Ecological Niche of Some Wetland Microbes

A. O. Ajayi[1*]

[1]*Department of Microbiology, Adekunle Ajasin University, P.M.B. 01, Akungba-Akoko, Nigeria.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

This study was focused on identifying and characterizing the ecological microbial communities in soil samples from Akoko communities in Akoko South West Local Government area, and Akure, the State capital, Ondo State, Nigeria. Parameters such as soil temperature, pH, and some biochemical characteristics of the microbial communities were determined. The total viable bacterial counts estimated for this purpose ranged from 58×10^7 cfu gm^{-1} in Borehole soil sources to 98×10^7 gm^{-1} in Stream sediment source. Similarly, some physiologic studies show that the temperature of the soil samples ranged from 28.0°C to 30.1°C, while the pH for borehole sample is pH6.09, stream, pH5.82 and river, pH6.25. The bacterial population of fish pond sources range from 20×10^7 cfu gm^{-1} to 55×10^7 cfu gm^{-1}. Twenty three bacterial isolates were obtained from Akoko communities and this includes *Bacillus* spp., *Acinetobacter* spp., *Eubacterium* spp. *Staphylococcus* spp., *Proteus* spp. *Acidobacteria* spp., *Escherichia coli, Klebsiella* spp., *Flavobacterium* spp. and *Pseudomonas* spp. In addition to this were six (6) bacterial and two (2) fungal isolates obtained from pond soil sources in Akure, Ondo State, Nigeria. This is constituted of *Salmonella* spp. *Bacillus* spp., *Clostridium* spp., *Streptococcus* spp., *Enterococcus feacalis* and *Lactobacillus* spp. While the fungal isolates include *Aspergillus niger* and *Aspergillus flavus.* The study will add to our knowledge of the microbial communities from different ecological sites in Nigeria and can be helpful in environmental management and site improvements after disturbances due to human interference and industrial developments.

Corresponding author: E-mail: olajide.ajayi@aaua.edu.ng;

1. INTRODUCTION

The soil forms the basic substrate for various microbial activities. It is a mixture of inorganic and organic materials that covers the earth and support the growth of plants. Mineral particles in most soil are compounds of silicon, aluminium and iron and lesser amounts of other minerals including calcium, magnesium, potassium, titanium, manganese, sodium, nitrogen clay particles (0.0002m or less) to large pebbles and gravels. Generally, these properties differ in various soil sources [1,2]. The physical structure, aeration, water holding capacity and availability of nutrients are determined by the proportion of these particles which are formed by the weathering of the rock and degradative activity of microorganisms. The plants and animal remains deposited on or in the soil contribute to organic substances. In the last stage of decomposition, such materials are referred to as humus, a dark colored amorphous substance composed of residual organic matter not readily decomposed by microorganisms. Microbial populations of both dead and living cells contribute significantly to the organic matter of soil, as this have impact on their ecology and survival [3].

There are various forms of microorganisms that constitute the soil microbial ecology, including bacteria, actinomycetes, protozoa, fungi, algae and of course viruses especially in aquatic environment. Bacteria are primary decomposers and make soil fertile by providing nutrients for plant growth. This is a very complex process that involves many types of bacteria. The heterotrophic bacteria are functional groups of bacteria that utilize oxygen in growth and represent a very diverse and important cross-section of soil microorganisms. Its enumeration is a good indicator of general soil conditions with the number of these bacteria decreasing with increasing depth in the soil. The upper 6 inches of agricultural soil generally contains between ten million and one billion (1 x 10^7 and 1 x 10^9) colony forming units (CFU) of heterotrophic bacteria per gram of soil. Finished compost will contain higher microbial load up to 1 x 10^{10} CFU/g [4].

In a similar way, Actinomycetes are a large group of bacteria that grow as hyphac-like fungi under certain environmental conditions and capable of degrading many complex chemical substances including chitin at neutral soil pH and are intolerant of waterlogged soils. This is based on microbial physiological state within the soil microbiome niche [4,5]. Most soil Actinomycetes are in the genus Streptomycetes and are well known for the production of antibiotics [5]. However, the presence of antibiotic substances in the soil can rarely be detected except active in a micro-environment. Agricultural soil Actinomycetes enumerations are typically in the range of 100 thousand to one million (1 x 10^5 to 1 x 10^6) CFU per gram of soil as in finished prepared with woody substrate like sawdust [4]. Such substrate can be used to culture some microbes and living things like mushrooms.

Soil microbial ecology plays a significant role in global ecosystems. Environmental impact of a proposed development project is assessed prior to activity taking place [4,6]. EIA is a powerful tool which ensures that the environmental and cultural impacts of proposed development activities are assessed, taken into account in decision-making and mitigation. Oil exploration is one of the environmental challenges facing society today. It has a major focus because of its importance to the oil producing communities and the society at large. It has a negative impact on the environment influencing food chains, destroying habitat and injuring or even killing species [7].

Bacteria from borehole soil have an ecology that is relative to some underground water microbes. Boreholes soil can be obtained down to 620 m below the ground level. Previous studies shows organisms obtained to include, *Acinetobacter, Bacillus, Desulfovibrio* or *Thiomicrospira*. The 16S RNA genes from 20 selected isolates were closely related to the sulphate reducers *Desulfomicrobium baculatum* or *Desulfovibrio* spp., the iron reducer *Shrewanella putrefaciens*, or distantly related to the genus *Eubacterium*. Viable counts confirmed the presence of sulphate-reducing bacteria. Borehole water is in many ways like well water, but is usually cleaner and easier to access [8].

Various forms of bacteria thrive well within stream soil. Similarly, soil bacteria and fungi play pivotal role in various biogeochemical cycles (BGC) [9]. The study of Ernebjerg and Kishony [2] stressed the complexity of soil microorganisms and the need to invent exemplified way of identifying them. Chemo organotrophic microorganism metabolized

available organic material recycling nutrient within the ecosystem. Autotrophic micro-organisms grow using the mineral released from the organic matter. This lead to the production of oxygen during the daylight hours; respiration occurs at night, resulting in diurnal oxygen shifts. Thus when the amount of organic matter added to the stream does not exceed the systems oxidative capacity, productive streams and rivers are maintained [10].

In this study, the occurrence and nature of organisms that constitute various ecological niche of the soil in human environment was determined. This will help to prospect for valuable soil microbes of industrial importance as well as control possible outbreak or epidemic in the various sites which may occur as a result of Zymogenous (intruders) bacteria which may arise due to some environmental influence. Various aquatic sources (including borehole soil zone, stream soil and river soil) and some terrestrial sources were taken into consideration for this purpose.

2. MATERIALS AND METHODS

Materials used for this study were washed with detergent and adequately sterilized appropriately at 121°C for 15 minutes in an autoclave, in case of culture mediums, or in hot oven 170°C for two hours for glassware and related materials. The medium routinely used for the study includes Nutrient agar, Mueller Hinton Agar and Potato Dextrose Agar. The general purpose and enriched medium were used in this study in correlation to the methods of Szewzyk et al. [8] who used some enriched saline medium for culturing similar soil microbes in boreholes and aquatic zones. Thereafter they were allowed to cool for use. Laminar flow chamber and work benches were sterilized by swabbing with cotton wool soaked in 70% ethanol.

2.1 Study Site and Collection of Samples

Soil and sediment samples were collected from four sites in Ondo State: Akungba-Akoko, Ayepe-Iwaro, River Ose and Akure in Ondo State, Nigeria. Akungba-Akoko, Ayepe-Iwaro and River Ose are located in Akoko South West Local government area of Ondo State that is located between longitude 5.440E and 5.450E and latitude 7.24 N and 7.28N. The samples were collected using sterile containers (super bottles), sterile spatula and hand glove from Akungba-Akoko stream, Ayepe-Iwaro borehole and River

Ose. In Akure town, the State capital, Ondo State, Nigeria [11,12], located in coordinates 7°15′0″N 5°11′42″E, samples were collected in areas such as Oshinle street, Roadblock area Akure, NEPA street and Ijoka road pond soil sources. The farm managers and their assistants assisted in collecting the soil samples usually below 5 meters deep from ground level. Samples were collected in triplicates from sampling sites.

Samples collected from study sites were immediately transferred to the laboratory for analysis. Sterile sample bottles used for this study were labeled with the date of collection, site and serial number of soil sample from various sites. In borehole, the soil sample was collected at a depth of about 20 meters at Ayepe Agbadotun Street using sterile spatula into a sterile super bottle. This was intensified with the assistance of technicians constructing borehole who brought out some of the soil samples removed during their construction process. This approach has correlation with the method of Abiola et al. [13] who explored some geoelectric conditions of one of the Akoko communities within the study area. While in streams, the sediment sample was collected close to the water at a depth of about 1 meter at Akungba-Akoko beside AUD secondary school manually into sterile polythene bag. In similar way, sediment sample was collected for river such as Ose river bank along Owo township road with the assistance of some divers at a depth lesser than 5 meters from surface. Modified method of Hazell et al. [14] and Roger et al. [15] was adopted in this sample collection system by taking into consideration some environmental factors. The pH of each sample was recorded. Collection of samples from different parts of Ondo State, Nigeria was randomly intensified in this study to enhance coverage of relatively large ecological zone in this part of the country.

2.2 Methods of Identification of Microorganism

Preliminary characterization of bacteria isolates was based on some standard microbiological methods including Gram stain, morphological and cultural characteristics. Further characterization was carried out with various biochemical tests such as catalase, coagulase, indole production, motility, gelation, fermentative reactions to sugars like glucose, sucrose, lactose, maltose, mannitol and growth characterization on MSA, MacConkey and Nutrient agar. Media used were prepared

according to manufacturer's specification and sterilized, unless where stated otherwise at 121°C for 15 minutes.

3. RESULTS

This study shows various aquatic soils sources from where samples were obtained for analysis (Fig. 1). Different types of microorganisms were isolated from these sources. In Table 1, total viable bacterial counts estimated ranged from 58 x 10^7 cfu gm^{-1} in borehole to 98 x 10^7 cfu gm^{-1} in site stream. Similarly, some physico-chemical parameters of samples determined includes the temperature of the soil samples which was 30.1°C, while the pH for borehole sample in Akungba-Akoko was pH6.09, stream in Supare, pH5.82 and Ose river, pH6.25 (Table 1). The Standard Deviation of 17.613 and 12.134 for Total bacterial count (107x cfu gm-1) and Enteric organisms (%) occurrence respectively shows a relatively wide range of dispersion of microorganisms in this sources compared to low deviation of 3.771 and 0.5 in Enteric pathogens (107x cfu gm-1) and Anaerobic organisms (107x cfu gm-1) respectively. The microbial load in pond water sources range from 20 x 10^5 cfu gm^{-1} in roadblock area, Akure to 55 x10^5 cfu gm^{-1} in NEPA street zone in Akure (Table 2).

Twenty three bacterial isolates were obtained from aquatic soil sources during the study from some Akoko communities, Nigeria. They were categorized into genus *Bacillus* spp., *Acinetobacter* spp., *Eubacterium* spp., *Staphylococcus* spp., *Proteus* spp. and *Acidobacteria* spp. Others were *Escherichia coli, Klebsiella* spp., *Flavobacterium* spp. and *Pseudomonas* spp. (Table 3). Similarly, six (6) bacterial and two (2) fungal isolates were obtained from selected pond soil sources in Akure Township, Ondo State, Nigeria (Tables 3 and 5). The percentage occurrence of this bacterial isolates in wetland sources studied was determined as shown in Table 4. The bacterial isolates in this context were *Salmonella* spp. *Bacillus* spp., *Clostridium* spp., *Streptococcus* spp., *Enterococcus feacalis* and *Lactobacillus* spp. While the fungal isolates were *Aspergillus niger* and *Aspergillus flavus* (Tables 3 - 5).

4. DISCUSSION

This study shows relatively high microbial population of selected aquatic zone soil ecological niche (Fig. 1). This is consistent with the findings of Itoh et al. [16] which show that changes in bacterial community composition can be correlated with geographic distance along a stream, for comparison reasons based on different streams and rivers environmental conditions. Complimentary to this, Kataja-aho, et al. [17] shows that soil microbial activity (CO_2 production) was higher in the stump removal plots but similar difference was not found in sieved soil samples incubated in the laboratory.
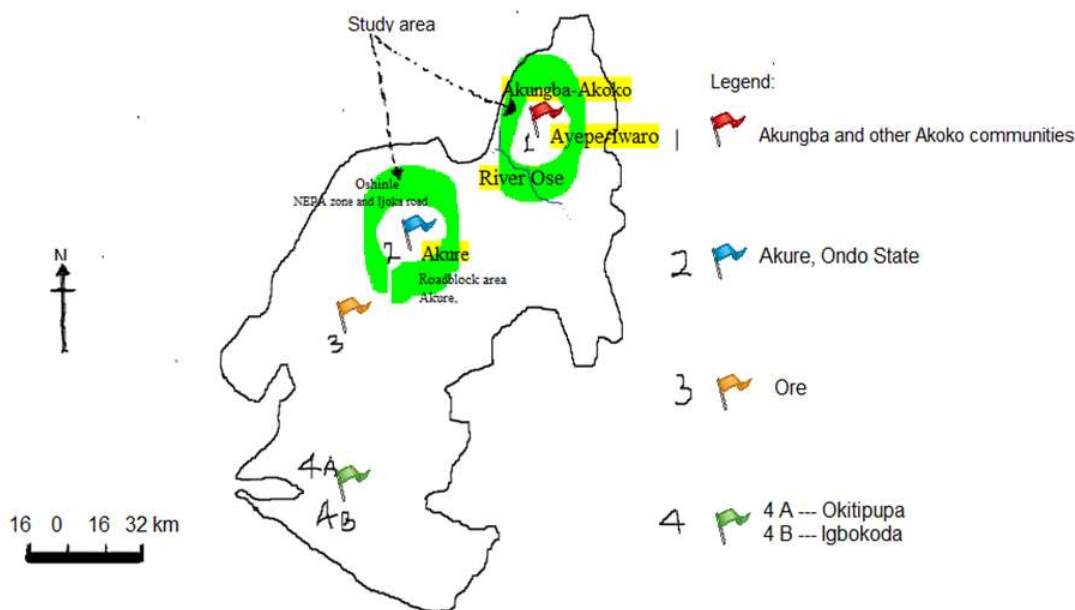

Fig. 1. Map of Ondo State, Nigeria showing sampling sites

Table 1. Total viable bacterial counts and some physico-chemical parameters of samples

Sample code	Total bacterial count (on NA) (10^7x cfu gm^{-1})	Enteric pathogens (on MAC) (10^7x cfu gm^{-1})	Enteric organisms (%) occurence	Anaerobic organisms (on MSA) (10^7x cfu gm^{-1})	pH	Temperature	Odour	Colour
Aq1	58	30	51.72	None	6.09	30.1	Odourless	Brown
Aq2	98	22	22.45	9	5.82	30.1	Odourless	Dark brown
Aq3	92	30	32.61	8	6.25	30.1	Odourless	Brown
Standard deviation	17.613	3.771	12.134	0.5	0.177			

Legend: Aq1 – Borehole, Aq2 – Stream soil, Aq3 – River soil, NA – Nutrient Agar, MAC – MacConkey Agar, MSA – Mannitol Salt Agar

Table 2. Total viable bacterial counts of fish pond soil sources (cfu/mL)

Sample code	Total viable bacterial counts (x10^5 cfu gm^{-1})
A1	32
A2	45
B1	20
B2	25
C1	55
C2	30
D1	28
D2	35

Legend: Sample A: Oshinle street, B: Roadblock, Akure; C: NEPA street; D: Ijoka road

Table 3. Bacterial isolates from wetland sources

Sample code	Bacterial isolates from borehole, stream and river soil zone.	Sample code	Bacterial isolates from pond sediment
1. Aqa	*Staphylococcus aureus*	B1	*Bacillus* spp.
2. Aqa	*Klebsiella* spp.	B2	*Clostridium* spp.
3. Aqa	*Acidobacteria* spp	C1	*Lactobacillus* spp.
4. Aqa	*Escherichia coli*	C2	*Salmonella* spp.
5. Aqa	*Aerobacter aerogene*	D1	*Enterococcus feacalis*.
6. Aqb	*Flavobacterium* spp*.*	D2	*Streptococcus* spp.
7. Aqb	*Proteus* spp.		
8. Aqb	*Pseudomonas* spp.		
9. Aqb	*Bacillus subtilis*		
10. Aqb	*Acinetobacter* spp.		
11. Aqb	*Bacillus subtilis*		
12. Aqb	*Acinetobacter* spp.		
13. Aqb	*Eubacterium* spp.		
14. Aqb	*Proteus* spp.		
15. Aqb	*Bacillus* spp.		
16. Aqb	*Acinetobacter* spp*.*		
17. Aqb	*Acidobacteria* spp.		
18. Aqc	*Psuedomonas aeruginosa*		
19. Aqc	*Escherichia coli*		
20. Aqc	*Klebsiella* spp*.*		
21. Aqc	*Psuedomonas aeruginosa*		
22. Aqc	*Staphylococcus* spp.		
23. Aqc	*Flavobacterium* spp*.*		

Legend: Aqa – Borehole, Aqb – Stream soil, Aqc – River soil, Sample A: Oshinle street, B: Roadblock, Akure; C: NEPA street; D: Ijoka road

This implies that, soil disturbance by stump pulling and/or differences in the microbial communities and quality of soil organic matter in the differently treated soil significantly influence ecology and microbial population of a wetland zone. The estimated populations' range of

58 x 10^7 cfu gm^{-1} in borehole sample source to 98 x 10^7 cfu gm^{-1} in stream sources (Table 1) is consistent with previous investigations [6,16].

River soils and some wetlands typically contain 10^9 to 10^{10} microorganisms per gram (dry weight), which may represent more than a million bacterial and fungal species [18]. This has some relevance to the microbial load of 9.2 and 9.8 x 10^8 cfu/mL obtained (Table 1) in both river and stream sediment sources respectively during the study. In correlation with the study of Strickland and Rousk [18], soil microbial communities are an integral component of many ecosystem processes which are productive in nature. However, characterization of the small fraction of microbes that has been cultivated provides only a glimpse of their potential physiological capacity and influence on soil ecosystems. The Standard Deviation of 17.613 and 12.134 for Total bacterial count (107x cfu gm-1) and Enteric organisms (%) occurrence respectively which shows a relatively wide range of dispersion of microorganisms in this sources compared to low deviation of 3.771 and 0.5 in Enteric pathogens

(107x cfu gm-1) and Anaerobic organisms (107x cfu gm-1) respectively is significant. The reason for this is that there are wide variations in that total microbial population and pathogens of the wetland sources based on the zone studied compared with generally low anaerobic isolates recovered from all sources.

The absence of pure cultures or genome sequences makes it difficult to ascertain the roles of specific microbes in soil environments: this is particularly true for bacteria in the phylum *Acidobacteria*, which are broadly distributed in soils but poorly represented in culture. This is unique but have relevance with nitrogenous and plant growth supporting attributes of rhizobacteria widely distributed in some agricultural soil zones [19,20]. The phylum *Acidobacteria* is now officially recognized in Bergey's Manual of Systematic Bacteriology and includes three genera with cultured representatives: *Acidobacterium*, *Geothrix*, and *Holophaga*. The genus Solibacter was recently proposed as the fourth genus in this phylum [16,21]. The Gram positive organisms were

Table 4. Occurrence of bacterial isolates from wetland sources (%)

Serial no.	Bacterial isolates from wetland sources	Number and percentage (%) occurrence of isolates
1.	*Staphylococcus aureus*	2 (6.67)
2.	*Klebsiella* spp.	2 (6.67)
3.	*Acidobacteria* spp	2 (6.67)
4.	*Escherichia coli*	2 (6.67)
5.	*Aerobacter aerogene*	1 (3.33)
6.	*Flavobacterium* spp.	2 (6.67)
7.	*Proteus* spp.	2 (6.67)
8.	*Pseudomonas* spp.	1 (3.33)
9.	*Bacillus subtilis*	2 (6.67)
10.	*Acinetobacter* spp.	3 (10)
11.	*Eubacterium* spp.	1 (3.33)
12.	*Bacillus* spp.	2 (6.67)
13.	*Psuedomonas aeruginosa*	2 (6.67)
14.	*Staphylococcus* spp.	1 (3.33)
15.	*Clostridium spp.*	1 (3.33)
16.	*Lactobacillus* spp.	1 (3.33)
17.	*Salmonella* spp.	1 (3.33)
18.	*Enterococcus feacalis*.	1 (3.33)
19.	*Streptococcus* spp.	1 (3.33)
20.	Total isolates	30 (100)

Table 5. Cultural and morphological characteristics of fungal isolates

Sample code	Spores and conidia arrangement	Colour	Hyphae	Probable isolates
A	Conidia had radical surface containing many phallides and chains of conidia collumella is absent.	Yellowish green colony	Non-septate	*Aspergillus flavus*
B	Conidia heads are black in colour and conidiophores are very long.	Black powdery colony	Non-septate	*Aspergillus niger*

Legend: Fungal isolate sample from A: Oshinle street, B: Roadblock, Akure

unable to grow on MacConkey agar due to their inhibition by the presence of bile salt and crystal violet [10]. Apart from the microbial population of soil ecological zones, physiological characteristics of both bacterial and fungal isolates from pond sources were also determined.

5. CONCLUSION

In conclusion, it was observed that the microbial load of borehole soil sources was low compared with that of river and stream sources including some enteric pathogens (Table 1). This can be result of some sedimentation by gravity of these microbes. Nevertheless, some of this group of organisms may not survive for long based on low nutrient nature and portability of borehole water sources, but it send signals to caution against underground water pollution. Complementary to this observation, this study helps to clarify various types of organisms that can be encountered in some soil ecological niche. It is important to note that some fractions of enteric pathogens constitute a niche of the wetland microbiomes which is significant healthwise. This serves as a challenge for the treatment of some of these ecologic zones with appropriate disinfectants. It also enhances some future prospects in assessing our wetland sources qualitatively for sustainable scientific and economic development. The report obtained in this context can serve as environmental bench mark for environmental amelioration, management and industrial purposes.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13637