

Variation of Lipase, Catalase and Dehydrogenase Activities during Bioremediation of Crude Oil Polluted Soil.

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Abstract

Crude oil pollution pervades the communities of the Niger Delta region of Nigeria. The present study examines the changes in lipase, catalase and dehydrogenase activities in crude oil polluted soils remediated with *arthrobacter* and *bacillus* species isolated from a crude oil polluted soil in Ikarama-Okordia community of Bayelsa State, Nigeria. A total of ten polythene bags containing 10kg soil were used. The experimental control (natural attenuation), remediated A (*arthrobacter* species bioaugmented) and remediated B (*bacillus* species bioaugmented) had three polythene bags polluted with 100, 200 and 400ml crude oil respectively and one unpolluted polythene bag (control). Remediation lasted for 90days and analysis was carried out every 30days. The results showed a significant ($p < 0.05$) variation in the activities of the enzymes studied. Lipase activity increased as the volume of crude oil (contaminant) increased relative to time, however, the increment was more pronounced in the soil samples remediated with *arthrobacter* species than *bacillus* species. In all the soils (irrespective of the volume of contaminant), it was observed that there was a significant reduction in the activity of catalase between the unremediated (undergoing natural attenuation) and remediated sites (*arthrobacter* and *bacillus* species) from the unpolluted soil (control); this reduction was pronounced in the unremediated soil and the soil remediated with *bacillus* species. However, as bioremediation proceeded from days 0 to 90, there was resurgence in catalase activity especially in the soils remediated with *arthrobacter* species. Interestingly, dehydrogenase activity reduced relative to time in all the soil samples. This study concludes that crude oil contamination in soil has adverse effects on the activities of lipase, catalase and dehydrogenase; however, microorganisms in polluted soils use their biochemical machinery including enzymes to degrade pollutants.

KEYWORDS: Lipase, Catalase, Dehydrogenase, Bioremediation, *Bacillus* species, *Arthrobacter* species

Introduction

Crude oil (often referred to as “black gold”) (Odebunmi, Ogunsaki and Ilukhor, 2002) is a naturally occurring mixture consisting predominantly of complex combination of hydrocarbons with other elements such as sulphur, nitrogen and oxygen appearing in the form of organic compounds which in some cases form complexes with metals such as nickel and vanadium (Odebunmi, Ogunsaki and Ilukhor 2002; TCPA, 2008). They are a continuum of tens of thousands of different hydrocarbons molecules some of which include paraffinic (straight and branched-chain alkanes), naphthenic (cycloalkanes or cycloparaffins), alkenes (olefins) and aromatic hydrocarbons which have carbon numbers that range from four to large molecules containing more than sixty carbons (Yasin *et al.*, 2013; Alinnor and Nwachukwu, 2013; The American Petroleum Institute, 2011; Aske, 2002). Pollution arising from crude oil is a recurrent anomaly in the Niger Delta communities. The soil and water are a primary recipient of most of the environmental “nonsense” that occurs in the ecosystem (Hodson, 2008). Pyagbara (2007) identified oil spills, gas flares, effluent and waste discharges as the major causes of crude oil pollution. The major causes of the spill incidences include pipelines and flow lines leakage/blowouts, blowouts from well-heads due to poor maintenance and damage and spills from flow stations. Oil spills involve

the release of dangerous hydrocarbons such as benzene, toluene, ethylmethylene and xylene (BTEX) and polycyclic aromatic hydrocarbon into the soil and water sources. These spillages affect vast stretches of land and waterways thus polluting not only crops but also marine life and the sources of water for domestic uses. When a complex substance such as crude oil is released into the environment, the individual constituents partition to different environmental compartments and degrade in accordance with their own physicochemical properties. In soils, crude oil will absorb into the soil matrix and volatile components will gradually partition to the atmosphere. Over time hydrocarbons available for microbial attack may be slowly degraded. In aquatic environments, crude oil will spread as a film on the surface of the water facilitating the loss of volatile components. Components that enter the troposphere will not likely persist as interactions with hydroxyl radicals leads to indirect photo-degradation (API, 2011). Pyagbara (2007) and Chang *et al.* (2014) asserts that the most profound and adverse impact of oil pollution in the Niger Delta with far-reaching implications on all other aspects of the traditional lifestyles and livelihoods, is the total loss of biodiversity (ecosystem) and destruction of habitats largely due to soil degradation.

Oil exploration activities started in the Niger Delta in 1908. However, commercial oil production began at the Ijaw community of Oloibiri, in the present day Bayelsa State by Shell British Petroleum (now Royal Dutch Shell) in 1956 but oil exportation started in 1958. Today, the inhabitants of this community are compelled to cope with damaged farmlands and polluted rivers with no electricity, potable drinking water and other basic social amenities. Like the Oloibiri situation, the locals in many of the oil-bearing communities of the Niger Delta still live in primitive conditions akin to that of the Stone Age, side by side with the high tech and modern facilities of the multinational community that they accommodate (Emuedo, Anoliefo and Emuedo, 2014; Oviasuyi and Uwadiae, 2010; Amnesty International, 2009; Kadafa, 2012). In 2006, the United Nations Development Programme (UNDP) described the region as suffering from “administrative neglect, crumbling social infrastructure and services, high unemployment, social deprivation, abject poverty, filth and squalor, and endemic conflict.” Suffice to say that the natural endowment of the peoples of this region is a typical example of “resource curse” (Amnesty International, 2009). These environmental and economic malaises pervade most oil-bearing communities of the Niger Delta even as a growing number of communities which were hitherto unaffected are being dotted with one environmental hazard or the other.

The need for the restoration of the environment can therefore not be over-emphasized. Conventional methods to remove, reduce, or mitigate toxic substances introduced into soil or ground water via anthropogenic activities and processes suffers from recognizable drawbacks and may involve some level of risk (Donlon and Bauder, 2008; **Mohsenzadeh, Rad and Akbari, 2012**). Researches using bioremediation to mitigate the effects of harmful pollutants has shown immense promise. Bioremediation (a process invented by George M. Robinson) (Sonawdekar, 2012) is a relatively low-cost, low-technology technique that uses natural biological processes/activity to degrade, transform, and/or essentially remove contaminants or impairments of quality from soil and water (Vidali, 2001; Sharma, Kumar and Rehman, 2014). Bioremediation basically uses indigenous oil consuming microorganisms (petrophiles) by enhancing and fertilizing them in their natural habitats. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source (Ajao, Oluwajobi and Olatayo, 2011).

Some types of microorganism are able to degrade petroleum hydrocarbons and use them as source of carbon and energy. The specificity of the degradation process is related to the genetic potential of the particular microorganism to introduce molecular oxygen into hydrocarbon and to generate the intermediates that subsequently enter the general energy yielding metabolic pathway of the cell (**Mohsenzadeh *et al.*, 2012**; Donlon and Bauder, 2008). For example, petroleum hydrocarbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water. Most organic chemicals and many inorganic ones are subject to enzymatic attack through the activities of living organisms (Thapa, Kumar and Ghimire, 2012). Vidali (2001) found that for bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products and the lack of an appropriate enzyme according to Thapa *et al.* (2012) will either prevent attack or will act as a barrier to complete hydrocarbon

degradation. Microorganisms used to perform the function of bioremediation are known as bioremediators (Sharma, 2012).

Bacteria that can degrade petroleum products are *Pseudomonas*, *Aeromonas*, *Moraxella*, *Beijerinckia*, *Flavobacteria*, *Chrobacteria*, *Nocardia*, *Corynebacteria*, *Atinetobacter*, *Mycobacteria*, *Modococci*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, *Streptomyces*, *Bacilli*, *Arthrobacter*, *Cyanobacteria*, etc. (Ukaegbu-Obi and Mbakwem-Aniebo, 2014; Thapa *et al.*, 2012). *Arthrobacter* species have been reported to be a good biodegrading organism (Smitha *et al.*, 2012). They have been shown to degrade various aromatic hydrocarbons such as phenanthrene and others (Seo *et al.*, 2006; Oil-Degrading Bacteria, 2008; Smitha *et al.*, 2012). *Bacillus* species demonstrate great catabolic and biosynthetic versatility. It is only recently, however, that significant progress has been made in understanding how synthesis of enzymes is regulated (Slepecky and Hemphill, 2006). Several findings (Khodadadi *et al.*, 2013; Mekuto, Jackson and Ntwampe, 2013; Devaraja *et al.*, 2013) have shown that *Bacillus* species are effective bioremediators. Microbial lipase, catalase and dehydrogenase activities have been extensively reported to be a useful indicator parameter for testing hydrocarbon degradation in soil (Karigar and Rao, 2011; Verma *et al.*, 2012; Ajao *et al.*, 2011). Thus, the aim of this study is to monitor the changes in the activities of soil microbial lipase, catalase and dehydrogenase during the bioremediation of a crude oil polluted soil relative to bioremediation time.

Materials and methods

Materials

Bonny light crude oil, cheesecloth, centrifuge, distilled water, water bath, test tubes, glass rod, incubator, pasteur pipettes, mortar and pestle, glass-separating funnel, Erlenmeyer flask, Rotary shaker, Filter (Whatman No. 40 filter paper), natural matrix soil certified reference material CRM131-100 (manufactured by R.T. Corporation Limited, Salisbury, United Kingdom), Soxhlet extractor, refrigerator, conical flasks, rotary evaporator (Büchi, Switzerland), aluminium foil.

Reagents

Phosphate buffer, acid reagent (dichromate/acetic acid mixture), 3% aqueous (w/v) 2, 3, 5-triphenyl tetrazolium chloride, ethanol, 0.1M NaOH, Fresh palm oil, 0.1% alcoholic phenolphthalein, acetate buffer (0.107 M), hexane, concentrated nitric acid, concentrated perchloric acid, 2.0 M HCl.

Research design

Soil samples from a crude oil polluted soil were collected and two species of microbial organisms were isolated from it. Thereafter, topsoil (0-5 cm depth) from an unpolluted site was used to fill 10 perforated black polythene bags. Each soil bag weighed 10 kg. Each soil bag was sprayed with 100, 200 and 400ml of crude oil, except the control. Bio-augmentation with the remedying organisms (isolated organisms), in the 10 polythene bags is described in table 1. The activities of lipase, catalase and dehydrogenase were assayed on days 0, 30, 60 and 90.

Table 1. Research Design

Soil samples (10kg)	Volume of pollution with crude oil and treatment with remedying organisms		
Unpolluted	NIL		
Unremediated	100ml	200ml	400ml
Remediated A	100ml + A	200ml + A	400ml + A
Remediated B	100ml + B	200ml + B	400ml + B

A and B are the remediating organisms; A is *Arthrobacter* species (cell concentration of 3.0×10^7 cfu/ml), B is *Bacillus* species (8.8×10^7 cfu/ml).

Sample collection

Soil samples from a polluted site in Ikarama-Okordia, Yenagoa Local Government Area of Bayelsa state were collected by adopting the Food and Agriculture Organization (FAO) guideline (2006), for soil sample collection where a sterilized soil auger was used to make a depth of 5cm using a grid. The samples for analyses were collected with unused and sterilized plastic bags sealed with rubber bands. All samples were labelled with a permanent water resistant marker and were taken to the laboratory within 1 hour of collection for analysis. The samples were collected from both the control and the polluted sites.

Bacteria Isolation, Biochemical Tests, Characterization and Identification

The bacteria used in this work were isolated from the top 5cm sandy-loamy soil of Ikarama-Okordia, Yenagoa Local Government Area of Bayelsa State that was polluted by crude oil arising from spillage. 1.0g of sample was weighed and introduced into a sterile solution under aseptic condition. Serial dilution was carried out. The sample was diluted to 10^{-4} and 10^{-5} respectively. Thereafter, 0.1ml aliquot of inoculum was collected using a sterile pipette and inoculated on nutrient agar medium. The plates were incubated at 37°C for 24hours. Distinct colonies that developed were counted to obtain the colony forming unit per gram (CFU/g) of the sample.

Again, two colonies having different cultural characterization were picked and sub-cultured on freshly prepared nutrient agar plate and incubated at 37°C for 24hours. Distinct colonies from the sub-cultured plate were picked and inoculated on nutrient agar slant to make stock-pure culture which was characterised and identified using biochemical tests. The results are presented in table 2. The two isolates were identified as *Bacillus* and *Arthrobacter* species.

Table 2. Results of biochemical characterization and isolation of bacterial isolates.

Test	Result	
Gram reaction	+	+
Cell morphology	Rod	Rod
Spores	+	-
Catalase	+	+
Oxidase	-	+
Indole	-	-
Citrate	+	+
Methyl red	-	+
Voges proskauer	-	-
Hydrogen sulphide	+	-
Gas	-	+
Slant	A	A

Butt	A	B
Glucose	A	A/G
Mannose	-	A
Possible identification	<i>Bacillus</i> spp.	<i>Arthrobacter</i> spp.

Enzyme Assays

Lipase activity was determined as described by Ugochukwu *et al.* (2008). A quantity of 20ml of each soil microcosm treatment cells of soil sample was introduced into a mortar and pestle and ground gently for 5 min, to release the extracellular microbial enzyme in a liquid medium. The whole solution was then transferred into a glass-separating funnel and allowed to stand for 30 min at room temperature. A quantity of 3ml each of the supernatant was used as source of crude enzyme solution. Lipase activity was measured by titrating the fatty acid released with 0.1M NaOH using 0.1% alcoholic phenolphthalein as indicator. Fresh palm oil was used as the source of glyceride. The oil (0.5 ml) was taken in a glass stopper Erlenmeyer flask and 10 ml of acetate buffer (0.107 M) and 1 ml of hexane were added. The contents were vortexed for 5min (for thorough mixing). Then 1ml of the enzyme solution was added with vigorous shaking. The set up was allowed to stand for 20 min for hydrolysis to take place with continuous shaking on a rotary shaker at $30 \pm 1^\circ\text{C}$. At the end of the hydrolysis, 20ml of ethyl alcohol was added. The liberated fatty acid was titrated against 0.1M NaOH. The determination of the blank was also carried out with the only difference that the enzyme solution was added after 20 min. The activity was expressed in arbitrary units—one arbitrary unit of enzyme activity corresponds to 1ml of 0.1M NaOH required to neutralize the fatty acid liberated during the incubation period of 20 min at 30°C . Appropriate controls were always run to compensate for spontaneous hydrolysis and the inherent acidity of the enzyme preparation. Zero time control titres were subtracted from each of the determinations and the averages of duplicate analyses were reported.

After preparation of extract, catalase activity was determined as described by Rani *et al.* (2004). Catalase breaks down hydrogen peroxide to give oxygen that oxidizes potassium dichromate. The oxidation of chromate gives a chromophore that absorbs maximally at 610nm. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.05M phosphate buffer (pH 7.5), 0.5ml of 0.2 MH_2O_2 , 0.4ml H_2O and incubated for different time period t_1 , t_2 and t_3 for 1, 2 and 3 minutes respectively. The reaction was terminated after each time interval by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes in boiling water and the absorbance was read at 610nm. Catalase activity was expressed in terms of moles of H_2O_2 consumed/minute.

Dehydrogenase activity was determined using the method described by Tabatabai (1982). Dehydrogenases convert 2, 3,5-triphenyl tetrazolium chloride to formazan. The absorbance of formazan was read spectrophotometrically at 485nm. Sieved soil (1g) was placed in test tubes, mixed with 1ml of 3% aqueous (w/v) 2, 3, 5-triphenyl tetrazolium chloride and stirred with a glass rod. After 96 hour of incubation (27°C), 10 ml of ethanol was added to each test tube and the suspension was vortexed for 30seconds. The tubes were then incubated for 1hour to allow suspended soil to settle. The resulting supernatant (5ml) was carefully transferred to clean test tubes using Pasteur pipettes. Absorbance was read spectrophotometrically at 485nm. Extinction coefficient of 15433 Mol/cm was used for evaluating the concentration of formazan formed (Dushoff *et al.* 1965).

Statistical Analysis

Results of all the studies are expressed as means \pm standard error of triplicate determination. In order to detect a significant difference between the groups, statistical analysis was carried out using one way analysis of variance (ANOVA). Data between groups were analyzed by the least significant difference (LSD) test using Statistical Package for the Social Science (SPSS®) Version 20 statistics software at 95% ($P < 0.05$) confidence level.

Results and Discussion

The results of variation in the concentration of lipase (mg/g/30nm) in 100ml, 200ml and 400ml crude oil polluted soils over a 90 days period are presented in figures 1a, b and c respectively. Lipase concentration increased ($p < 0.05$) as the volume of crude oil (contaminant) increased, however, the increment was more pronounced in the samples remediated with *arthrobacter* species than *bacillus* species. This finding corroborates studies by Frankenberger *et al.* (1982), Margesin *et al.* (2000) and Waarde *et al.* (1995) where it was found that increasing contaminant concentration increased microbial extracellular lipase activity, thus fronting lipase as a good option for study of contaminated soil bioremediation. In the 100ml, 200ml and 400ml crude oil polluted bags it was observed that on the 90th day of bioremediation, there was a significant ($p < 0.05$) reduction in the concentration of lipase, although the 400ml crude oil polluted sample remediated with *arthrobacter* species did not show any definite trend. A similar study by Lin *et al.* (2009) it was found that lipase activity in a petroleum polluted site decreased after bioremediation. Other works (Margesin *et al.*, 1999 and Riffaldi *et al.*, 2006) have shown that lipase is closely related with the organic pollutants present in the soil. Lipase activity has also been reported to be the reason behind the drastic reduction of total hydrocarbon from contaminated soil and its activity has been found to be a very useful indicator parameter for testing hydrocarbon degradation in soil (Margesin and Schinner, 1997; Karigar and Rao, 2011). Lipase degrades lipids and other lipid-like compounds derived from a large variety of microorganisms, animals and plants. Lipases can catalyze various reactions such as hydrolysis, inter-esterification, esterification, alcoholysis and aminolysis of organic pollutants (Karigar and Rao, 2011) laying credence to their avowed role in bioremediation. Lipases producing bacteria such as *bacillus* and *arthrobacter* species have been found in diverse habitats (Bayoumi *et al.*, 2012; Verma *et al.*, 2012).

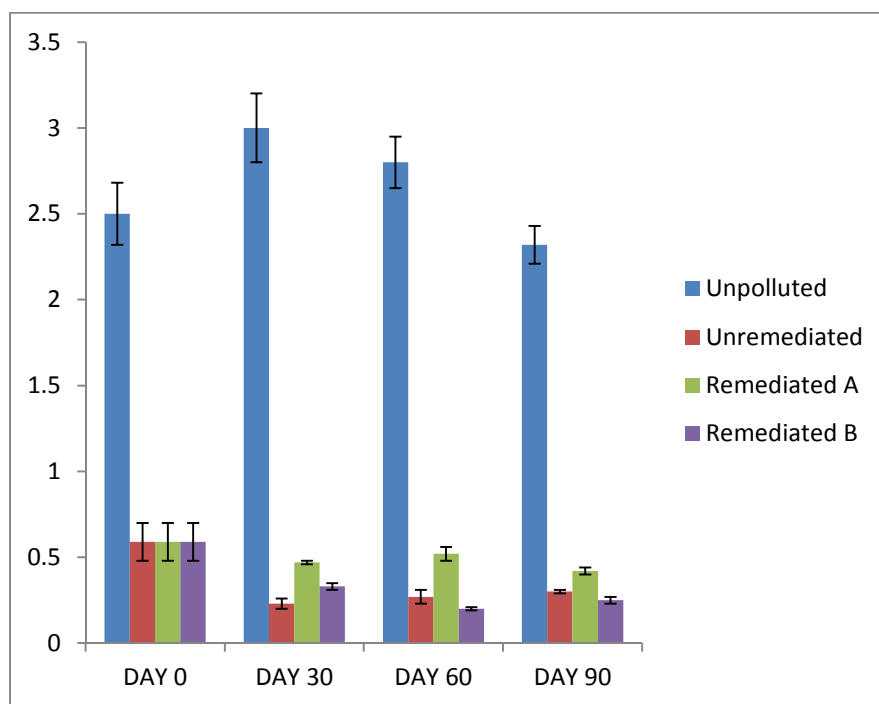


Figure 1a Result showing the concentration of lipase (mg/g/30nm) in soil samples polluted with 100ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$

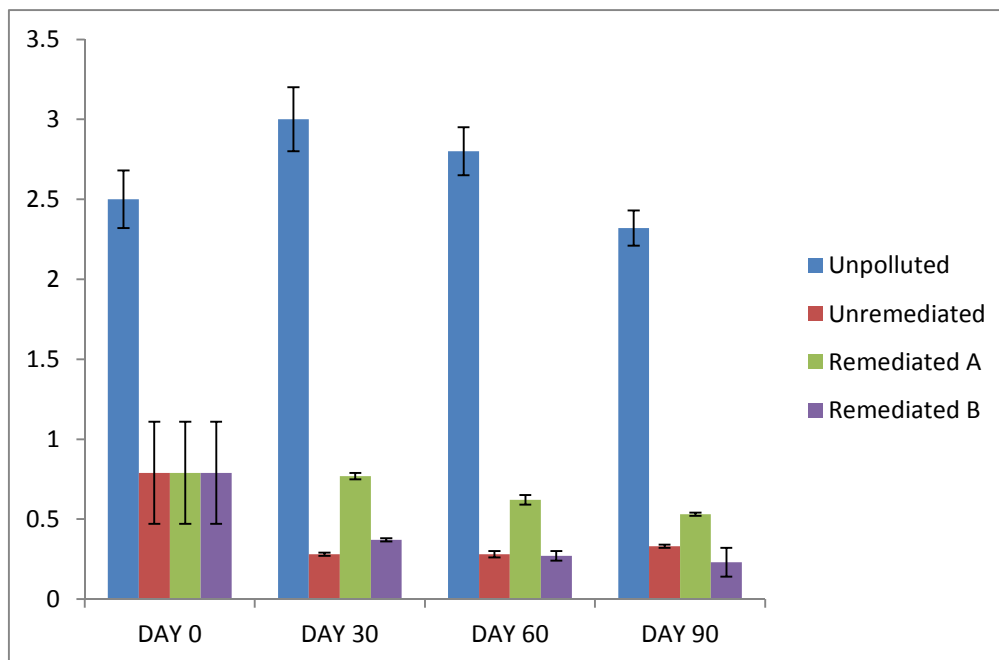


Figure 1b Result showing the concentration of lipase (mg/g/30nm) in soil samples polluted with 200ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$

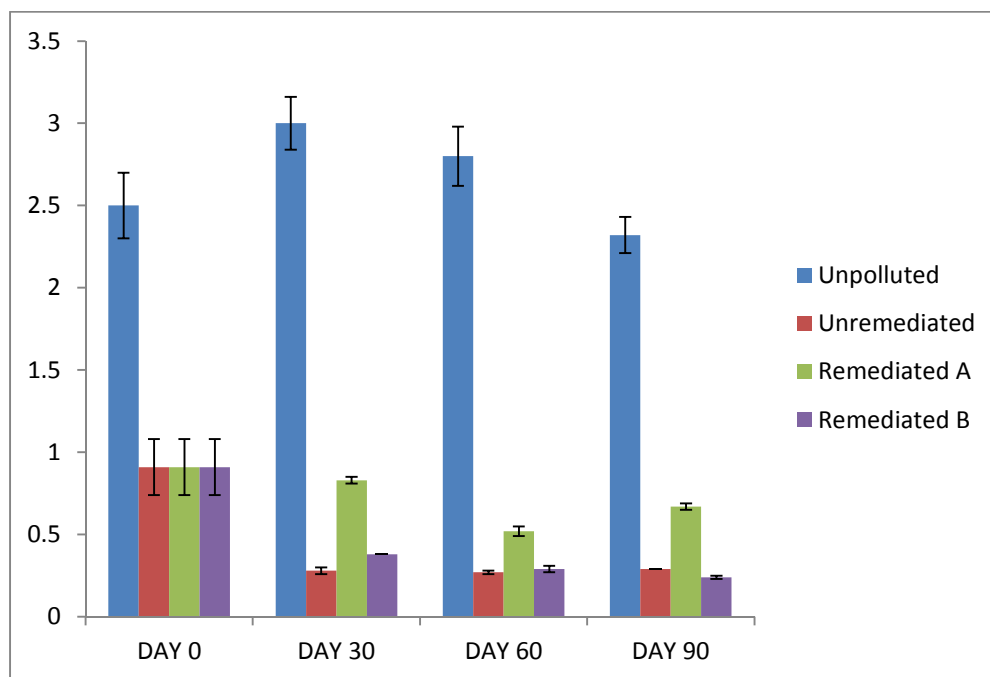


Figure 1c Result showing the concentration of lipase (mg/g/30nm) in soil samples polluted with 400ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$

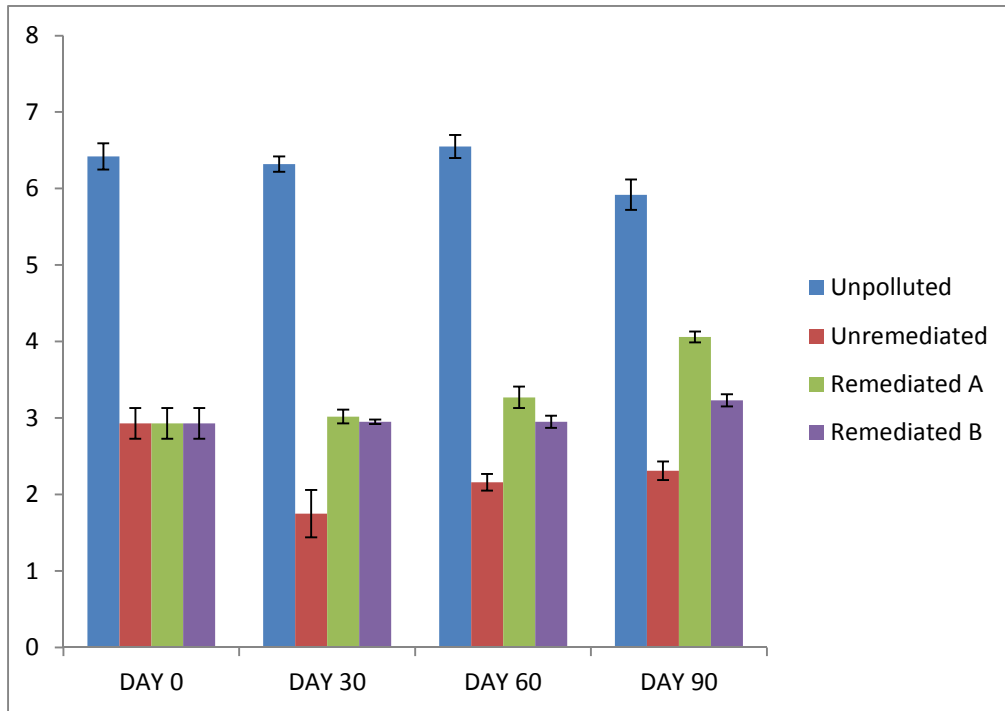


Figure 2a Result showing the concentration of catalase (nmol/min) in soil samples polluted with 100ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$

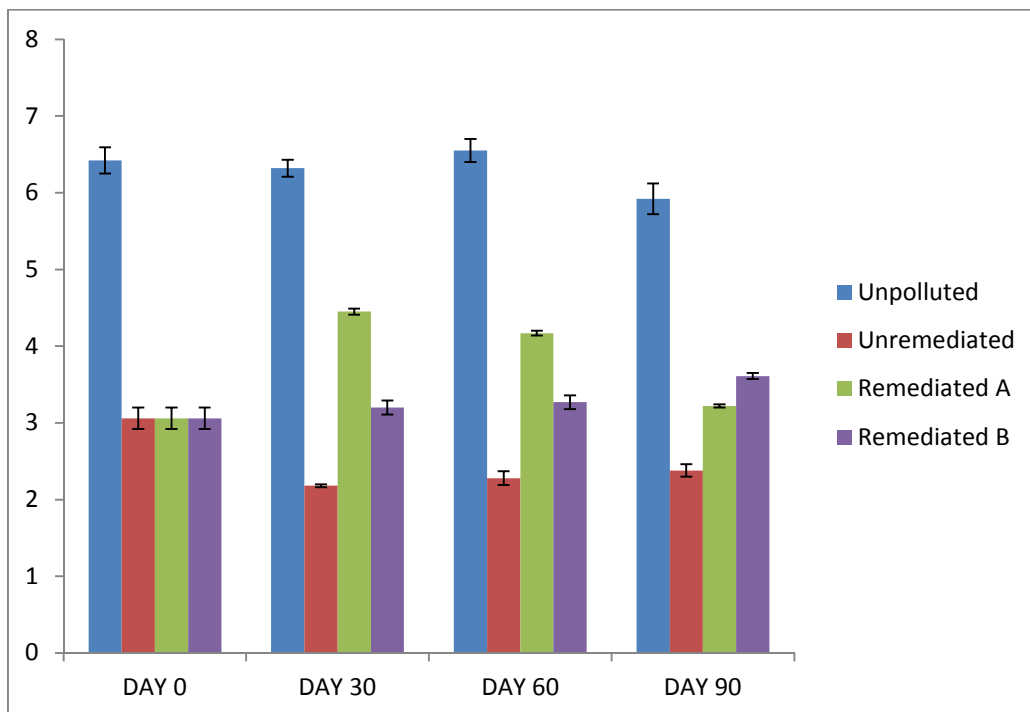


Figure 2b Result showing the concentration of catalase (nmol/min) in soil samples polluted with 200ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$

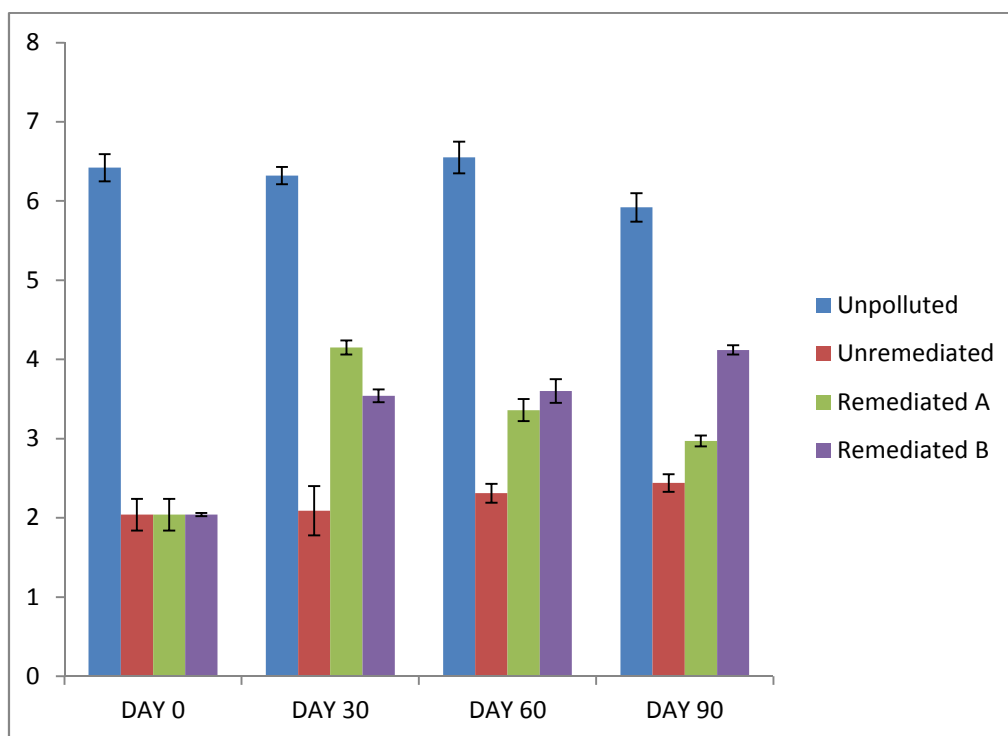


Figure 2c Result showing the concentration of catalase (nmol/min) in soil samples polluted with 400ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$.

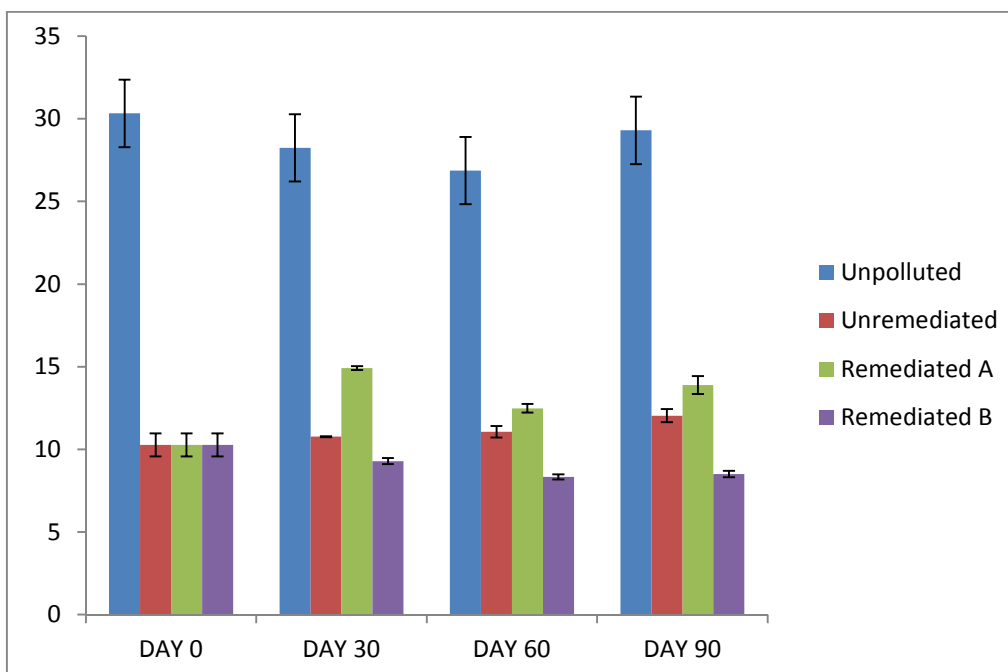


Figure 3a Result showing the concentration of dehydrogenase (mg/g/6h) in soil samples polluted with 100ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$.

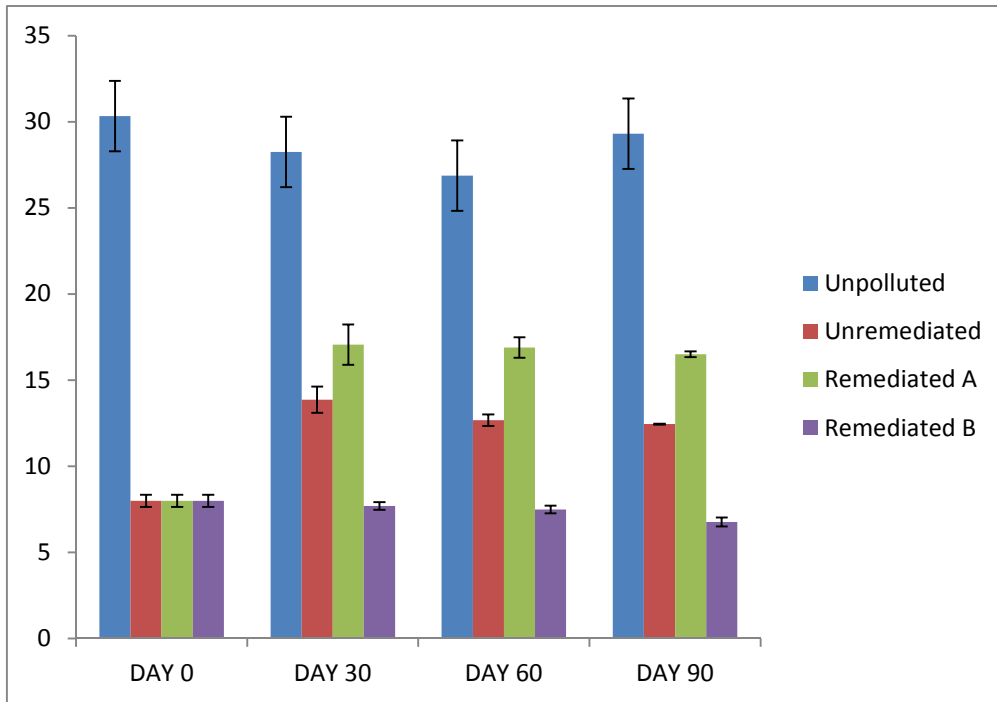


Figure 3b Result showing the concentration of dehydrogenase (mg/g/6h) in soil samples polluted with 200ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$.

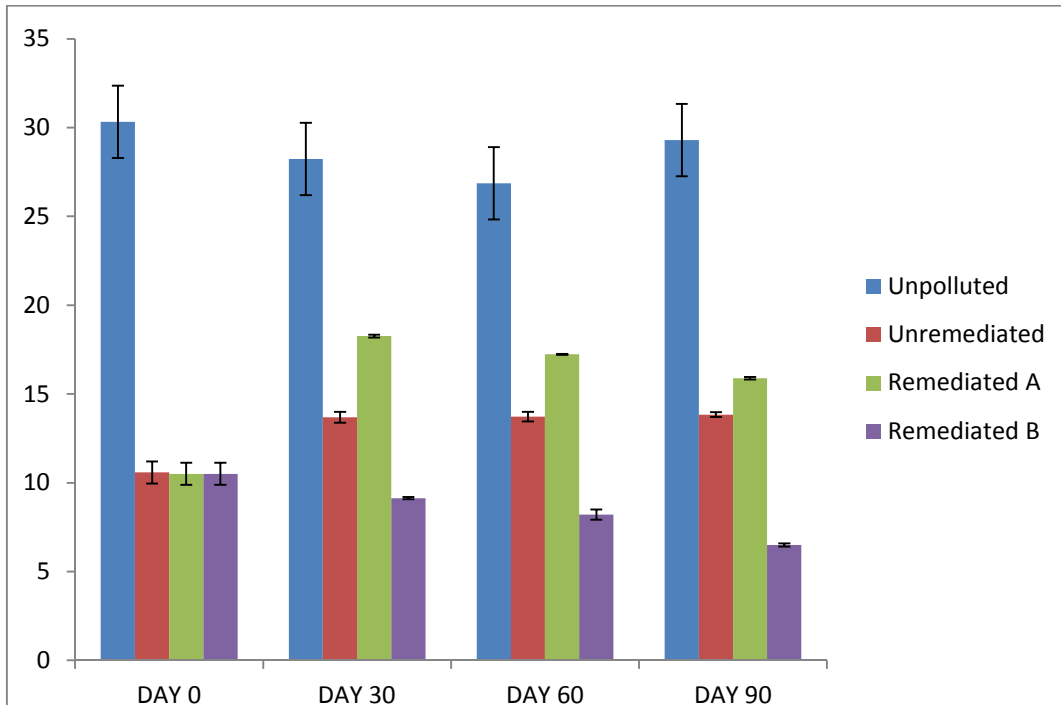


Figure 3c: Result showing the concentration of dehydrogenase (mg/g/6h) in soil samples polluted with 400ml of crude oil.

Values plotted are means \pm standard errors of triplicate determinations at $p < 0.05$.

The catalase activity of unpolluted soil and soils polluted with 100, 200 and 400ml crude oil are captured in figures 2a, b and c respectively. In all the soils (irrespective of the volume of contaminant), it was observed that there was a significant ($p < 0.05$) reduction in the activity of catalase between the unremediated (undergoing natural attenuation) and remediated sites (*arthrobacter* and *bacillus* species) from the unpolluted soil (control); this reduction was pronounced in the unremediated soil and the soil remediated with *bacillus* species. However, as bioremediation proceeded from days 0 to 90, there was resurgence in catalase activity especially in the soils remediated with *arthrobacter* species. This finding corroborates that of Achuba and Okoh (2014), which found that there was altered activity of soil catalase activity when petroleum products were added to the soil, however, its activity increased few days later which they predicated on the increased microbial activity towards biodegradation of available petroleum hydrocarbon. Achuba and Peretiemo-Clarke (2008) also asserted that the initial reduction of catalase activity could be because being an enzyme its activity is altered by unfavourable conditions, such as hypoxia, unavailability of nutrient and changes in pH. This finding place catalase in a good stead as a useful biomarker for indicating the onset of the biodegradation process as their activities decline after the rate of biodegradation has decreased (Ajao *et al.*, 2011). *Arthrobacter* species have been reported to be a good biodegrading organism (Smitha *et al.*, 2012). They have been shown to degrade various aromatic hydrocarbons such as phenanthrene and others (Seo *et al.*, 2006; Oil-Degrading Bacteria, 2008).

Dehydrogenase activities in the 100ml crude oil polluted soil (figure 3a) indicates that the soil sample remediated with *arthrobacter* species showed the highest increment ($p < 0.05$) on the 30th day of bioremediation, however, the *bacillus* species bioaugmented soil rather showed a reduction in dehydrogenase activity. This could be as a result of the ability of *arthrobacter* species to secrete more of this enzyme in the presence of pollutants. Similar trend was observed in the samples polluted with 200 and 400ml crude oil (figures 3b and 3c respectively). In all the samples, the activity of dehydrogenase reduced at the end of bioremediation (day 90), this is attributable to a reduced microbial population of the remediating organisms and a subsequent reduction in the biodegradation of the crude oil. Findings (Frankenberger and Jonhanson, 1982; Waarde *et al.*, 1995; Margesin and Schinner, 1997) have shown that dehydrogenase exhibit this trend in polluted soils. From the foregoing, it will be safe to assert that dehydrogenases are useful for indicating the onset of biodegradation process as its activity decline after the rate of biodegradation has decreased (Ajao *et al.*, 2011). Dehydrogenase occurs in all viable microbial cells and it functions as a measurement of the metabolic state of soil microorganisms and reflects the total range of oxidative activity of soil microflora (Liang *et al.*, 2014; Achuba and Okoh, 2014; Jarvan *et al.*, 2014). Dehydrogenase activity is one of the most adequate, important and one of the most sensitive bioindicators, relating to soil fertility and is often used as a measure of any disruption caused by pesticides, trace elements and can also indicate the type and significance of pollution in soils. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors (Wolinska and Stepniewska 2012; Das and Varma, 2011; Kumar, Chaudhuri and Maiti, 2013).

Microorganisms such as *bacillus* and *arthrobacter* produce enzymes in the presence of carbon sources which are responsible for attacking the hydrocarbon molecules (Thapa *et al.*, 2012). Bacterial activity is the major process involved in the hydrolysis of organic pollutants. Extracellular enzyme activity is a key step in degradation and utilization of organic polymers, since only compounds with molecular mass lower than 600 daltons can pass through cell pores (Vasileva-Tonkova and Galabova, 2003). Lipases, catalases and dehydrogenases may produce extensive transformations of structural and toxicological properties of contaminants, and even their complete conversion into innocuous inorganic end products. The secretion of these enzymes by the microbes makes them possible tools for review of bioremediation of contaminated soil (Margesin and Schinner, 1999; Okoro *et al.*, 2014; Erdogan and Karaca, 2011).

Conclusion

Crude oil has adverse effects on the environment. Interestingly, microorganisms secrete degradative enzymes to ameliorate the effects of contaminants in crude oil polluted soils. Lipase, catalase and dehydrogenase activities have been shown to vary with crude oil pollution relative to time. These microbial enzymes are effective in

degrading the crude oil components as seen in their variations in the different polluted soil samples. It is suggested that further research be done with a higher concentration of the microbial isolates and a study using a consortium of the remediating organisms will help expose the ability of these microorganisms in co-metabolism of pollutants.

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