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(RESEARCH ARTICLE)

Microbiological analysis of Udo Anwankwo river in Ikot Ekpene, South-South Nigeria

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Abstract

The microbiological study of water samples obtained from Udo Anwankwo River was investigated. Water samples were collected from three different sampling points along the course of the river and analyzed using standard procedures. The total bacterial counts, total coliform counts and total fungal counts of the water samples ranged from 2.6×10^5 to 4.8×10^5cfu/ml, 1.2×10^4 to 1.8×10^5cfu/ml and 0.24×10^3 to 1.9×10^3cfu/ml respectively. A total of nine bacteria species belonging to the following genera, *Bacillus, Salmonella, Escherichia, Pseudomonas, Micrococcus*, *Staphylococcus*, *Vibrio, Enterobacter* and *Streptococcus* were isolated and identified from the samples, while five fungal isolates including members of the genera *Aspergillus niger*, *Mucor*, *Penicillum, Rhizopus* and *Fusarium* were isolated. The study through microbial analysis has revealed that the river water sample was not free from pathogens and thereby not suitable for potable use. There is need to put adequate measures towards the control of pollution and proper treatment of the water before usage as it contains pathogenic organisms.

Keywords: Water samples; Coliform; Bacteria; Microbial analysis; Pathogens

1. Introduction

Water is fundamental to the biochemistry of all living organisms. It drives plant growth and provides a permanent habitat for many species. The earth ecosystem is being held and maintained by water [1]. Water makes up the aquatic ecosystem and covers about 70.9% of the earth surface [2].

Though water is abundant, suitable drinking water is limited by geography, demography and affordability [3]. The quality of water and its accessibility remains global challenge especially potable water. Improving access to safe drinking water can result in significant benefits to health [4].

The increase in population in Nigeria and the high rate of urbanization in the country have brought about high change in water quality. The burning of fuel, increase in agricultural activities, industrialization and environmental degradation constitute the change in water quality. Waste such as municipal, industrial, agricultural wastes enters water bodies [5]. Rivers are very important drinking water sources, recreational and sporting activities such as water sports and fishing is also carried out here. These activities have impacts on both human and other aquatic lives [5].

Contamination of water is a serious environmental problem as it affects human health and biodiversity in the aquatic ecosystem adversely [6]. Indicator bacteria like the faecal coliforms and faecal streptococci are widely used for assessment of faecal pollution and possible water quality deterioration in fresh water sources [7].

Generally, the greatest microbial risks are associated with the ingestion of water that is contaminated with human or animal faeces. Also, water is also contaminated with the release of chemicals from industries, fertilizers and other anthropogenic activities [3]. Contaminants ingested into water supply causes many diseases. Examples of such

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pathogens are *Salmonella spp., Shigella spp*, *Vibrio cholera* and *E. coli* [8]. The transmission of disease through drinking water is one of the primary concerns for a safe drinking water. Fecal pollution of drinking water may introduce a variety of intestinal pathogens which may cause disease from mild gastro-enteritis to severe and sometimes fatal dysentery, diarrhoea, cholera, typhoid, hepatitis, giardiasis etc [9].

The microbial quality of drinking water is of paramount importance and monitoring must be given priority [10]. Conformation with physiological and microbiological standards is of special interest because of the capacity of water to spread disease within a large population. Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading water born disease [11].

Udo Anwankwo River is located in Ikot Osurua, Ikot Ekpene and has a close proximity with the state polytechnic and the old sunshine battery factory. Activities carried out around the bank include laundry, dredging, bathing, swimming and fishing. These activities contribute significant amount of organic nutrients and other inputs to the river. Because of the associated dangers of contaminated waters, this study was carried out to assess the microbial quality as well as identify the groups of microorganisms implicated.

2. Material and methods

2.1. Study Area

Udo Anwankwo River is located beside the popular Akwa Ibom State Polytechnic, Ikot Osurua, Latitude 5.160° and Longitude 7.67 in the raffia city of Ikot Ekpene, Nigeria. It receives input from the old sunshine battery factory, Akwa Ibom State Polytechnic, Ikot Ekpene and also from the domestic activities carried out by residents of the community. Three sampling points were chosen along the course of the River. These points were strategically chosen because of the influx of wastewater and/or human activities/influence around the course of the river.

2.2. Water Sampling

Water samples were collected from three points in pre-cleaned 1 liter capacity plastic bottles and immediately delivered to the Microbiology and Biotechnology Laboratory, Department of Biological Sciences, Ritman University, Ikot Ekpene - Akwa Ibom State, Nigeria in ice packs. The samples were processed immediately.

3. Microbiological analysis

3.1. Sterilization of Glass wares

The glassware's (pipettes, test-tubes, beakers, measuring cylinders, conical flasks, McCartney bottles and Durham tubes) were sterilized in the hot air oven at 160°C for 1 hour. Pipettes were dried and kept in a canister before sterilization. Test-tubes were wrapped in aluminum foil; scalpels were wrapped in aluminum foil while the beakers, measuring cylinders and conical flasks were plugged with cotton wool before sterilization.

3.2. Media used in the experiment

These include MacConkey agar (MCA), Nutrient agar (NA), and Sabouraud dextrose agar (SDA).

3.3. Preparation of Media

Media were prepared by weighing approximate amount of the powder and dissolved in 1 litre of distilled water in a conical flask as stated by the manufacturer. The media were then homogenized by boiling before sterilizing in the autoclave at 121°C for 15minutes.The sterile media were allowed to cool to about 45°C before being poured into sterile Petri-dishes and allowed to set [12].

3.4. Isolation of Pathogenic Bacteria

The water samples were analyzed on different culture media using the standard spread plate technique. Nutrient agar (NA) was used for the heterotrophic bacteria count while Sabouraud dextrose agar (SDA) was used for fungal counts. The bacterial cultures were incubated at 37°C for 24 hours. The samples were also cultured using a differential/selective culture media namely MacConkey agar for *Enterobacter* and other coliforms; this method was adopted from [13, 14].

3.5. Enumeration and Identification of Bacterial

A serial dilution method was used for total viable count. Dilutions of 10^{-1} to 10^{-5} were carried out for all samples by transferring 1ml of the sample to 9mls of sterile diluents to make a 10-fold dilution. For direct counting, spread plate technique was performed as described thus; 0.1 ml of the river water sample was transferred by a micro pipette and spread on agar plate in duplicates with a sterile bent glass rod. All the plates were inoculated at 37°C for 24 hours. Only 30–300 colonies on a plate were considered for enumeration from the various dilutions. Total counts were expressed as colony forming unit per ml (cfu/ml) [15].

The bacteria isolates were characterized using microscopic techniques, morphological and biochemical tests. The identities of the isolates were determined by comparing their characteristics with those of known taxa as described by Bergey's manual of Determinative Bacteriology [16, 12]. The cultures used for biochemical test were between 18 to 24 hours old.

3.6. Enumeration and Identification of Fungi

Heterotrophic fungi were estimated using the dilution plate count method. Sterile physiological saline was used as diluents for inoculum preparation. One ml of the effluent was aseptically transferred into a sterile test tube containing 9.0ml of the diluents giving a 10^{-1} dilution. Subsequent dilutions up to 10^{-5} serial dilutions were prepared from the 10^{-1} dilution. A 0.1millilitre aliquot of the dilution from the effluent sample was aseptically removed with a sterile pipette and spread plated using a flame sterilized glass spreader on the SDA plates in duplicates. The cultures were incubated for 5-7 days at room temperature. After incubation, the colonies that develop on the SDA plates were counted and recorded as total heterotrophic fungi. Identification was based on their colony evaluation, colour, texture, shape and arrangement of conidia. Their characteristic hyphal and reproductive structures during the period of incubation were observed. They were then aseptically picked using sterile inoculating needle and sub-cultured onto fresh SDA to obtain pure cultures. Then respective mycelia were transferred onto lactophenol cotton blue solution with an inoculating needle for 2 minutes and then covered carefully with grease free cover slips. They were then observed microscopically under the low, middle and high-power objectives of the microscope [17].

4. Results

4.1. Microbial Loads and Type of Microorganisms

The microbial loads in the water sample at the various sampling points as shown by their colony forming units (cfu) is as represented in table 1 and figure 1 respectively. The groups of microorganisms represented include total bacterial count using nutrient agar, total coliform counts using macConkey agar and total fungal counts using sabouraud dextrose agar. The microbial counts ranged from 2.6×10^5 to 4.8×10^5cfu/ml for total bacterial count, 1.2×10^4 to 1.8×10^5cfu/ml and 0.24×10^3 to 1.9×10^3cfu/ml. The highest microbial counts were recorded at point 2 for all the groups of microorganisms investigated.

Table 1 Total count of microorganisms in the water samples

water Samples sampling points	Total bacterial count on NA (cfu/ml)	Total coliform count on MAC (cfu/ml)	Total fungal count on SDA (cfu/ml)
Point 1	26×104	4.9×104	1.9×103
Point 2	48×104	18×104	3.6×103
Point 3	38×104	1.2×104	0.24×103

Key: Nutrient Agar =NA, MacConkey Agar = MAC, Sabouraud Dextrose Agar = SD

Table 2 Results of Morphological, Biochemical Characterization and Sugar Fermentation of Isolates

Isolated strain	Morphology	Gram's reaction	Catalase	Oxidase	Motility	Citrate Spore stain	Indole	Methyl red	Voges proskauer	H_2S production	Starch hydrolysis	Urease	Glucose	Galactose	Sucrose	Fructose	Lactose	Maltose	Mannitol	Probable organism
001	C	+	-	-	+	+-	+	+	-	+	+	-	+	-	+	+	+	+	+	*Streptococcus spp*
002	R	-	+	-	+	+-	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterobacter aerogenes*
003	R	-	+	-	+	+-	+	-	+	+	-	-	+	-	+	+	+	+	+	*Vibrio spp*
004	R	-	+	-	+	--	-	+	-	+	+	-	+	+	-	+	-	+	+	*Salmonella sp.*
005	C	+	+	-	+	+-	+	-	-	-	+	+	+	-	+	-	-	-	-	*Micrococcus spp*
006	R	+	+	-	+	-+	-	+	+	-	+	-	+	-	-	+	-	-	-	*Bacillus spp*
007	R	-	+	+	+	+-	-	-	-	+	-	-	-	-	+	-	-	-	-	*Pseudomonas spp*
008	R	-	+	-	+	--	+	+	-	-	+	-	+	+	+	-	+	-	+	*Escherichia coli*
009	C	+	+	+	-	+-	-	+	+	-	+	+	+	+	+	+	+	+	+	*Staphylococcus aureus*

Key; C = Cocci, R = rods

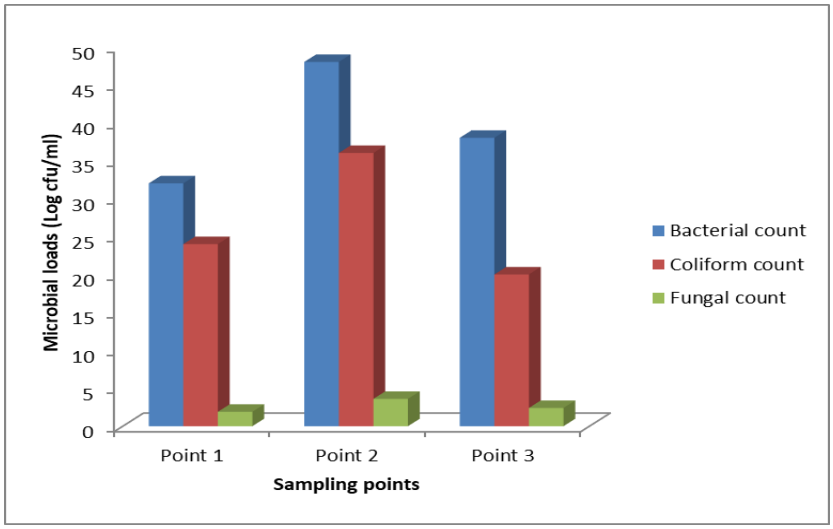

Figure 1 Counts of various groups of microorganisms from the water samples collected from the three sampling points

Morphological and biochemical characteristic of the bacteria and fungi isolated from the water samples at the different points are shown in Tables 2 and 3.

Table 3 Morphological characteristics of fungi isolates

Isolates	Cultural characteristics	Morphological features	Microscopy	Probable organism
F1	Yellowish-green mycelium	Conidia in long chains, branched cells	Branched smooth conidiophores, brush-like conidia head	*Penicillin* spp
F2	White to greenish grey with edge	Smooth walled erect Conidiophores	Septate, Dome gradually Enlarging	*Aspergillus niger*
F3	White to grayish brow	Rhizoids Ovoid Sporangiophores	Coenocytic, Filamentous Stolon	*Rhizopus* sp.
F4	White and pale Grey	Sporangiophores	Unseptate, Erect Sporangiophore	*Mucor* sp.
F5	White cottony with felty colony	Macro-conidia with light periphery in chains	Septate hyphae with branched conidiophores	*Fusarium sp*

5. Discussion

The quality of Udo Anwankwo River was investigated and the results vary in water quality at different sampling points. The total viable counts for the water samples collected from the three sample locations were generally high exceeding the WHO limit of $1.0x10^2$ cfu/ml which is the standard limit of bacterial counts for drinking water as total bacterial counts are indicative of the presence of high organic matter in the water [18]. The primary sources of these bacteria in water could be attributed to animal and human activities. The high coliform counts obtained from the samples, is an indication that the water sources have received faecal contamination [19, 20]. None of the sampling locations of the water sources complied with WHO standard for coliform in water and this could be supported by evidence advanced by Aboh EA et.al., [21] who reported high coliform counts.

A total of nine bacteria species belonging to the following genera, *Bacillus, Salmonella, Escherichia, Pseudomonas, Micrococcus*, *Staphylococcus*, *Vibrio, Enterobacter* and *Streptococcus* and a total of five fungal species were isolated from the samples. These include *Aspergillus niger*, *Mucor*, *Penicillum, Rhizopus* and *Fusarium* species. Most of the isolated bacteria and fungi have been confirmed in similar study by Atoyebi B et.al [6]. The isolated bacteria species were identified to be same with those commonly encountered in water and aquatic environments as was also reported in a study on streams surface water in Wyoming in U.S.A. reported by Okonko IO et.al., [22] and reviewed by Banwo K [23]. On the bacterial load, pathogenic bacteria were isolated from the three locations. Presence of enteric bacteria like *Salmonella* spp., *Vibrio* spp. and *E. coli* can be attributed to high level of faecal and municipal waste contamination which may constitute health hazard to the people drinking or using the water for domestic activities or both. A similar conclusion was drawn by Atoyebi B et.al, [6] The total viable counts for the water samples collected from the three sample points were generally high exceeding the WHO limit of $1.0x10^2$cfu/ml which is the standard limit of bacterial counts for drinking water as total bacterial counts are indicative of the presence of high organic matter in the water [18]. The high coliform counts obtained from the samples, is an indication that the water sources have received faecal contamination [19, 6]. None of the sampling locations of the water sources complied with WHO standard for coliform in water and this could be supported by evidence advanced by Aboh EA et.al [21] who reported high coliform counts. Every water sample that contains coliform, should be investigated for the presence of faecal coliforms, *E. coli* [24]. Other bacteria isolated from all sampling points are also of public health significance.

6. Conclusion

This study concluded that the Udo Anwankwo River needs a serious effort in limiting the numbers of microorganisms released into the body. The high microbial load in the river renders it to be unfit for human consumption though they can be used for other purposes. Water should meet different quality specifications depending on the particular uses. Potable and domestic water should be harmless for human health and other domestic uses. The water body needs urgent measures to control pollution by controlling anthropogenic activities such as washing, dredging etc. that goes on the river unabated so as to prevent sewage from entering the water body which is the key to avoid bacterial contamination of the water, and thus provide means of safe water for use, thereby protecting the water body.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no conflict of interest exists.

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