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Effect of Indomie industrial effleunt discharge on microbial properties of new Calabar River

Akaninwor, J. O.*, Anosike, E. O. and Egwim, O.

Biochemistry Department, University of Port-Harcourt, Port-Harcourt, Nigeria.

Water quality assessment of certain microbial pollution indicators arising from the industrial effluent of Indo-Food Company (Indomie) on water samples collected at five different sampling points of new Calabar Rivers was carried out. The sampling points were upstream, effluent fall out points, 200, 400 and 600 m distances from the fallout point. Using the control (upstream) as an index of comparism to determine extent of pollution caused by the industrial effluent discharge, the results of the analysis indicated a significant difference in the microbial parameters assessed between the control and fallout points, indicating that this sampling point was polluted as a result of the direct effluent discharge at the sampling at this sampling point. The microbial analysis indicated that the viable bacterial counts of the samples ranged from 5.4 x10^3 to 26 x 10^3 cfu(Table 1) suggesting that the water samples contain heavy microbial load. The biochemical identification of the isolate in the water samples showed that *Staphylococcus aureus, Shigella* sp*., Proteus vulgaris, Escherichia coli* and *Citrobacter* sp. pre dominated the water samples while the fungal microscopy identified *Candida* species, *Fusarium, Circinotrichum* and *Cephaliophora* in the water samples (Table 3). The elevated levels of microbial pollution indicators would invariably affect the taste, small, appearance and aesthetic properties of the river water and thus pose a potential health hazard of varying degrees to various life forms that depend on the water for survival and recreational purposes. A routine treatment of the effluent before discharge is therefore highly recommended to maintain safe levels of the microbial pollutants in the immediate and extended environment.

Key words: Microbial parameters, effluent, pollution and environment.

INTRODUCTION

Water has acquired added importance on the last few decades due to increased cost and demand, coupled with increased pollution. Natural waters are relatively dilute aqueous solutions (Obire et al., 2003). Pollution of the aquatic environment has been defined by UNESCO / WHO/UNEP as the introduction by man directly or indirectly of substances or energy into the marine environment which results in such deleterious effects as harm to the living resources, hazards to human health, hindrance of marine activities including fishing and impairment of quality for use of sea water. In other words, water is polluted when its acceptable quality has been altered by man's activities through anthropogenic imputes such that its intended usage for commercial or domestic purpose is hampered.

Water quality can be assessed using a number of lines of investigation, namely chemical, biological and bacteriological (Hodgkiss, 1998). Each line has its own uses and yields information not otherwise obtainable. Chemical investigation of the water quality of some Nigerian rivers (Ajayi and Osibanjo, 1981; Adeniji and Mbagu, 1983; Imevbore, 1970; Asuquo, 1989) reveals that water that was once an abundant natural resource is rapidly becoming scarce in quantity (high demand) and the quality is deteriorating in many places, owing to population increase, rapid industrialization and rural/urban migration. Almost all water used any man is returned as wastewater and requires proper disposal to prevent it from reaching and contaminating water resources. In most cases, this is not in practice due to lack of technical know how and treatment plant (Asuquo, 1989).

Pollution of freshwater bodies (rivers, lakes, pond and streams) by nutrients is mostly experienced as a result of

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*Corresponding author E-mail: akaninworj@yahoo.com.

industrial discharge, municipal domestic sewage disposals, surface runoff from agricultural kinds, underground water and salt-water intrusion and inundation (Asuquo, 1989).

Dissolved nutrient in natural waters are usually low (Asuquo and Okorie, 1987) and composition of surface waters is derived from the atmosphere, soil and rock sources. The relative contribution to surface waters from each of those sources is a function of the climate being modified increasingly by human activates (Zheng et al., 1995).

Industrial activities and urbanization in developing countries including Nigeria has gradually led to the deterioration in recent years. This situation has invariably increased the problem of waste disposal. Untreated wastes from processing factories located in cities are discharged into inland water bodies resulting to stench, discoloration and a greasy oily nature of such water bodies (Mombeshora, 1981). Increase in crude oil exploration, refining and activities of other industrial establishments in the Niger Delta has resulted in the wide-scale contamination of most of its creeks, swamps and rivers with hydrocarbon and dispersant products. The contamination of creeks, swamps and rivers has been shown to constitute public health and socioeconomic hazards (Kobayashi and Rittman, 1982). Industrial effluents contain toxic and hazardous materials from the wastes that settle in river water as bottom sediments and constitute health hazards to the urban population that depend on the water as source of supply for domestic uses. The levels of chemicals including those of heavy metals are concentrated in the organic matter of sediments which influence the adsorption of metallic elements (Tada and Suzuki, 1982).

Moore and Sutherland (1981) reported a significant correlation between the levels of lead, manganese, mercury, nikel and the organic matter content of bottom sediments (Akaninwor and Egwin, 2006).

A large proportion of the rural population in the developing world takes water from natural sources directly for drinking (WHO, 1973; Faehem Mc Garry and Mara, 1977). These sources are exposed to contamination with faecally derived organisms. The water is usually not treated at all or treated insufficiently to ensure acceptability according to international guidelines (WHO, 1983). Natural waters are therefore never pure, and water being a universal solvent dissolves many chemical substances and also carries in suspension many impurities (WHO, 1998). It contains small plants and animals, many of which are not visible to the human eye. Water quality is adversely affected when it is polluted by various pollutants and if these pollutants exceed certain limits, the water will be harmful to human health (WHO, 1990).

Portable water for domestic use should be free from pathogenic microorganisms and toxic substances such as heavy metals and hydrocarbons. Drinking water should be odorless, tasteless, colorless and devoid of particulate matter (Emile et al., 1999). The protection of public health requires that people be supplied with water of adequate quality which satisfies the minimum quality standard. An effective programme to control drinking water quality requires that adequate supportive legislation is available, quality standards have been evolved for the country and that surveillance is carried out regularly. An environmental demand from human activities due to urban industrialization and development increasingly affect the water quality of surface waters; there is an acute societal and global need to monitor water quality characteristics of some rivers.

The aim of this study is to carry out water quality assessment of the New Calabar River where the Industrial Effluent of the Indo-Food (Indomie) industry is discharged to ascertain the safety levels of the microbial load.

MATERIALS AND METHOD

Collection of samples

The study area is New Calabar Rivers in Choba, Rivers State. The Industrial Effluent is from Indo-Food Company, Nigeria Limited (Indomie). Indo Food Company is a food manufacturing company situated in Choba. The inhabitants, if Choba make nice of New Calabar for various domestic needs, such as bathing, cooking, swimming and even fishing. Also, the river hosts the affluent waste water discharged from Indo- Food Nigeria Limited. It is therefore pertinent to ascertain the quality of this source of water, which serves several purposed to rural drillers.

The sampling material used was sterile screw capped bottles and the water samples were carefully collected from about 4 - 6 cm below the water surface using sterile disposable hand gloves. The process of opening of the bottle caps was quickly done to avoid contamination. The samples were taken to the laboratory for analyses in coolers containing ice packs. The water samples were taken from five different sampling points; upstream, fallout points, 200, 400 and 600 m down stream.

Media preparation

The various culture media used were mineral salt agar (MSA), namely nutrient agar (NA), Sabouraud Dextrose Agar, Peptone iron agar, etc. These were obtained in the commercially prepared powered (hydrated) forms and prepared according to the manufacturer's specification. The specified quantities were dissolved in specified volume of distilled water and sterilized by autoclaving at $121^{\circ}C$ and 15 psi for 15 min in well stoppered conical flask. Each flask and its contents was allowed to cool at about 45 - $50^{\circ}C$ before dispensing into pre-sterilized Petri-dishes and allowed to gel on flat surfaces.

Microbiological analyses

Bacterial count was determined using the spread plate method as described by Cruick Shank et al. (1982). The Cruicks Shank et al. (1982) method was also used for sub-culturing of the isolates. Gram staining technique used was that described by Kirl et al. (1975). The method as described by Cruick Shank et al. (1982) was used for morphological identification of fungi.

Table 1. Total heterotrophic plate count of the primary culture of new Calabar River at Indomie industrial effleunt discharge.

Source	Bacterial count (cfu/100 ml)	Fungal count (cfu/100 ml)
Upstream (control)	$3.1 \times 10^3 \pm 50$	6.00 ± 0.20
Fallout point	$26.2 \times 10^3 \pm 200$	113.00 ± 14.70
200 m downstream	$28.0 \times 10^2 \pm 50$	13.00 ± 2.00
400 m downstream	$5.4 \times 10^0 \pm 0.1$	15.00 ± 2.60
600 m downstream	$3.2 \times 10^2 \pm 52$	12.00 ± 1.70

Table 3. Morphological and microscopic identification of fungi.

Samples	Colonial morphology	Colony counting	Possible fungus
Upstream (control)	Various colounies with whitish, brownish, greenish colours. Some colonies have two layers, colonies about 4cm in diameter.	6.00 ± 0.20	Candida, fusarium circinotrichum, cephaliophora
Fallout point	Different colonies with whitish brownish and greenish colours. Colonies are fluffy, 6cm in diameter	113.00 ± 14.70	Candida, fusaarium, circinotrichum cephaliophora
200 m from fallout	Colonies are whitish, brownish and greenish in colour with dark centers, about 4cm in diameter.	13.00 ± 2.00	Candida, fusarium, circinotrichum, cephaliophora
400 m from fallout	Whitish, brownish, greenish and fluffy colonies about 3cm in diameter,	15.00 ± 2.60	Candida, fusarium circinotrichum, cephaliophora
600 m from fallout	Whitish, brownish and greenish colonies, about 6cm in diameter.	12.00 ± 1.70	Candida, fusarium circinotrichum, cephaliophora

Biochemical tests

The bacterial isolates were tested for their ability to ferment certain sugars such as glucose and lactose and equally tested for the presence of enzymes such as catalase, oxidize etc. Catalase, methyl and motility tests were as described by Kirl et al. (1975). The procedure described by Collins and Lyne (1976) was used for indole production test, while the methods as described by Cruick Shank et al. (1982) were used for oxidation-fermentation, Voges-proskauer, citrate utilization, sugar fermentation, oxidase and hydrogen sulphide production tests.

Statistical analysis

Data were analyzed using the computational method of analysis (Student T-test). Significance was accepted at 5% level or 95% confidence limit (P = 0.05).

RESULTS AND DISCUSSION

Characterization of bacteria and fungal isolates

The results of heterotrophic plate count of primary culture are depicted in Table I. The fallout point registered the highest number of bacteria with the value of $26.2 \times 10^3 \pm 200$ cfu, while the 400 m sampling point had the lowest mean value of bacteria with the value 5.4 ± 0.1 cfu. The fallout point also recorded the highest fungi count of 113.00 ± 14.70 cfu (Table 1).

The various sampling points showed different morphological characteristics when cultured on nutrient agar plates and reacted differently to the diagnostic biochemical tests. However, all the isolates from the different sampling points were gram negative, catalase positive, and oxidation fermentation positive, oxidase negative and have the ability to ferment sugar (Table 2). The isolates showed different behavioral patterns to indole test, motility test, Voges-proskauer test, hydrogen sulphide production test and ability to utilize citrate. The biochemical activities of the isolates showed that they are enterobacteriaceae.

Bacteriological analysis

The results of the numerical estimates of bacteria from the primary culture revealed that the water samples contain heavy microbial load except 400 m sampling point. The fallout point registered the highest microbial load and this could be attributed to rapid proliferation of microorganisms at this samples point which aid in the degradation of organic matter present in the industrial effluent. The levels of total coliform bacteria as high as 26.2×10^3 cfu/100 ml was obtained in the fallout point. The results of the biochemical characteristics of the isolates suggest contamination from diverse sources. These sources of contamination include Indo Food Industrial effluent discharge.

Table 2. Morphological and Diagnostic Biochemical Characteristics of the Isolates.

Sampling points	Cultural characteristics	Gram RX	Catalase	Indole test	Oxidation fermentation	Methyl test	Motility test	Citrate utilization	Fermentation of sugar	Oxidase test	VP test	H$_2$S production	Possible organism
Upstream (control)	Colonies are small, about 0.5cm in diameter, spherical in shape	-ve cocci in clueters	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	*Staphylococcus aureus*
Fallout point	Colonies have a fishy odour, milky colonies on nutrient agar- plates, slimmy colonies are about 1mm in diameter,	-ve rods	+ve	-ve	+ve	+ve	-ve	-ve		+ve	+ve	+ve	*Shigelia specie*
200m downstream	Colonies have cremated edges, roundish, about 6cm in diameter.	-ve short rods	+ve	+ve	+ve	+ve	+ve	-ve		+ve	+ve	+ve	*Proteus vulgaris*
400m downstream	Smooth, glossy Translucent colonies, irregular shaped with thicker edges,	-ve rods	+ve	+ve	+ve	+ve	+ve	-ve	+Ve	+ve	+ve	+ve	*Escherichia coli*
600m downstream	Colonies are roundish, thicker in centers, 3mm in diameter,	-ve rods in singles	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	*Citrobacter specie*

+ = Positive, - = Negative, VP = Voges Proskauer and H$_2$S = Hydrogen Sulphide.

Morphological and biochemical characterization of the isolates identified the following organisms; *Staphylococcus aureus, Shigella* sp., *Protues vulgaris, Escherichia coli* and *Citrobacter* sp. The presence of these organisms in water can change the quality of water. Their presence could be attributed to the ubiquitous nature of microorganisms and the contaminated state of the river by industrial effluent which increases the organic content of the river thereby providing excellent nutritional source for the propagation of microorganism. The presence of fecal coliform bacterial is an indicator that a potential health hazard exists for individuals exposed to this source of water.

Fungal analysis

The study revealed that all the samples analyzed contained fungi in relative proportions. Heavy fungal load was obtained in the fallout point. The fungi isolates include *Candida* sp. and *Circinotrichum cephaliophora* (Table 3). Health problems associated with these organisms can cause test and odor problems thereby affecting the aesthetic properties of water (WHO, 1984).

Conclusion

The results of the microbiological analyses of New Calabar River in Choba investigated have shown that Indo Food Industrial Effluent is a major source of environmental pollution through the discharge of the effluent into this water body. Elevated levels of these pollution indicators when compared to the control would invariably affect the taste, smell, appearance and aesthetic properties of the water or could pose a potential health hazard of varying degrees to various life forms, which depend on the water for domestic and recreational purposes. Constant treatment of the effluent is recommended.

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