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Effects of Pollution on *Vibrios* in Woji River

OJESANMI, A S; IBE, S N

Department of Microbiology, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Rivers State, Nigeria

ABSTRACT: The effect of pollution on *Vibrio spp.* in five sampling stations along Woji River in Port Harcourt was studied in the months of April and November 2010. *Vibrio vulnificus, V. parahaemolyticus* and *V. alginolyticus* were isolated. The Plate count technique on Thiosulphate Citrate Bile Salt agar revealed a high population density of vibrios in the sampling stations than the Most Probable Number (MPN) technique. The average population density of vibrios ranged from 21MPN/100ml at Oginigba (station 1) to 1100MPN/100ml at Trans Amadi by slaughter (station 3) in April and 43MPN/100ml to 1100MPN/100ml in November respectively compared to plate counts that ranged from 2.2 x 10^5cfu/100ml to 1.6 x 10^8cfu/100ml in April and 3.2 x 10^5cfu/100ml to 2.6 x 10^8cfu/100ml in November respectively. The percentage proportion of *Vibrio spp.* to other heterotrophic bacteria ranged from 0.01 to 5.44% in April and 0.03 to 9.96% in November. The concentration of Total Dissolved Solids (TDS), Calcium, Magnesium, Hardness and chloride were much higher than the DPR/WHO limits and were not related to increase in presence of vibrios or their relative densities except for total dissolved solids. However, heterotrophic counts were high irrespective of the sampling station. There is therefore an urgent need to curtail the continued negative anthropogenic activities along the river course. @JASEM

The increasing and non-stop pollution of Woji River by industrial, abattoir and sewage effluents and the susceptibility of the inhabitants of the water fronts along the river course to water borne diseases and other environmental hazards have become a source of concern due to poor environmental and waste management initiatives. *Vibrios spp.* are autochthonous organisms of estuarine environments (Kaysner *et al*., 1987) and are commonly found in coastal marine waters and sea food throughout the world (TDSHS, 2009).

Several species of vibrios include clinically important human pathogens. *V. parahaemolyticus* is associated with gastroenteritis from eating undercooked seafood (Ndon*, et al,* 1992), *V. cholerae* causes cholera which is highly fatal and is associated with drinking contaminated water (WHO, 2001). *V. vulnificus* causes wound infection and septicaemia. Cholera outbreaks in Rivers State are rare. However, few cases were reported in Akuku Toru (15), Opobo/Nkoro (10), Andoni (5) and Degema (8) in 2010 (RSMH, 2010).

This research will therefore establish the levels of vibrios along the river course especially in areas of intense activity and help ascertain the rate of susceptibility of inhabitants to various water borne diseases associated with members of the vibrios.

MATERIALS AND METHODS

Study Area: Woji River is estuarine tidal water, a tributary of the upper Bonny River located between longitudes 7°00'' E and 7°15'' N and latitudes 4°28'' E and 4°40'' N. It arises from the bifurcation to the left of the Okpoka River, which drains into Bonny River. The area has a mean water depth of 4.8m, which is tidal and gradually transits from fresh to salt water at the head. Woji River receives industrial effluent discharges from the Nigerian Bottling Company, Schlumberger, Halliburton and Rivers State Vegetable Oil Company and transverses through several communities among which include Azuabie, Woji, Okuru-ama, Abuloma, Kalio-ama and Oba-ama. The Trans Amadi slaughter house and market generate wastes and faeces entering the River. *Water Sample Collection and Preservation:* Water samples were collected at five different sampling stations about 500meters apart along the river course and sampling was conducted twice in the year 2010 (April and November).

The sampling stations include: Station 1: Oginigba (Control), Station 2: Trans Amadi by Schlumberger, Station 3: Trans Amadi by slaughter, Station 4: Azuabie and Station 5: Okujagu-ama. One litre new plastic bottle was used to collect samples for microbiological analysis at a depth of 0.5m below the water surface and preserved in a picnic box with ice, before delivery to the laboratory and analysed within 3 hours. Some of the physicochemical water quality parameters that were determined on site (*in-situ*) include pH (pH meter, Hanna Instruments (HI) 9813), Conductivity (Conductivity meter, HI 9813), Dissolved Oxygen (Extech instrument, Model 407510A), Total Dissolved Solids (TDS meter, HI 9813), and Temperature (HI 9813). Samples were collected and preserved (<4^{o}C) for Anions, Cations, Total hardness, Salinity and Alkalinity analysis. Also, samples for BOD_5, Heavy metals (acidified using nitric acid pH<2), Oil and grease and Chemical Oxygen Demand (acidified using sulphuric acid pH<2) were collected and preserved separately before delivery to the laboratory.

Enumeration of Vibrios and Heterotrophs by Plate Count Method: Spread plate technique was used for the enumeration of *vibrios* and Total Heterotrophic count after ten-fold serial dilution with sterile distilled water as diluent. Aliquots of 0.1ml from each dilution were spread on Thiosulphate Citrate Bile Salt agar (TCBS) for vibrios and on nutrient agar plates in

***Email**: ojesanmiadesola_s@yahoo.com

duplicates for heterotrophs. Plates were incubated at 35°C for 48hours and colonies counted with a colony counter. Suspected *Vibrio sp* strains were transferred from TCBS media and identified with heterotrophs using a standard series of biochemical tests and the Bergey's Manual of Determinative Bacteriology, 1994.

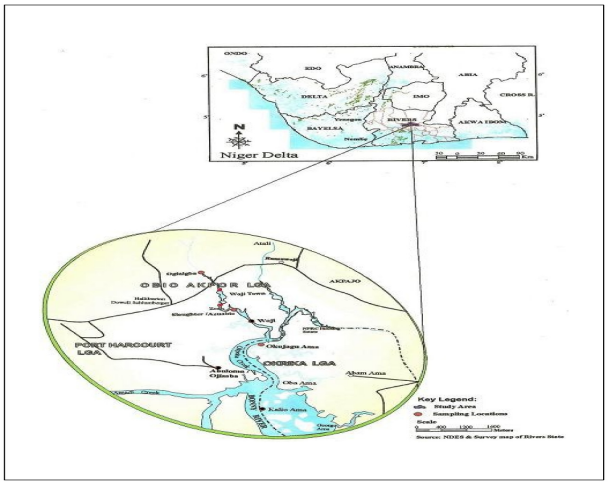

Fig 1: Map of study area showing the sampling stations

Enumeration of Vibrios by Most Probable Number Technique: Enumeration of *Vibrio spp.* was conducted using a 3-tube Most Probable Number (MPN) technique using Glucose Salt Teepol Broth (GSTB). The three sets of test tubes containing GSTB medium (purple) were as follows: 3 x 10ml (double strength), 3 x 10ml (single strength), 3 x 10ml (single strength). The tubes were inoculated with 10ml, 1ml and 0.1ml aliquots of water sample respectively and incubated overnight at 37°C and checked for turbidity and colour change to yellow. Most Probable Number (MPN) table was used to calculate the presumptive number of organisms per 100ml (MPN/100ml). In the confirmatory test, the positive tubes were streaked on Thiosulphate Citrate Bile Salt Agar (Fluka 86348) plates and incubated at 37°C. The completed test was conducted on colonies by series of biochemical characterization which include Gram's reaction, Motility test, Catalase test, Oxidase test, growth in 7% NaCl, growth in 0% NaCl, Indole test, Triple Sugar Iron agar test, Urease test, Citrate test, Methyl red test, Voges Proskauer and Starch hydrolysis tests (Speck, 1976).

RESULTS AND DISCUSSION

The results of the biochemical characteristics of *Vibrio* isolates are shown in Table 1. The population density of vibrios revealed that MPN technique gave a much lower number for vibrios than the plate count method. The *Vibrio spp.* ranged from 21MPN/100ml (Station 1/Oginigba) to 1100MPN/100ml (station 3/Trans amadi by Slaughter) in April and 43MPN/100ml to 1100MPN/100ml in November respectively. Similarly, average count of *Vibrio spp.* in the thiosulphate citrate bile salt agar ranged from 2.2 x 10^5cfu/100ml to 1.6 x 10^8cfu/100ml in April and 3.2 x 10^5cfu/100ml to 2.6 x 10^8cfu/100ml in November respectively at the two seasons (Table 2).

Likewise, the population density of the heterotrophs in April and November ranged from 1.8 x 10^9cfu/100ml to 2.94 x 10^9cfu/100ml and 1.03 x 10^9cfu/100ml to 2.61 x 10^9cfu/100ml respectively at the two seasons. Furthermore, the population of *Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio alginolyticus* were higher in the dry season than in the wet season due to dilution effect of rain water on the river water. The heterotrophic bacterial isolates identified in the water sample included: *Micrococcus sp, Salmonella sp, Shigella sp, Escherichia coli, Bacillus spp., Klebsiella spp., Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter spp., Serratia spp.,* and *Proteus spp.* The proportion of *Vibrios* to other heterotrophs in Woji River in April and November ranged from 0.01 to 5.44% and 0.03 to 9.96% respectively.

Oil and grease concentration of 25.9mgL^{-1} was recorded in station 2 (Trans Amadi by Schlumberger) due to the discharge of industrial effluents. Similarly, there was no significant effect of pH on vibrios as this was near neutral at all stations. The heavy metals recorded the highest concentration in station 2 (Trans Amadi by Schlumberger) and station 3 (Trans Amadi by Slaughter) with average concentrations of: Copper (2.85 mgL^{-1}, 1.20 mgL^{-1}), Iron (1.6 mgL^{-1}, 1.26 mgL^{-1}), Zinc (16.75 mgL^{-1}, 12.5 mgL^{-1}) and Lead (0.10 mgL^{-1}, 0.03 mgL^{-1}).

Table 1: Biochemical characteristics of *Vibrio* isolates

	Vibrio spp.		
	Vibrio parahaemolyticus	*Vibrio alginolyticus*	*Vibrio vulnificus*
Characteristics			
Pigment on TCBS	Green	Yellow	Green
Gram reaction	Negative rod	Negative rod	Negative rod
TSI	Alkaline/Acid	Acid/acid	Acid/acid
Urease	-	-	-
Citrate	-	-	-
Methy red test	-	+	-
Starch hydrolysis	-	+	-
Glucose	+	+	+
Lactose	-	+	-
Growth at 37ºC	+	+	+
Catalase	+	+	+
Motility	+	+	+
Indole	+	+	+
Voges Proskauer	-	+	-
Oxidase	+	+	+
Growth in 0% NaCl	-	-	-
Growth in 7%NaCl	+	+	+

Note: - Negative test, + Positive test

Table 2: Total viable count of *Vibrio spp.* at various sampling stations

	Plate count method							
	April				November			
Station	Total count (cfu/100ml)	*Vibrio alginolyticus* (cfu/100ml)	*Vibrio vulnificus* (cfu/100ml)	*Vibrio parahaemolyticus* (cfu/100ml)	Total count (cfu/100ml)	*Vibrio alginolyticus* (cfu/100ml)	*Vibrio vulnificus* (cfu/100ml)	*Vibrio parahaemolyticus* (cfu/100ml)
1 (Oginigba)	2.2×10^5	0.6×10^5	0.9×10^5	0.7×10^5	3.2×10^5	0.3×10^5	1.4×10^5	1.5×10^5
2 (Trans Amadi by Schlumberger)	6.3×10^5	2.30×10^5	1.5×10^5	2.50×10^5	1.24×10^6	0.2×10^5	0.7×10^5	0.34×10^5
3 (Trans Amadi by Slaughter)	1.6×10^8	0.3×10^8	0.5×10^8	0.8×10^8	2.60×10^8	0.5×10^8	0.8×10^8	1.3×10^8
4 (Azuabie)	2.6×10^5	0.7×10^8	0.8×10^8	1.1×10^8	3.6×10^5	0.3×10^5	1.6×10^5	1.7×10^5
5 (Okujagu-ama)	2.8×10^5	0.4×10^5	1.1×10^5	1.3×10^5	1.10×10^6	0.1×10^5	0.4×10^5	0.6×10^5

The average values of the physicochemical parameters and bacterial load examined at the various stations are shown in Table 3. Furthermore, the values for some parameters namely: Total Dissolved Solids (TDS), Calcium, Magnesium, Hardness and chloride were much higher than the DPR/WHO limits (Table 4).

The overall data was comparable to the values previously reported on physicochemical quality of Trans-Amadi (Woji) creek by Davis *et al*. (2008), showing high pollution of the river. It was observed that the increase in these values for the various stations were not related to increase in presence of vibrios or their relative densities except for total dissolved solids that the counts of vibrios were increased three fold from station 1 (Table 3). Heterotrophic counts were high irrespective of the sampling station but higher at the abbatoir point.

Studies have shown that organic nutrients (sulphates, nitrates and phosphates) from industrial and abbatoir wastes have been responsible for a significantly high microbial type both in the effluent and receiving water body (Ezeronye and Amogu, 1998), as the highest vibrio counts was observed at sampling stations with proximity to the discharge point of industrial and abbatoir effluents.

However, there was no consistent trend in the increase of vibrios across the sampling stations in both climatic regimes. Biochemical Oxygen Demand, Dissolved Oxygen, Chemical Oxygen Demand and Salinity did not significantly affect the vibrio count in the sampling stations.

Table 3: Average Physicochemical parameters and Bacterial load at sampling stations of Woji River

	Stations					WHO/DPR limits
Parameters (Average)	1	2	3	4	5	
Vibrio spp. (MPN/100ml)	71	290	1100	152	41	
Vibrio spp. (cfu/100ml)	2.7×10^5	9.9×10^5	2.1×10^8	3.1×10^5	6.4×10^5	-
Heterotrophs (cfu/100ml)	1.5×10^9	1.6×10^9	2.8×10^9	2.3×10^8	1.5×10^8	-
pH	6.57	6.73	6.33	6.51	6.47	6.5 – 9.2
Electrical conductivity (µS/cm)	14525.5	14758.5	14962	14885	15075	
DO (mgL^{-1})	4.61	5.07	4.85	4.82	4.92	
BOD_5 (mgL^{-1})	3.23	3.28	3.11	3.29	3.17	
COD (mgL^{-1})	6.52	6.61	6.28	6.62	6.39	
Magnesium (mgL^{-1})	3908	4035	3848	4097	4175	0.5
Chloride (mgL^{-1})	18032	18215	18840	18705	18430	600
Hardness (mgL^{-1})	4415	4636	4662	4785	4542	500
Salinity (‰)	13.61	11.79	9.95	13.55	11.62	
TDS (mgL^{-1})	4625	7395	10170	9260	7525	1500
Oil and grease (mgL^{-1})	<0.01	25.9	1.9	<0.01	<0.01	
Ammonium (mgL^{-1})	0.24	0.29	0.27	0.27	0.25	
Nitrate (mgL^{-1})	0.64	0.73	0.89	0.78	0.69	
Phosphate (mgL^{-1})	580.17	592.13	594.25	575.37	571.95	
Sulphate (mgL^{-1})	381.65	404.97	412.01	394.39	375.84	400
Alkalinity (mgL^{-1})						
Copper (mgL^{-1})	<0.01	2.85	1.20	0.38	0.15	1.5
Iron (mgL^{-1})	<0.01	1.6	1.26	0.05	0.08	1
Zinc (mgL^{-1})	1.17	16.75	12.5	8.15	2.21	15
Lead (mgL^{-1})	<0.01	0.10	0.03	<0.01	<0.01	-

Table 4: Seasonal variation of physicochemical parameters and bacterial load

Parameter	Mean values of stations		DPR/WHO limit
	April	November	
Vibrio spp. (MPN/100ml)	275^b	387^a	-
Vibrio spp. (cfu/100ml)	$3.23x10^{7a}$	$5.26x10^{7a}$	-
Heterotrophs (cfu/100ml)	$1.46x10^{9a}$	$1.02x10^{9a}$	-
Ph	$6.3±0.16^{*a}$	$6.78±0.12^a$	6.5-9.2
Temperature (°C)	$27.78±0.47^a$	$28.39±0.40^a$	-
Electrical conductivity (µS/cm)	$15205±244.35^a$	$14477±199.57^b$	-
DO (mgL^{-1})	$4.38±0.17^b$	$5.18±0.13^a$	-
Salinity (‰)	$9.83±0.83^b$	$14.37±3.40^a$	-
TDS (mgL^{-1})	$8468±24.2^a$	$7122±1937.01^b$	1,500*
BOD_5 (mgL^{-1})	$3.10±0.08^a$	$3.33±0.09^a$	-
COD (mgL^{-1})	$6.26±0.16^a$	$6.17±0.18^a$	-
Calcium (mgL^{-1})	$851.09±6.34^a$	$894.64±12.54^a$	1.50*
Magnesium (mgL^{-1})	$3807.4±147.34^b$	$4217.4±164.57^a$	0.5*
Hardness (mgL^{-1})	$3673.4±121.44^b$	$5542.6±220.42^a$	500*
Chloride (mgL^{-1})	$18366.8±430.42^a$	$18521.6±249.97^a$	600*
Alkalinity (mgL^{-1})	$85.59±6.56^a$	$83.58±3.37^a$	-
Ammonia (mgL^{-1})	$0.26±0.07^a$	$0.26±0.04^a$	-
Nitrate (mgL^{-1})	$0.77±0.14^a$	$0.72±0.07^a$	-
Phosphate (mgL^{-1})	$680.91±9.46^a$	$484.66±14.32^b$	-
Sulphate (mgL^{-1})	$439.88±14.90^{*a}$	$347.67±18.16^b$	400
Oil and grease (mgL^{-1})	$9.48±4.58^b$	$13.31±6.54^a$	-
Copper (mgL^{-1})	$1.13±0.79^a$	$1.24±1.04^a$	1.5
Iron (mgL^{-1})	$0.76±0.57^a$	$0.62±0.28^a$	1.0
Zinc (mgL^{-1})	$7.59±6.23^b$	$8.72±7.12^a$	15
Lead (mgL^{-1})	$0.022±0.04^a$	$0.028±0.04^a$	-

*Exceeds the maximum permissible limits by DPR/WHO; Mean values with the same letter in the column are not significantly different (P>0.05)

In the light of the aforementioned, the need for concerted environmental surveillance and usefulness of vibrios as a sensitivity index for organic pollution in brackish ecosystems is therefore encouraged.

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