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Phytoremediation of diesel oil contaminated soil using seedlings of two tropical hardwood species (*Khaya senegalensis* and *Terminalia superba*)

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Abstract- Phytoremediation of diesel oil contaminated soils by tree species could be a cheap, effective and sustainable means of rehabilitating ecosystems in the tropics. However, little is known about tropical tree species with phytoremediation capabilities. In this study, we determined the effect of different levels of diesel oil contamination (25 ml (T_1), 50 ml (T_2), 75 ml (T_3) and 100 ml (T_4) of diesel oil per kg of soil) on seedlings of *Khaya senegalensis* and *Terminalia superba.* For 12 weeks, the growth performance (number of leaves, seedling collar diameter and height) and biomass accumulated by roots, stem and leaves of seedlings in each treatment were measured, fortnightly. At the end of the study, heavy metal analysis was done to determine the concentration of Lead (Pb) and Nickel (Ni) in the above and belowground parts of seedlings in each treatment. Data were analysed using descriptive statistics and anova at $p < 0.05$. The diesel oil contamination had no significant effect on leaf production, collar diameter, height or biomass accumulated by both hardwood species. The *K. senegalensis* seedlings in the T_1 (29.47 ± 13.69 g) had the highest biomass while T_3 (16.33 ± 0.14 g) had highest for *T. superba.* The accumulation patterns showed that *T. superba* accumulated more heavy metals (Ni: 5.62 - 7.52 ppm; Pb: 12.63 – 17.82 ppm) than *K. senegalensis* (Ni: 4.71 – 6.34 ppm; Pb: 11.24 – 14.26 ppm) with roots retaining more than 50% of each metal in most of the treatments. The tolerance of diesel oil contamination by the two hardwood species indicates their potential for phytoextraction of heavy metals from hydrocarbon polluted areas in the tropics and further studies will be required to identify their tolerance limits. The two species are widely accepted timber species whose use for reforestation/phytoremediation of crude oil damaged sites could be beneficial for many oil producing tropical countries.

Index Terms: growth inhibition, heavy metal contamination, *Khaya senegalensis*, petroleum hydrocarbons, *Terminalia superba*

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1. INTRODUCTION

THE continuous drive for modernisation and mechanisation has resulted in a corresponding increase in the use of petroleum hydrocarbons and petroleum-based products. Consequently, water and soil contamination with crude oil and refined hydrocarbon products are becoming an increasing problem with serious environmental and health impacts [1], [2]. To tackle this challenge, environmental management experts employ a wide range of methods to remediate petroleum contaminated soil and groundwater (for example, the 'dig and dump' or encapsulation methods) [3]. However, the huge costs associated with such methods discourage their use for the removal of contaminants from soils, water and sediments during the implementation of rehabilitation projects [2], [4], [5].

Diesel oil is a product of crude oil which causes serious environmental pollution in Nigeria [6], [7], [8]. It is phytotoxic to plants at low concentrations, reduces soil fertility, soil microflora population and could cause a significant reduction in soil organic carbon [5], [9].

Recently, soil contamination by diesel oil is becoming a challenge owing to the increasing use of this hydrocarbon in engines of power generators, small and articulated vehicles. This increase in use is resulting in accidental spillage of diesel oil on agricultural lands, forests and water sources [6], [7], [10]. In addition, diesel oil contaminants are introduced to the environment through leakages from damaged storage containers, wrecks of oil tankers and warships; when refuelling vehicles as well as through improper handling and disposal of diesel oil by automobile technicians [9].

Excess concentrations of some heavy metals found in petroleum hydrocarbons, such as diesel oil has caused disruptions in the functioning of many aquatic and terrestrial ecosystems. Therefore, metals such as Zinc (Zn), Lead (Pb), Copper (Cu), Nickel (Ni) and Cadmium (Cd) cause non-degradable pollution in numerous sites. This heavy metal contamination from petroleum-derived substances result in changes in soil physical and chemical properties, reduce fertility and restrict availability of nutrients such as nitrogen and phosphorus [11], [12]. However, plants through phytoremediation can play an important role in decontaminating and rehabilitating such sites, making them environmentally safer [4], [13], [14].

Phytoremediation is a cheap, low technology, non-destructive, visually unobtrusive, in-situ approach to site restoration, partial decontamination and maintenance of the biological activity and physical structure of soils [3]. It can be applied in sites containing organic nutrients or metal pollutants that can be accessed, sequestered, degraded, immobilized or metabolized by specific plant species. These plants have the capacity to render harmless, extract or stabilize contaminants present in soil, or support populations of hydrocarbon degrading microorganisms in their rhizosphere. Through these actions, such plant species make contaminants unavailable to cause harm to other organisms and reduce environmental hazards [2], [15], [16].

Phytoremediation of hydrocarbon-contaminated soils by tree species could be a cheap, effective and sustainable means of rehabilitating ecosystems in the tropics. However, little is known about tropical tree species that could be used for the clean-up of petroleum-oil contaminated soil [5], [14], [16], [17]. Nevertheless, the potential of this technique in the tropics is favoured by the climatic conditions which facilitate rapid plant growth and stimulate high microbial activity. In addition, biomass production is high in the tropics, provided growth is not limited by nutrient availability. Therefore, screening and evaluation of tree species for their ability to grow, establish in contaminated soil, bio accumulate and degrade petroleum hydrocarbons are essential, especially with the continuous desire to manage the ecological impact of oil spillage on terrestrial and aquatic ecosystems [4], [11], [16]. The ideal candidate species for phytoremediation of heavy metals in contaminated site should be rapid growing, high biomass producing and with a tendency for extensive root systems that can both tolerate and accumulate the contaminants of interest. In addition, growth inhibition which is a nonspecific symptom of heavy metal stress, needs to be investigated in phytoremediating plants [2], [13].

Some studies have evaluated the effects of different heavy metal concentrations on live plants [5], [13], [14] [16], [18]. Most of these studies have been conducted using seedlings or adult plants and three types of heavy metal tolerance strategy have been identified. They include; accumulation (where metals are concentrated in the aboveground parts of the plant); exclusion (where low metal concentrations are maintained by the plant in its shoot over a wide range of soil concentrations, up to a critical value above which the plants physiological mechanism breaks down resulting in unrestricted transport of the metals through the plant); and indication (where uptake and transport of metals to the shoot are regulated so that internal concentration reflects external levels [18].

Candidate tree species for phytoremediation should show the potential for either phytoextraction (uptake and recovery of metals into above-ground biomass), or phytostabilization (stabilizing wastes by hydraulic and erosional control at the site) or phytofiltration (filtering of metals from water into root systems). For instance, organic chemicals that pass through membranes and are translocated to stem and leaf tissues could be converted (e.g. oxidized by cytochrome P450s), conjugated by

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glutathione or amino acids, and compartmentalized in plant tissues as bound residue [2], [4].

During the evaluation of candidate plants for phytoremediation of contaminated soils it is essential to examine their survival, tolerance, growth and biomass accumulation [11] [19]. Moreover, the response of seedlings or cuttings to heavy metal exposure remains the most common way of assessing the ability of different species, or clones of the same species, to take up, tolerate and survive such stress [3].

Various plants have been effectively used to remediate inorganic and organic contaminants in soil and groundwater. For example, canola (*Brassica napus* L.), oat (*Avena sativa*), barley (*Hordeum vulgare*) and Alamo switchgrass (*Panicum virginatum*) tolerate and accumulate metals such as selenium, copper, cadmium and zinc [20], [21]. However, the use of tree species rather than smaller plants allows the treatment of deeper contamination because tree roots penetrate more deeply into the ground [3], [4]. Many trees that were not initially selected for metal tolerance have been observed to survive in metal-contaminated soil, though with reduced growth rate. Thus, it has been suggested that the lack of reported heavy metal toxicity symptoms in trees could be as a result of a tolerance mechanism that allows trees to withstand higher heavy metal concentrations than agricultural crops [3], [18].

In this study, we evaluated the phytoremediation potentials of two tropical hardwood species (*Khaya senegalensis* (Desr.) A. Juss. and *Terminalia superba* Engl. et Diels), by conducting a 3 month experiment to determine their survival, growth performance and biomass accumulation in soil contaminated with different amounts of diesel oil. The study specifically, (i) determined the effect of various amounts of the pollutant on collar diameter, total height and number of leaves of seedlings of the two tree species, (ii) determined the biomass accumulated in leaves, shoot and roots, and (iii) determined the concentration of Nickel (Ni) and Lead (Pb) accumulated in the above and belowground parts of the seedlings after 12 weeks.

2. MATERIALS AND METHODS

2.1. Study area

This study was carried out in the nursery section of the Department of Forest Resources Management, University of Ibadan, Nigeria. The institution is located in the South western region of Nigeria about 160 km from the Atlantic Ocean at latitude 7°28′N and longitude 3°52′E with altitude 277 m above the sea level. The climatic condition is typical of West Africa monsoon with distinct dry and wet seasons. The dry season is from November through March, while the wet season starts from April to October. The annual rainfall is about 1300 mm while mean annual temperature is between 22°C and 34°C [22].

2.2. Seedling selection and experimental procedure

Soil was sieved through a 2 mm wire mesh in order to remove debris, and then mixed and thoroughly homogenized with diesel oil in order to achieve the following contamination levels: 25 ml (T_1), 50 ml (T_2), 75 ml (T_3) and 100 ml (T_4) of diesel oil per kg of soil, while uncontaminated soil served as control (T_0). Then, 2 kg each of T_0, T_1, T_2, T_3 and T_4 soil treatments was filled into 20cm by 15 cm polythene pots.

One year old seedlings of *Khaya senegalensis* and *Terminalia superba* were obtained from the nursery of the Department of Forest Resources Management, University of Ibadan. A total of 140 seedlings of uniform height were selected for each species and transplanted into polyethene pots containing the 5 soil treatments. The seedlings were watered daily according to the water holding capacity of the pots and monitored for growth and biomass accumulation. For growth assessment, four replicates per treatment were selected and monitored for 12 weeks. The seedling height, collar diameter was measured and number of leaves counted at the commencement of the experiment and every fortnight for 12 weeks after which the experiment was terminated. A vernier mini calliper was used to measure collar diameter, while a measuring tape was used to measure height.

To determine biomass allocation and distribution, four replicates were destructively sampled from each treatment after 2, 4, 6, 8, 10 and 12 weeks making a total of 120 seedlings. The sampled seedlings were uprooted, cleaned and separated into three parts (roots, stems, and leaves). The different parts were oven dried at 60°C and the constant dry weight determined using an analytical balance.

2.3. Heavy metal (Nickel and Lead) analysis of plant parts

After 12 weeks, oven-dried samples of roots, stems, and leaves were collected from each treatment and ground into fine powder using a commercial blender. Each milled component was thoroughly mixed, homogenized and stored in small polyethylene bags until acid digestion following the method of Allen [23]. The samples were placed in 250 ppm digestion tube and 10 ml of Nitric acid (HNO_3) was added. The sample was heated for 45 minutes at 90°C, then the temperature was increased to 150°C, and the sample boiled for 8 hours until a clear solution was obtained. Then the solution was filtered and the filtrate was transferred quantitatively into a 25 ml volumetric flask by adding distilled water. Heavy metal analysis was carried out using an Atomic Absorption Spectrophotometer (Buck Scientific Model 210 VGP) to determine the presence and concentration of Ni and Pb in seedlings growing in each soil treatment.

2.4. Statistical Analysis

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A two way anova was used to determine the effect of time and diesel oil contamination levels on the number of leaves, height, stem collar diameter and biomass accumulation of seedlings of each hardwood species. Holm Sidak Multiple Comparison Test was used to separate significant means at $p < 0.05$ level of significance. Because number of leaves, seedling height, collar diameter and biomass variances were not homogenous, these data were transformed using natural logarithm. All statistical tests were performed using SigmaStat 11 and Microsoft Excel 2010 software.

3. Results

3.1. Growth response of *Khaya senegalensis* and *Terminalia superba* seedlings to diesel oil contamination

A 100% survival rate was observed for all four replicates of both *K. senegalensis and T. superba* in different diesel oil contamination levels.

Number of leaves

The duration of study had a significant effect ($p = <0.001$) on number of leaves of *K. senegalensis* seedlings while the level of diesel oil contamination ($p = 0.901$) and its interaction with time ($p = 0.507$) were not significant. The posthoc analysis revealed that number of leaves at 10 and 12 weeks (which were not different from each other) differed from all the other time periods. The number of leaves did not significantly change until after 10 weeks (Fig. 1a). After 12 weeks, the highest number of leaves (52) was recorded in *K. senegalensis* seedling growing in the T_2 soils while the lowest (9) was observed in the T_4.

On the contrary, only the main effect of diesel oil contamination significantly influenced number of leaves in *T. superba* seedlings ($p = 0.011$). However, the post hoc analysis showed that the observed difference was due to the number of leaves on T_3 seedlings, which differed slightly from T_0 at the commencement of the study (Fig. 1b). The effect of time ($p = 0.400$) and its interaction ($p = 0.723$) with diesel oil amounts had no influence on number of leaves. After 12 weeks, there were averagely 31, 19, 24, 23 and 26 leaves on *T. superba* seedlings in T_0, T_1, T_2, T_3 and T_4 treatments, respectively. Generally, the diesel oil contamination did not have a serious impact on the leaf production of both hardwood species (Fig. 1a and b).

Stem collar diameter

The diesel oil contamination ($p = 0.241$) as well as its interaction with time ($p = 0.441$) had no significant effect on stem collar diameter development of *K. senegalensis* seedlings (Fig. 2a). However, the main effect of time ($p < 0.001$) on collar diameter was significant. The post hoc analysis revealed variations in the collar diameter in response to increase in time (Fig. 2a). At the end of the experiment, mean collar diameters were 9.26 ± 1.18 mm, 9.88 ± 2.28 mm, 7.05 ± 0.43 mm, 7.73 ± 1.77 mm and 6.93 ± 1.26 mm for *K. senegalensis* seedlings in T_0, T_1, T_2, T_3, T_4 treatments, respectively.

Similarly, *T. superba* seedlings were significantly affected by time ($p < 0.001$), but the level of diesel oil contamination ($p = 0.686$) as well as its interaction with time ($p = 0.895$) had no significant influence on the collar diameter development. There was a significant increase in the mean collar diameter of *T. superba* seedlings in each treatment as time progressed (Fig. 2b). After 12 weeks, the mean collar diameters were 7.9 ± 0.8 mm, 7.1 ± 0.5 mm, 8.2 ± 0.6 mm, 7.8 ± 0.7 mm and 7.4 ± 0.5 mm in T_0, T_1, T_2, T_3 and T_4, respectively.

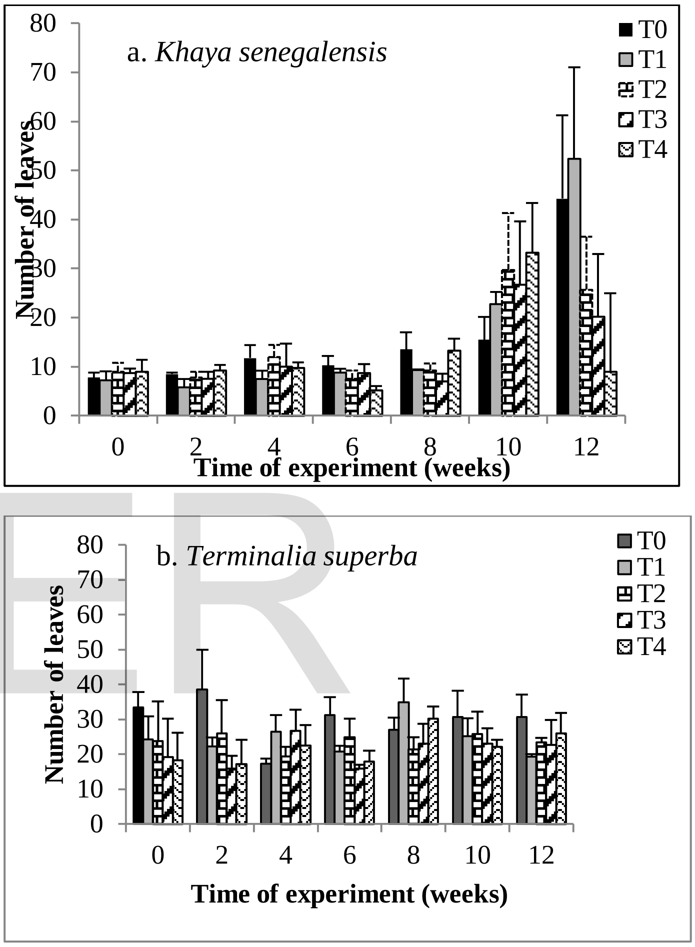

Fig. 1a and b. Number of leaves produced by *Khaya senegalensis* and *Terminalia superba* seedlings growing for 12 weeks in soil contaminated with different amounts of diesel oil (Mean + S.E error bars)

Seedling height

The heights of *K. senegalensis* seedlings were significantly affected by the level of diesel oil contamination ($p < 0.001$), time ($p < 0.001$) and the interaction of both factors ($p = 0.048$). However, the post hoc analysis on the effect of level of diesel oil contamination showed that only the mean height of seedlings in T_0 was significantly lower than all other treatments. This treatment effect was observed after 6 weeks, when the increase in height became significant for seedlings in other treatments. The variation in height of *K. senegalensis* seedlings in response to the effect of time is as shown in Fig. 3a. After 12 weeks, the total height were 43.1 ± 6.03 cm, 52.67 ± 13.51 cm, 31.15 ± 4.15 cm, 34.63 ± 6.62 cm and 36.2 ± 9.37 cm for *K. senegalensis* seedlings exposed to T_0, T_1, T_2, T_3 and T_4 treatments, respectively.

On the contrary, the level of diesel oil contamination ($p = 0.116$), time ($p = 0.469$) and their interaction (0.159) had no significant effect on the seedlings of *T. superba*. There was no significant increase in the height of seedlings during the study (Fig. 3b). After 12 weeks, the mean height was 71.25 ± 5.11 cm, 84.60 ± 4.21, 85.12 ± 8.92, 81.72 ± 3.71 cm and 71.05 ± 2.81 cm, for *T. superba* seedlings exposed to T_0, T_1, T_2, T_3 and T_4 treatments, respectively.

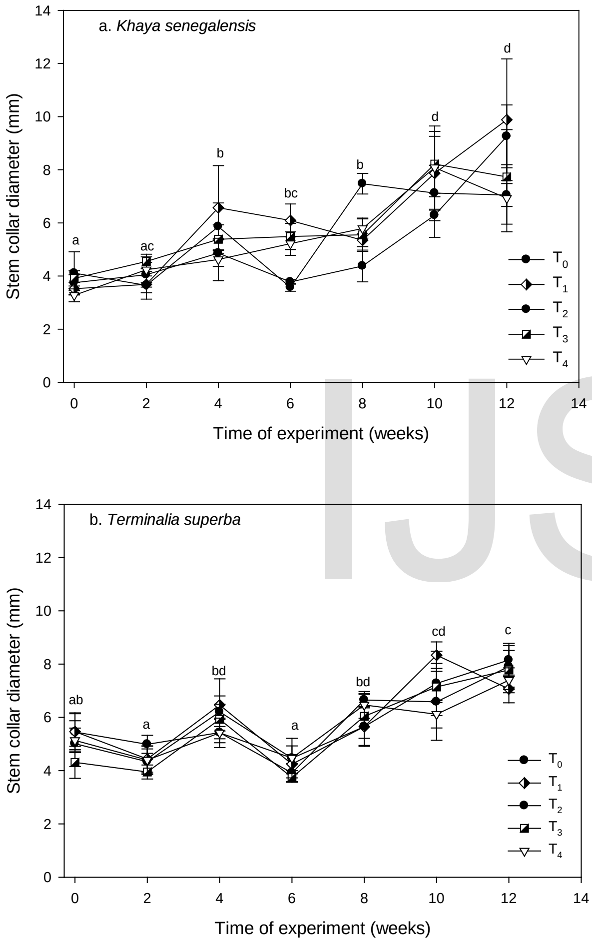

Fig. 2a and b. Stem collar diameter growth of *Khaya senegalensis* and *Terminalia superba* seedlings monitored for 12 weeks in soil contaminated with different amounts of diesel oil (Mean + S.E error bars). The letters in each figure indicate differences in the effect of time (weeks) on collar diameter for each species, while level of diesel oil contamination had no significant effect. Data points with the same letters were not significantly different at p < 0.05.

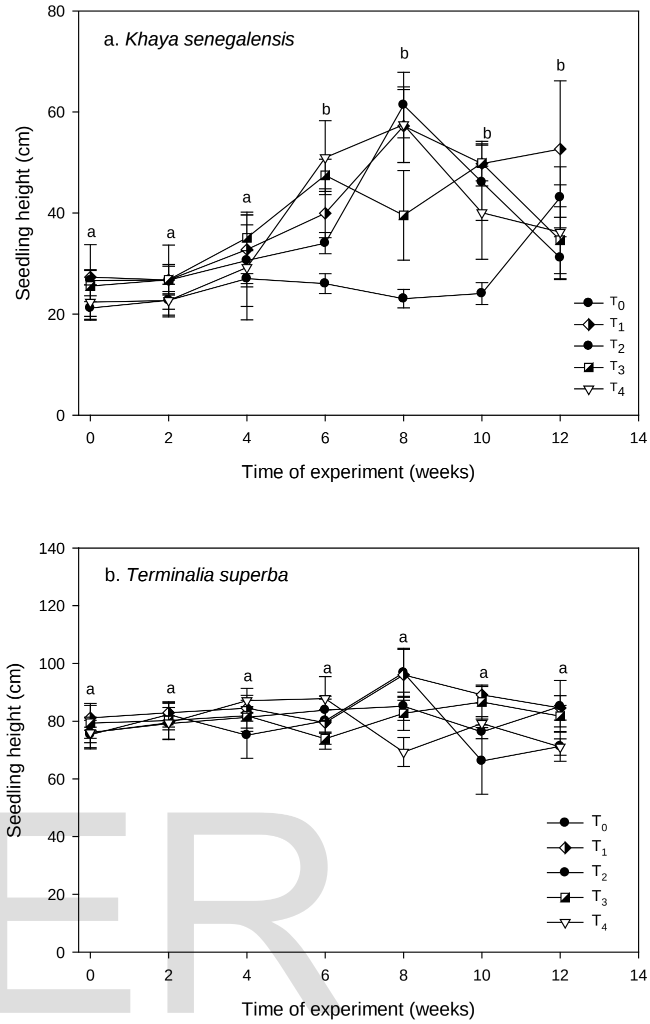

Fig. 3a and b. Total height of *Khaya senegalensis* and *Terminalia superba* seedlings monitored for 12 weeks in soil contaminated with different amounts of diesel oil (Mean ± S.E. error bars). The letters in each figure indicate differences in the fortnight measurements of total height for each species. Data points with the same letters were not significantly different at p < 0.05

Biomass assessment of seedlings of *Khaya senegalensis* and *Terminalia superba*

Leaf biomass

The level of diesel oil contamination ($p < 0.001$), time of experiment ($p < 0.001$) and their interaction ($p = 0.033$) had significant effects on leaf biomass of *K. senegalensis* seedlings. However, the post hoc analysis on the effect of level of diesel oil contamination revealed that only T_0 differed from other treatments, while T_1 differed from the T_4. After 12 weeks, *K. senegalensis* seedlings in T_1 had the highest mean leaf biomass (5.30 ± 1.64 g), while T_4 had the lowest (1.33 ± 0.90 g) (Table 1b).

On the contrary, level of diesel oil contamination ($p = 0.191$), and its interaction with time ($p = 0.731$) had no significant influence on leaf biomass of *T. superba.* However, time ($p = 0.014$) had a slightly significant influence on leaf biomass, though post hoc analysis

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revealed that only Week 4 differed from Week 8. After 12 weeks, *T. superba* seedlings in the T_2 and T_3 (1.78 ± 0.63 and 1.78 ± 0.43g, respectively) had the highest mean leaf biomass while T_4 (1.03 ± 0.34g) had the lowest (Table 1a).

Stem biomass

The main effect of level of diesel oil contamination ($p < 0.001$); time ($p < 0.001$) as well as their interaction ($p = 0.037$) had significantly effects on stem biomass of *K. senegalensis* seedlings. The post hoc analysis showed that T_0 seedlings differed significantly from T_1, T_2 and T_3. Also, the post hoc analysis on the effect of time showed Weeks 2 and 4 differed from the other time periods. After 12 weeks, *K. senegalensis* seedlings in T_1 had the highest mean stem biomass (14.33 ± 6.84 g), while those in T_3 (3.18 ± 0.65 g) had the lowest (Table 1b).

On the contrary, level of diesel oil contamination ($p = 0.914$), time ($p = 0.158$) and the interaction of both factors ($p = 0.722$) had no significant influence on stem biomass of *T. superba* seedlings during the study. After 12 weeks, *T. superba* seedlings in T_2 had the highest mean stem biomass (9.98 ± 1.83 g), while T_4 had the lowest (5.85 ± 0.38 g) (Table 1a).

Root biomass

The root biomass in *K. senegalensis* seedlings was significantly affected by the level of diesel oil contamination ($p = <0.001$) and time of experiment ($p = 0.007$) but there was no interaction effect for both factors ($p = 0.085$). The post hoc analysis showed that T_0 differed from T_1, T_2 and T_3 treatments, while Week 2 differed from Week 10. After 12 weeks, T_1 (9.83 ± 5.29 g) had the highest mean root biomass, while T_4 (2.3 ± 1.50 g) had the lowest (Table 1b).

On the other hand, the level of diesel oil contamination ($p = 0.750$), time ($p = 0.630$) and their interaction ($p = 0.810$) had no significant influence on root biomass of *T. superba* seedlings during the study. After 12 weeks, *T. superba* seedlings in T_3 had the highest mean root biomass (4.85 ± 1.33 g) while T_4 (2.35 ± 0.39 g) had the lowest (Table 1a).

Total biomass

The level of diesel oil contamination ($p < 0.001$) and time of experiment ($p = 0.004$) significantly affected the total biomass produced by *K. senegalensis* seedlings, however no statistically significant interaction ($p = 0.053$) was observed. The post hoc analysis showed that T_0 differed T_1, T_2 and T_3, while the total biomass at Week 2 differed from values at Weeks 6 and 8. After 12 Weeks, the *K. senegalensis* seedlings in the T_1 (29.47 ± 13.69 g) produced the highest mean total biomass while the T_3 (6.95 ± 2.13 g) produced the lowest (Table 1a).

However, the level of diesel oil contamination ($p = 0.856$), time ($p = 0.521$) and the interaction of the two factors ($p = 0.837$) did not significantly affect total biomass of *T. superba* seedlings over the 12 week period. At the end of the experiment, T_3 (16.33 ± 0.14 g) had highest mean total biomass while T_4 (9.23 ± 0.31 g) produced the lowest (Table 1b).

3.2. Nickel and Lead accumulation in seedling parts

After 12 weeks, no trace of Ni and Pb were found in the control treatments (T_0) for both species. However, *K. senegalenesis* seedlings in T_1, T_2, T_3 and T_4 had Ni concentrations: 4.71 ppm, 4.80 ppm, 5.03 ppm and 6.34 ppm, respectively, while *T. superba* seedlings bio accumulated 5.62 ppm, 5.89 ppm, 6.23 ppm and 7.52 ppm, respectively in the four polluted soils (Fig. 4a and b). Similarly, the Pb concentrations followed the same pattern as Ni with *K. senegalensis* having less amounts (11.24 ppm, 11.42 ppm, 13.51 ppm and 14.26 ppm) than *T. superba* seedlings (12.63 ppm, 14.50 ppm, 16.89 ppm and 17.82 ppm) in T_1, T_2, T_3 and T_4 soils (Fig. 5a and b). The roots of *K. senegalensis* accumulated 54.6%, 64.6%, 67.9% and 65.9% of Ni, while *T. superba* accumulated 55.2%, 56.2%, 62.8% and 60.1% from T_1, T_2, T_3 and T_4 soil treatments, respectively. In addition, the *K. senegalensis* roots accumulated 75.8%, 49.2%, 52.6% and 65.9% of Pb while *T. superba* roots accumulated 67.1%, 64.4%, 64.5% and 53.0% from T_1, T_2, T_3 and T_4 soil treatments, respectively.

Table 1a and b. Leaf, stem, root and total biomass of *Terminalia superba* and *Khaya senegalensis* seedlings exposed to different levels of diesel oil contamination (mean ± S.E.). No significant differences were observed in the main and interaction effects of time and diesel oil contamination on biomass accumulation in *Terminalia superba*. However, for *Khaya senegalensis* seedlings, the small letters (superscript) indicate significantly different means for values in each column (within treatment), under each biomass subheading, while capital letters (subscript) indicate significantly different means for values in each row (among treatments), under each subheading. All test were performed at p < 0.05 level of significance.

a. Terminalia superba

Time	T_0	T_1	T_2	T_3	T_4
Leaf biomass					
2	1.85 ± 0.83	2.20 ± 0.60	2.13 ± 0.58	1.70 ± 0.57	**1.63 ± 0.20**
4	2.60 ± 0.41	2.00 ± 0.64	2.95 ± 0.74	2.63 ± 0.38	**1.83 ± 0.54**
6	2.13 ± 0.57	2.28 ± 0.33	2.38 ± 0.53	1.75 ± 0.21	**1.88 ± 0.39**
8	1.57 ± 0.30	1.35 ± 0.23	1.75 ± 0.26	0.87 ± 0.26	**0.98 ± 0.14**
10	1.08 ± 0.35	3.40 ± 1.13	1.80 ± 0.38	2.20 ± 0.39	**1.63 ± 0.31**
12	1.75 ± 0.50	1.28 ± 0.35	1.78 ± 0.63	1.78 ± 0.43	**1.03 ± 0.34**
Stem biomass					
2	10.58 ± 2.89	10.10 ± 4.47	6.48 ± 2.24	7.63 ± 2.84	**6.55 ± 1.64**
4	6.45 ±	9.70 ±	8.35 ±	10.4 ±	**9.68 ±**

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	1.25	3.40	1.40	2.65	**2.12**
6	5.38 ± 1.03	5.40 ± 1.79	6.70 ± 1.06	3.88 ± 0.30	**9.23 ± 4.17**
8	10.73 ± 0.92	8.60 ± 2.08	9.20 ± 2.97	8.75 ± 1.86	**8.30 ± 2.64**
10	6.80 ± 3.49	11.58 ± 3.04	7.03 ± 1.68	8.23 ± 1.24	**5.75 ± 1.37**
12	6.70 ± 1.51	8.05 ± 1.76	9.98 ± 1.83	9.70 ± 2.12	**5.85 ± 0.38**
Root biomass					
2	5.30 ± 1.67	5.70 ± 3.57	3.95 ± 1.77	4.23 ± 1.79	**2.83 ± 0.62**
4	3.40 ± 0.42	4.65 ± 1.53	4.33 ± 0.86	5.08 ± 1.26	**3.30 ± 1.05**
6	3.43 ± 0.90	2.30 ± 0.84	3.63 ± 0.60	1.83 ± 0.13	**4.83 ± 2.05**
8	5.35 ± 0.55	3.40 ± 0.84	4.18 ± 1.58	4.23 ± 0.81	**4.25 ± 1.43**
10	3.95 ± 2.28	5.35 ± 1.48	3.73 ± 1.44	3.90 ± 0.91	**2.60 ± 0.90**
12	3.33 ± 0.96	3.23 ± 0.92	4.08 ± 0.96	4.85 ± 1.33	**2.35 ± 0.39**
Total biomass					
2	17.73 ± 0.13	18.00 ± 0.34	12.55 ± 0.26	13.55 ± 0.21	**11.00 ± 0.14**
4	12.45 ± 0.16	16.35 ± 0.16	15.63 ± 0.10	18.10 ± 0.07	**14.80 ± 0.37**
6	10.93 ± 0.18	9.98 ± 0.18	12.70 ± 0.05	7.45 ± 0.19	**15.93 ± 0.05**
8	17.25 ± 0.08	13.35 ± 0.15	15.13 ± 0.14	13.63 ± 0.13	**13.53 ± 0.33**
10	11.83 ± 0.33	20.33 ± 0.04	12.55 ± 0.24	14.33 ± 0.38	**9.98 ± 0.78**
12	11.78 ± 0.30	12.55 ± 0.21	15.83 ± 0.21	16.33 ± 0.14	**9.23 ± 0.31**

b. *Khaya senegalensis**

Time	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
Leaf biomass					
2	$1.2 \pm 0.19^{ab}_{A}$	$1.35 \pm 0.41^{ab}_{A}$	$2.00 \pm 0.71^{ab}_{A}$	$1.83 \pm 0.37^{a}_{A}$	$1.03 \pm 0.32^{a}_{A}$
4	$1.18 \pm 0.29^{ab}_{A}$	$4.68 \pm 2.62^{ab}_{A}$	$3.83 \pm 1.92^{ab}_{A}$	$3.53 \pm 1.16^{a}_{A}$	$2.08 \pm 1.12^{a}_{A}$
6	$1.15 \pm 0.09^{ab}_{A}$	$6.80 \pm 1.47^{b}_{B}$	$5.43 \pm 0.81^{b}_{B}$	$6.18 \pm 0.88^{a}_{B}$	$2.08 \pm 0.73^{a}_{AB}$
8	$0.9 \pm 0.34^{ab}_{A}$	$3.70 \pm 0.81^{ab}_{B}$	$7.13 \pm 2.33^{b}_{B}$	$3.95 \pm 1.30^{a}_{B}$	$5.23 \pm 0.74^{a}_{B}$
10	$0.63 \pm 0.17^{a}_{A}$	$2.90 \pm 0.89^{ab}_{A}$	$0.73 \pm 0.15^{a}_{A}$	$2.40 \pm 0.95^{a}_{A}$	$1.88 \pm 1.21^{a}_{A}$
12	$4.78 \pm 1.80^{b}_{A}$	$5.30 \pm 1.64^{ab}_{A}$	$2.13 \pm 0.76^{ab}_{A}$	$1.60 \pm 0.32^{a}_{A}$	$1.33 \pm 0.90^{a}_{A}$
Stem biomass					
2	$1.60 \pm 0.27^{ab}_{A}$	$2.63 \pm 0.49^{a}_{A}$	$3.75 \pm 1.92^{a}_{A}$	$2.30 \pm 0.49^{a}_{A}$	$1.93 \pm 0.47^{a}_{A}$
4	$1.15 \pm 0.33^{a}_{A}$	$3.70 \pm 1.46^{a}_{A}$	$4.13 \pm 2.70^{a}_{A}$	$3.75 \pm 0.87^{a}_{A}$	$2.75 \pm 1.53^{a}_{A}$
6	$1.58 \pm 0.17^{ab}_{A}$	$5.95 \pm 1.32^{a}_{AB}$	$4.77 \pm 0.38^{a}_{AB}$	$8.20 \pm 2.50^{a}_{B}$	$4.83 \pm 1.51^{a}_{AB}$
8	$1.10 \pm 0.41^{a}_{A}$	$7.43 \pm 1.35^{a}_{B}$	$11.68 \pm 1.49^{a}_{B}$	$5.20 \pm 1.43^{a}_{B}$	$7.47 \pm 2.33^{a}_{B}$
10	$3.57 \pm 1.13^{ab}_{A}$	$7.50 \pm 1.00^{a}_{A}$	$4.67 \pm 1.09^{a}_{A}$	$7.00 \pm 1.31^{a}_{A}$	$5.03 \pm 1.40^{a}_{A}$
12	$6.88 \pm 2.27^{b}_{A}$	$14.33 \pm 6.84^{a}_{A}$	$3.73 \pm 0.94^{a}_{A}$	$3.18 \pm 0.65^{a}_{A}$	$3.77 \pm 1.76^{a}_{A}$
Root biomass					
2	$1.20 \pm 0.15^{ab}_{A}$	$2.40 \pm 0.75^{a}_{A}$	$3.85 \pm 2.43^{a}_{A}$	$2.43 \pm 0.57^{a}_{A}$	$1.63 \pm 0.43^{a}_{A}$
4	$1.35 \pm 0.46^{ab}_{A}$	$4.40 \pm 2.22^{a}_{A}$	$2.90 \pm 1.49^{a}_{A}$	$4.73 \pm 0.84^{a}_{A}$	$1.73 \pm 0.80^{a}_{A}$
6	$1.45 \pm 0.32^{ab}_{A}$	$7.00 \pm 2.44^{a}_{A}$	$5.20 \pm 1.40^{a}_{A}$	$5.20 \pm 0.88^{a}_{A}$	$5.95 \pm 2.55^{a}_{A}$
8	$0.78 \pm 0.23^{a}_{A}$	$4.55 \pm 0.62^{a}_{B}$	$7.10 \pm 1.55^{a}_{B}$	$3.85 \pm 1.19^{a}_{AB}$	$6.77 \pm 1.03^{a}_{BA}$
10	$2.27 \pm 0.85^{ab}_{A}$	$5.93 \pm 1.31^{a}_{A}$	$4.30 \pm 1.40^{a}_{A}$	$8.40 \pm 2.41^{a}_{A}$	$6.00 \pm 2.05^{a}_{A}$
12	$5.75 \pm 2.40^{b}_{A}$	$9.83 \pm 5.29^{a}_{A}$	$3.55 \pm 1.28^{a}_{A}$	$2.58 \pm 1.14^{a}_{A}$	$2.3 \pm 1.50^{a}_{A}$
Total biomass					
2	$4.00 \pm 0.60^{ab}_{A}$	$6.38 \pm 1.17^{a}_{A}$	$9.60 \pm 5.03^{a}_{A}$	$6.55 \pm 1.13^{a}_{A}$	$4.58 \pm 1.01^{a}_{A}$
4	$3.68 \pm 1.05^{ab}_{A}$	$12.78 \pm 6.28^{a}_{A}$	$10.85 \pm 6.09^{a}_{A}$	$12.00 \pm 2.25^{a}_{A}$	$6.55 \pm 3.44^{a}_{A}$
6	$4.18 \pm 0.47^{ab}_{A}$	$19.73 \pm 5.09^{a}_{B}$	$15.40 \pm 2.42^{a}_{AB}$	$19.58 \pm 4.13^{a}_{B}$	$12.85 \pm 4.73^{a}_{AB}$
8	$2.78 \pm 0.95^{a}_{A}$	$15.68 \pm 1.06^{a}_{B}$	$25.90 \pm 5.29^{a}_{B}$	$13.00 \pm 3.78^{a}_{B}$	$19.47 \pm 4.06^{a}_{B}$
10	$6.47 \pm 2.13^{ab}_{A}$	$16.33 \pm 1.74^{a}_{A}$	$9.70 \pm 2.37^{a}_{A}$	$17.80 \pm 4.25^{a}_{A}$	$12.90 \pm 3.89^{a}_{A}$
12	$17.40 \pm 6.43^{b}_{A}$	$29.47 \pm 13.69^{a}_{A}$	$9.40 \pm 2.53^{a}_{A}$	$6.95 \pm 2.13^{a}_{A}$	$7.40 \pm 4.11^{a}_{A}$

*Interaction effects of time and level of diesel contamination

T_0 = control, T_1= 25ml diesel oil kg^{-1} of soil, T_2 = 50ml diesel oil kg^{-1} of soil, T_3 = 75ml diesel oil kg^{-1} of soil, T_4 = 100ml diesel oil kg^{-1} of soil

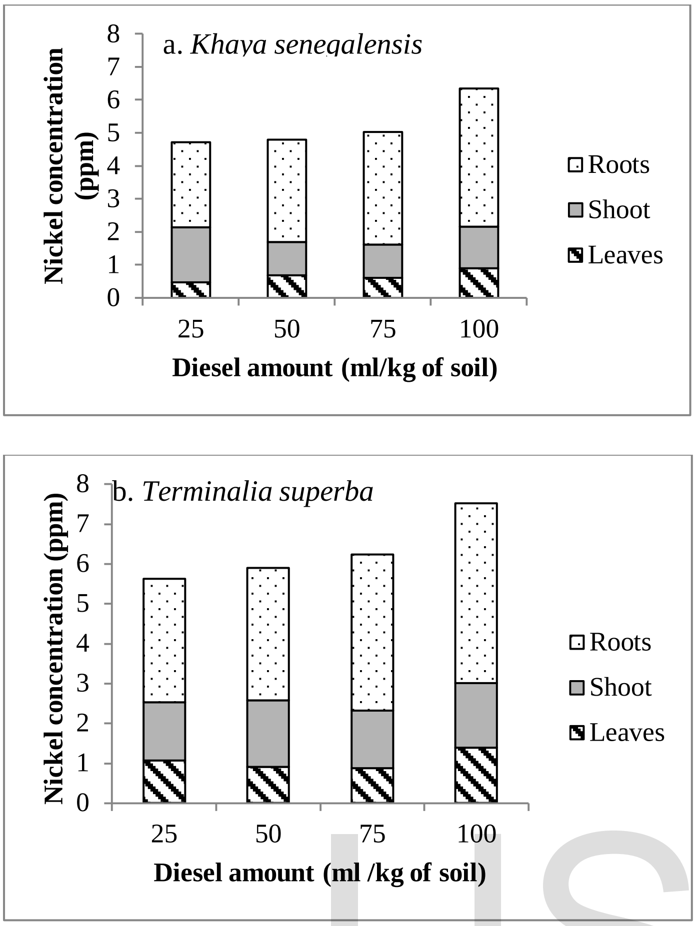
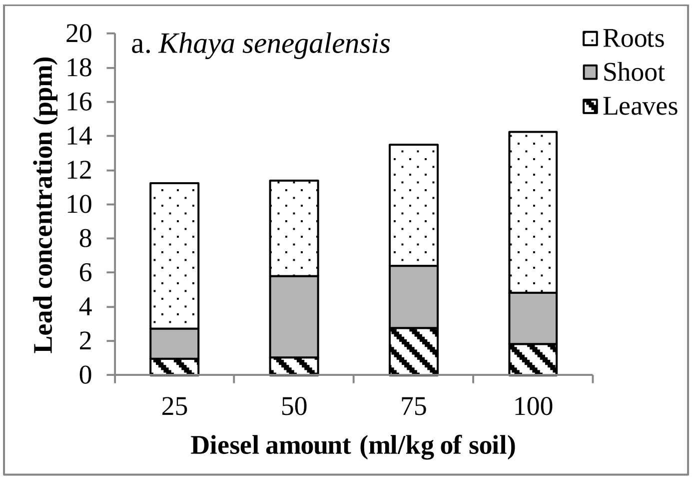


Fig. 4a and b. Nickel concentrations in different plant parts of *Khaya senegalensis* and *Terminalia superba* seedlings after 12 weeks



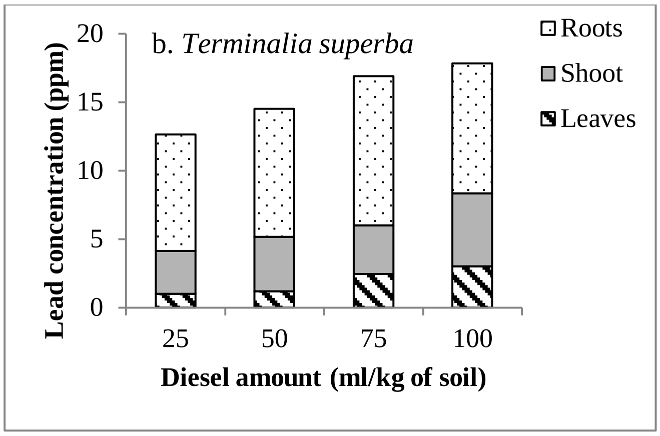


Fig. 5a and b. Lead concentrations in different plant parts of *Khaya senegalensis* and *Terminalia superba* seedlings after 12 weeks

4. Discussion

4.1 Growth of seedlings in diesel oil contaminated soil

Hydrocarbons from petroleum products affect plants directly, smearing roots with oily substances and therefore limiting respiration and transpiration by plants. These toxic pollutants reduce cell membrane permeability, disrupt metabolic activities and result in changes to chemical composition of products of photosynthesis. In addition, these products adversely reduce yields of various plant species and in high doses they can depress germination of plants and cause necrosis of seedlings [5], [24] However, these extreme effects were not clearly evident over the three months during which this study was conducted. Therefore, the ability of both *K. senegalensis* and *T. superba* to recover, tolerate and survive in the varying degrees of diesel oil contamination suggests that both species may be potential phytoremediators. Tolerance refers to the ability of the two tree species to grow in diesel oil contaminated soil; it does not necessarily mean the plant is healthy [4].

The diesel oil contamination did not have a significant influence on leaf production in both species. The foliage on the *T. superba* seedlings was generally higher than the *K. senegalensis* foliage until the 12th week (Fig. 1a and b). These findings suggest that tree species foliage may be able to better tolerate increasing levels of hydrocarbon contaminants than smaller plants. As Njoku *et al.* [7] observed significant reductions in leaf area of *Vigna unguiculata* growing in diesel oil/gasoline mixtures while Odjegba and Sadiq [25] observed a similar trend in *Amaranthus hybridus* growing in spent engine oil. The ability of the tree species to produce foliage, despite the increasing diesel oil pollution suggests that the contaminant may not have significantly hindered photosynthetic activity in the seedlings. The growth response of both species suggests that the photosynthetic efficiency of both plants may not have been adversely affected by the pollution as the main effect of level of diesel oil contamination was not significant. This highlights the potential that the tree species may be able to tolerate the hydrocarbon contamination at the seedling stage when compared with smaller, annual crops [7], [25]

All treatments showed fluctuations (increases and decreases) in collar diameter, height and number of leaves during the experiment (Figs. 1, 2 and 3) and this may be attributed to the toxic effect of diesel oil on the trees as suggested by Majid *et al.* [14]. Plant growth inhibition can be caused by toxic compounds of petroleum hydrocarbons such as molecules like aromatics, which can enter and permeate cell membranes leading to reduced, membrane integrity, damage or death of cells [4], [16], [18], [26]. However after 12 weeks, the mean height of *K. senegalensis* increased by 103%, 93%, 17%, 3% and 62% in the T_0, T_1, T_2, T_3 and T_4 treatments while slight reductions were observed in *T. superba seedlings* in T_0 (-4%) and T_4 (-5%) treatments, but collar diameter of both species increased over the 12 week period (Fig. 2a and b).

The reduced plant height and biomass production, for *K. senegalensis* and *T. superba* observed at certain points of the study are similar to results of previous experiments [16], [17]. A reduction of the rhizosphere directly affects the spatial extent of microbial stimulation and consequently may reduce biodegradation of pollutants with indirect effects on growth. This reduction in growth could also be attributed to the contamination limiting the flux of carbon to the root thereby, decreasing soil-derived resources such as water, nutrient supply, oxygen, and temperature [27]. However, this ability to change carbon fluxes in candidate species could be an advantage in phytoremediation as it reveals the capacity of the plant to respond to altered environmental conditions (water and nutrient deficiency) [17]. However, when evaluating species for phytoremediation, the decrease in plant growth and especially root biomass should be as low as possible.

Another explanation for the fluctuations in growth could be as a result of the hydrophobic behaviour of petroleum related oils, which change soil properties, thereby reducing water and nutrient availability [28]. Eventually, the soil condition tends to stabilize thus allowing for steady improvement in plant growth after a certain period of time. In this circumstance, plants produce more leaves and at the same time height and collar diameter growth improve with period of exposure [3], [16]. The increase in height, collar diameter and number of leaves and biomass was observed to be higher for plants growing in contaminated soil than in uncontaminated soil, and this supports the assumption of Merkl *et al.* [17] and Robson *et al.* [19] that petroleum hydrocarbons influence plant growth patterns and development, especially during the seedling stage.

Over time, petroleum derived contamination such as diesel oil can cause growth stimulation as observed for higher biomass, height and collar diameter of *K. senegalensis* and *T. superba* seedlings in polluted soils when compared with control treatment. This possibly can be explained by a hormonally influenced stress response [18]. The presence of diesel oil may have enhanced biomass accumulation for *K. senegalensis* in comparison to *T. superba*. This can be interpreted as stress response or as a strategy applied by some plants facing nutrient deficiency as increasing root biomass, increases nitrogen uptake [16].

After 3 months, the highest total biomass produced by the *K. senegalensis* was recorded in T_2 (Table 1b). This treatment also recorded the highest biomass in leaves, shoot and roots. Biomass accumulation plays a vital role in the absorption of heavy metals from soil, with the success of phytoaccumulation depending on the ability of plants to absorb, accumulate and tolerate metals in their shoots and roots. Thus, plants used as phytoremediators must possess a high capacity to absorb elements from soil or water as well as large biomass production [5], [14] [29]. Sun *et al.* [29] showed that fast growing tropical tree species such as *Prosopis pallida, Thespesia populnea, Casuarina equisetifolia, Cordia subcordata, Erythrina variegata, Myoporum sandwicense, Scaevola sericea,* were able tolerate the combined adverse effects of moderate salinity (1% NaCl) and 10 g diesel fuel/kg soil. And they reported that three species (*Prosopis pallida, Cordia subcordata* and *Thespesia populnea)* significantly accelerated the degradation of petroleum hydrocarbons.

Easy accessibility and availability of selected plant material is a key factor if phytoremediation is to be efficiently applied to different types of contaminated substrate (soil, water, sludge etc.) [26]. These two popular hardwood species (*K. senegalensis and T. superba)* due to their growth, genetic and cultural characteristics, could be potential candidates in the remediation of contaminated substrates. Though previous authors have highlighted the damage and growth inhibition caused by petroleum hydrocarbons to agronomic crops such as *Abelmoschus esculentus* (okro), *Lycopersicon esculentum,* (tomato), *Capsicum annuum* (pepper), *Solanum melongena* (eggplant) and *Solanum incanum, Arachis hypogaea, Vigna unguiculata* (cowpea), *Sorghum bicolor and Zea mays* (maize) [10], [30], [31]. This study has shown the potential capability of tropical hardwood species to survive and tolerate diesel oil contamination and offers a large potential for reforestation of hydrocarbon polluted lands [14]; especially in the Niger-Delta region of Nigeria. However, long term studies and increased period of exposure to higher contamination levels are important to confirm these assertions.

4.2. Heavy metal accumulation

The two species were able to bio accumulate both Pb and Ni in their tissues, with heavy metal concentration increasing in the order leaves < shoots < roots. The roots were able to retain over 50% of the heavy metals in most of the treatments studied (Fig. 4 and 5). Numerous studies have shown differentiations in heavy metal accumulation and translocation in actively growing tissues such as roots, stems and young leaves. For example, Baker [18] and Majid *et al*. [14] reported higher Cu absorption in roots than shoots of various plants.

Plants are known to present differential tolerance for various metals and their toxicity [13]. For both tree species, it was observed that the level of accumulation increased with increasing level of diesel oil contamination. The most commonly visible, but nonspecific symptom of heavy metal stress was growth inhibition has observed in T_4 seedlings which had the lowest biomass and growth performance (Table 1) but the highest concentrations of both heavy metals. Heavy metals interact with essential macro- and microelements, and these interactions influence plant nutrient uptake. They also affect plant–water relationships, by inhibiting formation of root hairs and causing a direct reduction in the root absorption surface [28].

In this preliminary study, the two tree species were able to translocate Pb and Ni to their aerial parts. The accumulation patterns showed that the trees accumulated more Pb than Ni, while *T. superba* accumulated more heavy metals (Ni: 5.62 - 7.52 ppm; Pb: 12.63 – 17.82 ppm) than *K. senegalensis* (Ni: 4.71 – 6.34 ppm; Pb: 11.24 – 14.26 ppm). Kim *et al*. [32] suggested such variations in amounts phytoextracted by different plants depend on heavy metal concentration, form of metal present, and plant species. Nickel, although essential for plants at low concentrations, is, however, toxic at higher concentrations, while Pb is highly toxic [13], [32]. However, the heavy metal form and concentration in the contaminated soil was not determined.

As observed in this study, heavy metal translocation to the above ground organs is a crucial biochemical process in any effort to effectively use plants for phytoremediation of polluted sites. It would reduce the damaging effects exerted by pollutants on root physiology and biochemistry while ensuring effective metal removal from the substrate, over time [26], [33]. *Khaya senegalensis* and *T. superba* showed their potential for heavy metal tolerance, bioaccumulation and translocation.

Conclusion

This study provided evidence of the ability of tropical hardwood tree species to tolerate and grow in diesel oil contaminated soil. Although oil degradation was not measured, rehabilitation of contaminated soils is likely to occur with time as the both species were able to phytoextract and translocate heavy metals from belowground to aboveground parts. The study also should that growth inhibition was not pronounced in the contaminated soil, while the growth performance and biomass accumulated by seedlings in uncontaminated soil did not significantly differ from those in polluted soil. Since perennial plants are more desirable for phytoremediation, *K. senegalensis and T. superba* are promising species that could be further evaluated for phytoremediation of petroleum-oil contaminated soils. The two species are widely accepted timber species and their use for reforestation/phytoremediation of crude oil damaged sites could be beneficial for many oil producing tropical countries. Further investigations into the tolerance of higher pollutant concentrations, possibilities of improving growth using fertilizers as well as identifying oil degrading microbial populations in the rhizospheres, in order to optimize growth and biomass accumulation, are recommended.

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