Universal Journal of Environmental Research and Technology
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Available Online at: **www.environmentaljournal.org**
Volume 3, Issue 2: 300-310

Open Access **Research Article**

Influence of Soil Particle Size and Arbuscular Mycorrhizal Fungi (AMF) in the Performance of *Phaseolus vulgaris* Grown under Crude Oil Contaminated Soil

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Abstract:

Biotic processes represent the major route responsible for the ecological recovery of hydrocarbon contaminated sites. An experiment was conducted to assess the influence of soil textural class and arbuscular mycorrhizal (AM) fungi in the performance of *Phaseolus vulgaris* under crude oil contaminated soil. Three soils of different textural class viz: clay loam soil (clay 52%), sandy clay soil (clay 30%), sandy loamy soil (clay 8%) were used to grow *P. vulgaris* under 2%, 4% and 8% (v/w) crude oil contamination. The experimental units were biostimulated with 2 g NPK fertilizer pot^{-1} and were inoculated with 12 g AM inoculum pot^{-1}. Non inoculated pots served as control. The results obtained showed that AM inoculated pots recorded higher and significantly ($P<0.05$) different dry matter yields and chlorophyll content than non AM inoculated pots. Residual total petroleum hydrocarbon (TPH) increased as percent crude oil contamination increased at each soil textural class. Total petroleum hydrocarbon decomposition and removal was highest at 52% clay textural class (2.57mg/g) and significantly differed from 30% clay (3.26 mg/g) and 8% (4.26 mg/g). With AM colonization, physiological characteristics of *P.vulgaris* and TPH decomposition improved. This is evinced by the linear regression analysis between colonization and TPH (R^2=0.77).

Keywords: Arbuscular mycorrhiza, crude oil decomposition, phytoremediation and soil textural class.

1.0 Introduction:

Crude oil contamination of agricultural soil often put severe stress to soil health and productivity. Record shows that crude oil spillage on arable land has been on the increase since the 20th century when global production doubled (Onosode, 2003). In the Niger delta region of Nigeria alone, about 1.8 million barrels of crude oil have been lost to the environment from 1976 to 1996 (DPR, 1999). Soil, under this circumstance, is much constrained to deliver the ecosystem goods and services. These constraints include soil moisture stress, low nutrient capital, erosion risks, low pH with aluminium (Al) toxicity, high phosphorus (P) fixation, low levels of soil organic matter, reduced soil aeration, poor soil permeability, bulk density and loss of soil biodiversity. The challenge for the next 50 years is to double food production in a more sustainable approach that will ensure public health and safety.

Bioremediation is a low cost approach towards soil restoration. It involves the use of natural processes to contain, reduce and degrade contaminants. In this case, the contaminants are left on site and the naturally occurring processes are left to clean up the site. The natural processes include biological degradation, volatilization, dispersion, dilution, radioactive decay, and sorption of the contaminant onto the organic matter and clay minerals in the soil. Various soil characteristics are essential to achieve comprehensive bioremediation of contaminated soil. Soil physical, chemical and biological properties are important in developing a biodegradation potential for contaminated soil (Rogers et al., 1993). For instance, the soil pH should be adjusted within the range 6–8 to enhance microbial activity (Hicks and Caplan, 1993, Gogoi et al., 2003). The levels of nitrogen and phosphorus in the soil may also be very critical as these may limit the biodegradation rates because of an interactive process occurring between the nutrients (Walworth and Reynolds, 1995). Major soil physical characteristics that may influence the

bioremediation process are porosity, bulk density and air permeability. The permeability determines the rate of transfer of electron acceptors to the contaminated soil. It is believed that the reduction of permeability because of microbial biofilms in the soil macrovoids, as well as in the smaller pores of the soil matrix (microvoids). The soil particle size distribution defines the air permeability and total soil porosity.

The soil pore size distribution is one factor determining the microbial habitat since it is assumed that microorganisms mainly live in pores of a certain size. Considering the size of bacterial cells, pores of a neck diameter of 2-6 mm are favourable microhabitats for soil bacteria (Hattori and Hattori, 1976). Hassink (1992) also found a good correlation between the habitable pore size fraction and N mineralization. Killham *et al*. (1993) showed that substrate utilization by microbes in soil was strongly affected by its location, both in terms of pore size and the matric water potential under which turnover takes place. It should, however, be noted that only a very small fraction of organic material in soil is likely to be at close proximity to soil organisms at any one time (Adu and Oades, 1978).Microbial degradation represents the major route responsible for the ecological recovery of hydrocarbon contaminated sites (Boonchan et al., 2000). Biologically and biochemically mediated processes in soils are of the utmost importance to ecosystem function. Soil microbes are the driving force behind many soil processes including the transformation of organic matter, nutrient release and degradation of petroleum hydrocarbons (Zabaloy et al., 2008). Soil enzymes can be used as potential soil quality indicators for sustainable management because they are sensitive to ecological stress and land management practices (Nwoko and Ogunyemi, 2010; Tejada, 2009). Enzymes may react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes (Kucharski et al., 2000; Andreoni et al., 2004; Zabaloy et al., 2008).

The fungi that are probably most abundant in agricultural soils are arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota). They account for 5–50% of the biomass of soil microbes (Olsson et al., 1999). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10–20 (Rillig et al., 2001). The external mycelium attains as much as 3% of root weight (Jakobsen and Rosendahl, 1990). Mycorrhizal fungi contribute to soil structure by (1) growth of external hyphae into the soil to create a skeletal structure that holds soil particles together; (2) creation by external hyphae of conditions that are conducive for the formation of micro-aggregates; (3) enmeshment of microaggregates by external hyphae and roots to form macroaggregates; and (4) directly tapping carbon resources of the plant to the soils (Miller and Jastrow, 2000). This direct access will influence the formation of soil aggregates, because soil carbon is crucial to form organic materials necessary to cement soil particles. Arbuscular Mycorrhiza is an important microflora in the rhizosphere of plants and thus improve overall microbial activity in the root zone. Gao et al., (2011) observed that optimized microbiota in mycorrhizal association was responsible for PAH degradation in AM Phytoremediation. Wu et al., (2011) suggested that the hyphae and extraradical mycelium of AM fungi could play important roles in the uptake and translocation of phenanthrene (PHE) and pyrene (PYR) in plants. This present research examined the influence of soil particle size distribution and arbuscular mycorrhizal fungi in the remediation of crude oil contaminated soil under African bean (*Phaseolus vulgaris)* grown pot experiment.

1.0 Materials and Methods:

2.1 Soil sampling:

The soil samples were randomly collected from agricultural top soil to represent three soil textural groups i.e. A: Clay loam soil (clay 52 %) and B: Sandy clay loamy soil (clay 30 %), C: Sandy loamy soil (Clay 8%). The idea is to create a range of textural class resulting in different soil pore size distribution (Table 1.0). Percentage porosity was determined by compacting each of the soil sample into a mould of volume (Vt=100cm^3). The mass of the sample, Mt, determined. The sample was then dried in an oven at 115^{o}C and then re-weighed to give the mass of solid Ms. The mass of water, Mw, volume of solid, Vs and volume of voids, Vv, were all calculated. From these, porosity values were then calculated from the formular (Brandom, 1986). $Porosity\ (\%) = \frac{Vv}{Vt}\ x\ 100$, where Vv=Vt-Vs and $Vs = \frac{Ms}{GsIw}$ Gs specific gravity of solids=2.66 and density of water, Iw=1g/cm^3. Sampling was done from 0 – 15 cm depth and bagged separately in plastic pots (diameter;13cm, Height 6cm) after drying and removal of the surface litter to make up to 9kg soil.

The bulk densities of the three soil textural class A, B and C are 1.55 $g^{-1}cm^3$, 1.49 $g^{-1}cm^3$ and 1.48 $^{-1}cm^3$, respectively. Other chemical characteristics of the soil were determined using standard methods (Table 1).

2.2 Soil microcosm experiment:

Each of the textural class was spiked with 2%, 4% and 8% (v/wt) of crude oil (*Nigerian bonny light*) and inoculated with 12 g of mycorrhizal inoculum (16 spores g^{-1} *Glomus mosseae*), in addition to soil resident microbes and was thoroughly mixed. Non inoculated pots were steam sterilized at 121°C for 2 h using the autoclave and this served as control. Four Seeds of African bean (*Phaseolus vulgaris* L.) were planted per pot and thinned to two after germination. These were laid out in a simple randomized block design and replicated thrice. The pots were biostimulated by adding 2 g of NPK fertilizer pot^{-1}. The moisture content was routinely monitored and maintained at 50% water holding capacity (WHC) and average room temperature of (25± 1^oC). The experiment was left for 10 weeks in a screen house.

2.3 Analytical methods:

Soils were randomly collected from each experimental unit, homogenized, crushed and dried in the dark at room temperature under a fume hood. The soil physicochemical properties were determined using methods generally applied in soil chemistry laboratories. pH by a potentiometric method in 1:2.5 (w/v) soil water ratio. Organic carbon content was determined by wet oxidation. Total petroleum hydrocarbon (TPH) was determined using a modified EPA 8015 technique.

2.4 Mycorrhizal colonization:

To assess AM fungi colonisation, the fresh fine root sub-samples were cut into approximately 1 cm pieces, heated in a pressure pan at 120 ^oC in 10% KOH and stained using an adaptation of Phillips and Hayman (1970) protocol including a longer incubation in 2% HCl (Oliveira et al., 2001). Stained root samples were examined microscopically to assess the percentage of mycorrhizal colonisation using the grid-line intersect method (Giovannetti and Mosse, 1980).

To estimate the percentage of mycorrhizal colonization (x), intensity of infection (I) and arbuscular development (A) in the infected region of the roots were estimated in *P.vuglaris* root samples.

2.5 Plant analysis:

Dry weight of roots and shoots were determined by drying at 70^oC for 24hrs. Chlorophyll content of plants was measured according to Harbon, (1984). The moisture content of plant tissues was determined as, an aliquot of plant sample was weighed, dried at 105 ^oC for 24 h, and weighed again; the difference gave the percent moisture.

2.6 Statistical Analysis:

The data obtained were subjected to analysis of variance (ANOVA) using Minitab software 16 edition and significant means grouped by Tukey's significant different at 5% confidence level.

2.0 Results and Discussion:

The growth and development of *Phaseolus vulgaris* as influenced by crude oil contamination and AM inoculation is shown in table 2.0. On the average, dry matter yield as influenced by percentage clay did not differ in this experiment (Fig 2.0) however, 52 % clay content recorded the highest dry matter yield while 8% clay had the least. Mycorrhizal inoculation generally influenced dry matter yield. Arbuscular mycorrhizal inoculated pots recorded higher and significantly different ($p<0.05$) yields compared to non AM inoculated pots except on 52% clay at 2% crude oil contamination where dry matter yield on AM was more than non AM inoculated pots. Percent crude oil contamination did not significantly affect dry matter yield on the average, low crude oil contamination (2%) had higher yields than 8%. Chlorophyll content of *P. vulgaris* was significantly affected by the crude oil contamination and mycorrhizal inoculation. *P. vulgaris* grown under high clay content (52%) gave higher and significantly different tissue chlorophyll concentration than those grown under 8% clay (Fig 2.0).

Table 1.0 Initial soil Characteristics

Property	Soil A	Soil B	Soil C	Analytical methods
Soil textural class	Sand-12% Silt -36% Clay-52% (Clay)	Sand-22% Silt-48% Clay-30% (loamy)	Sand-60 Silt-32% Clay-8% (Sand)	Sedimentation and decantation method
Porosity(%)	60.2	51.2	43.0	Brandom (1986).
Org.C (%)	0.75	0.62	0.54	Wet oxidation
Total N (%)	0.31	0.41	0.32	Micro kjeldhal
pH (1:2) H_2O	5.7	5.4	5.6	pH meter.

Shoot chlorophyll content decreased as percentage crude oil contamination increased. Mycorrhizal inoculation significantly enhanced chlorophyll concentration of *P.vulgaris* irrespective of the level of crude oil contamination and soil particle size (Table 2.0).

Table 2.0 Effect of soil particle size and AM on the performance of *P.vulgaris* on various concentration of crude oil.

Parameter	52% clay soil					
	2% (crude oil contamination)		4%		8%	
	AM	nonAM	AM	nonAM	AM	nonAM
Dry weight(g)	35.1± 0.6a	36.2± 0.95a	33.51±.08a	29.7± 0.43b	35.03 +1.06a	24.8 +1.68c
Chlorophyll(µg/g)	234.6 ±10.5a	179.3 ± 5.77b	158.3± 5.86c	151.0± 4.36c	165.6 ± 10.02 bc	109.0 ±6.08d
	30% clay soil					
	2%		4%		8%	
	AM	nonAM	AM	nonAM	AM	nonAM
Dry weight(g)	35.4± 0.87a	31.4± 1.61ab	33.3± 0.95a	27.67± 1.58b	34.6± 2.62a	16.4 ± 0.55c
chlorophyll(µg/g)	230± 15.39a	170± 5.8b	140.6±7 5.86c	94.07 ± 8d	121.6 ± 10.0c	72.67 ± 6.8d
	8%clay soil					
	2%		4%		8%	
	AM	nonAM	AM	nonAM	AM	nonAM
Dry weight(g)	25.3± 0.58a	15.4 ±1.62b	24.5± 1.91a	14.7± 1.41b	22.1 ± 1.155a	12.56 ± 0.56b
chlorophyll (µg/g)	155.7 ±48.9a	52.0 ± 11.2b	73.6 ±5.86b	61.0 ± 6.08b	77.0 ± 9.54b	32.6 ± 1.15b

Org.C= Organic carbon, TPH= total petroleum hydrocarbon , AM= arbuscular mycorrhiza
Rows bearing the same letters are not significantly different

The soil chemical characteristics as influenced by the crude oil contamination and soil particle size is presented in Table 3.0. Soil pH was not significantly affected by all the treatments. Soil moisture content was appreciably high at 52% clay and differed with that of 8%. Percent crude oil contamination did not affect percentage moisture of the residual soil. Residual organic carbon was significantly affected by 52% and 30% clay under various crude oil contaminations except at 8% clay content that compared well with all the crude oil contamination levels. The overall effect of crude oil contamination on soil organic carbon indicated lowest (0.76%) organic carbon at 8% crude oil, 0.81% at 4% crude oil and the highest (0.89%) at 2% crude oil contamination (Fig 1.0). Total residual petroleum hydrocarbon concentration was significantly affected at the different textural classes (Table 3.0). Residual

TPH concentration increased as percent crude oil contamination increased at each soil textural class. The soil particle size significantly affected residual TPH concentration on the average. Fifty two percent clay soil had the lowest TPH concentration (2.57 mg/g) followed by 30% clay with 3.26 mg/g TPH and 8% with 4.26mg/g. Total petroleum hydrocarbon decomposition and removal was highest at 52% clay textural class and significantly differed with the lowest at 8 % clay (Fig. 1.0). Arbuscular mycorrhizal inoculation significantly affected the decomposition of crude oil in this experiment. This is evidenced in the decrease in TPH concentration when compared to non-AM inoculated pots at different crude oil contaminations (Table 3.0). The overall assessment of impact of percent crude oil soil contamination showed no significant difference on all parameters at different levels of contamination (table 4.0).

The arbuscular mycorrhizal colonization of *P.vulgaris* roots is shown in Table 5. The textural class and crude oil contamination significantly affected the level of AM colonization, development and severity of infection in this experiment. The levels of root colonization by *G. moseae* are expressed in three ways: (1) frequency of root segments (X %) reflecting the proportion of roots colonized with mycorrhizal fungi. (2) Intensity of mycorrhizal colonization in root tissues (I %). (3) The rate of arbuscular formation in root segments (A %) reflecting the potentiality of exchange with the symbiosis. From the result, the root segmentation, intensity of colonization and arbuscular formation decreased as crude oil contamination increased irrespective of the textural class (Table 5).

Table 3.0 Residual soil chemical characteristics as influenced by soil particle size and AM under *P.vulgaris* grown pot experiment

Parameter	52% clay soil					
	2%(crude oil contamination)		4%		8%	
	Am	nonAM	AM	nonAM	AM	nonAM
pH	5.67± 0.06a	6.23± 0.31a	6.0 ±0.6a	5.7± 0.1a	6.1± 0.3a	5.7± 0.1a
Org.C(%)	1.23a	0.98ab	0.87b	0.85b	1.23a	0.93ab
TPH (mg/g)	1.32± 0.1c	4.06± 0.4b	3.3± 0.43c	6.0± 0.6a	3.7± 0.66b	6.1 ± 0.58a
%moisture	57.9± 0.5b	42.3 ±2.6d	58.4± 0.5b	46.1±3 0.4c	63.46± 1.05a	46.8± 1.4c
	30% clay soil					
	2% crude oil contamination		4%		8%	
	Am	nonAM	AM	nonAM	AM	nonAM
pH	5.7± 0.1a	5.8 ±0.05a	5.7 ±0.05a	5.7 ±0.05a	5.8± 0.11a	5.63± 0.2a
Org.C (%)	1.16a	0.78b	0.84ab	0.98ab	0.91ab	0.90 ab
TPH(mg/g)	2.23± 0.21e	4.1± 0.05c	2.96± 0.208d	5.66± 0.11b	4.6± 0.26c	7.13± 0.21a
%moisture	57.2± 0.72 a	40.6± 0.5c	52.9± 1.2b	36.6± 0.8d	53.3 ±1.05b	32.2± 0.52e
	8% clay soil					
	2% crude oil contamination		4%		8%	
	Am	nonAM	AM	nonAM	AM	nonAM
pH	5.7 ±0.1a	5.76 ±0.11a	5.7± 0.1a	5.73± 0.11a	5.6± 0.11a	5.6 ±0.05a
Org.C(%)	34.0 a	0.72 a	0.76a	0.84a	0.87a	0.73a
TPH (mg/g)	3.23b± 0.37d	4.13± 0.21c	4.4± 0.1 b	5.4± 01b	4.7 ±0.52c	7.9± 0.2a
%moisture	34± 1.57a	25.6± 1.91b	35.1± 0.49a	32.8± 1.10a	33.9± 0.68a	32.16± 1 a

Org.C= Organic carbon, TPH= total petroleum hydrocarbon, AM= arbuscular mycorrhiza
Rows bearing the same letters are not significantly different

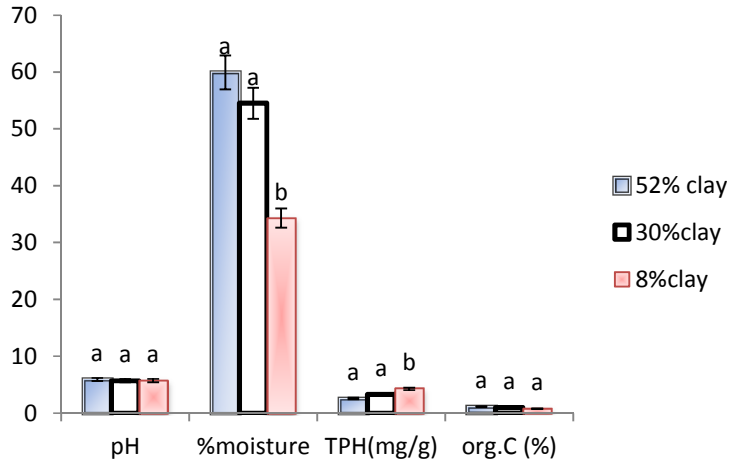

Fig. 1.0. Effect of soil particle size on soil chemical characteristics as influenced by crude oil contamination. Error bars represent standard error of means

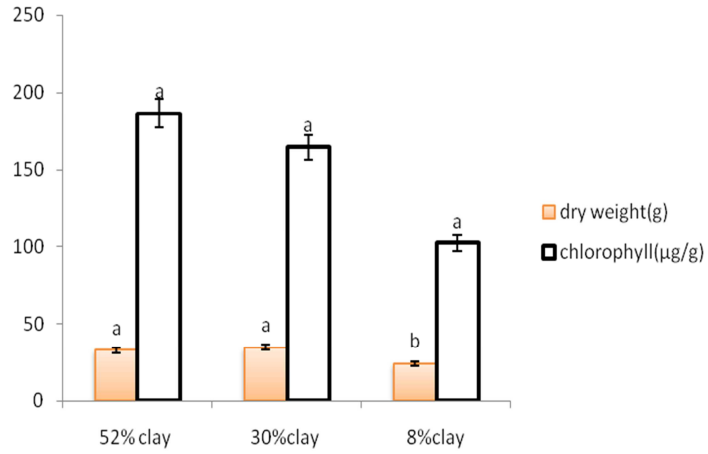

Fig. 2.0. Effect of soil particle size on *P. vulgaris* performance as influenced by crude oil contamination. Error bars represent standard error of means.

Table 4.0 Overall assessment of crude oil contamination on *P. vulgaris* and soil characteristics as influenced by arbuscular mycorrhizal inoculation.

% crude oil	pH	%moisture	TPH(mg/g)	Org.C(%)	Dry weight(g)	Chlorophyll(µg/g)
2%	5.68±0.02a	49.7 ±13.6a	2.48± 1.3a	0.89± 0.28a	31.9 ±5.7a	206.7± 44.5a
4%	5.8± 0.17a	48.7± 12.4a	3.78± 1.6a	0.81± 0.06a	30.4± 5.1a	124.0 ±44.3a
8%	5.83± 0.2a	50.2± 14.9a	4.3± 0.5a	0.76 ±0.19a	28.7± 6.3a	121.4 ± 44.3a
P<0.05	ns	ns	ns	ns	ns	ns

The correlation matrix between AM infection on soil and plant characteristics is shown in Table 6.0. There is positive correlation (p>0.05) between percentage organic carbon and arbuscular mycorrhizal development and infection in the crude oil contaminated soil. Dry matter yield and chlorophyll content of *P. vulgaris* had significant positive correlation with mycorrhizal colonization and intensity of infection. Thus, with AM colonization, the physiological characteristics of *P. vulgaris* was greatly improved. Total petroleum hydrocarbon concentration showed significant

negative correlation with the mycorrhizal colonization (P=0.001), intensity of infection (p=0.0001), arbuscular development (P=0.04). At higher degree of AM infection and severity, the crude oil degradation and removal was enhanced. This is evinced by the linear regression analysis between TPH and colonization, %porosity and textural class (Table 7.0). There was strong negative relationship between the AM root colonization of *P.vulgaris* and residual TPH concentration (R^2=0.77). Percentage clay also positively influenced TPH concentration in the soil (R^2=0.96, P=0.0001). Soil porosity measures the total volume of pore spaces and this negatively influenced soil residual TPH concentration (R^2=0.77, p=0.002).

Table 5.0 Percentage of AM colonization on the root of *P.vulgaris* as influenced by crude oil contamination and soil particle size.

Level	52% clay		
	2% crude oil	4%	8%
X%	77.0a	61b	46b
I%	32.3a	21.0b	18.3b
A%	46.0a	33.0b	22.0c
	30% clay		
	2% crude oil	4%	8%
X%	87a	66.3b	49.3c
I%	30.6a	26.3ab	22.6b
A%	41.0a	30.3b	20.3c
	8% clay		
	2% crude oil	4%	8%
X%	84.6a	71.0b	48c
I%	38.7a	25.0b	13.6c
A%	36.3a	24.6b	12.0c

X%= Frequency of mycorrhizal root segments, I%= intensity of mycorrhizal infection, A%= rate of arbuscular development. Rows bearing the same letters are not significantly different

Table 6.0. Correlation matrix of AM infection on soil and plant characteristics

Measurement	X %	I %	A %
Org. C(%)	0.44	0.54	0.210
	(0.2)	(0.13)	(0.58)
TPH (mg/g)	-0.89	-0.92	-0.684
	(0.001***)	(0.00***)	(0.04**)
Dry weight(g)	0.734	0.86	0.317
	(0.02**)	(0.003**)	(0.406)
Chlorophyll(µg/g)	0.876	0.831	0.80
	(0.002**)	(0.006**)	(0.01**)

X=Percentage mycorrhizal colonization, I%= intensity of AM infection, A%= arbuscular development.

Table 7.0 Linear Regressions for residual TPH concentration between percentage clay, porosity and AM colonization.

Variable X	Dependent variable Y	Intercept	Slope	R^2	P-value
% clay	TPH	2.06	0.047	0.96	0.0001
%colonization		6.20	-0.044	0.77	0.002
%porosity		6.20	-0.045	70.72	0.002

This study investigated the influence of soil particle size and arbuscular mycorrhizal fungi in the early development of *P.vulgaris* grown under crude oil contaminated soil. The overall significant dry matter yield and chlorophyll content observed in mycorrhizal inoculated pots may be attributed to improved nutrient acquisition, water relations, pollutant tolerance and sequestration potentials of AM infected roots of *P. vulgaris* . One mechanism that may be involved is the oxidation of contaminant by activated oxygen species and concomitant enhancement of oxidoreductases to protect the plant from oxidative stress. Satzer *et al.,* (1999) noted enhanced levels of hydrogen peroxide in AM roots as well as enhanced levels of peroxidase activity in mycorrhizal roots and the rhizosphere which may lead to enhanced oxidation of crude oil around AM colonized root (Criquet et al., 2000). One peculiarity of crude oil polluted soil that may be overcome by AM plant is the hydrophobicity and resulting limitations in uptake of water dissolved inorganic nutrients (Leyval and Binet , 1998). There are good reasons to believe that mycorrhizal infection of roots of tropical plant species induces tolerance against abiotic and biotic stresses. Decomposition of crude oil was significantly improved in the mycorrhizal inoculated pots as evidenced in the concentration of residual total petroleum hydrocarbon in this experiment. This important finding could be explained in the light of root physiology modification by AM that tends to increase enzyme activity level and root exudation which directly stimulates crude oil degradation. Indirect mechanisms rely on root surface properties or rhizosphere soil properties that act on crude oil availability through adsorption and co-metabolism (Jones and Leyval, 2003).

The levels of root colonization by AM were decreased with increasing concentration of crude oil in soil. Of note in this study is the behaviour of mycorrhizal activity in the contaminated soil. For example, the intensity of colonization in the root tissues and rate of arbuscular formation in root segments showed significant positive and negative correlations with dry matter yield, chlorophyll content and residual TPH, respectively. This observation reflects enhanced degradation of crude oil and symbiotic activity of AM fungi with *P. vulgaris* plant. Houngnandan *et al.,* (2000) concluded that farmers' management practices that allow a buildup of AM fungal inoculums would alleviate P-deficiency and hence increase N-fixation which will ultimately increase physiological development of the plant species. Similar interactions between AM fungi and rhizobia have been demonstrated for soybean (*Glycine max*) in low-P soils of the savanna in Nigeria (Nwoko and Sanginga, 1999; Sanginga *et al.,* 1999). AM fungal and rhizobial responses might show positive feedback. Rhizobial inoculation increased AM colonization in soybean (Sanginga *et al*., 2000) and mucuna (Houngnandan *et al*., 2001). *P. vulgaris* , a leguminous plant, may have played significant role in nitrogen fixation that further improved rhizodegradation of the crude oil contaminant.

In this experiment, the textural class significantly affected the decomposition and overall removal of total hydrocarbon. Degradation of TPH was highest at 52% clay textural class and lowest at 8% clay content. This result collaborated with the work of Walpola and Arunakumara, (2010) who noted that decomposition of *Gliricidia* leaves was significantly influenced by the soil textural class and that carbon mineralization in loamy sand soil was significantly higher than sandy clay loam soil throughout the incubation period. Hassink, (1992) also noted that total clay content, porosity, bulk density and pore-volume (0.75 to 6 μm in diameters) were all positively correlated to $^{14}CO_2$-C evolution (a measure of decomposition) of organic matter amended soils. Two mechanisms have been put forward to explain the influence of soil texture on organic matter and PAH decomposition (1) the protective action by clays against organic matter degradation through the formation of complexes between metal ions associated with large clay surfaces and high CEC (Giller *et al*., 1997), and (2) accessibility by soil microbes (van Veen and Kuikman, 1990). Clay particles are believed to protect some of the more easily decomposable organic compounds from rapid microbial breakdown through encrustation and entrapment (Anderson, 1979, Tisdall and Oades, 1982). Ladd *et al*., (1985) found a significant linear relationship between residual labelled C in topsoil and clay contents ranging from 5 to 42%. The higher the clay content in the different soils, the higher the residual C content after 8 years of experimentation. Also , Owabor and Ogunbor, (2006) observed increased PAH degradation in high clay soil than silt

fine soil. Hamarashid et al., (2010) noted that carbon mineralization, using CO_2 respiration method under laboratory conditions indicate that rates of CO_2 in fine soil textures (viz: clay loam, loam and silty clay loam), are significantly ($p \leq 0.01$) higher than coarser soil textures (silty loam, loamy sand and sandy loam).

4.0 Conclusions:

The physiological development of *P.vulgaris* under abiotic stress may be improved through soil biological improvement strategies such as arbuscular mycorrhizal inoculation. Arbuscular mycorrhizal tends to ameliorate unfavourable conditions posed by crude oil contamination by enhanced production of oxidative enzymes and overall improvement in the soil aggregation. Soil textural class is an important factor in soil microbial activity as observed in this study. Soil with 52% clay content enhanced crude oil decomposition more than 8% clay as evidenced by the low residual TPH concentration. The capacity of soils to preserve soil organic matter and total nitrogen in clay and silt sized particles is greater than sandy one.

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