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MICROBIAL LOAD AND PHYSICO-CHEMICAL PROPERTIES OF SOIL IN NATIONAL CEREAL RESEARCH INSTITUTE RICE FIELD, BADEGGI, NIGERIA

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ABSTRACT
This study was carried out to evaluate the microbial load and pyhsico-chemical properties of the soil in National Cereal Research Institute rice field, Badeggi, Nigeria. The soil samples collected from fadama, hydromorphic and uncultivated field of NCRI were serially diluted and plated on Potato Dextrose Agar and Nutrient Agar. The incubation was done at 27^{o}C and the associated bacteria and fungi were isolated and enumerated. The physico-chemical properties of the soil were also done. A total of seven (7) bacteria; *Echericha coli, Staphylococcus aureus, Enterobacter aerogenes, Chromobacterium* sp. *Pseudomonas aureginosa, Micrococcus luteus*, and *Bacillus cereus* were isolated and identified. In Fadama, *Chromobactreium* sp. has the highest percentage frequency (21.89%), *Micrococcus luteus* in hydromorphic (24.99%) and *Pseudomona aerugenosa* (23.79%) in uncultivated soil. A total of eight fungi belonging to five general were isolated, identified and characterized to be *Aspergillus niger, Ppenicillium* sp.*, Fusarium* sp.*, Rhizopus* sp., *Aspergillus flavus, Neurospora sitophilia, Mucor* sp. and *Aspergillus fumigatus.* In Fadama, *Aspergillus niger* has the highest percentage frequency (24.28%) while *Aspergillus flavus* has the highest percentage frequency (23.70% and 25.07%) in hydromorphic and uncultivated field respectively. The moisture content of Fadama and hydromorphic are 8.80$\pm$0.01 and 8.00$\pm$0.01 respectively which were significantly different ($p<0.05$)from Uncultivated soil(3.00$\pm$0.01) The results on mineral content of the three soils revealed that the organic carbon, phosphorus, exchange acidity, sand and clay were not significantly different ($p>0.05$) however, the percentage Nitrogen content were significantly different ($p<0.05$) with Fadama soil having the highest percentage of (0.43%) from hydromorphic soil and uncultivated soil which were not significantly different. The calcium, potassium and sodium were significantly different ($p<0.05$).Therefore, The analysis showed that soil microbes and its physico-chemical properties play very vital role in soil nutrient in rice cultivated field as the uncultivated soil showed less microbial activities and physic-chemical properties hence low nutrients.

KEY WORDS: Fadama, Hydromorphic, Uncultivated, Bacteria, Fungi, Rice field

INTRODUCTION
Rice (*Oryza sativa*) is one of the daily staple food crops in Nigeria. As the main sources of energy, rice forms the foundation of diet for many people. It is also the major source of protein to

those who cannot afford to buy meat, fish or other protein foods (Chandi and Sogi, 2006). Furthermore, rice contains vitamins, minerals, dietary fiber, low fat and also has no cholesterol (FAO, 2006; Ibukun, 2008). However, the optimum rice production is hampered by soil nutrient availability and pathogens. It is therefore pertinent to identify the effects of microbes in soil and the physio-chemical properties on the nutritional content of the soil *vis - a -vis* the production of rice (Bateman *et al.* (2000).

Soil is a complex mixture of organic and inorganic material. Soil is a product of rock weathering by agents of denudation and has physical, chemical and biological components. Soil is essentially a suitable medium for the growth and development of plants. To a great extent, soil quality determines the nature of the plant ecosystems and capacity of land to support animal life (Marinara *et al*., 2000). Results of physical and chemical tests provide information about the capacity of soil to supply the required mineral nutrients. The status of available microbes in soils and their relationship with various physico-chemical properties have been attempted by several investigators (Krishnadas, 2009).

The role of soil microbes in ecological processes and their functionality relates to 90% of all biological functions in the soil including water dynamics, nutrients cycling and disease suppression (Ritz and Young, 2004; and Hoorman *et al.*, 2011). They are a major decomposer in the ecosystem They also allow better assimilation and absorption of nutrients by plants through mycorrhizal association alter the soils natural micro flora, have a strong bearing on vegetation and correlated soil profile (Puangsombat *et al*., 2010; Sharma *et al*., 2011). According to Hoorman *et al.* (2011), the ability of Microbes to utilize nutrients such as carbon, nitrogen and phosphorus in the soil allow plants to increase their nutrients uptake capacity. The deficiency of microbes in soils has become a major constraint to productivity, stability and sustainability of soils, since soils with low microbial activity cause low growth and development in plant (Bell and Dell, 2008). This research was therefore carried out to evaluate the status of the available microbial population and the physico-chemical properties in rice field of National Cereal Research Institute (NCRI), Badeggi, Nigeria.

MATERIAL AND METHODS

Study Area

The research was carried out at National Cereal Research Institute (NCRI), Badeggi, Niger State, Nigeria (9.0568° N, 6.1434° E) rice field. The NCRI Badeggi is one of the fifteen (15) commodities Agricultural Research Institute in Nigeria under the aegis of the Federal Ministry of Agriculture and Rural Development.

Collection of Soil Samples

The soil samples were obtained from Fadama, Hydromorphic and Uncultivated (control) fields at NCRI, Badeggi Niger State, Nigeria. Samples were collected from cleared soil surface into sterile polythene bags with soil augar at the depth of 15cm. The samples were taken to the NCRI central services laboratory for all the analysis.

Preparation of Media

Potato Dextrose Agar (PDA)

Thirty nine (39) gram of PDA (Hi-media) was suspended in 1000ml distilled water and heated to dissolve the powder completely, the medium was sterilized by autoclaving at 121^{O}C for 15minutes (Aremu *et al.,* 2013).

Nutrient Agar

Nutrient agar was prepared by dissolving 28g of dehydrated nutrient agar powder into 1 liter of distilled water. It was heated to homogenize it and then sterilized by autoclaving at 121°C for 15 minutes. The Nutrient agar was used for isolation of bacteria (Aremu *et al.,* 2013).

Isolation and Identification of Bacteria

Twenty five grams (25) of samples was mixed thoroughly with 225ml of Buffer peptone water (BPW) and serially diluted to about 10 dilution factor. Already prepared nutrient agar of about 15ml (kept at about 45± 1^{o}c in a water bath) was poured into already sterilized petri dishes and allowed to solidify. 0.1ml from the seventh dilution tube was poured onto the surface of the solidified media (NA) spread evenly on the surface of the media in a petri dish and incubated at 32 ± 1^{o}c for 24 to 48hrs. After incubation, the bacterial colonies were counted and recorded. Streaking was done to get pure cultures of the different bacterial colonies. From pure culture isolated, biochemical tests (gram staining, catalase reaction, glucose fermentation, oxides test) were carried out to identify the bacteria.

Isolation of Fungi

Serial dilution technique was used. Ten (10g) of each sample was aseptically transferred into sterile distilled water in test tubes. It was shaken properly to allow even distribution of microorganisms present in the sample. Dilution factors 10^{-1} and 10^{-2} were used as stock solution. 1ml of each dilution was aseptically taken from the suspension and transferred into sterile Petri dishes. 10ml of PDA was pour into the petri dish with 1ml chlorophenicol. The plates were swirled gently to allow even distribution of the sample. Incubation was done at room temperature 28±2^{o}C for 24hrs. Subcultures were made until pure cultures were obtained. Identification of isolated fungi was done using their morphological features and mycological monograghs (Onion *et al.,* 1985. Cannon and Kirk, 2007; Adebola and Amadi, 2012).

Physico-Chemical Parameter of Soil Samples

Physical Properties

The colour of the soil sample was determined using munsel colour chart. Soil texture was determined by feel method (AOAC, 2009). Soil moisture was determined by weighing method**.**

Chemical Properties

The soil sample was air dried for about 24hrs, crushed gently and sieve with 2mm sieve before using for analysis. Soil pH was measured using digital pH meter, organic carbon was determined by dichromate titration method and nitrogen content was determined by dichromate titration method and nitrogen content was measured by micro-kjeldahl digestion method. Available phosphorus was determined by bray no 1 extraction method. Atomic absorption spectrophotometer was used to measure the calcium and mangnesium content in the extract, while sodium and potassium was determined by flame photometer method. The effective cation exchange capacity was evaluated by the summation method (AOAC, 2009).

Analysis of Data

All data generated were subjected to analysis of variance (ANOVA) and New Dunca Multiple range test was used to separate the means.

RESULTS

A total of seven (7) bacteria; *E. coli, Staphylococcus aureus, Enterobacter aerogenes, Chromobacterium* sp., *Pseudomonas aureginosa, Micrococcus luteus* and *Bacillus cereus* were isolated and identified.

Table 1: Biochemical Characteristics and Microscopic Identification of Bacterial Isolates

Biochemical test	Isolated Bacteria						
	E.coli	*S.aureus*	*M.* sp	*B. cereus*	*C.* sp	*E. aerogenes*	*P. aeruginosa*
KOH Solubility	+	_	_	_	+	+	+
Gram staining	_	+	+	+	_	_	_
Shapes	rods	coccus	coccus	rods	rods	rods	rods
Catalase test	+	+	+	+	+	+	+
Mannitol	+	_	_	_	+	+	_
Glucose	+	+	_	+	+	_	_
Lactose	+	+	_	_	_	+	_
Indole rxn	+	_	_	_	_	_	_
Spore stain	_	_	_	+	_	_	_
Oxidase test	_	_	+	_	+	_	+
Citrate test	_	_	_	+	_	+	+

+ =Positive - =Negative

While a total of eight fungi belonging to five general were isolated and identified to be *Aspergillus niger, Penicillium* spp., *Fusarium* spp., *Rhizopus* spp., *Aspergillus flavus, Neurospora sitophilia, Mucor* spp. and *Aspergillus fumigatus* (Table 2).

Table 2: Morphological Properties and Microscopic Identification of Fungal Isolates

Macroscopic characteristic	Texture	Microscopic characteristics	Organisms
Whitish to yellowish pigment that latertum, brownish red on the reversed side	Velvety	Septate hyphae, unbranched conidiophores from the foot of species	*Aspergillus niger*
Dark green colony with white ring powdery that grows moderately. The reverse side is cream	Powdery	Septate hyphae, branched conidiophores with secondary branches metulas. Sterigmata bears round conidia in chain	*Penicillium* sp.
Orange colony with white ring at the edge that is orange in the reversed	Floppy	Sepate hyphae, side shaped macroconidia, conidiophores bears conidia containing conidiospores	*Fusarium* sp.
Creamy colony that later turns black	Powdery	Aseptate hyphae, unbranched, sporangiophores arose from foot of rhizoid. Scattered spores which submerged in agar	*Rhizopus* sp.
Light green colony with white to cream	Velvety	Sepate hyphae, unbranched conidiophores scanty sterigmata	*Aspergillus flavus*
Orange to yellow colony that turns dark brown in complete dark	Fluffy	Sepate hyphae and unbranched sporangiophores	*Neurospora sitophilia*
Grayish white colony that later turns black	Powdery	Non-septate hyphae, unbranched sporangiopores with smooth appearance	*Mucor* sp.
White colony that turns dark green with white edge	Floppy	Conidiophores is one direction only and unbranched	*Aspergillus fumigatus*

In Fadama soil, seven bacteria were isolated out of which *chromobacterium* sp. has the highest percentage frequency (21.89%) followed by *Micrococcus luteus* (19.93%). *E.coli* has the least percentage (6.72%). In Hydromorphic soil seven bacteria were isolated out of which *Micrococcus luteus* has the largest percentage frequency (24.99%) followed by *Chromobacterium* sp. (18.70%). *E. coli* has the least percentage frequency (4.32%). In uncultivated soil, the same number of bacteria were isolated out of which *Pseudomonas aureginosa* has the highest percentage frequency (23.79%)followed by *Bacillus cereus*(19.00), *Staphylococcus aureus* (5.84%) and *Chromobacterium* sp. (18.7%).The results were significantly different ($p<0.05$) from their respective uncultivated soil (Table 3).

Table 3: Frequency of Bacterial Isolates from Rice field Soil

	Frequency of Bacterial in Soil Plots (%)		
Isolated Bacteria	Fadama	Hydromorphic	Uncultivated
E. coli	6.72^{a}	4.32^{a}	9.23^{b}
Bacillus cereus	15.84^{c}	15.89^{c}	19.00^{d}
Micrococcus luteus	19.93^{d}	24.99^{d}	18.39^{d}
Pseudomonas aeruginosa	16.52^{c}	17.35^{c}	23.79^{e}
Staphylococcus aureus	7.12^{a}	8.75^{b}	5.84^{a}
Chromobacterium spp	21.89^{d}	18.70^{c}	13.24^{c}
Enterobacter aerogenes	11.98^{b}	10.00^{b}	10.42^{b}

Mean in column followed by the same superscript are not differ significantly at ($p<0.05$)

Eight (8) fungi were isolated in Fadama soil out of which *A. niger* has the highest percentage frequency (24.28%) followed by *A. flavus* (23.33), however *Mucor* sp. has the least percentage frequency (4.47%). Out of eight (8) fungi isolated from hydromophic soil, *A. flavus* has the highest percentage frequency of (23.70%) followed by *A. niger* (20.50%) however, *Fusarium* sp. has the least percentage frequency (4.40%).

Table 4: Frequency of Fungi Isolates from Rice field Soil

	Frequency of fungi in plots		
Isolated Fungi	Fadama	Hydromorphic	Uncultivated
A. flavus	23.33^{d}	23.70^{d}	25.07^{d}
A. niger	24.28^{d}	20.50^{c}	22.49^{c}
A. fumigatus	9.08^{b}	6.44^{a}	8.39^{b}
Fusarium sp.	5.68^{a}	4.40^{a}	8.37^{b}
Neurospora sp.	8.77^{b}	13.41^{b}	8.03^{b}
Penicillium sp.	16.36^{c}	19.71^{c}	19.47^{c}
R. stolonifer	8.06^{b}	6.23^{a}	4.09^{a}
Mucor sp.	4.47^{a}	5.61^{a}	4.11^{a}

Mean in column followed by the same superscript are not differ significantly at ($P<0.05$)

From the physical appearance, the colour of the soil sample from Fadama and uncultivated sites were grayish, while that of hydromorphic was brown (Table5). All soil texture of the farm sites were fine sand except in uncultivated sample that was granular. Moisture content of the Fadama and hydromorphic site ranged between (8.80 ± 0.01 – 8.00 ± 0.01) which was significantly different ($p<0.05$) from the uncultivated (3.00 ± 0.01). Electrical conductivity of the soil samples for Fadama, hydromorphic and uncultivated field were (200,240, and 90µs/cm) respectively which are significantly different ($p<0.05$) (table5).

Table 5: The physicochemical properties of NCRI rice field soil

Sample	Colour	Texture	Moisture content	Conductivity
Fadama	Gray	fine sand	8.00 ± 0.01^{b}	200^{b}
Hydromorphic	Brown	fine sand	8.00 ± 0.01^{b}	240^{c}
Uncultivated	Gray	Granular	$3.00+0.01^{a}$	90^{a}

Mean in column followed by the same superscript are not differ significantly at ($P<0.05$).

The results on mineral content of the three soils revealed that their pH were not significantly different ($p<0.05$). The same results was obtained for organic carbon, phosphorus, exchange acidity, sand and clay, however, the percentage Nitrogen content were significantly different ($p<0.05$) with Fadama soil having the highest percentage of (0.43%). The hydromorphic soil and uncultivated soil were not significantly different. The calcium, potassium and sodium were significantly different ($p<0.05$) (table 6).

Table 6: Mineral content of the three types of soil from NCRI

SAMPLS	PH	Organic	carbon %	Nitrogen %	Available	Phosphorus	PPM	$CaCmolkg^{-1}$	$MgCmolkg^{-1}$	$KCmolkg^{-1}$	$NaCmolkg^{-1}$	Exchangeable	Acidity %	SAND %
Fadama	6.03^{a}	2.81^{a}	0.43^{b}	21.78^{a}	1.88^{b}	1.46^{a}	0.22^{a}	1.7^{b}	1.4^{a}	50.4^{a}	15.75^{b}	4.1^{a}		
Hydrmorphic	4.6^{a}	4.07^{a}	0.39^{a}	22.87^{a}	1.82^{b}	2.28^{b}	0.24^{a}	1.78^{b}	1.18^{a}	54.2^{a}	14.78^{b}	4.3^{a}		
Uncultivated	5.15^{a}	3.47^{a}	0.32^{a}	22.33^{a}	0.51^{a}	1.27^{a}	0.18^{a}	0.52^{a}	1.58^{a}	52.5^{a}	12.76^{a}	5.07^{a}		

Values along the column followed by the same superscript are not significantly different at (P<0.05)

DISCUSSION

The prevalence pattern of micro-organisms is related to the nature of the microbial resident in the particular type of soil. The high population density of micro-organisms in the studied rice cultivated field could be mainly due to the availability of nutrient content in the soil that determine the level of activity play by the microorganism in soil health, as earlier reported Akare (2003).

The dominant bacteria isolated; *E. coil, Bacillus cereus, Micrococcus luteus, Pseumodonas aureginosa, Staphylococcus aureus, Chromobacterium* sp. *Enterobacter aerogenes* and fungi; *Aspergillus flavus, A. niger A. fumigates, Fusarium* sp.*, Neurospora* sp.*, Penicillium* sp.*, rhizopus stolonifer and Mucor* sp. was clear indication that they also play a significant role in nutrient retention used by rice plant.

Plant Growth Promoting Rhizobacteria (PGPR) is one of the numerous beneficial microorganisms i.e. free living bacteria that colonize rhizosphere soil and enhance plant growth by a wide variety of mechanisms (Malleswari and Bagyanarayana, 2013). Such PGPR strains use one or more direct or indirect mechanisms to improve the growth and health of plants. Direct mechanisms include fixation of atmospheric nitrogen, solubilization of phosphorous, synthesis of plant growth hormones such as indole acetic acid (IAA), gibberellins and cytokinn and production of siderophores, while indirect mechanisms involve biological control of pathogens and deleterious microbes through the production of antibiotics, hydrogen cyanide, lytic enzymes and catalase or through competition for nutrients and space can improve significantly plant growth and health as evidenced by increase in seedling emergence, vigour and yield (Kloepper and Beauhamp 1992 ; Glick 1995; Verma *et al.,* 2010; Silini-cherif *et al.*, 2012). However most of the bacteria isolated in this study were among the group of PGPR.

It is clear from the data that fungal population of Fadama which has the highest amount of *Aspergillus niger* (24.28%) was probably because of the farming activities carried out and the used of fertilizer. The beneficial effect of fertilizer in increasing fungal population was reported by many workers (Karmegan and Daniel, 2000; Marinara, 2000; ICRISAT and APRLP, 2003) and ascribe the higher nutrient supply as the reason for it.

The observation on the selected parameter such as: colour texture moisture, electrical conductivity, pH and mineral content such as organic carbon, Nitrogen, phosphorus, potassium, exchangeable Acidity, sodium greatly affected the diversity of these micro-organisms. Moisture content plays an important role in microbial activity in the soil. Increase hydromorphic while the uncultivated recorded lower content (3.00±0.01). The moisture content of the hydromorphic soil was slightly lower than the one from Fadama soil, this is because Fadama soil has much water content and the activities carried on Fadama soil were both during dry and raining season, while at hydromorphic soil, activities were done during the raining season. The uncultivated field soil was low compared to the other soil samples due to less activity. Moisture content is chiefly responsible for the colonization of microorganisms (Marianara *et al.,* 2000).

Fadama soil colour was gray with fine texture with electrical conductivity was 200µs/cm. While hydromorphuc rice soil colour was black, the texture fine sand which is the same with the Fadama soil, but it has the highest electrical conductivity of 240µs/cm. Among all the three soil samples analyses, the uncultivated field soil was also gray colour but the texture was granular and the electrical conductivity was 90µs/cm which was also lower than that of the Fadama and hydromorphic soils samples. The Fadama soil and hyromorphic soil have the same soil texture, this is because their soil texture, that is fine sand hold a lot of water as earlier reported by Hole *et al.* (2005) who pointed out the important role of soil texture in the retention water and availability of nutrient in soil.

Soil pH is considered a master player in agriculture as it affects nutrient availability and other important chemical processes. In this study, the pH of sampling site range from 6.03 for Fadama soil, followed by uncultivated soil with 5.15 and the hydromorphic soil has the lowest pH of4.60 which has stronger acidity than the other soil. This seemingly wide range of pH values among sampling sites might be due to the differences in soil types and the fertilizers used by farmers. This, however is not apparently detrimental to the cultivation of rice since the crop is well adopted to different soil conditions including pH as reported by Lal (1998) who reported that pH less than 6.6 is best for growing rice and can produce higher yields than neutral and alkaline soil. This implies that fungi abundance increase slightly as soil pH decreases. Rousk and colleagues (2010) explained that fungi, unlike bacteria have wide pH tolerance for optimum growth and though are less affected by pH gradients.

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