Phytoextraction of Heavy Metals from Diesel Oil Contaminated Soil using *Terminalia ivorensis* A. Chev. Seedlings

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Abstract

This study investigated the growth and heavy metal extraction potential of Terminalia ivorensis in diesel oil contaminated soil. Using a completely randomized design, seedlings were transplanted into pots containing soil contaminated with different amounts of diesel oil (0, 50, 100 and 150 ml/kg). The growth performance: height and collar diameter were monitored fortnightly for 16 weeks. The Relative Growth Rates (RGRs), biomass accumulation were determined monthly. Lead and Nickel accumulation in different plant parts were determined at the end of the experiment. Data were analysed using descriptive statistics and ANOVA at P<0.05. Growth and biomass accumulation were significantly reduced with increasing level of contamination. Control treatment had the highest height (44.13 ± 1.02 cm) and collar diameter (5.46 ± 0.14 mm) while seedlings in 150ml/kg of soil had the least height (34.82 ± 2.08 cm) and collar diameter (3.27 ± 0.14 mm). The RGRs were 0.061 g g^{-1} $week^{-1}$, 0.060 g g^{-1} $week^{-1}$, 0.053 g g^{-1} $week^{-1}$ and 0.055 g g^{-1} $week^{-1}$ for plants grown in soil containing 0, 50, 100 and 150 ml/kg diesel, respectively. Highest biomass (7.86 g) was observed in pots treated with 0 ml/kg while least (2.39 g) was observed in pots treated with 150 ml/kg. No traces of Lead and Nickel were found in the control while; roots accumulated the highest Lead (1.58 mg/kg, 1.86 mg/kg, 2.45 mg/kg) and stem recorded the highest Nickel (0.26 mg/kg, 0.31 mg/kg and 0.46 mg/kg) in seedlings growing in 50 ml/kg, 100 ml/kg and 150 ml/kg treatments. Growth inhibition and low heavy metal extraction suggest that Terminalia ivorensis seedlings may not be a good candidate for phytoremediation.

Keywords: Growth inhibition, heavy metals, hydrocarbon pollution, oil spill, terminalia.

Introduction

Soil contamination with petroleum derivatives is commonly observed in soils around industrial plants and in areas where petroleum products and natural gas are obtained (Chaudhry *et al.*, 1998; Pulford and Watson, 2003). It reduces water, oxygen and nutrients available for plant use, as well as poses a threat to human and animal health in such environments. As a matter of fact, these pollutants increase the concentration of heavy metals such as Zinc (Zn), Lead (Pb), Vanadium (V), Nickel (Ni), Copper (Cu) and Cadmium (Cd) in such environments (Amadi *et al.*, 1996; Wyszkowski and Ziólkowska, 2008; Ogbo, 2009). Hence, if soil contaminated by petroleum products is not properly managed, it could result in environmental degradation, loss of biodiversity, loss of habitat, and death of organisms (Wyszkowska and Kucharski, 2000; Wyszkowski *et al.*, 2004).

Diesel oil is one of the major derivatives from crude oil, causing pollution in the Nigerian environment (Nwaogu *et al.*, 2008; Njoku *et al.*, 2009). The increasing rate of industrialization and the epileptic power supply in Nigeria have resulted in a corresponding increase in the demand for diesel oil for powering car engines, industrial trucks and generators (Ogbo, 2009; Olajuyigbe and Aruwajoye, 2014). This petroleum derivative could be introduced into the environment through leakages of damaged storage containers, accidental spillage; during refueling of vehicles and destruction of warships as well as through inappropriate disposal by automobile mechanics (Nwaogu *et al.*, 2008). Diesel spills on agricultural lands reduce the growth of plants; and cause a reduction in soil fertility and microflora populations (Barcelo and Poschenrieder, 1990; Nwaogu *et al.*, 2008; Wyszkowski and Ziólkowska, 2008). Diesel oil has been confirmed to contain high amounts of heavy metals, whose sources include additives applied during the refining process, absorption of the metals from storage tanks and supply vessels as well as natural presence of the metals in the source rock from which the crude was obtained (Akpoveta and Osakwe, 2014; Idodo-Umeh and Ogbeibu, 2010). Consequently, heavy metal contamination through diesel oil spillage could result in changes in soil physical and chemical properties, as well as restrict availability of nutrients such as nitrogen and phosphorus (Robson *et al.*, 2004a; Wyszkowska and Wyszkowski, 2006).

Unfortunately, the huge amount of money required for cleaning up and removing these heavy metal contaminants from soils, water and sediments make their management difficult and sometimes unsustainable (Lone *et al.*, 2008). Phytoremediation has been widely proposed as a viable, environment friendly approach for remediating sites contaminated by organic and inorganic pollutants (Chaudhry *et al.*, 1998 ; Pulford and Watson, 2003). Plants, through phytoremediation, could potentially decontaminate and rehabilitate petroleum polluted sites making them safer and productive (Frick *et al.*, 1999; Sai Kachout *et al.*, 2009; Majid *et al.*, 2011). The high tolerance mechanisms and lack of toxicity symptoms exhibited by many trees make them suitable phytoremediators of hydrocarbon pollutants from soils and groundwater; when compared with herbaceous crops (Frick *et al.*, 1999; Pulford and Watson, 2003; Majid *et al.*, 2011; Olajuyigbe and Aruwajoye, 2014). Therefore, this study assessed the phytoextraction potentials and growth performance of *Terminalia ivorensis* seedlings planted in diesel oil contaminated soil.

Materials and Methods

Study Area

This experiment was conducted under nursery conditions at the Department of Forest Resources Management, University of Ibadan, Nigeria. The University is located in the Southwestern part of Nigeria about 160 km from the Atlantic Ocean between latitude $7^{o}26'45''$-$7^{0}27'31''$N and longitude $3^{o}53'31''$-$3^{0}54'14''$E, at an altitude of 277 m above sea level. The tropical climate has distinct wet (April – October) and dry (November-March) seasons, with the mean annual temperature ranging from 22^{o}C and 34^{o}C while the annual rainfall is approximately 1300 mm.

Experimental Procedure

Sieved top soil was thoroughly mixed and homogenized with diesel oil in order to achieve four contamination levels: 50, 100 and 150 ml/kg, while uncontaminated soil served as control (0 ml/kg) treatment. Afterwards, 2 kg of each soil treatment was filled into 20 cm by 15 cm polythene pots. Then, 120 (two-months old) *Terminalia ivorensis* seedlings were transplanted into the polythene pots and watered daily or as required. The experiment was set up using a completely randomized design with 30 replicates in each of four treatments. The growth of the seedlings in each treatment was assessed for 16 weeks with fortnight measurements of seedling height (cm) and collar diameter (mm). A vernier caliper was used to measure collar diameter, while a measuring tape was used to measure the height. Biomass accumulation was assessed through destructive sampling of three seedlings per treatment at the 6th, 10th and 16th week. The samples were oven dried at 60°C for 24-48 hours until constant dry weight was achieved. The oven dried seedlings were separated into three parts: roots, stems, and leaves.

Determination of Lead and Nickel in the plant parts

Lead and Nickel content of the seedlings' roots, stem and leaves in each of the treatments was assessed after 16 weeks. The plant samples were milled to powdery form, using a blender in the laboratory. Each milled component was mixed and homogenized before storing in small plastic bags until acid digestion was done following the procedure of Allen *et al.* (1974). Heavy metal analysis was carried out using an Atomic Absorption Spectrophotometer (Buck Scientific Model 210/211 VGP) to determine the presence and concentration of Ni and Pb in seedlings grown on soil contaminated with diesel oil.

Statistical Analysis

The data were not homogenous, so they were normalized by transformation using natural logarithm. Analysis of Variance (ANOVA) was used to determine the effect of period of exposure and diesel oil contamination levels on seedling height, stem collar diameter and biomass accumulation. All significantly different means were separated using Holm Sidak Multiple Comparison Test at $P < 0.05$ level of significance. After 16 weeks, the Relative Growth Rates (RGRs) were estimated using Equation 1.

Equation 1:

$$RGR = \frac{\ln TDW_2 - \ln TDW_1}{t_2 - t_1} \quad \text{……….} (1)$$

Where, RGR = Relative Growth Rate (g g^{-1} week^{-1}), TDW_1 = Initial total dry weight (g), TDW_2 = Final total dry weight (g), t_1 = Initial time (weeks), t_2 = Final time (weeks) and ln = Natural Logarithm.

Results

There were significant differences in the effect of period of exposure ($P<0.001$) and level of diesel oil contamination ($P<0.001$), on seedling height. Particularly, the 150 ml/kg treatment caused stunted growth after 8 weeks when compared to the control treatment (Table 1). Similarly, the collar diameters were negatively affected by the period of exposure ($P<0.001$) and level of diesel oil contamination ($P<0.001$). It was observed that 100 ml/kg and 150 ml/kg treatments had similar limiting effects on collar diameter growth.

Root biomass accumulation was significantly affected by the level of diesel oil contamination, although no difference was observed over the period of exposure (Figure 1). But, stem biomass was signify-cantly reduced with increasing level of contamination and over the period of exposure (Figure 2). Also, leaf biomass reduced significantly with increasing level of contamination and period of exposure (Figure 3). The diesel oil contamination had a significantly negative effect on foliage production with wilting of stem and loss of leaves observed in 100 ml/kg and 150 ml/kg treatments. Nevertheless, only the level of contamination appeared to have a cumulative effect on total biomass production, while the period of exposure had no significant effect (Figure 4). After 16 weeks, the Relative Growth Rates (RGRs) were estimated as 0.061 g g^{-1} $week^{-1}$, 0.060 g g^{-1} $week^{-1}$, 0.053 g g^{-1} $week^{-1}$ and 0.055 g g^{-1} $week^{-1}$ for seedlings in the 0 ml/kg, 50 ml/kg, 100 ml/kg and 150 ml/kg treatments, respectively.

Table 1. Seedling height and collar diameter of *Terminalia ivorensis* growing in soils subjected to varying levels of diesel oil contamination for 16 weeks

Period of exposure (weeks)	Control (0 ml diesel kg^{-1} of soil)	50 ml diesel kg^{-1} of soil	100 ml diesel kg^{-1} of soil	150 ml diesel kg^{-1} of soil
		Seedling total height (cm)		
2	$38.91 \pm 0.90^{a}_{A}$	$41.16 \pm 1.14^{ab}_{A}$	$44.71 \pm 0.96^{b}_{A}$	$42.62 \pm 0.99^{ab}_{A}$
4	$39.61 \pm 0.90^{a}_{A}$	$41.47 \pm 1.14^{ab}_{A}$	$44.73 \pm 0.95^{b}_{A}$	$42.92 \pm 0.93^{ab}_{A}$
6	$40.34 \pm 0.89^{a}_{A}$	$42.30 \pm 1.14^{a}_{A}$	$45.23 \pm 0.95^{a}_{A}$	$43.02 \pm 1.00^{a}_{A}$
8	$42.48 \pm 0.84^{a}_{A}$	$43.22 \pm 1.11^{a}_{A}$	$43.92 \pm 1.15^{a}_{A}$	43.38 ± 1.01^{a}
10	$42.87 \pm 0.92^{a}_{A}$	$42.68 \pm 1.47^{a}_{A}$	$43.13 \pm 1.36^{a}_{A}$	$42.29 \pm 1.15^{a}_{AB}$
12	$43.03 \pm 0.92^{a}_{A}$	$42.34 \pm 1.59^{ab}_{A}$	$41.65 \pm 1.35^{ab}_{A}$	$37.89 \pm 1.71^{b}_{BC}$
14	$43.63 \pm 1.01^{a}_{A}$	$41.92 \pm 1.83^{a}_{A}$	$39.55 \pm 1.50^{a}_{A}$	$34.62 \pm 2.09^{b}_{C}$
16	$44.13 \pm 1.02^{a}_{A}$	$42.40 \pm 1.79^{ab}_{A}$	$39.61 \pm 1.54^{b}_{A}$	$34.82 \pm 2.08^{c}_{C}$
		Seedling collar diameter (mm)		
2	$2.71 \pm 0.09^{a}_{A}$	$2.98 \pm 0.11^{ab}_{A}$	$3.17 \pm 0.12^{b}_{A}$	$3.05 \pm 0.11^{ab}_{A}$
4	$3.15 \pm 0.10^{a}_{B}$	$3.20 \pm 0.11^{a}_{AB}$	$3.29 \pm 0.11^{a}_{A}$	$3.18 \pm 0.11^{a}_{A}$
6	$3.63 \pm 0.09^{a}_{C}$	$3.51 \pm 0.11^{a}_{B}$	$3.47 \pm 0.12^{a}_{A}$	$3.36 \pm 0.11^{a}_{A}$
8	$4.13 \pm 0.10^{a}_{CD}$	$3.92 \pm 0.12^{ab}_{BC}$	$3.65 \pm 0.12^{b}_{A}$	$3.52 \pm 0.12^{b}_{A}$
10	$4.51 \pm 0.11^{a}_{DE}$	$3.91 \pm 0.16^{b}_{BC}$	$3.54 \pm 0.15^{b}_{A}$	$3.51 \pm 0.14^{b}_{A}$
12	$4.87 \pm 0.12^{a}_{E}$	$4.13 \pm 0.17^{b}_{C}$	$3.59 \pm 0.16^{c}_{A}$	$3.53 \pm 0.15^{c}_{A}$
14	$5.09 \pm 0.14^{a}_{E}$	$4.25 \pm 0.19^{b}_{C}$	$3.34 \pm 0.17^{c}_{A}$	$3.32 \pm 0.13^{c}_{A}$
16	$5.46 \pm 0.14^{a}_{E}$	$4.44 \pm 0.19^{b}_{C}$	$3.32 \pm 0.17^{c}_{A}$	$3.27 \pm 0.14^{c}_{A}$

Mean ± S.E., the small letters (superscript) indicate significantly different means for values across each row (among treatments) while capital letters (subscript) indicate significantly different means for values in each column (within treatments).

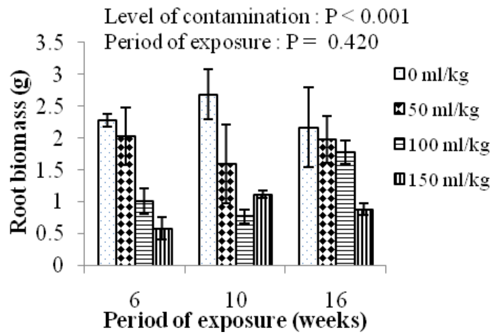

Figure 1. Root biomass accumulated by *Terminalia ivorensis* seedlings grown for 16 weeks in soil contaminated with varying amounts of diesel oil (inset: P-values for treatment factors).

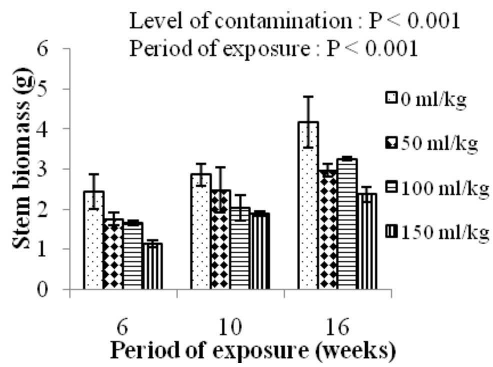

Figure 2. Stem biomass accumulated by *Terminalia ivorensis* seedlings grown for 16 weeks in soil contaminated with varying amounts of diesel oil (inset: P-values for treatment factors).

There was a reduction in the contribution of leaves to total biomass from 36.1%, 32.12%, 23.33% and 29.23% to 19.25%, 12.58%, 2.27% and 9.02% in 0 ml/kg, 50 ml/kg, 100 ml/kg and 150 ml/kg treatments, respectively. However, the proportion of stem to total biomass increased to 53.10%, 52.48%, 71.67% and 99.50% in 0 ml/kg, 50 ml/kg, 100 ml/kg and 150 ml/kg treatments, respectively. Consequently, total biomass decreased with increasing level of diesel oil with mean dry matter of 7.86g, 5.66g, 4.55g and 2.39g.

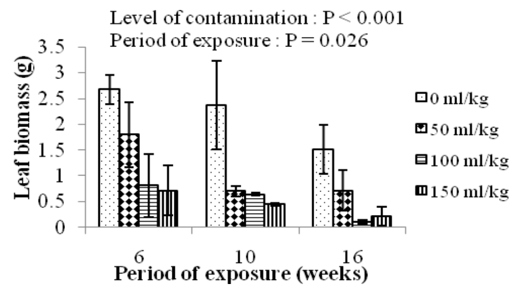

Figure 3. Leaf biomass accumulated by *Terminalia ivorensis* seedlings grown for 16 weeks in soil contaminated with varying amounts of diesel oil (inset: P-values for treatment factors).

There was a bio-accumulation of Pb and Ni in the stem, roots and leaves of the *T. ivorensis* seedlings (Table 2). After 16 weeks, no trace of Ni and Pb were found in seedlings growing in the control treatment. Seedlings grown in soil contaminated with 50 ml/kg, 100 ml/kg and 150 ml/kg diesel oil had 0.64 mg/kg, 0.90 mg/kg and 1.03 mg/kg of Ni. Similarly, Total Pb concentrations in the contaminated treatments were 3.51 mg/kg, 4.77 mg/kg and 5.74 mg/kg, respectively. The roots accumulated 45%, 39% and 42.7% of the total Pb content found in seedlings growing in 50 ml/kg, 100 ml/kg and 150 ml/kg treatments, respectively.

Also, the roots contained 40.6%, 35.6% and 39.0% of Ni at the end of the study.

Table 2. Heavy metal accumulation (Lead and Nickel) in above and belowground parts of *Terminalia ivorensis* seedlings after 16 weeks of growth in soil contaminated with varying amounts of diesel oil

Treatment	Leaves (mg/kg)	Stem (mg/kg)	Roots (mg/kg)	Total biomass (mg/kg)
		Lead (Pb)		
0 ml/kg	0.00	0.00	0.00	0.00
50 ml/kg	0.74 ±0.30	1.19±0.75	1.58±0.36	3.51±1.41
100ml/kg	1.28±1.07	1.63±0.08	1.86±0.4	4.77±1.55
150 ml/kg	1.31±0.51	1.98±0.27	2.45±0.39	5.74±1.17
		Nickel (Ni)		
0 ml/kg	0.00	0.00	0.00	0.00
50 ml/kg	0.12±0.07	0.26±0.22	0.26±0.18	0.64±0.47
100 ml/kg	0.28±0.01	0.31±0.16	0.31±0.14	0.90±0.31
150 ml/kg	0.22±0.04	0.46±0.23	0.35±0.08	1.03±0.35

Discussion

Trees selected for potential phytoremediation must be naturally adapted to the biotic and abiotic factors of the environment where they grow (Robson *et al.*, 2004a; Robson *et al.*, 2004b). This necessitated the choice of *Terminalia ivorensis* as a test species for phytoextraction of heavy metals in diesel oil contaminated soil. The candidate species showed an ability to tolerate and survive despite its exposure to varying amounts of diesel oil contamination. Tolerance in this context refers to the ability of a plant species to grow in contaminated soil; it does not necessarily mean the plant is healthy (Frick *et al.*, 1999). Although, the heavy metal presence in the topsoil was not determined at the start of the experiment; no traces of heavy metal (Ni and Pb) were found in the control seedlings suggesting that the source of heavy metal found in the contaminated seedlings was the diesel oil. These toxic pollutants from petroleum products have direct effects on plants by smearing roots with oily substances and thus inhibiting respiration and transpiration, limiting cell permeability and disrupting metabolic activities in the plants (Amadi *et al.*, 1996; Wyszkowski *et al.*, 2004; Olajuyigbe and Aruwajoye, 2014). This consequently causes leaf abscission, depression in growth and yield.

The growth and development of *T. ivorensis* seedlings was limited by increasing concentration of diesel oil. This growth inhibition became evident after eight weeks with yellowing of leaves, as well as retardation in height and diameter development. Previous studies have also reported such effects in *Vigna unguiculata* (Njoku *et al.* 2009), *Khaya senegalensis* and *Terminalia superba* (Olajuyigbe and Aruwajoye, 2014), *Jatropha curcas*

(Agbogidi *et al.*, 2013). In this study, control seedlings had the highest height and collar diameter growth after 16 weeks. This corroborates the assertion that RGR is indirectly correlated with increasing levels of soil contamination (Robson *et al.*, 2004a).

The negative impact of diesel oil on plant growth has been attributed to the blockage of air pores that allow air and water seepage into the soil. This blockage results in soil compaction and deterioration in the soil physical, chemical and biological properties, thus reducing biomass production (Wyszkowski and Ziólkowska, 2008). It has also been suggested that diesel oil contamination limits the flow of carbon to the roots thereby, decreasing the supply of resources (water, nutrients, oxygen) to the above ground parts of plants (Nwoko *et al.*, 2007). A limitation in this study is that the soil physical, chemical and biological properties were not assessed before and after the experiment to highlight the impact of the pollutant on soil conditions.

The greasy texture of hydrocarbons further inhibits the conversion of mineral and organic nitrogen compounds in soil, thus depressing the processes of ammonification and nitrification (Amadi *et al.*, 1996). In addition, the microbial communities (bacteria and fungi) growing on soil contaminated with hydrocarbons consume all available nitrogen and therefore restrict the uptake of these elements by plants (Wyszkowski *et al.*, 2004). After 16 weeks, seedlings in the control treatment recorded the highest total biomass accumulated by the plant while diesel oil contamination reduced biomass accumulation in the other treatments. There was a reduction in the leaf biomass in all treatments due to leaf abscission resulting probably from a combination of environmental factors and level of contamination. Notwithstanding, it has been confirmed that reduced biomass production accompanies an increase in the level of diesel oil contamination in soil (Adam and Duncan,1999; Wyszkowski *et al.*, 2004). For example, Olajuyigbe and Aruwajoye (2014) reported that an increase in the level of diesel oil contamination resulted in a reduction in biomass accumulation in *Terminalia superba* and *Khaya senegalensis* seedlings while Al-Yemeni *et al*. (2010) reported similar effects in *Phaseolus vulgaris* seedlings. The reduced biomass could be attributed to the toxic environment created by the presence of the contaminant in the soil.

Plants used for phytoremediation must possess the capability to absorb, sequester and translocate heavy metals into different parts of their biomass (roots, stem and leaves). Many studies have confirmed that different mechanisms of metal accumulation, exclusion and compartmentation exist in various plant species (Lone *et al*., 2008; Pulford and Watson, 2003; Wyszkowski *et al.*, 2004; Majid *et al.*, 2011). The *T. ivorensis* species was able to phytoextract small amounts of Pb and Ni and store them in its tissues with a consequent translocation from below ground to the above ground parts. However, the roots accumulated more Pb than the leaves and stem in all treatments. This corroborates the assertion that there are differences in heavy metal accumulation and storage in actively growing tissues of plants (Majid *et al.*, 2011; Olajuyigbe and Aruwajoye, 2014). For instance,Baker (1981) and Majid *et al.*

(2011) both reported higher Cu absorption in roots than stems of various plants.

Furthermore, the level of heavy metal accumulation increased with increasing level of diesel oil contamination in all plants, except for leaves in the 150 ml/kg treatment. This difference may be attributed to the possible damage the higher concentration might have caused to the leaf structure of seedlings in this treatment (Lone *et al*., 2008). In addition, the accumulation pattern revealed that the test plants accumulated more Pb which is highly toxic than Ni which is an essential nutrient at low concentrations.

Globally, for plant species to be classified as hyper accumulators they must have the ability to bioaccumulate >1000 mg/kg (dry weight) of one or more metals (Reeves, 2003). In this study, the accumulation patterns showed that the trees bioaccumulated very low amounts of the heavy metals. This is an indication that the process of phytoremediation was slow and limited and the species may not be able to rapidly phytoextract substantial amounts of heavy metals from diesel oil polluted lands.

Conclusion

Findings in this study revealed that growth inhibition and reduced biomass accumulation increased with increasing levels of contamination across all treatments. *Terminalia ivorensis* was a poor phytoextractor of heavy metals from diesel oil contaminated soil. However, long term assessments and possible soil amendments are required to categorically confirm the phytoremediation potential of the species.

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