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MICROBIAL ABUNDANCE, PHYSICOCHEMICAL PROPERTIES AND HEAVY METALS OF MANGROVE SWAMP WATER OF RIVER ETHIOPE SAPELE IN COMPARISON WITH THE SOURCE AT UMUTU, DELTA STATE

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ABSTRACT

Mangrove swamps are distinct and diverse coastal ecosystems located in tropical and subtropical regions. This study was carried out to evaluate the microbial abundance, heavy metals, and physicochemical properties of mangrove swamp water of River Ethiope Sapele, in comparison with the source at Umutu, Delta State. The bacterial and fungi isolates were identified using standard microbiological techniques and the values for physicochemical and heavy metal parameters were obtained using standard methods. The total heterotrophic bacterial counts ranged from 5.7±0.9 x 10^5 cfu/mL to 10.3±1.2 x 10^5 cfu/mL, while the total fungal counts ranged from 3.3±0.3 x 10^5 cfu/mL to 5.0±1.5 x 10^5 cfu/mL. The probable bacterial isolates identified were *Pseudomonas aeruginosa, Acinetobacter calcoaceticus, Rhodococcus pneumonia, Bacillus subtillis, Proteus mirabilis, Chromatium okenii, Serratia marcescens, Pseudomonas fluorescens, Escherichia coli.* While the probable fungal isolates were *Aspergillus niger*, *Candida utilis.*, *Epicoccum* spp., *Aspergillus flavus and Rhodotorula glutinis.* The Physicochemical analysis carried out revealed values for respective parameters to include pH, ranging from 5.07±0.21 to 6.47±0.39, temperature (26.37±0.09 ºC to 26.70±0.51 ºC), and BOD (1.08±0.02 mg/L to 2.67±0.16 mg/L). While the heavy metal analysis revealed values to include Cadmium ranging from 0.15±0.03 mg/L to 2.59±0.07^{a} mg/L, Chromium (0.16±0.02 mg/L to 1.23±0.04 mg/L), and Zinc (0.46±0.02 mg/L to 8.47±0.07^{ab} mg/L). Most of the physicochemical and heavy metals parameters analyzed did not fall within the standard values set by World Health Organization for drinking water. This study was able to reveal that there was more pollution at the Sapele axis of River Ethiope than its source at Umutu, due to human activities, including the release of untreated waste into the river from surrounding industries and municipalities.

Keywords: Heavy metals, Microbial, Physicochemical, River Ethiope
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INTRODUCTION

Water is an immensely vital resource for human survival and stands as the most unique natural compound on our planet [1]. While water plays a crucial role in supporting human life and biodiversity, it can also be a significant source of disease transmission when contaminated [2]. As a universal solvent, water has the capacity to dissolve a wide array of substances, some of which can either benefit or harm living organisms. The quality of water is influenced by various physical, chemical, and biological parameters, as well as factors like vegetation, land usage, and geological structure, which dictate its beneficial utility [3, 4]. In Nigeria, natural water bodies have been used for the disposal of refuse, human sewage, and untreated wastewater from residential, agricultural, and industrial areas [5, 6]. The primary physical and chemical parameters affecting the aquatic environment encompass temperature, rainfall, pH, salinity and dissolved oxygen, along with total suspended and dissolved solids, total alkalinity and acidity, and the presence of heavy metal contaminants. These parameters serve as limiting factors for the survival of aquatic organisms, including both flora and the human body [7]. Mangrove swamps are distinct and diverse coastal ecosystems located in tropical and subtropical regions. Mangrove swamps play a critical role in protecting, stabilizing, and providing a secure habitat for aquatic organisms, while also contributing to pollution remediation in the ecosystem [8, 9].

Additionally, they offer various goods and services to humans, including aquaculture, forestry, shoreline erosion protection, wood for burning and household items, as well as other products like wax and honey [10]. Beyond erosion protection, mangroves mitigate the impact of tsunamis and act as crucial nurseries for juvenile fish [11]. Despite the recognized benefits of maintaining healthy environment, these ecosystems face significant threats and are currently vanishing at a rate of 1% to 2% annually across their habitats [12]. The microbial communities within mangroves, including freshwater, marine, and terrestrial soil microbiota, play substantial roles in biogeochemical cycles, such as nitrogen fixation, nitrification,

denitrification, phosphate solubilization, carbon cycling, reduction of sulfur, and other elements, as well as cellulose decomposition [13, 14]. The primary hazardous components of water pollutants are heavy metals, including lead, arsenic, cadmium, manganese, chromium, and mercury [15]. Due to activities like burning of fossil fuels, agricultural pesticide production, and domestic and industrial sewage, mangrove swamps have become exposed to heavy metal pollution. These pollutants disrupt the food chain, posing harm to both humans and microorganisms. However, in microbial communities, there is rapid responses to environmental shifts, and this serves as pivotal indicators of environmental pollution [16]. Due to the constant increase in anthropogenic activities in rivers, ongoing environmental surveillance and reporting are crucial to promote economic stability, environmental protection, and sustainability [17].

The Ethiope River, which is widely utilized for recreational and economic activities such as fishing, swimming, washing, bathing, and other domestic activities, has been significantly impacted by pollution and degradation. Various human activities, including dredging, burning wood planks, logging, as well as agricultural and residential runoff, occur in the rivers, particularly around the study areas, negatively affecting the aquatic environment. Local inhabitants wash clothes along the river course, and some of the farmers, who are mainly cassava producers, release chemicals (cyanide) from cassava processing and washing into the stream, posing a significant threat to human health. Also, during the rainy season, the river overflows its banks, conveying domestic waste and rain runoff into the river. Continuous monitoring of the river's water quality parameters is essential to determine if they fall within acceptable ranges for the survival of aquatic organisms [18]. This study was carried out to evaluate the microbial abundance, heavy metals, and physicochemical properties of mangrove swamp water of River Ethiope, Sapele in comparison with the source at Umutu, Delta State.

MATERIALS AND METHODS

Study site

The site used for this study was between Okirigwhre and Amukpe part of the mangrove swamp of River Ethiope, Sapele which is about 85 km from the source of the river at Umuaja, Umutu, Delta State, which was the second sampling site. The Ethiope River is the longest river in Delta State, and it finds its way through various parts of this coastal West African States, bordered by the great Atlantic Ocean to the West. Samples were taken three times from two distinct random locations within Sapele axis and from one random location within Umutu axis. The sampling sites were designated as A, B and C.

Collection of water samples

Water samples were collected from each study site from a distance of 30 meters apart by scooping technique, using the targeted random sampling method described by Ogbeibu and Ogboghodo *et al.* [19, 20]. The collected samples were introduced into 500 mL sterilized screw capped bottles. At each spot, A, B and C samples were collected in triplicates denoted as A1, A2, A3, B1, B2, B3, C1, C2, and C3 respectively. Samples collected were transported right after to the laboratory for immediate microbiological analyses and a second part of each sample was sent for physicochemical, elemental and heavy metal analyses [20, 21, 22].

Enumeration and isolation of associated microorganism

The enumeration of microbial population in water samples was done using serial dilution and pour plate method Scott *et al.*; Cheesebrough; Ehiagbonare *et al*. [23, 24, 25]. Respectively, 1 mL of each sample was added to 9 mL of sterilized distilled water and gently swirled to mix, after which nine-fold serial dilution was made and aliquots (0.1 mL) of the serially diluted suspensions at dilution factor 10^{-4} was separately inoculated and plated on Nutrient agar (N.A.) medium containing nystatin (5 µg/mL) and Potato dextrose agar (PDA) medium containing Ciprofloxacin (1 µg/mL). All experiments were done in triplicates and N.A plates were incubated at 38ºC for 24 hours, while PDA plates were incubated at 27±2ºC for 72 hours. Following incubation, emerging discrete bacterial and fungal colonies of the isolates were observed, counted, and recorded as colony forming unit per milliliter (cfu/mL) of the water samples.

Characterization and identification of isolates

The bacterial and fungal isolates were characterized and identified based on their colonial appearance, microscopy, and biochemical characteristics. 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 Cheesebrough [24] and the Bergey's manual of determinative bacteriology were duly referred to. The preparation of pure fungal cultures for microscopy was done by degreasing clean glass slides, adding drops of ethanol, and fragments of pure cultures were transferred onto the slide. With the aid of a sterile Pasteur pipette, a drop of lactophenol blue stain was added, made even on the slide with flamed blunted needle for about 60 seconds, slides were separately covered with a cover slip and viewed under the microscope, and appropriate mycological atlas consulted [24, 26].

Physicochemical analyses:

Determination of pH values
The pH of the water samples was determined using the pH meter (*in-situ)* according to the methods described by Barry *et al.*; A.O.A.C. [27, 28]. The digital pH meter was inserted using the sensitive tip into the spots of sample collections and the reading were taken and recorded.

Determination of temperature values
The temperature (*in-situ*) at each sampling spot was determined using the mercury in glass thermometer, with respect to the method of A.O.A.C. [28]. The temperature was determined, and temperature measurements recorded in ^oC.

Determination of colour
Colour determination of the water samples was done in comparison with Munsel colour chart by direct observation of a group of three researchers (*in-situ*) and the colour recorded on a scale of zero to three as: 0 = colourless, 1 = mildly coloured, 2 = highly coloured, and 3 = dark turbid.

Determination of odour
This was carried out by observation of a group of three sensitized researchers using sensory omparison (*in-situ*), and the odour was scored on a scale of zero to three. The odour was rated from 0 to 3 as: 0 = Odourless, 1 = mild odourous, 2 = strongly odourous, and 3 = very strongly odourous [28].

Determination of turbidity
Turbidity determination of the water samples was done using nephelometer also known as turbidity meter and readings recorded in nephelometric turbidity units (NTU) according to the method of A.O.A.C. [28]. In the process, the turbidity meter was calibrated with standard cuvettes to 0 NTU and the samples were introduced respectively into cuvettes which were then placed inside the receiving chamber and the readings recorded.

Determination of nitrate
The nitrate content of the water samples was determined according to the method of A.O.A.C. [28]. Summarily, 10 mL of aliquot of the water samples was added to 2 mL brucine reagent and sulphuric acid in 250 mL volumetric flask. The solution was mixed for 30 seconds and dispensed into smaller tubes which were allowed to stand for 5 minutes and the transmittance was measured at 470 nm using spectrophotometer.

Determination of sulphate in water
Twenty (20) mL aliquot of water sample was pipetted into 250 mL volumetric flask. Thereafter, 1 mL of gelatin barium chloride reagent was added and the content mixed thoroughly and allowed to stand for 30 minutes. The transmittance and optical density were measured at 420 nm using spectrophotometer [27].

Determination of phosphate in water
The phosphate content of water samples was spectrophotometrically evaluated by the method of A.O.A.C. [28]. In the process, 20 mL of aliquot was added to 20 mL of 0.002N H_2SO_4. The mixture was stirred and the suspension filtered using Whatman No. 40 filter paper to obtain a clear solution. Two (2) mL of ammonium molybdate solution and 5 drops of stannous chloride reagent were added to 50 mL of the filtrate, to develop blue colouration. The intensity of the colour was measured by using spectrophotometer at 690 nm.

Determination of dissolved Oxygen (DO) content of samples
The dissolved oxygen was determined (*in-situ)* using DO meter (Extech 407510A), using the methods of A.O.A.C. [28] and Wu *et al.* [29]. The spots for sample collection were randomly selected at each site and homogenized by a localized stirring for 7 minutes using a sterilized spatula and allowed to settle for 3 minutes. The DO meter sensor was then immersed, and readings recorded.

Determination of biological oxygen demand (BOD)
The biological oxygen demand (BOD) was determined using the method described by A.O.A.C. [28]. The 5-day BOD was computed from difference in the DO value of day 1 and day 5. Biological Oxygen Demand (BOD) was done using the relationship BOD = DO - DO5.

Determination of total oil and grease
The oil and grease content of each water sample was determined using non-volatile oil and grease extraction method of Gulla and Waghray; A.O.A.C. [30, 28]. Exactly 100 mL of sample measured into pre-weighed crucibles was re-weighed (weight of crucible and content) and refrigerated at 4^oC overnight to precipitate visible oil and grease which were separated into a different pre-weighed crucible. All crucibles and content were evaporated to dryness, removed from the oven cooled in a desiccator and the weight was recorded. The non-volatile oil and grease extract were expressed as a percentage of the sample weight loss given by:

$$\text{Oil and Grease (mg/L)} = \frac{\text{Weight loss x 1000}}{\text{Weight of sample}} \quad (1)$$

Or

$$\text{O and G (mg/L)} = \frac{W_1 - W_2 \text{ x } 1000}{W_0} \quad (2)$$

Where: W_0 = weight of sample
W_1 = weight of empty oven dried thimble plus weight of sample
W_2 = weight of oven dried thimble and residue after extraction

Determination of total suspended solids (TSS)
The total suspended solids of the water sample were determined according to the method described by Wu *et al.* [29]. In the process, about 100 mL of the sample was measured, weighed and filtered through a pre-weighed filter. The residue retained together with the filter was then dried in an oven at 103°C to 110°C until the weight of the filter no longer changes. The increase in weight of the filter represents the total suspended solids. This can be represented as:

$$TSS\ (mg/L) = \frac{(Mf_2 - Mf_1)}{V} \quad \text{_ _ _ _ _ _ _ _ _ _ (3)}$$

Where: Mf_1 = Mass of filter prior to filtration
Mf_2 = Mass of filter after filtration and heating
V = volume of sample filtered (L)

Determination of total hardness
Total Hardness was determined by titrating with EDTA using Eriochrome black T as indicator [28].

Determination of total dissolved solids
Total dissolved solid (TDS) was determined gravimetrically by filtration and drying of known weight of milli filter. In summary, a known volume of samples was filtered through a pre weighed 0.40 to 0.50 mm pore size membrane filter. The filtrate was then evaporated to dryness in a pre-weighed crucible to a constant weight and the TDS estimated as the weight difference of the crucible before and after drying [28].

Heavy metal analysis
The analysis of the following heavy metals and elements were carried out: Zinc (Zn), Chromium (Cr), Manganese (Mn), Lead (Pb), Iron (Fe), Cadmium (Cd), Arsenic (As), Mercury (Hg), Copper (Cu), Magnesium (Mg) and Calcium (Ca). The water samples of up to 100 mL in a volumetric flask and the concentration of the elements/ metals was measured with the atomic absorption spectrophotometer (AAS) according to the method of A.O.A.C. [28].

Statistical analysis
Statistical analyses of the data were analyzed using SPSS software version 20 (SPSS Inc. Chicago) and results presented as described by Ogbeibu [19]. Data obtained were also subjected to descriptive (mean and standard error of mean) analysis and charts made using Microsoft Excel, as well as inferential (analysis of variance) analysis.

RESULTS AND DISCUSSION

Water serves as a reservoir for numerous contaminants and a natural habitat for many microorganisms. Despite the escalating pressure on water resources in both developed and developing nations, comprehending the presence of pollutants in these aquatic environments holds great significance.

Table 1: Total heterotrophic bacteria count of the water samples

Water samples	Mean counts ($x10^5$) (cfu/mL)
A	5.7±0.9
B	10.0±1.5
C	10.3±1.2

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

Table 2: Total fungi count of the water samples

Water samples	Mean counts ($x10^5$) (cfu/mL)
A	3.3±0.3
B	5.0±1.5
C	4.3±0.3

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

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

Characteristics	**Colony A**	**Colony B**	**Colony C**	**Colony D**	**Colony E**	**Colony F**	**Colony G**	**Colony H**	**Colony I**
Elevation	Flat	Flat	Raised	Raised	Flat	Flat	Raised	Flat	Convex
Margin	Smooth	Rough	Smooth	Rough	Rough	Rough	Rough	Smooth	Smooth
Shape	Circular	Circular	Circular	Circular	Circular	Irregular	Circular	Circular	Circular
Colour	Creamy green	Dirty cream	Creamy white	Cream	Cream	Purplish red to cream	Reddish orange	Creamy green	Metallic green
Motility	+	-	-	+	+	+	+	+	+
Gram reaction	-	-	+	+	-	-	-	-	-
Cell type	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Clusters	Clusters	Clusters	Chains	Chains	Clusters	Clusters	Clusters	Single
Biochemical test									
Catalase	+	+	+	-	-	-	+	+	+
Urease	+	-	+	+	+	+	+	+	-
Oxidase	+	-	-	+	+	-	-	+	-
Citrate	+	-	-	-	-	-	-	+	-
Coagulase	-	-	-	-	-	-	-	-	-
Indole	-	+	-	-	-	-	+	-	+
Sugar fermentation									
Glucose	+	+	+	+	+	-	+	-	+
Maltose	-	+	+	+	+	-	+	-	+
Sucrose	+	+	+	+	+	-	+	+	+
Galactose	+	+	+	+	+	-	-	+	-
Lactose	+	+	+	-	-	-	+	+	+
Probable isolates	*Pseudomonas aeruginosa*	*Acinetobacter calcoaceticus*	*Rhodococcus pneumonia*	*Bacillus subtilis*	*Proteus mirabilis*	*Chromatium okenii*	*Serratia Marcescens*	*Pseudomonas fluorescens*	*Escherichia coli*

Key: N.A = Nutrient agar, + = Positive, - = Negative

Table 4: Occurrence of bacteria isolates in samples

Bacteria isolates	Sample A	Sample B	Sample C	Number of isolates	% frequency
Pseudomonas aeruginosa	0	1	1	2	7.1
Acinetobacter calcoaceticus	1	2	1	4	14.3
Rhodococcus pneumonia	1	1	1	3	10.7
Bacillus subtillis	0	1	1	2	7.1
Proteus mirabilis	1	2	1	4	14.3
Chromatium okenii	1	1	0	2	7.1
Serratia marcescens	1	1	1	3	10.7
Pseudomonas fluorescens	1	1	2	4	14.3
Escherichia coli	0	2	2	4	14.3

Table 5: 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	*Aspergillus niger*	*Candida utilis*	*Epicoccum* sp.	*Aspergillus flavus*	*Rhodococcus glutinis*

Key: CMA = Corn meal agar, PDA = Potato dextrose agar, SDA = Sabouraud dextrose agar

Table 6: Distribution of occurrence of fungi isolates in samples

Fungi isolates	Sample A	Sample B	Sample C	Number of isolates	% frequency
Aspergillus niger	1	1	1	3	21.4
Candida utilis	0	1	1	2	14.3
***Epicoccum* spp.**	1	1	2	4	28.6
Aspergillus flavus	1	1	0	2	14.3
Rhodotorula glutinis	1	1	1	3	21.4

Key: Sample A = Control sample, Sample B = Sapele site A, Sample C = Sapele site B

Table 7: Physicochemical properties of water samples

Parameters	Sample A	Sample B	Sample C	WHO std.
Colour	0.00±0.00	2.00±0.04	2.00±0.04	0
Odour	0.00±0.00	1.00±0.00	1.00±0.00	0
pH	6.47±0.39	5.07±0.21	5.33±0.33	6.5-8.5
Temp. (^{o}C)	26.70±0.51	26.37±0.09	26.57±0.41	25
Turbidity (NTU)	1.77±0.02	5.33±0.22^{a}	6.00±0.13	5.0
TDS (mg/L)	7.67±0.08	118.33±3.40^{ab}	120.33±4.20^{ab}	500
TSS (mg/L)	31.83±1.30	873.00±4.55^{ab}	772.33±6.40^{ab}	30
DO (mg/L)	15.17±0.30	5.33±0.05	6.33±0.09	14
BOD (mg/L)	1.08±0.02	2.67±0.16	2.30±0.14	4.0
T. Oil and Grease (mg/L)	1.30±0.06	13.67±0.29^{a}	12.00±0.43^{a}	10
Total hardness (mg/L)	1.83±0.02	12.00±0.49^{a}	12.67±0.52^{a}	500
Magnesium (mg/L)	1.68±0.22	6.20±0.14^{a}	5.77±0.09	0.2
Calcium (mg/L)	0.25±0.01	3.13±0.04^{a}	2.97±0.03	150
Phosphate (mg/L)	0.68±0.05	3.38±0.11	3.55±0.13	0.3
Sulphate (mg/L)	0.61±0.03	3.41±0.05	4.91±0.18^{a}	250
Nitrate (mg/L)	0.05±0.01	0.42±0.02	0.47±0.06	10

The values are the mean ± SEM for the physicochemical properties analyzed for the water samples significant at $p<0.05$ with respect to normal control.

Key: A = Sampling site A (Umutu - Control sample), B = Sampling site B (Sapele), C = Sampling site C (Sapele), a = Significant, ab = Highly significant, WHO std. = World Health Organization standard.

Table 8: Heavy metal properties of water samples

Parameters (mg/L)	Sample A	Sample B	Sample C	WHO std.
Cadmium (Cd)	0.15±0.03	2.59±0.07^{a}	2.46±0.04^{a}	0.005
Arsenic (Ar)	1.13±0.02	2.90±0.09	2.65±0.06	0.05
Lead (Pb)	0.01±0.00	0.13±0.01	0.16±0.02	0.01
Chromium (Cr)	0.16±0.02	1.23±0.04	1.17±0.02	0.05
Iron (Fe)	0.98±0.02	6.00±0.05^{a}	6.67±0.05^{a}	0.3
Copper (Cu)	1.25±0.02	4.87±0.05^{a}	3.77±0.04	1.0
Mercury (Hg)	0.01±0.00	0.03±0.01	0.05±0.01	0.001
Zinc (Zn)	0.46±0.02	8.47±0.07^{ab}	7.97±0.06^{ab}	5.0
Manganese (Mn)	0.36±0.04	1.93±0.03	2.17±0.04	0.1

The values are the mean ± SEM for the heavy metal properties analyzed for the water samples significant at $p<0.05$ with respect to normal control.

Key: A = Sampling site A (Umutu - Control sample), B = Sampling site B (Sapele), C = Sampling site C (Sapele), a = Significant, ab = Highly significant, WHO std. = World Health Organization standard.

The results in Tables 1 revealed the total heterotrophic bacteria count (THBC) which ranged from (5.7±0.9 cfu/mL x 10^5 to 10.3±1.2 x 10^5 cfu/mL), while that of Table 2 revealed the total fungi counts which ranged from (3.3±0.3 x 10^5 cfu/mL to 5.0±1.5 x 10^5 cfu/mL), just like it has been reported by other researchers such as [31, 32, 33], who reported THBC of 6.66 x 10^7 cfu/mL, 8.22 x 10^7 cfu/mL and 6.02 x 10^7 cfu/mL from water samples collected from Nembe waterside at Port Harcourt. The presence of bacterial and fungal growth in the contaminated water samples demonstrates these organisms' ability to adapt to such environments. This adaptation may be linked to the role of River Ethiope as a repository for pollutants and waste from both human and industrial activities. The study by Zhang *et al*. [34] revealed how heterotrophic activities among microorganisms enable them to derive many benefits similar to multicellular life. 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. According to Abbas *et al.* [35], the high concentration of these 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 cultural, morphological, and biochemical characteristics of the identified bacterial isolates, while Table 4 outlined their percentage frequency of occurrence. A total of nine distinct bacterial isolates (*Pseudomonas aeruginosa* (5.9%), *Acinetobacter calcoaceticus* (11.8%), *Rhodococcus pneumonia* (11.8%), *Bacillus subtilis* (8.8%), *Proteus mirabilis* (14.7%), *Chromatium okenii* (8.8%), *Serratia marcescens* (11.8%), *Pseudomonas fluorescens* (11.8%), *and Escherichia coli* (11.8%) were recorded from the studied samples. These results are similar to findings reported by Kwadzah and Iorhemen [36] at River Kaduna, Edjere *et al.* [37] at Ikpoba River in Benin City, and Deborah *et al.* [38] at Ngadda River in Maiduguri. It was generally observed that *P. mirabilis* and *E. coli* had the highest frequency of occurrence in the samples and this could be due to their adaptability and ability to survival more in this environment, while *P. aeruginosa* had the lowest frequency of occurrence.

The presence of these coliforms such as *E. coli* in the analyzed water samples, indicates the water's contamination with fecal material of man or other animals. This reaffirms the study of Ibiene *et al.* [39]. The cultural and morphological characteristics of the fungi isolates were revealed in Table 5, while their percentage frequency of occurrence was revealed in Table 6. A total of five distinct isolates (*Aspergillus niger* (21.1%), *Candida utilis* (15.8%), *Epicoccum* spp. (26.3%), *Aspergillus flavus* (21.1%) *and Rhodotorula glutinis* (15.8%) were recorded from the water samples studied. The most common fungi isolate in the current study was the genus *Aspergillus (A. niger* and *A. flavus*). This reaffirms the findings of Atuanya *et al.*; Akatah *et al*. [40, 41] at Ikpoba River in Edo State. The presence of fungi in water samples analyzed could probably be due to the contaminated water which favours the growth of fungi and also, as a result of the exposure of the river to fecal materials or organic manure. The high presence of these microbes indicated the unsafe nature of the river as it is used by indigenes for their everyday activities, like drinking, bathing and washing. This is similar to observations reported by Deng *et al*. [42]. The result of the physicochemical parameters for various sampling points on River Ethiope were presented in Table 7. Among these parameters, the hydrogen ion concentration (pH) is crucial, influencing chemical reactions and biological activities in the water [43, 44].

The study of Morrison *et al.* [45] noted that significant alterations in water pH can profoundly impact aquatic life, which is finely attuned to specific pH levels, and even slight deviations can lead to fatalities. In this study a pH range of 5.07±0.21 to 6.47±0.39 was recorded, which was below the World Health Organization standard for drinking water (ranging from 6.5 to 8.5). There was no significant difference between the pH of the control sample and those of the test sites. This finding aligns with Odekina *et al.* [46], who reported similar pH ranges in water sources at Isaka Bundu waterfront in Rivers State. Though the research of Edokpayi *et al.* [47] recorded lower pH values at Ibiekum, Ekpoma, and Adofi Rivers. The research of Asibor *et al*. [48] also reported lower pH levels in Ogun. These differences could be linked to diverse land use activities, such as cassava milling centers and rubber processing factories, which discharge effluents into the river, all located in the riparian forest at Sapele axis. The research of Wu *et al*. [49] indicated that these activities tend to elevate acidity and ionic levels. A low pH in this environment will distort the natural ecosystem of the environment as will alter the natural metabolic activities of microbes.

The results of Arimoro *et al.* [50] in Warri River, who reported a water temperature range of 26.3^{o}C to 30.3^{o}C, falls in line with the temperature range of this present study (26.37±0.09^{o}C to 26.7 ±0.51^{o}C). This result however, was not in agreement with findings of Ezekiel *et al.* [51] in Sombrero River, who reported a higher temperature range of 27.90^{o}C to 29.20^{o}C. The range of surface water temperature recorded in this study is conducive to supporting aquatic organisms, as indicated by Iloba [52]. The total dissolved solids (TDS) values observed in this study, ranging from 7.67±0.08 mg/L to 120.33±4.20^{ab} mg/L were lower compared to the range reported by Shaikh *et al.* [53], who categorized TDS levels per liter for brackish water to fall between 1500 to 5000 mg/L and saline water to be greater than

5000 mg/L. This outcome suggests a fluctuation between fresh and brackish waters within the mangrove swamps. The elevated TDS value documented at the Sapele axis might be attributed to agricultural runoff and human activities such as domestic washing. The findings of Akankali *et al.* [54] suggests that water containing over 2000 mg/L of dissolved solids should not be utilized when alternative less mineralized water sources are available. Heavy metals are often used as indicators of pollution due to their significant toxicity to both human and aquatic life. Ogbeibu and Omoigberale [55] associated the elevated levels of heavy metals in aquatic ecosystems with discharges from industries, waste, and sewage. The concentrations of heavy metal parameters in the water samples analyzed are presented in Table 8. In this study, Mercury (Hg) and Lead (Pb) concentrations were below the detectable level (BDL). The concentration of Zinc in Sapele axis (8.47 ± 0.07^{ab} mg/L) was above the allowable value of 5.0 mg/L released by WHO [56], and higher than results reported by Iwegbue *et al.* [57] at Ase River in Niger Delta. Among the heavy metals analyzed in this study, only Zn showed statistical significance in comparison with normal control. High accumulation of Zn in the body for long leads to high decease of copper absorption and this is because zinc can inhibit copper absorption. This condition leads to hypocupremia, anemia, leukopenia and neutropenia [57]. The research of Igic *et al.* [58] suggests that all these factors play a significant role in the metal contamination of the river.

CONCLUSION

It has been observed that due to human activities in these communities with water bodies around them, there is a gradual increase in pollution of water sources around these communities. This research showed high level of contamination of the water source at Sapele of River Ethiope when compared to the source at Umutu. This is because there is more traffic and human activities due to higher population at the Sapele axis than the Umutu axis. These human activities leading to pollution of water sources pose a great potential risk to the health and well-being of man and animals living along and around the riverbanks. Community's heads and government agencies must rise to the task to checkmate this constant pollution going on as it will further make the environment unsafe for the wellbeing of the surrounding communities.

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