COLIFORMS AND ENTEROCOCCI AS INDICATORS OF FAECAL POLLUTION OF WOJI RIVER RECEIVING ABATTOIR EFFLUENTS IN PORT HARCOURT, RIVERS STATE NIGERIA.

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ABSTRACT

This study assessed the influence of the abattoir effluents and other human centred activities on the microbial quality of the Woji Trans-Amadi River of Port Harcourt, using coliforms and enterococci as indicators of faecal pollution. Four sites on the Woji River namely: the abattoir effluent, the waste/faeces disposal, upstream and downstream sites were monitored for total coliforms, enterococci, total heterotrophs, biochemical oxygen demand (BOD), pH and salinity. Total coliform numbers varied from 2.0×10^6 MPN/100ml to 1.70×10^{10} MPN/100ml with the highest values of 1.70×10^{10} MPN/100ml and 4.70×10^8 cfu/100ml at the abattoir and waste sites respectively and the lowest values of 2.0×10^6 MPN/100ml and 3.10×10^5 cfu/100ml at the upstream and downstream respectively. Lower numbers ranging from 3.00×10^5 cfu/100ml to 2.00×10^7 cfu/100ml were obtained for enterococci similarly at the respective sites, and the range of values from 3.7×10^5 to 1.8×10^{10} cfu/100ml were obtained for total heterotrophs. Bacterial numbers were higher at all the sites during the dry season compared with the rainy season. The BOD varied from 2mg/l at the upstream site to 360mg/l at the abattoir effluent site; and similarly the pH level varied from 6.22 to 7.36 and the salinity from 2879mg/l to 13898mg/l at both sites respectively. None of the sites achieved internationally accepted standards for water quality of Rivers. The results of this investigation revealed that Woji River is subject to sewage pollution and is impacted from the abattoir effluent site and waste/faeces site.

INTRODUCTION

Worldwide, indicator bacteria such as coliform bacteria, *Escherichia coli*, faecal streptococci has been used for assessing faecal pollution and possible deterioration in fresh water sources such as lakes, rivers, underground waters and streams (APHA,1995). Obiri-Danso et al., (2005) monitored coliform bacteria and enterococci in Subin, an urban river located in Kumasi Ghana, to determine the microbial pollution in the river. Also, Shawky et al.,

(2007) monitored coliform bacteria, *E.coli* and other heterotrophic bacteria to determine the microbial quality of river Nile (located at the Damietta branch, Egypt).

Faecal contamination of rivers is a major water quality issue in many fast growing cities where population growth far exceeds the rate of development of wastewater collection and treatment (Obiri- Danso *et al*., 2005).

Despite global efforts during the United Nations water and sanitation decades of 1980's,

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improvement in water and sanitation infrastructure has not kept pace with population growth and urbanization in most developing countries. (Obiri-Danso *et al*., 2005).

Studies in village settings carried out in Ulasi River, Okija, Anambra State in which activities such as cassava processing, laundry, bathing and swimming are carried out, showed low pollution level (Anazoo and Ibe 2005) when compared with rivers in urban settings such as studies carried out in Elechi Creek in Port Harcourt, which showed high level of pollution (Obire *et al*., 2005). This showed that urban population has high impact on river pollution.

The Woji River is an urban river which runs through the commercial centre of Port Harcourt city in the oil producing Rivers State, the fifth largest and one of the fastest growing city in Nigeria with a population of 1,148, 665 (Geonames,2010) .

The Woji River receives untreated waste effluents from an abattoir, waste /faeces disposal site, the slaughter market, indiscriminate refuse dumping, residential buildings and industries namely Coca –Cola, Nigerian Breweries Limited (NBL) and Baker-Hudges .

FEPA (Federal Environmental Protection Agency) has guidelines for physiological parameters for effluent from industries and not microbial contamination for inland water quality (FEPA, 1991).

FEPA is unlike GEPA (Environmental Protection Agency of Ghana) which has set limits for permitted level of microbial contamination in liquid effluent discharged into water bodies (GEPA Act 490 1994), where total coliforms should not exceed 400per 100ml and *E.coli* 10 per 100ml.

WHO (1992) guidelines are 500 per 100ml for total coliforms and 100 per 100ml for enterococci.

This study examined the influence of abattoir effluents, urban waste, industrial and commercial waste on pollution of the Woji River in Trans-Amadi using coliforms and enterococci as indicators using two methods of assay, the most probable number technique (MPN) and the spread plate method for comparison. It also evaluated the effect of seasonal variation on the bacteriological and physicochemical parameters of the river.

MATERIALS AND METHODS
Study Area:
The Woji River is an urban river which runs through the commercial and industrial centre of Port Harcourt, one of the largest city in Nigeria. The Woji river is located between longitude 7^0 00" and 7^0 15" and latitude 4^0 28"E and 4^0 40"N. It is a tributary of the upper bonny estuary in Port Harcourt. It passes through many communities namely: Oginigba, Woji, Azubiae, Okujagu, Okuru-ama, Abuloma, Ojimba, Oba, Kalio and Okirika. The water flows from Bonny river down to Rumukpakoluosi, Eliozu at high tide and low tide flows back to Bonny river. Many human activities going on within and around this river include bathing and swimming, fishing, boating, navigation, laundry, disposal of waste and excreta. This aquatic body receives effluent discharge from the Trans-Amadi abattoir, slaugther markets, residential buildings and industries.
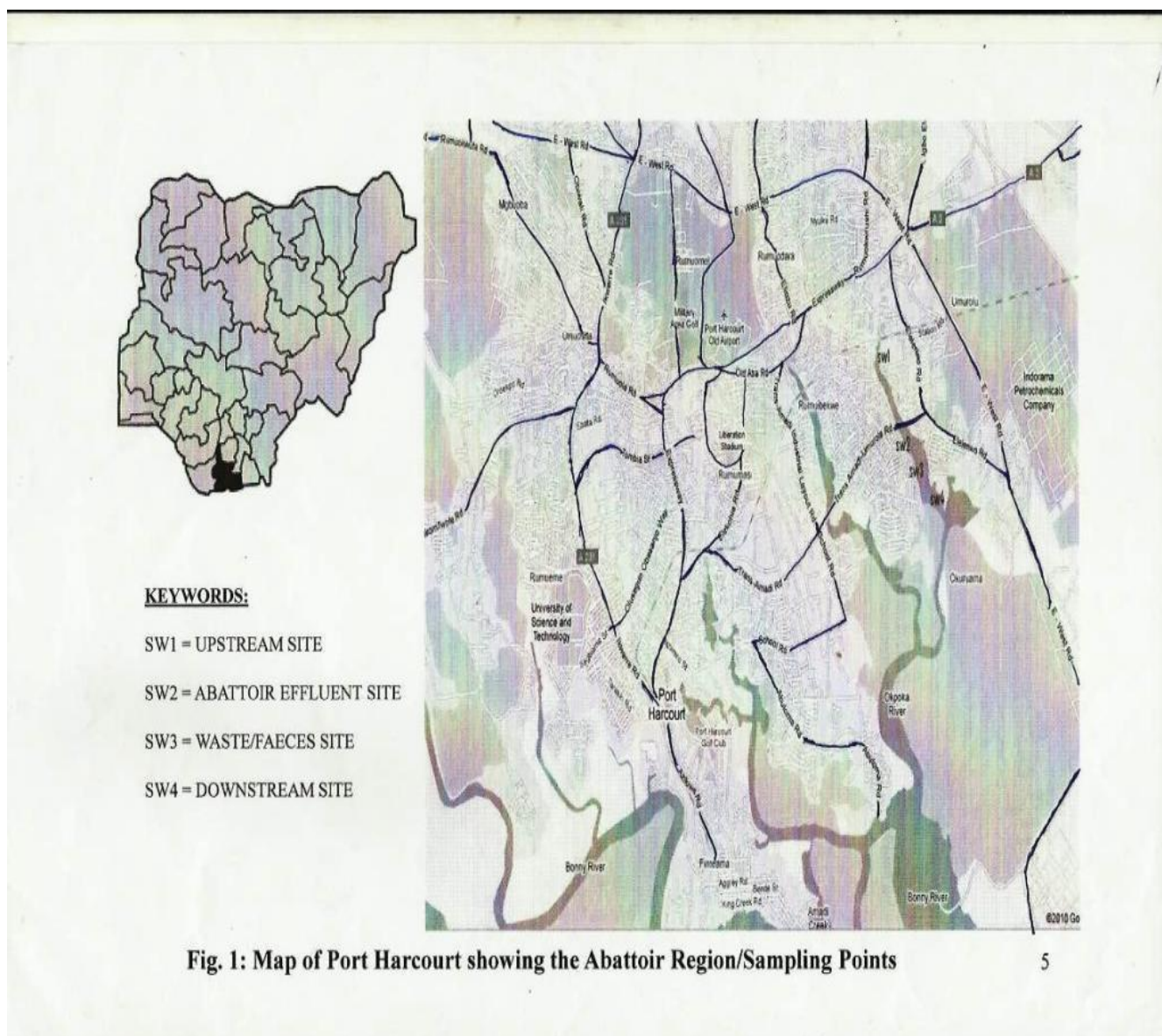
Sampling Sites
The four main sites sampled on the Woji River were the upstream site (US), the abattoir effluent site (AE), waste/faeces disposal site (WS) and downstream site (DS) as in Fig.1.

Sample Collection
Water samples for dry season were collected in March and April 2010, while samples for rainy season were collected in July and August 2010. The shoreline sampling method as described by Milne (1996) was employed. The collection of sample was accomplished from a boat. The samples were taken from approximately 20 –

30cm below water surface using 250ml sterilized sample bottles (Obire *et al*., 2005). Samples were stored in a portable ice box after collection and transported to the laboratory for microbiological and physiochemical analysis same day.

KEYWORDS:

SW1 = UPSTREAM SITE

SW2 = ABATTOIR EFFLUENT SITE

SW3 = WASTE/FAECES SITE

SW4 = DOWNSTREAM SITE

Fig. 1: Map of Port Harcourt showing the Abattoir Region/Sampling Points

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Microbiological Analysis of Water Sample
Total Heterotrophic Counts

Water samples were extracted and diluted serially up to 10^{-9} dilution and 0.1ml aliquots were inoculated in duplicates onto nutrient agar medium using the spread plate method and incubated at 37^0C for 24hrs for total heterotrophic bacteria (APHA, 1995).

Colonies were counted, Gram stained and identified using standard biochemical tests and keys provided in the Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1993).

Total Coliform Counts

Coliforms were estimated using both the MPN method and spread plate method. For the MPN method a three tube most probable number technique was adapted using standard procedures (APHA, 1992). Water samples were pipetted under aseptic condition and diluted serially up to 10^{-9} in physiological saline and 1ml aliquots of 3 consecutive dilutions were used for MPN process. Each dilution was inoculated into three sets of tubes containing 10ml of single strength MacConkey broth (Oxoid) with Durham tubes and incubated at 37^0C for 48 hrs. Tubes showing acid and gas were considered as positive for presumptive total coliforms. Confirmatory test was carried out by plating loopfuls onto MacConkey agar (Oxoid) and Eosine ethylene blue agar plates followed by incubation at 37^0C for 48h and examining for typical coliform colonies. Presumptive counts per 100ml were calculated from Most Probable Number table. Colonies were identified by using Gram staining and biochemical tests (Bergey and Holt, 1993).

For spread plate method, 0.1ml at three consecutive dilutions was plated in duplicates on MacConkey agar plates. Plates were incubated at 37^0C for 48hrs. Red colonies were counted and expressed as cfu/100ml.

Enterococci Counts

The standard plate method and most probable number methods were used. Azide broth and Azide agar was compounded using sodium chloride 5g, peptone 10g, dipotassium hydrogen phosphate 5g, potassium dihydrogen phosphate 2g, D – glucose 5g, yeast extract 3g, sodium azide 0.25g, bromocresol purple 3ml, distilled water 1000ml and 15g agar for the Azide agar. The pH was adjusted to 6.6 – 6.8 before sterilization of media at 121^0C for 15 min at 15 p.s.i (Harrigan and Margaret 1985). For MPN, 10ml, 1ml and 0.1ml aliquots of undiluted water samples were inoculated appropriately into three sets of tubes with one set containing 10ml double strength azide broth and two sets single strength azide broth. The tubes were incubated at 37^0C for 48hrs and tubes with turbidity were considered as positive and were use for presumptive enterococci enumeration. Loopfuls of culture from positive tubes were inoculated into azide broth tubes and incubated at 44.5^0C for 48hrs for turbidity to occur and loopfuls were streaked on azide agar and incubated for colony identification. For plate count, azide agar plates were inoculated with 0.1ml aliquots of 3 consecutive dilutions of water sample and incubated at 37^0C for 72hrs. Light purple colonies were counted and expressed as cfu/100ml and positive tubes as MPN/100ml. Morphological identification and biochemical tests were performed on colonies. (APHA, 1995; Treagan and Pulliam, 1982).

Physico- chemical Determination:

The physicochemical parameters determined for the Woji water samples were pH, salinity and five- day biochemical oxygen demand (BOD_5). The pH of the water samples were determined on arrival at the laboratory using a pH glass kem-H- electrode meter (Model No.210).

The salinity levels of the water samples were determined in mg/l using standard laboratory procedures (APHA, 1985). The

Winkler's method and standard laboratory method as described by APHA (1985) was used for the five- day biochemical oxygen demand (BOD$_5$) to estimate the amount of oxygen required by microorganisms to breakdown the organic compound in the water samples.

RESULTS

Quality of Woji Trans-Amadi River Indicator Bacteria Counts:

The microbial population values at the four sites ranged from 4.76 x10^6 to 1.50x10^{10}cfu/100ml for total coliforms, 1.1x10 to 1.13x10^7 for enterococci and 9.1x10^6 to 1.65x10^{10}cfu/100ml for total heterotrophs as shown in Figures 2-5.

Figs. 2-5 show that the abattoir effluent site recorded the highest microbial load with the range of 1.63x10^6 to 1.50x10^{7}MPN/100ml for total coliforms, 4.00x10^7 to1.65x10^{10}cfu/100ml for total heterotrophic bacteria and 1.0x10^2 to 1.13x10^{7}cfu/100ml for enterococci, while the upstream and downstream recorded the lowest values.

The MPN method gave higher counts for total coliforms (Figs. 4 and 5), but lower counts for enterococci. Plate count was used for assessing enterococci as indicator organisms. There was a consistent increase in bacteria loading as the river flowed downstream from the upstream site. Comparative analysis of bacterial loads in the two seasons showed that dry season counts were higher than rainy season counts
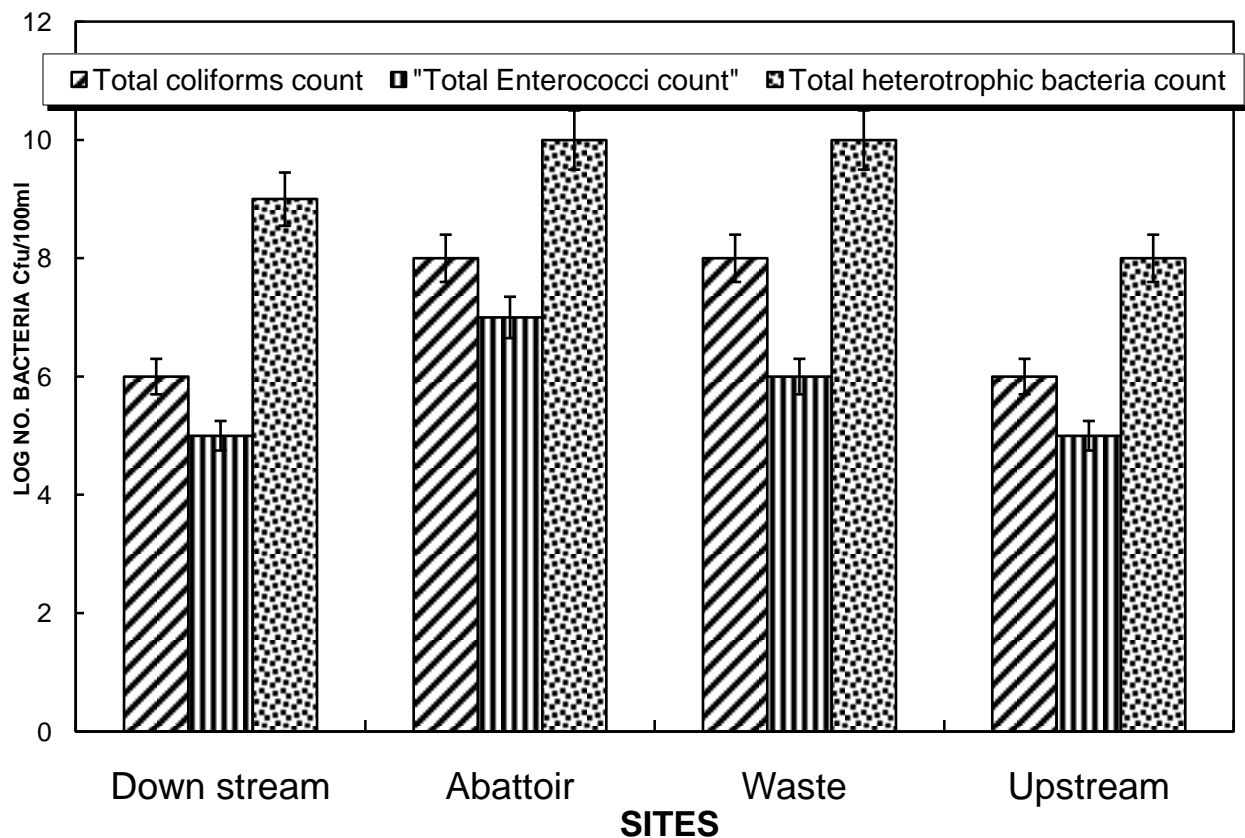

Figure 2: Mean bacteria indicator counts at the four sites during the dry season
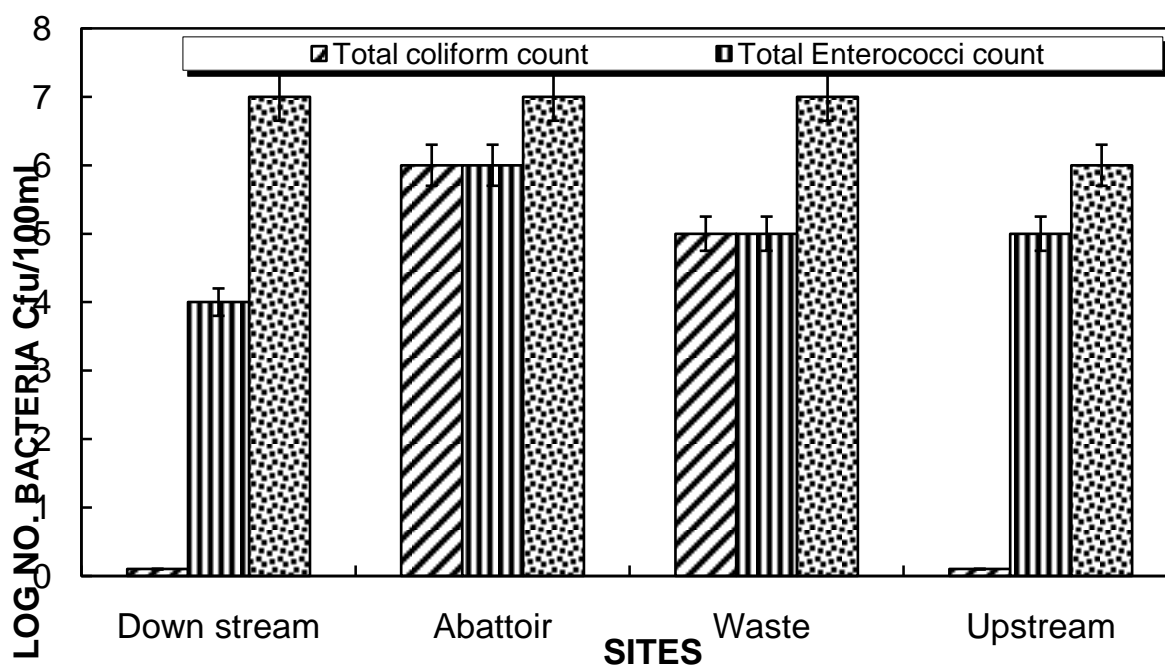
Figure 3: Mean bacteria indicator counts at the four sites during the rainy season
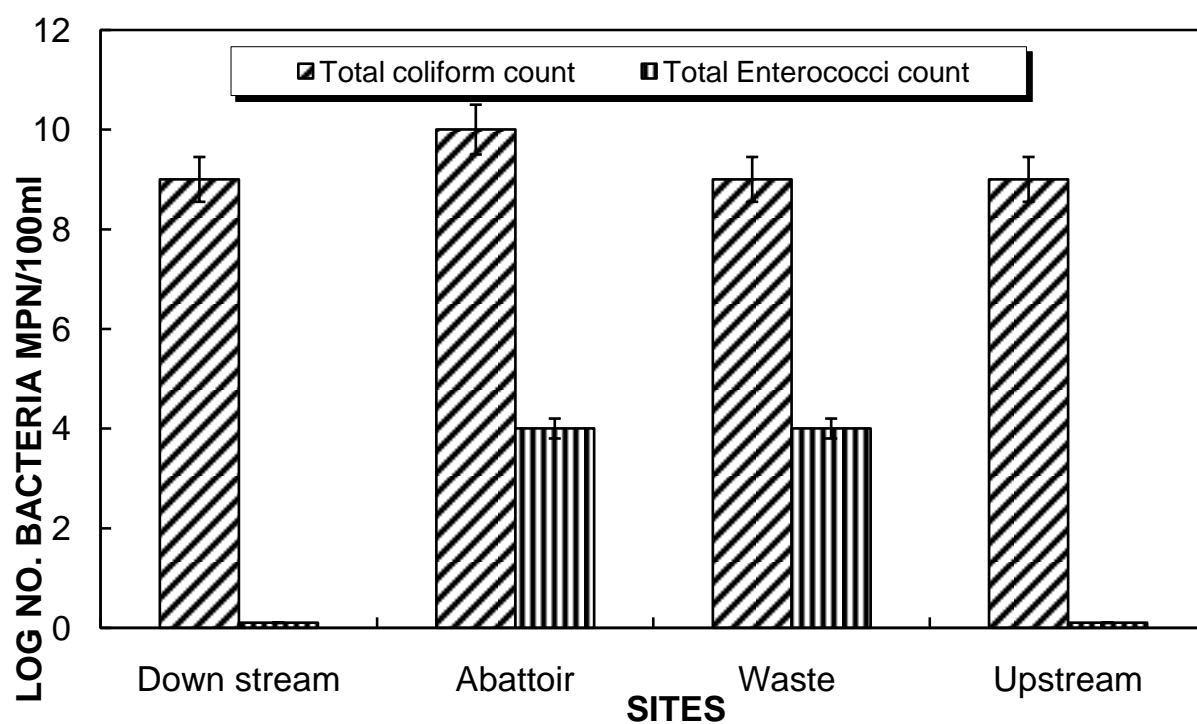

Figure 4: Mean bacteria indicator counts at the four sites during the dry season
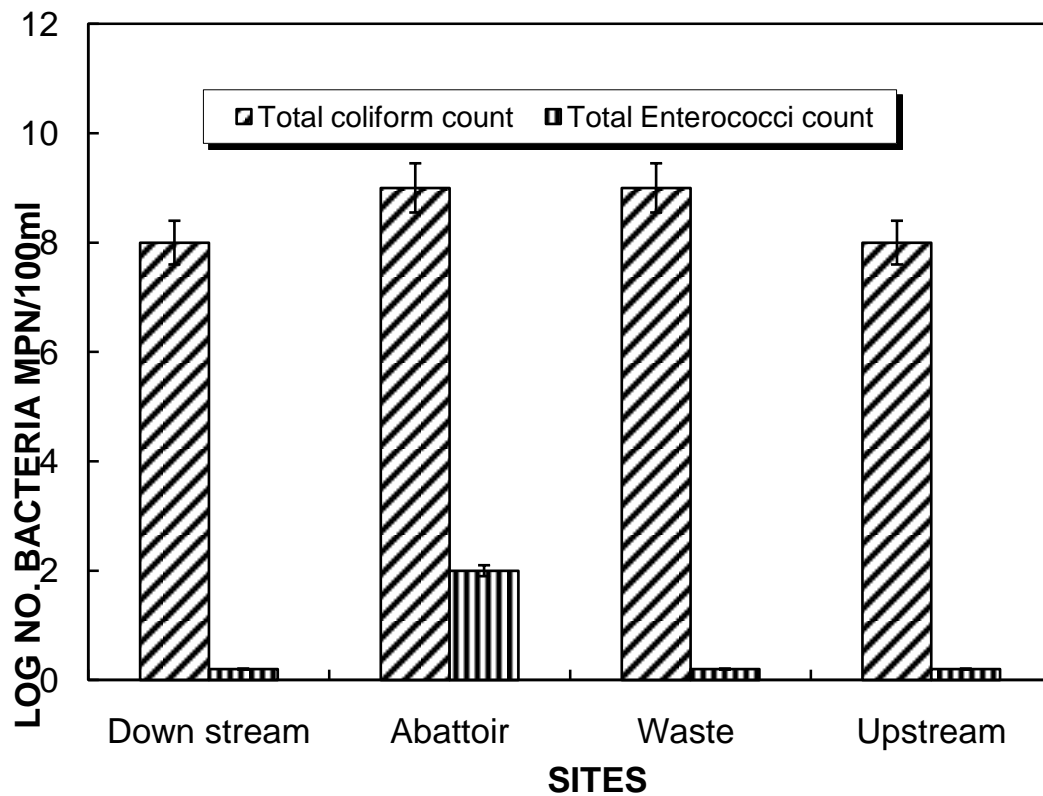
Figure 5: Mean bacteria indicator counts at the four sites during the rainy season

Biochemical Oxygen Demand Levels

Mean BOD levels ranged between 2mg/l and 360mg/l (Fig.7). In general, BOD levels increased as the river flowed downstream with the abattoir effluent site and waste disposal site recording the highest value of 216mg/l and 48.5mg/l respectively. Only abattoir site showed seasonal variation (Fig.7) with low BOD in rainy season.

pH Value Level

The pH level ranged from 6.22 to 7.36. The pH decreased as the river flowed downstream with the abattoir effluent site and waste site recording the lowest value of 6.22 to 6.72 and upstream recording the highest value of 6.67 – 7.36 as in Fig.6.

Salinity Level

Salinity ranged from 2879mg/l – 13708mg/l as shown in Fig.8 with highest level at the upstream site. Seasonal variation was pronounced with lower salinity of water samples in the rainy season (Fig.8).
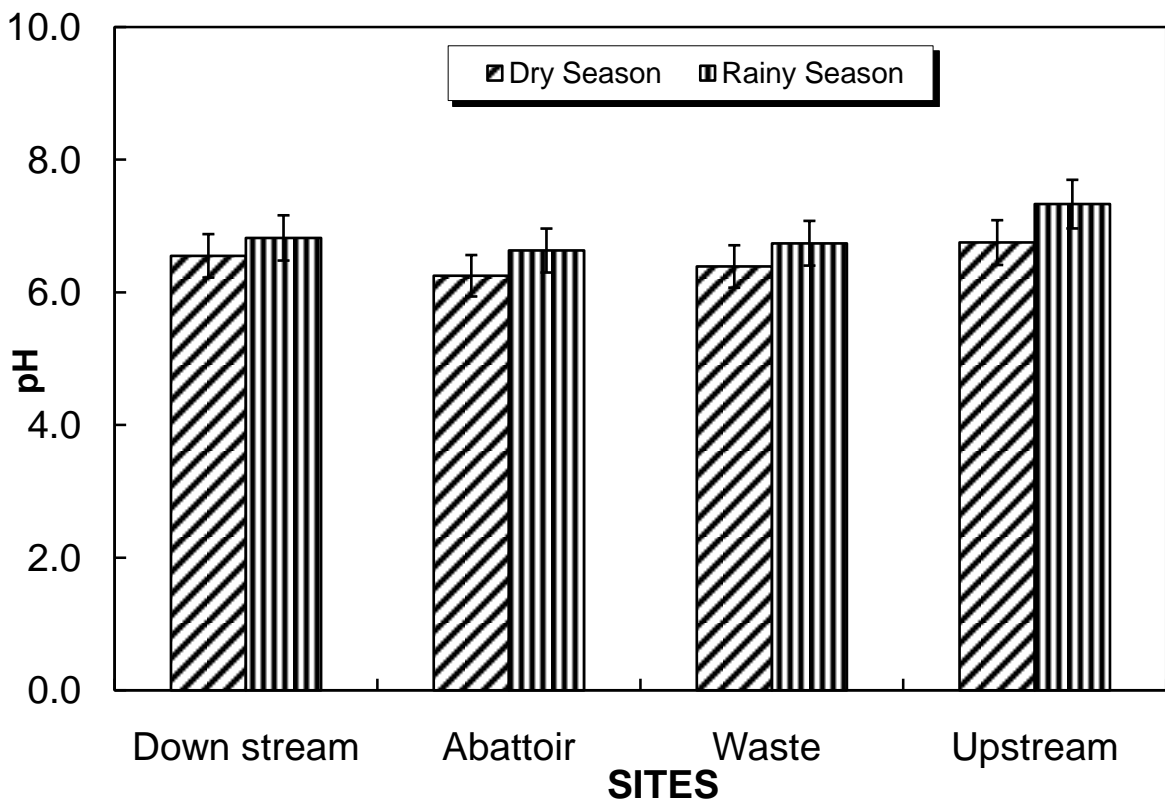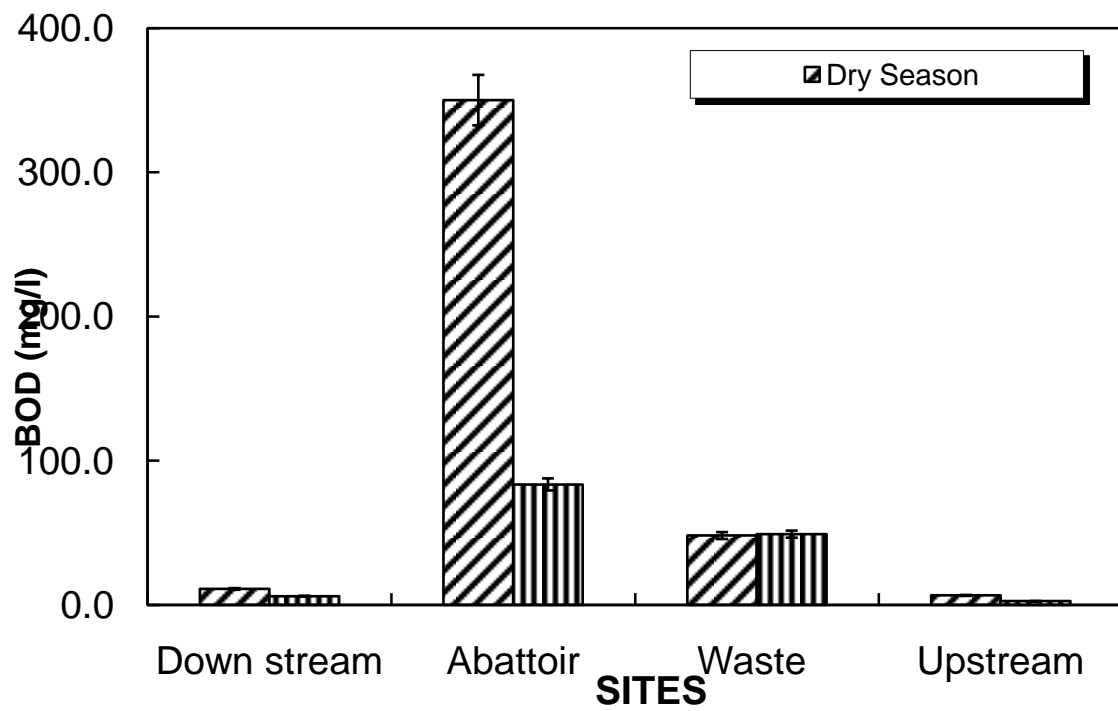
Figure 6: Mean pH at the four sites

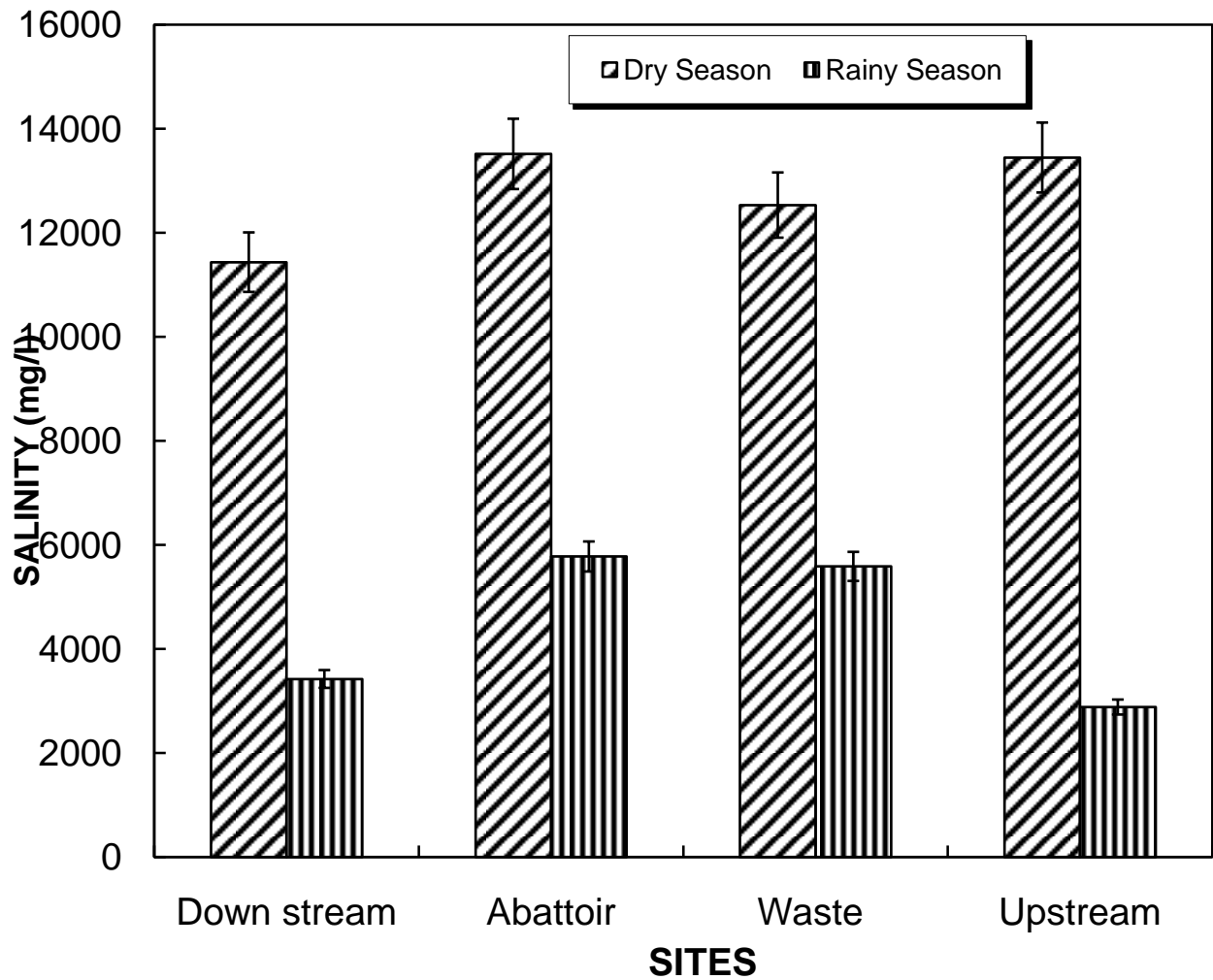**Figure 7: Mean Biochemical Oxygen demand (BOD) at the four sites**

Figure 8: Mean salinity values at the four sites

Table1: Biochemical characteristics of coliforms, enterococci and some heterotrophs isolated

Isolates Characteristics	Ws10	Ws1	Ws5	Ws2	Es3	Ws7	Us9	Ds7	WS6	Ae3	C1	C2
Indole	+	-	-	-	-	-	-	+	-	-		
M.R	+	+	+	+	-	-	-	+	-	+		
V.P	-	-	-	-	+	+	-	-	+	-		
Citrate	-	+	-	-	+	+	-	-	+	-		
TSia												
Slant/Butt	Y/Y	R/Y	Y/Y	R/R	R/Y	Y/Y	R/R	Y/Y	Y/Y	R/Y		
Gas	+	+	-	-	-	+	-	+	+	-		
H_2S	-	+	-	-	-	-	-	+	-	-		
Lactose	+	+	-	-	-	+	-	-	+	-	+	+
Glucose	+	+	+	-	+	+	-	+	+	+	+	+
Urease	-	-	-	+	-	-	+	+	-	-		
Motility	+	+	+	-	+	+	-	+	-	-		
Catalase	+	+	+	+	+	+	+	+	+	+	-	-
Oxidase	-	-	+	-	-	-	-	-	-	-		
pH 9.6											+	-
NaCl 6.5											-	-
Starch Hydrolysis											-	+
Glycerol											+	-
Sucrose											+	+

Key Words

Y = Yellow

R = Red

Isolate	Possible Organisms
Ws10	*E. coli*
Ws1	*Citrobacter freundii*
Ws7	*Enterobacter sp*
Ws6	*Klebsiella pneumoniae*
Es3	*Bacillus cereus*
Ae3	*Shigella flexneri*
Ws5	*Pseudomonas aeruginosa*
Ws2	*Yersinia sp*
Us9	*Micrococcus sp*
Ds7	*Proteus vulgaris*
C2	*Streptococcus bovis*
C1	*Streptococcus faecalis*

Table 2: Total coliform count at four samples sites

Sites	Dry season average Cfu/100ml	Rainy Season average Cfu/100ml	Dry season average MPN/100ml	Rainy season average MPN/100ml
Down stream	4.76×10^6	0	1.10×10^9	3.5×10^8
Abattoir effluent site	3.3×10^8	1.63×10^6	1.50×10^{10}	4.7×10^9
Waste site	1.85×10^8	6.55×10^5	6.85×10^9	1.7×10^9
Upstream site	8.05×10^6	0	1.20×10^9	1.0×10^8

Table 3: Total streptococcal count at four samples sites

Sites	Dry season average Cfu/100ml	Rainy season average Cfu/100ml	Dry season average MPN/100ml	Rainy season average MPN/100ml
Down stream	3.60×10^5	3.20×10^4	2.25×10	1.9×10
Abattoir effluent site	1.13×10^7	1.10×10^6	1.75×10^4	1.0×10^2
Waste site	6.70×10^6	3.90×10^5	1.2×10^4	3.0×10
Upstream site	3.80×10^5	3.00×10^5	4.3×10	1.1×10

Table 4: Total heterotrophic culturable bacteria load at four samples sites

Sites	Dry season Average Cfu/100ml	Rainy season average Cfu/100ml
Down stream	8.85×10^9	2.1×10^7
Abattoir effluent site	1.65×10^{10}	4.00×10^7
Waste site	1.01×10^{10}	5.45×10^7
Upstream site	9.35×10^8	9.1×10^6

Table 5: Variations in pH, salinity and BOD at the four sites in dry season and raining season

		March	April	Mean dry season	July	August	Mean raining season
DS	pH	6.33	6.76	6.55	6.84	6.80	6.82
	BOD	12	10	11	5	7	6
	Salinity	11,322.0	115525	11,437.25	3429.5	3417	3423.25
AES	pH	6.28	6.22	6.25	6.66	6.59	6.63
	BOD	340	360	350	86	81	83.5
	Salinity	13,500	13,537.5	13,518.75	5776	5780	5778
WS	pH	6.32	6.45	6.39	6.76	6.72	6.74
	BOD	52	44	48	40	58	49
	Salinity	12,435	12,635	12,535	5595.5	5580	5587.7
US	pH	6.67	6.83	6.75	7.36	7.29	7.33
	BOD	8	5	6.5	3	2	2.5
	Salinity	13,708.5	13,189.5	13,449	2888	2879	2883.5

DISCUSSION

The results of the study show that the Woji River is extremely polluted with faecal material and the microbial quality substantially deteriorates as it flows from upstream, through the commercial centre of Trans-Amadi Port Harcourt. The abattoir effluent point recorded the highest bacterial count with a range for total coliforms 1.63×10^6 to 1.5×10^{10} MPN/100ml, total heterotrophs $4.00 \times 10^7 - 1.65 \times 10^{10}$, enterococci 1.0×10^2 to $1.13.0 \times 10^7$ cfu/100ml (Fig.2-5). This could be attributed to untreated faecal waste from the slaughtered cows, goats and vultures attracted to the site. The loading of ruminant faeces with coliforms and enterococci is similar to human (Obiri-Danso *et al*., 2005). Secondly, a large flock of white-headed vultures (*Trigonoceps occipitalis*) and hooded vultures (*Necrosyrtes monarchus*) seen around the abattoir contribute to the high counts.

Birds shed substantial amounts of indicator bacteria in their faeces (Jones and Obiri-Danso 1999; Obiri-Danso *et al*., 2005). Ghanaian hooded vultures have been shown to shed enterococci at a rate of 1.27×10^4 to 1.37×10^7 cfu/ml into abattoir effluents (Obiri-Danso and Jones 2002).

The second site contributing to pollution was the waste/faeces disposal site. The abattoir has no toilet facility for the abattoir workers and the large daily influx of traders. Consequently, the workers, buyers and traders resort to unsanitary practices as they urinate and defecate into open spaces, gutters or polythene bags which are dumped into the river. Apart from the abattoir effluent and faecal pollution, the bulk of the market refuse is carried into the Woji River. This explains why the downstream site had high bacteria count for total heterotrophs of 3.0×10^{10} cfu/100ml, total coliforms 1.7×10^9 MPN/100ml and enterococci 3.6×10^5 cfu/100ml. None of the sites met the WHO criteria of 100

enterococci per 100ml, and 500 total coliforms per 100ml for rivers (Chukwura, 2001).

The Woji River like other surface water bodies has been subject to various contaminating material capable of initiating the impairment of the water quality.

The pH ranged from 6.28 to 7.36 with the highest value obtained at the upstream site and the lowest at the abattoir site (Fig.6). Okokoye and Rim-Rukey (2003) reported lower values ranging from 6.20 to 6.50 for three polluted sections of Orogbodo River, Agbo, while the unpolluted section had a pH of 6.90 and Obire *et al*., 2005 reported a pH range between 6.4 to 7.7 and noticed lower pH values at the downstream site.

Abattoir effluent sites and waste/faeces disposal sites were favourable for bacteria multiplication this could be as a result of the faecal pollution from the sites, while the neutral pH at the upstream site was favourable for lower bacteria counts at the site.

The mean value for BOD ranged from 2.5mg/l to 350mg/l (Fig.7) with the highest mean BOD levels at the abattoir effluent site and waste site, which is attributable to the presence of higher degradable organic matter. The difference and lower indicator numbers at downstream is most likely due to die-off and dilution predicted by the distance-decay relationships of bacteria indicators (Kay and McDonald 1980). Imevbor (1979) reported that the contamination of water with faeces increases the biochemical oxygen demand because it contains mainly organic matter, which makes oxygen less available to desirable organisms. BOD levels of 8.00 to 419mg/l, 80 – 153mg/l and 1.50 to 6mg/l has also been reported by Obiri *et al* (2005); Biney (1986); Jonnalagadd and Mhere (2001). No site met the physiochemical standard for water quality of BOD of 2mg/l (Obiri-Danso *et al*., 2005; FEPA 1991).

The bacteria species isolated at the four sites were numerous. Of the fourteen species isolated *E.coli, Citrobacter freundii, Micrococcus* sp., *Bacillus cereus, Pseudomonas aeruginosa, Streptococcus faecalis, Streptococcus bovis* occurred in all the four sites sampled. The other species occurred in some sites and not in other sites. *Shigella flexneri* occurred only in the abattoir effluent site and downstream sites. There is relationship between environmental factors and natural microbial numbers and types. Atlas (1988) reported that the population of any aquatic system is to a large extent influenced by the water body. Hollaway *et al.* (1980) stated that establishing the presence of bacteria in waterbodies is important, as they have been identified as the major organisms which initiate the breakdown of introduced waste to various metabolic intermediates. *Salmonella* was not recovered. Morinigo *et al.* (1989); Tobias and Heinemeyer (1994) has also reported difficulty in culturing *Salmonella* from environmental samples.

The observed high coliform counts and presence of *Escherichia coli, Streptococcus faecalis, Streptococcus bovis* was sufficient to suspect the waterbody was contaminated with pathogenic bacteria and therefore not fit for drinking or other domestic purposes.

Indicator bacterial counts signifying faecal pollution in Woji River was significantly higher in the dry season than the raining season presumably due to the dilution of rain water during raining season. This agrees with the result of Obire *et al* (2005).

According to the guideline criteria for faecal indicator organism of (WHO report 1992) which accepts the guided value of the investigated bacteria batween 10 to 500/100ml for total coliforms and 100/100ml for enterococci, the survey of the indicator bacteria at the Woji river revealed that Woji river is subject to faecal pollution and the untreated abattoir effluent has high impact on the river.

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This Study has revealed that the abattoir effluent site has high impact on the Woji River ranging from high bacteria load, to high biochemical oxygen demand which thus reduces the amount of oxygen in the environment due to high demand of oxygen for breakdown of the organic compounds. Therefore, the Federal Ministry of Environment should see this as a great task since the river is not safe for domestic activities and recreational activities there is need for treatment of the abattoir effluents. The Rivers State government likewise has a role to play in ensuring the construction of a standard abattoir with effluent treatment facilities, provision of toilet facilities in the area and establishing effective policy on management of water bodies.

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