

Bacterial Diversity and Abundance in Wetland Soils of Itu Akwa Ibom State, Nigeria

Bassey, Maria. E.^{*}, Umana Seyene. I., Iwatt, M. U.,
and Ekanem Ekaette. U.

Department of Microbiology, Akwa Ibom State University, Ikot Akpaden, Mkpatt Enin L.G.A, Nigeria

Corresponding author: **Bassey, Maria** | E-mail: mariabassey@aksu.edu.ng

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Abstract

*This study investigated bacterial diversity and abundance in wetland soils of Itu, Akwa Ibom State Nigeria. The study aimed at assessing the relationship between the distribution of bacterial communities and their abundance using standard microbiological and analytical techniques. The study was carried out to identify the bacterial communities inhabiting the wetland soil in Itu, Nigeria. The study area was randomly demarcated using line transects. Soil samples were collected using soil auger from the depth of 0-30cm in each sites to represent the vertical distribution and four different locations to represent the horizontal distribution and transported in iced packed containers to the laboratory for bacterial analysis using standard procedures Serial dilution technique and streaking method were used to inoculate the samples on Nutrient Agar plates and incubated at 37°C for 24 hours. Bacterial counts were determined using colony counter and Colonies observed were identified using gross morphology, gram staining reaction and biochemical tests. The results obtained revealed significant difference ($P \leq 0.05$) in the bacterial load present in the four study sites. The results revealed high bacterial loads (1.67×10^6 – 1.42×10^6) in areas close to the water bodies with high amount of debris (station A). The bacterial species identified were *Bacillus subtilis*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The result implies that, the conservation status of the study area is at risk as most decomposers were found deep in the soil as the distributions related with variations in organic carbon, clay content and soil water characteristics at the field scale.*

Keywords: Diversity, Wetland, Bacterial and Soils.

INTRODUCTION

Wetlands are renowned for their ecosystem services such as purifying polluted water, remediating storm water and supporting diverse wildlife communities. It is therefore referred to any geographical area with characteristics of both dry land and bodies of water [1]. A Wetland is a terrestrial habitat in which the soil is saturated with moisture either permanently or seasonally such areas may also be covered partially or completely by shallow pools of water [2]. The physicochemical properties of wetlands vary widely in accordance with the multiplicity and diversity of environmental, lithological and pedogenetic factors with which the wetlands are associated [3]. This implies that wetlands are characterized by certain water regimes, plant species and soil characteristics [4]. In most cases the water level in wetlands called the water table is usually at above or just below the soil surface for enough time to restrict the growth of plants to those adapted to wet condition and promote the development of soil characteristics of wetland environment [2].

Soil microorganisms play crucial roles in various biogeochemical processes [5], facilitating the decomposition of excess nutrient in wetland

ecosystems under both aerobic and anaerobic soil conditions and are the foundation of the wet ecosystems [5]. Studies have shown that microbial nitrification and denitrification processes regulate about 80-90 % of the total nitrogen in wetlands. In addition phosphorus accumulating organisms may absorb phosphate from wastewater under alternating aerobic and anaerobic conditions [6]. However, the nutrient removal as well as other ecosystem functions of wetlands can differ due to factors that alter physicochemical properties and microbial compositions [6].

Factors such as soil depth gradients, water depth, soil organic carbon and nitrogen concentrations, soil water contents and pH can substantially influence microbial communities [7]. In addition soil microbial communities are likely to shift over time, as the soil total organic carbon in wetlands may gradually increase with wetland age. Therefore understanding the composition and diversity of microbial communities and their relationships with environmental characteristics that shape this ecosystem is necessary [9]. Wetland soils constitute vast underexploited and sometimes undiscovered ecologies in many countries including Nigeria.

The acidic nature of the wetlands soils can influence the soil biological community; a consequence of the acidic nature of the parent rock and the influence of the leached profile under high annual rainfall condition. Studies have shown that wetland soils have considerable agricultural potentials for the production of rice, maize, dry season vegetables cocoyam and early yam species [10-11]. Wetland serves as transition between terrestrial and aquatic ecosystems, therefore functions as buffers for terrestrial runoff thereby preventing eutrophication of inland as well as coastal waters. Wetland is essential for hydrological and ecological process and the area supports a rich floristic diversity and fauna. Wetland also serves as a water filter nutrients and sediments are abundant and that makes it possible for many species to live [12]. The close proximity of oxic-anoxic conditions in this area often created by the wetland plant roots facilitates the simultaneous activity of aerobic as well as anaerobic microbial activities. Wetland soils possess unique physicochemical characteristics, including high moisture content, abundant organic matter, fluctuating redox conditions, and varying nutrient availability [13]. These properties can significantly influence the nutritional and anti-nutritional composition of plants and fruits grown in such environments. The notational properties of plant have greater potentials on human health [14 15]. Wetlands receiving industrial effluents, agricultural runoff, or urban wastes may contain elevated concentrations of toxic metals, including lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As). These metals can be absorbed and accumulated by plants and subsequently enter the human food chain through consumption [16]. The bioaccumulation of these contaminants may adversely affect human health [17], leading to various toxicological effects depending on the level and duration of exposure [18-20].

MATERIALS AND METHODS

SAMPLE COLLECTION

Soil samples were collected from two depths (0-15cm and 15- 30 cm) from four locations/ sites (A,B,C,D.) in Itu, Akwa Ibom State. The soil samples were collected using methods of Anderson and Ingram [21] during the wet and dry season into labeled sterile polythene bags and taken in ice packed coolers to the laboratory for microbiological analysis.

MICROBIOLOGICAL ANALYSIS

Serial Dilution

Tenfold serial dilution of the soil samples were made using standard methods.

Inoculation and Incubation

One milliliter of appropriate tenfold serial dilutions of the soil samples was inoculated onto nutrient agar (Oxoid CM 314) in five replications using pour plates method.

Inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 18 – 24 hours at 32°C for the enumeration of total heterotrophic bacteria count Visible discrete colonies in incubated plate were counted using colony counter and expressed as colony forming unit per gramme (Cfu/g) of soil sample.

The organisms were identified using gross morphology Gram staining reaction and biochemical tests. Data obtained for bacterial counts was analyzed using Analysis of Variance (ANOVA) with Duncan's New Multiple. Range test used to separate significant means at 5% level.

Maintenance of Pure Culture

Discrete colonies were purified by repeated subculture unto nutrient agar. Pure cultures were preserved on nutrient agar slants and stored in the refrigerator ($4^\circ\text{C} \pm 2^\circ\text{C}$) at ambient temperature ($28^\circ\text{C} \pm 2^\circ\text{C}$) for further analysis.

Characterization and identification of Bacterial Isolates

The pure bacterial strains were identified using gross morphology, Gram staining reaction and biochemical tests including carbohydrate utilization [22]. Identification of bacterial isolates was accomplished by comparing the characteristics of the cultures with that of a known taxa as in Domsch *et al.*, [23].

RESULTS AND DISCUSSION

The result of the total heterotrophic bacteria count obtained in this study ranged from 1.10×10^6 – 1.67×10^6 Cfu/g and a significant difference ($P \leq 0.05$) in bacterial loads present in four study area. The distribution of these organisms in the soil of study is presented in Table 1.0. The results showed high bacterial counts in the area close to the water bodies with high amount of organic matter. The bacteria isolates were *Bacillus cereus*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Similar observations were reported by Udotong *et al.* [13], who observed higher bacterial populations in wetland soils with elevated organic matter content.

Table 1: Bacterial Counts at Different Soil Locations in Itu L.G.A

Study Location	Soil depth /Bacterial load (CFU/g)	
	0-15cm	15- 30cm
A	1.67×10^6	1.42×10^6
B	1.26×10^6	1.10×10^6
C	1.18×10^6	1.16×10^6
D	1.15×10^6	1.17×10^6

Key:

Station A – Ntak Inyang

Station B – Ikot Ebom

Station C – Mbak

Station D – Odiok

Table 2: Biochemical characterization of bacterial isolates from Itu wetland

Isolate code	Gram's reaction	Cell Morphology	Spore	Ind	Mr	Vp	Cit	Mot	Cat	Glu	Oxi	lacs	Suc	Gal	Man	Probable organism
ITW1	+ve	Rod	+	-	-	+	+	+	+	A	-	-	A	A	A	<i>Bacillus subtilis</i>
ITW 2	-ve	Rod	-	+	+	+	+	-	+	A G	-	-	AG	A	-	<i>Proteus vulgaris</i>
ITW3	-ve	Rod	-	-	-	+	+	-	+	AG	-	AG	-	-	A	<i>Enterobacter aerogenes</i>
ITW4	-ve	Rod	-	-	-	+	+	-	+	AG	-	A	A	A	A	<i>Klebsiella pneumoniae</i>
ITW5	+ve	Cocci	-	-	+	-	-	-	+	AG	-	AG	AG	AG	AG	<i>Staphylococcus aureus</i>
ITW6	-ve	Rod	-	+	+	-	-	+	+	AG	-	AG	AG	AG	AG	<i>Escherichia coli</i>
ITW7	-ve	Rod	-	-	-	+	-	-	+	AG	+	-	A	A	AG	<i>Pseudomonas aeruginosa</i>

KEY: Ind= indole; Mr=methyl red; Vp= Voges Proskauer; Cit=Citrate; Mot= Motility; Cat= Catalase; Glu = Glucose; Oxi = Oxidase; Lac = Lactose, Suc= sucrose; Gal= Galactose; Man = Mannose

The occurrence and abundance of bacteria obtained in this study is shown in Table 1. The location that shows high bacteria count was the one near the body of water (River). This shows that the conservation state of the area is at risk as most of the decomposers were found deep in the subsoil as distribution of nutrient and microorganisms is related with the variations in organic carbon, clay content and soil water characteristics at the field scale. Moreover, Perz and Restrepo, (2008) reported that, water from such type of wetland is not bacteriologically safe for human consumption. The bacteria species identified were grouped into 6-7 species. Out of this, 64 % were *Bacillus* species, 12% were species of *E. coli*, *Klebsiella* sp (10 %), *Pseudomonas* sp (8%) and *Enterococcus* (6%). Location A had the highest bacterial counts followed by Location C while location B had the least. The distribution of total heterotrophic bacteria count with depth revealed that 0-15 cm had the highest count in all the locations. This is due to the fact that surface soil had higher biomass population than the sub soil as reported by Akpan and Ayegba, [24].

Bacterial community in the soil of Itu wetland can degrade several types of organic compound [13], [25]. The relative occurrence of these degrading bacteria in Itu wetland soil provides a good surface for agricultural activities. The presence of different bacteria species in Itu wetland soils have indicated the relative significance of the soil sustaining floral diversity despite being useful in bioremediation of pollutants reported by Macaulay [26]. The presence *Enterococcus* spp in the wetland soil in Itu is in line with the findings of Akpan and Ayegba. [24], and Udotong *et al.*, [13] who reported *Klebsiella* among the predominant soil inhabiting bacteria in Eket wetland in Nigeria. However, the bacterial species identified in Baturiya wetland differed from the ones identified by Ballesteros *et al.*, [27] from some wetland in Columbia. This difference could be as a result of variation in climate change and edaphic factors in the study area. Therefore organic matter degrading bacterial species are ubiquitous in many environments [28]. Diversity of microorganisms is a key factor for soil structure, fertility and microbial metabolism and these were identified in this study. The diversity of bacteria in the wetland soils of Itu highlights the ecological importance of these environments as reservoirs of microbial communities. The high bacterial abundance and diversity observed in the wetland soils of Itu demonstrate the widespread distribution of microorganisms in natural environments and their ability to thrive under varying ecological conditions [5], [29].

The bacterial diversity observed in the wetland soils of Itu reflects the ecological richness of the environment and its capacity to support a wide range of microbial species [30-31], the occurrence of diverse bacterial populations in wetland soils, water, fish samples and waste dumpsite suggests that environmental reservoirs such as wetlands may influence the microbial communities [5] [9], [25]

The occurrence of bacterial species with known biodegradation potentials further suggests that the wetland possesses natural capacity for the attenuation of organic pollutants and environmental contaminants [32]. Therefore, the conservation and sustainable management of these wetlands are essential to preserve their ecological functions, maintain microbial diversity, and support agricultural productivity and environmental health in the region.

CONCLUSION

The wetland soil under study had abundant microbial populations. High bacterial loads were found mostly in flooded areas. It was concluded that seven bacterial species were found inhabiting the wetland soil in Itu and their load varied with locations and soil depth. The high diversity of species present in the study area ensures great potential and soil resilience in the degradation process.

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