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Appraisal of The Impact of RENA And Mycoremediation Techniques In Petroleum Hydrocarbon Impacted Soil Using Maize (Zea Mays L.) As Test Crop

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Abstract: Field trial was carried out in Clay loamy soil within University of Port Harcourt environment, to comparatively appraise the growth and development of Maize (*Zea mays* L.) in crude oil impacted plots that have selectively undergone remediation using Remediation by Enhanced Natural Attenuation (RENA) and Mycoremediation (*Pleurotusostreatus*). Seven and half (7.5)litres of Bonny light crude was used to spike each triplicate plots of RENA, Mycoremediation, combination of RENA/Mycoremediation and untreated control treatment technique. After five months of remediation treatment period, the treated plots were planted with viable Maize (*Zea mays* L.) seeds and then assessed for germination and growth parameters within an 8 week study period.Results obtained from the current study indicated that Maize (Zea mays L.) showed crude oil dose-dependent response in the impacted soil. There was significant difference in the germination and growth parameters of maize (*Zea mays* L.) in all the remediation treatment techniques investigated. The combination of RENA/Mycoremediation technique showed the highest maize (*Zea mays* L.) crop yield in all the parameters assessed while the untreated crude oil impacted control plots showed the lowest maize (*Zea mays* L.) cropyield in all the growth parameters studied. Hence the study has shown that crude oil contamination hasadverse effectsongermination and growth of maize (*Zea mays* L.) crops but this adverse effect is greatly reduced when crude oil impacted plot is innoculated with *Pleurotusostreatus*and treated in conjunction with application of RENA technique.
Keywords:RENA, Mycoremediation, Pleurotusostreatus, Crude Oil and Maize (*Zea mays* L.)

I. INTRODUCTION

Petroleum hydrocarbon is a key pollutant of the environment in oil producing nations because of the extraction and processing of oil [1]. When crude oil losses its containment in pipelines, flowlines, storage tanks, barges, refineries and wells, it leads to spill causing deleterious effect to the land, water and air environment. In someinstances the pollutants may infiltrate the groundwater thereby affecting its quality. Crude oil when spilled into an environment causes changes to the biological and physiochemical properties of soil. The toxic effect of crude oil has both short term and long term effect on the environment[2].Again the toxicity of crude oil is harmful to the existence of microorganisms and plants in the environment [3].The infiltration of crude oil into the soil pores causes the reduction of oxygen, moisture content and other nutrients that are essential for the survival and growth of microorganisms and plants[4; 5]. Oil spill thereby makes farmlands non-productive leading to abandonment of farmlands and increased agitation by local communities[6]. Maize (*Zea mays*) is a widely cultivated crop and it is consumed in most homes in Nigeria.

It also serves as a major source of raw materials for numerous industries in Nigeria [7]. Niger Delta area of Nigeria has been the epicenter of oil exploitation and production in Nigeria. It has had its fair share of environmental contamination emanating from crude oil spills[8].[9]reported that since 1976 about 550 recorded incidences of oil spillage introducing over 3 million barrels of crude oil into the NigerDelta environment. The use of improved technology to remediate the polluted Niger Delta environment is the only viable option to the issues of socio-economic and environmental dislocation of the Niger Delta area[10]. Over the years attempts has been made to clean up the crude oil polluted NigerDelta environment like the use of Land farming or Remediation by Enhanced Natural Attenuation (RENA) which is a form of bioremediation that employs the use of physical, chemical and biological processes in order to achieve site restoration [11; 12]. On the otherhandmycoremediation, a term coined by [13]is the use of fungi to bioremediate contaminated soil. Several researchers have implicated mushrooms including the oyster mushroom (*Pleurotusotreatus*) in the removal of harmful toxins like dyes, herbicides, heavy metals, crude oil from the environment[14; 15; 16; 17; 18; 19]. *Pleurotusostreatus* is cultivated in many countries including Nigeria and it can colonise several substrates including agricultural waste products[20; 21; 22].This study aims to use Maize (*Zea mays* L.) as a test crop in comparatively assessing the bioremediative abilities of RENA and Mycoremediation using *Pleurotusostreatus* in crude oil impacted soil.

II. MATERIALS AND METHOD

2.1 Experimental Site description: This study was carried out within Abuja campus of University of Port Harcourt, located in Obi-Akpor Local Government Area of Rivers State. The area is a flat plain with the top soil type being clayey loamy. The area records very high relative humid for the most part of the year. The area records monthly rainfall of 180 mm and mean temperature varying between 24 ^{o}C and 32 ^{o}C. Two seasons exist namely; rainy and dry seasons. The rainy season is between March and October while the dry season is between November and February[23].

2.2 Study area:

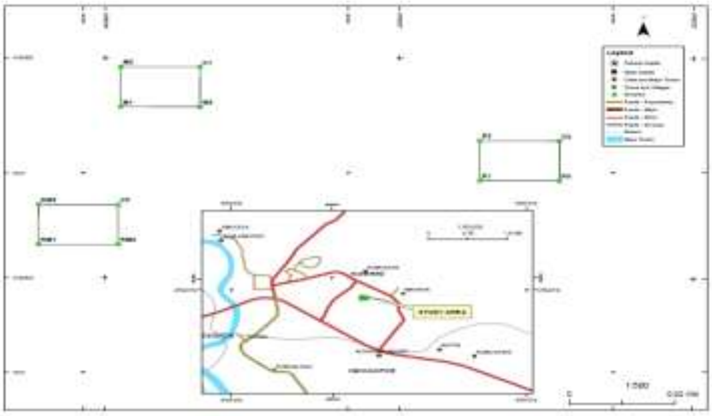

Plate 1:Study area map

2.3 Source of Research materials: Bonny light crude oil used for the study was gotten from Shell Petroleum Development Company of Nigeria while the Federal Institute for Industrial Research, Oshodi (FIIRO), Lagos provided the spawn of *Pleurotusostreatus*. Seeds of Maize (*Zea mays* L.) were purchased from Songhia Farm in Bunu, Tai Local Government Area, Rivers State.

2.4 Research Design: The experimental site was divided into 12 plots of 1 meter by 1 meter dimension using wooden planks. The study was in two phases.

2.4.1 Phase One: Each plot was spiked with 7.5 Litres of Bonny light-crude. The impacted soil was left undisturbed and allowed for stabilization for two weeks. Four treatment options were selected. They include RENA, Mycoremediation, combination of RENA/Mycoremediation and Control techniques respectively. Each treatment option has triplicate plots. The polluted plots were subjected to the treatment option for a five month period. Samples were collected at 0-15cm and 15-30cm depths for each of the plots. Samples were collected once a month and taken to the laboratory for analyses.

2.4.1.1 Remediation Treatment Techniques

2.4.1.1.1* RENA technique*:

Initial Tilling: Plots were tilled using shovels two weeks after spiking with Bonny Light Crude.

Secondary Tilling: Seven days after the initial tilling, the lumps of soil in the crude oil impacted plots were broken down and homogenized. The plots were tilled and allowed to stand for another 7 days. The aim of this stage was to increase surface area and enhance distribution of the contaminant.

Windrow Construction: Soil ridges of about one foot high and one foot wide were built after the secondary tilling on the study site. The ridges were made to enhance porosity and improve soil aeration and enhance moisture content that would promote biodegradation activities of resident microorganisms. The windrows were allowed to stand for about two weeks.

Breakdown of Windrows: Shovels and rakes were used to breakdown ridges. This was done two weeks after windrows construction. Then samples were collected and composited for laboratory analyses.

2.4.1.1.2 RENA/Mycoremediation Technique*:* Plots which have already been spiked with bonny light crude oil were inoculated with 400grams of *Pleurotusostreatus*. This mixture was spread out in the crude oil impacted plots and allowed to go through the process of RENA technique for the duration of the study.

2.4.1.1.3 Mycoremediation technique*:*Plots which have already been spiked with 7.5 litres of Bonny Light Crude oil were inoculated with 400 grams of *Pleurotusostreatus*. This mixture was spread out in the crude oil impacted plots and allowed for the duration of the study.

2.4.1.1.4 *Control:*These plots were left unattended to after spiking 7.5 litres of Bonny Light Crude oil. The plots were left to stand like this for the duration of the study.

2.4.2 Phase Two: After the remediation treatment of the plots, each plot was planted with20 viable Maize (*Zea mays* L.) seeds. The plots were non-destructively monitored weekly for some plant growth parameters which include: rate of seed emergence, plant height, number of leaves and leaf surface area. At the end of the 8 week study period the crop were destructively harvested for shoot/root weight biomass study.This was thinned down to one germinated seed per plot after 14 days of monitoring for rate of germination. The seeds were planted 30 cm apart. Weeding was done manually as required and the plots were watered when necessary.

The following plant growth parameters were investigated during the study period:

2.4.2.1 Seed Emergence Percentage (SEP): This was done at 2 weeks of planting by counting number of emerged seeds against number of planted seeds and expressed in percentage on treatment basis.
Seed Emergence Percentage (SEP)= $X/N \times 100$; where X = total number of emerged seeds and N = total number of planted seeds.

2.4.2.2 *Plant Height (cm):*Plant height was recorded from soil surface level to the tip of the terminal bud using long meter rule. This was expressed in (cm)

2.4.2.3 *Number of leaves:*This was done by visual counting of the number of leaves of the tagged crop.

2.4.2.4 *Leaf Area (cm^2):*Non-destructive method of leaf area measurement was used for leaf area estimation. Leaf area was derived from the measurements of Length and Breadth of the longest leaf per plant and correction factor value 0.75 was used[24].

2.4.2.5 *Wet/Dry Shoot/Root biomass Weight:*This was done destructively by uprooting the crop from the root and each crop component (shoot and root) were detached from one another and the yield from each plot was weighed fresh in an electronic weighing balance; this gave the wet weight; then each harvested plant component (shoot and root) was placed in a brown envelope and air dried until a particular weight was obtained. This gave dry biomass weight and this was expressed in (g).

2.5 *Statistical analysis:*The relationship between treatment techniques and time was established using one way analyses of variance (ANOVA) and post hoc analysis for the multiple comparisons of data collected from field and laboratory. SPSS version Excel 20.0 was employed for this purpose.

III. RESULT AND DISCUSSION

3.1 Effect of bioremediation treatment techniques on Total Petroleum Hydrocarbon (TPH)

The initial concentrations of Total Petroleum hydrocarbon (TPH) were 3870.67, 3862.03, 3926.48 and 3920.37 mg/kg for RENA, Mycoremediation, Combination of RENA/Mycoremediation and Control respectively (Figure 1). As bioremediation progressed to the 20^{th} week the concentration of TPH reduced to 1135.85, 1517.8, 383.43 and 2688.6 mg/kg for RENA, Mycoremediation, Combination of RENA/Mycoremediation and Control respectively. The rate of loss of TPH (87 %) was highest in plots treated with the combination of RENA/Mycoremediation technique. This can be attributed to the effect of tilling and homogenization on contaminated soil leading to improved aeration and enhancing the forces of volatilization. Another reason for the highest losses in TPH observed in RENA/Mycoremediation treatment might have been as a result of the abilities of *Pleurotusostreatus* to secrete extra-cellular Lignin Modifying Enzymes which degrade the hydrocarbon in the impacted soil. [17]reported that *Pleurotusostreatus*releases extracellular enzymes that degrade petroleum hydrocarbon. Similar to the finding in this study, [18]reported that after 6 months of inoculating a crude oil polluted soil with *Pleurotusostreatus*, the total petroleum content (TPC) recorded 91.2 % loss.

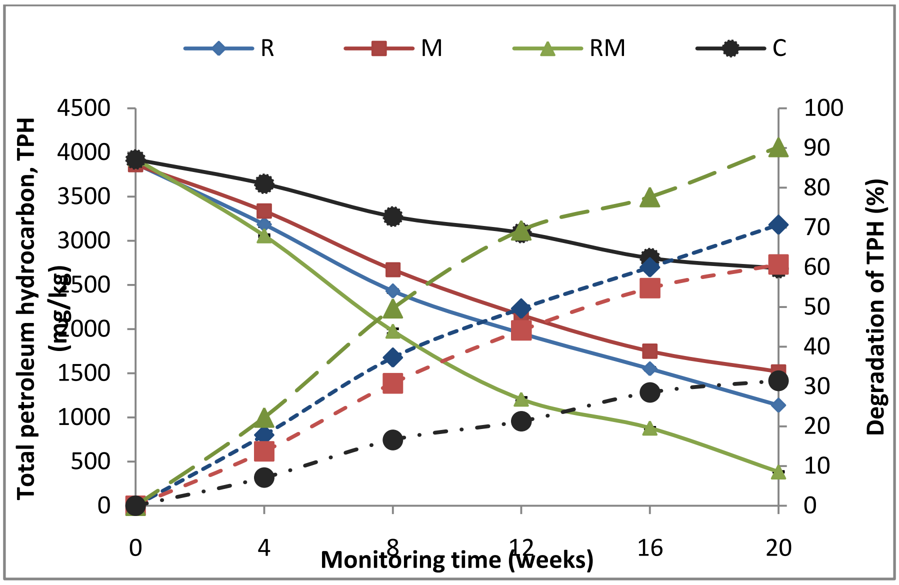

Figure 1: Trend of Total Petroleum Hydrocarbon (TPH) (mg/kg) during the study period

3.2 Effect of bioremediation treatment techniques on Maize (*Zea mays* L.)

3.2.1 Percentage emergence (%): Germination rate was monitored for two weeks after planting (2 WAP). The results of the observations are shown in (Figure 2). The percentage emergence was observed for the different treatments options.The percentage emergence recorded 87, 78, 97 and 33 % in RENA, Mycoremediation, RENA/Mycoremediation and Control respectively. The combination treatment of RENA/Mycoremediation recorded the highest percentage emergence of 97 %.This result may have been as a result of the depletion of toxic compounds like TPH in the treated soils.[25] reported otherwise that maize (*Zea mays* L.) can overcome the inhibition of crude oil in oil contaminated soil, although this is not without some defects like stunted growth and the effect of crude oil on maize (*Zea mays* L.) germination is dependent on crude oil dose in the soil. The untreated control plots showed the least emergence of 33 %. This can be attributed to reduced metabolic and biochemical activities caused by the high concentration of crude oil in the untreated control plots. This result is in line with the works of [26] that seed embryo was endangered by crude oil contamination. This affect could be because of polar compounds dissolved in moisture which may have infiltrated the seed coat causing polar narcosis[27; 28]. Generally, the germination and growth of plant in crude oil contaminated soil is concentration dependent as has been soon in this study. [29]reported that plant growth could be inhibited by toxic compounds in crude oil.[30]reported that crude oil pollution affects germination of seeds.

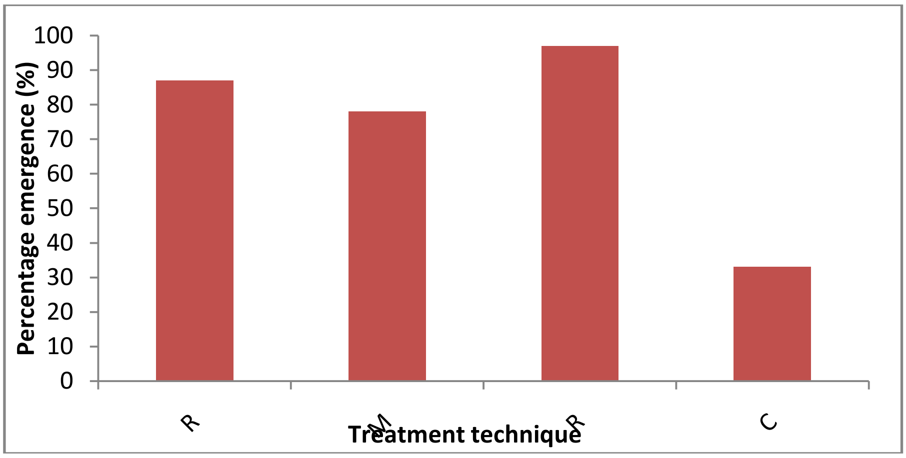

Figure 2: Trend of percentage emergence (%)

3.2.2 Number of leaves: Seeds planted in plots treated with a combination of RENA/Mycoremediation recorded the highest number of leaves per plant over the eight (8) weeks study period with 13.67±0.58 (Figure 3). The least number of leaves was recorded in untreated polluted control with mean values recorded 5.67±0.58. RENA recorded 9.67±0.58 while Mycoremediation recorded 7±0.0. The result showed a significant difference p<0.05 in the number of leaves from week one to week 8 across the various treatment methods applied in this study. The smaller number of leaves as observed in the control plots could be as result of toxic levels of hydrocarbon or the inhibition of the roots by crude oil from uptake of minerals in the soil. It could also be as a result of displacement of water and air in the soil thereby creating stressed condition in the soil. These conditions would have impacted on the photosynthetic system of the plant which would have led to more yield like increased number of leaves. This is in line with the findings of [31] who observed that hydrocarbon impacted soil may have deleterious consequences on the leaves and stem girth of Okra and fluted pumpkin. This collaborates with the observation of [32; 33] that luxuriant plant parameters indicate improved nutrient availability. [34]reported that crops grown in hydrocarbon polluted soils are retarded with chlorosis of leaves and dehydration of the crop. [35]reported significant decrease in number of leaves of Air potato owing to the adverse effect of high TPH concentration on Chlorophyll content of Air Potato compared with uncontaminated soil.

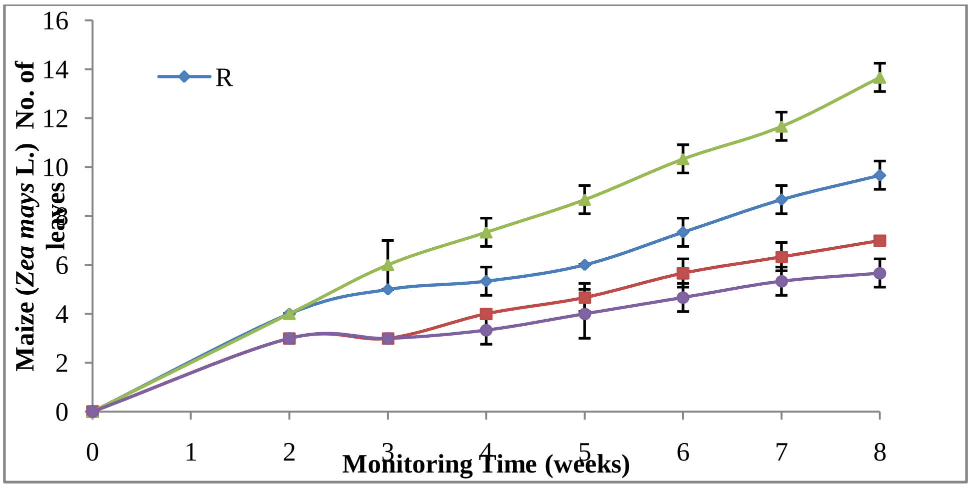

Figure 3: Changes in number of Maize (Zea mays L.) leaves during the study

3.2.3 ***Plant Height:***The height per plant was investigated for the eight (8) weeks after planting (8WAP). At the eight week, Maize (*Zea mays* L.) crop recorded heights of 120.1cm±7.31, 75.03cm±3.37, 137.53cm±2.42 and 53.20cm±1.78 representing RENA, Mycoremediation, Combination of RENA/Mycoremediation and Control respectively (Figure 4). The plots that were treated with combination of RENA/Mycoremediation recorded the highest value in plant height from 2nd week to the 8th week.This could be attributed to the lot of factors, amongst include the reduced hydrocarbon load of the treated soil. It has been reported that *Pleurotusostreatus* are able to degrade short chain alkanes and reduce the toxin in contaminated soil [36; 37; 38; 39; 40]. In addition the tilling, homogenization and loosely placed in windrows helped to increase the needed aerobic conditions that is required by microbes to degrade the hydrocarbon and improve the nutrient level of the impacted soil. The untreated Control recorded the least in plant heights. This result agrees with the works of [41; 42] that hydrocarbon contamination negatively affected the height of maize (*Zea mays* L.) and *Vignaunguiculata* respectively.

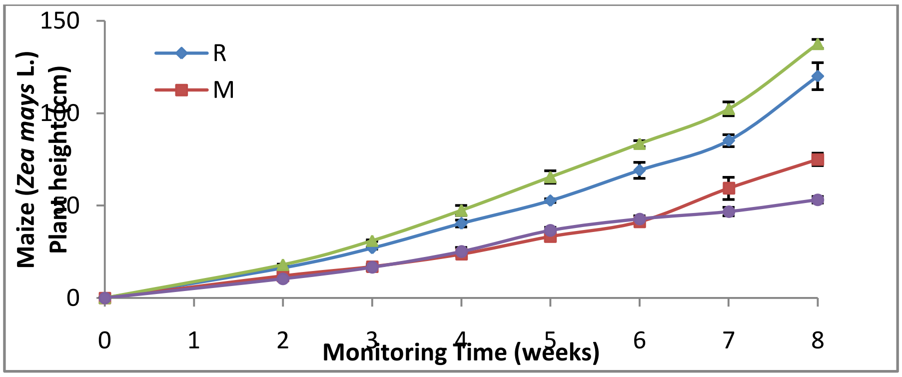

Figure 4: Changes in the height of maize (*Zea mays* L.) plant during the study period

3.2.4 ***Fresh/Dry Shoot Biomass:***Fresh crop shoot biomass were destructively harvested and investigated.The biomass weight is as follows: RENA 84.27g±3.08, Mycoremediation 51.54g±2.38, RENA/Mycoremediation121.73g±3.45 and 37.59±3.63 untreated control respectively. See figure 5. The descending order of gain in shoot fresh biomass weight is as follows: combination techniques of RENA/Mycoremediation>RENA>Mycoremediation>Control. The shoots were dried and then investigated for dry shoot biomass weight. Just as in fresh shoot biomass weight the crops planted in plots treated with the combination technique recorded the most weight in dry biomass (23.18g±1.95). This result is in line with the works of [43] the crop yield is ultimately determined hugely by the leaf surface area. The control plots recorded the least gain in dry root biomass weight (5.48g±1.27). The reduction in the plant yield recorded in the untreated control crops could be attributed to non-availability of nutrient in the soil due to the high TPH values as reported by [44]. Some researchers [45; 46]observed that the level of crude oil pollution affects negatively the plant yield.

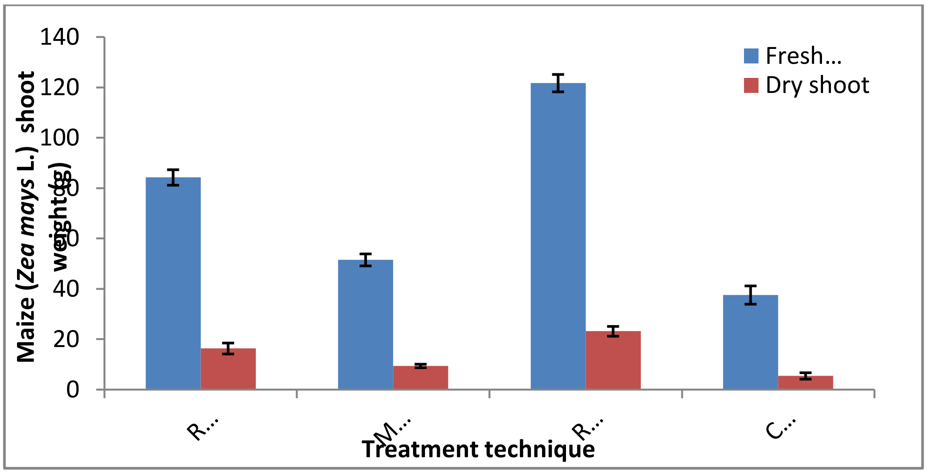

Figure 5: Changes in fresh and dry Shoot weight biomass during the study period

3.2.5 Fresh/Dry Root Biomass: The fresh root were harvested eight (8) weeks after planting (8 WAP) and examined for its gain in root biomass weight. See figure 6. The most weight gain in fresh root biomass was observed in crops planted in the plots treated with the combination of RENA/Mycoremediation techniques 23.94g±0.1. The least weight gain in fresh root biomass was observed in crops planted in untreated plots control plots 10.12g±0.79.The roots were dried and investigated for dry root weight biomass measurement. Just as in fresh root biomass weight, the plots treated with the combination technique of RENA/Mycoremediation gained the most weight 4.37g±0.4. The least gain in weight was observed in untreated plots (control) 2.56g±0.26. This result is in line with the works of [47]that natural forces can attenuate low level of soil pollution thereby remediating the soil and improving soil organic matter and fertility.

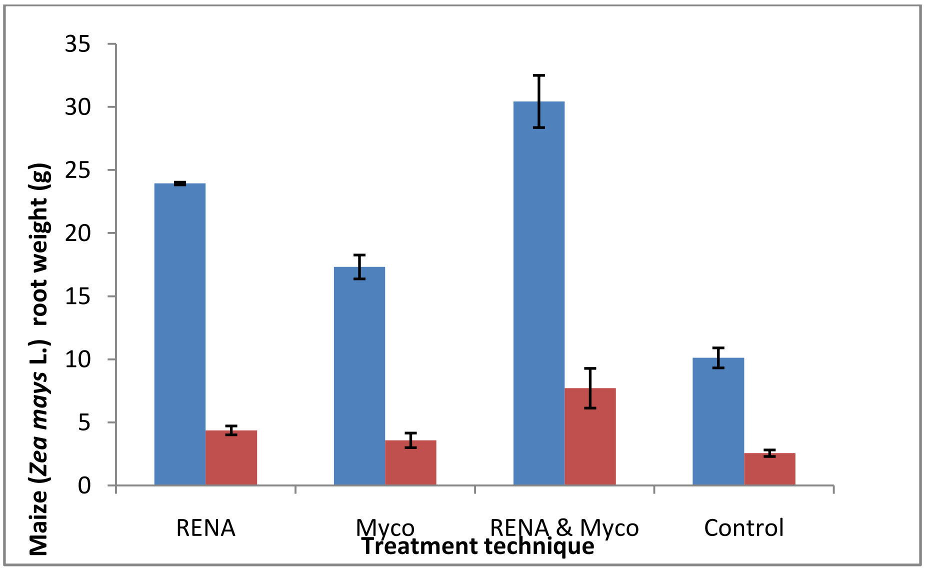

Figure 6: Changes in fresh and dry Root weight biomass during the study period

3.2.6 Leaf Surface Area: This was non-destructively monitored from the second week to the eight week after planting (8 WAP). See details in Figures 7. At the eighth week the plots treatment with combination of RENA/Mycoremediation technique recorded the largest leaf area 310.24cm^2±9.88.The least in Leaf surface area size was in untreated polluted control with mean values recorded as 65.49±2.8. RENA recorded leaf area of 209.46±13.35while Mycoremediation recorded leaf area of 153.65cm^2±3.93. This result is in line with the work of [48] that crude oil contamination caused anatomical and morphological defects leading to differential changes in the rate of leaf development and growth.

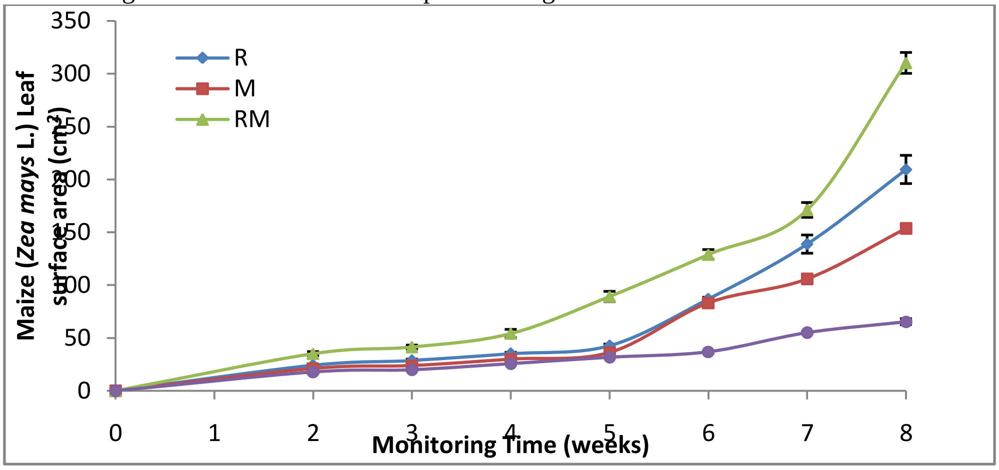

Figure 7: Changes in Maize (*Zea mays* L.) leaf Surface area during the study period.

IV. CONCLUSION

The use of *Pleurotusostreatus* in conjunction with application of RENA techniques improves the soil fertility and conditions that is favourable to microorganisms and leads to enhanced crop growth and yield. This combination techniques RENA/Mycoremediation should be employed in remediation of crude oil contaminated soils and to better agricultural yield from farmlands.

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