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Growth Response of Alternanthera brasiliana (L.) Kuntze in a Waste Engine Oil-Polluted Soil

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Abstract

The indiscriminate dumping of waste engine oil which has led to the loss of arable land, forests and potable water is on the rise. Phytoremediation which is the use of plants in the clean-up of polluted environment is an emerging technology, which is environmentally friendly and cheaper than the conventional technology. About 400 plant spp. are able to remediate polluted environments and more are being discovered. This study therefore, was carried out to determine the impact of waste engine oil-polluted soil on some growth parameters of *Alternanthera brasiliana* (L.) Kuntze. Sun-dried top soil (0-10cm) was measured into buckets. Waste engine oil (WEO) was added to soil and mixed thoroughly to obtain various concentrations on weight basis: 0, 1, 3, 6, 9 and 12 % w/w oil-in-soil. One month after soil was amended with WEO, sprouted shoot cuttings of *A. brasiliana* were transplanted from a nursery. There was significant ($p<0.05$) decreases in plant growth parameters in the oil-polluted soil. The sprouted plants had a shoot growth rate of 58.6 % in the 1 % w/w oil-polluted soil, while plants in the 12 % w/w oil-polluted soil had a growth rate of 36 % by the 2nd week after transplant (WAT). In the 1 % w/w oil-polluted soil, plants grew to an average height of 100 cm while plants in the 12 % w/w oil-polluted soil had average height of 41.67 cm. It was generally observed that in spite of the duration of the study (4 months) and the impact of oil on the vegetative growth of *A. brasiliana*, the plants survived even at 12 % w/w oil-in-soil treatment. The persistence of *A. brasiliana* in the polluted soil provides the possibility for its being a candidate for phytoremediation.

Keywords: waste engine oil, polycyclic aromatic hydrocarbon, physicochemical, phytoremediation, Alternanthera brasiliana,

INTRODUCTION

The United States Environmental Protection Agency (2001) defined waste engine oil as any oil that has been produced from crude oil or, any synthetic oil and as a result of usage has been contaminated by chemical or physical impurities. Pollution from spent engine oil is as a result of anthropogenic activities such as; indiscriminate disposal in trash, on land or into sewers and has the potential for contaminating soil, ground water and surface water. This type of pollution is one of the environmental problems in Nigeria and is more widespread than crude oil pollution (Odjegba and Sadiq, 2002).

Waste engine oil consists of a base lubricating oil which is a mixture of hydrocarbons, 80 % to 90 % by volume, and performance enhancing additives (detergents, anti-corrosion/rust, anti-oxidants and metal deactivators) are present in engine oils (Anders, 2006). Waste engine oil contains more polycyclic aromatic hydrocarbons than new motor oil. In new motor oil, lighter hydrocarbons which may cause acute toxicity to aquatic organisms are present. This is in contrast with used motor oil which possesses heavy metals and more polycyclic aromatic hydrocarbons, which cause chronic hazards and cancer (Mandri and Linn, 2006). Polycylic aromatic hydrocarbons may be photo-oxidized, leached, volatilized, adsorbed to soil particles, bioaccumulated, chemically oxidized or microbially degraded when present in the environment (Olesczuk, 2008). In waterways, used motor oil is toxic to plants and animals living in the water, and its film can disrupt natural processes like oxygen replenishment and photosynthesis (Joan, 2006). Soils polluted with waste engine oils have low oxygen concentration, reduced fertility and reduced microbial composition.

Poisonous contaminants are further introduced into the soil by physicochemical clean-up methods (Ikhajiagbe and Anoliefo, 2011) thus; environmentally safe and cost effective methods for the cleanup of these polluted soils have been sought. Some plants, together with some microorganisms through the process of phytoremediation have been found to remediate waste engine oil-polluted soils. These plants includes; Poplar (*Populous sp.*), Sunflower (*Helianthus annus*), Indian mustard (*Brassica juncea*) and *Jatropha curcas* (Burken and Schnoor, 1996). The present study hopes to investigate the capability of *Alternanthera brasiliana* to survive in a waste engine oil-polluted soil, as an indicator for its suitability as a phytoremediator.

Alternanthera brasiliana (L.) kuntze originates from tropical and sub-tropical regions of Australia and

South America. It is a perennial plant and belongs to the family Amaranthaceae. *Alternanthera* are grown for edging beds and for beddings for animals. These plants can withstand trimming as they are hardy plants and are propagated easily by cutting or by division (Anonymous, 2005). The plant *Alternanthera brasiliana* is an ornamental, as well as a medicinal plant, found growing wild in bushes and along the road sides. Several species are grown for their ornamental leaves which may be variegated green and yellow, or bronze and green, or red and pinkish brown. It could be a noxious and/or an invasive weed whose seeds cannot be stored for long. *Alternanthera brasiliana* has been implicated as an indicator of pollution (Salvador *et al*., 2004). Results showed that it had elemental concentrations which were higher than 1% of dry weight plant tissues. The present study is therefore undertaken to investigate the growth response of the plant to waste engine oil-polluted soil, and also to ascertain the usefulness of the plant in phytoremediation strategies.

MATERIALS AND METHODS

Top soil (0-10 cm), of known physicochemical properties (Table 1), was collected randomly from an area marked in the Department of Plant Biology and Biotechnology Botanic Garden, University of Benin, Benin City, Nigeria. Thereafter, 10kg sun-dried soil was each placed into large perforated bowls with 5 random perforations made with 2 mm diameter nails at the bottom of each bowl. Waste engine oil (WEO) was added to soil in the bowls and mixed thoroughly to obtain different concentrations on weight basis: 0, 1, 3, 6, 9 and 12 % w/w oil-in-soil. Treatments were replicated 3 times. The buckets were transferred into a well ventilated screen house with inherent room temperature (27 – 35^{o}C).

The entire setup was left for one month without any physical disturbance, though the buckets were constantly ridded of weeds (Ikhajiagbe and Anoliefo, 2011). Buckets were also watered every evening with just enough, to moisten the soil in the screen house. After one month, soil was tilled, using a hand trowel, and watered before planting. The soil samples were arranged in randomized block design.

Plant Collection and Cultivation

Young stem cuttings of about 15 cm of *Alternanthera brasiliana* were collected from the wild. These were sown with stem nodes, deep into unpolluted nursery soil and watered, to encourage rooting. The stem cuttings were left to establish in the nursery for 7 days, after which they were transplanted into the polluted soil treatments. Five sprouted stem cuttings were sown in each bucket. Plants were watered every evening throughout the course of the experiment

Soil Physicochemical Analyses

Soils were dried at ambient temperature (22-25^{o}C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm fraction was used for the determination of heavy metal fractions by atomic absorption spectrophotometry (APHA, 1985).

Determination of Soil Total Hydrocarbon Content (THC)

The method of Wang *et al.*, 2011 was used to determine the total hydrocarbon content of the soil.
Absorbance was read at 460 nm, using a spectrophotometer through a set of working standard series of the order 0, 5.0, 10.0, 15.0, 20.0 and 25.0 ppm respectively.
Mg/kg THC (ppm)=

$$\frac{A \times S.R \times V_1}{W\ (kg)} \quad (1)$$

S.R= slope reciprocal; A= absorbance; V_1- volume extracted; W- weight of sample

Determination of Soil Nitrate

One hundred (100) grams of sodium acetate salt was dissolved in 400 ml of distilled water into which, 30 ml of acetic acid (glacial) was added, adjusted to pH 8.2 and diluted to 1 liter. Fifteen grams (15 g) of the processed air dried soil was weighed into a 250 ml plastic bottle and 20 ml of the Morgan's reagent was added into the plastic bottle containing the sample. This was placed on a mechanical shaker and shook for 1hr and was filtered with Whatman filter paper No. 42.

Determination of Nitrate in the Filtrate

Five milliliter (5 ml) aliquot (portion) of the soil extract was transferred into a clean plastic bottle into which, 2 ml of brucine reagent was added, and rapidly, 10 ml of concentrated sulphuric acid was also added. This was mixed for about 30 seconds and allowed to stand for 15 minutes.
Absorbance was measured at 470 nm through a set of standard of the order 0, 0.4, 1.2, 1.6 and 2.0 M.

$$\frac{A \times S.R \times V_1\,(ml) \times V_2\,(ml)}{W_1\,(kg) \times V_3\,(ml)} \quad (3)$$

A- Absorbance; S.R- slope reciprocal; W_1- Weight of sample; V_3- volume of aliquot; V_1- volume of extract; V_2- volume of colour extracted

Determination of Available Phosphorus

The Bray and Kurtz (1945) method was used in determining the available phosphorus is the soil.

Soil moisture determination

Soil moisture was determined using the gravimetric method by Black (1965).

$$\%\ \text{moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad (4)$$

W_0- weight of empty can; W_1- can + soil sample; W_2- dried soil sample + can

RESULTS AND DISCUSSION

Physiochemical contents of waste engine oil used in the presented study are presented on Table 1. The heavy metals nickel, lead, copper and cadmium were not detected as they were present in amounts below the sensitivity level of the Atomic Absorption Spectrophotometer. Mercury was not present in the waste engine oil sample. Zinc had the highest concentration amongst the heavy metals (180.85 mg/kg).

Table 1: Heavy metal contents of waste engine oil used in the study

Parameters	Concentration (mg/kg)
Nickel (Ni)	0
Lead (Pb)	0
Copper (Cu)	0
Cadmium (Cd)	0
Zinc (Zn)	180.85
Iron (Fe)	40.42
Chromium (Cr)	19.01
Total	**240.28**

From Table 2, the total organic carbon content and the total hydrocarbon content were observed to increase as the concentration of the waste engine pollutant increased in the soil. The total organic carbon (TOC) at 0 % was 0.770 mg/kg and 1.283 mg/kg in the 1 % w/w oil-polluted soil; whereas TOC in the 12 % w/w oil-polluted soil was 1.663 mg/kg. Soil nitrate was highest in the 9 % w/w oil-polluted soil (2.227 mg/kg). Soil phosphate was highest in the 3 % w/w oil-polluted soil (8.576 mg/kg), while the soil moisture content was highest in the 1 % w/w oil-polluted soil (25.92 mg/kg). Increased heavy metal content of soil was indicative of the increased concentrations of oil in soil (Table 2). Chromium was 15.51 mg/kg in 1%w/w treatment, compared to 39.93 mg/kg in the 12%w/w treatment respectively. Cd and V were undetected.

Table 2: Physicochemical properties of soil one month after pollution with waste engine oil, before planting

Parameter	0 %	1 %	3 %	6 %	9 %	12 %
Total organic carbon (mg/kg)	0.770	1.283	1.717	2.027	1.407	1.663
Total hydrocarbon (mg/kg)	5.530	6.273	18.183	61.447	109.290	151.050
Soil Nitrate (mg/kg)	1.577	0.437	0.980	0.597	2.227	1.103
Soil phosphate (mg/kg)	5.663	2.763	8.576	5.777	6.160	7.367
Soil moisture content	21.350	25.920	21.140	10.22	10.80	12.400
Heavy metals						
Chromium (Cr)	1.350	15.507	25.01	31.601	33.906	39.932
Nickel (Ni)	0.000	0.000	0.000	0.000	1.086	2.294
Lead (Pb)	6.303	24.957	25.033	24.202	43.521	52.485
Copper (Cu)	0.000	0.000	0.629	0.685	17.177	2.291
Cadmium (Cd)	0.000	0000	0.000	0.000	0.000	0.000
Zinc (Zn)	6.058	42.770	58.517	88.770	108.980	135.110
Iron (Fe)	415.325	7699.000	9857.300	9716.800	10295.000	10990.000
Vanadium (V)	0.000	0.000	0.000	0.000	0.000	0.000

The pH of the soil at 1, 3, 9 and 12 % w/w oil-polluted soils at 4 months after pollution were higher than values at 1 week after pollution (WAP), except in the control soil (0 % w/w) and 6 % w/w as obtained in Table 3. The control soil had a pH of 6.01 at 1 WAP and 5.70 at 4 months after pollution. The 1 % w/w oil-polluted soil had a pH of 5.69 at 1 WAP and 6.10 at 4 months after pollution; the 12 % w/w oil-polluted soil had a pH of 7.22 and 7.75 at 1 WAP and 4 months after pollution respectively. ANOVA revealed that there was no significant difference in pH at 1 WAP and 4 months after pollution ($P > 0.05$).

Table 3: pH values of polluted soil at 1 WAP and 4 months after pollution

	0 %	1 %	3 %	6 %	9 %	12 %
1 WAP	6.01	5.69	5.87	6.70	6.62	7.22
1 MAP	6.01	5.97	6.03	6.70	6.89	7.32
4 MAP	5.70	6.10	6.30	6.60	7.00	7.50

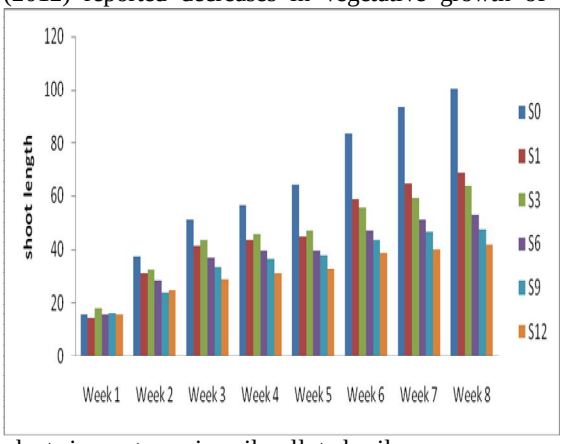
WAP- Week after pollution; MAP- month after pollution

Generally, plants growth rate was higher in the lower oil-in-soil concentrations. However, the lowest growth rate recorded in the present study was 35 % within the first 2 weeks. This may be as a result the waste engine oil pollutant present in the soil which contains heavy metals and polycyclic aromatic hydrocarbons. Apart from these constituents which inhibit plant growth by a variety of ways, the viscose nature of waste engine oil limits the penetration of water, which ionizes nutrients, thus the plant is starved of nutrients; and oxygen, which is required for respiration (Atlas, 1977).

From Figure 1, it can be observed that there was a consistent increase in the shoot length in all concentrations. Growth was slowest in plants in 12 % w/w oil-in-soil treatment and fastest in 0 % w/w oil-polluted soil. The control soil (0 % w/w), had the highest growth rate of 60 % between the 1st and 2nd WAT, which later slowed down to 7 % between the 7th and 8th week of the experiment. Shoot length was15.30 ± 0.754 cm and 37.33 ± 1.202 cm at week 2 in 0 % w/w oil-polluted soil. At week 8, shoot length

was 100.33 ± 5.069 cm in the control soil. Plants in the 1 % w/w oil-polluted soil had a growth rate of 53.3 % within the first two weeks. This growth rate slowed down at the 7th and 8th week to 6.6 %, while the growth rate of plants in the 12 % w/w oil-polluted soil within the 1st and 2nd week was 38.3 %. This later slowed down to 4 %, at the end of the experiment.

The mean height of the plants decreased with increasing oil in soil treatments. Beginning from the 1st to the 5th WAT, the stem lengths were not synchronized with the treatments. At 5 weeks after transplant, the mean shoot length of plants in the 1 % w/w oil-polluted soil (44.83 ± 4.419 cm) was lower than at 3 % w/w oil-polluted soil (47 ± 2.598 cm), which ought not to be. Statistical analysis using ANOVA revealed that, there was a significant difference at $P < 0.05$, in plant height within 8 weeks of growth at the various oil-in-soil treatments. This implies that, the waste engine oil in the soil had a profound effect on the growth rate of the plants. Anoliefo *et al.* (2010); Ikhajiagbe and Anoliefo (2012) reported decreases in vegetative growth of



plants in waste engine oil-polluted soil.
S0- 0% polluted soil; S1- 1% polluted soil; S3- 3% polluted soil; S6- 6% polluted soil; S9- 9% polluted soil; S12- 12% polluted soil
Fig 1: Shoot length of *Alternanthera brasiliana* in polluted soil between one to eight weeks of growth.

Alternanthera brasiliana root length at 2 weeks after transplant was generally higher than before transplant at all concentrations as seen in Table 4. Root length in the control soil, 17.67 ± 2.68 cm was higher than root lengths at all other concentrations. Root growth in the 1 % w/w oil-polluted soil at 2 weeks after transplant, 15.0 ± 0.50 cm, was higher than in the 3 % w/w oil-polluted soil (13.67 ± 0.17 cm) even though the initial root length in the 3 % w/w oil-polluted soil, 10.0 ± 0.58 cm was higher than in the 1 % w/w oil-polluted soil, 9.92 ± 0.96 cm. This shows the effect of the pollutant on the root growth. The root growth rate in the 0 % w/w oil-polluted soil was 35.9 %, while in the 12 % w/w oil-polluted soil; it was 18.8 % at 2 weeks after transplant.

The statistical analysis showed that there was a significant difference in the root length at the various treatments at $P < 0.05$.

Table 4: Root length before transplant and 2 weeks after transplant into polluted soil

	0 %	1 %	3 %	6 %	9 %	12 %
R1(cm)	11.33 ± 2.35	9.92 ± 0.96	10.0 ± 0.58	11.50 ± 1.16	11.00 ± 1.26	10.83 ± 0.44
R2(cm)	17.67 ± 2.68	15.0 ± 0.50	13.67 ± 0.17	13.50 ± 0.93	14.17 ± 0.93	13.33 ± 0.44

R1- Root length before transplant; R2- Root length 2 weeks after transplant; Mean ± standard error

The weeds in the control soil had chlorophyll content which increased between 1 and 4 weeks after transplant from 33.73 ± 1.40 chlorophyll concentration index (CCI) to 39.23 ± 6.35 CCI (Table 5). This is in opposition to the values obtained from plants in 1 to 12 % w/w oil-in-soil treatments which dropped within this period. Chlorophyll content in the 1 % w/w oil-polluted soil was 40.27 ± 4.54 CCI at 1 WAT and these values decreased to 15.93 ± 2.41 CCI at 4 weeks after transplant. The chlorophyll content of plants in all the treatments dropped between 4 to 8 weeks after transplant. ANOVA at $P < 0.05$ shows that there was a significant difference in chlorophyll content between 1, 4, and 8 weeks after transplant of *Alternanthera brasiliana.* The leaves of *A. brasiliana* plants in higher concentrations of 9 and 12 % w/w oil-polluted soils had reddish-purple colorations, especially the leaves present at the base of the plant. These reddish-purple leaves later dropped. At 14 weeks after transplant, flowering buds were noticed in all *A. brasiliana* plants at all treatment levels. The reduction in chlorophyll content in the leaves of *Alternanthera brasiliana* may be linked to the presence of zinc (180.85 mg/kg) in the waste engine oil used to pollute the soil. In the work by Doncheva *et al*. (2001), a high zinc concentration was observed to reduce the chlorophyll content in pea plants and also alter the chloroplast structure thus, reducing the granal thylakoids (Sivasankar *et al.*, 2012). The chlorophyll content of the plants in the control soil increased between 1 to 4 weeks after transplant, because there was no pollutant in the soil. Meanwhile, the chlorophyll concentration at other concentration levels decreased between 1 to 4 weeks after transplant. It has been demonstrated that intact and photo-modified polycyclic aromatic hydrocarbons affects the primary photochemical processes in different plants. This occurs when they (photo-modified polycyclic aromatic hydrocarbons) accumulate in the thylakoids of the chloroplasts and also of the microsomes, damaging the photosynthetic apparatus of the plants (Duxbury *et al.*, 1997; Huang *et al.*, 1997).

Table 5: The chlorophyll content of leaves of *Alternanthera brasiliana* at 1, 4 and 8 weeks after transplant

	0 %	1 %	3 % (CCI)	6 %	9 %	12 %
1 WAT	33.73±1.40	40.27±4.54	33.17±3.42	34.97±3.90	40.53±2.07	37.10±3.04
4 WAT	39.23±6.35	15.93±2.41	10.53±0.44	13.10±2.40	17.37±3.03	13.57±1.69
8 WAT	13.67±0.88	8.67±0.33	7.67±1.21	13.00±4.16	6.33±0.33	12.30±2.90

CCI- Chlorophyll Concentration Index; WAT- Week after transplant; mean values ± standard error

Table 6: ANOVA summary table for some selected parameters

ANOVA:pH

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.099008	1	0.099008	2.04612	0.211995	6.607891
Columns	3.585275	5	0.717055	14.81876	0.005093	5.050329
Error	0.241942	5	0.048388			
Total	3.926225	11				

ANOVA: Chlorophyll concentration index

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	2190.364	2	1095.182	25.49331	0.000119	4.102821
Columns	224.5309	5	44.90619	1.045313	0.443174	3.325835
Error	429.5958	10	42.95958			
Total	2844.49	17				

ANOVA: Shoot length

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	10307.76	7	1472.537	27.12984	2.38E-12	2.285235
Columns	4850.017	5	970.0034	17.87123	8.89E-09	2.485143
Error	1899.709	35	54.27739			
Total	17057.48	47				

ANOVA: Root length

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	43.16813	1	43.16813	32.13461	0.002377	6.607891
Columns	8.9477	5	1.78954	1.332144	0.380332	5.050329
Error	6.716767	5	1.343353			
Total	58.8326	11				

CONCLUSION

This study revealed that *Alternanthera brasiliana* L. Kuntze. could withstand a waste engine oil-polluted soil of 12 % w/w oil-in-soil treatment for over four months making it a possible candidate for phytoremediaton. Most plants survive a hydrocarbon-polluted soils by a number of mechanisms like hyperaccumulation, production of enzymes, rhizospheric interactions, etc; but the mechanism of tolerance of *Alternanthera brasiliana* to waste engine oil-polluted soil is still to be ascertained as further study would be required to determine this.

IMPORTANCE OF STUDY

In recent times, over 400 plant species of phytoremediating plants have been discovered. This study effectively tests the tolerance of the plant in a WEO polluted soil of up to 12 % w/w oil-in-soil treatment, and as can be seen in the results, the plant survived. Thus, *Alternanthera brasiliana* can be added to the list of plants which can tolerate WEO polluted environments and further studies would be required to determine its mechanism of survival and its phytoremediation capabilities.

LIMITATION OF THE STUDY

This study was limited by equipments and inaccessible funds.

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