



Influences of twosome artisanal wastes on nodulose and root growth of *Sphenostylis stenocarpa* (Hochst. ex A. Rich. Harms) and some loamy-soil chemical properties

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Abstract

The effects of twosome artisanal wastes on root development, root length and nodulose biomass of *Sphenostylis stenocarpa* and selected soil chemical properties of the study area (Faculty of Agriculture teaching and research farm) were carried out using randomized complete block design (RCBD). The study was carried out in the Screen house at the Centre for Ecological Studies University of Port Harcourt Rivers State, Nigeria between July 2017 and March, 2018. The Carbide Sludge (CS) and Waste Engine Oil (WEO) used for the study were collected from a roadside mechanic workshop, and the Seeds (of *S. Stenocarpa*) used were purchased from mile 3 market in Port-Harcourt. The Soil baseline analysis prior vegetation with *S. Stenocarpa* (SBAV) was obtained, and the loamy soil was filled into 25 planting bags, each bag weighted 5000g. Carbide Sludge (CS) and Waste Engine Oil (WEO) were measured in relative amounts of 150g of CS, 100g of CS and 50ml of WEO, 50g of CS and 100ml of WEO, and 150ml of WEO alongside a Control soil and replicated five times. Each treatment has five replicates in RCBD. Three seeds of *S. stenocarpa* were planted in each bag and later thinned down to one seedling per bag at one week after germination. Results revealed that CS and WEO were markedly reduced ($P < 0.05$) in the below-the-ground parts of *S. stenocarpa* in terms of root length, root biomass, nodule count, and the chemical properties of the soil in the study area. The effect varies with pollutant concentration and type. The least reduction of mean nodule count (2 ± 0.9), root length (9.04 ± 0.2 cm), root fresh weight (2.7 ± 0.7 g) and root dry weight (1.21 ± 0.5 g) was observed in the 150 ml WEO as compared to their respective controls of root nodule (118.4 ± 3.2), root length (59.78 ± 3.4 cm), fresh weight (32.25 ± 2.1 g) and root dry weight (21.48 ± 0.4 g). CS and WEO also changed the chemical properties (pH, Total Organic Matter, Total Hydrocarbon, Electrical Conductivity, Organic Nitrogen, Organic Phosphorus and Potassium ion concentration) of the soil. These increased with increase in the concentrate of the pollutants. The study reveals that WEO and CS had adverse effect on the below-the-ground parts of the investigated plant and selected soil chemical properties with more toxicity by WEO than CS especially at high concentration.

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Introduction

In most developing countries the incidences of disposal of artisanal wastes in soil are common occurrences, and plant life forms around such environments suffer setback and defect in growth due to soil contamination. Carbide Sludge (CS) and Waste Engine Oil (WEO) are the main soil contaminants generated from automobile mechanics, panel beaters (who work alongside) and other related artisans workshops (Ihinmikaiye, and Tanee, 2014). CS is a by-product of calcium carbide; produced when calcium carbide is converted to acetylene (C_2H_2) in a generator controlled reaction with water (Carbide Industries, 2015). The acetylene when

combine with oxygen in an oxyacetylene torch, produce a flame use by panel beater for metal fabrication and construction purposes. CS (the by-product of the process) is discarded as waste due to high content of impurities, a result of the reactants and reaction processes (Chukwudibolu *et al.* 2013). Abdulhadi *et al.* (2016) described WEO as any lubricating oil that has served its service properties in a vehicle withdrawn from the meant area of application and considered not fit for its initial purpose. WEO is a mixture of different chemicals usually obtains after serving and subsequent draining from automobile and generator engines (Wang *et al.*, 2000 and Sharifi *et al.*, 2007). The Nigeria

Automobile Technicians Association (NATA) with 4.2 million registered members in Nigeria (The Nation, 2009) and panel beaters with average membership of twenty five thousand (Chukwudibolu *et al.*, 2013), scattered all over the country generate and deposit large proportion of wastes into soil. Evan (1989) reported that soil is the repository of society wastes. CS and WEO are indiscriminately disposed to the environment after serving their purposes and this act often leads to soil contamination. Kayode *et al.* (2009) revealed that WEO pollution in soil makes the soil condition unsatisfactory for plants and affected soil physical and chemical compositions, reflecting on poor forest stands and composition (Unanaonwi and Anonum, 2017). Kinako and Amadi (1997) study cited in (Tanee, 2011) found that dumped CS delays water infiltration into soil, reduced vegetation regeneration and biomass accumulation of plants. At high concentration, CS and WEO in soil reduced the amount of water and oxygen available for plant growth (Irwin *et al.* 1997; Tanee, 2011).

Sphenostylis stenocarpa (Hochst. Ex. A. Rich. Harms) is an annual leguminous crop of family Fabaceae grown in humid tropical region (Amoatey, 2000). It is a vigorously climbing herbaceous vine whose height can reach 2 meters or more depending on the height of the stakes and cultivar. The seeds can be brown, white, speckled or marbled with the hilum having a dark-brown border (Oshodi *et al.*, 1995). The crop is a rich source of protein and the seeds are eaten by human as important substitute for the more widely eaten cowpea; and with ability to fix atmospheric nitrogen through its root nodules.

This study aimed to investigate the effect of twosome artisanal wastes on root length, root biomass, and nodule production of *S. stenocarpa* and selected soil chemical properties of the study area.

Materials and Methods

The study was carried out in a Screen house at the Centre for Ecological Studies University of Port Harcourt Rivers State, Nigeria (Lat. 4°65'N; Long. 7°05'E). The area enjoys an annual rainfall of about 2500mm, a relative humidity of 82% and temperature varies from 21°C to 35°C annually. Carbide Sludge (CS) and Waste Engine Oil (WEO) used were collected from a roadside mechanic workshop at Aluu area of Port-Harcourt City. Seeds of *S. stenocarpa* used were obtained while they are yet in their pods from mile 3 market in Port-Harcourt, Nigeria. The CS used was sun-dried for seven days and pulverized to obtain the actual weight as a necessary condition of homogeneity with the soil according to (Tanee, 2011), and (Ihinmikaiye and

Tanee, 2014). Sandy loam soil was collected from four years fallowed plot in the University Demonstration Farm within a depth of 0-15cm, organic debris were sieved out through a 2mm sieve pan; and 1000g of the soil was weighed in a crucible, labelled F₁, kept aside and later sun-dried to serve as a baseline for the treatment soils. Subsequently, 5000g of the soil was repeatedly weighted into a crucible on a weighing scale (Camry SQ, China) and transferred into 25 planting bags. The planting bags were labelled and arranged into groups (A₁, A₂...A₅, B₁, B₂...B₅, C₁,C₂...C₅ and D₁ D₂...D₅) and were contaminated with 150g of CS only, 100g of CS and 50ml of WEO, 50g of CS and 100ml of WEO and 150ml of WEO only. Group (E₁, E₂...E₅) however, serves as control. Each treatment set-up was replicated five times. The treatments were watered (with 750 ml) and left for a week for proper draining and setting of the soil after which 3 seeds of *S. stenocarpa* were planted in each bag and later thinned down to one seedling per bag at one week after germination (1 WAG). The treatments were watered at an interval of 84 hours and the experiment was monitored for 12 weeks; however, the plant started fruiting at week 10 and nodules were seen visibly on roots that projected out of the soil, yet, treatment groups which received higher doses of pollutants started dying prompting the termination of the experiment at twelve weeks after planting (12 WAP). The roots were harvested by carefully loosing the bags, scattered its contents and treated in basin containing water to wash off soil attached to the roots. Subsequently, the nodules on the roots were extracted and counted accordingly. The root lengths were obtained with the aid of a measuring tape calibrated in centimetres. Root biomass (fresh and dry weight) was determined on an electronic (SF-400c) compact scale measured in grams. The fresh root biomass was determined immediately after harvest, the roots were labelled accordingly and sundried for 5 days before the dried root biomass was determined using the earlier procedures.

pH of the soil in each treatment was determined according to Ibitoye, (2006). 5g of air dried soil sample was weighed into a clean 100ml beaker. 20ml of distilled water was added (in ratio 1:2 soil to water) and the suspension was stirred with electromagnetic stirrer for 10minutes and allowed to stand for 30minutes. The pH meter electrode was immersed in the beaker and the figure on the pH meter screen was allowed to stabilize before reading was taken on each treatment soil. And Total Organic Nitrogen was determined by Kjeldahl method as described by (Gresshoff *et al.*, 2008). Phosphorus in each treatment was determined according to (Bray and Kurtz, 1945) and analysed spectrophotometrically.

5g air-dried soil was weighted into a 250ml beaker, 35ml ammonia chloride (NH_4Cl) was added. The mixture was stirred for 10 minutes and filtered. 10ml of the filtrate was pipetted into 50ml flask and 8ml ascorbic acid in Murphy and Railey reagent was added and makeup to 50ml with distilled water the solution turned blue after allowing to stay. Subsequently, standard solutions were prepared and soil phosphorus absorbance was read at a wavelength of 660micron using spectrophotometer. THC was determined by spectrophotometer method. 1g of air-dried soil was extracted with 10ml of chloroform in a test tube. The extraction was partitioned between distilled water in a separator flask. Chloroform layer (lower phase) was taken into a clear test tube and was dehydrated by adding a spoonful of anhydrous sodium sulphate. The clear extraction solution was absorbed at 420nm wavelength with thermospectromic spectrophotometer and the concentration of THC extrapolated. Potassium content in the soil samples were determined according to (Jenway, 2016) using Jenway PFP7 flame photometer.

Statistical Analysis: Data obtained from nodule counts, root-length, root biomass of *S. stenocarpa* in CS and WEO treatments, and the control setup as well as, data from soil parameters (Soil pH, TOM, THC, EC, N, P and K) were analysed through descriptive statistics represented by Bar graphs and a table. Besides, the data collected were also subjected to ANOVA, to test the degree of difference and significant means were separated with least significant different (LSD) at $p > 0.05$ using Microsoft excel version 2007 and SPSS version 20 (Ogbeibu, 2005).

Result: The study shows that differences occurred in nodule count in *S. stenocarpa* in CS and WEO treatments. Figure 1 showed that nodule count of the control was significantly different ($p < 0.05$) compare to nodule count in treatments with 150 ml WEO, 150 g CS, 100 ml:50 g (WEO:CS), and 50 ml:100 g (CS:WEO). A comparison between nodule count in 150 g CS treatment and 150 ml WEO treatment, shows that nodule count in 150 ml WEO treatment was significantly reduced ($p < 0.05$). However, when nodule count in 100 ml: 50 g (WEO: CS) treatment and 50 ml: 100 g (WEO: CS) treatment were compared, the results were not statistically significant ($p > 0.05$).

The result of root length of *S. stenocarpa* in CS and WEO are shown in Figure 2. The root length of *S. stenocarpa* in 150 ml WEO treatment was significantly reduced compared to the other treatments. It was observed that the different treatment either in single or combinations drastically affected the fresh weight of the test plant. Highest root fresh weight yield was recorded at the control treatment while the least was in the 150 ml WEO treatment (Figure 3). Also the root fresh weight yield of the plant in the 150 ml WEO was significantly reduced as compared to the other polluted treatments (150 g CS, 100 ml:50 g (WEO:CS), and 50 ml:100 g (CS:WEO)). Similarly, in Figure 4, the control treatment showed significant improvement ($p < 0.05$) in root biomass (dry weight) compared to the biomass of *S. stenocarpa* in the other treatments. However, highest reduction in root biomass was observed in 150 ml WEO treatment when compared to other treatments.

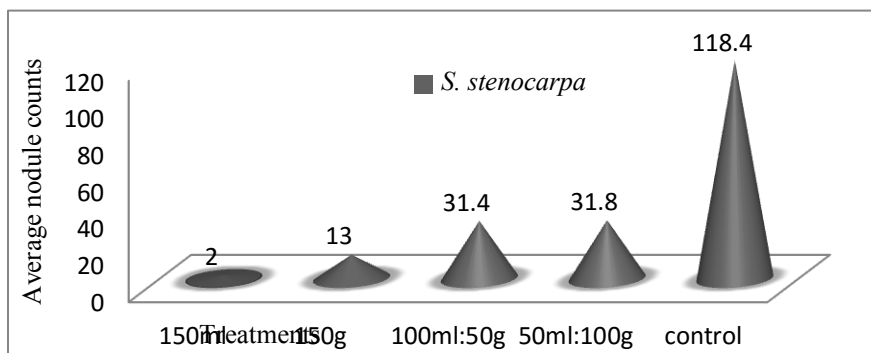


Figure 1: Mean counts of *S. stenocarpa* in CS and WEO treatments

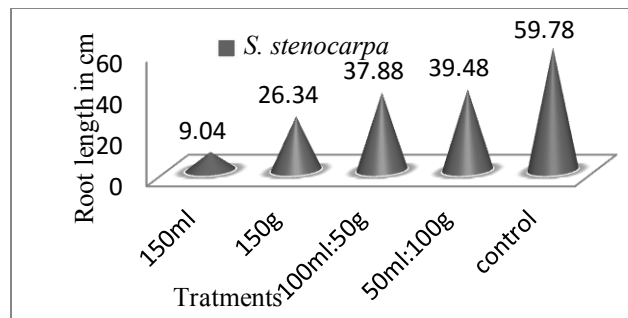


Figure 2: Root length of *S. stenocarpa* in CS and WEO treatments

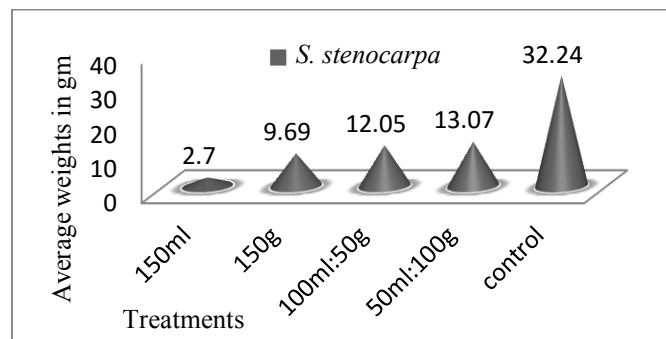


Figure 3: Root biomass (fresh weight) of *S. stenocarpa* in SC and WEO treatments

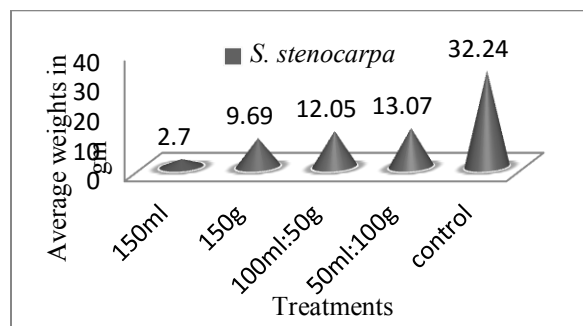


Fig. 4: Root biomass (dry weight) of *S. stenocarpa* for CS and WEO treatments

The results of the soil chemical characteristics are presented in Table 1. It was observed that the soil pH of 150 g CS treatment significantly increased ($p < 0.05$) compared to the other treatments while 150 ml WEO had the least soil pH compared to treatments. Total Organic Matter (TOM), electrical conductivity (EC) and Total Hydrocarbon Content (THC) of treatment 150 ml WEO was significantly higher ($p < 0.05$) compared to those of the control, treatments 150 g (CS), 100 g:50 ml (WEO:SC) and 50 g:100 g (WEO:CS) respectively. Nutrient status of the experimental site soil was low. Although, addition of pollutants (CS and WEO) caused an appreciable increase in nitrogen and phosphorus, especially in 150 ml WEO as compared to the control and initial and baseline results. However, the reverse was the case with potassium content which decreases with increase with WEO concentration.

Discussion

The presence of CS and WEO in soil had adverse influence on the root length and biomass, number of nodules of *S. stenocarpa* and soil chemical properties. The result of root length, root biomass and nodulose count of *S. stenocarpa* as well as the soil chemical properties in twosome artisanal wastes revealed that CS and WEO affect the chemical properties of soil and subsequently the root development and nodulose formation of *S. stenocarpa*. Decrease in nodule production and counts observed suggest that CS and WEO suppressed nodule formation in legumes, with the effect more pronounced in WEO at high concentration in contrast to CS ($p < 0.05$). Similar results were reported by John *et al.* (2011) who observed that crude oil contaminated wet land ultisol exhibit gross reduction in the nodulation of legumes. Also (Ilango and Vivekanandan, 1992) asserted that number of root nodules/plant leghaemoglobin content in root nodules of *Vigna mungo* (L) Hepper grown in polluted soil decreased significantly due to persistence of hydrocarbon.

Reduction in root length and root biomass indicated that root development of *S. stenocarpa* in CS and WEO polluted soil decreased with increase in the concentration of the pollutants in soil, and this subsequently affect root development in soil. This result agrees with (Tanee and Ochekwu, 2010), who reported significant differences in root length and projected root area among soil treated with toxic substances.

Table 1: Chemical characteristics of CS and WEO polluted soil planted with *S. stenocarpa*

	pH	TOM [†]	THC ^{††} (Mg/kg)	EC ^{†††} (Us/cm)	N (%)	P (Mg/kg)	K (Mg/kg)
150g	11.77±0.18a	0.65±0.02a	101.06±0.68a	70.8±1.99a	0.181±0.005a	49.19±0.25a	0.126±0.003a
100g:50ml	6.01±0.78b	0.69±0.03b	104.21±1.16b	59.2±1.99b	0.282±0.017b	50.9±1.096b	0.062±0.006b
50g:100ml	6.99±0.07c	0.53±0.001c	156.52±1.15c	41.6±0.49c	0.172±0.002c	48.17±0.824b	0.107±0.003c
150ml	6.98±0.03c	0.57±0.01d	173.77±0.09d	40.9±0.49c	0.241±0.002c	42.6±0.748c	0.104±0.002c
Control	6.72±0.01c	0.46±0.01e	57.91±0.37e	33.8±0.35d	0.131±0.003e	34.01±1.343d	0.146±0.003d
SBAV*	6.70	0.42	30.00	34.03	0.090	34.01	0.210

Mean with the same letter in each column are not significant (p>0.05)

[†]Total organic matter; ^{††}Total Hydrocarbon Content oil organic carbon; ^{†††}Electrical conductivity

*Soil baseline analysis prior vegetation with *S. stenocarpa*.

Soil analysis results showed that CS and WEO influenced and altered soil chemical properties. The mean pH value of the experimental setup (control (E) and polluted treatment (A, B,C and D) increase compare with the pH value of the soil prior vegetation (SBAV) . However, the mean value of treatment (A) was significantly high (p<0.05) compare to the mean pH values of the other treatments (B, C and D) and the control experiment (E). The increase in soil pH values were due to high concentration of CS and conforms to the reports of (Freney *et al.*, 1992), who earlier reported increase in soil pH in spent carbide waste polluted soil and this might be responsible for scanty regeneration leading to low tree population and poor plant growth in carbide polluted soil. Also low pH value observed in treatment D might not be unconnected with the high concentration of WEO and this agree with (Agbogidi and Enujeke 2012) and (Obazuaye and Obueh, 2014) who asserted that soil contamination with WEO have lower pH value. Electrical conductivity EC is a measure of ionic concentration in the soil (Osuji and Nwoye, 2007), thus increases in the level of EC in the treatments suggested that the contaminants impact soil with ions. A similar result was reported by (Abdulfattah *et al.*, 2016) that EC of oil polluted soil increased in contrast to control soil, and the high value of EC in polluted soil may refer to a high presence of charged ions (cat ions and anions) in the soil. Healthy growth leads to the absorption of more nutrients from soil (Anoliefo and Edegbai, 2000): this might have accounted for the low nutrient content of the soil planted with *S. stenocarpa*, due to the utilization of soil nutrients by the plant. However, the presence of CS and WEO might have also impeded full utilization of potassium, phosphorus and nitrogen in the treated soil leading to poor root development of the test plant. Soil contaminants interfere with nutrient and water absorption by plants and affect normal growth and development (Ogbuehi *et al.*,

2011; Ekpo *et al.*, 2012; Anoliefo *et al.*, 2006; Benka-Coker and Ekundayo, 1995). There was a slight increase in TOM and THC of control (E) when compare with SBAV, yet a significant build up (p<0.05) of TOM occurred in treatments ABCD when compared, and when THC values of treatments A,B,C and D are compared also. High TOM level in treatment (A) corroborate (Osuji and Nwoye, 2007). The relative increased THC values corresponding to increased WEO concentration and this is expected since it has been reported that WEO contains high amount of total hydrocarbons. This accord with (Benka-Coker and Ekundayo, 1995), who reported that soil treated with oil correlates with increase THC.

Conclusion

The presence of contaminants such as CS and WEO in soil affects soil physico-chemical properties, plant growth and development especially the below-ground development as evident in this study. Thus, effort should be made to enlighten the artisans and other end-users of CS and WEO against indiscriminate disposal of these contaminants and thereby safeguard the soil, the vegetation and environment at large from contamination. Regulations on environmental pollution must be made effective to provide partial alleviation of environmental pollution.

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