Responses of *Rhizophora mangle* to simulated crude oil pollution

Victor Johwo Odjegba

Department of Botany, University of Lagos,Akoka, Lagos, Nigeria.
jodjegba@unilag.edu.ng

Abstract

Physiological and biochemical responses induced by crude oil stress were investigated in pot-grown seedlings of the mangrove, *Rhizophora mangle*. Plants were grown for 4 weeks in soils amended with Bonny light crude oil to achieve 0, 1, 3, and 5 % v/w, oil/soil concentrations. Growth, physiological, biochemical, as well as metabolic parameters representative of oxidative stress and enzyme activities were measured at the end of the treatment period. It was observed that crude oil treatments resulted in low biomass accumulation, relative water content, and chlorophyll level. The treatments however led to a significant increase in lipid peroxidation, catalase, and ascorbate peroxidase activities. It was concluded that *R. mangle* is among the plant species in the mangrove ecosystem that are susceptible to crude oil toxicity.

Keywords: Rhizophora mangle; crude oil; environment, pollution; ecosystem

Abbreviations: ANOVA- One-way analysis of variance; AOAC- Association of analytical chemist; APX- Ascorbate peroxidase; CAT- Catalase; MDA- Malondialdehyde; ND- Not detected; RWC- Relative water content; ROS- Reactive oxygen species; SE- Standard error; TCA- Trichloroacetic acid; THC- Total hydrocarbon.

1. Introduction

The mangrove ecosystem is one of the vegetation zones in Nigeria. It is found in the coastal region and it consists of tidal swamps, interspersed with creeks and lagoons. It covers about 10,000 square km forming 15 – 45 km vegetation belt along the Nigeria's coast line. The region is very rich in biodiversity with mangrove species like *Rhizophora mangle* and *Avicenia germinans* as dominant species.

The mangrove forest is an important vegetation belt known for its numerous beneficial roles in the tropical and subtropical regions of the world. Different mangrove species protect and stabilize low lying coastal lands and provide protection and food sources for estuarine and coastal fishery food chains. It serves as habitat to a numbers of animals and other living organisms.

It is now a common knowledge that the mangrove ecosystem in Nigeria is inundated with various factors that threatened its existence. Most of these factors are anthropogenic and if not well managed could lead to the disappearance of this important ecosystem from the coastal region of Nigeria.

Pollution caused by oil spills presents a number of harmful effects on the coastal environment. Past studies have shown that mangrove growth and sustainability are affected by various oil treatments [1], but little information are available regarding the mechanisms of effects of crude oil on the mangrove. It is important to understand the basics of crude oil effects on mangrove through biochemical and physiological responses of mangrove species under oil-induced stress. Due to the paucity of information concerning the responses of mangrove species to crude oil here in Nigeria, the author has evaluated some biochemical and physiological responses of *Rhizophora mangle* to simulated crude oil pollution in order to add to the existing data concerning mangrove and oil pollution in Nigeria.

2. Materials and methods

2.1 Plant growth and treatments

Mature propagules of *R. mangles* were collected from Majidun Estuary (06^{o} 61´ N, 03^{o} 43´ E) in Ikorodu area of Lagos, Nigeria. Enough propagules were collected from the same area in a single batch. Twelve propagules were planted in each plastic bucket (4) that contained sediment collected in the same area for acclimation and growth for 8 weeks before treatments were effected. After the acclimation period, relatively equal height seedlings (15 cm, measured from the top of the propagule to the terminal bud of the

seedling) were selected for the study.

Bonny light crude oil was collected from Warri Refinery and Petrochemical Company in Delta State, Nigeria. The physicochemical property of the oil was determined according to AOAC [2]. Soil treatment was done by manual mixing of weighed sediment with known volume of crude oil to achieve the required concentrations of 1, 3, and 5% v/w oil/soil [3]. Sediment without oil application served as control. One seedling was transplanted into each nursery bag representing each treatment, and replicated 8 times. The experimental set up was completely randomized and stationed under natural light in the Botanical garden of the University of Lagos, Akoka. Samples were kept moist by adding water when necessary. The seedlings were allowed to grow in the treated soil for 4 weeks before they were harvested for analyses.

2.2 Dry weight determination

Plants were carefully uprooted after the treatment period and rinsed with tap water. The shoot was carefully separated from the upper part of the propagule, while the roots were severed from the bottom of the propagules. The corresponding shoot and roots were placed in labeled paper bags and oven dried at 70 ^{o}C until constant weight was achieved. The dried samples were weighed using a digital top loading weighing balance (Mettler AE 100) to determine the dry weight.

2.3 Leaf area measurement

The leaf area was determined by comparing the weight of leaf traces with a standard paper of known weight according to the method described by Eze [4].

2.4 Relative water content of leaves

The second leaves were harvested for the determination of relative water content (RWC). The RWC of each leaf was determined according to the method of Turner [5] by using the formula RWC (%) = [(fresh weight - dry weight)/(turgid weight – dry weight)] x 100.

2.5 Determination of total chlorophyll

Plant leaves (0.5g) were ground in 10ml 80% acetone in the dark. After centrifugation at 4000 g for 5 min, the absorbance of the supernatant was read at 645 and 663 nm [6]. The total chlorophyll content was calculated using the formula given by Machlachlan & Zalik [7].

2.6 Lipid peroxidation assay

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following a modified procedure of Wang & Jin [8]. About 0.3 g of excised roots measuring 1 cm from the tip were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 g for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as $6.45(A_{532}-A_{600}) – 0.56\ A_{450}$.

2.7 Enzyme assay

For enzyme analysis, fresh samples of leaves (100 mg each) were ground in a ceramic mortar and extracted with 5 ml of 100 mM potassium phosphate buffer pH 7.5, with 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 rpm for 5 min. The supernatant was used for the estimation of antioxidant enzyme activities. Catalase (CAT) activity was determined according to Aebi [9], by monitoring the decrease in the absorbance at 240nm as a consequence of H_2O_2 disappearance. Ascorbate peroxidase (APX) activity was assayed according to the method of [10]. The oxidation of ascorbate was determined by the change in absorbance at 290nm.

2.8 Statistical analysis

Means of four replicates as well as their standard errors (SE) were determined. The test of significance between the treatments was done using a one-way analysis of variance (ANOVA).

3. Results

The physicochemical analyses of Bonny light crude oil showed that the oil has no detectable levels of nickel and lead but contained trace amount of sulphur. The ash content was 0.112% while its density was 0.61 cm^3 (Table 1).

Table.1 *Physicochemical properties of Bonny light crude oil used in this study.*

Parameters	Level detected
Density	0.61 cm3
THC	89.26 %
Ash	0.112 %
Sulphur	0.006 %
Nickel	ND
Lead	ND

ND denotes not detected; THC, total hydrocarbon

Treatments of *R. mangle* seedlings with crude oil inhibited the growth of the plant as indexed by root and shoot biomass of the plant. The inhibitory effect was concentration dependent and biomass accumulation decreased with increase in oil concentration (Figure 1). While the control plants had a mean root biomass of 2.13 ± 0.06 g, those that were treated with 3 and 5 % crude oil respectively had 1.33 ± 0.06 and 0.78 ± 0.04 g.

Fig.1. Root and shoot biomass of R. mangle seedling treated with different concentrations of crude oil. Error bars represents standard error (n=4).

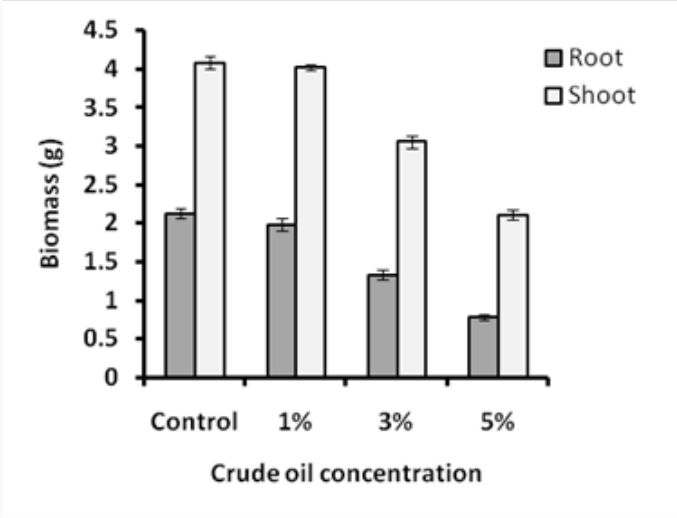
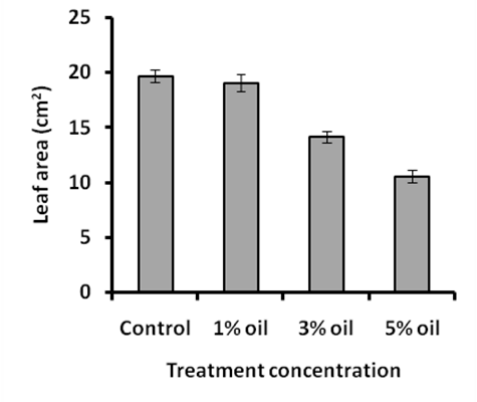


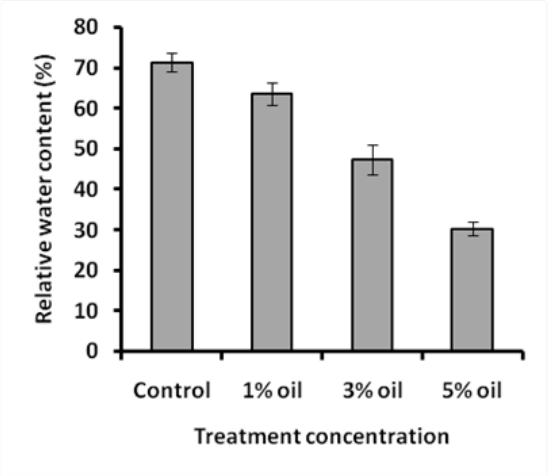
Figure 2 shows the effect of crude oil treatment on the leaf area of *R. mangle* seedlings. It was observed in this study that crude oil concentration above 1% caused a significant reduction in the leaf are of the plant. Seedlings that were grown in soil containing 5% oil had a mean leaf area of 10.6 ± 1.7 cm^2 compared to the control plants which had 19.4 ± 1.3 cm^2.

Fig.2. Effects of crude oil on the leaf area of R. mangle seedlings at the end of the 4 wk treatment period. Means and standard errors of 4 replicates are presented.



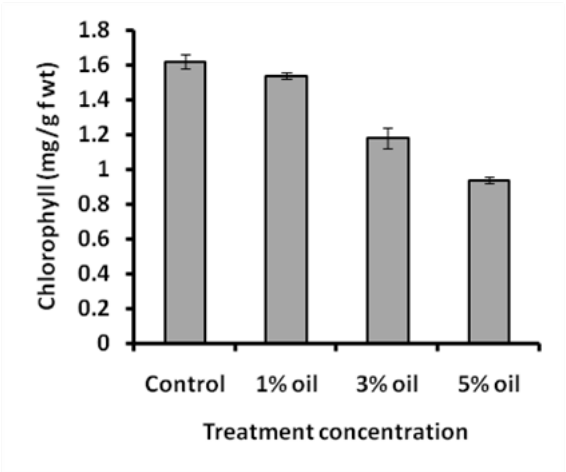
Data showing the effect of crude oil treatment on the relative water content of *R. mangle* is represented in figure 3. It was observed that crude oil treatment significantly ($p < 0.05$) reduced the relative water content of the plant. The control plants had a mean RWC of 71.33 ± 3.26 % as against 30.33 ± 3.76 % observed for plants treated with 5% crude oil.

Fig.3. Relative water content of R. mangle leaves as affected by crude oil treatment. Means and standard errors of 4 replicates are presented.



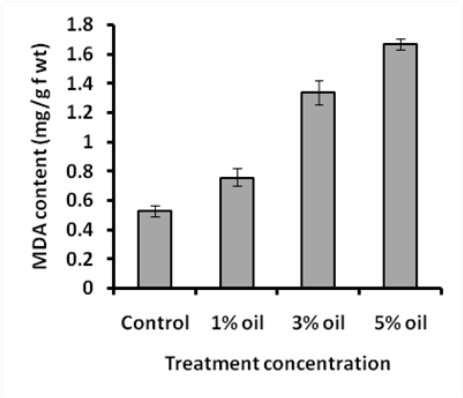
The chlorophyll content is regarded as a useful *in vivo* indicator of toxicity caused by pollutants. In this study, the total chlorophyll in *R. mangle* was quantified to know the effect of crude oil treatment on the photosynthetic pigment level. It was observed that crude oil consistently reduced the amount of chlorophyll present in the leaves of the experimental plant. While the control plants had a mean value of 1.62 ± 0.04 mg/g fresh wt, seedlings that were treated with 1, 3, and 5 % crude oil respectively had 1.54 ± 0.02, 1.18 ± 0.06, and 0.94 ± 0.02 mg/g fresh wt (Figure 4).

Fig.4. The effect of crude oil on the total chlorophyll content in the leaves of R. mangle seedlings after 4 weeks of exposure. Means and standard errors of 4 replicates are presented.



To determine the impact of Bonny light crude oil on lipid peroxidation, the malondialdehyde in the root tissue of *R. mangle* seedlings was measured. It was observed in this study that the crude oil treatment caused lipid peroxidation in the plant as it led to more than 100 % increase in MDA when plants were exposed to crude oil compared with the control (Figure 5). The control plants had a mean MDA content of 0.53 ± 0.04 mg/g fresh wt as against 0.76 ± 0.06, 1.34 ± 0.08, and 1.67 ± 0.04 mg/g fresh wt observed for plants treated with 1, 3, and 5 % crude oil respectively.

Fig.5. Malondialdehyde (MDA) content of R. mangle seedlings after a 4 week exposure to crude oil. Error bars represents standard errors (n=4).



The activities of catalase (CAT) and ascorbate peroxidase (APX) were measured as representative enzymes involved in antioxidant metabolism. In this study, it was observed that the activities of the two enzymes followed the same trend when *R. mangle* seedlings were treated with crude oil (Figures 6 & 7). The activities of the enzymes significantly increased with increase in oil concentration, indicating oil-induced oxidative stress. The control plants had a mean value of 15.41 ± 0.67 µmol/min/g f wt as CAT activity, while plants treated with 5 % crude oil had 31.41 ± 0.41 µmol/min/g f wt. In the same vein, *R. mangle* seedlings that were exposed to 5 % crude oil had the highest APX value of 0.094 ± 0.002 µmol asc/mg prot/min, compared to 0.051 ± 0.002 µmol asc/mg prot/min observed for the control plants (Figure 7).

Fig.6. Catalase activity of R. mangle seedlings as affected by crude oil treatment. Means and standard errors of three replicates are presented.

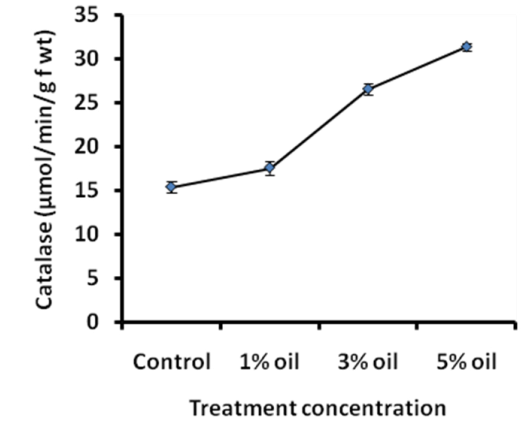
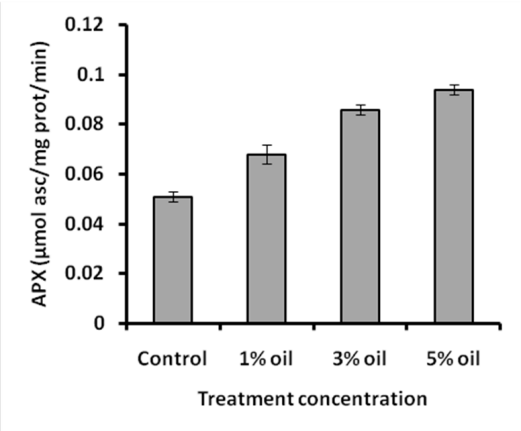


Fig.7. Ascorbate peroxidase (APX) activities of R. mangle seedlings at the end of the 4 weeks of treatment. Means and standard errors of 3 replicates are presented.



4. Discussion

The present research was conducted to evaluate the physiological and biochemical responses that could accompany crude oil pollution in *Rhizophora mangle* seedlings. The low biomass observed in plants treated with crude oil could be as a result of the oily nature of the soil which consequently repels water making it difficult for the plants to absorb water and dissolved nutrients. Sub-optimal water and nutrient supply retarded the growth of the plant as indexed by reduced leaf area and biomass accumulation. Similar observations were reported by past investigators [11, 12, 13, 14].

The oily nature of the soil disrupted the normal plant-water relations and prevented optimal absorption of water by plant roots. This explained why plants grown in crude oil polluted soils had low RWC compared to the control plants. This finding is in conformity with the earlier report by Odjegba & Badejo, [14] that *Celosia argentea* exposed to crude oil had significantly low RWC compared to the control plants.

Generally, crude oil treatment led to a significant reduction in chlorophyll content of the experimental plant. The low pigment content could be related to lack or sub-optimal supply of nitrogen and magnesium which are essential for chlorophyll synthesis. The chlorophyll has a nitrogen-containing porphyrin ring structure, with a magnesium atom at its center. The inadequate supply of these nutrient elements would have direct effect on chlorophyll synthesis since they form the integral part of this important photosynthetic pigment.

Oil pollution and other environmental stresses may lead to induction of oxidative stress by generating oxygen radicals known as reactive oxygen species (ROS). The ROS cause damage to biomolecules including proteins, lipids, nucleic acids [15]. Malondialdehyde assay is popularly used to assess lipid peroxidation in biological systems, and is a valid indicator of free radical formation [16]. The significant increase in MDA content following crude oil treatment was an indication that crude oil pollution induced oxidative stress in this plant. This result agreed with the observations made by Eriyamremu & Asagba, [17]; Odjegba & Badejo, [14].

Plants possess complex antioxidative defense system comprising of non-enzymatic and enzymatic components to scavenge ROS. Under normal conditions, potentially toxic oxygen radicals are generated at a low level and are adequately detoxified by different antioxidant enzymes present in the plant cells. The balance between production and quenching of ROS may be perturbed by a number of adverse environmental factors, giving rise to rapid increases in intracellular ROS levels [18]. In order to avoid the oxidative damage, higher plants raise the level of endogenous antioxidant defense and such response reflects an adaptation of a plant to its environment [19]. In the present study, it was observed that the activities of catalase and ascorbate peroxidase increased significantly when *R. mangle* seedlings were treated with crude oil. The increase in activity of these antioxidant enzymes as well as MDA content, underscored the fact that crude oil pollution caused oxidative stress in *R. mangle*.

5. References

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