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Spent Lubricating Oil Decreased Vegetative Growth Parameters and Rhizosphere Microbial Abundance of Groundnuts (*Arachis hypogea*) and Maize (*Zea mays*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author TLA designed the study, wrote protocol, managed the analyses and performed the statistical analysis. Author ODO wrote the first draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Research Gap: there are little or no information on effect of oil pollution on groundnuts and maize.
Aim: to ascertain effect of spent engine oil (SEO) on plants' growth, chlorophyll content, growth and survival of rhizosphere microorganisms in the contaminated soil.
Study Design: experiment was laid out as a completely randomized design in 3 replications.
Place and Duration of Study: Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun/six months.

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Methodology: farm top soil was collected and physico-chemical analysis was done. Viable seeds were determined by flotation method. Four treatments of SEO (5, 10, 15, 20ml) were applied to soil with plants at 2 weeks after planting. Shoot length, dry matter, leaf area, chlorophyll content of uprooted plant samples, rhizosphere bacterial and fungal counts were estimated biweekly using standard methods.

Results: different responses to SEO concentrations were observed. Leaf area was most affected (20ml SEO) at week 6 for groundnuts (0.0075±0.002m^2) and maize (0.583±0.006m^2). Application of SEO to soils had reductive effects on dry matter content of all plants; content values for groundnuts and maize at week 6 were 167±0.006g and 368±0.004g while controls were 0.567±0.007g and 0.860±0.002g, respectively. Bacterial counts increased in groundnuts from 1.21 ×105 CFU/g to 1.69×105 CFU/g (5ml), 0.7 x 10^5 CFU/g to 1.19 ×10^5 CFU/g (20ml), 2.03×10^5CFU/g to 2.56 ×10^5CFU/g (control) at weeks 2 and 6. Counts in maize were 1.35 ×10^5 CFU/g to 2.25×10^5 CFU/g (5ml), 0.8 ×10^5 CFU/g to 1.19×10^5 CFU/g (20ml), 2.15×10^5CFU/g to 2.96×10^5 CFU/g (control), respectively at weeks 2 and 6. There were statistical differences between the monitored parameters and treatments with respects to days.

Conclusion: study revealed plants may suffer great growth inhibition and perform poorly when exposed to SEO at early stages of growth. Therefore, enforcement of strict disposal regulations and legislations against irregular and indiscriminate disposal of spent engine oil into the environment is imperative.

Keywords: Spent engine oil; rhizosphere; microorganisms; groundnuts; maize.

1. INTRODUCTION

“Engine oil is defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine” (Nwite & Alu 2015, Adeleye et al. 2018). “Spent engine oil is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain. They are lubricants that have been used to operate an automobile machine and considered not fit for initial purpose. Spent engine oil also referred to as used or waste motor oil is a mixture of low and high molecular weight aliphatic and aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products, heavy metal contaminants that come from engine parts as they wear down” (Nwachukwu et al. 2020).

“In Nigeria, indiscriminate discharge of spent engine oil into water drain, open vacant plots of lands is becoming an acute environmental problem in Nigeria, particularly when large areas of agricultural land are contaminated. Sometimes, they get into the environment via cautious or unintentional release by auto mechanics” (Ozomata et al. 2022). Regrettably, these contaminants are released widely by artificers knowing little or nothing of the consequential harm to the environs (Ojo et al. 2020). “In most cases, the soil may remain unsuitable for crop growth for months or years, until oil is degraded to a tolerable level” (Johnbosco et al. 2020). “Spent engine oils contain higher percentage of toxic compounds and metals than fresh oils, some of these metals can dissolve in water, move through the soil easily to pollute surface and groundwater” (Orji et al. 2018, Azorji et al. 2021a, Nishitha et al. 2022). “It causes great damage to soil and soil micro flora. It creates an unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients and lowering of soil pH. It leads to significant reduction of soil moisture. It inhibits the activities of soil catalase and dehydrogenase enzymes, delays germination of seeds and causes reduction in the growth of plants” (Adeleye et al. 2018, Adeleye et al. 2017, Azorji et al. 2021b).

“Soil is the key component of natural ecosystem and environmental sustainability depend largely on sustainable ecosystem. Soil contain very large number of microorganisms including hydrocarbons utilizing bacteria and fungi” (Huang et al. 2021, Ataikiru & Okorhi 2024). “Microorganisms are capable of breaking down many complex molecules by adaptation of their degradative enzymes system. Bioremediation is the application of naturally occurring process by which microorganisms transform environmental contaminants into harmless end products” (Ataikiru & Ajuzieogu 2023). “Spent engine oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and an induction of a limitation in soil nitrogen and phosphorus" (Echiegu et al. 2022, Njoku et al. 2021); these conditions generally cause unsatisfactory seed germination, growth and yield in soil contaminated with spent engine oil (Azorji et al. 2023).

"Microorganisms are found in large numbers in the soil usually between one and ten million which are present per gram of soil with bacteria and fungi being the most prevalent" (Fu et al. 2023). "Soil bacteria are the primary digestive system of the soil and their activity is responsible for almost 90% of all biological and chemical actions. The toxicity of the spent oil varies widely, depending on their composition, concentration, environmental factors and the biological state of the organisms at the time of contamination" (Nwachukwu et al. 2020, Azorji et al. 2021a, Emoyan et al. 2020).

"Maize (*Zea mays*) is a plant belonging to the family of grasses (*Poaceae*). It is cultivated globally, being one of the most important cereal crops worldwide" (Liao et al. 2015, Vescio et al. 2021). "Maize is not only an important human nutrient but a basic element of animal feed and raw material for production of industrial products. In Nigeria, maize is a major food and an industrial crop grown both commercially and at subsistence level by most farmers" (Azorji et al. 2023). "Unlike wheat and triticale, maize is more tolerant to abiotic and biotic stress factors, temperature resistant, and able to grow on nutrient-poor sandy soils with a low pH" (Miedaner 2019).

"Groundnut (*Arachis hypogea*) is an important legume crop which forms a source of cheap food on the table of the average Nigerian. It belongs to the family *Fabaceae* and is a native to regions like South America, Mexico and Central America. It is one of the world's principal oilseed crops. Groundnut kernels are consumed directly as raw, roasted or boiled kernels and oil extracted from the kernel is used as culinary oil" (Wang 2016). "The nuts are also used as animal feed and industrial raw materials (oil, cake and fertilizer). Both domestic and foreign trade multiple use of groundnut makes it an excellent crop for developing and developed countries' commerce. Groundnut is rich in vitamins, contains at least 13 different types of vitamins that include Vitamins A, B, C and E together with 26 essential minerals like calcium, zinc, iron, boron, potassium, phosphorous, manganese, magnesium, copper, fat, sodium, water, protein, carbohydrate and fiber" (Bao et al. 2013, Sales et al. 2014).

"Most of the earlier investigations, focused on oil pollution effect on plant growth and resistance, observing plants' growth in oilfield areas and phytoremediation potential" (Sales et al. 2020, Hatami et al. 2019, Gospodarek et al. 2019, Grifoni et al. 2020). There are relatively little data accessible on consequence of oil pollution on these plants especially, groundnuts. The current study will be one of the earliest studies aimed at investigating the effects of different concentrations of crude oil in the soil on growth response in groundnuts plants. The outcomes of the present-day study will improve the understanding of growth and physiological reactions of the plants to several levels of oil pollution in soil.

Maize and groundnut plants were used as test crops in this study because of their multifarious uses among farmers in the study area (Delta State, Nigeria). Although, some works have been done on spent engine oil pollution on the growth of maize and groundnut in some States in Nigeria but the information is not sufficient enough due to the different type of soil and for proper documentation. Hence, this study was done to ascertain the adverse effects on the growth of maize, groundnuts and rhizosphere microbes due to the unfavourable conditions created in the soil by the spent engine oil.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted at the research Laboratory of the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources Effurun, Delta state (Lat. 5°38'33.07''E, Long. 5°38'23.32''N).

2.2 Sample Collection

The surface soil samples were collected at 0 - 15cm from agricultural farms and mixed to form a composite sample at each location. Soil samples were sorted to remove stones, plant and root debris. All soil samples from the four locations were pooled together and stored. Soils were properly mixed, put into perforated polythene bags to a weight of 1kg, watered and allowed to settle.

Healthy groundnut and maize seeds were obtained from Effurun Market, Uvwie Local Government Area, Delta State, Nigeria.

The spent engine oil (SEO) was obtained as pooled used engine oil from heavy-duty vehicles at different motor mechanic workshops in Effurun, Uvwie Local Government Area, Delta State.

2.3 Soil Physico-Chemical Analysis

Soil physical and chemical analyses were determined using standard methods. Analysis of pH, moisture content, electrical conductivity, total organic carbon (TOC), nitrates and phosphates were assessed according to standard methods (Sales 2014). Cadmium, lead, calcium, magnesium, potassium and sodium were detected by the flame analysis method 7000B using the Atomic Absorption Spectrophotometer (Buck Scientific Model 210 VGP) following the protocol described by the American Public Health Association (APHA 2018).

2.4 Seed Viability Test

The viable seeds were determined by the flotation method (Ajuzieogu et al. 2015). Five viable maize and groundnut seeds were planted in each bag and thinned down to two seedlings after two weeks.

2.5 Experimental Setup

The experiment were laid out as a completely randomized design in 3 replications. The modified method of Nwite & Alu (2015) was adopted. Four treatments of SEO; 5 ml/kg, 10 ml/kg, 15 ml/kg, 20ml/kg (v/w) were applied to the soil with maize and groundnut plants at 2 weeks after planting. Manual watering was done weekly. Plant physical characteristics were assessed every forthnight. The shoot length, dry matter, leaf area and chlorophyll content of the uprooted plant samples were the characteristics monitored. Also, rhizosphere soil samples collected from the plant roots were used for microbiological (bacteria and fungi) analysis.

2.6 Measurement of Shoot Length, Leaf Area and Dry Matter

The plants shoot length were determined by measuring the plants from the base of each plant to the tip while the dry matter content were determined by drying. Plant samples were oven dried at 60°C to constant weight for 24h after which the weights of the dry samples were determined using a sensitive weighing balance. The leaf area was determined following the method described by Pearcy et al. (1989). This was done by measuring the length of the longest part of the leaf, the width of the widest part of leaf and calculating the area using the formula 0.5 × L ×B (L= length and B= breadth).

2.7 Chlorophyll Test

The chlorophyll content of the plant was determined using the method of Heidcamp (2003). It involved the extraction of the chlorophyll of 1g of leaves with 10ml of 80% acetone. The 80% aqueous acetone solutions was prepared by mixing distilled water and reagent-grade acetone in a ratio (distilled water: acetone) of 2:8 (v/v).

A gramme of leaves was weighed from each sample, cut into small pieces using scissors, placed in the mortar and 2-mL of extraction solution was added using 5-mL pipette. The material was carefully ground with a pestle while kept chilled over ice. After grinding for approximately 30 seconds (until the tissue is a fine slurry), extraction solution (acetone solution) was used to wash any sample material adhering to the pestle by pouring 2 mL of extraction solution over the pestle. Thereafter, the extract was carefully poured into a centrifuge tube. It was ensured that the chlorophyll was extracted completely from the tissue (i.e, the tissue was devoid of green colour).

Samples extract in centrifuge tubes, were centrifuged for twenty minutes at high speed (approximately 500 × gravity). The supernatant solution was decanted into a 10mL graduated cylinder and the volume brought to 10mL with 80% aqueous acetone. Dilution was required when the initial reading was out of the linear range of instrument detection. The supernatant was diluted by adding 80% aqueous acetone. The 80% aqueous acetone was used as the blank to zero the instrument initially and after every wavelength resetting.

Dilutions were accurately done and recorded to calculate the chlorophyll concentration in the original tissue sample. Calculations for chlorophyll concentrations were made after the absorbance were read at 663nm wavelength using the UV Spectrometer (UV-VIS SPEC Model UV 1800).

2.8 Microbial Counts

The population count of microorganisms was carried out by traditional viable cell counts following the method of Ataikiru & Okorhi (2024). One (1) gram of each rhizosphere soil sample was suspended in 9 ml of sterile distilled water. Serial dilution was done aseptically. Aliquots (0.1ml) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Plate count agar (PCA) was used for the enumeration of total heterotrophic bacteria and Potato dextrose agar (PDA) was used for the enumeration of fungi. Cultured plates were inverted, incubated at 28±2^{0}C for 48 hours (bacteria) and 5days (fungi). The individual colonies were counted and recorded as colony forming units (CFU/g).

2.9 Statistical Analysis

Data were subjected to two way analysis of variance (ANOVA) for significance in responses of growth parameters with respect to treatments and weeks.

2.10 Safety

All procedures and associated reagents used during the research had risk assessment protocols specified by the Analytical Investigation Group of the Environmental Management and Toxicology Laboratory. All risks assessment were strictly followed.

3. RESULTS AND DISCUSSION

3.1 Physico-Chemical Analysis

Table 1 shows the results of the physical, chemical analysis and heavy metals present in the soil used for the experiment.

The soil had a pH value of 4.84 indicating that the soil was slightly acidic. Most farmyard soils have pH between 5.5 and 8.0 but under different agricultural practices, soils' pH values may increase or reduce. The solubility of necessary soil macronutrients, micronutrients or trace elements are influenced by soil pH (Tudararo-Aherobo & Ataikiru, 2020). The electrical conductivity (EC) of the soil sample was 53µs/cm. The EC value is a function of level of contamination of a polluted site. The total organic carbon (TOC) of pooled soil sample was 3.43%. The TOC was low probably due to the absence of hydrocarbon pollutants. The total petroleum hydrocarbon (TPH) for the pooled soil sample was 0.000mg/kg. Our results were in conformation to the findings of Johnbosco et al. (2020). They reported that levels of TOC and TPH in the soils of the auto-mechanic villages were higher than that of the control unpolluted soils. This implies that introduction of used oil and other carbonated fluid from mechanic workshops increased the organic matter content and total petroleum hydrocarbons in soils. The moisture content of pooled soil sample used for the study was 10.0%. The amount of nitrate was 17.82mg/kg and phosphate was 9.20 mg/kg in the soil sample. The growth rate of microorganisms is often constrained by the availability of nutrients like nitrogen and phosphorus (Adeleye et al. 2018). These nutrients are the basic foundation of life and facilitate the environment for necessary enzymes production by the microorganisms. The cation exchange capacity (CEC) which is the calculation from the absorbance obtained from the various cations and the activities of the individual ions was 16.52. The concentration of lead and cadmium were <0.002mg/kg and <0.01mg/kg in the tested pooled soil. The values were less than the recommended limits by Federal Ministry of Environment (FMEnv). According to Eshalomi-Mario & Tanee (2015), the increase in the concentration of heavy metals in soil may be due to the hydrocarbon pollution which alters the physico-chemical parameters of the soil as well as increasing the concentration.

Table 1. Physical and chemical characteristics of experimental soil (0-15 cm)

Parameters	Value
pH	4.84
Electrical conductivity (EC), us/cm	53
Moisture content	10.0
Cation exchange capacity (CEC), meq/100g	16.52
Lead, mg/kg	<0.002
Cadmium, mg/kg	<0.01
Nitrates, mg/kg	17.82
Phosphates, mg/kg	9.20
Total organic carbon (TOC), %	3.43
Total petroleum hydrocarbons (TPH), mg/kg	0.000

3.2 Shoot Length, Leaf Area and Dry Matter

The shoot length of the groundnut and maize seedlings exposed to the different concentrations of spent oil during their growth are shown in Fig. 1 and Fig. 2, respectively. There were noticeable reductions of the shoot length of plants exposed to the spent oil. The control for groundnuts and maize plants increased in length from 24cm to 39cm and 38cm to 52cm, respectively. This was an indication of higher growth due to the absence of the spent oil unlike other plants exposed to different concentrations of the spent oil. At high concentrations of the spent engine oil in the soil, most plants suffered remarkably decreased growth rates. Results showed that shoot length of all plants were affected at the various levels of contamination from those grown in non-contaminated soil. The control (non-contaminated soil) had a mean height of 39 ± 0.005cm, whereas the contaminated soils with 5, 10, 15, and 20 concentrations had mean heights of 30 ± 0.002cm, 27 ± 0.002cm, 22 ± 0.003cm and 20 ± 0.006cm, respectively for groundnuts at the end of study indicating reduction in height with increased concentration of the spent engine oil. Also, there were significant differences in the shoot length at the various concentrations. Measured length of the maize plants were 52 ± 0.008cm, 45 ± 0.002cm, 34 ± 0.004cm, 26 ± 0.003cm and 21 ± 0.006cm for 0ml (control), 5ml, 10ml, 15ml and 20ml, respectively. Our findings were in line with the reports of Nwachukwu et al (2020). They reported that spent oil in soil creates an unsatisfactory conditions for plant growth ranging from heavy metals toxicity to insufficiency in aeration. High oil concentrations in soil decreased seedlings height and this was in corroborations with the findings of Liao et al (2015). According to several reports, “spent engine oil has the ability to prevent the uptake of nutrients, water and oxygen required for growth as a result of volatile fractions of oil which have the high wetting capacity and penetrating power” (Saraeian et al. 2015, Zhang et al. 2015, Hussain et al. 2019). “Oil pollution modifies the permeability and structure of the plasma membrane, alters the shape and size of the parenchyma tissue, reduces intercellular space in the cortex of the stem and roots, and inhibits the mitotic activity of the root meristem” (Bellout et al. 2016). Furthermore, “air displacement from the pore spaces between the soil particles (insufficient aeration) leads to root stress and low water accessibility” (Hussain et al. 2019). Also, “it decreases the amount of organic matter obtainable by plants and diminishes the quantity of mineral nutrients such as sodium, phosphates, potassium, sulfates, and nitrates” (Otitoju et al. 2017). Also, Fernandes et al. (2020) recounted the occurrence of used engine oil in the soil-plant micro-environment upsets typical soil chemistry reducing nutrient release and uptake in addition to the amount of water.

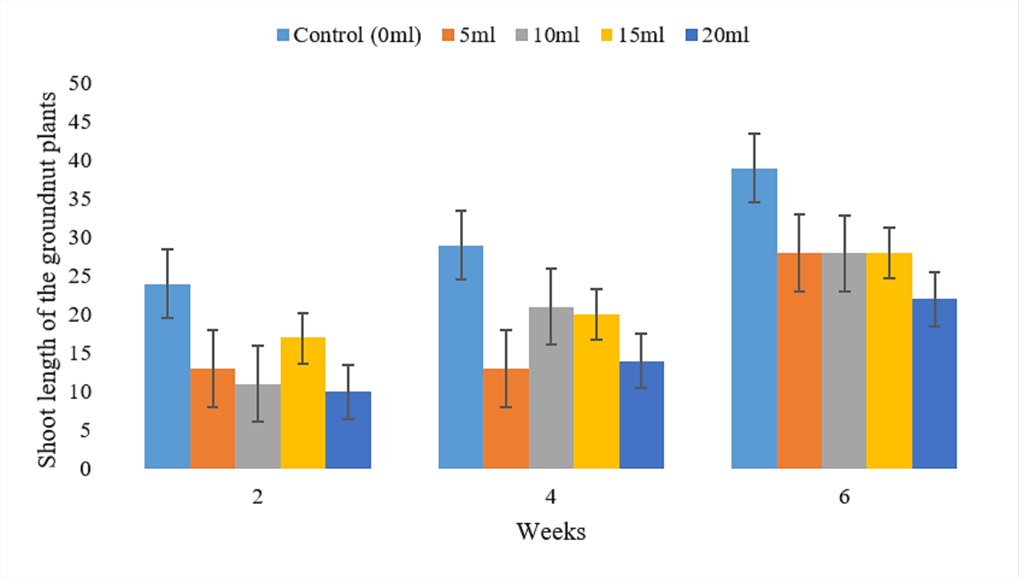

Fig. 1. shoot length of the groundnuts during the study.
** Results are in mean ± standard deviation*

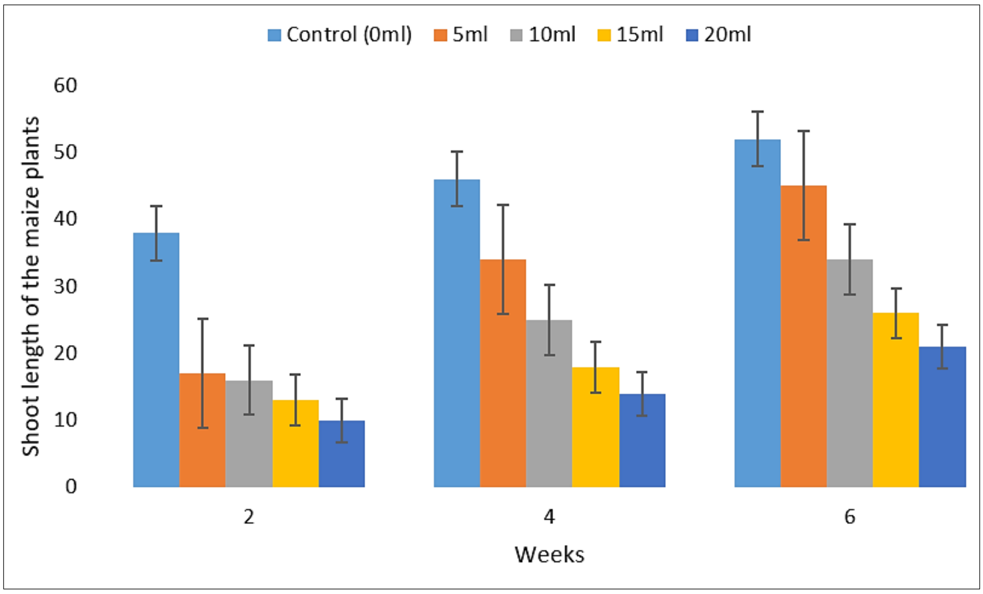

Fig. 2. Shoot length of maize plants during study

** Results are in mean ± standard deviation*

The values of the measured leaf area are shown on Fig. 3 & Fig. 4. The leaf area of plants treated with low levels of spent oil were larger than the leaf areas of those treated with higher concentrations. There were significant difference among the measured areas of plants during the experiment. The leaf areas of plants with 20ml spent oil were smallest throughout the study. After the application of spent oil, smaller leaves were observed in plants with 15ml and 20ml spent oil suggesting that these concentrations had greater impacts on the leaf area of plats than lower concentrations. The application of spent engine oil had a dose-dependent inhibitory effects on leaf area. The leaf area of groundnuts was generally more affected by all treatments than other maize used in the bioassay at the end of study. The measured leaf areas were 0.0521±0.003m^2 (control), 0.02±0.002m^2 (5ml), 0.02±0.001m^2 (10ml), 0.015±0.003m^2 (15ml) and 0.0075±0.002m^2 (20ml) for groundnuts while 1.46±0.008m^2 (control), 1.06±0.002m^2 (5ml), 1.05±0,001m^2 (10ml), 0.74±0.003m^2 (15ml) and 0.583±0.006m^2 (20ml) were recorded for maize. Our findings were in corroboration with the reports of Azorji et al. (2023). They reported that spent engine oil had significant effects on the test crops (*Vigna unguiculata* TGm-50, *Glycine max* and *Zea mays* TZm-30181) in a dose-dependent manner which we also observed from our research. “The small leaf area observed may be due to the decline in soil aeration due to development of thin film layer on the topsoil by the applied used oil thus, reducing air passage through the soil pores, initiating the suffocation of plants, for that reason, reduction in the leaf areas” (Odiyi et al. 2020).

Figs. 5 & 6 showed the dry matter content of the maize and groundnut plants under investigation. The dry matter content of the plants were significantly affected by the quantity of spent oil added to the soil. The application of 15ml and 20ml of the spent oil led to a significant reduction. The presence of spent engine oil contamination decreased the dry matter of the plants. Plants grown in non-contaminated soils had significantly higher dry matter than those grown in oil contaminated soils. Dry matter content strongly correlated with contamination. For control, it increased from 0.507±0.001g (week 2) to 0.567±0.007g (week 6), 0.380 ±0.009g (week 2) to 0.403±0.003g (week 4) to 0.205±0.004g (week 6) at 5ml contamination, 0.380±0.009g (week 2) to 0.403 ±0.003g (week 4) to 0.205±0.004g (week 6) at 10ml but reduced from 0.218 0.001g (week 2) to 0.167 0.006g (week 6) at 20mls for groundnut plants. Similarly, dry matter content for maize are shown on Fig. 6. The content measured were 0.693±0.002g (5ml), 0.40±0.004g (10ml), 0.382±0.003g (15ml), 0.368±0.004g (20ml) while control was 0.860±0.002g at the end of trial (week 6). The matter content decreased as the concentration of spent engine oil in soil samples increased in agreement with Azorji et al (2023), who observed similar effects of oil pollution on both wet and dry weight of *Vigna unguiculata*, *Glycine max* and

Zea mays. Skrypnik et al. (2021) reported that oil contamination stimulated the dry matter content of the rye plant at low concentrations but had a negative impact at higher concentrations. “Some researchers have linked reduction of the wet and dry plant biomass with the negative impact of environmental stressors (including petroleum products) present in soil” (Hussain, et al. 2019, Cui et al. 2016, Han et al. 2016 Lacalle et al. 2018).

3.3 Chlorophyll Content

The chlorophyll content (µg/g) of the leaves of groundnuts and maize treated with different concentration of spent oil are as seen in Fig. 7 and Fig. 8. At the end of the tests, chlorophyll content measured were 0.014±0.002µg/g (5ml), 0.012±0.004µg/g (10ml), 0.008±0.003µg/g (15ml), 0.005±0.006µg/g (20ml) while control was 0.042±0.003µg/g for groundnut plants. In maize, chlorophyll content values were 0.018±0.001µg/g (5ml), 0.016±0.001µg/g (10ml), 0.009±0.001µg/g (15ml), 0.003±0.002µg/g (20ml) while control was 0.051±0.007µg/g. The control recorded the highest mean chlorophyll content in all the test plants. There was a decrease with increased concentration of spent oil. Our results were in confirmation with the results of Skrypnik et al. (2021). They found out that oil contamination resulted in a significant decrease in chlorophyll content at all concentrations (including low concentrations) when they investigated the effect of crude oil on two rye varieties. Similar findings were reported by Osuagwu et al. (2013) in their study with air potato (*Dioscorea bulbifera*).

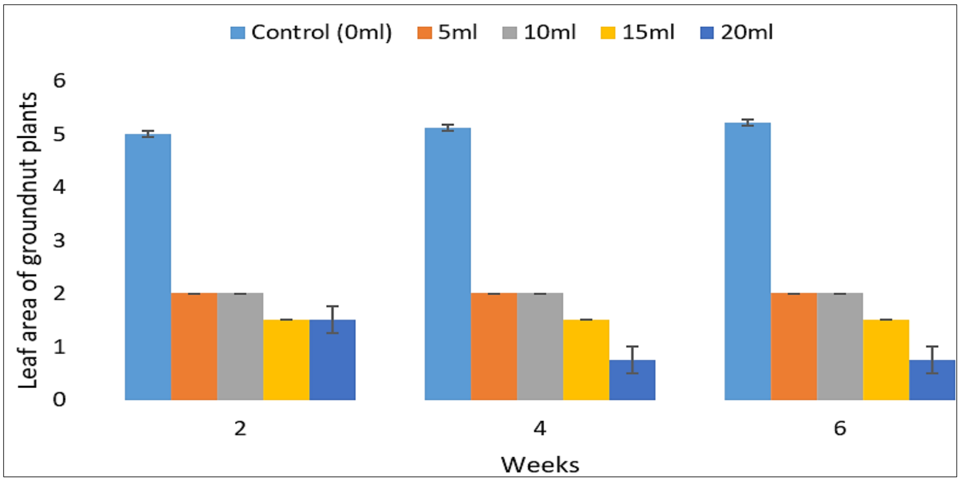

Fig. 3. Leaf area of groundnuts plants (m^2)
** Results are in mean ± standard deviation*

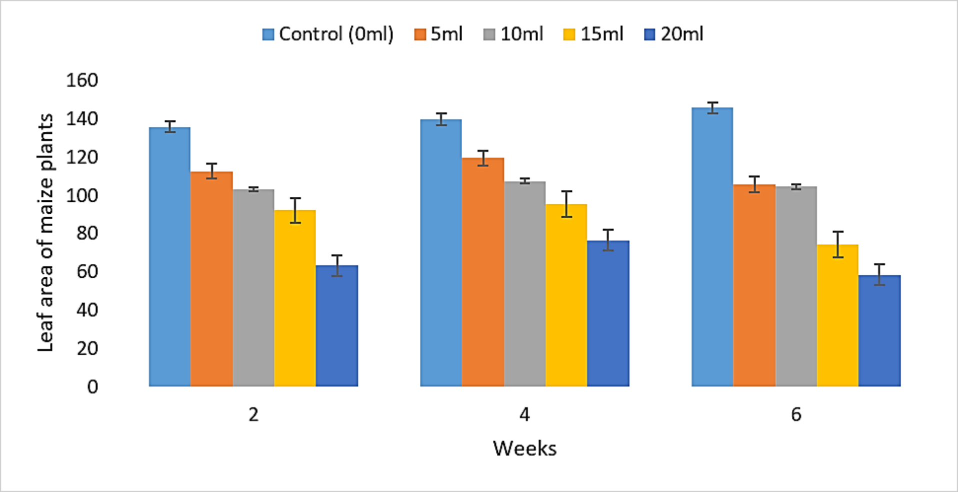

Fig. 4. Leaf area of maize plants (m^2)
** Results are in mean ± standard deviation*

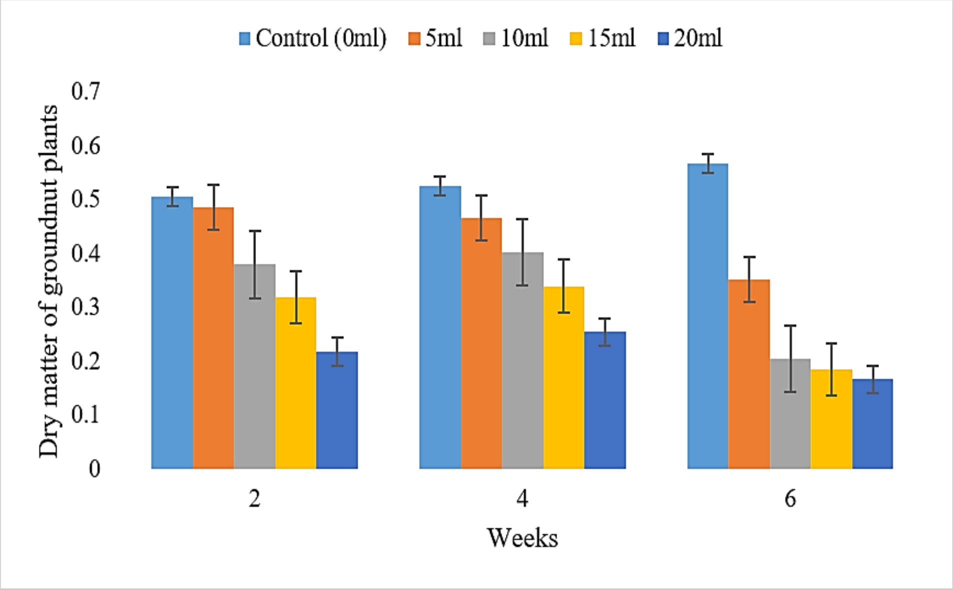

Fig. 5. Dry matter of groundnut plants (g)
** Results are in mean ± standard deviation*

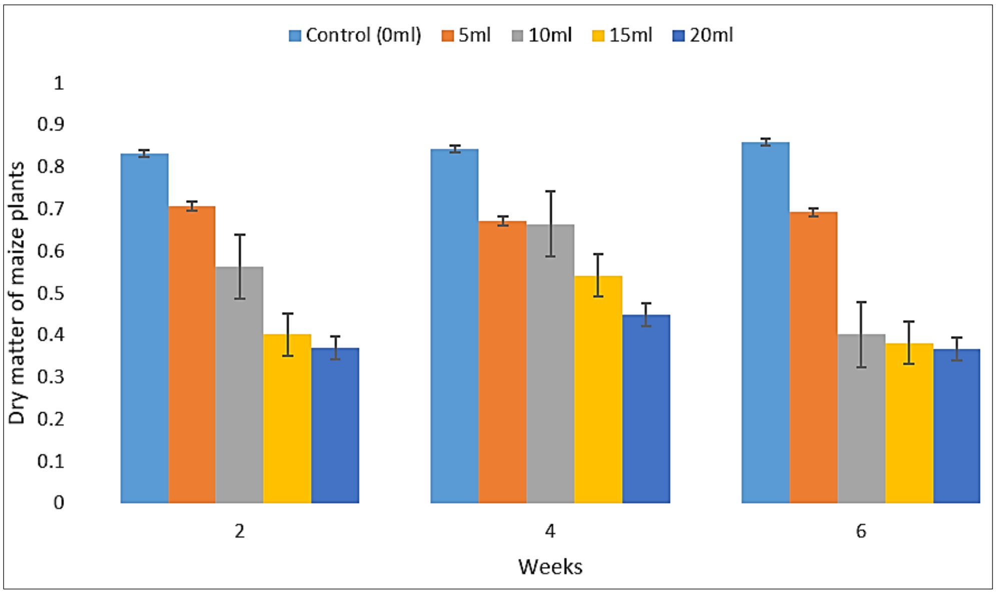

Fig. 6. Dry matter of maize plants (g)
** Results are in mean ± standard deviation*

Chlorophyll content, an indicator of photosynthetic activity and timing of fertilizer application of crop is crucial in plant growth and establishment. This pigment is critical as an index of plant growth and production of organic matter. The chlorophyll content of the plant could be reduced by oil interference on the ability of the plant to absorb some of these mineral nutrients; magnesium, iron, boron and manganese that are essential for chlorophyll synthesis (Odiyi et al. 2020). Some researchers (Athar et al. 2016, Tahseen et al. 2016, Achuba et al. 2018) have reported that oil pollution reduces photosynthetic activities and chlorophyll content in plants. Again, da silva Correa et al. (2022). opined that oil pollution leads to environmental stress, morphological, physiological and anatomical changes in plants depending on the characteristics of the oil and soil. Our result also showed that maize was more tolerant than groundnut plants.

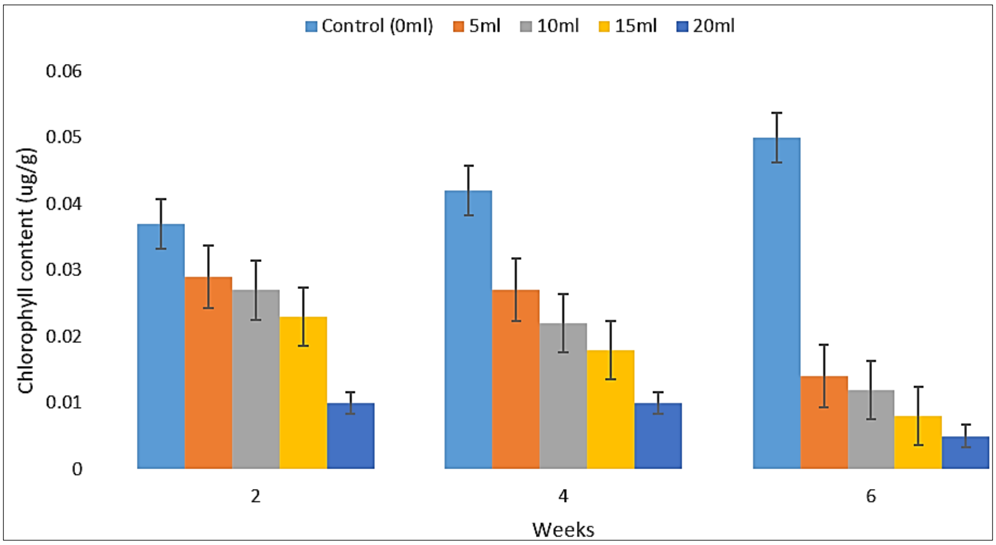

Fig. 7. Chlorophyll content of groundnut plants
** Results are in mean ± standard deviation*

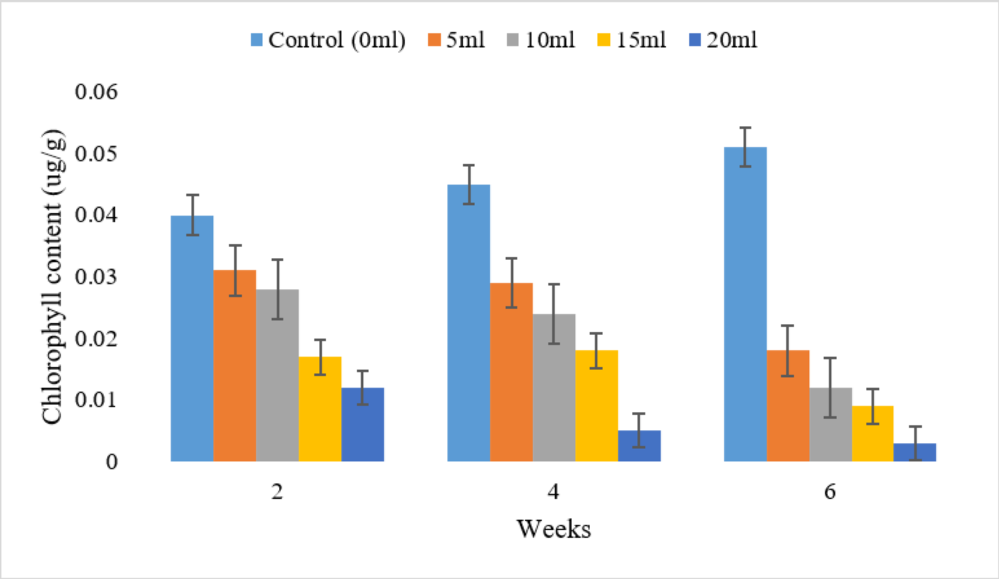

Fig. 8. Chlorophyll content of maize plants
** Results are in mean ± standard deviation*

Estimating chlorophyll affords vital evidence about the influence of toxicants on plant growth. Researchers have recommended it as the utmost index directly applicable to estimation of plant yield (Hussain et al. 2019, Cui et al. 2016, Han et al. 2016). In plant researches, the quantity of photosynthetic pigments affords basic facts on the plants' biological and functional status. Consequently, chlorophyll investigation has been done in various studies owing to its significance in plant physiology. It can be distorted in response to biotic and abiotic stressors such as pathogen infection (Mur et al. 2017) and light stress. Thus, as an indicator for crop growth and development, precise assessment of chlorophyll concentration is indispensable. However, it has been established that the influence of used oil contamination/pollution depends on species and variety of the plants.

3.4 Microbial Counts

Figs. 9 and 10 showed the bacterial counts for groundnuts and maize during the study, respectively. There were increases in the bacterial counts at all concentrations as the study

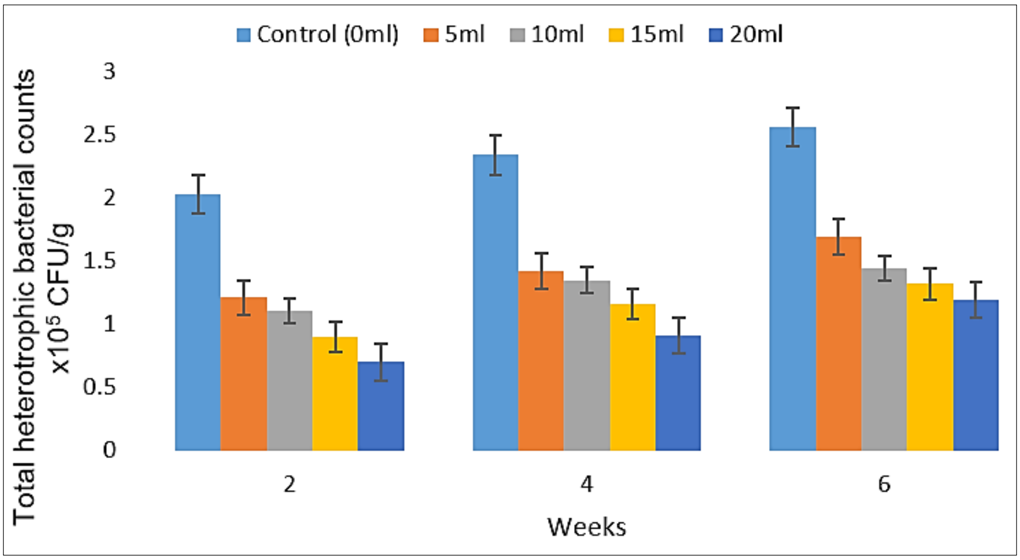

Fig. 9. Total heterotrophic bacterial counts for groundnuts
** Results are in mean ± standard deviation*

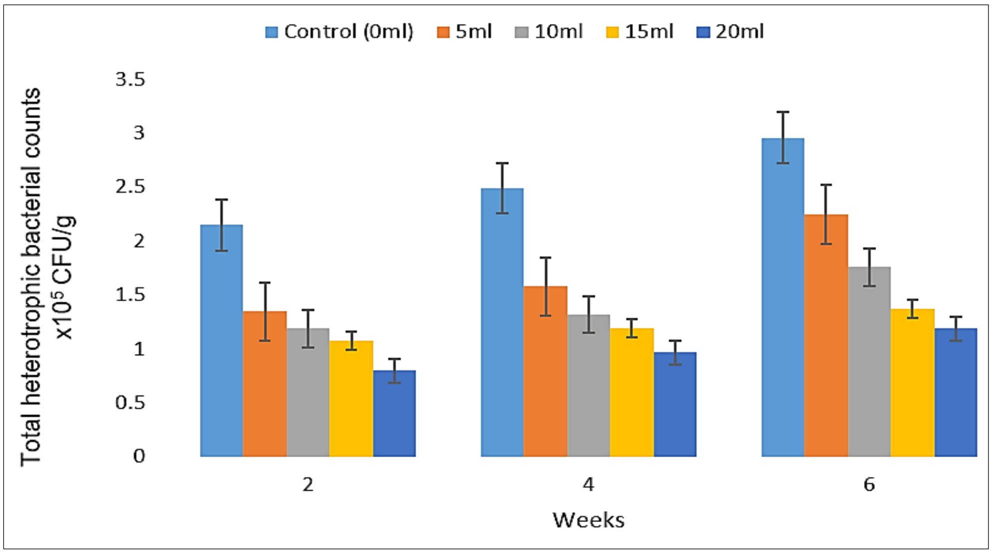

Fig. 10. Total heterotrophic bacterial counts for maize
** Results are in mean ± standard deviation*

progressed. The highest counts (2.03×10^5 CFU/g to 2.56×10^5 CFU/g in groundnuts and 2.15×10^5 CFU/g to 2.96×10^5 CFU/g in maize) were recorded from the control soil. The reverse was observed in the treatments with spent oil, the higher the oil concentration the lower the counts. There were reductions in total heterotrophic bacterial counts in treatments at week 2 and increases, thereafter from week 4 to week 6. Counts increased from 1.42×10^5 CFU/g to 1.69×10^5 CFU/g (5ml), 1.35×10^5 CFU/g to 1.44×10^5 CFU/g (10ml), 1.16×10^5 CFU/g to 1.32×10^5 CFU/g (15ml) and 0.91×10^5 CFU/g to 1.19×10^5 CFU/g (20ml) in the groundnuts microcosms. Also, the THB counts in maize increased from 1.58×10^5 CFU/g to 2.25×10^5 CFU/g (5ml), 1.32×10^5 CFU/g to 1.76×10^5 CFU/g (10ml),1.19×10^5 CFU/g to 1.37×10^5 CFU/g (15ml) and 0.97×10^5 CFU/g to 1.19×10^5 CFU/g (20ml), respectively as shown in Fig. 10.

The fungal counts in both treatments increased throughout the study after the decrease observed (10ml, 15ml and 20ml only) at week 2. Fig. 11 showed increases were from 5.9×10^6 CFU/g to 7.3×10^6 CFU/g (5ml), 1.62×10^6 CFU/g to 2.2×10^6 CFU/g (10ml),1.17×10^6 CFU/g to 1.40×10^6

CFU/g (15ml) and 0.90 × 10^6 CFU/g to 1.20 × 10^6 CFU/g (20ml) in treatments containing groundnuts plants. Increases were observed from 6.1 × 10^6 CFU/g to 9.8 × 10^6 CFU/g (5ml), 1.72 × 10^6 CFU/g to 2.32 × 10^6 CFU/g (10ml),1.21 × 10^6 CFU/g to 1.61 × 10^6 CFU/g (15ml) and 0.85 × 10^6 CFU/g to 1.43 × 10^6 CFU/g (20ml) in treatments with maize plants as seen in Fig. 12. Counts increased throughout the study for the control soils from 3.1 ×10^6 CFU/g to 4.8 × 10^6 CFU/g for groundnuts and 3.8 ×10^6 CFU/g to 5.2 × 10^6 CFU/g for maize.

The microbial counts assessment indicated a decrease in the microbial population of bacteria and increase in population of fungi in the contaminated soils at 5ml. Nwachukwu et al (2020) reported an increase in the microbial population of bacteria and decrease in population of fungi in the polluted soils and a decrease in the microbial population of bacteria and fungi in the control which was contrary to our findings, as we observed increases in fungal counts at low concentration of the spent engine oil and decreases for both fungal and bacterial counts at

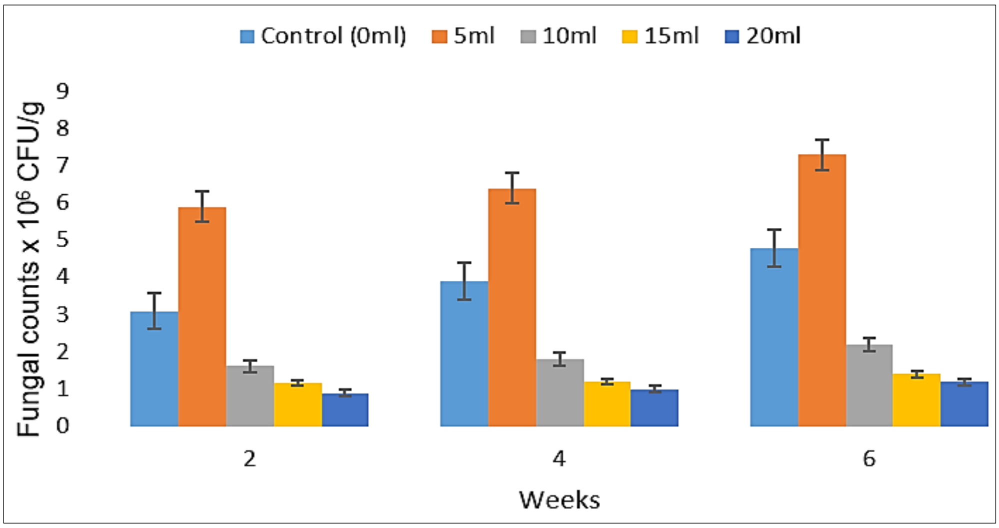

Fig. 11. Fungal counts for groundnuts
** Results are in mean ± standard deviation*

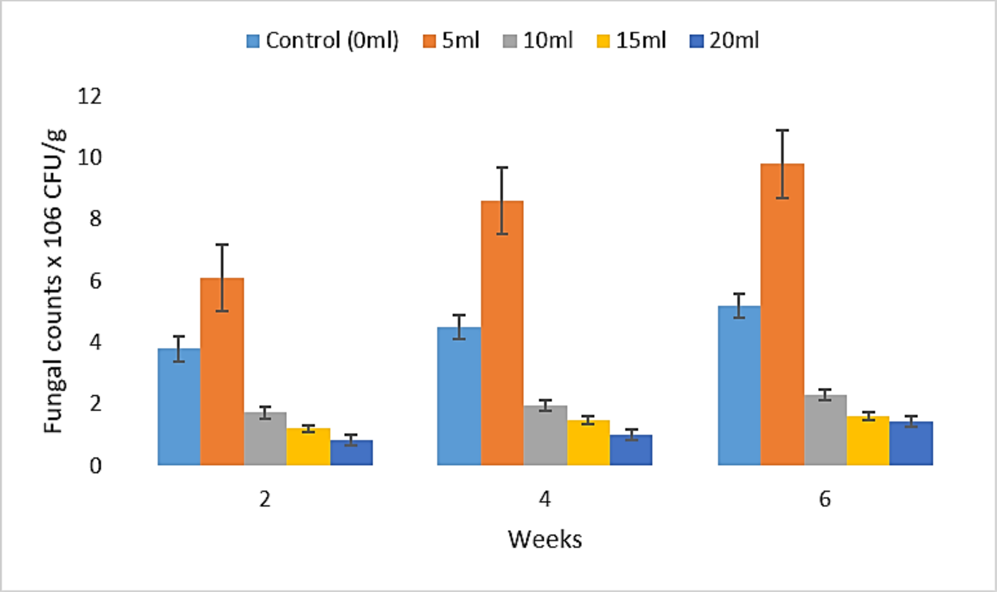

Fig. 12. Fungal counts for maize
** Results are in mean ± standard deviation*

all other concentrations of used oil. The preliminary decline observed in their numbers could be traceable to the noxious effect of the spent engine oil on exposure. The adaptability of microbial genes to acclimatize and reduce toxicants possibly will be responsible for resultant increases. Furthermore, existence and extraordinary records of these microbes ratifies they are ever-present, heterogeneous and as well, can adjust to any unfavorable ecosystem (Mur et al. 2017, Joshi et al. 2021, Edekor 2021, Ataikiru & Okorhi 2022). Furthermore, we recommend a detailed research on the influence of used oil on plants via assessment of hydrogen peroxide, monodehydroascorbate, superoxide dismutase, catalase, ascorbate peroxide among others.

4. CONCLUSION

This indiscriminate disposal of wasted engine oil adversely influence plants, microorganisms and aquatic lives due of the vast amount of hydrocarbons and highly poisonous polycyclic aromatic hydrocarbons found in the oil. Heavy metals such as vanadium, lead, aluminium, nickel and iron which are found in considerable quantities in old motor oil may be retained in soil, in form of oxides, hydroxides, carbonates, exchangeable cation and/or attached to organic materials in the soil. This research has shown that spent engine oil has phytotoxic effects on growth and establishment of some cultivable crops in Nigeria; the environmental problem of indiscriminate disposal of used engine oil vis-à-vis land pollution, its attendant effects on soil, plants and public health. The polluted soils with spent engine oil showed an adverse effect on the test crops, *Arachis hypogea* and *Zea mays*. Reduction in shoot length, leaf area, chlorophyll content and dry organic matter were recorded but there were increases in these growth parameters with the control plants. The used engine oil inhibited these crops in a dose dependent manner. The adverse effect of spent oil may be due to the adverse conditions presented by drought conditions and the non-availability of nutrients. Hence, to reduce plant loss, it is important to prevent their exposure to this toxicant. Any condition that disrupts the normal plant-water relationship of the roots within the soil will negatively affect the normal growth of the plants. Also, study showed that indiscriminate disposal of engine oil had significant effects on both bacterial and fungal population in the rhizosphere. This study highlights the need for legislations against agricultural soil pollution with used engine oil. Therefore, it’s our opinion that greater environmental consciousness be instilled into petroleum product marketers and auto mechanics through educational workshops on the consequences of spills and the unhealthy disposal of used engine oil.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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