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SEASONAL CHANGES IN THE HEAVY METAL RESISTANT BACTERIAL POPULATION OF THE NEW CALABAR RIVER, NIGERIA

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ABSTRACT

Seasonal changes and the contribution of industrial effluent discharges to the population of heavy metal – resistant bacteria (HMRB) in the river water and sediment of the New Calabar River, were examined. On exposure of river water microflora to 2µg of heavy metals, the HMRB population ranged from 55 to 78% in the rainy months and 100% in the dry months. The HMRB of the river water ranged from 0.4 to 0.8% and 11 to 21% in the rainy and dry months respectively, on exposure of river water microflora to 20µg of heavy metals. The HMRB in the sediment ranged from 86 to 100% and 20 to 43% in the dry months and 55 to 100% and 1.7 to 2.4% in the rainy months when sediment microflora were exposed to 2µg and 20µg of heavy metals respectively. Higher concentrations of heavy metals resulted in reduced populations of HMRB. The total aerobic heterotrophic bacterial (THB) count ranged from 1.0×10^7 to 5.8×10^8 cfu/ml and 2.2×10^6 to 5.3×10^7 cfu/ml for surface water, 5.5×10^6 to 7.4×10^8 cfu/ml and 1.4×10^5 to 4.0×10^6 cfu/ml for subsurface water and 1.0×10^5 to 1.1×10^9 and 2.5×10^5 to 6.8×10^7 cfu/g for sediment during the rainy and dry months respectively. Physicochemical parameters such as conductivity and BOD showed higher values in the rainy season than in the dry season. Dissolved oxygen and pH showed no significant difference between their rainy and dry season levels indicating the absence of seasonal variations with these parameters. Heavy metals levels of river water and sediment showed higher values in the dry season than in the rainy season. There was no significant difference between the heavy metal levels of industrial effluent discharges with those of the river water and sediment levels during the rainy season. During the dry season, the heavy metal levels of the industrial effluents were less than those of the river water and sediment. Thus their contribution to seasonal variation of these metals in the river water and sediment is negligible. Results also suggested that the percentage of heavy metal resistant bacteria and the heavy metal levels of the river water and sediments were higher in the dry season than in the rainy season.

Key words: Seasonal changes, effluent discharges, heavy metal resistant bacteria, New Calabar River.

INTRODUCTION

Heavy metals have a great ecological significance, due to their toxicity and accumulative behaviour. In the Niger Delta, the Upstream and Downstream activities of the petroleum industry, may have introduced high concentrations of heavy metals into rivers, streams and other waterways.

High concentration of heavy metals in the effluents from waste water treatment plants, located in some of the refineries petrochemical plants, may have led to increased heavy metal concentration in low land rivers, from which some urban water supplies are abstracted.

The effect of metals on microbial ecosystems are primarily described in terms of the toxicity of heavy metals to microorganisms (Collins and Stotzky 1989; Gadd, 1992) and their impact on microbial community, structure and function (Baath, 1989; Dean-Ross, 1991). In polluted environments, the transfer of heavy metal up the food chain is of great concern. Though some of the metals (Zn) are necessary to the biota at trace levels. Others have no known function, and are highly toxic to prokaryotic and eukaryotic organism (Babich and Stotzky, 1997).

Microorganisms have developed several mechanisms at the cellular and molecular levels to overcome changes arising from heavy metal toxicity in their external environment. Such changes include interference with microbial metabolism, or alteration of the physicochemical environment of cells (Gadd, 1992; Gadd and White, 1997). Heavy metal resistance in microorganism can occur by a variety of mechanisms, including physical sequestration, exclusion and/or efflux, reduced uptake detoxification and synthesis binding proteins. They remove toxic metal via absorption to cell surfaces (Mullen *et al.*, 1989), complexations by exopolysaccharides (Scott and Palmer, 1988), binding with bacterial cell envelopes (Hoyle and Beveridge, 1984), intracellular accumulation

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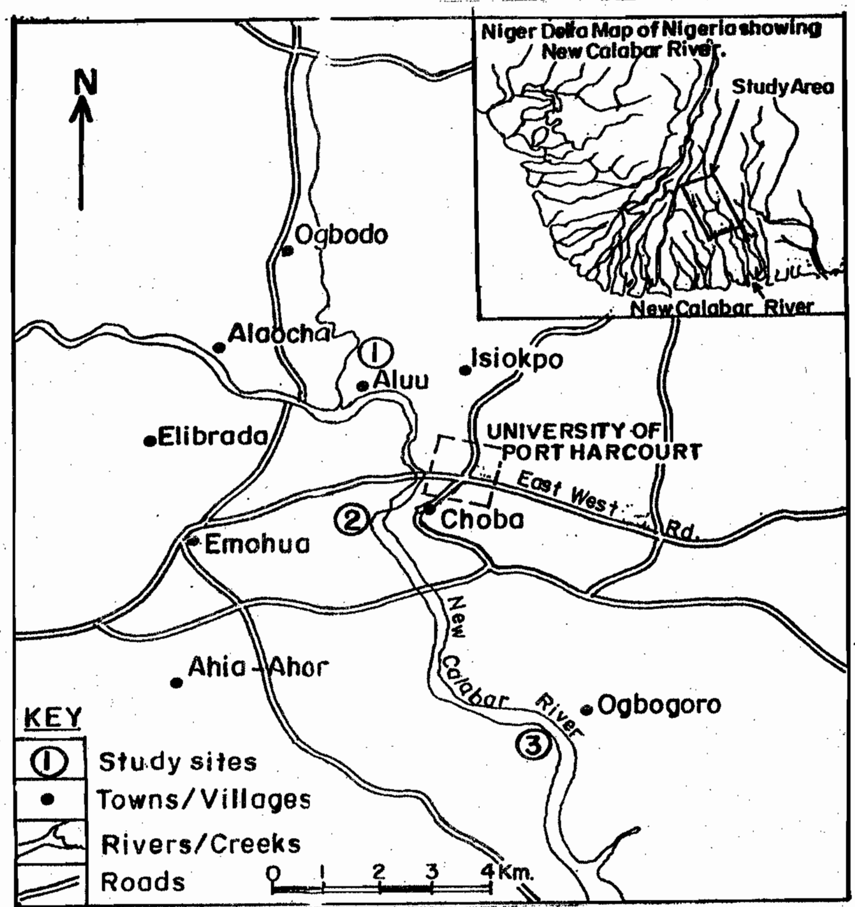

Fig 1: A map showing the three sampling sites (1-3) of the New Calabar River.

(Laddaga and Silver, 1985), biosynthesis of metallothineins and other proteins that trap metals (Higham *et al.*, 1984), precipitation (Aiking et al., 1985), and transformation to volatile compounds (Meissner and Falkinham, 1984).

Although many different chemical methods have been employed to remove metals from wastewater, for example hydroxide and sulphide precipitation, ion exchange, membrane processes (Metzner, 1977) addition of natural and synthetic polymers and acid treatment (Wheatland *et al.*, 1975). The technique of selective bioaccumulation of heavy metals by microbial systems offers the best possible approach for the remediation of contaminated water bodies.

This study was thus conducted to investigate seasonal changes in the distribution of heavy metal resistant bacteria and the probable contribution of inputs of heavy metals by industrial effluent discharges into the New Calabar River water and sediment.

MATERIALS AND METHODS

STUDY AREA

The New Calabar River is found in the lower Niger Delta, near Port Harcourt, Nigeria (Figure 1), with its source at Elele Alimini Umeozoro. It is freshwater from source to Aluu and brackish beyond this point. The river water is soft and acidic with pH 5.5-6.5 (Odokuma and Okpokwasili 1993a; Odokuma and Okpokwasili 1993b; Odokuma and Okpokwasili, 1997).

SAMPLE COLLECTION AND PRESERVATION

Three sampling points were chosen for this study. At Choba (site 2), an oil servicing company (WB), a fibre processing industry (HF) and a market are located. At Ogbogoro (site 3), a construction company (SPC), and a mini-market are present. Aluu (site 1) has no industrial activity.

Samples were collected using 2-litre sterile water containers. Containers were pre-washed with detergents, then with distilled water, soaked with 95% alcohol and allowed to dry. At points of collection, containers were rinsed severally with the river water and then used to collect samples from the surface (0-15cm) and sub-surface (15-30cm) of the river. Eckman grab sampler was used for collection of sediments samples.

A total of nine sampling trips were

conducted from May 1998 to January 1999 and samples were collected between the 15th and 20th of each month when there are average climatic conditions. All samples collected were transported to the laboratories and stored in the refrigerator at 4°C. Analysis was carried out within 24h-48h.

PHYSICOCHEMICAL ANALYSIS

The following parameters were determined: dissolved oxygen (DO) biochemical oxygen demand (BOD), chloride, total hardness, total suspended solids, conductivity, Alkalinity, pH and temperature. Methods used for the determination of these parameters were adapted from APHA (1985).

MICROBIOLOGICAL ANALYSIS

Nutrient Agar used alone or supplemented with 2 and 20μg of salts of heavy metals (Pb, Cu, Zn, Fe and Cd) per milliliter was used for pour plate counts of total and heavy-metal-resistant bacteria respectively and for propagation of isolates (modified method of Timoney *et al.*, 1978). Weighed fractions of sediments (1g) and 1 millilitre of water samples were introduced into tubes containing 9ml of 0.85% NaCl solution in deionised water (diluent). The contents were mixed thoroughly and 0.1ml transferred into Nutrient Agar plates as well as to medium supplemented with 2-2μg of metal/ml 100/1% Isolation = Number of Colonies on Nutrient Agar.

Frequency of Isolation of each isolate was obtained as in Timoney *et al.*, (1978) on medium amended with 20μg metal per ml by the formula:

$$\frac{\text{Frequency of Isolation (number) of counts of each Isolate}}{\text{Total Count}} \times \frac{100}{1}$$

Heavy metal – resistant bacteria count is the mean of all counts on plates supplemented with Cu, Cd, Zn, Fe and Pb salts. Salts of metals employed were as follows: $CuCl_2 2H_2O$, Pb_3O_4, $CdCl_2.6H_2O$, $ZnSO_4.7H_2O$, and $FeSO_4.7H_2O$.

Isolates showing growth on Nutrient Agar amended with 20μg of metals per ml were purified by sub-culturing unto Nutrient Agar plates. Pure Colonies were aseptically transferred unto this-minimal medium (Heavy metal resistant medium of Diels, 1997). Isolates that grow on this medium are confirmed to be metal resistant and were transferred unto Nutrient Agar slants and stored at 4°C in the refrigerator for later identification of isolates.

For identification, isolate were examined by colonial morphology of cell micromorphology and biochemical characteristics. Pure culture from the stock were transferred to Nutrient Agar plates

Table 1: Total aerobic Heterotrophic bacteria Count of the New Calabar River on between the month of May, 1998 to January, 1999.

Site	Position	May	June	July	August	September	October	Nov.	Dec.	Jan.
Aluu	Surface(cfu/ml)	3.6x10⁸±1x10²	1.0x10⁷±6x10	2.2x10⁸±6.10	1.6x10⁷±5x10	13x10⁸±5x10	1.2x10⁷±5x10	4.1x10⁷±5x10	3.2x10⁶±5x10	2.2x10⁶±3x10
	Sub-surface(cfu/ml)	2.4x10⁸±5x10	1.5x10⁷±7x10	2.0x10⁷±7x10	2.2x10⁶±9x10	2.1x10⁷±1x10	5.5x10⁶±6x10	4.0x10±6x10	2.4x10⁶±7x10	1.4x10±4x10
	Sediment(cfu/ml)	2.0x10⁸±7x10	1.5x10⁷±6x10	1.0x10⁵8x10	1.1x10⁷±7x10	3.4x10⁷±6.10	1.8x10⁷±5x10	5.5x10⁶7x10	3.0x10⁶±5x10	2.5x10⁶±1x10
Choba	Surface(cfu/ml)	6.9x10⁸±8x10	6.0x10⁷±7x10	3.6x10⁸±5x10	3.7x10⁷±7x10	1.8x10⁸±1x10	3.4x10⁷±4x10	3.2x10⁷±5x10	3.0x10⁶±6x10	1.0x10⁶±6x10
	Sub-surface(cfu/ml)	7.4x10⁸±7x10	5.5x10x10⁷±5x10	6.1x10⁷±v5x10	5.6x10⁶±5x10	5.7x10⁷±5x10	5.6x10⁸±1x10	3.0x10⁵±5x10	3.0x106±8x10	2.0x10⁵±5x10
	Sediment(cfu/ml)	4.0x10⁹±10	2.5x10⁸±9x10	8.2x10⁷±v7x10	2.0x10⁷±9x10	3.1x10⁷±6x10	2.4x10⁷±5x10	7.0x10⁶±6x10	3.2x10⁶±7x10	4.0x10⁵±1x10
Ogbogoro	Surface(cfu/ml)	5.5x10⁸±1x10	4.0x10³±1x10	3.0x10⁸±2x10	2.1x10⁹±7x10	2.0x10⁹±7x10	2.4x10⁷±6x10	5.3x10⁷±8x10	4.0x10⁷±5x10	4.0x10⁶±1x10
	Sub-surface(cfu/ml)	5.8x10⁸±5x10	7.5x10⁷±5x10	1.0x108±1x10	3.1x10⁷±6x10	4.5x10⁷±5x10	4.2x106±7x10	3.5x106±v7x10	2.1x106±6x10	2.0x105±2x10
	Sediment(cfu/ml)	1.1x10⁹±110²	1.2x10⁸±1x10	5.2x10⁸±1x10	4.8x10⁷±1x110	7.5x10⁷±1x10	2.8x107±5x10	6.8x107±6x10	3.4107±5x10	1.4x106

Results represent means and standard deviation of triplicate samples.

and incubated for 24h. Microscopic examinations of colonies were carried out for colonial morphology. Gram stain and some biochemical tests were carried out for effective characterization of isolates. Identification was carried using the criteria contained in *Bergey's Manual of Determinative Bacteriology* (1985).

RESULT AND DISCUSSION

In Table 1 the total aerobic heterotrophic bacteria (THB) count of the New Calabar River water between the month of May 1988 to January 1999 are presented. The THB counts were higher in the rainy season than in the dry season. The THB count of the surface water ranged from 1.0×10^7 to 5.8×10^8 cfu/ml in the rainy months and 2.2×10^6 to 5.3×10^7 cfu/ml in the dry months. The THB of the subsurface water ranged from 5.5×10^6 to 7.4×10^8 cfu/ml and 1.4×10^5 to 4.0×10^6 cfu/ml for the rainy and dry months respectively. The THB of the sediment ranged from 1.0×10^5 to 1.1×10^9 cfu/g in the rainy months and 2.5×10^5 to 6.8×10^7 in the dry months. These results may be due to the increase in nutrient load of the river during the rainy season. For instance the biochemical oxygen demand (BOD) of the river water (Table 8) in station 1 ranged from 0.75 to

Table 2: Total Heterotrophic bacteria counts and the Percentage of Heavy metal –resistant aerobic bacteria obtained at the 3 Sites in May, 1998 and Janury, 1999.

HEAVY METAL-RESISTANT AEROBIC BACTERIA OF WATER AND SEDIMENT							
Month	Site	Position	THB on Nutrient Agar (cfu/ml) or (cfu/g)	Nutrient Agar +2μg metal/ml	Percentage of Total	Nutrient Agar + 20μg metal/ml	Percentage of Total
May, 1998	Aluu	Water	$3.6x10^8 \pm 6x10$	$2.8x10^8 \pm 1x10$	78	$2.3x10^6 \pm 6x10$	0.64
		Sediment	$2.0x10^8 \pm 7x10$	$1.1x10^8 \pm 2x10$	55	$3.8x10^7 \pm 5x10$	1.9
	Choba	Water	$6.9x10^8 \pm 3x10$	$3.5X10^8 \pm 3X10$	51	$3.0X10^6 \pm 2X10$	0.4
		Sediment	$4.0x10^9 \pm 2x10$	$3.1x10^9 \pm 1x10$	78	$6.7x10^7 \pm 8x10$	1.7
	Ogbogoro	Water	$5.5x10^8 \pm 5x10$	$3.0x10^8 \pm 4x10$	55	$4.2x10^7 \pm 7x10$	0.8
		Sediment	$1.1x10^9 \pm 1x10$	$2.9x10^9 \pm 5x10$	>100	$2.7x10^7 \pm 5x10$	2.4
January 1999	Aluu	Water	$2.2x10^6 \pm 1x10$	$4.6x10^6 \pm 2x10$	>100	$2.x10^5 \pm 7x10$	11
		Sediment	$2.5x10^5 \pm 3x10$	$2.5x10^5 \pm 3x10$	100	$4.9x10^4 \pm 5x10$	20
	Choba	Water	$1.0x10^6 \pm 5x10$	$3.3x10^6 \pm 5x10$	>100	$2.1x10^5 \pm 8x10$	21
		Sediment	$4.2x10^5 \pm 2x10$	$4.8x10^5 \pm 4x10$	>100	$1.8x10^5 \pm 5x10$	43
	Ogbogoro	Sediment	$1.4x10^6 \pm 5x10$	$1.2x10^6 \pm 2x10$	86	$4.6x10^5 \pm 1x10$	33

Results represent means and standard deviation of triplicate samples.

Table 3: Frequency of Bacteria Genera Isolated from different locations in the river water (Nutrient Agar + 20μg metal)

	Aluu		Choba		Ogbogoro	
Genus	May 1998	January 1999	May 1998	January 1999	May 1998	January 1999
Bacillus	36	65	40	72	38	92
Arthrobacter	14	9	15	8	22	2
Proteus	14	16	8		4	
Alcaligenes	26	6	13	18	23	6
Lactobacillus	-	-	10	-	-	-
Salmonella	-	-	2	-	-	-
Staphylococcus	10	4	12	2	13	

Percentage of Isolation was obtained by Dividing mean number of Isolates on Nutrient Agar amended with 20 μg/ml salts of heavy metals by mean total of all isolates obtained.

Table 4: Genera Isolated from different locations in the sediment and their Frequency of Isolation. (Nutrient Agar + 20μg Metals) on Sediment Samples.

Genus	May	January	May	January	May	January
Bacillus	33	67	35	83	43	96
Arthrobacter	14	3	8	7	12	-
Proteus	12	32	5	-	5	
Alcaligenes	15	20	10	5	16	4
Lactobacillus	-	-	10	-	-	-
Salmonella	2	-	-	-	-	-
Staphylococcus	24	7	27	5	24	-

Percentage of Isolation was obtained by dividing mean number of isolates on Nutrient Agar Amended with 20μg/L salts of heavy metals by mean total of all isolates obtained.

Salts of metals used are: Pb_3O_4, $CdCl_2.6H_2O$, $ZnSO_4.7H_2O$, $CuCl_2.2H_2O$ and $FeSO_4 7H_2O$

Table 5: Heavy metal concentration of the water and sediments of the New Calabar River during rainy month of 1998 and dry month of Jan, 1999.

		MEAN CONCENTRATION OF HEAVY METALS (mg/l)				
Month	Site	Cd	Cu	Zn	Pb	Fe
May, 1998	Aluu Surface	<0.001	0.030±0.001	0.067±0.002	0.007±001	29.64±0.05
	Sub-surface	<0001	0.0101±0.001	0.083±0.002	0.007±0.001	30.02±0.05
	Sediment	<0.001	1.093±0.001	0.270±0.005	0.034±0.001	109.6±10.6
January 1999	Aluu Surface	0.060±0.005	0.220±0.001	0.400±0.001	0.230±001	44.12±5.4
	Sub-surface	0.030±0.001	0.200±0.002	0.48±0.02	0.230±0.001	42.53±7.0
	Sediment	0.030±0.002	0.090±0.002	0.710±0.01	0.560±0.04	413.5±5.0

Result represent means and standard deviation of triplicate samples

2.3mg/L in the rainy season and 0.30 to 1.5mg/L in the dry season. In station 2 the BOD ranged from 2.1.to 2.8mg/L in the rainy season and 0.54 to 1.62mg/L in the dry season. In Stations 3 the BOD ranged from 2.0 to 3.2mg/L and 0.22 to 1.78mg/L in the rainy and dry season respectively. The BOD represents the fraction of the total organic carbon of the river water that is susceptible to oxidation (biodegradation) by resident microflora. High BOD levels in aquatic systems have been known to support high heterotrophic microbial populations (Odokuma and Okpokwasili, 1993a; Odokuma and Okpokwasili, 1993b; Odokuma and Okpokwasili, 1997). Surface run-off from the river banks also contributed to increasing the nutrient load of the river during the rainy season.

The percentage of heavy metal resistant aerobic bacteria (% HMRB) in the river, water and sediment at the three stations on the river are presented in Table 2. The percentage of HMRB in both the river water and sediment were greater in the dry season than in the rainy season. The % HMRB n the river water ranged from 100% and 11 to 21% in the dry months and 55 to 78% and 0.4 to 0.8% in the rainy months when exposed to 2µg and 20µg of heavy metals respectively.
In the sediment the % HMRB ranged from 86% to 100% and 20 to 43% in the dry months and 55 to 100% and 1.7 to 2.4% in the rainy months when sediment microflora were exposed to 2µg and 20µg of heavy metal respectively.

In Tables 5,6 and 7 the heavy metal levels of the river water and sediment are presented. The heavy metal levels of the river water and sediment were higher in the dry seasons than in the rainy season. Surface water ranges of heavy metals were, Cd (0.020 to 0.060mg/L), Cu (0.070 to 0.22mg/L), Zn (0.19 to 0.40mg/L), Pb (0.23 to 0.42mg/L), Fe (36.68 to 44.12mg/L) in the dry

Tables 6.: Heavy metal concentration in water and sediments of the New Calabar River during the rainy month of May, 1998 and dry month of January, 1999.

		MEAN CONCENTRATION OF HEAVY METALS (mg/l)				
Month	Site	Cd	Cu	Zn	Pb	Fe
May, 1998	Choba Surface	<0.001	0.014±0.001	0.009±0.001	0.015±0.002	38.20±5.4
	Sub-surface	<0001	0.029±0.001	0.013±0.002	0.014±0.003	34.69±2.0
	Sediment	<0.009	1.348±0.001	0.313±0.002	0.066±0.001	101.2±15.0
January 1999	Choba Surface	0.020	0.070±0.001	0.300±0.001	0.230±002	40.74±2.0
	Sub-surface	0.023	0.090±0.001	0.220±0.01	0.440±0.003	45.34±5.0
	Sediment	0.040	0.120±0.002	0. 490±0.05	0.560±0.05	122.18±6.0

Results represent means and standard deviation of triplicate samples

Table 7: Heavy Metal concentration of river water and sediments of the New Calabar River during the rainy month of May, 1998 and dry month of Jan. 1999

		MAY, 1998		JANUARY,1999		
Month	Site	Cd	Cu	Zn	Pb	Fe
May, 1998	Ogbogoro Surface	<0.001	0.055±0.005	0.009±0.003	0.013±0.002	30.24±5.0
	Sub-surface	<0.001	1.201±0.001	0.056±0.001	0.220±001	30.01±2.0
	Sediment	<0.001	1448±0.002	1.269±0.003	0.057±0.001	62.63±8.0
January 1999	Ogbogoro Surface	0.040±0.001	0100±0.003	0.190±0.01	0.420±0.001	36.68±3.5
	Sub-surface	0.007±0.001	0.072±0.001	0.194±0.002	0.411±0.001	38.26±4.5
	Sediment	0.050±0.001	0.180±0.002	0.520±0.003	0.410±0.002	64.50±9.4

Results represent means and standard deviation of triplicate samples

season and Cd (<0.001mg/L) Cu (0.014 to 0.055mg/L), Zn (0.009 to 0.075mg/L) Pb (0.007 to 0.015mg/L), Fe (29.64 to 38.2mg/L) in the rainy season. Sediments ranges of heavy metals in the dry season were Cd (0.030 to 0.04mg/L), Cu (0.09 to0.18mg/L), Zn (0.49 to 0.71mg/L) Pb (0.41 to 0.56mg/L) and Fe (64.5 to 413.5mg/L). In the rainy season the ranges were Cd (<0.001mg/L), Cu (1.093 to 1.45mg/L), Zn 90.27 to 1.27mg/L), Pb (0.034 to 0.066mg/L) and Fe (62.63 to 109.6mg/L).

The lower %HMRB in the rainy season may be due to the higher levels of heavy metals in the dry season than in the rainy season. The higher the concentrations of metals in the river and sediment the greater the probability of contact with resident microflora and the greater the population of organisms developing resistance to these metals (Hoyle and Beveridge, 1984; Laddaga and Silver, 1985; Scott and Palmer 1988; Mullen *et al.*, 1989).

In Tables 3 and 4, the major heavy metal-resistant bacterial genera isolated from the river water and sediment are presented. Bacillus was the most prevalent throughout the year. Other prevalent bacterial genera were Arthrobacter and staphylococcus. Results suggest that some of these organisms showed a seasonal variation in the population.

Bacillus was more prevalent in the dry season (January) than in the rainy season (May) in both sediment and river water. *Arthrobacter*, *Alcaligenes* and *Staphylococcus* were generally more prevalent in rainy season than in the dry season in the both sediment and river water in all stations.

The physiochemical characteristics of temperature, conductivity, pH, dissolved oxygen hardness and biochemical oxygen demand, chloride, alkalinity and total suspended solids of the river water in the 3 sampling stations are presented in Table 8.

The river water temperature ranged from 24^{0}C to 28^{0}C. There was no apparent seasonal variation in temperature. Similar results have been obtained by Odokuma and Okpokwasili (1993b). Effect of temperature on microbial interaction is observed in humic uptake in adsorption experiments. By fluctuation in temperature heavy metals adsorbed unto humic coatings serve as metal sink through which metals are released to the water body or tightly bound to the sediment.

The pH of the river was slightly acidic. It ranged from 4.8 to 7.0. There was no apparent seasonal variation in the pH of the river water at the three sampling stations. Similar observations have been made by Odokuma and Okpokwasili (1993b). water pH affects metal toxicity in two ways, firstly the speciation and bioavailability of the metals may change, and secondly, the uptake and toxicity of the metals can be affected by changes in the sensitivity of the cell surfaces as well as heavy metals (Campbell and Stokes, 1985). Effect of pH on humic coating showed that the higher pH (the more alkaline), the lower the adsorption and vice versa (Jun *et al.*, 1994). The study revealed that the pH of the river water favoured the adsorption process.

There was seasonal variation in the conductivity values in the river water at the three sampling stations. Conductivity values were greater in the rainy months than in the dry months. The increase in nutrient load as a result of surface run-off during the rainy season may have introduced anions and cations into the river water thereby influencing the conductivity values. Similar results have been obtained by Odokuma and Okpokwasili (1993b).

Some physicochemical properties of the three industries located at site 2 in the New Calabar River are presented in Table 9. Results suggest that during both rainy and dry season,

Table 8: Physicochemical characteristics of the New Calabar river water.

0	Site	May	June	July	August	September	October	November	December	January
Temp°C	Aluu	29.0±1.0	27.0±1.0	26.1±10	26.0±1.0	23.0±2.0	26.8±1.0	27.0±2	28.0±1	28.0±1.0
	Choba	29.0±2.0	27.0±1.0	26.1±1.0	26.5±±1.0	23.6±1.0	27.6±1.0	27.5±1	29.4±1	28.9±1.0
	Ogbogoro	28.4±2.0	28.9±2.0	27.2±1.0	27.2±1.0	30.6±2.0	28.4±1.0	29.0±1.0	30.1±2.0	31.0±2.0
pH	Aluu	5.48±0.2	5.54±0.1	5.45±0.1	5.61±0.1	5.74±0.3	6.03±0.2	5.40±0.2	5.30±0.1	4.75±0.2
	Choba	5.36±0.3	5.62±0.2	6.98±0.3	6.02±0.1	5.84±0.2	5.84±0.3	5.81±0.3	5.23±0.2	5.05±0.1
	Ogbogoro	5.63±0.2	5.94±0.3	6.36±0.2	6.08±0.2	6.97±0.1	5.68±0.2	6.52±0.2	6.82±0.2	5.74±0.3
TSSmg/l	Aluu	0.52±0.3	0.44±.01	1.00±.05	0.11±.01	1.13±0.7	0.24±01	0.22±.02	0.25±.02	0.11±0.03
	Choba	0.66±02	0.61±.01	1.25±08	0.19±.03	1.55±06	0.54±..02	0.36±.01	0.21±.01	0.23±0.02
	Ogbogoro	0.89±03	1.03±.02	1.60±.05	0.25±0.1	1.61±.05	0.68±.03	0.49±0.2	0.28±.01	0.33±0.01
Conductivity	Aluu	105±10.5	167±20.1	184±30.1	0.66±1.5	2.15±30.1	040±.5.5	027±6.9	068±.5.0	015±2.0
μS/m	Ogbogoro	167±50.1	207±50.1	213±20.1	0.82±10	239±60.1	099±.10	085±7.1	089±4.5	079±11.0
	Choba	122±25.2	192±40.1	156±30.1	061±8.5	224±40	051±.7.5	021±2.5	019±1.5	029±2.0
Dissolved	Aluu	6.22±.5	6.83±.2	6.99±.2	6.00±.2	6.92±.1	5.66±.1	6.00±.1	5.20±.2	5.00±.2
Oxygen (DO) mg/i	Choba	6.04±.3	5.32±.3	6.48±.2	5.80±.1	6.24±.2	6.00±.2	6.02±.1	5.08±.3	4.00±.2
	Ogbogoro	610±.1	5.82±.2	6.73±.2	6.20±.3	6.58±.1	5.80±.2	6.13±.1	4.14±.2	4.00±.3
BOD mg/l	Aluu	1.30±3	2.30±.2	0.75±.1	0.42±.1	2.50±.2	2.30±.2	1.20±.2	1.50±.1	0.30±
	Choba	2.40±5	2.80±.2	2.20±.2	0.88±.1	2.10±.1	2.60±.3	1.62±.1	1.00±.3	0.54±.2
	Ogbogoro	2.80±16	3.20±.3	2.50±.2	0.22±.1	2.00±1	2.60±.1	1.78±.3	1.20±.2	0.62±.1
Chloride	Aluu	12.23±.5	13.79±.2	8.24±.2	5.66±.1	3.26±.1	2.22±.5	1.47±.2	1.94±.5	6.02±.2
mg/l	Choba	22.92±.1	20.27±.5	8.62±.2	10.50±.5	3.00±.2	3.08±.6	4.30±.5	16.80±.5	20.55±.5
	Ogbogoro	33.65±.8	36.80±.2	18.18±.3	44.91±.2	10.20±.1	7.73±.3	11.00±.2	30.00±.3	36.99±.5
Alkalinity	Aluu	25.6±5.0	22.0±6.0	17.2±2.0	11.8±7.0	12.8±9.0	11.0±3.0	10.89±3.0	8.2±7.0	6.0±5.0
mg/l	Choba	34.2±7.0	29.6±5.0	18.2±5.0	15.4±5.0	18.4±7.0	18.4±5.0	11.4±2.0	9.6±6.0±6.0	6.0±6.0
	Ogbogoro	40.0±8.0	28.4±4.0	23.4±6.0	15.2±3.0	18.8±6.0	18.0±±2.0	16.6±4.0	14.6±1.0	14.8±7.0
Hardness mg/l	Aluu	30.04±5.0	3.36±5.0	40.26±10.0	36.26±2.0	26.03±2	26.00±	22.00±	21.65±	22.00±
	Choba	41.18±10.0	46.21±7.0	62.80±20.0	40.69±5.0	40.02±5.0	38.30±5.0	36.08±7	34.08±9	37.76±10.0
	Ogbogoro	43.20±9.0	46.00±10.0	63.13±15.0	44.42±5.0	40.04±6	39.00±10	36.22±8	36.22±5	32.00±7.0

Results represent means and standard deviation of triplicate samples.

Table 9: Physicochemical properties of the Effluents of three industries located along thNew Calabar River Collected during the rainy month of May, 1998 and dry month of January, 1999

	MEAN CONCENTRATION OF HEAVY METALS (mg/l)					
Parameters SPÇ	WB		HF	SPC	WB	HF
DO mg/l	1.10±0.01	1.40±0.01	2.04±0.02	0.85±0.05	0.63±0.02	1.72±0.02
BOD mg/l	0.44±0.02	0.50±0.01	0.23±0.03	1.27±0.03	1.53±0.01	0.26±0.05
pH	6.93±0.01	9.20±0.02	7.30±0.05	7.03±0.03	7.46±0.02	6.84±0.50
Salinity mg/l	1.21±0.03	0.32±0.01	0.36±0.02	0.10±0.01	0.14±0.03	0.42±0.01
Conductivity μS/M	186±20.1	122±0.03	520±30.1	320±205	158±30.5	840±50.5

WB, HF, SPC= Code Names for industries
Results represent means and standard deviation of triplicate samples

Table 10: Heavy Metal content of Industrial Effluent during the rainy month of May, 1998 and dry month of January, 1999.

		MEAN CONCENTRATION OF HEAVY METALS (mg/l)				
Industry	Month	Cd	Cu	Pb	Fe	Zn
WB	May	0.07±0.012	0.061±0.01	0.22±0.03	0.421±0.02	0.280±0.01
	Jan	0.06±0.02	0.05±0.02	0.50±0.02	0.860±0.01	0.361±0.02
HF	May	0.10±0.01	0.09±0.01	0.147±0.01	1.080±0.01	0.416±0.03
	Jan	0.120±0.01	0.06±0.01	0.420±0.01	1.640±0.02	0.542±0.01
SPC	May	0.02±0.02	0.011±0.01	0.09±0.02	1.940±0.02	0.261±0.04
	Jan	0.03±0.03	0.03±0.01	0.352±0.03	2.310±0.01	0.465±0.02

WB, HF, SPC=Code Name for Industries
Results represent means and standard deviation of triplicate samples

the dissolved oxygen (DO) levels of the industries were very low (0.63 to 2.04mg/L). this may indicate the presence of high concentrations of dissolved solids in these effluents. There was no apparent seasonal variation in the DO levels of the effluent. The biochemical oxygen demand (BOD) levels of the effluents were low (0.23 to 1.53mg/L) indicating reduced levels of biodegradable organic matter in the effluents. These results also suggest that the effluents may not serve as a source of readily utilizable nutrients for bacteria at sample station 2. There was no apparent seasonal variation in BOD levels. The pH of the effluents ranged from slightly acidic to slightly alkaline (6.84 to 9.20). Bacteria prefer alkaline environments. Thus the discharge of effluents in the river at the these points may create a local rise in the pH of the river water (creating an alkaline condition) thus stimulating bacterial growth. Seasonal variation in the pH of the effluents were not apparent. The low salinity levels of the effluents did not show any seasonal variation. Conductivity values of the effluents (122 to 840µS/m) were similar to those obtained by Odokuma and Okpokwasili (1993).

In Table 10 the heavy metal content of industrial effluents during the rainy month and dry month are presented.

Heavy metal ranges were Cd 90.02 to 0.07mg/L), Cu (0.011 to 0.061mg/L), Pb (0.02 to 0.22mg/L), Zn (0.261 to 0.416mg/L), Fe (0.42 to 1.9mg/L) in the rainy season and Cd (0.03 to 0.12mg/L), Cu (0.011 to 0.05mg/L), Pb (0.03 to 0.06mg/L), Zn (0.35 to 0.5mg/L), Fe (0.86 to 2.3mg/L) in the dry season. Results show that there was no seasonal variation in the heavy metal levels of the industrial effluent discharges. These values were generally similar to those obtained in the river water and sediment except for iron. The levels of iron in the river water and sediment were much higher than most of the effluents. Odokuma and Okpokwasli (1993b) observed relatively higher concentration of parameters such as BOD, alkalinity, total dissolved solids (TSS) chloride and nitrate in effluent of most of these very industries sited along the river when compared with the river water levels, suggesting their high pollution potential especially in the absence of appropriate effluent treatment measures. However, in this present study, the similarity in effluent parameters with those of river water parameters may suggest the introduction of effluent treatment measures by these industries. It may also be that the industries are using the water from the river without actually introducing heavy metals into the waste water. Whatever the case may be results suggest that the pollution potential of these effluent discharges is reduced and they do not contribute to the seasonal variation in the heavy metal content of the river water and sediment.

CONCLUSION

This study has revealed that there was seasonal variation in the percentage of heavy metal – resistant bacteria in the river water and sediment of the New Calabar river. While the percentage of heavy metal resistant bacteria was greater in the dry season than in the rainy season, the converse was however the case for the total aerobic heterotrophic bacterial population. The higher % HMRB during the rainy season was attributed to higher heavy metal levels in the river water and sediment during this season. High BOD levels of the river water during the rainy season was suggested as partly been responsible for the high THB counts during this season.

Predominant HMRB genera encountered in both the river water and sediment include *Bacillus*, *Proteus*, *Alcaligenes*, *Staphylococcus* and *Arthrobacter*. *Bacillus* was the most predominant of all the bacteria. The heavy metal levels of the industrial effluent discharges were similar to the levels in the river water and sediment. They did not show any seasonal variation. Thus their contribution to seasonal variations of these metals in the river water and sediment was considered negligible.

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