Advance Research in Agriculture and Veterinary Science
ISSN 2348-5353
Adv. Res. Agri. Vet. Sci. Vol. 1, No. 2, 2014: 88-94
Academy for Environment and Life Sciences, India
Available at www.aravs.org

ORIGINAL ARTICLE

EVALUATION OF SEASONAL VARIATION IN THE MICROBIAL AND HEAVY METAL CONCENTRATIONS OF IMO RIVER ESTUARY OF THE NIGER DELTA MANGROVE ECOSYSTEM

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KEYWORDS
Microbial,
Heavy metals,
Surface,
Sub-surface,
Sediment.

Article History:
Received on
23 May 2014

Accepted on
8 May 2014

Published on
10 June 2014

ABSTRACT: Evaluation of seasonal variation in the microbial and heavy metal content in Imo River estuary of the Niger Delta mangrove ecosystem using standard analytical methods was carried out on samples collected. The study was conducted during the wet and dry seasons (August and November). The results obtained showed that the sediment samples had a significantly ($P < 0.05$) higher counts of total heterotrophic bacteria (THB) than the surface and sub-surface water samples. However, the sediment samples during the dry season had a significantly ($P < 0.05$) higher counts of THB than the wet season (2.55 ± 2.34 107 cfug-1 and 2.46 ± 2.20 107 cfug-1) respectively. There was no significant difference ($P > 0.05$) in the population of THB in the surface water during the wet and dry seasons (2.23 ± 2.23 106 cfuml-1 and 2.39 ±1.63 106 cfuml-1) respectively, while there was a significant difference ($P < 0.05$) in the population of total heterotrophic bacteria (THB) in the sub-surface water during the dry and the wet seasons (2.27 ±2.00 106 cfuml-1 and 2.13 ± 1.84 106 cfuml-1) respectively. The total fungal (TF) densities in the surface water were 1.17 ± 0.93 x 105 cfuml-1 and 1.38 ± 0.63 x 105 cfuml-1 during the wet and dry seasons respectively, the mean densities of 1.15 ± 0.63 x 105 cfuml-1 and 1.30 ± 0.48 x 105 cfuml-1 were observed in the sub-surface water during the wet and dry seasons respectively, while in the sediments, the mean densities observed were 1.42 ± 1.19 x 106 cfug-1 and 1.60 ± 1.05 x 106 cfug-1 during the wet and dry seasons respectively. The results obtained also indicate that the water and sediment samples show a remarkable variation in the concentrations of heavy metals during the wet and dry seasons.

INTRODUCTION

The detection of microbial diversity and their variation in water and sediments is of great practical and scientific relevance, especially in coastal ecosystems (Zhang *et al.*, 2008). If the microbial community structure in soft-benthic habitat is determined by their environment, then pollution loading or organic enrichment is expected to shift their composition, and a counter shift toward the original community should be evident after the abatement of pollution discharge (Yoza *et al.*, 2007). The contamination of the aquatic system with heavy metals has been on the increase since the last century due to industrial activities (Asaolu and Olaofe, 2004). Heavy metals are taken up as cations. Among the heavy metals detected by water soluble fraction (WSF) are lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), nickel (Ni), chromium (Cr), and vanadium (V). Heavy metals are non-biodegradable and are toxic under certain conditions (Asaolu and Olaofe, 2004). Although some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all branches of life, including microbes, by forming complex compounds within the cell. Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (by efflux, complexation, or reduction of metal ions) or to use them as terminal electron acceptors in anaerobic respiration (Ober *et al.*, 1987).

Thus far, tolerance mechanisms for metals such as copper, zinc, arsenic, chromium, cadmium, and nickel have been identified and described in detail. Most mechanisms studied involve the efflux of metal ions outside the cell, and genes for this general type of mechanism have been found on both chromosomes and plasmids. Because the intake and subsequent efflux of heavy metal ions by microbes usually includes a redox reaction involving the metal (that some microorganisms can even use for energy and growth), microorganisms that are resistant to and grow on metals also play

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an important role in the biogeochemical cycling of those metal ions (Ober *et al.*, 1987). This is an important implication of microbial heavy metal tolerance because the oxidation state of a heavy metal relates to the solubility and toxicity of the metal itself. When looking at the microbial communities of metal-contaminated environments, it has been found that among the microorganisms present, there is more potential for unique forms of respiration. Also, since the oxidation state of a metal ion may determine its solubility, many scientists have been trying to use microbes that are able to oxidize or reduce heavy metals in order to remediate metal-contaminated sites (Ober *et al.*, 1987).

In high concentrations, heavy metal ions react to form toxic compounds in cells (Ukabiala *et al.*, 2010). Because some heavy metals are necessary for enzymatic functions and microbial growth, uptake mechanisms exist that allow for the entrance of metal ions into the cell. There are two general uptake systems — one is quick and unspecific, driven by a chemiosmotic gradient across the cell membrane and thus requiring no ATP, and the other is slower and more substrate-specific, driven by energy from ATP hydrolysis. While the first mechanism is more energy-efficient, it results in an influx of a wider variety of heavy metals, and when these metals are present in high concentrations, they are more likely to have toxic effects once inside the cell (Nies, 1999).

MATERIALS AND METHODS

2.1. Study Site

The study site for this research work was Imo River estuary in the Niger Delta region of Nigeria. Imo River estuary lies between latitude 04^0 $34^0$52N and longitude 007^0 $32^0$59E, with an elevation of 11 m above sea level.

2.2. Sample Collection

Using a decontaminated shovel and a scoop, the desired thickness and volume of sediment from the sampling area was collected and transferred into an appropriate sample or homogenization container. Surface water was decanted from the sample prior to sealing and transfer, care was taken to retain the fine sediment fraction during this process. Collection of surface and sub-surface water samples was done aseptically into clean one (1) liter capacity plastic bottles. The bottles were open to fill and closed below the water surface. All containers were rinsed at least three times with water that was to be analyzed (APHA, 1998). After collection, the samples were refrigerated at about 4 C during the transport to the laboratory (Radojevic and Bashkin, 1999; APHA, 1998). All measurements were done in triplicates and the means obtained.

2.3. Microbiological Analysis

Samples for microbial analysis were collected aseptically, labeled and stored in ice packed plastic coolers and transported to the laboratory where analysis within 24 hours of collection was carried out. Ten-fold serial dilution of the samples was carried out for enumeration of densities of the different microbial groups. The methods and media used for the enumeration of the various microbial groups are described in the following sections. The densities of the following microbial groups were determined: total heterotrophic bacteria (THB), total heterotrophic fungi (THF), crude oil-utilizing bacteria (CUB), crude oil-utilizing fungi (CUF).

2.4. Characterization and Identification of Bacterial Isolates

Various indices were employed to characterize and identify the isolates. These were colonial appearance on solid media, changes in the surrounding medium, pigment production, Gram reaction, microscopic appearance, sugar fermentation and other biochemical tests. Distinct or representative colonies from the culture plates were selected for characterization. The test results for bacteria were evaluated using characteristics presented in *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994). Representative colonies of fungal isolates were characterized and identified based on their cultural and morphological features as described by Barnett and Hunter (1987).

2.5. Estimation of Densities of Heterotrophic Microorganisms

The counts of total heterotrophic bacteria were determined by the pour plate techniques (Chikere *et al.*, 2009) using nutrient agar (NA). The NA medium was amended with nystatin ($50\mu gml^{-1}$) in order to prevent the growth of fungal contaminants. The total heterotrophic fungi count was

determined by pour plate technique using Sabouroud dextrose agar (SDA) supplemented with streptomycin (50µgml^{-1}) to inhibit the growth of bacterial contaminants (Martini *et al.*, 1980; Barnett and Hunter, 1987). Inoculated NA plates were incubated at 28^{0}C for 24 hours, while the SDA plates were incubated at room temperature for 3 days before enumeration of microbial colonies.

2.6. Enumeration of Crude Oil-utilizing Microorganisms

The counts of crude oil-utilizing bacteria and fungi were enumerated by pour plate techniques (Dean-Ross and Mills, 1989; Ober *et al.*, 1987) using vapour phase transfer technique on mineral salts medium (MSM). For the enumeration of oil-degrading bacteria, the medium was supplemented with 50µgml^{-1} fungizol miconazole nitrate to prevent the growth of fungal contaminants. On the other hand, mineral salts medium supplemented with 50µgml^{-1} streptomycin to inhibit the growth of bacterial contaminants was used to ensure the enumeration of oil-degrading fungi. In both cases the crude oil (Bonny light) used was sterilized by Millipore filtration (0.45 µm pore size) and stored in sterile bottles. The plates were incubated at room temperature for 5 days before enumeration.

2.7. Heavy Metal Analysis

The analysis of the following heavy metals were carried out: Nickel (Ni), Chromium (Cr), Lead (Pb), Vanadium (V), Cobalt (Co), and Copper (Cu). Analysis was done after finely grounding the sediment samples to facilitate the digestion of 1 g of the sample with perchloric acid and nitric acid. Heavy metal content of the sediment digest and water samples were determined using atomic absorption spectrophotometer (Model UNICAM 939).

2.8. Statistical Analysis

All statistical analysis of data from various treatments was carried out using analysis of variance (ANOVA) test using 3-factor factorial experiment. Means were separated using least significant difference (LSD) test.

RESULTS

3.1. Total Heterotrophic Microorganisms

It was observed that the sediment samples produced significantly ($P<0.05$) higher total heterotrophic bacterial (THB) counts than the surface and sub-surface water samples. However, the results showed that sediment samples during the dry season produced significantly higher ($P < 0.05$) THB with a mean density of 2.55 ± 2.34 10^7 cfug^{-1} than the wet season with a mean density of 2.46 ± 2.20 10^7 cfug^{-1}. The results showed that there was no significant difference ($P > 0.05$) in THB of the surface water during the wet and dry seasons (2.23 ± 2.23 10^6 and 2.39 ±1.63 10^6) respectively, while a significant difference ($P < 0.05$) was observed in the sub-surface water (2.27 ± 2.00 10^6 and 2.13 ± 1.84 10^6) respectively. The total heterotrophic fungi (THF) density revealed that in the surface water, the mean densities observed were 1.17 ± 0.93 x 10^5 cfuml^{-1} and 1.38 ± 0.63 x 10^5 cfuml^{-1} during the wet and dry seasons respectively, the mean densities of 1.15 ± 0.63 x 10^5 cfuml^{-1} and 1.30 x 10^5 ± 0.48 cfuml^{-1} were observed in sub-surface water during the wet and dry seasons respectively, while in the sediments, the mean densities observed were 1.42 ± 1.19 x 10^6 cfug^{-1} and 1.60 ± 1.05 x 10^6 cfug^{-1} during the wet and dry seasons respectively (Table 2).

3.2. Crude Oil-utilizing Microorganisms

The results revealed that there was a significant difference ($P < 0.05$) in population of crude oil-utilizers with respect to season. The mean densities of crude oil-utilizing bacteria observed were 1.22 ± 1.20 x 10^5 cfuml^{-1} and 1.32 ± 1.05 x 10^5 cfuml^{-1} in surface water during the wet and dry seasons respectively, in the sub-surface water, the mean densities observed were 1.18 ± 1.06 x 10^5 cfuml^{-1} and 1.32 ± 0.98 x 10^5 cfuml^{-1} during the wet and dry seasons respectively, while for the sediments, the mean densities observed were 1.52 ± 2.03 x 10^6 cfug^{-1} and 1.63 ± 1.34 x 10^6 cfug^{-1} during the wet and dry seasons respectively. The mean densities of crude oil-utilizing fungi (CUF) observed for the surface water were 7.2 ± 0.23 x 10^3 cfuml^{-1} and 8.9 ± 0.63 x 10^3 cfuml^{-1} during the wet and dry seasons respectively. In the sub-surface water, the mean densities were 7.4 ± 0.78 x 10^3 cfuml^{-} and 8.8 ± 0.84 x 10^3 cfuml^{-1} during the wet and dry seasons respectively, while for the sediments, the mean densities observed were 9.9 x 10^4 ± 0.94 cfug^{-1} and 1.15 ±0.98 x 10^5 cfug^{-1} during the wet and dry seasons respectively (Table 1).

3.3. Heavy Metals Concentrations of the Water and Sediments

The mean concentrations of heavy metals in surface water were 0.16 ± 0.02 mgl^{-1} Pb, 0.122 ± 0.01 mgl^{-11} Cu, 0.144 ± 0.02 mgl^{-1} Ni, 0.022 ± 0.01 mgl^{-1} V, 0.094 ± 0.01 mgl^{-1} Cr, and 0.038 ± 0.01 mgl^{-1} Co. The mean concentrations in sub-surface water were 0.17 ± 0.01 mgl^{-1} Pb, 0.244 ± 0.03 mgl^{-1} Cu, 0.164 ± 0.03 mgl^{-1} Ni, 0.017 ± 0.01 mgl^{-1} V, 0.047± 0.02 mgl^{-1} Cr, and 0.049± 0.01 mgl^{-1} Co, while the mean values for sediments were 0.88 ± 0.03 mgkg-1 Pb, 0.95 ± 0.08 mgkg-1 Cu, 1.11 ± 0.10 mgkg-1 Ni, 0.385 ± 0.03 mgkg-1 V, 1.04± 0.08 mgkg-1 Cr, and 0.64 ± 0.03 mgkg-1 Co (Figure 3). The result revealed that the mean concentrations of Pb was significantly ($P < 0.05$) higher in sediments than the surface and sub-surface water. The result further revealed that there was no significant difference ($P > 0.05$) in the mean concentrations observed in surface and sub-surface water. However, the result showed that Pb was significantly ($P < 0.05$) higher in sediments in the dry season than wet season. There was no significant difference ($P > 0.05$) in surface and sub-surface water during each season with the concentrations during the dry season significantly ($P < 0.05$) higher than in the wet season. The sediments had a significantly ($P < 0.05$) higher Pb content than the water samples (Figure 1).

Whereas Cu and Ni contents were significantly ($P < 0.05$) higher in sediments during the dry season than the wet season and were significantly ($P < 0.05$) higher in sediment samples than water samples (Table 3). Sub-surface water showed a significantly ($P < 0.05$) higher Cu and Ni content than surface water during the dry season. Surface and sub-surface water samples during the first study had the lowest Cu and Ni content and there was no significant difference ($P > 0.05$) between the two microhabitats. The result also shows that V was significantly ($P < 0.05$) higher during the dry season than wet season in all the microhabitats with the sediment samples producing a significantly ($P < 0.05$) higher content than the water samples. There was no significant difference ($P > 0.05$) in V content in surface and sub-surface water throughout the study, while Cr content was significantly ($P < 0.05$) higher during the dry season than the wet season and was significantly ($P < 0.05$) higher in sediment samples than water samples (Figure 2).

Table 1: Influence of source of sample collection and sampling points on microbial populations

	Surface water (cfuml^{-1})					Sub-surface water (cfuml^{-1})					Sediment (cfug^{-1})					
	P	SP1	SP2	SP3	SP4	SP5	P	SP1	SP2	SP3	SP4	SP5	P	SP1	SP2	SP3	SP4	SP5
THB	$1.95^{c}\pm$	$2.39^{b}\pm$	$2.33^{b}\pm$	$2.46^{b}\pm$	$2.39^{b}\pm$	$2.33^{b}\pm$	$1.16^{c}\pm$	$2.36^{b}\pm$	$2.37^{b}\pm$	$2.28^{b}\pm$	$2.36^{b}\pm$	$2.30^{b}\pm$	$2.36^{b}\pm$	$2.63^{a}\pm$	$2.43^{a}\pm$	$2.58^{a}\pm$	$2.50^{a}\pm11.04$	$2.52^{a}\pm$
	$2.30x10^{5}$	$1.98\ x10^{6}$	$0.84\ x10^{6}$	$0.16\ x10^{6}$	$0.59\ x10^{6}$	$0.23\ x10^{6}$	$0.15\ x10^{5}$	$1.53\ x10^{5}$	$2.32x10^{6}$	$0.98\ x10^{b}$	$0.56x10^{6}$	$0.18\ x10^{6}$	$0.17\ x10^{6}$	$0.10\ x10^{7}$	$0.94x10^{7}$	$1.32x10^{7}$	$x10^{7}$	$0.95\ x10^{7}$
CUB	$1.21^{c}\pm$	$1.43^{b}\pm\ 0.94$	$1.22^{b}\pm$	$1.33^{b}\pm$	$1.24^{b}\pm$	$1.18^{b}\pm0.0$	$1.04^{c}\pm$	$1.31^{b}\pm$	$1.21^{b}\pm$	$1.14^{b}\pm$	$1.41^{b}\pm1.90$	$1.38^{b}\pm$	$1.49^{b}\pm$	$1.51^{b}\pm$	$1.62\pm$	$1.73^{a}\pm$	$1.53\pm$	$1.58^{a}\pm$
	$0.18\ x10^{4}$	$x10^{5}$	$0.23\ x10^{5}$	$0.58\ x10^{5}$	$0.19\ x10^{5}$	$.26\ x10^{5}$	$1.54\ x10^{4}$	$0.91\ x10^{5}$	$0.83\ x10^{5}$	$0.43\ x10^{5}$	$x10^{5}$	$0.0.24x10^{5}$	$1.90\ x10^{5}$	$0.18\ x10^{6}$	$0.17\ x10^{6}$	$0.23\ x10^{6}$	$0.95x10^{6}$	$1.60\ x10^{6}$
TF	$9.6^{c}\pm\ 0.98$	$1.38^{b}\pm$	$1.36^{b}\pm$	$1.27^{b}\pm0.28$	$1.07^{b}\pm$	$1.61^{b}\pm$	$7.5^{c}\pm$	$1.18^{b}\pm$	$1.16^{b}\pm$	$1.17^{b}\pm$	$1.48\pm$	$1.60^{b}\pm$	$1.34^{b}\pm$	$1.44^{a}\pm$	$1.52\pm$	$1.55^{a}\pm$	$1.46\pm$	$1.74^{a}\pm$
	$x10^{3}$	$0.23\ x10^{5}$	$0.20\ x105$	$x10^{5}$	$0.34\ x10^{5}$	$0.56\ x10^{5}$	$0.19\ x10^{3}$	$0.51\ x10^{5}$	$1.06\ x10^{5}$	$0.82\ x10^{5}$	$0.19\ x10^{5}$	$1.52\ x10^{5}$	$1.63\ x10^{5}$	$0.54\ x10^{5}$	$0.23\ x10^{6}$	$0.91\ x10^{6}$	$0.60x20^{6}$	$1.08\ x10^{6}$
CUF	$7.1^{d}\pm\ 0.30$	$8.0^{c}\pm$	$7.9^{c}\pm$	$9.3^{C}\pm$	$8.0^{c}\pm0.19$	$8.6^{c}\pm$	$5.0^{d}\pm$	$8.9^{c}\pm$	$9.7^{c}\pm$	$9.7^{c}\pm$	$8.0^{c}\pm$	$8.6^{c}\pm$	$1.11^{b}\pm$	$1.08^{b}\pm$	$9.1^{b}\pm$	$1.08^{a}\pm$	$1.21^{a}\pm$	$1.04^{a}\pm$
	$x\ 10^{2}$	$0.18\ x10^{3}$	$0.10\ x10^{3}$	$0.63x10^{3}$	$x10^{3}$	$0.20\ x10^{3}$	$0.15\ x10^{2}$	$0.22\ x10^{3}$	$0.24\ x10^{3}$	$0.12\ x10^{3}$	$0.18\ x10^{3}$	$0.21\ x10^{3}$	$0.43\ x10^{4}$	$0.50\ x10^{4}$	$0.26x10^{4}$	$0.32\ x10^{5}$	$0.12x10^{5}$	$0.20\ x10^{5}$

Means with the same superscript along the horizontal array represent no significant difference (P>0.05).
KEY: THB = total heterotrophic bacteria, THF = total heterotrophic fungi, CUB = crude oil utilizing bacteria, CUF = crude oil utilizing fungi, cfu = colony forming unit, P = pristine, SP_n = sampling point number. LSD: THB =2.53, CUB =1.48, THF =2.64, CUF = 1.28

Table 2: Influence of season and source of sample collection on the microbial population of the samples

	Wet season (August)			Dry season (November)			
	SW (cfuml^{-l})	SSW (cfuml^{-l})	SED (cfug^{-l})	SW (cfuml^{-l})	SSW (cfuml$^{-l)}$	SED (cfug^{-l})	LSD
THB	$2.23^{c}\pm2.23\ x10^{6}$	$2.13^{d}\pm1.84\ x10^{6}$	$2.46^{b}\pm2.20\ x10^{7}$	$2.39^{c}\pm1.63\ x10^{6}$	$2.27^{c}\pm2.00\ x10^{6}$	$2.55^{a}\pm2.34x10^{7}$	1.46
CUB	$1.22^{a}\pm1.20\ x10^{5}$	$1.18^{b}\pm1.06\ x10^{5}$	$1.52^{a}\pm2.03\ x10^{6}$	$1.32^{b}\pm1.05\ x10^{6}$	$1.32^{b}\pm0.98\ x10^{5}$	$1.63^{a}\pm1.34\ x10^{6}$	8.5
THF	$1.17^{c}\pm0.93\ x10^{5}$	$1.15^{c}\pm0.63\ x10^{5}$	$1.42^{b}\pm1.19\ x10^{4}$	$1.38^{c}\pm0.63\ x10^{3}$	$1.30^{c}\pm0.48\ x10^{3}$	$1.60^{a}\pm1.05\ x10^{5}$	1.52
CUF	$7.2^{c}\pm0.23\ \ 10^{3}$	$7.4^{c}\pm0.78\ \ 10^{3}$	$9.9^{b}\pm0.94\ \ 10^{4}$	$8.9^{c}\pm0.63\ \ 10^{3}$	$8.8^{c}\pm0.84\ \ 10^{3}$	$1.15^{a}\pm0.98\ 10^{5}$	1.05

Means with the same superscript along the horizontal array represent no significant difference (P>0.05).
KEY: SW = surface water, SSW = sub-surface water, SED = sediment, THB = total heterotrophic bacteria, THF = total heterotrophic fungi, CUB = crude oil utilizing bacteria, CUF = crude oil utilizing fungi, cfu = colony forming unit.

Table 3: Influence of season and source of sample collection on heavy metal concentration of the samples

	Wet season			Dry season			
	SW	SSW	SED	SW	SSW	SED	LSD
(Pb) mgkg^{-l-})	$0.011^{d}\pm0.01$	$0.012^{d}\pm0.01$	$1.51^{b}\pm0.08$	$0.21^{c}\pm0.07$	$0.25^{c}\pm0.02$	$2.31^{a}\pm0.11$	0.08
Cu (mgkg^{-l})	$0.02^{e}\pm0.01$	$0.02^{e}\pm0.0$	$1.49^{b}\pm0.18$	$0.20^{d}\pm0.03$	$0.34^{c}\pm0.08$	$2.41^{a}\pm0.15$	0.06
Ni (mgkg^{-l})	$0.01^{e}\pm0.01$	$0.01^{e}\pm0.02$	$0.92^{b}\pm0.06$	$0.14^{d}\pm0.02$	$0.22^{c}\pm0.01$	$1.79^{a}\pm0.10$	0.02
V (mgkg^{-l})	$0.003^{d}\pm0.01$	$0.002^{d}\pm0.01$	$0.141^{b}\pm0.02$	$0.026^{c}\pm0.01$	$0.023^{c}\pm0.01$	$0.262^{a}\pm0.01$	0.04
Cr(mgkg^{-l})	$0.014^{e}\pm0.01$	$0.007^{e}\pm0.01$	$0.83^{b}\pm0.03$	$0.095^{c}\pm0.01$	$0.053^{d}\pm0.01$	$1.737^{a}\pm0.01$	0.02
Co (mgkg^{-l})	$0.004^{d}\pm0.01$	$0.005^{d}\pm0.01$	$0.11^{b}\pm0.02$	$0.075^{c}\pm0.01$	$0.077^{c}\pm0.01$	$1.09^{a}\pm0.02$	0.02

Means with the same superscript along the horizontal array represent no significant difference (P>0.05).
KEY: SW = surface water, SSW = sub-surface water, SED = sediment, LSD = least significant difference

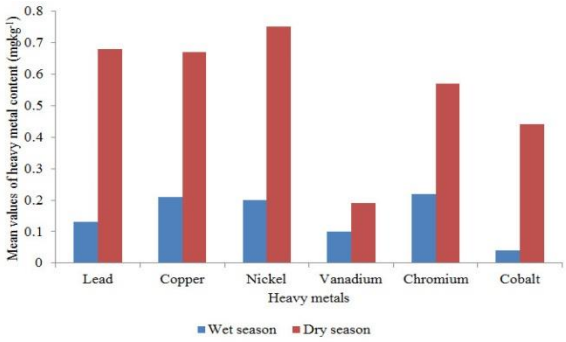

Figure 1: Mean concentrations and distribution of heavy metals in Imo River estuary

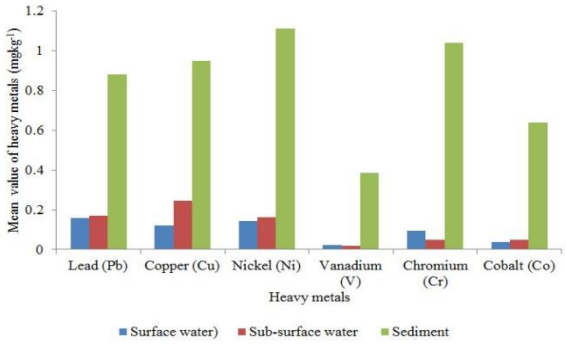

Figure 2: Mean concentrations and distribution of heavy metals in the surface water, sub-surface water and sediments of Imo River estuary.

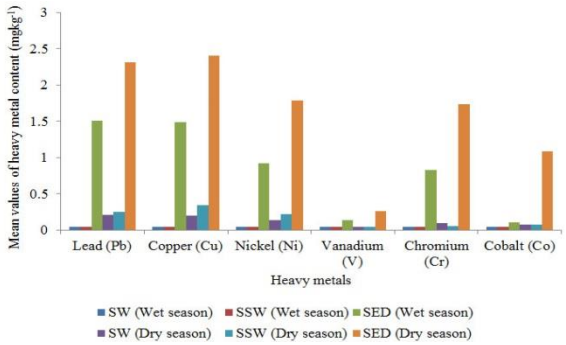

Figure 3: Mean concentrations and distribution of heavy metals in surface water, sub-surface water and sediments during the wet and dry seasons.

DISCUSSION

Water and sediment microbial communities are a major component of microbial food webs, biogeochemical cycles and energy flow. Bacteria and fungi are the predominant organisms in these microhabitats (Youssef *et al.*, 2010). Their biodiversity is structured and determined by the temporal and spatial variability of physicochemical and biotic parameters and thus, can reflect local environmental conditions (Zhang *et al.*, 2008). In this study, microbial loads were significantly high ($P < 0.05$), but varied with microhabitats. The main reason for high abundance of microbial populations in estuaries is the high productivity rate because estuaries provide habitats for a large number of organisms and the presence of phytoplankton (mainly the diatoms and dinoflagellates) which are the primary producers in estuaries. The densities of crude oil-utilizing microorganisms were low compared to total heterotrophic counts. The results showed that there was a significant difference ($P < 0.05$) in crude oil-utilizers with respect to season and microhabitats. In general, the sediment shows high average density of heterotrophic and crude oil-utilizing microorganisms than water. This may be due to the fact that microorganisms tend to attach and grow on the surface of sediments. The humic substances and other elements in sediment phase could serve as nutrients for microorganisms, stimulating their growth. The high densities of crude oil utilizing microorganisms in sediments than the water may be due to low heterotrophic activities of oil degraders in the microhabitat. In this study, eight (8) species of bacteria and five (5) species of

fungi were isolated, characterized and identified. The bacteria species were *Flavobacterium* sp, *Micrococcus* sp, *Vibrio* sp, *Pseudomonas* sp, *Klebsiella* sp, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* sp. The fungi species isolated were *Cladosporium* sp, *Penicillium* sp, *Aspergillus* sp, *Monilia* sp and *Fusarium* sp.

The contamination of the aquatic system with heavy metals has been on the increase since the last century due to industrial activities (Florea and Busselberg, 2006). The mean concentrations of heavy metals obtained during the dry season were significantly ($P < 0.05$) higher than the mean concentrations during the wet season (Figure 1). The heavy metals analyzed in the present study were all detected throughout the study. The significantly ($P < 0.05$) higher concentrations of heavy metals in sediments than in surface and sub-surface water may be ascribed to the sedimentation, percolation and flocculation patterns in the aquatic ecosystem. Microorganisms that are resistant to and grow in metals also play an important role in the biogeochemical cycling of those metal ions. This is an important implication of microbial heavy metal tolerance because the oxidation state of a heavy metal relates to the solubility and toxicity of the metal itself.

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