Global Journal of Environmental Sciences. ISSN 1596-6194. Vol. 2, No 2, 2003. 98 - 101

HEAVY METAL BIOSORPTION BY THREE BACTERIA ISOLATED FROM A TROPICAL RIVER

*L. O. ODOKUMA AND *A. E. ABAH

(Received 29 January 2002; Revision Accepted 30 May 2003)

ABSTRACT

Bioaccumulation (bioconcentration) of four heavy metals cadmium, lead, zinc and nickel by three bacteria *Bacillus*, *Staphylococcus* and *Pseudomonas* as a tool for the decontamination of heavy metal impacted aquatic systems was investigated . The bacteria were obtained from the New Calabar River. Monitoring of the physicochemical parameters of the river water and sediment revealed upstream downstream increases in their levels. Ranges for riverwater parameters were temperature (23 to 27°C), pH (5.34 to 5.84), alkalinity (12.0 to 18.8mg $CaCO_3$/L), dissolved oxygen (6.24to 6.92mg/L), total suspended solids (1.13 to 1.61mg/L), conductivity (2.15 to 2.39 S/m), chloride (3.0 to 10.20mg/L), biochemical oxygen demand (2.0 to 2.5mg/L) and chemical oxygen demand (4.0 to 10.0mg/L). Ranges for sediment parameters were temperature (20 to 25°C), pH (5.3 to 6.97), conductivity (2.55 to 4.22 S/m), dissolved oxygen (3.2 to 4.22mg/L), chloride (3.5 to 15.3mg/L), biochemical oxygen demand (5.5 to 10.5mg/L) and chemical oxygen demand (20.7 to 100.6 mg/L). River water heavy metals levels were lower than sediment levels. The river water ranges were; Cu (0.01 to 0.05mg/L), Pb (0.01 to 0.02mg/L), and Zn (0.01 to 0.07 mg/L). Sediment heavy metal ranges were Cu (1.09 to 1.45 mg/L), Pb (0.03 to 0.07mg/L) Cd (nd to 0.009mg/L) and Zn (0.27 to 1.27mg/L). The percentage accumulation of heavy metals by *Bacillus*, *Staphylococcus and Pseudomonas* after 24h of exposure to heavy metals were Cadmium; 68.6% , 58.4% and 28.3%, Nickel; 94.5% , 85.7% and 90.8%, Lead; 91.6%, 68.1% and 52.9%, Zn; 71.6% 72.1% and 77.0% respectively. The ease of bioaccumulation of the metals by bacteria showed the following trend N_i > Zn > Pb > Cd. Cadmium was the most toxic of the metals to the bacteria. Lead and Zinc displayed similar levels of toxicity, while Nickel was the least toxic. Bioaccumulation potentials of the three bacteria indicated that *Bacillus* showed the highest potential this was followed by *Staphylococcus* while *Pseudomonas* showed the least potential. These results indicate that bacteria especially *Bacillus* may be employed in the bioremoval of heavy metals from tropical aquatic environments impacted with heavy metals.

Key Words: Bioaccumulation, Bioconcentration Heavy metals, Decontamination, Bacteria.

INTRODUCTION

Anthropogenic activities have resulted in elevated concentrations of metals in many aquatic environments (Ankley *et al.*, 1994). Chronic and acute heavy metal pollution arises from a number of anthropogenic sources, including petroleum industry activities, leaching of metals from garbage and solid waste dumps, metal mining and refining, manufacturing, fossil fuel combustion, industrial emissions, agriculture pesticides, and domestic and industrial effluent discharges (Van Loosdrecht *et al.*, 1987). Heavy metal concentrations in polluted environments vary considerably e.g. the concentration of zinc in polluted sediments are often between 0.5 and 600ppm McEdlowney, 1994). Heavy metals have a great ecological significance due to their toxicity and accumulative behaviour (Numberg, 1983) and they constitute a large class of inorganic chemicals. The metals may exist in several oxidation states each with different reactive toxicological, physiological and bioconcentration potentials. Many metals such as cadmium, lead and zinc are toxic in their original cationic forms while others require biochemical transformation to organic forms prior to been toxic. These elements contrary to most organic pollutants are not biodegradable and undergo a global ecobiological cycle (Numberg, 1983) in which natural waters are the main pathways.

Bacteria occur as free living cells in aquatic and terrestrial ecosystems or as monolayers and biofilms attached to solid substrata. Binding of metals to cells is closely related to the composition of cell walls and to the nature of the cation. Accumulation is also dependent on speciation of the metal, which may be highly variable in the environment. Furthermore uptake of metals by bacterial cells is affected by the presence of other cations that compete with metals for anionic sites and for tranport (Boularbah *et al.*, 1992). The metabolism of elements occurs in many species of bacteria. Four major type of metal transformation in organisms have been observed. These are: chelate formation by the binding of metals to organic ligands, shifts in metal valences, substitution of one metal for another and biomethylation of metals. Where metals are chelated to substrate molecules the adsorption of these molecules may represent a means, of accumulating metals within a cell. The oxidation of extracelluar polymers could also result in metal accumulation within cells. Biomethylation may also result in accumulation of metals.

In aquatic systems heavy metals are taken up by microorganisms, fauna and flora either directly or indirectly as in the case of fauna through the consumption of smaller flora and fauna. This uptake could provoke an increase in the concentration of the metal in the organism. If the excretion phase is slow, this can lead to bioaccumulation. When these metals move up the food chain, the process results into biomagnification. Studies have been conducted in the use of bacteria to bioconcentrate and thereby subsequently clean-up heavy metal polluted waters. In this study three bacterial

*Dept. Of Microbiology, Faculty of Science, University of Port Harcourt, Nigeria

genera *Bacillus*, *Staphylococcus* and *Pseudomonas* commonly found in the New Calabar River, a river that has been continuously subjected to industrial effluent discharges were examined for their ability to accumulate metals predominantly found in the industrial effluents and in the river water and sediment. Thus the organism with the highest bioaccumulation potential or a mixed population of many of such microbial species may subsequently be employed in the clean-up of heavy metal impacted tropical aquatic systems.

MATERIALS AND METHODS AREA OF STUDY

River water and sediment samples were collected from three sites along the New Calabar River (Fig.1) the sites are marked 1, 2, and 3, in Fig 1. The river is a short coastal river about 150 200km in length. It consists of brackish water (Odokuma and Okpokwasili 1993a, b, Odokuma and Okpokwasili 1997) and it is under the influence of tidal cycles. The river is subjected to effluent discharges from industries sited along its bank, surface run-off, soil erosion, lumbering activities and domestic sewage impacts. Three main industries discharge their effluents into the river; an oil service industry whose activities include building and repair of oil pipelines; a fibre industry; and the third industry involved in the building and servicing of barges, tugboats and other smaller river craft.

WATER SAMPLE COLLECTION

Water (0-30cm depth) and sediment samples were collected from each of the three stations. Water samples for microbiological analysis were collected in sterile 1.5L plastic cans previously rinsed with 95% ethanol. Sediment samples were collected with an Eckman grab sampler, into plastic polyethene bags. Samples were collected during the low tide. Water samples for physicochemical analysis were also collected in clean 1.5L plastic cans. However these were not rinsed with alcohol.

Water temperatures were recorded in the field using mercury in glass thermometer. All other parameters (microbiological and physicochemical) were analyzed on reaching the laboratory.

DIGESTION OF SEDIMENT SAMPLES

Sediment samples were digested with a mixture of concentrated nitric acid, perchloric acid and sulphuric acid (APHA 1998).

HEAVY METALS

The salts of heavy metals used in this study were $PbCl_2$, $CdCl_2$, $NiNO_3$ and $ZnSO_4$. One gram (1g) equivalent of each of the metals in the different metal salts were weighed out (1.343g, 1.631g, 2.056g and 2.469 of $PbCl_2$, of $CdCl_2$, N_iNO_3, and ZnSO, respectively) and dissolved in 1000ml of deionized water to make 1000mg/L. Tenfold serial dilutions were now performed to obtain 100mg, 10mg 1mg and 0.1mg per litre of each metal.

MICROBIAL ANALYSIS

Pure cultures of *Bacillus*, *Staphylococcus* and *Pseudomonas* were obtained by inoculating river water (0.1ml) on selective medium, Bacillus agar for *Bacillus*, Pseudomonas selective agar for *Pseudomonas* and Mannitol salt agar for *Staphylococcus*. Isolates from these media were transferred to slants of these media and further characterized by the criteria of Krieg and Holt (1994).

PRELIMINARY RANGE FINDING TESTS

A loopful of a 24h pure culture of the isolates was introduced into about 100ml of sterile normal saline. This was adjusted to bring the cell concentration to about 1x 10^5Cfu/ml. One millilitre each of the standard inoculum was pipetted into the different concentrations of the heavy metals. Nutrient agar plates were immediately inoculated using spread plate technique (APHA 1998). This was for zero hour. Plates of various salt concentrations were inoculated after 2,4,12 and 24h. Both plates and tubes were incubated at room temperature 282°C for 24h. The median 50% lethal concentration (LC_{50}) was determined by plotting mortality against concentration of metal on a Probit graph. Regression analysis was used to obtain the line of best fit (Finney, 1978). Analysis of variance and the Least Significant test were also performed (Finney, 1978). A tenth (0.1) of the LC_{50} was used for bioaccumulation studies.

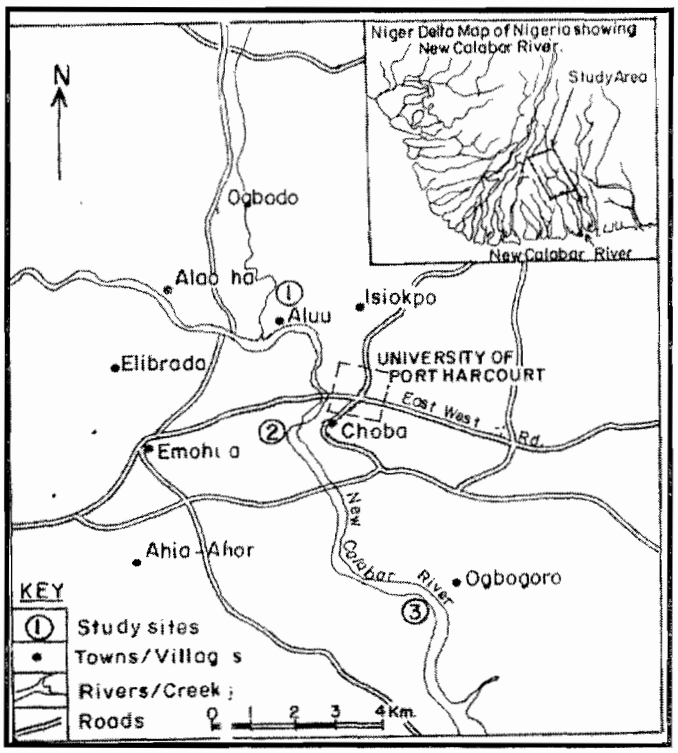

Fig 1: A Map showing the three sampling sites (1-3) of the New Calabar River.

BIOACCUMULATION TEST

The method of Bauda and Block (1985) and Langely and Beveridge (1999) were employed for bioaccumulation tests. Pure cultures of *Bacillus*, *Staphylococcus and Pseudomonas* were used. The test organisms were grown in nutrient broth medium for 15h and then harvested by centrifugation at 6000 x g for 10 min in a centrifuge (Model 114 Shanghai Analytical Instrument Co.). The cells were washed thrice with sterile 0.1m Tris (hydroxyl/methyl amino methane) buffer at pH 7.3 and the supernatant decanted. Three milliliters of the cells were resuspended in appropriate concentration (one tenth of the LC_{50}) of the metal solution for 24h at room temperature (282°C). After incubation with the metals, the cells were centrifuged at 6000 x g for 5 minutes. and the supernatant solution removed acidified with 0.2% v/v HNO_3 and stored at 4°C. The washing procedure was repeated twice and each was

acidified and stored at 4°C. The concentration of metals in the supernatant before incubation of cells with metals and after incubation of cells with metals were determined using model AA 320 atomic absorption spectrophotometer (Shanghai Analytical Instrument Co).

The percentage (% of metal accumulated) was calculated by using the formula of Langely and Beveridge (1999).

$$\frac{I - F}{I} \cdot 100$$

Where I = The initial metal concentration in the supernatant

F= Final metal concentration in the supernatant.

PHYSICO-CHEMICAL ANALYSIS.

Parameters such as dissolved oxygen (DO), biochemical oxygen demand (BOD), chloride, conductivity, alkalinity, and pH were determined employing methods from APHA (1998). Lead, cadmium nickel and zinc were determined using model AA320 atomic absorption spectrophotometer (Shanghai Analytical instrument Co). Chemical oxygen demand (COD was determined by using dichromate oxidation method APHA (1998).

RESULTS AND DISCUSSION

Upstreamdownstream increases in levels of physicochemical parameters of river water and sediment are presented in Tables 1 and 2. Ranges for river water parameters were temperature (23 to 27°C), pH (5.34 to 5.84), alkalinity 12.0 to 18.8mg $CaCO_3$/L) dissolved oxygen 6.2 to 6.9mg/L), total suspended solids (1.13 to 1.61 mg/L), conductivity (2.15 to 2.39 S/m), chloride (3.0 to 10.2mg/L), BOD (2.0 to 2.5mg/L) and COD (4.0 to 10.0mg/L). Ranges for sediment parameters were temperature (20 to 25°C), pH (5.3 to 6.97), conductivity (2.55 to 4.22S/m), dissolved oxygen (3.2 to 4.22mg/L), chloride (3.5 to 15.3 mg/L), BOD (5.5 to 10.5mg/L) and COD (20.7 to 100.6mg/L). These observations are similar to those made by Odokuma and Okpokwasili (1993; 1997) and Odokuma and Ijeomah (2003). They attributed these observations to increased nutrient load resulting from inputs from industrial discharges, erosion and surface run off. In the case of temperature the insulating effect of increased load with progress downstream may have raised the temperature by several degrees (Alabaster and Lloyd, 1980). Sediment values for most parameters were generally higher than river water values. This may be because the sediment served as a sink for most inorganic and organic constituents of the river, which influences the physico-chemical characteristics of the river. Also the relative mobility of the river water over the sediment does not allow for the accumulation of these constituents in the river water.

The higher heavy metal levels of sediment over that of river water (Tables 3 and 4) may also be attributable to the same reasons above. The river water heavy metal ranges were Cu (0.01 to 0.05mg/l), Pb (0.01 to 0.02 mg/L), and Zn (0.01 to 0.07mg/L). Sediment heavy metal ranges were Cu (1.09 to 1.45mg/L), Pb (0.03 to 0.07mg/L), Cd (nd to 0.009mg/L) and Zn (0.27 to 1.27mg/L). The heavy metal levels of the river water are similar to those observed by Odokuma and Ijeomah (2003) in the same river. Odokuma and Ijeomah (2003) have suggested that these heavy metal levels in the river water may not result from anthropogenic activities but rather be of allochthonous (intrinsic) origin. They observed that the effluent levels of heavy metals from industries sited along the river were much smaller than the existing river water heavy metal levels.

The 24h LC_{50} of the four heavy metals to *Pseudomonas*, *Staphylococcus* and *Bacillus* are presented in Table 5. The 24h LC_{50} of Cd, Ni, Pb and Zn to *Pseudomonas were* 0.48, 14.0, 2.2 and 2.3mg/L respectively. The 24h LC_{50} of Cd, Ni, Pb and Zn to *Staphylococcus* were 0.63, 47.0, 1.2 and 1.50 mg/L, while the 24h LC_{50} of Cd, Ni, Pb and Zn to *Bacillus* were 0.52, 15.0, 1.3 and 16.0 mg/L respectively. These results indicated that cadmium was the most toxic of the metals while nickel was the least toxic. Lead and zinc displayed similar levels of toxicity. Toxicity of heavy metals to microbial systems is attributable to a number of factors including solubility in aqueous systems (Gadd, 1990). Studies by Doelman and Haaustra (1979), Gadd, (1990), Silver and Walderhang (1992) have observed the order of toxicity of heavy metals to follow the trend Cd> Pb > Ni > Zn. A similar trend Cd > Pb Zn > Ni was observed in this study.

The percentage accumulation of heavy metals by the three organisms was a reflection the degree of toxicity of the metals (Table 6). The percentage accumulation of Nickel by the three organisms was the highest of the four metals. Percentage accumulation followed the trend Ni> Zn Pb > Cd. This was the reverse of toxicity. Percentage accumulation was therefore related to toxicity in aqueous medium. *Bacillus* generally accumulated more metals than the other two organisms. This was followed by *Staphylococcus*. The percentage accumulation by *Pseudomonas* was significantly less than *Staphylococcus* for cadmium and lead at 95% probability level. These results may suggest that Gram-positive organisms (*Bacillus* and *Staphylococcus*) accumulated these metals (Cd and Pb) better than the Gram-negative bacteria (*Pseudomonas*). The trend was however different in the accumulation of zinc, where there was no significant difference at 95% probability level in the accumulation of this metal by the three organisms. This may mean that specific organisms are better off for specific heavy metal accumulation. Similar observations have been made by Boularbah *et al.*, (1992). In this study it seems that Gram positive bacteria seem to accumulate more of these metals than Gram negative bacteria. Reasons for this trend are not yet known. However studies have revealed that bacteria remove toxic metals via adsorption to the cell surface binding with bacterial cell envelopes and complexation by exopolysaccharide, (Hider, 1984; Lester *et al.*, 1984; Wood and Wang 1983). Gram-positive organisms virtually do not have Lipopolysaccharides (Stanier *et al.*, 1982) however the peptidoglycan layer of the cell wall is very thick and multilayered (Stanier *et al.*, 1982). This may assist the organism to accumulate these metals better than metals better than Gram negative bacteria.

Table 1: Some Physicho-Chemical Parametres of the River Water

PARAMETER	LOCATION 1	2	3
Temperature (°C)	23±0.2	25±0.1	27±0.15
PH	5.34±0.1	5.6±0.1	5.84±0.1
TSS (mg/L)	1.13±0.01	1.55 ±0.01	1.61±0.01
Conductivity (n$/m)	2.15±0.01	2.24±0.01	2.39±0.01
Dissolved Oxygen (DO) (mg/L)	6.24±0.5	6.58±0.5	6.92±0.5
Biochemical Oxygen Demand (BOD) (mg/L)	2.0±0.01	2.20±0.01	2.50 ±0.01
Chemical Oxygen Demand (COD) (mg/L)	4.0±0.2	7.0 ±0.3	10.0±0.2
Chloride (mg/L)	3.00±0.01	3.26±0.1	10.20±0
Alkalinity (mg $CaCO_3$/L)	12.0±0.8	16.4 ±0.5	18.8±0.5

TABLE 2: PHYSICO-CHEMICAL PARAMETERS OF THE SEDIMENT.

PARAMETER	LOCATION		
	1	2	3
Temperature (°C)	20±0.1	23±0.2	25±0.2
PH	5.3±0.1	5.86±0.1	6.97±0.1
Conductivity (mS/m)	2.55±0.04	3.25±0.01	4.22±0.01
Dissolved Oxygen (DO) (mg/L)	3.2±0.2	3.5±0.3	4.22±0.2
Biochemical Oxygen Demand (BOD) (mg/L)	5.5±0.5	7.4±1.0	10.5±1.5
Chemical Oxygen Demand (COD) (mg/L)	20.7±2.0	50.5±5.5	100.6±0.10
Chloride (mg/L)	3.5±0.3	4.0±0.8	15.30±0.1
Alkalinity (mg $CaCO_3$/L)	16.5±2.5	20.3±3.5	28.4±5.5

TABLE 3: HEAVY METAL STATUS OF THE NEW CLABAR RIVER WATER.

Location	Metal (mg/Kg)			
	Cu	Pb	Cd	Zn
1	0.01	0.02 ±0.01	nd	0.01
2	0.03 ±0.01	0.01	nd	0.07 ±0.01
3	0.05 ±0.01	0.01	nd	0.07 ±0.01

nd = Not detected (<0.001)

TABLE 4 HEAVY METAL STATUS OF THE NEW CALABAR RIVER SEDIMENT

Location	Metal (mg/Kg)			
	Cu	Pb	Cd	Zn
1	1.35 ±0.01	0.07 ±0.01	0.009 ±0.001	0.31 ±0.0
2	1.09 ±0.01	0.03 ±0.01	nd	0.27 ±0.0
3	1.45 ±0.01	0.06 ±0.01	nd	1.27 ±0.0

nd = Not detected (<0.001)

TABLE 5: 24h LC_{50} OF HEAVY METALS TO THREE BACTERIAL ISOLATES

ISOLATE	Cd	Ni	Pb	Zn
Pseudomonas	0.48±0.05	14.0 ±1.5	2.2±0.5	2.3±0.8
Staphylococcus	0.63±0.06	47.0±2.5	1.2±0.8	1.5±0.5
Bacillus	0.52±0.05	15.0±0.6	1.3±0.53	16.0±1.5

TABLE 6: PERCENTAGE (%) ACCUMULATION OF HEAVY METALS BY THREE BACTERIAL ISOLATES.

ISOLATE	Cd	Ni	Pb	Zn
Pseudomonas	28.3±0.6	90.8±1.0	52.9±05	77.1±0.5
Staphylococcus	58.4±08	85.7±0.8	68.1±0.5	72.1±0.5
Bacillus	68.6±0.5	94.5±0.0	91.6±0.8	71.6±0.5

CONCLUSION

The three bacteria genera isolated from the New Calabar river water (*Bacillus*, *Staphylococcus* and *Pseudomonas*) have the ability to bioaccumulate heavy metals. This accumulation potential is however greatest with *Bacillus*. Of the four heavy metals (Ni, Zn, Pb and Cd) cadmium was the most toxic to the three bacteria while nickel was the least toxic. The converse was the case for bioaccumulation. Nickel was the most bioaccumulated while cadmium was the least bioaccumulated. These results suggest that removal of heavy metals from heavy metal impacted tropical aquatic environments can be effected by bacteria especially *Bacillus*.

REFERENCES

Alabaster, J. S., and Lloyd, R., 1980. Water Quality for fresh fish. 1st ed. Butterworths London, p. 283.

Ankley, G.T., Mattson, V.R., Leonard, E.N., 1994. Prediction of bioaccumulation of metals from contaminated sediments by the Oligochaete. L. *Variegatus*. Water Research 28:1071-1076.

APHA, 1998. American Public Health Association (APHA), Standard Methods for the Examination of Wastewater and Water Washington DC, USA.

Bauda, P. and Block, J.C., 1985. Cadmium Biosorption and Toxicity to Laboratory Grown Bacteria. Environment and Technology Letters 6:445 454.

Boularbah, A., Morel, I.L., Bitton, G. and Guckert A., 1992. Cadmium Biosorption and Toxicity to six cadmiumresistant, grampositive bacteria isolated from contaminated soil. Environmental Toxicology and Water Quality 7:237-246.

Doelman, P. and Haaustra, L., 1979. Effects of Lead on the soil bacteria microflora Soil Biology and Biochemistry 11:487-491.

Finney, D.J., 1978. Statistical Methods in Biological Assay 3rd Edition. Charles Griffin, London. pp1-252.

Gadd, G.M., 1990. Metal Tolerance: In Environmental Biotechnology, Microbiology of Extreme Environments Edward I (ed) McGraw Hill, New York pp178 210

Hider, R.C., 1984. Siderophore mediated absorption of iron. Structure Bonding 58:26.

Krieg and Holt (eds.), 1994. Bergey's Manual of Determinative Bacteriology. 9th Edition. Williams and Wilkins, Baltimore Maryland, USA.

Langley, S. and Beveridge T.J., 1999. Effect of *o*-side chain lipopolysaccharide chemistry on metal binding. Applied Environmental Microbiology, 65: 489 498.

Lester, J.N., Sterret, R. M., Rudd, T. and Brown, M.J., 1984. Assessment of the role of bacterial extracellular polymers, in controlling metal removal, in biological waste water treatment. In; Microbiological Methods of Environment Biotechnology Grainger, I. M. and Lynch, J.R. (eds.) Academic Press London pp 440 451.

McEdlowney, S., 1994. Effect of cadmium and zinc on attachment and detachment interactions of *Pseudomonas*, *Flourescens* H_7 with Glass. Applied and Environmental Microbiology, 60:2759 2765.

Numberg, H.W., 1983. Investigations on heavy metal speciation in natural waters by voltametric procedure Analytical Chemistry 316: 557 565.

Odokuma, L.O. and Okpokwasili, G.C., 1993a Seasonal ecology of hydrocarbon utilizing microbes in the surface waters of a River. Environmental Monitoring and Assessment 27:175 199.

Odokuma, L.O. and Okpokwasili, G.C., 1993b Seasonal influences on inorganic anion monitoring of the New Calabar River, Nigeria. Environmental Management 17(4): 491 496.

Odokuma, L.O and Okpokwasili G.C., 1997 Seasonal influences of the organic pollution monitoring of the New Calabar River Nigeria. Environmental Monitoring and Assessment 45: 43-56.

Odokuma, L.O. and Ijeomah, S.O., 2003: Seasonal changes in the Heavy metal resistant bacterial population of the New Calabar River. Global Journal of Pure and Applied Sciences Vol.9, No.2 (in press).

Silver, S. and Walderhang, M., 1992. Gene regulation of Plasmid and Chromosome determined inorganic ion transport in bacteria. Microbiological Reviews 56: 195 228.

Stanier, R. Y., Adelberg, E. A. and Ingramham, J. L., 1982. General Microbiology. Macmillan, New York, NY, 4th ed.

Van Loosdrecht, M.C.M., Iyklema, J. Noode, W., Schraa, G. and Zelinder, A.J.B., 1987. The role of bacteria Cell Wall hydrophobicity in adhesion. Applied Environmental Microbiology 53:1893 1897.

Wood, J.M. and Wang, H., 1983. Microbial Resistance to Heavy metals. Environmental Science Technology 17: (12): 583A 590A.