Phytoremediation of Soil Contaminated with spent Engine Oil using *Corchorus olitorus* Linn.

[1]Kekere, O., Olarewaju, T. and [2]Ogbemudia F. O.
[1]Department of Plant Science & Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State Nigeria.[2]Department of Botany and Ecological Studies, University of Uyo, P.M.B. 1017, Uyo Akwa Ibom State, Nigeria.
Corresponding author: kekereekunnoi@yahoo.com Phone: +2348067646215

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Abstract

Phytoremediation is an alternative low cost approach for in situ treatment of polluted soils. This study was carried out to evaluate growth performance, nutrient composition and heavy metal uptake of Corchorus olitorus Linn. in soil contaminated with spent engine oil (SEO), to assess phytoremediation potentials of the plant. Plants were subjected to 0 (control), 2, 4, 6 and 86% w/w of oil in a pot experiment laid out in a completely randomized block design replicated 7 times. Significant treatment effects were observed in plant height, number of leaves, total leaf area per plant and root growth. Lower dry weight of plant parts and total biomass were obtained in polluted seedlings relative to the control. There was decrease in nutrient composition and total chlorophyll content of oil-polluted C. olitorus, suggesting stress infliction on the plants. Survival was not affected by oil pollution except at 16 % w/w. Results show that C. olitorius is tolerant to spent engine oil and was able to take up heavy metal contaminants at high concentrations with little phytotoxic effect. It is therefore a good candidate for phytoremediation of oil-polluted sites.

Oil pollution resulted in higher concentration of heavy metals in plant tissues of polluted than non-polluted plants.

Keywords: *Phytoremediation,* ***Corchorus olitorus****, spent engine oil contamination, pollution.*

Introduction

The contamination of the natural environment by petroleum-derived substances contributes to the degradation of land (Njoku *et al.*, 2009). Engine oil is one of the products of the fractional distillation of crude petroleum oil. It is used to reduce friction between moving parts of engines, which helps to reduce wear and tear and loss of power and prevents corrosion of engine parts. Disposal of spent engine oil into gutters, watercourses, open vacant plots and farmlands are common practices among automobile operators, which increases incidence of oil contamination of agricultural soils. Heavy metal contamination has also been found to be associated with spent engine oil pollution as there is presence of higher concentrations of heavy metals like vanadium, lead, aluminum, nickel and iron in used lubricating oils than in unused ones. Oil contaminated soils are of environmental concern because they are unsuitable for agricultural and recreational use and are potential sources for surface and ground water contamination (Nwoko *et al.*, 2007).

Topsoiling and other methods for oil removal are expensive and do not give permanent solution to the problem (Sung *et al.*, 2004). Phytoremediation is an attractive low cost to traditional remediation strategies. Plants can absorb, translocate or sequester contaminants, thus removing them from soil (Cunningham *et al.*, 1995). Aeration within plant root areas resulting from direct release of O_2 from roots and O_2 diffusion along old root channels which stimulates microbial activity within the region also enhances bioremediation of hydrocarbons (Nwoko *et al.*, 2007). Roots can also reduce movement of contaminant in soil by extracting excess water, thus reducing the downward flow of water (Viamis *et al.*, 1985).

Previous findings have shown that phytoremediation had good performance in bioremediation of crude oil contaminated soil (Anoliefo and Vwioko, 2003). Issoufi *et al.* (2006) reported significant percentage emergence and incineration as possible clean up methods in *Zea mays* L.and *Glycine max* L. Merr.when grown in oil-contaminated soil. Similarly, petroleum hydrocarbonwas significantly reduced by *Lolium perenne* L.compared with an unvegetated control in a laboratory study (Gnther *et al.*, 1996). Plant growth performance and survival has been used to evaluate the potential of plants to grow and act as phytoremediating agents (Spiares *et al.*, 2001). Kekere *et al.* (2006) reported higher concentration of heavy metals in the leaves and stems of fluted pumpkin. *Telfaria occidentalis* Hook. F. grown in soil polluted with spent engine oil compared with control.

Cochorus olitorus belongs to the family Tiliaceae. It is widely grown in Africa especially in Nigeria where the leaves are eaten as vegetable, raw or cooked. Young leaves are added to salads while older leaves and immature fruits are cooked as a potherb. It is widely eaten in Nigeria and is locally called 'ewedu' soup. It is commonly grown around home stead where there is high likelihood of being polluted with spent engine oil. Ability of plant species to absorb and sequester contaminants, with little expression of phytotoxic effects has been identified to be a quality of plants used for phytoremediation (Nwoko *et al.*, 2007). Therefore, the present study examines the growth performance and heavy metal uptake of *Corchorusolitorus* grown in soil contaminated with spent engine oil.

Materials and Methods

The experiment was carried out in the Greenhouse of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria (Lat. 7^0 N 28^1, Long. 544^1 E). Sandy loam soil collected from a nearby field with 5.60pH, 6.1% clay, 4.2% silt, 89.7% sand, 2.89% C, 0.14% N, 9.02mg/kg P, 6.24mg/100g Ca, 1.84mg/100 Mg, 0.35mg/100g Na, 0.23 mg/100g K, 0.20mg /100g H and 8.8mg/100CEC was used for planting. Perforated plastic pots (9 cm height and 23 cm diameter) were filled with about 3 kg of soil treated with spent engine oil (SAE 40) to achieve concentrations of 0, 2, 4, 8 and 16% weight/weight (w/w) of oil/soil, respectively. These were replicated 7 times and laid out in a completely randomized block design.

Cochorus olitorius seeds were obtained from the local farmers and three seeds were planted per pot and later thinned to one after emergence. Growth parameters including plant height, leaf number, stem diameter and total leaf area per plant were determined at 2, 4, 6 and 8 days after pollution (WAP). Root number, root length, dry weights of plant parts and total biomass were recorded at harvest. Total leaf chlorophyll content and heavy metal concentration of dry leaves were also analyzed. Meter rule was used to measure plant height from soil level to terminal bud while root length was from the base to the root tip. Total leaf area was measured by comparing the weight of a cut-out traced area with standard paper of known weight to area ratio (Eze, 1965).

Stem diameter was measured at 10cm from ground level by an electronic digital caliper (model 0-200mm digital caliper). Dry weight was measured by carefully oven drying at 80°C until weight was constant. Total chlorophyll of plants was extracted in 80% (v/v) aqueous acetone and absorption was measured in spectrophotometer at 645 and 663 nm (Arnon, 1949). Chlorophyll content (mg l^{-1} fresh leaf weight) was calculated using the following formula: Total chlorophyll = $(20.2 \times D_{645} + 8.02 \times D_{663}) \times (50/1000) \times (100/5) \times ½$, where D = absorbance.

Dried plant leaves were placed in porcelain crucibles and ashed in a muffle furnace for 24 h at 500 ^{0}C. Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+} and Fe^{2+} content were analyzed using a Perkin Elmer model 360 atomic absorption spectrophotometer after acid digestion. Na^+ and K^+ concentrations were assayed by flame emission spectrophotometry, while total nitrogen content was by Micro-Kjedahl method. Data were subjected to single factor analysis of variance and means were separated by Duncan's MRT at a significant level of $P \leq 0.05$.

Results

The results show the influence of spent engine oil on the growth of *C. olitorius*.Plants maintained high level of survival under oil pollution except at 16% w/w where some seedlings died at 4 weeks after treatment and 75.71% survival was finally obtained (Figure 1). Table 1 shows data on the effect of SEO on plant height, leaf area and stem diameter of *C. olitorius*. Plant height and leaf area and stem diameter decreased with increasing concentration of oil. Reduction in plant height became significant ($p\leq 0.05$) between treatments from 2 weeks after pollution while leaf area was from 4 weeks after treatment application. At the end of the study, leaf area and plant height of oil-treated seedlings were significantly different from that of control. The highest reduction was obtained at concentration of 16% w/w. However, difference in stem girth between treatments became significant ($p\leq 0.05$) only at the highest level of pollution after 8 weeks. There was no significant difference ($P \leq 0.05$) between the control plants and those grown in soil with 2-8% of oil. Figure 2 shows the number of leaves of the seedlings as affected by oil treatment. Number of leaves increased with time but it decreased significantly ($p\leq 0.05$) in comparison with untreated control. At the end of the study, the highest reduction (26.23%) in number of leaves was obtained at 16% w/w relative to the control treatment. Root growth was also reduced with increasing concentration of oil as shown in Figure 3. Significant ($p\leq 0.05$) reduction was obtained at 16% for root length while number of roots decreased significantly at 8-16%.

Dry weight of plant parts decreased with increase in oil intensity as shown in Figure 4. The least weight was obtained at 16% w/w. Total biomass reduced significantly ($p\leq 0.05$) in seedlings grown in polluted soil compared with the unpolluted control (Figure 5). Total chlorophyll was negatively affected by oil pollution particularly at high oil intensity (Figure 6). Oil pollution led to a reduced nutrient uptake in the leaves of *C. olitorius* as the concentrations of all the essential elements were lower in polluted plants than in unpolluted ones (Table 2). Table 3 shows the heavy metal concentration in the leaves of *C. olitorius* grown in soil with different concentrations of SEO. There was an increase in the level of heavy metals with increasing concentration of oil which was remarkably higher than the control.

Discussion

Although, the presence of SEO in the soil had negative effect on the growth of *C. olitorius*, it was still highly tolerant to oil pollution. The presence of SEO in the soil-plant microenvironment appears to have affected the normal soil chemistry wherein nutrient release and uptake as well as amount of water have been reduced (Nwoko *et al.*2007). The growth of the plant was not significantly affected except at very high concentration of 16% w/w. This means that *C olitorius*can tolerate low and high levels of SEO contaminations as evidenced by plant height, leaf number, leaf area and stem diameter in low and high SEO contamination, which gave high values comparable to the control. This is similar to the findings of Adenipekun *et al.* (2009) that growth parameters were reduced in *A. esculentus* grown in soil contaminated with spent engine oil. Also,

Agbogidi (2010) showed that six cultivars of cowpea grown in 25 ml of spent engine oil gave consistently significant high values in growth parameters.Mineral ions absorbed by the root are finally received by the mesophyll cells of the leaves which can cause reduction in leaf area if toxic mineral ions are absorbed. Growth reduction under oil pollution has also been reported by Anoliefo Edegbai (2000) on *Solanummelongena* and *Solaumincanum*, Odjegba and Sadiq (2002) on *Amarantushybridus*, Vwioko and Fashemi (2005) on *Ricinuscommunis*, Kekere *et al.*,(2006) on *Telfaria occidentalis*, Adenipekun *et al.* (2009) on *A. esculentus* and. Njoku *et al.* (2009) on *Zeamays*. Recently, Agbogidi (2010) recorded reduction in the growth parameters of 6 cultivars of cowpea grown in soil contaminated with spent engine oil. The presence of oil in the soil plant micro- environment appears to have affected normal soil chemistry where nutrient release and uptake as well as amount of water have been reduced (Nwoko *etal.*,2007).

The SEO treatment had adverse effect on the total chlorophyll content which decreased with increase in SEO concentrations. This is in conformity with the findings of Odjegba and Sadiq (2002) where reduction in chlorophyll and protein levels were reported in *Amaranthus hybridus* grown in soil contaminated with spent engine oil. Likewise, Adenipekun *et al.* (2009) reported that spent engine oil led to a decrease in the chlorophyll content of *A. esculentus* relative to the control. Reduced chlorophyll production might be connected with the low level of essential nutrients required for normal growth. Reduction in root development in the presence of oil could be due to water unavailability and oxygen deficiency which resulted in less optimal functioning of the roots. Plant mortality obtained at very high concentration of oil can be attributed to water and oxygen stress, nutrient deficiency and uptake of toxic substances such as heavy metals. Agbogidi (2010) showed build up of heavy metals in engine oil-impacted soil and stated that it had toxic effects on plants. Dry weight reduction by oil pollution in this study is in conformity with the results of Omosun *et al.* (2008) who found that dry weight was reduced in *Amaranthus hybridus* grown in oil-contaminated soil. Total biomass reduction in *C. olitorius* can be attributed to the negative influence of oil pollution on growth parameters. Reduced number of leaves and total leaf area must have led to reduced interception of light for photosynthesis, hence an overall effect on biomass production.

This investigation shows that *C. olitorius* is tolerant to spent engine oil pollution as evidenced by high survival and growth comparable to control even at high concentration. The plant was able to take up heavy metals at high concentrations with little phytotoxic effect. Although, spent engine oil has potential adverse effect on the growth of *C. olitorius* at very high concentration with negative effect on the nutritional composition of the leaves, but it is a good candidate for the phytoremediation of oil polluted sites. It is therefore necessary to discourage indiscriminate disposal of spent engine oil into the environment.

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Table 1: Growth parameters of *Corchorus olitorius* as affectd by spent engine oil pollution

Growth Parameters	WAP	Spent engine oil concentration (% w/w)				
		0	2	4	8	16
Plant height (cm)	2	28.57[a]	23.44[b]	22.88[b]	20.75[b]	18.13[b]
	4	35.00[c]	25.89[b]	24.13[b]	24.63[b]	18.25[a]
	6	38.14[c]	25.88[b]	26.38[b]	27.88[b]	18.25[a]
	8	40.43[b]	30.00[b]	30.50[b]	27.88[b]	20.25[a]
Leaf area (cm^2)	2	29.22[a]	20.51[a]	20.14[a]	20.57[a]	18.40[a]
	4	31.53[b]	24.24[ab]	20.02[ab]	21.02[ab]	18.68[a]
	6	35.14[b]	24.97[ab]	23.86[ab]	22.72[ab]	14.50[a]
	8	37.60[b]	24.46[b]	19.07[a]	15.01[a]	15.08[a]
Stem diameter (cm	2	0.34[a]	0.31[a]	0.31[a]	0.31[a]	0.30[a]
	4	0.37[a]	0.33[a]	0.30[a]	0.30[a]	0.28[a]
	6	0.39[a]	0.33[a]	0.30[a]	0.28[a]	0.20[a]
	8	0.43[b]	0.33[b]	0.31[b]	0.28[b]	0.17[a]

WAP= weeks after pollution.
Means followed by the same letter(s) within a column are not significantly different at $P \geq 0.05$. (DMRT).

Table 2: Effect of spent engine oil on the nutrient composition (%) in the leaves of *C. olitorius*

Nutrient composition (%)	Spent engine oil concentration (% w/w)				
	0	2	4	8	16
N	1.98	1.50	0.790	0.43	0.12
P	0.11	0.09	0.07	0.05	0.02
K	2.11	1.23	0.92	0.38	0.12
Ca	2.37	1.99	0.63	0.32	0.14
Mg	0.48	0.35	0.28	0.15	0.07
Na	0.04	0.04	0.04	0.03	0.03

Table 3:Effect of spent engine oil on heavy metal content (%) of *C. olitorius*

Heavy metal content (%)	**Spent engine oil concentration (% w/w)**				
	0	2	4	8	16
Fe	15.04	23.06	27.34	36.99	37.00
Zn	10.12	28.27	35.97	38.12	40.56
Pb	5.72	11.71	13.89	14.14	14.94
Cd	0.14	1.78	2.12	2.98	23.16

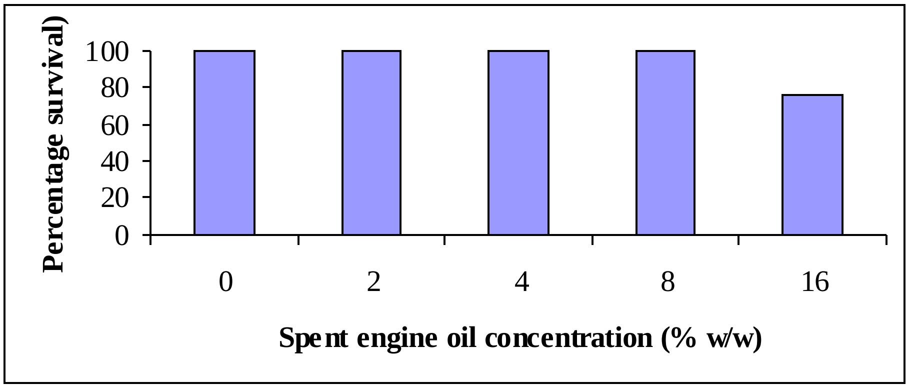

Figure 1: Percentage survival of *C. olitorius* after 8 weeks of growth in soil polluted with spent engine oil

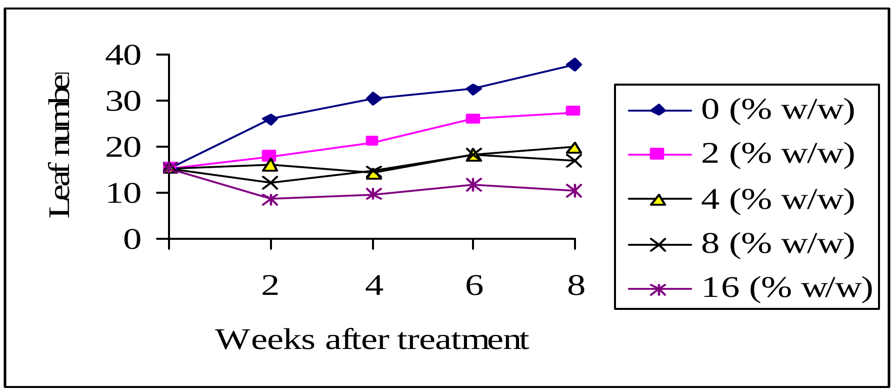

Figure 2: Leaf number of *C. olitorius* grown in soil contaminated with spent engine oil

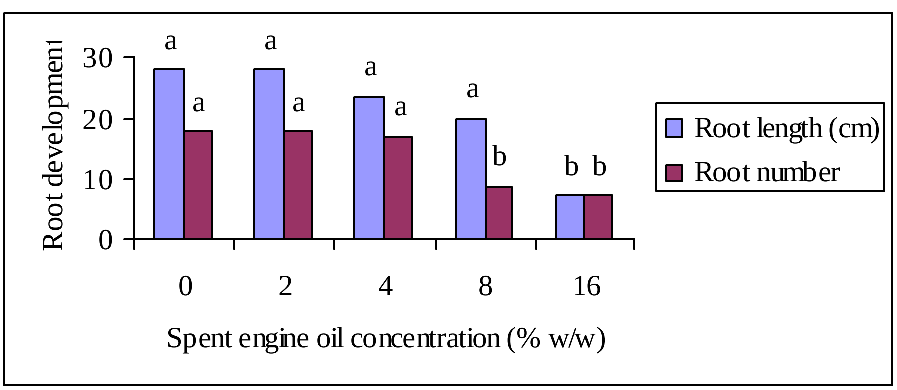

Figure 3: Root growth of *C. olitorius* after 8 weeks of growth in soil polluted with spent engine oil

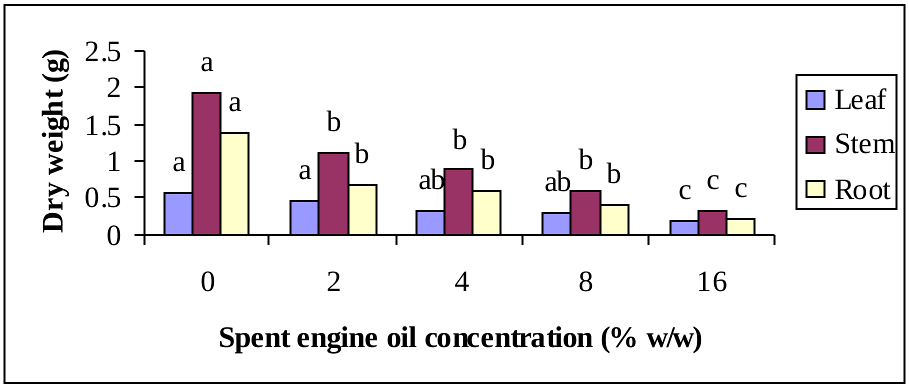

Figure 4: Dry weight of *C. olitorius* after 8 weeks of growth in soil polluted with spent engine oil

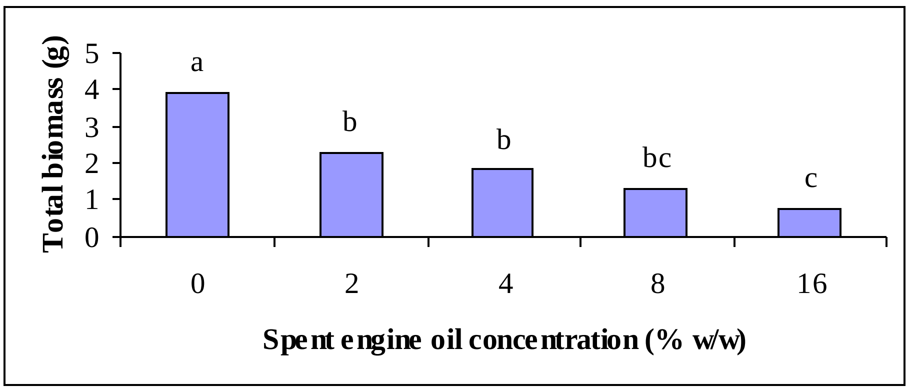

Figure 5: Total biomass of *C. olitorius* after 8 weeks of growth in soil polluted with spent engine oil.

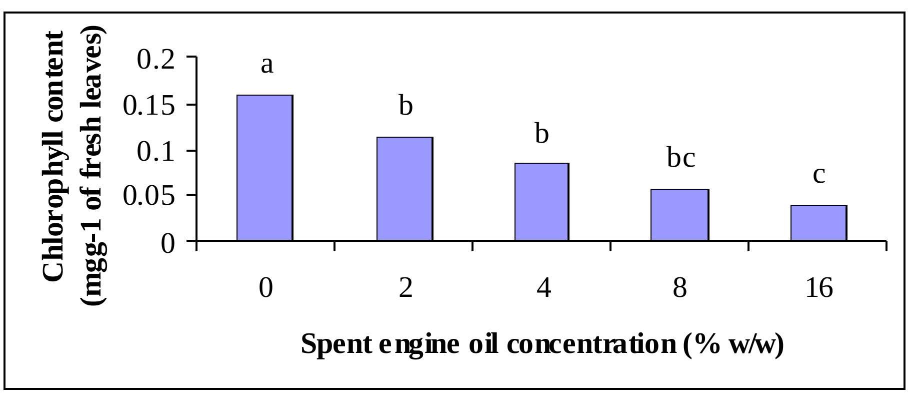

Figure 6: Total chlorophyll content of *C. olitorius* after 8 weeks of growth in soil polluted with spent engine oil.