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EFFECT OF NICKEL TOXICITY ON MIXED MICROBIAL POPULATION FROM NEW CALABAR RIVER, PORT HARCOURT NIGERIA.

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ABSTRACT: The effect of nickel on the percentage survival of the mixed culture of the NRew Calabar River showed that there was reduced toxicity. The culture tolerated concentration of the metal up to 0.01mg/l throughout the duration of the toxicity study while between 0.1mg/l to 1000mg/l resulted to a slight reduction in colony count after the first 6 hours. This is probably because the organism was able to utilize nickel at these concentrations while increasing the concentration of nickel as toxicant slightly affected enzyme and biological activities of the mixed culture. The apparent low sensitivity of mixed culture to various concentrations of nickel is due to the short exposure time as used. It follows that biological damage can be minimized by removing the metals from the effluent as required by environmental regulations before discharge into water bodies. These organisms are known to play very important roles in biogeochemical cycle of the environment.

KEY WORDS: Nickel toxicity, Percentage survival, New Calabar River, Mixed culture, Biological activities, Biogeochemical cycle.

INTRODUCTION

Biogenic and petrogenic waste materials are ubiquitous due to human activities. Higher concentrations of these wastes in water, sediments and organisms are found in near shore waters, particularly in urban and industrialized parks and inlets because of the proximity to chronic inputs. This waste water when discharged into water bodies without pretreatment may contain high concentrations of heavy metals that may have adverse effects on the biological activity.

It has long been known that certain microbes can alter the redox potential or valence state of some of the metals. This information led to the development of processes for removal of heavy metals from waste. Many important toxic metal contaminants can be transformed to less-soluble or non-volatile species by micro-organisms. In these altered oxidation state mediated through microbial action, the metal chemistry is changed and often any metal toxicity is destroyed (King *et al.,* 1998). However, exposure of microbes to inhibitory metals can result to the occurrence of a variety of abnormalities such as interference with cell wall synthesis, decreased enzyme activity and deactivation of DNA and RNA (Sato *et al.,* 1986; Boularbah *et al*., 1992).

Although toxicity can be followed by monitoring the variation of internal pools of ATP or DNA, the inhibition of some microbial vital function are usually easier to measure. Blessing and SuBmuth (1992) reported that as a consequence of the high demand, rapid inexpensive and relatively simple screening tests for evaluating the acute toxicity of metal in the environment, the use of bacteria and other micro-organisms in biological short-term assays has recently received increased attention.

Aquatic ecosystems have the ability to assimilate certain amounts of waste and maintain near normal function. However, when these wastes are discharged in excess, the natural cleansing process of a river ceases, causing damage and death to micro-organisms and higher organisms (Amanchukwu and Okpokwasili, 1996, Zhu *et al.*, 2001). Sediments play an important role in elemental cycling in the aquatic environment. They are responsible for transporting a significant proportion of many nutrients and contaminants such as toxic heavy metals. They also mediate their uptake, storage, release and transfer between environmental compartments (Bartram and Ballace, 1996).

Microorganisms especially bacteria have evolved mechanisms to maintain low intracellular concentrations of toxic heavy metals. These strategies include: active expulsion of metals after they have entered the cell, complexation of metal by biologically synthesized ligands, oxidation/reduction resulting in precipitation, immobilization or volatilization (Williamson and Johnson, 1981; Dutka and Kwan, 1994). Nevertheless, these mechanisms become inefficient in the face of increasing concentrations of these toxic heavy metals in their environment (Amanchukwu and Okpokwasili, 1996).

It is therefore in the scope of this study to examine the effect of these waste toxic heavy metals with reference to Nickel on microorganisms in their niche thus generating information from the toxicity test, that can be of use in the management of pollution for the purposes of prediction of environmental effects of a waste, comparison of toxicants or test condition and regulations of discharge.

MATERIALS AND METHODS

Sources of microbial inoculum

Sources of inoculum for this study was from brackish river water, obtained from New Calabar River located in the Niger Delta, about 1km Southwest of the University of Port Harcourt. The river was selected due to waste discharges from Wilbros Nigeria Limited (WNL), an oil servicing firm. Surface water and sediment samples were collected using sterilized bottles and grab, respectively in March, 2009. These were transported to the Environmental Microbiology Research Laboratory, Department of Microbiology, University of Port Harcourt, for analysis in an ice cooled box.

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Detection, enumeration and identification of different microorganisms

The total heterotrophic plate count of the samples was determined using nutrient agar. Serial ten-fold dilution using sterile normal saline (0.85% NaCl) as diluent was prepared and 0.1ml aliquot of appropriate dilutions were spread-plated in triplicate, incubated for period of 3 5 days at room temperature. After inoculation, the plates were observed for growth and plate count taken.

The total coliform was done by membrane filtration employing Membrane Lauryl Sulphate Broth (MLSB). Appropriate volumes of sample were filtered and the filters incubated on absorbent pads soaked in MLSB. The organisms produce characteristic yellow colonies on MLSB at 37˚C. Confirmation of isolates was carried out by subculturing on sterile nutrient agar followed by incubation for 24hours at 37˚C and appropriately tested for the production of acid from lactose at 37˚C. *Vibrio* species were detected by membrane filtration followed by growth on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar. Typical colonies were confirmed by gram-stain reaction, oxidase, catalase, and citrate and sugar fermentation.

Microbial colonies from the various methods were randomly picked, isolated and purified by streaking on nutrient agar. They were examined for their biochemical and phenotypic characteristics such as gram-stain reaction, colonial appearance, motility, catalase, indole, oxidase, citrate, methyl-red and Voges-Proskauer, sugar fermentation and starch hydrolysis. The test were done following the procedures of Gehardt (1994) and identification based on Holt (1994).

Preparation of standard inoculum

A mixed culture of isolates from New Calabar River was prepared by aseptically transferring discrete colonies from six plates into 200ml Erlenmeyer flaks containing sterile physiological saline. This was allowed to stand for 1 hour at room temperature to enable the organisms to acclimatize. Serial ten-fold dilution of the mixed culture was made and appropriate dilutions were spread-plated to nutrient agar in triplicates for 24 hours at room temperature. The reference number of colonies in each 0.1ml aliquot of the mixed culture served as standard inoculum.

Toxicant preparation

A solution of 0.5g Nickel per 500ml of deionized water was autoclaved at 121 C for 15 minutes and 100ml was transferred aseptically into 200ml Erlenmeyer flasks. Toxicant solutions of 1000, 100, 10, 1.0, 0.1, 0.01 and 0.001mg/litre were prepared in the nickel solution. The pH was adjusted to 7.2.

Toxicity test procedure

The various toxicant concentrations were inoculated with 0.1ml of standard inoculum of mixed culture. Following inoculation, 0.1ml was plated out at 0h, then at 6h, 12h, 18h, 24h, 30h, 36h, 42h and 48h, on nutrient agar. Plates were incubated at room temperature for 24hrs, and those with colonies between 30 - 300 were chosen and counted. The percentage survival was calculated using the methods of Williamson and Johnson (1981).

Physicochemical analysis

The temperature of the river was directly measured using a standard mercury thermometer. The pH was determined by using Model 291 MK pH meter. The biological oxygen demand (BOD) was determined by the Azide modification method; oil and grease by partition-gravimetric method; nickel, chromium, and iron were determined using Atomic Absorption Spectrometry (AAS) method. All tests were conducted according to APHA (1998).

RESULTS AND DISCUSSION

The observed high microbial load of New Calabar river can be attributed to industrial and municipal activities going on along the river that generate such enormous effluents (Table 1). The presence of total coliform and *Vibrio* species is suggestive of possible human faecal contamination of the river. The objective of treating is to reduce the level of toxic components such as ammonia, phenols, polycyclic aromatic hydrocarbons and heavy metals, hence making it conducive for aquatic biota and its dependents.

The effect of total coliform and *Vibrio* species could be a point source of infectious diseases to the inhabiting communities (WHO, 1993). The genera of the following indigenous microorganisms were identified; *Baccillus, Enterobacter, Escherichia, Proteus, Pseudomonas, Klebsiella, Serratia, Shigella, Staphylococcus, Vibrio* species while fungal genera include; *Aspergillus, Geotricum, Penicillum, Trichoderma, Candida and Saccharomyces*. These different genera of bacteria made up the community of the mixed culture.

The effect of nickel on the percentage survival of the mixed culture of bacteria from the New Calabar River is presented in Figure 1. The result of the study showed that there was reduced toxicity of nickel to the mixed culture of bacteria isolates (Wang and Paulo, 1985; Okpokwasili and Odokuma, 1993; Dutka and Kwan, 1994). The toxicity of this metal to the mixed culture increased with increased concentration and increased exposure time. Toxicity of nickel to the mixed culture became apparent at concentration of 0.1mg/l. This is comparable to the findings of Sato *et al.* (1986) who with copper, cadmium and nickel observed a reduction in the growth of *Nitrosomonas europaea* in the organic medium at concentration as low as 0.04mg/1.

The culture tolerated concentration of the metal up to 0.01mg/l throughout the duration of the toxicity study while 0.1mg/l to 1000mg/l resulted to a slight reduction in colony count after the first 6h. The metal showed a slight effect on the mixed culture at concentrations of 0.001mg/l to 0.01mg/l. This is probably because the organism was able to utilize nickel at these concentrations. Between 0.1mg/l to 1000mg/l, toxicity of the metal was more pronounced for the last 6h because; the bacteria could not utilize the metal during this time. A decrease in percentage survival with increase in exposure time was also observed (Okpokwasili and Odokuma, 1996).

Table 1: Microbiological results of samples from the New Calabar River

Parameter	Surface	Sediment
Total heterotrohic count (cfu/ml)	8.2 (±0.2) x 10^6	8.6 (±0.2) x 10^6
Total fungal count (cfu/ml)	8.4 (±0.3) x 10^3	1.0 (±0.4) x 10^4
Total coliforms (cfu/ml)	6.0 (±0.1) x 10^2	9.0 (±0.1) x 10^2
Vibrio species (cfu/ml)	3.2 (±0.2) x 10^2	4.4 (±0.2) x 10^2

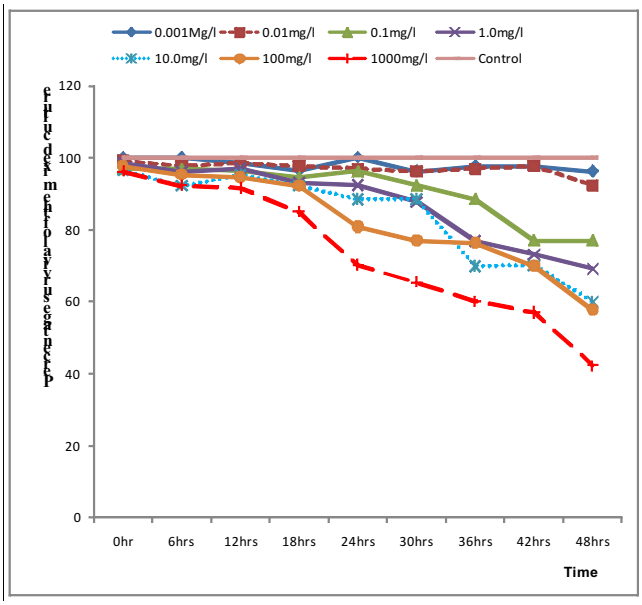

Figure1: Effect of Nickel on the percentage survival of the mixed culture of Bacteria from the New Calabar River over time.

The reduced toxicity of nickel observed may be that it precipitated and have sorbed on fine particles thereby reducing its availability and toxicity (Wang and Paulo, 1985; Sato *et al.,* 1986; Dutka and Kwan, 1994). The apparent low sensitivity of mixed culture to various concentrations of nickel is due to the short exposure time as used. This can minimize any toxic effect on enzyme synthesis, cell reproduction, interference with cell wall synthesis and deactivation of DNA and RNA (Boularbah *et al.* 1992; Laroche, 1992). The low toxicity could also be attributed to the concentration of the metal used, form of this metal, species of organism used in preparation of the mixed culture, traces of other cation present. Toxicity screening of microbial tests are often reported in terms of 50% inhibition concentration.

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The temperature of the river falls within the ambient temperature of the region while the pH is slightly acidic as a result of effluents discharged into the river. Industry which discharges effluent including petroleum related waste shall treat the effluent to a uniform level to ensure assimilation by the receiving water. To confirm compliance to this, parameters like temperature, pH, biological oxygen demand, oil and grease, nickel, chromium and iron were determined and compared with Federal Environmental Protection Agency (FEPA, 1991) standards. The comparison revealed that these parameters fall below the acceptable level of the standard. Therefore it follows that industries that discharge effluents into the river with such a reckless neglect should be meant to comply with effluent limitation guidelines.

Results indicated that increasing the concentration of nickel as toxicant slightly affected enzyme and biological activities of the mixed culture. This stimulated the real life occurrence in the New Calabar River when certain heavy metals are allowed indiscriminately into the river, especially nickel. Given that microorganisms are usually some of the most affected when untreated waste is discharged. It follows that biological damage can be minimized by removing the metals from the effluent as required by environmental regulations before discharge into water bodies. These organisms are known to play very important roles in biogeochemical cycles of their environment.

REFERENCES

Amanchukwu, S.C. and Okpokwasili, G.C. (1996). Microbial ecology of petroleum refinery effluent outfall sites. *African Journal of Ecology*. *34*: 239 245.

APHA (American Public Health Association) (1998) *Standard Methods for the Examination of Water and Wastewater,* 20th edn, APHA, Washington DC.

Bartram, J. and Balance, R. (1996). *Water Quality Monitoring: A practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes.* Published on behalf of UNEP and WHO. Chapman and Hall, London.

Blessing, B. and SuBmuth, R. (1992). Stimulating effects in bacteria toxicity tests: a source of error in evaluating the risk posed by chemicals. *Water Resources*. *27*: 225 229.

Boularbah, A., Morel, J.L., Bitton, G. and Guckert, A. (1992). Cadmium biosorption and toxicity to six cadmium-resistant gram-positive bacteria isolated from contaminated soil. *Environmental Toxicology and Water Quality*. *7*: 237 246.

Dutka, B.J. and Kwan, K.K. (1994). Studies on synthesis activated sludge toxicity screening procedure with comparison to three microbial tests. In: *Toxicity Screening Procedures using Bacteria Systems*. D., Liu and B.T., Dutka (ed). Dekkar, New York. Pp 125 138.

FEPA (Federal Environmental Protection Agency) (1991). *Guideline and Standards for Environmental Pollution Control in Nigeria*. FEPA Publisher pp. 46 59.

Gehardt, P. (1994). *Methods for General and Molecular Bacteriology* (ed) ASM Press, Washington, DC.

Holt, J. G. (ed.) (1994) *Bergey's Manual of Determinative Bacteriology*, 9th ed. Williams and Wilkins Co., Baltimore.

King, R.B., Long, G.M. and Sheldon, J.K. (1998). *Practical Environmental Bioremediation: The Field Guide*. 2nd ed. Lewis Publishers CRC Press, Boca Raton, FL.

Laroche, G. (1992). Biological effects of short term exposure to hazardous materials. In: *Control of Hazardous Materials Spills*. Environmental Protection Agency, University of Houston, Texas. pp. 199 - 206.

Okpokwasili, G.C. and Odukuma, L.D. (1993). Tolerance of *Nitrobacter* to toxicity of some Nigerian crude oils. *Bulletin of Environmnet Contamination and Toxicology. 53*(3): 388 - 395.

Okpokwasili, G.C. and Odukuma, L.D. (1996). Response of *Nitrobacter* to toxicity of drilling chemicals. *Journal of Petroleum Science and Engineering. 16*: 81 87.

Sato, C., Schoor, J.L. and McDonald, D.B. (1986). Effect of copper and nickel on the growth of *Nitrosomonas europaea*. *Toxic Assessment International*. 1: 357 376.

Wang, W. and Paulo, R.P. (1985). *Nitrobacter* bioassay for aquatic toxicity. *Environment International*. *13*: 35 39.

WHO (World Health Organiztaion) (1993). *International Standards for Drinking Water*. (3rd ed.). Geneva.

Williamson, K.J. and Johnson, O.G. (1981). A bacterial assay for assessment of waste water toxicity. *Water Resources*. *15*: 383 390.

Zhu, X., Venosa, A.D., Suidan, M.T. and Lee, K. (2001). Guidelines for the bioiremediation of marine shorelines and freshwater wetlands. Report under a contract with office of research and development, U.S. Enviroinmnetal Protection Agency.