

Studies on Seasonal Variation and Effect of Heavy Metal Pollution on Microbial Load of Marine Sediment

Esther Ebah, Ichor Tersagh*, Gideon C. Okpokwasili

Department of Microbiology, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria

*Corresponding author: smartichor@uam.edu.ng

Abstract Sediment samples were collected from 15 stations at Onne Port, Rivers State, Nigeria, during the dry and rainy seasons of 2012 to determine the spatial distribution, seasonal and temporal variation and effect of different heavy metal contents on microbial load. The heavy metals; chromium, cadmium, copper, nickel, zinc, mercury, tin and arsenic were analyzed using atomic absorption spectrophotometer and compared with different standard and reference values of TELS (threshold effect levels) and PELS (probable effect levels). Total heterotrophic bacterial and fungal counts were evaluated using the spread plate technique. Heavy metals accumulated in the sediment during the dry season in the order As > Hg > Zn > Ni > Sn > Cr > Cd > Cu and in the wet season in the order As > Sn > Zn > Ni > Hg > Cr > Cd > Cu and ranged from 0.001 to 15.1 mg/kg and 0.001 to 13.2 mg/kg for dry and wet seasons respectively. The concentrations of heavy metals demonstrated a unique seasonal pattern with the highest concentration during the dry season and lowest during the wet season. The log count of total heterotrophic bacteria varied between 5.81 cfu/g and 5.37cfu/g for dry and wet season respectively and total fungal counts varied between 4.95cfu/g and 4.80cfu/g for dry and wet seasons respectively. There was no significant correlation between heavy metal concentration and microbial load. Although the levels of the heavy metals determined were within regulatory limits, destruction of wetland biomass will release the heavy metals into the environment with the risk of metals entering the food chain. To check the pollution in marine sediment, the anthropogenic sources of pollution should be at minimal level.

Keywords: heavy metals, microbial load, sediment, concentration

Cite This Article: Esther Ebah, Ichor Tersagh, and Gideon C. Okpokwasili, "Studies on Seasonal Variation and Effect of Heavy Metal Pollution on Microbial Load of Marine Sediment." *American Journal of Marine Science*, vol. 4, no. 1 (2016): 4-10. doi: 10.12691/marine-4-1-2.

1. Introduction

In recent decades, the problem of pollution from heavy metals has caused increasing concern in marine sediments and water. Heavy metals in the marine environment have received much attention because they are toxic, non-biodegradable in the environment and easy to accumulate and magnify in organisms. It is difficult to measure the input of metals in the environment as a result of anthropogenic activities. Concentration of heavy metals in aquatic ecosystem has increased considerably due to the inputs of industrial waste, sewage, runoff and agricultural wastes [31,41]. In other words, heavy metal pollution may likely go with the rapid economic development [12,16].

The analysis of heavy metal in marine sediment is widely used to assess longer term anthropogenic inputs, into the marine environment. In many places, it started several years ago and contamination level increases daily without any major process to control the level of pollution [22,29]. This type of contamination disturbs the aquatic environment and also affects the adjacent coastal zone area with major ecological degradation [18,19,36].

Furthermore, there is increasing evidence that presence of heavy metals is linked to the exacerbation of some

microbial diseases in aquatic organisms. At sufficiently high concentrations, heavy metals appear to be toxic to the organism and so it is important to know by how much their concentration may be increased above the normal range in the environment before the effects on marine organisms [39].

Microbial communities in soils and sediments are key players involved in metal mobility (Ford and Ryan, 1995). Consequently, relationships between metals and microbial variables such as total biomass, bacterial diversity and activity have been investigated in various sedimentary environments. However, it is very difficult to draw general conclusions and it seems that each microbial community is unique and reacts in a different way. For instance, in some environments trace metals were shown to negatively affect microbial biomass [2,14,25], microbial diversity [11] or microbial activities [15,23,40]. However, for other environments microbial communities were found to be relatively insensitive to high metal loads. This was the case for biomass [8,37], bacterial diversity [5,14,32] and microbial activity [3,23]. In some studies, communities within polluted environments were even more active with higher bacterial abundance or higher diversities, than the corresponding reference ecosystems [8,41].

Heavy metals are among the most harmful of the elemental pollutants and are of particular concern because

of their toxicity to human. They include essential elements like iron as well as toxic metals like cadmium and mercury. Most of them show significant affinity to sulphate and disrupt enzyme function by forming bonds with sulphur groups in enzymes cadmium, copper, lead and mercury ions bind to the cell membrane hindering transport process through the cell wall. Qi *et al.* [33] reported that some of the metalloids, elements on the borderline between metals and non-metals are significant water pollutant.

The heavy metals entering the marine environment are transported by prevailing currents. The main source of this marine pollution are power and desalination plants, sewage treatment facilities, agricultural facilities, port facilities, agricultural activities, coastal constructions, mining and quarrying activities [33].

The aim of this work is to investigate the distribution of heavy metals (Cu, Cd, Cr, Ni, Zn, Hg, Sn and As), evaluate their seasonal variation and their effect on the microbial population of Onne port sediment.

2. Materials and Methods

2.1. Sampling Site

The sediments used for this study were collected from Onne sea port, Rivers State, Nigeria. Onne port is located in the south south zone of Nigeria where many ships anchor. It is located on Ogu creek near the Bonny River 19 km from Port Harcourt. It cuts across three local government areas of Rivers State, Eleme, Ogu-Bolo and Bonny. The port consists of two major facilities, the federal ocean terminal and federal lighter terminal. Onne port has been designated as an oil and gas free zone by the Nigerian Government but as at present over 100 companies have licenses to work at Onne port, as an economic free zone. It serves as a hub for oil and gas operations.

2.2. Collection of Samples

Sampling was carried out for two seasons. Dry season sampling was done in February and wet season was in July. Sediment samples were collected from a depth of 20m from 15 different points.

Sediments from these 15 sampling points were contracted into 5 samples by taking a range of 3. Sediments were collected with an Eckman grab (Wildlife Supply Co. Nig.) into sterile polythene bags and transported immediately to the laboratory for analysis.

2.3. Analysis of Heavy Metals

The heavy metals studied were Ni, Cu, Zn, Cr, Cd, Hg, Sn, and As. The sediment samples were oven-dried in Petri dishes at 800°C for 48 hours and thereafter ground with a rolling pin to disaggregate the samples but not break down the grains themselves. Sieved sediments were collected and 2.0g of each samples was dried in a beaker and the concentration of aqua-regia- extractable heavy metals were released to 0.03ml of HNO₃(65%) and 6.0ml of HCl (37%) as described by Rasmuseen and Soresen [34] and Miroslav and Vladimir [26]. The mixture was heated to near dryness and allowed to cool before 20ml and 5M

HNO₃ solutions were added. The solutions were allowed to stay overnight and filtered. The filtrates were transferred into 100ml volumetric flask and made up of mark with 0.5 < HNO₃ [4]. The heavy metals concentrations were measured using a flamed atomic absorption spectrophotometer (Optima 3000 – Perkin-Elmer).

2.4. Microbiological Analysis

The total heterotrophic bacterial and fungal counts in sediment were determined by the pour plate technique [35] using nutrient agar (NA) Sabouraud dextrose agar (SDA) respectively. The NA medium was amended with lactic acid to inhibit the growth of fungi and to adjust the pH to 5.5. Inoculated NA plates were incubated at 28°C for 24 hours and SDA for 48 hours before enumeration.

2.5. Statistical Analysis

Statistical analysis was performed on the data generated from the concentration of heavy metals and their variations between the dry and wet season using One-Way Analysis of Variance (ANOVA) and the Post Hoc Tool pack of the Statistical Package for Social Sciences (SPSS) version 20.0.

3. Results

3.1. Dry Season Variation of Concentration of Heavy Metals in Onne Port Sediment

The heavy metal concentration of sediment from Onne port was analyzed for two seasons. Their concentration in the dry season is recorded in Table 1. The concentration of Arsenic showed the highest for the sediments from the 15 sampling stations. Sampling stations 1-3 represented as 'A' exhibited the highest concentration with a value of 15.1mg/kg and sampling stations 12-15 designated as 'E' recorded the lowest concentration. These were followed up by mercury recording the highest concentration at stations 10-12 known as 'D' with a value of 2.4mg/kg and lowest concentrations at 1-3 and 4-6 as 'A' and 'B' with similar values of 1.0mg/kg. The concentration of nickel and zinc showed higher values compared to copper, chromium, tin across the sampling stations with values ranging from 0.444mg/kg- 0.348mg/kg and 0.444mg/kg- 0.3505mg/kg respectively.

The concentration of heavy metals analyzed recorded lower values during the wet season; this is shown in Table 2. Arsenic showed an elevated concentration than the other metals. The values obtained for station 1-3 represented as 'A' recorded higher mean values of 13.2mg/kg and stations 7-9 as 'C' had the lowest concentrations of Cu, Cd, Cr, Ni, Zn, Hg, and Sn had much lower values compared to Arsenic with values 0.001mg/kg- 0.001mg/kg, 0.001mg/kg – 0.001mg/kg, 0.001mg/kg – 0.001mg/kg, 0.162/mg/kg – 0.301mg/kg, 0.294 mg/kg – 0.30/mg/kg, 0.294mg/kg- 0.2mg/l, 0.1mg/kg – 0.1mg/kg, and 3mg/kg – 3mg/kg respectively.

The seasonal variation of Ni, Zn, Hg and As exhibited a higher concentration during the dry season in all the sampling stations as compared to their concentration in the wet season. Only Tin showed a higher concentration in the wet season. This is shown in Figure 2 –Figure 6.

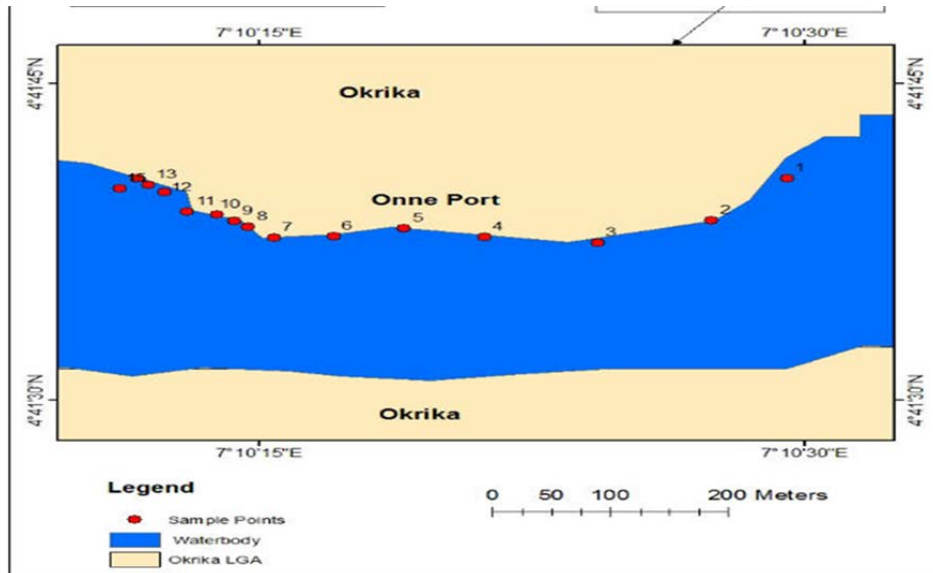


Figure 1. Map of the study area

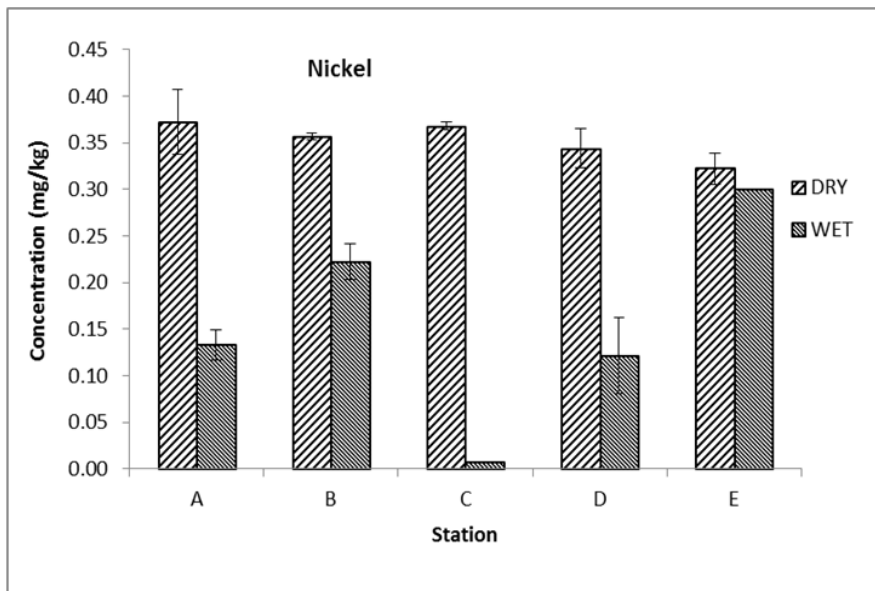


Figure 2. Seasonal variation of nickel in sediment

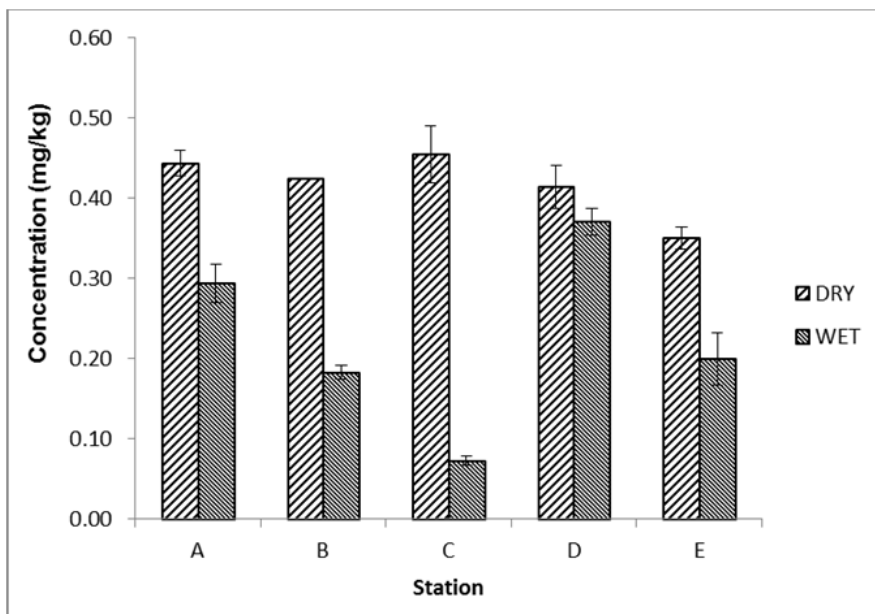


Figure 3. Seasonal variation of zinc in sediment

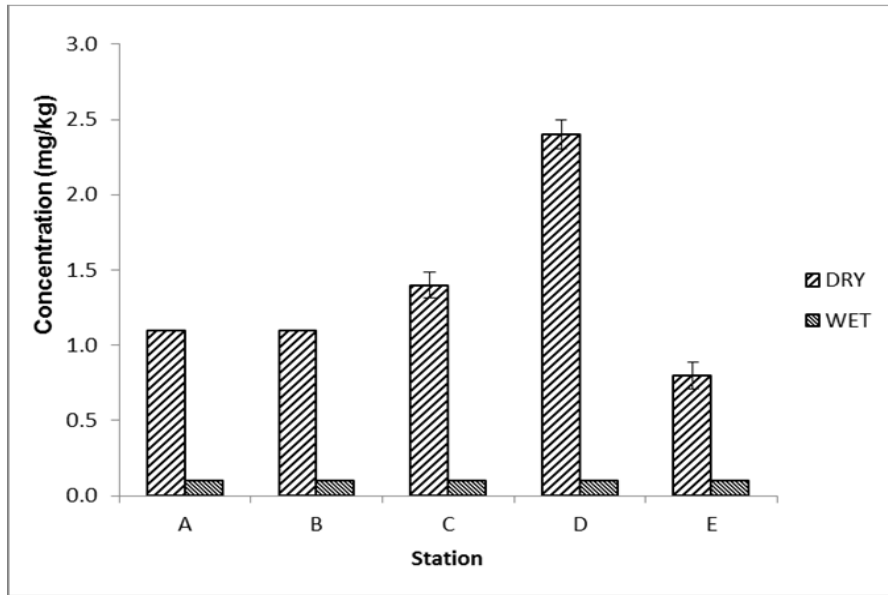


Figure 4. Seasonal variation of mercury in sediment

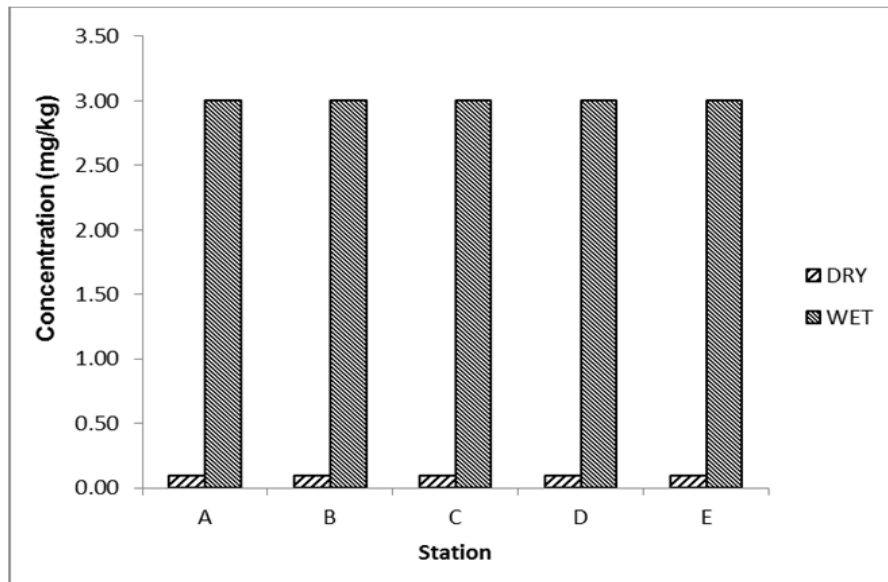


Figure 5. Seasonal variation of tin in sediment

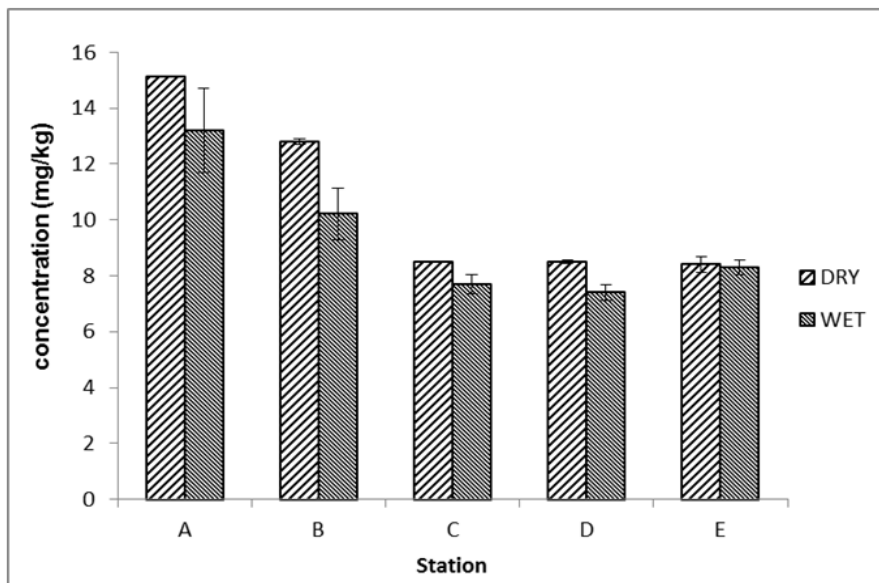


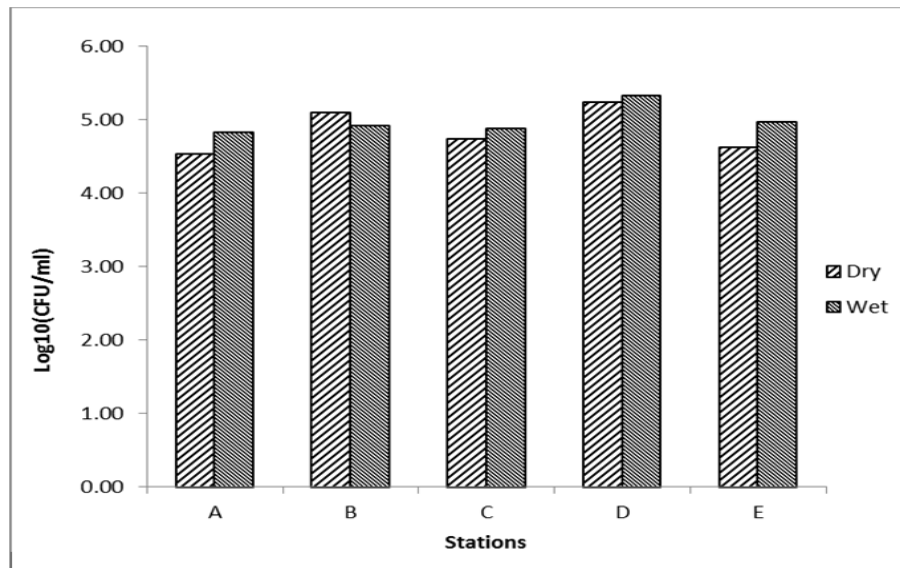
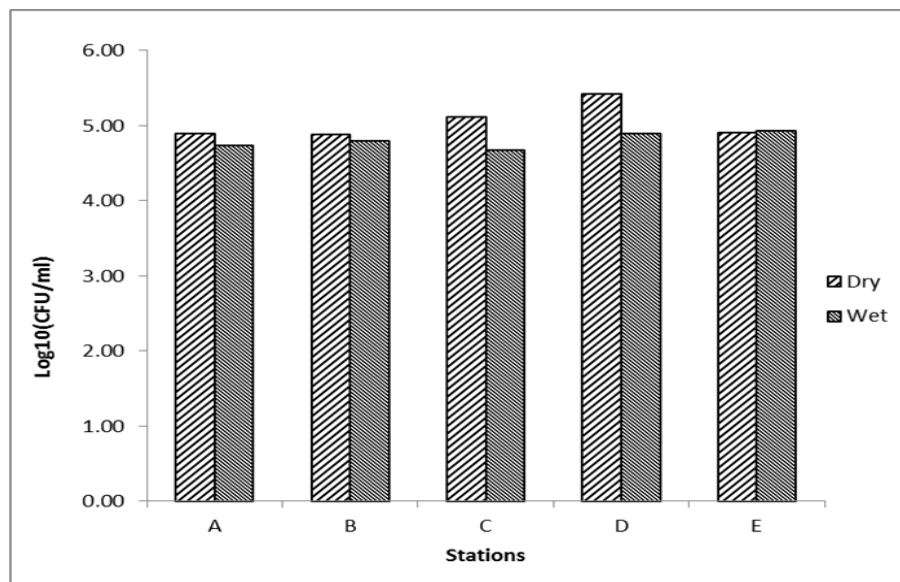
Figure 6. Seasonal variation of arsenic in sediment

Table 1. Dry season variation of heavy metal concentration in sediment samples

| METALS | A | B | C | D | E mg/kg |
|----------|-------|-------|--------|--------|---------|
| Copper | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Cadmium | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Chromium | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Nickel | 0.441 | 0.364 | 0.37 | 0.381 | 0.348 |
| Zinc | 0.444 | 0.424 | 0.4547 | 0.4145 | 0.3505 |
| Mercury | 1.1 | 1.1 | 1.4 | 2.4 | 0.8 |
| Tin | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Arsenic | 15.1 | 12.8 | 8.5 | 8.5 | 8.4 |

Table 2. Wet season variation of heavy metal concentration in sediment sample

| METALS | A | B | C | D | E mg/kg |
|----------|-------|-------|-------|-------|---------|
| Copper | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Cadmium | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Chromium | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Nickel | 0.164 | 0.257 | 0.008 | 0.199 | 0.301 |
| Zinc | 0.294 | 0.183 | 0.073 | 0.371 | 0.2 |
| Mercury | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Tin | 3 | 3 | 3 | 3 | 3 |
| Arsenic | 13.2 | 10.2 | 7.7 | 7.4 | 8.3 |

**Figure 7. Seasonal variation of bacterial count in sediment****Figure 8. Seasonal variation of fungal count in sediment**

3.2. Seasonal Distribution of Microbial load in Sediment

The bacterial and fungal counts of sediment were enumerated for both dry and wet seasons. The total heterotrophic bacteria count exhibited the highest count in station A with a log count of 5.81 cfu/g and 5.37cfu/g

during the dry and wet season respectively. Total fungal count recorded highest count in station D with log count of 4.95cfu/g and 4.80cfu/g for dry and wet season respectively as shown in Figure 7 and Figure 8. The concentration of heavy metal in bottom sediment was observed to be lower during the wet season than during dry season.

4. Discussion

The concentrations of Cu, Cr, Cd, Zn, Ni, Sn, Hg, and As in the sediments of Onne port exhibited a seasonal and station base oscillation. In this present study, heavy metal accumulated in the sediment during the dry season in the order of As > Hg > Zn > Ni > Sn > Cr > Cd > Cu and in the wet season in the order of As > Sn > Zn > Ni > Hg > Cr > Cd > Cu. The concentrations of heavy metals demonstrated a unique seasonal pattern with highest concentration during the dry season and lowest during the wet season. This is in agreement with the studies of Mohiuddinni *et al.* [27].

Significant spatial variation of heavy metal concentrations in Onne port were observed between the sampling stations. These reflect the adverse impacts of crude oil leakages, urbanization, industrialization and discharge of untreated wastes. Heavy metals are integral components of crude oil and are usually introduced into any environment impacted by hydrocarbons [1,6,28]. The variation may also be attributed to increased absorption, sedimentation and flocculation dynamics that takes place in the estuary [24]. Once heavy metals are in the environment, a number of dynamic transformations may occur [17]. Hydrological flow pattern of the wetland, autochthonous production, re-suspension and salinity [24] are significant facilitators in sedimentation and flocculation, which bear direct influence on enhanced concentrations of heavy metals down the sediment profile. Contamination with hydrocarbons and tidal influences may have been responsible for the high heavy metal loads particularly arsenic.

Heavy metals are environmental pollutants [13,30] and could be increasingly introduced anthropogenically as co-products, by-products and finished products into aquatic ecosystem [10,24]. Heavy metals in aquatic environment are principally associated with geochemical cycles and biological processes, and could be greatly influenced by man mediated activities such as industrial activities, agricultural practices and waste disposal [28,42].

In wet lands, metals may be introduced anthropogenically through the use of organic and chemical pesticides and fertilizers [42], mining, smelting and metaliferous ores, electroplating and gas exhausts [20,21] and crude oil spillages. It may as well be inherited from parent material [38].

The concentration of heavy metals in this sediment when compared with suggested guidelines namely; PELS (probable effects levels) and TELS (threshold effect levels) are well below the screening value as given by FDEP [7]. Although, the levels of the heavy metals determined were within regulatory limits, deterioration of the marine ecosystem and environ eco-quality can be expected with increased input of wastes, domestic and industrial sludge and crude oil leakages.

There was no significant effect of heavy metal concentration on the microbial load in the Onne port sediment. This was evident with the increased microbial load observed during the dry season. The number of bacterial cell is usually high where and when waters are not deep and where organic matters are concentrated. This observation could be attributed to the fact that the concentration of heavy metal was far below the screening value as given by FDEP [7] guidelines. It could also be

due to the fact that microorganisms in the sediment have acclimatized or insensitive to the heavy metal concentration of the area under study. Microorganisms remove heavy metal directly from wet lands by two mechanisms; the first is by metabolism-dependent uptake of metals into their cells at low concentrations, the second is biosorption which is a non-active adsorption process binding metal ions to the extracellular charged materials or the cell walls [24]. Therefore, it is obvious that the destruction or harvesting of wetland biomass will release the heavy metals into the environment with the risk of metals entering the food chain.

5. Conclusion

The knowledge of heavy metal concentrations in marine sediment is very important with respect to nature management, aquatic ecology and human health. Routine monitoring of the pollution status of the aquatic foods with the capability of accumulating heavy metals including dangerous metal like arsenic is vital.

Onne port is one of the very busy ports in Nigeria with various activities. Its strategic location in the crude oil region of the country, subjects this port to the possibilities of accumulating heavy metals. Although the Nigeria Port Authority has put some restriction and law in place to check some of these anthropogenic factors and practices, a regular monitoring is very important.

References

- [1] Amadi, E. N. and Braide, S. A. (2003). Distribution of petroleum hydrocarbon degraders around petroleum related facilities in a mangrove swamp of the Niger Delta. *Journal of Nigeria Environmental Society*. 1(2):187-192.
- [2] Aoyama, M., Nugumo, T., (1997). Effect of heavy metal accumulation in apple orchard Soils on Microbial biomass and microbial activities. *Soil Science Plant Nutrition*. 43: 601-612.
- [3] Barajas – Aceves, M., (2005). Comparison of different microbial biomass and activity measurement methods in metal-contaminated soils. *Bioresource Technology*. 96: 1405-1414.
- [4] Binning, K. and D. Barrd (2001). Survey of heavy metals in the sediments of Swatkop Rivers Estuary, Port Elizabeth, South Africa. *Water South Africa*. 24(4): 461-466.
- [5] Bouskill, N.J., Barko-Finkkel, J., Galloway T.S, Handy, R.D, Ford, T.E, (2010) Temporal bacterial diversity associated with metal-contaminated river sediments *Ecotoxicology* 19 :317-328.
- [6] Dibble, J. T. and Bartha, R. (1979). Effect of Environmental Parameters on the biodegradation of oil sludge. *Applied Environmental Microbiology*. 37: 729-739.
- [7] Federal Department of Environmental Protection (FDEP) 1994.
- [8] Feris, K., Ramsey, P., Frazar, C., Moore, J.N., Gannon, J.E., Holben, W.E., (2003). Differences in hyporheic-zone Microbial Community Structure along a heavy-metal contamination gradient. *Applied and Environmental Microbiology*. 69: 5563-5573.
- [9] Ford, T., Ryan, D., (1995). Toxic metals in aquatic ecosystems: a microbiological perspective. *Environmental Health Perspect*. 103 (suppl 1), 25-28.
- [10] Frostegard, A., Tunlid, A., Baath, E. (1993). Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology*. 59: 3605-3617.
- [11] Ganguly, S. and Jana, B.B. (2002). Cadmium induced adaptive responses of certain biogeochemical cycling bacteria in an aquatic system. *Water Research*. 36: 1667-1676.
- [12] Geo, X.L, Zhou, F.X. and Chen, C.T.A. (2014). Pollution status of the Bohaisea, China. An overview of the environmental quality assessment related trace metals. *Environment International*. 62: 12-30.

- [13] Gratani, L. S., Taglioni, F. and Crescente, M. F. (1992). The accumulation of lead in agricultural soil and vegetation along highway. *Chemosphere*. 24:941-949.
- [14] Grilan, D.C., Danis, B., Pernet, P., Joly, G. and Dubios, P. (2005). Structure of sediment associated microbial communities along a heavy metal contamination gradient in the Marine environment. *Applied and Environmental Microbiology* 71: 679-690.
- [15] Irha, N., Slet, J. and Petersell, V. (2003) Effect of heavy metals and PAH on soil accessed via dehydrogenase assay. *Environment International*. 28: 779-782.
- [16] Janaki – Roman, D., Jonathan, M. P., Srinivasalu, S., Armstrong-Alban, J. S., Mohan, S. P., and Rammohan, V. (2007). Trace metal enrichments in core sediments in Muthupet mangroves, S. E. coast of India. Application of the acid leachable technique. *Environmental Pollution*. 145:245-257.
- [17] Johnson, B. (1993). *Interactions in Marine Shallow Water Sediment with Emphasis on Microalgae*. Goteborg: Goteborg University Press.
- [18] Jonathan, M. P. and Rammohan, V. (2003). Heavy metals in sediment of the inner shelf Off the Gulf of Mannar, South East Coast of India. *Marine Pollution Bulletin*. 46: 258-268.
- [19] Jonathan, M. P., Rammohan, V. and Srinivasulu, S. (2004). Geochemical variation of major and trace elements in recent sediments off the Gulf of Manner, the south east coast of India. *Environmental Geology*. 45:466-480.
- [20] Kaata-Pendias, A. and Pencia, A. (1984). *Trace Elements in Plants and Soil*. CRC Press, Boca Raton, Florida.
- [21] Lasat, M. M. (2000) Phytoextraction of metals from contaminated soils. A review of plant/soil metal interaction and assessment of pertinent agronomic issues. *Journal of Hazardous Substances Research*. 2:1-25.
- [22] Li, R., Shu, K., Luo, Y. and Shi, Y. (2010). Assessment of Heavy metal pollution in Estuarine surface sediments of Tangxi River in Chaohu Lake Basin. *Chinese Geographical Science*. 20: 9-17.
- [23] Magalhaes, C., Costa, J., Teixeira, C. and Bordalon, A.A. (2007). Impact of trace metal on denitrification in estuarine sediments of the Douro Rivers Estuary. Portugal. *Marine Chemistry*. 107:332-341.
- [24] Matagi, S. V., Swai, D. and Mugabe, R. (1998). Heavy metals removal mechanisms in wetlands. *African Journal of Tropical Hydrobiology Fisheries*. 8:23-35.
- [25] McGrath, S.P., Chauri, A.M. and Giller, K.E. (1995). Long-term effects of land application of sewage sludge, soils, microorganism and plants. *Journal of Industrial Microbiology*. 14:94-104.
- [26] Miroslav, R. and Vladimr, N. B. (1999). *Practical Environmental Analysis* Cambridge: The Royal Society of Chemistry.
- [27] Mohiuddini, K. M., Ogawu, Y., Zakir, H. M., Otomo, K. and Shikazono, N. (2011). Heavy metals contamination in water and sediment of an urban river in a developing country. *Autumn* 8(4):723-736.
- [28] Odu, C. T. I. (1972). Microbiology of soils contaminated with petroleum hydrocarbons. Extent of contamination and some soil microbial properties after contamination. *Journal of the Institute Petroleum*. 58: 201-208.
- [29] Ogilive, L.A. and Grant, A. (2008). Linking pollution induced community tolerance (PICT) and microbial Community structure in chronically metal polluted estuarine sediment. *Marine Environmental Resource*. 65: 187-198.
- [30] Onyari, J. M., Wandiga, S. O., Njenga, G. K. and Nyetebe, J. O. (1991). Lead contamination in street of Nairobi city and Monbosa Island, Kenya. *Bulletin of Environmental Contamination Toxicology* 46:789.
- [31] Prica, M., Dalmacija, B., Ron Cevic, S. and KrCmarBecelic, M. (2008). A comparison of sediment quality results with acid volatile sulphite (AVS) and simultaneously extracted metals (SEM) ratio in vojvodina (Serbia) Sediments. *Science of the Total Environ*. 389: 235-244.
- [32] Pringault, O., Viret, H. and Duran, R. (2010). Influence of Microorganisms on the removal of nickel in tropical marine. *Pollution Bulletin*. 61: 530-541.
- [33] Qi, S., Leipe, T., Rueckert, P., DI, Z. and Harff, J. (2010). Geochemical sources deposition and enrichment of heavy metals in short sediment cores from the pearl River Estuary, Southern China. *Journal of Marine System*, 82:528-542.
- [34] Rasmuseen, L.D. and Soresen, S.J. (2001). Effects of Mercury contamination on the culturable heterotrophic functional and genetic diversity of the bacterial community in soil. *FEMS Microbiology Ecology*. 36: 1-9.
- [35] Schindler, P.W. (1991). The regulation of heavy metals in natural aquatic systems. In vernal JP (Ed). *Heavy Metals in the Environment*. Elsevier, Amsterdam. Pg 95-123.
- [36] Selvaraj, K., Rammohan, V. and Szefer, P. (2004). Evaluation of metal contamination in coastal sediments of the Bay of Bengal India: geochemical and statistical approaches. *Marine Pollution Bulletin*. 49:174-185.
- [37] Shi, W., Becker, J., Bischoff, M., Turco, R.F. and Konopoka, A.G. (2002). Association of Microbial community composition and hydrocarbon contamination. *Applied and Environmental Microbiology*. 68: 3859-3866.
- [38] Udosen, E. D. (2001) Determination of trace metals and fluxes in sediments along a segment of Qua Ibo River in Southern Nigeria. *Journal of Natural and Applied Science* 2(1): 82-90.
- [39] Varol, M. (2011). Assessment of heavy metal contamination in sediments of the Tigris River 9Turkey) using pollution indices and multivariate statistical techniques. *Hazardous Materials*. 195: 350-364.
- [40] Wong, K.W., Toh, B.A., Ting, Y.P., Obbard, J.P. (2005). Biodegradation of phenantherene by the indigenous microbial biomass in a zinc amended soil. *Letters in Applied Microbiology* 40:50-55.
- [41] Yang, YQ, Chem, FR, Zhang, L., Li, U., JS, WU, SJ., (2012). Comprehensive assessment of heavy metal contamination in sediment of the Pearl River estuary and adjacent shelf. *Marine Pollution Bulletin* 64: 1947-1955.
- [42] Zhang, C., Zhang, S., Zhang, L., and wang, L., (1995). Background contents of heavy metals in sedments on the Yangtze River system and their calculation methods. *Journal of Environmental Sciences* 7:422-429.