Comparison of the Efficacy of Remediation by Enhanced Natural Attenuation with Phytoremediation in the Recovery of Crude-Oil Polluted Soils

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Abstract

The efficacy of remediation by enhanced natural attenuation (RENA) and phytoremediation using the leguminous crop, *Vigna unguiculata* (PVU) in the recovery of crude oil polluted soils was compared. The potency of both remediation techniques was evaluated by the activities of selected soil enzymes serving as soil quality indicators. The rate and progress of the remediation processes were monitored at two weeks interval for a period of six weeks, beginning from the 7th day of pollution. The crude oil polluted soil experienced a significant (P<0.05) increase in soil acid phosphatase activity with a corresponding decrease in soil alkaline phosphatase and amylase activities respectively. The remediation processes showed that with RENA, the soil's acid phosphatase activity decreased from 7.23±0.56 to 5.66 ± 0.50 (U/L) but with PVU, its activity increased from 7.60 ± 0.64 to 21.13 ± 7.8 (U/L). Both RENA and PVU showed progressive significant (P<0.05) decreases in alkaline phosphatase activity. However, RENA recorded a marked increased activity from 8.13 ± 5.40 on day 35 to 10.95 ± 5.71 (IU/L) on day 49. Soil amylase showed a decreased activity from 4.71 ± 0.77 and 4.76 ± 0.34 (U/L) on day 7 to 3.81 ± 1.03 and 3.65 ± 1.15 (U/L) on day 21 for RENA and PVU respectively. The findings of this study strongly suggest that remediation by enhanced natural attenuation and phytoremediation with *V.unguiculata* are suitable in the recovery of crude oil polluted soils. However, remediation by enhanced natural attenuation showed greater efficiency in the remediation of polluted agricultural lands.

Key words: Efficacy, Enhanced Natural Attenuation, Phytoremediation, Enzyme activities.

1.0 Introduction

Increased awareness of the deleterious effects of pollutants on the environments by the public has led to resistance against contamination of the Niger Delta environments in Nigeria. In recent times, there have been remarkable increases in urbanization and industrial activities (Eze and Okpokwasili, 2010). This has resulted in an ever-increasing reliance on petrochemicals, which in turn has resulted in the contamination of a significant number of sites with petroleum (crude oil) and petroleum by-products (Bauman, 1991).

Contamination of the environment by petroleum and petroleum by-products could be as a result of equipment failure, operational mishap (e.g., high pressure pipelines, seepages, tanker accidents) and/or intentional damages to oil production facilities otherwise known as sabotage (Atlas, 1981; Osuji and Onojake, 2004; Osuji and Onojake, 2006).

Contamination of soil with petroleum hydrocarbon has adverse effect on soil microflora, all of which assist in soil fertility (Torstenssen *et al.*, 1998). Microbial presence in the soil is of great importance in maintaining soil fertility, as it was reported by Torstenssen *et al.*, (1998), that soils which maintain a high level of microbial biomass are capable of not only storing more nutrients but also cycling more nutrients through the system. The sustainability of soil fertility, quality and productivity is of immense interest and concern to man, since there is direct reliance and dependence of man's existence on the soil. Therefore indices that serve as indicators for assessing soil quality and fertility must be continually maintained and monitored.

The impact of oil spillage is often assessed from changes in the physical, chemical and biological components of the ecosystem. Soil enzymes are a group of enzymes whose usual habitats are the soils and are continually playing an important role in maintaining soil ecology, physical and chemical properties, fertility and soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al.*, 1991). They are important in catalyzing several vital reactions necessary for life processes of micro-organisms in soils, the stabilization of the soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling, hence playing an important role in agriculture (Dick *et al.*, 1994; Dick, 1997). These enzymes include amylase, arylsulphatases, β -glucosidase, cellulose, phosphatases, proteases, ureases, chitinase, and dehydrogenases released from both living and dead plantsand animals (Kanfer *et al.*, 1974), organic compounds and micro-organisms (Richmond, 1991) and soils (Ganeshamurthy *et al.*, 1995).

Enzymes are the direct mediators for biological catabolism of soil organic and mineral components. Thus, these catalysts provide a meaningful assessment of reaction rates for important soil processes. Soil enzyme activities are often closely related to organic soil matter, soil physical properties and microbiological activity or biomass, It's activities changes more sooner than other parameters, thus providing early indications of changes in soil health and can be used as measures of microbial activity, soil productivity and inhibiting effects of pollutants (Dick *et al.*, 1996). Thus, some enzymatic activities which can be determined quite promptly and precisely are a reliable indicator reflecting the current biological state of the soil (Wyszkowska *et al.*, 2002).

The objective of this study was to compare the efficacy of RENA with PVU in the recovery of crudeoil contaminated soils. Phytoremediation is the use of plants to detoxify, transform and extract pollutants from its site to a form that is less harmful or harmless to man (Frick *et al.*, 1999). Its principle is based on certain plants' natural ability to bioaccumulate, degrade or render contaminants harmless in soil, water or air. Natural attenuation processes however, include a variety of physical, chemical or biological processes that under favourable conditions, act without human intervention to reduce the mass, toxicity, morbidity, volume or concentration of contaminants in soil or ground water (NSCEP, 1999). Thus, remediation by enhanced natural attenuation, works with the principle that all things in nature ultimately succumb to decay (microbial breakdown) but at a very slow rate. It therefore focuses on what can be done to enhance or accelerate this natural process of remediating contaminated soil, water or air without any adverse effect. These technologies are ecologically friendly, solar energy driven and is based on "using nature to clean up nature".

2.0 Materials and methods

2.1Experimental design:

The study area was divided into four equal plots, labeled: A, B, C and D respectively.

Plot A Normal control (NC) \rightarrow uncontaminated site

Plot B

Experimental control (EC) \rightarrow crude oil contaminated site.

Plot C

Crude oil contaminated site planted with cowpea (undergoing phytoremediation with *Vigna unguiculata*). Plot D

Crude oil contaminated site undergoing RENA technique (remediation by enhanced natural attenuation).

Each site is a widely spaced plot measuring 2.10m by 2.10m, respectively. Preliminary preparation of the land was carried out to remove weeds, grasses, cans and other litters that may interfere with the research. The land was further tilled to loosen the soil and stimulate microbial activity. Each plot (with exception of normal control) was polluted with equal volumes of bonny light crude petroleum (6 litres each). Samples for post-contamination analyses were collected 7 days after pollution. The study was performed in triplicates.

2.2 Planting of the cowpea (Vigna unguiculata)

Planting of the cowpea was performed seven (7) days after contamination. The seeds were soaked in a bowl of water for about two (2) minutes before planting. Seeds viability was determined by floatation. All seeds that floated on water were discarded while those that remained at bottom were considered viable. Five seeds were planted per hole to an approximate depth of 2cm as reported by Achuba (2006).

2.3 Land treatment technique

After 7 days of contamination, the site to be remediated by RENA was prepared by tilling followed by homogenization and then heaped in form of a pile. The rhizosphere of an unpolluted area was tilled and homogenized also. An approximately equal quantity of unpolluted soil was transferred to the prepared contaminated sites. The soils were continually tilled and thoroughly mixed for about 3 - 4 times weekly, throughout the experimental process. The mixing was done manually using shovels and hoes.

2.4 Sampling techniques

The contaminated soil was left for seven days. Sample collections started at the 7th day prior to the administration of the remediation techniques, and ran at 14days intervals – day 7, 21, 35 and 49 respectively. Day 7 readings represent the base line for all readings obtained.

2.5 Determination of soil enzymes' activity

Procedure

10 grams of soil samples was introduced in a previously washed and dried measuring cylinder and diluted to 100ml with distilled water. The constituent solution was then filtered and the filtrate was kept in a plain bottle. The measurement of the enzyme activity was carried out using an aliquot of the filtrate as reported by Jeroh *et al.*, (2011).

Soil Alkaline Phosphatase activity was determined by the method of Kochmar and Moss (1976), while that of Acid Phosphatase and Amylase was by the method of Young (1995).

2.6 Method of data analysis

Results were expressed as Mean \pm Standard error of mean (SEM), of three replicates, n=3. Significant difference between the group means was determined by one way analyses of variance (ANOVA) followed by Post hoc Turkey's test using statistical package for social science (SPSS) version 16 for windows. P<0.05 were considered statistically significant while P>0.05 were considered statistically non-significant.

3.0 Results

The mean enzymes activities obtained for the uncontaminated (NC: normal control), contaminated (EC: experimental control) and remediated (PVU: phytoremediation with *vigna unguiculata* and RENA: remediation by enhanced natural attenuation) are shown in figure 1, 2 and 3. The NC soil had an initial mean acid phosphatase activity of 3.20 ± 0.78 U/L, while the crude oil contaminated soils had a mean activity of 7.08 ± 0.93 , 7.60 ± 0.64 and 7.23 ± 0.93 (U/L) for EC, PVU and RENA respectively, indicating that crude oil contamination of soil significantly (P<0.05) increased acid phosphatase activity. Remediation process led to a progressive increase in acid phosphatase activity but at different rate in both the soil samples remediated with RENA and PVU respectively, giving rise to mean acid phosphatase activities of 11.27 ± 0.83 , 5.66 ± 0.50 and 21.13 ± 4.8 (U/L) for EC, RENA and PVU soil samples at day 49 respectively.

The NC soil had an initial mean alkaline phosphatase activity of 29.20 ± 5.0 IU/L and an initial mean activity of 23.88 ± 4.36 , 23.77 ± 5.98 and 25.98 ± 2.19 (IU/L) for EC, RENA and PVU soil samples respectively, indicating that contamination of soil causes a reduction in soil alkaline phosphatase activity. When subjected to remedial treatments, the EC, RENA and PVU soils decreased progressively in alkaline phosphatase activity, with an exception of the RENA soil, which recorded a markedly non-significant (P>0.05) increase on day 49. Mean alkaline phosphatase activity of 11.93 ± 2.97 I/UL for the EC was obtained as against 10.95 ± 5.71 , 7.09 ± 1.03 (IU/L) for RENA and PVU soils respectively.

In the case of amylase, the NC soil had an initial activity of $5.72\pm0.76U/L$, as against 3.65 ± 0.50 , 4.71 ± 0.77 and $4.76\pm0.34(U/L)$ for the contaminated soil samples of EC, RENA and PVU respectively, indicating that crude oil contamination reduces soil amylase activity. Both remediation by RENA and PVU experienced a decrease in activity, followed by an increase in activity. This amounted to an activity of 9.54 ± 0.75 and 7.43 ± 1.75 (U/I) for RENA and PVU soil samples respectively as against $1.79\pm0.20U/I$ for EC on day 49. This indicates that remediation of crude oil polluted soil increases soil amylase activity in both remedial treatments, and that RENA recorded the highest mean activity.

4.0 Discussion

When crude oil spills on land, it affects the soil in diverse ways. Such that a once rich agricultural soil becomes devastated and impoverished due to reduction in nutrient availability in the soil as a result of increased soil acidity and toxicity of crude oil fraction (Andrade *et al.*, 2004). All soils contain a group of enzymes that determines soil metabolic processes depending on its physical, chemical, microbiological and biochemical properties (McLaren, 1975). Thus, the efficacy of different remediation measures can be evaluated by some enzymatic activities which can be determined quite promptly and precisely acting as reliable indicator reflecting the current biological state of the soil (Wyszkowska, *et al.*, 2002).

The mean activities of alkaline phosphatase and Amylase activities decreased non-significantly (P>0.05) in the contaminated soil when compared to the NC soil, while the mean activity of acid phosphatase increased non-significantly (P>0.05) in the contaminated soil, relative to the NC soil. Since enzyme activity depends on the population and kinds of microbes that colonize an area (Ratul and Samatha, 2012), the decrease in mean alkaline and amylase activities on contamination may be as a result of the depression of microbial densities and activities caused by contamination of soil by crude oil, even in relatively low contamination (Odu, 1972). Nwaugo *et al.*, (2007a) in a similar work observed that soil pollution reduces soil enzymatic activities. Also, Achuba (2006) stated that crude oil contamination induced changes in starch degrading enzymes, thus inducing stress which leads to a decrease in the level of amylase activity in soil. The observed increase in acid phosphatase activity could be due to the fact that crude oil contamination of soil reduces soil pH. This reduced pH creates an acidic environment which favours the activity of the enzyme since the prevailing pH has a profound influence on the abundance of microorganisms (Fenchal and Blackburn, 1979), and acid/alkaline phosphatase activity is the product of microbial secretion of this enzyme to its nearest soil particles (Ratul and Samantha, 2012).

From the result obtained, mean alkaline phosphatase activity for the EC decreased consistently, but at a very slow rate. Also, that of acid phosphatase increased consistently still at a very slow rate throughout the course of the experiment, indicating a very slow rate of remediation. Both RENA and PVU remediated soil samples show a steady and significant (P<0.05) decrease in mean alkaline phosphatase activity on day 35 when compared with EC, with RENA remediated soil having a more decreased activity of 8.13 ± 5.40 , relative to PVU of 10.27 ± 1.49 . While the latter experienced a further significant decrease (P<0.05) in activity, the former

recorded a significant (P<0.05) increase in activity on day 49. In the same vein, RENA remediated soil and PVU remediated soil showed a significant (P<0.05) increase in mean acid phosphatase down to day 35 of the remediation period. While PVU remediated soil showed a further increase in activity on day 49, RENA remediated soil experienced a marked decrease in activity. The increase/decrease in mean acid phosphatase and alkaline phosphatase activity respectively, describes the state of the soil in terms of pH, which in turn determines which of the two enzyme activities dominates. While alkaline phosphatase is active in alkaline pH, acidic pH favours the other (Ratul and Samantha, 2012). The further significant (P<0.05) increase in acid phosphatase activity and the corresponding increase in alkaline phosphatase activity on day 49 in RENA remediated soil speaks more of not just further degradation of the hydrocarbons but tends towards recovery of the once contaminated soil (Njoku *et al.*, 2009). This is further buttressed by McBridge (1994), who stated that increased acidity (which signifies increased acid phosphatase activity) is a problem for agricultural soils.

Amylase activity recorded an increase as the remediation process continued after the initial decrease experienced on contamination, relative to the EC. The increase in activity could be attributed to the bioremediation potential of both remedial mechanism (Anigboro and Tonukari, 2008). The activity of amylase in the PVU remediated soil sample $(7.43\pm1.75U/L)$ shows a significant (P<0.05) decreased mean activity when compared to RENA remediated soil sample (9.53±2.22U/L). The decreased mean activity of the former is supported by the findings of Anigboro and Tonukari (2008), which revealed that crude oil contamination in soils planted with *V. unguiculata* reduces its invertase and amylase activities, which may be as a result of reduction in nutrient mobilization. The significantly (P<0.05) increased mean amylase activity in RENA remediated soil may be attributed to the findings of Kathiresan and Manivannan (2006), that carbon and organic nitrogen sources are preferred for the production of amylase. Decomposition of biological materials like dry leaves and dead organisms is an alternative carbon source that can assist in the readjustment of the carbon to nutrient ratio to a level or close to that recommended for an agricultural soil to maintain balanced soil nutrition (Leo and Iruka, 2007). The activities of amylase (a hydrolyzing enzyme) produce glucose among other components. Since the addition of glucose encourages crude-oil degradation (Okolo *et al.*, 2005) RENA can be said to be a more efficient soil remedial option when compared with PVU.

5.0 Conclusion

The findings of this work revealed that remediation by enhanced natural attenuation (RENA) and phytoremediation with *V. unguiculata* (PVU) have proven to be potential mechanisms for the remediation of petroleum hydrocarbon (PHC) contaminated soils with remediation by RENA technique being more efficient.

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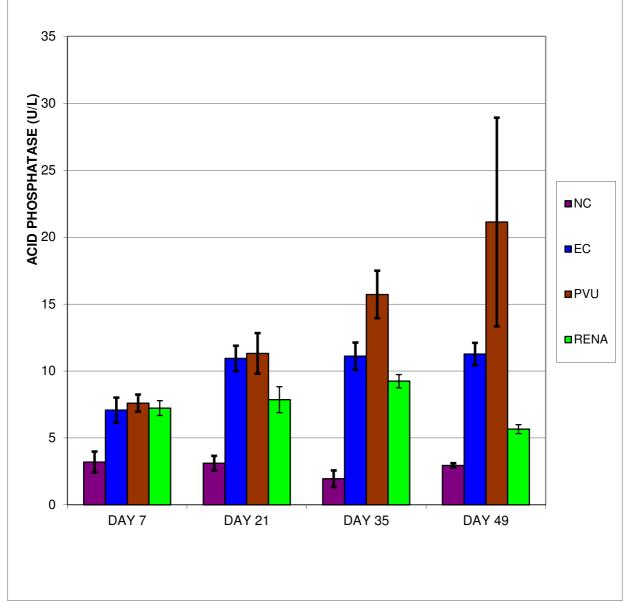
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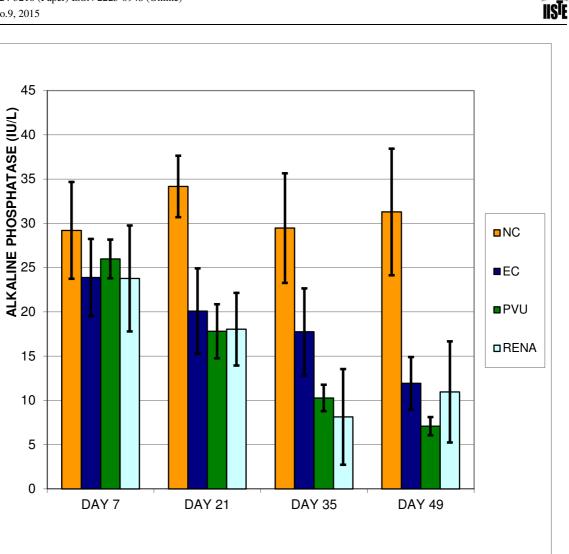
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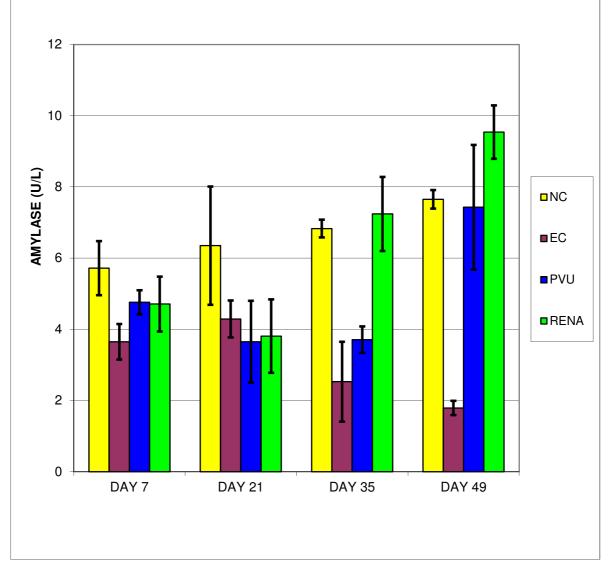
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Figure 1: Bar chart showing the Mean ± S.E.M of acid phosphatase (ACP) activity of uncontaminated (NC: Normal control), contaminated (EC: Experimental control), and remediated (phytoremediation with *Vigna unguiculata*: PVU and remediation by enhanced natural attenuation: RENA) soil samples.



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Figure 2: Bar chart showing the Mean ± S.E.M of alkaline phosphatase (ALP) activity of uncontaminated (NC: Normal control), contaminated (EC: Experimental control), and remediated (phytoremediation with *Vigna unguiculata*: PVU and remediation by enhanced natural attenuation: RENA) soil samples.



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Figure 3: Bar chart showing the Mean ± S.E.M of amylase activity of uncontaminated (NC: Normal control), contaminated (EC: Experimental control) and remediated (phytoremediation with *Vigna unguiculata*: PVU and remediation by enhanced natural attenuation: RENA) soil samples.

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