International Journal of Animal Breeding and Genetics Vol. 5 (7), pp. 001-018, July, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of nutrient supplementation on biodegradation and metal uptake by three bacteria in crude oil impacted fresh and brackish waters of the Niger Delta

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Accepted 17 March, 2016

The effect of nutrients supplements (NPK inorganic fertilizer and poultry litter) on the biodegradation of crude oil and metal uptake by three bacterial isolates (*Bacillus sp*, *Pseudomonas sp* and *Aeromonas sp*) in crude oil impacted fresh and brackish water aquatic systems of the Niger Delta were investigated. The abilities of these single cultures to reduce the contaminating oil levels as well as to bioconcentrate-associated heavy metals (Fe, Pb, Cd, Zn, Ni and Cu) were carried out for a period of 120 days. The effect of the nutrient supplements were monitored and enhanced by periodic re- addition to desired levels. Habitat water samples without nutrient supplements served as controls. Nutrient supplementation resulted in both increased pH as well as increase in biomass. In microcosms containing *Bacillus sp* and *Pseudomonas sp*, total viable cell counts increased while hydrocarbon, pH and the inorganic nutrients decreased. Significant reductions (64 and 82%) in oil and grease levels were obtained in fresh and brackish water respectively that received NPK. for *Bacillus* and *Pseudomonas.* Application of poultry litter resulted in 59.7 and 78.7% reductions in fresh and brackish water options respectively for both organisms. However, no significant changes occurred in microcosms containing *Aeromonas sp* except that counts declined considerably with time. *Bacillus* and *Pseudomonas* proved suitable for the uptake of the heavy metals present *Aeromonas* was unsuitable though it was resistant to the heavy metals. Bioconcentration of heavy metals followed the pattern: *Pseudomonas* > *Bacillus*. The pattern of heavy metal uptake by the three bacterial isolates was different in the various treatment options. The uptake of these metals was enhanced by increases in microbial biomass of the isolates. Peak uptake of metals occurred in the exponential phase for *Bacillus* while for *Pseudomonas* peak uptake of metals corresponded with the stationary phase. No significant difference in the amounts of heavy metals bioconcentrated when NPK fertilizer was used as supplement than when poultry litter was employed. The initial concentration of metals, pH of the medium as well as the cultural status of the isolates influenced metal uptake. Results indicated that addition of NPK inorganic fertilizer or poultry litter promoted both biodegradation of crude oil by *Bacillus* and *Pseudomonas* and heavy metal uptake by *Bacillus* and *Pseudomonas* in fresh and brackish water aquatic systems of the Niger Delta.

Key words: Nutrient supplements, bioconcentration, microcosms, heavy metals.

INTRODUCTION

Rehabilitation of polluted environments in the Niger Delta has been a major challenge. This has led to the development of a wide range of clean-up techniques including physical, chemical and biological techniques (Atlas, 1981; Odokuma and Ibor, 2002; Odokuma and Dickson, 2003a, b). Of the three methods biological methods are regarded as the best because it is environmentally friendly and less expensive though it is time consuming and high concentrations of the pollutant reduces the population of degrading and microorganisms and slows down the process. Biological methods (Bioremediation) exploit the diverse degradation abilities

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of microorganisms to convert the complex chemical components of crude oil to harmless products by mineralization (Alexander, 1981; Atlas, 1981). Bioremediation makes use of three processes, biodegradation, bioaccumulation and biosorption. Crude oil biodegradation is a slow but natural process limited mainly by scarcity of nitrogen and phosphorus in the environment (Ladousse and Tramier, 1991; Odokuma and Ibor, 1992). Studies have shown that crude oil biodegradation can be accelerated by the addition of nitrogen and phosphorus- containing fertilizers (whether organic or inorganic) in aqueous, terrestrial or sediment environments (Stevens, 1991; Ladousse and Tramier, 1991; Odokuma and Ibor, 1992).

There is a continuous influx of heavy metals into the biosphere from both natural and anthropogenic sources (Perelomov and Prinsky, 2003). Crude oil is also a source of these metals. In aquatic terrestrial ecosystems their bioavailability depends on their chemical forms. These heavy metals are known to cause severe damage to aquatic and terrestrial life (Odiete, 1999; Odokuma and Emedolu, 2005). A specific problem associated with metals in the environment is their accumulation in the food chain and persistence in the environment (Malekzadeh et al., 1996).

Microorganism's uptake metal either actively (bioaccumulation) and/or passively, (biosorption) (Foureset and Roux, 1992; Shummate and Strandberg, 1995). Thus the use of microorganisms for the recovery of metals from waste streams has received growing attention.

In this study the effect of nutrient (NPK fertilizer and organic poultry litter) supplementation on bioaccumulation of heavy metals and biodegradation of Bonny light crude oil in a brackish water aquatic system of the New Calabar River water of the Niger Delta was examined. The objectives were to examine; the effect of both organic and inorganic fertilizers on both metal uptake and biodegradation. Two nutrient supplements-NPK (Nitrogen, Phosphorus and Potassium) (15, 15, 15) fertilizer and organic poultry litter were used in the study. The test conditions were used to assess:

1.) The effect of NPK fertilizer in enhancing biodegradation of crude oil and bioconcentration of it's associated heavy metals.
2.) The effect of the organic poultry litter in enhancing biodegradation of crude oil and bioconcentration of its associated heavy metals.
3.) The effect of periodic replenishment of the above nutrients in enhancing biodegradation of crude oil as well as the bioconcentration of it's associated heavy metals.

Three bacterial (*Bacillus, Pseudomonas* and *Aeromonas*) isolates from the river water were obtained for metal uptake (bioaccumulation) studies. The test organisms, *Bacillus, Pseudomonas* and *Aeromonas* were chosen after screening for their resistance to heavy metals found in Bonny light crude oil from a population of nine bacterial isolates which were the most predominant indigenous species in the New Calabar River water. Also supporting their use as test organisms for bioaccumulation tests were the results from related studies confirming their resistance to these heavy metals (Odokuma and Abah, 2003; Odokuma and Ijeomah, 2004; Odokuma and Emelodu, 2005).

MATERIALS AND METHODS

Preparation of stock solution of heavy metal salts

The heavy metal salts employed in this study include: Nickel tetraoxosulphate (vi) salt ($NiSO_4$), copper (ii) tetraoxosulphate (vi) salt ($CuSO_4$), lead trioxonitrate (v) salt ($PbNO_3)_2$, iron (ii) tetraoxosulphate (vi) salt ($FeSO_4$), cadmium tetraoxosulphate (vi) salt ($CdSO_4$) and zinc tetraoxosulphate (vi) $ZnSO_4$ salt. A weight of each of these heavy metal salts that gave a corresponding 1g of each of the respective heavy metal was weighed and dissolved in 1000 ml of deionised water. These were left to stand for 30mins to obtain complete dissolution. This was followed by sterilization by membrane filtration (0.2 m pore size Aerodisc).

Isolation of heavy metal resistant bacteria from the river water

The test organisms, *Bacillus sp*, *Pseudomonas sp* and *Aeromonas sp* were isolated from the brackish water sample collected from the New Calabar River, Port -Harcourt, Rivers State. The spread plate technique (APHA, 1998) using nutrient agar (Oxoid) was employed for their isolation. The plates were incubated at 37°C for 18 - 24 h. Pure bacterial isolates were characterized and identified using various criteria as described by Krieg and Holt (1994). Pure isolates were transferred into nutrient agar slants stored at 4°C and served as the stock cultures for subsequent tests.

Nine predominant bacterial genera; *Achromobacter*, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* were identified.

Preparation of standard inoculum of isolates

A loopful of cells from the respective stock cultures were incubated into 100ml sterile nutrient broth contained in 250 ml Erlenmeyer flasks. The flasks were incubated at 37°C for 24 h with intermittent shaking. At the end of the incubation period, cells were harvested by centrifugation at 4000 rpm for 30 min and re- suspended in 100ml sterile physiological saline. The total viable counts were carried out to estimate the number of viable organisms. During this process, the cultures were subjected to serial dilutions up to 10^6 dilutions. An amount (0.1ml) from each dilution was inoculated by spread plate technique into freshly prepared nutrient agar plates, which were incubated at 37°C for 24 h. The dilutions that produced between 30 - 300 colonies were chosen and served as inoculum for preliminary screening experiments.

Preliminary screening test

This was carried out to determine the isolates that possess resistance to some of the heavy metals associated with the crude oil. One hundred millilitres of 1mg/l of the respective heavy metal solutions were prepared as earlier described. Nine millilitres were dispensed into test tubes and sterilized. Controls contained 9 mls of

physiological saline. One millilitre of respective standardized isolates' inoculum was then added and incubation followed immediately at a temperature of 25^{o}C±2 for duration of 24 h. At the end of the incubation period, 0.1ml were withdrawn and plated onto the surface of freshly prepared nutrient agar plates using the spread plate technique as described by APHA (1998). Incubation followed immediately at 25^{o}C±2 for 18-24 h. Colonies formed were counted and percent log survival were calculated according to Williamson and Johnson (1981).

$$\% \log survival = \frac{\log of\ count in toxicant concentration}{x\ 100\ Log of\ count in control}$$

Based on the results, *Pseudomonas, Bacillus* and *Aeromonas* were chosen for further studies.

Production of inoculum for biodegradation experiments

A loopful of each stock culture of *Bacillus sp*, *Pseudomonas sp* and *Aeromonas sp* were respectively inoculated into 100 ml of freshly prepared sterile nutrient broth contained in 250 ml Erlenmeyer flask. Incubation followed at room temperature for 18 - 24 h. This was then transferred aseptically into 1000 ml of sterile nutrient broth amended with 0.5% crude oil and incubated at room temperature for 7 days. The cultures were centrifuged at 4000 rpm for 30 min using 800 Dmodel centrifuge. The cells were washed thrice in sterile physiological saline. The washed cells were suspended in 750 ml of physiological saline contained in 1L flask. The total viable counts of the cell suspension were 1.81×10^5, 7.9×10^4 and 1.51×10^5 for *Bacillus sp*, *Pseudomonas sp* and *Aeromonas sp* respectively. This gave the inocula introduced into the various treatment options.

Water sample source and collection

Composite surface water (0 - 15 cm) samples were collected and pooled together in a clean pre-sterilized 20 L container from Abonnema Wharf (brackish water) and Omuhuechi River (fresh water) both in Port Harcourt, Rivers State, Nigeria. Abonnema Wharf is associated with anthropogenic/xenobiotic pollution while Omuhuechi River is subjected to anthropogenic discharges, soil erosion, surface run-off and other human activities. These two types of aquatic habitats are characteristic of crude oil producing areas of the Niger Delta. Physiochemical analyses of both types of water samples were determined prior to crude oil contamination as shown in Table 1.

Crude oil

Bonny light crude oil (45° API) obtained from Nigerian Agip Oil Company (NAOC) Port Harcourt was used in this experiment.

Nutrient supplements

Two nutrient supplements- NPK (Nitrogen, Phosphorus and Potassium) (15, 15, 15) fertilizer and organic poultry litter were used in the study. The NPK 15, 15, 15 fertilizer was obtained from the Rivers State Ministry of Agriculture Port Harcourt Rivers State, Nigeria.

The organic nutrient supplement (poultry litter) was obtained from a poultry farm cited in University of Port-Harcourt, Port Harcourt Rivers State Nigeria.

The bedding material in the poultry farm was composed of shavings and saw dust. Litter was collected from top 5 cm following the standard stratified sampling procedure (Peterson and Levine, 1986). The litter was sieved using a mesh of approximately 1mm size and 10 g of filtrate were added to 500 ml-deionized water. This was mixed using a blender and stirred at 150 rpm for 1 h. The poultry litter mixture was centrifuged at 4000 rpm for 10 min and the supernatant withdrawn using a pipette.

Amounts (5 ml) of the supernatant were added to appropriate assay flask at day 0. At day 35, 5 ml of the poultry litter was re-introduced. On analyses the poultry litter contained 30.1 mg/g and 2.02 mg/g of Nitrogen and Phosphorus respectively.

The fertilizer (0.1 g) was added to the appropriate flasks. The concentration was arrived at after performing a preliminary toxicity test using NPK as toxicant. Also at day 35, 0.1 g of the fertilizer was re-introduced into the appropriate flasks.

Biodegradation / bioconcentration tests

Erlenmeyer flasks (250 ml) were employed in this experiment. The assay vessels comprised 198 flasks and on each analysis day, the entire content of the desired flask was completely utilized for the analysis. The respective water samples were dispensed in 89 ml amounts into appropriately labelled flasks and sterilized by autoclaving at 121°C for 10 - 15 min. On cooling, 1ml of crude oil and 10 ml of appropriate test organism (for *Pseudomonas sp* it contained 7.9×10^4 cfu/ml, 1.81×10^5 cfu/ml of *Bacillus sp* and 1.51×10^5 cfu/ml of *Aeromonas sp*) were introduced. However, there were some exceptions in that microcosms that received poultry litter supplement consisted of 84 ml of appropriate water sample and 5 ml poultry litter. Detailed descriptions of the treatment option are presented in Table 1.

The flasks were attached to a shaker operated at a speed of 150rpm and a temperature of 25°C ± 2. Changes in bacterial growth, pH, phosphate, nitrate, and residual hydrocarbon were monitored periodically on Days 0, 7, 14, 21, 28, 35, 42, 49, 63, 90 and 120. On the above day 1 ml of sample was withdrawn aseptically from desired flask for enumeration of total viable bacterial count while the remaining content were centrifuged at a speed of 4000 rpm for 30 min. The sediment was washed thrice in phosphate buffered saline and digested for heavy metal analysis.

The test tubes used for centrifugation were flushed thoroughly with xylene to remove all traces of crude oil that adhered during centrifugation. This was then poured back into the supernatant. The various physiochemical analyses were done using the supernatant

Physicochemical analyses

The pH was determined using the Jenway pH meter (3015 model). The ascorbic acid method as described in APHA (1998) was employed in the determination of available phosphorus while the nitrate content of samples was determined using the Brucine method (APHA, 1998). Total hydrocarbons (oil and grease) levels were determined by employing the photometric method (APHA, 1998).

Bacteriological analyses

Viable bacteria present in each flask were enumerated on nutrient agar (oxoid) using the spread plate technique (APHA, 1998).

Digestion and bacterial biomass

The wet oxidation method (APHA, 1998) was adopted. Harvested

Table 1. Treatment options of crude oil impacted freshwater and marine water samples.

S/N	Treatment option	Description
1	CFW/PS	Crude oil + freshwater sample + *Pseudomonas*
2	CFW/PL/PS	Crude oil + freshwater + poultry litter + *Pseudomonas*
3	CFW/NPK/PS	oil + freshwater + NPK + *Pseudomonas*
4	CFW/Ba	Crude oil + freshwater + *Bacillus*
5	CFW/PL/Ba	Crude oil + freshwater + Poultry litter + *Bacillus*
6	CFW/NPK/Ba	Crude oil + freshwater + NPK + *Bacillus*
7	CFW/Ae	Crude oil + freshwater + *Aeromonas*
8	CFW/PL/Ae	Crude oil + freshwater + Poultry litter + *Aeromonas*
9	CFW/NPK/Ae	Crude oil + freshwater + NPK + *Aeromonas*
10	CSW/Ps	Crude oil + brackish water + *Pseudomonas*
11	CSW/PL/PS	Crude oil + brackish water + Poultry litter + *Pseudomonas*
12	CSW/NPK/Ps	Crude oil + brackish water + *Pseudomonas*
13	CSW/Ba	Crude oil + brackish water + *Bacillus*
14	CSW/PL/Ba	Crude oil + brackish water + Poultry + *Bacillus*
15	CSW/NPK/Ba	Crude oil + brackish water + NPK + *Bacillus*
16	CSW/Ae	Crude oil + brackish water + *Aeromonas*
17	CSW/PL/Ae	Crude oil + brackish water + Poultry litter + *Aeromonas*
18	CSW/NPK/Ae	Crude oil + brackish water + NPK + *Aeromonas*
19	Control (Ci	Crude oil + Freshwater
20	Control (Cii)	Crude oil + brackish water

Table 2. Heavy metals present in the crude oil impacted water samples.

Heavy metal	Concentration (mgl^{-1}) in fresh water	Concentration (mgl^{-1}) in brackish water
Iron	1.981	2.006
Lead	1.269	1.68
Cadmium	0.603	1.405
Zinc	0.692	1.724
Nickel	0.38	1.45
Copper	0.502	0.912

and washed bacterial biomass was mixed with 1 ml of trioxonitrate (v) acid, perchloric acid and tetraoxosulphate (VI) acid. The mixture was heated for 10 - 15 min. This caused lysis of the cells releasing the cytoplasmic content.

Heavy metal analyses

The crude oil samples obtained were digested using the wet oxidation method and the heavy metals present were determined using the atomic absorption spectrophotometer (UNICAM 929 AA Spectrometer). Also on each analysis day (0, 7, 14, 21, 28, 35, 42, 49, 63, 90 and 120), the harvested, washed and digested bacterial biomass were analysed for the accumulation of the heavy metals present in the crude oil sample.

Statistical analyses of data

The two-way analysis of variance (ANOVA) and correlation analysis were employed (Finney, 1978).

RESULTS

Results of the analysis of heavy metals present in the crude oil impacted water samples are given in Table 2. The isolate obtained from the river water sample were *Achromobacter*, *Alkaligens*, *Aeromonas*, *Bacillus*, *Corynebacterium*, *Chromobacterium*, *Micrococus*, *Pseudomonas* and *Serratia*. The results of the preliminary screening for the resistance of isolates to the toxicity of the various heavy metals are presented in Table 3. Three of the test isolates (*Aeromonas*, *Bacillus* and *Pseudomonas*) showed resistance to the six heavy metal salts.

Figure 1a shows the growth profiles of all three organisms in fresh and brackish-water treatment options. Lower counts were obtained in flasks without nutrient supplements. In fertilized options, there was a general increase in the counts from Day 0 to Day 14 followed by

Table 3. Response of Isolates to the toxicity of the various heavy metals.

Isolates	Fe	Zn	Cd	Cu	Ni	Pb	Control
Alcaligenes	++	++	-	-	-	-	+++
Aeromonas	+++	+++	++	+++	++	++	+++
Bacillus	+++	+++	+++	+++	+++	+++	+++
Achromobacter	+++	++	-	-	+	-	+++
Chromobacterium	++	+	-	+	-	-	+++
Corynebacterium	++	++	-	+	+	-	+++
Micrococcus	+++	++	+	++	+	-	+++
Pseudomonas	+++	+++	+++	+++	++	++	+++
Serratia	+++	+	-	+	+	-	+++

Key:
+++ = > 70% log survival
++ = 50-69% log survival
+ = 30-49% log survival
- = < 29% log survival

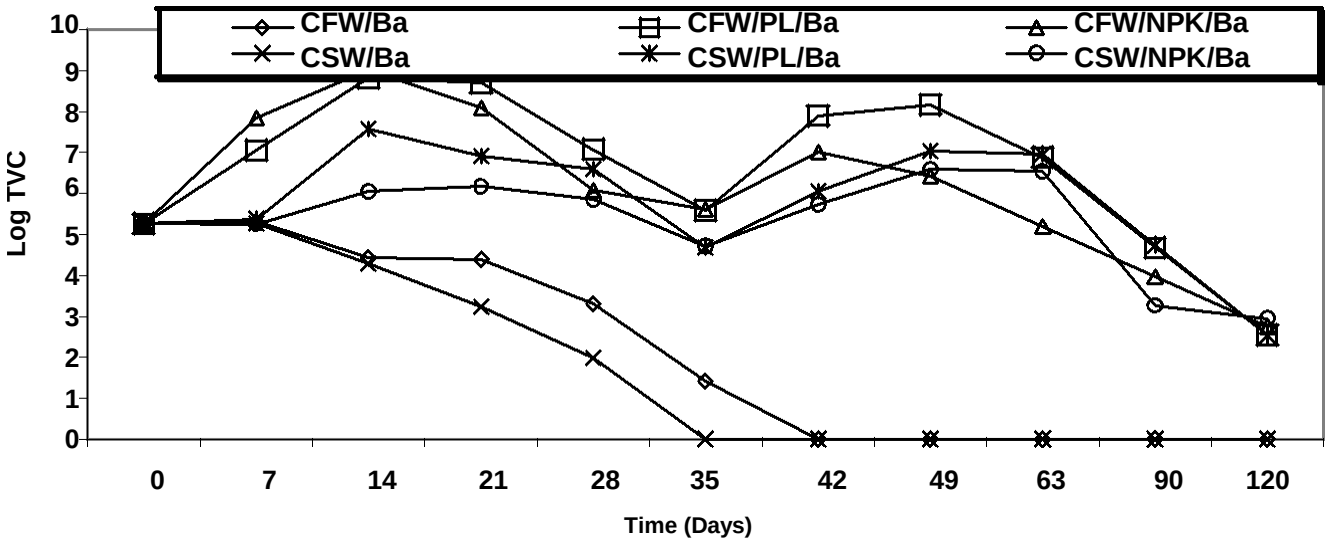

Figure 1a. Log total viable *Bacillus* count in various set-ups.

a reduction from Day 25 to Day 35. The re-application of nutrients at Day 35 resulted in another increase in growth of the organisms. This is evident in Figs 1a (*Bacillus)* and 1b (*Pseudomonas*). The application of nutrients in Figure 1c (*Aeromonas* did not stimulate any further increase in growth in the options containing nutrient supplements.

In Figures 2a and b there was a decrease in hydrocarbon levels with time. The decrease was however greater in fresh water systems supplemented with nutrients than in brackish water systems supplemented with nutrients. Hydrocarbon losses were greater in *Bacillus* and *Pseudomonas* containing test systems. Hydrocarbon losses in *Aeromonas* containing test systems were insignificant. Test systems without nutrient supplementation showed insignificant decreases in hydrocarbon levels.

The changes in nitrate and phosphate levels presented in Figures 3a - b and 3c - d is a reflection of reapplication of nutrients by Day 35 in nutrient supplemented test systems. From Day 0 to day 28 and Day 35 there was a decrease in concentrations of these nutrients. However following reapplication of fresh nutrients by Day 35 there was an increase to Day 42, which was followed by a decrease till Day 120. Test systems without fertilizer/poultry litter application did not show this sequence.

The pH of nutrient supplemented test systems (4a - b) showed a decrease with time. This decrease was evident in both fresh and brackish water test systems. The pH of systems without nutrient supplementation showed insignificant decreases with time.

The abilities of the three bacteria *Bacillus, Pseudomonas*

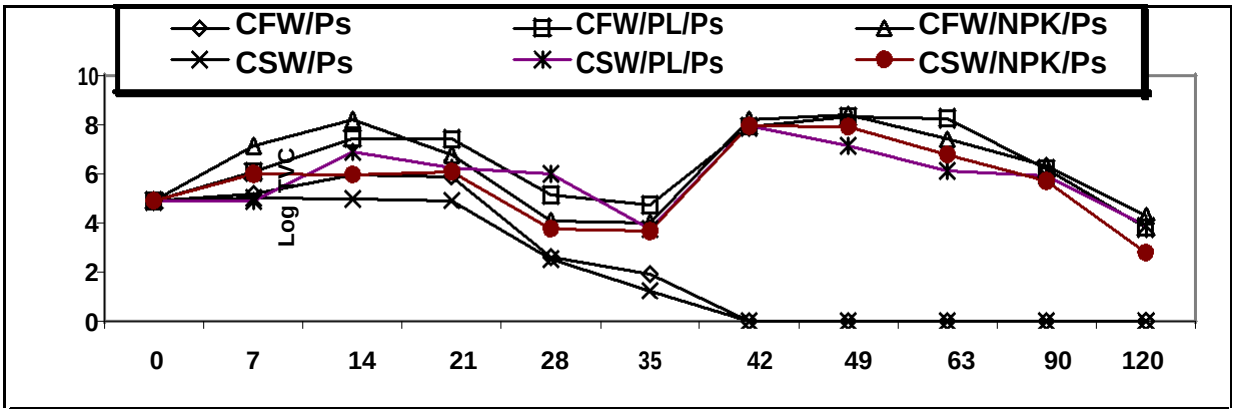

Figure 1b. Total viable *Pseudomonas* count in various set-ups.

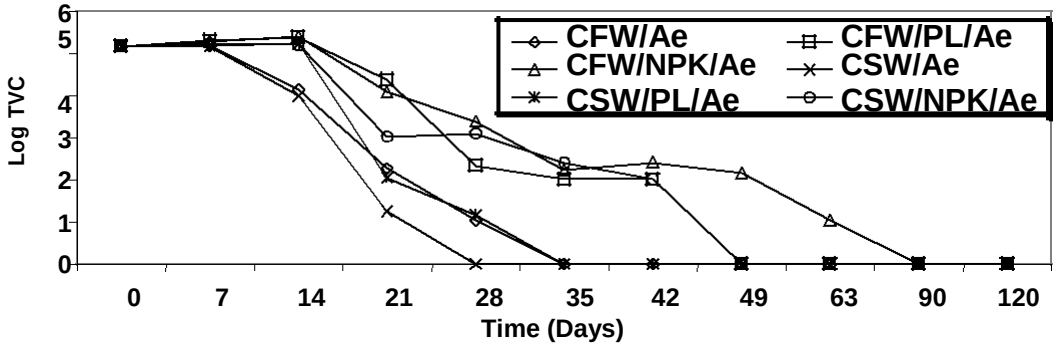

Figure 1c. Total viable Aeromonas count in various set-ups.

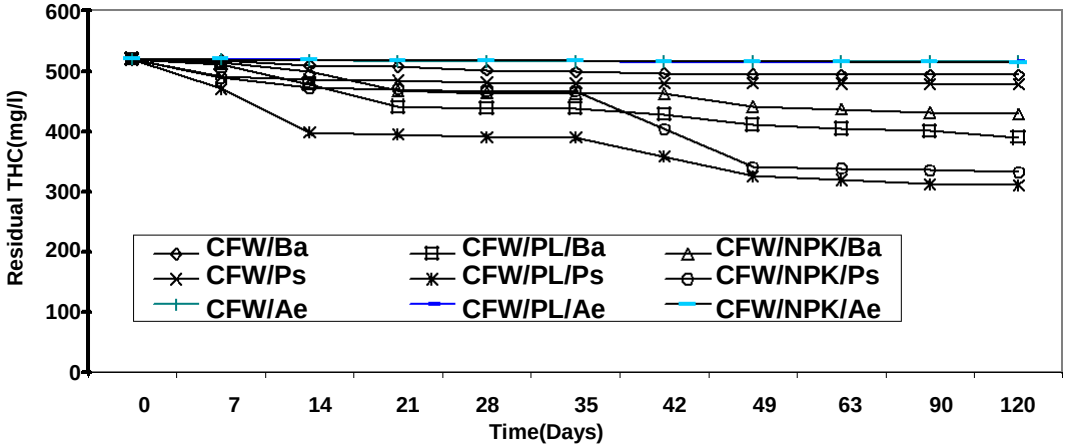

Figure 2a. Changes in total hydrocarbon content (THC) in various freshwater treatment options.

and *Aeromonas* to bioconcentrate the six test heavy metals are presented in Figures 5a - d, 6a - d and 7a - d respectively. The relationship between hydrocarbon loss pH and microbial counts is also shown in these figures. The results showed that in test systems containing nutrient supplements bioconcentration of heavy metals increased with time. The pH, the total hydrocarbon levels and microbial counts also decreased with time. This was evident in systems containing *Bacillus* and *Pseudomonas.* Bioconcentration increased with increase

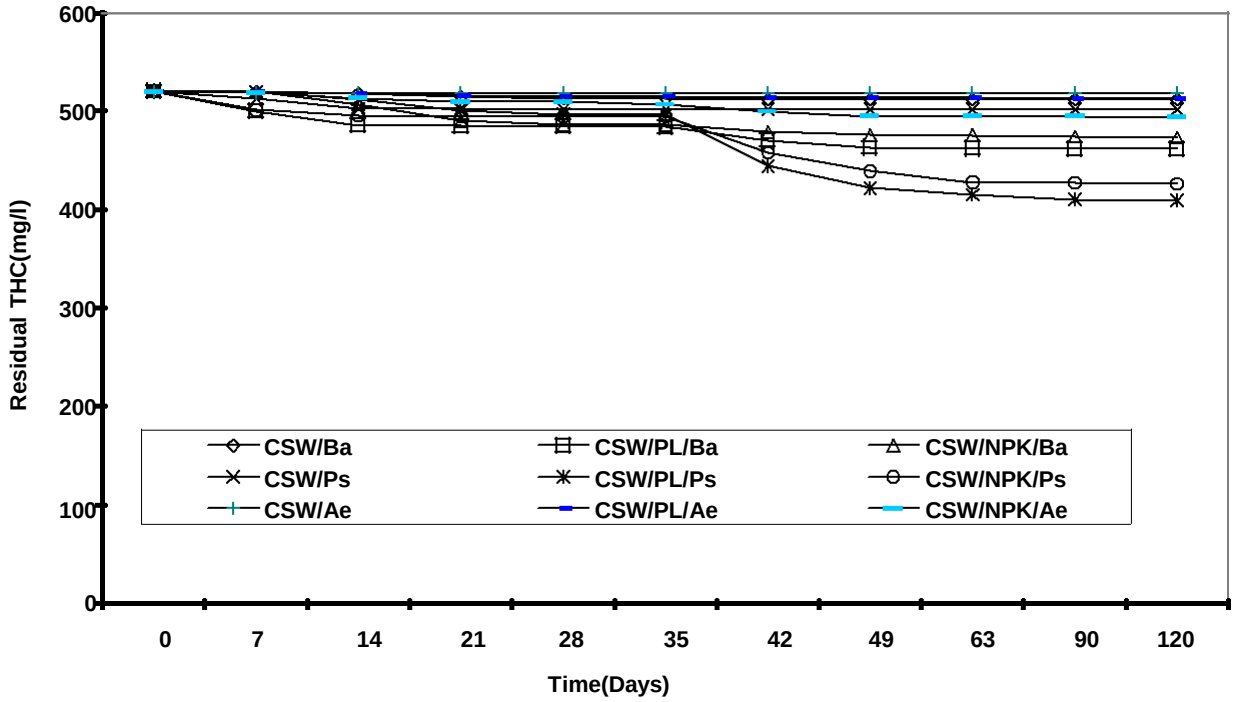

Figure 2b. Changes in total hydrocarbon content in various saltwater treatment options.

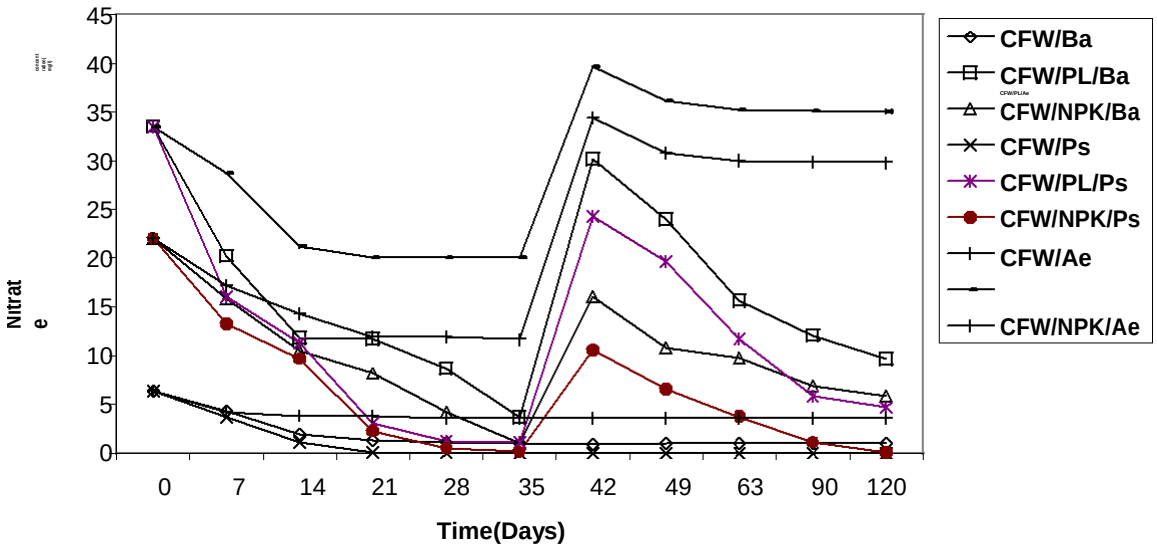

Figure 3a. Changes in nitrate concentration in various fresh water treatment options.

in microbial biomass (microbial counts). Bioconcentration occurred within pH maxima of 8b (optimum pH was within the acidic range). In test systems without nutrient supplements a decrease in bioconcentration of heavy metals was observed with time. In these systems insignificant decreases in pH and total hydrocarbons also occurred with time. This pattern was also evident in systems containing *Aeromonas*.

DISCUSSION

The results of this study showed that the inoculation of crude oil contaminated fresh water and brackish water with two of the three isolates (*Bacillus* and *Pseudomonas*) may be employed as a bioremediation (biodegradation of hydrocarbons and bioconcentration of heavy metals) strategy. These isolates have been

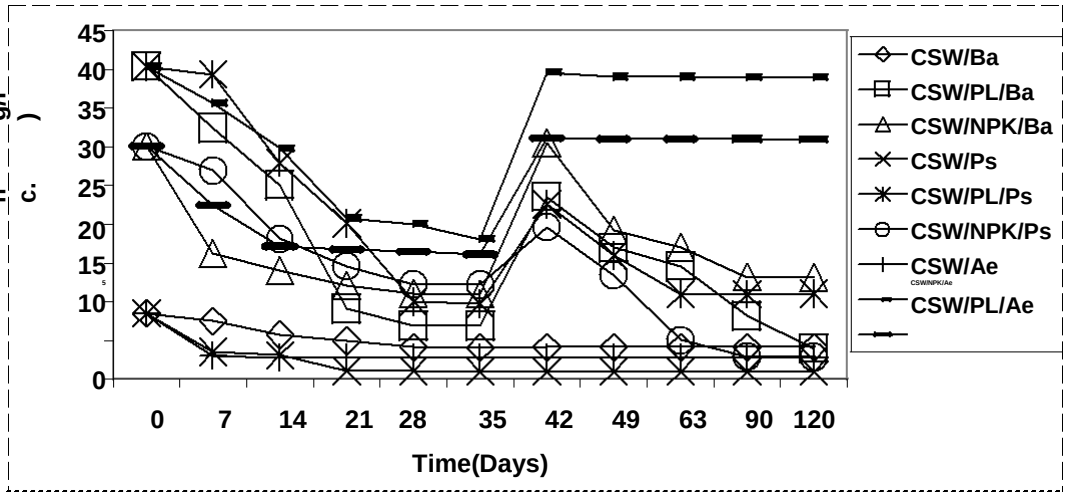

Figure 3b. Changes in nitrate concentrations in various saltwater treatment options.

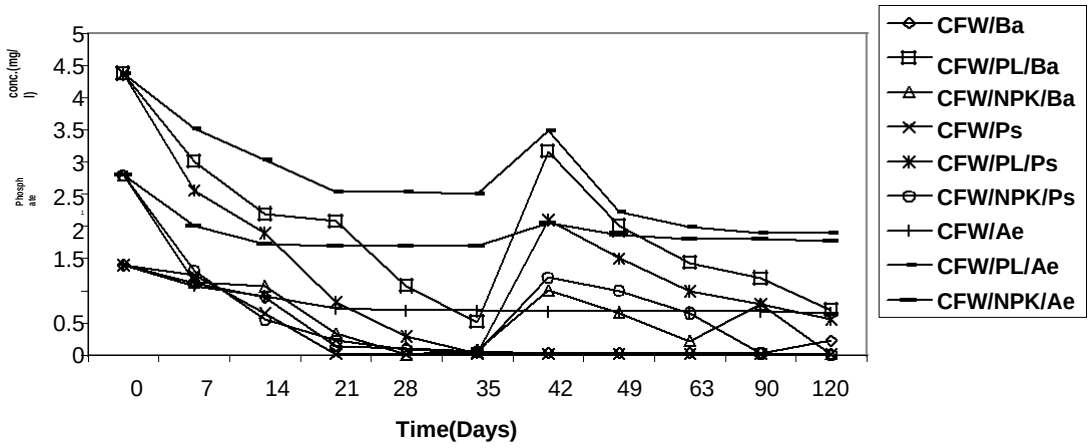

Figure 3c. Changes in phosphate concentration in various freshwater treatment options.

associated with petroleum product degradation (Nwachukwu and Gurney, 2000; Odokuma and Ibor, 2003; Okerentugba and Ezeronye, 2003) in the Niger Delta.

The increase in counts with addition of fresh nutrients (corresponding to Day 0 - 14 and Day 35 - 49) indicates the importance of additional nitrogen and phosphorus to microbial growth in a batch system. The results of inorganic nutrient analysis indicated that phosphate and nitrate became limiting at day 28 - 35. The activities of the isolates in the experimental samples were slowed down probably because of the depletion of these inorganic nutrients. This may have resulted in the reduction in both hydrocarbon levels as well as heavy metal accumulation. This is further supported by the fact that the re- addition of nutrients at day 35 resulted in the resumption of activities by the isolates. This process corrected the nutritional imbalance thereby leading to a further decrease in hydrocarbon levels and an increase in heavy metal accumulation. Microorganisms require phosphorus as phospholipids in synthesizing cell membranes, as components of nucleic acids and for sugar phosphorylation (Andrew and Jackson, 1996). They also exploit nitrate sources to meet their protein and nucleic acid requirement. NPK fertilizer and poultry litter contain these inorganic nutrients and when they were added to the crude oil contaminated water samples active growth of *Bacillus sp* and *Pseudomonas sp* were obtained with proportional decrease in hydrocarbon levels. The insignificant changes in options containing *Aeromonas sp* may be due to the fact that the isolate lacked required enzymes (genetic constitution) and hence incapable of degrading the complex crude oil components and not as a result of limiting nutrient supply. These observations emphasizes the importance, in all bioremediation programmes for monitoring routinely the

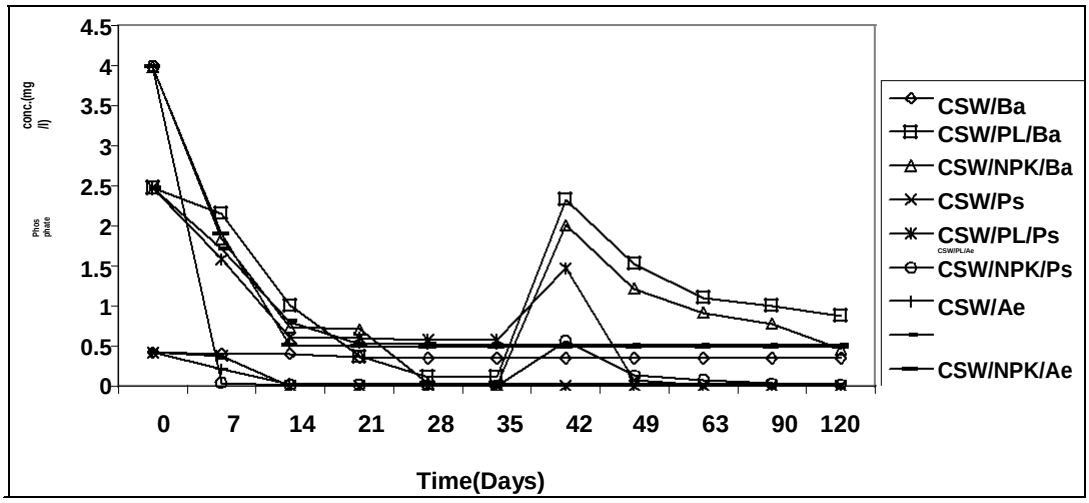

Figure 3d. Changes in phosphate concentrations in various saltwater treatment options.

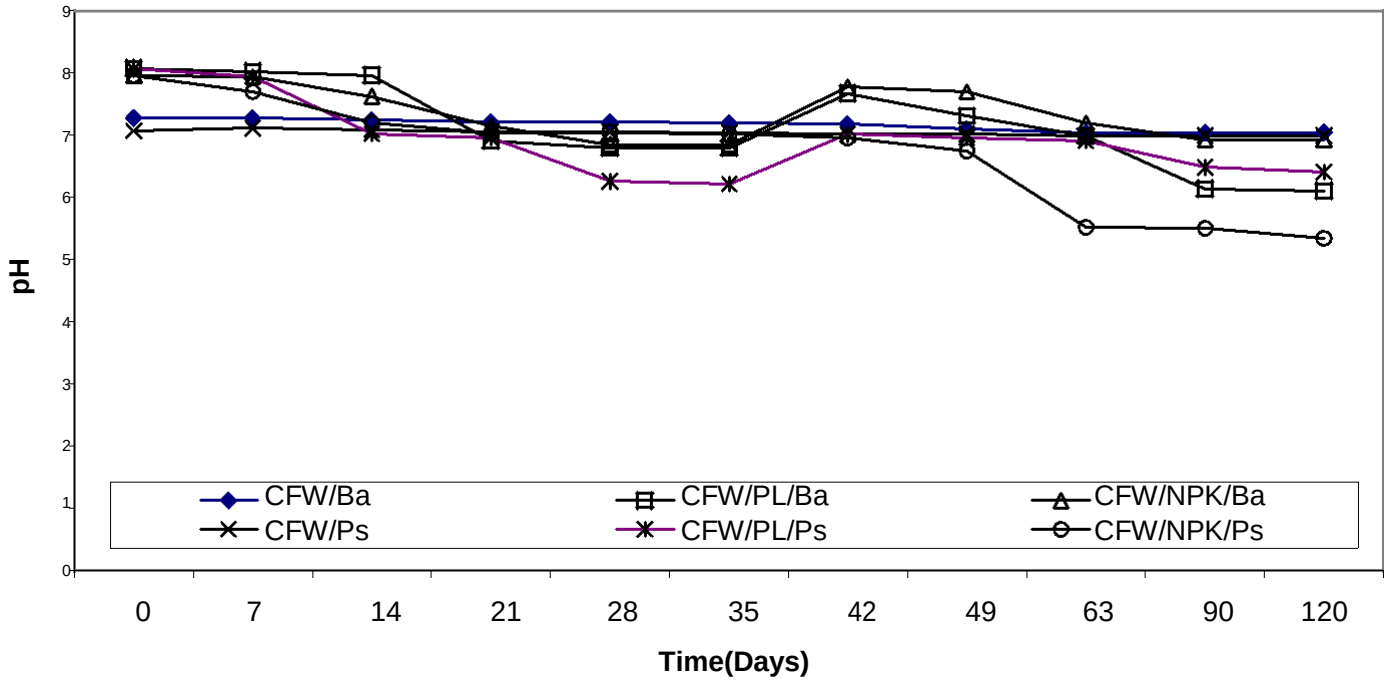

Figure 4a. Changes in pH values in the various freshwater options.

nutritional variables known to influence the biodegradation of pollutants and to correct any imbalances and hence accelerate the recovery rate of the hydrocarbon contaminated environments.

Bioremediation strategies for crude oil impacted water bodies in oil producing regions of developing countries like Nigeria need to be cost- effective and also, able to apply indigenous technology. Thus the appropriate technology may include appropriate strain development in addition to the application of readily available and inexpensive nutrient supplements. *Pseudomonas* and *Bacillus* that showed effectiveness in oil degradation are predominant in aquatic habitats in the Niger Delta. *Pseudomonas* has been used extensively in the efficient biodegradation of crude oil and petroleum products (Amund and Igiri, 1990; Rocha et al., 1992; Nwachukwu, 2000; Nwachukwu and Gurney, 2000). However, in chronic oil pollution incidents, there is usually the occurrence of loss of microbial diversity and reduced microbial population leading to the persistence of the contaminant (Atlas, 1991; Wang et al., 1994). Therefore, prospecting for and the inoculation of organisms having

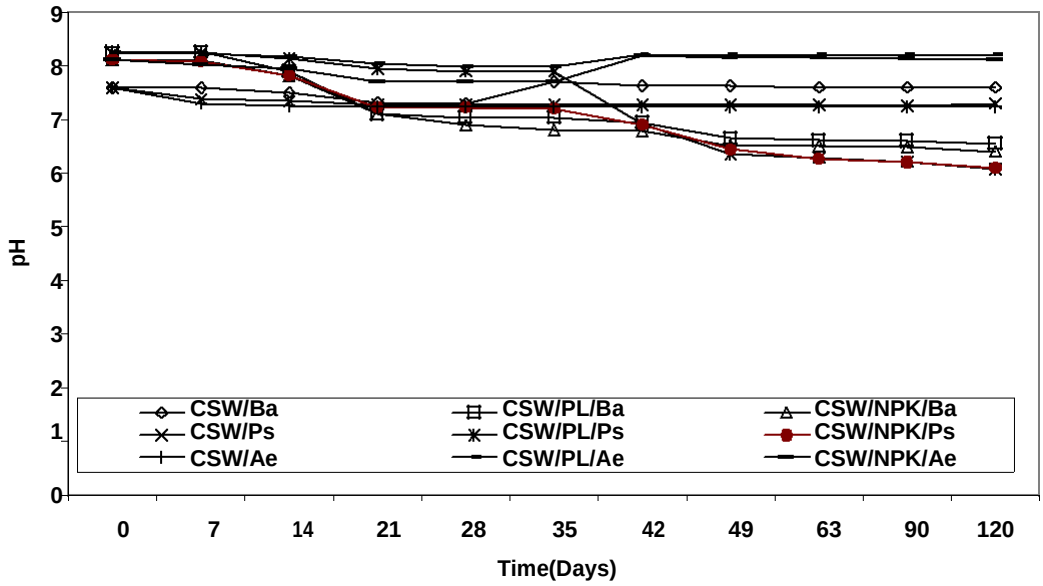

Figure 4b. pH changes in various saltwater treatment options.

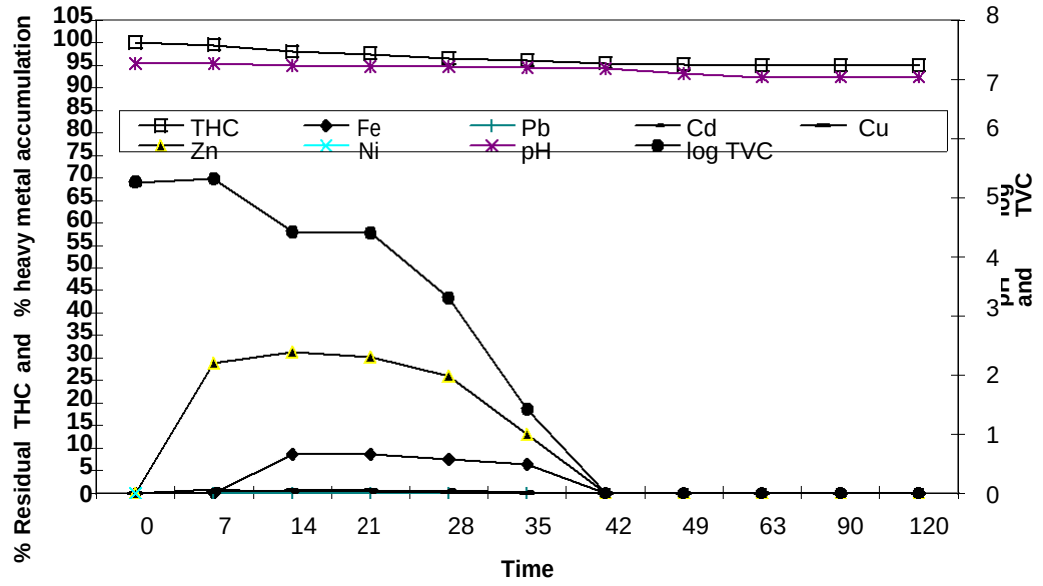

Figure 5a. Activities of *Bacillus* in CFW/Ba.

genes with crude oil degrading potentials as well as employing methods that increase their biomass have been known to promote oil biodegradation in contaminated regions.

Bacillus and *Pseudomonas* revealed great potentials in accumulating the heavy metals associated with crude oil. Similar observations had been made by other investigators demonstrating the capabilities of several bacteria in removing heavy metals such as lead, cadmium, copper, nickel and other heavy metals from polluted effluents (Higham et al., 1985 and Malekzadeh et al., 1995). In this study an increasing uptake pattern was observed for all metals in *Pseudomonas* and *Bacillus* test systems which must have resulted from the genetic make up of both organisms and increases in biomass generated due to the periodic addition of appropriate nutrient supplements. Saturation of biomass with heavy metals was not observed, indicating that available sites for heavy metal adhesion probably exist. The decreases in values of bioaccumulation obtained corresponded to periods when the population of organisms was reducing. However, determination of saturation levels was not the purpose

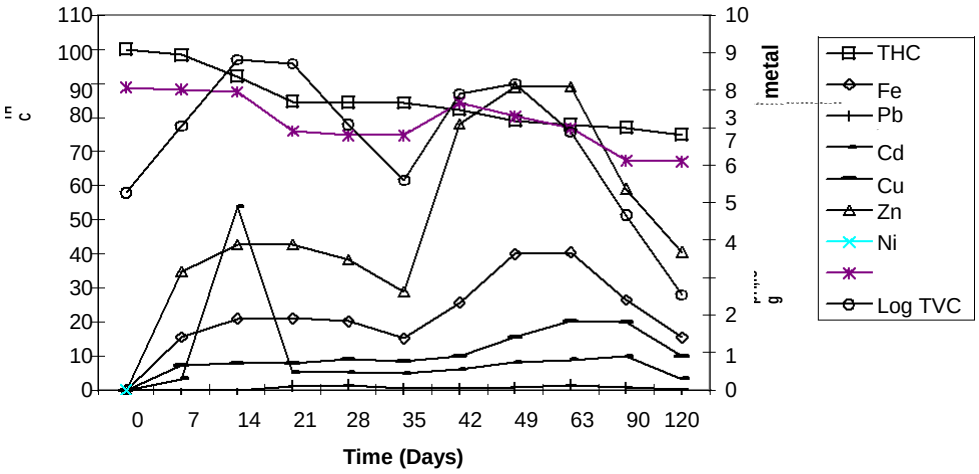

Figure 5b. Activities of *Bacillus* in CFW/PL/Ba.

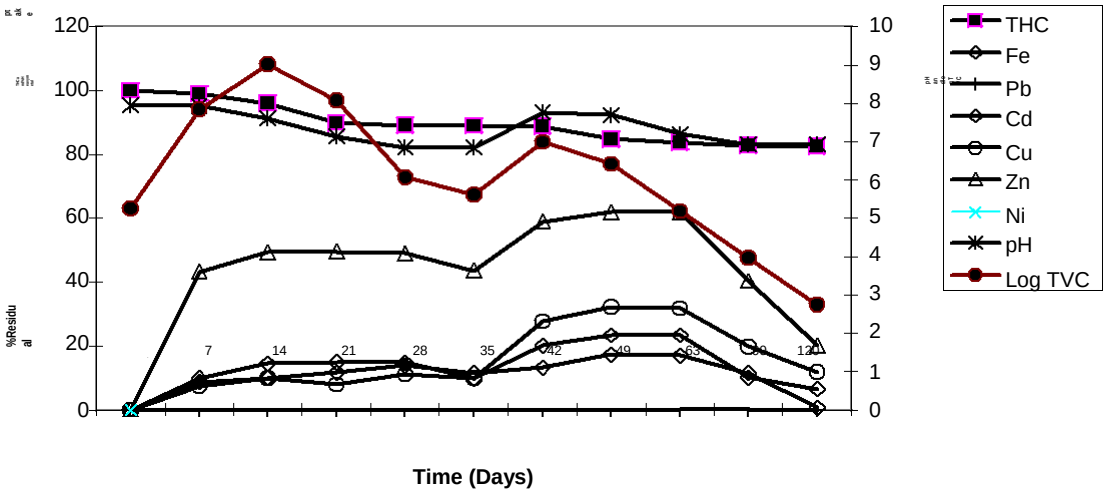

Figure 5c. Activities of *Bacillus* in CFW/NPK/Ba.

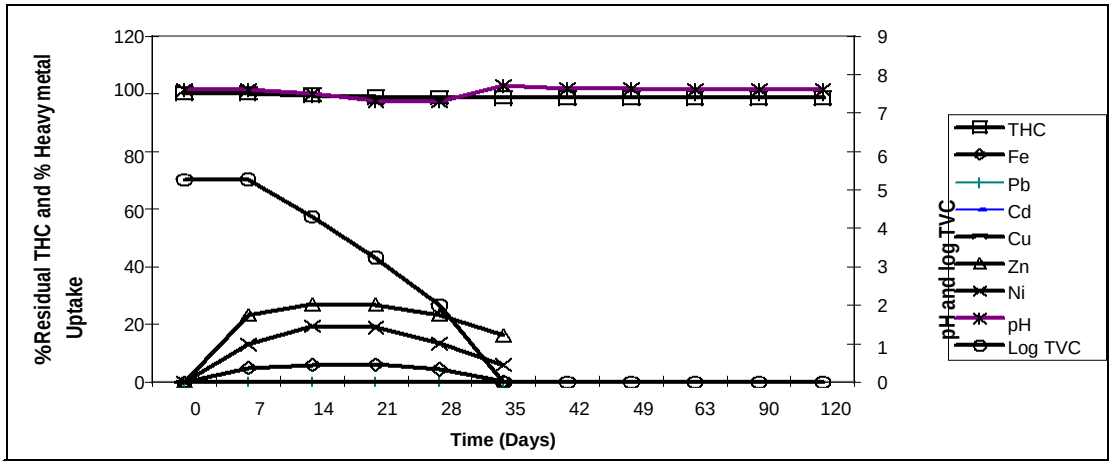

Figure 5d. Activities of *Bacillus* in CSW/Ba.

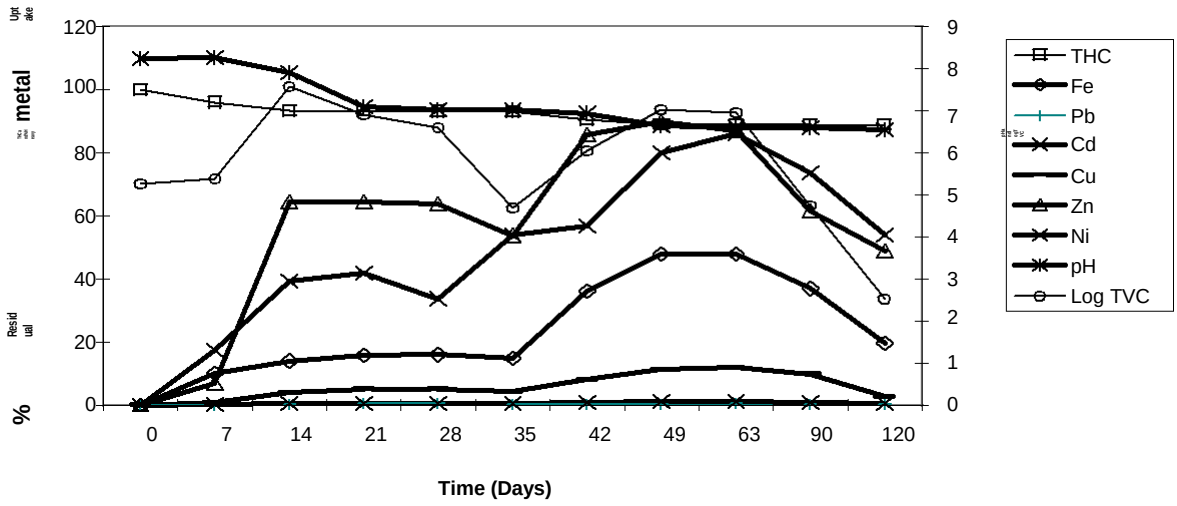

Figure 5e. Activities of *Bacillus* in CSW/PL/Ba.

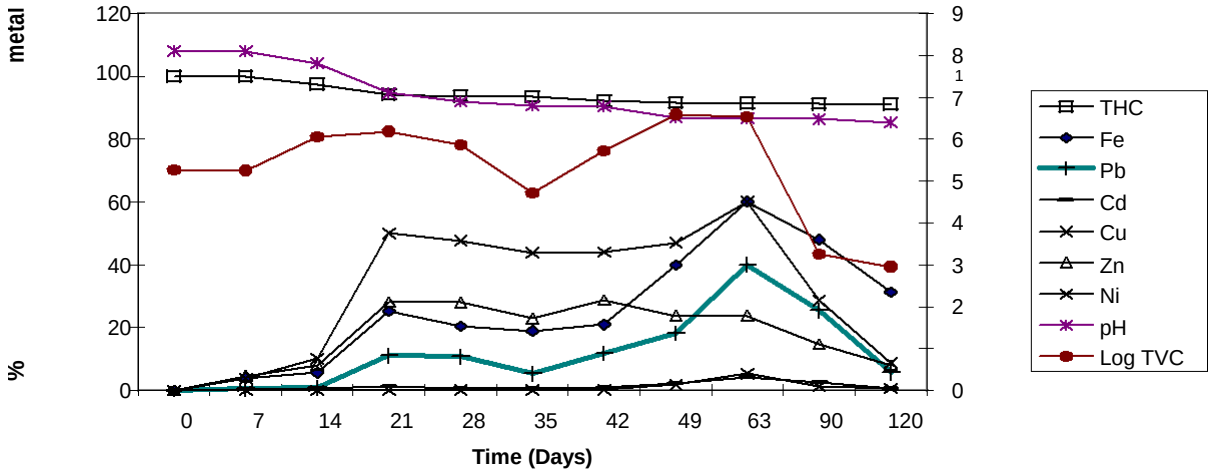

Figure 5f. Activities of *Bacillus* in CSW/PL/Ba.

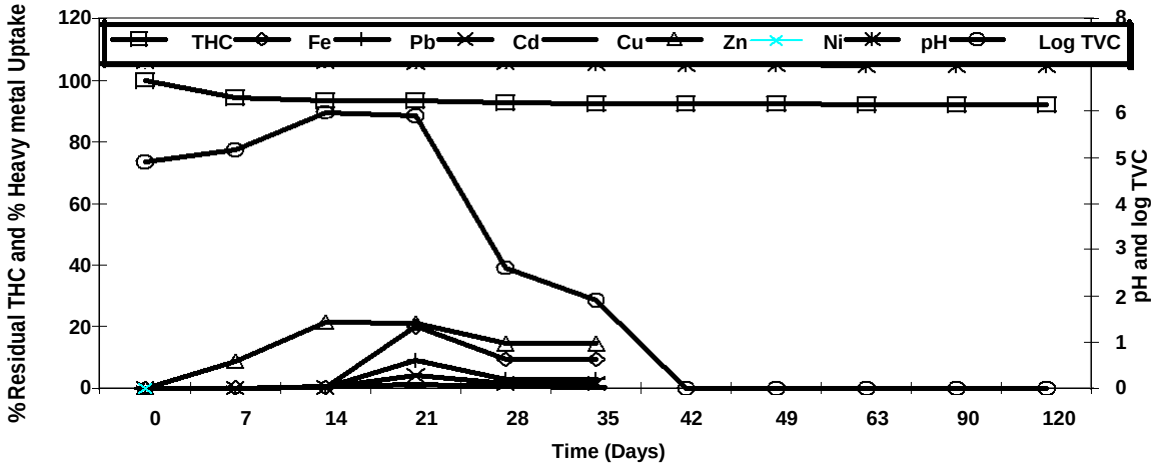

Figure 6a. Activities of *Pseudomonas* in CFW/Ps.

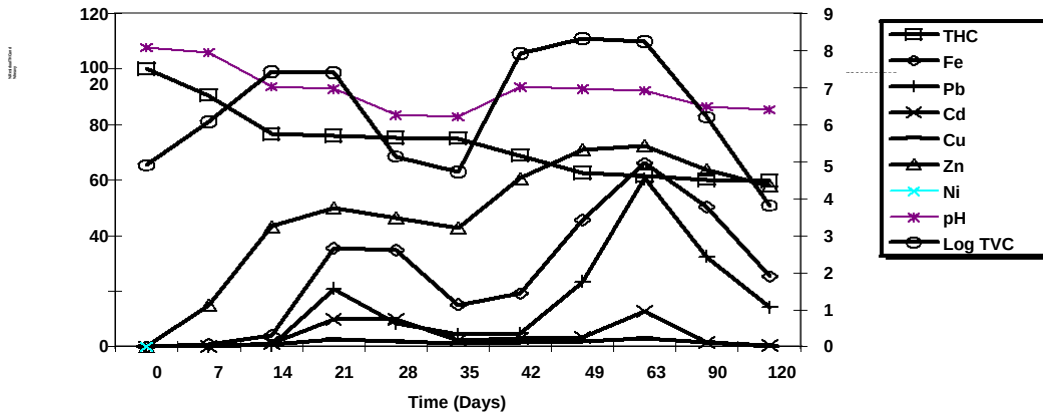

Figure 6b. Activities of *Pseudomonas* in CFW/PL/Ps.

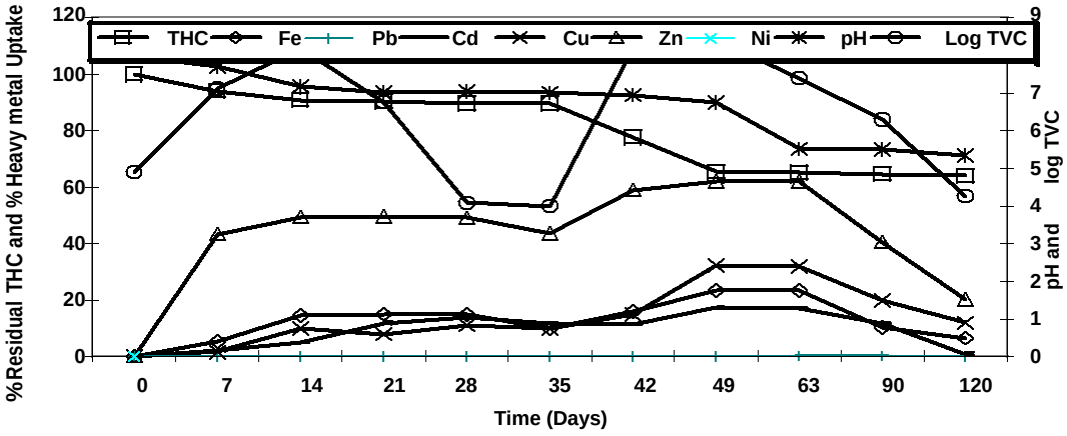

Figure 6c. Activities of *Pseudomonas* in CFW/NPK/Ps.

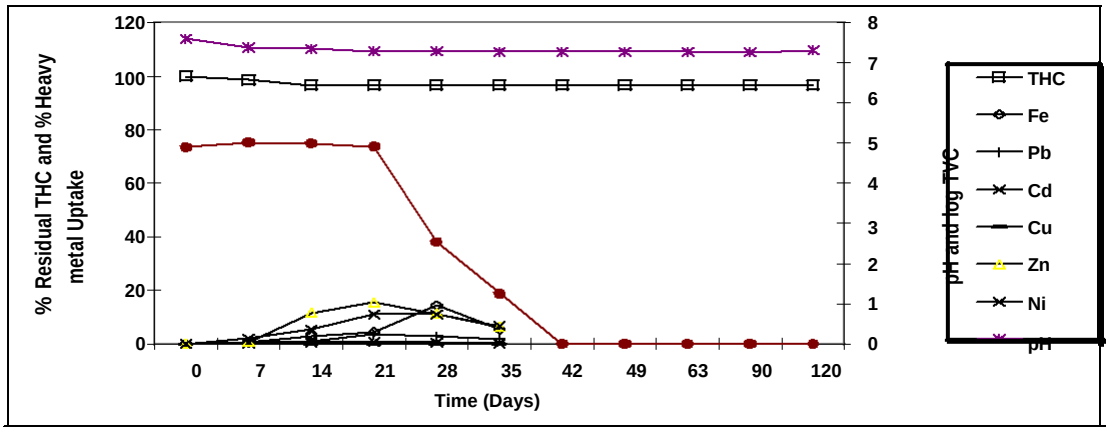

Figure 6d. Activities of *Pseudomonas* in CSW/Ps.

of this study, but the determination of the abilities of the cells to accumulate heavy metals in the presence of other contaminants and hence their use as biosorbents.

Although, the same qualitative results were described for the two test isolates, quantitative bioaccumulation of heavy metals were statistically distinct and followed the

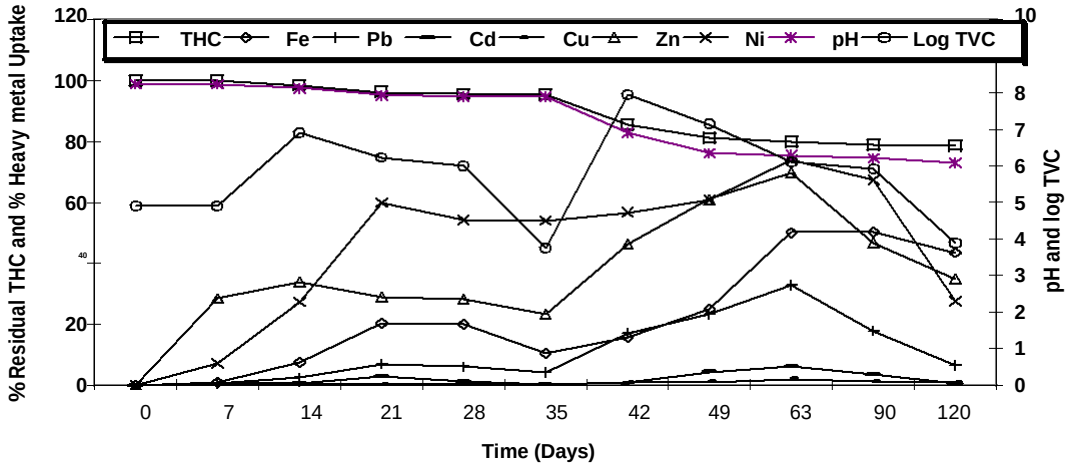

Figure 6e. Activities of *Pseudomonas* in CSW/PL/Ps.

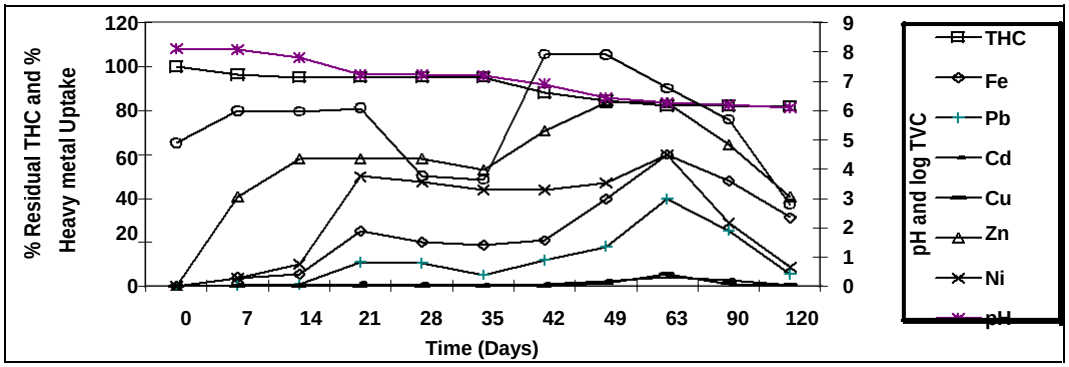

Figure 6f. Activities of *Pseudomonas* in CSW/NPK/Ps.

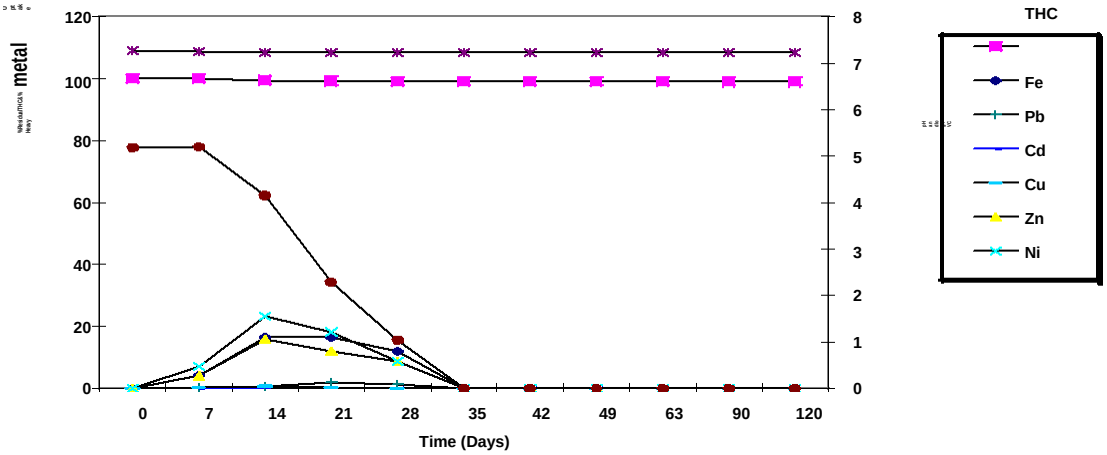

Figure 7a. Activities of *Aeromonas* in CFW/Ae.

pattern: *Pseudomonas* > *Bacillus*. The reduced ability of *Aeromonas* in accumulating heavy metals may be attributable to its low survival rate in the contaminated test systems probably from its genetic make up, which may have resulted from its inability to utilize crude oil as its sole carbon source or it's toxicity to the organism (Not toxicity to heavy metals). The profile for bioaccumulation for the gram negative isolates was such that higher

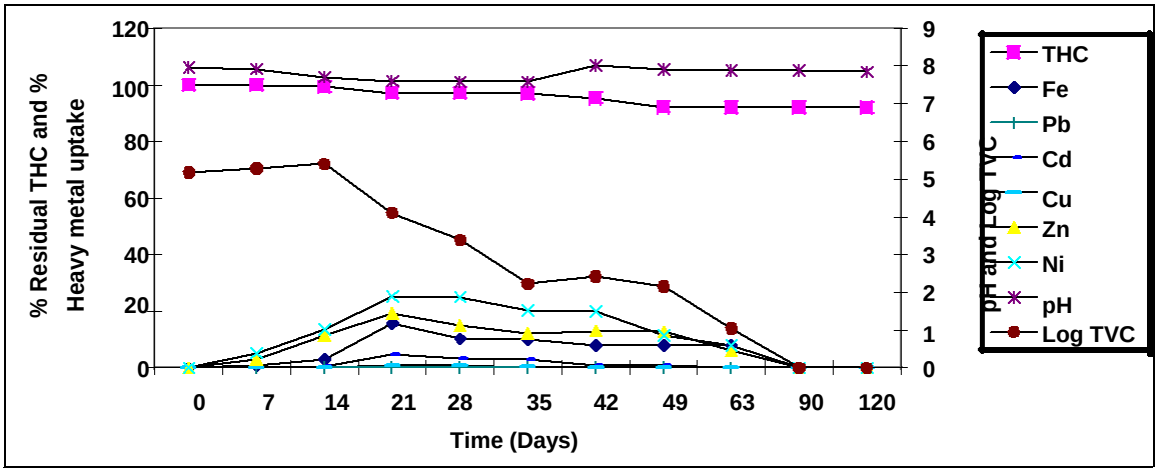

Figure 7b. Activities of *Aeromonas* in CFW/NPK/Ae.

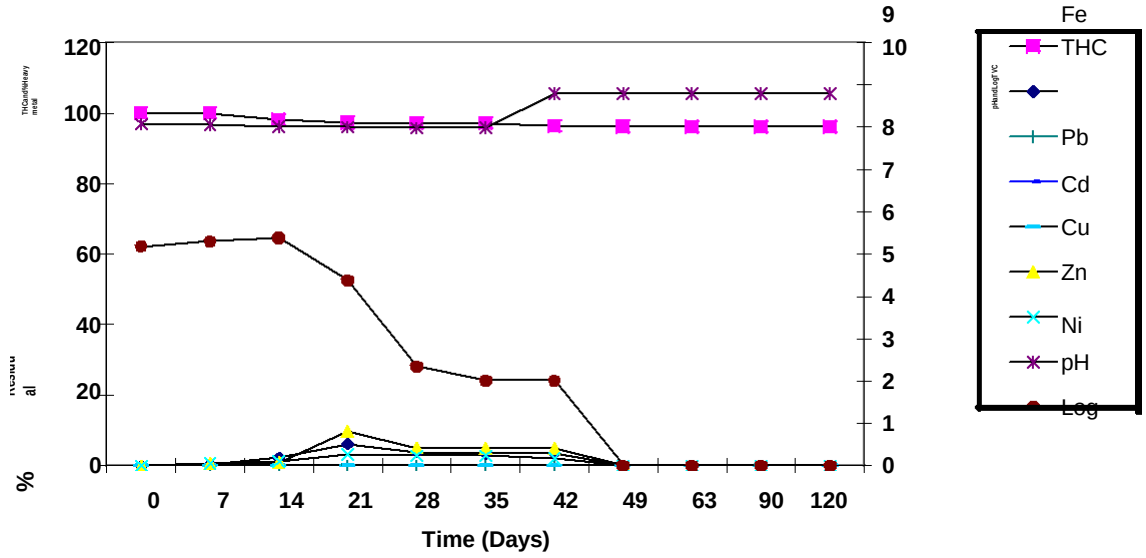

Figure 7c. Activities of *Aeromonas* in CFW/PL/Ae.

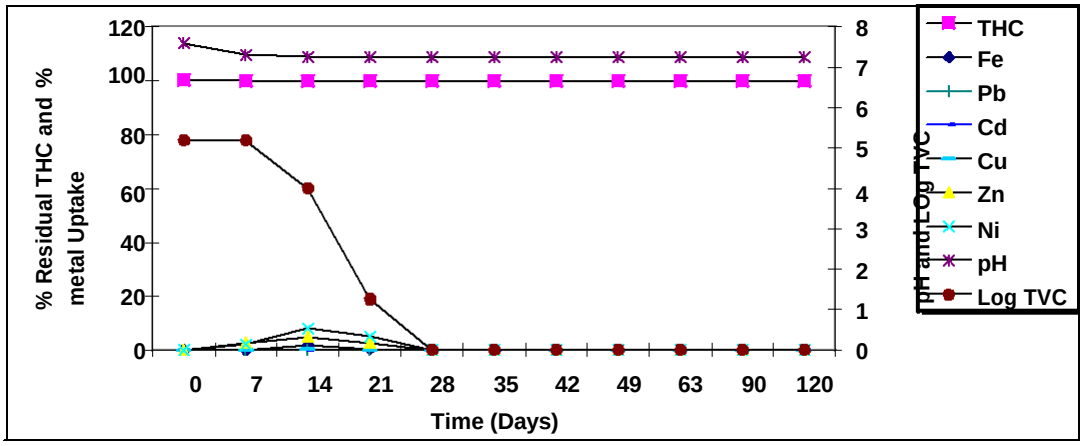

Figure 7d. Activities of *Aeromonas* in CSW/Ae.

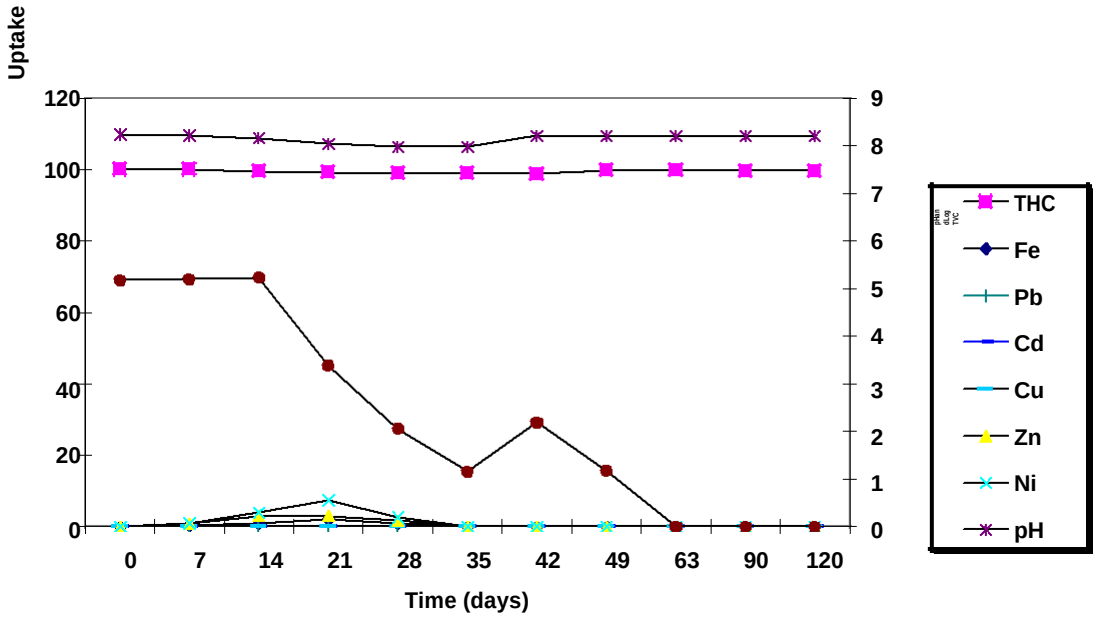

Figure 7e. Activities of *Aeromonas* in CSW/PL/Ae.

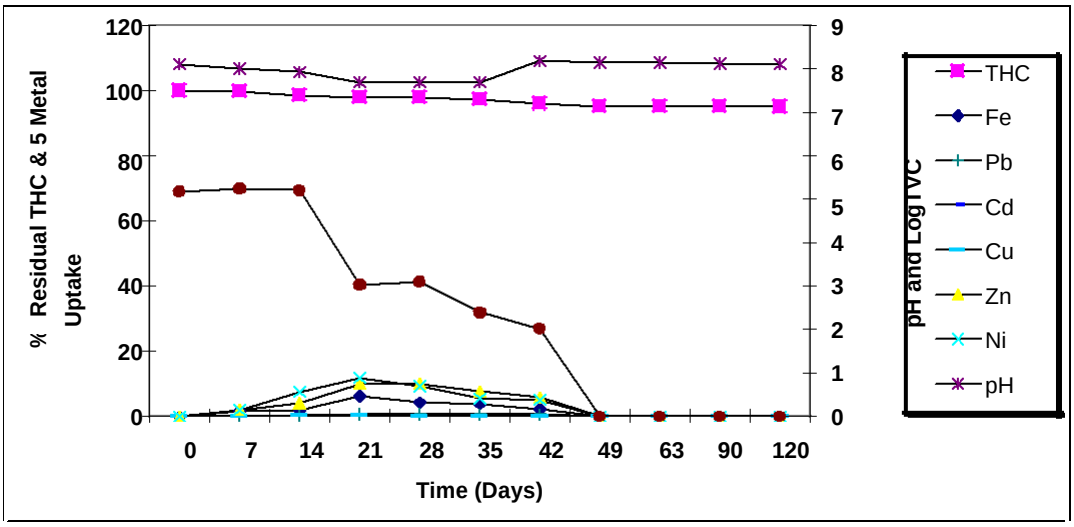

Figure 7f. Activities of Aeromonas CSW/NPK/Ae.

concentrations were accumulated when bacteria growth remained more or less constant corresponding to the stationary growth phase. However, for *Bacillus*, peak accumulation was observed during active growth or exponential growth phase. This findings suggest that metal uptake by *Pseudomonas sp* involves not only diffusion but also surface adsorption which is metabolism independent. In *Bacillus sp*, however, the most likely phenomenon may be diffusion which may result from increased membrane permeability.

Maximum uptake of heavy metals except zinc and nickel by *Pseudomonas* were observed when pH values were in the acidic zones. This may account for the high rates observed in the accumulation of these two heavy metals especially in brackish water environments where pH values remained in the alkaline range for a longer period of the study. However, lead, copper and cadmium uptake when compared to other metals, were highly repressed in test options containing *Bacillus* and *Pseudomonas* . Their low concentrations in combination to the high pH values in the set-ups might account for this phenomenon. Previous authors have reported that prevailing pH value is one of the main factors in bioaccumulation efficiency by different organisms (Leung et al., 2000; Lopez et al., 2000; Al- Garni, 2005). They reported that low pH affects the network or chemistry of cell wall as well as physiochemistry and hydrolysis of the heavy metal. It was also reported that at low pH values, lead ions, compete with hydrogen ions on the binding sites of the microbial cell. The accumulation of lead,

copper and cadmium were in accordance to these findings. However, the inability of *Bacillus sp* to accumulate these three metals could be due to the observation that its accumulation potential was impaired by age of cultures (stationary phase) and it was during this period that pH values reduced to the acidic zone.

The overall result however, showed that higher concentrations of each metal were accumulated beyond Day 35. This may be as a result of the increased biomass yield obtained due to the periodic addition of appropriate nutrient supplements. The study proves that *Bacillus* and *Pseudomonas* are capable of utilizing hydrocarbon contaminants and may be employed in the future for the removal of heavy metals immobilized on waste materials. However, it is important to note that some species of these bacteria are opportunistic pathogens Thus, it is necessary to screen for pathogenicity to confirm their environmental friendliness before field trials.

Conclusion

Periodic nutrient supplementation with NPK fertilizer and poultry litter promoted both biodegradation of crude oil and heavy metal uptake in both crude oil impacted fresh and brackish water test systems containing *Bacillus* and *Pseudomonas.* The hydrocarbon levels of test systems containing *Aeromonas* were fairly constant throughout the duration of the study probably due to toxicity of crude oil components to this organism or the inability of the organism to utilize the oil as sole carbon source (genetic make up). Heavy metal uptake by *Aeromonas* was much reduced when compared with *Bacillus* and *Pseudomonas* probably for the same reason. *Aeromonas* did not serve as a good tool for biodegradation and bioconcentration. Bioconcentration capabilities of organisms revealed the following trend *Pseudomonas* > *Bacillus* > *Aeromonas.* The pattern of heavy metal uptake by the three bacterial isolates was different in the various treatment options. Bioconcentration was also affected by biomass concentrations and pH of the test systems. The study revealed that there was no significant difference in bioconcentration values when comparing NPK fertilizer with poultry litter. The study has shown that a combination of bioaugumentation with indigenous species of *Bacillus* and *Pseudomonas* and biostimulation with inorganic NPK fertilizer or poultry litter may be employed to bioremediate (biodegrade and bioconcentrate) crude oil impacted fresh and brackish water systems of the Niger Delta.

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