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Research Article

Microbiological and Physicochemical Properties of Krakrama (Brackish) Water in Rivers State, Niger Delta, Nigeria

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Abstract

Background: Microbiological and Physicochemical properties of brackish water ecosystem is influenced by a lot of ecological and anthropogenic factors. These factors in turn impact on the distribution, productivity and microbial community as well as the integrity of the ecosystem.

Objective: Assessment of Microbiological and physicochemical properties of Krakrama (brackish) water in Rivers State.

Method: A 2-week sampling protocol at four different points along the brackish water course was carried out for three (May-July) months. Samples were analysed for Microbiological and Physicochemical parameters using standard analytical methods highlighted in the study.

Results: Physicochemical parameters ranged as follows pH (6.29 - 6.71), temperature (26.9 - 28.4°C), electrical conductivity (EC 950.77 - 1514.15 µS/cm), Nitrate (0.51 - 1.34 mg/mL) and salinity (16.20 - 22.50%) values were within statutory permissible limits, but total suspended solids (TSS 28.03-76.17) and total dissolved solids (TDS 570.8 - 915.30 mg/mL) were below whereas turbidity (30.80 - 40.00 NTU), biochemical oxygen demand (BOD_5 9.68 - 15.20 mg/L), Chemical oxygen demand (COD 154.2 - 214 mg/mL), dissolved oxygen (DO 5.19 - 6.65 mg/L) and phosphate exceeded the acceptable limits though most of them do not directly apply to brackish water. In contrast, fifteen (15) genera of bacteria were identified and six (6) genera of fungi. Total heterotrophic bacterial counts (THBCs) ranged from 6.65 ± 0.1-6.92 ± 0.1 x 10^4 CFU/mL as highest and least total *Shigella-Salmonella* counts (TSSCs 3.82 ± 0.1 x 10^1 CFU/mL). Total faecal/thermotolerant coliform bacteria ranged from 900-≥1600/100 mL whereas total fungal counts ranged from 2.12 ± 01 - 2.28 ± 0.1 x 10^2 with *Penicillium* being predominant. Of the isolates obtained *Escherichia coli, Bacillus cereus, Vibrio cholerae, V. parahaemolyticus, Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* as well as Candida albicans, Fusarium and Aspergillus niger are known aetiologic agents of various human and animal pathologies.

Conclusion: The water samples revealed elevated levels of physicochemical parameters and requires continuous surveillance due to natural and anthropogenic activities. The microbial community is diverse with high incidence of faecal coliforms and opportunistic pathogens rendering the water unfit for recreation and consumption.

Keywords: Anthropogenic; Faecal Coliforms; Physicochemical Parameter; Brackish Water; Microbial Community

Introduction

Water, doubles as a source of life as well as disease when it is polluted due to increased human population, soil erosion, runoff, agricultural waste, atmospheric deposition, municipal and industrial effluents, discharge of sewage and harmful contaminants [1,2]. Coastal waters are one of the nation's most important natural resources, valued for their ecological richness as well as for the many human activities they support. The possibility of transboundary transportation with concomitant discharge of pollutants may consequently constitute a public health challenge, thus, determination of water quality and environmental surveillance/monitoring especially in the Niger Delta is critical. Water quality is a topical global issue, especially as it impacts on humans in terms of water-borne diseases, deterioration and perturbation of the aquatic ecosystem [3,4].

Water quality is described by its physical, chemical and microbiological characteristics. However, microbiological examination of river waters is obligatory for use-related purposes such as potable water production, irrigation and recreation to assess for potential human pathogens. Such analysis requires bioindicators; plants, planktons, animals and microbes to screen the health of the natural ecosystem [5]. Microbes such as coliform bacteria, *Escherichia coli,* faecal streptococci, enterococci, etc. have been used to assess faecal pollution and deterioration in fresh water sources; lakes, rivers, underground waters and streams [6]. Polluted water is the major rea-

son for the spread of many endemic diseases like gastroenteritis, skin and eye infections, cholera, tuberculosis, typhoid, diarrhoea, viral hepatitis A and even death. Mortality in children of less than five years due to water related diseases is estimated at 3.4 - 4 million annually in under developed countries [7,8].

Brackish water is mostly found in estuarine areas where seawater mixes with freshwater and refers to water with salinity of 500 - 17,000 mg/L; conductivity of 1000 - 80,000 µS/cm and total dissolved solid of 1000 - 5000 mg/L [9,10].

The discharges from industrial effluents containing dissolved salts, leachates of saline soils and the runoff of salt deposits such as halite or gypsum present in the sedimentary rocks also increase the amounts of total dissolved salts and thus lead to the formation of brackish water [11].

Due to the strategic significance of brackish water ecosystem in the Niger Delta, extensive research on the microbiological and physicochemical profiles had been well documented [2,6,12]. This ecosystem is the habitat for a wide range of biodiversity including macrofauna/flora and microorganisms [3,13]. Several workers have also reported that, the physicochemical parameters of the habitats are major determinants of the distribution, composition and productivity of microbial communities with salinity being one of the crucial parametric variables [14,15].

The site for this study is Krakrama River in Asari-Toru Local government Area, Rivers State. The rivers is a tidal brackish water ecosystem and this research is the first of its kind in this coastal community. Human activities include fishing, defaecation, lumbering, recreation (canoeing, bathing and swimming), laundering, sabotage to facilities, trafficking in crude oil and refined products and incriminate waste disposal are prevalent where there are human settlements along the coast line. Apparently, these anthropogenic activities have resulted in direct or accidental discharge of organic and inorganic substances which may have impacted negatively into the surrounding water bodies. This study is designed to investigate the microbiological and physicochemical properties of Krakrama (brackish) water as to isolate, identified potential human pathogens and assess the of level of pollution.

Materials and Methods

Study area

Krakrama is a community situate in Asari Toru Local Government Area in Rivers State, Niger Delta, Nigeria. Krakrama is approximately 17.2 km from Buguma and 52.1 km from Port Harcourt the Rivers State capital. The community is located between latitude 4°45′15″N and longitude 6°47′0″E and it is a tributary of New Calabar River (Figure 1). The study site was selected based on anthropogenic activities such as defaecation (by pier toilet system), washing, swimming/bathing, lumbering, incriminate disposal of domestic and industrial wastes, etc., into the river.

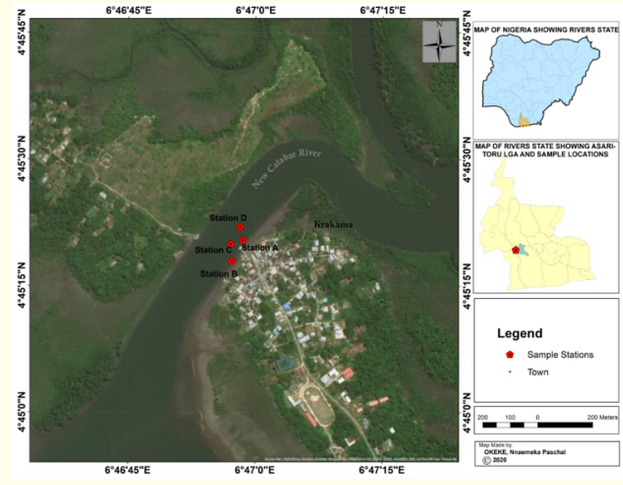

Figure 1: The study site: Krakrama in Asari Toru Local Government Area of Rivers State, Nigeria.

Collection of water samples

Brackish water samples from the aforementioned community were collected at four (4) points at about 20 metres away from each other fortnightly for three months (May-July, covering only rainy season). Model bottles used for the water sample collection were labelled and transported in an ice-box for laboratory analyses.

Physicochemical analysis

Water samples were analysed for the following parameters: Temperatures, pH, Turbidity, Salinity, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Total suspended solids (TSS), Total dissolved solids (TDS), Electrical conductivity (EC), Phosphates and Nitrates.

pH (Hydrogen ion concentration)

pH of the water was measured as described in [16-APHA, 2005] with pH meter model 291 Mk2 after standardization and calibration with buffered solutions at pH 7, 4, 10. The pH meter electrode was lowered into the beaker and the reading was recorded and repeated as required.

Electrical conductivity (EC)

EC was measured using a standard solution of potassium chloride of known conductivity cell (0.01NKCl, 745.6 mg in 1.0L deionized water = 1413 µmhos/cm) in accordance with [16]. Thus, the conductivity cell/electrode was inserted thrice into 0.01N KCl solution and washed, then conductivity of the solution was measured. Finally, the conductivity cell was immersed into the sample and results were recorded.

Dissolved oxygen (DO) and biochemical oxygen demand (BOD)

Airtight 300 ml capacity BOD bottles were filled to the brim with the samples. The initial DO in the sample was determined as described [16]. The diluent was prepared by measuring out 22.5 g/L $MgSO_4.7H_20$, 27.8 g/L $CaCl_2.2H_2O$, and 0.25 g/L $FeCl_3.6H_2O$, Phosphate buffer: 8.5g $KHPO_4$; 21.7g of $K_2HPO_4.7H_2O$; 1.7g of NaCl and pH 7.2, into a measuring container making up volume to 1L with distilled water. The contents of the flask were mixed by swirling gently and covered. The dilution water was first saturated with dissolved oxygen by shaking in a partially filled bottle before using to dilute the samples. BOD bottles were then filled with the diluted samples and another two bottles with the dilution water to serve as blank. The bottles were stoppered carefully to avoid the entrapment of air. The blank and one experimental BOD bottles were used for the initial dissolved oxygen (DO) determination. The remaining two BOD bottles were water-sealed by filling the flared neck of the bottles with distilled water from a wash bottle. The cover cap supplied with the BOD bottles was used to retain the water. The bottles were incubated at 20°C for 5 days. At the end of this period, the final DO was determined. The BOD_5 in mg/L of the sample was estimated using the formula:

BOD_5 (mg/L) = D_1 - D_2/P .....Equation 1

D_1 = Represents Dissolved Oxygen (mg/L) of sample 15 minutes after preparation.

D_2 = Represents Dissolved Oxygen (mg/L) of sample 5 days after incubation at 20°C.

P = Represents Decimal volumetric fraction of sample used.

Chemical oxygen demand (COD)

This is a measure of oxygen proportion of the untreated matter content of a sample that is susceptible to oxidation by a strong chemical oxidant [17]. A 50 ml brackish water sample was collected in clean glass bottles and dispensed into 500 ml Erlenmeyer flask with 24/40 ground glass joint. Clean boiling clips were added to prevent bumping, 5 ml of sulphuric acid and 1.0g of mercuric sulphate were added to the flask. The solution was mixed to dissolve while cooling by immersing in ice water and 25 ml of standard potassium dichromate (0.25N) added to it from a 50 ml burette. The flask was then connected to the condenser and cooling water turned on again. 70 ml of sulphuric acid were slowly added to the flask through the open end of the condenser. The flask was thoroughly mixed by swirling as the sulphuric acid was being added. The hot plate was brought into positions and turned on. The temperature was adjusted to allow the refluxing liquid to rise at least half the height of the condenser. Foreign materials were kept away by placing a beaker at top end of the condenser. Refluxing was carried out for 2hours, after which the flask was allowed to cool. The condenser was washed down with distilled water using a wash bottle. The content of the flask was made up to 300 ml with distilled water and allowed to cool to room temperature. About 3 drops of ferroin indicator were added to the flask. The excess potassium dichromate was titrated with standard ferrous ammonium sulphate (0.1N), to the first appearance of the reddish-brown colour end-point. The volume of the titrant was taken at the end. The COD in mg/L was estimated as follows:

COD\ (mg/L)=(A-B)Nx\ 8000/*S*.....Equation 2

A = Represents ml of titrant used for the blank

B = Represents ml of titrant used for the sample

N = Represents Normality of titrant

S = Represents ml of sample used for the test.

Turbidity

Turbidity affects light penetration, scattering and absorption in the aquatic environment and it is measured with a standard Formazin polymer as a reference to compare intensity of light scattered by the sample. Distilled water was used to calibrate nephelometer (0.0 NTU). Hydrazine sulphate 1.0g was dissolved in 100 ml of distilled water to form solution 1. Hexamethylenetetramine 10.0g was dissolved in distilled water and made up to 100 ml in volumetric flask to obtain solution 2. Then 5 ml of solutions 1 and 2 were mixed in a volumetric flask and kept for 24h at about 25°C. The mixture was diluted to 1000 ml with distilled water to give a 400 NTU stock suspension. Afterwards, 10 ml of the stock solution was diluted to 100 ml with distilled water to give 40NTU standard solution [16]. Turbidity was calculated using the formula below:

Turbidity (NTU) = Nephelometer readings x Dilution factor..... Equation 3

If turbidity of the sample is > 40 NTU, then the sample is diluted and the dilution factor is accounted for in final calculations.

Total suspended solids

This parameter for the water sample was determined as described in [16]. Vacuum pump with distilled water was applied to wash the membrane filter (pore size 0.45 µm). The membrane filter was carefully separated, placed in the crucible and dried in the oven at 103°C for 1h. During the analysis, the dried filter paper was wetted with a small volume of distilled water and placed in the filtration unit. About 50 ml of homogenously mixed test sample was filtered through the membrane, the contents with membrane filter was transferred to the crucible and oven dried to a constant weight at 103°C.

Total dissolved solids

These are solids that is regularly dissolved in water making it unhealthy for consumption. It is calculated by subtracting weight of total suspended solids from total solids as represented in equation 7.

$$Total\ Dissolved\ Solids\ \left(\frac{mg}{L}\right)=((W_1\text{-}W_2))\ x\ 1000\ \text{.....}\ \text{Equation 4}$$

Where: W_1 represents Weight of total solids.

W_2 represents Weight of total suspended solids.

Temperature

Temperature of the water sample was measured at the sampled site immediately after collection using mercury in glass thermometer graduated in centigrade (°C). The thermometer remained immersed for 5 minutes to stabilize and the thermometric readings was recorded

Salinity

The salinity of the water sample was measured in the laboratory as described in Horiba Instruction Manual [18]. About 20 ml of water was dispensed into a beaker and the salinity was read by immersion of the probe after standardization for about 3-minutes.

Phosphate

A simple qualitative method was used to determine the presence of phosphate ions in the water sample. A small amount of the sample was acidified with concentrated nitric acid, to which a little ammonium molybdate was added. The presence of phosphate ions was indicated by the formation of a bright yellow precipitate layer of ammonium phosphomolybdate. The appearance of the precipitate can be facilitated by gentle heating. This test is also used to detect arsenic, a yellow precipitate being formed.

Nitrate

The brown ring test was performed by adding iron (II) sulphate to the water sample containing nitrate, then slowly concentrated sulphuric acid was added such that the acid forms a layer below the aqueous solution. A brown ring will form at the interface of the two layers, indicating the presence of the nitrate ion. The overall reaction is the reduction of the nitrate ion by iron (II) which is oxidized to iron (III) and formation of a nitrosonium complex where nitric oxide is reduced to NO^- (nitrite).

$$2HNO_3 + 3H_2SO_4 + 6FeSO_4 \longrightarrow 3Fe_2(SO_4)_3 + 2NO + 4H_2O$$

$$[Fe\ (H_2O)_6]SO_4 + NO = [Fe(H_2O)_5(NO)]SO_4 + H_2O$$

This test is sensitive up to 2.5 µg and a concentration of 1 in 25,000 ppm.

Microbiological analysis

Total heterotrophic bacterial count (THBC) were determined when serial dilutions were made from the samples and 0.1 aliquots were spread-plated in duplicate on nutrient agar (NA; Titan Biotech Ltd, India). Total coliforms including *Escherichia coli* count (TCC) were determined on pre-poured, surface-dried MacConkey agar (Oxoid Ltd., UK) and incubated for 37°C for 24h. Total faecal coliform count (TFCC) was obtained from 1 ml of dilution 10^1 of sample inoculated on surface-dried Eosin Methylene Blue Agar at 44.5°C for 24h. Most probable number technique (MPN) was also performed using fifteen (15) test tubes for each sample of 10 ml, 1 ml and 0.1 ml with double or single strength [17,19]. Total *Shigella* and *Salmonella* Count (TSSC) was determined on pre-poured *Shigella-Salmonella* agar whereas total *Vibrio* count (TVC) was determined in duplicates on surface-dried thiosulphate-citrate bile-salt-sucrose (TCBS) agar (Lab M Ltd, UK) using spread-plate method and plates were incubated at 37ºC for 24h. Representative colonies (30 - 300) were enumerated as colony forming units (CFUs) and identification of bacterial isolates was carried out based on cultural, morphological and biochemical characteristics [20-22] and ABI identification software.

Total fungal count (TFC) was determined with 0.1ml aliquots of decimal dilutions and inoculated onto prepoured solidified Sabouraud's dextrose agar (SDA; Titan Biotech Ltd, India) and incubated at 27 ± 2°C for 1 - 4 days. Viable representative colonies were picked at random, streaked and subcultured for purification and stored in the refrigerator at 2 - 4°C. Then, isolates were identified after staining with lactophenol cotton blue and compared with observed cultural and morphological characteristics as described [22,23].

Statistical analysis

The analyses were carried out after two and six determinations and means calculated manually. ANOVA used was based on software of SPSS version 22 for Windows and the significance of the mean differences determined at $p < 0.05$.

Results

Physicochemical parameters of Krakrama River at different points of assessment are presented in table 1. The mean pH values of the water samples at different points ranged from 6.29 - 6.71, with sample A as the highest and D least. Temperature mean values ranged from 26.9 - 28.4°C, with samples at points A and D being the highest and B lowest. DO mean values ranged from 6.65 - 5.19 mg/L, the highest being sample A, and B lowest. Mean BOD values ranged from 9.68 - 15.20 mg/mL the highest occurred at sampled point C and lowest at D. COD mean values ranged from 154.23 - 214.41 mg/mL, highest at point B and lowest at D. Salinity mean

Parameter	Point A	Point B	Point C	Point D	Permissible limit of: USEPA	Permissible limit of: WHO
pH	6.71	6.58	6.37	6.29	6.5 - 8.5	
Temperature	28.4	26.9	28.3	28.4	40.0	20.0 - 30.0
DO (mg/L)	6.65	5.19	5.83	5.41	40.0 - 60.0	≥ 5.0
BODs (mg/L)	13.52	10.82	15.20	9.68	10.0	2.0 - 6.0
COD (mg/L)	177.09	214.41	197.98	154.23		7.5
Salinity (%/ppt)	22.50	17.00	16.50	16.20	NA	
EC (µS/cm)	1514.15	950.77	1125.53	989.90	400.0	600.0
Turbidity (NTU)	40.00	39.50	30.80	32.50	5.10	5.0
TSS (mg/L)	45.57	28.03	76.17	54.10		500.0
TDS (mg/L)	915.30	570.84	673.74	897.19	2000.0	500.0 - 1000.0
Phosphates	3.89	9.60	5.45	4.96		
Nitrate (mg/L)	1.34	1.08	0.51	1.02		5.00

Table 1: Physico-chemical parameters of Krakrama River at different sample collection.

Legend: WHO: World Health Organisation, 2011; (USEPA): United States Environmental Protection Agency, 2000; Values are means of duplicates. NA = Not applicable.

values ranged from 16.20 - 22.50 ppt, highest value was observed at point A and lowest at D. Mean EC levels was between 950.77 and 1514.15 µS/cm with sampled point A being the highest and B lowest. Turbidity mean values ranged from 30.80 - 40.00 NTU, highest value was observed at point B and least C. Mean TSS ranged from 28.03 - 76.17 mg/L, highest value occurred at point C and lowest at B. Mean TDS level ranged from 570.84 - 915.30 mg/L, highest was observed at point A and lowest at B. Mean phosphate values ranged from 3.89 - 9.60 mg/L, the highest being at sampled B and least at A. Mean nitrate level ranged from 0.51 - 1.34 mg/L, sampled point A had the highest whereas the lowest occurred at sampled point C. However, fluctuations in these parameters are not unconnected with anthropogenic activities.

Microbial group counts for THBC, TCC, TFCC, TVC, TSSC and TFC of samples from different points in Krakrama River are shown in table 2. THBC mean values ranged from 6.65 ± 0.13 - 6.92 ± 0.08 x 10^4 CFU/mL at point A which had the highest and point B, lowest. Mean level of TCC ranged from 3.98 ± 0.10 - 4.26 ± 0.33 x 10^1 with sample at point A being the highest and point C the least. TFCC mean values ranged from 3.88 ± 0.09 - 4.14 ± 0.38 x 10^1, highest mean value occurred at point B and point D being the lowest. TVC mean values was between 3.89 ± 0.06 and 4.04 ± 0.00 x 10^1 with highest mean value at sampled point A, the lowest being at point D. Mean values of TSSC ranged from 3.82 ± 0.09 - 4.07 ± 0.09 x 10^1 highest mean value occurred at point A and lowest at C. Total fungal count (TFC) mean values ranged from 2.12 ± 0.05 - 2.28 ± 0.05 x 10^2, sampled point A had the highest whereas point D least.

TFCCs of water from the sampled points (A-D) using the Most Probable Number (MPN) method are depicted in table 3. Counts ranged from 900 - ≥ 1600. Sampled points A and B had higher counts than points C and D (Table 3).

Microbial isolates of water from the sampled points were fifteen (15) genera of bacteria and six (6) of fungi table 4 and 5. Percentage occurrence of identified bacteria at the different sampled points are highlighted in table 4. Point A had the highest bacterial load and

Points	THBC (x10^4)	TCC (x10^1)	TFCC (x10^1)	TVC (x10^1)	TSSC (x10^1)	TFC(x10^2)
A	6.92 ± 0.08^{c}	4.26 ± 0.33^{b}	4.05 ± 0.17^{ab}	4.04 ± 0.00^{b}	4.07 ± 0.09^{b}	2.28 ± 0.05^{a}
B	6.81 ± 0.09^{bc}	4.06 ± 0.05^{ab}	4.14 ± 0.38^{a}	3.98 ± 0.05^{ab}	4.01 ± 0.09^{b}	2.22 ± 0.05^{a}
C	6.74 ± 0.16^{a}	3.98 ± 0.10^{a}	3.93 ± 0.16^{a}	3.92 ± 0.11^{a}	3.87 ± 0.09^{a}	2.13 ± 0.06^{a}
D	6.65 ± 0.13^{a}	3.99 ± 0.04^{a}	3.88 ± 0.09^{a}	3.89 ± 0.06^{a}	3.82 ± 0.09^{a}	2.12 ± 0.05^{a}

Table 2: Microbial group counts (CFU/mL) of water at different sampled points.

Legend: $^{a,b \text{ and } c}$: Means of values with the same superscript (alphabet) across the column. Shows no significant difference at ($p > 0.05$). Values are means of duplicates ± standard deviation (SD) of the mean. TCC = 10/100 mL and THBC = 100000/mL WHO, 2011.

Points/Duration Index/100mL	Dilutions (mL)/Number of Positive Tubes			MPN
2-Weeks	5 of 10mL each	5 of 1 mL each	5 of 0.1 mL each	
A (Toilet/defaecation+)	5	5	5	≥ 1600
B (Domestic waste+)	5	5	5	≥ 1600
C (Bathing+)	5	5	3	900
D (Bathing/Washing+)	5	5	3	900

Table 3: Estimation of TFCC by MPN for various combinations of Positive and Negative Results with five 10.0 mL portions, five 1.0 mL portions and five 0.1 mL portions.

Legend: +: Anthropogenic activities at various sampled points (A-D) in Krakrama river. Value represents mean of six determinations.

Isolates	A	B	C	D
Bacillus cereus	1.25	0	1.25	0
B. subtilis	1.25	1.25	0	0
B. siamensis	1.25	1.25	1.25	0
B. tequilensis	0	1.25	1.25	0
B. niacin	0	1.25	1.25	1.25
Bifobacterium bifidum	1.25	1.25	1.25	0
Citrobacter freundii	1.25	1.25	0	1.25
C. koseri	1.25	1.25	0	0
Corynebacterium xerosis	1.25	1.25	0	0
Escherichia coli	2.50	2.50	1.25	1.25
Enterobacter clocae	1.25	1.25	0	0
Klebsiella pneumoniae	1.25	1.25	1.25	0
K. aerogenes	0	0	1.25	1.25
K. oxytoca	1.25	1.25	0	0
K. singaporensis	1.25	0	1.25	0
Proteus mirabilis	1.25	0	0	1.25
P. vulgaris	1.25	1.25	0	0
Providencia regretti	1.25	1.25	0	0
Pseudomonas aeruginosa	2.50	2.50	1.25	1.25
Serratia marcescens	1.25	1.25	0	0
S. odorifera	1.25	1.25	0	0
Shigella flexneri	1.25	0	1.25	1.25
Shewanella putrefaciens	1.25	0	0	0
Staphylococcus aureus	1.25	1.25	0	1.25
S. deverisei	1.25	1.25	0	0
S. epidermidis	1.25	0	1.25	1.25
Streptococcus pneumoniae	1.25	0	0	0
Vibrio cholerae	1.25	1.25	0	0
V. parahaemolyticus	1.25	0	0	1.25
V. aerogenes	1.25	0	1.25	0
V. alginolyticus	1.25	1.25	0	0
V. fluvialis	1.25	0	0	0
V. metschnikovii	1.25	1.25	0	0

Table 4: Percentage occurrence of bacterial isolates in water from the Sampled points (A-D).

diversity/heterogeneity followed by point B and the lowest at point D. The predominant single species were *Escherichia coli* and *Pseudomonas aeruginosa* obtained at sampled points A and B whereas the most predominant genera on the basis of diversity were *Bacillus* (15%) and *Vibrio* (13.7%) followed by *Staphylococcus* (10%) and *Klebsiella* (7.5%) (Table 4).

Percentage occurrence of identified fungi from different sampled points are highlighted in table 5. Sampled points A and C had the highest fungal load and diversity with *Penicillium* being the most dominant genera.

Isolates	A	B	C	D
Penicillium chrysogenum	4.0	4.0	4.0	4.0
P. italicum	4.0	4.0	4.0	4.0
Aspergillus niger	4.0	4.0	4.0	4.0
Fusarium solani	4.0	4.0	4.0	4.0
Mucor spp.	4.0	4.0	4.0	0
Candida albicans	4.0	0	4.0	4.0
Saccharomyces cerevisiae	4.0	4.0	4.0	0

Table 5: Percentage occurrence of fungal isolates from each Sampled points (A-D) from Krakrama River

Discussion

Physicochemical and microbiological characteristics of brackish water systems are driven by several environmental and human activities. In this water system, intense biogeochemical transformations cause pronounced pH fluctuations due to the uptake of anthropogenic emissions and high loads of dissolved organic matter [24]. The mean pH values recorded were near neutral and varied at sampled points. Such variations in pH at very small distances have been reported to be linked to atmospheric and anthropogenic emissions (defaecation, disposal of domestic wastes, etc), buffering and dilution effects of heavy rains. This acidic-alkaline pH of the brackish water may not only relief physiological stress but best suited to support aquatic life, and corroborates earlier reports [3,25,26].

The temperature drives the biochemical and chemical reactions of water system and impacts on the rate of all biological activities. Therefore, it can be used as an initial step to predict the effects of human activities on aquatic lives [1]. The mean temperature results ranged from 26.9 - 28.4ºC which is within statutory permissible limit. Slight fluctuations were observed at sampled points which may be attributed to insulating effects of increased nutrient load, evaporation, fresh water influx and anthropogenic inputs which is in consonance with those reported by other investigators [2,27].

Dissolved oxygen (DO) is a fundamental factor for metabolism of the aerobic aquatic organisms, determines natural depuration capacity or freshness of a river [28]. Although, mean DO values were slightly above the threshold limits for drinking water as recommended [7] higher concentrations have been reportedly used agricultural purposes in brackish water aquaculture [29].

Both BOD_5 and COD are key indicators of the environmental health of a surface water supply, they are rarely in general water treatment [1]. Mean BOD values of all the sampled points exceeded recommended maximum permissible limits of 2 - 6.0 mg/mL [9] obviously due to discharge of domestic wastes (sewage) and poorly executed agricultural activities near the river banks. The impact of these activities may result in the accumulation of suspended particulate matter with insulation effect on the estuary and such seasonal ranges had earlier been reported [12,30]. Unpolluted waters typically have values of 2 mg/L or less, whereas those receiving wastewaters may have values up to 10 mg/L or more.

COD is another measure of organic material contamination, the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. Mean COD values ranged from 154.23 - 214.0 mg/L indicative of the presence of decaying organic matter and inorganic pollutants respectively. This range fell within the purview of polluted waters 20 - 200 mg/L [25] which may be associated with the discharge of untreated or incompletely treated industrial effluents, run-off, microbial activity and domestic wastes into the river as earlier reported [31].

Seasonality of salinity in the New Calabar River are driven by rainfall regime, tidal cycle, soil erosion, dilution effect, anthropogenic and ecological factors which may result in changes in the ratios of main phylogenetic groups: Archaea, Bacteria, Algae and Cyanobacteria [14]. Salinity values were within the range recorded for growth and best sustainability of brackish water aquaculture [15,26]. Despite the frequency of fluctuations in salinity there should be monitoring and control of the water environments to safe and sustain aquatic life forms.

EC and TDS are frequently used as water quality parameters, especially in the coastal area and these parameters are indicators of salinity level which makes them very useful in studying seawater intrusion [32]. Mean EC values suggest addition of dissolved solids from atmospheric and anthropogenic emissions as well as erosion activities which corroborate reports of other workers on related water bodies [4,6,33]. EC in water is due to ionization of dissolved inorganic solids and becomes a measure of total dissolved solids. It is used as a primary index to select the suitability of water for all purposes [34].

The TSS reported in the study were below permissible limit [9] whereas TDS was near maximum, accentuated by the location of open toilet facility, domestic waste and suspended materials discharged at points A and D. The occurrence of such levels of TSS and TDS in recent times have been reported in literatures [35,36] with evidence of survival of aquatic biota.

High turbidity water has an altered odour, taste and negatively impacts the aesthetic values as well as light penetration into the water column necessary for survival and sustainability of aquatic life. High mean turbidity values obtained during the study may be attributed to the effects of torrential rains, wind and increased flow velocity with more suspended particles from sewage wastes, surface runoff and soil erosion. The turbidity values were astronomically higher than that stipulated for domestic use [9].

Increasing loads of nitrogen (N) and phosphorus (P) in water bodies has become one of the major environmental problems facing the world. Nitrates indicate the presence of fully oxidized organic matter. The mean nitrate values were lower than the permissible limit earlier reported [7,37] which implies that the analysed water samples contained low levels of oxidized organic matter. Although, nitrates are relatively non-toxic at the concentration recorded, in excess, they may result in eutrophication and impede overall survival and growth of plants and cause methemoglobinemia in infants, i.e. blue baby syndrome [8,38,39]. In contrast, phosphate in two sampled points (B and C) exceeded the WHO permissible limit for drinking water whereas points A and D within the limit. Phosphorus is an essential element for all life and an integral part of the DNA molecule. But in excess phosphorous stimulates algal growth which can endanger other aquatic lives. However, phosphate pollution levels at sampled points may be attributed to geologic weathering, phosphate detergent, fertilizer application and animal wastes, rainfall patterns as well as anthropogenic impacts which concurs with [40,41]. Since Nigeria has no regulation criterion for phosphates, concentrations tend to always exceeded maximum contaminant levels (MCLs) of foreign regulation agencies such as Swaziland Water Service Corporation (SWSC) - i.e. 1.0 mg/L

for drinking water, 2.0 mg/L for rivers and industrial effluents; South African criterion of 1.0 mg/L PO_4-P for sewage effluents and Zimbabwe Water (Waste and Effluent Disposal) Regulation [39,42] so, emergency control measures are required to avoid hyper-eutrophication.

The diversity of microbial groups observed in the sampled points (Table 4 and 5) may have been favoured by the interplay of the various ecological factors and anthropogenic activities. Such plethora of microorganisms have also been reported by other workers [3,13,43]. THBC were slightly below statutory criterion but TCC and TFCC (Table 2) exceeded acceptable limits [9]. Such high *E. coli* and coliform load may be linked to open defaecation, soil erosion, discharge of industrial and domestic wastes, bathing/washing and recreational activities, thus resulting in proliferation of waterborne pathogens as earlier reported in different rivers [44,45]. The absence of *Salmonella* species however, may be due to the prevailing tropical temperature and level of salinity in brackish habitat. With respect to the various sampled points, the means of TCC (Table 2) at sampled point A was significantly different ($p > 0.05$), the elevated faecal coliform counts (Tables 3) further validates obvious waterborne faecal pollution as commonly reported in developing countries [45,46]. On the other hand, the relatively high total fungal count/load (TFC) observed in this study apart from factors such as human activities, may be attributed to their physiological and morphological adaptation to this habitat. However, water with such magnitude of microbial pathogen contamination including *B. cereus, K. pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumoniae, P. aeruginosa*, *V. cholerae, V. parahaemolyticus, C. albicans, Fusarium* and *A. niger)* poses a serious threat to public health, is unfit for domestic use and consumption and incommodious for recreation.

Conclusion

This study demonstrates that Krakrama water was impacted by a variety of physicochemical and anthropogenic factors which may become a prolific source of microbial diversity. Elevated levels of total faecal coliforms and pathogenic microflora of public health significance highlights the magnitude of the problem of contamination. However, most of the physicochemical parametric values were within the permissible limit but BOD, COD and phosphate concentrations require emergency intervention measures so that the water would not pose only ecological and socio-economic problems but a threat to public health.

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