Global Journal of Environmental Research 10 (1): 14-21, 2016
ISSN 1990-925X

DOI: 10.5829/idosi.gjer.2016.10.01.10342

Reciprocal Transplant of Mangrove (*Rhizophora racemosa*) and Nypa Palm (*Nypa fruticans*) Seedlings in Soils with Different Levels of Pollution in the Niger River Delta, Nigeria

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Abstract: Years of oil and gas exploration and spread of exotic nypa palm has converted the mangroves into a disturbed system. It is hypothesized that polluted soils will have adverse effect on the growth of mangrove and Nypa palm seedlings. This hypothesis was tested using a reciprocal transplant experiment on soils with different levels of pollution. Soil and seed samples were collected in-situ, cross-planted and monitored for sixteen months. Stem diameter, height of seedling, number of leaves and number of leaf scars was measured and survivorship curves plotted. We found that for mangroves the source of the soil had a significant effect on height, but no effect on diameter and number of leaves. Furthermore, the source of the seed had effect on both the height and the number of leaves. Both the soil- and the seed- source had no effect on leave scars, even though more scars were found on mangrove grown in highly polluted soil than on mangroves grown in lowly polluted soil. We found that for nypa palm both soil- and seed- source had effect on height and number of leaves, but had no effect on diameter. Mangrove propagules grown in lowly polluted soil had higher survival than those grown in highly polluted soil. Nypa palm seedlings had higher survival rate than mangrove propagule. Thus, pollution had little effect on seedling growth within species, but had effect across species.

Key words: Hydrocarbon Pollution · Invasive Species · *Rhizophora* · Mangrove · Nypa Palm · Transplant

INTRODUCTION

Mangroves are habitat specialists that grow along the coast mostly in the tropics [1] and sometimes in the temperate regions. They provide ecosystem services that have great economic value to humans and the environment [2-4]. Globally, mangroves are threatened by tsunami [5], aquaculture [6], deforestation [7] hydrocarbon pollution [8, 9] and invasive species [10]. In Africa, mangroves do not only serve as sanctuaries for plants and animals but also serve as homes to natives due to over population and poverty [11].

Oil and gas exploration cause the greatest damage to mangrove in the Niger Delta [12]. Exploratory activities such as the use of dynamite to explore for crude oil, clearing of forest, laying of pipelines, construction of oil-rigs and seismic camps affects mangroves. Hydrocarbon pollution contaminates mangroves [8] and other members of the mangrove community [13] leading to early deaths [14, 15]. The nypa palms like the mangroves are also affected by pollution, but their resistance to pollution varies with that of the mangroves, a fact that is yet to be investigated in our study system. Hydrocarbon pollution cause significant negative physiological effect on mangroves [16, 17] leading to long-term changes [18, 19]. Several studies have used growth parameters [20, 21] post oil spill impact [22] and life history stages [21] to characterize the impact of crude oil on mangrove health.

Nypa palms were introduced into Nigeria to fight coastal erosion [23] but have rapidly expanded their range. They are well adapted and in most cases out-compete the mangroves [24]. However, whether the invasive ability of the palms translates into a higher survival rate in a polluted environment remains.

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Specifically our objectives were: (i) to compare growth of mangrove and nypa palms in soils with varying levels of pollution; (ii) to compare the growth of mangrove and nypa palms collected at varying levels of pollution; (iii) to compare the growth of nypa palms in different forests types; and (iv) to compare the survivorship of mangrove and nypa palm seedling in soils with varying levels of pollution.

MATERIALS AND METHODS

Study Area: We conducted fieldwork in mangrove forests of the town of Okrika located around a major oil refinery in the Niger Delta, Nigeria. The coordinates of the plots include: (Plot 1: N 04°43. 509′; E007° 05. 259′; Plot 2: N 04°43. 484′; E007° 05. 325′). The area has tropical monsoon climate with rainfall occurring all through the year except in December through February. The mean annual precipitation ranges from 2500-4500mm [25] and the mean annual temperature ranges from 26-30°C. The soil is swampy and grades from red to brown while the soil pH ranges from 5.8-7.0. A detailed description of the hydrology is given by NEDECO [26].

Study Species: Rhizophora racemosa Meyer (Rhizophoraceae) is the most abundant of the mangrove species in the Niger Delta [27] and in estuarine wetlands [28, 29]. It has the largest propagule size with an average length of 22.3 cm and a fresh mass of 0.0175 kg [30].

Nypa palm (*Nypafruticans*, Wurmb) is of the group Arecaceae or palmae [31]. They are mainly tropical and occur in all habitats such as the mangrove swamp. Nypa is of the nypoid line and a single monotypic genus that inhabits salt water [32, 33]. It is the only palm that is sometimes considered as mangrove [24] but not regarded as true mangroves [34]. They have frond-like leaves (~7 m) with inflorescent flower arrangement. The fruit is enclosed in a gold colored leaf-like bract, 20-30 cm in diameter. The individual one-seeded woody fruit breaks off when ripe and germinates in the swamp.

Data Collection: Mangrove propagules were collected in-situ from low, medium and highly polluted plots and cross-planted in soils from all three plots to give a total of 90 replications. Similarly, 90 nypa palm seedlings were collected from mangrove forest (PM), mixed forest (PMF) and open forest (PO) and then transplanted in soils derived from low, medium and highly polluted plots.

Table 1: Mean ± 1 SE soil properties of highly and lowly polluted mangrove (*R. racemosa*) in Niger River Delta, Nigeria

	Pollution level			
Soil properties	High	Low	*F*	*P*
THC ($mgkg^{-1}$)	2362.1± 388.6	344.6 ±1.9	58.77	0.01
N (%)	0.28 ±0.002	0.23 ±0.02	0.11	0.76
CO_4^+ ($mgkg^{-1}$)	660.5 ±19.5	63 ±40	78.11	0.001
NO_4^+($mgkg^{-1}$)	4.35 ±0.15	0.8 ±0.4	19.5	0.01
NH_4^+($mgkg^{-1}$)	5.6 ±0.2	1.00 ±0.5	23.47	0.02
Fe^{2+} ($mgkg^{-1}$)	1504.1 ±647.5	283.1±116.3	24.07	0.02

The mangrove and open forest are in the highly and medium polluted plots, while the mixed forest is in the lowly polluted plot. Open areas don’t have canopy covers, the mixed forests have mixture of mangrove and nypa palm trees, while mangrove forest is exclusively mangroves.

The pollution gradients were determined by characterizing five soil samples from each forests stands for physicochemical analysis of: THC, TOC, N, CO_4^+, NO_4^+, NH_4^+ and Fe^{2+} (Table 1). We evaluated the differences in pollution gradients (i.e. soil physicochemical qualities) between highly and lowly polluted plots using one-way ANOVA.

The reciprocal transplant experiment was performed in a common garden. Soil from low, medium and highly polluted plots were put in small polyethylene bags (0.3m × 1.5m) and tagged with place, date and time of collection. The seedlings were then reciprocally planted. In order to create a semi-natural condition the seedlings were exposed to sunlight and rainfall and then watered daily with sea water collected in-situ [35, 36]. This is because similarity of growth to a semi-natural condition facilitates growth [34] and increases the statistical power of the experiment [20]. The growth measurements (i.e., height of seedlings, number of leaves, stem diameter and number of leaf scars) for mangrove and nypa palm seedlingswere recorded at five, six, seven, ten and sixteen months after planting.

Statistical Analysis: The growth parameters of mangrove and nypa palm seedlings were used as the dependent variables for the statistical analyses, while the soil and seed sources were used as the independent variables [37]. We evaluated differences in growth using two-way ANOVAs. The diameter and height was log transformed while the number of leaves and leave scars were square root transformed. For zero variables we added one i.e. Log (Y+1). We also evaluated seedling deaths using life tables to derive survivorship curves. Survivorship was calculated as the number of group surviving multiplied by 1000 divided by the original number (n).

RESULTS

Overal Growth Indices: The study plots were characterized into different levels of pollution based on physico-chemical content of the soil (Tables 1). Highly polluted area has a significantly higher amount of THC (2362.1± 388.6) than lowly polluted area (344.6 ±1.9; $F = 58.77$, $P < 0.05$). THC is 7 times higher in highly polluted plot compared to the lowly polluted plot. In most cases physico-chemical properties of the highly polluted plot were higher than those in the lowly polluted plot. This is also indicated in other related studies conducted in the same area [38]. High physicochemical properties of mangrove soils have also been found in other areas of the

Table 2: Mean sizes of propagules ± 1 SE of mangrove (*R.racemosa*) grown at different levels of pollution in Okrika, Niger River Delta, Nigeria. Different letters indicate significant differences ($P < 0.001$). Values are presented in centimeters

Months	Character	Low	Medium	High
Five	Propagule height	13.7 ±5.9 ^{a}	8.2 ± 2.3^b	12.4 ± 3.3^c
	Propagule diameter	1.4 ± 0.3^a	1.4 ± 0.3 ^{a}	1.5 ± 0.4 ^{a}
	No. of leaves	5 ^{a}	4^b	6^c
	No. of scar	1 ^{a}	1^b	3^c
Six	Propagule height	7.1 ± 5.9 ^{a}	8.3 ± 2.3^b	12.8 ± 3.0^c
	Propagule diameter	1.4 ± 0.3 ^{a}	1.5 ± 0.3 ^{a}	1.6 ± 0.5 ^{a}
	No. of leaves	7 ^{a}	6^b	9^c
	No. of scar	0	0	0
Seven	Propagule height	14.5 ± 6.4 ^{a}	8.7 ± 2.3^b	12.6 ± 3.1^c
	Propagule diameter	1.3 ± 0.3 ^{a}	1.5 ± 0.3 ^{a}	1.4 ± 0.4 ^{a}
	No. of leaves	6 ^{a}	5 ^{a}	5 ^{a}
	No. of scar	1 ^{a}	1^b	3^c
Ten	Propagule height	15.8 ± 4.5 ^{a}	8.7 ± 3.5^b	9.6 ± 6.9^c
	Propagule diameter	1.1 ± 0.7 ^{a}	1.1 ± 1.7 ^{a}	1.2 ± 0.8 ^{a}
	No. of leaves	4 ^{a}	4 ^{a}	5 ^{a}
	No. of scar	2 ^{a}	1^b	3^c
Sixteen	Propagule height	-	-	1.4 ± 4.5
	Propagule diameter	-	-	0.2 ± 0.5
	No. of leaves	-	-	1
	No. of scar	-	-	1

Table 3: Mean sizes of seedlings ± 1 SE of nypa palm (*N. fruticans*) at different time intervals in open, mangrove and mixed forest in Okrika, Niger River Delta, Nigeria. Values are presented in centimeters

Months	Character	Open	Mangrove	Mixed
Five	Propagule height	15.0±5.9 ^{a}	14.9± 4.3^b	17.2± 2.3^c
	Propagule diameter	2.4 ± 0.7 ^{a}	2.3± 0.6 ^{a}	2.5 ± 0.5 ^{a}
	No. of leaves	5 ^{a}	5 ^{a}	5 ^{a}
	No. of scar	-	-	-
Six	Propagule height	15.3± 4.3 ^{a}	13.0± 2.5^b	17.6± 2.6^c
	Propagule diameter	2.3± 0.4	2.1± 0.3	2.4± 0.3
	No. of leaves	4 ^{a}	4 ^{a}	5 ^{a}
	No. of scar	-	-	-
Seven	Propagule height	18.7±4.6 ^{a}	15.7± 2.9^b	19.8± 4.7^c
	Propagule diameter	2.7± 0.5	2.7± 0.4	2.5± 0.7
	No. of leaves	6 ^{a}	5 ^{a}	6 ^{a}
	No. of scar	-	-	-
Ten	Propagule height	23.4± 4.1	21.7± 8.3	22.6± 5.0
	Propagule diameter	2.7± 0.9	2.7± 1.0	2.5± 0.4
	No. of leaves	6 ^{a}	6 ^{a}	7 ^{a}
	No. of scar	-	-	-
Sixteen	Propagule height	10.2 ± 12.5 ^{a}	8.1 ± 11.6^b	11.2 ± 13.3^c
	Propagule diameter	1.5 ± 1.6	1.1 ± 1.6	1.2 ± 1.4
	No. of leaves	4 ^{a}	3 ^{a}	4 ^{a}
	No. of scar	-	-	-

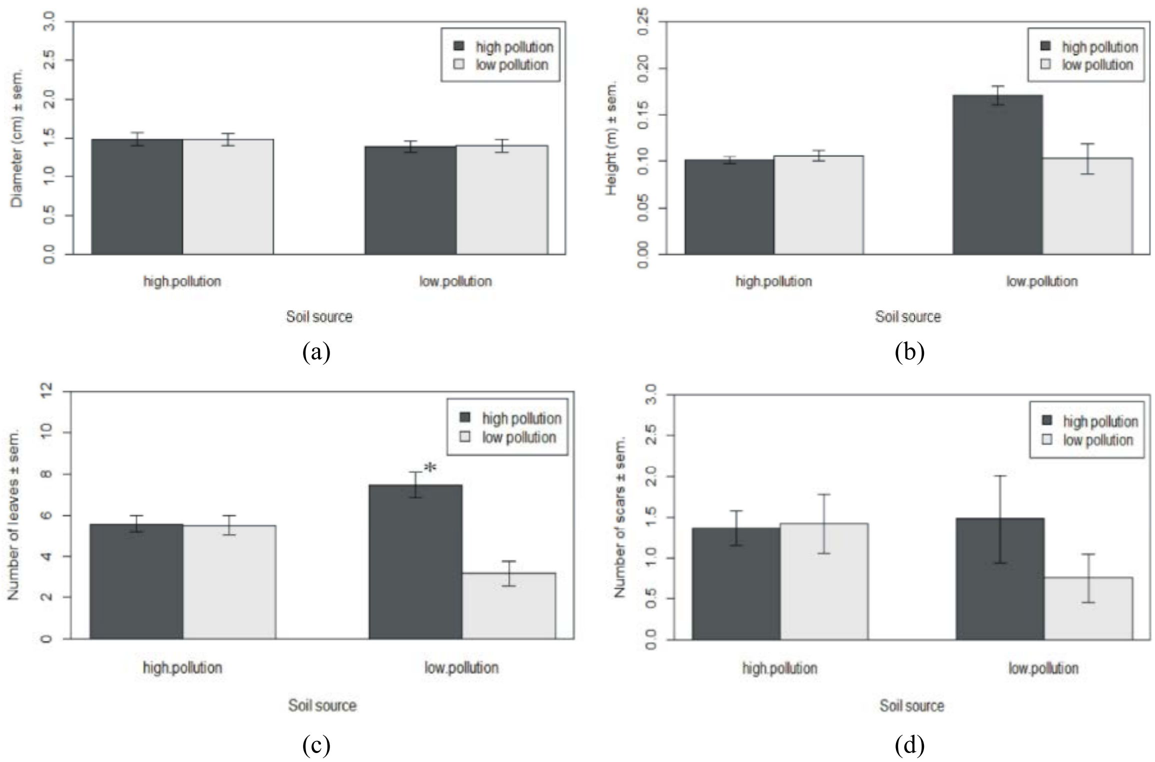

Fig. 1: Mean (a) diameter; (b) height; (c) Number of leaves; and (d) number of scar of propagule in highly and lowly polluted soil as a function of seed source in a common garden in the Niger River Delta. Tukey's HSD test (*) shows that propagule from highly polluted plot planted in lowly polluted soil did better. Vertical lines show 1standard error of the mean.

world [39, 40]. We observed significant differences in the growth of mangrove propagules amongst plots at different months intervals (Table 2). Propagules from highly polluted plot had the largest and longest growth period than those from medium and lowly polluted plot. Similarly, for the nypa palms the largest growth was recorded in seedlings from mixed forests (Table 3). In addition, nypa palm seedlings survived longer than the mangrove propagules (Tables 2 and 3).

Growth Estimates Based on Soil Source

Mangrove: The soil source has no significant effect on the stem diameter ($F_{1, 483} = 3.31$, $P = 0.0697$, Figure 1a) and number of leaves ($F_{1, 483} = 1.173$, $P = 0.279$, Figure 1c), but has significant effect on height ($F_{1, 483} = 120.06$, $P < 0.0001$, Figure 1b). Propagulesgrown in lowly polluted soils were slightly taller than propagules grown in highly polluted soils.

The seed source has significant effect on the height ($F_{1, 483} = 20.52$, $P< 0.0001$, Figure 1b) and the number of leaves ($F_{1, 483} = 23.01$, $P < 0.0001$, Figure 1c), but don't have siginificant effect on the stem diameter ($F_{1, 483} = 0.12$, $P = 0.7301$, Figure 1a). Similarly, propagules grown in highly polluted soil had more leaves than propagules grown in lowly polluted soil.

Both soil source ($F_{1, 483} = 1.651$, $P = 0.199$) and seed source ($F_{1, 483} = 0.916$, $P = 0.339$) have no effect on the seed scar (Figure 1d). Soil source and seed source are not independent of each other, as they interact with each other in leave production ($F_{1, 483} = 53.188$, $P < 0.0001$).

Nypa Palm: Both soil source ($F_{1, 487} = 0.68$, $P = 0.4106$) and seed source ($F_{2, 487} = 2.66$, $P = 0.0713$) have no significant effect on stem diameter. On the contrary, soil source ($F_{1, 487} = 28.86$, $P < 0.0001$) and seed source ($F_{2, 487} = 6.65$, $P < 0.001$) have a significant effect on height of seedlings. Similarly, soil source ($F_{1, 487} = 4.84$, $P < 0.0284$) and seed source ($F_{2, 487} = 13.29$, $P < 0.0001$) have significant effect on the number of leaves. The Tukey's HSD test indicates that mixed forest was the most significant variable amongst the different forests. Nypa palm seedlings did not have seed scar throughout the study period. This is because nypa palm don't have litter-fall like mangroves [41].

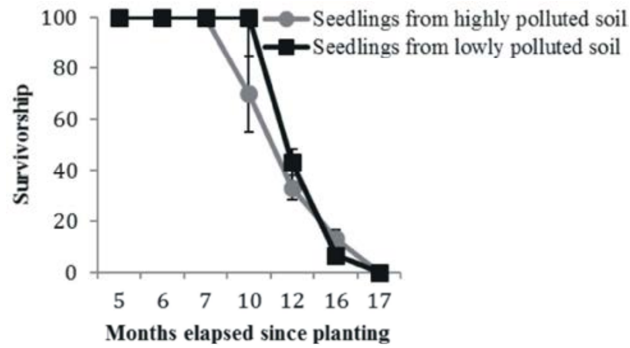

Fig. 2: Mean survival of mangrove propagules at different month interval. Survivorship curve of mangrove propagules (*R.racemosa*) from highly and lowly polluted soils that survived over time in a common garden in the Niger River Delta, Nigeria. The curve shows early decline for propagules growing in highly polluted soils. Bars represent 1 SE of the mean

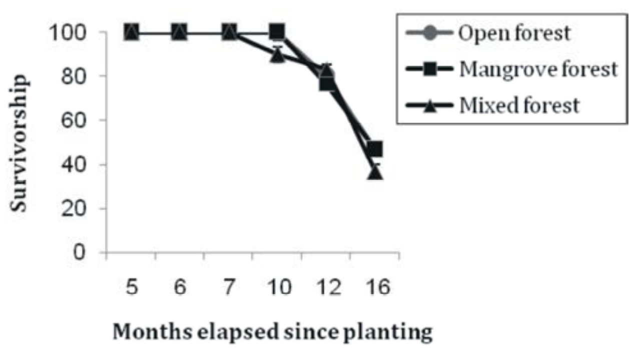

Fig. 3: Mean survival of Nypa palm seedlings at different month intervals. Survivorship curve of Nypa palm (*Nypafruticans*) seedlings from open, mangrove and mixed forest that survived over time in a common garden in the Niger River Delta, Nigeria. The curve shows early decline for mixed forests when compared to the other forests. Bars represent 1 SE of the mean.

Survival Curve: Mangrove propagules in lowly polluted soil showed higher survival rate than propagules in highly polluted soil (Figure 2). Furthermore, nypa palm seedlings had higher survival rate than mangrove propagules (Figure 3). Amongst the different forest types, seedlings from the mixed forest (i.e. low pollution) had the highest survival.

DISCUSSION

Growth Indices

Mangrove Growth in Soils with Different Levels of Pollution: In previous studies, stem diameter was used to assess plant growth in polluted soils [20] because it acts as an indicator of local growth conditions [42]. But in our study there was no significant difference in stem diameter. However, propagule height was influenced by the source of soil and those from highly polluted plot grew taller than propagules from lowly polluted plot (Figure 1a). The effect of pollution might vary as a result of differences in root tolerance to pollution [18]. Mangrove roots can prevent the passage of pollutants to sensitive parts of the plant [43]. In contrast, reduced growth can be caused by nutrient limitation as a result of pollution [34, 44]. In previous studies, low phosphorus limited growth in dwarf *R. mangle* [45]. But still in other studies *Avicennia marina* survived anaerobic condition by using its pneumatophores to absorb atmospheric oxygen [46]. Physiological acclimation or adaptation through selection could also give mangroves the ability to grow in polluted soil [20] as observed in our study where highly polluted soil had the best growth quality. Since seeds planted in polluted soils have better growth qualities, it then means that hydrocarbon pollution may have some antiseptic or growth enhancing effect on mangroves [47-49] which will require further studies. Leaves are used to determine the health status of mangrove trees [49]. This is because crude oil spillage can lead to leave yellowing and leave loss in mangrove seedlings [50-52]. Our study showed that the number of leaves was greatly influenced by the source of the seeds than the source of the soil (Figure 1c). We found that propagules collected or grown in highly polluted soil had more leaves than propagules collected or grown in lowly polluted soil (Figure 1c). Increase in number of leaves in propagules grown in polluted soils might be an adaptation to replace defoliated leaves. Leaf scar is used as a signature of pollution and for estimating the age of seedlings of *Rhizophora* [49, 53]. This study showed that the source of the soil and seed did not influence the number of leaf scars for mangroves (Figure 1c). However, we observed that more scars were recorded in propagule grown in highly polluted soil than propagules grown in lowly polluted soil (Table 2). *Rhizophora* species are viviparous and starts to germinate while still on the tree [30] which means soil has little influence at the initial stage of growth.

Nypa Palm Growth in Soils with Different Levels of Pollution: Mixed forests is located in the lowly polluted plot and our study showed that seedlings grown in soils from this forests had the best growth quality as compared to the open and mixed forests. Origin of the soil did not influence the diameter of nypa palm seedlings. Both the seed- and soil- source influenced the height and number of leaves. In the highly polluted soils, seedlings from mangrove forest and open forest had more leaves than seedlings from mixed forest. We observed that nypa palm seedling did not have leaf scar because none of its leaves dropped during the study (Table 3).

Survivorship Curves: Mangrove propagules planted in highly polluted soil had lower survival than propagules planted in lowly polluted soil (Figure 2). All the mangrove propagules from both kinds of soils died during the seventeenth month. But the nypa palm seedlings continued growing beyond the seventeenth month (Figure 3). The source of the soil had little effect on the growth of nypa palm, when compared to the source of the seed. This is because Nypa palms can grow in any kind of soil whether swampy or sandy, which had given them a better competitive advantage over the native mangroves [54].

CONCLUSION

In conclusion, our study showed that mangrove propagules that survive a polluted environment developed better growth quality than mangroves that grow in a lowly polluted environment while nypa palms seedlings derived from mixed forest have better growth quality than seedlings from other kinds of forest. We also found that in a polluted environment nypa palm seedlings are likely to survive longer than the mangrove propagules, which gives the palms a higher competitive advantage over the mangroves. Thus for the sake of conservation nypa palm encroachment into mangrove forests should be checked to prevent displacement.

ACKNOWLEDGEMNTS

We thank Chimezie and Sokari for assistance with sample collections. The project was supported by Idea Wild, a Saint Louis University Brennan Summer Fellowship and the Educational trust fund by the University of Port Harcourt, Nigeria.

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