Original Research

Effect of Crude Oil on the Development of White Mangrove Seedlings (Avicennia germinans) in the Niger Delta, Nigeria

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Abstract

Crude oil is a complex hydrocarbon whose introduction into the environment may be hazardous to aquatic and human life, and consequently a threat to natural resources. The severity of the impact of crude oil depends on a variety of factors, including characteristics of the oil itself, natural conditions such as water temperature and weather, and the sensitivities of receiving or impinging biota. As a result, various biological resources such as mangrove seedlings have different sensitivities to oil spills. The long-term persistence of oil in the environment may cause defoliation and possibly death. Recruitment of seedlings into the oiled area may also be affected. This study is designed to evaluate the acute and chronic effects of crude oil (Bonny Light) on the growth performance of mangrove seedlings in the laboratory; monitoring critical plant growth attributes such as stem height and diameter, leaf length, width and numbers of leaves (leaf production), senescence, and seedling survival, for sixteen weeks.

The study revealed that mangrove seedlings responded differently in growth attributes with varying treatment. Evidence of crude oil effects were remarkably demonstrated between control and acute (Wilcoxon sign – rank t-test $(1.0) > P(0.29)_{0.05}$ and chronic (Wilcoxon sign – rank, t-test $(1.0) > P(0.47)_{0.05}$ exposure. The critical growth response by mangrove seedlings as a result of the treatments was further explained by the cluster and correspondence analyses.

Keywords: Avicennia germinans, Mangrove seedling, crude oil, Niger Delta, Nigeria

Introduction

The Niger delta of Nigeria has four main ecological zones: coastal barrier islands, mangrove swamp forests, freshwater swamps, and lowland rainforests. These ecologocal zones are incredibly well-endowed with high biodiversity in addition to supporting abundant flora and fauna. In the lower delta, within the brackish water limits, is an

expanse of mangrove forests estimated to cover approximately 5,000 to 8,580 km² [1,2]. There are three main mangrove families: (*Rhizophoraceae*, *Avicenniaceae*, and *Combretaceae*) comprising six species, namely: *Rhizophora racemosa*, *Rhizophora mangle*, *Rhizophora harrisonii*, *Languncularia racemosa*, *Avicennia germinans*, and *Conocarpus erectus*, and the exotic family Palmae (Arecaceae) *Nypa fruticans* van Wurmb that is now spreading fast across the Niger Delta [1, 3-5, 33].

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Poor land management upstream, caused by human impacts, coupled with oil industry activities and associated pollution of oil has caused land loss and mangrove forest clearing, severe habitat fragmentation, and damage to the zonal ecosystem [6-15]. These have facilitated and provided a springboard for a non-native invasive species of palm, *Nypa fruticans*, that has quickly colonized the mangrove system. The shallow root system and poor litter generation potential help to destabilize the normal bank sediment distribution, resulting in declining nutrient processes, poor recruitment potentials, decreases in biodiversity and reduction of ecosystem functions.

The degradation of the ecosystem is responsible for lower economic value of highly important resources utilized by indigenous people, such as logs, fuel wood, charcoal, wood-chips, paper pulp, scaffold poles, piling, construction material, stakes for fish traps and fishing platforms, railway sleepers, wood for furniture and carvings, material for roof thatching, bark for tannin, medicinal products, sugar, alcohol, acetic acid, and dye [16]. The poor yield of other resources, including fin and non-fin fishes, in addition to the poor natural regeneration of mangrove after such ecological abuse, has generated apprehension and great concern among various stakeholders. This has led to their demand for re-vegetation of the degraded mangrove areas. This study evaluated the development, in crude oil treatments, of mangrove - Avicennia germinans seedling. This mangrove species does not grow on prop roots, but possesses pneumatophores that allow its roots to breathe even when submerged. It is a hardy species and expels absorbed salt mainly from its leathery leaves.

Materials and Methods

Description of Study Area

The study was conducted at Eagle Island near Rivers State University of Science and Technology, Port Harcourt, located at the upper reach of Bonny estuary of the eastern Niger Delta, Nigeria, and lies within longitude 4°-35" – 4°.5N" and latitude 7°.00" – 7°.53" E (Fig. 1).

The vegetation of the area is predominantly mangrove, with the dominant species being red mangrove (*Rhizophora mangle, R. racemosa*, and *R. harsonii*), white mangrove (*Avicennia germinans*), and black mangrove (*Laguncularia racemosa*). There are other plants such as the fern (*Achrostichum aureum*), and grass (*Paspalum varginatum*). Animals include *Periophthalmus papilo* (mud skipper), *Uca tangeri* (fiddler crabs), and *Tympanotonus fuscatus* (Periwinkles), whose presence, among others, provides a significant contribution to the dynamics of the mangrove community as a whole.

The climate of the area is basically one where rainfall occurs almost year-round, except the mouths of December, January, and February. These three months are not completely rain-free in some years. Mean annual rainfall of the area is about 2,405.2 mm [17]. Annual mean air temperature is 31.3°C with the highest monthly mean of 29.7°C (in August), and the lowest monthly mean temperature of 27.5°C (in January). The seawater surface temperature values range between 25.9.9°C and 30.6°C, with salinity ranging between 8 and 20‰. The tidal range is between 0.43 m and 1.67 m, with a mean tidal variation of 0.9 m. The cur-



Fig. 1. Map of Africa, Nigeria, and Elechi Creek showing the study area.

rent flows are unidirectional flooding (inundation) during high tide and receding at low tide regime. The mud (sediment) has a dark appearance with hydrogen sulphide as the major byproduct of sulphur bacteria. The soil type is clay (Chikoko). Economic activities in this area are mainly fishing, trading, and transportation.

Nursery Preparation

The sampling sites were established in a relatively undisturbed tidally inundated mangrove wetland next to the Rivers State University of Science and Technology, Port Harcourt. Surface soil form the study area was collected (0-15 cm depth) during tidal recession. The wet surface soil samples (4 kg) were weighed and potted in polyethylene bags (40 x 50 cm), leaving 10 cm at the upper end for irrigation of water. Each bag was labeled.

Mangrove seedlings (*Avicenna*) in good condition were carefully uprooted using hand trowel and transplanted into the potted bags, ensuring that the there was no root damage. Seedlings were grown for 60 days (2 months) to eliminate effects of transplant shock. The seedlings were arranged in 10 rows of parallel triplicates at 1 m intervals for each treatment (chronic, acute, and control) Plate 1.

Treatments

The seedlings were subjected to acute and chronic treatment levels of crude oil (Bonny Light) using indicative growth parameters such as stem growth, leaf production and growth and leaf drop – senescence, and seedling survival as measures.

Treatment commenced after the 60-day stabilization period.

For acute treatment, a one-time addition of 120 ml of crude oil was placed at the base of the seedlings, on the surface of the mud. For chronic treatment a smaller aliquot of 15 ml (crude oil) was added weekly. The crude oil for each treatment was delivered after measuring it in a graduated cylinder that was allowed to drain for at least 1 minute.

Shoot height, diameter of stem at the first inter-node, number of nodes, number of leaves, and leaf area were measured individually using veneer caliper and the fate and growth of seedlings were monitored for 16 weeks.

Data analyses on seedling growth rate were run for height, diameter, leaf length, and width, number of leaves, yellowing of leaves, and seedling survival for 16 weeks.

The calculation for RGR (Relative growth rate) was adapted from Hunt's classical approach [18]:

$$RGR = \frac{In(L_2) - In(L_1)}{t_2 - t_1}$$

...where L_1 and L_2 is the growth at time t_1 and t_2 , respectively.

The response patterns of mangrove seedlings among treatments were examined by hierarchical cluster analysis on log (x + 1) transformed data using JMP IN analytical

software [19, 20]. Group average sorting (=unweighted pair-group method; [21] was used as the clustering method and Bray-Curtis similarity [22] for resemblance measure.

Results were expressed as a dendogram in which samples were ordered into groups. Correspondence analyses on growth responses were carried out using Kovach Computing Services-MultiVariate Statistical Package (MVSP) version 3.1.

Results

Chronic Treatment

The response of seedlings to chronic exposure with respect to stem growth (height) indicated a steady growth for both control and treatment plants. Initial height (184 mm) for the chronic treatment of seedlings increased rapidly for the first 10 weeks to 327.5 mm). Thereafter, the growth continued but slowed, reaching a final height of 350.5 mm at the end of the 16th week. The seedlings under control condition rapidly continued to grow while treatment seedling growth relatively stagnated from the 12th week to the end of the study (16th week). However, the control (R²=0.92) recorded relatively high regression value than the treatment (R²=0.92) (Table 1 and Fig. 2).

A similar growth trend was observed for the stem diameter, with increases being observed from week 0 to the 11th week before growth stagnation was observed for the treatment plant to the end of the experiment while the control relatively continued growth to the end of the experiment, but no difference was observed in the regression values between the control (R²=0.93) and treatment (R²=0.93) (Table 1 and Fig. 2).

Leaf length for the treatment plant tended to decline almost steadily with pulses at the 2^{nd} (46.12 mm), 8^{th} (37.44 mm), and the 14^{th} week (23.73 mm). While the control seedlings had almost an exponential growth trend from start (40.4 mm) to finish (69.1 mm), and there were no differences in the regression values for the control ($R^2=0.85$) and treatment ($R^2=0.85$) (Table 1 and Fig. 2).

Senescence in seedlings for the control commenced from the 3^{nd} week and continued uniformly to the 7^{th} week before an increase from the 8^{th} week that continued exponentially to the 12 week. It then maintained a steady value before a slight increase in the 16^{th} week. However, the treated seedlings started senescence from the 6^{th} week and increased exponentially to the end of the experiment (16^{th} week). Regression values for control (R^2 =0.78) and treatment were the same (R^2 =0.78) (Table 1 and Fig. 2).

The control seedlings had 100% survival from start to the end of the experiment while in treated seedlings a reduction on survival commenced on the 13th week but stabilized in the 15th week.

Acute Treatment

The mangrove seedlings subjected to acute treatment showed stem growth (height) in an exponential manner from start (203.1 mm) to the end of the experiment (week

Table 1. Regression equations for relationships for each treatment on *Avicennia germinans* seedling growth characteristics.

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	54 54					
Leaf length Treatment $-1.6384x + 46.239$ $0.8384x + 46.239$ Leaf width Control $-0.5118x + 14.924$ $0.688x + 14.924$ Treatment $-0.5118x + 14.924$ $0.688x + 14.924$	54					
Leaf width Treatment -0.5118x + 14.924 0.6	14					
Treatment $-0.5118x + 14.924$ 0.6	1.7					
	14					
Leaf Control 4.7843x - 22.529 0.7	75					
senescence Treatment 4.7843x - 22.529 0.7	75					
Seedling Control -0.152x + 10.838 0.5	17					
survival Treatment -0.152x + 10.838 0.5	17					
Leaf Control -0.1277x + 9.5199 0.00	53					
production Treatment -0.1277x + 9.5199 0.00	53					
Acute	Acute					
$\begin{array}{c c} \text{Treatment} & \begin{array}{c} \text{Model equation,} \\ Y = \end{array} & R^2 \end{array}$	=					
Stem height Control 14.265x + 154.73 0.98	557					
Treatment 5.6757x + 214.6 0.88	327					
Control 0.1945x + 2.1673 0.95	47					
Treatment 0.0531x + 3.4077 0.87	78					
Control 1.9881x + 39.169 0.95	73					
Lent length	-					
Leaf length Treatment $-1.6384x + 46.239$ 0.83	54					
Treatment -1.6384x + 46.239 0.85 Control 0.3352x + 17.015 0.72						
Treatment -1.6384x + 46.239 0.83	271					
Treatment $-1.6384x + 46.239$ 0.8: Control $0.3352x + 17.015$ 0.72	.37					
	37					
Treatment	271 37 398 578					
Treatment	271 37 398 578					
Treatment	271 37 398 578					

16,295 mm) as was observed for the control and treatment from start (169 mm) to the end (388 mm) of the study, and the control was higher in magnitude (R^2 =0.98) than that of the treatment seedlings (R^2 =0.88). The observation the stem diameter followed similar treatment as observed for stem height and recorded higher regression value for control (R^2 =0.95) than the treated (R^2 =0.88) (Table 1 and Fig. 3).

Table 2. The relative growth rate (RGR) of *Avicennia germinans* seedlings exposed to different crude oil treatments.

Variables	Chronic	Acute	Control	
Leaf production	1.23	0.42	2.61	
Seedlings survival	1.85	0.86	2.24	
stem height	5.83	5.64	5.22	
stem diameter	1.57	1.36	0.21	
Leaf fallout	4.42	4.20	3.26	
leaf length	1.46	2.48	3.16	
leaf width	2.39	1.67	2.16	

The leaf length seemingly increased from week 0 (42.8 mm) to the 2nd week (46.1 mm), declined rapidly in the 3rd week (34.3 mm) and maintained a consistent length from the 4th week (35.6 mm) to the 7th week (35.6 mm), and then increased slightly in the 8th week (37.4 mm), declined slightly in the 9th week (36.2 mm), and continued to the end of the study (16th week – 14.6 mm). While the control increased steadily to the end of the study with pulses at the 7th and 11th weeks and had higher regression value (R²=0.96) than the treatment (R²=0.85). The observation on leaf width did not vary with that for the leaf length with control (R²=0.73) having higher regression value than the treatment (R²=0.61) (Table 1 and Fig. 3).

Leaf senescence commenced from the 2nd week (3 leaves) for the treatment plants and sharply increased in the 3rd week (21 leaves) and 5th week (22 leaves), and then increased exponentially to the end of the experiment (67 leaves). In the control, leaf senescence commenced from the 3rd week (2 leaves) and thereafter maintained a stable number to the 7th week (2 leaves), with increase resumed in the 8th week (5 leaves) to the 12th week (19 leaves), stabilising again to the 15th week (19 leaves) before a another leaf fall in the 16th week (26 leaves). Contrary to the observed trend in stem height, stem diameter, leaf length, and leaf width, the treated seedling had higher regression value (R²=0.97) than the control (R²=0.89) (Table 1 and Fig. 3).

Seedling survival for the treated plant showed 5 pulses, with the first being in weeks 0-2 with 100% survival, the second pulse was in-between weeks 3 and 4 (70%), the third pulse between weeks 5 (60%) and week 10 (60%), the fourth pulse at week 11 (50%), while the fifth pulse was between weeks 12 and 16 (30%), while the control had 100% survival throughout the duration of the study (Table 1 and Fig. 3).

The production for the treatment declined almost consistently from the start (6 leaves) to the end of the study (1.9 leaves), in contrast to the control increasing in leaf production to the end of the study (Table 1 and Fig. 3).

The differences in seedling attributes for different treatments showed differing responses with acute treatment demonstrating a declining response pattern of stem height – RGR=5.64 > leaf fall – RGR=4.20 > leaf length – RGR=2.48 = leaf width – RGR=1.67 > stem diameter –

RGR =1.36 > seedling survival RGR = 0.86 > leaf production RGR =0.42 and chronic with stem height – RGR=5.83 > leaf fall – RGR=4.42 > leaf width – RGR=2.39 > seedling survival – RGR=1.85 > leaf length – RGR = 1.46 > leaf production – RGR = 1.23. In the control, the pattern was stem height – RGR = 5.22 > leaf fall – RGR = 3.26 > leaf length – RGR= 3.16 > leaf production – RGR = 2.61>

seedling survival – RGR 2.24 > leaf width – RGR = 2.16 > stem diameter – RGR = 0.21 (Table 2). However, the pooled data of all the seedling growth attributes using Wilcoxon sign – rank t-test for treatment comparison demonstrated non significance between acute and chronic (Wilcoxon sign – rank, t-test (-7.0) < $P(0.85)_{0.05}$, but this was significantly different between control and chronic (Wilcoxon sign –

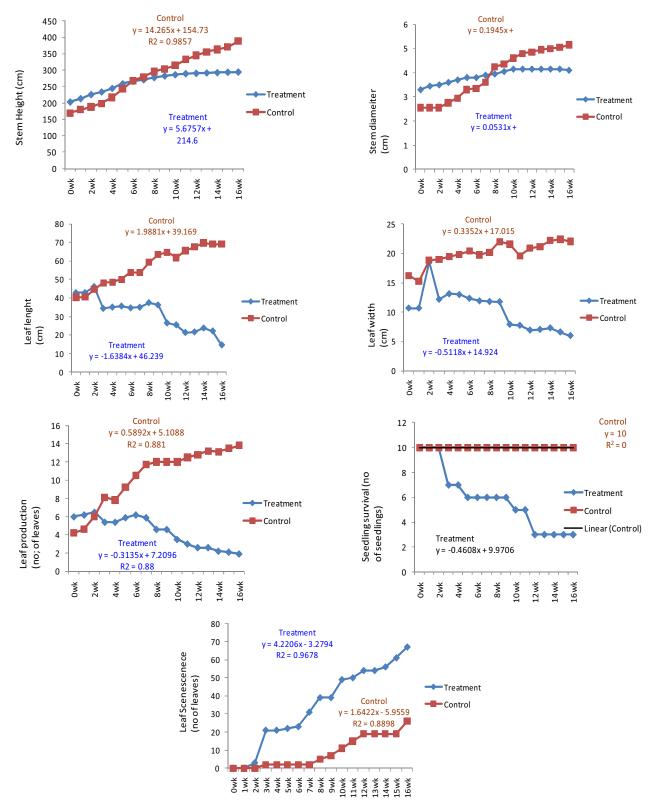


Fig. 2. The growth responses of different growth descriptors of white mangrove seedlings under acute exposure to crude oil.

rank, t-test $(1.0) > P(0.47)_{0.05}$ and between control and acute (Wilcoxon sign – rank t-test $(1.0) > P(0.29)_{0.05}$.

At each step, the two clusters that are closest together are combined into a single cluster.

Similarity analysis using the average method and Euclidean distance measure for acute and chronic treatment examined responses of the plant attributes to different exposures (Figs. 4 and 5). The analyses revealed 3

major responses, denoted as A, B, and C of the attributes of the mangrove seedlings that yielded for the acute treatment, the highest affinity amongst the attributes was between stem diameter and leaf production (A-1, 99.5%), and stem diameter and seedling survival (A-2, 99.1%), followed by leaf length and yellowing of leaf (B, 92.3%), and then stem height (C, 20%) in that decreasing response (Fig. 4).

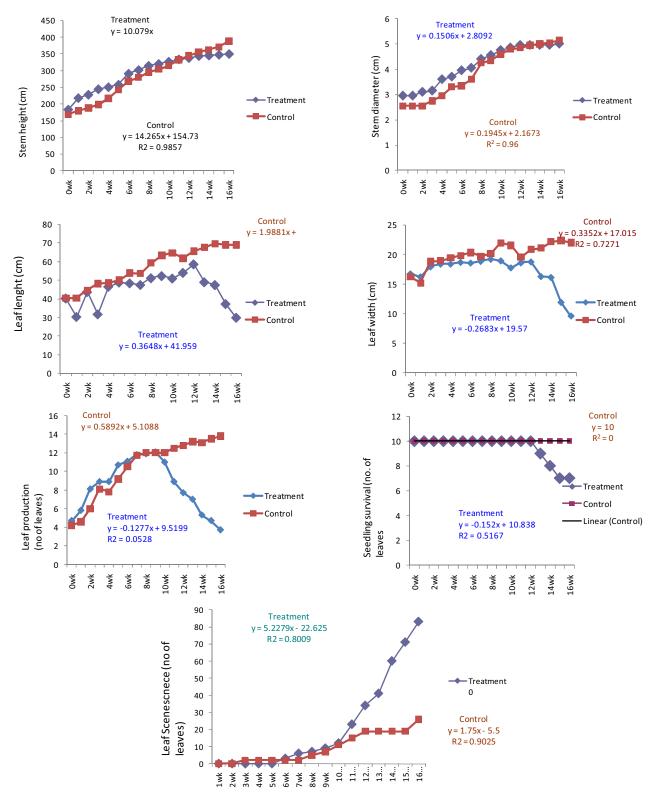


Fig. 3. The growth responses of different growth descriptors of white mangrove seedlings under chronic exposure to crude oil.

Type of analysis	Treatment						
	Control		Acute		Chronic		
Correspondent analysis	Axis -1	Axis -2	Axis -1	Axis -2	Axis -1	Axis -2	
Percentage contribution (%)	96.95	1.42	98.06	1.1	93.15	5.95	
Cum. Percentage	96.95	98.37	98.06	99.16	93.15	99.1	
Cases	Response estimate from scores Response estimate from sco		ate from scores	Response estimate from scores			
Stem height acute	moderate		moderate		moderate		
Stem diameter acute	moderate		moderate		moderate		
Leaf size length	high		moderate		high response		
Leaf size width	high		low		high response		
Leaf fall (Senescence)	low		high		low response		
Seedlings survival	high		high		high response		
Leaf production	moderate		moderate		moderate		

Table 3. Summary of structure of the correspondence analysis for control, acute, and chronic exposures.

The chronic treatment indicated the strongest affinity between seedling survival and leaf production (A-1, 99.3%), followed by seedling survival and leaf width (A-2, 99.0%), stem diameter and leaf width (A-3, 98.2%), stem diameter and leaf length (B, 91.6%), and stem height and stem diameter (19.1%) in that decreasing order of response (Fig. 5).

The control response gave rise to 5 cluster groups among the seedling growth attributes such as the declining affinity between seedling survival and leaf production (A, 99.1%), stem diameter and leaf fall (B, 98.4%), leaf width and stem diameter (C, 97.9% with groups A and B), leaf length and stem diameter (D, 96.1%), stem diameter and leaf length (86.1%), and stem height and stem diameter (E, 22.4%) in that respective order (Fig. 6).

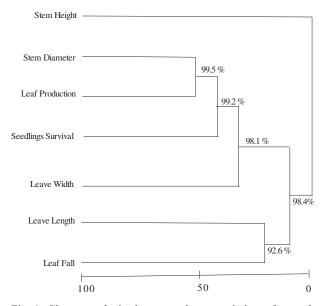


Fig 4. Cluster analysis demonstrating association of growth attributes of seedlings exposed to acute treatment.

The correspondent analysis for the first and second axes for acute, chronic, and control were 98.1% and 1.1%, 93.2 and 5.95%, 93.03%, and 5.17%, respectively(Table 3). The cumulative percentages for acute, chronic, and control were 99.16%, 99.1%, and 98.2%, respectively (Table 3). The correspondence analysis provided further insight indicating differences in mangrove seedling response patterns for the different crude oil exposure (acute and chronic treatments) and control (Figs. 7-9). Seedling survival demonstrated high response pattern for the three series of treatments, while stem height, stem diameter, and leaf production demonstrated moderate response for the 3 treatments

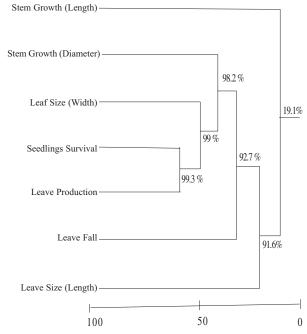


Fig. 5. Cluster analysis demonstrating association of growth attributes of seedlings exposed to chronic treatment.

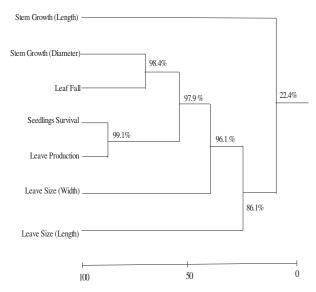


Fig. 6. The cluster analysis demonstrating association of growth attributes of seedlings exposed to no treatment.

(acute, chronic and control), However, leaf length demonstrated a high response pattern for control and chronic exposure but moderate for acute exposure. Leaf width shared the same high response pattern for control and chronic, but differed with acute that exhibited low response. Similarly, leaf fall for the control and chronic exposure had low response, contrary to high response observed for acute exposure.

Discussion

Human development activities cause particular risks for plant habitat, wildlife and human communities that involve intrusion into relatively pristine environmental areas, disrupting sometimes the habitat and facilitating decline in habitat quality and biodiversity loss.

Utilization and dependence on natural resources also may skew the ecobalance and alter inter-relationship among them. Efficiency in resource management options and regeneration could transform the current declining quality of the ecosystem into one that is healthier, more resilient and productive. The mangrove ecosystem, which is more ecologically sensitive to human perturbation and natural reestablishment processes, also demonstrates exceedingly poor potential for rejuvenation of natural vegetation. This peculiar characteristic is further exacerbated by contamination from crude oil spills and other human activities. The rehabilitation of degraded habitats through replanting exercise requires considerable understanding of the factors associated with the growth processes and seedling survival. This is crucial particularly as it relates to the predominant environmental concern in the region with is presence of elevated hydrocarbon in the environment.

In our study on the different exposure levels (acute and chronic) of crude on mangrove seedling growth, responses were demonstrated. Our observations elucidated disparity

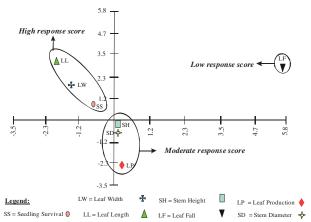


Fig. 7. The arch effect (relationship between the first two axes) of the correspondence analysis indicating response of seedling attributes for control.

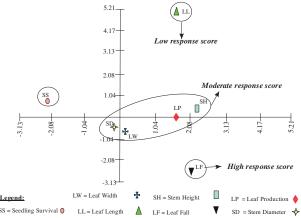


Fig. 8. The arch effect of the correspondence analysis indicating response of seedling attributes for acute treatment.

in mangrove seedling growth response with crude oil treatments (acute and chronic). For instance, the improved or enhanced growth performance for each of the attributes by the control against the treatments is an indication of the obtrusive and interfering role of crude oil on seedling development as demonstrated by comparison (Wilcoxon sign – rank t-test) between treatments (acute and chronic and control) where variation between the two exposure levels were not significant (Wilcoxon sign – rank, t-test (-7.0) $< P(0.85)_{0.05}$, whereas the relationship between control and chronic exposure (Wilcoxon sign - rank, t-test (1.0) > P (0.47)_{0.05} and that between control and acute (Wilcoxon sign – rank t-test $(1.0) > P(0.29)_{0.05}$ both demonstrated significant differences. However, the response amongst the treatments projected the mangrove seedlings as having responded better under chronic conditions than in acute exposure as demonstrated by the trend observed on stem and leaf growth attributes (Figs. 2 and 3). The cluster analysis also grouped similarly the same attributes as having the highest affinity for chronic (between seedling survival and leaf production – 99.3%) treatment (Table 3) and control conditions (between seedling survival and leaf production –

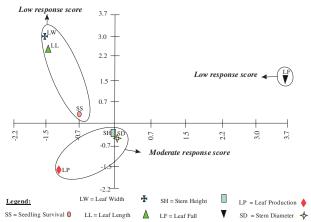


Fig. 9. The arch effect of the correspondence analysis indicating response of seedling attributes for chronic treatment.

99.1%) between seedling survival and leaf production (Table 3). This relationship is further corroborated by the correspondence analysis with chronic and control having similar response patterns for high (leaf size length, leaf size width, and seedling survival), moderate (stem height, stem diameter, and leaf production) and low (leaf fall). Nonetheless, mangrove seedlings under acute exposure recorded two attributes for high responses (leaf fall and seedlings survival), four attributes for moderate response (stem height, stem diameter, leaf size length, and leaf production) and leaf size width for low response (Figs. 7-9).

These underscore the fact that acute exposure of seedling had more damaging effects on seedlings than chronic exposure. This observation is in consonance with the work of Proffit et al. [23] on mangrove seedlings subjected to different exposure levels (acute and chronic).

Chindah et al. [24] attributed the observed declining growth tendencies in red mangrove seedling *Rhizophora racemosa* to the hash and stringent physiological conditions imposed by polycyclic aromatic components associated with crude oil. This scenario was also demonstrated for mangrove seedlings under chronic exposure. For instance the development of stem (height and girth) and leaf (length and width) based on the relative growth rate suggests that the acute exposure of seedling had more damaging effects on seedlings than chronic exposure. A similar observation was made on mangrove seedlings by Proffit et al. [23] at different exposure levels (acute and chronic) and demonstrated linear growth but less than that of the control.

Other analogous studies have indicated such adverse consequences of the negative crude oil effect on mangrove seedlings [2, 7, 8, 23, 25-27]. This process may have been achieved by the crude oil altering the sediment quality (properties), and by reducing sediment porosity and gaseous exchange that in turn may have negative consequences on the physiological function of the plant [6, 28]. Secondly, crude oil may possibly lower mineral-nigrogen by the process of immobilisation of mineral-nitrogen by the activities of soil micro-organisms during degradation of crude oil (petroleum hydrocarbons) impacted soil [29], thus making nitrogen unavailable to the plant.

Thirdly, petroleum hydrocarbons induce stress in saltextracting plants such as the mangroves plants, by disrupting the ability of the roots to exclude ions from sea or brackish waters [30]. Oil stress in salt-excluding halophytes, such as Mangroves, results from interference by hydrocarbons in this process [31]. Chloride ion exclusion in the roots of Mangrove seedlings is disrupted by exposure to other hydrocarbons such as diesel fuel, and toluene [32]. In effect oil stress in Mangroves is an artificially induced hypersalinity syndrome in which the oil-exposed trees are less able to exclude salt from their root tissues. Thus concentrations of sodium, the principal seawater cation, would be elevated in the tissues of Mangrove plants unable to exclude salt efficiently in their roots. Potassium ion, a major physiological cation, serves as a reference. In a healthy tree, the ratio of sodium to potassium would be smaller than in a tree unable to exclude salt effectively. The responses in trends provide concrete and imperative contrivance for understanding the consequences of crude oil on mangrove seedling development.

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