British Journal of Applied Science & Technology
4(2): 399-422, 2014

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Effects of Heavy Metals on Microbial Function in Water and Sediment around Abonnema Island at Lower Sombriero River, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author TJKI edited the proposal, supervised the field sampling, carried out the laboratory and statistical analyses of the study and wrote the final manuscript. Author SDO proposed and financed the study managed the literature searches and wrote the first draft of the manuscript. Both the authors read and approved the final manuscript.

Original Research Article

Received 5th September 2012
Accepted 23rd November 2012
Published 22nd October 2013

ABSTRACT

Aims: The interrelationships between heavy metals Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr) and Zinc (Zn) and microorganisms in sediment and overlying water along the shoreline of Abonnema Island were investigated by determining the levels of Carbon Dioxide (CO_2), Ammonia (NH_3), and Sulphate (SO_4^{2-}).
Study Design: Water and sediment samples were collected from seven stations approximately 500 metres apart using plastic containers and Erkman grab sampler respectively. Glucose, palm oil, and cowbell powdered milk were separately added to each set of water and sediment samples and each inoculated with 0.05mg/l, 0.15mg/l and 0.5mg/l of copper, zinc, lead, chromium and cadmium and incubated for seven days at about 30ºC.
Place and Duration of Study: The study was undertaken in the Institute of Geosciences and Space Technology, University of Science and Technology, Port Harcourt, Nigeria between January 2007 and November 2008.
Methodology: Heavy metals were determined using Atomic Absorption

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Spectrophotometer. Carbon dioxide, Ammonia, Sulphate in water and sediment were determined by titration, phenate, turbidimetric and KH_2PO_4 extraction methods using Spectrophotometer. Total heterotrophic counts were carried out on Nutrient agar plates. Total coliform and Total faecal coliform counts were determined using the most probable number (MPN) technique using Mac – Conkey broth. Petroleum degrading bacteria was isolated in petroleum agar.

Results: The results showed that heavy metals had stimulatory effect on microbial functions in some cases and inhibitory effect in others. The stimulatory effect on CO_2 respiration was more in the water than in the sediment while the stimulatory effect on NH_3 respiration was more in the sediment than in the water. The stimulatory effect on SO_4^{2-} respiration was more in the sediment than in the water. The inhibitory effects on NH_3 and SO_4^{2-} respiration were more in the water than in the sediment. The concentrations of CO_2 and SO_4^{2-} were within permissible limit while NH_3 exceeded permissible limit.

Conclusion: The inhibitory effects of the heavy metals on the levels of CO_2, NH_3 and SO_4^{2-} resulting from the biodegradation of carbohydrate (glucose) and protein (cowbell powdered milk) show that the rate of cycling of carbon, Nitrogen and Sulphur in the coastal waters is being affected by the heavy metals. Thus effluents should be treated before discharge into the river while direct discharge of other heavy metal containing wastes into the river should be discouraged.

Keywords: Heavy metals; microbial function; water; sediment; Sombriero river; inhibition; stimulation; Abonnema Island.

1. INTRODUCTION

Microbiological processes in coastal areas contribute in ecosystem changes at larger scales. Where human induced changes have been adjudged to be negative, the natural micro biota or introduced surrogates may be a useful means for some level of restoration through bioremediation [1].

Changes in microbial functions (i.e. modification of communities' functions or shift in the liberation of CO_2 and NH_3) in respect to the toxic heavy metals interactions become an indicator of the effect of toxic heavy metals on the microbial functions. Microbial function can then be potentially used as a biomarker of contaminant availability and increase in concentration. Microbial function may be a responsive marker of contaminant stress in the coastal water. The purpose of microbial activities from the microorganisms' point of view is to get energy for cellular activities but from the human point of view is to clean up organic wastes of man, domestic, industry and agriculture by processes of oxidation and reduction [2].

Contamination of soils with toxic metals is a major problem world wide; of particular interest to the field of bioremediation is the selection of biological or biochemical markers for the end point of remediation [3]. Biomarkers indicate that sufficient pollutant is available for enough time to elicit a response or effect [4]. Because biochemical changes generally are detectable before adverse effects are seen at higher levels of biological organization, the biochemical marker approach is often an early warning or proactive tool. This is great advantage because responses at higher levels such as the ecosystem are usually measurable only after significant or permanent damage has occurred. The biomarker can be used as monitoring tool for environmental degradation. The biochemical markers are also useful to

monitor the shift back to a normal state after clean up of a contaminated environment. It can also serve as evaluating remediation alternatives for contaminated aquatic system [5,6].

The effects on the microorganisms, the interactions of contaminants and the resident microorganisms are almost overlooked in many environmental toxicological studies [1]. However, environmental contaminants may affect microbiological activities. Micro biota effects can range from stimulation to inhibition depending on the form and concentration of the contaminant. If contaminants are either acutely or chronically toxic to part or all of the microbial population, alterations in the normal path ways of carbon energy flow and productivity within the system may be expected.

At present, however, sufficient information of the effects on the microorganisms of interactions between the natural microorganisms of coastal areas and environmental contaminants especially toxic heavy metals is relatively sparse, making it difficult to predict the fate and effects on the microorganisms of contaminants compounds in affected areas [1]. By the breakdown of organic wastes, microorganisms contribute decisively to the natural self- purification of coastal waters. During this process the concentration of organic nutrients is diminished so much that eventually the bacterial content of the water decreases correspondingly [7]. Natural self-purification of coastal waters is extremely important because this process keeps removing contaminants from the water so that rivers are reasonably clean again some kilometers away from the point of the organic waste outlet. This process keeps contaminants concentration in sea foods low.

The decisive role is played by bacteria and fungi however, they can break down organic compounds both those which are present in solid form and in solution [7,8].

Heavy metals discharged into aquatic ecosystems are likely to be scavenged by particles leading to their accumulation in sediments [9]. A large reservoir of metals in the sediments can also act as a source to the overlying water column after their input to the ecosystem has ceased [10], potentially leading to adverse ecologic effects [9]. However, the extent of the risks is difficult to accurately assess because of the complexity of biologic and chemical interactions that alter the bioavailability of metals. Release from sediments may not only result from re-suspension of particulates, but also through the activities of microorganisms within the sediments and at the sediment-water interface, resulting in biotransformation to more volatile/soluble forms [11]. These soluble forms of the heavy metals may be incorporated in crustaceans, finfish and shellfish [12].

The shoreline of Abonnema having served as harbour for more than two decades, before it was abandoned at the wake of the Nigerian Civil war in 1967 by the Nigerian ports Authority, by implication has the toxic heavy metal pollutants found around harbours. These heavy metals get adsorbed onto the sediment surface at different pH. Heavy metals sop onto suspended particulates; this is a concern because filter feeding organisms such as shellfish are most likely to bioconcentrate the metals associated with these particulates [12].

The shoreline of Abonnema is biologically productive, as artisanal fishing activities are always carried out in the coastal waters. The inhabitants of Abonnema depend largely on sea foods as the major source of their protein need. Therefore it is important to keep surveillance by the biomarker approach over increase in concentration of the toxic heavy metals and the purity of the coastal waters where these sea foods are obtained; so that bioavailable toxic heavy metals do not disable the community structure and functions of the major self-purification instruments (the micro biota) of the coastal waters by anthropogenic

activities. The shoreline of Abonnema is occasionally inundated by spilled oil from oil spillage when it occurs as it leads to other oil and gas bearing communities like Idama and Soku where oil and gas exploitation activities are at the peak. An oil spill can cause not only hydrocarbon contamination but also heavy metal contamination in extremely small concentrations such as inorganic salts, metal soaps and organic metal complex compounds [13]. The building of the Abonnema - Degema bridge contributed its share of toxic heavy metals. The heavy metal contaminants may be released from the sediment into the water column; leading to the possible contamination of benthic organisms living in contact with them and finally of all the benthic food chain [14].

This study aims to determine the effects of lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr) and Zinc (Zn) on microbial functions by determining the levels of carbon dioxide (CO_2), Ammonia (NH_3), and Sulphate (SO_4^{2-}) liberated in water and sediment around the shorelines of Abonnema island.

2. MATERIALS AND METHODS

2.1 The Study Area

Abonnema along whose shoreline the samples for the study were collected is located on the lateritic clay residual deposits in the transition or mangrove (Middle Delta) zone of the Niger Delta along the Sombriero River of Rivers State [15] Fig. 1. This is the mangrove brackish water zone that has numerous intertidal flats and mangrove vegetation subsoil here is characterized by a typical fibrous pervious clayey mud that shows a large value of compressibility and consolidation [15].

The climate of the zone is basically that of tropical monsoon with rainfall occurring almost all through the year except the months of December, January and February, which are not completely free from rainfall in some years [15].

The zone experiences diurnal ebb and flow of the tides with maximum values obtained during the once- a-year spring tide [15]. The surface water of the Sombriero River along the shoreline of Abonnema is the sodium chloride (NaCl) subtype of the rock dominated type [16], because during the dry season a slight sparking in the river water is noticed when the water is disturbed at night by the observer in a boat. When the river water is collected and evaporated to dryness, sodium chloride (NaCl) crystals form [17].This salt can be used as table salt after recrystallizations to improve the purity, as practiced by most coastal communities located along the Sombriero River [17].

FIG.I : MAP OF THE STUDY AREA SHOWING SAMPLING POINTS

2.2 Sampling Site Selection and Location

The sampling site was selected based on representativeness of the site of wastes and metal components discharge and easy accessibility. Water and sediment samples were collected from seven stations approximately 500 metres apart. Station 1 (Owusara) is situated between longitude 006º 46′ 23″ E and latitude 04º 44′ 24″ N, station 2 (Bulk oil produce, BOP, company area) is situated between longitude E006º 46′ 11″ E and latitude 04º 43′ 48″ N, station 3 (Alice-Okolo creek junction) is situated between longitude 006º 46′ 11″ E and latitude 04º 43′ 48″ N, station 4 (Quick-Penny water side in Georgewill compound) is situated between longitude 006º 46′ 9″ E and latitude 04º 43′ 59″ N, station 5 (Timber Poku i.e. Timber water side) is situated between longitude 006° 46′ 19″ E and latitude 04º 45′ 32″ N, station 6 (Alice Okolo, Bridge Area) is situated between longitude 006º 46′ 36″ E and latitude 04º 43′ 31″ N, station 7 (Okolobio, between Jack and Georgewill compounds) which served as control, is situated between longitude 006º 46′ 30″ E and latitude 04º 43′ 59″ N. The water and sediment samples were labeled (a) and (b) respectively at each station.

2.3 Sample Collection and Preparation

The water samples were collected after recording the pH in-situ using portable digital pH meter and temperature using thermometer, plastic containers were used to collect the water sample. The containers were rinsed thoroughly with the water twice before collection. The sediments samples were collected with an 'Erkman grab' sampler.

Dissolved oxygen (DO) contents of the water samples were fixed in the field by the addition of manganous sulphate solution (Winkler I) and alkaline iodide azide reagent (Winker II). The sediment was dried, ground, sieved, weighed and stored in polyethylene bags.

The parameters determined are Carbon dioxide, Nitrogen (ammonia), Sulphate, pH, Temperature, and Dissolved Oxygen.

2.4 Materials

Water and sediment samples from Abonnema shoreline for the sources of the bacteria, glucose for carbohydrate, palm oil for lipid, cowbell powdered milk for protein, lead nitrate (Pb $(NO_3)_2$), copper nitrate (Cu $(NO_3)_2$), and zinc nitrate (Zn $(NO_3)_2$), cadmium nitrate (Cd $(NO_3)_2$), chromium chloride ($CrCl_3$)for the sources of the toxic heavy metals. Solar series Atomic Absorption Spectrophotometer (AAS), Spectronic 2ID, Erlenmeyer flasks, centrifuge tubes, 2mm sieve, Analytical balance, Whatman No 42 filter paper, conical flasks, funnels, mortar and pestle, photometer, Burettes, pipettes, mechanical shaker, Erkman grab sampler, polythene bags, plastic bottles, DO bottles and thermometer. Palm oil and 'Cowbell' powdered milk were used in this study because they are cost effective, readily available and locally consumed.

2.5 Experimental Methods

2.5.1 Decomposable organic matter

Water samples, 200ml were measured into each of six sets of seven 250ml plastic containers, representing the sampling stations, with lids. Into two sets were inoculated 0.9375g of carbohydrate (glucose), and into the second two sets were inoculated 2ml of lipid (palm oil) and into the third two sets were inoculated 0.7930g of protein (cowbell powdered milk).

Ten grams of sediment samples was measured into six sets of seven 250ml plastic containers with lids. Into two sets were inoculated 0.9375g of carbohydrate (glucose) and into the second two sets were inoculated 2ml of lipid (palm oil) and into the third two sets were inoculated 0.7930g of protein (cowbell powdered milk).

Ten grams of sediment samples was measured into six sets of seven 250ml plastic containers with lids. Into two sets were inoculated 0.9375g of carbohydrate (glucose) and into the second two sets were inoculated 2ml of lipid (palm oil) and into the third two sets were inoculated 0.7930g of protein (cowbell powdered milk).

One set of the water and sediment samples inoculated with the decomposable organic matter were incubated simultaneously for seven days at about 30ºC. At the end of the incubation period all the samples containing carbohydrate and lipid were tested for carbon

dioxide. And all the samples containing protein were tested for carbon dioxide, Ammonia and Sulphate.

2.5.2 Metal inoculation

One set of water samples containing carbohydrate, lipid, and cowbell powdered milk, were inoculated with 0.0277g of ($Cu(NO_3)_2$) containing 0.05mg/l of copper. One set of sediment samples containing carbohydrate, lipid and cowbell powdered milk were inoculated with 0.0277g of ($Cu(NO_3)_2$). Both the water and sediment samples containing the heavy metal were incubated for seven days at about 30ºC. At the end of the incubation period all the samples containing carbohydrate and lipid were tested for carbon dioxide and all the samples containing proteins were tested for carbon dioxide, Ammonia and Sulphate.

The differences between the chemical parameters tested for in the samples with heavy metal and those without heavy metals were noted. The sets of containers containing metals were similarly and separately treated (incubated) with the following concentrations of heavy metals: (0.05mg, 0.15mg and 0.5mg)/l of copper; (0.05mg, 0.15mg, 0.5mg)/l of zinc; (0.05mg, 0.15mg, 0.5mg)/l of lead; (0.05mg, 0.15mg, 0.5mg)/l of chromium; (0.05mg, 0.15mg, 0.5mg)/l) of cadmium. For each of the incubations, the upper limit of the toxic heavy metals used was 10mg/l. This concentration limit was taken for this study because most of the compounds of Pb and Cu have aquatic toxicity TLM 96, 1-10mg/l. The toxic heavy metals concentrations were taken as 1mg/l (0.05mg), 3mg/l (0.15mg) and 10mg/l (0.5mg) for the seven samples of water and seven samples of sediments respectively. The mg of Pb from $Pb(NO_3)_2$ was obtained via the mole fraction of Pb in $Pb(NO_3)_2$. For instance 0.05mg Pb was given by 0.0265g $Pb(NO_3)_2$.

2.6 Analytical Methods

2.6.1 Heavy metals

Heavy metals were determined using solar S. series Atomic Absorption Spectrophotometer (AAS).

2.6.2 Carbon dioxide

Carbon dioxide was determined by titration method. The sample was titrated with sodium hydroxide solution to the pink colour and point using phenolphthalein indicator.

2.6.3 Ammonia

Ammonia was determined by phenate method. 50ml of the sample solution was treated with 2ml each of phenol and sodium nitroprusside, 5ml each of hypochlorite, trisodium citrate and sodium hydroxide solutions to produce bluish colour. The mixture was measured in spectrophotometer, spectronic 2ID at 630nm.

2.6.4 Sulphate

Sulphate in the water samples was determined by turbidimetric method and sulphate in the sediment by KH_2PO_4 extraction method.

2.6.4.1 Turbidimetric method

Sample aliquot, 20ml, was measured into conical flask and 1ml of conditioning reagent added. $BaCl_2$ crystals were added to the sample and shaken. The absorbance of the sample solution was measured before and after addition of $BaCl_2$ with spectrophotometer spectronic 2ID [18].

2.6.4.2 KH_2PO_4 extraction method

Five grams (5gm) of the sediment sample was treated with 25ml of KH_2PO_4 solution and filtered through Whatman No. 42 filter papers. Sulphate content was determined by the turbidity method and using spectrophotometer. pH was determined by pH meter. Temperature was determined by Thermometer.

2.6.5 Dissolved oxygen (DO)

Dissolved oxygen (DO) was determined by the modified Winkler method [18]. The sample was collected into 75ml DO bottle; 0.5ml of Winkler I solution was added followed by 0.5ml of Winkler II solution. The sample was corked and shaken, and then 0.5ml by 0.5ml of concentrated sulphuric acid H_2SO_4, was added and shaken. 25ml of the fixed sample was titrated against 0.025 N $Na_2S_2O_3$ with starch indicator.

2.6.6 Microbiological analysis

The samples were investigated for Total heterotrophic count (THC), Total coliform count (TCC) and Total faecal count (TFC). Petroleum degrading Bacteria count (PDBC). The total heterotrophic counts were carried out on Nutrient agar plates and incubated at 37ºC for 48 hours.

Total coliform and Total faecal coliform count were determined using the most probable number (MPN) technique using Mac – Conkey broth in tubes and incubating at 37ºC for 24 hours.

Petroleum degrading bacteria was isolated in petroleum agar. The sediment was added to diluents i.e. 1g of the solid to 10ml of H_2O and 0.1ml was plated directly on the petroleum agar. The solid nutrient is petroleum and agar for solidification. Bacteria characterization was by Gram staining method.

3. RESULTS AND DISCUSSION

3.1 Results

The Results of the study are presented in Table 1 below.

Table 1. Levels of physico-chemical parameters measured in water and sediment along the shoreline of abonnema before incubation

S/No.	Sample No.	pH	DO (mg/l)	SO_4^{2-}(mg/l)	NH_3 (mg/l)	CO_2 (mg/l)
1	1a	7.0	15.71	196.10	0.173	12.49
2	1b	6.2	9.32	52.83	0.710	12.49
3	2a	7.1	14.61	163.12	0.034	19.02
4	2b	5.9	5.67	29.11	0.820	17.48
5	3a	7.2	12.42	132.68	0.174	15.68
6	3b	6.1	7.88	88.98	0.560	14.98
7	4a	6.81	5.7	269.7	0.07	16.48
8	4b	6.5	5.62	551.2	1.02	19.76
9	5a	6.9	7.3	373.7	<0.02	16.28
10	5b	7.1	6.82	424.4	0.91	14.78
11	6a	7.06	8.1	287.4	<0.02	15.98
12	6b	6.3	6.78	728.8	0.49	16.0
13	7a	6.99	8.1	218.9	<0.02	10.88
14	7b	6.1	5.93	459.9	1.76	13.98

a = water b = sediment

Table 1 shows the levels of physico-chemical parameters determined in water and sediment before incubation in the decomposable organic matter (glucose, palm oil and cowbell powdered milk) and heavy metals. pH range of 6.81 – 7.2 was determined in the water and 5.9 – 7.1 in the sediment. Dissolved oxygen range of 5.7 – 15.71mg/l was determined in the water and 5.62 – 9.32mg/l in the sediment. The levels of NH_3 ranged from < 0.02 to 0.174mg/l in the water and 0.49 to 1.76mg/l in the sediment. The levels of CO_2 ranged from 10.88 to 19mg/l in the water and 12.49-19.76mg/l in the sediment. The levels of SO_4^{2-} ranged from 132.68 to 373.7mg/l in the water and 29.11 to 728.8mg/l in the sediment.

3.2 Discussion

3.2.1 Physico-chemical parameters

In Table 1, the lower limits of the pH measured at stations 7 and 5 were slightly acidic; this may be as a result of high organic carbon load oxidation in those stations which produced large volumes of carbon dioxide that dissolved in the water to produce carbonic acid which lowered the pH. The pH of the water was higher than that of the sediment. Statistical analysis showed significant difference (P = 0.05) between the pH in the water and sediment. This was due to organic carbon breakdown in the sediment. The pH range was within the permissible limit of 6.5 – 8.5 [19]. The relative changes in pH were affected by the buffer capacity of the water and amount of substrate utilized by the microorganisms [8,20].

The level of dissolved oxygen in the water was higher than that in the sediment. This may be due to photosynthesis by phytoplankton taking place in the water and limited dissolved oxygen penetration into the sediment. Statistical analysis showed significant difference (P = 0.05) between the levels of dissolved oxygen in the water and sediment Dissolved oxygen concentrations at all the stations were within the permissible limit of 5mg/l [19].

The concentrations of NH_3 were higher in the sediment than in the water but statistical analysis showed that there was no significant difference (P > 0.05) between the

concentrations of NH_3 in the water and sediment. The concentrations of NH_3 in the water and the sediment were higher than the permissible limit of 0.0125mg/l [19].

The levels of CO_2 did not follow regular pattern. For instance the levels did not vary at station 1 but were higher at stations 2, 3 and 5 in water and stations 4, 6 and 7 in sediment. Statistical analysis showed that there was no significant difference (P>0.05) between the concentrations of CO_2 in the water and in the sediment. The concentrations of CO_2 were within the permissible limit of 0 – 15ppm [19].

The concentrations of SO_4^{2-} were higher in the sediment than in the water but statistical analysis showed that there was no significant difference (P>0.05) between them. The mean concentrations of SO_4^{2-} in the water and the sediment were within the permissible limit of 500mg/l [19].

3.2.2 Effect of incubation

The levels of CO_2 determined in the water after incubation with glucose were higher than those in the water before incubation (Fig. 1a). This was as a result of higher population of bacteria produced by the incubation. Statistical analysis showed significant difference (P = 0.05) between them with high positive correlation coefficient (0.7164). The correlation coefficients indicate how closely the variable pair of data are linearly related and show whether the slope of the regression line is positive or negative. The levels of CO_2 measured in the sediment after incubation with glucose were higher than those in the water before incubation (Fig. 2a). This was as a result increased microbial activities resulting from incubation which increased the population. Statistical analysis showed no significant difference (P>0.05) between them with negative correlation coefficient (- 0.1973). The levels of CO_2 measured in the water after incubation with palm oil were similar with those before incubation (Fig.1b). Statistical analysis showed no significant difference (P>0.05) between them with negative correlation coefficient (-0.1000). There was also no significant difference (P>0.05) between the levels of CO_2 measured in the sediment after incubation with palm oil and those before incubation with negative correlation coefficient (-0.2429) (Fig. 2b).

The levels of CO_2 measured in the water after incubation with protein were higher than those in the water before incubation (Fig. 1c), but statistical analysis showed no significant difference (P > 0.05) with low positive correlation coefficient (0.0527). The levels of CO_2 measured in the sediment after incubation with protein were higher than those before incubation (Fig. 2c) but statistical analysis showed no significant difference (P > 0.05) with high positive correlation coefficient (0.7061).

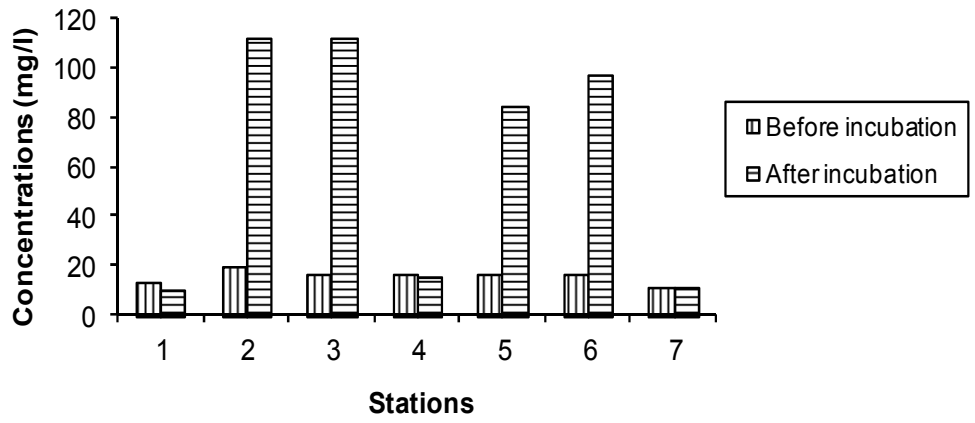

FIG.1a Concentrations of CO_2 in water before and after incubation with glucose

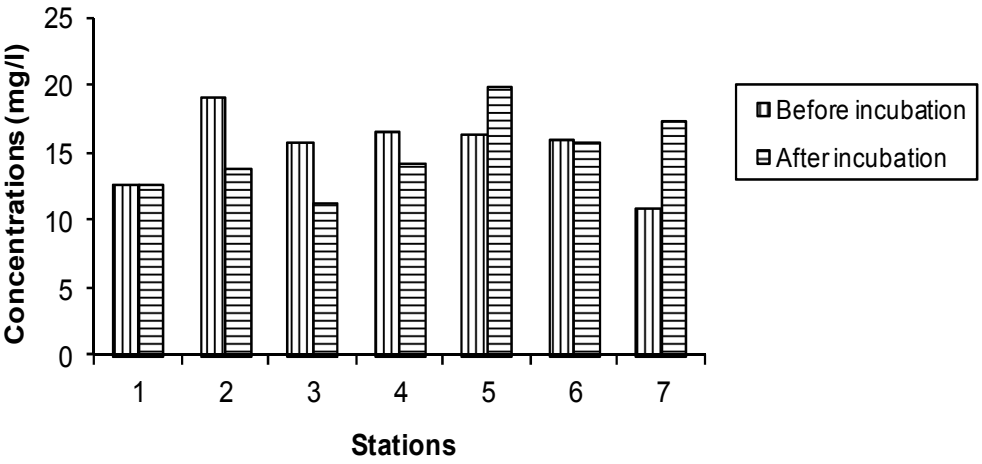

FIG. 1b Concentrations of CO_2 in water before and after incubation with palm oil

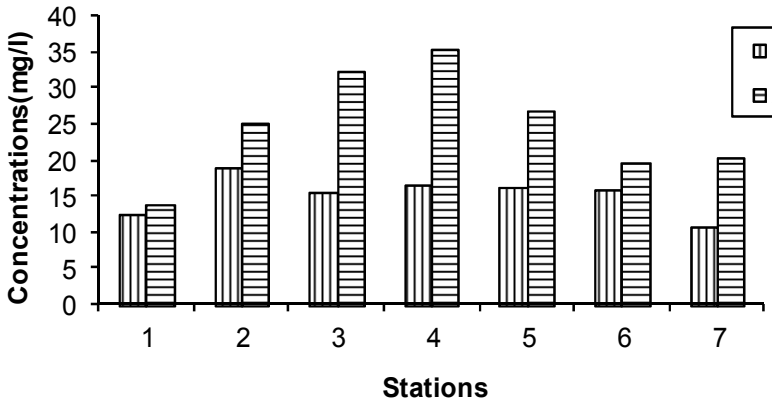

FIG. 1c Concentrations of CO_2 in water before and after incubation with protein

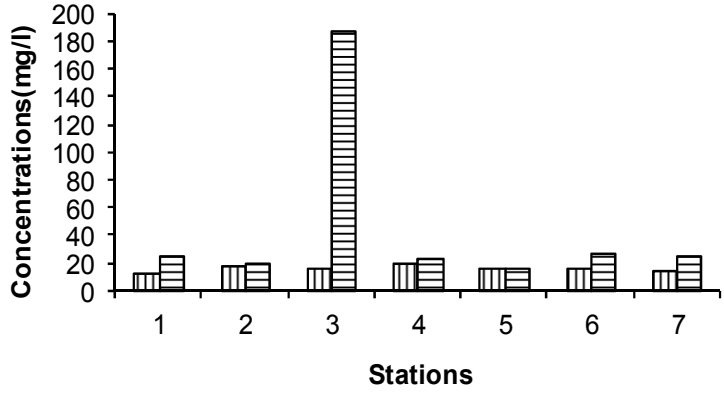

FIG. 2a Concentrations of CO_2 in sediment before and after incubation with glucose

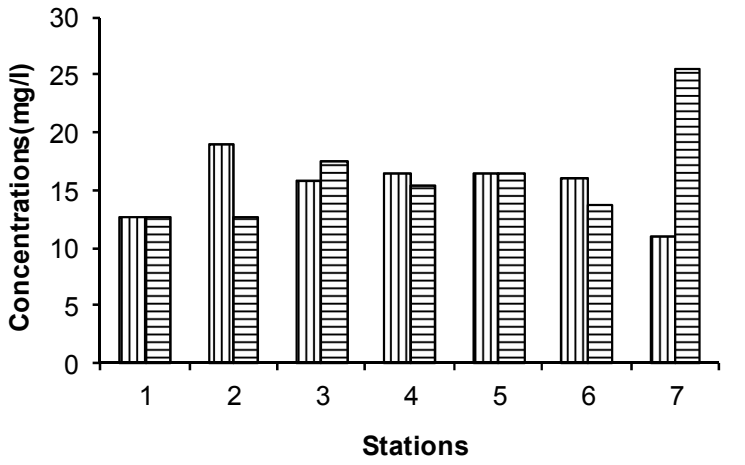

FIG. 2b Concentrations of CO2 in sediment before and after incubation with palm oil

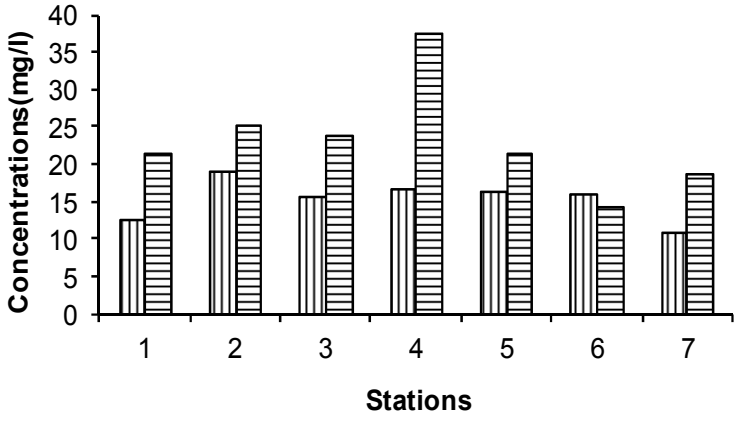

FIG. 2c Concentrations of CO2 in sediment before and after incubation with protein

The concentrations of NH_3 determined in the water after incubation with cowbell powdered milk were higher than those in the water before incubation (Fig. 3). This was as a result of bacteria population increase due to incubation. Statistical analysis showed no significant difference ($P > 0.05$) between them and positive correlation coefficient (0.2499). The concentrations of NH_3 measured in the sediment after incubation with cowbell powdered milk

was higher than those in the sediment before incubation (Fig. 4) but statistical analysis showed that there was no significant difference ($P > 0.05$) between them with negative correlation coefficient (-0.2747).

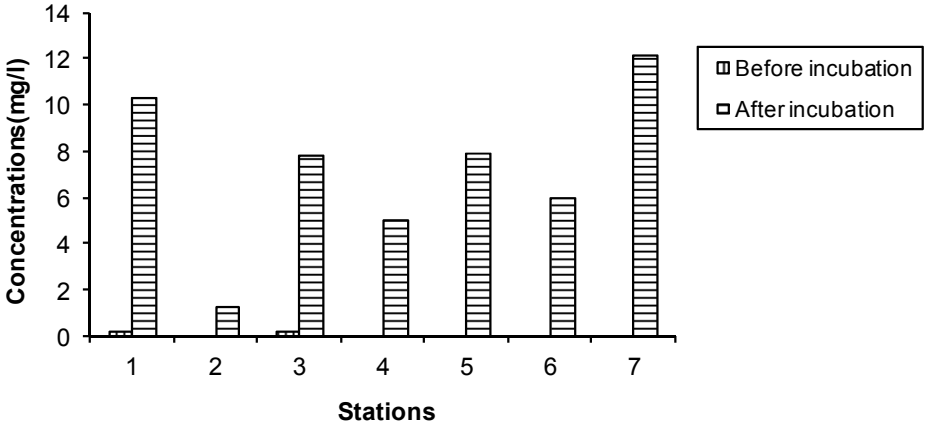

FIG. 3 Concentrations of NH_3 in water before and after incubation with protein

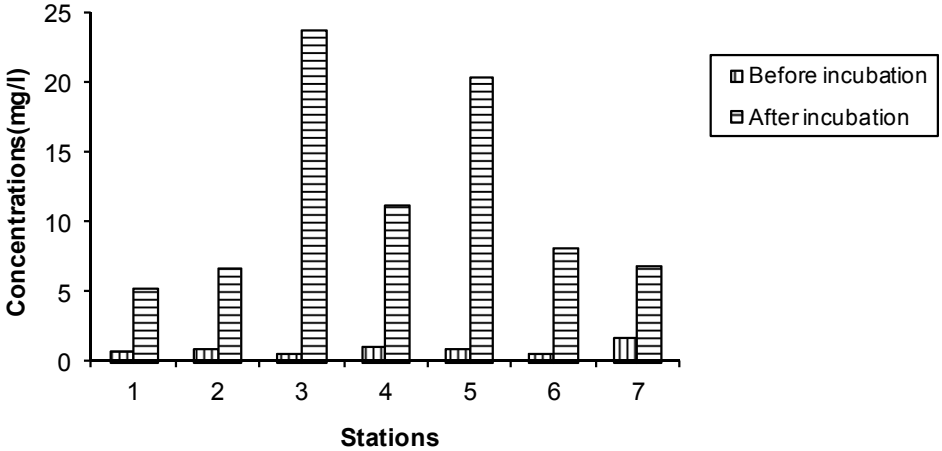

FIG. 4 Concentrations of NH_3 insediment before and after incubation with protein

The concentrations of SO_4^{2-} measured in the water after incubation with cowbell powdered milk was lower than those in the water before incubation (Fig. 5). This was as a result of low

concentrations of the protein for the incubation compared to the high protein load in the shoreline. Statistical analysis showed that there was significant difference (P = 0.05) between them with negative correlation coefficient (-0.1053). The concentrations of SO_4^{2-} measured in sediment before incubation was higher than those in the sediment after incubation with cowbell powdered milk (Fig. 6). Statistical analysis showed that there was significant difference (P = 0.05) between them with high positive correlation coefficient (0.6841). This was as a result of low concentration of SO_4^{2-} used for the incubation compared with the high protein load along the shoreline.

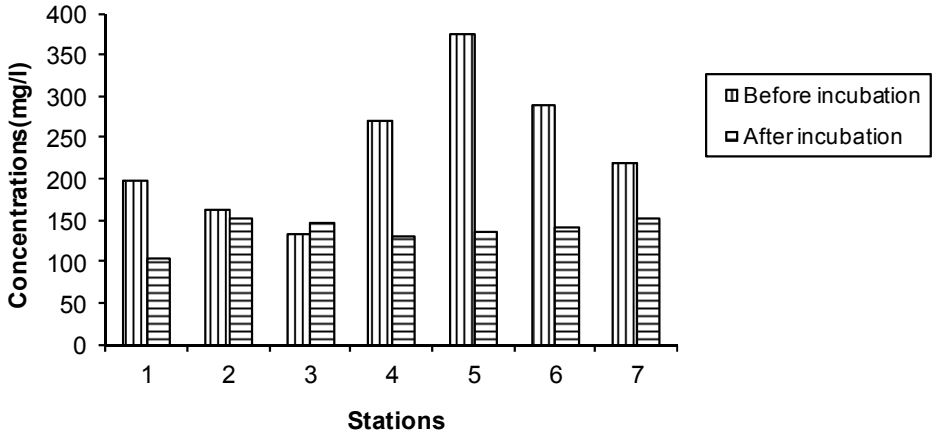

FIG. 5 Concentrations of SO_4 in water before and after incubation with protein

3.2.3 Effects of heavy metals

The levels of CO_2 measured in the water containing glucose before incubation were higher than those in the water after incubation with a mixture of glucose and Cu (Figs. 7a – 7b). Statistical analysis showed that there was no significant difference (P > 0.05) between them with negative correlation coefficient (-0.2377). The levels of CO_2 in the sediment treated with glucose before incubation was higher than those in the sediment after incubation with a mixture of glucose and Cu (Fig 10a – 10b) but statistical analysis showed that there was no significant difference (P>0.05) between them with negative correlation coefficient (-0.3823).

The levels of CO_2 in the water after incubation with palm oil and Cu was higher than those in the water treated with palm oil (Figs. 7a – 7b) but statistical analysis showed no significant difference (P > 0.05) with positive correlation coefficient (0.6336). The levels of CO_2 in the sediment containing palm oil and Cu were higher than those treated with palm oil after incubation (Fig. 10a – 10b) but statistical analysis showed that there was no significant difference (P>0.05) between them with positive correlation coefficient (0.6888). The levels of CO_2 in water containing cowbell powdered milk and Cu after incubation were higher than those in the water treated with cowbell powdered milk before incubation (Fig. 7a – 7b) but statistical analysis showed significant difference (P=0.05) with positive correlation coefficient

(0.3846). The concentrations of CO_2 in the sediment containing cowbell powdered milk and Cu after incubation were higher than those in the sediment treated with cowbell powdered milk before incubation (Figs. 10a – 10b). Statistical analysis showed that there was no significant difference (P>0.05) between them with low positive correlation coefficient (0.0025).

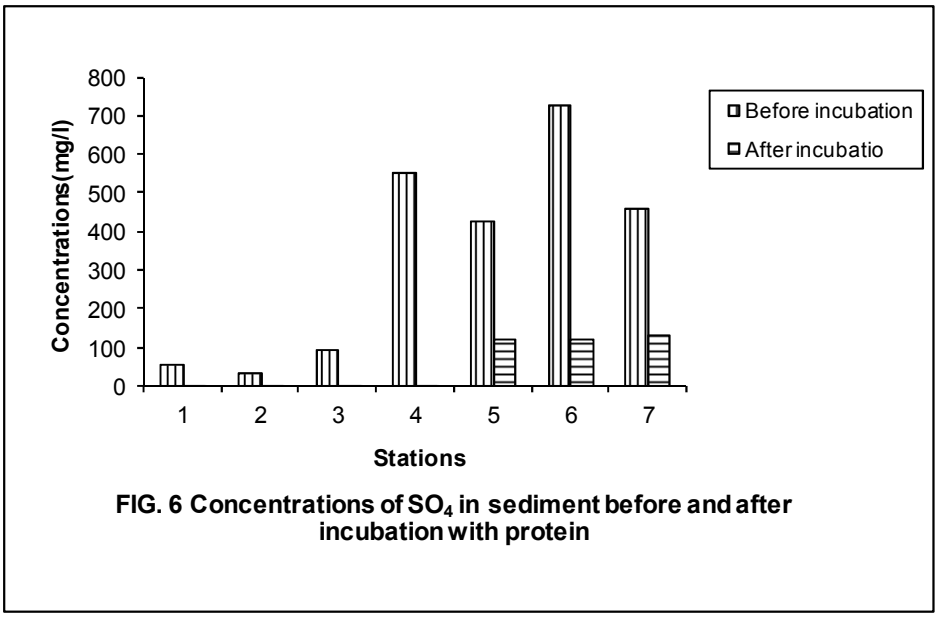

FIG. 6 Concentrations of SO_4 in sediment before and after incubation with protein

The concentrations of NH_3 in the water containing cowbell powdered milk and Cu after incubation were higher than those in the water treated with cowbell powdered milk before incubation (Fig. 8a – 8b) but statistical analysis showed significant difference (P=0.05) between them with low positive correlation coefficient (0.2471). The concentrations of NH_3 in the sediment treated with cowbell powdered milk and Cu after incubation were higher than those in the sediment treated with cowbell powdered milk after incubation (Figs. 11a – 11b) but statistical analysis showed significant difference (P = 0.05) between them with low positive correlation coefficient (0.2707).

The concentrations of SO_4^{2-} in the water treated with cowbell powdered milk and Cu after incubation was higher than those in the water treated with cowbell powdered milk before incubation (Figs. 9a – 9b) but statistical analysis showed there was no significant difference (P>0.05) between them with low positive correlation coefficient (0.1626). The concentrations of SO_4^{2-} in the sediment treated with cowbell powdered milk and Cu before incubation were higher than those in the sediment treated with cowbell powdered milk before incubation (Figs. 12a – 12b) but statistical analysis showed that there was no significant difference (P>0.05) between them but the correlation coefficient (0.8950) between them was positive and high.

Cu inhibited the oxidation of glucose to produce CO_2 in the mixture of glucose and Cu in the water and sediment but stimulated CO_2 production in the mixture of palm oil and Cu in the water and sediment; Cu also stimulated CO_2 and NH_3 production in the mixture of cowbell powdered milk and Cu in the water and sediment but inhibited SO_4^{2-} in the sediment and

stimulated SO_4^{2-} oxidation in the water. This observation agrees with the report of [1]. Similar observations were made with Zn, Pb, Cd and Cr.

From the results of this research work it was observed that the interfaces of toxicity did not depend on heavy metals concentration gradients but on bioavailability of the metals. In some cases lower concentrations of the metals had higher toxic effects on the microorganisms which were either stimulatory or inhibitory than the higher concentrations of the metals. This is attributed to the fact that toxicity is related to bioavailability of a particular metal for uptake and agreed with the reports of [1,12].

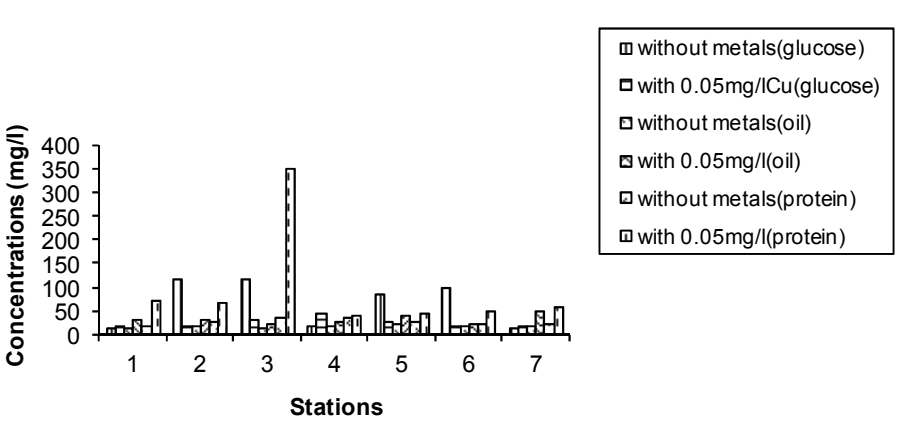

FIG. 7a Concentrations of CO_2 in water after incubation with and without metal

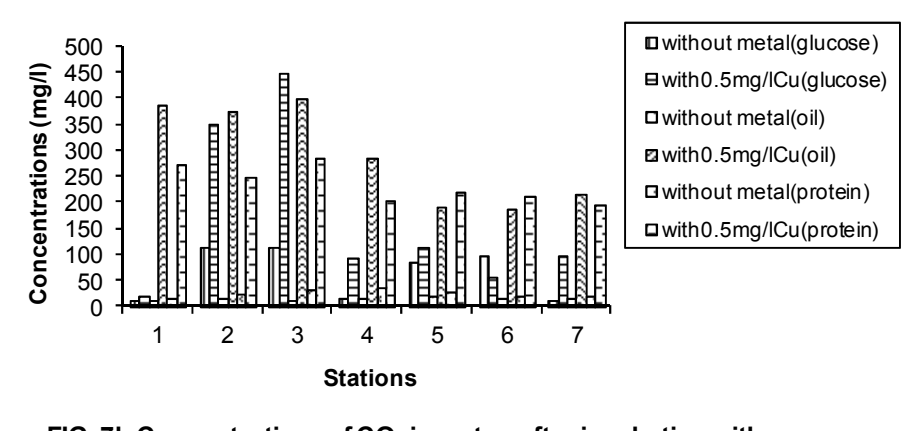

FIG. 7b Concentrations of CO_2 in water after incubation with and without metals

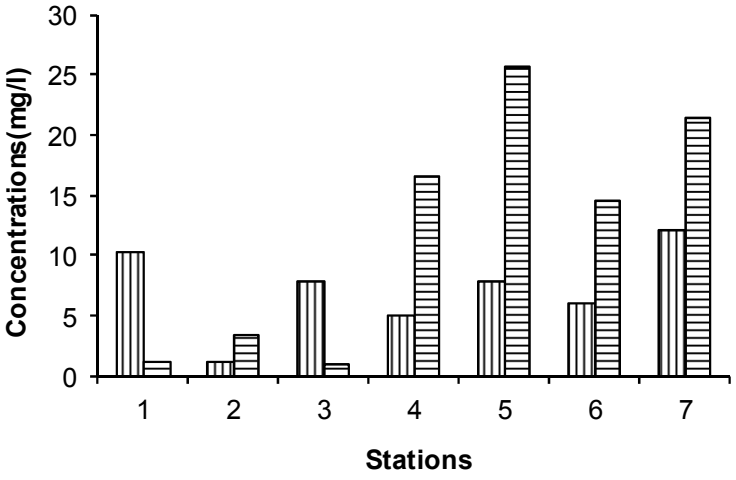

FIG. 8a Concentrations of NH_3 in sediment after incubation with and without metal

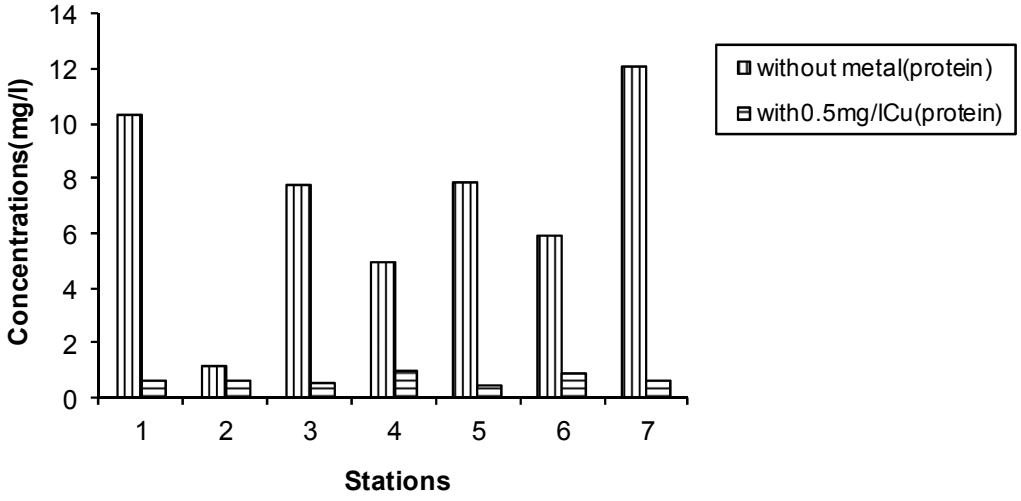

FIG. 8b Concentrations of NH_3 in sediment after incubation with and without metal

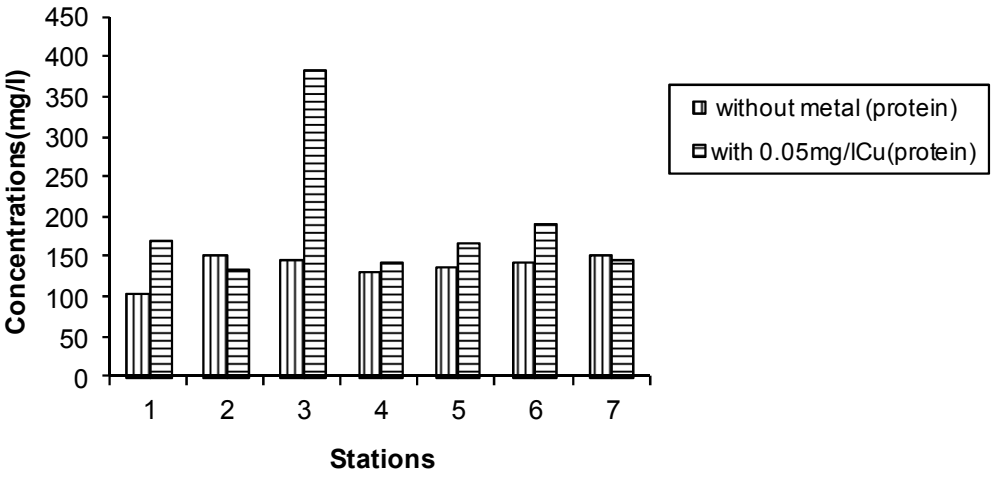

FIG. 9a Concentations of SO_4^{2-} in water after incubation with and without metal

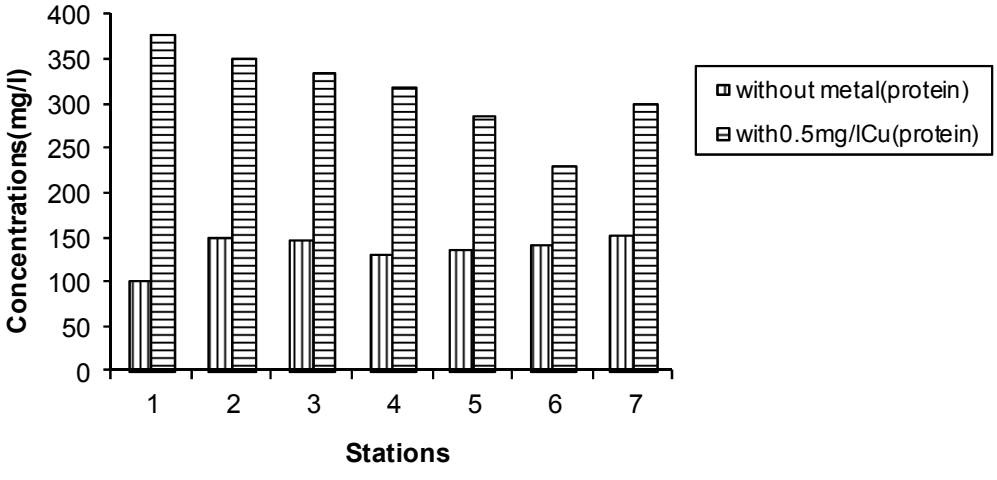

FIG. 9b Concentrations of SO_4^{2-} in water after incubation with and without metal

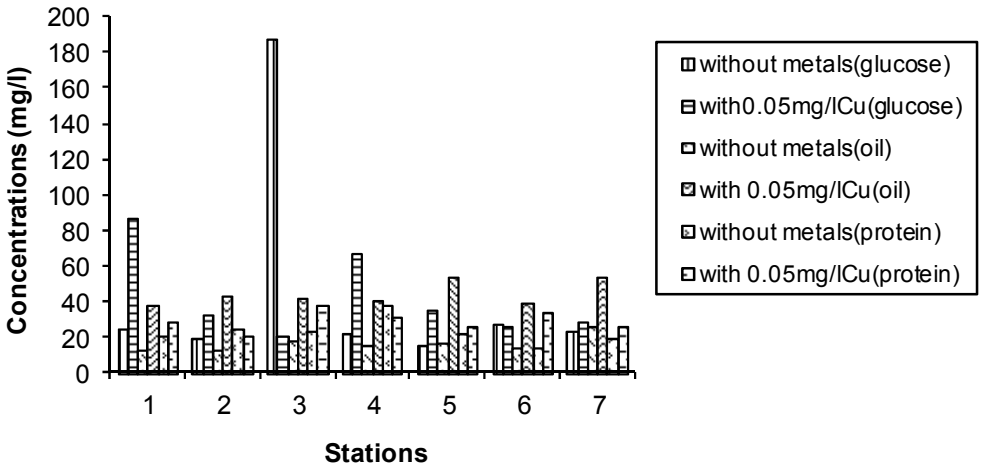

FIG. 10a Concentrations of CO_2 in water after incubation with and without metal

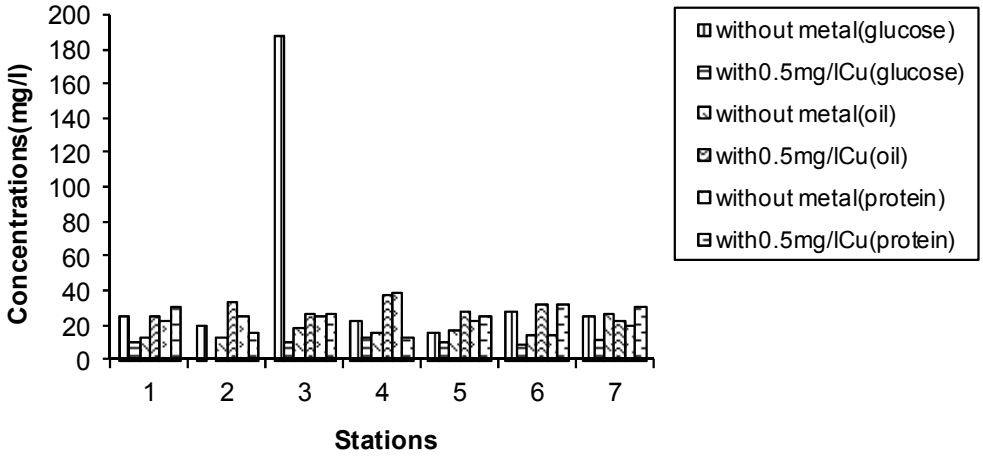

FIG. 10b Concentrations of CO_2 in water after incubation with and without metal

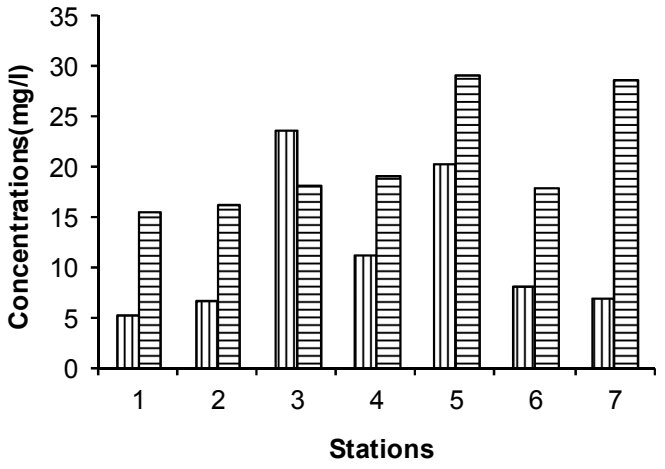

FIG. 11a Concentrations of NH_3 in sediment after incubation with and without metal

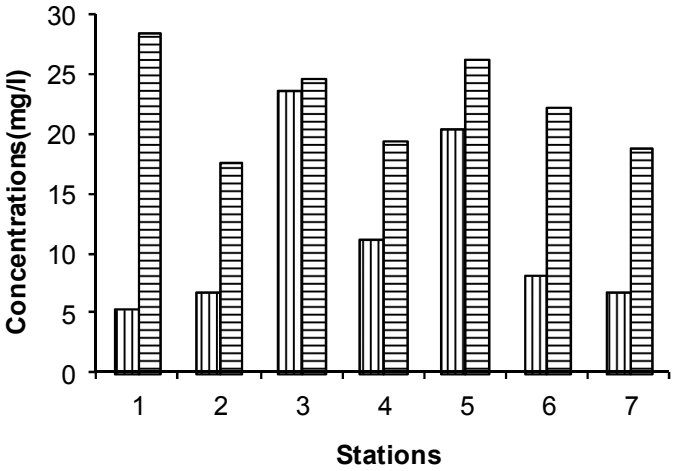

FIG. 11b Conentrations of NH_3 in sediment after incubation with and without metal

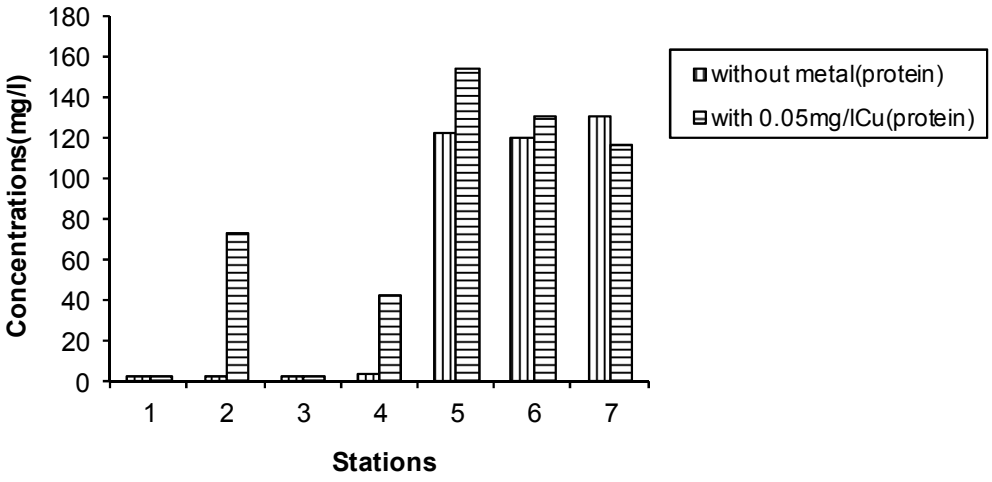

FIG. 12a Concentations of SO_4^{2-} in sediment after incubation with and without metal

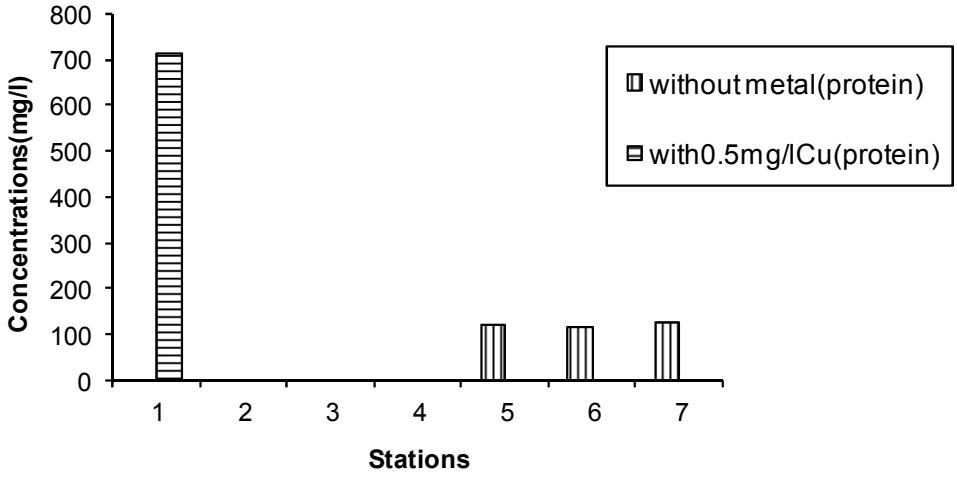

FIG. 12b Concentrations of SO_4^{2-} in sediment after incubation with and without metal

4. CONCLUSION

The inhibitory effects of the heavy metals on the levels of CO_2, NH_3 and SO_4^{2-} resulting from the biodegradation of carbohydrate (glucose) and protein (cowbell powdered milk) show that the rate of cycling of Carbon, Nitrogen and Sulphur in the coastal waters is being affected by the heavy metals.

The Government should introduce domestic waste treatment methods so that only treated wastes will be discharged into the coastal waters.

ACKNOWLEDGEMENT

We acknowledge the Institute of Pollution Studies and Department of Applied and Environmental Biology of the Rivers State University of Science and Technology Port Harcourt for the use of their laboratories and Grace Livinus for typing the manuscript. The study was financially sponsored and supervised by the authors.

COMPETING INTERESTS

The authors declare that no competing interests exist.

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