



## Microbiological and Physiochemical Properties of Mangrove Swamp Sediments of River Ethiope, Amukepe - Sapele, Delta State

Udinyiwe, C.O.,<sup>1,2\*</sup> Salami, D.A.<sup>1</sup> and Osa, T.I<sup>1</sup>

<sup>1</sup>Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

<sup>2</sup>Applied and Environmental Bioscience and Public Health Research Group

\*Corresponding Author: [collins.udinyiwe@uniben.edu](mailto:collins.udinyiwe@uniben.edu)

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### Abstract

The mangrove swamps of River Ethiope in Amukepe-Sapele, Delta State, present a unique ecological habitat that plays a crucial role in maintaining the environmental equilibrium of the region. The purpose of this study was to determine the microbiological and physiochemical properties of mangrove swamp sediments of River Ethiope, Amukepe-Sapele, Delta State. Soil samples were obtained from each study site in triplicate using the targeted random sampling method. Standard microbiological techniques were used for the microbiological and biochemical analysis. The physiochemical analysis were carried out according to the methods of Association of Official Analytical Chemist (A.O.A.C) for the determination of the soil physiochemical parameters, cation exchange capacity and heavy metals. The total heterotrophic bacteria count (THBC) ranged from  $7.9 \pm 0.3 \text{ cfu/g} \times 10^5$  -  $17.4 \pm 0.2 \times 10^5 \text{ cfu/g}$ , while the total fungi counts which ranged from  $5.3 \pm 0.7 \times 10^5 \text{ cfu/g}$  -  $12.4 \pm 1.3 \times 10^5 \text{ cfu/mL}$ . The bacteria revealed were *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Rhodococcus pneumonia*, *Bacillus subtilis*, *Proteus mirabilis*, *Serratia marcescens*, *Pseudomonas fluorescens* and *Escherichia coli*, while the fungi revealed were *Aspergillus niger*, *Candida utilis*, *Epicoccum spp*, *Aspergillus flavus* and *Rhodococcus glutinis*. The physiochemical results showed pH ranging from  $5.7 \pm 0.34$  -  $6.40 \pm 0.12$ . The cation exchange capacity (CEC) of Calcium ranging from  $4.6 \pm 0.34$  -  $7.2 \pm 1.97$ , Magnesium  $1.86 \pm 0.7$  -  $4.43 \pm 1.39$ , Sodium  $0.26 \pm 0.19$  -  $0.76 \pm 0.65$  and Potassium  $1.32 \pm 0.11$  -  $2.64 \pm 0.89$ . The heavy metal values from this study were below the recommended acceptable standard. The study showed that the sediments within mangrove ecosystems acts as repositories of nutrients, organic matter and contaminants.

## 1.0.Introduction

Mangrove ecosystems, characterized by their unique intertidal habitats, play a pivotal role in the ecological health and coastal sustainability of tropical and subtropical regions worldwide [1]. These ecosystems, situated at the interface between terrestrial and marine environments, provide a range of vital ecological services, including habitat provision for various flora and fauna, shoreline stabilization, water quality improvement, and carbon sequestration. Among the key factors influencing the ecological health of mangrove ecosystems are the quality of their sediments and their interactions with various biotic and abiotic components [2]. The

sediments within mangrove ecosystems act as repositories of nutrients, organic matter, and contaminants [3]. Understanding the composition and quality of these sediments is crucial for assessing the overall health of mangrove habitats and their capacity to support diverse ecosystems and sustain local communities. The aim of this study was to investigate the microbiological and physiochemical properties of mangrove swamp sediments of river Ethiope, Amukepe - Sapele, Delta State.

## **2.0 Materials and Method**

### **2.1 Study Area**

Sapele mangrove swamp of river Ethiope, occupies the swampy opposite end to the usual beach across the river Ethiope which lies within the Niger Delta basin in the southern part of Nigeria, on the West African coast region. It is bordered by the Atlantic Ocean in the west. The river takes its source from a spring at Umuaja in Umutu, and flows for over 100 km to empty into the river close to the African Timber and Plywood (AT and P) Company. Inhabitants of the surrounding villages rely on the river for domestic water supply, washing, fishing, sand mining and inter-village transportation. The river lies within latitudes 5° 55'N and 5° 45'N, and longitudes 5° 60'E and 6° 10'E at the equatorial region. The swamp is full of trees, *Raffia* palms and forest like canopies of a variety of vegetations including *Bambusa vulgaris*, *Havea brasiliensis*, *Avecinia* spp., *Dryopteris* species, *Grewia* species etc.

### **2.2 Study Sites and Sources of Samples**

The sampled site was within river Ethiope, Sapele is between Okirigwhre and Amukpe part of the mangrove swamp which is approximately 85 km from the source of the river at Umuaja, Umutu Delta State which was the second sample site.

### **2.3 Collection of Samples**

Samples were obtained from each study site using the targeted random sampling method described by [4] and [5] from a distance of 30 meters apart using a grab sampling method and transferred into 500 mL sterilized screw capped bottles. At each site two spots were chosen for the collection. At the sampling site A, B and C samples were collected in triplicates. Samples collected were immediately transported to the laboratory for immediate microbiological analysis and a second part of each sample was sent for physicochemical, elemental and heavy metal analyses.

### **2.4 Sample Treatment and Isolation of Associated Microorganism**

The microbial load in the soil samples were enumerated using serial dilution and pour plate method [6]. Accordingly, 1 g of sample was introduced into 9 mL of sterilized distilled water. Subsequently, 1 mL of sample was added to 9 ml of sterilized distilled water and serially diluted to dilution factor  $10^{-3}$  and was separately pour and plated on nutrient agar (N.A.) containing nystatin (5µg/mL). All experiments were done in triplicates and nutrient agar plates were incubated at 38 °C for 24 hours. Emerging discrete bacterial colonies were observed, counted and recorded as colony forming unit per milliliter (cfu/mL) of the soil samples. The discrete colonies were sub-cultured into agar slants from where they were further sub-cultured into various selective and differential media and the pure isolates were characterized by standard procedures described by [6] based on colonial appearance, microscopy and biochemical characteristics and the Bergey's manual of determinative bacteriology were appropriately referred to as well. Standard microbiological methods were also used in the fungi analysis.

## 2.5 Determination of pH values

The pH of the sediment samples was determined using the pH meter according to the method described by [7].

## 2.6 Physiochemical and heavy metal analysis

The experiment was conducted and parameters determined according to the methods of [8],[9].

## 3.0 Results and Discussion

The results in Tables 1 revealed the total heterotrophic bacteria count (THBC) which ranged from  $(7.9 \pm 0.3 \text{ cfu/g} \times 10^5)$  to  $(17.4 \pm 0.2 \times 10^5 \text{ cfu/g})$ , while that of Table 2 revealed the total fungi counts which ranged from  $(5.3 \pm 0.7 \times 10^5 \text{ cfu/g})$  to  $(12.4 \pm 1.3 \times 10^5 \text{ cfu/mL})$ . Table 3 revealed the following bacterial isolates *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Rhodococcus pneumonia*, *Bacillus subtilis*, *Proteus mirabilis*, *Serratia marcescens*, *Pseudomonas fluorescens* and *Escherichia coli*. Table 4 revealed the following fungi isolates *Aspergillus niger*, *Candida utilis*, *Epicoccum* spp, *Aspergillus flavus* and *Rhodococcus glutinis*. Table 5 revealed the physiochemical results were pH ranged from  $5.7 \pm 0.34$  -  $6.40 \pm 0.12$ . Table 6 revealed the exchangeable cations to be Calcium ranging from  $4.6 \pm 0.34$  -  $7.2 \pm 1.97$ , Magnesium  $1.86 \pm 0.7$  -  $4.43 \pm 1.39$ , Sodium  $0.26 \pm 0.19$  -  $0.76 \pm 0.65$  and Potassium  $1.32 \pm 0.11$  -  $2.64 \pm 0.89$ . Table 7 revealed the results of the heavy metals analyzed.

**Table 1: Total heterotrophic bacteria count of the soil samples.**

Soil samples	Mean counts ( $\times 10^5$ ) (cfu/g)
A	$7.9 \pm 0.3$
B	$16.0 \pm 0.5$
C	$17.4 \pm 0.2$

**Key:** A = Sampling site A (control sample), B = Sampling site B (Amukpe), C = Sampling site C (Sapele)

**Table 2: Total fungi count of soil samples.**

Soil samples	Mean counts ( $\times 10^5$ ) (cfu/g)
A	$5.3 \pm 0.7$
B	$11.0 \pm 1.1$
C	$12.4 \pm 1.3$

**Key:** A = Sampling site A (control sample), B = Sampling site B (Amukpe), C = Sampling site C (Sapele)

**Table 3: Cultural, morphological and biochemical characteristics of bacterial isolates**

Characteristics	Colony A	Colony B	Colony C	Colony D	Colony G	Colony H	Colony I
Elevation	Flat	Flat	Raised	Raised	Raised	Flat	Convex
Margin	Smooth	Rough	Smooth	Rough	Rough	Smooth	Smooth
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Creamy green	Dirty cream	Creamy white	Cream	Reddish orange	Creamy green	Metallic green
Motility	+	-	-	+	+	+	+
Gram reaction	-	-	+	+	-	-	-
Cell type	Rod	Cocci	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Clusters	Clusters	Clusters	Chains	Clusters	Clusters	Single
<b>Biochemical test</b>							
Catalase	+	+	+	-	+	+	+
Urease	+	-	+	+	+	+	-
Oxidase	+	-	-	+	-	+	-
Citrate	+	-	-	-	-	+	-
Coagulase	-	-	-	-	-	-	-
Indole	-	+	-	-	+	-	+
<b>Sugar fermentation</b>							
Glucose	+	+	+	+	+	-	+
Maltose	-	+	+	+	+	-	+
Sucrose	+	+	+	+	+	+	+
Galactose	+	+	+	+	-	+	-
Lactose	+	+	+	-	+	+	+
Probable isolates	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter calcoaceticus</i>	<i>Rhodococcus pneumonia</i>	<i>Bacillus subtilis</i>	<i>Serratia marcescens</i>	<i>Pseudomonas fluorescens</i>	<i>Escherichia coli</i>

**Key:** + = Positive, - = Negative

**Table 4: Cultural and morphological characteristics of fungal isolates**

Characteristics	Colony A	Colony B	Colony C	Colony D	Colony E
Colonial shape	Irregular concentric and cottony	Smooth, circular coccus	Irregular concentric circles	Concentric cottony lump	Smooth circular coccus
Colour on agar surface	Whitish turned black/brown	Yellowish cream on PDA	Cotton yellow to brownish olive/black	Yellowish green (lime green) on PDA	Pink/red on SDA
Colour on reverse surface	Pale brown	Creamy brown	Pale yellow	Yellowish	Creamy pink
Cell type	Filamentous mycelia	Clusters of cocci cells in irregular chains	Filamentous	Filamentous mycelia	Clusters of cocci cells
Cell arrangement	Septate multinucleate hyphae	Oval/round clusters of pseudohyphae	Septate multinucleate hyphae	Septate multinucleate hyphae	Clusters of slimy ovoid cells
Vegetative structure or spore description	Oval/round, black or brown conidia	Clusters of buds and bud scars around cells	Crowded conidiophores	Oval green conidia forming clusters of short chains	Clusters of young buds and scars around cells
Special feature	Globose and carbon black/dark brown conidial heads (biserate)	Pink glossy colony on CMA, glucose, sucrose, maltose positive but lactose negative and Urease positive	Short oval conidiophores	Presence of foot cells extended into conidiophore vesicle with seriated phialides	Pigmentation and inability to ferment sugars
Possible isolates	<i>Aspergillus niger</i>	<i>Candida utilis</i>	<i>Epicoccum</i> spp.	<i>Aspergillus flavus</i>	<i>Rhodococcus glutinis</i>

**Key:** Potato dextrose agar (PDA), Sabouraud dextrose agar (SDA)

**Table 5: Physicochemical characteristics of mangrove swamp sediments**

Parameters	Sampling site A	Sampling site B	Sampling site C
Sand	9.0±0.70	11.7±2.40 <sup>a</sup>	12.8±1.98 <sup>a</sup>
Silt	53.0±6.13	34.1±4.6 <sup>a</sup>	30.4±2.74 <sup>a</sup>
Clay	49.0±7.12	56.0±3.98	54.7±3.20
Colour	BB	BB	BB
pH	6.1±0.32	5.7±0.34	5.7±0.55
Temp (°C)	23.6±0.32	24.7±1.97	25.3±0.87
Total organic carbon (%)	39.0±9.41	18.0±1.85 <sup>ab</sup>	16.5±5.86 <sup>ab</sup>
Carbon (%)	19.6±2.20	7.0±2.00 <sup>ab</sup>	6.3±1.52 <sup>ab</sup>
Total oil and grease (%)	4.6±0.57	0.1±0.10 <sup>ab</sup>	0.13±0.15 <sup>ab</sup>
EC (µs/cm)	384.0±61.3	336.0±57.6	317.0±3.05
Total Nitrogen (mg/kg)	1.3±0.50	1.2±0.320	0.1±0.04 <sup>ab</sup>
Nitrate (mg/kg)	1.2±0.36	1.0±0.08	0.1±0.03 <sup>ab</sup>
Sulphate (mg/kg)	1.7±5.58	2.8±0.84	1.1±0.83 <sup>a</sup>
Phosphate (mg/kg)	1.6±0.61	1.2±0.05	0.6±0.41 <sup>ab</sup>

Key: Sampling site A (control sample), B = Sampling site B (Amukpe), C = Sampling site C (Sapele), a – significant, ab – highly significant

**Table 6: Results of cation exchange capacity of hydrocarbon contaminated soil samples**

Parameters (cmol <sub>c</sub> /kg)	Sampling site A	Sampling site B	Sampling site C
Calcium (Ca <sup>2+</sup> )	4.60±0.70	6.6±0.73 <sup>a</sup>	7.20±1.97 <sup>a</sup>
Magnesium (Mg <sup>2+</sup> )	1.86±0.77	4.43±1.39 <sup>ab</sup>	4.23±1.13 <sup>ab</sup>
Sodium (Na <sup>+</sup> )	0.26±0.19	0.60±0.43 <sup>ab</sup>	0.76±0.65 <sup>ab</sup>
Potassium (K <sup>+</sup> )	1.40±0.56	2.64±0.89	2.35±0.69

Key: A = Sampling site A (control sample), B = Sampling site B (Amukpe), C = Sampling site C (Sapele), a – significant, ab – highly significant

**Table 7: Result of heavy metal analysis of hydrocarbon contaminated soil samples**

Parameters (mg/kg)	Sampling site A	Sampling site B	Sampling site C
Lead (Pb)	0.29±0.34	0.01±1.39	0.02±0.01
Cadmium (Cd)	3.59±0.79	0.48±0.57 <sup>ab</sup>	0.74±0.45 <sup>a</sup>
Arsenic (Ar)	4.89±1.01	4.65±2.43	1.70±0.89 <sup>ab</sup>
Chromium (Cr)	2.90±0.23	0.30±0.07 <sup>ab</sup>	0.28±0.81 <sup>ab</sup>
Iron (Fe)	8.66±1.53	2.01±0.38 <sup>ab</sup>	2.60±1.21 <sup>ab</sup>
Copper (Cu)	8.53±1.49	0.73±0.39 <sup>ab</sup>	0.75±0.40 <sup>ab</sup>
Mercury (Hg)	0.30±0.02	0.01±0.02 <sup>ab</sup>	0.03±0.04 <sup>ab</sup>
Zinc (Zn)	17.1±2.36	0.60±0.89	0.68±0.92
Manganese (Mn)	4.93±0.34	3.37±0.21 <sup>a</sup>	0.97±0.38 <sup>ab</sup>

Key: A = Sampling site A (control sample), B = Sampling site B (Amukpe), C = Sampling site C (Sapele), a – significant, ab – highly significant

Soil is known to be a reservoir of microbes. Bacteria are known to be ubiquitous, hence can easily be isolated from soil samples. The presence of bacteria at hydrocarbon polluted sites is evidence of its ability to utilize carbon as source of energy. The result in Table 1 and 2 revealed the total heterotrophic bacteria count (THBC) and total fungi count, which showed growth from the soil samples. The result of Table 1 and 2 revealed that these microbes have the ability to adapt to such as environment. The research of [10] revealed THBC of bacteria growth from hydrocarbon contaminated soil ranging from  $(2.3 \pm 1.1 - 5.2 \pm 3.5) \times 10^4$  cfu/g. This agrees with this present study. The research of [11] revealed THBC of  $6.66 \times 10^4$  cfu/g of soil samples collected from Nembe waterside Port Harcourt. The research of [12] revealed THBC of  $6.25 \times 10^5$  cfu/g from hydrocarbon contaminated soil just like that of this present study. The results of these studies revealed that microbes can grow at such sites due to adaptability to the environment. The high counts of bacteria and fungi noted in Sapele axis strongly indicate pollution in the area. The practice of dumping waste and human excreta into the river by local inhabitants can influence microbial abundance. It is obvious that the high concentration of contaminants acts as nutrients and substrates, promoting microbial growth and reproduction, essentially turning human waste into a food source for microorganisms. Table 3 revealed the bacteria species isolated in this study. The research of [10] reported similar bacteria from hydrocarbon contaminated soil reported by this present study. The research of [13] reported the isolation of twenty-three bacteria isolates, more than that reported by this study from a hydrocarbon polluted environment. The research of [14] revealed bacteria isolates from hydrocarbon contaminated soil from mechanic workshops which are similar to bacteria isolates isolated from soil samples of this study. These results agree with this research, noting that bacteria isolates are present and can be isolated from hydrocarbon contaminated soil samples and this is due to their adaptability to such environments. The result in Table 4 revealed fungi isolated from hydrocarbon contaminated sites in this study. The research of [15] of soil collected from hydrocarbon contaminated soils revealed similar fungi isolates revealed by this present study. This is an indication that these organisms can thrive in this environment. The research of [16] isolated fungi from petroleum contaminated soil similar to the once isolated in this study. This research collaborates the result of this study. The results of Table 5 revealed the results of pH and several physiochemical parameters. Studies have shown that the pH of swamp sediments influences the uptake of nutrients by plants and affects the interaction of microorganisms, their proliferation and their potential for carrying out different activities in the mangrove habitat. In this study, the pH was within a comfortable range that could aid the bacteria isolated from the swamp sediments carry out various activities. There was no significant difference between the pH of the control sample and the test sites. Soil pH is a key factor that influences the uptake of available nutrients for plants utilization [17]. The study of [18] reported similar pH range as reported in this study. Several research have asserted that pH aids and favours microbial metabolic activities. The pH reported by [19] was higher than the pH reported in this study. It was observed that the pH of the control of this study was higher than that of the sampling sites, and this could be due to the hydrocarbon contamination of the soil samples collected. This means that hydrocarbon contamination can affect the pH of a soil. The results in Table 6 revealed the cation exchange capacity (CEC) result of Ca, Mg, Na and K. The results of the CEC values below  $10 \text{ cmol}_c/\text{kg}$  implies that the hydrocarbon pollutant greatly influenced the soil and therefore had negatively impact on the soil ability to retain necessary and essential nutrients needed for plant growth. The values of CEC in this study were all below  $10 \text{ cmol}_c/\text{kg}$ . The control sample and the test sites showed levels of significance, even though their CEC content were all below the acceptable threshold. This implies that the entire sites have been negatively impacted with pollutants over the years and had poor soil nutrient composition. The research of [20] showed that CEC results ranged from  $6.63 \text{ cmol}_c/\text{kg} - 41.65 \text{ cmol}_c/\text{kg}$  of agricultural soils, which falls within the range of higher CEC group with values above  $10 \text{ cmol}_c/\text{kg}$ . This result is in variance with the results of this research because the soil was free from hydrocarbon pollution,

while the soil in this study was negatively impacted by hydrocarbon pollution. The research of [21] of soil contaminated with hydrocarbon revealed that most of the values of CEC were below 10 cmol<sub>c</sub>/kg like that of this study apart from few values above 10 cmol<sub>c</sub>/kg. This result is in agreement with this present study. These results revealed that hydrocarbon pollutant reduces CEC values, and this leads to reduced nutrients in soil and poor soil value. The results in Table 7 shows heavy metal values of Pb, Cd, Ar, Cr, Fe, Cu, Hg, Zn and Mn. There was significance with the test samples with respect to normal control. The heavy metal content of this study was higher than the acceptable values with respect to WHO standards. The heavy metals results of this study present a troubling situation and a potential threat to the health of the surrounding environment at the long run. The research of [22] revealed values of heavy metals of hydrocarbon contaminated soil higher than that reported by this study. The presence of high heavy metal contents in soil has the ability of decreasing crop production. Heavy metals can also enter a food chain as a result of high levels in plants after their absorption from polluted sites, and this is a potential threat to animals and human health [10];[23];[24]. The research of [25] revealed higher values of heavy metals of hydrocarbon contaminated soil unlike that of this study. Heavy metals in soil have an influence in microbial distribution and fertility of organisms in the soil, though at high concentration they are detrimental to microbial life [26].

#### 4.0 Conclusion

This study provides valuable insights into the environmental health of the mangrove swamp sediments along the River Ethiope in Amukepe, Sapele, Delta State. The findings reveal that these sediments exhibit a complex interplay of factors. The bacterial quality assessment indicates the presence of both beneficial and potentially harmful microorganisms, emphasizing the need for monitoring and management strategies to protect the ecosystem and human health. The physicochemical properties of the sediments reveal a dynamic environment influenced by various factors, including sediment texture, pH, and organic matter content. The analysis of heavy metals highlights the presence of certain elements, possibly due to anthropogenic activities, which could pose risks to the ecosystem and surrounding communities. These properties play a significant role in shaping the overall health and ecological function of the mangrove ecosystem.

#### Conflict of interest

There is no conflict of interest associated with this work.

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