African Journal of Plant Science Vol. 3 (5), pp. 102-106, May, 2009
Available online at http://www.academicjournals.org/AJPS
ISSN 1996-0824 © 2009 Academic Journals

Full Length Research Paper

Screening of four common Nigerian weeds for use in phytoremediation of soil contaminated with spent lubricating oil

Ogbo Erute Magdalene[1]*, Avwerosovwe Ufuoma[2] and Odogu Gloria[3]

[1]Department of Botany and Microbiology, University of Lagos, Akoka-Yaba, Lagos, Nigeria.
[2]Department of Botany, Delta State University, Abraka, Nigeria.
[3]Department of Botany, University of Benin, Benin City, Nigeria.

Accepted 13 May, 2009

Phytoremediation is a non destructive and cost effective *in situ* technology that can be used for the cleanup of contaminated soils. The potential for this technology in the tropics is high due to the prevailing climatic conditions which favour plant growth and stimulates microbial activity. The present study investigated the use of use of 4 common weeds in Nigeria - (*Phyllanthus amarus* Schum and Thonn., *Hyptis spicigera* Lam., *Sida rhombifolia* L. and *Mariscus alternifolius* Vahl.) for their reaction to spent lubricating oil contamination and subsequent reduction of the contaminant by the plants. Shoot length, leaf area and root length and chlorophyll contents were determined for these plants grown in spent lubricating oil contaminated soils. The residual hydrocarbons were extracted from soil and percentage degradation was gravimetrically determined for the total hydrocarbons and saturated hydrocarbons present in the spent lubricating oil. The contamination caused a reduction in the shoot length, leaf area, root length and total chlorophyll content of the test plants used. A statistically significant influence of spent lubricating oil on the test plants could not however be established. The degradation of total petroleum content was low as the highest degradation recorded was 35.30% for the plant *P. amarus*, however appreciable degradation of the saturated hydrocarbons as the plant *S. rhombifolia* and *M. alternifolia* removed over 60% of the saturated hydrocarbons present. *H. spicigera* recorded the least degradation for the saturated hydrocarbons (39.04%). The growth of the plants- *S. rhombifolia* and *M. alternifolius* which caused a reduction of over 60% of the saturated hydrocarbons makes these 2 plants choice plants for the remediation of spent lubricating oil from contaminated soils. The 2 plants are unwanted plants indigenous to Nigeria and can be found growing abundantly in the wild.

Key words: Phytoremediation, phyto-assessment, contamination, spent lubricating oil.

INTRODUCTION

Contamination of soil by oil spills is a wide spread environmental problem that often requires cleaning up of the contaminated sites (Bundy et al., 2002). Disposal of oil based wastes, oil spills from well blow outs and pipeline ruptures are the most common sources of petroleum contamination (Reis, 1996). The indiscriminate disposal of spent lubricating oil by motor mechanics is a common source of spent lubricating oil contamination of soil in countries like Nigeria that do not enforce strict compliance to environmental laws. Crude oil and its products' spills affect plants adversely by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. It has been recorded that oil contamination causes slow rate of germination in plants. According to Adam and Duncan (2002) this effect could be because the oil acts as a physical barrier preventing or reducing access of the seeds to water and oxygen.

Some plants can render harmless, extract or stabilize a contaminant in soil, thus making it unavailable for other organisms and reducing environmental hazards in a process termed phytoremediation (Cunningham et al.,

*Corresponding author. E-mail: erute70@yahoo.com.

1996). Current phytoremediation techniques require that plants live in the zone of contamination. Consequently plants' viability is a critical issue in the successful application of phytoremediation. Understanding plants responses to contaminant stress defines whether a plant has the potential to transform and tolerate a wide range of organic chemicals which would enhance phytoremediation. If the contaminant in its present concentration is not phytotoxic, cultivation of plants can be a valuable tool in soil remediation (Merkl et al., 2004). The mechanisms and efficiency of this technology called phytoremediation depend on the type of contaminant, bioavailability and soil properties (Cunningham and Ow, 1996). The mechanism believed to be responsible for most of the degradation of petroleum hydrocarbons in vegetated soil is the stimulation of growth and activity of degrading microorganisms in the rhizosphere (Frick et al., 1999). There are several approaches to selecting candidate plants for phytoremediation of soils contaminated with organic pollutants. These approaches have been based on the occurrence of plants under specific climatic conditions (Gudin and Syratt, 1975; Banks et al., 2003) their resistance to pollutant phytotoxicity (Kirk et al., 2002), the presence of phenolic compounds in the plant root exudates (Hegde and Fletcher, 1996; Liste and Alexander, 1999) or their capability to reduce the pollutant concentration in soil. Most studies on the phytoremediation of petroleum hydrocarbon contaminated soils have employed grasses (poacae) and legumes (legu-minosae) (Aprill and Simms, 1990; Gunther et al., 1996, Merkl et al., 2005; Kirkpatrick et al., 2006; Qui et al., 1997; Schwab et al., 2006).

Some weeds of the grass family are considered to be particularly suitable for phytoremediation since they offer an increased rhizosphere zone because of their multiple ramified root systems. This gives room for more microbial activity and growth around the root zone (Aprill and Sims, 1990). Researchers like, Adam and Duncan, (1999); Merkl et al., (2004, 2005) have concluded that grasses and legumes are the best candidates for the process of phytoremediation or rhizoremediation because of their root systems. Bioremediation is generally considered a promising technology for the tropics because climatic conditions favour microbial growth and activity (Merkl et al., 2004). For example grasses like *Panicium maximum* and *Brachiara brizantha* were able to degrade 55 and 63% of oil and grease present in the contaminated soils in the tropics (Merkl et al., 2004). Also biomass production is high in the tropics provided adequate nutrients are available. Consequently phytoremediation of hydrocarbon contaminated soils, a composite effect of microorganisms and plants can be thought to be particularly effective in the tropics. The screening of plant species for their ability to grow and establish in contaminated soil is one of the first steps in the selection of species for phytoremediation in the tropics, followed by the evaluation of their influence on the degradation of petroleum hydrocarbons in soil (Merkl et al., 2004). However little is known about tropical species that could serve for the cleanup of oil contamination. This investigation therefore choose weeds commonly found in abundance in Nigeria for screening for the purpose of phytoremediation in a country like Nigeria where environmental contamination with petroleum based products occur frequently. The plants chosen are *Phyllanthus amarus* Schum and Thonn., *Hyptis spicigera* Lam., *Sida rhombifolia* L. and *Mariscus alternifolius* Vahl. *Phyllanthus amarus* belongs to the family Euphorbiaceae it is a common weed of cultivated fields which is very wide spread in West Africa (Akobundu and Agyakwa, 1998). *H. spicigera* Lam. of the family Lamiaceae is a widespread weed in the Guinea-savannah zone. It occurs mostly in moist soils in bush fallows and by the road side. It is cultivated as a food flavouring agent and used locally as an insect repellent for protecting cowpea seeds and other grains during storage (Akobundu and Agyakwa, 1998). *S. rhombifolia* of the family Malvaceae called wireweed is also wide spread in West Africa and commonly found on roadsides, grasslands, pastures and disturbed areas in both the derived savannah and Guinea savannah zones (Akobundu and Agyakwa, 1998). *Mariscus alternifolius* of the family Cyperaceae is common weed of cultivated crops and wastes. It is very widespread in west Africa, especially Nigeria (Akobundu and Agyakwa, 1998).

MATERIALS AND METHODS

The plants *P. amarus, H. spicigera, S. rhombifolia* and *M. alternifolius* were collected from the wild from fallow farmlands in Abraka Delta State, Nigeria. The roots and stems were cropped leaving behind 5 cm of stems. Soil samples were collected from a fallow plot of land in Abraka, Nigeria. The soils were air dried and filtered using 2 mm mesh gauze to remove debris. A composite mixture was made and mixed homogenously. Spent lubricating oil got from a mechanic workshop (112.35 ml) was mixed homogenously with 1000 g of soil to get a 10.00% level of contamination. There were 2 controls; one had spent lubricating oil but no plant was introduced into the contaminated soil, the other had no spent lubricating oil but had each plant planted in the pots used. Chlorophyll content of all harvested test plants was spectrometrically got after extraction with acetone. Total petroleum content was got gravimetrically by weighing after extraction with chloroform from the soil. The saturated hydrocarbons were got from fractionation of the total petroleum content using silica gel with mesh size 60 - 100 in a column and eluting with n-hexane. The fraction got was weighed using the Metler's analytical balance.

5 replicates were made for each treatment. Differences (≤ 0.05) among treatments were tested using analysis of variance using Duncan's multiple range tests and LSD (SPSS for windows version 13.00)

RESULTS

All the test plants used grew successfully in the 10.00% spent lubricating oil contaminated soil. Spent lubricating oil contamination caused reduction in the growth of the 4 test plants. The shoot height, root length and leaf areas of the plants were consistently lower in contaminated soils compared to the control (Tables 1, 2 and 3). There was significant reduction only in the shoot height of the *P.*

Table 1. Plant height of plants grown in spent lubricating oil contaminated soils (cm).

Weeds	Contaminated soil	Control (no contamination)
P. amarus	24.17 ± 4.02[a]	31.39 ± 3.10[b]
H. spicegera	8.68 ± 0.45[c]	15.44 ± 0.46[d]
S. rhombifolia	7.33 ± 0.00[c]	9.88 ± 1.61[c]
M. alternifolius	9.43 ± 0.23[c]	13.43 ± 1.77[cd]

Means followed by same letters do not differ significantly according to LSD (P≤ 0.5)

Table 2. Leaf area of plants grown in spent lubricating oil contaminated soils (cm^2).

Weeds	Contaminated soil	Control (no contamination)
P. amarus	15.41 ± 0.49[a]	24.38 ± 2.74[b]
H. spicegera	2.59 ± 0.17[c]	4.49 ± 0.40[c]
S. rhombifolia	2.28 ± 0.00[c]	3.54 ± 0.52[c]
M. alternifolius	8.64 ± 0.78[a]	12.19 ± 0.89[a]

Means followed by same letters do not differ significantly according to LSD (P ≤ 0.5)

Table 3. Root length of plants grown in spent lubricating oil contaminated soils.

Weeds	Contaminated soil	Control (no contamination)
P. amarus	7.65 ± 1.45[a]	12.05 ± 0.44[a]
H. spicegera	3.11 ± 0.52[b]	3.88 ± 0.38[b]
S. rhombifolia	3.12 ± 0.00[b]	3.73 ± 0.81[b]
M.alternifolius	8.20 ± 1.22[a]	12.07 ± 1.19[a]

Means followed by same letters do not differ significantly according to LSD (P ≤ 0.5)

Table 4. Effect of spent lubricating oil on chlorophyll A content of test plants (mg/g).

Weeds	Contaminated soil	Control (no contamination)
P. amarus	0.1266 ± 0.03[a]	0.2101 ± 0.04[a]
H. spicegera	0.0336 ± 0.01[a]	0.0951 ± 0.01[a]
S. rhombifolia	0.6410 ± 0.00[a]	0.9237 ± 0.46[a]
M. alternifolius	-	-

Means followed by same letters do not differ significantly according to LSD (P ≤ 0.5)

Table 5. Effect of spent lubricating oil on Chlorophyll B of test plants (mg/g).

Weeds	Contaminated soil	Control (no contamination)
P. amarus	0.1863 ± 0.04[a]	0.2889 ± 0.05[a]
H. spicegera	0.0347 ± 0.03[a]	0.1059 ± 0.04[a]
S. rhombifolia	0.8888 ± 0.00[a]	1.2626 ± 0.67[a]
M. alternifolius	-	-

Means followed by same letters do not differ significantly according to LSD (P ≤ 0.5)

Table 6. Effect of spent lubricating oil on total chlorophyll content of test plants (mg/g).

Weeds	Contaminated soil	Control (no contamination)
P. amarus	0.3128 ± 0.09[a]	0.4988 ± 0.08[a]
H. spicegera	0.6830 ± 0.02[a]	0.2009 ± 0.05[a]
S. rhombifolia	1.5293 ± 0.00[b]	2.921 ± 1.25[b]
M. alternifolius	-	-

Means followed by same letters do not differ significantly according to LSD (P≤ 0.5)

amarus and *H. spicegera.* There was significant reduction in the leaf area of the plant *P. amarus.* The highest shoot height was recorded in *P. amarus* and the least in *S. rhombifolius* (Table 1). Leaf area was also highest in *P. amarus* and least in *S. rhombifolia* using the contaminated soil values (Table 2). Root length was however highest in *M. alternifolius* and least in *H. spicigera* and *S. rhombifolia* (Table 3). The reduction in root length was however not significant.

Spent lubricating oil caused a general reduction in the chlorophyll a, b and total chlorophyll content of all but one of the test plants (Tables 4, 5 and 6). The total chlorophyll content of *H. spicegera* was higher in the contaminated soil treatment than the control (Table 6). There was however no significant reduction in the chlorophyll content caused by spent lubricating oil in the soil (Tables 4, 5 and 6). The increase in chlorophyll content recorded in the plant *H. spicegera* was also not significant (Table 6).

There was reduction in the total hydrocarbon content and the saturated hydrocarbon content in the contaminated soil. The percentage degradation which was dependent on the control and the various treatments varied with the type of plant species used. The percentage degradation of total petroleum content for all test plants was not appreciable as the highest recorded was 35.26% with *P. amarus* (Table 7). The plants however performed better in the removal or reduction of saturated hydrocarbons. The plant *S. rhombifolia* removed 87.94% of the saturated hydrocarbons in spent lubricating oil contaminated soils (Table 7). *P. amarus* which recorded the highest percentage degradation for total petroleum content however recorded the least degradation for saturates (39. 04%) (Table 7).

DISCUSSION

The assessment of the *plants P. amarus, H. spicigera, S. rhombifolia* and *M. alternifolius* for phytoremediation was done by measuring the effect of the contaminant on the continued growth of the plants in the contaminated soils.

Table 7. Percentage degradation of residual total and saturated hydrocarbon content of soil exposed to different weeds.

Weeds	Percentage degradation of Total Petroleum Hydrocarbons (%)	Percentage degradation of saturated hydrocarbons
P. amarus	35.26[a]	45.89[a]
H. spicegera	23.21[b]	39.04[a]
S. rhombifolia	12.30[c]	87.94[b]
M. alternifolius	27.00[b]	61.34[c]

Means followed by different superscript letters are significantly different within the same column (P≤ 0.05)

The plants grew successfully in the 10.00% level of spent lubricating oil contamination tested. There was however reduction in the shoot height and leaf area and lateral root length of the test plants. Chaineau et al., (1997) recorded similar results with a growth rate reduction of beans and wheat by 80.00%. This report agrees with that of Molina-Barahona et al., (2005) who recorded reduced elongation of stems and roots caused by diesel fuel contamination in the species of plants used. They inferred that this could be due to the impermeability effect of the fuel or the immobilization of nutrients mainly nitrogen or by inhibitory effects of some of the polycyclic aromatic hydrocarbon components. This reason can also be adopted here since the similar chemical components apply. Baud-Grasset et al. (1993) however reported that a reduction in root length is a sensitive plant response to exposure to chemical substances. The reduction of stem length plant relative to control may have resulted from a systemic toxic effect of translocation of long chain alkanes to stems (Molina-Barahona et al., 2005). The experimental data on the uptake of alkanes by higher plants (Palmroth et al., 2002) and simulations of a plant uptake model (Trap et al., 1994) indicated that there is rapid uptake of long chain (heavy) alkanes into fine roots but slow translocation to stems and leaves because of their low solubility in water but high sorption to roots. The reducetion in plant growth may also be an effect of the small aliphatic, aromatic and naphtha and phenolic like compounds that may reduce respiration, transpiration and photosynthesis system II (Trap et al., 2005, Vouillamoz and Milke, 2001). These compounds are present in crude oil, diesel fuel and spent lubricating oil and can account for the effects recorded in this study. Vega-Jarquin et al., (2001) reported however that the physiological responses of a plant are species dependent. In this study however a statistically significant influence of spent lubricating oil on the growth of the test plants could not be established for all growth parameters measured. Based on this the 4 test plants have potentials for phytoremediation of spent lubricating oil contaminated soils.

The study also had the objective of seeking out species that can be used for phytoremediation by their response to the contaminant in question. Kirk et al. (2002) confirms that the phytotoxicity assay to assess plant species for phytoremediation of petroleum contaminated sites reduces the number of pot or green house degradation studies that need to be conducted before plant species can be chosen for petroleum phytoremediation. This assay also provides a rapid, efficient mechanism of pre-screening potential plant species and eliminating those not able to germinate and establish in soil conditions present in the contaminated sites (Kirk et al., 2002). Plant bioassays in combination with an assay measuring the biodegradation potential may be useful in screening the efficacy of phytoremediation for a particular site. *S. rhombifolia* and *M. alternifolius* are thus presented as plants that have high potential for phytoremediation of crude oil contaminated soils. This is in line with Kirk et al. (2002) they selected alfalfa as the best candidate for phytoremediation on the basis of its high resistance to the phytotoxicity of oil contaminated soils. The choice of the two plants above emanates from the fact that they were able to enhance the removal of over 60% of the saturated hydrocarbons present compared to the other two test plants used. The two species are also indigenous to Nigeria and can be found growing abundantly in the wild as weeds or unwanted plants.

In conclusion, these species are worthy of further study with respect to its use in phytoremediation of crude oil polluted sites which abound in oil producing countries like Nigeria, Venezuela, Ecuador and Indonesia that are found in the tropics.

REFERENCES

Adam G, Duncan H (2002). Influence of diesel on seed germination. Environ. Pollut. 120: 363-370

Akobundu IO, Agyakwa CW (1998). A hand book of West African Weeds. Ibadan. International Institute of Tropical Agriculture p. 162.

Aprill W, Sims RC (1990). Evaluation of the use of prairie grass for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere 20:253-265.

Banks MK, Schwab P, Liu B, Kulakow PA, Smith JS, Kim R (2003). The effects of plants on the degradation and toxicity of petroleum contaminants in soil: A field assessment. Adv. Biochem. Eng. Biotechnol. 78: 75-96.

Baud-Grasset F, Baud-Grasset S, Safferman SI (1993). Evaluation of the Bioremediation of a Contaminated Soil with Phytotoxicity Tests. Chemosphere 26:1365-1374.

Bundy JG, Paton GI, Campbell CD (2002). Microbial communities in different soil types do not converge after diesel contamination. J.

Appl. Microbiol. 92:276-288.
Chaineau CH, Morel JL, Oudot J (1997). Phytotoxicity and plant uptake of fuel oil hydrocarbons. Environ. Qual. 26:1478-1483.
Cunningham SD, Anderson TA, Schwab PA, Hsu FC (1996). Phyto-remediation of soils contaminated with organic pollutants. Adv. Agron. 56:55-114.
Cunningham SD, Ow DW (1996). Promises and prospect of phytoremediation. Plant Physiol. 110: 715-719.
Frick CM, Farrell RE, Germida JJ (1999). Assessment of phytoremediation as an in situ technique for cleaning oil contaminated sites. Petroleum Technology Alliance Canada: Calgary, Canada p. 88.
Gallegos Martinez M, Gomez Santos A, Gonzalez Cruz L, Montes de Oca Garcia MA, Yanez Trujillo L, Zermeno Eguia Liz JA, Gutierrez-Rojas M (2000). Diagnostic and resulting approaches to restore petroleum-contaminated soil in a Mexican tropical swamp. Wat. Sci. Tech. 42:377-384.
Gudin C, Syratt WJ (1975). Biological aspects of land rehabilitation following hydrocarbon contamination. Environ. Pollut. 8:107-112.
Gunther T, Dornberger U, Fritsche W (1996). Effects of ryegrasss on biodegradation of hydrocarbons in soil. Chemosphere. 33:203-215.
Hegde RS, Fletcher JS (1996). Influence of plant growth stage and season on the release of root phenolics by mulberry as related to development of phytoremediation technology. Chemosphere 32: 2471-2479.
Kirk JL, Klironomos JN, Lee H, Trevors JT (2002). Phytotoxicity Assay to Assess Plant Species for Phytoremediation of Petroleum Contaminated Soil. Bioremed. J. 6: 57-63.
Kirkpatrick WD, White PM Jr, Wolf DC, Thoma GJ, Reynolds CM (2006). Selecting plants and nitrogen rates to vegetate crude-oil-contaminated soil. Int. J. Phytorem. 8: 285-297.
Liste HH, Alexander M (1999). Rapid screening of plants promoting phenanthrene degradation. J. Environ. Qual. 28:1376-1377.
Merkl N, Schultze-Kraft R, Infante C (2004). Phytoremediation in the Tropics- The Effect of Crude Oil on the Growth of Tropical Plants. Bioremediation J. 8:177-184.
Merkl N, Schultze-Kraft R, Infante C (2005). Assessment of tropical grasses and legumes for phytoremediation of petroleum contami-nated soils. Water Air Soil Pollut. 165:195-209.
Molina-Barahona L, Vega-Loyo L, Guerrero M, Ramirez S, Romero I, Vega-Jarquin C, Albores A (2005). Ecotoxicological Evaluation of Diesel-Contaminated Soil Before and After a Bioremediation Process. www.interscience.wiley.com pp.100-109.
Palmroth MRT, Pichtel J, Puhakka JA (2002). Phytoremediation of subarctic soil contaminated with diesel fuel. Bioresource Technol. 84: 221-228.
Qui X, Leland TW, Shah SI, Sorensen DL, Kendall EW (1997). Field study: Grass remediation of for clay soil contaminated with polycyclic aromatic hydrocarbons. In: Kruger EL, Anderson TA, Coats JR (eds) Phytoremediation of Soil and Water Contaminants, ACS Symposium Series 664, pp.186-199.
Reis JC (1996). Environmental Control in Petroleum Engineering. Houston, TX, Gulf.
Schwab P, Banks MK, Kyle WA (2006). Heritability of phytoremediation potential for the alfalfa cultivar Riley in petroleum contaminated soil. Water Air Soil Pollut. 177:239-249.
Trap S, McFarlane C, Mathies M (1994). Model for uptake of xenobiotics into plants-validation with bromacil experiments. Environ. Toxicol. Chem. 13: 413-422.
Trap S, Kohler A, Larsen LC, Zambrano KC, Karlson U (2005). Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. J. Soil and Sediments. 1:71-76.
Vega-Jarquin C, Dendooven L, Magana-Plaza L, Thalasso F, Ramos-Valdivia A (2001) Biotransformation of hydrocarbon by cells cultures of Cinchoma robusta and Dioscorea composite. Environ. Toxicol. Chem. 20:2670-2675.
Vouillamoz J, Milke MW (2001). Effect of compost in phytoremediation of diesel-contaminated soils. Water Sci. Technol. 45: 291-295.