Physicochemical and Microbiological Quality of a Creek in the Niger Delta Region of Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author WJO designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors WJO and ME managed the analyses of the study. Author ME managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

An investigation was carried out on the microbiological and physicochemical quality of Amadi-ama Creek in Rivers State. Samples taken from the Creek were subjected to some physicochemical analyses like pH, DO, BOD, Hardness, temperature, sulphate, phosphate, turbidity, electrical conductivity, Total dissolved solids, nitrate, ammonia, etc. pH ranged from 7.14 to 7.16, temperature, 26.4°C to 27.6°C while salinity was from 13.6 mg/l to 15.9 mg/l. Dissolved Oxygen (DO) ranged from 7.20 mg/l to 7.40 mg/l. Chemical oxygen demand was from 21.2 mg/ml to 26.1 mg/l . The total heterotrophic bacterial counts ranged from $3.0x10^5$ - $4.0x10^5$ cfu/ml which exceeded the limit of $1.0 x 10^2$ cfu/ml for natural water. The total Yeast and Mould counts ranged from $2.3x10^4$ –$2.7x10^4$ sfu/ml and $2.0x10^4$ -$2.2x10^4$ sfu/ml respectively. The total coliform counts were higher in point A for weeks 1 and 2 with 400 and 420 MPN Index/100 ml respectively while Point B had 390 and 400 MPN Index/100 ml for weeks 1 and 2. The total thermotolerant coliform for points A and B for both weeks was 85 MPN Index/100 ml. The genera of bacteria isolated included *Salmonella* sp.*, Shigella* sp.*, Bacillus* sp.*, Klebsiella* sp.*, E.coli, Staphylococcus* sp.*, Streptoccocus* sp.*, Corynebacterium* sp. and *Enterobacter* sp. The study revealed few species of fungi and they include *Penicillium* sp., *Fusarium solani, Mucor* sp. and *Rhizopus* sp. These are a group of fungi that

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invade the superficial layer of the skin and degrade the keratinized tissues of skin, hair and nails in living animals including man, causing skin diseases. *Aspergillus* was also present in this study and it is a group of moulds found everywhere worldwide. Only a few of these molds can cause illness in humans and animals. Most people are naturally immune and do not develop disease caused by *Aspergillus.* However, when disease occurs, it takes several forms. Any Water body contaminated with some of `these pathogenic microorganisms represent an unhealthy livelihood (poor personal and household hygiene) in the environment.

Keywords: Amadi-ama Creek; water quality; bacteria; fungi.

1. INTRODUCTION

Water occupies about 70% of the earth surface yet it is one of the scarcest commodities especially in the developing countries of the world. The need for water has had serious socio-economic and health influences on urban development in developing countries where population concentrations have put serious strains on available resources [1].

Surface water can be contaminated by several ways. In farming areas, the routine application of agricultural fertilizers is the major source [2]. In rural areas, careless disposal of industrial effluents and other wastes may contribute greatly to the poor water quality [3].

Microorganisms mainly found in river water are bacteria and fungi which sometimes cause gastrointestinal tract infection. In the last three to four decades, many investigators have conducted research on the Niger Delta aquatic ecosystem with a view to understanding the characteristics of the various water types and the attendant flora and fauna [4].

In advanced countries, environmental monitoring agencies and laws are strictly effective and followed. General environmental quality monitoring is compulsory and the monitoring of the quality of water resources is done on a regular basis [5]. As a result, any abnormal environmental or water quality can easily be detected and appropriate actions taken before the outbreak of epidemics. The case is quite opposite in many developing countries [6].

Industries such as NLNG (Nigerian Liquefied Natural Gas), Julius Berger Construction Companies and others are located around the Amadi-Ama Creek; hence, most of their waste materials are discharged into this Creek. Such information is important for the authorities to take proper action against the pollution of the environment for the good health of the population [7].

This study was caried out to evaluate the quality of Amadi-ama Creek using microbiological and physicochemical analyses in Port Hacourt, Rivers State of the Niger Delta region of Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Amadi-ama Creek, Port Harcourt; Rivers State in the Niger Delta region of Nigeria and the coordinates are 4°46`0″ N and 7°3`0″E in DMS (Degrees Minutes Seconds). It is surrounded by some communities in Phalga Local Government Area in Rivers State. Along the Riverside, there are some companies like LNG and Julius Berger Construction Company. The vegetation had been cleared and then modified to suit the inhabitants. The area is very significant since it serves as dump site for these companies. Apart from dump site, there are various human activities including fishing, boat movement and social gathering etc. taking place in this location.

The study area is mainly along the drainage runoff from the companies located along the river and house hold waste. The areas of study were referred to as location Points A and B.

Fig. 1. LNG drainage system (waste discharge point) (point A)

Fig. 2. Amadi-Ama village (dump site) (point B)

2.2 Sample Collection

Water samples were collected with sterile one (1) litre containers. After collection of the water samples, the containers were appropriately labeled with the sample codes (i.e sample A and B) and transported immediately to the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria for microbiological and physicochemical analyses.

2.3 Physicochemical Parameters of the Samples

The physicochemical parameters were measured using standard analytical procedures [8] AOAC, 2000). The pH meter used was pocket-sized HANA pHep + HI 98108 with automatic temperature compensation. Nitrate content was determined using the macro Kjeldahl digestion method of Brady and Weil [9] and available phosphorus was determined using the method reported by Olsen and Sommers [10]. Sulphate was determined using the turbidometric method. Standard methods were used for the determination of Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand(COD), turbidity, electrical conductivity, ammonia, total dissolved solid, AOAC[8].

2.4 Enumeration and Identification of Bacteria and Fungi

Sampling from each point was carried out for the enumeration of total Heterotrophic Bacteria (THB). Samples were serially diluted and an aliquot from each sample was placed on nutrient agar medium (Oxoid) for isolation of THB with the addition of 50μg/ml nystatin to suppress the growth of fungi. Plates were incubated at 30°C for 24 hours before the colonies were counted. The bacterial isolates were characterized using microscopic techniques (Gram staining) and biochemical tests [11].

Acidified potato dextrose agar plates containing streptomycin (1 mg/100 ml) were used to obtain fungal isolates. The plates were incubated at 30°C and observed after 48 hours for yeasts and 96 hours for mould, after this, isolation of pure isolates was done [11].

2.5 Estimation of Coliform and Faecal Coliform Bacteria in the River Water Samples

Coliform bacteria in the River water samples were estimated using the Most Probable Number (MPN) technique. Reactions to MPN technique and thermo tolerant coliform bacteria MPN index/100 ml of each water sample was done using double strength Mac Conkey broth for 10ml of sample and single strength Mac Conkey broth for 1 ml and 0.1 ml of the sample . The test for the estimation of coliforms involves the following steps: Presumptive, confirmatory and completed test. It was performed as described by [12,11].

2.6 Identification and Characterization of Isolates

The methods described in [13] were adopted in characterization of isolates. Isolates were identified by standard methods [14].

2.7 Biochemical Identification of Bacterial Isolates

Identification of the isolates was done using conventional methods namely; gram stain, motility test, catalase, oxidase, coagulase, indole, methyl red, voges proskauer, citrate, sugar fermentation test for glucose, lactose and mannitol [15].

2.8 Gram's Reaction

Gram staining reaction was carried out on the isolates to differentiate gram's positive bacteria from gram negative bacteria based on the differences in the nature of their cell walls. This reaction also makes it possible to observe and determine the shapes, and relative sizes of the obtained organism i.e. whether in chains, bunches, singly etc.

2.9 Motility (Diffusion Method)

The motility of the truly motile organisms, of the isolates was studied appropriately using half strength nutrient agar(that is,14 g of nutrient agar was added to 1000 ml of distilled water and 9 ml of the mixture) was dispensed in each test tube to be used and then autoclaved as usual. The test-tubes were then allowed to cool and set (gel-like), after which each isolate was inoculated into each test-tube by the stabbing method (diffusion method). This was then incubated at 35°C for 24, hours.

2.10 Catalase Test

This test demonstrates the presence of catalase in organism. Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide to water and oxygen. A drop of hydrogen peroxide was placed on a clean microscope slide. A sterile wire loop was used to pick up a colony of the investigated organism and smeared on the drop of hydrogen peroxide; a catalase positive organism will produce bubbles of oxygen, while catalase negative bacteria will not.

2.11 Citrate Utilization Test

Citrate Utilization test is used to determine the ability of a microorganism to convert citrate into oxaloacetate. Citrate is an intermediate of the Kreb's cycle. This was carried out by inoculating the test organism in test tube containing Simon's citrate agar which contains ammonium salts, citrate as the only carbon source and bromthymol blue, a pH indicator which is green at neutral pH and blue at alkaline pH. A colony of the investigated isolate was inoculated in Simon's citrate agar with a sterile inoculating needle and incubated at 35°C for 24 hours. The development of deep blue colour after incubation indicates a positive result.

2.12 Oxidase Test

This test is used to determine if a bacterial isolate contains cytochrome (oxidase-an enzyme which catalyzes the transport of electrons from donor compounds (usually NADH) to electron acceptors (usually oxygen). This encompasses a redox reaction. The redox indicator, tetramethyl-*p*-phenylendiamine di-hydrochloride is used in the test. This test is carried out by moistening a filter paper with few drops of freshly prepared 1% tetramethyl-*p*-phenylenediamine dihydrochloride. The colony of the investigated isolate was picked using a sterile wire loop and smeared on the filter paper. The development of a purple colour on the filter paper signifies a positive test.

2.13 Indole Test

The indole production test is done to determine if bacteria can breakdown the amino acid, tryptophan into indole, a nitrogen-containing compound. Only bacteria possessing the enzyme, tryptophanase can breakdown tryptophan. This test is carried out by dispensing tryptone broth in test tubes and sterilizing them in the autoclave. After the broth had cooled down, 2-3 colonies of the investigated isolate were inoculated and incubated for 48hours at 35°C. The formation of a red/pink layer on the broth indicates a positive result.

2.14 Coagulase Test

This is a test used to detect the presence of the enzyme, coagulase. Coagulase catalyzes the conversion of fibrinogen, a soluble blood plasma protein to fibrin which results in blood clotting. This test was done by dropping two drops of normal saline on a clean microscope slide. A colony of the investigated isolate was collected using a sterile wire loop and emulsified on the microscope slide. A drop of blood serum was added on the inoculated saline drop, mixed with the sterile wire loop and rocked for about 10 seconds. A macroscopic clumping on the test slide within 10 seconds indicated a positive result.

2.15 Vogues Proskauer Test

This test is used to determine if glucose can be converted to acetoin. Acetoin is a chiral molecule which is colourless or pale yellow to green yellow liquid with a pleasant, buttery odour. This test was carried out by dispensing the MRVP broth into test tubes and sterilizing for 10minutes with the autoclave. After the test tubes had cooled down, 2-3 colonies of the investigated isolates were added and incubated at 37°C for 48 hours. After incubation, ten drops of freshly prepared Barritt's reagent A was added to the broth culture in a clean test tube, immediately ten drops of Baritt's reagent B was added, agitated for 30 seconds and allowed to stand. The formation of a red colour indicates a positive result while the formation of a yellowish or brown colour indicates a negative result.

2.16 Methyl Red Test

Methyl red test is used to determine if glucose can be converted to acidic products like lactate, acetate and formate. This test was carried out by dispensing the MRVP broth into test tubes and sterilizing for 10minutes with the autoclave. After the test tubes had cooled down, 2-3 colonies of the investigated isolates were added and incubated at 37°C for 48hours. After incubation, 3-5 drops of freshly prepared methyl red solution was added to the broth culture. The formation of a red colour indicates a positive result while the formation of a yellow colour indicates a negative result.

2.17 Sugar Fermentation Test

The test was performed to detect the reduction in pH, as a result of acid production which would occur if fermentation of the given sugar occurred. Two sugars (glucose and lactose), peptone water and phenol red (pH indicator) is dispensed into test tubes in which Durham tubes have been placed. The broths are then sterilized by autoclaving at 115°C for 10 minutes. After sterilization colonies of the investigated isolates are inoculated into the tubes and incubated at 35°C for 24 hours. The result is recorded accordingly; AG- acid and gas produced, A- acid produced and no gas.

2.18 Statistical Analysis

Results were analyzed using Student T-test.

3. RESULTS OF THE PHYSICO-CHEMICAL ANALYSIS

The physicochemical properties of Points A and B in Amadi-ama Creek, Port Harcourt Local Government Area in Rivers state for week 1 are shown on Table 1.

The physicochemical properties of Points A and B in Amadi-ama Creek, Port Harcourt Local Government Area in Rivers state for week 2 are shown on Table 2.

The mean values of the total heterotrophic bacterial counts obtained ranged from 3.0×10^5 cfu/ml to 4.0×10^5 cfu/ml. Week 1 had a higher mean of bacterial count than week 2. The reaction of the River water samples from points A and B to MPN techniques to total coliform counts showed that point B recorded a higher coliform count of 400 - 420 MPN Index/100 ml while point B had 390 - 400 MPN Index/100 ml for weeks 1 and 2 respectively. The River water samples' reaction to MPN technique of Thermo tolerant Coli form count showed that both rivers recorded the same value (85 MPN Index/100 ml).

The mean values of the total Yeast counts obtained ranged from 2.3×10^4 cfu/ml to 2.5×10^4 cfu/ml for weeks 1 and 2 in point A and 2.5×10^4 cfu/ml and 2.7×10^4 cfu/ml for both weeks in point B. The mean values of total Mould counts were 2.0×10^4 cfu/ml and 2.1×10^4 cfu/ml for weeks 1 and 2 in point A while point B had 2.11×10^4 cfu/ml and 2.2×10^4 cfu/ml for both weeks. .

Using Student T-test on the data obtained showed that there was no significant difference at $p < 0.05$ between the microbiological (bacteria, yeast, mould, total and thermo tolerant coli form bacteria) characteristics at points A and B. Using Student T-test, there was no significant difference in the physicochemical characteristics at both points.

4. DISCUSSION

The findings of this study revealed that the Amadi-Ama Creek is brackish, saline and polluted due to influx of waste discharged. [14] had a similar finding in Eagle Island River. The pH of the water ranged from 7.15 to 7.16. The pH measured in both points were within permissible limit of 6.5 -8.5 [16], which is associated with the fresh water emptying into the Creek from the adjoining swamp forests, streams and municipal [7]. The dissolved oxygen ranged from 7.20 mg/l to 7.40 mg/l. This observation might be attributed to the floods and municipal drains depositing wastes (inorganic, organic and debris) into the Creek thereby leading to increased fouling and degradation [17]. The BOD concentration obtained ranged from 9.7 mg/l and 16 .6 mg/l for both points. These values varied slightly across the points, which is indicative of influx of contaminations into the water body. This can be attributed to the influx of biodegrdable materials from external source, possibly due to urban run-off and anthropogenic factors in the area. Generally, the variation across points indicate possible contamination of the water from external sources with the BOD in point B recording the highest value of 16.6 mg/l [14]. The salinity varied from 14.6 mg/ml to 15.9 mg/l. This indicates higher level of carbonates, bicarbonates and hydroxides [18]. [14] had lower values in Eagle island River with a range of 9.0 mg/l to 9.75 mg/l. The electric conductivity (Ec) ranged from 1210 μs/cm to 1220 μs/cm. These

high Ec values show the presence of high concentration of total dissolved solid from municipal and industrial effluents and to a large extent, the incursion of brackish water [19]. The values obtained could also be attributed to the combined influence of sea water particularly during rainy reason. Chemical Oxygen Demand (COD) ranged from 22.2 mg/l to 26.1 mg/l. This result suggests that efficient and proper sanitary check on water and environment has to be executed regularly in view of its great public

health significance and at the same time, good observation of personal and household hygiene has to be emphasized. The total hardness level of the water was high (1130–1135 mg/l), hence, the Creek is classified as hard water. Alkalinity is an evaluation of weak acid and their salts present in water or as the acid neutralizing capacity of water body. The alkalinity values of the points in Amadi Creek for both weeks were 60 mg/l. The alkalinity values correlated with the pH values obtained and was within World Health
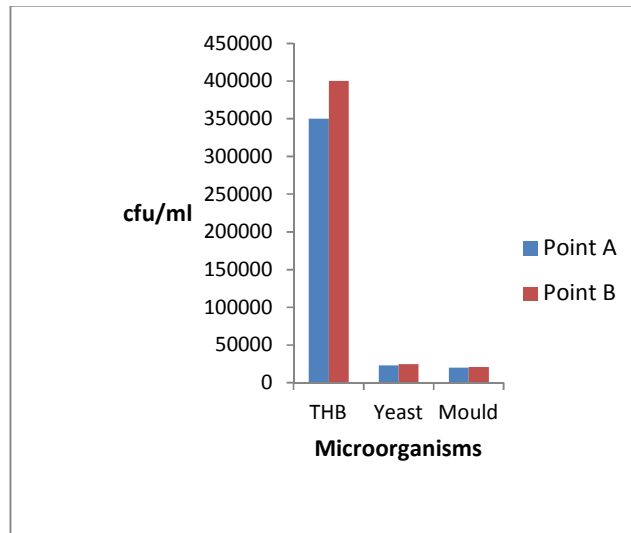

Fig. 1a. Bacterial and fungal counts from points of collection during week 1
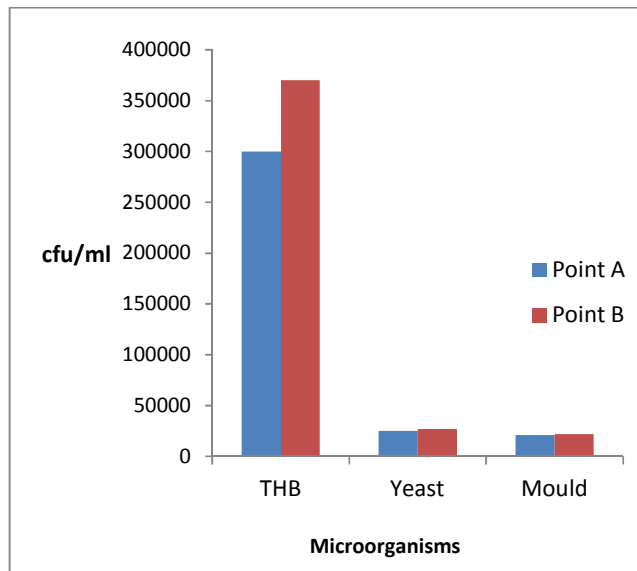*Key: THB-Total Heterophic Bacteria*

Fig. 1b. Bacterial and fungal counts from points of collection during week 2
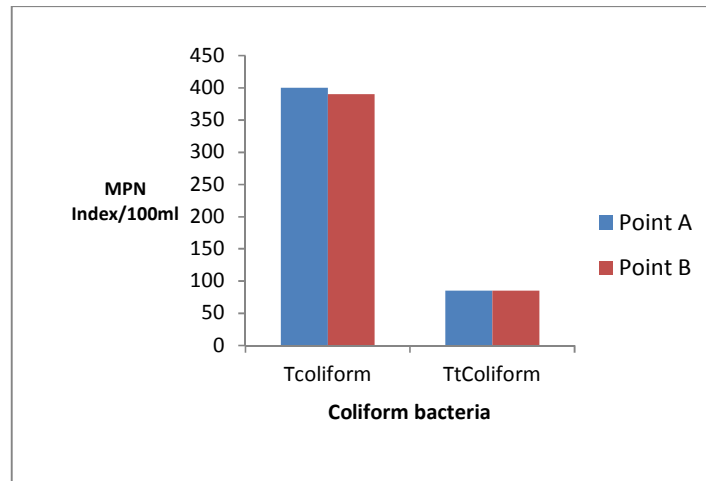*Key: THB-Total Heterophic Bacteria*

Fig. 2a. Total and thermo tolerant coliform bacterial counts from points of collection during week 1

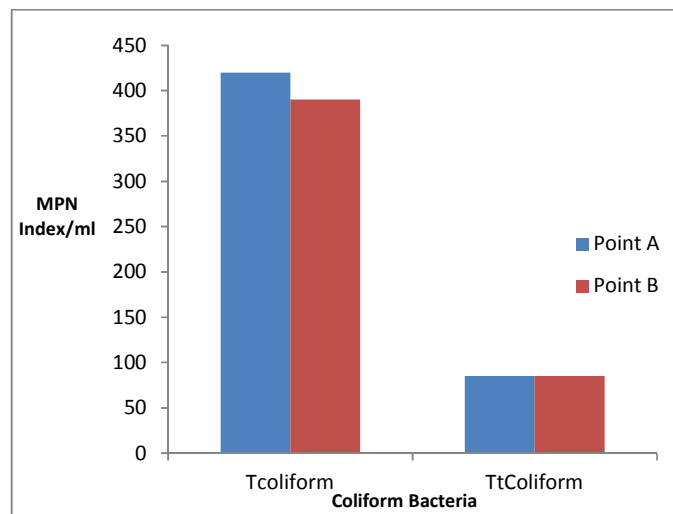*Key: Tcoliform-Total Coliform; Tt Coliform-Total thermotolerant Coliform*

Fig. 2b. Total and Thermo tolerant Coliform Bacterial counts from points of collection during week 2

Key: Tcoliform- Total Coliform; Tt Coliform- Total thermotolerant Coliform

Organization permissible limit for alkalinity of domestic and water for industrial purpose (30-500 mg $CaCO_3$/l). The sulphate values obtained for both weeks and points ranged from 760-780 mg/l which is lethal to human and the aquatic life. The high levels of sulphate in Amadi-ama Creek could be as a result of depletion of oxygen due to activities of human and respiration by aquatic life. In areas where the rate of consumption equates the rate of supply, the aquatic environment becomes anoxic or devoid of adequate oxygen [20]. Phosphate can be found as a free ion in water systems and as a salt in terrestrial environments used in detergents as water softeners. Phosphates can be in organic forms (organically-bound phosphates) or inorganic forms (including orthophosphates and polyphosphates). There was no significant (P>0.05) difference in all the physicochemical parameters values for both weeks as well as all the points of sampling. The values were below the allowable limit of 10 mg/l [16]. Presence of nitrate could be caused by the discharge of human waste and sewages. Surface runoff and organic matter decomposition in water bodies also produced inorganic nutrients such as ammonia, nitrate and phosphates with resultant effects of eutrophication and other serious

ecological impairments of such water body [21]. High nitrate levels (above 45 mg/l) in water have grave health implications and could result in several diseases. Nitrate is generally the cause for concern in drinking water at levels greater than 10 mg/l. At higher concentration levels, there is an increased risk of babies developing infant methaemoglobinaemia, a disease commonly known as 'blue baby' syndrome [14].

This investigation showed the presence of total heterotrophic bacteria, Yeast, Mould, total Coliform and Thermotolerant Coliform bacteria counts in the water samples obtained from Amadi-ama Creek, Rivers State.The faecal coliform count obtained was far above recommended standards. The detection of faecal coliform indicates faecal pollution of drinking water. The presence of faecal indicators showed the presence of *Escherichia coli* and *Shigella sp.* Other enteric pathogens such as *Enterobacter* indicated that the water source was significantly polluted with faecal matter. The international

standards for drinking water states that potable water should not contain 100 cells of Total Heterotrophic Bacteria per 100 ml of water, but unfortunately, the bacterial counts obtained in this study exceeded the standard [22]. If the water is used for drinking, it would pose threat to public health causing gastrointestinal diseases.

The study revealed few species of fungi and they include *Penicillium* sp., *Fusarium solani, Mucor* sp. and *Rhizopus* sp*.* These are a group of fungi that invade the superficial layer of the skin and degrade the keratinized tissues of skin, hair and nails in living animals including man, causing skin diseases. *Aspergillus* was also present in this study and it is a group of moulds found everywhere worldwide. *Aspergillus* is also common in the home, including beddings. Only a few of these molds can cause illness in humans and animals. Most people are naturally immune and do not develop disease caused by *Aspergillus.* However, when disease occurs, it takes several forms.

Table 1. Physicochemical properties of points A and B in Amadi-ama creek for week 1

Parameter	Point A	Point B
pH	7.15	7.14
Temperature (°C)	27.6	27.6
Electrical conductivity (µs/cm)	1210	1220
Sulphate (mg/l)	780	765
Phosphate (mg/l)	0.02	0.01
Total Hardness (mg/l)	1130	1135
Total alkalinity (mg/l)	60	60
Nitrate (mg/l)	0.60	0.61
Total dissolved solids (mg/l)	12220	11940
Biochemical oxygen demand (mg/l)	9.7	6.5
Chemical oxygen demand (mg/l)	22.2	26.0
Salinity (%)	13.6	15.9
Dissolved oxygen (mg/l)	7.20	7.40

Table 2. Physicochemical properties of points A and B in Amadi-ama creek for week 2

Parameter	Point A	Point B
pH	7.16	7.15
Temperature (°C)	26.4	26.4
Electrical conductivity (µs/cm)	1210	1210
Total dissolved solids (mg/l)	11950	11950
Biochemical oxygen demand (mg/l)	9.75	16.6
Chemical oxygen demand (mg/l)	22.2	26.1
Salinity (%)	14.6	15.9
Dissolved oxygen (mg/l)	7.20	7.40
Sulphate (mg/l)	780	760
Phosphate (mg/l)	0.02	0.01
Total Hardness (mg/l)	1130	1135
Total alkalinity (mg/l)	60	60
Nitrate (mg/l)	0.58	0.63

5. CONCLUSION

The findings of this study revealed that the water in Amadi-Ama Creek is brackish, saline, hard and polluted due to influx of waste discharge. The water is unfit for drinking but can sustain aquatic life. Human population, development and rain water have remarkable effect on the water quality. Further more, the various activities going on by the shores of the Creek such as human sewage discharge, drainage construction, and others have been identified as major sources of nutrients and other elements introduced into the aquatic environment. These activities affected the microbial load of the aquatic system which resulted in slight variation in microbial counts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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