EVALUATION OF THE EFFECT OF CRUDE OIL ON EARLY GROWTH OF SOURSOP (*Annona muricata* L) AND AFRICAN BREADFRUIT (*Treculia africana* Decne) SEEDLINGS

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ABSTRACT
The contamination of soils in the Niger Delta Region of Nigeria due to crude oil pollution has continued unabated with grave consequences for agro-ecosystems and rural livelihoods. This study was carried out to ascertain the performance of *Annona muricata* and *Treculia africana* seedlings in crude oil contaminated soils. The experiment was laid out in a Completely Randomized Design (CRD) with five replicates, for each of the species. Seedlings of *A. muricata* and *T. africana* about the same height and with the same number of leaves for each species were introduced into polypots containing 5kg of bulked topsoil contaminated with different levels of crude oil –25.00 ml, 50.00 ml, 75.00 ml and 100.00 ml, and a Control (0.00 ml). Seedling growth was evaluated at the various crude oil levels for a period of 12 weeks. Growth in seedling height, collar diameter, leaf area, leaf production, and seedling biomass were significantly reduced ($p < 0.05$) by addition of the various crude oil levels in both *A. muricata* and *T. africana*. Growth in seedling height and leaf area varied significantly ($p < 0.05$) between *A. muricata* and *T. africana* at Control (0.00 ml) but did not vary significantly ($p > 0.05$) between the two species at the various crude oil levels. Number of leaves produced was significantly different ($p < 0.05$) between *A. muricata* and *T. africana* both at the Control and the various crude oil levels. The study shows that the various levels of crude oil used significantly reduced growth in both *A. Muricata* and *T. africana* seedlings. Crude oil levels below 25ml/5kg soil are recommended for further studies to ascertain the performances of seedlings of both species at lower crude oil levels.

Key Words: Soil, pollution, *A. muricata*, *T. africana*, Seedling growth

INTRODUCTION

One of the major problems of the Niger Delta region is crude oil pollution and degradation of the arable lands and water bodies on which the people depend for their livelihoods. Thus, the need for continuous research on innovative and environmentally friendly remediation approaches which will help to reclaim agricultural lands from the menace of increasing pollution and degradation had been observed by Oyedeji *et al.* (2012). Apart from phytoremediation – the decontamination of soils using plants, trees also help in the restoration of degraded ecosystems through other means like the improvement of soil structure by stabilizing soil aggregates using their roots and root exudates, nutrient pumping and recycling, and nitrogen fixation especially by the members of the leguminoceae family.

A successful effort at using trees to enhance the restoration of crude oil polluted soils, will to a large extent depend on the identification of tree species that are tolerant to crude oil to some reasonable extent, since remediated crude oil polluted soils are not 100% free of crude oil. Earlier attempts to ascertain the tolerance level of plant species to crude oil pollution had been made mainly with arable crops such as *Zea mays* (Agbogidi *et al*., 2007), *Hibiscus exculentus* (Cara *et al*., 2014), and *Abelmoschus esculentus* (Oyedeji*et al*., 2012); a mangrove species *Avicennia germinans* (Chinda *et al*., 2011); and *Persea americana* (Agbogidi *et al*., 2009).

There is paucity of information on the crude oil tolerance level of trees generally and fruit trees in particular. In addition, the acceptance of tree species for incorporation into crude oil polluted and remediated or partially remediated agro-ecosystems and their management by the rural farmers, will depend to a very large extent on their nutritional and economic values to them.

The fruit tree –*Annona muricata*, commonly known as soursop is a member of Annonaceae family comprising of approximately 130 genera and 2,300 species (Mishra *et al*., 2013). All portions of *Annona muricata* tree are tremendously used as traditional medicine for ailment such as cancer and parasitic infections; and for treating malaria, dysentery, arthritis, fever and many other ailments (Mishra *et al*., 2013). In the same vein, *Treculia africana* (African breadfruit) in the Moraceae family is an important fruit tree that is widely used for various purposes in the Niger Delta region of Nigeria and across tropical Africa. The seeds can be used to produce a variety of baked foods and the species canopy contributes to soil conservation by preventing erosion; the wood is suitable for pulp and paper making and firewood; and its medicinal uses include treatment for wooping cough, leprosy and as a laxative (Orwa *et al*., 2009).

Hence, this study sought to ascertain the crude oil tolerance level of seedlings of *Annona muricata* and *Treculia africana*, which are a very important and widely consumed fruit trees in the Niger Delta region of Nigeria with a view to ascertaining if they can thrive in crude oil polluted sites or partially remediated crude oil polluted sites.

The specific objectives of the study were to determine the effect of different crude oil levels on seedling height, collar diameter, leaf area and number of leaves produced, and seedling biomass.

MATERIALS AND METHODS

Study Area

The study was carried out at the Department of Forestry and Wildlife Management Nursery, located near its arboretum at the main campus of the University of Port Harcourt. The University of Port Harcourt (Latitudes 4.90794 and 4.90809 N and longitudes 6.92413 and 6.92432 E) is located on a land area of about 400 hectares in Obio/Akpor Local Government Area of Rivers State, Nigeria. The area is characterized with two seasons, the dry and the wet seasons with a nearly all year round rainfall (Aiyeloja *et al*, 2014). The arboretum covers a total land area of about $4226.2581m^2$ with tree species including *Gmelina arborea, Tectona grandis, Nauclea diderrichii, Khaya grandifoliola* and *Irvingia gabonensis*. These tree species are of great importance in wood production, paper making, food production, among several other uses (Chima *et al*., 2016).

Seed Collection, Processing and Viability Test

The fruits of *A. muricata* and *T. africana* were collected from a single healthy growing mother tree (for each species) within Rivers State and processed by depulping them manually. Seed viability was tested by floatation method where the seeds were soaked for three (3) hours and the seeds that floated were discarded.

Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD) and replicated five times.

Collection of Soil Sample, filling of polypots and transplanting of seedlings

Topsoil was collected from 0 – 20 cm depth at the University of Port Harcourt Arboretum. Leaf litters from the forest floor were removed before the collection of topsoil. The topsoil was collected using a soil auger and was properly bulked to enhance its homogeneity before the application of treatments (different crude oil levels).

Soils contaminated with different levels of crude oil (25.00 ml, 50.00 ml, 75.00 ml and 100.00 ml), and a control (0.00 ml), were used to fill 5kg-polypots. Three weeks old seedlings of *Annona muricata* and *Treculia africana* with equal number of leaves were transplanted into separate polypots. Each of the treatments (crude oil levels) was replicated five times for each species.

Watering

The seedlings were watered two times (early in the morning and late in the evening) daily throughout the period of the experiment with equal volume (100 mls for the first 4 weeks and 200 mls for the last 8 weeks) of water for each replicate/polypot.

Data Collection

Baseline data were collected immediately after transplanting the seedlings into the polypots contaminated with different levels of crude oil in order to ascertain the effect of crude oil on the considered growth attributes. Data collection continued on weekly basis for a period of twelve (12) weeks. At the end of the experiment, the initial value of each of the growth attributes at each crude oil level was subtracted from the value at week 12 to ascertain the growth increment during the period of study. The growth increments of the different attributes at the different crude oil levels were subjected to analysis of variance to determine if they were significantly different. Data were collected on seedling height, number of leaves, collar diameter, and leaf area.

Data Analysis

Analysis of Variance

One way analysis of variance (ANOVA) was used to test for significant differences in the measured seedling growth attributes amongst the different treatments (crude oil levels) for each tree species. Duncan Multiple Range Test (DMRT) was used for mean separation where significant difference occurred. The T-test was used to ascertain if growth for specific seedling attributes varied significantly between *A. muricata* and *T. africana* at each crude oil level. These analyses were performed using the Statistical Package for Social Sciences (SPSS) following Oloyo (2001).

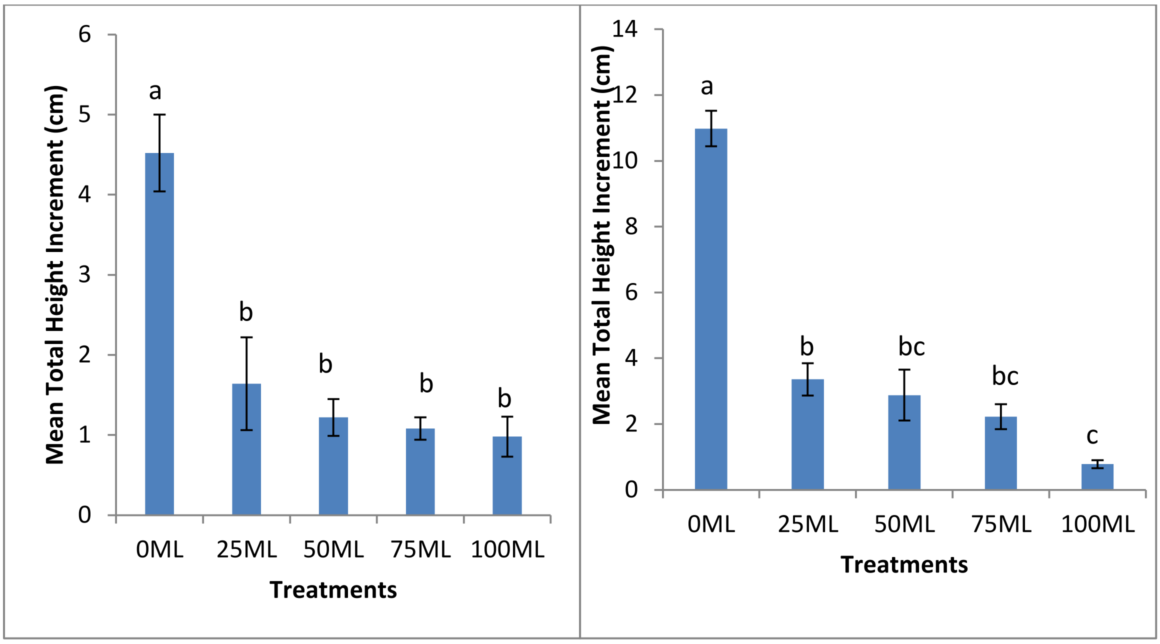
RESULTS

Effect of different crude oil levels on average total height of seedlings

Mean increment in total height of seedlings was significantly affected ($p < 0.05$) by the addition of different crude oil levels both in *A. muricata* (Figure 1a) and *T. africana* (Figure 1b). However, there was no significant difference ($p > 0.05$) in total height increment amongst the various crude oil levels in *A. muricata* while it varied significantly ($p < 0.05$) between the 25ml and 100ml crude oil levels in *T. africana*. Between *A muricata* and *T. africana*, growth in total height varied significantly ($p < 0.05$) at 0.00ml (control) but did not vary significantly at 25ml, 50ml, 75ml and 100ml (Table 1).

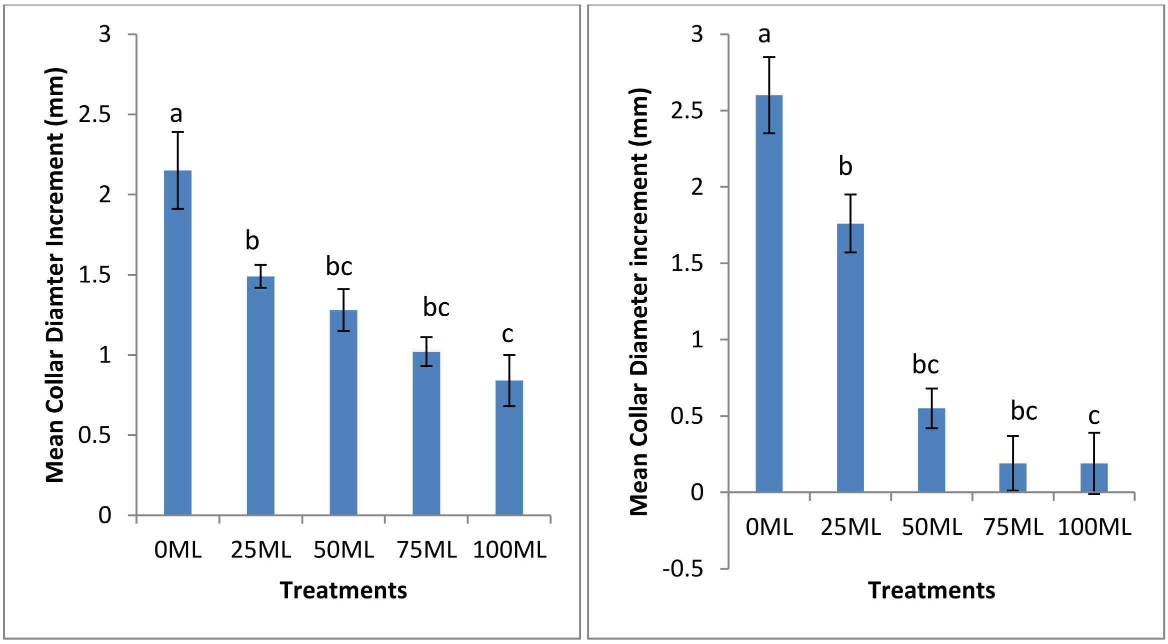
Effect of different crude oil levels on average collar diameter of seedlings

Growth in collar diameter was significantly reduced ($p < 0.05$) by addition of the various crude oil levels in both *A. muricata* (Figure 2a) and *T. africana* (Figure 2b). However, there was no significant difference ($p > 0.05$) in growth of collar diameter amongst the various crude oil levels in both species except between 25ml and 100ml. Growth in collar diameter did not vary significantly (p > 0.05) between *A. muricata* and *T. africana* both at control (0ml) and each of the various crude oil levels (Table 1).



(a) (b)

Figure 1: Increment in average total height (cm) of *A. muricata* (a) *and T. africana* (b) seedlings at different crude oil levels twelve weeks after transplanting
Means with the same alphabet on each column chart are not significantly different (p>0.05)

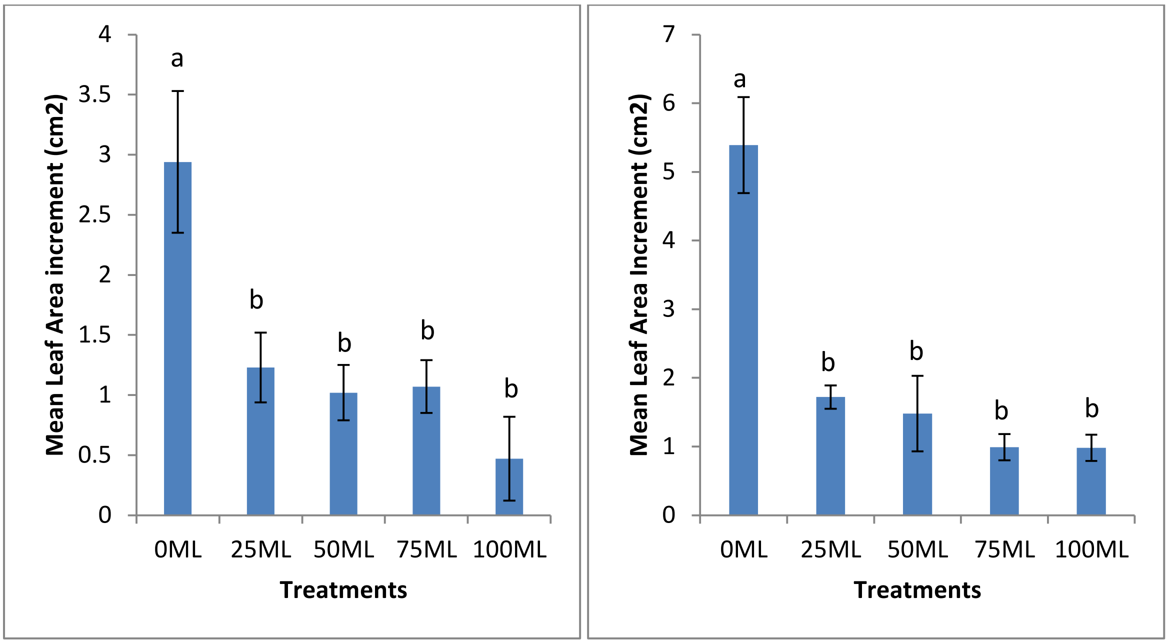


(a) (b)

Figure 2: Total increment in the average collar diameter (mm) of *A. muricata* (a) *and T. Africana* (b) seedlings at different crude oil levels twelve weeks after transplanting
Means with the same alphabet on each column chart are not significantly different (p>0.05)

Effect of different crude oil levels on average leaf area of seedlings

Addition of the various crude oil levels significantly reduced ($p < 0.05$) growth in leaf area of both *A. muricata* (Figure 3a) and *T. africana* (Figure 3b). However, there was no significant difference ($p > 0.05$) in leaf area increment amongst the various crude oil levels in both species. Between *A. muricata* and *T. africana*, growth in leaf area was significant ($p < 0.05$) only at the 0ml (Table 1).



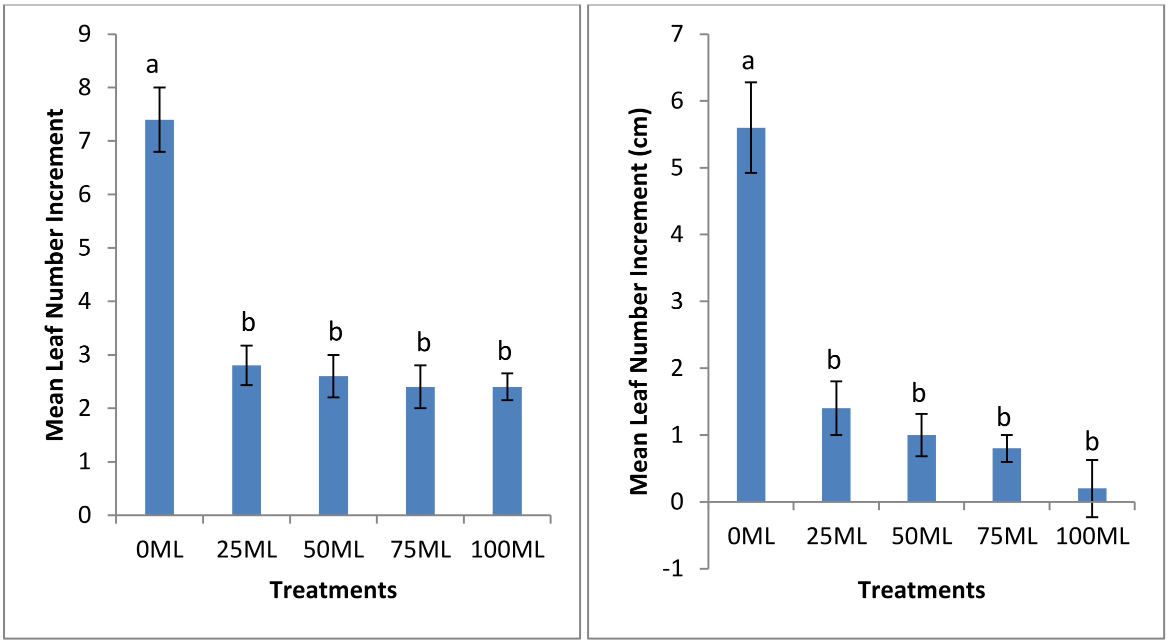
(a) (b)

Figure 3: Total increment in the average leaf area (cm^2) of *A. muricata* (a) *and T. africana* (b) seedlings at different crude oil levels twelve weeks after transplanting

Means with the same alphabet on each column chart are not significantly different ($p>0.05$)

Effect of different crude oil levels on leaf production

Increase in average number of leaves was significantly reduced ($p < 0.05$) by the various crude oil levels in both *A. muricata* (Figure 4a) and *T. africana* (Figure 4b). However, growth in average number of leaves did not vary significantly ($p > 0.05$) amongst the various crude oil levels in both species. Between *A. muricata* and *T. africana*, growth in number of leaves was significantly different ($p < 0.05$) at the Control (0ml) and at the various crude oil levels (Table 1).



(a) (b)

Figure 4: Total increment in the average leaf number of *A. muricata* (a) *and T. africana* (b) seedlings at different crude oil levels twelve weeks after transplanting

Means with the same alphabet on each column chart are not significantly different (p>0.05)

Table 1: T-test for growth performances of *Annona muricata* and *Treculia africana* seedlings at different crude oil levels

	0.00 ml		25.00 ml		50.00 ml		75.00 ml		100.0 ml	
	A. muricata	*T. africana*	*A. muricata*	*T. africana*	*A. muricata*	*T. africana*	*A. muricata*	*T. africana*	*A. muricata*	*T. africana*
Total height(cm)	$4.52±0.48^{b}$	$10.98±0.54^{a}$	$1.64±0.58^{a}$	$3.36±0.49^{a}$	$1.22±0.23^{a}$	$2.88±0.77^{a}$	$1.08±0.14^{a}$	$2.22±0.38^{a}$	$0.98±0.25^{a}$	$0.78±0.12^{a}$
Collar diameter(mm)	$2.15±0.24^{a}$	$2.60±0.25^{a}$	$1.49±0.07^{a}$	$1.75±0.19^{a}$	$1.28±0.13^{a}$	$1.48±0.13^{a}$	$1.03±0.09^{a}$	$1.25±0.18^{a}$	$0.84±0.16^{a}$	$1.08±0.20^{a}$
Leaf area (cm^2)	$2.94±0.59^{b}$	$5.39±0.7\ 0^{a}$	$1.23±0.29^{a}$	$1.72±0.17^{a}$	$1.02±0.23^{a}$	$1.48±0.05^{a}$	$1.07±0.422^{a}$	$0.99±0.19^{a}$	$0.47±0.35^{a}$	$0.98±0.19^{a}$
No. of leaves	$7.40±0.60^{a}$	$5.60±0.68^{b}$	$2.80±0.37^{a}$	$1.40±0.40^{b}$	$2.60±0.40^{a}$	$1.00±0.32^{b}$	$2.40±0.40^{a}$	$0.80±0.20^{b}$	$2.40±0.25^{a}$	$0.20±0.20^{b}$

Means on the same row with same alphabet for each crude oil level are not significantly different ($p > 0.05$)

Effect of elevated crude oil levels on the biomass of *A. muricata* and *T. africana* seedlings

The biomass (shoot and root) of *Annona muricata* seedlings and *Treculia africana* seedlings under the different crude oil levels is shown in Tables 2 and 3 respectively. Biomass of *Annona muricata* seedlings varied significantly ($p < 0.05$) between the 0.00 ml and each of the different crude oil levels (Table 2). However, there was no significant difference ($p > 0.05$) in *Annona muricata* seedling biomass amongst different crude oil levels. Shoot biomass of *T. africana* (Table 3) varied significantly ($p < 0.05$) between 0.00 ml and each of the crude oil levels. However, there was no significant difference ($p > 0.05$) between 0.00 ml and 75.00 ml for fresh root weight; and between 0.00 ml and each of 50 ml/75.00 ml, for dry root weight.

Table 2: Biomass of *A. muricata* at different crude oil levels

	Crude Oil Levels				
	0.00 ml	25.00 ml	50.00 ml	75.00 ml	100.00 ml
FSW (g)	1.72±0.48[a]	0.60±0.10[b]	0.58±0.06[bc]	0.48±0.09[bd]	0.52±0.07[be]
DSW (g)	0.68±0.16[a]	0.30±0.03[b]	0.34±0.05[bc]	0.24±0.02[bd]	0.32±0.04[be]
FRW (g)	1.20±0.33[a]	0.62±0.11[b]	0.78±0.10[ab]	0.78±0.07[ab]	0.78±0.08[ab]
DRW (g)	0.20±0.05[a]	0.14±0.02[abd]	0.14±0.02[acd]	0.10±0.00[d]	0.14±0.02[ade]

Means with the same alphabet on the same row are not significantly different (p > 0.05)
FSW = Fresh Shoot Weight; DSW = Dry Shoot Weight; FRW = Fresh Root Weight; DRW = Dry Root Weight

Table 3: Biomass of *T. africana* at different crude oil levels

	Crude Oil Levels				
	0.00 ml	25.00 ml	50.00 ml	75.00 ml	100.00 ml
FSW (g)	5.38±2.61[a]	0.84±0.22[b]	0.98±0.15[bc]	0.80±0.07[bd]	0.66±0.08[be]
DSW (g)	1.88±0.97[a]	0.34±0.07[b]	0.40±0.06[bc]	0.34±0.04[bd]	0.36±0.04[be]
FRW (g)	2.28±0.18[a]	0.74±0.14[b]	0.90±0.18[bc]	0.92±0.06[abd]	0.60±0.09[be]
DRW (g)	0.40±0.18[a]	0.14±0.02[b]	0.18±0.03[abc]	0.16±0.02[abd]	0.10±0.00[be]

Means with the same alphabet on the same row are not significantly different (p > 0.05)
FSW = Fresh Shoot Weight; DSW = Dry Shoot Weight; FRW = Fresh Root Weight; DRW = Dry Root Weight

Comparative Evaluation of *Annona muricata* and *Treculia africana* seedlings biomass at different crude oil levels

A comparative evaluation of the biomass of *Annona muricata* and *Treculia africana* seedlings at each crude oil level is presented in Table 4. Fresh shoot weight, dry shoot weight, fresh root weight and dry root weight did not vary significantly (p >0.05) between *Annona muricata* and *Treculia africana* except at 75.00 ml crude oil level.

Table 4: T-test results for variation in biomass of *Annona muricata* and *Treculia africana* seedlings at different crude oil levels

	0.00 ml		25.00 ml		50.0 ml		75.00ml		100.00 ml	
	A. muricata	*T. africana*	*A. muricata*	*T. africana*	*A. muricata*	*T. africana*	*A. muricata*	*T. africana*	*A. muricata*	*T. africana*
FSW (g)	1.72±0.48[a]	5.38±2.61[a]	0.60±0.10[b]	0.84±0.22[b]	0.58±0.06[c]	0.98±1.15[c]	0.48±0.09[d]	0.80±0.07[e]	0.52±0.07[e]	0.66±0.08[e]
DSW (g)	0.68±0.16[a]	1.88±0.97[a]	0.30±0.03[b]	0.34±0.07[b]	0.34±0.05[c]	0.40±0.06[c]	0.24±0.02[d]	0.34±0.04[e]	0.32±0.04[e]	0.36±0.04[e]
FRW (g)	1.20±0.33[a]	2.28±1.01[a]	0.62±0.11[b]	0.74±0.14[b]	0.78±0.10[c]	0.90±0.18[c]	0.78±0.07[d]	0.92±0.06[e]	0.78±0.08[e]	0.60±0.09[e]
DRW (g)	0.20±0.05[a]	0.40±0.18[a]	0.14±0.02[b]	0.14±0.02[b]	0.14±0.02[c]	0.18±0.04[c]	0.10±0.00[d]	0.16±0.02[e]	0.14±0.02[e]	0.10±0.00[e]

Means on the same row for each crude oil level are not significantly different ($p > 0.05$)
FSW = Fresh Shoot Weight; DSW = Dry Shoot Weight; FRW = Fresh Root Weight; DRW = Dry Root Weight

DISCUSSION

The significant reduction in the average total height, collar diameter, leaf area, number of leaves produced, and biomass of seedlings of both *A. muricata* and *T. africana*, is indicative of the negative impact of crude oil on the growth of their seedlings. The fact that average seedling height which varied significantly between *A. muricata* and *T. africana* without the application of crude oil, was found not to be significantly different at the various crude oil levels, lends credence to the ability of crude oil to retard growth in seedling height, since *T. africana* naturally grows taller; with the possibility of reaching a height of 30m (Salami, 2002).

Several reasons have been advanced by several authors, for the negative impact of crude oil on plant growth. These include: the disruption in water and nutrient uptake owing to the effects of oil in soil, and the depletion of soil nitrogen and phosphorus content (Baran *et al.*, 2002); creation of conditions in soil that makes water and nutrients unavailable (Agbogidi and Nweke, 2005); inhibition of cell division and nutrient availability which could negatively affect the anatomical and physiological structures of the plant and hence growth reduction (Agbogidi and Eshegbeyi, 2006); insufficient aeration caused by displacement of air from pore spaces (Rowell, 1977); disruption in nutrient level and flow due to immobilisation (Agbogidi *et al.*, 2009); increased demand for oxygen by oil decomposing organisms (De Jong, 1980); and dehydration indicating water deficiency due to increase in soil toxic levels (Agbogidi *et al*., 2007).

Although the leaf area was significantly different between *A. muricata* and *T. africana*, with the latter having higher mean values, the addition of crude oil significantly reduced the leaf area in both species. Besides shrinkage of leaves following the addition of various levels of crude oil, yellowing of leaves was observed in both species. This probably could have been due to the inhibitory effect of crude oil on chlorophyll which is essential for photosynthesis. Oil pollution has been reported to physically act by absorbing light wavelengths essential for photosynthesis (Baker, 1970; Odejegba and Sadiq, 2002). Differential changes in the rate of leaf growth following the application of crude oil may equally be associated with anatomical and morphological changes caused by the crude oil (Agbogidi *et al*., 2005). However, the significant difference observed in leaf area between seedlings of *A. muricata* and *T. africana* without the application of crude oil is attributable to the inherent differences in the genetic makeup of both species, as *T. africana* naturally has broader leaves than *A. muricata*.

The significant reduction in leaf production following the addition of various crude oil levels can be attributed to a host of factors including blockage of conducting tissues thereby preventing water and nutrients into the plant and limiting their ability to produce more leaves (Osuagwu *et al.*, 2013). Agbogidi *et al.* (2009) attributed reduction in the number of leaves of *Persea americana* following crude oil application to reduced movement of nutrients and photosynthates probably caused by disruption or obstruction in the xylem and phloem vessels from the sources of production to sites where they are required. However, the significant higher production of leaves by *A. muricata* at both the Control (0ml) and the various crude oil levels was expected as *A. muricata* produces more leaves than *T. africana*, by nature.

The significant difference observed in the shoot biomass of seedlings of both species between the Control (0 ml) and the various crude oil levels can, to some extent, be ascribed to the significant effect of crude oil on root biomass which must have distorted water and nutrient uptake from the root to the above-ground parts of the seedlings. Agbogidi (2010) posited that a reduction in shoot growth is a direct result in the root growth as roots are input organs for the absorption and translocation of water and mineral nutrients.

CONCLUSION

The addition of crude oil (25 – 100ml/5kg soil) significantly affected the early growth of both *A. muricata* and *T. africana* in terms of seedling height, collar diameter, leaf area, number of leaves produced, and biomass. There was no clear distinction in the performances of the two species at different/elevated crude oil levels.

RECOMMENDATIONS

The performances of *A. muricata* and *T. africana* at crude oil levels lower than 25ml/5kg soil, should be evaluated to ascertain if they can thrive in remediated sites than may contain very low levels of crude oil. More studies should be carried out to ascertain the tolerance of other fruit tree species that contribute to rural livelihoods, to crude oil pollution.

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