Microbiological and Physico-Chemical Studies of Water in Nduetong Oku Streams of Akwa Ibom State.

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Abstract: The importance of routine assessment of water quality available for consumption, domestic and industrial uses cannot be overemphasized. Hence, studies on microbiological and physico-chemical characteristics of Nduetong Oku streams (Idim Afia and Idim Etuk) were carried out to assess their pollution status. Microbiological analysis showed mean bacterial count of $8.05x10^5$ CFU/mL for Idem Afia and $7.86x\ 10^5$ CFU/mL for Idem Etuk; the mean coliform count for two streams were $3.57x10^5$CFU/mL and $3.4xl0^5$ CFU/mL , while the mean fungal counts was $5.43xl0^4$ CFU/mL and $4.52xl0^4$ CFU/mL for Idem Afia and Idem Etuk, respectively. Isolates identified included *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Vibro* sp. which are considered pathogenic, other were *Enterococcus faecalis* and *Escherischia coli,* notable pollution indicators and pathogens. Fungi isolates obtained were *Aspergillus candidus, Absidia* sp, *Chaetomium* sp, *Candida Iropicalis, Candida pseudotropicalis, Penicillium frequentans, Eurotium herbariorum.* All the fungal isolates and *Escherichia* sp:, *Bacillus* sp., and *Pseudomonas* sp. exhibited hydrocarbon degradation capability. The physico-chemical analysis of the water sources showed that some parameters were higher, some below and others within the specified range of WHO (World Health Organization) standard. From these baseline studies conducted in Nduetong-Oku streams, it has been noted that the streams contain a number of tested parameters that do not conform to the WHO standard for potable water.

Key Words: Water, Natural water sources, Pathogenic microorganisms, Pollution, potable water.

Introduction

Water is a common name used to refer to the liquid state of the hydrogen-oxygen compound H_2O. The ancient philosophers regarded water as a basic element typifying all liquid substances. Scientist did not discard that view until the latter half of the 18th century. In 1781, the British Chemist, Henry Cavendish synthesized water by detonating a mixture of hydrogen and air. However, the result of this experiment were not clearly interpreted until two years later, when the French Chemist Antoine Laurent Lavoidier proposed that water was not an element but a compound of oxygen and hydrogen (Mascetta, 2007).

Water is the most abundant and widely distributed of all chemical compounds and is most important to man's survival. Seventy percent (70%) of the earth's surface is covered with water even though not all are suitable for human consumption (Williams, 2014). Without water, life would not be possible. Living organisms uses water as their solvent. Since water is an excellent solvent, even in its natural state in streams, rivers, lakes and from underground aquifers, it is never pure water but contains varieties of inorganic and organic compounds (Porteous 2000). Water can also carry large amounts of insoluble material in suspension.

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Rainfall, Agricultural, Domestic and Industrial effluent (sewage) impose a variety of effects on the natural characteristics of the water courses (Rivers, Streams and Lakes) into which they are discharged (Akpor and Muchie, 2011). The quality of water from these sources varies greatly. Surface waters generally contain larger quantities of particles and bacteria than ground water, but ground water contains higher concentrations of dissolved chemicals. Seawater contains high concentrations of dissolved chemicals and microscopic organisms as well (Khatri and Tyagi, 2014).

All waters are subjected to a degree of natural pollution. The major sources of water pollution which change the quality of water can be classified as municipal, industrial and agricultural. The measurement and control of water quality is therefore of crucial importance in the interest of public health and maintenance of the environment.

Most of our fresh waters (Rivers and Streams) have been contaminated by natural processes, and human activities in several ways. Commonly observed in our water bodies are suspended solids, oxygen-demanding materials, dissolved inorganic compounds (particularly compounds of phosphorus and nitrogen) and harmful/pathogenic bacteria. Wastes from water can also contain chemicals which are toxic to both aquatic life and man, some having insidious harmful effects at all levels of food chain (Tchounwon *et al.*, 2012). Many researchers recognized water as a medium for transmission of enteric bacteria, which in effect reduces the quality of water in many communities.

The principal ecological functions of microorganisms in fresh water habitats however, include, decomposition of dead organic matter, liberating mineral nutrients for primary production, assimilation and re-introduction into the food web of dissolved organic matter; they perform mineral cycling activities which is a system of chemical transformation of elements by living system (Biogeochemical cycle) (Lahiri *et al.*, 2018). They serve as a food source of grazers. Nonetheless, the negative impact of microorganisms and other pollutants in fresh water bodies can never be overemphasized. In fact, human health is really involved when water quality is been considered (Meybeck *et al.*, 1996). There is need to constantly evaluate fresh water sources to know their pollution status with time.

In view of this, streams around Uyo metropolis, the Capital city of Akwa Ibom State, Nigeria need to be subjected to microbiological and physicochemical studies to ascertain their current pollution status. Many human, natural and environmental unfriendly activities such as industrial effluent and domestic wastes impact water bodies because Uyo is an industrially evolving town. Nduetong-Oku streams in Uyo metropolis is chosen for this study as it is strategically located and serves as the major source of water for people leaving in that environment.

The aim of this study was to isolate microorganism associated with these streams; determine the current pollution status of the streams and its public health significance, and to determine the physio-chemical qualities of these streams standard.

Materials and Methods

Description of the Study Area

Idim Etuk and Idim Afia are located in Nduetong oku village in Uyo metropolis. Physical features of the area show that it is hilly with steep slopes (gorges). These slopes were carved as a result of gully erosion. Semi-intensive farming is practiced around this area with yam, cassava, cocoyam and vegetables as their major crop of plantation. Environmental unfriendly human activities are common on this environment as it can be clearly seen around where human sewage are disposed, bathing, but of all the most worrisome is its use as a refuse dump (stream) site. This had not only reduced the velocity of the stream but also poised generally ecological hazard to the inhabitant of its suburb who rely on this streams for their domestic purposes.

Sample Collection

Upstream and down streams samples labeled A-E were collected from the two streams. Sterile screw-capped bottles were used to collect the samples adopting the method of Collins and Lyne (1976). The water samples were transported immediately to the laboratory for analysis. Physical parameters like the temperature were determined at the sampling site using the thermometer. Some of the water samples were used for physic-chemical analysis.

Materials for Microbial Analysis

For bacteriological and mycological examination of the water samples, the following culture media were used: Nutrient Agar (NA), MacConkey Agar (McA), Eosin Methylene Blue Agar (EMBA) and Sabouraud Dextrose Agar (SDA). These media were prepared according to their manufacturer's instructions and were sterilized by autoclaving at 121° C for 15 minutes at 15 p.s.i. Other reagents and chemicals used were of analytical grade.

Microbiological Analysis

A 10- fold serial dilution technique was carried out up to 10^{-4}. One (1ml) aliquots of the samples were pipette into sterile petri-dishes in duplicate and about 10-15ml of sterile Nutrient Agar, MacConkey Agar and Sabourand Dextrose Agar that had been cooled to 45°C was poured into the plates, gently swirled and allowed to solidify (pour plate method). After solidification, the plates were inverted and incubated at 370c for 24 hours to obtain bacteria growth while Sabourand Dextrose Agar for fungi was kept for 4 to 5 days to check morphological characteristics (ambient temperature) (Cruichshank *et al.*, 1980). Pure cultures obtained by repeated sub-culturing were stocked in McCartney's bottles. They were stored in the refrigerator at 4°C for identification and further studies.

Characterization and Identification of the Isolate

Colonial Morphology

Isolates were observed morphologically as described by Holt *et al.* (1994). They were further characterized and identified based on the following biochemical tests. Gram stain, Spore stain, Motility test, Catalase test, Coagulate test, Indole test, Oxidase test, methyl Red (MR) test, Voges Proskauers (VP) test, Citrate utilization test and Carbohydrate fermentation test.

Total Count of Petroleum Hydrocarbon Degrading Microorganisms

Determination of total hydrocarbonoclastic organisms was done using the modified mineral salt agar medium of Zajic and Supplison (1975). The mineral salt agar was supplemented with 1% crude oil and poured into sterile petri-dishes. The plates were allowed to solidify and the isolated organisms were picked with the aid of a sterile wire loop and streaked on the plate for the bacterial isolates while the spores of the fungi isolates were stabbed on the surfaces of appropriate medium. The hydrocarbon degrading microorganisms were enumerated after incubation for 10-14 days at 27±3°C.

Physico-Chemical analysis of Water Samples

The physical parameters determined included appearance, odour, colour, turbidity, temperature, total dissolved solids (TDS) and dissolved oxygen. Some chemical radicals like NO_3, NO_2, chloride Cl, were analysed. Other metals analysed were sodium and potassium. Other characteristics also studied were acidity, alkanity, salinity, free carbondioxide CO_2, total hardness, total iron. The methods of chemical analysis employed were according to Field and Kealey (1986).

Results

Total Heterotrophic Bacterial Counts

The total heterotrophic counts determined using standard plate count technique ranged from 3.10 x $10^5$to 1.94 x 10^6CFU/mL, with mean count 8.05 x10^5 CFU/mL for Idem Afia. Idem Etuk had total bacterial count range of 3.45 x $10^5$to 1.94 x 10^6 CFU/mL with a mean count of 7.86 x 10^5CFU/mL. The mean coliform bacilli and fungal counts obtained from samples from both streams were 3.57 x10^5CFU/mL and 5.43x10^4CFU/mL respectively, for Idem Afia and 3.47 x 10^5CFU/mL and 4.52 x 10^4 CFU/mL respectively, for Idem Etuk. Idem Afia generally had higher burden of microorganisms than Idem Etuk (Table 1). The bacterial isolates from the water sample included *Staphylococcus* sp, *Escherichia* sp., *Enterrococcus* sp., *Bacillus* sp., *Klebsiella* sp. and *Pseudomonas* sp. In Idim Afia, *Escherichia* sp. and *Enterococcus* sp. had the highest occurrence of 24.9% and 20.7%, respectively, followed by *Bacillus* sp. with 18.4% and the least was *Stapholococcus* sp. with 7.9% while in Idim Etuk *Enterococcus* sp. had the highest occurrence of 23.7% and the lowest was *Staphylococcus* sp. with 10.0% occurrence.

***Morphological, Structural Characteristics and* Occurrence of Fungi Isolates**

Morphological and structural characteristics of the obtained fungal isolates are presented in Table 2. The Fungal isolates from the water samples included: *Aspergillus candidus*, *Absidia* sp., *Chaetomium* sp., *Candida tropicalis*, *Candida pseudotropicalis*, *Penicillium frequentans* and *Eurotium herbariorum*. In Idim Afia, *Pencicillium frequentans* had the highest occurrence with 28.6% and *Eurotium herbariorum* had the least occurrence with 7.1% while in Idim Etuk, *Aspergillus candidus* and *Candida tropicalis* have the highest occurrences with 26.9% while *Absidia* sp had the least occurrence with 7.1%.

Screening for Hydrocarbon Biodegradability of Test Organism

The hydrocarbon biodegradation capabilities of the fungal and bacterial isolates are shown in Table 3. For bacterial isolates in Idim Afia, *Pseudomonas* sp. had the highest crude oil biodegradability followed by *Bacillus* sp. while *Escherichia* sp. had the lowest; in Idim Etuk, *Bacillus* sp. and *Pseudomonas* sp.were majorly the crude oil degraders. For the fungi isolates, in Idim Afia, *Candida pseudotropicalis* had the highest crude oil biodegradability and the organisms with the least ability to utilize crude oil were *Penicillium frequentans* and *Eurotium herbariorum* while in Idim Etuk, *Absidia* sp. and *Eurotium herbariorum* had moderate growth in the presence of 1% crude oil. Lowest growth in the presence of crude oil observed in *Aspergillus candidus* and *Candida pseudotropicalis* in this same stream.

Comparison of Physico-Chemical Analysis of Idim Afia and Idim Etuk (Nduetong-Oku Streams) with W.H.O. Standard 1984.

Table 4 shows the physic-chemical properties of the two different stream waters in Nduetong-Oku village in Uyo metropolis. The colour and turbidity reveals that the streams do not meet the WHO standard; some other parameters were below or others within the range recommended by WHO.

TABLE1: Mean Microbial Count Of Water Samples Obtained From Nduetong-Oku Streams (Idem Afia And Idem Etuk).

Bacterial Counts	Idem Afia	Idem Etuk
Means Total Heterotrophic Bacterial Counts	8.05 x 10^5 CFU/mL	7.86 x 10^5 CFU/mL
Mean Coliform Bacterial Counts.	3.57 x 10^5 CFU/mL	3.47 x 10^5 CFU/mL
Mean Fungal Counts	5.43 x 10^4 CFU/mL	4.52 x 10^4 CFU/mL

Table 2: Morphological, Structural Characteristics and Identity of Fungal Isolates Obtained from Idem Afia and Idem Etuk.

Isolate Code	Somatic Nature	Colony Colour Pigment-ation	Hyphae	Aerial Repro-ductive Hyphae	Reproduc-tive Spores/ Features	Vesicle Shape Columar	Probable Isolate
F_1	Filamentous	White mycelium	Septate	Conidio-shore	Conidia	Dome shape	*Aspergillus candidus.*
F_2	Filamentous	White mycelium	Coenocytic	Branched Sporangio spore	Sporangio-spore	Spherical sporangium	*Absidia* sp.
F_3	Filamentous	Light to Dark gray	Septate	Elongated perithecia	Ascospores	-	*Chaetomium* sp.
F_4	Filamentous	Wet white mycelium that remain white	Septate	Conidio spore	Chlamydo Spores/conidia	-	*Candida tropicalis*
F_5	Filamentous	Wet white Mycelium, milky white with age	Septate	Conidio spore	Chlamydo Spores/conidia	-	*Candida Pseudotro-picals*
F_6	Filamentous	Greenish Spreading mycelium	Septate	Conidio spore	Conidia phialides	-	*Penicillium frequentans*
F_7	Filamentous	Blue green	Septate	Conidio spore	Conidia phialides	-	*Eurotium harbariorum*

Table 3: Screening For Hydrocarbon Biodegradability Among the Obtained Organisms from both Streams Cultured in 1% v/v Crude Oil.

ORGANISMS	GROWTH	
	IDIM AFIA	IDIM ETUK
BACTERIA		
Staphylococcus sp.	-	-
Escherichia sp.	+	++
*Enterroccus*sp.	-	-
Bacillus sp.	++	++
Pseudomonas sp.	+++	++
*Klebsiella*sp.	-	-
FUNGI		
Aspergilluscandidus	++	+
*Absidia*sp	++	++
*Chactomium*sp	-	-
Candida tropicalis	-	-
Candida pseudotropicalis	+++	+
Penicilliumfrequentans	+	-
Eurotiumherbariorum	+	++

KEY:

+	Low growth
++	Moderate growth
+++	Profuse growth

TABLE 4: Comparison of Physico-Chemical Analysis of Idim Afia and Idim Etuk (Nduetong-Oku Streams) with WHO Standard.

PARAMETERS	IDIM AFIA	IDIM ETUK	WHO STANDARD
Appearance	Slightly cloudy	Clear with suspended particles	-
Colour (Hazen unit)	60.00	50.00	15.00
Odour	Inoffensive	Inoffensive	Inoffensive
Temperature (0c)	24.95	24.85	27-28
Total Iron Fe3+ mg/L	0.12	0.04	0.30
Turbidity (NTU)	35.15	1.24	5.00
Electrical Conductivity (Us/cm)	15.65	19.20	1000mg/L
Sodium (Na+) mg/L	2.50	1.55	200.00
Potassium (mg/L)	0.45	0.20	150
Total Alkalanity (m/L)	2.5	1.00	100-200
Chloride cl (mg/L)	10.00	10.00	250
Salinity (Nacl) %	0.29	0.41	-
Total Hardness mg/L	14.5	5.5	500
Dissolved oxygen (mg/L)	0.11	0.11	1.00-5.00
Total Dissolved solid (mg/L)	8.10	0.95	1000
Free Carbondioxide C02 (mg/L)	1.02	1.00	-
Nitrate (No3) mg/L	0.10	0.12	10.00
Acidity mg/L	0.41	1.50	4.5-8.2
Nitrite (No2) mg/L	0.01	0.02	<1
Total suspended solid (mg/L)	0.03	0.02	-
Ph	6.85	6.50	6.50-8.50

Discussion

From the samples analyzed, the microbial loads were relatively high. This is probably because as the rain falls, soil microbiota and microorganisms adhering to vegetation, municipal sewage, garbages, domestic and industrial wastes are washed into these water bodies thereby polluting them (Ita *et al.*, 1996). Microbiologically, the presence of enteric organisms like *Escherichia* sp., *Klebsiella* sp., *Enterococcus* sp. and the Gram-positive *Staphylococcus* sp. in these streams is due to various human activities and dumping of human wastes into the streams by the people of Nduetong-Oku village. The *Bacillus* sp. and *Pseudomonas* sp. are present in this waters because of the nearby soil and moist organic matter which are in contact with the streams. This agrees with the report of Bedard *et al.* (2016). The Presence of Coliform bacilli, particularly, *Escherichia* sp., in the waters is an indication of recent feacal contamination. *Escherichia* sp. and *Enterococcus* sp. are of public health significance, having the ability to cause urinary tract infection, gastroenteritis, septicemia etc. (Abat *et al.*, 2016).

The results reveal that some of the isolates could tolerate 1% w/v crude oil, an indication that the organisms found in these streams may have at some point be exposed to hydrocarbon containing substances as waste discharged into the water.

A careful comparison of the results of the physic-chemical parameters as obtained with the WHO (World Health Organization) Standards reveal that the colour and turbidity did not meet the WHO Standards; however, pH, Dissolved solid, Dissolved oxygen, Nitrate, Sodium, Potassium were all within the acceptable WHO benchmarks for water bodies. The Trace elements considered in this research were all of nutritional importance to humans.

Conclusion

From the baseline studies conducted in Nduetong-Oku streams, it has been noted that the streams contain a number of coliform bacteria and thus do not conform to the WHO standard for potable water. The major pollution observed has been microbiological due to faecal contamination and disposal of waste.

Recommendation

1. There is need to for the government of this local environment to organize sanitation and also create awareness to members of the public as regards the dangers of indiscriminate dumping of domestic and industrial waste into water bodies.
2. The water from these streams should be properly treated before use at home or in the industrial organizations sourcing from these water bodies so as to guard against outbreak of water borne infections.

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