Impacts of Human Perturbations on the Physico-chemistry and Biological Parameters on the Water Quality of Cross River Estuary, South Eastern Nigeria.

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Abstract: Studies on the impacts of human perturbations on the physico-chemistry and biological parameters on the water quality of cross river estuary, south eastern Nigeria was investigated between January and December, 2014 to assess the water quality of Cross River Estuary with emphasis on the physical, chemical and biological parameters. Samples were collected from three stations namely Calcemco, James Island and Parrot Island and were analyzed using standard laboratory method. Analysis of the physicochemical parameter shows that some samples were within the WHO guideline value for portable water, while others were above WHO standard. Temperature, salinity, dissolved oxygen (DO), conductivity, total dissolved solutes (TDS), Chemical Oxygen demand (COD), nitrate, phosphate, sulphate and alkalinity values of the water samples were within WHO guideline values for drinking water. BOD_5 in station one was within WHO permissible limits but values obtained in station 2 and 3 were above WHO standard for portable water. Turbidity, total suspended solids (TSS) values were above WHO standard. Result shows that high total heterotrophic bacteria counts were observed in all the three station with the lowest count ($3.8x10^4$) recorded in station 1 and 3 during dry season and the highest count ($5.8x10^4$) observed in station 3 during wet season. Values obtained for THB during the study exceeds WHO permissible limits for drinking water. Enumeration of coliform counts revealed that water samples from station 1, 2 and 3 had 23MPN/100ml and 26MPN/100ml, 22MPN/100ml and 25MPN/100ml and 20MPN/100ml and 29MPN/100ml respectively for total coliform during the dry and wet season and 13MPN/100ml and 15MPN/100ml, 18MPN/100ml and 19MPN/100ml and 17MPN/100ml and 20MPN/100ml each for faecal coliform during the dry and wet season respectively. Values obtained for total coliform and faecal coliform were above WHO permissible limits. Bacteriological identification of the 74 isolates obtained from the samples showed the presence of the genera: *Pseudomonas* 8(10.81%), *Escherichia coli* 12 (16.22%), *Proteus* 8 (10.81%), *Enterobacter* 6 (8.12%), *Salmonella* 6 (8.12%), *Shigella* 4 (5.41%), *Streptococcus* 8 (10.81%), *Vibrio* 4 (5.41%), *Staphylococcos aureus* 6 (8.12%), and Bacillus 12 (16.22%). This study indicates that this water source is highly polluted due to high presence of faecal coliform and other parameters that were above WHO standard for drinking water. We therefore recommend adequate treatment before consumption in order to avoid epidemic of water related diseases.
[George, U. U., Atakpa, E. O. **Impacts of Human Perturbations on the Physico-chemistry and Biological Parameters on the Water Quality of Cross River Estuary, South Eastern Nigeria.** *Rep Opinion* 2015;7(11):49-55]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 6. doi:10.7537/marsroj071115.06.

Keywords: Impacts, human perturbations, biological parameters, physicochemical parameters, water quality, Cross River Estuary.

1. Introduction

Water quality is a major economic and environmental issue in developing countries. With increase in human population, our interactions with the water resources on which we are completely dependent become more and more critical. It is important to note that the major cause of environmental degradation is as a result of human induced activities which include industrial, mining, agriculture, house hold waste production, road construction and other human related activities that is capable of increasing the concentration of heavy metal and pathogenic organisms in the environment, thereby altering the status of the aquatic ecosystem which may in turn affects fish stocks, extinction of many economic species and possibly alteration in the quality of water which hampered the use of such system for domestic, , irrigation, fish production, farming and recreation purposes (Ekpo, *et. al*., 2015).

Water is the most known and most abundant of all known chemical substances, which occur naturally on the surface of the earth. It is fundamentally important to all plants, animals and man (Ajewole, 2005). It is a prime solvent and its properties determine many natural phenomena, making water a universal solvent. Water can be derived from a number of sources, which includes streams, lakes, rivers, ponds, rain, springs, ocean and wells.

Generally, water resource problems are of three broad categories: too little water, two much water and

polluted water (Ayoade, 1988; Adebola, 2001). About 20 % of the world's population does not have access to safe and potable drinking water (UN, 2006) and most of the affected communities are found within Asia and sub-Saharan Africa of which Nigeria is inclusive. The provision of portable water to the rural and urban population is of utmost importance to prevent health hazards (Nikoladze and Akastal, 1989; Lemo, 2002). Before water can be described as potable, it has to meet compliance with certain physical, chemical and microbiological standards, which are designed to ensure that the water is safe for drinking.

The problem associated with water especially in the Niger Delta is not that of availability but portability (Efe *et al.,* 2005). However, the presence of certain chemicals such as iron, copper, zinc, cobalt, magnesium, manganese, selenium, chromium, aluminum, ammonia, nitrite, calcium, phosphate, sulphate and nitrate in water can also be detrimental to human health (Hazelnot, 2000). It is estimated that 80% of all illness in developing countries is related to water and sanitation, and that 15% of all child deaths under the age of 5 years in developing countries results from diarrhoea diseases (WHO, 2003, 2004). The presence of faecal coliform of Escherichia coli is used as an indicator for the presence of any of these water borne pathogens (Okpokwasili and Akujobi, 1996; Chukwural, 2001).

The presence of these undesirable biological and chemical parameters in drinking water affects the pH, total dissolved solids, salinity, dissolved oxygen, total suspended solutes, alkalinity, turbidity and conductivity of the water beyond WHO specified tolerable permissible limits. The presence of these environmental pollutants in water will lead to various water borne related diseases including diarrhea, cholera, typhoid fever, shigellosis, giardiasis, schistosomiasis, hepatitis, cryptosporidiosis, onchocerciasis and dracuncuculiasis.

In an industrialized society, maintaining completely unpolluted water in all drains, streams, rivers, and lakes is probably impossible. But we can evaluate the water quality of a body of water through constant monitoring of aquatic bodies and take steps to preserve or improve its quality by eliminating sources of pollution. The presence study seeks to assess the water quality of Cross River Estuary with emphasis on the physical, chemical and biological parameters.

2. Material and Methods

2.1 Study Area

The Cross River Estuary is a tropical brackish ecosystem located between $4^0$30'5.15'N of the equator, and between $8^0$00'8.40'E of the Greenwich meridian. It is a part of South-eastern Nigeria rainforest characterised by shallow depth (4-10m) and 5.5km width, and extensive intertidal mud with salinity fluctuating between fresh and brackish water depending on the tidal phase and season (Akpan, 1994). The climate is marked by alternating dry and wet seasons- a long wet season between April and November and a relatively short dry season from December to March (Akpan, 1994). The mean annual air temperature is 28^0c and the mean precipitation is 500mm, surface water temperature varies between 22^0c and 30^0c (Etim, 1991).

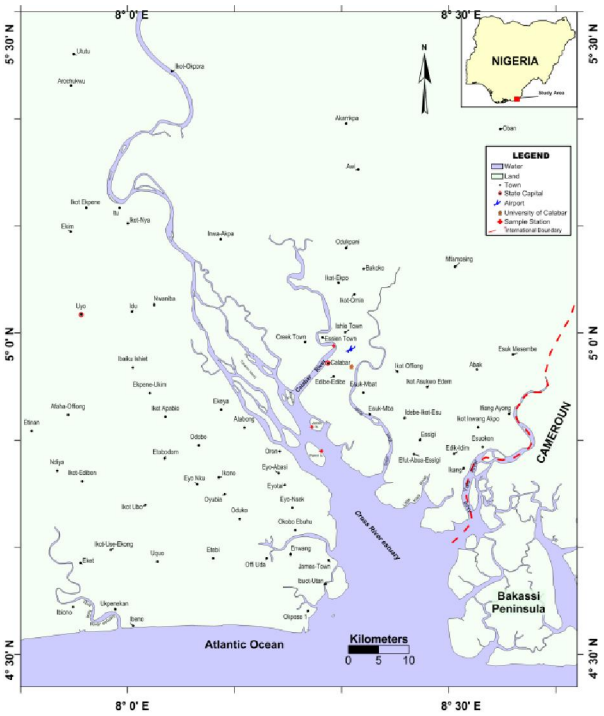

Fig. 1: Map of the study area

2.2 Sample collection

Water samples were collected from each sampling station using washed and sterilized plastic containers (1 litre). Water sample was collected by carefully lowering the sample bottle in the water to fill. Once the bottle was full, it was pulled out of the water and corked firmly.

2.3 Analysis of Samples

2.3.1 Physicochemical Analysis

Physico-chemical parameters, such as temperature, pH, turbidity, dissolved oxygen, conductivity, transparency as well as total dissolved solids were measured *in situ* during sampling. The physico-chemical parameters were assessed using standard methods for examination of water and wastewater (APHA, 1998). A digital thermometer from "EuroLab" was used in the determination of water temperature. A hand held pH meter from

HANNAH Instruments was used for the determination of the H^+ ion index (acidity or alkalinity) of the water. The DO was measured with hand held (portable meter) from "Search Tech Instrument". A hand held instrument from HENNAH was used in determining the conductivity (in mS / cm) of the water. Total Dissolved Solid (TDS) was measured using a portable digital meter from "HENNAH" Instruments. The turbidity of the water was determined with the use of turbidity meter from SEARCHTECH instruments, UK. Transparency of the water was measured using Sechi disc tied to a graduated line. Water samples for BOD_5, COD, Alkalinity, phosphate, chloride, Total suspended solids, Nitrate, sulphate and THC was collected in 250ml glass specimen bottles. The bottles was filled with water and stoppered under water, ensuring that no air bubbles were trap in it. 2ml each of Winkler's solution A and B (Manganous sulphate and potassium iodide) was introduced into the sampling bottles. The contents of the bottles was then thoroughly agitated and transported to the laboratory. In the laboratory (Devine Concept Integrated Laboratory) Port Harcourt, the parameters will be determined using standard laboratory methods according to (AOAC, 2000).

2.3.2 Bacteriological Analysis

2.3.2.1 Enumeration of Total Heterotrophic Bacteria count:

Total heterotrophic bacteria in the water samples were obtained using the spread plate method. Dilutions of 10-1 to 10-4 of the samples were prepared in 0.1% buffered peptone water (oxoid) and 0.1ml aliquots of each dilution was inoculated into the surface of dried nutrient agar plate in triplicates and incubated at 37ºC for 24 hours. Petri-dishes from dilutions containing between 30 and 300 discrete colonies were counted and the result expressed as colony forming unit per milliliter (Krieg and Holt, 1994).

2.3.2.2 Examination of Total and Feacal Coliform:

Presumptive test: Total coliform and faecal coliform were enumerated by multiple tube fermentation tests as described by APHA, (1995). Coliform count was obtained using the three tube assay of the Most Probable Number (MPN) technique. Presumptive coliform test was carried out using MacConkey broth (Oxoid). The first set of the five tubes had sterile 10ml double strength broth and the second and third sets had 10ml single strength broth. All the tubes contained Durham tube before sterilization. The three sets of the tubes received 10ml, 1ml and 0.1ml of water samples using sterile pipettes. They were carefully labeled and incubated at 37ºC for 24-48 hours for estimation of total coliforms and at 44.5ºC for faecal coliforms for 24-48 hours and examined for acid and gas production. Acid production was determined by colour change in the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tube. The MPN was then determined from the MPN table for the three set of tube.

Confirmed test: Confirmed test was carried out by transferring a loopful of culture from a positive tube from presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth (oxoid) with Durham tubes. The tubes were incubated at 37ºC for 24-48 hours for total coliform and 44.5ºC for faecal coliforms and observed for gas production.

Completed test: Completed test was carried out by streaking a loopful of broth from a positive tube onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37ºC for 24-48 hours. Colonies developing on EMB agar were further identified as faecal coliforms (*Escherichia coli*). Colonies with green metallic sheen were confirmed to be faecal coliform bacteria with rods shape.

2.3.2.3 Isolation of *Salmonella / Shigella* species

Salmonella and *Shigella* species were isolated using *Salmonella/Shigella* agar (SSA). The media was prepared following the manufacturer's directive and 0.1ml aliquot of ach water sample was transferred onto the surface of a dried sterilized SSA plates. The plates were inoculated in triplicates and incubated at 37°C for 24 to 48 hours. Pure cultures were obtained through sub-culturing and the colonies were identified using standard procedures (Cheesbrough, 2002).

2.3.2.4 Isolation of *Vibrio* species

Thiosulphate citrate bile salt (TCBS) agar was used to screen for the presence of *Vibrio* species. The media was prepared according to manufacturer's directive, poured into sterilized Petri dishes and allowed to solidify. Then, 0.1ml of each water sample was transferred onto the dried TCBS agar plates in triplicates using a 1ml pipette and spread evenly with a hockey stick. The plates were incubated at 35°C for 24 to 48 hours. Thereafter, yellow colonies were counted and identified following standard procedures (Cheesbrough, 2002).

2.3.2.5 Identification of Isolates

The cultural, morphological and biochemical characteristics of the isolates in a pure culture were determined following the procedures of Bergey`s manual of determinative bacteriology (Krieg and Holt, 1994).

2.4 Statistical Analysis

Results of the water samples from the different stations collected were subjected to student T-test to test if there is any significant difference between the physico-chemical parameters and biological parameters obtained during the study. We considered p-value less than 0.05 as statistically significant. All statistical analyses were carried out using SPSS 17.0 window package. Data obtained from each bacteria

isolates from the water sample were empirically analyzed using the formula:

$\% R_a = {}^{n}/_{N} \times 100$ (Ali *et al.*, 2003).

Where: $\%_{Ra}$ = relative abundance

n = number of individuals

N = total number of all individuals.

3. Result

The physicochemical parameters of the water samples are shown in Table 1. Temperature value ranged from 27.8-29.24°C. The pH of the water ranged from 6.7 in wet season at station 1 to 7.2 in dry season at station 3. Dissolved Oxygen value ranged from 3.9 to 4.71mg/l. The result of the Salinity, Turbidity, Transparency, Conductivity, Total Dissolved Solid, Total Hydrocarbon, Total Suspended Solid, BOD_5, COD, Alkalinity, Phosphate, Nitrate, Sulphate levels of the water samples from all the three stations and the corresponding WHO guideline values for drinking water are presented in Table 1. Statistical analysis using T-test showed significant different in some parameters in all the three stations and some were not significantly different. Temperature, Dissolved Oxygen, Ph, salinity, transparency, BOD_5, COD, alkalinity, phosphate, nitrate and sulphate showed no significant ($P>0.05$) in all the stations during the study, while conductivity, TDS and turbidity were statistically different ($P<0.05$) in all the stations during the study. THC was statistically different ($P<0.05$) in station 2 and 3 and showed no significant at station 1.

The enumeration of total heterotrophic bacteria (THB) from the water samples obtained from the three stations shows that the THB counts ranged from $3.8x10^4$cfu/ml at station 1 to $5.8x10^4$cfu/ml at station 3. Result shows that total coliform and faecal coliform counts ranged from (22-29 MPN/100ml and 13-20 MPN/100ml respectively) during the study (Table 2). Statistical analysis showed no significant ($P>0.05$) in the values obtained for THB, total coliform and faecal coliform in all the stations during the study.

The result of the bacteriological analysis showed their numerical abundance and percentage relative abundance. The results revealed the presence of *Pseudomonas* sp with 8 counts representing (10.81), *Escherichia coli* 12 (16.22), *Proteus* sp 8 (10.81), *Enterobacter* sp 6 (8.12), *Salmonella* sp 6 (8.12), *Shigella* sp 4 (5.41), *Streptococcus* sp 8 (10.81), *Vibrio* sp 4 (5.41, *Staphylococcus aureus* 6 (8.12) and *Bacillus* sp with 12 counts representing (16.22) (Table 3).

Table 1: Mean values of Physicochemical Parameters obtained from the Water Samples during the study period

Parameters	Station 1		Station 2		Station 3		WHO Limits
	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet season	
Tempt. (°C)	28±1.47	27.8±1.81	29.24±1.25	28.15±2.24	29.06±1.16	28.2±2.15	24-30 °C
pH	6.9±0.36	6.7±0.13	7.0±0.17	6.8±0.22	7.2±0.30	7.0±0.41	6.5-9
DO (mg/l)	4.1±0.54	4.63±0.42	4.12±0.37	4.71±0.44	3.9±0.38	4.16±0.2	8-10mg/l
Salinity ($\%_{00}$)	0.825±1.72	1.048±1.63	1.967±3.53	2.170±3.24	3.152±4.25	3.309±4.40	NI
Turbidity	68±40.28	46.6±40.09	64±34.78	56.14±32.99	103±158.13	86.3±115.04	1-5NTU
Transparency	36.3±10.52	32.71±5.68	34.8±6.14	33.43±6.32	37.8±19.59	36.6±17.51	NI
Conductivity ms/cm^3	0.69±0.55	0.09±0.02	0.87±0.44	0.08±0.03	0.89±0.44	0.22±0.37	250 ms/cm^3
TDS	0.012±0.00	3.78±0.49	0.95±1.28	3.83±0.22	0.01±0.00	3.65±0.51	0-500mg/l
THC	1.40±0.37	1.75±3.14	1.13±0.29	0.37±0.39	0.82±0.37	0.17±0.15	NI
TSS	0.012±0.004	0.018±0.001	0.016±0.02	0.17±0.02	0.014±0.001	0.018±0.001	0.01mg/l
BOD_5	4.06±0.002	4.87±0.001	4.02±0.002	6.02±0.001	3.25±0.002	6.87±0.001	1-5mg/l
COD	6.82±0.02	7.86±0.01	7.52±0.01	6.64±0.00	7.12±0.001	8.06±0.01	10-20mg/l
Alkalinity	37.0±2.00	42.0±6.08	29.3±0.01	31.0±0.00	35.0±0.1	47.0±0.2	200mg/l
Phosphate	0.19±0.1	0.16±0.1	0.17±0.01	0.15±0.02	0.25±0.001	0.20±0.001	200mg/l
Nitrate	0.28±0.01	0.30±0.02	0.32±0.02	0.38±0.01	0.25±0.1	0.37±0.1	50mg/l
Sulphate	10.92±0.01	8.46±0.02	9.07±0.01	8.26±0.01	10.99±0.02	13.26±0.01	250mg/l

Table 2: Mean Values of the Biological Parameters obtained from the water samples during the Study period.

Parameters	Station 1		Station 2		Station 3	
	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet season
THB (cfu/ml)	$3.8x10^4$	$4.8x10^4$	$4.5x10^4$	$5.6x10^4$	$3.8x10^4$	$5.8x10^4$
Total coliform (MPN/100ml)	23	26	22	25	20	29
Faecal coliform (MPN/100ml)	13	15	18	19	17	20

Table 3: Bacteria isolated from the Water Samples during the Study period

Isolates	NumericalAbundance (n=74)	RelativeAbundance (%n)
Pseudomonas sp	8	10.81
Escherichia coli	12	16.22
Proteus sp	8	10.81
Enterobacter sp	6	8.12
Salmonella sp	6	8.12
Shigella sp	4	5.41
Streptococcus sp	8	10.81
Vibrio sp	4	5.41
Staphylococcus aureus	6	8.12
Bacillus sp	12	16.22

4. Discussion

The examination of the physicochemical parameters showed that the pH of all the samples collected from the three stations were below WHO permissible limit of 6.5 - 8.5 (WHO, 2003, 2006). Result shows that pH values ranged from 6.7 to 7.2, indicating that the water sources were slightly alkaline. The range observed during the study could be attributed to the presence of alkaline metabolites. Temperature, salinity, dissolved oxygen (DO), conductivity, total dissolved solutes (TDS), Chemical Oxygen demand (COD), nitrate, phosphate, sulphate and alkalinity values of the water samples were within WHO guideline values for drinking water. BOD_5 in station one was within WHO permissible limits but values obtained in station 2 and 3 were above WHO standard for portable water. Turbidity, total suspended solids (TSS) values were above WHO standard. Turbidity and total suspended solids (TSS) relatively measures the physical or visual observable dirtiness of a water resource and are indicators of water pollution. The high values could be attributed to direct emptying of waste materials into the water source, a phenomenon that is common in Nigeria and Africa at large.

Analysis of the THB count in the water samples revealed the presence of heterotrophic bacteria in all the three stations were sampling was carried out. World standard as recommended by WHO for heterotrophic bacteria in potable water observe that the total heterotrophic bacteria count should not exceed 100cfu/ml (WHO, 2003, 2006). The presence of counts exceeding this limit indicates the presence of high concentration of bacteria that could make the water unsafe for drinking and domestic purpose. Result obtained from the study shows that the values of THB count ranged from $3.8x10^4$ in station 1 during the dry season to $5.8x10^4$ in station 3 during the wet season. These values exceeds WHO permissible limits for drinking water. This finding agrees favourably with the results of Uzoigwe and Agwa (2012) who reported high counts of total heterotrophic bacteria during their studies in some borehole water samples in Port Harcourt, Nigeria. THB counts that were higher than WHO permissible limits was also reported by Erah *et al*. (2002) in a separate research during their studies on the quality of groundwater in Benin City, Nigeria. Also, in a related studies by Akubuenyi *et. al*., (2013) who also reported high counts of THB during their studies on microbiological and physicochemical assessment of major sources of water for domestic uses in Calabar Metropolis, Cross River, Nigeria.

The concentration of total coliform and faecal coliform observed during the studies from the water samples exceeds WHO standard of 10MPN/100ml and 0MPN/100ml respectively (Table 1). This indicates that the water is not safe for drinking. The results of the present findings corroborates with the findings of Tula *et al*., (2013) in a related studies who reported high concentration of total coliform and faecal coliform. Obiri-Danso *et. al*., (2009), also observed high faecal coliform from wells and boreholes water in some peri-Urban communities in Kumasi, Ghana. Eniola *et. al.,* (2007) in a related study isolated some members of coliform in stored borehole water samples.

The bacteriological identification of 74 isolates obtained from the water samples revealed the presence of these genera: *Pseudomonas* 8(10.81%), *Escherichia coli* 12 (16.22%), *Proteus* 8 (10.81%), *Enterobacter* 6 (8.12%), *Salmonella* 6 (8.12%), *Shigella* 4 (5.41%), *Streptococcus* 8 (10.81%), *Vibrio* 4 (5.41%), *Staphylococcos aureus* 6 (8.12%), and Bacillus 12 (16.22%). These organisms are important human pathogens associated with a variety of infectious diseases such as typhoid fever, dysentery, cholera, gastroenteritis, and urinary tract infections etc (Orji, *et al.* 2006; Nwidu *et. al.,* 2008). Their presence raises serious public health concern because they are known causative agents of many water borne diseases and indicates that these water sources are not potable and safe for drinking. Their entry into water sources could be attributed to indiscriminate discharge of sewage

into waterways, surface run off from nearby communities around the study area. Although, deliberate and indiscriminate discharge of animal waste and human faeces into rivers are commonly observed in riverine areas. This can be attributed to lack of sanitary system in these communities, thereby encouraging open defecation into nearby rivers. Petridis *et. al*., (2002) opined that the presence of *Escherichia coli* which is the most common indicator of faecal pollution in a water sample is an indication of the presence of other enteric pathogens. The greatest risk to humans from water sanitary point of view is from faecal contamination of water. The sanitary quality of water therefore is based on the presence and density of faecal coliform or *E. coli* (WHO, 2003).

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11/10/2015