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Influence of a mycorrhizal fungus and mineral fertilizer on the performance of *Costus lucanusianus* under crude oil contaminated soil

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Abstract

Considering the detrimental effects of crude oil pollution on plants, and its implications on food security and environmental safety, it becomes imperative to screen for plants with strong tolerance to crude oil contaminated soil. This study was conducted to assess the influence of arbuscular mycorrhizal fungus (AM) and a mineral fertilizer (NPK 15 15 15 grade), on the performance of African spiral ginger (*Costus lucanusianus*) plant, under crude oil contaminated soil. About 10 kg of sterilized soil was contaminated with Bonny light crude oil at different concentrations of; 0, 200, 300 and 500 ml/ pot. Moreover, a fertilizer (i.e. NPK) was applied at three different levels (0, 0.7 and 1.2 g/ pot) to the crude oil contaminated soil in the pots, and then inoculated with arbuscular mycorrhizal (AM) fungus; consisting of 20 g of *Glomus clarum*. After that, *Costus lucanusianus* was planted in the pot soil by stem cuttings. Results of this greenhouse assay involving; the residual total petroleum hydrocarbon (TPH) content of the soil, plant height, number of leaves, fresh and dry weights, percent of mycorrhizal root colonization, and fungal total colony count, were collected at 4, 8 and 12 weeks after planting (WAP). Current results showed that mycorrhizal inoculation and fertilizer application enhanced the tolerance and growth of *C. lucanusianus* plant to the crude oil contaminated soil. Mycorrhizal inoculation and NPK fertilizer application at 1.2 g/ pot recorded higher and significantly (p <0.05) different plant height, number of leaves, fresh and dry weights of *C. lucanusianus*. The TPH degradation and removal was higher in soil inoculated with AM, compared to non AM inoculated control treatments. Similarly, NPK fertilizer application also resulted in lower residual TPH content in the crude oil contaminated soil, compared to the control. Combined AM colonization and NPK fertilizer application resulted in improved physiological parameters of the *C. lucanusianus* plant.

Keywords: *Costus lucanusianus*, Arbuscular mycorrhiza, NPK fertilizer, Oil contaminated soil, Bioremediation

1. Introduction

Crude oil is a complex substance that consists of various aliphatic, branched and aromatic hydrocarbons, and other organic compounds (Riser-Roberts, 1998; Yuniati, 2018). Soil pollutions/ contaminations associated with crude oil are widespread. This is due to the over dependence on petroleum hydrocarbons as a major source of energy for homes and industries, thus resulting in the pollution of the agricultural lands, due to oil spillage during exploration and processing operations. Previous studies conducted by Schwab *et al*., (1999); Adipah, (2019) reported that crude oil contaminated soils are not suitable for agricultural and recreational uses, and are potential sources of surface and ground water contamination. Dale *et al*., (2006) documented that due to its lead (Pb) contents, crude oil pollution renders soils unproductive for many years after oil spillage, thereby reducing the growth performance of plants.

According to Frick *et al*., (1999); Dadrasnia *et al*., (2015), incineration, thermal desorption, soil washing, etc. are some of the physico-chemical techniques used to treat petroleum hydrocarbon polluted soil. However, Frick *et al*., (1999); Gong, (2012) reported that these techniques are often expensive and have limited local application, due to the complex scientific knowledge needed to operate the component units. Biological treatments are better alternative methods for the treatment of contaminated soils. Previous studies of Soleimani *et al*., (2010); Graj *et al*., (2013) revealed that these methods are economic, eco-friendly, and have shown promising results in the treatment of organic compounds contaminated soil, especially petroleum hydrocarbons.

The concept of bioremediation techniques is based on the enhancement of microbial populations in the soil either through biostimulation and\or bioagumentation, and the use of plants (phytoremediation) to degrade the soil contaminants (i.e. hydrocarbons). This is very important since the natural reduction of crude oil in the environment is a slow process, due to the nutritional imbalance or deficiency created by crude oil spills. Results of Ijah and Ndana, (2003); Adenipekun and Fasidi, (2005); Iquatt *et al*., (2006); Hamoudi-Belarbi *et al*., (2018) reported that addition of nutrients in the form of inorganic fertilizer such as NPK, is effective in dealing with crude oil spills in soils. The added fertilizer provides nitrogen and phosphorus to the hydrocarbonoclastic microorganisms in the soil, thereby promoting the growth and crude oil- degrading capability of the microorganisms.

Mycorrhiza, an association between plants and fungi that colonize the cortical tissue of roots during the active periods of the plant growth have been suggested to improve biodegradation of the organic pollutants, due to its large size and very high surface interface with soil (Sharma *et al*., 2007). Several studies such as those of Alarcon *et al*., (2008); Liu and Dalpe, (2009) have suggested that mycorrhizal fungi collaborate with some of the soil microorganisms during the phytoremediation process. The green plants and associated microbiota, soil amendments and the different agronomic techniques help in ameliorating the impact of contaminants in the soil. The crude oil pollution affects the soil properties and growth of plants depending on their concentrations in this soil. Chukwu and Udoh, (2014) reported that at higher concentrations, crude oil becomes increasingly detrimental to the functional capability of the soil and to the plant growth.

Considering the detrimental effects of crude oil pollution on plants and its implications on food security and environmental safety, it has become necessary to screen for plants with strong tolerance to crude oil contaminated soil. The objectives of the present study were to: 1) determine the impact of mycorrhiza and mineral fertilizer (NPK) on the growth of African spiral ginger (*Costus lucanusianus*) in crude oil contaminated soil, and 2) determine the combined

effects of mycorrhizal fungi and mineral fertilizer (NPK) on the remediation of crude oil contaminated soil planted with the African spiral ginger.

2. Materials and methods

2.1. Experimental site and planting materials

This study was carried out at the Department of Botany Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The AM fungus used in the current study was provided by the Soil Microbiology Laboratory, Department of Agronomy, University of Ibadan, Ibadan, Oyo State, Nigeria, while the mineral fertilizer (NPK 15 15 15) was purchased from the open agricultural market. The Bonny light crude oil was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt refinery, Rivers State, Nigeria. Finally, the African spiral ginger was supplied by the Department of Botany Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

2.2. Soil preparation

Soil samples at depth 0-15 cm were collected, air dried, sieved through a 2 mm sieve, sterilized, and then filled into 10 kg capacity pots.

2.3. Planting and management

The crude oil at 200, 300 and 500 ml/ pot, was mixed thoroughly with the soil and allowed to stand for two weeks. NPK fertilizer was applied after two weeks at the rate of 0.7 and 1.2 g /pot, and then mixed with the soil for even distribution. After another two weeks, an African spiral ginger was planted by stem cuttings (10 cm long). Treatment of soil with *Glomus clarum* consisted of 20 g of *G. clarum* blended into the central third of the pot soil at planting, according to Carling *et al*., (1978).

2.4. Data collection

Plant height; number of leaves, fresh and dry weights were determined at 4, 8 and 12 weeks after planting (WAP). The plant height was measured in centimeter with the aid of a meter rule. The number of leaves was counted visually. The fresh biomass yield of *C. lucanusianus* was determined by uprooting the fresh plant from the pot soil, rinsing the root in clean water to get rid of soil, and then weighed. On the other hand, the dry weight was evaluated by oven-drying of this fresh biomass of the plant at 65ºC to a constant weight. The TPH content of the soil was also determined at 4, 8 and 12 weeks after planting (WAP), using Soxhlet extraction method (USEPA. 2017).

2.5. The experimental design

The experiment was designed in a completely randomized design (CRD), and replicated three times. It was a factorial experiment with two factors; Mycorrhizal inoculation at two levels (with and without), and mineral fertilizer at 3 levels making a total of 6 treatment combinations. The treatments were:

Factors (a): Mycorrhizal inoculum (20 g)

i. *Glomus clarum* inoculation

ii.Without *Glomus clarum* inoculation

Factor (b): Mineral fertilizer

i. No NPK (15-15-15) 0 kg NPK/ ha (0 g NPK/pot)

ii. NPK (15-15-15) at 70 kg NPK/ ha (0.7 g NPK/pot)

iii. NPK (15-15-15) at 120 kg NPK/ ha (1.2 g NPK/pot)

2.6. Determination of the physicochemical properties of the soil sample

The soil mechanical analysis was estimated by the hydrometer method of Bouyoucos, (1951), while the soil pH was determined according to Udo and Ogunwale, (1986). The organic carbon was estimated by the modified procedure of Walkley-Black (Nelson and Sommers, 1996). The total nitrogen and available phosphorous content of the soil were evaluated by the

micro Kjeldahl digestion and distillation method, as described by Udo and Ogunwale, (1986), and Bray P 1 method (Bray and Kurtz, 1945). The exchangeable bases (K, Na, Ca and Mg) were estimated according to Jackson, (1958); Ca and Mg were read off using atomic absorption spectrophotometer (AAS), while K and Na were read off using a flame photometer. Similarly, the effective cation exchange capacity was determined by the summation method of Juo *et al*., (1976), while the exchangeable acidity was estimated by the method of Mclean, (1982).

2.7. Determination of the percentage of mycorrhizal root colonization

The percentage roots colonization was determined using the grid-line intersect method of Kormanik and McGraw, (1982).

2.8. Microbial enumeration of fungi

The number of viable fungi were estimated using the plate count technique (Ochei and Kolhatkar, 2008).

2.9. The total petroleum hydrocarbon (TPH)

The soxhlet extraction method was used to estimate the TPH content. Analytical determinations of the hydrocarbons in the soil extracts were performed by Infrared spectrophotometer (IR). Quantification of the TPH content was carried out using the procedure described by the USEPA SW-846 series method 3540 (USEPA. 2017). After collection of the extract, it was passed through sodium sulfate and silica gel to remove water and polar constituents. An aliquot of the soil extract was then placed in the infrared spectrophotometry (IR) analyzer. The TPH value was determined by comparing to a three-point calibration curve constructed from dilutions of a stock solution of a 2:3:3 volume ratios of chloro- benzene, isooctane, and n-hexadecane made up in perchloroethylene (PCE), according to USEPA. (2017).

2.10. Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) by Proc. GLM of GenStat version 17, and significant means were separated using least significant different (LSD), and Duncan's multiple range test (DMRT).

3. Results

3.1. Soil characteristics

The pre-experimental characteristics of the soil sample with a pH of 4.9 are demonstrated in Table (1). The values of the major nutrient elements are as follows: total N: 1.0 g/ kg, available P: 28.70 mg/ kg and exchangeable K: 0.12 cmol/ kg. The textural class of the soil is sandy.

3.2. Effects of the mycorrhizal and NPK fertilizer application on the height and number of leaves of *C. lucanusianus* plant

In the mycorrhizal inoculated soil, the *C. lucanusianus* plants have higher plant heights of 44.16 cm, 58.29 cm, compared to the non-mycorrhizal inoculated plants recording heights of 41.72 cm, 55.00 cm, at the 8 and 12 WAP; respectively. However, at the 4th WAP, there is no noticeable difference in the height of *C. lucanusianus* plant.

Moreover, mycorrhizal inoculation resulted in higher number of plant leaves recording; 18.94, 34.14 and 47.14, at the 4, 8 and 12 WAP; respectively, compared to the non-mycorrhizal inoculation (16.03, 28.97 and 42.39), as shown in Table (2). Treatment of pot soil with 1.2 g NPK/ pot resulted in higher plant heights of 37.17, 47.41 and 66.21 cm, at the 4, 8 and 12 WAP; respectively, compared to the other treatments. However, treatment with 0 g NPK/ pot recorded lower heights of 28.28, 38.56, and 47.76 cm, compared to that of 0.7 g NPK/ pot (32.67, 42.85 and 55.96 cm). Fertilizer application at 1.2 g NPK/ pot caused pronounced increase in the number of plant leaves (19.54, 36.04 and 51.83) at the 4, 8 and 12 WAP, compared to the other treatments levels. On the other hand, application of the fertilizer at 0 g NPK/ pot had noticeably lower number of leaves (26.04 and 35.08), except at the 4th WAP, compared to the treatment at 0.7 g NPK/ pot, as clear in Table (2).

Table 1: Physical and chemical properties of the soil sample used in the different experimental assays

Soil properties	Value
pH (H_2O)	4.9
C (g/kg)	12.4
N(g/kg)	1.0
Av. P (mg/kg)	28.70
Moisture content (%)	7.8
Exchangeable cations (cmol/kg)	
Ca	6.0
Mg	1.2
K	0.12
Na	0.08
Al	0.16
H^+	0.82
ECEC (cmol/kg)	8.4
Particle size (g/kg)	
Sand	840
Silt	110
Clay	50
Textural class	Sandy

Where: three replicates were used for detecting each of the physical and chemical properties of the soil sample

Table 2: Effects of mycorrhiza and NPK application on the height and number of leaves of *C. lucanusianus* plant at different WAP

Treatments	Plant height (cm)			Number of leaves		
	4WAP	**8WAP**	**12WAP**	**4WAP**	**8WAP**	**12WAP**
Mycorrhiza						
M+	32.92	44.16	58.29	18.94	34.14	47.14
M-	32.49	41.72	55.00	16.03	28.97	42.39
LSD (0.05)	NS	1.25	1.55	0.91	1.12	1.18
SE (±)	0.36	0.45	0.54	0.32	0.39	0.42
NPK (g/pot)						
S0	28.28	38.56	47.76	17.17	26.04	35.08
S1	32.67	42.85	55.96	15.75	32.58	47.38
S2	37.17	47.41	66.21	19.54	36.04	51.83
LSD (0.05)	1.24	1.53	1.89	1.12	1.27	1.45
SE (±)	0.44	0.54	0.67	0.39	0.48	0.51

Where; M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; LSD = least significant difference; SE = standard error; NS = not significant. N/B: 3 replicates per treatment

3.3. Effects of mycorrhiza and NPK fertilizer on the fresh and dry weights of *C. lucanusianus* plant

At all the weeks evaluated (except at the 12 WAP), mycorrhizal inoculation resulted in appreciably higher fresh weight of Costus plant compared to the non-mycorrhizal inoculation (Table 3). Fertilizer application at 1.2 g NPK/pot resulted in higher fresh weight of Costus plant at the 4, 8 and 12 WAP recording; 50.31, 64.29 and 96.38 g; respectively, compared to the other application levels (Table 3). On the other hand, treatment with 0 g NPK/pot have significantly lower fresh weight of Costus plant (50.31, 64.29 and 96.38 g), compared to that of 0.7 g NPK/ pot. Mycorrhizal inoculation resulted in noticeably higher dry weight of the tested plant (24.56, 28, 51 and 42.34 g) at the 4, 8 and 12 WAP; respectively, compared to the non-mycorrhizal inoculation (Table 3). Significantly higher dry weights of Costus (24.71, 30.80 and 47.90 g) at the 4, 8 and 12 WAP; respectively, are obtained with the fertilizer application at 1.2 g NPK/ pot, compared to the other application levels (Table 3). However, fertilizer application at 0 g NPK/ pot resulted in appreciably lower dry weights of the plant, compared to that of 0.7 g NPK/pot.

3.4. Effect of interaction of mycorrhiza with NPK fertilizer on the height and number of leaves of *C. lucanusianus* plant

The interaction of mycorrhizal inoculation with 1.2 g NPK/ pot at the 4, 8 and 12 WAP resulted in noticeably higher plant heights of; 37.61, 48.15 and 67.21 cm, compared to the other treatments (Table 4); however, this is not significantly different from the interaction between 1.2 g NPK/ pot and treatment without the mycorrhizal inoculation. Lower Costus plant heights (28.01, 37.83 and 46.74 cm) across the weeks evaluated is recorded from the combination of 0 g NPK/ pot and treatment without the mycorrhiza inoculation. At all the weeks examined, appreciably higher number of leaves are obtained from the interaction of mycorrhiza with 1.2 g NPK/ pot, compared to the other treatments (Table 4), while significantly lower number of leaves (14.33, 23.58 and 34.33) are obtained from the combination of 0 g NPK/pot and treatment without the mycorrhizal inoculation.

3.5. Effect of the interaction of mycorrhiza with NPK fertilizer application on the fresh and dry weights of *C. lucanusianus* plant

The interaction of mycorrhiza with 1.2 g NPK/pot resulted in magnificent higher fresh weights of Costus plant across the weeks examined, compared to the other treatments (Table 5). But this is not significantly different from the interaction of 1.2 g NPK/pot and treatment without the mycorrhizal inoculation. The combined application of 0 g NPK/pot and treatment without the mycorrhiza application resulted in noticeably lower Costus fresh weights (37.01, 46.74 and 67.93 g) at the 4, 8 and 12 WAP; respectively, compared to that of mycorrhiza application with 0 g NPK/pot. Appreciable higher dry weights across the weeks evaluated are obtained from the interaction of mycorrhiza inoculation with 1.2 g NPK/pot, compared to the other treatments (Table 5). However, this is not noticeably different from the interaction of 1.2 g NPK/pot and treatment without the mycorrhizal inoculation, except at the 12 WAP. Similarly, the interaction of 0 g fertilizer and treatment without the mycorrhizal inoculation resulted in magnificently lower dry weights of Costus plant (22.30, 23.27 and 31.22 g), compared to the interaction of mycorrhiza inoculation with 0 g fertilizer except at the 4 WAP.

3.6. Effects of mycorrhiza and NPK fertilizer application on the TPH content of soil planted with *C. lucanusianus*

At the 4, 8 and 12 WAP, mycorrhizal treatments recorded significantly lower total petroleum hydrocarbon (TPH) content of the soil (65.75, 57.94 and 53.84 g/ kg), compared to the non-mycorrhizal treatment (Table 6). Fertilizer application at 1.2 g NPK/pot resulted in appreciably lower TPH content of the contaminated soil (63.81, 51.95 and 47.93 g/ kg) across the weeks examined, compared to the other

application levels (Table 6). On the other hand, fertilizer application at 0 g NPK/ pot has noticeably higher TPH content of the contaminated soil (70.92, 65.39, and 61.30 g/ kg), compared to that of 0.7 g NPK/ pot.

Table 3: Effects of mycorrhiza and NPK application on the fresh and dry weights of *C. lucanusianus* plant at different WAP

Treatments	Fresh weight (g)			Dry weight (g)		
	4WAP	**8WAP**	**12WAP**	**4WAP**	**8WAP**	**12WAP**
Mycorrhiza						
M+	46.02	56.11	83.64	24.56	28.51	42.34
M-	44.24	54.68	82.50	22.37	26.31	39.65
LSD (0.05)	1.19	1.19	NS	0.62	0.72	0.76
SE (±)	0.42	0.42	0.52	0.22	0.24	0.27
NPK (g/pot)						
S0	38.48	45.73	67.56	22.75	24.25	32.53
S1	46.60	56.15	85.28	22.95	27.19	42.57
S2	50.31	64.29	96.38	24.71	30.80	47.90
LSD (0.05)	1.46	1.46	1.80	0.77	0.88	0.93
SE (±)	0.51	0.51	0.63	0.27	0.31	0.33

Where; M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; LSD = least significant difference; SE = standard error; NS = not significant. N/B: 3 replicates per treatment

Table 4: Effects of the interaction of mycorrhiza with NPK application on the height and number of leaves of *C. lucanusianus* plant at different WAP

Mycorrhiza	NPK (g/pot)	Plant height (cm)			Number of leaves		
		4WAP	**8WAP**	**12WAP**	**4WAP**	**8WAP**	**12WAP**
M+	S0	28.56^{d}	39.29^{cd}	48.78^{d}	18.00^{b}	28.50^{d}	35.83^{c}
M+	S1	34.02^{b}	45.03^{b}	58.88^{b}	17.17^{b}	34.33^{b}	48.17^{b}
M+	S2	37.61^{a}	48.15^{a}	67.21^{a}	21.67^{a}	39.58^{a}	57.42^{a}
M-	S0	28.01^{d}	37.83^{d}	46.74^{d}	14.33^{b}	23.58^{e}	34.33^{c}
M-	S1	31.31^{c}	40.66^{c}	53.04^{c}	16.33^{b}	30.83^{c}	46.58^{b}
M-	S2	36.74^{a}	46.67^{ab}	65.21^{a}	17.42^{b}	32.50^{bc}	46.25^{b}
	SE (±)	0.62	0.76	0.94	0.56	0.68	0.72

Where; Means with the same letter(s) in the same column under the same variable are not significantly different from each other at $p< 0.05$, using Duncan's multiple range test. N/B: 3 replicates per treatment. M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; SE = standard error

Table 5: Effects of the interaction of mycorrhiza with NPK application on the fresh and dry weights of *C. lucanusianus* plant at different WAP

Mycorrhiza	NPK (g/pot)	Fresh weight (g)			Dry weight (g)		
		4WAP	**8WAP**	**12WAP**	**4WAP**	**8WAP**	**12WAP**
M+	S0	39.94^{c}	44.72^{d}	67.18^{c}	23.19^{b}	25.23^{c}	33.84^{e}
M+	S1	50.30^{a}	60.23^{b}	86.42^{b}	24.73^{a}	29.40^{b}	44.09^{c}
M+	S2	50.75^{a}	65.22^{a}	97.31^{a}	25.77^{a}	30.91^{a}	49.10^{a}
M-	S0	37.01^{d}	46.74^{d}	67.93^{c}	22.30^{b}	23.27^{d}	31.22^{f}
M-	S1	42.46^{b}	52.07^{c}	84.13^{b}	20.13^{c}	24.98^{c}	41.04^{d}
M-	S2	50.32^{a}	63.37^{a}	95.44^{a}	24.68^{a}	30.69^{a}	46.71^{b}
	SE (±)	0.73	0.72	0.89	0.38	0.44	0.46

Where; Means with the same letter(s) in the same column under the same variable are not significantly different from each other at $p< 0.05$, using Duncan's multiple range test. N/B: 3 replicates per treatment. M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; SE = standard error

Table 6: Effects of mycorrhiza and NPK application on the TPH content of the soil planted with *C. lucanusianus* plant at different WAP

Treatments	Total petroleum hydrocarbon (g/kg)		
	4WAP	**8WAP**	**12WAP**
Mycorrhiza			
M+	65.75	57.94	53.84
M-	67.42	58.33	52.68
LSD (0.05)	0.27	NS	1.35
SE (±)	0.09	0.29	0.47
NPK (g/pot)			
S0	70.92	65.39	61.30
S1	65.03	57.07	50.54
S2	63.81	51.95	47.93
LSD (0.05)	0.33	0.99	1.65
SE (±)	0.12	0.35	0.58

Where; M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; LSD = least significant difference; SE = standard error; NS = not significant. N/B: 3 replicates per treatment

3.7. Effect of the interactions of mycorrhiza with NPK fertilizer application on the TPH content of soil planted with *C. lucanusianus*

The interaction of the mycorrhizal inoculation with 1.2 g NPK/ pot resulted in lower TPH content of the contaminated soil (62.88, 50.64 and 47.66 g/ kg) at the 4, 8 and 12 WAP; respectively, compared to the other treatments. However, at the 12 WAP, the interactions between mycorrhizal inoculation with 1.2 and 0.7 g NPK/pot and treatment without the mycorrhizal inoculation are not too much different (Table 7). Across the weeks evaluated, higher TPH contents of the contaminated soil are recorded from the combination of 0 g NPK/ pot and treatment without mycorrhizal inoculation, compared to the interaction of the mycorrhizal inoculation with 0 g NPK/ pot (Table 7).

3.8. Effect of mycorrhiza and NPK fertilizer application on the mycorrhiza root colonization of *C. lucanusianus* plant

At the 4, 8 and 12 WAP, mycorrhiza inoculated plants have higher mycorrhizal root colonization of; 49.32, 53.14 and 58.80 %, compared to the non-mycorrhizal inoculated plants (Table 8).

Across the weeks tested, treatments with 1.2 g NPK/pot have higher mycorrhizal root colonization of; 38.79, 40.95 and 47.14 %, compared to the other treatments (Table 8). On the other hand, fertilizer application at 0 g NPK/ pot resulted in appreciably lower mycorrhizal root colonization, compared to that of 0.7 g NPK/ pot.

3.9. Effect of the interaction of mycorrhiza with NPK fertilizer application on the mycorrhizal root colonization of *C. lucanusianus* plant

The combination of mycorrhizal inoculation with 1.2 g NPK/ pot at the 4, 8 and 12 WAP, resulted in higher mycorrhizal root colonization of; 56.45, 59.71 and 69.73 %, compared to the other treatment combinations (Table 9).

3.10. Effect of mycorrhizal and NPK fertilizer application on total fungal colony count (CFU/g soil)

Results in Table (10) demonstrate the effect of mycorrhiza and NPK fertilizer application on the total fungal colony count (CFU/ g soil), and the microorganisms identified in the experimental soil. The microorganisms recovered include; *Aspergillus* spp., *Mucor* spp., and *Rhizospus* spp. The total fungal colony count (CFU) range from 4×10^4 to 8×10^4 CFU/ g soil, where the mycorrhizal inoculated treatments have the highest total fungal colony count.

Table 7: Effects of the interaction of mycorrhiza with NPK application on the TPH content of soil planted with *C. lucanusianus* plant at different WAP

Mycorrhiza	NPK (g/pot)	Total petroleum hydrocarbon (g/kg)		
		4WAP	**8WAP**	**12WAP**
M+	S0	70.47^{b}	64.51^{b}	59.93^{b}
M+	S1	63.91^{e}	56.93^{c}	51.19^{c}
M+	S2	62.88^{f}	50.64^{e}	47.66^{d}
M-	S0	71.37^{a}	66.27^{a}	62.68^{a}
M-	S1	66.15^{c}	57.22^{c}	49.89^{cd}
M-	S2	64.73^{d}	53.26^{d}	48.21^{d}
	SE (±)	0.16	0.49	0.82

Where; Means with the same letter(s) in the same column are not significantly different from each other at $p< 0.05$ using Duncan's multiple range test. M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; SE = standard error. N/B: 3 replicates per treatment

Table 8: Effects of mycorrhiza and NPK application on the mycorrhizal root colonization of *C. lucanusianus* plant at different WAP

Treatments	Mycorrhiza root colonization (%)		
	4WAP	**8WAP**	**12WAP**
Mycorrhiza			
M+	49.32	53.14	58.80
M-	18.13	20.14	22.59
LSD (0.05)	0.57	0.62	0.62
SE **(±)**	0.20	0.22	0.216
NPK (g/pot)			
S0	27.63	31.77	34.95
S1	34.75	37.20	39.99
S2	38.79	40.95	47.14
LSD (0.05)	0.69	0.76	0.75
SE **(±)**	0.25	0.27	0.265

Where; M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; SE = standard error; LSD = Least significant different. N/B: 3 replicates per treatment

Table 9: Effects of the interactions of mycorrhiza and NPK application on the mycorrhizal root colonization of *C. lucanusianus* plant at different WAP

Mycorrhiza	Soil amendment (g/pot)	Mycorrhiza root colonization (%)		
		4WAP	**8WAP**	**12WAP**
M+	S0	40.37^{c}	44.98^{c}	49.33^{c}
M+	S1	51.15^{b}	54.72^{b}	57.34^{b}
M+	S2	56.45^{a}	59.71^{a}	69.73^{a}
M-	S0	14.90^{f}	18.56^{f}	20.57^{f}
M-	S1	18.36^{e}	19.68^{e}	22.63^{e}
M-	S2	21.13^{d}	22.18^{d}	24.56^{d}
	SE (±)	0.346	0.377	0.374

Where; Means with the same letter(s) in the same column are not significantly different from each other at $p< 0.05$ using Duncan's multiple range test. M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK; SE = standard error. N/B: 3 replicates per treatment

Table 10: Effects of mycorrhiza and NPK fertilizer application on the total fungal colony count (CFU/ g soil), and on the fungi recovered in the treated soil under pot experiment

Treatments	Fungal total colony count (cfu/g soil)	Fungi species identified
M+S0	6×10^{4}	*Aspergillus* spp., *Mucor* spp.
M+S1	6×10^{4}	*Rhizospus* spp.
M+S2	8×10^{4}	*Aspergillus* spp. ,*Mucor* spp.
M-S0	4×10^{4}	*Aspergillus* spp., *Mucor* spp.
M-S1	4×10^{4}	*Rhizospus* spp., *Aspergillus* spp.
M-S2	5×10^{4}	*Aspergillus* spp., *Mucor* spp.

Where; M+ = with *Glomus clarum*; M- = without *Glomus clarum*; S0 = 0 g NPK; S1 = 0.7 g NPK; S2 = 1.2 g NPK. N/B: 3 replicates per treatment

4. Discussion

Results of this study showed that mycorrhizal inoculation enhanced the height, number of leaves, fresh and dry weights of *C. lucanusianus* plant. This could be attributed to the gainful impact of mycorrhizal inoculation as observed on different root crops (Salami *et al*., 2005; Dare *et al*., 2007; Prabawardani *et al*., 2012; Yamawaki *et al*., 2013). Mycorrhizal inoculation also help in reducing the adverse effects of petroleum hydrocarbons on plants growth, leading to enhanced plant growth as observed in the current study compared to non-mycorrhizal inoculated plants, as reported by Quinones-Aguilar *et al*., (2003). Moreover, it also enhanced the degradation of various levels of total petroleum hydrocarbons (TPH) in the contaminated soil. These results are in agreement with the previous findings of Alarcon *et al*., (2008); Khan *et al*., (2013); Gao *et al*., (2014), where they reported an enhanced degradation of TPH in crude oil contaminated soils, due to mycorrhizal inoculation.

The rate of TPH degradation within the crude oil contaminated soil was significantly enhanced with the mycorrhizal inoculation, compared to the non-inoculated soil. This is in accordance with the previous findings of Harrier and Watson, (2004), where they reported that mycorrhizal fungi favor the activities of some soil microorganisms, thus the quantity of pollutants remediated through mycorrhizal assisted remediation (MAR) is increased, due to the enhanced activities of these microorganisms. In addition, results of the present study corroborate with the findings of Weyens *et al*., (2009); where they observed improved soil remediation following inoculation of phyto-remediators with suitable microorganisms. Furthermore, findings of the current study are in agreement with the previous work conducted by Parrish *et al*., (2005), who reported that reduction of petroleum hydrocarbon toxicity is faster in soils containing plants and mycorrhizal associations. This reveals that mycorrhizal inoculation not only improves the resilience of plants to crude oil polluted soil, but also leads to additional changes in the root-zone (rhizosphere) conditions, therefore increasing the degradation or removal efficiency.

The application of NPK (15:15:15) fertilizer significantly enhanced the survival, the growth and yield parameters of *C. lucanusianus* in the crude oil contaminated soil. This supports similar studies conducted by Ayotamuno and Kogbara (2007); Eremrena and Akonye (2013); Ukpaka and Amadi, (2016), where they reported the enhanced growth in beans, cassava and maize crops planted in crude oil contaminated soil, due to soil amendments. The growth parameters increased with increasing the NPK fertilizer application rate. This was in accordance with the previous results of Ayotamuno and Kogbara, (2007), who reported that application rates in the order of 26,000 kg NPK/ ha, may be very effective in supporting plants grown on crude oil polluted agricultural soils, as in this case both the hydrocarbon degrading microorganisms and the plants are competing for available nutrients. A recent study of Fubara-Manuel *et al*., (2017) also reported the increased growth and yield parameters of maize grown in crude oil contaminated soil amended with higher rate of NPK fertilizer.

Additionally, NPK fertilizer application significantly enhanced TPH degradation in the crude oil contaminated soil compared to the control (0 kg NPK/ ha). This supports the observations of Adenipekun and Fasidi (2005); Isitekhale *et al*., (2013); Ukpaka and Amadi, (2016); Ekpobari *et al*., (2019), who reported a significant reduction in TPH level in crude oil contaminated soil with the application of soil amendments either singly or in combination. This observed decrease in TPH could be attributed to the fact that the added NPK fertilizer provides nitrogen and phosphorus to the hydrocarbonoclastic microorganisms in the crude oil contaminated soil, thereby promoting the growth and crude oil degrading capability of the microorganisms,

thus leading to improved plant growth in this crude oil contaminated soil. Previous findings of Liu *et al*., (2009) also recorded that soil amendment through biostimulation/ bioagumentation could be far better than natural attenuation.

Current fertilizer application at 1.2 g NPK/ pot (similar to 120 kg NPK/ ha) had the least residual TPH contents in the crude oil contaminated soil compared to the other application levels. This corroborates with the previous findings of Ayotamuno and Kogbara, (2007); Isitekhale *et al*., (2013); Ukpaka and Amadi (2016), who reported higher reduction in TPH levels in the crude oil contaminated soils treated with higher rates of NPK fertilizer. An earlier study conducted by Ayotamuno *et al*., (2006) reported that increased concentrations of nutrients in crude oil contaminated soil lead to greater rate of biodegradation. A recent study of Agarry, (2018) also reported that extra addition of inorganic fertilizer extensively improved crude oil biodegradation. This implies that the higher the fertilizer application rate, the more N and P in the crude oil contaminated soil will be available to the crude oil degrading microorganisms, thus better remediation process is observed.

The combined application of mycorrhiza with 120 kg NPK/ ha (1.2 g NPK/ pot) had the least residual TPH in the crude oil contaminated soil. This enhanced degradation observed is credited to the fact that mycorrhizal fungal mycelia and the surrounding soil provided suitable habitats for differing network structure of microorganisms, which increased the accessibility of high-energy metabolic substrates and their surfaces for colonization (Sen, 2003). This is in addition to the stimulatory impact of NPK fertilizer that offered nitrogen (N) and phosphorus (P) to the microorganisms associated with the breakdown of the hydrocarbons (Paudyn *et al*., 2008; Si-Zong *et al*., 2009). Moreover, the reduction in TPH could be ascribed to the fact that mycorrhiza produces an enzyme which can decompose petroleum contained in oil contaminated soil. Results of the current study are in agreement with the findings of Amalia *et al*., (2017) who observed a decrease in TPH levels in petroleum contaminated soil inoculated with mycorrhizal fungi. Current findings are in agreement with those of Gogoi *et al*., (2003), where they observed that addition of commercial oleophilic fertilizers containing nitrogen (N) and phosphorus (P) to the hydrocarbon contaminated soil increased the hydrocarbon degrading microbial abundance, and the corresponding total petroleum hydrocarbon degradation. Recently, Agarry, (2018) also reported that addition of a relatively high amount of inorganic fertilizer led to a considerable decrease in TPH content in the crude oil contaminated soil.

It was also observed that mycorrhizal inoculation combined with 120 kg NPK/ ha enhances the height, number of leaves, fresh and dry weights of the tested plant. This could be attributed to the mycorrhizal effect, which assisted the plant to overcome the physiological stress caused by the crude oil contamination. This is done by increasing the rate of nutrient uptake by the plant, especially phosphorus which is a macronutrient needed by plant for the different metabolic and respiratory processes. Similar results were also observed by Bucking and Shacker-Hill, (2005); Smith *et al*., (2003), where they reported an increase in the growth parameters of Amaranth plant due to mycorrhizal inoculation. An earlier study conducted by Bolan, (1991) referred this increase in nutrient to the expanded surface area of the contact soil, the increased movement of nutrients into the mycorrhizae, an alteration of the root environment and to the increased in nutrient storage. Generally, the hydrocarbonoclastic microorganisms that use hydrocarbons as sole sources of carbon and energy are diverse and widely distributed in nature. Current results of microbial analysis showed that hydrocarbonoclastic fungi were generally higher in the crude oil contaminated soils, compared to the non-contaminated ones. This increase in microbial population could be attributed to the presence of petroleum in the soil as suggested by Agbor *et al*., (2012); Okolo *et al*., (2005).

The fungal species isolated and identified in this study includes: *Mucor* spp., *Aspergillus* spp.,

Penicillium spp. and *Rhizospus* spp. This is in agreement with previous works of Agbor *et al*., (2012); Dawoodi *et al*., (2015); who also recovered the same fungal species in their crude oil contaminated soil. Currently, the dominant fungal species is *Aspergillus* spp. This authenticates the results of Chaillan *et al*., (2004) that *Aspergillus* and *Penicillium* spp. are the most commonly recovered fungi in tropical soils, which are able to degrade hydrocarbons. The dominance of *Aspergillus* spp. in this study could be attributed to its efficiency in degrading petroleum hydrocarbons.

Conclusion

Current results demonstrated that mycorrhizal inoculation and NPK fertilizer application significantly enhanced the tolerance of *C. lucanusianus* plant to crude oil contamination, as well as the plant survival in crude oil contaminated soil. Moreover, improved growth parameters of the same plant were detected from mycorrhizal inoculated and NPK fertilizer amended soil treatments, either singly or combined. In addition, crude oil removal from contaminated soil was observed to have occurred in all the studied soil samples. However, the quantity of crude oil removal was higher in mycorrhizal inoculated and NPK fertilizer amended treatments, compared to that of natural attenuation. Accordingly, due to the significant TPH removal recorded, the combined application of mycorrhizal inoculation with 1.2 g NPK/ pot (120 kg NPK/ ha) in addition to planting in presence of African spiral ginger is recommended as a valuable option for the bioremediation of crude oil polluted soil. Finally, there is an urgent need for further studies to identify and determine the role of the plant specific exudates present in the rhizosphere during the remediation process.

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Conflict of interest

The authors declare that they have no financial or non-financial conflict of interests with regard to the current manuscript.

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Ethical approval

This article does not contain any studies with human participant's and\ or animals performed by the authors.

5. References

Adenipekun, C.O. and Fasidi, I.O. (2005). Bioremediation of oil-polluted soil by *Lentinus subnudus*, a Nigerian white-rot fungus. African Journal of Biotechnology. 4(8): 796-798.

Adipah, S. (2019). Introduction of Petroleum Hydrocarbons Contaminants and its Human Effects. Journal of Environmental Science and Public Health. 3(1): 1-9.

Agarry, S.E. (2018). Evaluation of the Effects of Inorganic and Organic Fertilizers and Activated Carbon on Bioremediation of Soil Contaminated with Weathered Crude Oil. Journal of Applied Science and Environmental Management. 22(4):587-595.

Agbor, R.B.; Ekpo, I.A.; Osuagwu, A.N.; Udofia, U.U.; Okpako, E.C. and Antai, S.P. (2012). Biostimulation of microbial degradation of crude oil polluted soil using cocoa pod husk and plantain peels. Journal of Microbiology and Biotechnology Research. 2(3): 464-469.

Alarcon, A.; Davies Jr, F.T.; Autenrieth, R.L. and Zuberer, D.A. (2008). Arbuscular mycorrhiza and petroleum-degrading microorganisms enhance

phytoremediation of petroleum-contaminated soil. International journal of phytoremediation. 10: 251-263.

Amalia, R.G.; Cauny, N.A.; Ahmad, D.F.; Ghaisani, N.P.; Anwary, M.N. and Purbajanti, E.D. (2017). Phytoremediation of Petroleum Wastes: Use of Mycorrhiza and Hydrocarbonoclastic Bacteria in Phytoremediation with *Samanea Saman* Plant in Petroleum Contaminated Soil. International Journal of Scientific and Technology Research. 6(8): 128-131.

Ayotamuno, J.M. and Kogbara, R.B. (2007). Determining the tolerance level of *Zea mays* (maize) to a crude oil polluted agricultural soil. African Journal of Biotechnology. 6 (11): 1332-1337.

Ayotamuno, J.M.; Kogbara, R.B.; Ogaji, S.O.T. and Probert, S.D. (2006). Bioremediation of a crude-oil polluted agricultural soil at Port Harcourt, Nigeria. Applied Energy. 83(11): 1249-1257.

Bolan, N.S. (1991). A critical review of the role of mycorrhizae fungi in the uptake of phosphorus by plants. Plant and Soil. 134: 189-207.

Bouyoucos, C.H. (1951). A recalibration of hydrometer method for making mechanical analysis of soils. Agronomy Journal. 43(9): 434-438.

Bray, R.H. and Kurtz, L.T. (1945). Determination of Total Organic and Available Forms of Phosphorus in Soils. Soil Science. 59: 39-45. http://dx.doi.org/10.1097/00010694-194501000-00006

Bucking, H. and Shacker-Hill, Y. (2005). Phosphate uptake, transport and transfer by arbuscular Mycorrhizal fungus is increased by carbohydrate availability. New Phytologist. 165: 889–912.

Carling, D.E.; Richle, W.G.; Brown, M.F. and Johnson, D.R. (1978). Effect of a vesicular arbuscular mycorrhizal fungus on nitrogen reductase and nitrogenase activities in nodulating and non-nodulating soybeans. Phytopathology. 68: 1590-1596.

Chaillan, F.; Flèche, A.L.; Bury, E.; Phantavong, Y.; Grimont, P.; Saliot, A. and Oudot, J. (2004). Identification and biodegradation potential of tropical aerobic hydrocarbon degrading microorganisms. Research in Microbiology. 155: 587-595.

Chukwu, E.D. and Udoh, B.T. (2014). Effect of crude oil and industrial wastes pollution on some soil chemical properties in Ikot Abasi, Niger Delta Area, Nigeria. In Proceedings of the 38th Annual Conference of the Soil Science Society of Nigeria, 10–14th March 2014. pp. 83-88.

Dadrasnia, A.; Salmah, I.; Emenike, C.U. and Shahsavari, N. (2015). Remediation of oil contaminated media using organic material supplementation. Petroleum Science and Technology. 33: 1030-1037.

Dale, P.; Repekine, J.; Levrnskaite, L. and Lugauskas, A. (2006). Growth and metal accumulation ability of plants on soil polluted with Cu, Zn and Pb. Ekologija. 1: 48-52.

Dare, M.O.; Abaidoo, R.C.; Fagbola, O. and Aseidu, R. (2007). Variability of yam (*Dioscorea* spp.) genotypes in root colonization by Arbuscular mycorrhiza in the yam belt of Nigeria. African Crop Science Proceedings. 8: 629-638.

Dawoodi, V.; Madani, M.; Tahmourespour, A. and Golshani, Z. (2015). The Study of Heterotrophic and Crude Oil-utilizing soil fungi in crude oil contaminated regions. Journal of Bioremediation and Biodegradation. 6: 270.

Ekpobari, N.; Edmund, E.N. and Emeka, E.O. (2019). Effectiveness of NPK Fertilizer-Saw Dust Amendment on Biodegradation of Crude Oil in Polluted Soil. International Journal of Advances in Scientific Research and Engineering. 5(5): 56-67.

Eremrena, P.O. and Akonye, L.A. (2013). Growth and Biochemical performance of Cassava-*Manihot esculenta* Crantz to Crude oil Polluted soil amended with *Centrosema pubescens* Benth and NPK. Journal

of Applied Science and Environmental Management. 17(2): 195-201.

Frick, C.M.; Farrell, R.E. and Germida, J.J. (1999). Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Report submitted to Petroleum Technology Alliance of Canada (PTAC). pp. 88.

Fubara-Manuel, I.; Igoni, A.H. and Jumbo, R.B. (2017). Performance of irrigated maize in a crude-oil polluted soil remediated by three nutrients in Nigeria's Niger delta. American Journal of Engineering Research. 6(12): 180-185

Gao, Y.C.; Guo, S.H.; Wang, J.N.; Li, D.; Wang, H. and Zeng, D.H. (2014). Effects of different remediation treatments on crude oil contaminated saline soil. Chemosphere. 117: 486-493.

Gogoi, B.K.; Dutta, N.N.; Goswami, P. and Krishna-Mohan, T.R. (2003). A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site. Advances in Environmental Research. 7: 767-782.

Gong, X. (2012). Remediation of weathered petroleum oil-contaminated soil using a combination of biostimulation and modified fenton oxidation. International Bio-deterioration and Bio-degradation. 70: 89-95.

Graj, W.; Lisiecki, P.; Szulc, A.; Chrzanowski, Ł. and Wojtera-Kwiczor, J. (2013). Bioaugmentation with petroleum-degrading consortia has a selective growth-promoting impact on crop plants germinated in diesel oil-contaminated soil. Water, Air and Soil Pollution. 224: 1-.15.

Hamoudi-Belarbi, L.; Hamoudi, S.; Belkacemi, K.; Nouri, L.; Bendifallah, L. and Khodja, M. (2018). Bioremediation of Polluted Soil Sites with Crude Oil Hydrocarbons Using Carrot Peel Waste. Environment. 5(11): 124.

Harrier, L.A. and Watson, C.A. (2004). The potential role of Arbuscular Mycorrhizal (AM) fungi in the bio-protection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Management Science. 60: 149-157.

Ijah, U.J.J. and Ndana, M. (2003). Stimulated biodegradation of crude oil in soil amended with periwinkle shells. The Environmentalist. 23: 249-254.

Iquatt, C.B.; Oyewole, O.A. and Abioye, O.P. (2006). Bioremediation of petroleum polluted soil: a review. International Journal of Natural and Applied Sciences. 1(1): 21-25.

Isitekhale, H.H.E.; Aboh, S.I.; Edion, R.I. and Abhanzioya, M. (2013). Remediation of Crude Oil Contaminated Soil with Inorganic and Organic Fertilizer Using Sweet Potato as a Test Crop. Journal of Environment and Earth Science. 3(7): 116-121.

Jackson, M.L. (1958). Soil Chemical Analysis. Prentice Hall Inc. Eaglewood Cliffs, New Jersey United States of America. pp. 59 -67.

33 Juo, A.S.R.; Ayanlaja, S.A. and Ogunwale, J.A. (1976). An evaluation of cation exchange capacity measurements for soils in the tropics. Communication in Soil Science and Plant Analysis. 7(8): 751-781.

Khan, S.; Afzal, M.; Iqbal, S. and Khan, Q.M. (2013). Plant–bacteria partnerships for the remediation of hydrocarbon contaminated soils. Chemosphere. 90: 1317-1332.

Kormanik, P.P. and McGraw, A.C. (1982). Quantification of vesicular-arbuscular mycorrhizal in plant roots. In: Methods and Principles of Mycorrhizal Research. (ed Schenck, N.C.). American Phytopathological Society, St. Paul Minnesota, USA, pp. 37-45.

Liu, A. and Dalpé, Y. (2009). Reduction in soil polycyclic aromatic hydrocarbons by arbuscular mycorrhizal leek plants. International Journal of Phytoremediation. 11: 39-52.

Liu, L.; Chen, H.S.; Cai, P.; Liang, W. and Huang, Q.Y. (2009). Immobilization and phytotoxicity of Cd in contaminated soil amended with chicken manure compost. Journal of Hazardous Mater. 163: 563-567.

McLean, E. (1982). Soil pH and lime requirement. Methods of soil analysis. Part 2. Chemical and microbiological properties. 199-224.

Nelson, D.W. and Sommers, L.E. (1996). Total carbon, organic carbon and organic matter. In: Methods of Soil Analysis, Part 2, 2nd ed., A.L. Page et al., Ed. Agronomy. 9:961-1010. American Society of Agronomy, Inc. Madison, WI.

Ochei, J.O. and Kolhatkar, A.A. (2008). Medical Laboratory Science: Theory and Practice. Tata McGraw Publishing Company Limited. 7th Edition. pp. 820-821.

Okolo, J.C.; Amadi, E.N. and Odu, C.T.I. (2005). Effects of Soil Treatments Containing Poultry Manure on Crude Oil Degradation in Sandy Loam Soil. Applied Ecology and Environmental Research. 3(1): 47-53.

Parrish, Z.D.; White, J.C.; Isleyen, M.; Gent, M.P.N.; Iannucci-Berger, W.; Eitzer, B.D.; Kelsey, J.W. and Mattina, M.I. (2005). Accumulation of weathered polycyclic aromatic hydrocarbons (PAHs) by plant and earthworm species. Chemosphere. 64: 609-618.

Paudyn, K.; Rutter, A.; Rowe, K.R. and Poland, J.R. (2008). Remediation of hydrocarbon contaminated soils in the Canadian arctic by land farmin. Cold Regions Science and Technology. 53(1): 102-114.

Prabawardani, S.; Wasgito, D.P. and Nouke, L.M. (2012). The effectiveness of AM fungal in improving the tolerance of sweet potato plants to drought stress. International Conference on Agricultural, Environment and Biological Sciences (ICAEBS2012). Phuket.

Quiñones-Aguilar, E.E.; Ferrera-Cerrato, R.; Gavi-Reyes, F.; Fernandez-Linares, L.; Rodriguez-Vazquez, R. and Alarcón, Y.A. (2003). Emergence and growth of maize in a crude oil polluted soil. Agrochemical. 37: 585-594.

Riser-Roberts, E. (1998). Remediation of Petroleum Contaminated Soils. CRC Press, Boca, Raton, FL.

Salami, A.O.; Odebode, A.C. and Osonubi, O. (2005). The use of arbuscular mycorrhiza (AM) as a source of yield increase in sustainable alley cropping system. Archives of Agronomy and Soil science. 51(4): 385-390.

Schwab, A.P.; Su, J.; Wetzel, S.; Pekarek, S. and Banks, M.K. (1999). Extraction of petroleum hydrocarbons from soil by mechanical shaking. Environment Science and Technology. 33: 1940-1945.

Sen, R. (2003). The root-microbe-soil interface: new tools for sustainable plant production. New Phytologist. 157: 391-394.

Sharma, J.; Ogram, A.V. and Al-Agely, A. (2007). Mycorrhizae: Implications for Environmental Remediation and Resource Conservation. University of Florida IFAS, Florida A and M. University Cooperative Extension Program, Gainesville, Florida. ENH1086/EP351.

Si-Zong, Y.; Hui-June, J.; Zhi, W.; He, R.X.; Yan-Jun, J.; Xiu-Mei, L. and Shao-Peng, L. (2009). Bioremediation of oil spills in cold environments: a review. Pedosphere. 19(3): 371-381.

Smith, S.E.; Smith, F.A. and Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology. 133:16-20.

Soleimani, M.; Afyuni, M.; Hajabbasi, M.A.; Nourbakhsh, F.; Sabzalian, M.R. and Christensen, J.H. (2010). Phytoremediation of an aged petroleum contaminated soil using endophyte infected and non-infected grasses. Chemosphere. 81: 1084-1090.

Udo, E.J. and Ogunwale, J.A. (1986). Laboratory Manual for Analysis of Soil, Plant and Water Samples.

2nd Edition, Department of Agronomy, University of Ibadan, Nigeria. University Press Ibadan. pp. 151-162.

Ukpaka, C.P. and Amadi, S.A. (2016). Effect of organic and inorganic fertilizer on bioremediation of crude oil polluted land. Current Science Perspectives. 2(4): 83-94.

USEPA. (2017). Test method for evaluating solid wastes, physical and chemical methods. First Update. (3rd Edition). https://inis.iaea.org/search/searchsinglerecord.aspx?recordsFor=SingleRecord&RN=20065272

Weyens, N.; van der Lelie, D.; Taghavi, S.; Newman, L. and Vangronsveld, J. (2009). Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends in Biotechnology. 27: 591-598.

Yamawaki, K.; Matsumura, A.; Hattori, R.; Tarui, A.; Hossain, M.A.; Ohashi, Y. and Daimon, H. (2013). Effect of inoculation with arbuscular mycorrhizal fungi on growth, nutrient uptake and curcumin production of turmeric (*Curcuma longa* L.). Agricultural Sciences. 4(2): 66-71.

Yuniati, M.D. (2018). Bioremediation of petroleum-contaminated soil: A Review IOP Conference Series: Earth and Environmental Science. 118 012063