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Physico-chemical properties of soil polluted with petroleum crankcase oil and chlorophyll concentration of *Abelmoschus esculentus* (okra).

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Abstract

Effect of petroleum crankcase oil (PCO) on soil physico-chemical parameters and chlorophyll content of *Abelmoschus esculentus* (Okra) was investigated. Garden topsoil was collected from Obingwu, Ohii in Owerri West Local Government Area of Imo State, Nigeria tested for soil physico-chemical parameters, weighed and polluted with different volumes of PCO to give the various percentage pollutions. Five viable seeds of *A. esculentus* were planted in each. An unpolluted soil sample served as the control for the investigation. Harvest of the leaves and fruits was done immediately after fruiting and taken to the laboratory for the assessment of soil physico-chemical parameters and chlorophyll concentration. Results obtained for soil pH and soil phosphate show there was no significant difference ($p>0.05$) at the various levels of PCO pollution. However, there was a significant increase ($p<0.05$) in the percentage moisture content and calcium carbonate with respective increase in PCO pollution while a significant decrease ($p<0.05$) was observed in organic carbon, nitrogen and chlorophyll content of the PCO polluted samples when compared with the control. Furthermore, at 6% PCO pollution, only two seeds sprouted and leaf growth was not sustained till the end of the experiment. These results indicate that soil physico-chemical parameters and chlorophyll concentration of *A. esculentus* leaves were responsive to the detrimental effects of PCO in areas where it is disposed indiscriminately.

Keywords: *Abelmoschus esculentus*, pollution, petroleum crankcase oil (PCO), soil, chlorophyll.

1. Introduction

Nigeria is a major world producer of crude oil. Pollution of the environment due to crude oil spillage and its allied products has steadily increased as a result of oil exploration, industrialisation and incessant disposal of crude products and wastes by road side automobile mechanics, maintenance workshops, and dealers (Kori – Siakpere, 1998). The role of oil and gas industries in improving the quality of life and economic development in Nigeria cannot be overemphasized. Alongside the good things that brought an enhancement in the standard of living was the emergence of deleterious substances in the environment following oil and gas activities (Nwaogu *et al* 2012). These industrial pollutants, including CO_2 from exhausts of automobiles and other pollutants such as heavy metals, constitute an important source of environmental pollution. In oil and gas operations, crude oil, corrosive acid waste, toxic chemicals and other harmful industrial wastes are intermittently released into the environment (air, soil and water) (Rowell, 1977). Sulphur and other toxic gases by oil companies are usually released into the atmosphere. These, together with injected particulates and unburned hydrocarbons undergo series of chemical reactions in the presence of sunlight, resulting in dense characteristic smog (Bamidele, 2000). The implication of petroleum hydrocarbon pollution in agricultural production system and the environmental impact associated with the exploration and exploitation of crude oil has been an area of great interest in the last three decades. The increase in demand for petroleum and its products as a source of energy and primary raw material for petrochemical industries has resulted in a corresponding increase in the harm caused by crude oil and its allied products like petroleum crank case oil (Odiete, 1999).

Abelmoschus esculentus (okra), known in many English-speaking countries as lady's fingers or gumbo is a flowering plant in the mallow family. It is one of the most important fruit vegetable crops and a source of calorie (4550kcal/kg) for human consumption and ranks first before other vegetable crops (Babatunde *et al*. 2007). It is an important vegetable and widely distributed from Africa to Asia, Southern Europe and America (Khomsug *et al*. 2010). *Abelmoschus esculentus* is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. It is among the most heat- and drought-tolerant vegetable species in the world and will tolerate soils with heavy clay and intermittent moisture, but frost can damage the pods (Okafor & Fernandes 1987), Okra cultivation and production has been widely practiced because of its importance to the economy development and can be found in almost every market in Africa. Varieties vary by plant height, size of fruit, colour, early or late maturing etc., (Udoh *et al*. 2005).

Germination occurs between six days (soaked seeds) and three weeks. Seedlings require ample water. The seed pods rapidly become fibrous and woody, and, to be edible, must be harvested within a week of the fruit having been pollinated. The fruits are harvested when immature and eaten as a vegetable.

Soil pollution especially through oil spillage has been shown to have significant impact on plant growth, yield and performance (Inoni *et al.* 2006; Ngoku *et al.* 2008; Agbogidi, 2009; Agbogidi, and Enujeke, 2012). Previous study has revealed that petroleum crankcase oil often resulted in insufficient aeration of the soil due to the displacement of air from the spaces between the soil particles; retarded the growth of plants, resulted in the chlorosis of leaves, and the dehydration of plants (Rowell, 1977). This research was therefore, carried out to investigate the effects of petroleum crankcase oil on soil physico-chemical parameters and the chlorophyll level of *Abelmoschus esculentus* (okra).

2. Materials and Methods

2.1 Collection of soil samples

Viable okra seeds were bought from.Garden top soil was collected from Obingwu, Ohii in Owerri-West Local Government Area of Imo State, Nigeria. The soil sample was tested for soil physicochemical parameters (moisture content, pH, nitrogen content, phosphorus, organic carbon, carbonate, etc.) to ensure that *A. esculentus* suffers no other stress except that induced by the PCO pollution. 4900g of the soil was weighed out into four places labelled A, B, C and D respectively. 49mL, 147mL and 294mL of PCO obtained from an electric generating plant were added into A, B and C and stirred thoroughly to represent 1%, 3% and 6% polluted soil samples respectively and poured into nursery containers. Sample D had no PCO and thus served as the control for the experiment. The entire set up was kept for 3 days and adequately watered. In each of the containers, six viable okra seeds were planted, and seed germination potential and growth were monitored. The nursery containers were labelled accordingly.

2.2 Preparation of Extract

The leaves of *A. esculentus* were thoroughly washed with distilled water and then homogenized separately with respect to their percentage PCO pollution in sodium-phosphate buffer at pH 7.4 using Kenwood homogenizer. Extraction was done as described by Levine *et al.* (1990). The homogenates were stored at $4^{O}C$.

2.3 Determination of soil physicochemical parameters

Soil pH was measured using Beckman's glass electrode pH meter (AOAC, 2003). While moisture content was calculated on the basis of the air dry weight as described by Buurman, *et al.* (1996), the percentage organic carbon was calculated by the method described by Walkely and Black, (1934). One gram of soil was weighed into a 500ml conical flask and 10ml of 1.0M $k_2Cr_2O_7$ and 20ml of concentrated H_2SO_4 were added simultaneously. The flask and its content was swirled and allowed to stand for 30mins. Two hundred ml of distilled water was added slowly followed by 10ml H_3PO_4. Then, 1ml diphenylamine indicator was added and titrated against 0.5M ferrous ammonium sulphate solution until the appearance of green colour which indicated the end point. A blank was run simultaneously.

% Organic carbon = (10(B-S) x 0.39 x mcf)/(B x W)

Where

B = number of mls of ferrous ammonium sulphate solution used for blank.

S = number of mls of ferrous ammonium sulphate solution used for sample.

Mcf = Moisture Correction Factor

W = Sample weight (g)

0.39 = Conversion factor (including a correction factor for a supposed 70% oxidation of organic carbon.

Nitrogen concentration was determined according to the method described by Dhyan Singh *et al.* (1999). The sample was digested in a mixture of H_2SO_4, K_2SO_4 and selenium which converted all nitrogen into ammonium sulphate. The distillation of ammonia (liberated after sodium hydroxide was added to ammonium sulphate), over boric acid and titrated against standard acid to determine the nitrogen content.
Total Nitrogen in soil (mg/kg) = ((S-B) x N x 14)(1000)/ Sample weight (g).
Where

S = Volume of acid used against sample.

B = Volume of acid used against blank.

N = Normality of the acid.

The Piper method by Dhyan Singh *et al.* (1999) was used to determine the carbonate content in the soil. Five g of the soil sample was weighed into 250ml conical flask and 100ml of 1M HCl was added using a pipette, this was thoroughly shaken and kept to stand overnight. It was shaken again for two hours and allowed for the suspension to settle. Five ml of the supernatant was pipette into a 100ml conical flask and 10ml of distilled water was added. Three drops of phenolphthalein indicator were added and titrated against 0.25M NaOH until there was a colour change to purple.

$CaCO_3$ (%) = ((B-S) x N x 100) x Mcf)/ Sample weight (g)

Where

B = ml NaOH used for blank

S = ml NaOH used for sample

N = Normality of NaOH

Mcf = Moisture Correction Factor

100 = Conversion factor

Phosphorus was determined by the method of Lim, (2011). Ten mL of the acid digest of the soil sample was placed in a 50ml volumetric flask, 10ml of vanadate-molybdate reagent was added and diluted to 50ml with distilled water. The contents were mixed thoroughly and the absorbance read after 10mins in a spectrophotometer at 420nm as a function of phosphorous concentration. Zero, 1, 2, 3, 4 and 5ml of the 100mg/l phosphorus solutions were taken in 50ml volumetric flask and colour developed in identical manner. The spectrophotometer was calibrated with known phosphorus concentration and the individual sample concentrations read with the standard.

2.4 Preparation of Vanadate - molybdate reagent

Solution A: A 2.5g of ammonium molybdate $(NH_4)_6\,Mo_7O_{24}.4H_20$ was dissolved in 30.0mL of distilled water. Solution B: A 0.125g of ammonium metavanadate was dissolved by heating to boiling point in 30mL of distilled water. This was cooled and 33mL concentrated HCL was added. Solution C: After cooling to room temperature, Solution A was mixed with Solution B and diluted to 100mL.

2.5 Determination of chlorophyll content

Chlorophyll content was estimated using the method described by Singh and Rao (1981). Fifteen g representative of the leaf sample was crushed and homogenized with quartz sand in a quartz mortar and pestle with 80% acetone. The suspension was quantitatively placed in large centrifuge tubes and centrifuged for 5mins at maximum speed of 4500rpm. The supernatant was decanted into a 25ml volumetric flask. The pellet was extracted with 5ml of 80% acetone and centrifuged again at 3000rpm for 5mins. This was repeated until the green colour of the pellet disappeared. The samples were left to warm up by bringing them out from the refrigerator and kept at room temperature and the supernatant in the volumetric flask was made up to 25ml with 80% acetone. The absorption of the chlorophyll extracts was measured with a spectrophotometer at the wavelengths of 663, 646 and 710nm respectively. The zero absorption with 80% acetone was determined at every wavelength whereas the wavelength of 710nm was used to determine the absorption of possible compounds that may interfere with the measurements of the chlorophyll. The absorption at 652nm wavelength was sufficient for total chlorophyll content. Results were all taken in triplicates.

2.6 Statistical analyses

Data were analyzed using one-way student's T-Test while results were expressed as mean ± SEM and the probability tested at 95% level of significance ($p<0.05$).

3. Results

3.1 Germination Analysis

Results obtained from germination sequence of *A. esculentus* in various percentages of PCO polluted soil samples (Table 1) show that two seeds of *A. esculentus* sprouted on the 6th day while the remaining four sprouted on the 7th day in the unpolluted soil sample. However, sprouting was observed in the 1% polluted soils on day 7 (two seeds), 8 (three seeds), and 9 (one seed), while for the 3% polluted soil, day 8 (one seed), 9 and 10 (two seeds each). Similarly, for 6% polluted soil, on day 9 and 10 (one seed each) while two seeds sprouted on day 11.

Table 1: Germination sequence of *A. esculentus* in different percentage PCO polluted soil samples

% Pollution	Day of sprouting					
	6th	7th	8th	9th	10th	11th
0	2	4	-	-	-	-
1	-	2	3	1	-	-
3	-	-	1	2	2	-
6	-	-	-	1	1	2

3.2 Soil physico-chemical parameters

3.2.1 Soil pH

The values obtained for soil pH before planting and after harvest are shown in Fig. 1. There was no significant difference ($p>0.05$) between the mean values obtained. However, there was a gradual decrease ($p<0.05$) in pH as the percentage of the pollutant increased from 0% to 6%.

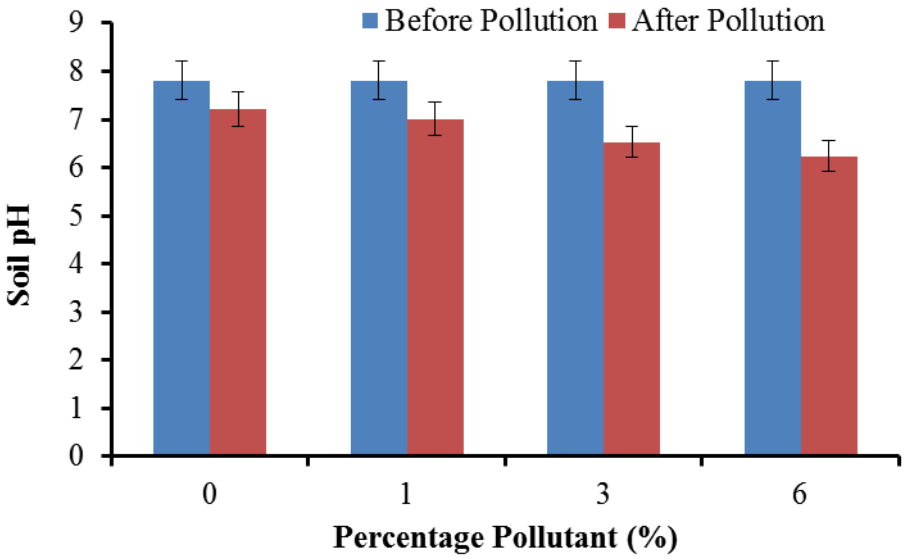

Figure 1: pH of the soil samples before planting and after harvest

3.2.2 Soil Moisture Content

The results obtained for percentage moisture content before planting and after harvest are shown in Fig. 2. The mean values obtained show there was significant difference ($p<0.05$) in all levels of pollution. The percentage moisture content increased with respective increase in PCO pollution.

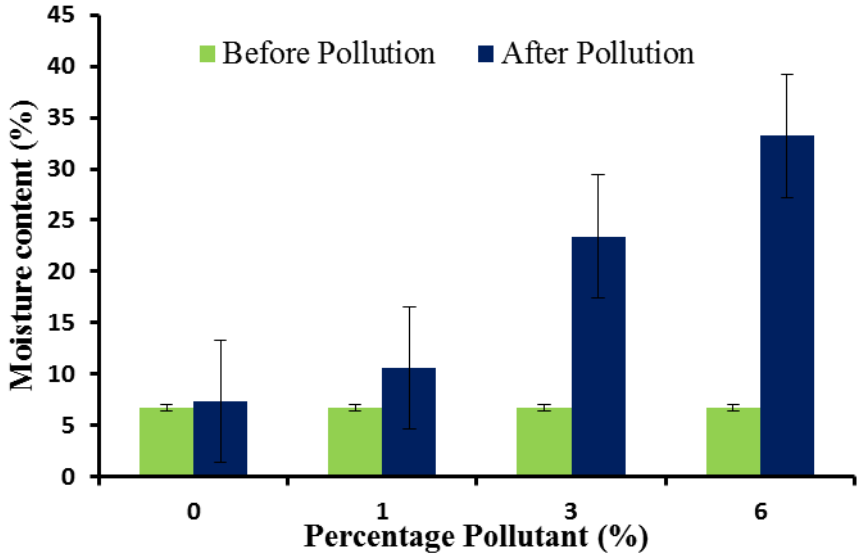

Figure 2: Percentage moisture content of the PCO soil before planting and after harvest

3.2.3 Soil Percentage Organic Carbon

The results obtained for soil organic carbon concentration in various percentages PCO polluted soil samples are shown in Fig. 3. There was significant difference ($p<0.05$) from the mean values obtained at the various levels of PCO pollution. However, soil organic carbon concentration increased progressively with respective increase in PCO pollution.

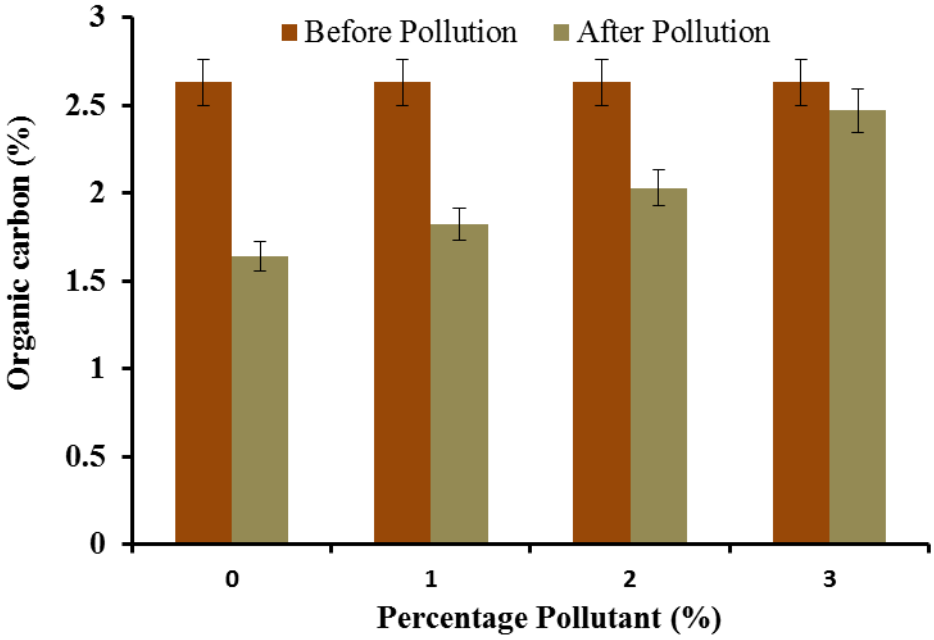

Figure 3: Percentage organic carbon concentration of the soil before and after PCO pollution

3.2.4 Soil Total Nitrogen Concentration

Results obtained for soil nitrogen content before and after PCO pollution are shown in Fig. 4. The mean values obtained at different percentage of soil pollution with PCO show that there was significant decrease ($p<0.05$) in Total nitrogen concentration as the percentage PCO in the soil increased progressively.

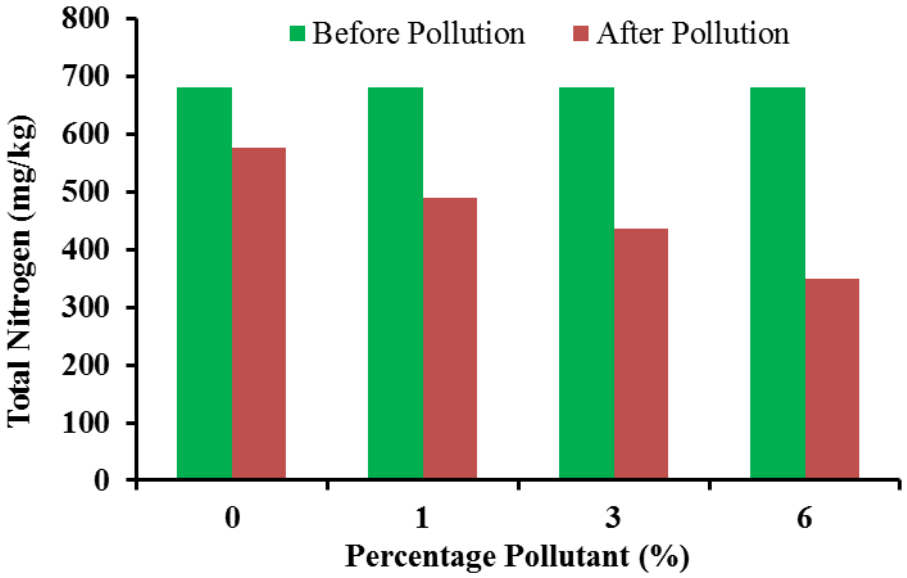

Figure 4: Soil Nitrogen concentration before planting and after harvest

3.2.5 Percentage Soil Carbonate

The results obtained for percentage soil calcium carbonate concentration before and after PCO pollution are shown in Fig. 5. Mean values obtained at various concentrations of PCO pollution show a significant increase ($p<0.05$) in PCO polluted soils when compared with the control.

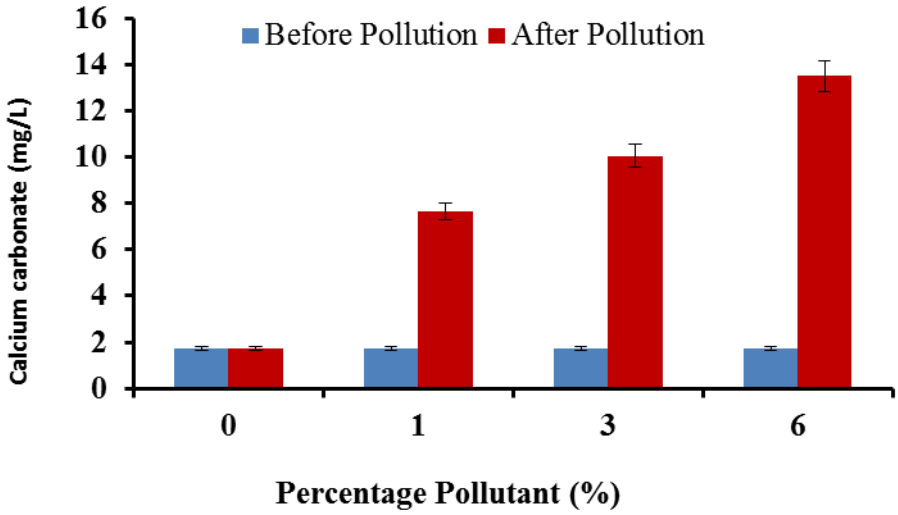

Figure 5: Percentage Calcium Carbonate concentration of the soil before planting and after harvest

3.2.6 Percentage Soil Phosphate concentration

Results obtained for soil phosphate (Fig. 6) showed that there was no significant difference ($p>0.05$) between the mean values obtained at all levels of pollution when compared to the control.

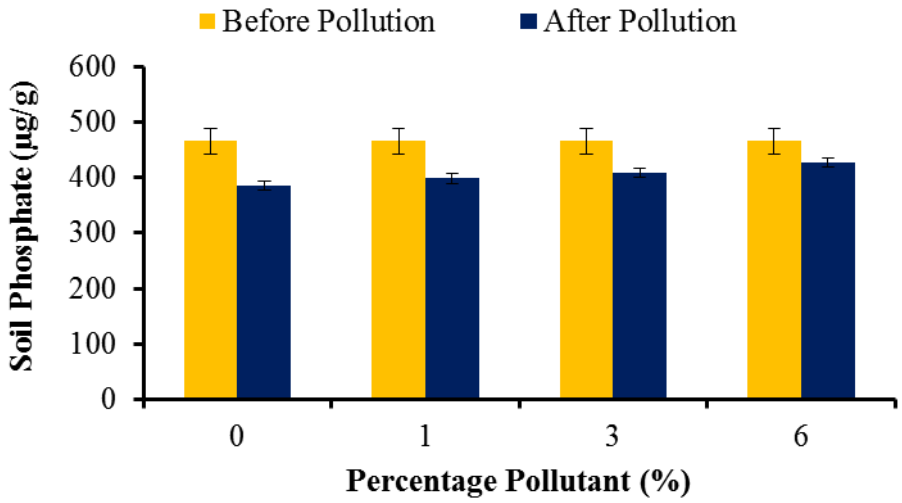

Figure 6: Soil phosphate concentration before planting and after harvest

3.2.7 Chlorophyll concentration

The results obtained for the chlorophyll concentration of *A. esculentus* in various percentages PCO polluted soil samples are shown in Fig. 7. The mean concentration values show that there was significant decrease ($p<0.05$) in the chlorophyll concentration of leaves of *A. esculentus* that germinated on soil with 1% and 3% PCO pollution when compared with the control. The concentration of chlorophyll in the leaves decreased respectively with increasing PCO pollution. However, there was no result of chlorophyll concentration for the 6% pollution group since germination of leaves was not sustained till the end of the experiment.

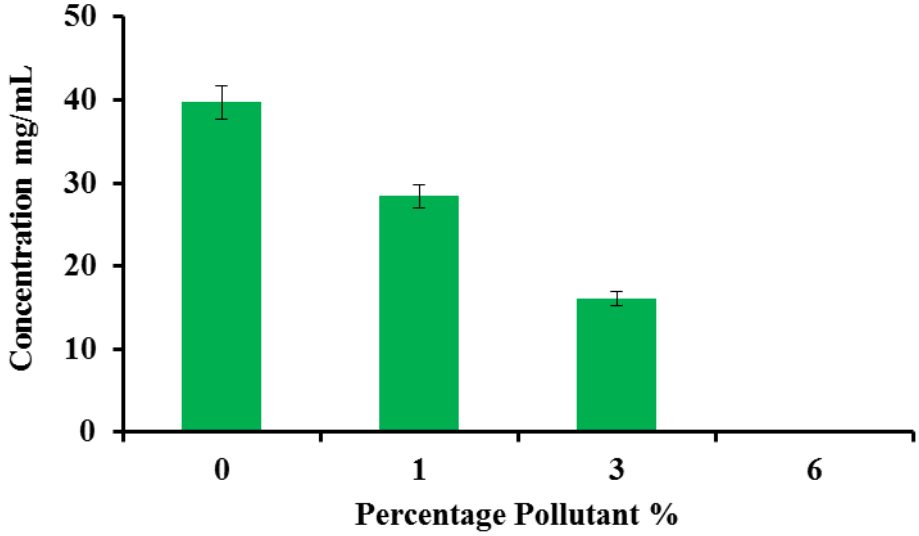

Figure 7: Chlorophyll concentration of *A.esculentus* leaves from PCO polluted soil samples.

4. Discussion and Conclusion

The results obtained from the germination sequence of the various seedlings of *A. esculentus* in the various polluted soil samples show that germination was delayed in the respective PCO oil polluted soils. The number of germinated seedlings was also affected by increased PCO pollution. This delay in germination and viability of seedlings over time could be attributed to inadequate air space (poor aeration) and increased temperature posed by high concentration of PCO in the soil samples. Plants grow well in soils that are well aerated, not water-logged; that have adequate water-holding capacity (Atuanya, 1987). Ekundayo *et al.* (2001) reported that PCO reduced germination by coating on seed surfaces thereby affecting physiological functions within the seed while Agbogidi *et al*. (2005b) showed that crude oil has a deleterious effect on plant growth. This finding supports the reports of Anoliefo and Vwioko (2001) on *Chromolaena odorata*, Sharifi *et al.* (2007) on six plant species, Agbogidi (2009a) on cowpea and Agbogidi & Enujeke (2012) on Arachis hypogaea L.

The mean value of the pH of soil sample before pollution (7.81±0.02) was slightly alkaline. The pH values of the polluted soil samples however decreased to 7.01±0.02, 6.53±0.01 and 6.24±0.03 in 1%, 3% and 6% pollution

respectively. Soil pH was slightly alkaline in the unpolluted soil sample, and became progressively acidic as the percentage pollution with PCO increased. The pH values fall within FEPA, (1997) permissible standard of 6-9. The lower mean pH values of polluted soils may be attributable to the higher concentrations of particulates from PCO. These particulates would normally settle on the top soil, and hence, affect its acidity.

Soil moisture is necessary for the proper germination of seeds, burrowing of soil by soil organisms and growth of plants (Page *et al.*1982). There was significant difference ($p<0.05$) in the mean values obtained for soil percentage moisture content in the three levels of pollution. Pollution by PCO may have resulted in the corresponding increase in water holding ability of the polluted soil samples with respect to PCO concentration. The high values obtained in percentage soil moisture content with respect to PCO concentration may be attributed to the PCO pollution in the soil samples, hence encouraging leaching of soil nutrients and creating unfavourable conditions for the proper growth and development of *A. esculentus* seeds.

Organic carbon is a major component of the soil organic matter. Organic matter in soil is derived from residual plant and animal materials decomposed by micro-organisms under the influence of temperature, moisture and optimal soil conditions (Amadi *et al.* 1993). The percentage organic matter is usually expressed first in terms of percentage organic carbon before being converted to organic matter by a factor of 1.729. Results from these physicochemical parameters indicate that percentage organic carbon was significantly ($p<0.05$) higher with corresponding PCO pollution than the control sample. This could be attributed to the high PCO pollution in the soil samples. Amadi *et al.* (1993) reported a value of 3.69% from crude oil-polluted soil as percentage organic carbon content. This disparity in percentage organic carbon may be due to the fact that composition of crude oil pollution and that of PCO differs.

Nitrates are essential for normal plant growth. Crops grown in soils with low nitrogen content can exhibit necrosis, wilting and chlorosis (Nwaogu *et al.* 2012). There was a gradual decrease in total nitrogen concentration after harvest when compared to their initial concentrations. The plant may have utilized nitrogen for its metabolic processes and hence, the corresponding decrease observed in the various samples.

Phosphates are required by living organisms for their normal metabolic and physiological processes (Nwaogu *et al.* 2012). This high energy compound is translocated via the root to other parts of the plant with other soil nutrients. There was no significant difference ($p>0.05$) at the three levels of PCO pollution but there was significant difference ($p<0.05$) between the polluted samples and the control sample after harvest. There was a significant reduction ($p<0.05$) in the concentration of soil phosphate in the control soil when compared to the polluted soils. The uptake of phosphate by plants via translocation might have been restricted accordingly with increasing PCO pollution in the soil samples hence resulting in the accumulation of phosphate in the polluted soils. This high phosphate concentration in the polluted soils may be attributable to the exposure of the soils to pollution by PCO.

Chlorophyll plays an important role in the absorption of light energy of the sun by plants and in the manufacture of plant food (Woodward *et al.*1990). There was significant difference ($p<0.05$) at the three levels of pollution in the mean chlorophyll concentration of *A. esculentus* leaves treated with different concentrations of PCO. Results of chlorophyll concentration in the respective samples show that chlorophyll synthesis in *A. esculentus* is inhibited as the concentration of PCO increased, hence the wilting and necrosis observed in 6% polluted soil sample. The unavailability of chlorophyll for the plant physiological processes however affected the yield and performance of *A. esculentus*.

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