Effect of crude oil on the development of mangrove (*Rhizophora mangle* L.) seedlings from Niger Delta, Nigeria

Efecto del petróleo crudo sobre el desarrollo de plántulas de mangle (*Rhizophora mangle* L.) en el Delta de Niger, Nigeria

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Received: 09/04/2007 First reviewing ending: 10/24/2007 First review received: 11/10/2007
Second reviewing ending: 11/21/2007 Second review received: 12/11/2007 Accepted: 12/13/2007

ABSTRACT

This study was designed using randomized block design to evaluate the acute and chronic effects of crude oil (Bonny Light) on the growth performance of mangrove seedlings in a 16-week laboratory experiment monitoring critical plant growth attributes such as stem height and diameter, leaf length , width and numbers of leaves (leaf production), senescence and seedlings survival. Two treatments were compared with the control (no oil added); they were: 150 mL crude oil applied once and 15 mL crude oil applied weekly. The results showed differences in response of seedling attributes exposed to the different treatments with acute exposure having a declining response pattern of stem height > stem diameter > leaf length = leaf and chronic exposure with leaf length > stem height > leaf width > stem diameter. These results were further corroborated by cluster and correspondence analyses, and demonstrated affinities of the attributes and extent and sensitivity of each attributes. This suggests that the mangrove seedlings respond differently to various crude oil exposures which has implications for restoration activities. The present study demonstrated that mangrove seedlings are negatively impacted by both acute and chronic exposure but more so with seedlings under acute exposure and further provided insight on the potential ecological risk associated with mangrove seedling development exposed to crude oil contamination.

Key words: *Rhizophora mangle*, mangrove seedling, Bonny light crude oil, Niger Delta, Toxicity

RESUMEN

Este estudio se diseñó para evaluar los efectos agudos y crónicos del petróleo (Bonny Light) sobre el comportamiento del desarrollo de plántulas de mangle bajo condiciones de laboratorio, monitoreando las características críticas del crecimiento de las plantas tales como altura y diámetro del tallo, longitud de hojas, ancho y número de hojas (producción foliar), senescencia y sobrevivencia de plántulas durante 16 semanas. Los resultados mostraron diferencias en la respuesta de las características de las plántulas expuestas a los diferentes tratamientos con el efecto agudo teniendo un patrón de respuesta descendente de la altura del tallo - tasa de crecimiento relativo (TCR) = 0,17 > diámetro del tallo - TCR = 0,01 > longitud de hojas – TCR = 0 = ancho de hojas – TCR = 0 y el efecto crónico con longitud de hojas - TCR = 0,20 > altura de tallo – TCR = 0,19 > ancho de hojas – TCR = 0,15 > diámetro del tallo – TCR = -0.03. Estos resultados fueron adicionalmente corroborados mediante análisis de agrupamiento y correspondencia. Los resultados sugieren que las plántulas de mangle respondieron diferentemente a las varias exposiciones de petróleo y suministraron evidencia del comportamiento de las plántulas, la supervivencia y la implicación de las actividades de restauración a varios niveles de exposición de petróleo.

Palabras claves: *Rhizophora mangle*, plántulas de mangle, petróleo crudo, Delta del Niger, Nigeria

INTRODUCTION

Nigeria has the third largest mangrove forest in the world and the largest in Africa (9,730 km^2) occupying and the lower stretches of the southern limit of the Niger Delta and covering between 5,400 km^2 and 6,000 km^2 (NDES, 2000). There are three main mangrove families (Rhizophoraceae, Avicenniaceae and Combretaceae) comprising six species, namely: *Rhizophora racemosa* G. Mey, *Rhizophora mangle* L., *Rhizophora harrisonii* Leechem., *Languncularia racemosa* Gaertn, *Avicennia germinans* L. and *Conocarpus erectus* L., and the exotic family Palmae (Arecaceae) that is rapidly spreading across the Niger Delta (RPI, 1985; NDES, 1996 and 2000; NDDC, 2004). Another important component of the mangrove vegetation is the exotic Nypa palm (*Nypa fruticans* Wurmb) of the family Palmae introduced from Singapore Botanical Gardens to Calabar in 1906 and Oron in 1912 (Keay *el al*., 1964). The red mangrove constitutes over 60% of the mangrove area cover in the region.

The mangrove plants (*Rhizophora mangle* L.) are salt tolerant species that grow on sheltered shores in the tropics and sub-tropical estuaries (IPIECA, 1993), where they provide ecosystem functions and several human utility benefits especially for coastal communities of Niger Delta (Nigeria). Their halophytic nature and ability to compensate for low oxygen in the soil allows them to flourish in the environment (Choudhry, 1997). However, their complex breathing roots make them vulnerable to crude oil that can block the openings of the breathing roots. This has posed serious threats to mangrove plants.

Crude oil plays an important role in the economy of Nigeria and about 70% of oil exploration and exploitation activities take place in the mangrove areas of the Niger Delta. However, mangrove forest clearing and oil spills from operational failures and vandalism of pipelines, oil well blowouts, tanker seepages and accidents and deblasting operations contribute to mangrove species loss and degradation of the ecosystem (Imevbore, 1979 and 1981; Baker, 1981a,b; Ekweozor 1985 and 1989, Snowden and Ekweozor, 1987; Nnyong and Antia, 1987, Amadi *et al*., 1996).

The crude oil spilled into the mangrove environment through tidal influences that characterize the ecosystem provides for wider dispersal and distribution in the intertidal flat areas resulting in the deposition of crude oil on the aerial roots and sediment (Baker, 1981a). Thus, crude oil covers the breathing roots and pores, thereby asphyxiating the sub-surface roots that depend on the pores for oxygen transfer (Odu *et al*., 1985). This in turn impairs the normal salt exclusion process resulting in accumulation of excess salt in the plant contributing to enhancing the stress condition of the plant and ultimately, to death, loss of mangrove plants, habitat destruction and degradation (Imevbore, 1979).

Of the four main ecological areas in the Niger Delta (mangrove, freshwater swamp forest, lowland and barrier island swamp forest) the mangrove is the most affected by oil exploration and exploitation as it has very poor regeneration potential. This scenario generates concern among the different stakeholders on the need of revegetating the degraded mangrove habitat

On account of this, mangrove plants are vulnerable and undergo steady unpalatable declining quality and functions in the integrity of the ecosystem. The continuous oil activity in the region and accidental crude oil spills into the mangrove ecosystem are the genesis of the scientific motivation to examine the acute and chronic effects of Bonny light crude oil on the development of mangrove seedlings of *Rhizophora mangle* using growth attributes (such as stem growth, seedlings survival, leaf production and senescence, as surrogates).

MATERIALS AND METHODS

Description of Study Area

The study was conducted at Eagle Island located at the upper reach of Bonny estuary of the eastern Niger Delta, Nigeria and lies within longitude 4º 35” and 4º 5” N and latitude 7º 00” and 7º 53” E (Figure 1).

Vegetation in the area was characteristically mangrove, with the dominant types being red mangrove (*Rhizophora racemosa*), white mangrove (*Avicennia africana*) and black mangrove (*Laguncularia racemosa*). The area was also inhabited by other plants (e.g fern -*Achrostichum aureum* and grass-*Paspalum varginatum*) and animals (e.g. mud skipper *Periophthalmus* sp., fidder crabs *Uca tangeri* and Periwinkles).

The climate of the area was basically that of equatorial tropical rainfall occurring throughout most of the year except for the months of December, January and February which comprised the dry season. The annual rainfall in the area was about 2,405.2 mm (Gobo, 1988). Annual mean air temperature was 29.7 ºC with the highest monthly mean temperature at 31.3 ºC (in August), and the lowest monthly mean temperature at 27.5 ºC (in January). The surface seawater temperature values ranged between 25.9 ºC and 30.6 ºC, and the salinity of the seawater ranged between 8‰ and 20 ‰. The tidal variations ranged between 0.43 and 1.67 m with a mean tidal variation of 0.9 m. The current flows were unidirectional flooding (inundation) during high tide and receding at low tide regimes. The mud (sediment) had a dark appearance with hydrogen sulphide as the major byproduct of sulphate reducing bacteria. The soil type was mainly clay (Chikoko) with acidic pH of 5.3, with brackish conductivity of 18,000µS/cm and organic content of 26.4%. Cation concentrations of soil had decreasing order of Na (87.9 meq/100g of soil) > Mg (65 meq/100g of soil) > Ca(3.3 meq/100g of soil) > K (2.6 meq/100g of soil) and nutrient concentrations for NH_4-N, NO_2-N , NO_3-Nand PO_4^{3-} being 35.5 mg/g, 28.5 mg/g, 18.1mg/g, 5.4mg/g respectively.

Economic activities by human in this area were mainly, fishing, trading and transportation.

The sampling sites (10 x 15 m^2) included a relatively undisturbed tidal inundated mangrove wetland, beside the Rivers State University of Science and Technology, Port Harcourt. Surface soil (0 - 15 cm) from the study area was collected 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. The matured healthy mangrove seedlings in good conditions that tend to settle down in the substrate were carefully uprooted using hand trowel and transplanted into the potted bags ensuring that

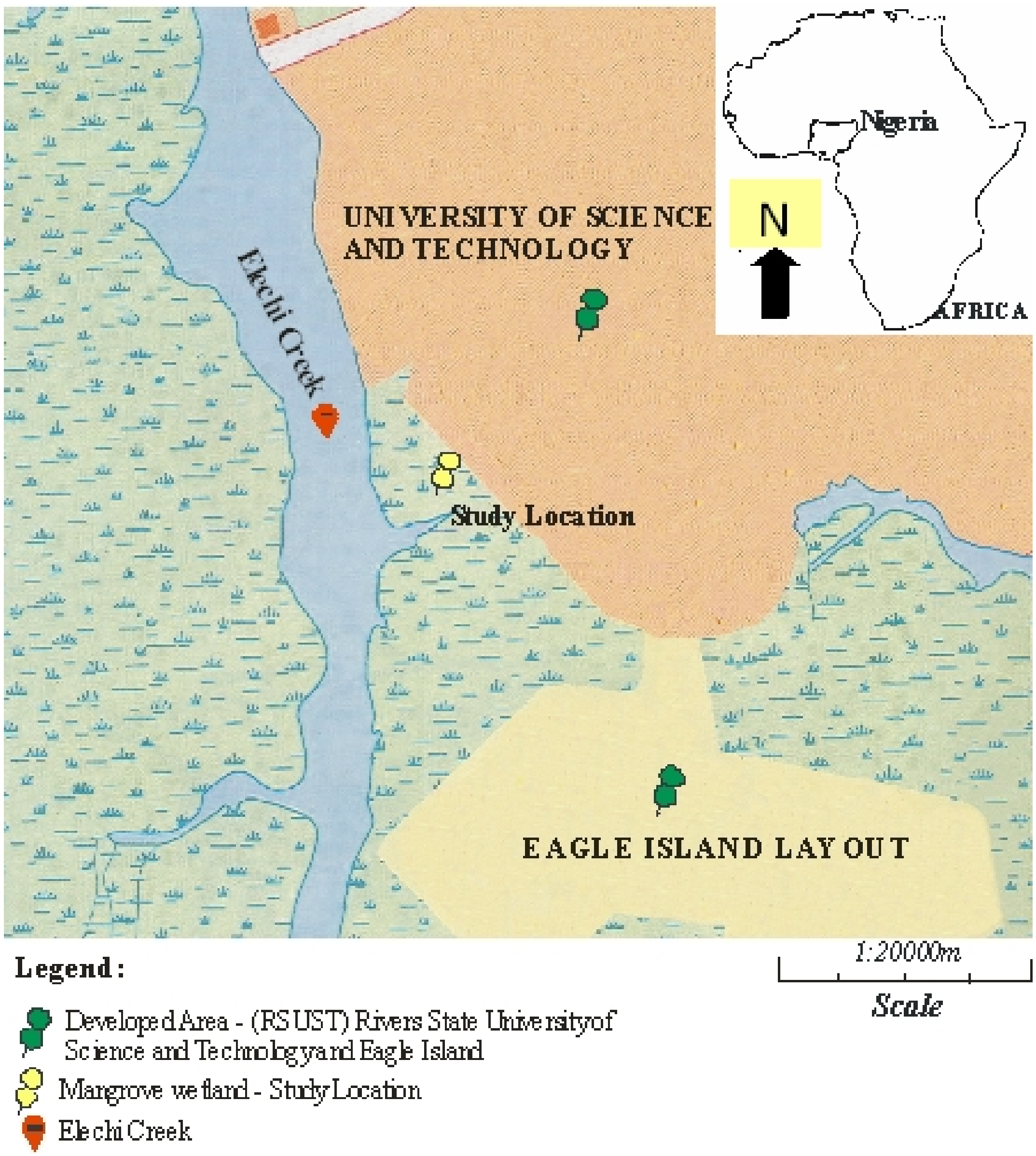

Figure 1: The location of the study site of the nursery preparation in Elechi Creek, Nigeria Africa.

there was non to minimum root damage. These seedlings were allowed to acclimate for 60 days (2 months). The seedlings were arranged in 10 rows of parallel triplicates laid at 1 m intervals for each treatment (chronic, acute and control).

Treatments

Treatment was by applying the Bonny light crude oil (BLC) that commenced at the end of 60-day acclimation period. The crude oil (Bonny Light Crude -BLC*)* constitutes of *n*-alkane-containing oil such as saturates (56%), aromatics (31%), polars (11%), and asphaltenes (2%), it also has 35.3° API gravity and contains 0.1% sulphur content (Norman *et al*., 2004).

The acute treatment, consisted of a one-time application of 120 mL crude oil (Bonny Light crude oil) added on the surface of the mud. The chronic treatment consisted of weekly application of smaller amount (15ml) of the same crude oil (Monaghan and Koons, 1975 and Proffitt *et al*., 1995).

Stem height, stem girth (diameter) at the first inter-node, number of nodes, number of leaves, and leaf area (length, and width), were measured individually using vernier calipers; the fate and growth of seedlings were monitored weekly for 16 weeks. Any yellowing of leaves and seedling survival were recorded. 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 (Clarke and Gorley, 2001, 2006). Group average sorting (= unweighted pair-group method; (Sneath and Sokal, 1973) was used as the clustering method and Bray–Curtis similarity for resemblance measure (Bray and Curtis, 1957). Results were expressed as a dendrogram in which samples were ordered into groups.

RESULTS

Seedling Survival

Acute treated plants demonstrated seedling mortality on 2nd and 3rd week corresponding to 90% and 80% survival, respectively. No further mortality occurred until the 9th week when 30% loss was observed culminating in 70% survival (Table 1, Figure 2a). The relationship within the acute treated seedlings was not significant (r = 0.02), as well as the difference between treated and control seedlings (Wilcoxon sign rank Z = 60.3 > P = $0.21_{(0.05)}$). Seedlings under chronic treatment did not show any mortality such that 100% survival was observed at the end of the experiment (16 weeks). Similarly, 100% survival pattern was observed for the control seedlings (Table 1, Figure 2a).

Stem Growth (Height)

R. mangle seedlings exposed to acute crude oil treatment exhibited a 7.93% increase in height during the first nine weeks of the study; but showed little growth thereafter. Stem growth (height) increased slowly, but steadily in a near-linear fashion (Table 1, Figure 2b), achieving a total increase of 11.76% after 16 weeks.

While seedlings exposed to chronic crude oil treatment demonstrated increases in stem growth (height) from start (71.2 mm) to the end of the experiment (93.7 mm), achieving 24.01% growth increase. Similarly, the control seedlings demonstrated increases in height as was observed for the chronic treated plants with increases recorded from start (52.5 mm) to the termination of the experiment (68.6 mm) thus achieving 23.32% growth (Table 1, Figure 2b). A strong relationship was observed for both treated seedlings (acute $r^2 = 0.97$; chronic $r^2 = 0.99$) and control ($r^2 = 0.98$). Comparison between the treated and control seedlings showed that the differences were not statistically significant for acute (Wilcoxon sign rank Z = 76.5. P = $0.21_{(0.05)}$) and chronic exposures (Wilcoxon Sign-Rank t < $P_{(0.05)}$).

Stem Girth (Diameter)

Stem girth for acute exposed seedlings increased slightly from start (4.2 mm) to the 1st week (4.25 mm). Thereafter, the values remained unchanged to the 4th week (4.25 mm), then increased again slightly in the 5th week (4.35 mm) and maintained the same girth size to the 7th week (4.35 mm) before increasing almost steadily to the end of the experiment (5.1 mm). At the end of the experiment, it achieved 17.64% increase in girth (Table 1, Figure 2c).

For the chronic exposed seedlings there was no observable increase in girth size until the 4th weeks (3.70 mm) and this was maintained till the 7th week (3.70 mm), with slight increase in the 8th week (3.90 mm). Another increase in girth size was observed in the 10th week (3.95 mm), then it remained unchanged till the 13th week (3.95 mm) before increasing almost uniformly to the end of the experiment (4.30 mm), achieving 15.12% increase in girth (Table 1, Figure 2c).

There was no observable change in girth from start to the 3rd week (3.45 mm) for the control, a but slight increase was initially observed from the 4th week (3.60 mm) which continued to the 5th week (3.65 mm). A lag in growth was maintained for a period of one week (6th week) thereafter, growth in girth resumed almost steadily to the 11th week (4.9 mm). Another dormant growth period for two weeks (week 11 to 13) was observed before gradual increase was observed to the end of the experiment (5.25 mm), achieving 34.29% increase in girth (Table 1, Figure 2c).

Statistical assessments between treated seedling and control demonstrated great similarity (acute r^2 = 0.94; chronic r^2 = 0.95, Table 1, Figure 2) and differences between treated and control seedlings were not statistically significant (Acute - Wilcoxon Sign-Rank z = < $P_{(0.05)}$; chronic Wilcoxon Sign-Rank z = 42.0 > P = $0.043_{\mathbf{(0.05)}}$).

Leaves

Leaf Production (Number of Leaves)

Leaf production for the acute crude oil treatment on *R. mangle* demonstrated an unsteady pattern, but an increase in the number of leaves was observed starting from week 1 (40) to the end (week 16) of the experiment (48). Thirty-eight percent of *Rhizophora* seedlings produced new leaves while 62% did not record leaf production. Leaf development (sprouting) started in the 3rd week; the maximum production was not until the 3rd and 8th week (Table 1, Figure 2d).

Table 1. Linear regression equations for relationships for each treatment on mangrove (*Rhizophora mangle* L.) growth characteristics of seedlings exposed to different crude oil (Bonny Light) treatments (acute, chronic and control) in the Niger Delta, Nigeria.

Plant Attributes		Relationship	R^2
Leaf production	Control	y = 0.1105x + 6.08	R^2 = 0.58
	Acute	y = 0.2819x + 4.82	R^2 = 0.89
	Chronic	y = 0.1105x + 6.08	R^2 = 0.58
Seedling survival	Control	y = 10	R^2 =0.00
	Acute	y = -0.174x + 9.45	R^2 = 0.78
	Chronic	y = 10	R^2 = 0.00
Stem height	Control	y = 0.9429x + 51.26	R^2 = 0.98
	Acute	y = 0.8814x + 116.66	R^2 = 0.97
	Chronic	y = 1.3306x + 69.21	R^2 = 0.99
Stem diameter (girth)	Control	y = 2.2377x - 9.43	R^2 = 0.75
	Acute	y = 2.174x + 1.08	R^2 = 0.95
	Chronic	y = 2.4191x - 5.13	R^2 = 0.95
Leaf length	Control	y = 1.6441x + 45.06	R^2 = 0.97
	Acute	y = -0.2292x + 54.10	R^2 = 0.06
	Chronic	y = 1.6349x + 42.58	R^2 = 0.97
Leaf width	Control	y = 0.7968x + 16.14	R^2 = 0.97
	Acute	y = -0.0572x + 20.07	R^2 = 0.04
	Chronic	y = 0.6734x + 13.91	R^2 = 0.98
Senescence	Control	y = 2.4191x - 5.125	R^2 = 0.95
	Acute	y = 2.174x + 1.08	R^2 = 0.95
	Chronic	y = 2.4191x - 5.125	R^2 = 0.95

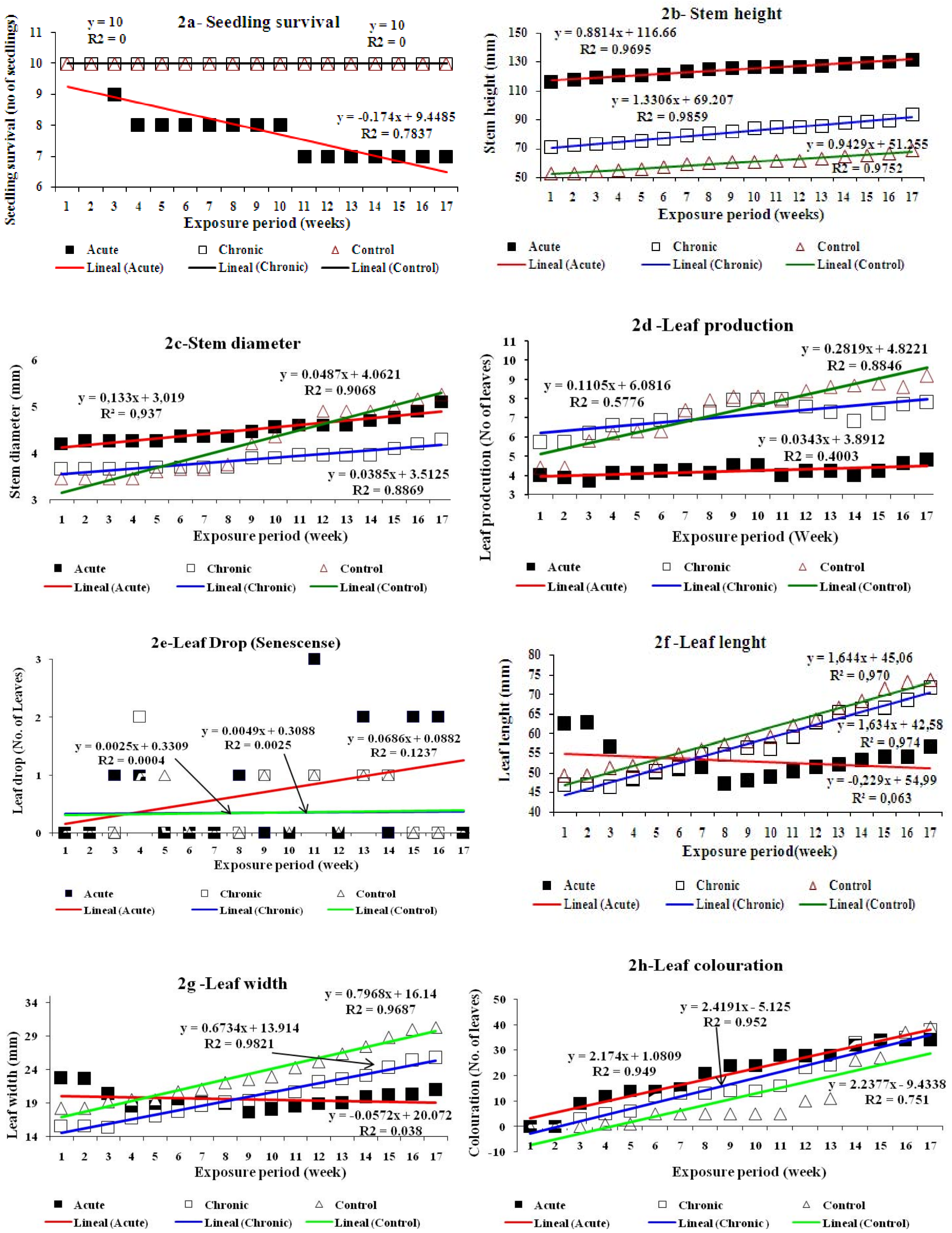

Figure 2a-h. Response of several characters of mangrove (*Rhizophora mangle* L.) seedlings to different crude oil (Bonny Light) treatments (acute, chronic and control) in the Niger Delta, Nigeria.

Leaf production for chronic crude oil treatment on *Rhizophora mangle* showed an unsteady pattern, with steady increase in the number of leaves from week one (57) to the 10^{th} week (80), thereafter a decline to 78 at the end of the experiment with thirty two percentages of treated seedlings producing new leaves and sixty eight (68%) percentages did not record leaf production. Peak production was in the 8^{th} week (Table 1, Figure 2d).

However, the control had consistent leaf production increasing from week one (44) to the end of the experiment (92), more leaves was produced by the control seedlings. Twenty eight percentages (28%) of *Rhizophora* seedlings produced new leaves while 72% did not record leaf production. Leaf development (sprouting) started from the second week and peak production was observed on the 3^{rd} week (Table 1, Figure 2d).

Leaf drop (Senescence)

Leaf drop for acute treated mangrove plant (seedlings) was between (9 and 34 leaves) with senescence commencing at the early stages of the experiment (week 2). The number of shading increased almost exponentially to the end of the experiment (16^{th} week) and maximum shading of leaves was observed on the 14^{th} week (Table 1, Figure 2e).

Leaf drop for chronic treated seedlings lies between (3 and 38), while the control values ranged from (1- 39). For the treatment, seedlings started shading leaves from week three. The number shaded increased at intervals of six almost the same number of leaves was observed to be shaded between weeks 3 to 4 (5), weeks 5 to 7 (13), weeks 8 to 9 (14), weeks 11 to 12 (24), weeks 13 to 14 (33 and 34) and finally weeks 15 to 16 (35 and 38). Maximum shading of leaf was observed at the end of the experiment (Table 1, Figure 2e).

The control demonstrated the same pattern. Shading increased at interval of five almost the same number of leaves was observed to be shaded at each of the intervals between weeks 3 to 4 (1), weeks 5 to 10 (5), week 11 to 12 (10 and 11), weeks 13 to 14 (26 and 27), and finally weeks 15 to 16 (37 and 39). Maximum shading was recorded on the 16^{th} (Table 1, Figure 2e). The correlation coefficient were moderately high ($r = 0.93$) and not significant (Wilcoxon Sign-Rank, $z = 57 > P = 0.01_{(0.01)}$).

Leaf Length

Chronic exposure of the mangrove seedlings to the crude oil had no significant effect on leaf length (Table 1, Figure 2f). Indeed, seedlings receiving a chronic exposure to the oil exhibited a growth rate (1.64 mm wk^{-1}) that was equal to that of the control plants. Conversely, acute exposure of the seedlings to crude oil produced significantly shorter leaves during the initial stages (i.e., the first seven weeks) of the test exposure. Starting at about week eight, however, the plants began to show signs of recovery, with the leaves increasing in length at a rate of about 0.90 mm/week.

Changes in leave length in treatment plants fluctuated widely during the study period. There were noticeable changes in the leave length from start (week 0) to first week (46.97 mm), before a slight decline in the second week (46.54 mm), and subsequent increase to the 8^{th} week (56.50 mm). Thereafter, a sudden decline in length was observed in the 9^{th} week (56.09 mm), before increasing again gradually to the end of the experiment (71.89 mm) achieving 34.66% increase in leaf length (Table 1, Figure 2f).

However, the control plants demonstrated a rather steady growth pattern with increases from start week 0 (49.37 mm) to the end of the experiment (73.85 mm), achieving 33.15% increase in leaf length (Table 1, Figure 2f). The affinity between treated and control were moderately close to unity for acute treated seedling ($r = 0.94$) than chronic treated seedlings ($r = 0.77$) and comparatively both treatments (acute Wilcoxon Sign-Rank, $t = 54.5 < P = 0.016_{\mathbf{(0.05)}}$ and chronic Wilcoxon Sign-Rank, $z = 57 > P = 0.01_{(0.05)}$) were not statistically significant with the control.

Leaf Width

Similar *R. mangle* seedlings exposed to acute treatment decline in leaf width as reported for leaf length from start week (22.69 mm) to the third (18.25 mm). Thereafter, values increased slowly to the sixth week (19.15 mm) before another decline were observed to the eight week (17.62 mm). Subsequently from the ninth week a steady increase was observed to the end of the study (20.96 mm). However, the overall decline was 7.6% of the initial value (Table 1, Figure 2g).

Changes in leave width in the chronic treated plant started from the 1st week (15.62 mm), and decline suddenly in the second week (15.44 mm), thereafter increased again almost exponentially to the 16th week end of the study (25.77 mm), achieving 39.39% increase in leave width (Table 1, Figure 2g). The control demonstrated observable changes in leave width from the first week (18.20 mm) which continued gradual through the third week (19.23 mm) to the end of the study (30.30 mm), achieving 39.93% in leaf width (Table 1, Figure 2g). The strong correlation coefficient were observed for acute and chronic treatments (r = 0.99) and differences between treated seedlings and control were not significant (acute - Wilcoxon Sign-Rank t = 59.5 > P = $0.003_{(0.05)}$) and chronic Wilcoxon Sign-Rank z = 76.5 > P = $0.01_{(0.05)}$).

Leaf Colouration

R. mangle seedlings exposed to acute treatment demonstrated 10% yellowish colouration (chlorosis) which was commenced from the second week. Also, seedlings exposed to chronic treatment had 19% yellowish colouration (chlorosis), while the control had 5% yellowing of leaves (Chlorosis). The yellow colouration for chronic and control commenced from the third week to the end of the 16th week (Figure 2h)

Relative growth rate (RGR)

The relative growth rate for the seedling treatments indicated a better growth performance by the chronic than the acute treatment with respect to the control (Table 2). The RGR response value for acute treatment follow a pattern of stem height (0.17) > stem diameter (0.01) > leaf length (0) = leaf width (0) ,while chronic and control had similar RGR pattern of leaf length (RGR = 0.20) > stem height (RGR = 0.19) > leaf width (RGR = 0.15) > stem diameter (RGR = -0.03) and leaf length (RGR = 0.20) > stem height (RGR = 0.17) > leaf width (RGR = 0.16) > stem diameter (0.04) respectively (Table 2).

Table 2. Relative growth rate of mangrove (*Rhizophora mangle* L.) seedlings exposed to different crude oil (Bonny Light) treatments (acute, chronic and control) in the Niger Delta, Nigeria.

	Treatment		
Parameter	Acute	Chronic	Control
Stem height	0.17	0.20	0.17
Stem diameter	0.01	-0.03	0.04
Leaf length	0.00	0.20	0.20
Leaf width	0.00	0.15	0.16

Similarity analysis carried out with the use of the average method and Euclidean distance measure for acute and chronic treatment examined responses of the plant attributes on the different exposure. There was a relative divergent response of the attributes on the mangrove seedlings which yielded four major results, denoted as A, B C and D. For the acute treatment, the highest response was between stem girth and leaf length (A-1, 81.1%) followed by stem height (A-2, 59.6%), yellowing of leaf (B, 40.5%), leaf width (C 21.6%) and seedling survival (D, 0%) in that decreasing response (Figure 3). While the chronic treatment indicated leaf length and width (B, 67.6%) followed by stem height and stem girth (A, 63.5%), yellowing of leaf (C, 27.0%) and seedling survival (0%) in that decreasing order of response (Figure 4).

The correspondence analysis corroborated the findings observed with the cluster analysis and reveal high homogeneity between stem girths and leaf length that had high response score, with stem height and yellowing of leaf having moderate response score while seedling survival had very low response score for acute treatment (Table 3 and Figure 5). Correspondingly, leaf length, leaf width and stem height and girth for chronic treatment with relatively high response score, while yellowing of leaf and seedling survival had low response score (Table 3 and Figure 6).

DISCUSSION

For the past three decades, the Niger Delta mangrove wetland had consistently has been subjected to ecological abuse owing primarily to crude oil exploration and exploitation activity. Indeed uncontrolled exploitation of natural resources in the eco-region has resulted in declining habitat quality and biodiversity loss. The mangrove ecosystem is ecologically very sensitive to human perturbation and natural reestablishment processes have been exceedingly slow. This is reflected both in the poor rejuventation potential of the natural vegetation and the effects of contamination from crude oil spill. The rehabilitation of crude oil impacted habitats will require replanting strategies and a considerable understanding of the factor associated with the growth processes, in addition to seedling survival under the prevailing degraded environmental conditions in the region.

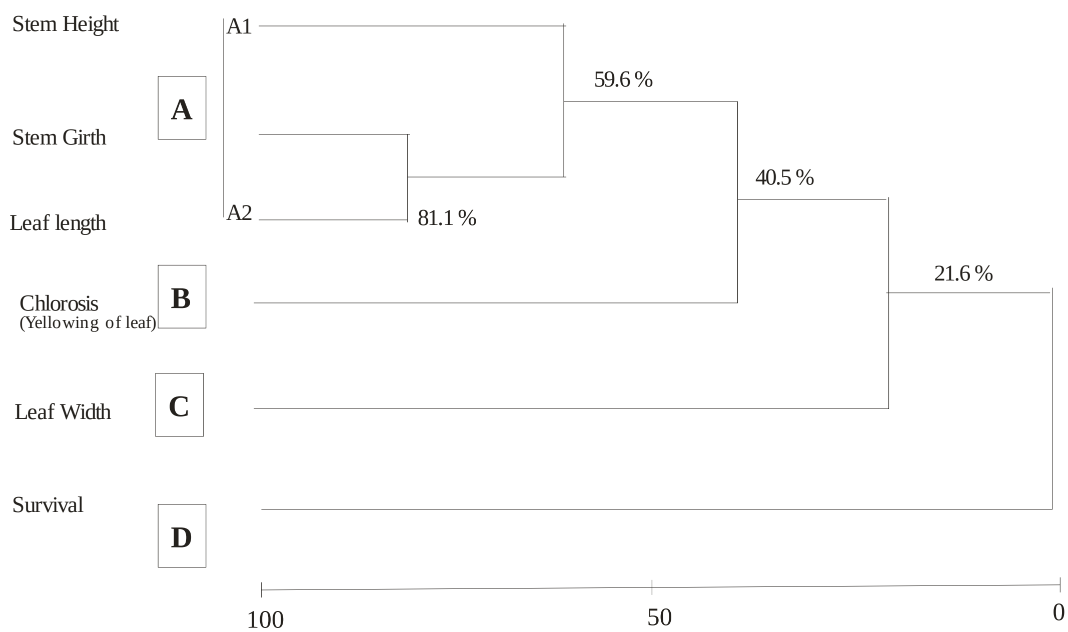

Figure 3. Cluster analysis of mangrove (*Rhizophora mangle* L.) seedlings exposed to acute crude oil (Bonny Light) treatment in the Niger Delta, Nigeria.

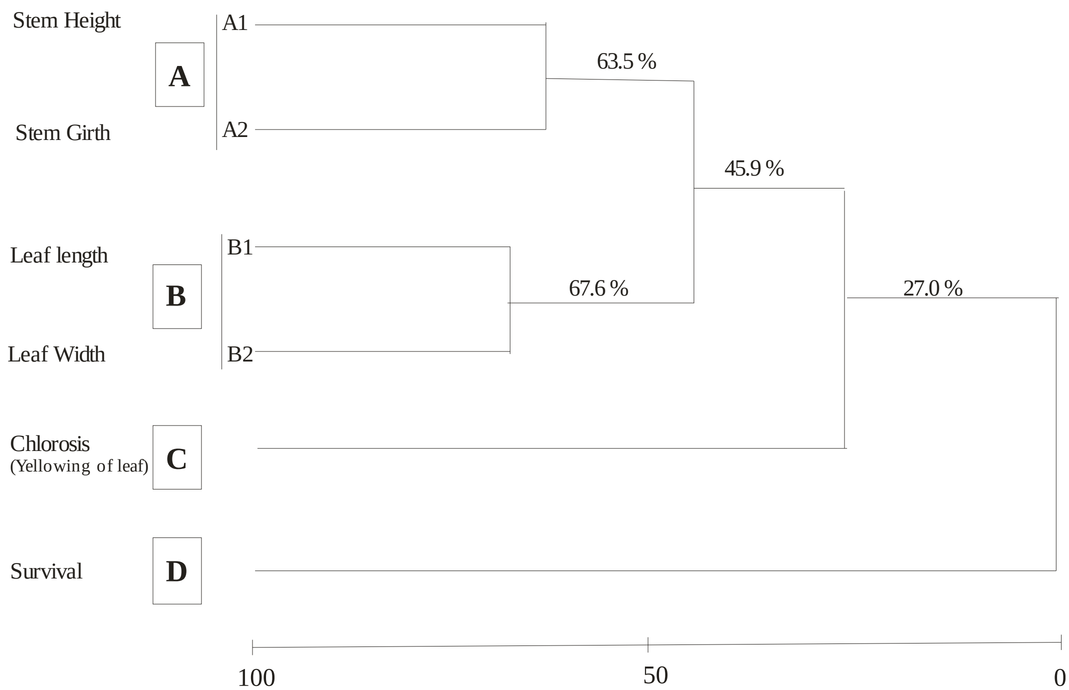

Figure 4. Cluster analysis of mangrove (*Rhizophora mangle* L.) seedlings exposed to chronic crude oil (Bonny Light) treatment in the Niger Delta, Nigeria.

Table 3. Total structure coefficients of mangrove (*Rhizophora mangle* L.) seedlings exposed to acute and chronic crude oil (Bonny Light) treatments in the Niger Delta, Nigeria

	Acute	
Variable	C-1	C-2
Stem growth (height)	-0.50	-0.91
Girth (diameter)	-1.35	0.98
Leaf length	-0.58	1..01
Leaf width	-0.25	0.94
Colouration	-0.42	-0.18
Survival	-1.18	-1.99
	Chronic	
Variable	C-1	C-2
Stem growth (height)	2.22	-1.98
Girth (diameter)	-0.01	0.02
Leaf length	2.48	-1.91
Leaf width	2.03	-0.67
Colouration	1.21	1.25
Survival	1.48	1.92

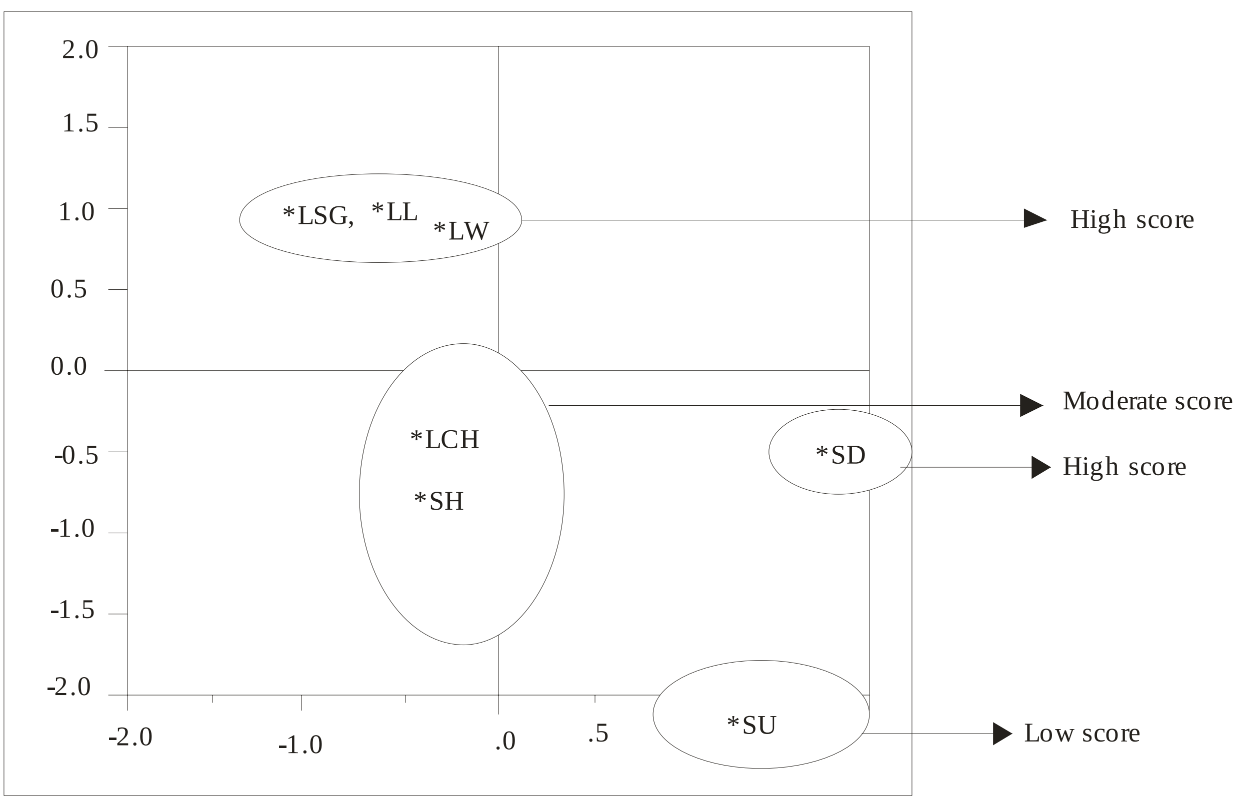

Figure 5. Correspondence analysis of mangrove (*Rhizophora mangle* L.) seedlings exposed to acute crude oil (Bonny Light) treatment in the Niger Delta, Nigeria.
Legend: LSG = Stem girth; LL = Leaf length; LCH = Chlorosis (Yellowing of leaf); SD = Stem diameter; LSU = Leaf survival; LW = Leaf width and SH = Stem height.

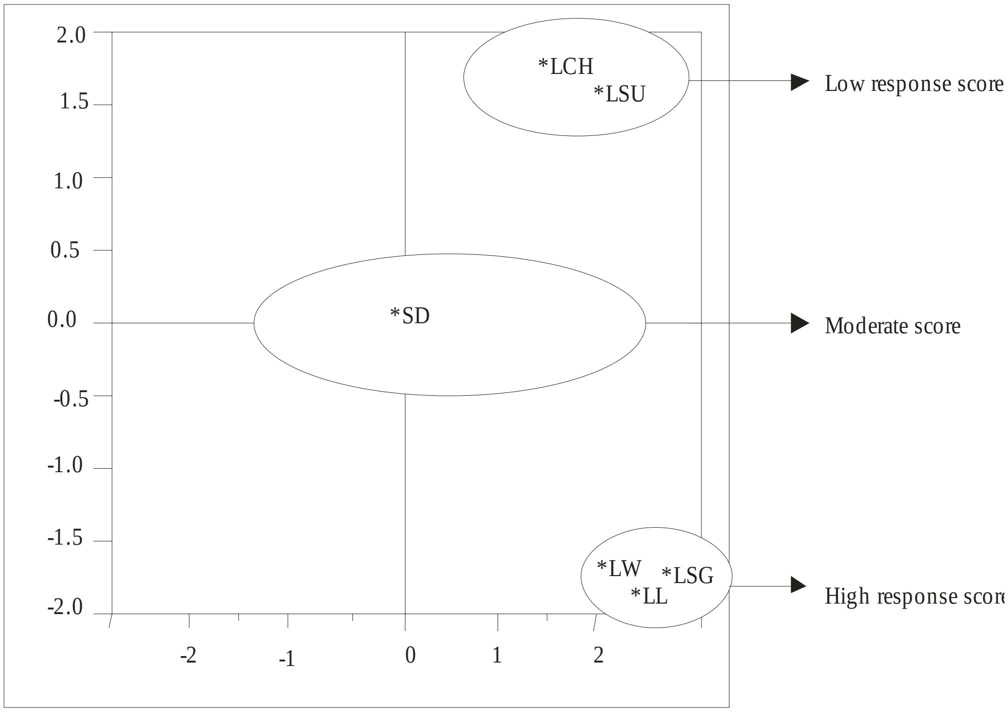

Figure 6. Correspondence analysis of mangrove (*Rhizophora mangle* L.) seedlings exposed to chronic crude oil (Bonny Light) treatment in the Niger Delta, Nigeria.
Legend: LSG = Stem girth; LL = Leaf length; LCH = Chlorosis (Yellowing of leaf);
SD = Stem diameter; LSU = Leaf survival; LW = Leaf width and SH = Stem height.

Our study on the effects of crude oil exposed to different treatments (acute and chronic) indicated considerable variation in seedling reaction ranging from growth responses such as stem height, stem girth, leaf length, leaf width, yellowing of leaves (colouration), leaf loss (senescence), leaf production and seedling survival.

Our findings from these experiments on mangrove seedlings exposed to different crude oil treatments demonstrated hampered growth with respect to the stem growth -height and girth, leave development including leaf length, width and yellowing of leaves against the control (that demonstrated greater development for stem growth - height and girth, leaves and survival of seedlings) in spite of the non statistical significant difference observed between various treatments and control. This situation is attributed to the stringent 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 effect on seedlings than the chronic exposure. Similar observation was made on mangrove seedlings by Proffitt *et.al*. (1995), at different exposure levels (acute and chronic) and demonstrated linear growth but was less than that of the control.

The observed differences in response of seedling attributes exposed to the different treatments with acute having a declining response pattern of stem height - RGR = 0.17 > stem diameter - RGR = 0.01 > leaf length - RGR = 0 = leaf width - RGR = 0 and chronic with leaf length - RGR = 0.20 > stem height - RGR = 0.19 > leaf width - RGR = 0.15 > stem diameter - RGR = -0.03 were further corroborated by cluster and correspondence analysis. These suggest that mangrove seedling respond differently to crude oil exposure. Similar studies have indicated such adverse consequences of the negative crude oil effect on mangrove seedling (Proffitt *et al*. 1995, DeLaune *et al.* 1979, DeLaune *et al*. 1990, Duarte *et al.*, 1998). This response trend provide veritable and important tool for considering effect of crude oil on mangroves.

The observed difference between the treated seedling (acute) and control indicates evidence of negative role of crude oil on mangrove seedling development. This observed retardation in seedling development particularly on stem height, stem girth, and yellowing of leaf (chlorosis) with over 50% reduction in growth against the control is relatively in support of similar studies on the deleterious effect of crude oil on plant development (Baker, 1981a,b; Duarte et al 1998). The decline in leaf width is evidence adduced to the effect of acute treatment on the seedling. Generally mangrove seedlings exposed to chronic faired better than the acute against the control treatment.

The crude oil level may have also altered the sediment quality (attributes) firstly; crude oil in the soil may reduce sediment porosity and gaseous exchange that in turn may have a negative effect on the physiological function of the plant (Amadi *et al.* 1997, IPS 1989). Also other possible effect may be hinged on one of the characteristic of soils polluted by crude oil (petroleum hydrocarbons) contributing to their low mineral-nitrogen content. This is based on the fact that in the immobilisation of mineral-nitrogen by soil micro-organisms during the process of degrading the polluting crude oil (petroleum hydrocarbons). Reduction in mineral-nitrogen contents after oil pollution as a result of microbial immobilisation has been reported (Odu, 1972). Oil pollution adversely affects the availability of mineral nitrogen by encouraging the rapid growth of soil micro-organisms which immobilise soil mineral nitrogen and this may be responsible for the yellowing of leaves observed.

Secondly, petroleum hydrocarbons induce stress in salt-extracting plants such as the red Mangroves, by disrupting the ability of the roots to exclude ions from sea or brackish waters (Page *et al*, 1985). Oil stress in salt-excluding halophytes, such as Mangroves, results from interference by hydrocarbons in this process (Scholander, 1968). Chloride ion exclusion in the roots of Mangrove seedlings is disrupted by exposure to diesel fuel, and toluene (Teas, 1979).

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 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.

Non the less, other studies on crop plants have indicated similar negative growth pattern on plant survival and biomass production. Merkl *et al*., (2005) observed death of leguminous plants and reduced biomass production of grasses exposed to oil contaminated soil. Adoki and Orugbani (2007) observed that non-nutrient supplemented oil polluted soil recorded low percentage germination; contrary to, contaminated soil treated with fertilizer supplement that demonstrated enhanced percent germination. Similarly, reduction in crop yield, declined land productivity and depressed farm income in oil spill farmland in Delta State of Nigeria had been observed (Inoni *et al*.; 2006).

These scenarios suggest that crude oil have negative consequences both on mangrove plants and agricultural crops.

ACKNOWLEDGEMENTS

We are indebted to Ifiesimama Oluka, Hanson Uyi, and Nathan Nario for their kind assistance and advice while we carried out these experiments at the Institute of Pollution Studies Laboratory, Rivers State University of Science and Technology, Port Harcourt. We are indeed grateful to Udonna Ikoro, the chief laboratory technologist of the Institute of Pollution Studies for invaluable advice and the use of equipment and facilities for analysis of samples. More thanks are also due to the eight unanimous reviewers for the helpful comments and suggestions on the manuscript.

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