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Phytoremediation potential of *Nauclea diderrichii* (De Wild and Th. Dur.) seedlings grown in spent engine oil contaminated soil

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Abstract

Remediation of environmental pollution has been of great concern in recent years. This study was carried out to investigate the potentials of *Nauclea diderrichii* seedlings to remediate heavy metals from spent engine oil (SEO) contaminated soil. SEO from diesel and petrol engines in different quantities were applied to each plant using 2 kg soil. Plant height, collar diameter, number of leaves, root length were assessed. Amount of lead and cadmium present in the leaves, stem and root of the seedlings were assessed at the end of the experiment. Result showed that 100 % of the seedlings subjected to various treatments survived for the duration of 5 months. There was no significant difference ($\alpha >_{0.05}$) in the height of *N. diderrichii* seedlings. Significant ($\alpha =_{0.05}$) negative reductions were observed in the seedlings collar diameter, no. of leaves and root length. There was significant difference ($\alpha >_{0.05}$) in the amount of lead and cadmium extracted by *N. diderrichii* seedlings. This study has been able to show that *N. diderrichii* has the ability/potential to phyto-extract heavy metals from soil.

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Introduction

Among the indigenous tree species that have been successfully raised in plantations in Nigeria, *Nauclea diderrichii* is the most dominant (Onyekwelu, 2007). *Nauclea diderrichii* is a species of plant in the Rubiaceae family. It is found in Nigeria and some other African countries. Its natural habitat is subtropical or tropical moist lowland forest. The wood of this tree is known as Bilinga, or sometimes Aloma in Germany and Opepe in England and Nigeria. It is a very fast growing tree species. The fast growing ability of this tree species makes it a good candidate specie for remediation of environmental pollution.

Pollution is one of the most important problems around the world today in which thousands of millions of world inhabitants suffer health problems related to industry and atmospheric pollutants. Recent years have witnessed significant attention to the problems of environmental contamination by wide variety of chemical pollutants including heavy metals (El-Demerdash and Elagamy, 1999). Environmental pollution by heavy metals is now a global issue that requires considerable attention. Soils contaminated with heavy metals usually lack established vegetation cover due to the toxic effects of the heavy metals (Salt *et al*., 1995).

Spent engine oil (SEO) is a product of crude oil which causes serious environmental pollution in Nigeria (Nwaogu *et al*., 2008; Njoku *et al*., 2009; Agbogidi *et al*., 2013). Recently, soil contamination with SEO is becoming a challenge owing to the increasing use of this hydrocarbon in engines of power generators, and small and articulated vehicles. This increase

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in use is resulting in accidental spillage and/or purposeful discharge of SEO on agricultural lands, forests and water sources (Nwaogu *et al*., 2008; Njoku *et al.,* 2009; Ogbo, 2009). SEO is phytotoxic to plants at low concentrations, reduces soil fertility, soil micro flora population and could cause a significant reduction in soil organic carbon (Wyszkowski *et al*., 2004; Wyszkowski and Ziólkowska, 2008; Iqbal *et al*., 2016).

Heavy metals such as lead and cadmium are trace elements present in petroleum products which are toxic to both plants and animals. In the majority of studies, grasses and legumes have been singled out for their potential in remediating contaminated soil. Trees however also have attributes to phytoremediate but not many studies have been carried out on assessing the phytoextracting potentials of tropical trees. The potential uses and selection of suitable plant species for phytoremediation research and implementation is one of the challenges that has not been met and a pre-requisite for successful phytoremediation research. Heavy metals cannot be broken down, the only possible means is extraction from contaminated soil and translocation to harvestable portions. Hence, seedlings of *Nauclea diderrichii* were studied to assess the potential of this species to extract and store heavy metals from soil polluted with spent engine oil with a view to provide safe method to reclaim crude oil degraded lands in Nigeria and other parts of the world.

Materials and method

Experimental site

This study was carried out in the Silviculture nursery, Department of Sustainable Forest Management, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. FRIN is located on the longitude 07^{0}23'18"N to 07^{0}23'43"N and latitude 03^{0}51'20"E to 03^{0}51'43"E. The climate of the study area is the West African monsoon with dry and wet seasons. The dry season is usually from November through March and is characterized by dry cold wind of harmattan. The wet season usually starts from April to October with occasional strong winds and thunderstorms. Mean annual rainfall is about 1548.9 mm, falling within approximately 90 days. The mean maximum temperature is 31.9^{0}C, minimum 24.2^{0}C while the mean daily relative humidity is about 71.9% (FRIN, 2016).

Germination and raising of N. diderrichii seedlings

Seeds of *N. diderrichii* were sown in a medium composing of river sand and topsoil at ratio 1:1. After 12 days, germination occurred. The germinated seeds were watered daily and transplanted into polypots filled with topsoil soil after full development of 2 leaves. The seedlings were watered daily for the period of 3 months. Three months old *N. diderrichii* seedlings of equal height, with 4 leaves and good vigour were randomly selected from the batch of seedlings raised in the Silviculture nursery, Silviculture section of Department of SFM, FRIN.

Collection of spent engine oil

Spent engine oils were collected from two cars from Aleshinloye Mechanical Workshop, off Alalubosa Estate, Ibadan, Oyo State. Details on the type of the car, age of the cars, type of engine, brands of the engine oil and period the oil was used by car were documented (Table 1). Four (4) liters of spent engine oil from both diesel engine and petrol engine were collected into different containers and transported to FRIN.

Table I. Information on the SEO samples collected

Details	SEO D	SEO P
Car type	Land rover LR4 2015	Acura TL 2007
Age of car	2 years	10 years
Engine	Diesel V6	Petrol V6
Brand of oil	Visco 2000 API SL/CF-4	AZ Crown Super API/SJ/CF-4
Period oil was used	3 months	4 months

Experimental procedure

Preparation of contaminated soil

Forest topsoil was collected from FRIN arboretum and sieved using 2mm sieve to remove stones, roots and other materials that may be detrimental to the growth of the seedlings. To this soil, different levels of spent engine oil 0 ml (Control), 50, 100, 150 and 200 ml of SEO from diesel and petrol engines per 2 kg of soil were deliberately added to contaminate the soil before filling in polythene pots. Measuring cylinder was used to measure the spent engine into their various concentrations while weight balance was used to measure the soil to 2 kg into different polythene pots.

Introduction of seedlings to contaminated soil

After preparation of soil samples, a total of 45 seedlings were transplanted into the contaminated soils with different concentrations and without contamination (control). Five seedlings were used per treatment. The seedlings were watered daily to field capacity. The growth of the seedlings wasobserved and data was collected fortnightly for 5 months. The mean maximum and minimum temperature of the screen house during the experiment was (29.5^{0}C and 23.9^{0}C) respectively while mean daily relative humidity was 71.5 %.

Data collection

Number of survived seedlings was recorded after 5 months, growth variables (height (cm), collar diameter (mm), root length (cm) and leaves production) were assessed fortnightly.

Physicochemical analysis of soil

The soil was sieved through a 2mm sieve size and then analysed in the laboratory to determine the physicochemical properties. Particle size distribution was determined by hydrometer method (Bouyoncous, 1962).

Chemical analysis of the soil

Soil pH was determined using glass electrode pH meter in distilled water according to Thomas (1996) at 1:2 soil /water ratio, available P was determined by Bray-I method as described by Kuo (1996), the organic carbon was measured by wet combustion method (Walkley, 1947) and converted to organic matter by multiplying the values of organic carbon by a factor of 1.724 following the procedure of Jackson as reported by Sparks (1996). Total nitrogen concentration was determined by Macro-kjeldahl method according to Bremner and Mulvanery (1982) and exchangeable Ca, Mg and K were extracted with neutral normal ammonium acetate buffer according to Sparks (1996). K was determined using Flame Photometer (Gallenkamp Model FH 500) and exchangeable Ca and Mg by Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Model 210 VGP).

Heavy metals analysis of the soil

To determine the heavy metals (Pb and Cd) present in the topsoil, the method of Dewis and Freitas (1970) was used. Sample of 5 g of the soil was air dried for several days, after which alcoholic magnesium nitrate was added. It was then heated to dryness and conc. HCl was added to the cooled sample. The soil sample was then heated again and after cooling, conc. HCl and water was added to the dried sample and solution was boiled. After boiling, the solution was cooled, shaken and filtered. Atomic Absorption Spectrometry (AAS) (Buck Scientific Model 210 VGP) was then used to determine the presence and concentration of lead and cadmium.

Heavy metal analysis of plant samples

Heavy metal analysis was done at the end (5 months) of the experiment. At the end of the experiment, all the seedlings were removed from the soil and washed thoroughly with distilled water. Afterwards, the seedlings were oven dried for 24 h at 70^{o}C. Dried seedlings were separated into roots, stem and leaves for each treatment, and each part of the seedlings was milled into powdery form. Each milled component were thoroughly mixed and stored in small polythene bags until acid digestion following the method of Allen (1974). Then 5 g of each sample was placed in 25 ml digestion tubes and 10 ml of conc HNO_3 was added. The samples were heated for 45 minutes at 90^{o}C, then the temperature was increased to 150^{0}C, and the sample was boiled for 8 h. until a clear solution is obtained. The solution was then filtered and filtrate was transferred quantitatively into 25 ml volumetric flask by adding distilled water. Heavy metal analysis was carried out using an AAS (Buck Scientific Model 210 VGP) to determine the presence and concentration of lead, cadmium and arsenic in samples of plantlets and seedlings grown in each contaminated tissue culture and soil treatment. Values obtained for the heavy metal analysis were expressed as mg/kg.

Data analysis

Data collected were subjected to descriptive analysis and one-way Analysis of Variance (ANOVA) and means found to differ significantly were subjected to follow-up test procedure using Duncan Multiple Range Test (DMRT).

Results and discussion

Physical and chemical properties of soil

Results obtained showed that pH of the soil was 6.22 while electrical conductivity of the soil was 295 (ms/cm). Total organic matter was 0.57 % while total nitrogen was 0.16 %. Proportion of sand was higher than clay and silt (77.48, 9.4 and 12.87 %). Calcium (10.81) was higher than magnesium (9.88) and potassium (1.33) in the soil. Phosphorus content in the soil was very high (142.21 mg/kg). lead in the soil was 0.03 mg/kg and cadmium was 0.01 mg/kg while arsenic was below detection limit (Table II).

Chemical properties of spent engine oil

Result obtained showed that pH of SEO from diesel engine was 5.85 while pH of SEO from petrol engine was 5.83. Lead content of SEO from diesel engine was 19.47 while lead content of SEO from petrol engine was 19.65. Cadmium content of SEO from diesel and petrol engines was 1.25 and 1.38 respectively (Table III).

Table II. Physical and Chemical properties of soil

Parameters	Soil
pH	6.22
Electrical conductivity (ms/cm)	295
Total organic matter (%)	0.57
Total nitrogen (%)	0.16
Exchangeable acidity (meq/100 g soil)	0.23
K (meq/100 g soil)	1.33
Ca (meq/100 g soil)	10.81
Mg (meq/100 g soil)	9.88
P (mg/kg)	142.21
Clay (%)	12.87
Silt (%)	9.4
Sand (%)	77.48
Pb (mg/kg)	0.03
Cd (mg/kg)	0.01

Table III. Chemical properties of spent engine oil (Selected components only were determined)

Parameters	Spent engine oil (Diesel engine)	Spent engine oil (Diesel engine)
pH	5.85	5.83
Pb (mg/kg)	19.47	19.65
Cd (mg/kg)	1.25	1.38

Effect of spent engine oil on the survival rate of N. diderrichii seedlings

Result showed that 100 % of the seedlings subjected to various treatments survived for the duration of 5 months (Table IV).

Effect of spent engine oil on the mean height of N. diderrichii seedlings

At the end of the 5th month, the mean height of *N. diderrichii* seedlings subjected to various treatments ranged from 10.87 to 13.96 cm. Seedlings without SEO (control) had the highest height of 13.96 cm while seedlings treated with 150 ml SEO from petrol engine had the lowest height of 10.87 cm (Table V). The result of ANOVA revealed no significant difference (p>0.05) in the height of *N. diderrichii* seedlings subjected to various treatments (Table VI). However, the height of seedlings untreated was higher than the seedlings treated with SEO (Table V).

Table IV. Survival rate of seedlings of *N. diderrichii*seedlings

Treatment	No. of survived seedlings	Survival rate %
Control	5	100
50 mL SEO D	5	100
100 mL SEO D	5	100
150 mL SEO D	5	100
200 mL SEO D	5	100
50 mL SEO P	5	100
100 mL SEO P	5	100
150 mL SEO P	5	100
200 mL SEO P	5	100

where, SEO = spent engine oil, D = diesel engine,
P = petrol engine

Table V. Mean height of *N. diderrichii* seedlings after 5 months

Treatment	Height (cm)
Control	13.96±1.80[a]
50 ml SEO D	12.26±0.82[a]
100 ml SEO D	12.56±0.59[a]
150 ml SEO D	11.33±0.68[a]
200 ml SEO D	12.07±0.22[a]
50 ml SEO P	12.08±1.22[a]
100 ml SEO P	11.62±1.07[a]
150 ml SEO P	10.87±1.49[a]
200 ml SEO P	11.06±0.98[a]

mean±SE followed by the same superscripts in column are not significantly different (p>0.05)
where, SEO = spent engine oil, D = diesel engine,
P = petrol engine

Effect of spent engine oil on the mean collar diameter of N. diderrichii seedlings

At the end of the 5th month, the mean collar diameter of *N. diderrichii* seedlings subjected to various treatments ranged from 2.20 to 3.19 mm. Seedlings without SEO (control) had the highest collar diameter of 3.19 mm while seedlings treated with 200 mL SEO from diesel engine had the lowest collar diameter of 2.20 mm (Table VII). The results of ANOVA revealed a significant difference ($p \leq 0.05$) in the collar diameter of *N. diderrichii* seedlings subjected to various treatments (Table VI). Mean separation showed that collar diameter of seedlings untreated with SEO was significantly different ($p \leq 0.05$) from the collar diameter of seedlings treated with SEO while seedlings treated with SEO were not significantly different ($p > 0.05$) from each other (Table VII).

Effect of spent engine oil on the mean No. of leaves of *N. diderrichii* seedlings

At the end of the 5th month, the mean leaf production of *N. diderrichii* seedlings subjected to various treatments ranged from 6 to 9 leaves. Seedlings without SEO (control) had the highest leaves produced while seedlings treated with 150 ml SEO D had the lowest leaves produced (Table VIII). The result of ANOVA revealed a significant difference ($p \leq 0.05$) in the leaf production of *N. diderrichii* seedlings subjected to various treatments (Table VI). Mean separation showed that leaf production

Table VI. ANOVA results for growth parameters of *N. diderrichii* seedlings

Parameter	SV	df	SS	MS	F	Sig.
Height	Spent engine oil	8	34.92	4.36	0.74	0.66^{ns}
	Error	36	212.07	5.89		
	Total	44	246.99			
Collar diameter	Spent engine oil	8	3.44	0.43	3.42	0.01*
	Error	36	4.53	0.13		
	Total	44	7.98			
Leaf production	Spent engine oil	8	35.62	4.45	5.51	0.00*
	Error	36	29.12	0.81		
	Total	44	64.74			

ns- not significant ($p > 0.05$) *- significant ($p \leq 0.05$)

Table VII. Mean collar diameter of *N. diderrichii* seedlings after 5 months

Treatment	Collar diameter (mm)
Control	3.19±0.24^{a}
50 ml SEO D	2.34±0.12^{b}
100 ml SEO D	2.51±0.23^{b}
150 ml SEO D	2.45±0.10^{b}
200 ml SEO D	2.20±0.07^{b}
50 ml SEO P	2.32±0.25^{b}
100 ml SEO P	2.42±0.13^{b}
150 ml SEO P	2.35±0.05^{b}
200 ml SEO P	2.28±0.05^{b}

mean±SE followed by the same superscripts in column are not significantly different ($p > 0.05$)
where, SEO = spent engine oil, D = diesel engine, P = petrol engine

Table VIII. Mean no. of leaves of *N. diderrichii* seedlings after 5 months

Treatment	Number of leaves
Control	9.16±0.12^{a}
50 ml SEO D	7.48±0.26^{b}
100 ml SEO D	6.88±0.39^{bc}
150 ml SEO D	6.04±0.42^{c}
200 ml SEO D	6.24±0.28^{bc}
50 ml SEO P	6.80±0.44^{bc}
100 ml SEO P	6.56±0.19^{bc}
150 ml SEO P	6.28±0.54^{bc}
200 ml SEO P	6.88±0.67^{bc}

mean±SE followed by the same superscripts in column are not significantly different ($p > 0.05$)
where, SEO = spent engine oil, D = diesel engine, P = petrol engine

of seedlings untreated with SEO was significantly different ($p \leq 0.05$) from the leaf production of seedlings treated with SEO. Leaf production of seedlings treated with 50 ml SEO D was not significantly different ($p > 0.05$) from seedlings treated with 150 ml SEO D but significantly different ($p \leq 0.05$) from other seedlings treated with SEO (Table VIII).

Table IX. Mean root length of *N. diderrichii* seedlings after 5 months

Treatment	Root length (cm)
Control	35.38 ± 0.25^{a}
50 ml SEO D	28.50 ± 0.32^{b}
100 ml SEO D	19.55 ± 0.27^{d}
150 ml SEO D	13.48 ± 0.23^{g}
200 ml SEO D	9.30 ± 0.27^{i}
50 ml SEO P	24.44 ± 0.33^{c}
100 ml SEO P	17.58 ± 0.29^{e}
150 ml SEO P	16.50 ± 0.29^{f}
200 ml SEO P	12.50 ± 0.27^{h}
Sig.	0.00

** significant ($p \leq 0.05$)
mean± SE followed by the same superscripts in column are not significantly different ($p > 0.05$)
where, SEO = spent engine oil, D = diesel engine, P = petrol engine

Effect of spent engine oil on the mean root length of N. diderrichii seedlings

At the end of 5th month, the root length of the seedlings ranged from 9.30 to 35.38 cm (Table IX). Seedlings untreated with SEO had the longest root system while the seedlings treated with 200 mL D had the shortest root system (Table IX). ANOVA results revealed significant difference ($p \leq 0.05$) in the root length of the seedlings subjected to various treatments. Mean separation results showed that the root length of seedlings subjected to various treatments were significantly different ($p \leq 0.05$) from each other. The root length of *N. diderrichii* seedlings subjected to 50 mLSEO D (28.50 cm) was higher than the seedlings subjected to 50 ml SEO P (24.44 cm). Root length of seedlings subjected to 100 mL SEO D (19.55 cm) was higher than that of 100 mL SEO P (17.58 cm). Root length of seedlings subjected to 150 ml SEO P (16.50 cm) was higher than the root length of seedlings subjected to 150 ml SEO D (13.48 cm) while root length of seedlings subjected to 200 mL SEO P (12.50 cm) was higher than the root length of seedlings subjected to 200 ml SEO D (9.30 cm). Moreover, increase in SEO caused decrease in the root length and vice visa (Table IX; Figs. 1 and 2).

Amount of lead (Pb) extracted by N. diderrichii seedlings

Result revealed a significant difference ($p \leq 0.05$) in the mean amount of Pb in leaves, stem, root and total mean Pb of the seedlings subjected to various treatments (Table X). Mean Pb in the leaves ranged from 2.0 to 2.1 mg/kg. Seedlings treated with 50 ml SEO D had the lowest Pb in leaves while seedlings treated with 200 ml SEO D and 50 ml SEO P had the highest Pb in leaves (Table X). Mean Pb in the stem of the seedlings ranged from 1.0 to 2.6 mg/kg. Seedlings untreated with SEO had the lowest Pb in stem while seedlings treated with 100 ml SEO P had the highest Pb in stem (Table X). Mean Pb in the root of the seedlings ranged from 6.0 to 31.0 mg/kg. Seedlings untreated with SEO had the lowest Pb in root while seedlings treated with 50 ml SEO D had the highest Pb in root (Table X). Result showed that the mean total Pb in the seedlings ranged from 10.0 to 56.0 mg/kg. Seedlings untreated with SEO had the lowest total Pb while seedlings treated with 50 ml SEO P and 200 ml SEO D had the highest Pb (Table X).

Amount of cadmium (Cd) extracted by N. diderrichiiseedlings

Result revealed a significant difference ($p \leq 0.05$) in the mean amount of Cd in leaves, stem, root and total mean Cd of the seedlings subjected to various treatments (Table XI). Mean Cd in the leaves of seedlings ranged from 0.00 to 2.00 mg/kg. Seedlings treated with 100 ml SEO D and 50ml SEO P had the lowest Cd in the leaves while seedlings treated with 100, 150 and 200 ml SEO D had the highest Cd in leaves (Table XI). Mean Cd in the stem of seedlings ranged from 0.00 to 2.00 mg/kg. Seedlings treated with 50 ml SEO D and control had the lowest Cd in the stem while seedlings treated with 100 and 200 ml SEO P had the highest Cd in the stem (Table XI). Mean Cd in the root of the seedlings ranged from 0.00 to 2.00 mg/kg. Seedlings treated with 100 ml SEO D and control had the lowest Cd in the root while seedlings treated with 100, 150 and 200 ml SEO P had the highest Cd in the root (Table XI). Result showed that the mean total Cd in the seedlings ranged from 1.00 to 7.00mg/kg. Seedlings treated with 100 ml SEO D and control had the lowest total Cdwhile seedlings treated with 200 ml SEO P had the highest total Cd (Table XI).

The textural class of the soil used for this study was sandy loam. The pH value of the soil was slightly acidic compared

Fig. 1. Root of seedlings without SEO (control)

to the pH of the SEO. Organic carbon and total nitrogen recorded low values according to Landon (1991) rating. Available phosphorus was high (Enwezor *et al*., 1982). Heavy metal concentration in SEO indicated that the values of Pb and Cd were within the normal safe range in soils (Alloway, 1990).

Pollution caused by petroleum and its derivatives is the most important problem in the environment (Iqbal *et al*., 2016) and it is receiving worldwide attention (Millioli *et al*., 2009). Survival rate of the seedlings of *N. diderrichii* was 100 % after growth monitoring for the period of 5 months. Although, the seedlings of *N. diderrichii* that were subjected to different concentrations of SEO had showed lower growth characteristics than the seedlings grown in uncontaminated soil. This implies that the seedlings did not have a good condition for growth and also there was no easy access to nutrients in the soil.

The observed increase in the seedlings grown in the uncontaminated soils could be seen as the pure nature of the soil structure which allowed normal good growth of the plants. Growth reduction in the seedlings of *N. diderrichii* subjected to SEO could be interpreted as being due to the detrimental effects of SEO which could have shown up either in the distortion or reduction in the number of stomata per unit area of the leaf, thereby, affecting photosynthetic process and consequently, the amount of photosynthates produced. Growth reductions in some plant species following oil pollution of soil have earlier been reported by some researchers (Anoliefo and Vwioko, 1995; Anoliefo *et al*., 2003; Vwioko and Fashemi, 2005). Interference with the soil-water-relation as well as, nutrient immobilization and the presence of heavy metals could also be responsible for the observed reduction in plant parameters observed as seen in this study.

The result obtained from this study showed that SEO inhibited seedling growth as evidenced by reduction in plant height. The findings on height of *N. diderrichii* seedlings are in correlation with the work reported for *Jatropha curcas, Moringaoleifera*, *Parkinsonia aculeate* (Agbogidi and Eruotor, 2012; Agbogidi and Ilondu, 2013; Iqbal *et al*., 2016).

Mean collar diameter of *N. diderrichii* seedlings grown in soil polluted with higher concentration of SEO was adversely affected which is a pointer to the fact that the contaminant (SEO) at higher level is phytotoxic. The findings on the collar diameter of *N. diderrichii* in this study is in correlation with the findings of Agbogidi and Eruotor (2012) who reported significant difference in the collar diameter of *M. oleifera* seedlings as influenced by different levels of SEO.

The highest leaf production of *N. diderrichii* seedlings was obtained from control treatment. Significant reduction in leaves of *N. diderrichii* seedlings grown in soil polluted with SEO implies that SEO is inhibitory to plant growth and this could be attributed to large amount of hydrocarbons in used oil, including the highly toxic poly-aromatic hydrocarbons (PAHs) as reported by Wang *et al*. (2000).

The negative effects of SEO contamination on reduction of the root length of *N. diderrichii* showed that the higher the concentration of SEO the lower the growth of the root of the seedlings. This shows the toxicity level of this pollutant in soil or water (Petukhov *et al.,* 2000).

Yellowness of leaves of *N. diderrichii* seedlings subjected to SEO of varying concentrations could be due to nutrient immobilization as oil pollution has been reported to cause unavailability of some essential nutrients while some toxic ones like Pb as observed in this study may be more readily available (Benka-Coker and Ekundayo, 1995; Benka-Coker and Ekundayo, 1997; Agbogidi and Ejemete, 2005). The observed leaf drop/fall and wilting could be linked to the inability of the seedlings to absorb water because they were watered like other seedlings in the uncontaminated soils. This could be due to the alteration

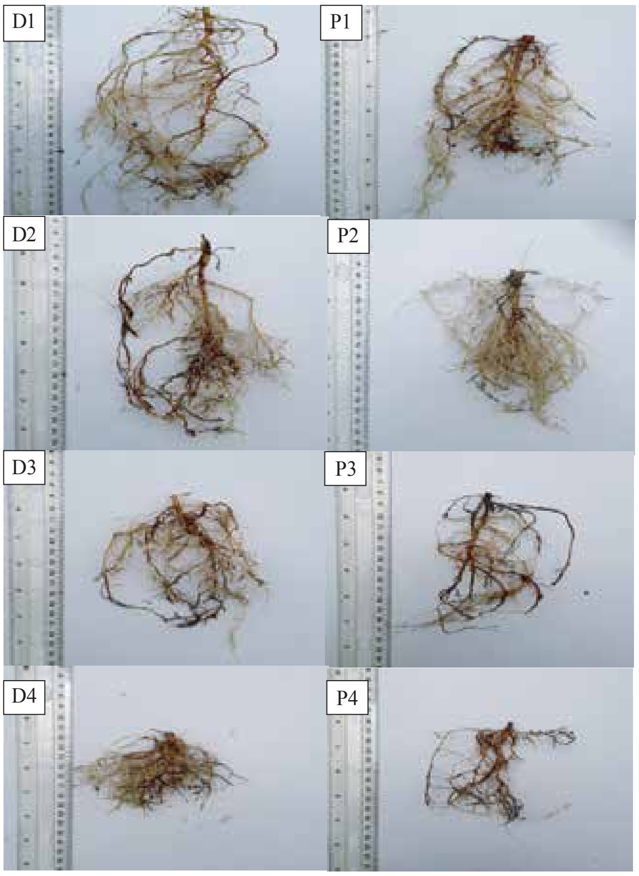

Fig. 2. Root samples of N. diderrichii seedlings from different SEO

caused by the presence of the oil in soil and this observation is in agreement with the findings of (Atuanya, 1987; Ekundayo and Obuekwe, 1997; Agbogidi and Eruotor, 2012) that oil in soil affects the physical, chemical and biological properties of the soil. Growth retardation could have been attributed to both soil-plant-water interrelations as well as a disruption in the xylem and phloem vessels due to the adulterated soil structure (Agbogidi and Eruotor, 2012). This observation correlates with the reports of Agbogidi and Ofuoku, (2005); Agbogidi and Eshegbeyi, (2006) that oil level seemed to exert the greatest influence on plant growth and yields.

The presence of other toxic components of the SEO as well as heavy metals could have also accounted for the growth reduction and stagnation (Agbogidi and Eruotor, 2012). Plant tissue analyses (leaves, stems and roots) showed higher amounts of Pb and Cd in seedlings subjected to vary concentration of SEO compared with tissues obtained from seedlings subjected to uncontaminated soils. These findings agree with earlier reports (Osubor and Anoliefo, 2003; Okafor andNwajei, 2006; Agbogidi *et al*., 2007a and b; Agbogidi and Egbuchua, 2010) that heavy metals abound in soils contaminated with petroleum hydrocarbons.

The Pb and Cd content in the tissue of seedlings subjected to SEO from petrol engine was higher than that of seedlings subjected to SEO from diesel engine. Agbogidi and Eruotor (2012) also reported that presence of any metal varies from site to site depending on the source of individual pollutants. Achuba and Peretiemo (2008) reported that excessive uptake of metals by plants may produce toxicity in human nutrition. For example, Cd can lead to acute gastrointestinal and respiratory damages and acute heart, brain and kidney damages. High concentration of heavy metals in soil can negatively affect plant growth as these metals interfere with metabolic functions in plants including physiological and biochemical processes, inhibition of photosynthesis and respiration (Osubor and Anoliefo, 2003; Achuba and Peretiemo, 2008). Other effects of heavy metals in plants according to Onweremadu and Doruigbo (2007), Nwuche and Ugoji (2008) and Nwachukwu *et al*. (2010) include degeneration of main cell-organelles and even leading to death. Oboh *et al*. (2002) reported that Pb is known to cause some biochemical and structural changes in fresh water snail when accumulated in the flesh of snail. Soil contamination with heavy metals may also cause changes in the composition of soil microbial community and other biological and biochemical properties as well as the physical and chemical properties of the soil following the lowering of soil pH (Agbogidi and Eruotor, 2012).

Conclusion

The growth of *N. diderrichii* seedlings were affected by the application of SEO from both diesel and petrol engines. Increase in the concentration of SEO from both diesel and petrol engines caused decrease in the rooting system of the seedlings. Yellowness of the seedlings' leaves revealed the nutrient immobilization in the seedlings. The study revealed that contamination of the soil medium with SEO from both diesel and petrol engines can lead to a gradual accumulation of heavy metals which have a direct effect on the tissues of the plants grown in such environment. Pb and Cd were easily extracted by seedlings of *N. diderrichii* in SEO collected from petrol engine than the SEO collected from diesel engine. Although the amount of Pb and Cd observed in this study are below the tolerable limits and gradual accumulation and bio-magnification of these non-biodegradable elements, a rise to a dangerous level with their inherent health hazard could be envisaged in man and animals. This study indicated that organisms including plants

Table X. Mean Pb in the seedlings of *N. diderrichii*

Treatment	Leaf Pb (mg/kg)	Stem Pb (mg/kg)	Root Pb (mg/kg)	Total Pb (mg/kg)
Control	3.00±0.00[g]	1.00±0.00[g]	6.00±0.00[i]	10.00±0.00[h]
50 ml D	2.00±0.00[h]	2.00±0.00[f]	31.00±0.00[a]	35.00±0.00[e]
100 ml D	6.00±0.00[f]	8.00±0.00[c]	27.00±0.00[b]	40.00±0.00[d]
150 ml D	13.00±0.00[b]	15.00±0.00[b]	24.00±0.00[c]	52.00±0.00[b]
200 ml D	21.00±0.00[a]	15.00±0.00[b]	20.00±0.00[d]	56.00±0.00[a]
50 ml P	21.00±0.00[a]	15.00±0.00[b]	20.00±0.00[e]	56.00±0.00[a]
100 ml P	9.00±0.00[d]	26.00±0.00[a]	10.00±0.00[g]	45.00±0.00[c]
150 ml P	7.00±0.00[e]	6.00±0.00[d]	7.00±0.00[h]	20.00±0.00[g]
200 ml P	10.00±0.00[c]	4.00±0.00[e]	15.00±0.00[f]	29.00±0.00[f]
Sig.	0.00*	0.00*	0.00*	0.00

** significant at ($p \leq 0.05$)
mean± SE followed by the same superscripts in column are not significantly difference ($p>0.05$)
where, SEO = spent engine oil, D = diesel engine, P = petrol engine

Table XI. Mean Cd in the seedlings of *N. diderrichii*

Treatment	Leaf Cd (mg/kg)	Stem Cd (mg/kg)	Root Cd (mg/kg)	Total Cd (mg/kg)
Control	1.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	1.00±0.00[e]
50 ml SEO D	1.00±0.00[b]	0.00±0.00[c]	1.00±0.00[b]	2.00±0.00[d]
100 ml SEO D	0.00±0.00[c]	1.00±0.00[b]	0.00±0.00[c]	1.00±0.00[e]
150 ml SEO D	1.00±0.00[b]	1.00±0.00[b]	1.00±0.00[b]	4.00±0.00[c]
200 ml SEO D	1.00±0.00[b]	1.00±0.00[b]	1.00±0.00[b]	2.00±0.00[d]
50 ml SEO P	0.00±0.00[c]	1.00±0.00[b]	1.00±0.00[b]	2.00±0.00[d]
100 ml SEO P	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]	5.00±0.00[b]
150 ml SEO P	2.00±0.00[a]	1.00±0.00[b]	2.00±0.00[a]	5.00±0.00[b]
200 ml SEO P	2.00±0.00[a]	2.00±0.00[a]	2.00±0.00[a]	7.00±0.00[a]
Sig.	0.00*	0.00*	0.00*	0.00

** significant at (≤ 0.05)
mean±SE followed by the same superscripts in column are not significantly different ($p>0.05$)
where, SEO = spent engine oil, D = diesel engine, P = petrol engine

accumulate metals from their environments. Oil spillage can cause reduction in the root length and root hairsas observed from this study thereby limiting the nutrients and water available for plants. Furthermore, Pb was stored more in the roots than other parts of the seedlings while Cd is almost evenly distributed to different parts (leaves, stem and root) of the seedlings. The seedlings of this species has been able to survive and extract heavy metals from soil contaminated with SEO for the period of 5 months which shows that this species has the potential of harvesting and storing heavy metals thereby reducing environmental degradation.

A further research is needed on the recovery possibility of *N. diderrichii* after the effect of pollutants. Addition of fertilizers (organic or inorganic) to contaminated soil can be examined with the test species. Chlorophyll content and stomata conductance of the leaves of seedlings subjected to SEO should be examined. Nutrient translocation in plant tissue parts after exposure to SEO should be examined. This will help in environmental stability of parts of the world where crude oil pollution is widely found.

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