Microbiological Quality of Sediment and Water Samples from Selected Surface Waters in Anambra State, Nigeria

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Abstract: Water occupies about 70% of the earth's surface and is one of the most demanded of all urban and rural amenities, thus indispensable for human activities. Microbiological properties of water and sediment samples from four surface waters in Anambra state Nigeria were investigated for the evaluation of their pollution level. A total number of twenty (24) samples, with three (3) samples per sampling station were collected and examined in this study. Standard plate counts method was used to estimate the total aerobic, heterotrophic and coliform counts. The total aerobic heterotrophic bacterial counts obtained varied in each station from 3.6 x 10^4 cfu/ml – 2.23 x 10^5cfu/ml in the rainy season and from 3.2 x 10^4cfu/ml – 2.1 x 10^5cfu/ml in the dry season. The microorganisms found included *Escherichia coli, Micrococcus* sp., *Pediococcus* sp., *Citrobacter* sp., *Planococcus* sp., *Flavobacterium* sp., *Mucor* sp., *Allescheria* sp. *and Saccharomyces* sp. The average high iron content ranging from 6.4 – 52.55 mg/l was greater than the World Health Organization (0.3 mg/l) permissible range for drinking water. There was a negative correlation between the alkalinity value with total coliform of water, but positive correlation between the alkalinity values and the total coliform counts of the sediment and water. There was a significant difference at $P < 0.05$ between the mean seasonal alkalinity values of the water and sediments. The findings revealed that the selected surface waters are highly contaminated with pathogenic microorganisms with poor physicochemical characteristics and remains unsafe for human consumption.This necessitates the need for primary prevention measures to be identified.
Key word: Water, sediment, surface waters, analysis

INTRODUCTION

Surface water is a natural water source which collects from water running across the surface of the ground. As this water runs across the ground surface, it picks up microorganisms, organic matter and minerals. The type and number of microorganisms is a reflection of the condition of the water. Some of the microorganisms found in water may be regarded as indigenous, while others may be considered as contaminants (Okaka and Ene, 2005). Microorganisms are widely distributed in nature and are found in most natural waters. Their abundance and diversity may be used as a guide to the suitability of water for fish, animals, recreational and amenity purposes. Sediment is matter (Sand, dirt, gravel) that settles to the bottom of a water body after euthrophication. Euthrophication is any increase in the concentration of available nutrients: it may be man-made as in sewage discharge into stream or natural as with rain water washings (Chukwura, 2001). Generally, excessive amount of organic matter produced in eutrophic bays, high amounts of primary production results in appreciable amounts of organic matter input into the sediment where active involvement of biological, microbiological and chemical processes decompose the organic matter (Smetacek *et al*., 1991). Microbiologically mediated decomposition process play a vital role in the mineralization of organic matter and is expected in sediments receiving high load of organic matter because the oxygen transport within the sediment is limited. Hence, these sediments are always under reduced conditions because of stratification and high organic matter input (Blackburn, 1991).

Water pollution is one of the most important environmental problems faced by the third world countries (Barry, 2000). The use of bacteria as water quality indicators can be viewed in two ways: first the presence of such bacteria can be taken as an indication of faecal contamination of the water and thus as a signal to determine why such contamination is present, how serious it is, and what steps can be taken to eliminate it:

second, their presence can be taken as an indication of the potential danger of health risks that faecal contamination poses. The higher the level of indicator bacteria, the higher the level of faecal contamination and the greater the risk of contracting the disease (Villa,2000). Increasing industrialization and urbanization has led to a wide scale contamination of surface waters from industrial effluents, domestic sewage discharge and excessive usage of fertilizers and pesticides (Haruvy, 1997). Domestic wastes vary in composition and often contain millions of bacteria per milliliters. The continuous discharge of untreated sewage around homes into surface water source is a serious health hazard because the causative agents of bacillary dysentery, cholera, enteric fever and other diarrheal diseases are carried in sewages (Chukwura, 2001).

MATERIALS AND METHODS

Sample sources and collection: Eight samples on average (4 of water and 4 of sediment) were collected from the following locations; Obizi sediment, Obizi water, Nkisi sediment, Nkisi water, Ebenebe river sediment, Ebenebe river water, Agulu lake sediment and Agulu lake water. Each sample for physiochemical analysis was collected using a clean 2-litre plastic container with screw cap and at the point of collection, the container was rinsed with the sample.

Microbiological Analysis –

Isolation: Isolation was carried out by spread plate method according to the method of Ogbulie *et al*. (1998). Bacteria were isolated from sediment and water after serial dilution using nutrient agar plates. The plates were incubated at room temperature for 24 hours after which the colonies developed on the plates were sub-cultured on fresh nutrient agar. The purified isolates were maintained on nutrient agar slant for further tests. Moulds were isolated from the sediments and water after serial dilution using Sabouraud dextrose agar (SDA) plates. The plates were incubated at room temperature for 48 hours after which the developed colonies were sub cultured. Yeasts were isolated from the sediment and water using yeast extract agar (YEA). The plates were incubated at room temperature for 48 hours, developed colonies were sub-cultured on fresh yeast extract agar plates for purification and purified isolates maintained on slants.

Estimation of Escherichia coli counts using the membrane filteration technique:

Approximately 2 ml of membrane enriched Lauryl sulphate was added to the absorbent pad contained in a Petri dish. The Petri dish was covered until the sample was filtered through the membrane. The millipore filter was placed on a filtration unit and damped in position, and the samples (10 ml diluted with 90 ml of sterilized distilled water) were drawn through the millipore filter with the aid of vacuum pump. The filter disk handled with sterile forceps was placed on absorbent pad previously saturated with membrane enriched lauryl sulphate. Incubation was carried at 44^0C for 24-48 hours after which yellow colonies were counted (Chesbrough, 2006).

Identification of isolates: Identification of bacterial isolates was based on cultural, morphological and biochemical tests (Chesbrough, 2006). Moulds were identified based on colonial morphological features-lactophenol blue mount preparation and slide culture technique. Yeast isolates were identified based on colonial morphological features and sugar fermentation characteristics (Ogbulie *et al*., 1998).

RESULTS AND DISCUSSION

The result of the morphological and biochemical characterization of the bacterial isolates in rainy season were recorded as *Micrococcus* spp., *Escherichia coli*, *Pediococcus* spp., *Citrobacter* spp., *Planococcus* spp., and *Flavobacterium* spp. respectively (Table 1). *Escherichia coli* was found in all the sampling stations during this period. The dry season showed a decrease in the number of bacterial genera obtained and *E.coli* was found in most of the sampling stations during this period (Table 2). The

fungal genera isolated were *Mucor* spp. and *Allescheria* spp. (moulds) and *Saccharomyces* sp. (Yeast) (Table 3 and 4). The presence of enteric pathogens in water constitutes health hazards and the presence of *E. coli* in both seasons indicated that there was recent contamination of faecal origin as at the time of sampling. The results of the total aerobic heterotrophic, coliform and *E.coli* counts of the sediment and water (Tables 5 and 6) showed that total higher aerobic and coliform counts were recorded in sediment than in that of water for both seasons. This could be attributed to the fact that a high percentage of microorganisms are attached to solid particles which provide protection against environmental factors and these microrganisms could settle down towards the segments faster than the planktonic ones (Fang *et al*., 2018). Environmental conditions in sediment are quite different than in the water column due to reduced sunlight, lower temperature fluctuation, protection against predators, increased nutrient organic carbon availability and more colonizable surfaces enhancing microorganisms' persistence and survival (Fang *et al*., 2018).The higher total aerobic, heterotrophic and coliform counts recorded in the rainy season indicated that run- offs from land increased the bacterial load of the surface water sources during this period (Okpokwasili and Akujobi, 1996). Floods and run-offs represent greater pollution sources than that of humans since the frequencies of human trips to the streams and rivers for the purpose of fetching water for domestic purposes decrease in rainy season (Okpokwasili and Akujobi, 1996). Individuals without pipe-borne water in their homes resort to rain water harvesting from rooftops during the season. The fact that water sampled were obtained at the areas of the streams from which users fetched their water meant that these were the points where humans made direct contact with the water sources. This falls in line with the reports of Blum *et al.* (1987) and Okpokwasili and Akujobi (1996). The selected surface waters are highly contaminated with pathogenic microorganisms with poor physicochemical characteristics and remains unsafe for human consumption. This necessitates the need for primary prevention measures to be identified in order to reduce health risks associated with exposures to pathogenic microorganisms in surface waters and sediments.

Table 1: Morphological and biochemical characteristics of bacterial isolates (Rainy season)

Isolates	Morphological Characteristics	Gram stain	Catalase	Vogesproskauer	Methyl Red	Urease	Indole	Oxidase	Motility	Spore stain	Citrate	Glucose	Fructose	Manitol	Galactose	Identity
A	Smooth Sphercal Cells in pairs Clusters	+	+	+	+	+	+	+	+	-	+	**A**	**A**	**AG**	**AG**	***Micrococcus* spp.**
B	Moist small rods in Singles	-	+	-	+	+	+	-	-	-	+	**A**	**A**	**AG**	**AG**	***E. coli***
C	Small, Smooth Spherical cells in	+	-	-	+		+	+	-	-	+	**A**	**A**	-	-	***Pediococcus* spp.**
D	Smooth, Shiny Surface entire edge	-	+	-	+	+	+	-	+	+	+	**AG**	**AG**	**AG**	**AG**	***Citrobacter* spp.**
E	Smooth, Yellow, spherical Cells in pairs	+	+	-	-	+	-	-	+	-	+	**A**	**A**	-	**A**	***Planococcus* spp.**
F	Smooth, small Colonies, short Slender rods	-	+	-	-	-	+	-	-	-	+	**A**	-	**A**	**A**	***Flavobacterium* spp.**

Key: Source of Isolates, + Positive result A – 1b, 2a, 2b,3a,3b,4b, -Negative result B – 1a – 4b, A Acid Production C – 1b, 2b, 3b, 4b, G Gas production D – 1a, 2a, 2b, 3b, 4b, E – 1a, 1b, 2b, 3b, 4b, F – 1b, 2b, 3b, 4b

Table 2: Morphological and biochemical characteristics of bacterial isolates (Dry season)

Isolates	Morphological Characteristics	Gram stain	Catalase	Vogesproskauer	Methyl Red	Urease	Indole	Oxidase	Motility	Spore stain	Citrate	Glucose	Fructose	Mantol	Galactose	Identity
G	**Smooth, Moist Shiny surface Entire edge, small Short rods in Single pairs**	-	+	-	+	+	+	-	+	-	-	**A**	**AG**	**AG**	**AG**	***E. coli***
H	**Translusce nt Smooth Entire, small Colonies, short Slender rods**	-	+	-	-	-	+	+	-	-	+	**A**	**A**	**A**	**A**	***Flavobacteri um* sp.**
I	**Yellow, Smooth Spherical cells in Singles and pairs**	+	+	-	-	+	-	-	+	-	+	**A**	-	**A**	-	***Planococcus* sp.**
J	**Small, Smooth Cells in pairs of Cocci**	+	-	-	+	-	+	-	-	-	+	**A**	-	-	-	***Pediococcus* sp.**

Key: Source of Isolates, + Positive result G – 1a, 1b, 2b,,3b, 4b, -Negative result H – 1a, 1b, 2a, 2b, 3b,4b, A Acid Production I – 3b, 4b, G Gas production J – 1a, 2a, 2b,3a, 3b, 4b.

Table 3: Morphological and sugar fermentation characteristics of yeast isolates (Rainy and Dry season)

Isolates	Colour	Elevation	Shape	Glucose	Fructose	Maltose	Mannitol	Lactose	Sucrose	Galactose	Identity
X	Milkish	Raised	Ovoid	A	A	A	A	A	A	A	*Saccharomyces* sp.
Y	Milkish	Raised	Ovoid	AG	AG	-	A	A	A	AG	*Saccharomyces* sp.

Key: Source of Isolates, -Negative result X – 1b, 2b, 4b, A Acid Production Y – 2b, 3b, G Gas production

Table 4: Mould isolates (Rainy and Dry season)

Isolates	Macroscopic Characteristics	Microscopic Characteristics	Probable Identity
1	Rapid growth spreads and Fills the plate, whitish gray And fluffy mycelium	Spores are borne within their fruiting bodies enclosed in sac-like structure sporangia	*Mucor* sp.
2	Soft, mouse-grey, flur like Colony	coenocytic hypha, spores borne singly on branched conidiophores with each conidiophores ending in a single conidium	*Allescheria* sp.

Key to source of Isolates: Isolate 1- Both seasons, Isolate 2- Majorly in rainy season except one sample site

Table 5: Total aerobic heterotrophic coliform and *E. coli* counts of the sediment and water (Rainy season)

Sampling Stations	Total aerobic bacterial Count ($\times 10^3$ cfu/ml)	Total coliform count ($\times 10^3$ cfu/ml)	*E. coli* count (cfu/100ml)
1a	72	5	27
1b	36	2	20
2a	85	25	33
2b	54	6	15
3a	223	31	21
3b	105	17	10
4a	133	45	24
4b	69	19	13

Table 6: Total aerobic heterotrophic coliform and *E. coli* counts of the sediment and water (Dry season)

Sampling Stations	Total aerobic bacterial Count (× 10^3 cfu/ml)	Total coliform count (× 10^3 cfu/ml)	*E. coli* count (cfu/100ml)
1a	46	10	15
1b	32	4	8
2a	57	15	-
2b	31	10	6
3a	174	20	-
3b	55	12	4
4a	210	32	-
4b	62	14	10

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