.058	0.400 ^b ±0.058	0.900 ^c ±0.058
Sulphur	64.200 ^a ±0.115	69.000 ^b ±0.115	69.800 ^{bc} ±0.058

NB: Values are means of three replicates; ± = standard error of the mean; Means with the different superscript differ significantly ($P < 0.05$) from each other, means with the same superscript does not differ significantly ($P > 0.05$) from each other while means with two or three superscripts do no differ significantly ($P > 0.05$) from the two or three values.

DISCUSSION

The rhizosphere microbial counts for bacteria (aerobic and anaerobic), actinomycetes and fungi, are each significantly higher ($P < 0.05$) than the non rhizosphere microbial counts in weeks 4, 6 and 8; this is in line with the reports of Aliyu and Oyeyiola^[3] and may be attributed to the presence of organic substance from the root exudates and dead root cell; for rhizosphere was defined by Hiltner in 1904 as the portion of soil influenced by the root, where micro-organisms interact with plant roots and soil constituents.

There was significant difference ($P < 0.05$) in total aerobic bacteria count between the rhizosphere of okro, groundnut and the non rhizosphere soil in week 4 and 6 (TABLE 1); the count is higher in the rhizosphere soil of okro than groundnut which is in turn higher than the non rhizosphere soil and may be due to the fact that okro is a vegetables while groundnut is a legume. There was increase in count in all the rhizosphere soils at week 6, with further increase in the rhizosphere of groundnut but no increase in rhizosphere of okro at week 8; this increase in microbial count of rhizosphere of groundnut may be due to longer maturity period of the crop plant compare to okro; this is in line with the report of Aliyu and Oyeyiola^[3] who showed that as the crop approaches

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harvest the microbial load decline. This could also be the reason for the similar results in anaerobic bacteria count and actinomycetes counts in TABLES 2 and 4 respectively.

There was significant difference ($P < 0.05$) between bacterial count and fungi count for both rhizosphere and non rhizosphere; that is, the bacteria count is significantly higher ($P < 0.05$) than the fungi count at each of the weeks and this may be due the shorter generation time of bacteria compare to fungi. There was no increase in fungal count of the rhizosphere of okro at week 6 and 8 but that of groundnut decrease at week 6 and increase again at week 8, all of these may be due to the slow growth rate of fungi and effect of the exudates.

The rhizosphere of groundnut and okro have a wider range of isolates than the non rhizosphere soil that of TABLES 5, 6, and 8; this may be due to larger amount of exudates. *Bacillus megaterium*, *Bacillus mycoides*, *Micrococcus luteus*, *Alternaria* sp., *Aspergillus flavus*, *Penicillium* sp. *Actinomyces* sp. and *Nocardia* sp. were not found in the non rhizosphere soil but were found in the rhizosphere soil in agreement with those isolated by Aliyu and Oyeyiola^[3] in rhizosphere soil of groundnut; this may also be due to the root exudates secreted into the rhizosphere soil. *Streptococcus* sp. was isolated from the rhizosphere of groundnut but not found in the rhizosphere of okro and spinach; this may be due to the fact that *Streptococcus* is a proteobacteria that is involved in nitrogen fixation. *Alternaria* was only isolated from rhizosphere soil of okro and this may be due the high organic matter obtained from it when compared to that of groundnut as the plant ages. *Alternaria* also serves as a biocontrol agent against invasive plants.

The pH obtained from the rhizosphere and non rhizosphere soil ranges from 6.7 – 7.8 which is an optimum pH for bacteria growth while fungi thrive more in acidic pH. The pH of 7.3 obtained for the non rhizosphere soil is the same with that determined by Oyeyiola^[19] for non rhizosphere soil in the research on Rhizosphere Bacterial Flora of *Amaranthus hybridus*. The pH of each rhizosphere soil for week 4 and 6 is higher than that of the non rhizosphere soil but lower at week 8; this may be due to the microbial activities as the plant ages. The pH of soil affects specifically plant nutrient availability by controlling the chemical forms,

for instance in slightly to moderately alkaline soil, macronutrient availability is increased except phosphorus which is reduced and may adversely affect the growth of plant while in acidic soil most micronutrient is increased. The concentration of Nitrogen is less sensitive to pH than concentration of available Phosphorus which requires 6.0 – 7.5 to be available^[27].

The Electrical Conductivity of soils varies depending on the amount of moisture held by soil particles. Consequently, EC correlates strongly to soil particle size and texture^[26]. There is significant difference ($P < 0.05$) in the conductivity of rhizosphere of spinach as the plant ages; the decrease in conductivity was explained by Kajafu and Parsazadeh, (2011) that it may be due to increase in the nitrogen content of the soil as the plant ages which agrees with this research; in his research there was also significant increase ($P < 0.05$) in pH due to the effect of the nitrogen but there was no significant difference ($P > 0.05$) in pH in this research and this may be so because nutrient solution (nitrate - ammonium) was added to soil before the cultivation of the spinach by Kajafu and Parsazadeh, (2011).

Nitrogen, Phosphorus, Potassium and Sulphur that was determined, constitute part of the macronutrient required by plant from the soil to survive, while sodium is one of the micronutrient needed by plants for growth; these nutrients availability affects soil pH making it alkaline or acidic^[27]. The sodium level obtained in the research for rhizosphere and non rhizosphere soil ranging from 6 – 12 ppm is optimum due to the optimum pH obtained in the results, for pH level over 8.4 is usually indicative of high sodium level in the soil with high level of clay and organic matter which can lead to poor drainage and also impede the root from taking in important mineral such as calcium, potassium and magnesium from the soil^[27].

Nitrogen, phosphorus and potassium are primary macronutrients which are consumed by plant in large quantity. Potassium is very essential in soil for plant growth, it is known to activate sixteen enzyme needed for plant growth. There is significant increase ($P < 0.05$) in phosphorus in the rhizosphere of all the plants as the plant ages and this may be due the mineralization activities of the increasing microbial community. Organic and atmospheric Nitrogen can only be available for use by plant when they are converted to nitrate and this pro-

cess of mineralization is carried out by microorganisms. The nitrogen content of rhizosphere soil of groundnut increased significantly ($P < 0.05$) as the plant ages this may be due the activity of the nitrogen fixing bacteria (*Rhizobium*) found in the nodules and in the soil (*Streptococcus*) of leguminous plant.

Phosphorus is an immobilized nutrient and requires 6.0 – 7.5 to be available. *Actinomyces*, *Bacillus*, *Aspergillus* and *Penicillium* present in all the tested rhizosphere has the ability to solubilize Phosphorus.

There is significant difference ($P < 0.05$) between the moisture (%) of the non rhizosphere and each of the rhizosphere; the moisture of content of the non-rhizosphere is significantly higher due to loss of water by transpiration through the leaves and drainage of water by roots penetrating and loosening the soil thereby creating pores in the soil which encourage drainage in the rhizosphere soil as explained by Kelechi *et al.*, (2012). The moisture (%) also increased as the plant ages and this may be due to transition from dry to wet season with the evidence of steady rainfall.

CONCLUSION

There is a significant difference ($P < 0.05$) between the microbiological properties of rhizosphere of *Arachis hypogaea* (groundnut) and *Spinacia oleracea* (spinach) when compared with non-rhizosphere soil. There was also a significant difference ($P < 0.05$) in their physicochemical properties. The difference may be due to the influence of exudates from the roots of the different plant and absence of these exudates in the non-rhizosphere soil.

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