

Research Article

Microbiological Examination of Water and Sediment Samples Collected from the Imo River at the Onuimo Market Section in Obowo, Imo State, Nigeria

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Abstract: A study to assess the impact of wastes from Onuimo (Imo Rescue Mission) market on the microbiology of Imo River was carried out. Sediment and water samples were collected monthly and analysed quantitatively for faecal bacteria indicators of pollution and qualitatively for specific pathogens. The study reveals that the sites were heavily polluted with faecal bacteria (90 – 205 cfu/100 ml TVC and 17 – 64 cfu/100 ml TFC in water sample, and 51 – 216 cfu/100 ml TVC and 6 – 140 cfu/100 ml TFC in sediments) that consistently exceeded the World Health Organisation (WHO) recommended range for potability. These organisms often comprised the pathogens; *Shigella* spp., *Salmonella* spp., *Escherichia coli*, *Candida* spp., *Enterobacter aerogenes*, and *Staphylococcus* spp., which seemed to be pervasive. They all tended to be highest in water samples during rainy season and in sediment samples during dry season. There is spatial variation ($P>0.05$) with point B as the most impaired segment. The findings show that the river is under heavy pollution due to wastes from the market and households around the river; and thus does not meet the World Health Organisation standards for domestic, irrigation and aquaculture purposes. The water should therefore be treated properly before use.

Keywords: Imo River, market wastes, microbial examination, bacteria indicators, water, pollution.

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INTRODUCTION

Microbiological examination of river water is obligatory for use-related purposes such as drinking water production, irrigation and recreation (Csanyi, 2002). The common practise of unregulated waste disposal into water courses affects their normal use by municipalities and aquatic environments near cities are usually prone to over loading with a variety of pollutants, either through direct or indirect discharges (Olayemi, 1994). This situation may be worsened by the indiscriminate disposal of untreated wastes, often heavily laden with sewage into actively used streams. In addition to their characteristic micro flora, sewage-polluted water carries numerous sewage microflora, some of which pose public health risk. Several other researchers have documented the public health problems arising from faecal pollution of natural waters (Ukagwu *et al.*, 2014; Tita *et al.*, 2013; Olayemi, 1994; Olayemi, *et al.*, 1990; Anderson, 1968; Ogedengbe and Adeniye, 1978; Ekundayo, 1979).

Water pollution (Hogan, 2014) is the contamination of natural water bodies by chemicals, physical, radioactive or pathogenic microbial substances. While United States Environmental Protection Agency (US EPA, 2015) opined that water pollution is what happens when factories, wastewater treatment plants, construction sites, and people put things in the water that make it dirty.

The Municipal solid wastes (MSW) that can pollute a water body include those refuse from households, non-hazardous solid waste from industrial, commercial and institutional establishments (including hospitals), market waste, yard waste, and street sweepings as well as those of commercial institutions such as paper, vegetable matter, plastics, metals, textile, rubber and glass (Ogwueleka, 2009; Ayuba, *et al.*, 2013). Briggs (2003) confirmed that the problems are undoubtedly greatest in the developing world, where traditional sources of pollution such as industrial emissions, poor sanitation, inadequate waste

management, contaminated water supplies and exposures to indoor air pollution from biomass fuels affect large numbers of people. The widespread consequences of water pollution upon ecosystems as recorded (Hogan (2014) include species mortality, biodiversity reduction and loss of ecosystem services. And (Wagner *et al.*, 2002) warned that global fresh water resources are being increasingly polluted and depleted, thereby threatening sustainable development and human ecosystem. Pollutants include not only chemicals, but also living and viable organisms like bacteria and biological materials, as well as energy in its various forms (like noise, radiation, heat), wastewater from central sewers and vast array of endotoxins that can be released from the protoplasm of organisms after death (Briggs, 2003 and Royal Commission on Environmental Pollution, 2003).

These microscopic single cell or a multi-cellular living organisms are found everywhere in the biosphere (Mack, 2014; Fox, 2014; Glud, *et al.* 2013 and Choi, 2013). They reside in the sediment and other substrates, and in the water of aquaculture facilities, as well as in and on the cultured species (MOAWTS, 2002); and are used as indicators of faecal contamination of aquatic environment (Hach, 2000; Tortorello, 2003 and Staradumskyte & Paulauskas, 2012). These indicator microorganisms include the coliform bacteria, faecal streptococci (enterococci) and the sulphite-reducing clostridia (i.e., *Clostridium perfringens*). Most of the bacteria families such as the Enterobacteriaceae, like *Salmonella*, *Shigella*, *Yersinia enterocolitica*, *Klebsiella pneumoniae*, *Enterobacter* and the enterotoxigenic *Escherichia coli* (*E. coli*), *Vibrio cholerae* and *Campylobacter jejuni* found in contaminated water bodies (Hach, 2000) are also known to cause outbreak of enteric diseases (Tortorello 2003; Ukagwu *et al.* 2014 and Saxena *et al.*, 2015). Their presence also represents the faecal contamination of a water body with pathogens and quality deterioration (Saxena *et al.* 2015) and over the years due to their ubiquitous presence used to determine water quality (Tortorello 2003 and Marcheggiani and Mancini 2011). They are also the main source of fertility and of degradation of organic matter and pollutants in sediments (Marcheggiani and Mancini 2011).

The organisms are spread by water contaminated with faecal material from humans and

other warm blooded animals (NRC 2004; Toranzos *et al.* 2007 and WHO 2009.) and the wastewater discharged in fresh water and costal seawaters is attributed as a major source of pathogenic microbes (Fenwick 2006; WHO 2009).

The direct disposal of wastes from the market and surrounding communities into the river body makes the water body to become less available for recreation, agriculture, domestic and irrigation as well as aquaculture purposes because of the eminent contamination. Obviously, there is a dearth of scientific information on microbiology of the river at the locality for appropriate intervention.

The main objective of this study is to produce a baseline research report on the river at the early existence of the market. Specifically, the study is aimed to investigate the diversity of microorganisms of the river and to determine the effects of the wastes from the market on the microorganisms and physiochemical quality of the river. The result of the study will be helpful to relevant government and regulatory agencies to form a framework for practical measures to guide waste disposal into such aquatic environment thus, mitigate and control the impact of the pollution on the adjacent population.

MATERIALS AND METHODS

Area of Study

The Imo River is located in Imo State bounding Imo and Abia State in south eastern Nigeria and flows 241 km into the Atlantic Ocean. Its estuary is around 40km wide (FAO, 1997) and the river has annual discharge of 4km³ (Russell, 1993) with about 26,000 hectares of wetland. The Imo's tributary rivers are the Otamiri and Oramirukwa (Chukwu, 2005). The river has its source (head) at a spring in Isuochi (Isuikwuato Local Government Area, Abia State) runs through Aku (Okigwe Local Government Area, Imo State) where it is known as Ibii to Ihitte Ubom and Obowo (all in Imo state), down to Ukwa (Abia State) where it is mixed with the Aba River before emptying into the Atlantic Ocean. It transverses through the Onuimo industrial and general goods market (which came into full operation in July, 2013) with the sampling stations located at the coordinates: 5^o10'N – 5^o5'N and long 6^o35' E – 7^o28' E.

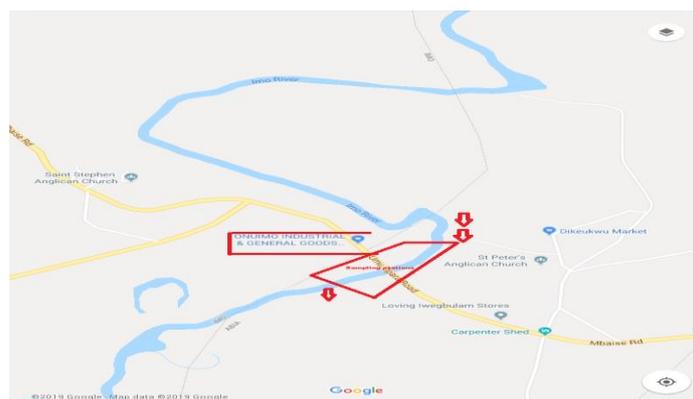


Figure 1. Diagram showing sampling stations

Sampling Methods and Station

There were three sampling points along the sampling station labelled A, B and C and were at least 500 m apart from each other. Point A served as control and located about 500m off the location of the market devoid of the market wastes while point B was located within the market area with adequate waste disposal and point C located also about 500 m from point B.

The sampling was carried out monthly from June to January for microbiological analysis. The water samples were collected from each sampling point during morning hours of the day (7 am – 9 am), under controlled temperature conditions using 1 litre properly labelled screw-capped sterile plastic containers. Sediment from each of the points was collected with the aid of hand excavator and saved in plastic plates with cover. Immediately, after taking a sample, it was put in a portable cooler box containing ice boxes so as to maintain the 4° C temperature before reaching the laboratory. They were transported to the laboratory in the ice box and analyzed within 5 hours of collection.

Physiochemical and Biological Parameters Determination

All the media used were autoclaved using wet steam technique at 121° C, 15 pounds pressure for 15 minutes and the glass wares were autoclaved using hot air technique in an oven at 160°C for 1 hour. The water sampling containers were sterilized with ethanol for 30 minutes and the sediment samplers were autoclaved using hot air technique in an oven at 160° C for 1 hour before taking to the site (Suzan *et al.*, 2019).

Most probable number technique was employed in the microbial examination with different agar to identify each target organism using pour plate method (Saxena *et al.*, 2015).

Pour plate method was used in performing the plate count. The laboratory procedure involved making serial dilutions of the sample (1:10, 1:100, 1:1000 etc.) in sterile water and cultivated on nutrient agar in sterilized petri dishes that was labelled with sample number, sealed and incubated. Typical media used include Plate count nutrient agar for a general count and MacConkey agar to count Gram negative bacteria such

as *E. coli*. Typically one set of plates was incubated at 22° C and for 24 hours and a second set at 37° C for 24 hours and the Eosin Methylene Blue (EMB) agar plates were incubate under room temperature for 24 hours (Society of American Bacteriologist, 1957 and Dagny *et al.*, 2001).

For characterization, Smears were made on slide from the plates containing EMB and (Urea agar base) UAB media, and observed for morphological features and appearance under electron microscope. pH, temperature, total suspended solids and dissolved oxygen were also determined using standard methods. Nutrient agar (Microgen DM 1001 – 500 g) was used for the cultivation of less fastidious microorganisms and was enriched with blood and other biological fluids (Sotir, 2009). Mannitol salt agar base (TM media M2A6BO01) was employed for the selective isolation of pathogenic staphylococci. UAB (TM media M3J4GO01) was used for the detection of urease producing bacteria (Ibe and Okpenye, 2005), Potato dextrose agar (Himedia M096 – 500 g) for the isolation and enumeration of yeasts and moulds (Tita *et al.*, 2013). MacConkey agar (Microgen DM 1008 g) was used for the selective isolation and differentiation of lactose fermenting and lactose non – fermenting enteric bacteria (Anderson, 2013 and Tita *et al.*, 2013). Eosin methylene blue agar, EMB (Microgen DM 1317) was used for the isolation and differentiation of Gram negative enteric.

RESULTS AND DISCUSSION

The result of the microbiological features of the analyzed water samples are presented in figures 2 and 3, which shows that the highest viable count of 205 was recorded in sample C in August and the least count of 90 was recorded in sample B January, it revealed that the highest total faecal count of 64 was recorded in sample B in August and the least count of 17 recorded in sample C in January.

The result shows that there are high counts in the months of July and August particularly in point B and point A. The average counts of all the months indicates that point B has the highest average viable

counts (147) followed by point A (127) counts and point B (118) count in descending order.

The faecal count in point B shows gradual increase from June through August and began to fall following the pattern of rain fall which has great

influence in the influx of faecal materials from the river banks. The result shows that microbes of faecal origin are more prevalent in point B with total counts of 387 followed by point A (263) and point C the least with total faecal counts of 243 during the survey period.

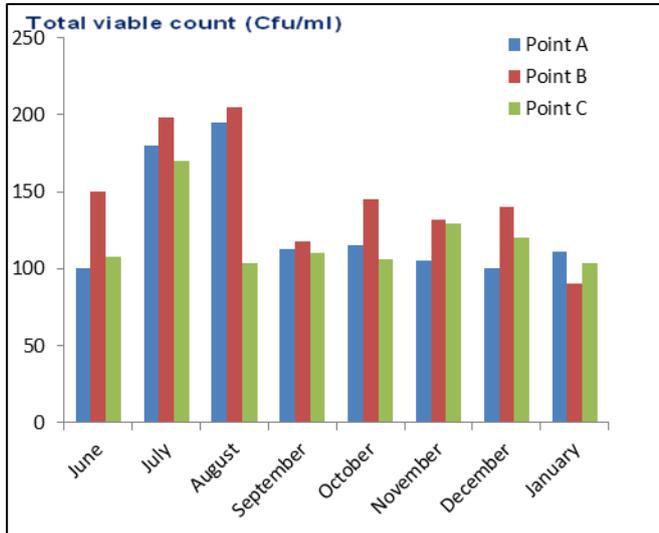


Fig 2: Total viable counts of water samples

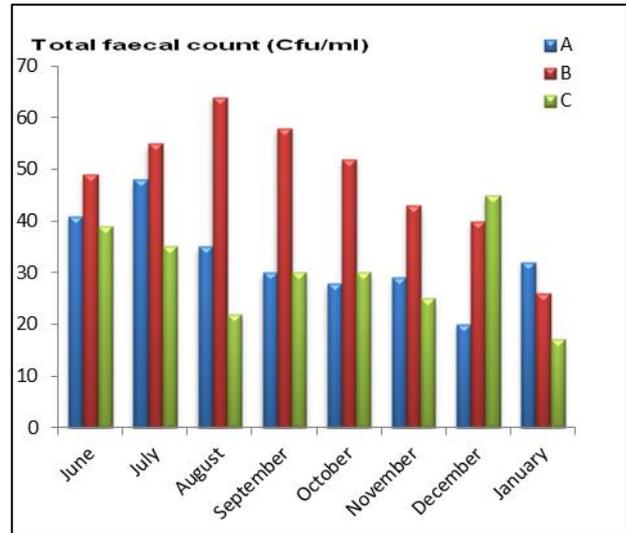


Fig 3: Total faecal count of water samples

The results of the microbiological features of the sediments are shown in figures 4 and 5. The result indicates that there was highest viable count of 216 at point C in the month of December and the least 52 recorded at point B in January. It also shows that the faecal count of 140 was recorded at point C in December and the least 6 recorded at point B in January.

sample A the least in these months. There was less growth recorded in January in all the samples examined; but in all, the average growth counts show that point C has the highest average (173) counts followed by point B (169) and point A the least. The microbial count of faecal origin in point C shows gradual increase from June through December followed by point B, but point A in contrast shows downward fall from June through December. In general, there is minimal growth in the month of January in all the sediment samples examined.

The result reveals that there are high viable counts observed in sediment samples of point B and C in June, July, August, September and December with

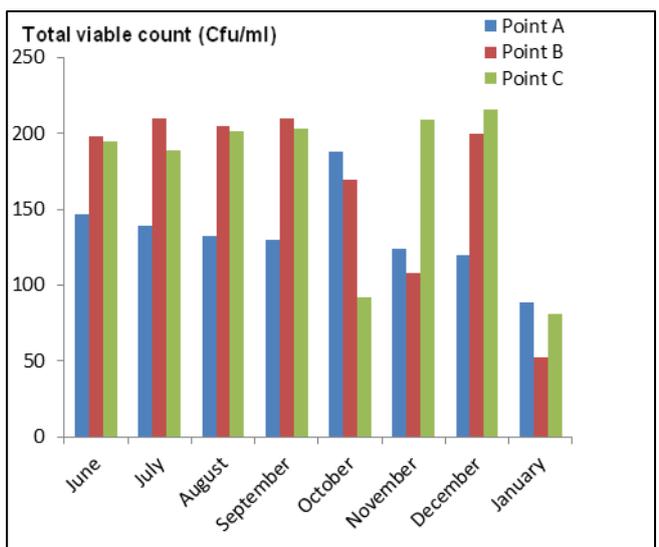


Fig. 4: Total viable count of sediment samples

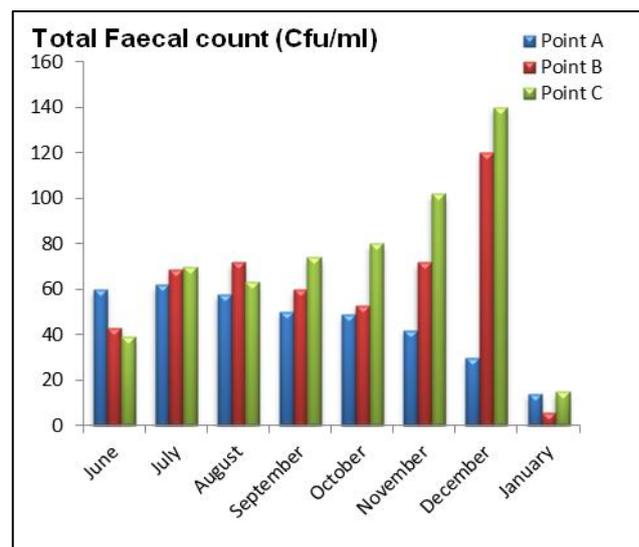


Fig. 5: Total faecal coliform count of sediment samples

The isolation and differentiation of specific microorganisms in both the water and sediment samples are presented in figures 6 and 7. The isolation in water

samples reveals that there were more lactose fermenting and lactose non – fermenting enteric bacteria in sample B, followed by Gram negative bacteria, urease

producing bacteria, yeast and staphylococci in descending order of abundance. Hence, revealing that there were more enteric bacteria like *E. coli*, *Salmonella*, *Shigella*, *Enterobacteriaceae*, *Pseudomonas*, *Moraxella*, *Helicobacter pylori* and *Legionella* in the sample.

Similarly, the isolation in sediment samples shows that there was dominance of enteric bacteria as in

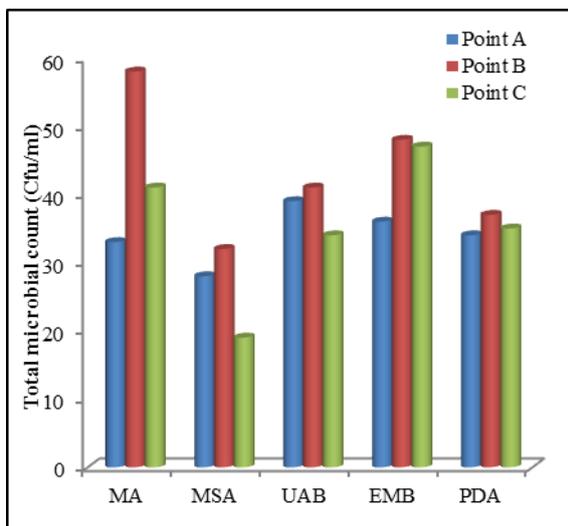


Fig.6: The means of microbial isolation of water samples with specific media.

The means of pH, total suspended solids (TSS) and temperature of the water samples (table 1) shows that the temperature was at the range of 23⁰ – 28⁰ C. The highest recorded in point A in January. The result further indicates that the TSS of the water sample is within the range of 0.6 and 15.6 as recorded in the month of January in point C and in June/July in the sample A respectively, while the pH ranges within 5.0 and 7.5 as recorded in point A in July and October respectively.

The result reveals that the river is slightly acidic fluctuating regularly towards being neutral and acidic irrespective of the season and time, indicating presence of acid forming compounds. The temperature of the river body still fall within the normal temperature range of water bodies in the tropics, the result shows that the temperature changes follow the trend of prevailing seasons – rising slightly during hot temperatures. It is also obvious from the result that the water samples contain less suspended solids during the dry season compared to the increased suspended solids during the rainy season due to influx of materials. That shows that the water is more transparent, that is, less turbid during the dry season than during the rainy season.

The values of the total suspended solids and the pH of the sediment samples (Table 2), reveals that the TSS of the sediment samples was at the range of 15.2 – 33.9 as obtained in point A during November

the water samples. Though there is considerable presence of yeasts in the sediments examined, with sample B accounting for the highest number of such microbes. The result also reveals the considerable growth of Gram positive bacteria such as *Staphylococcus aureus*, *S. epidermidis* and *S. saprophyticus* in the sample B.

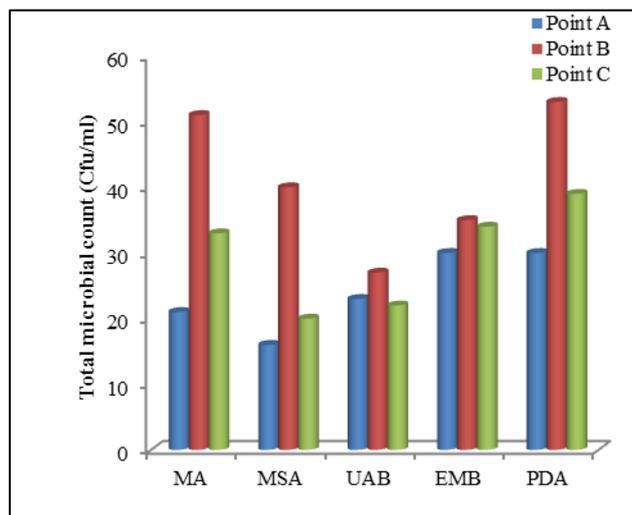


Fig. 7: The means of microbial isolation of sediment samples with specific media.

and in point B in the month of October respectively, while the pH was within the range of 5.0 and 7.5 as recorded in sample A in July and October respectively.

The pH mean values of the sediment samples show that the river sediments are more acidic than the water samples revealing that there was sedimentation and accumulation of more acid forming compounds in the sediments than in the water samples. And it also reflected in the result of the TSS showing that there was accumulation of materials that reduced the transparency of the samples, that is, increased turbidity level which is higher in sample C during the dry season.

Tables 3 and 4 show the results of the possible isolates of the samples analysed, they also show the features and characteristics of the organisms based on the different biochemical tests carried out. Based on the characteristics, morphology and the results of the biochemical tests conducted, six possible isolates were identified from both the water and sediment samples as *Escherichia coli*, *Candida Spp.*, *Enterobacter aerogenes*, *Salmonella Spp.*, *Shigella Spp.*, and *Staphylococcus aureus*. Four of the organisms are rod shaped bacteria (*Enterobacter aerogenes*, *Salmonella Spp* and *Shigella Spp.*) and one of the organisms is a spherical (cocci) shaped bacterial (*Staphylococcus aureus*). The *Candida Spp.* was the only yeast (of the fungi group) identified.

Table 3: Result of biochemical characteristics of bacterial isolates

Test/Probable Isolates					
	<i>Enterobacter aerogenes</i>	<i>Escherichia coli</i>	<i>Salmonella Spp</i>	<i>Shigella Spp</i>	<i>Staphylococcus aureus</i>
Catalase test	+	+	+	+	+
Fermentation of Lactose	-	+	+	+	-
Gram's test	-	-	-	-	+
Growth in Hugh and modified Liefson's medium	-	-	-	-	+
Growth in Indole – E. C. Broth	-	+	-	-	-
Growth in Indole methyl medium	+	-	-	-	-
Growth in Kohn's Two – tube medium	-	-	+	+	-
Growth in lactose medium	+	-	-	-	-
Growth in manitol salt agar	-	-	-	-	+
Growth in Salmonella/Shigella medium	-	-	+	+	-
Growth in Simmons citrate agar	-	+	-	-	-
Growth in Tetrathionate broth	-	-	+	+	-
Growth in urea agar base medium	-	-	+	+	-
Growth in Voges Proskaur medium	-	+	-	-	-
Motility test	+	+	+	+	+
Production of acid	+	+	+	+	+
Production of gas	+	+	+	+	+
Slide Coagulase test	-	-	-	-	+
Tube Coagulase test	-	-	-	-	+
Morphology	Rods Rods Rods Rods Cocci				

Table 4: Result of biochemical characteristics of *Candida Spp*

Test	Result
Gram's test	+
Budding test	+
Methylene blue reduction test	+
Glucose fermentation test	+
Lactose fermentation test	+
Presence of Pseudomycelia	+
Presence of Blasto spores	+

Water suitable for agricultural irrigation, fish rearing, human consumption and domestic uses should be free of disease-producing organisms or large amounts of non – pathogenic organisms. Freshwater quality criteria for domestic supply require that faecal bacteria levels should not exceed a geometric mean value of 100 cfu/100 ml while the drinking water criterion is <1 cfu/100 ml (WHO, 2001).

Faecal coliforms such as *E. coli* are prevalent in the digestive tracts of warm-blooded animals and it is believed that there is a correlation between their presence and pathogenic organisms since they have very similar survival characteristics to those of the well-known pathogenic members of the family, *Salmonella* and *Shigella* (Van Kessel *et al.*, 2004). They thus serve

as indicators for these pathogens and associated animal wastes that enter the water body, and their presence is definitive evidence of faecal contamination and the potential risk of zoonotic pathogens (Saxena *et al.*, 2015; Tortorello, 2003 & Entry and Farmer, 2001). Faecal streptococci, particularly *Enterococcus Spp* and *Clostridium* spores also show faecal contamination, the former indicating recent contamination as they do not multiply in the environment, and the latter representing more ancient contamination (Payment and Franco, 1993).

Since the water body (Imo River) is constantly used for domestic purposes, agriculture and aquaculture by the communities living along its banks, there is possibility of transferring these organisms to human

beings and eventual outbreak of their related diseases (Olayemi *et al.*, 1990; Tita *et al.*, 2013).

As a consequence of no proper waste management system in the market, a lot of faecal matter and associated market wastes accumulate on the drainage channels and land within the market due to the lack of rainfall during the dry season. The first rains wash them into the nearby water body and the upsurge in bacterial counts at this time could thus be a combination of contributions from such land stores and those re-suspended from sediments.

The result revealed that there are more counts in the water samples during the peak of the rainy season than during the dry season. But the reverse becomes the case during the dry season when organisms were found to accumulate more in the sediments (Olayemi, 1994).

This may be attributed to the fact that in the dry season, water levels are much reduced and bank vegetations cleared for the cultivation of market vegetables using the river water for irrigation. This makes the streams more accessible to both humans and animals. This may increase faecal bacteria levels in the water column through direct deposition of faecal matter and re-suspension from sediments, compounded by minimum dilution due to low river flow (Castillo *et al.*, 2004). It is also possible that as the water velocity reduces in the dry season, faecal bacteria accumulate and settle as a result of greater contact between water and sediment which enables significant sediment-water exchange (Mitsch and Gosselink, 2000).

The high faecal counts observed generally on the sampling station can also be attributed to the fact that greater percentage of Africans still defecate in the open due to lack of toilet systems and this can be said of the communities living along the river banks. Similarly, the market has no toilet facility exposing the individuals that do businesses in the market to defecate indiscriminately.

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