Inorganic Nutrient Ions (PO_4^{3-}, NO^-_3 and SO_4^{2-}) as Essential Requirements in Bioremediation of Soils Polluted with Crude Petroleum

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Abstract: The recovery rate of oil impacted agricultural soils by joint application of biostimualtion agents such as PO_4^{3-} and NO_3^- (inorganic nutrient ions) and *Bacillus subtilis* (BS) as a bioaugmentation agent was investigated for 12 weeks. The dissolved oxygen (DO), pH, inorganic nutrient ions by ion chromatography and residual oil content by a gravimetric method, were determined over the period, as well as the microbial population density (MPD) by standard plate count (SPC). MPD enumerated for both the experimental (EDS) and the control (CDS) between weeks 2 and 7 showed increasing trends with the corresponding decreases in the oil content, particularly in EDS inoculated with BS. However, at week 8, $P0_4^{3-}$ and $N0^{3-}$ depleted appreciably from 346 and 807 to 24 and 375 ppm respectively and simultaneously with a rapid decrease in MPD particularly the hydrocarbon utilizers (HCU). This apparently indicated the importance of nutrient ions in the bioremediation of oil polluted environments. The pH and DO values consistently decreased in both EDS and CDS over the period. The result of this study showed that biostimulation involving inorganic nutrients adjustment and bioaugumentation by application of BS were the two key factors responsible for the remarkable differences in the results obtained for EDS, particularly in the rapid oil content reduction when compared with CDS

Keywords: Bioaugmentation, biostimulation, inorganic nutrient ions, *Bacillus subtilis*, crude petroleum

Introduction

In Nigeria, exploration, production and processing of crude petroleum for export purposes are carried out in the oil-rich Niger Delta region. According to Chikere *et al.*, (2009), over 80% of the country's oil revenue comes from this region. Incidentally, the activities of the oil-producing industries operating in the region provide potential sources of oil pollution of agricultural land and domestic water sources causing enormous damage to biodiversity and economic growth of farmers in the region (Nwachukwu and Ugoji, 1995). Consequently, the oil pollutant persists for a very long time in the impacted environments following depletion of plants and microorganisms which could initiate the bioremediation process. In such devastated environments, both bioaugmentation to replace the lost biodiversity and biostimulation to reintroduce the lacking nutrients may be inevitable to restore the soil fertility and productivity.

Bioremediation has long been applied as a treatment technology that is cost-effective, ecologically friendly and efficient for the decontamination of agricultural soils polluted with hydrocarbons(Leahy and Colwell, 1990;Mercade, 1996; Rosenberg and Ron, 1996). In this study, both bioaugumentation using BS and biostimulation involving inorganic nutrient supplementation were applied to rehabilitate the crude petroleum polluted environment. BS was selected for this study because it is always very abundant in pristine soil samples in the Niger Delta region (Nwachukwu, 2000a) while the oil pollution causes leaching of nutrients required for the sustainability of microbial diversity.

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Materials and Methods

Sampling Site and Culture Collection

The microbial strain (BS used for this study was previously obtained from oil polluted agricultural soil in the Niger Delta region, Nigeria (Nwachukwu, 2000a).

Bioremediation Process

Garden soil (1200kg) was contaminated by mixing with 2000 ml of Nigerian crude oil and dispensed into four plastic containers (300 kg each).Two containers were designated Experimental Design Set-ups (EDS), each was inoculated with 500ml nutrient both containing approximately 1.51 x 10^7cfu/ml of BS giving a ratio of 1:2 to the value of total heterotrophs naturally present in soils (3.2 x 10^7 cfu/g) (Chikere *etal.*, 2009; Nwachukwu, 2001). The remaining two containers not inoculated with BS served as the Control Design Set –ups (CDS) to check the abilities of autochthonous organisms in the biodegradation of the oil pollutant. The four set-ups were watered (500 ml each) with sterile distilled and deionised water at weekly intervals. At week 8, 500ml sterile distilled and deionised water containing the modified version of Raymonds medium (rich in $P0_4^{3-}$ and $N0_3$- ions but no $S0_4^{2-}$ions) was added to EDS to replace the depleted ions (Raymond *et al.*, 1976).

Raymonds medium was not added to CDS since it was not inoculated with the bioaugmentation agent (BS) which utilized the inorganic ions and resulted in their rapid depletion. From each of the EDS and CDS, 25.0g samples were aseptically collected at weekly intervals for 12 weeks and used for analyses.

Gravimetric, Microbial and Physico-chemical Analysis

The mean changes in the residual oil content was evaluated by gravimetric method as described by Yveline *et al.* (1997) to determine levels of oil depletion in both EDS and CDS. Inorganic nutrient ions including PO_4^{3-}, NO^-_3 and SO_4^{2-} were determined by a computer optimized ion chromatography (Nwachukwu, 2001). Total heterotrophs (TH) and hydrocarbon utilizers (HCU) (Nwachukwu, 2001) were ascertained by standard plate count method. The pH of samples was determined with the use of pH meter (Model: S & M Mettler, Toledo 3201) while dissolved oxygen (DO) was determined using Orion Portable Dissolved Oxygen meter (Model: Hanna HI 9142, USA) after dilution of soil samples with oxygenated water as described by Umanu and Nwachukwu, (2010) over the study period.

Results and Discussion

Microbial strains isolated from both EDS and CDS included *Micrococcus* spp, *Corynebacterium* spp, *Pseudomonas* spp, *Bacillus* spp, *Flavobacterium* spp, *Rhizopus* spp, *Rhodotorula* spp and *Candida* spp (Fungi) with *Pseudomonas* spp and *Bacillus* spp occurring most abundantly in EDS. Tables 1, 2 and 3 show respectively the mean changes in TH, HCU and concentrations (ppm) of nitrate, sulphate and phosphate ions in soil samples polluted with crude petroleum over the study period of 12 weeks in both EDS and CDS. Between weeks 2 and 12, the TH for both EDS and CDS showed increasing trends (although these were much more remarkable in EDS apparently because of the inoculation with BS) with corresponding decreases in residual oil content (ROC) particularly in EDS. However, between weeks 6 and 8, the trends in ROC were not as rapid as between weeks 0 and 6. The inorganic nutrient ions namely PO_4^{3-}, NO_3^- and SO_4^{2-} present in the soil samples are summarized in ion chromatograms obtained for weeks 0, 8 and 12 (Figures 1 and 2). Thus, at week 8, the levels of inorganic nutrient ions were low particularly in EDS inoculated with BS with PO_4^{3-} and NO_3^- ions almost completely depleted (Table 3). This observation coincided with the period when the changes in microbial populations, particularly the HCU, slowed down with equivalent low proportions in oil reduction. This is an indication that the increase in TH was probably the key factor responsible for the remarkable oil reduction in EDS when compared with CDS and this could be attributed to the levels of the inorganic nutrients ions (biostimulation) and BS (bioaugmentation) introduced into EDS.

Thus the addition of Raymond's medium provided adequate inorganic nutrient ions required for the growth of BS inoculated into EDS as well as other autochthonous microorganisms present in the soils resulting to the increase in their population density. These ions became limiting in EDS at week 8 and were restored by the addition of the modified Raymond's medium which attracted a corresponding decrease in ROC observed for EDS. Essentially, microorganisms including BS inoculated into EDS utilize PO_4^{3-} ions to meet their energy metabolism and synthesis of cellular components such as phospholipids while NO_3^- ions serve as nitrogen sources for the synthesis of cellular nitrogenous compounds including amino acids, proteins and nucleic acids (Nwachukwu, *et al.* (2000b); Nwachuckwu, 2001). The changes in dissolved oxygen (DO) and in pH profiles are shown in Table 4. The trends observed for these variables were much more remarkable in EDS. The decreasing trends of DO obtained for both EDS and CDS emphasize the consumption of oxygen in the biodegradation of organic matter as reported by other workers (Singh *et al*; 1999; Chikere *et al*; 2009). Thus organic matter such as crude petroleum pollutant in both EDS and CDs was degraded and this probably caused depletion of oxygen, and hence, the reduction in DO over time in the oil impacted environments.

Most tropical soils are acidic with low pH values (Chikere *et al*; 2009; Nwachukwu, 2000a). Moreover, the biodegradation of crude oil in soil ecosystems causes production of acidic intermediates which could lower the pH values (Raymond *et al*; 1976). This phenomenon was probably the key factor responsible for the decreasing trends of pH values observed for both EDS and CDS.

Conclusion

The results of this study showed that biostimulation involving inorganic nutrient adjustment and bioaugmentation by application of *Bacillus subtilis* (a good hydrocarbon utilizer in the presence of oxygen) were probably the two key factors responsible for the remarkable differences in the results observed for EDS and CDS, particularly in the oil content reduction.

Table 1: Mean changes in the population density of heterotrophs in the soil samples polluted with crude petroleum.

Time (Weeks)	Population density (cfu/g) x 10^6	
	EDS	CDS
0	19.9	13.4
1	19.6	9.0
2	21.0	9.8
3	23.0	10.2
4	85.0	12.8
5	251.4	11.0
6	309.2	16.4
7	320.0	26.0
8	324.0	39.5
9	449.0	48.0
10	1296.0	60.1
11	1374.0	74.3
12	1450.0	95.0

EDS, experimental design set-ups; CDS, control design set-ups

Table 2: Total hydrocarbon utilizers present in the soil samples (x10^2 cfu/g)

Time (Weeks)	EDS	CDS
0	3.6	3.8
1	37.7	3.5
2	54.1	4.1
3	68.0	4.8
4	79.5	5.7
5	80.1	6.4
6	88.0	7.0
7	92.0	7.4
8	89.4	7.7
9	116.6	7.9
10	154.2	8.5
11	170.0	8.9
12	178.4	9.3

EDS, experimental design set-ups; CDS, control design set-ups

Table 3: Mean changes in the concentrations (ppm) of nitrate, sulphate and phosphate ions in soil samples polluted with crude petroleum.

Time(weeks)	Concentration of ions (PPM)					
	EDS			CDS		
	NO_3^-	SO_4^{2-}	PO_4^{3-}	NO_3^-	SO_4^{2-}	PO_4^{3-}
0	807	504	346	809	506	344
1	801	499	337	807	503	339
3	755	481	300	797	497	321
4	723	453	238	787	491	308
5	643	412	158	772	480	293
6	550	372	91	759	471	276
7	453	334	49	741	461	262
8	807 (375)	308	346 (24)	728	450	250
9	665	276	295	717	442	241
10	591	248	250	701	433	233
11	513	229	205	683	424	218
12	444	216	184	670	413	205

EDS, experimental design set-ups; CDS, control design set-ups. Figures in parenthesis represent new values of the respective ions after addition of Raymonds medium

Table 4: Changes in the hydrogen ion and dissolved oxygen concentrations in the soil samples polluted with crude petroleum.

Time (weeks)	Hydrogen ion concentration (pH)		Dissolved oxygen concentration (mg/kg)	
	EDS	CDS	EDS	CDS
0	6.6	6.7	7.1	6.9
1	6.5	6.7	6.9	6.9
2	6.5	6.6	6.7	6.8
3	6.3	6.5	6.4	6.8
4	6.1	6.5	6.1	6.6
5	5.8	6.4	5.6	6.5
6	5.5	6.4	5.3	6.5
7	5.4	6.4	5.2	6.3
8	5.4	6.3	5.1	6.2
9	5.1	6.1	4.7	6.0
10	4.6	5.8	4.5	5.8
11	4.3	5.7	4.3	5.6
12	4.3	5.5	4.1	5.4

EDS, Experimental Design set-up; CDS, Control Design set-up

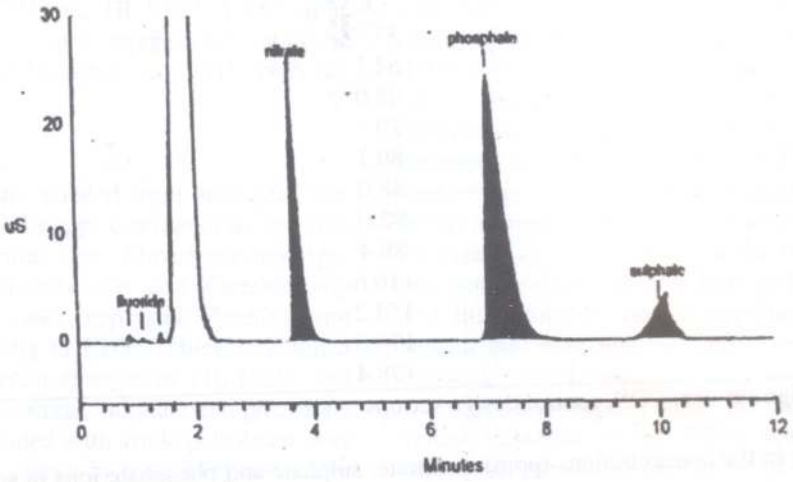

Fig. 1. Ion Chromatogram of experimental design set-up (EDS) at week 0 before addition of Raymonds medium showing high levels of inorganic ions

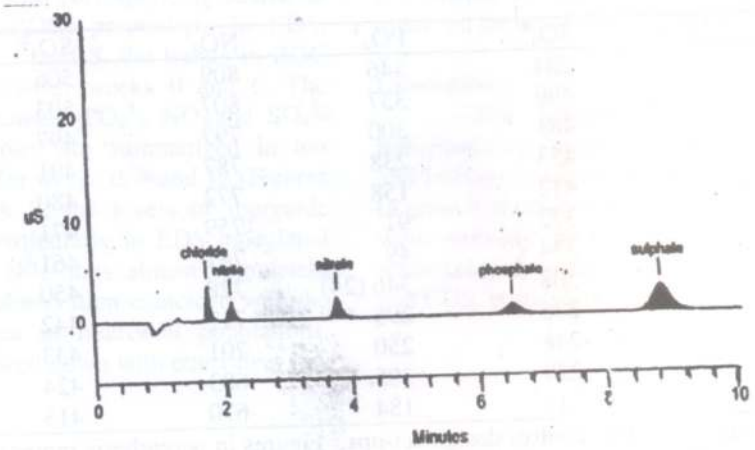

Fig. 2. Ion Chromatogram of experimental design set-up (EDS) at week 8 before addition of Raymonds medium showing depleted levels of various inorganic ions

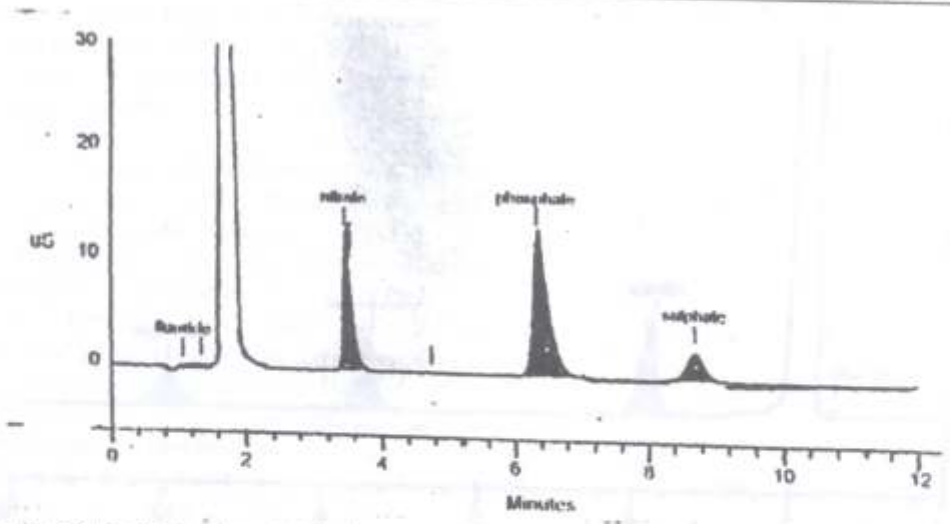

Fig. 3. Ion Chromatogram of experimental design set-up (EDS) at week 8 after addition of Raymonds medium

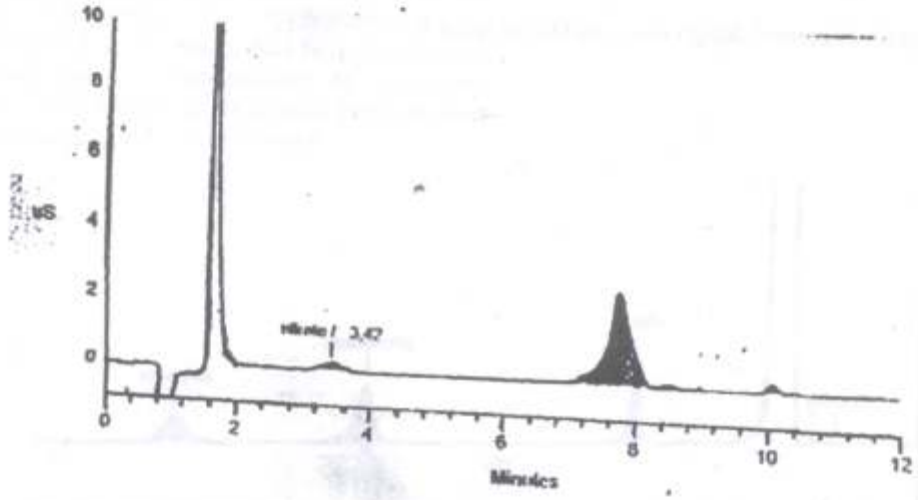

Fig. 4. Ion Chromatogram of experimental design set-up (EDS) at week 12 after addition of Raymonds medium

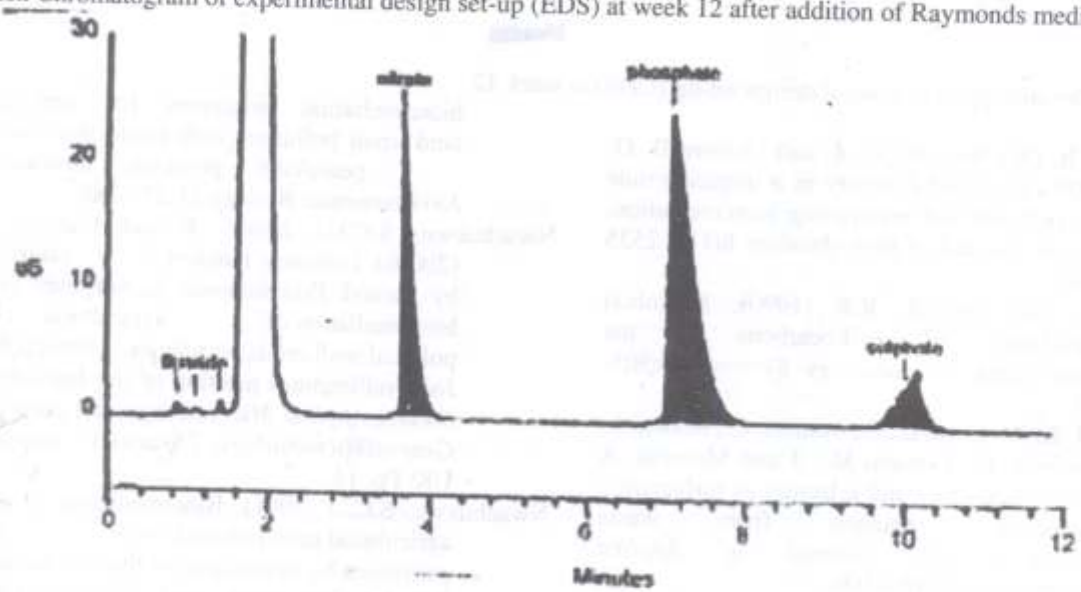

Fig. 5. Ion Chromatogram of control design set-up (CDS) at week 0

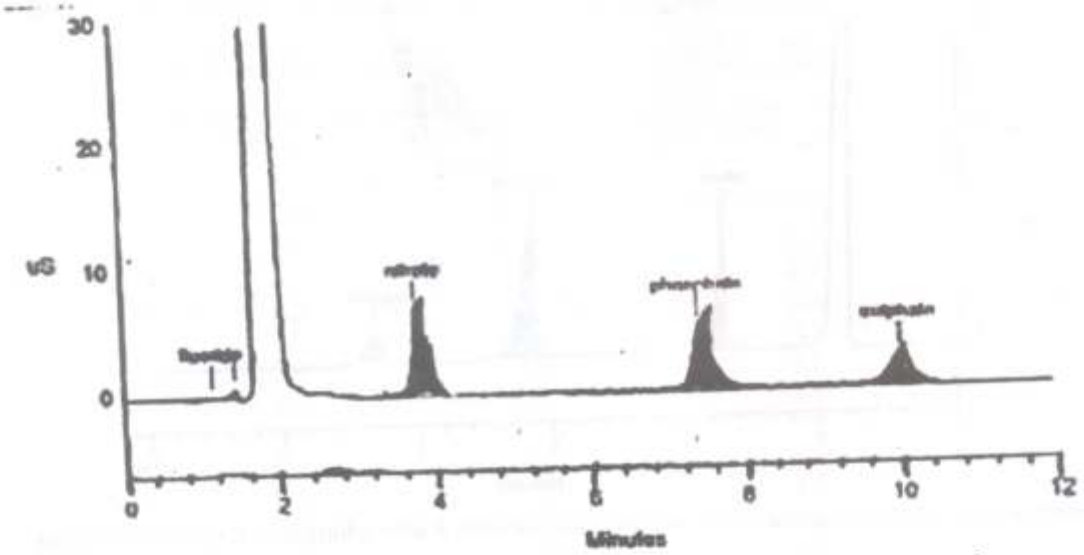

Fig. 6. Ion Chromatogram of control design set-up (CDS) at week 8

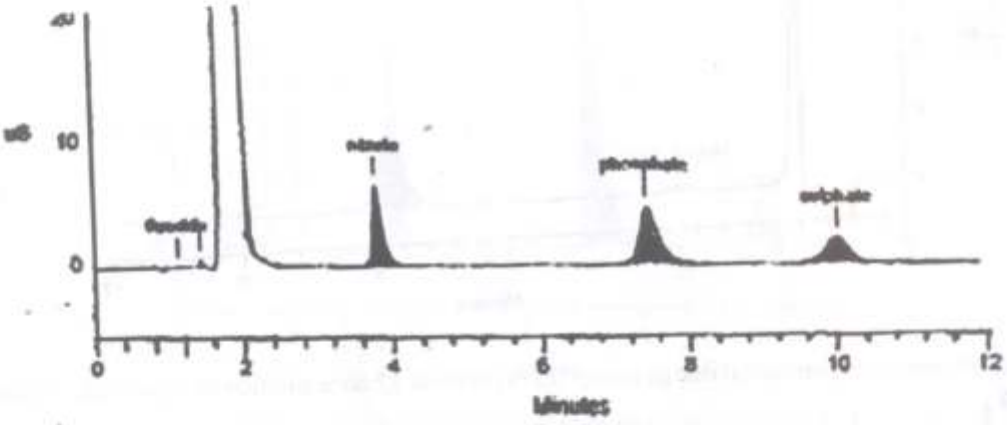

Fig. 7. Ion Chromatogram of control design set-up (CDS) at week 12

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Biodegradation of Photographic Effluent with *Bacillus megaterium*

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Abstract: Industrialization is a worldwide threat to public health. New initiatives for environmental restoration are therefore, required. This study investigates the potentials of *Bacillus megaterium* in the biodegradation of photographic effluent discharged indiscriminately in Lagos environs. Samples of photographic effluents were collected from Agbado-Oke (A), Ifako-Ijaye (B) and Mainland (C) local government areas (LGAs) of Lagos State Nigeria. The effluent samples were diluted with sterile distilled water at concentrations of 10, 25 and 50%. The experimental samples (ES) were inoculated with *B. megaterium* isolated from soil and effluent samples at a population density of 2.74×10^2 CFU/ml while the control samples (CS) had sterile distilled water in place of inoculum. At the end of a six day study period, gas chromatography (GC) analysis showed marked reduction in silver nitrate, elimination of ammonium thiosulphate and potassium fluoride in ES compared to the CS. The study showed possible degradative potential of *B. megaterium* as revealed by reduction in concentrations of the major toxic constituents of the photographic effluents.

Key Words: *Bacillus megaterium*, biodegradation, public health, photographic effluent

Introduction

One of the major challenges facing developing and densely populated countries like Nigeria is the pollution of the natural environments such as soils and water bodies with both domestic and industrial wastes known as effluents. Industrial effluents contain toxic, hazardous pollutants and wastes materials (Plumb *et al.*, 2001). As a result, water bodies which are major receptacles of untreated industrial wastes have become highly polluted. This pollution of natural water bodies negatively affect public health and the environment in great magnitude (Osibanjo *et al.*, 2011). The impact of industrial wastewaters on aquatic and terrestrial ecosystems has drawn a lot of attention worldwide because of its overwhelming environmental significance. Most industrial wastewater can be characterized as extremely complex mixtures containing numerous inorganic as well as organic compounds (Bougrier *et al.*, 2007). The complexity makes it almost impossible to carry out a hazard assessment based on chemical analysis.

Photographic processing involves the development or printing of paper prints, slides, negatives, enlargements, movie film, and other sensitized materials. Many photographic processes, including those in the graphic arts industries, utilize large amounts of water in various chemical solutions necessary for development and processing operations. This water is used for chemical reactants, preservatives, catalysts, accelerators and the likes. It has been conventional practice therefore, to discharge spent chemical solutions into municipal sewer systems and utilize fresh water to make new chemical solutions for use in industrial processes (Brooks *et al.*, 2006). This practice puts an enormous strain on the resources of the municipal water treatment plants. Presently, governments of many countries throughout the world are now carefully scrutinizing chemical discharge levels in industrial wastewater effluents (Kao *et al.*, 2001). This scrutinization has led to new legislations which bans or significantly reduces the discharge limits of chemicals into wastewater (Lemordant *et al.*, 1989; Kennedy *et al.*, 1992). Printing, photographic film and associated industries processes (activities) generate photographic wastes such as spent fixers and bleaches. Each year, thousands of liters of such wastes (which contain silver in solution) are collected and treated in countries where environmental laws are very strict mainly through the electrochemical methods (Farmer *et al.*, 1992). The remaining liquid from the electrolysis process is treated and neutralized in a waste water treatment plant before discharge into the sewers to generate environmentally acceptable effluents (Yazici *et al.*, 2011; Bas *et al.*, 2012). Many photographic processes also produce toxic gases. These gases may be released slowly from baths or stored chemicals as they age. In addition, these gases are usually generated at faster rates if the photo chemicals are heated or if certain chemicals are mixed with acid. The typical constituents of the photo processing wastewater stream include: organic chemicals, chromium compounds, ferricyanide, silver, thiocyanate, ammonium compounds, sulfur compounds, phosphate (Spyrou and Douglas, 1994). A wide variety of chemicals are used in black-and-white photographic processing including: developers, stop baths and fixer, intensifiers and reducers, hypo eliminators, toners, hardeners, hydroquinone and other hazards (Hosseini *et al.*, 2004).

Many hypo eliminators are skin and respiratory irritants. Some are corrosive to skin, eyes, nose and throat. Ammonia (both vapor and liquids) is especially hazardous to eyes and to the mucous membranes of the respiratory system. Organic solvents

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are used for a number of applications in the printing industry. In addition to general concerns about volatile organic compound (VOC) emissions, some potential solvent components like dyes which are highly structured polymers may be persistent in the environment or difficult to decompose biologically (Tzitzi *et al.*, 1994). Silver is present in small amounts in used developer and in moderate quantities (3 - 8 g/litre) in used fixer. Therefore some silver gets into the waste from the wash after fixing. Since photoprocessing solutions are used over and over, the effluents may contain high levels of silver. This silver can be recovered through hydrogen peroxide treatment (Bas *et al.*, 2012). Silver is one of the most toxic metals regulated by the regulatory bodies (Hosseini *et al.*, 2004) and the film processing effluents are classified as hazardous waste since they may cause soil and water pollution, if not properly disposed of or treated (Bas *et al.*, 2012). Besides the direct health effects, the subtle danger of such pollutants lies in the fact that they may be mutagenic or carcinogenic and lead to several human afflictions like cancer, cardiovascular diseases and premature ageing (Grover and Kaur, 1999). Receiving rivers such as estuaries and inland water bodies, which are the major sources of drinking water, are often contaminated by anthropogenic activities and industrial establishments (Phiri *et al.*, 2005). The river systems are the main means of waste disposal, especially the effluents, from industries that are near them. These effluents from industries have a profound influence on the pollution of the water body with which the effluents can alter the physical, chemical and biological properties of the receiving water body (Sangodoyin, 1995). As societies throughout the world become more aware of the issues involved in water pollution, there has been considerable public debate about environmental effects of effluents discharged into aquatic environments because of the many associated hazards (Wakelin *et al.*, 2008).

Industrial pollution remains one of the major problems facing Nigerian cities. In Nigeria, several types of solid and liquid wastes from the industries and individuals are discharged directly into the environment without any treatment (Adewole, 2009). Such polluted habitats may lose their capability to support both plant and animal life and thus constitute public health and socio- economic hazards as well as pose serious aquatic toxicity problems (Okerentugba and Ezeronye, 2003). There is paucity of information on the toxic constituents of photographic effluent and environmentally friendly solutions to its potential devastating effects on the environment. The present study investigates the potential biodegradative potential of *Bacillus megaterium* on the toxic components of photographic effluents.

Materials and Methods

Collection of samples

Samples of photographic effluents and surrounding soils were aseptically collected in screw-capped bottles from three Local Government Areas (L.G.As.) in Lagos State, South-west, Nigeria viz:. Agbado-Oke (A), Ifako-Ijaiye (B), and Mainland (C). They were transported to the Microbiology Laboratory, University of Lagos for subsequent microbial analysis.

Bacterial Culture and Identification

The soil samples were autoclaved to exclude all microorganisms except the heat tolerant spore formers. Subsequently, ten milliliter (10.0 ml) of the photographic effluent was added to 90.0g of each soil sample and the mixtures were allowed to stand for four days in conical flasks. One gram (1g) of each treated soil sample was serially diluted and aliquots (0.1ml) of selected dilutions plated on nutrient agar medium. Inoculated plates were incubated at 37^0C for 24 to 48hours. The developed colonies were purified by repeated streaking. Cultures were maintained on slants at refrigeration temperature of 4^0C. The pure bacterial isolates were identified to species level based on colonial morphology, biochemical tests and phenotypic characterizations using the analytical profile index (API 50CHB test kit, Biomerieux).

Preparation of Inoculum

Pure overnight cultures were suspended in sterile broth. The Optical Density (OD) of the pure broth culture against the sterile broth was determined at 600nm.

Experimental Set-up

The effluent samples collected from each LGAs were grouped into A, Ai, Aii, B, Bi, Bii and C, Ci, Cii where A, B, C are un-inoculated samples of the three LGAs which also served as controls; Ai, Bi, and Ci represented inoculated samples and Aii, Bii and Cii were the corresponding duplicates respectively. The samples were diluted at concentrations of 50%, 25% and 10% respectively. Two milliliter (2.0 ml) of culture suspension was inoculated aseptically at the population density of 2.78×10^4 CFU/ml (OD: 0.058) into the ES while CS had sterile distilled water in place of inoculum. Both ES and CS were kept at room temperature for six days.

Gas Chromatographic (GC) Analysis of effluents

The GC was carried out to determine concentration of ammonium thiosulphate, potassium fluoride, silver nitrate, silver oxide, sulfuric acid, phenols, boric acid, sodium bromide, acetic acid, ammonium bromide, potassium bromide and silver bromide in the photographic effluents before inoculation and six days after inoculation with *B. megaterium*. The effluents were injected into a gas chromatograph (GC) (GCSRI model 8640) while the

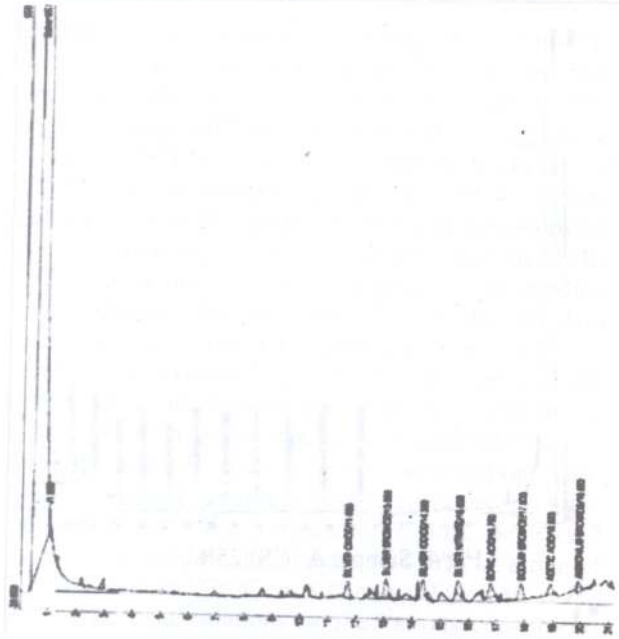

Fig 7. Sample A (CS) 10%

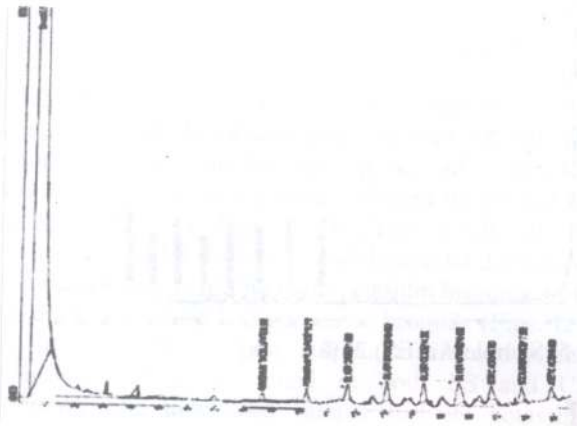

Fig 8: Sample Ai (ES) 10%

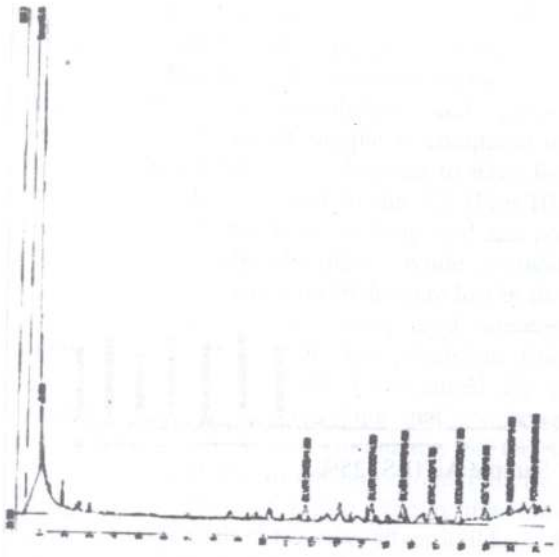

Fig 9. Sample Aii (ES) 10%

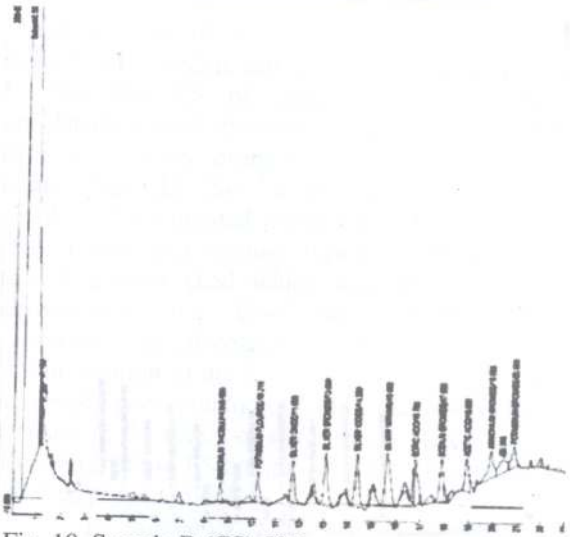

Fig. 10. Sample B (CS) 50%

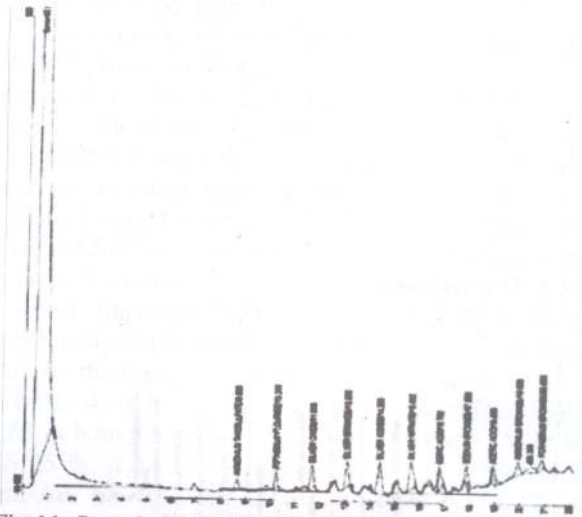

Fig 11. Sample Bi (ES) 50%

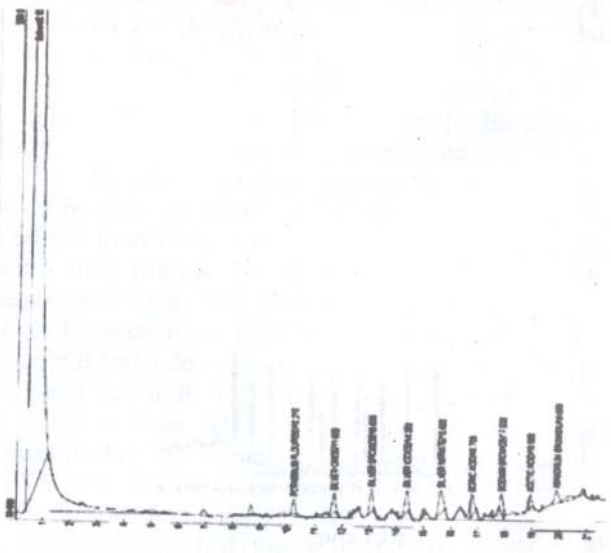

Fig. 12. Sample Bii (ES) 50%

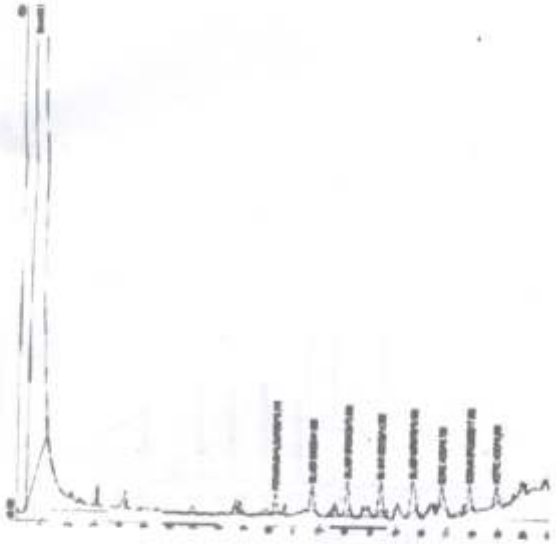

Fig. 13. Sample B (CS) 25%)

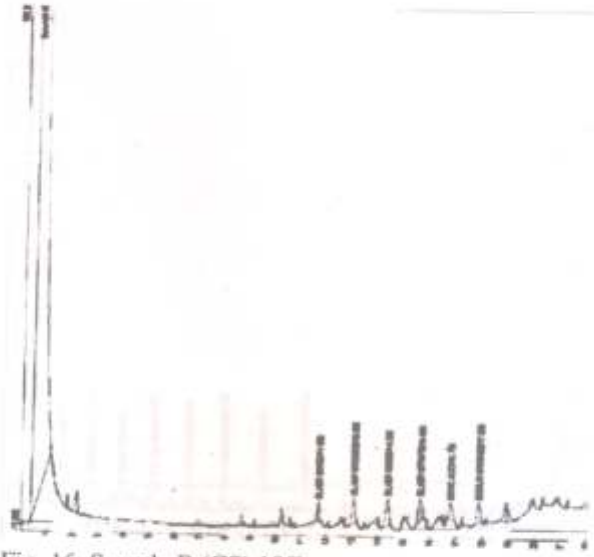

Fig. 16. Sample B (CS) 10%

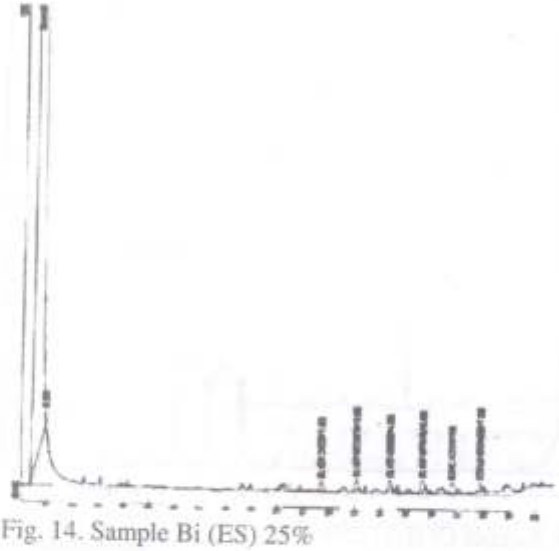

Fig. 14. Sample Bi (ES) 25%

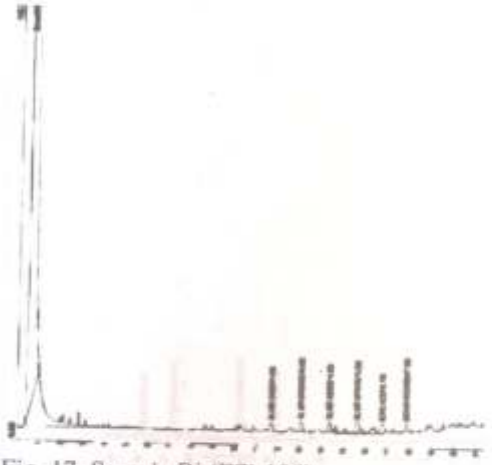

Fig. 17. Sample Bi (ES) 10%

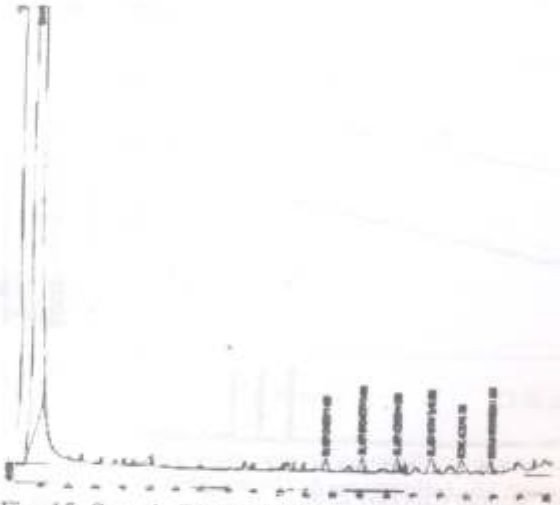

Fig. 15: Sample Bii (ES) 25%

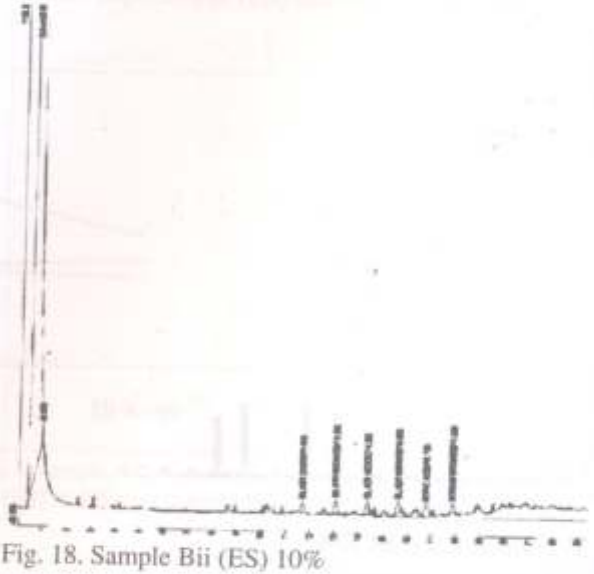

Fig. 18. Sample Bii (ES) 10%

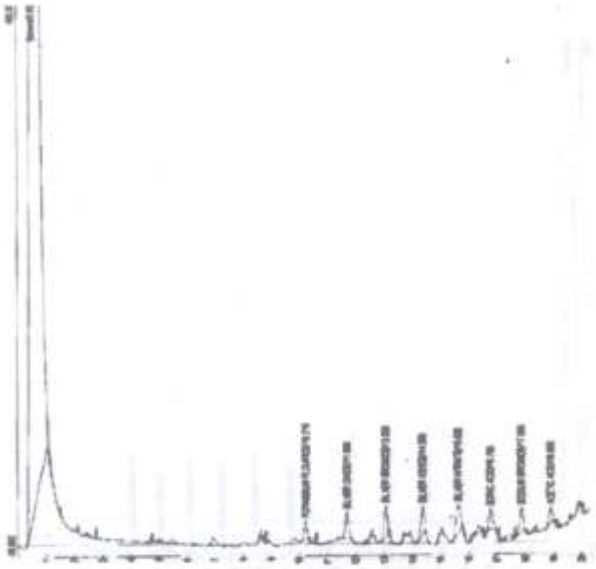

Fig 19. Sample C (CS) 50%

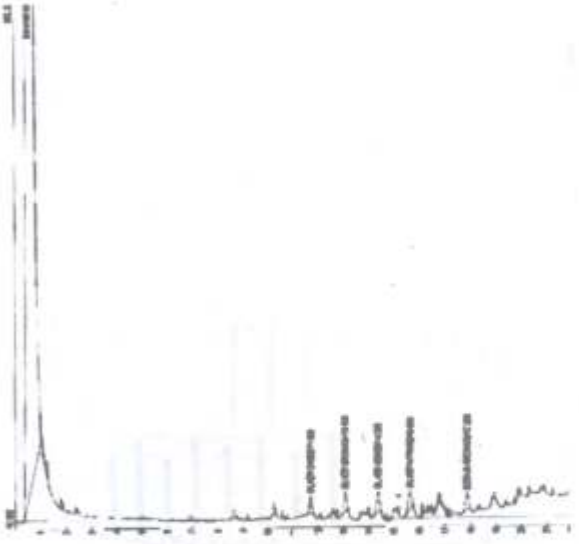

Fig. 22. Sample C (CS) 25%

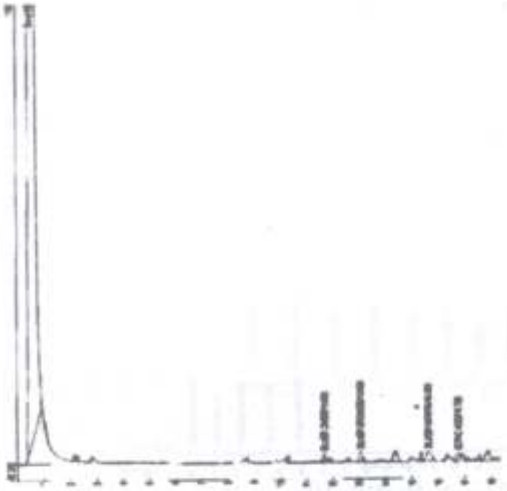

Fig. 20. Sample Ci (ES) 50%

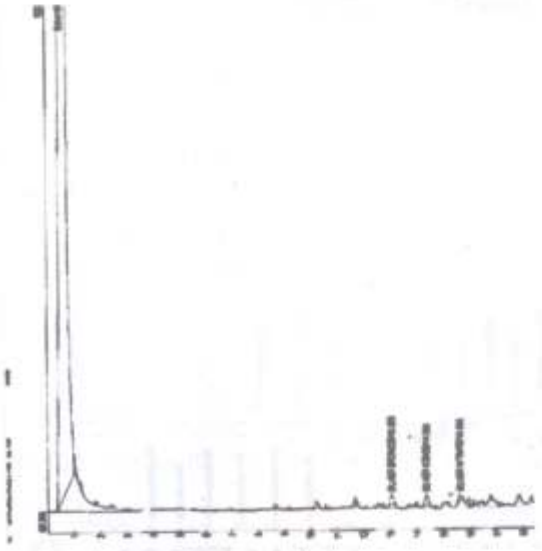

Fig 23: Sample Ci (ES) 25%

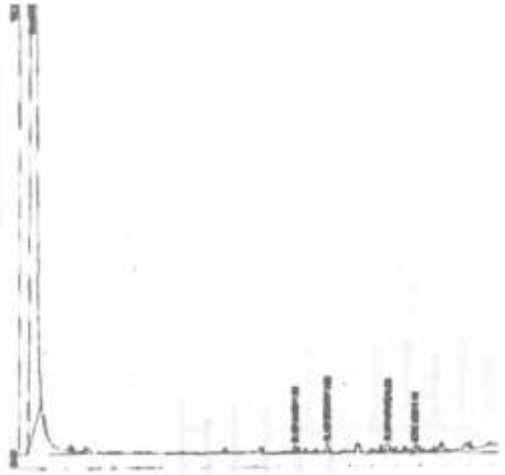

Fig 21. Sample Cii (ES) 50%

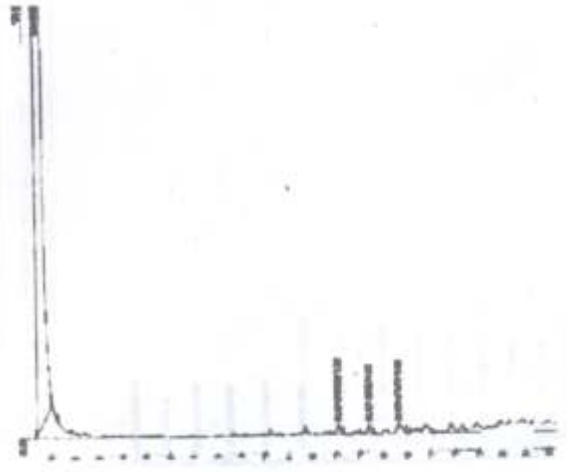

Fig. 24: Sample Cii (ES) 25%

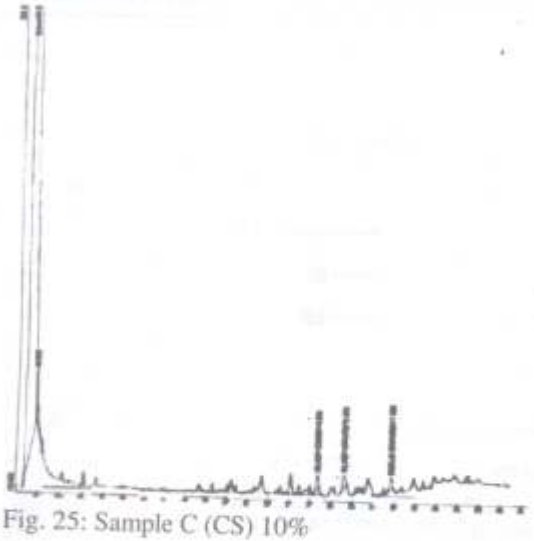

Fig. 25: Sample C (CS) 10%

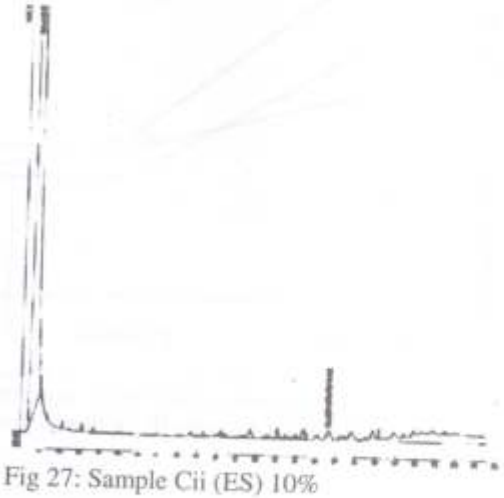

Fig 27: Sample Cii (ES) 10%

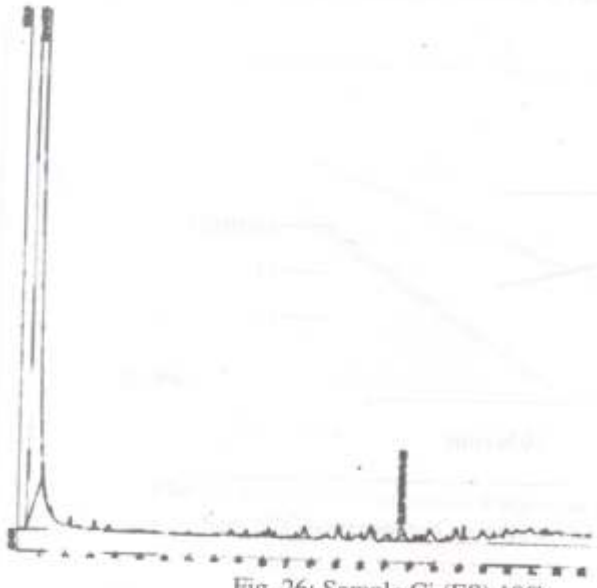

Fig. 26: Sample Ci (ES) 10%

Chromatograms showing peaks of individual components of the photographic effluent of samples A, B and C at concentrations 50, 25 and 10% respectively. Samples, (i) and (ii) are duplicates of (ES) treated concentrations. CS: Control samples

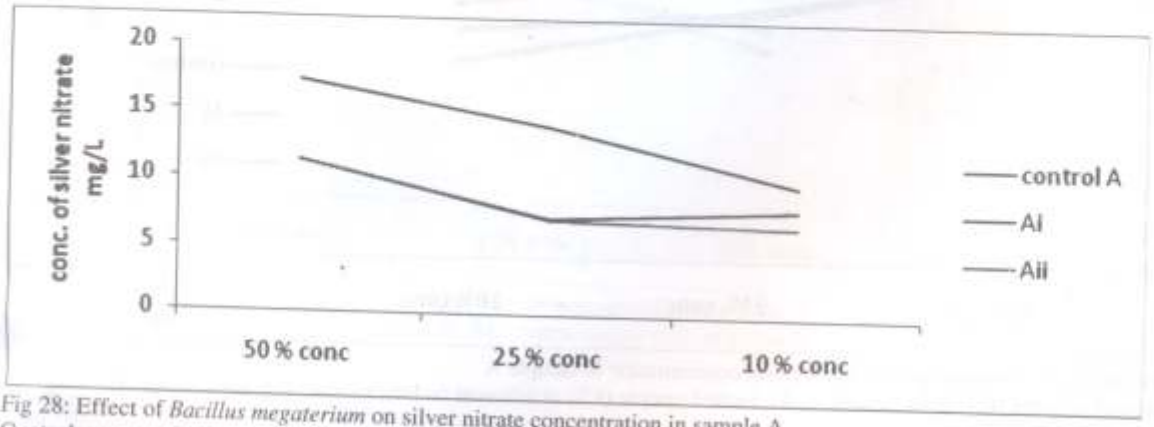

Fig 28: Effect of *Bacillus megaterium* on silver nitrate concentration in sample A
Control: untreated effluent (CS) from sample A, Ai: treated sample (ES) at different concentrations, Aii: duplicate of Ai

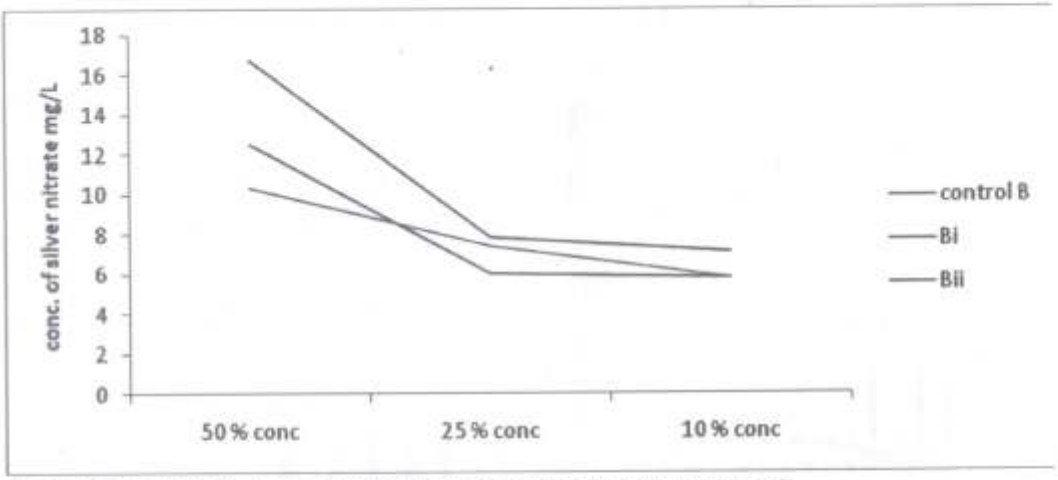

Fig. 29: Effect of *Bacillus megaterium* on silver nitrate concentration in sample B
Control: untreated effluent (CS) from sample B, Bi: treated sample (ES) at different concentrations, Bii: duplicate of Bi

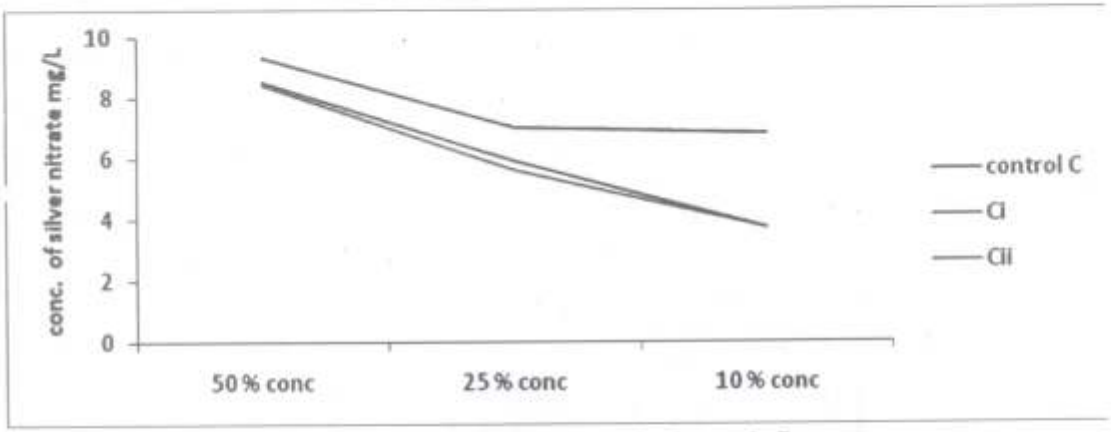

Fig.30: Effect of *Bacillus megaterium* on silver nitrate concentration in sample C
Control: untreated effluent (CS) from sample C, Ci: treated sample (ES) at different concentrations, Cii: duplicate of Ci

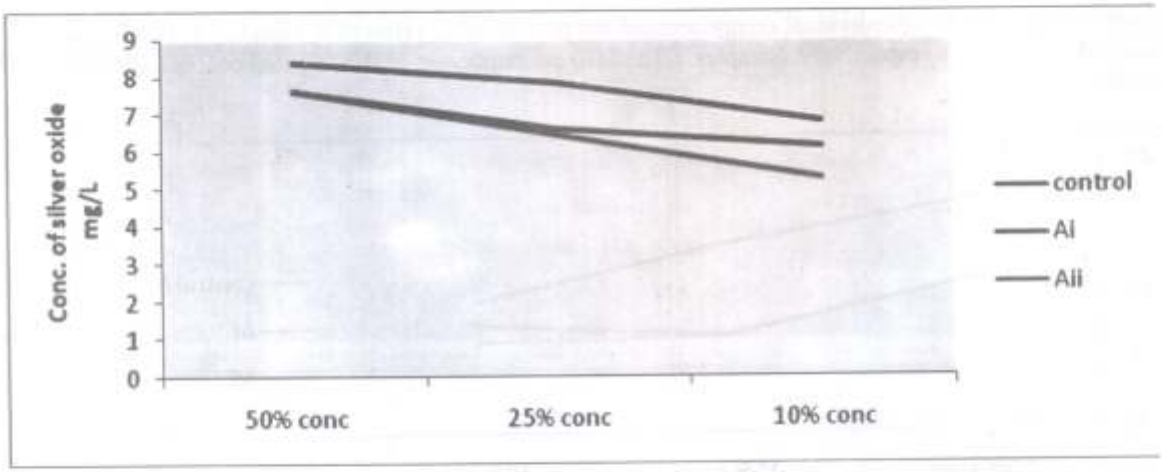

Fig. 31: Effect of *Bacillus megaterium* on silver oxide concentration in sample A
Control: untreated effluent (CS) from sample A, Ai: treated sample (ES) at different concentrations, Aii: duplicate of Ai

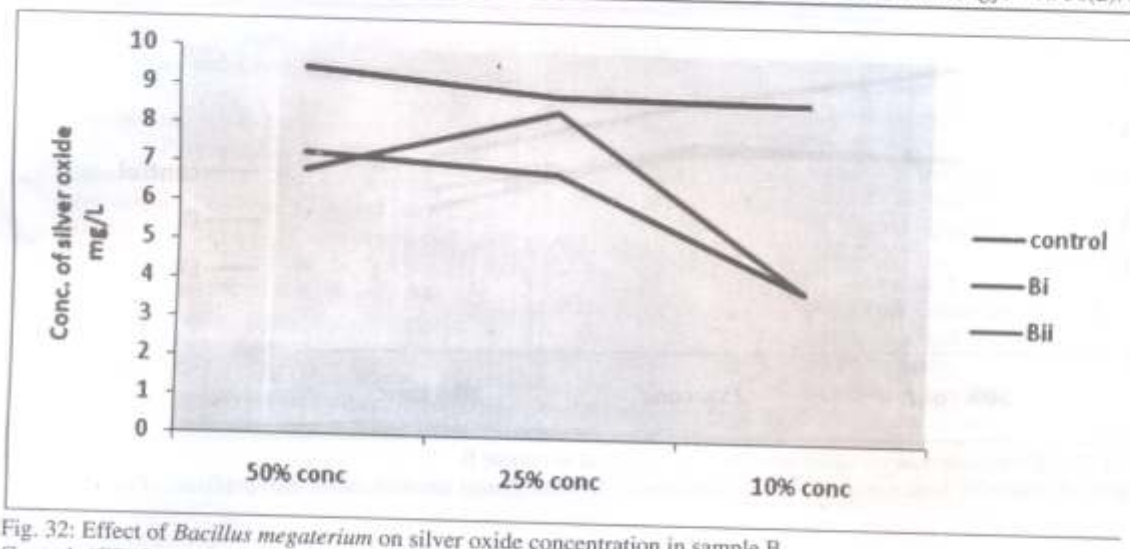

Fig. 32: Effect of *Bacillus megaterium* on silver oxide concentration in sample B
Control: (CS) from sample B, Bi: treated sample (ES) at different concentrations, Bii: duplicate of Bi

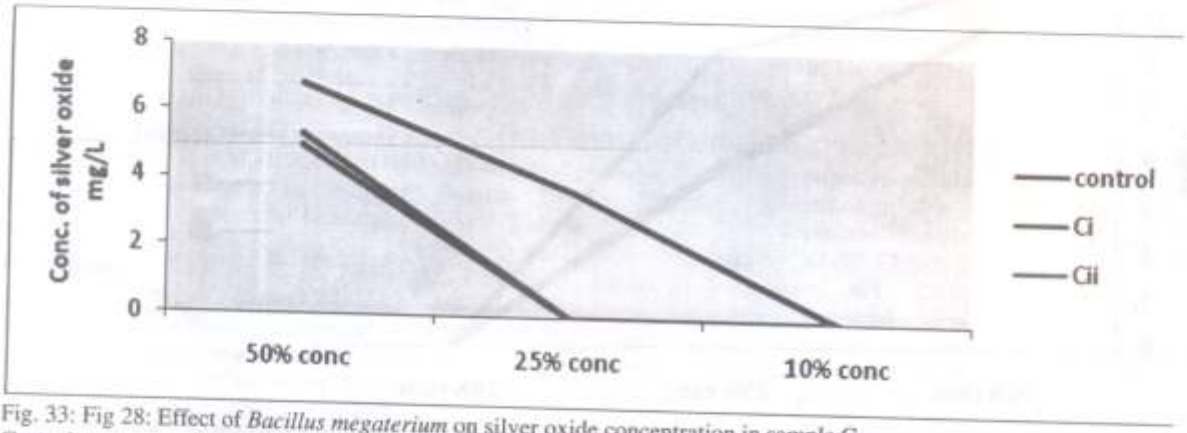

Fig. 33: Fig 28: Effect of *Bacillus megaterium* on silver oxide concentration in sample C
Control: untreated effluent (CS) from sample C, Ci: treated sample (ES) at different concentrations, Cii: duplicate of Ci

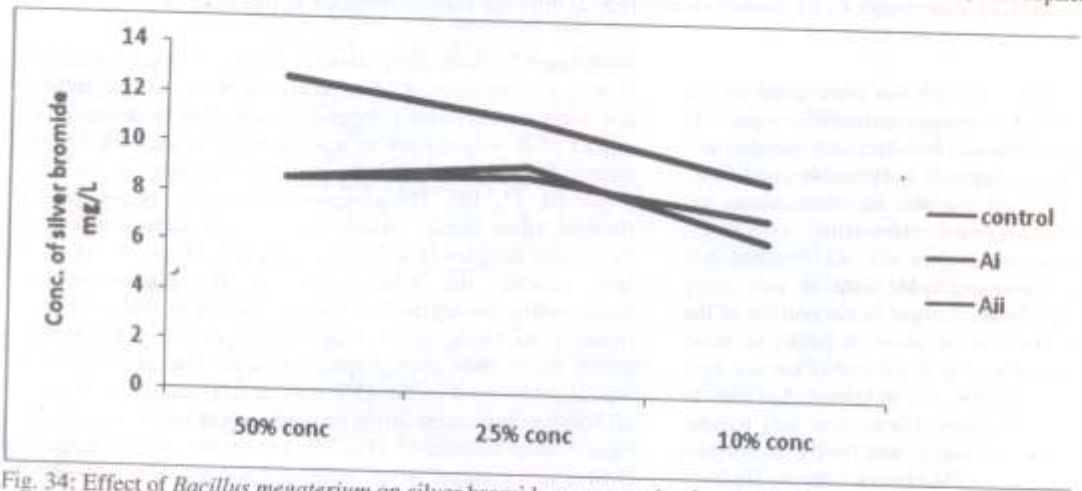

Fig. 34: Effect of *Bacillus megaterium* on silver bromide concentration in sample A
Control: untreated effluent (CS) from sample A, Ai: treated sample (ES) at different concentrations, Aii: duplicate of Ai

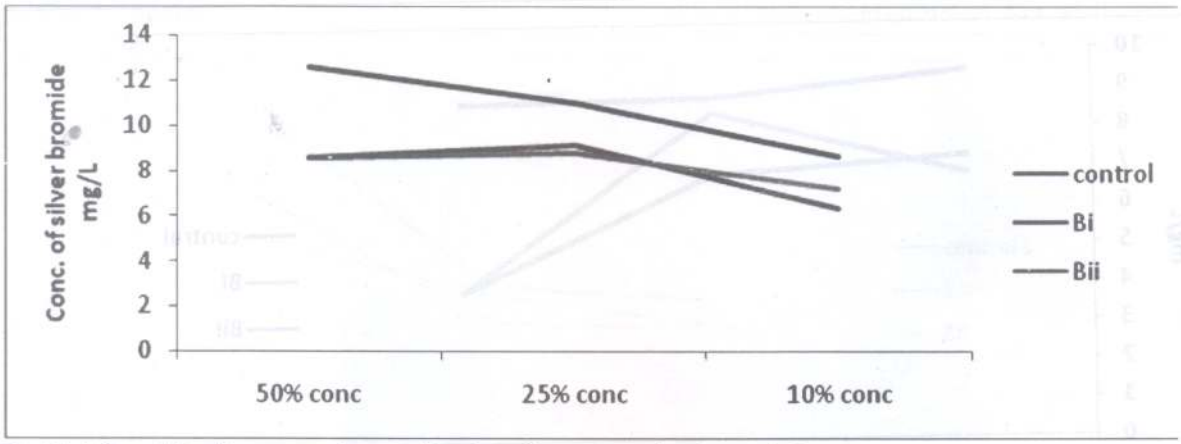

Fig. 35: Effect of *Bacillus megaterium* on silver bromide concentration in sample B
Control: untreated effluent (CS) from sample B, Bi: treated sample (ES) at different concentrations, Bii: duplicate of Bi

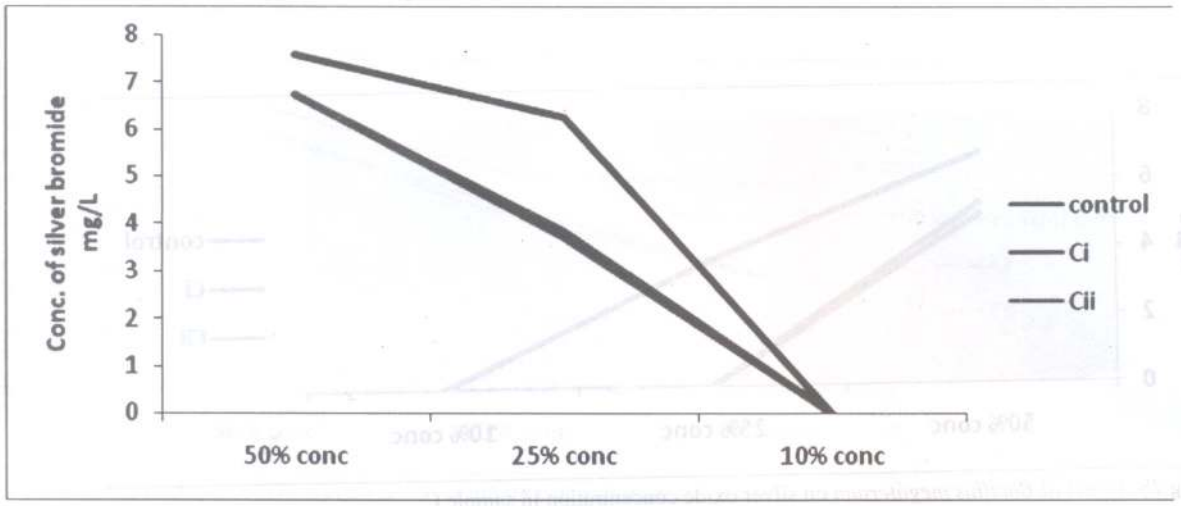

Fig. 36: Effect of *Bacillus megaterium* on silver bromide concentration in sample C
Control: untreated effluent (CS) from sample C, Ci: treated sample (ES) at different concentrations, Cii: duplicate of Ci

Discussion

A lot of scientific research has been done on the impact of wastewater on the receiving communities especially effluents of organic origin that can be effectively metabolized by microorganisms at the appropriate or favorable conditions. Pollutants believed to be biodegradable can contaminate the environment and cause long-term devastating effects on humans and the environment if they are not detected and eliminated. The gas chromatographic data in this study (Figures 1-27) shows significant changes in the profiles of the photographic effluent. The concentration or peaks of these constituents decreased significantly at the end of the six days exposure time. Phenol is toxic not only to humans but also, to animals, plants and microorganisms (Dean-Ross and Rahini, 1995). The peaks of phenol decreased and finally diminished at the end of six days exposure. This agrees with the findings of Otokunefor and Obiukwu, (2005) where *Bacillus* spp was used to bioremediate phenol in refinery effluent laced with various concentrations of phenol and monitored for twenty eight (28) days. Sulfuric acid, ammonium thiosulphate, potassium bromide and phenol were completely eliminated in the experimental sample (ES) 25% of sample B. It is noteworthy that the 25% ES had also lost acetic acid, ammonium bromide and potassium fluoride (Figs. 13, 14, 15). However, concentration of effluent was observed to correlate positively with effluent composition as well as biodegradative impact of *B. megaterium* as was observed in the 10% ES of sample B which retained the same components as the CS (Figs. 16, 17, 18). The complete elimination of potassium fluoride, silver iodide, sodium bromide and acetic acid in all the treated samples (ES) 50% of sample C (Figs. 19, 20, 21) also indicate the effectiveness of *B. megaterium* in biodegrading photographic effluent. This is in line with the report of KODAK, (1999) that *Bacillus megaterium* has the ability to degrade photographic effluent. This phenomenon was also observed in the 25% treated (ES) sample C, where all components except silver bromide, silver iodide and silver nitrate were eliminated (Fig.22) unlike the control sample (CS) which retained silver oxide and sodium. Metal toxicity negatively impacts all biological and cellular processes, influencing metabolism, genetic fidelity and growth (Patel *et al.*, 2007). *Bacillus megaterium* reduced the level of silver nitrate as seen in the peaks of the post inoculation of the effluent sample. The reduction in metal concentration was recorded in Figures 28-36. The result obtained in the present study revealed that *Bacillus megaterium* has the potential to

bioremediate pollutants like silver oxide and silver bromide which poses undesirable effect on the environment.

Conclusion

Photographic effluent that is released indiscriminately to the environment or as a matter of convenience, especially in the developing countries like Nigeria has a lot of environmental and public health consequences. This can be prevented with the use of microorganisms such as *B. megaterium.*

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