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PHYTOTOXIC RESPONSES OF *Cenchrus bilforus* Roxb AND *Chrysopogon aciculatus* [Retz.] Trin GROWN IN DIFFERENT LEVELS OF WASTE ENGINE OIL

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ABSTRACT
The potentials of grass species Cenchrus bilforus and Chrysopogon aciculatus to remediate waste engine oil polluted soil was investigated. Waste engine oil was added to four (4) kg soil samples at different concentrations on weight basis: 0% (control), 3%, 6% and 9% v/w oil-in-soil and allowed to stand for seven days before transplanting. They were three replicates. The plants were harvested after 8 weeks. The dried plants and the soil samples on which they were grown were analysed for four heavy metals, Chromium (Cr), Cadmium (Cd), Iron (Fe) and Arsenic (As). Results showed that there was reduced mean percentage reductions in the heavy metal contents of the soils. Cenchrus *bilforus and C. aciculatus showed maximum reductions of 110.6% and 209.4% for Pb. The shoots and roots of the grass species showed reduced heavy metal accumulation while soil showed increased heavy metal accumulation. C. biflorus showed maximum accumulation of Cr (0.05mg/kg), Cd (0.05mg/kg), As (0.22mg/kg) in the soil and maximum accumulation of Fe (10.13mg/kg) in the shoot. while C. aciculatus showed maximum accumulation of Cr (0.15mg/kg), Cd (0.09mg/kg) in the soil and maximum accumulation of Fe (10.9mg/kg) in the shoot and As (0.22mg/kg) in the root. General observation showed low concentrations of these metals in the roots and shoots when compared to the soil of the plant species. In order words the two grass species studied could not be recommended for phytoremediation of waste engine oil contaminated soil.*

KEYWORDS: *C. bilforus, C. aciculatus, Potentials, Polluted soil, Remediate*

INTRODUCTION
Various types of activities like industry, agriculture and transportation produce large amount of waste which are classified as either agricultural, industrial, municipal or nuclear waste are found in urban area (Onwuka *et al*., 2012). These waste from various sources are deposited on the soil environment either deliberately applied as fertilizer, sprays or pesticides (Lauhanen *et al.*, 2004) or through small or large leaks (Adesodun, 2014) as solids, plastics, crude oil or spent engine oil. Onwuka *et al.* (2012) opined that some of these wastes can be recycled into some important products that can be used to meet with the resulting

challenges from population increase in Nigeria. They can be recycled into manures and fertilizers for production of crops and animals among others (Onwuka *et al.,* 2012) while there are others that cannot be converted into any beneficial secondary use and therefore pose a serious threat to the environment and one of such is waste engine oil (Onwuka *et al.,* 2012).

Furthermore, engine oil contains fresh and lighter hydrocarbons that would be more of concern for short-term (acute) toxicity to living organisms, whereas waste engine oil contains more metals and heavy polyaromatic hydrocarbons (PAHs) that would contribute to long-term (chronic) hazards including carcinogenicity (Ugwu, 2015). Ugwu, 2015 wrote that waste engine oil is a contaminant of concern, with large volume entering aquatic ecosystems through water runoff. Several work support the fact that waste engine oil renders the environment unsightly and constitutes a potential threat to humans, animals and the vegetation at large (Adelowo *et al.,* 2006; Okerentugba and Ezeronye, 2003). Agbogidi and Ilondu (2013) stated that soil contaminated with waste engine oil has significant effect on reducing the germination response and subsequent performance including the biomass production of *Moringa oleifera* seedlings. However, as the use of petroleum hydrocarbon products increased, soil contamination with diesel and waste engine oil has become one of the major environmental problems (Mandarin and Lin, 2017). To investigate the counter measure to remediate soils contaminated with oils, phytoremediation provides an effective and efficient strategy to speed up the clean-up processes (Mandarin and Lin, 2017). Compared with conventional methods of soil remediation, the use of plants provides several striking advantages. It is cheap: after planting, only marginal costs apply for harvesting and field management, such as weed control (Dietz and Schnoor, 2001). This could be achieved by a relatively new technology known as phytoremediation, which uses plants to remove pollutants from the environment (Sharifi *et al.,* 2007). Many plants have been identified to have the capability for site clean-up and reclamation; among these are those identified to have abilities to enable phytoremediation of contaminants, particularly petroleum hydrocarbons (Reilley *et al.*, 1996). Hence, there the need to investigate *C. bilforus and C. aciculatus* grown waste engine oil contaminated soil and its ability to remediate contaminated environmental conditions.

The aim of this study was to investigate phytoremediation ability of *C. bilforus and C. aciculatus grown on waste engine oil* contaminated *soil.*

MATERIALS AND METHODS

Soil Samples

The soil samples used were collected from the experimental farm of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria. The waste engine oil was obtained as pooled engine oil from two different major mechanic workshops in the Mechanic Village, Ohiya, Umuahia, Abia State. The plant materials, *C. bilforus and C. aciculatus* used were collected from bush fallows within Umuahia metropolis. Top soil (0-10 cm) were collected from a marked area, air dried and sieved through a 2 mm mesh gauge (Ogedegbe *et al.*, 2013). Samples of the soil (4kg each) were introduced into different 4-litre perforated plastic buckets after which different concentrations (3%, 6% and 9%) of waste engine oil were added and labeled as T^1, T^2 and T^3. The mixing was done gradually to ensure thorough and even mixing and each treatment was in three (3) replicates. An untreated soil sample with 0% waste engine oil served as a control (T^c) (Adenipekun *et al.,* 2009). After mixing, the soil samples were left under shade for a period of seven days without planting to ensure uniformity of oil, moisture content, air content, constant temperature and effective activities of soil micro-organisms (Oyibo, 2013). They were artificially irrigated with water before the transplanting of the plant species and left for natural irrigation.

Plant Materials

The plant species *C. bilforus and C. aciculatus,* which has the potential for producing mass rooting system being investigated were propagated by tiller. The tillers of the different plants were separated and the same height (shoot 15 cm) were selected; the roots were soaked in water for 2 days to improve their rooting ability (Brandt, 2003). The tillers were transplanted into different treated soil samples, each with three tillers and allowed to stand for eight weeks. The plant samples were harvested and soil was washed off with water after which they were separated from the shoot and placed in labeled separate envelops for oven drying and heavy metal analysis.

Determination of Heavy Metal Concentrations in the Plant Samples

The root and shoot samples of the plant species were oven dried at 65°C for 8 hours, milled in a Thomas Willey Milling Machine, sieved through a 0.5mm sieve and stored in labeled containers. A portion (0.2g) of the samples (root and shoot) of the two plant species were weighed into 150ml conical flask, 5ml of the multiple nutrient extraction reagent (H2SO4-selenium powder salicylic acid) solution was added in each flask for digestion and allowed to stand for 20 hours. The samples were placed in a hot block plate at 32ºC for 2 hours, after which 5ml of 75% of perchloric acid was added to each sample and re-digested at 60ºC. The digestion continued until a clear digest were seen producing a profuse perchloric fumes. The digests were allowed to cool and 50ml of distilled water was added; the samples were filtered and the filtrates were analyzed using UNICAM Solar 969 atomic absorption spectrometer (AAS) which used acetylene- air flame to determine the heavy metals. The heavy metals determined were Chromium (Cr), Cadmium (Cd), Iron (Fe) and Arsenic (As).

Determination of Heavy Metal Contents of the Soil Samples

The soil samples were also prepared for heavy metal assay as above.

Statistical Analysis

The results were summarized using Descriptive Statistic Package of Microsoft Excel while one-way ANOVA was used to test for statistical differences among the treatments and Tukey's pairwise comparisons test was performed to determine the location of significant difference ($P<0.05$).

RESULTS AND DISCUSSION

Some of the physico-chemical properties of the soil samples are presented in Table 1. The control (0% concentration of waste engine oil) showed that the soil texture is sandy loam based on the USDA textural classes of soil i.e. 65%, 18.80% and 16.20% for sand, silt and clay respectively. The soil was acidic with pH of 4.90 while the soil nutrients: phosphorus, calcium, magnesium, potassium and sodium, were 40.90mg/kg^{-1}, 4.40Cmolkg^{-1}, 2.80Cmolkg^{-1}, 0.11Cmol/kg^{-1} and 0.10Cmolkg^{-1} respectively. The percentage organic carbon was found to be 1.90% which was within the topsoil ranges from 0.5 - 3.0% organic carbon for most upland soils. Based on MS1517 organic fertilizer specification (2012), both percentage organic matter and nitrogen have low values required for optimum plant growth values given as 3.28% and 0.18% respectively. Cation exchangeable capacity was moderate (8.86Cmol/kg^{-1}) which could be attributed to the acidic nature of the soil and the soil texture. Anion exchangeable capacity increases at low pH but different in this study. The value of 1.44 Cmol/kg^{-1} recorded could also be equally attributed to the type of soil texture. The soil texture at different concentrations of waste engine oil remains the same. The acidic nature of the soil was maintained but there was a gradual increase in pH. Soil nutrients (P, Ca, Mg, K and Na) were higher than the control but recorded a gradual increase in their concentrations with treatments. This could be as a result of pollution on soil which resulted in an imbalance in the carbon: nitrogen ratio. There will be net immobilization of the nutrients by the microbes leading to loss of soil fertility, if the ratio is greater than 17:1 in soils (Jobson *et al*., 1974). The percentage organic carbon had the highest value of 2.98% at 9 % treatment. The higher organic carbon content of the soil recorded with the treatment may be attributed to the high carbon content of the soil which is in line with Benkacoker and Ekundayo (1995). The percentage nitrogen was higher than the control but increased remain constant from 6 - 9% treatment levels (0.112%). Percentage organic matter was generally higher than in the control and the highest values (5.14%) were recorded in 4% and 10% treatment levels. The increment could also be due to soil simulations with oil. Some of the soil nutrients analyzed were not within the range required for plant growth. Exchangeable anion capacity (EAC) was lower in the different treatments compared to the control (0%) while effective exchangeable anion cation capacity (ECEC) was higher in the treatments compared to the control (0%).

Table 1: Physical properties of the soil samples before experiment

Parameters	Treatments %			
	0%	3%	6%	9%
% Sand	65.00	71.00	77.00	71.00
% Silt	18.80	16.80	10.80	14.80
% Clay	16.20	12.20	12.20	14.20
Texture	SL	SL	SL	SL
pH (H_2O)	4.90	6.00	6.20	6.70
P Mg/Kg	40.90	53.50	55.00	64.50
% N	0.18	0.21	0.28	0.28
% OC	1.90	2.46	3.17	3.52
% OM	3.28	4.23	5.46	6.08
Ca $Cmol/Kg^{-1}$	4.40	8.80	9.20	6.40
Mg $Cmol/Kg^{-1}$	2.80	4.00	3.60	4.00
K $Cmol/Kg^{-1}$	0.11	0.15	0.16	0.17
Na $Cmol/Kg^{-1}$	0.10	0.18	0.19	0.21
EA $Cmol/Kg^{-1}$	1.44	0.64	0.56	0.72
ECEC $Cmol/Kg^{-1}$	8.86	12.78	13.72	14.48
% BS	83.71	95.33	95.92	93.73

Legend: OC = Organic Carbon, OM = Organic Matter, EA = Exchangeable Anion, ECEC = Effective Cation Exchange Capacity

The concentrations of the heavy metals in the contaminated soils increased with the increasing concentrations of waste engine oil (Table 2). The 9 % treatment level recorded the highest concentrations and was significantly different ($P<0.05$) from the control (0 %). The order of increment for the heavy metals in the 9 % treatment level was as follows: As>Cd>Cr>Fe. Ifediora and Okwunodolu, (2018) reported that the order of increment for the heavy metals at the 10 % treatment level of spent engine oil polluted soil when compared with control was as follows: Ni>Pb>Cu > Zn.

Table 2: Initial heavy metal content of different concentrations of waste engine oil polluted soil one week after contamination

Treated Soil	Heavy metal concentration (mg/kg^{-1})			
	Cr	Cd	Fe	As
0%	0.013	0.054	2.2	0.041
3%	0.095	0.058	7.95	0.105
6%	0.116	0.038	8.07	0.116
9%	0.306	0.046	8.9	0.104

The Table 3 of this study showed that as the waste engine oil concentration increased in the soil, there was increased uptake of the heavy metals by the two grasses from the soil. At 9 % treatment level, *C. bilforus* showed maximum reduction of Cr (110.6%) while *C. aciculatus* showed maximum reduction of Cr (209.4%). *C. bilforus* had Cd>As> Fe>Cr and *C. aciculatus* had Cd >Cr>As> Fe *as* their order of reduction. In their own observation, Ifediora *et al.,* (2019) noted the following trends: Ni>Pb> Cu> Zn for B. deflexia and Ni > Pb >Cu> Zn for *P. scrobiculatum*. Also, for *D. horizontalis (Ni>Pb> Cu>Zn), E. indica (Ni > Cu >Pb> Zn) and S. barbata (Ni > Cu >Pb> Zn) (*Ifediora and Okwunodulu, 2018).

Table 3: Percentage reduction of chromium, cadmium, iron and arsenic in soil 8 weeks after planting of *C. bilforus* and *C. aciculatus*

Grass species	Waste engine oil Polluted soil (%)	Percentage reduction in the soil Cr	Cd	Fe	As	Mean
***C.** bilforus*	0%	69.2	71.3	143.6	53.7	84.3
	3%	11.6	84.5	51	41.9	47.2
	6%	12.9	136.8	45.7	40.7	59.0
	9%	49.7	150	48.8	36.5	71.2
	Mean	35.8	110.6	47.2	56.6	
C. aciculatus	0%	84.6	70.4	92.9	95.2	85.7
	3%	150.5	72.4	53.5	77.1	88.3
	6%	136.2	271.1	74.8	94.8	144.2
	9%	97.4	423.9	101.2	165.4	196.9
	Mean	117.1	209.4	80.6	108.1	

The concentrations of the heavy metals in the shoots and roots of the grass species and soil in which they were grown are shown in Figs.1- 4. Fig. 1 showed the uptake of chronium by the root and shoot of the grass species and soil. It was observed that the soil of *C. aciculatus* accumulated the highest concentration of chronium (0.15 mg/kg) while the lowest accumulation was observed in the shoot where *C. biflorus* (0.02mg/kg). Fig 2 showed the concentrations of the cadmium in soil, roots and shoots of the grass species and it was observed that the soil of *C. aciculatus* absorbed the highest concentration (0.09mg/kg) of cadmium while the shoot of *C. biflorus* showed the lowest concentration of cadmium (0.01mg/kg). Fig. 3 showed the concentration of Iron uptake in soil, roots and shoots of the grass species; the shoot of *C. aciculatus* accumulated the highest concentration of Iron (10.9mg/kg) while the soil of *C. biflorus* had the lowest concentration

(3.81mg/kg). Fig. 4 showed the concentrations of arsenic in soil, roots and shoots of the grass species; the highest uptake of arsenic was in the soil of *C. biflorus* and the root of *C. aciculatus* (0.22mg/kg) while the lowest concentration (0.01mg/kg) was recorded in the shoot of *C. aciculatus.*

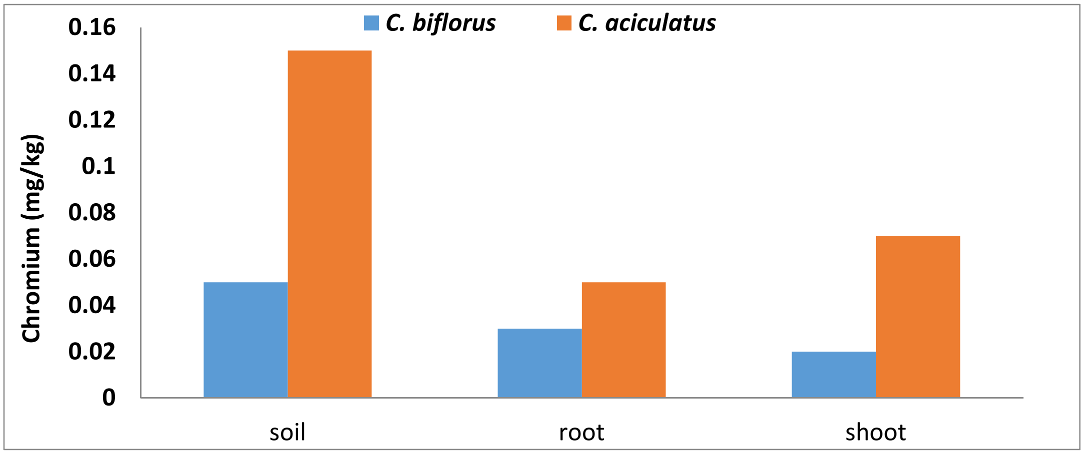

Fig. 1: The End Concentrations of the Chromium in Soils, Roots and Shoots of the Grass Species

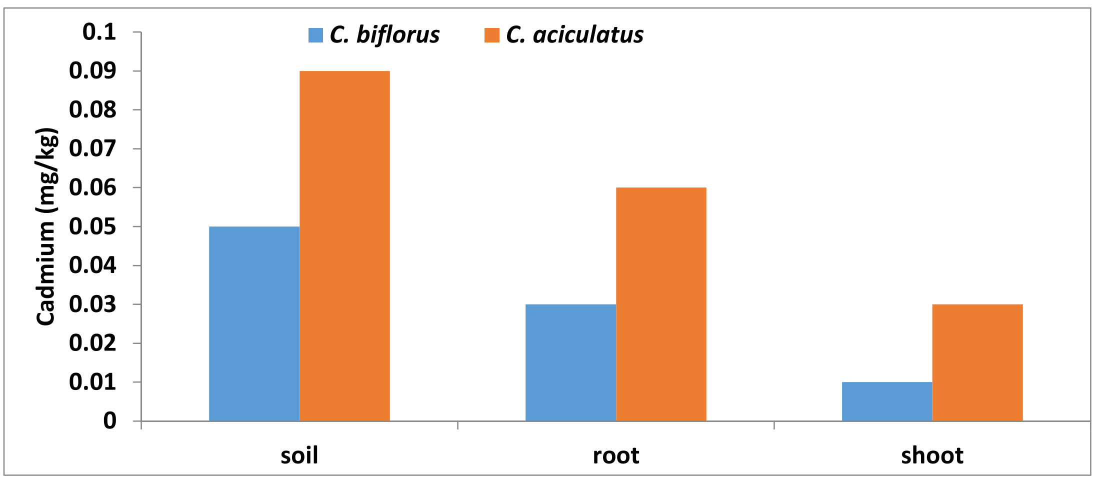

Fig. 2: The End Concentrations of the Cadmium in Soils, Roots and Shoots of the Grass Species.

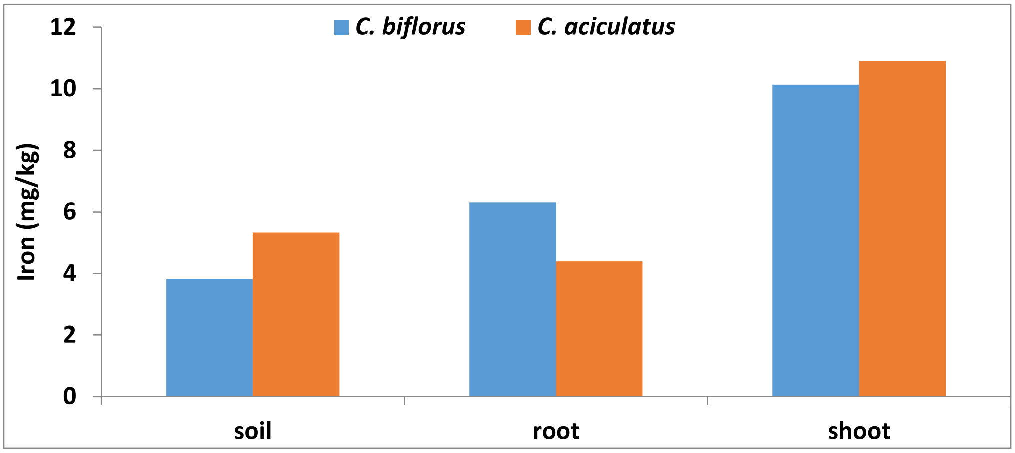

Fig. 3: The End Concentrations of the Iron in Soils, Roots and Shoots of the Grass Species

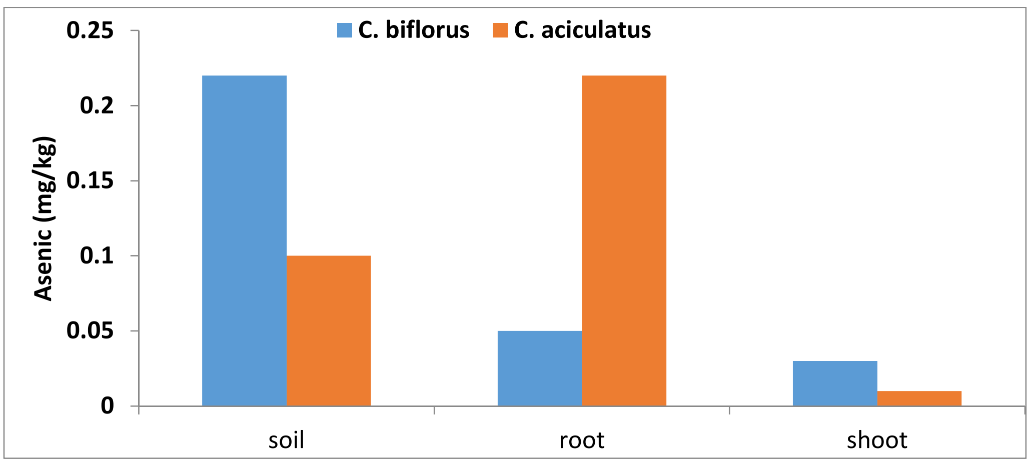

Fig. 4: The End Concentrations of the Arsenic in Soils, Roots and Shoots of the Grass Species

General observation showed low concentrations of these metals in the roots and shoots when compared to the soil on which the plant species were grown. Cr, Cd and As were highly accumulated in the soil while Fe accumulated more in the shoots. The observed decrease in accumulation or bioavailablity of metals to plants could be as a result of higher pH of polluted soils. Reichman, 2002, supported this by stating that the proportion of total metal which is in the soil solution is determine by the other soil parametres such as pH, organic matter, clay and redox conditions. The pH has a great impact on bioavailability in the soil (Egbenda *et al.,* 2015). At high pH, metals tend to form metal mineral phosphates and carbonates which are insoluble while at low pH they tend to occour as free ionic species or as soluble organometals and are more bioavailable (Naidu *et al* 1997; Twiss *et al*., 2001: Rensing and Maier, 2003; Sandrin and Hoffman, 2007). Furthermore, ITRC, 2009, observed that the essential inorganic plant nutrients are taken up by the root system as dissolved constituents in soil moisture and these elements are required by the plant for growth, development, or reproduction. This can be the reason for the high concentration of Fe in the shoots of the grass species studied.

CONLUSION

In conclusion, the grass species *C. bilforus* and *C. aciculatus* could not remediate the polluted soil by observing higher percentages of the heavy metals studied in the soil in which the two grass species grown when compared with thier roots and shoots.

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