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Soil chemical properties and heterotrophic bacterial population in the rhizosphere of oil palm plantations under different ages

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Abstract
This study was carried out to determine the soil chemical properties and heterotrophic bacterial population and species that are predominant in the rhizosphere of oil palms (Elaeis guineensis. jack) and to compare the bacteria population in the rhizosphere of matured (10 years and above) fruit bearing palms with those present in young (3 – 6 years) palms that are just bearing fruits for the first few years. Four plantations of different ages were sampled at the Nigerian Institute for Oil Palm Research (NIFOR) main station located in Ovia North Local Government Area of Edo State at soil depth of; 0 – 15 cm, 15 – 30 cm, 30 – 45 cm and 45 – 60 cm. The soil samples obtained were analyzed for their physical, chemical and microbial compositions and the bacteria counts found in the various oil palm plantations were tested for significant differences ($p \leq 0.05$) using Duncan multiple range test in a Completely Randomized Design. The soil texture of Field 52 (3 – 6 years) was sandy in both top and sub soil (0 – 60 cm) while the older plantations had sandy-loam and loamy-sand at some depth. The nitrogen, phosphorus and organic carbon content of the soil were also observed to increase with age. The investigation further showed a high presence of bacteria at the top soil (0 – 15 cm and 15 – 30 cm) than in the sub soils (30 – 45 cm and 45 – 60 cm). This was in close relationship with the high presence of carbon and other primary nutrients that were also more at the top soils. On the basis of plant age, the heterotrophic bacteria populations were greater in the older plantations than found in the young (3 - 6 years) plantations. The specific bacteria isolated from the rhizosphere soils were Pseudomonas aeroginosa, Micrococcus cereus, Staphylococcus aureus, and Coryrubacteria spp.

Key words: *Heterotrophic, Bacteria, Rhizosphere, Oil palm, Microbes*

Introduction

Generally, several compounds present in root exudates play important roles in biological process that occurs in the rhizosphere (Bais *et al.*, 2003). On the other hand, the specific content of root exudates may create a niche that influences which microorganisms are to colonize the rhizosphere, thereby altering the composition and diversity of microorganisms colonizing the rhizosphere in a plant specific manner. The loss of organic material from the roots provides the driving force for the development of active microbial populations around the root, known as the rhizosphere effect (Morgan and Whipps, 2001). The rhizosphere effect is beneficial to the plants in two ways; first, it helps in providing nutrients to the plant and secondly, it helps the plant in combating root diseases. As a result of these interactions, the rhizosphere is suggested to have a great potential for biotechnology exploitation (Barea *et al*., 2004) and the success of any biological control development for such a crop as oil palm which continues to gain increase cultivation in our environment will begin with the understanding of the rhizosphere microbial composition and interactions.

Although the number of microbial species present in the soil can vary from some

thousands to millions, many studies indeed suggest that, the proteobacteria and the actinobacteria forms the most common of the dominant population found in the rhizosphere of many different plant species (Singh *et al*., 2007). This group probably contains many 'cultured members' and as such constitute majority of the soil organisms investigated both as beneficial and pathogenic microbial inoculants.

Moreover, plant species, plant developmental stages, and soil type have been indicated as major factors determining the composition of the rhizosphere microbial communities but the extent to which the above cited factors contribute to microbial communities in the rhizosphere is not fully understood and there are several contrasting reports indicating either plant or soil as the dominant factor (Nunan *et al*., 2005). Owing to the above statement, it can be generalized that, the diversity and predominance of rhizosphere microbial population depend on a number of abiotic and biotic factors prevailing in that particular ecological niche.

Furthermore, the root architecture had also been thought to affect the structure of the microbial communities inhabiting the rhizosphere. It can therefore be expected that much metabolites will be released by the roots of oil palm considering the numerous numbers of root possessed by this plant and that, such metabolites will attract microorganism towards its rhizosphere. This investigation was carried out to reveal the population of the specific bacteria in the rhizosphere of matured-fruit-bearing oil palms (10 – 25 years and above) and to compare findings with the same composition in the rhizosphere of palms that are just bearing fruits for the first few years (3 – 6 years)

Materials and Methods

Experimental site

This study was conducted at the Nigeria Institute for Oil palm Research (NIFOR) main station located in Ovia North Local Government Area of Edo state. The site is situated at the Rainforest belt of the humid tropics and southern ecological zone of Nigeria, with distinct dry and wet seasons having an annual rainfall range of 1800 – 1900 mm, attaining its peak in July to September (NIFOR, 2013). The soils of this area are mainly Ultisols with pH between 4.0 and 6.5 and as such have been classify to fall within the subdivision of acids sands (Vine, 1956). The vegetation cover is mostly oil palm with few under growth of weeds that are mainly *Chromolaena odorata, Pennisetum purpureum, and Axonopus compressus.* The species of oil palm cultivated in this station is the *'tenera'*; this oil palm specie usually starts flowering and fruiting at age 3 – 4 years although the first harvesting is carried out by the institute when the palms are 5 – 6 years old.

Soil sampling and analysis

Four plantations of different ages; 3 – 6 years, 10 – 15 years, 16 – 20 years and above 25 years old plantations were sampled at the rhizosphere soil depth; 0 – 15 cm, 15 – 30 cm, 30 – 45 cm, 45 - 60 cm. The geographical coordinates of the sampled locations are presented in table 1 below.

Table 1.The Geographical Coordinates of the NIFOR Plantations Sampled.

NIFOR FIELD	Age (years)	Geographical Coordinates
52	3 – 6	Lat. 6˚33'13.9" - 6˚33'20.6" N Long. 5˚37'39.7" - 5˚37'32.4" E Elev.538ft ± 14 – 534ft ± 12
42	10 – 15	Lat. 6˚33'28.3" - 6˚33'17.1" N Long. 5˚37'28.1" - 5˚37'27.2" E Elev. 541ft ± 20 – 548ft ±20
16	16 – 20	Lat. 6˚32'42.8" - 6˚32'58.3" N Long. 5˚37'29.8" - 5˚37'31.6" E Elev. 469ft ± 25 – 478ft ±23
44	Above 25	Lat. 6˚32'28.5" - 6˚32'48.4" N Long. 5˚37'79.2" - 5˚37'98.5" E Elev.446ft ± 32 – 498ft ± 15

❖ Lat. (Latitude), Long. (Longitude), Elev. (Elevation).

The palm rhizosphere was sampled at two sides of each palm and the soils collected from three palms that were randomly selected within a particular plantation were composited into one sample representative and a total of 12 samples which were collected from each of the four plantations were carefully bagged and subjected to routine physical, chemical and biological analysis. The particle size distribution was determined by hydrometer method according to Gee and Or (2002). The soil's pH was determined at 1:1 soil to water using a glass electrode digital pH meter (Thomas, 1982) and available P was extracted using Bray-1 solution (Kuo, 1996). Total N contents were determined using micro-Kjeldahl digestion and distillation process (Jackson, 1962), chromic acid digestion method by Nelson and Sommers (1982) was adopted in the determination of organic carbon while the exchangeable bases (Ca, Mg, K and Na) were extracted with 1N ammonium acetate (NH_40Ac). Exchangeable calcium and magnesium were determined by ethylene diamine-tetraacetic acid (EDTA) titration method while exchangeable potassium and sodium were estimated by flame photometry (Jackson, 1962). Exchangeable acidity was extracted with KCl (1N) and measured titrimetrically according to the procedure of Maclean (1982). Nutrient agar (NA) was used in the culturing of the bacteria in a spread plate technique while the bacteria isolates were characterized based on their cultural, morphological and biochemical characteristics (Cowan and Steel 1970).

Statistical Analysis:

The Bacteria count were tested for significant differences between plantations using a two-way analysis of variance (ANOVA) procedure in a Completely Randomized Design as described by Alika, (2006). The variability of the microbial count was calculated using Genstat Software and the significant effects were expressed at 5% using Duncan Multiple Range Test.

Results and Discussion

The pH (1:1 H_2O) values of soil used in this study ranged from 4.10 – 6.60, and also had its particle sizes in the order as sand > clay > silt; an indication that the soils are still within the range of acid sands as earlier stated

by Vine (1956). These results coupled with the Total Nitrogen, available Phosphorus and organic carbon contents are presented in table 2. The amount of organic carbon present in Field 52 and Field 42 rhizosphere top soils (0 – 15 cm) ranged between 9.90 kg^{-1} and 13.1g kg^{-1} to 4.20 and 5.80g kg^{-1} in their sub soils (45 – 60 cm).while in Field 44, the organic carbon content at the top soil (0 – 15 cm) ranged between 13.1kg^{-1} – 13.8g kg^{-1}, this amount was higher than the content (5.7 – 5.9 g kg^{-1}) found at their sub soils (45 – 60 cm) and greater than the organic carbon concentration (10.9 – 13.4 g kg^{-1} and 3.9 – 4.6g kg^{-1}) present at the top and sub soils of Field 16 respectively. The high amount of these nutrients at the top soil can be traceable to the higher amount of organic carbon at that horizon relative to the sub soil. (Ingam, 2000 and Adaikwu *et al*., 2017)

The soil pH had higher values (5.90 – 6.40) at the top soil (0 – 15 cm) whereas, at the lower rhizosphere depth (45 – 60 cm), the pH values ranged between 4.50 -5.0. These results however, suggest an increase in acidity as the soil depth increases. This result agrees with the findings of Ogbemudia *et al*., (2016) who on the investigation of some chemical and microbial properties of soils in Edo State Nigeria, also observed a steady increase in soil acidity down the profile.

The soil available phosphorus and nitrogen also had a similar trend of being higher in amount at the top soils (0 -15 cm & 15 – 30 cm) than in the sub soils (30 – 45 & 45 – 60 cm). Nitrogen had a relatively higher amount that ranged between 1.12kg^{-1} – 0.78g kg^{-1}at the 0 - 15 cm depth in Field 52 whereas, in Field 42, its content ranges between 0.98 – 0.88g kg^{-1}atthe soil depth of; 0 – 15 cm. On the basis of the average amount; (0.56, 0.63, 0.60 & 0.68 g kg^{-1}) were found in Field; 52, 42, 16 and 44 respectively. The phosphorus in all of the fields appeared to be close in amount ranging between 12.30 – 19.70mg $kg^{-1}$and 13.88 – 17.16 mg kg^{-1} at their 0 – 15 cm and 45 – 60 cm respectively. However, the concentration of phosphorus in Field 44 extended richly to the 45 – 60 cm depth with values between 5.30 – 5.85 mg kg^{-1} while at same depth in the other fields, it ranged between 3.41 – 5.70 mg kg^{-1}. The phosphorus content in all the fields are considered to be moderate according to *Chude et al*., (2011)

Unlike the order of occurrences for organic carbon, phosphorus and nitrogen that had higher values at the top soils than in the sub soils, the soils clay fractions were greater in the sub soils. In Field 52, the clay fraction measured in g kg^{-1}ranged between 24 and 39 at the 0 – 15 cm depth whereas, at the sub soil (45 – 60 cm), clay values ranged between 79 - 149 g kg^{-1}. Although, the highest clay contents (154 - 159g kg^{-1}) were found at the 45 – 60 cm depth in Field 16 as against the other Fields' concentration that ranged between 59 - 129g kg^{-1}. In contrast to the order of the clay concentrations, the sand content of the rhizosphere soils were greater at the top soils (0 – 15 cm) which had values between 917 – 957 g kg^{-1}while at the sub soils (45 – 60 cm), their value ranged between 847 - 932g kg^{-1}. This result is suspected to be due to leaching effects as a result of the high amount of rainfall in the area (Ogunkule, 2007).

The amount of Calcium (Ca) present in Field 52 (a) (10.08, 6.24, 6.08 & 4.34 Cmol kg^{-1}) at the rhizosphere depth of; 0 -15, 15 – 30, 30 – 45 and 45 – 60 cm respectively were relatively higher than the content (5.60, 4.96, 3.84 and 2.88 Cmol kg^{-1}) present in Field 42 (a) following similar depth range. Whereas, in Field 44, the content ranged between 6.56 – 9.92 Cmol kg^{-1} at the top soils (0 – 15 cm) and it gradually reduce to 3.36 kg^{-1} – 5.44 Cmol kg^{-1} at the sub soils (45 - 60)cm. Comparatively, Field 52 and 44 almost had the same amount (10.08 - 4.34 Cmol kg^{-1}) at the 0 – 15 cm depth in terms of their calcium content while Field 16 had the lowest amount (5.70 – 5.90 Cmol kg^{-1}) at the same depth.

The magnesium content at the top soil (0 – 15 cm) in the entire fields ranged between 2.24 – 5.60 Cmol kg^{-1} while at the sub soils (45 – 60 cm), it ranged between 0.48 – 2.88 Cmol kg^{-1}indicated the magnesium content of the soil to reduce with an increase in soil depth. Its amount in Field 44 (3.36 – 5.60 Cmol kg^{-1}) suggested a higher amount when compared to the 2.40 – 2.56 Cmol kg^{-1} at a similar depth of; 0 – 15 cm in Field 16 and 42.

Table2: Selected physical and chemical properties of the oil palm rhizosphere soils

*NIFOR Field	Depth (cm)	pH (1:1) H_2o	P mg/kg	N g kg^{-1}	O.C g kg^{-1}	Exchangeable Cations Ca Cmol kg^{-1}	Mg Cmol kg^{-1}	Na Cmol kg^{-1}	K Cmol kg^{-1}	H^+ Cmol kg^{-1}	CEC Cmol kg^{-1}	Clay g kg^{-1}	Silt g kg^{-1}	Sand g kg^{-1}
52	0 – 15	5.60	16.00	0.95	11.5	7.44	3.60	0.65	0.07	0.30	12.06	31.5	21.5	947.0
(3 – 6 years)	15 – 30	5.45	6.65	0.53	7.05	5.20	2.16	0.43	0.04	0.60	8.43	51.5	14.0	934.5
	30 – 45	5.15	8.03	0.41	5.45	4.72	1.60	0.40	0.03	0.80	7.55	76.5	10.0	913.5
	45 – 60	4.75	5.03	0.33	4.35	3.77	1.28	0.33	0.02	1.20	6.60	102.0	6.6	889.5
42	0 – 15	6.30	15.52	0.93	10.90	5.12	2.40	0.40	0.60	0.60	8.58	44.0	29.0	927.0
(10 - 15 years)	15 – 30	5.70	10.86	0.69	8.95	3.76	1.52	0.32	0.04	0.80	6.44	69.0	24.0	907.0
	30 – 45	5.00	7.46	0.53	7.35	3.12	0.90	0.42	0.04	1.10	5.58	96.5	16.5	887.0
	45 – 60	4.80	4.39	0.37	5.60	2.40	0.64	0.46	0.03	1.40	4.93	119.0	9.0	872.0
16	0 – 15	5.80	10.33	1.00	12.15	4.00	2.48	0.33	0.07	0.40	7.28	31.5	26.5	942.0
(16 –20 years)	15 – 30	5.60	7.64	0.66	8.85	3.45	1.92	0.31	0.04	0.50	6.22	51.5	21.5	927.0
	30 – 45	5.35	6.13	0.42	7.25	3.04	1.52	0.34	0.04	0.60	5.54	111.0	9.5	879.5
	45 – 60	4.90	4.17	0.33	4.25	2.64	0.88	0.28	0.02	0.80	4.62	156.5	9.5	834.0
44	0 – 15	6.45	12.71	1.12	13.45	8.24	4.48	0.42	0.08	0.20	13.42	29.0	34.0	937.0
(>25 years)	15 – 30	6.15	9.31	0.71	9.25	5.52	3.12	0.30	0.05	0.40	9.39	61.5	24.0	926.0
	30 – 45	5.90	7.42	0.50	7.25	4.64	2.80	0.40	0.05	0.70	8.59	111.5	11.5	877.0
	45 – 60	5.45	5.58	0.38	5.80	4.40	1.92	0.37	0.02	1.20	7.91	139.0	4.0	857.0

NIFOR FIELD 52; 3-6years old oil palm plantation; *NIFOR FIELD 16; 16 – 20 years old oil palm plantation; *NIFOR FIELD 44; Plantation above 25 years; *NIFOR FIELD 42; 10-15years old oil pam plantation

The Rhizosphere Bacteria population/ differences between similar Depths across Fields

The results in table 3 shows the mean values for the heterotrophic bacteria colony forming unit (cfu) per the various rhizosphere depths while Table 4 below shows the mean comparison of the total heterotrophic bacteria colony forming units between the plantations sampled. The colony forming units of the heterotrophic bacteria in at rhizosphere depth 0 – 15 cm in Field 44 (palms above 25 years of age) was significantly different from the population level in Field 16, (16 – 20 years old palms), Field 42 (10 – 15 years old palms) and Field 52 (3 – 6 years old palms) at same rhizosphere depth. Whereas, the colony forming units of these bacteria at depth; 15 – 30 cm shows no significant differences between Field 44, 16 and 42 but were all significantly different ($p \leq 0.05$) from the population in Field 52 which had the least mean value. At the 30 – 45 cm depth of the rhizosphere, a significant difference in the comparison between Field 16 and Field 42, Field 16 and Field 52 with the highest mean values at Field 16 in both cases. This result of having more organism in older plantations may be due to the higher carbon deposits in the older plantations compared with the young plantations (Ingham, 2000). However, no significant differences existed in samples taken from the 45 – 60 cm depth across the fields. This observation agrees with the findings of Marschner *et al*., (2001) whose investigation revealed that, microorganisms are significantly greater in the matured root zone than the young root tips. The fact that the older oil palm

plantations had more bacteria population in its rhizosphere is also in line with the view expressed by Broeckling *et al*., (2008) that, plant species, plant developmental stage / age and soil type can be major factors that determine the composition of rhizosphere microbial communities. And in this case where the soil type is similar, the age of the plant and their developmental stages can be concluded to have resulted in the differences in terms of the bacteria population of the various plantations examined. The specific bacteria isolates were *Pseudomonas aeroginosa, Micrococcus cereus, Staphylococcus aureus, and Coryrubacteria spp.*

Table 3: The difference in the total Heterotrophic Bacteria Count (THBC) between similar Depths

Plantation	0 – 15 cm	15 – 30 cm	30 – 45 cm	45 – 60 cm
NIFOR FIELD 52	9.00_b	10.67_b	9.33_{ab}	6.67_a
NIFOR FIELD 42	13.00_b	20.33_a	5.67_{ab}	4.67_a
NIFOR FIELD 16	17.67_b	17.67_a	19.33_a	13.67_a
NIFOR FIELD 44	28.33_a	21.33_a	12.33_a	4.67_a
Grand Mean	17.00_a	17.50_a	11.67_b	7.42_b

❖ Values within a row having the same sub-script are not significantly different

Table 4: The Mean comparison for Heterotrophic Bacteria in the various plantations

NIFOR Field	Age of palms	Means
52	3-6years old	8.92_b
42	10-15years old	10.92_b
44	Above 25years old	16.67_a
16	16-20years old	17.08_a

❖ Mean values having the same letters are not significantly different from one another at 5% level of probability using Duncan multiple range test.

Conclusion and Recommendations

This investigation showed that, the microbial composition under the rhizosphere of oil palm varies with age with the older plantations having higher population than the younger plantations. The bacteria composition were highly concentrated at the top soil (0 – 30 cm) than in the sub soil (30 – 60 cm) and had its peak at 15 – 30 cm soil depth. Although the highest population of bacteria count was found with palms ranging between 16 and 20 years of age but on its comparison ($p \leq 0.05$), there were no significant differences between its count and those that were in the plantations above 25 years of age.

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