Bioremediation Potentials of a Petroleum Hydrocarbon Polluted Soil by *Aspergillus oryzae* (JQ675305.1) Isolated from Diseased *Irvingia gabonensis* Seeds

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ABSTRACT

Fungi associated with diseased conditions in *Irvingia gabonensis* (Baill) seeds were isolated from these seeds. A representative species of the fungi (*Aspergillus oryzae*) was deployed to a Spent Engine Oil (SEO) polluted field. By the 6th month, *A. oryzae* caused over a 99% reduction in the level of the initial Total Petroleum Hydrocarbon (TPH) present on the spillage site. In addition, this fungus also caused a significant ($P<0.05$) increase in the level of some soil macronutrients in the polluted soil.

(Keywords: mycoremediation, *Aspergillus sp*., oil pollution, total petroleum hydrocarbon (TPH), *Irvingia gabonensis*, soil macronutrients)

INTRODUCTION

The petroleum industry is one of the most powerful machines driving our modern world. In spite of the colossal blessings from the petroleum industry, there are various negative impacts on the human environment, some of which include hydrocarbon contamination of the soil, air, surface and underground aquifers.

In their respective opinions, Hallier-Soulier et al. (1999), Margesin and Schinner (2000), and Adekunle and Adebambo (2007) all noted that the dominance of petroleum products in the world economy has created a platform for the distribution of a large amount of toxins into populated areas and ecosystems around the globe.

On the terrestrial environment, oil spills cause extensive damage ranging from the destruction of terrestrial flora and fauna to biomagnifications of the toxic components of the petroleum, conversion of arable land to barren soils and the destruction of the aesthetic quality of the environment. Other environmental consequences of oil pollution include the adverse effects on the soil microflora and microfauna and ground water pollution (Obire and Anyanwu, 2009). Some hydrocarbon components have been known to belong to a family of carcinogenic and neurotoxic organopollutants (Hallier-Soulier et al., 1999).

The processes leading to the eventual removal of hydrocarbon pollutants from the environment has been extensively documented and involves the trio of physical, chemical and biological alternatives. According to Adekunle and Uma (1996), Adekunle and Oluyode (2005) and Adekunle and Adebambo (2007), pathogenic fungi isolated from melon- *Cucumeropsis mannii* (Naud), soybean- *Glycine max* (L.) Merr. and *Detarium senegalense* (G.F. Gmel.) seeds respectively were found to contain some enzymes (lipase enzymes) that possess the ability to degrade hydrocarbons compounds present in these oil seeds.

From the foregoing, Adekunle and Adebambo (2007) therefore pointed out that the most rational way of decontaminating an environment polluted with petroleum hydrocarbon derivatives is the application of methods that is based mainly on the metabolic activity of microorganisms-biodegradation, especially since these microorganisms possess the innate ability to degrade hydrocarbons present in the oils

contained in the seeds on which they are pathogenic.

Biodegradation is the use of naturally occurring microorganisms or genetically-engineered microorganisms (bacteria and fungi) by man, to detoxify man-made pollutants (Okoh, 2006). Its potential contribution as a counter measure biotechnology for decontamination of oil polluted systems could be enormous. The involvement of microorganisms in the degradation of petroleum hydrocarbons in the environment has been established as an economic, efficient, versatile, and environment friendly treatment method (Margesin and Schinner, 2000; Yakubu, 2007; George-Okafor et al., 2009; Agarry et al., 2010).

Microorganisms, particularly fungi, represent a promising, largely untapped resource for environmental biotechnologies. The ease of transportation, genetic engineering, and scaling-up makes fungi the organisms of choice in bioremediation. Fungi are particularly good at digesting complex organic compounds that are normally not degraded by other organisms (Zeyaullah et al., 2009). According to Covino et al. (2010), an important characteristic, which distinguishes filamentous fungi from bacteria and makes them an excellent candidate for bioremediation strategies is the penetration of the fungal hyphae into the polluted matrix and the excretion of oxidative enzymes. These oxidases, mainly laccase, lignin peroxidase and manganese peroxidase present very low substrate specificity and, being active in the extracellular environment, are able to attack scarcely bioavailable contaminants. Fungi can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacteria growth might be limited (Davis and Westlake, 1979).

In the Nigerian context, there is an urgent need to test fungi that are indigenous to our specific environment in bioremediation for oil pollution in our own environment. It should be noted that in Nigeria, no information is available regarding the commercial production of indigenous fungi inocula for use in bioremediation of oil polluted environments. This effort therefore may probably represent the necessary step in this regard. Besides, there is the need for putting to a constructive use (such as in cleaning up oil pollution) some fungal species that have been implicated in the pathogenicity of a useful oilseed such as *I. gabonensis*, thereby providing a platform for reversing some losses in our national economy due to the activities of these pathogenic fungal species.

Studies have shown that bioremediation agents may be effective in the laboratory but significantly less so in the field (Lee et al., 2002; Mearns, 1997; Venosa et al., 1996 and 2002). Therefore, field studies (which is generally regarded as the most convincing demonstration of the effectiveness of any bioremediation agent), was used in this research.

Aims and Objectives of Study

In view of the foregoing, the aim of this study was to investigate if the fungal species associated with diseased conditions in the *I. gabonensis* oilseeds (which were suspected to be capable of utilizing the vegetable hydrocarbons present in the *I. gabonensis* oilseeds) are capable of degrading hydrocarbons of petroleum origin outside of the host tissue- on the field, under a real life situation. The specific objectives of this research are therefore to evaluate the effectiveness of one of the fungal species (associated with the post- harvest deterioration of *I. gabonensis* seed) at remediating a petroleum hydrocarbon polluted field under real life conditions.

MATERIALS AND METHODS

Collection of Samples

Diseased seeds of *I. gabonensis* from 4 sources were sampled for fungi over a 30 month period (once in 2 months). All the sampling sites were in Lagos metropolis, Lagos State, Nigeria.

Isolation of Fungi from *I. gabonensis* Seed

The modified method of Adekunle and Oluyode (2005) was employed. Diseased seeds of *I. gabonensis* (from each of the sites) that had been cut into discs were surface sterilized by leaving them in a solution of common bleach (sodium hypochloride - NaOCl) and sterile distilled water in a ratio of 3:2 for one minute, then rinsed in three changes of sterile distilled water, and were thereafter placed (using sterile forceps) into the already prepared and solidified plates of sterile Potato Dextrose Agar (PDA).

This was thereafter incubated at room temperature (30^oC) in the Uniscope SM9082 (serial number L-809693) incubator. Inside the incubator, the plates were observed daily for fungal growth. To obtain a pure culture, resultant fungal cultures were repeatedly sub cultured into fresh sterile PDA plates until each plate contains only one type of fungal isolate.

Identification of Fungi

The first step in the identification of the fungal species was the morphological studies which are hinged on observing the shape, color, size and texture of fungal species in plates and time taken for each of the fungus to reach the maximum diameter (9cm) in plates. After this, slides of each isolate was prepared by teasing out a little portion of the growth in the plate on to the slide, this is then teased out and stained with lactophenol in cotton-blue, and observed under a compound light microscope. The photomicrographs were then compared with the descriptions given by Talbot (1971); Deacon (1980); Domoschet et al. (1980); and Bryce (1992) for identification.

The final confirmation of the identities of the key fungal species, and a comparison between these key isolates was done using molecular techniques such as PCR, electrophoresis gel analysis and DNA sequencing at the Biotechnology Centre, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria and Macrogene Inc., USA respectively.

Hydrocarbon Utilization Studies

A preliminary test for petroleum hydrocarbon utilizing ability of the fungal isolates via the growth test under a petroleum hydrocarbon source- crude oil fume was carried out in the laboratory using the modified method of Amund et al. (1987) as described by Adekunle and Oluyode (2005).

Sixty filter papers (Whatman No. 1001125) that had been previously sterilized were dipped in 400 ml of crude oil contained in 1000 ml beaker for about 25 seconds using sterile forceps. Using the same forceps, these filter papers were allowed to drain for another 60 seconds. Each of the crude oil treated paper was then placed on the cover of petri dishes containing freshly prepared solidified sterile PDA. This PDA was thereafter inoculated with fungal spores (from a pure plate) that were previously isolated from the diseased cotyledons of *I. gabonensis*. All this process was done under a sterile condition. All plates were incubated at room temperature and they were observed regularly for fungal growth.

This was carried out in order to determine the best candidates out of the many isolates, that posses the most ability to degrade petroleum hydrocarbon.

Field Trial of Mycoremediation Experiment

The next stage of this experiment was to take one of the promising fungal isolates from the preceding preliminary hydrocarbon utilization studies (done in the laboratory) for an actual field trial.

Following a modified approach of Egunjobi and Onweluzo (1979); Amund et al. (1993); and Egberongbe et al. (2010), a single experimental plot of land measuring $80.0m^2$ was mapped out. Some portion of this plot was deliberately polluted with spent engine oil (SEO) at the rate of 2 L/m^2, and/or fungal spores in different combinations called Treatments.

For some of the Treatments, weeding was done while for others weeding was not done. The Treatment was applied only once during the entire duration of the experiment. The fungal spores and SEO were applied to the soil at the same time using different pre-sterilized stainless steel, fine nozzle watering cans. The SEO spilled on the Treatment cells was not worked into the soil; the essence of which is to provide a scenario as close to real life as possible. Each of these Treatments were replicated 5 times. The eight Treatments as outlined below were assigned to this plot:

Treatment 1 (T_1) = not weeded (natural vegetation) with nothing.

Treatment 2 (T_2) = weeded with nothing.

Treatment 3 (T_3) = weeded + SEO + *A. oryzae*.

Treatment 4 (T_4) = not weeded + SEO + *A. oryzae*.

Treatment 5 (T_5) = weeded + SEO – *A. oryzae*.

Treatment 6 (T_6) = not weeded + SEO – *A. oryzae*.

Treatment 7 (T_7) = weeded – SEO + *A. oryzae*.

Treatment 8 (T_8) = not weeded – SEO + *A. oryzae*.

It is important to note that that the 8 Treatments above can be grouped into 4 pairs (with one of the pair acting as a control for the other Treatment in the pair) thus:

Pair 1: T_1 AND T_2 general control pair (where T_1 is acting as a control for T_4, T_6 and T_8 and as a baseline for all the other Treatments and as well as for T_2).

Pair 2: T_3 and T_5 (This is the only Treatment pair that is verifying in an exclusive manner (i.e. without the encumbrances of vegetation cover), the ability of *A. oryzae* to mycoremediate a petroleum hydrocarbon polluted field. Here T_5 is acting as a control to T_3).

Pair 3: T_4 and T_6 (this Treatment pair seeks to verify how efficient *A. oryzae* can mycoremediate a petroleum hydrocarbon polluted field that has some vegetation cover. Here, T_6 is acting as control to T_4).

Pair 4: T_7 and T_8 (this Treatment pair seeks to verify how *A. oryzae* can affect a terrestrial environment (with and without vegetation cover) in the absence of petroleum hydrocarbon pollution. This Treatment pair in addition further seeks to address the ethical considerations that are involved in introducing microorganisms from one environment to another.

Using the Randomized Complete Block Design (RCBD), the plot was divided into 5 blocks called Block 1 (B_1), Block 2 (B_2)….. Block 5 (B_5). Within each of the blocks, the eight Treatments were randomly assigned using the random number table as shown in Figure1.

On the field, data were taken on the following parameters:

(1) Soil nutrient namely total nitrogen (%N), available phosphorus (mg/kg), potassium (meq/100g) and magnesium (meq/100g) were all analyzed for in each Treatment. Data on the effect of *A. oryzae* as an agent to mycoremediate a SEO polluted soil in this experiment was collected 3 times thus: at baseline point, at the 3rd month and the 6th month after the application of Treatments. Soil for each Treatment was taken from each block, and this was thereafter bulked into one composite sample. Each composite sample was analyzed in 3 replicates, thus giving us the mean concentration of element in the soil for each Treatment.

(2) Amount of Total Petroleum Hydrocarbon (TPH) in the soil (using GC method) both as a baseline data (day 1) and at 6 months after the application of Treatment were determined for each Treatment. As above, sample for each Treatment was taken per block. This was thereafter bulked into one composite sample before being analyzed. The values of TPH in the soils of the different Treatments were obtained after triplicate analysis from electronic integration measurements using flame ionization detector.

Block 5	Block 4	Block 3	Block 2	Block 1
T_1	T_3	T_7	T_8	T_6
T_7	T_8	T_4	T_1	T_3
T_4	T_6	T_8	T_6	T_7
T_3	T_5	T_2	T_3	T_4
T_8	T_7	T_6	T_2	T_5
T_2	T_4	T_3	T_5	T_1
T_5	T_2	T_1	T_7	T_8
T_6	T_1	T_5	T_4	T_2

Figure 1: Visual Presentation of Field Layout.

Soil Nutrient Analyses (Digestion of Soil Samples)

A total of 5.0 g of the soil sample was weighed into the pre cleaned borosilicate 250 ml capacity beaker for digestion. Thereafter, 30 ml of the mixture of hydrochloric acid and nitric acid in the ratio 3:1 was added into the weighed sample in the beaker. The sample with the digesting solution was placed on the hot plate digestion in the fume cupboard. The beaker and its content after the digestion were allowed to cool. Another 20 ml of the digesting solution was added and digested further in the fume cupboard, and the mixture was allowed to cool to the room

temperature. The mixture was filtered into 250 ml volumetric capacity borosilicate container. The filtrate was made up to the mark with de-ionized water. All the digested samples were sub-sampled into pre-cleaned borosilicate glass containers for Atomic Absorption Spectrophotometer (AAS) analysis. In all cases, data analyzed and presented were the mean values from three replicates. The SAS statistical software was used in the analysis of the data, and the means were separated using the Duncan's multiple range test.

(i) Magnesium (Mg)

Standards of Magnesium solution of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/l were made from metal solution of 1000 mg/l stock solution of the analyte. The set of standard solutions and the filtrate of the digested samples were read by an AAS. The detection limit of the metal in the sample was 0.0001 mg/l. The model of the AAS is UNICAM 929 London, powered by SOLAAR software. Magnesium cathode lamp was used for the analysis of the heavy metal ion in the standards and the filtrate of the samples. Gas mixtures were used in the generation of the flame.

(ii) Potassium (K)

All the digested samples from the AAS analysis were sub-sampled into pre-cleaned borosilicate glass containers for the for Flame Photometric analysis of potassium in soil samples. Standards of potassium solutions of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l were made from the metal solution of 1000 mg/l stock solutions of the analyte. The set of standard solutions and the filtrate of the digested samples were analyzed by Flame Photometer. The model of the Flame Photometer is JENWAY PFP 7. Gas mixture of air and LPG was used in the generation of the flame, while the detection limit of the metal in the sample was 0.0001 mg/l.

(iii) Nitrogen Forms (Nitrate and Total Nitrogen)

Measurement of nitrogen in the soil samples was carried out as total nitrogen. The analyses of the various forms relied on the chemical reaction of the samples with the catalyzing agents. The extract now reacted with color forming agents (for example sulfanic acid in nitrate analysis) to form specifically colored complexes and/or compounds. Measurement of the concentrations of the nitrogen form is then made spectrophotometrically at specific wavelengths against standard concentration curves.

(iv) Phosphorus forms (phosphate and total phosphorus)

Soil samples were analyzed for forms of phosphorus as phosphate. Phosphorus analysis APHA 4500-PE (STD Methods, 19th ed.) consists of two general procedural steps:

(a) conversion of the phosphorus form of interest to dissolved orthophosphate, and

(b) spectrophotometric determination of dissolved orthophosphate.

The primary forms determined in this analysis were reactive phosphorus and total phosphorus. Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the samples are termed "reactive phosphorus". While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually was hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms. Total phosphorus (orthophosphate, condensed, and organically bound) was determined by acid oxidation with persulfate, followed by the reactive phosphorus test. Organically bound phosphate was then determined by subtracting the acid-hydrolyzable phosphorus.

ANALYSIS OF DATA

Data generated were analyzed for statistical significance at 95% confidence interval and means were separated using the Duncan's Multiple Range Test (DMRT) using the electronic statistical software package SAS.

RESULTS AND DISCUSSION

Preliminary Screening of Fungal Isolates for Petroleum Hydrocarbon Utilizing Ability

The results for the fungal isolates from the diseased seeds of *I. gabonensis* that 'passed' the preliminary hydrocarbon utilizing test and their

sources are as summarized in Table 1. These isolates were *A. niger* and *A. oryzae* from each of the different location (sources).

Confirmation Studies for the Effectiveness of *A. oryzae* as a Mycoremediation agent on a Petroleum Hydrocarbon Polluted Soil

The summary of the Gas Chromatographic (GC) reading for the Total Petroleum Hydrocarbon (TPH) in the soil at the initial point (on application of Treatments) and at the final point (6 months after the application of Treatments) are shown in Table 2.

The results from Table 2 show a very high initial level of TPH for all the Treatment soils that were polluted with SEO irrespective of the presence or the absence of *A. oryzae* and or vegetation cover (T_3, T_4, T_5 and T_6) when compared to those Treatment soils that were not polluted (T_1, T_2, T_7, and T_8). However, those Treatments that had the mycoremediation agent- *A. oryzae* - applied to the SEO pollution (T_3 and T_4) showed the most remarkable rate of reduction of 99.1% and 98.9%, respectively, in their final TPH levels as compared to those Treatments that had only SEO pollution and no *A. oryzae* (T_5 and T_6) which on their own had a 92.1% and 93.0% reduction in the TPH left in the soil 6 months after the application of the Treatments.

A further comparison of the amount of TPH that was removed from the soil after 6 months, using Treatments 3 and 4 as a comparison to some other Treatments (i.e., T_1, T_2, T_7 and T_8 as seen from Table 2) further shows the level of efficiency of *A. oryzae* at remediating a petroleum hydrocarbon polluted soil as the percentage removal of TPH from the soils that had neither *A. oryzae* nor petroleum hydrocarbon contamination (T_1 and T_2) ranged from between 2.7% and 6.3%, respectively, when compared to the between over 99% and 98% reduction in the TPH achieved for the soils that had *A. oryzae* as a mycoremediation agent in the oil pollution (T_3 and T_4).

Furthermore, results from Table 2 show that the presence of vegetation in T_4 and T_6 (as of the time when pollution occurred) compared to the absence of vegetation as of the same time (T_3 and T_5) caused a slight reduction in the amount of SEO (hence the amount of TPH) that was able to get to the soil, and that remained in the soil 6 months after

Table 1: Fungal Isolates Suspected to be Capable of Utilizing Petroleum Hydrocarbon, their location, and Sources.

s/n	Location	Source	Identity	Accession number
1	Bariga market	*I. gabonensis.*	*A. niger**	Na
2	Oyingbo market.	"	*A. niger.**	Na
3	"	"	*A. oryzae****	JN561274.1++
4	Alayabiagba market, Ajegunle.	"	*A. oryzae****	JQ675305.1++
5	"	"	*A. niger**	Na
6	Agege market	"	*A. niger**	Na

Key:
s/n Serial number
*** Identity confirmed using DNA sequence
* Identity not yet confirmed by DNA sequence
na: Not available
++: Accession number of the fungus in the present study.

Table 2: Total Petroleum Hydrocarbon (TPH) level in the soil at months 0 (initial) and 6 (final)

sample ID	Initial TPH in the soil (mg/kg)	Final TPH in soil(mg/kg)	Percentage TPH removed from the soil at 6 months
T_1	19,867.13*	18, 618.09	6.287
T_2	19,955.20	19, 402.44	2.770
T_3	2, 013,070	18, 156.87	99.100
T_4	1,749,360	18,909.03	98.919
T_5	1,820,700	120,748.59	92.983
T_6	1,756,400	122,759.95	93.011
T_7	20,131.38	19,444.88	3.410
T_8	20,129.00	19,585.54	2.700

*The values for TPH were the means of triplicate analysis from electronic integration measurements using flame ionization detector
Key:
TM Treatment
BL Baseline data
T_1 (not weeded not polluted and no *A. oryzae* added)
T_2 (weeded, not polluted and no *A. oryzae* added)
T_3 (weeded, polluted and *A. oryzae* added)
T_4 (not weeded, polluted and A. oryzae added)
T_5 (weeded, polluted, no *A. oryzae* added)
T_6 (not weeded, polluted, no *A. oryzae* added)
T_7 (weeded, no pollution, *A. oryzae* added)
T_8 (not weeded, no pollution, *A. oryzae* added)

EFFECT OF THE MYCOREMEMDIATION AGENT ON THE NUTRIENT STATUS OF THE SOIL

(i) Total Nitrogen (%)

The results in Tables 3 show the effect of the Treatments on the amount of total nitrogen (N) in the soil. This result further show that the spillage of SEO (irrespective of any of the other factors such as the presence or the absence of *A. oryzae* and or weed) caused a significant reduction in the total N in the soil at the baseline point (Table 3 Treatments 3, 4, 5 and 6). It can also be seen from Table 3 that the addition of *A. oryzae* (irrespective of the absence or the presence of vegetation- T_3 and T_4, respectively) to the SEO polluted field by the 2nd and 3rd sampling (at the 3rd and 6th months, respectively) caused a significant increase in the amount of total N in the soil from 0.07 to 0.11 and 0.12% at baseline point, month 2 and month 6 respectively for Treatment 3. These values obtained for T_3 especially at the 6th month was significantly higher than the values obtained from the 2 control Treatments (T_1 and T_2), and indeed all the other Treatments at this time. Comparing T_3 and T_4 at the 2nd sampling, it can be seen that the

presence of vegetation seem to have caused a reduction in the ability of *A. oryzae* to increase the amount of total N in the SEO polluted soil. This is because the amount of total N in T_4 was significantly lower than in T_3 (Table 3). By 3rd sampling at the 6th month however, this trend had been reversed (Table 3), as both soils that had *A. oryzae* added as a mycoremediation agent to the SEO polluted soil had a total N content that was significantly higher than all the other Treatments. Comparing T_3 with T_1, it is also evident from Table 3 that by the 3rd month after the application of the Treatment, *A. oryzae* had effectively restored the N in the soil to a normal level comparable to the control, and by the 6th month after the application of the Treatments, *A. oryzae* had left the soil better off than the control soils in terms of its N content.

(ii) Available Phosphorus (mg/kg)

The results in Table 3 (baseline values for T_3, T_4, T_5 and T_6) also show that SEO pollution (irrespective of the presence or absence of the *A. oryzae* and or vegetation cover) in the soil caused an initial significant reduction in the level of available phosphorus. The addition of *A. oryzae* as a mycoremediation agent however by the 3rd month caused a significant increase in the level of available phosphorus (Table 4, T_3 and T_4) over and above the control plots (Table 4, T_3 compared with T_1 and T_2), a trend which continued until the 6th month. In general terms however, it appears that the presence of vegetation cover on the Treatment plots significantly reduced the efficiency of this mycoremediation agent (*A. oryzae*) at increasing the amount of available phosphorus in the soil both at the 3rd and 6th months after the application of the Treatments (Table 4, comparison between T_3 and T_4).

(iii) Available Potassium (meq/100g)

The results from this research also show that SEO pollution caused a significant reduction in the level of potassium in the soil at the baseline point (Table 3, baseline values for T_3, T_4, T_5 and T_6 compared with T_1, T_2, T_7 and T_8). The addition of *A. oryzae* to a SEO polluted soil (with or without a vegetation cover) clearly caused a significant increase in the level of available potassium in the soil by the 3rd and 6th months (Table 3, T_3 and T_4 compared with T_1, T_2, T_7 and T_8).

(iv) Available Magnesium (meq/100g)

Results from this research also showed that the pollution of a soil with SEO (irrespective of the presence or the absence of a mycoremediation agent and or vegetation cover) caused a significant reduction in the amount of soil magnesium at the baseline point (Table 3, baseline values for T_3, T_4, T_5 and T_6 compared with T_1, T_2, T_7 and T_8). The effect of the addition of *A. oryzae* (on available soil magnesium) as a mycoremediation agent to the SEO polluted soil however shows clearly that by the 3rd and the 6th months after the application of Treatments, this fungus had caused a significant increase in the level of magnesium in the soil (Table 3, T_3 compared with T_1 and T_2). The presence of vegetation cover in a SEO polluted soil however appears to have caused a significant reduction in the ability of *A. oryzae* to increase the level of magnesium in the soil (Table 3, T_4 compared with T_3).

Discussion

With respect to the petroleum hydrocarbon utilizing ability of fungal species, the results from this work show that the *A. oryzae* associated with pathogenic conditions in the seed of *I. gabonensis,* is capable of degrading petroleum hydrocarbon. This finding is in agreement with many previous reports, where filamentous fungi in particular were severally reported to have degraded a whole array of hydrocarbon containing compounds (Zobell, 1946; Adekunle and Oluyode, 2005; Saratale *et al.,* 2007, George-Okafor *et al*., 2009) by producing capable enzymes. On account of their aggressive growth, greater biomass production and extensive hyphal growth in soil, fungi offer potential for mycoremediation technology (Kenneth, 1995; Saadoun 2002). It is of interest that a strain of *A. niger,* i.e. *A. oryzae,* from the results obtained from this work is being shown probably for the first time in any report to have the ability to utilize petroleum hydrocarbon compound and to be capable of efficiently mycoremediating a spent engine oil polluted soil.

Table 3: Effect of the Treatments on Total Nitrogen, Phosphorus, Potassium, and Magnesium in the Soil.

TM	Nitrogen (%)			Phosphorus (mg/kg)			Potassium (meq/100g)			Magnesium (meq/100g)		
	BL	3rd month	6th month	BL	3rd month	6th month	BL	3rd month	6th month	BL	3rd month	6th month
T_1	0.11^{a}	0.11^{a}	0.10^{bc}	16.93^{a}	13.00^{bc}	11.87^{a}	3.13^{a}	3.15^{a}	2.92^{c}	1.58^{a}	1.07^{b}	1.03^{a}
T_2	0.11^{a}	0.11^{a}	0.11^{b}	15.85^{b}	14.73^{a}	12.45^{a}	3.11^{a}	2.99^{cd}	2.72^{f}	1.53^{a}	1.07^{b}	1.02^{b}
T_3	0.07^{c}	0.11^{a}	0.12^{a}	1.23^{e}	14.75^{a}	11.94^{a}	0.81^{e}	3.14^{a}	2.85^{e}	0.55^{c}	1.07^{b}	1.03^{a}
T_4	0.07^{c}	0.10^{b}	0.12^{a}	1.16^{f}	13.89^{b}	10.84^{bc}	0.86^{d}	3.17^{a}	2.95^{b}	0.47^{d}	1.05^{c}	1.01^{bc}
T_5	0.07^{c}	0.10^{bc}	0.11^{b}	1.13^{g}	12.48^{c}	10.04^{c}	0.74^{f}	2.99^{cd}	2.69^{g}	0.45^{d}	0.93^{f}	0.79^{f}
T_6	0.07^{c}	0.10^{bc}	0.10^{bc}	1.12^{h}	14.11^{b}	11.76^{ab}	0.70^{g}	3.11^{ab}	2.99^{a}	0.45^{d}	1.13^{a}	1.00^{d}
T_7	0.10^{b}	0.09^{d}	0.11^{b}	14.04^{d}	12.42^{c}	10.62^{bc}	2.96^{c}	2.95^{d}	2.56^{h}	1.11^{b}	0.97^{e}	0.72^{g}
T_8	0.11^{a}	0.10^{b}	0.10^{bc}	15.42^{c}	13.16^{bc}	10.04^{c}	3.01^{b}	3.05^{bc}	2.86^{d}	1.08^{b}	1.03^{d}	0.96^{e}

Mean values for the same parameter along the same column carrying different superscripts are significantly ($P<0.05$) different
Key
TM: Treatment
BL: Baseline data
T_1: (not weeded not polluted and no *A.oryzae* added)
T_2: (weeded, not polluted and no *A.oryzae* added)
T_3: (weeded, polluted and *A.oryzae* added)
T_4: (not weeded, polluted and A.oryzae added)
T_5: (weeded, polluted, no *A.oryzae* added)
T_6: (not weeded, polluted, no *A.oryzae* added)
T_7: (weeded, no pollution,*A.oryzae* added)
T_8: (not weeded, no pollution, *A.oryzae* added)

A report by Ekundayo and Obire (1987) have shown that a marked change in properties occur in soils polluted with petroleum hydrocarbons, thus affecting the physical, chemical, biological and microbiological properties of the soil. According to Vwioto *et al*. (2006) oil pollution of soil leads to build up of essential (organic carbon, phosphorus, calcium, magnesium) and non-essential (magnesium, zinc, iron, cobalt, copper) elements in soil and their eventual translocation in plant tissues. This submission probably explains the trend seen in the results obtained in this work where after the baseline, there was a general increase in the level of all the elements that were tested for in the soils that were polluted with SEO over and above the control soils that were not polluted with SEO. Furthermore, between the soils polluted with SEO, those Treatments that had *A. oryzae* added to the SEO pollution generally showed a higher nutrient level than those Treatments that did not have *A. oryzae* added to their SEO pollution. These results seem to be highlighting the role of fungi in the soil mineralization process as reported by Covino *et al*. (2010).

With respect to the TPH residue and the nutrient level in the soil, occasionally, T_6 produced some results comparable to what was obtained for T_3 and T_4, and even better than what was obtained

for T_3 and T_4. The reason for this observation may probably be as a result of the activities of some microorganisms that are found associated with the rhizosphere of some of the weed species that were present on these Treatment plots. Microorganisms associated with plant rhizosphere have been severally reported in literature as contributing significantly to the degradation of petroleum hydrocarbon and other pollutants in the soil (Wang *et al*., 2011; Hrynkiewicz and Baum, 2012).

The observed increase in the level of some soil macro nutrients that was found in this work following the degradation of the SEO (as from 2 months after the application of the treatments) corroborates the position of earlier workers such as Odu (1979), Udo and Fayemi (1975). These workers all reported an increase in the organic matter content, total carbon and nitrogen in petroleum contaminated soils when compared with normal soils. Udo and Fayemi (1975) also reported increases in carbon: nitrogen ratio (C:N ratio) in oil contaminated soils. Adams and Ellis (1960) reported increases in the phosphorus content of oil contaminated soil, a position which agrees with the findings in this work. The general increases that were observed in the level of the investigated soil macronutrients especially on the SEO polluted plots that had *A. oryzae* added might be attributable to the large changes in the redox (oxidation and reduction) potential of oil contaminated soils as was first reported by Adams and Ellis (1960).

Another piece of evidence showing the ability of *A. oryzae* to mycoremediate a petroleum hydrocarbon polluted environment is a comparison of the results for the initial and final TPH levels in the soil between Treatments 3 and 5, and Treatments 4 and 6. To the exclusion of any other factor, the extent of reduction achieved in the final level of TPH in the soil can be adduced directly to the action of the *A. oryzae* that was added to the SEO polluted soil. This result agrees with the findings of Thangarajan *et al.* (2011) where the reduction in the TPH level as a result of bioaugementation of a hydrocarbon polluted soil with the fungus *Scedosporium apiospermum* consistently produced a lower TPH value from day 0 – day 35 than the naturally attenuated soils.

In addition, the action of *A. oryzae* in this work appears to further reinforce the belief in the role of fungi in the soil humification process (Covino *et al*., 2010). Furthermore, it appears that in this studies, the presence of vegetation appears to have caused a reduction in the efficiency of this fungus to mycoremediate the SEO pollution.

CONCLUSION

This work is most likely a first report at evaluating the efficiency of a pathogenic fungal species from *I. gabonensis* seed at remediating a petroleum hydrocarbon polluted field. Furthermore, the findings from this mycoremediation field trial of *A. oryzae* (isolated from the diseased seeds of *I. gabonensis*) showed that as a result of the addition of this *A. oryzae* to the SEO polluted soil, GC analysis of the TPH showed that there was a significant reduction in the TPH level in the soil 6 months after the application of the Treatment. In addition, mycoremediating a SEO polluted soil with this *A.oryzae* also resulted in a significant increase in the level of the following macronutrients N, P, K and Mg from the 3rd month up to the 6th month into the mycoremediation studies.

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