Effect of Crude Oil Polluted Soil, Amended with Leaf Litter and Hydrogen Peroxide on the Growth of Beans, Garden Egg and Cucumber

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Abstract
Studies on the growth parameters of beans, garden egg and cucumber, grown on crude oil polluted soil using the leaf litter of Terminalia catappa and hydrogen peroxide as amendments were carried out in Rivers State University Agricultural farm. A complete block design was employed for the experiment. A total of 70 bags were used for each plant with 14 treatments and 5 replicates. T1:100ml Crude Oil + 100ml H_2O_2, T2:100ml Crude Oil + 100g l/l, T3:200ml Crude Oil + 200ml H_2O_2, T4:200ml Crude Oil + 200g l/l, T5:300ml Crude Oil + 300ml H_2O_2, T6:300ml Crude Oil + 300g l/l, T7:400ml Crude Oil +300ml H_2O_2, T8:400ml Crude Oil + 400g l/l, T9:500ml Crude Oil + 500ml H_2O_2, T10:500ml Crude Oil + 500g l/l, T11:no pollution + 500ml H_2O_2, T12:no pollution + 500g l/l, T13:500ml Crude Oil + no amendment and T14:control. Garden egg, Beans and cucumber seeds were used as test crops. 10kg soil was used in each bag and crude oil was applied afterwards at various levels. Organic and Inorganic manure were applied at 1 month interval after pollution for 4 months, and planting was later done. Plant parameters assessed were Plant-height, Leaf-area, Stem-girth, and Leaf-number which were measured and analysed at 3weeks interval for 12 weeks. Results of plant parameters showed a significant increase in leaf litter amended soils whereas the hydrogen peroxide amended soils had little increase in all the weeks with poor yield generally. Application of Leaf-Litter Manure and H_2O_2 greatly degraded the PAHs in the soil. Beans performed better in the growth parameters in Leaf-Litter soils than in H_2O_2, while cucumber and garden-egg had poor growth in both leaf-litter and H_2O_2 soils which was obvious in the hydrogen peroxide amended soils. In general the use of leaf-litter manure for remediation of petroleum contaminated soil should be encouraged as this proved to amend the soils better than hydrogen peroxide.

Introduction

Pollution simply refers to the introduction of contaminants into the natural environment that causes adverse change (Webster, 2010). Ndukwu *et al*., (2012) viewed it as the production and release through human activities, of any substance into the environment, in quantities which are harmful to man, other living things or in some way reduce the quality of human life. These substances which make the environment impure are called pollutants.

When there is alteration in the composition or condition either directly or indirectly the environment is said to be polluted which is as a result of man's daily activities that makes it less favourable in its natural state (Ekweozor, 1987).

Solanum melongena **(eggplant)** is a species of night shade grown for its edible fruit. Eggplant is the common name in North America, Australia and New Zealand; in British it is *aubergine*, and in South Asia and South Africa, brinjal. As a member of the genus *Solanum*, it is related to the tomato and the potato (Doijode, S.D. 2001).

Phaseolus vulgaris **(bean or cowpea)** is an annual herbaceous legume from the genus *Vigna*. Due to its tolerance for sandy soil and low rainfall it is an important crop in the semi-arid regions across Africa and other countries. The size and shape of the leaves varies greatly, making this an important feature for classifying and distinguishing cowpea varieties (Pottorff, *et. al.*, 2012).

***Cucumis sativus* (cucumber)** is a widely cultivated plant in the gourd family, Cucurbitaceae. It is a creeping vine that bears cucumiform fruits that are used as vegetables. There are three main varieties of cucumber: slicing, pickling, and seedless. Within these varieties, several cultivars have been created. The cucumber is mainly from south Asia where a great many varieties have been observed (Doijode, S.D. 2001),

Materials and Methods

Experimental site

The study site is characterized by tropical monsoon climate with mean annual temperature of 32.15^0C, 66% humidity and 0.9948 atmospheric pressure, while, the soil is usually sandy or sandy loam underlain by a layer of impervious pan.

The study site was situated at the Rivers State University Agricultural Farm which functions under the Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria. An area of 20m x 10m was marked out with a measuring tape and then cleared to ground level.

Planting Materials

Treated seeds of garden egg (*Solanum melongena*), beans (*Phasseolus vulgaris*) and cucumber (*Cucumis sativus*) were the planting materials used for the experiment. They were obtained from ADP (Agricultural Development Programme) Rumuokoro, Port Harcourt in Rivers State, Nigeria.

Experimental bags

A total of 210 experimental bags filled with soil were used for the whole experiment. 70 experimental bags were used for each plant. The experimental bags were purchased from mile 3 market, Port Harcourt, and filled with 10kg soil. The bags were punctured on all sides and beneath to prevent water logging of the experimental bags.

Crude oil

100 litres of crude oil was purchased from the Port Harcourt Refinery, Eleme, Rivers State, and conveyed to the Rivers State University Research Farm and applied as a pollutant on the agricultural soil.

Fertilizer

Organic and inorganic fertilizers were used to carry out this experiment. The organic fertilizer used was *Terminalia catappa* (leaf litter) while the inorganic fertilizer was Hydrogen peroxide (H_2O_2). The *Terminalia catappa* was obtained from a site in the Rivers State University while the hydrogen peroxide was obtained from a scientific supply shop in Alakahia, Port Harcourt. The leaf litters were dried and analysed before use.

Amendment Materials and Treatment

The following materials were used as remediation agents: leaf litter and hydrogen peroxide. The experiment was in 3 blocks for each plant. Block 1; 10kg of soil was used with 100, 200, 300, 400 and 500ml of crude oil amended with leaf litter (100,200,300,400,500g) and hydrogen peroxide (100,200,300,400 and 500ml) with some unpolluted soils that served as the control. The same experiment was replicated for blocks 2 and 3 respectively.

Levels of Crude Oil Pollution and Amendments with Organic and Inorganic Fertilizers on Phaseolus Vulgaris, Cucumis Sativus and Solanum Melongena

T1	100ml crude oil + 100ml H_20_2
T2	100ml crude oil + 100g leaf litter
T3	200ml crude oil + 200ml H_20_2
T4	200ml crude oil + 200g leaf litter
T5	300ml crude oil + 300ml H_20_2
T6	300ml crude oil + 300g leaf litter
T7	400ml crude oil + 400ml H_20_2
T8	400ml crude oil + 400g leaf litter
T9	500ml crude oil + 500ml H_20_2
T10	500ml crude oil + 500g leaf litter
T11	NO POLLUTION + 500ml H_20_2
T12	NO POLLUTION + 500ml leaf litter
T13	500ml crude oil + NO AMENDMENT
T14	CONTROL

Planting of Garden Egg, Beans and Cucumber

Planting of garden egg, beans and cucumber was carried out 4 months after treatments were added to the soil.

Vegetative Growth Determination

Recovery of soil as indicated by growth of garden egg, beans and cucumber was investigated. The soil recovery potential was monitored by planting garden egg, beans and cucumber on experimental bags (treated and untreated) and observation was made on the growth parameters as the plants responded to the various treatments. The soil recovery was determined by measuring height, leaf area, stem girth and number of leaves produced by garden egg, beans and cucumber in each of the treatments. Growth parameters were measured every two (2) weeks for a period of 12 weeks.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA). The means were separated using Least Significant difference (LSD) at 5 % level of probability. The statistical tool employed was Duncan Multiple Range Test (DMRT) (Eckman, S. 2018).

Results

Plant Morphological Parameters

Mean Plant Heights of Garden Egg, Beans and Cucumber

The result for the total plant height of garden egg, beans and cucumber is as shown in figure 1. The results showed that beans plant had the highest plant height in all the treatments followed by cucumber, the least plant height was observed in garden egg. The results also showed that all the plants in the unpolluted soil had higher plant heights than those in the polluted and amended soils, especially in T12 (25.4, 798.0 and 220.8). T13 (polluted soil without amendment) had the lowest plant heights for the three plants (12, 294.4 and 18.8) except for garden egg with a mean height of 0.4. The results also showed no significant difference in height between T1 (100 ml crude oil + soil + 100 ml H_2O_2)., T2 (100 ml crude oil + soil + 100 g LL), T3 (200 ml crude oil + soil + 200 ml H_2O_2), T5 (300 ml crude oil + soil + 300 ml H_2O_2), T7, T9, T10, T11, T12, T13 and T14 in garden egg at $p= 0.05$, while there was an observable significant difference between T1, T4, T6, and T8 at $p=0.05$. In beans plant, the results showed that there were significant increases in height between T11 (629.2) and T12 (798.0), and all the other treatments. There was no significant difference in height between T13 (294.4), T3 (320.0), T7 (321.8), T9 (322.6), T5 (324.4), T14 (330.2) and T1 (334.4). For cucumber, the results showed that there was a significant increase in height between T12 and all the other treatments at p=0.05.

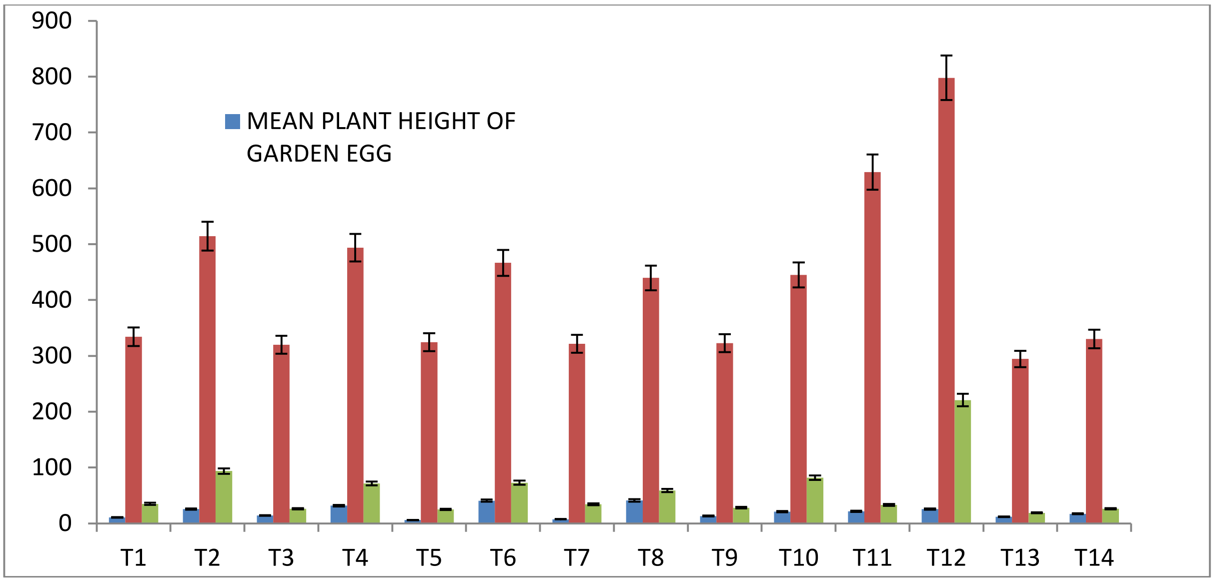

FIG 1: EFFECT OF ORGANIC AND INORGANIC MANURE ON MEAN PLANT HEIGHTS OF GARDEN EGG, BEANS AND CUCUMBER ON CRUDE OIL POLLUTED SOIL. T1 = (100ml crude oil + 100ml H_20_2), T2 = (100ml crude oil + 100g leaf litter), T3 = (200ml crude oil + 200ml H_20_2), T4 = (200ml crude oil + 200g leaf litter), T5 = (300ml crude oil + 300ml H_20_2), T6 = (300ml crude oil + 300g leaf litter), T7 = (400ml crude oil + 400ml H_20_2), T8 = (400ml crude oil + 400g leaf litter), T9 = (500ml crude oil + 500ml H_20_2), T10 = (500ml crude oil + 500g leaf litter), T11 = (no pollution + 500ml H_20_2), T12 = (no pollution + 500g leaf litter), T13 = (500ml crude oil + no amendment), T14 = (control).

Mean Stem Girth of Garden Egg, Beans and Cucumber

The result for the mean stem girth of garden egg, beans and cucumber is shown in figure 2. The results showed that beans plant had the highest stem girth in all the treatments followed by cucumber, while the least stem girth was seen in garden egg. The results also showed that T12 (Unpolluted soil + leaf litter) had the highest stem girth in beans (7.1) and cucumber (5.16), whereas T4 (200 ml crude oil + soil + 200 g LL) had the highest stem girth in garden egg (2.54) followed by T8 (2.3). The results also showed that T13 (Polluted soil without amendment) had the lowest stem girth in all the three plants analyzed (0.38, 4.1 and 1). From the ANOVA, T12 (Unpolluted soil + LL) recorded a significantly higher stem girth in beans (7.1) and cucumber (5.16) in all the treatments. However, garden eggplant showed no significant difference in stem girth in all the treatments. For beans, there were significant differences between T12 (7.1) and all the other treatments at p=0.05.

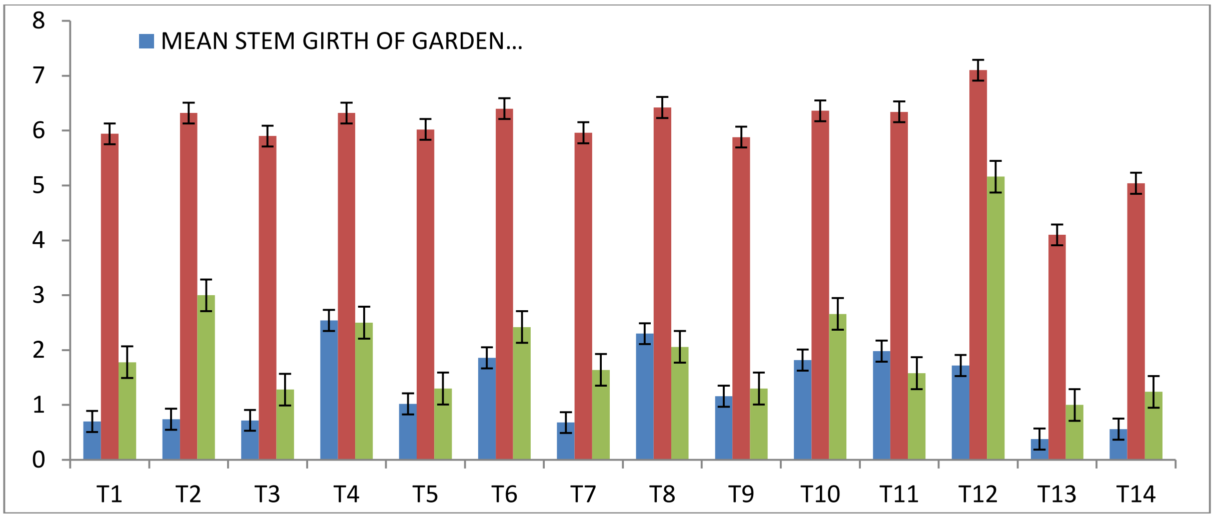

FIG 2: EFFECT OF ORGANIC AND INORGANIC MANURE ON MEAN STEM GIRTHS OF GARDEN EGG, BEANS AND CUCUMBER ON CRUDE OIL POLLUTED SOIL. T1 = (100ml crude oil + 100ml $H_2 0_2$), T2 = (100ml crude oil + 100g leaf litter), T3 = (200ml crude oil + 200ml $H_2 0_2$), T4 = (200ml crude oil + 200g leaf litter), T5 = (300ml crude oil + 300ml $H_2 0_2$), T6 = (300ml crude oil + 300g leaf litter), T7 = (400ml crude oil + 400ml $H_2 0_2$), T8 = (400ml crude oil + 400g leaf litter), T9 = (500ml crude oil + 500ml $H_2 0_2$), T10 = (500ml crude oil + 500g leaf litter), T11 = (no pollution + 500ml $H_2 0_2$), T12 = (no pollution + 500g leaf litter), T13 = (500ml crude oil + no amendment), T14 = (control).

Mean Leaf Number of Garden Egg, Beans and Cucumber

Figure 3 shows the mean leaf number of the three crops grown in crude oil polluted soil amended with hydrogen peroxide (H_2O_2) and leaf litter (LL). The results for garden egg showed that the highest leaf number was observed in T8 (63.6), followed by T6 (63.2). The results showed no significant difference in leaf number between T6 (63.2) and T8 (63.6) and between T1 (22.4) and all the other treatments at p=0.05. For beans plant, the highest mean leaf number was recorded in treatment 12 (64.4), followed by treatment 14 (62.2), while the least leaf number was seen in T 10 (29.6). There was however no significant difference between treatments 12 (64.4), and treatment 14 (62.2), and between treatments 1 (41.4) and all the other treatments. In cucumber, T12 had the highest mean leaf number (50.0), followed by treatment 2 (46.8). The least mean leaf number was observed in 13 (14.4). The result showed significant differences between treatments 12 (50.0) and all the other treatments. There was no significant difference between treatments 2 (46.8), 6 (34.2), and 10 (37.4). When the three plants analyzed were compared, it was observed that T13 had the least leaf number in all the plants (17.4, 19.2 and 14.4), while T12 had the highest mean leaf number in all the plants (20.8, 62.2 and 21.4).

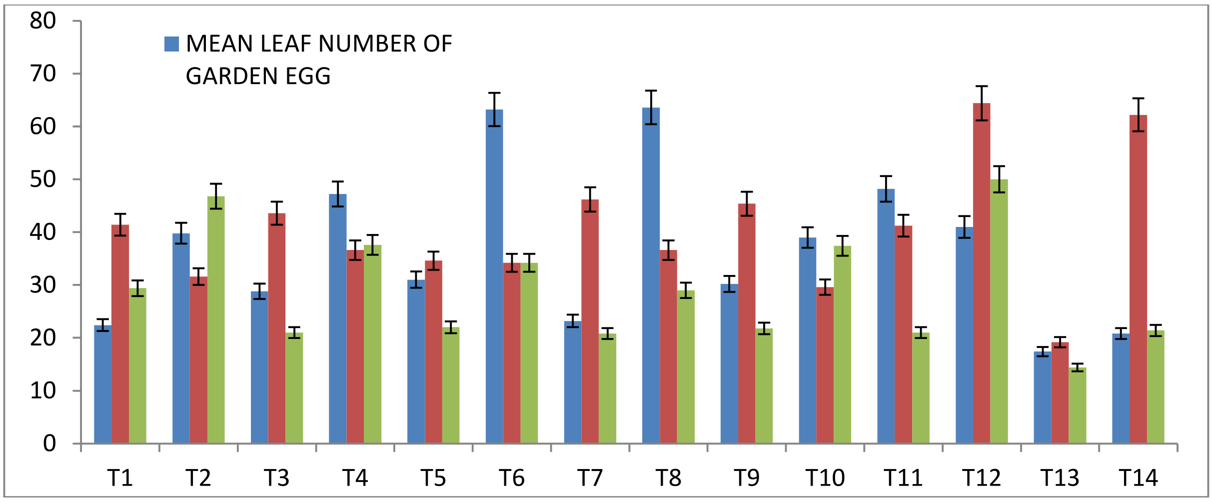

FIG 3: EFFECTS OF ORGANIC AND INORGANIC MANURE ON MEAN LEAF NUMBER OF GARDEN EGG, BEANS AND CUCUMBER ON CRUDE OIL POLLUTED SOIL. T1 = (100ml crude oil + 100ml H_20_2), T2 = (100ml crude oil + 100g leaf litter), T3 = (200ml crude oil + 200ml H_20_2), T4 = (200ml crude oil + 200g leaf litter), T5 = (300ml crude oil + 300ml H_20_2), T6 = (300ml crude oil + 300g leaf litter), T7 = (400ml crude oil + 400ml H_20_2), T8 = (400ml crude oil + 400g leaf litter), T9 = (500ml crude oil + 500ml H_20_2), T10 = (500ml crude oil + 500g leaf litter), T11 = (no pollution + 500ml H_20_2), T12 = (no pollution + 500g leaf litter), T13 = (500ml crude oil + no amendment), T14 = (control).

Mean Leaf Area of Garden Egg, Beans and Cucumber

Figure 4 shows the mean leaf area of garden egg, beans and cucumber grown in crude oil polluted soil amended with hydrogen peroxide (H_2O_2) and leaf litter (LL). In garden egg, it was observed that T12 (unpolluted soil + LL) had the highest mean leaf area (357.6) followed by T11 (293.8), while the least leaf area was seen in T13 (64.6). The result showed significant difference in all the treatments except for treatments 1 (146.2), T3 (145.6), T5 (145.0), T7 (142.8), T9 (151.8).T14 had low values (88.4). The results for beans plant showed that treatment 12 had the highest leaf area (991.4) followed by treatment 11 (968.4) while the lowest was seen in treatment 13 (292.8). The result showed significant difference in all the treatments at p=0.05. In cucumber, T12 had the highest leaf area (1019.0) amongst all the plants sampled, followed by T11 (1006.6) while the lowest leaf area in cucumber was observed in T5 (158.0). There was a significant difference in leaf area between T12 (1019.0) and all the other treatments in cucumber.

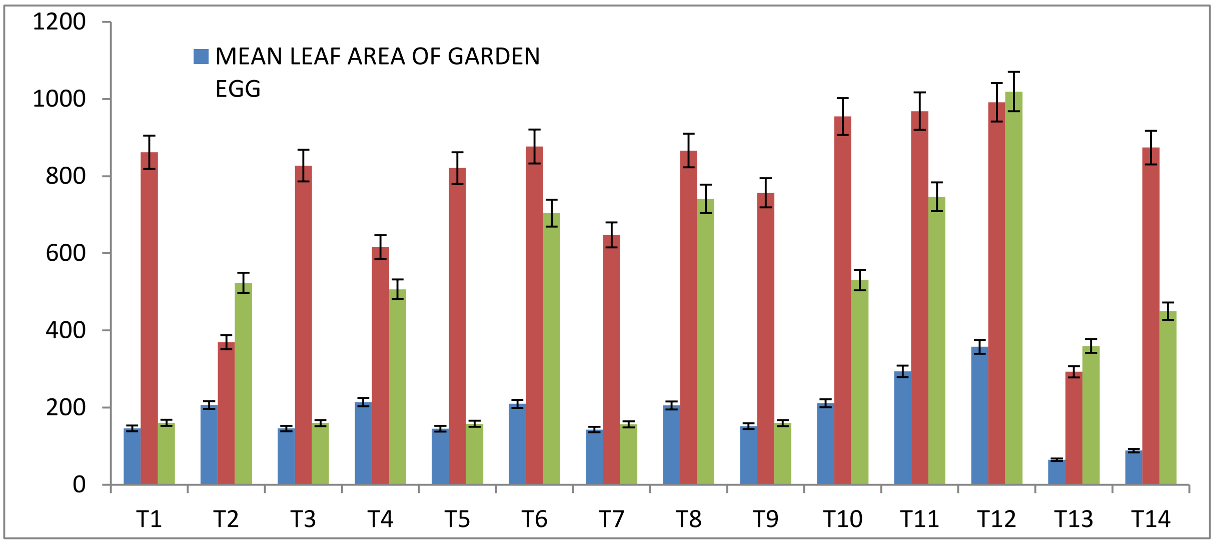

FIG 4: EFFECTS OF ORGANIC AND INORGANIC MANURE ON MEAN LEAF AREA OF GARDEN EGG, BEANS AND CUCUMBER GROWN IN CRUDE OIL POLLUTED SOIL. T1 = (100ml crude oil + 100ml H_20_2), T2 = (100ml crude oil + 100g leaf litter), T3 = (200ml crude oil + 200ml H_20_2), T4 = (200ml crude oil + 200g leaf litter), T5 = (300ml crude oil + 300ml H_20_2), T6 = (300ml crude oil + 300g leaf litter), T7 = (400ml crude oil + 400ml H_20_2), T8 = (400ml crude oil + 400g leaf litter), T9 = (500ml crude oil + 500ml H_20_2), T10 = (500ml crude oil + 500g leaf litter), T11 = (no pollution + 500ml H_20_2), T12 = (no pollution + 500g leaf litter), T13 = (500ml crude oil + no amendment), T14 = (control).

Discussion

Germination and Growth Parameters

The presence of crude oil and its derivatives in soil caused a delay in the growth of garden egg and cucumber and inhibition in seed germination in all the treatments except for those without crude oil pollution. Bean seeds germinated 4 days after planting but the garden egg and cucumber seeds had a delay in emergence in all the polluted soils except the unpolluted soils that germinated at 4 days. Bean seeds germinated on all the treatments (both polluted and unpolluted soils) at 4 days because of its nitrogen fixing capabilities and its ability to withstand polluted environments hence a hyper accumulator. Garden egg and cucumber seeds germinated only on some of the treatments but most of the samples died before harvest due to the presence of this petroleum hydrocarbon that had altered the growth of these plants. There was inhibition and numbers of seeds germinated were reduced and did not look healthy. This might have been as a result of seed viability losses. Cowpea (Beans) has the ability to withstand adverse environmental conditions and as such recorded better yield and yield components on both the polluted (T1-T10 and T13) and unpolluted soils (T11, T12, T14) in the whole experiment. However, garden and cucumber had no yield on the polluted soils except on unpolluted and amended soils which gave little yields for both plants. The least germination percentage observed in T13 (500ml crude oil + no amendment) might be due to the fact that soils polluted with crude oil are hindered from free flow of air (oxygen) (Chuku *et al.*, 2017). It has been reported that the ability of microbes capable of degrading crude oil which inhibit seed germination are suppressed due to crude oil pollution (Basra *et al.*, 2006). It also infers that petroleum delays and decreases seed germination especially at high concentrations (Chuku *et al.*, 2017). The result from this study agrees with the work of Amadi *et al.*, (1993) and Onuh *et al.*, (2008). Also, the inhibition of germination especially at the 400-500 vol/kg pollution level could be because the seed were soaked with oil which caused them to be swollen and slimy, as was observed when the un-germinated seeds were dug out from the soil. This also is in line with the study of Amakiri and Onofeghara (1983) whose work on the impact of petroleum contamination on *Zea mays* and *Capsicum fruitescens* observed a notable reduction in the germination rate. Leaf litter plays a fundermental role in the nutrient turnover and in the transfer of energy between plants and soil, the source of nutrients being accumulated in the uppermost layers of the soil (Singh, 1971).

The improvement in plant growth (height) in the nutrient supplemented soils could be as a attributed to the fact that the leaf litter and hydrogen peroxide acted as a means of nutrient supply to the soil which had obvious results in the soils with beans as sample crops.
The improved plant height of melon observed in the amended soils may be due to the enrichment of the soil resulting from the organic and inorganic amendment of the polluted soils. The same trend was also observed for plant height of beans, cucumber and garden egg with time. Madukwe *et al*., 2008, and Onuh *et al*., 2008 also reported the annexation of organic manure on the growth and morphology of crops and also to the reports of Rose *et al.* (1996) who observed that the continuous application of higher amounts of manure would lead to an increases plant nutrients and organic matter in the soil, which generally improves the physico-chemical characteristics of the soil. The poor performance and death of the garden egg and cucumber crops grown in polluted and amended soils (T1-T10 and T13) showed that petroleum pollution slows down the growth of plants as supported with the research of Agbogidi *et al.*, (2007). Furthermore, the high performance of beans could be as a result of its nitrogen fixing and hyper accumulative capabilities (Tanee and Akonye, 2009).

The leaf area of garden egg, beans and cucumber improved with the addition of the organic manure (leaf litter) and inorganic manure (hydrogen peroxide) which is an oxygen supplier. The improved leaf area of the crops in soil treated with leaf litter and hydrogen peroxide could be inferred to the stabilization of the essential nutrients needed for plant growth which are already present in the soil. The reduced leaf area in the densely polluted soils (T9, T10 and T13) in garden egg and cucumber soils shows how petroleum adversely affects plant leaf area at higher concentrations. This is in agreement with the research of Kelechi *et al.*, (2008).
The leaf number increased in the three test plants with the application of leaf litter and hydrogen peroxide (oxygen supplier) as the number of weeks increased. The increase could be inferred that the increase in leaf number in these treatments is dependent on the soil nutrient supply, though, the leaf number in the garden egg and cucumber plants were not appreciable which could be as a result of the fact that they do not have the capability to withstand or grow well in petroleum hydrocarbon polluted soils.

For organic amendments, the leaf number with the highest values occurred in the densely ammended soils (T9, T10, T11 and T12) while the polluted soils without amendment (T13) had the lowest which decreased with time 12 WAP. Amakiri and Onofegara, (1984) gave a report that oil physical characteristics inflicted some stressful conditions, which may have hindered water uptake and exchange of gases. Anoliefo and Edegbai, (2000) emphatically stated that physiological drought may occur as a result of the stressful conditions. Plant leaves in soil that have been contaminated curl upwards, gradually fade-off, and dies. Treatment 14 had reduced growth parameters in beans, garden egg and cucumber which could be due to the fact that most crops need extra supply of nutrient (either organic or in organic manure) for healthy growth for agriculture that is productive and profitable.

The stem girth of beans, garden egg and cucumber grown in both leaf litter and hydrogen peroxide amended soils increased with increase in time. The highest stem girth for the 3 plants were seen in the leaf litter amended soils with no pollution, while the other treatments performed well but not as much as the leaf litter amended soils. Organic fertilizer greatly enhanced the stem girth upon its addition.

Conclusion

The study has given appreciable details of how crude oil and its derivatives affect the soil physico-chemistry and all other parameters adversely, on productivity of crops which comprises of inhibition of germination, growth, yield and yield components.

From the revealed results, beans performed better when compared to cucumber and garden egg in the whole experiment because of its hyper accumulative and nitrogen fixing potentials. Cucumber and garden egg had very poor growth parameters and poor yield on all the treatments. From the results, both the polluted, unpolluted and polluted and amended soils performed extremely well in the beans experiment whereas unpolluted and leaf litter amended soils were the only soils that produced yield in the cucumber and garden egg soils, while the other treatments had no yield. From the results, the polluted and leaf litter amended soils performed extremely better than the control soils in the bean experiment which implies that regardless of the condition of the soil, beans requires organic amendments for maximum yield.

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