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Phytoremediation of Diesel Oil Polluted Soil by Fluted Pumpkin (*Telfairia Occidentalis Hook F.*) in Uyo, Niger Delta Region, Nigeria

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Abstract
Phytoremediation is an emerging technology for cleaning contaminated soils. In this study, the effect of fluted pumpkin (*Telfaira occidentalis Hook F.*) on the degradation of petroleum hydrocarbon in a diesel oil contaminated soil was investigated. Fluted pumpkin seeds were planted in each treatment (0.00, 1.11, 1.59, 2.50 and 3.06%) for a period of 18 weeks. The following parameters were determined: germination percentage, length of vine, number of leaves per vine, leaf yield and the dry weight of leaves and vines on the 3rd, 6th, 9th, 12th and 15th weeks after planting (WAP). Total petroleum hydrocarbon (TPH) was determined on the 2nd and 18th weeks after oil pollution (WAOP). Total bacterial and fungal counts were determined on the 3rd, 6th, 9th, 12th, 15th and 18th WAOP. The results demonstrated that diesel affected soil depressed seed germination. Length of vine and number of leaves per vine were observed to increase from 3rd to 15th WAOP. Leaf yield increased from 3rd to 9th WAOP and thereafter declined from 12th to 15th WAOP. The results further revealed reduction in dry matter of leaves as concentration of oil increases, whereas dry weight of vines increased with increasing oil pollution. The result also demonstrated that fluted pumpkin stimulated total bacterial and fungal number. Total petroleum hydrocarbon (TPH) removal in the polluted soil was observed to be 86.53, 94.38, 92.80 and 92.97% in 1.11, 1.59, 2.50 and 3.06% concentration respectively. Thus, fluted pumpkin has proved to be efficient for removal of TPH from oil-contaminated soil.
Keywords: Phytoremediation, diesel oil, fluted pumpkin, contaminated soils, petroleum hydrocarbon

1. Introduction

The presence of oil and refined petroleum products in the soil can lead to toxic effects on plants and soil microorganisms and acts as a source of ground water contamination (Scott, 2003). Petroleum hydrocarbon contamination of soil occurs through extraction, accidents, pipeline, raptures, consumption and refining (Scott, 2003). Most of the crude oil reservoirs and oil refineries in Nigeria are located in areas with agricultural activities and urban areas in the Niger Delta. It is believed according to UN reports, that an average riverine dweller of the Niger Delta is exposed to polluted air, polluted water and polluted food, hence facing health hazard resulting to reduced life expectancy (UN Report, 2001). Consequently, the remediation of soil impacted by oil production and transport is not only of importance considering environmental problems but also for the preservation of agricultural productivity and human health. Chemical and physical methods applied for remediation of petroleum-contaminated soils such as thermal treatment, soil washing, solidification and stabilization are expensive, disruptive to the environment and involved high-energy consumption (Kaimi et al., 2007). Therefore, natural remediation techniques have been developed to provide more environmentally friendly and cost effective cleanup of sites impacted by petroleum spills (Alkorta and Garbisu, 2001). Bioremediation has emerged as an effective technology for treatment of hydrocarbon contaminants in soils. A diverse consortium of microorganisms are capable of degrading a wide range of hydrocarbon molecules, however, biodegradation is often limited by extremes in pH, inadequate concentrations of oxygen, nutrients and high levels of contaminants such as metals. Addition of fertilizers and other amendments may accelerate the degradation rate (Bollag et al., 1994). Recent studies indicate that plant roots provide beneficial habitat for hydrocarbon degrading microbes. The use of vegetation to enhance microbial populations and activity is termed phytoremediation (Cunningham et al., 1991; US FEPA, 2000).

Phytoremediation is an emerging green technology that uses plants to remediate soil, sediment, surface and ground water contaminated with toxic metals, organic and radionuclides (Alkorta and Garbisu 2001, Gerhardt et al., 2009). This technique has been shown to be effective for petroleum-contaminated soils in several laboratory and field studies (Newman and Reynolds 2004, Euiios et al., 2008, Gerhordt et al., 2009). The plant roots seem to provide an ideal environment for degradation of organic compounds because of several mechanisms. Plant root system allows rapid movement of water and gases through the soil due to the improvement of soil structure. It also provides a biologically active soil region (that is, the rhizosphere), which encourages microbial activity and enhances bioavailability (Newman and Reynoids, 2004, Wenzel 2009). Hence, the use of plants and rhizosphere microorganisms is a promising green technology for remediation of

contaminated soils (Weyens et al., 2009). Although, phytoremediation of organic contaminated soils using endophytic bacteria, grasses (Poaceae) and legumes (leguminosae) have been a subject of several studies (April and SIMS 1990, Schwab et al., 2006, Phillips et al., 2008), there is no information about the use of *Telfairia occidentalis* (a mycorrhizal plant) on petroleum contaminated soils. Therefore, the objective of this study was to evaluate the suitability of fluted pumpkin (*Telfairia occidentalis*) for use in the phytoremediation of Diesel oil contaminated soil in Uyo, Niger Delta region, Nigeria. Fluted pumpkin was used because it is a mycorrhizal plant with taproot system having primary and extensive secondary roots. The plant can withstand stressed condition, source for water and nutrients during drought, broad leaf that can cover the entire land within a short period giving hydrocarbon degrading microorganisms favourable environment to act.

2. Materials and Methods

2.1 Study Area

The experiment was conducted at the University of Uyo Teaching and Research Farm, Uyo, Nigeria. Uyo lies between latitudes 5.17° and 5.27°N and longitudes 7.27° and 7.58°E (UCCDA, 1998) (Fig. 1). The area has distinct wet and dry seasons. The wet season starts from March to October, while the dry season starts from November to March. A short dry spell is normally noticed in August and is traditionally referred to as "August Break". The annual rainfall ranges from 2000-3000mm, and temperature is relatively high, about 28°C. The soil of this area is characterized by low organic matter arising from influence of high temperature and humid conditions. The soils are too sandy and are pruned to leaching and erosion. The soil is classified "Acid sand", because they are generally sandy and are generally acidic, with pH ranging from 4.0-6.0. They have low buffering capacity because of low activity clay.

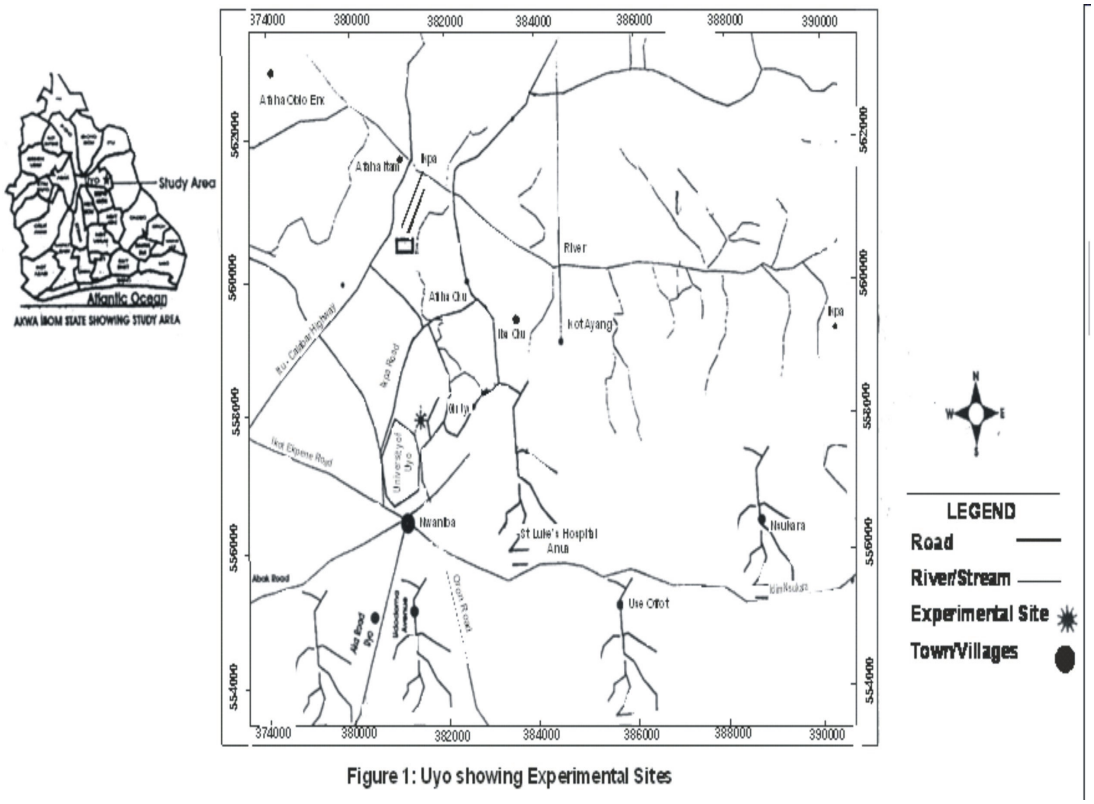

Figure 1: Uyo showing Experimental Sites

2.2 Field Study

An experiment to determine phytoremediation of diesel oil contaminated soils by fluted pumpkin (*Telfairia occidentalis Hook F.*) was conducted in 2008 for a period of 18 weeks. The experiment was laid out in completely randomized design (CRD) in triplicates. The size of the experimental plot was 216m^2 (0.0216 ha). The plot was sub-divided into three sub-plots each separated by one metre apart. Each of the plots was tilled into beds with the aid of shovel to loosen the soil and enhance aeration. The tilled soil was mixed using rakes; this was to further homogenize the soil in order to create favourable condition for plant growth. Diesel oil was then applied evenly using a fine hose watering can and properly worked into the soil using hand fork in each of the plot receiving the following treatments (0.0, 8.0, 11.5, 18.0 and 22.0 litres of diesel) in each of the replicate. Each of the treatment was converted into percentage pollution using the method of Yagodin (1982) and this

gives: 0.0, 1.11, 1.59, 2.50 and 3.06 percent pollution respectively. After treatment of the site with diesel, it was allowed to degrade for two weeks to reduce the toxicity of diesel on the test plant before planting the test crop (*Telfaira occidentalis Hook F.*). A total of 5 rows were planted in each replicate and *Telfaira occidentalis* planted at a distance of one by one (1 X 1) metre.

2.3 Percentage Emergence

This was done at 3 weeks after planting. It was done by counting total number of stands that emerged against total number of seeds planted and expressed in percentage on treatment basis.

2.4 Length of Vine

Three plants were randomly tagged from each treatment in the three plots and measurement taken on the 3, 6, 9, 12 and 15 week after planting (WAP) and results expressed in centimeter (cm).

2.5 Yield of Pumpkin Leaf

The leaves were harvested on the 3,6,9, 12 and 15 WAp and weighed using a weighing balance.

2.6 Dry Weight

This was done by partitioning the plant into leaves and vines on 3, 6, 9, 12 and 15 WAP. Oven dried, at 105^{o}C for 24 hours for corresponding dry weight.

2.7 Laboratory Studies

Soil samples were collected before pollution of the soil for physico-chemical properties of the study site. The soil was air-dried, ground and sieved with a 2mm mesh and analysed to know the nutrient status by standard methods. Particle size distribution was done by hydrometer method of Bouycoucos (1962), soil pH in 1.2.5 soil/water suspension was determined by the method of (Rowell, 1994), electrical conductivitiy (EC) in 1.5 soil/water suspension by an electrical conductivity meter (Rhoades, 1982), organic carbon was analysed by Wallkley and Black Method (1934) as modified by Nelson and Sommers (1982), total nitrogen by the Macro Kjeldahl method (Bremner and mulvaney, 1982). Avaiable phosphorus was determined by Bray P-1 method (Olsen and Sommers, 1982) and colour developed in soil extract using the ascorbic acid method (Murphy and Rilay 1962). Exchangeable bases (Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}) were extracted with IN NH_4OAC buffered at pH 7.0 (Thomas, 1982). Exchangeable Na^{+} and K^{+} were read on flame photometer, while Ca^{2+} and Mg^{2+} were read on atomic absorption spectrophotometer. Exchangeable acidity was extracted with IN KCl and determined by titration with 0.05 N NaOH using phenolphthalein indicator. Effective cation exchange capacity was taken as the summation of exchange bases ($Na^{+} + K^{+} + C^{2+} + Mg^{2+}$) and total exchangeable acidity (Chapman, 1965).

2.8 Determination of Total Petroleum Hydrocarbon (TPH)

Soil for the determination of TPH was collected on the 14th and 18th WAOP. One gramme of the soil samples was dissolved in 10ml of hexane and shaken for 10 minutes using a mechanical shaker. The solution was filtered using Whatman filter paper and the filtrate diluted by adding 1 ml of the extract into 50 ml hexane. The absorbance of this solution was read at 460 nm with spectrophotometer using n-hexane as blank. Total petroleum hydrocarbon was determined on the 2nd and 18th week after oil pollution (WAOP).

2.9 Microbiological Analysis

Soil samples from contaminated and uncontaminated soils were collected on the 2, 6, 9, 12, 15 and 18 WAOP and analysed for most probable number (mpm) of bacteria and fungi by using nutrient agar (oxoid) and *Sabrouroud Dextrose agar* (SDA) by standard plate counts technique (Alexander et al., 2005). No attempt was made to isolate and characterized any specific type of microorganism including hydrocarbon-degrading microorganisms. Total recoverable bacteria were counted on nutrient agar plates and fungi on SDA plates were recorded. The total number of colonies in each dilution was used to determine an average number per gramme of soil.

2.10 Statistical Analysis

The statistical analysis was conducted using the superior performance softwares system (SPSS) 15.0. One-way analysis of variance (ANOVA) was determined based on completely randomized design (CRD) with means separated using least significant difference (LSD). The test was used to study, number of leaf, length of vines and plant dry weight in the contaminated soil.

3. Results and Discussion

The selected physico-chemical properties of soil of the experimental site are as presented in Table 1. The results in Table 1 show selected physcio-chemical properties of soil of the experimental site with the textural class of sandy loam. It was observed that diesel oil contamination of the soil had a negative effect on fluted pumpkin (*Telfairia occidentalis Hook F.*) during the first four weeks of planting.

Table 1: Selected Physico-chemical Properties of Soil of the Experimental Sites

pH	4.90±0.003
EC dS/m	0.216
Total N (%)	0.05±0.0001
Organic C (%)	1.37±0.006
C/N	27.4±0.005
Available P (mg/kg)	120±2.0
Na+ (Cmol/kg)	0.12±0.0005
K+ (Cmol/kg)	0.08±0.00005
Mg^{2+} (Cmol/kg)	1.35±0.002
Ca^{2+} (Cmol/kg)	1.23±0.0012
Exchange Acidity (Cmol/kg)	2.88±0.003
Effective cation Exchange capacity	5.96±0.003
Base saturation (%)	51.48±1.5
Sand (%)	86±1.2
Silt (%)	6.6±0.001
Clay (%)	4.8±0.002

3.1 Seed Germination

Seed germination in some of the treatments were delayed up to 4 weeks, particularly the highest pollution level (3.06% concentration). Normally, the seeds germinate between 2-3 week, but at the end of 4 weeks, most of the seeds in 2.56 and 3.06 % pollution levels did not geminate (Fig. 2). The seeds when dug, the ungerminated seeds were observed to be swollen, indicating, that they might have absorbed the oil, which lead to reduction of germination vigour of most of the seeds. This may also be due to decreased nutrients availability to the seeds to germinate and have inhibitory effect on germination by physically impeding water and oxygen transfer between the seeds and the surrounding soil environment. This observation is similar to previous report by Udo and Fayemi (1975). They observed low maize germination due to the effect of petroleum contamination. In addition (Basalatpour et al., 2008) opined that petroleum hydrocarbon in the soil may decrease seed germination to tall fissure more than 50%.

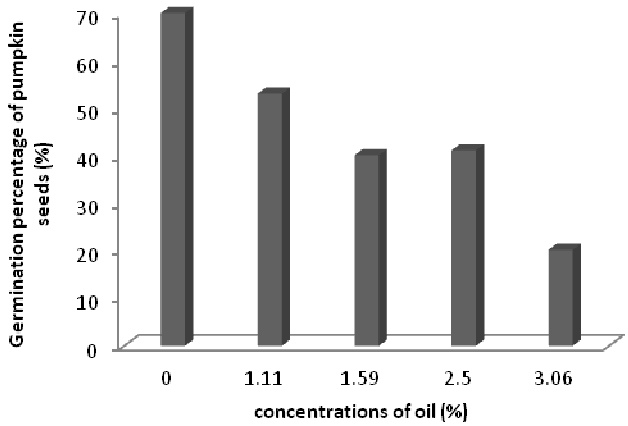

Figure 2: Effect of different concentrations of diesel oil on germination of pumpkin seeds

3.2 Length of Vine

The length of pumpkin vine increased with time and across the treatments. In 3 weeks after planting (WAP) the vine length per treatment were as follow: 1.11% = 3.66, 1.59% = 60.09, 2.50%= 62.10 and 3.06% = 65.22 cm compared to 27.77 cm of the control. In weeks 6, 12 and 15, vine length were longer in contaminated soil than uncontaminated (control), but were not significantly ($P \geq 0.05$) different from each other (Fig. 3). This result demonstrated that *T. occidentalis* increased in ability to grow in oil contaminated soil.

The result further demonstrated that *T. occidentalis* had shown to be more tolerant to stresses. The ability of *T. occidentalis* to tolerate stresses in hydrocarbon-contaminated soil may be attributed to its being a mycorrhizal plant. Mycorrhiza in plants enhances plant's ability to tolerate biotic and abiotic stresses and harsh environmental conditions in the soil. It may also be due to the effects of rhizosphere on the bioavailability and phytotoxicity of pollutants and release of secondary metabolites such as phenodic compounds into the rhizosphere, which can act as plant defense metabolites to biotic and abiotic stresses. These results corroborate

the observations by Soleimani et al., (2010), they reported that plants infected with endophyte increases plants ability to tolerate stress.

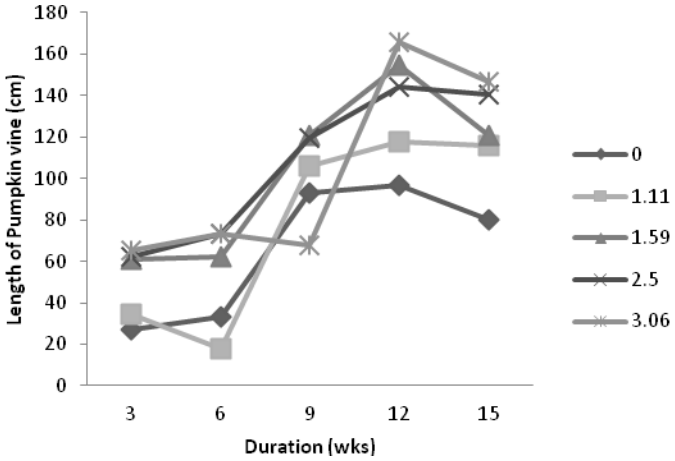

Figure 3: Effect of different concentrations of diesel oil polluted soil on length of pumpkin vine

3.3 Effect on Number of Leaves

The results show that on the 3rd and 6th WAP no significant (P≥0.05) difference were obtained in number of leaves per vine. In the 9th WAP, number of leaves increased as the concentration of diesel oil in the soil increases. The number of leaves in treatments 1.59, 2.50 and 3.06 percent of diesel oil concentrations were significantly ($P \leq 0.05$) more than the uncontaminated soil (control) (Fig. 4). The leaves in the soil with higher concentrations of diesel oil were larger succulent and greenish. The luxuriant leaf yield in the contaminated soil may be due to the effect of increased nitrogen in the soil. The large leaf surface area provides covering to the soil, acting as "life mulch". The mulching characteristic of the leaves provide conducive environment that stimulates the activity of soil organisms and hence the mineralization of organic substrates including the hydrocarbon. The leaf covering also aided in reducing erosion and this has an impact on the nutrients status of the soil. This encourages microbial activities, particularly hydrocarbonastic utilizing microorganisms.

These observations corroborate the previous report by Akpan and Ekpo (2006). They reported increased in nutrients contents of contaminated soil, which they said was due to the activities of free-living nitrogen fixing organisms in response to oil pollution of the soil.

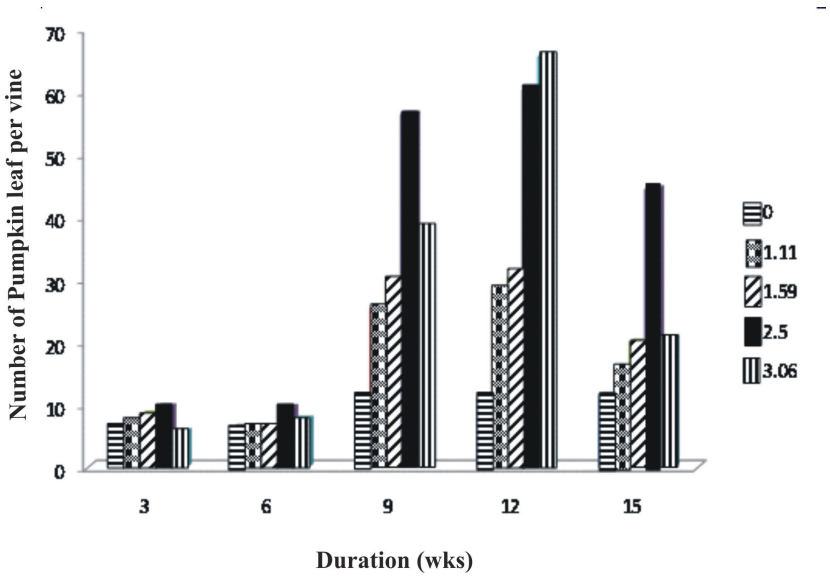

Figure 4: Effect of Different Concentrations of Diesel of Polluted Soils on Number of Pumpkin leaf per Vine

3.4 Effect on Pumpkin Vine Dry Weight

There was increase in vines dry weight as the concentration of oil in the soil increases. On the 3rd WAP dry weight of pumpkin vine decreased as the concentration of oil increases but not significantly ($P \geq 0.05$). Similarly, on the 9th WAP there was no significant ($P \geq 0.05$) change in dry weight of vines among treatments (Fig. 5).

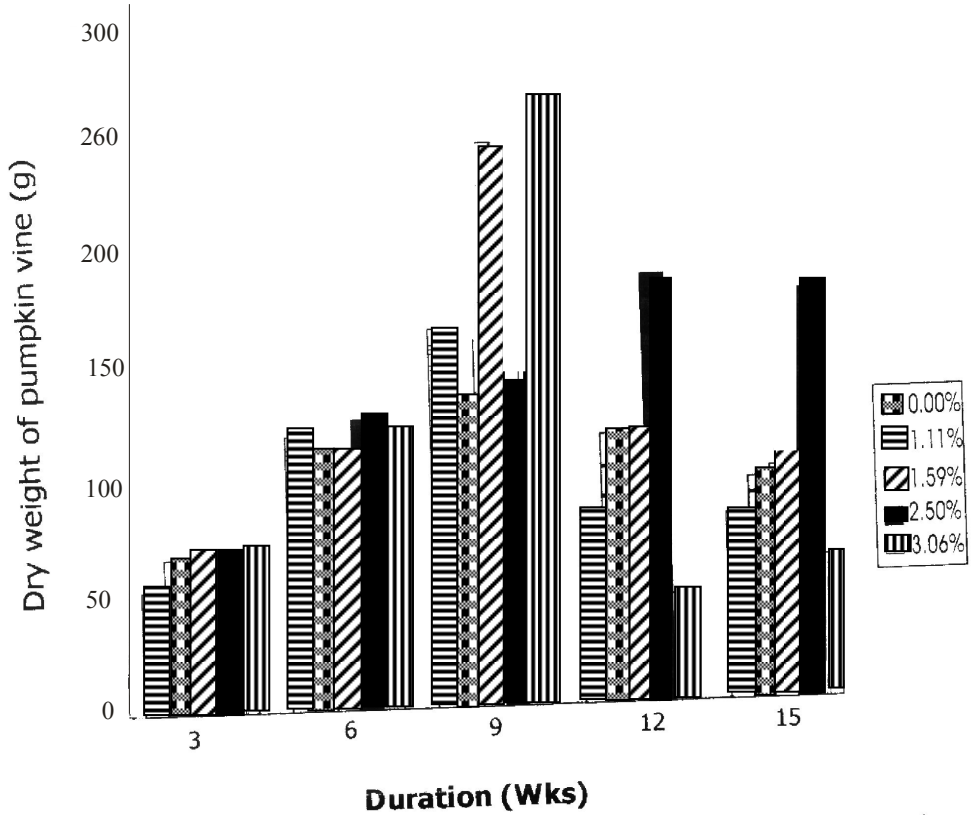

Figure 5: Effect of different concentrations of diesel polluted soils on dry weight of pumpkin vine (g)

3.5 Effect on Pumpkin Leaf Dry Weight

A consistent trend was observed on the 3rd and 6th WAP, the dry weigh was decreasing with increase in level of contamination of the soil. The control on 3rd and 6th WAP had mean values of 218.9g/plant and 205.2g/plant which were significantly ($P \leq 0.05$) higher than the treatment receiving 3.06% level of pollution with the mean values of 53.73g/plant and 85.11g/plant respectively. On the 9th, 12th and 15th WAP there was no significant difference ($P \geq 0.05$) among treatments. The reason for low dry biomass of pumpkin leaf may be due to loss in water content, because during harvest the leaves in the higher pollution levels were larger and succulent compared to the control (Fig. 6).

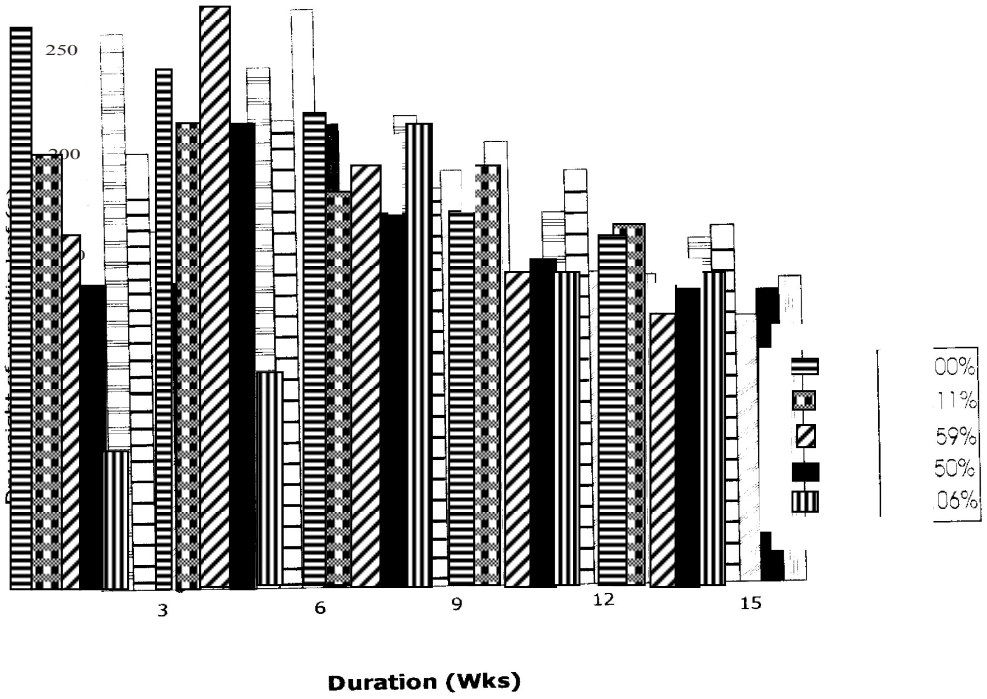

Figure 6: Effect of different concentrations of diesel polluted soils on dry weight of pumpkin leaf (g)

3.6 Effect on Leaf Yield

It was observed that the yield of pumpkin leaf increased with increase in diesel oil contamination on 3rd, 6th, 9th, 12th and 15th WAP. The highest leaf yield was recorded for the plots with 2.50% and 3.06% pollution levels with 34.33 tha^{-1} and 29.00tha^{-1} which were significantly ($P \leq 0.05$) higher than the control respectively, with 18.00^{th-1}. The reason for the increase in yield of fluted pumpkin with increase in diesel oil pollution of the soil may be due to increase in nutrients concentration in these plots. This observation was inline with previous reports by Akpan and Ekpo (2005). They recorded increase in cassava yield in plots with high concentrations of diesel oil pollution (Fig. 7).

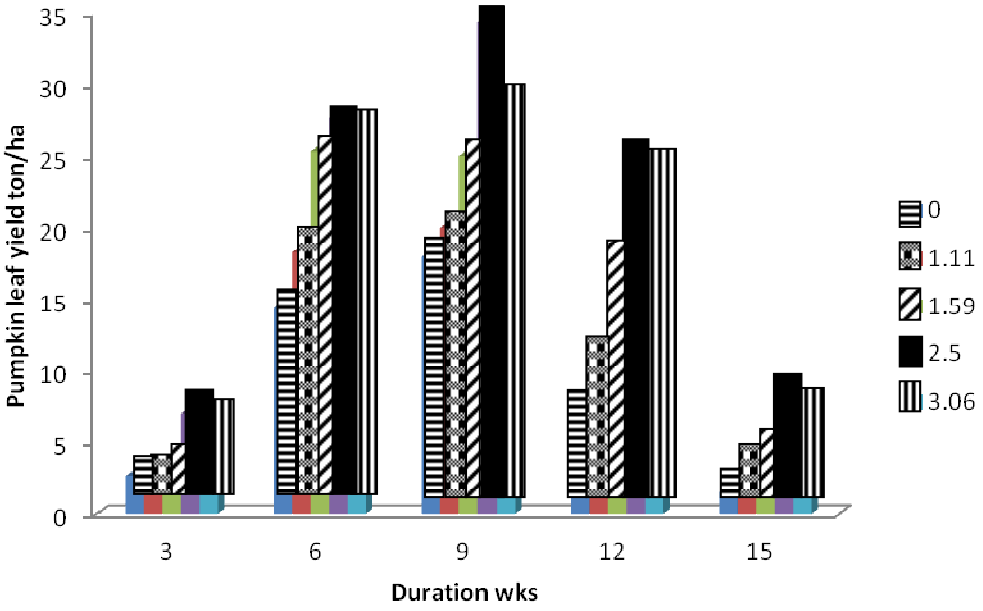

Figure 7: Effect of different concentrations of diesel of polluted soils on pumpkin leaf yield (ton/ha)

3.7 Effect on Microbial Populations

The growth of microorganisms was stimulated by the presence of plants roots, the leaf acting as "life mulch" giving favourable environment for microbial proliferation, nutrient contents due to mineralization of organic matter and the diesel. Plants can generally promote soil microbial activity through the release of organic compounds from the root system, example, amino acids, organic acids, sugars, enzymes and carbohydrates, which produce carbon source and energy for microbial growth.

This finding was in line with previous report by Van Hecke et al., (2005). Despite the stimulation of microbial community growth due to favourable environmental climate provided by the pumpkin leaves, release of carbohydrates and phenolic-like compounds from the roots of *Telfairia occidentalis* some of the rhizodeposits which are mostly found in the treatments receiving 1.59, 2.50 and 3.06% pollution levels can possibly act as microbial growth inhibitors (Fig. 8). The population of bacteria and fungi increased between treatments and with time. The number of culturable bacteria and fungi were more in the treated soil than the untreated soil (control), although not significantly particularly in week 3 and 6 due to inhibiting effect of some metabolites. But in weeks 6,9,12,15 and 18 bacterial populations in 1.59, 2.50 and 3.06% were significantly ($P \geq 0.05$) higher then those in the control plots.

Reverse was the case in fungal populations there was no significant difference ($P \geq 0.05$) among the treatments except in week 6 that the plots receiving 1.59, 2.50 and 3.06% concentrations were significantly ($P \leq 0.05$) higher than the control respectively (Fig. 9).

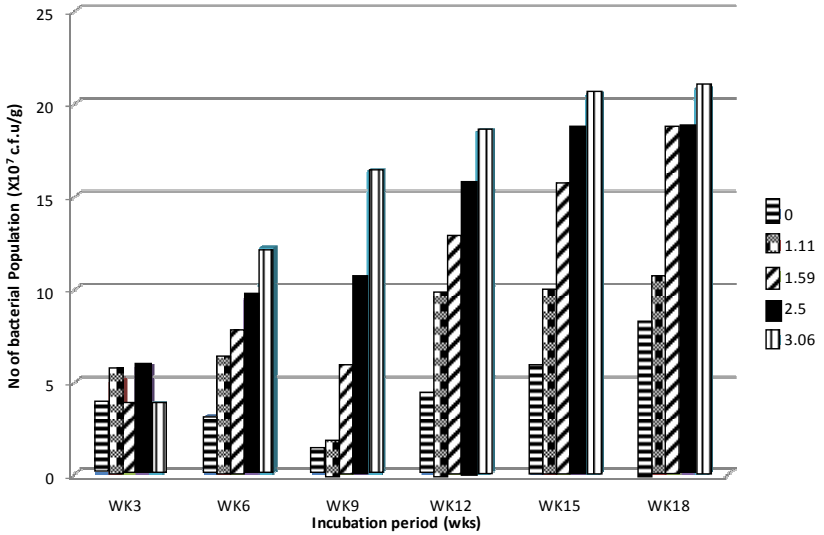

Figure 8: Effect of different concentrations of diesel oil polluted soils on bacterial population

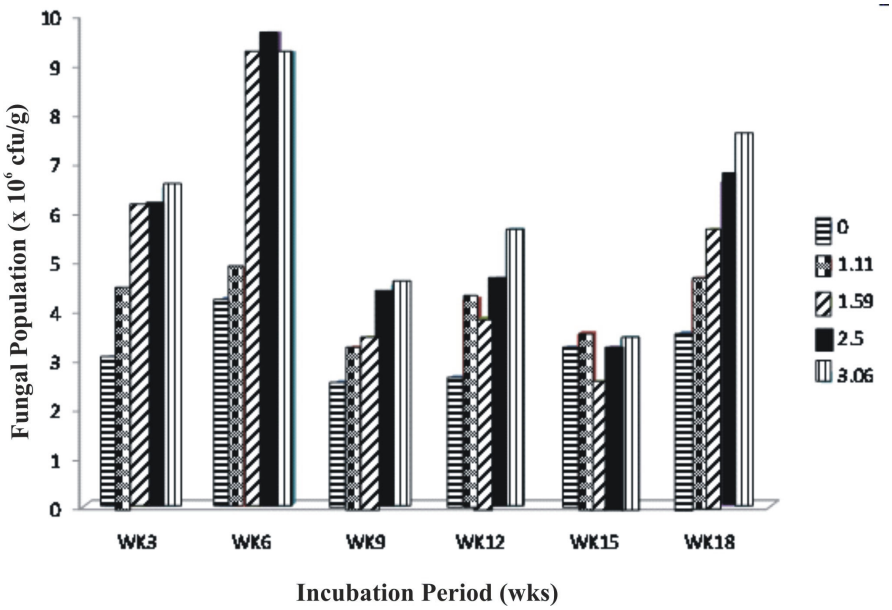

Figure 9: Effect of different concentrations of diesel oil polluted soils on fungal population

3.8 TPH Degradation in Soil

Degradation of diesel, a hydrocarbon product boiling between approximately 150^{o}C and 400^{o}C, with carbon chain length of G_5-C_{22} was determined after 2 and 18 weeks of pollution. It was observed that *Telfairia occidentalis* brought about 86.53, 94.38, 92.80 and 92.97% reduction of TPH respectively, in the 1.11, 1.59, 2.50 and 3.06% pollution levels (Table 2). It may be due to the long primary and extensive secondary root systems, root biomass and root surface area. This increased secretion of microbial enhancing metabolites such as water-soluble phenols, and therefore stimulated microbial activity in the soil. The oxidation of alkanes in the soil depends on enzyme classes which are mostly related to hydrocarbonastic degrading microorganisms. It could also be ascribed to the fact that *Telfairia occidentalis* with taproot system, that is made up of primary and extensive secondary root systems, make it possible for the plant to source for water and nutrients particularly during drought. These characteristics make *Telfairia occidentalis* suitable for phytoremediation in oil impacted soils.

Other factors that make *Telfairia occidentalis* a suitable plant for phytoremediation is its long vines and broad leaf system that covers the soil as ("life mulch") creating suitable environment for hydrocarbon degrading microorganisms activity. To the best of our knowledge, this is the first research using *Telfairia occidentalis* to remediate soil impacted with oil.

Table 2: Degradation of Total Petroleum Hydrocarbon

Concentration of Diesel (%)	**2 WAOP**	**18 WAOP**	**% Degradation**
0	ND	ND	ND
1.11	20.8mg/kg	2.8 mg/kg	86.53
1.59	60.5mg/kg	3.4mg/kg	94.38
2.50	80.6mg/kg	5.8mg/kg	92.80
3.06	82.5mg/kg	5.8mg/kg	92.97

ND = Not determined WAOP = Weeks after oil pollution

4. Conclusion

In this study it was observed that planting of *Telfairia occidentalis* in oil impacted soils could enhance dissipation of total petroleum hydrocarbon. *Telfairia occidentalis* increased the number of total and oil degrading bacteria and fungi in the contaminated soil. The plant showed more capability to stimulate degradation of TPHs in the C_{15}-C_{22} fraction and TPH in the rhizosphere. This observation might be due to enhancement of microbial degradation, which could be due to release of nutrients to the microbes. It might also be due to the favourable environment provided by the plant to enhance activity of microbial communities with certain enzyme to degrade C_{15}-C_{22} chain length alkanes.

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