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Phytotoxicity assessment of the effects of unused and spent engine oil on the germination and seedling growth of *Zea Mays* L

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ABSTRACT
The effects of unused and used engine oil on the germination and seedling growth of *Zea mays* L. were studied for phytotoxicity. Thirty poly bags containing 3kg of loamy soil were filled, with 24 bags containing varied amounts of spent and unused engine oil and 6 bags serving as controls. There were four distinct concentrations of used and unused motor oil, ranging from 50 to 200 mg/l, with three replicates for each treatment. The findings revealed a negative association between soil oil levels and the growth indices assessed (germination, plant height, number of leaves, leaf length, and leaf breadth). With increasing concentrations of the contaminant, the reductions and morphological effects in the growth properties of *Zea mays* L. with spent and unused engine oil increased. Spent engine oil was found to have more detrimental effects than the unused oil. This study therefore showed that the presence of the engine oil in the soil has great effect on the growth of *Zea mays* L.

Keywords: Bioremediation, Phytotoxicity, Germination, Engine oil, Pollution

1. INTRODUCTION
Plant development has been found to be unsatisfactory as a result of soil pollution, particularly after oil spills (Dejong, 1980). This oil, also known as spent lubricant or waste engine oil, is typically collected after servicing and then draining automotive and generator engines, with the majority of it being thrown into the earth. It is a common and harmful environmental contaminant that does not occur naturally (Dominguez-Rosado and Pitchel, 2004). Engine oil is made up of two main components: base stock and additive packages (Udonne, 2011). The base fluid makes up the majority of the oil (70-95 percent), while the additional chemicals are added to enhance the base stock's good attributes (Ogbeide, 2011). Engine oil base stocks are generated from petroleum or synthesized to meet certain quality requirements. Synthetic base stocks are chemically created from pure substances, whereas petroleum base stocks are purified from crude oil (Ogbeide, 2011). Branched alkanes, linear alkanes, polyaromatic hydrocarbons,

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cycloalkanes, calcium, zinc, sulfur, phosphorus and additives are all found in engine oil (Ayoola and Akaeze, 2012)

When a fresh mineral-based crankcase is subjected to high temperatures and mechanical strain, spent oil is created (ATSDR, 1997). It is a mixture of substances (Wang *et al*., 2000) that originate from engine parts as they wear away, including petroleum hydrocarbons, chlorinated biphenyls, chlorodibenzofurans, lubricative additives, breakdown products, and heavy metals (ATSDR, 1997). After going through many manufacturing stages, additives are frequently added to improve certain of the oil's properties, such as viscosity, heat and oxidation stability, and so on. When these additives are exposed to the air, they have a harmful effect on humans and animals who come into contact with them. As a result of discharge by generator and motor mechanics (Odjegba and Sadiq, 2002), and also from the engine leaks, exhaust system employed, spent engine oil ends up in the environment. The degradation of land is aided by the contamination of the natural environment by petroleum-derived pollutants (Sztompka, 1999; Osinakachukwu & Peter, 2020).

Many anthropogenic factors contribute to the formation of Polycyclic Aromatic Hydrocarbons in soil, including cooking plants, solid fuel household heating, airplane exhaust, forest fires and car exhaust (Smith, *et al*., 2006).

The oxidation of lubricating oil hydrocarbons at the point of application results in the production of free radicals, which then convert to peroxides, which then condense and polymerize to generate per acids, nephtenic acids, and other compounds (Evdokimov and Fooks, 1959). However, the type of soil elements present in the soil-water system and the local environmental circumstances are both factors. Because different petroleum products have varied compositions, they have different effects on the environment. This means that petroleum products can alter the growth and development of organisms that rely on such soils at varying rates.

Alterations in stomata mechanisms, chlorophyll degradation, and reduction in respiration and photosynthesis, increase in stress-related phytohormones production, decrease in size, and less biomass production accumulation of toxic substances or their by-products in vegetal tissue, are the most important and common symptoms observed in plants contaminated with oil and its by-products (Baker, 1970).

Maize or corn (Zea mays L.) is a plant belonging to the family (Poaceae). It is grown all over the world and is one of the most significant cereal crops. Maize is a vital human nutrient as well as a key component of animal feed and a raw material for a variety of industrial products. Corn starch, corn syrup, corn oil, maltodextrins, and fermentation and distillation products are among the goods. It's also being used as a biofuel recently. Maize is a flexible crop that can be produced in a variety of climate zones. In fact, no other crop compares to maize's adaptability to a variety of conditions.

Although some heavy metals are required micronutrients for plants at low quantities, at high concentrations, they can induce metabolic problems and growth suppression in the majority of plants (Fernandes and Henriques, 1991). Plants, on the other hand, react to contaminants in diverse ways. According to (Agbogidi, 2010), soil polluted by petroleum hydrocarbons undergoes a significant change in characteristics, impacting the physical, chemical, and microbiological aspects of the soil. Changes in soil qualities caused by petroleum-derived compounds can result in a lack of water and oxygen, as well as a lack of accessible nitrogen and phosphorus (Wyszokowska and Kucharski, 2000). As a result, this study was conducted to assess and compare the impacts of spent and unused engine oil on Zea mays L. germination and growth parameters.

2. MATERIALS AND METHOD

Sample Collection

This research was conducted at the green house of the Department of Botany and Ecological Studies University of Uyo, Uyo. A total of thirty poly bags were filled each with 3kg of loamy soil. The loam soil was obtained from the Botanical Garden of University of Uyo, Uyo. The soil was sieved with a mesh to separate the non-degradable materials out of the soil in order to keep the soil free of unwanted materials which could disturb the proper functioning of soil micro-organisms, proper stretch of the roots and proper organization of the soil. The sieved soil was air-dried before weighing in order to avoid apparent weight of soil which could be brought about by the weight of water. By this procedure, an accurate weight of soil was obtained in relation to the engine oil used.

Soil Contamination

The loam soil was weighed with a weighing balance and set in the respective perforated bags. The soil was mixed with different volumes of spent engine oil and unused engine oil. The volumes of engine oil used for this work were graded from 50ml, 100ml, 150ml, to 200ml to a constant level of 3 kg of soil. The engine oil was measured with the measuring cylinder graded up to 300±1ml. The engine oil was thoroughly mixed with the soil. The thirty poly bags of soil were divided into two groups of spent and unused engine oil respectively with each group having three replicates for control and for each volume of engine oil. The experiment was arranged in randomized complete block design. The soil was left for a period of one week without planting. This was to achieve uniformity of temperature, oil, air content, moisture and improved activities of micro-organisms in the soil. (Sanni, 2013).

Planting of Maize Seed

Healthy seeds were selected and sorted out. Viability tests were carried out on the seeds using floatation technique. Three seeds of *Zea mays* L. were sown in the soil and watered with 50ml of water. Watering of the plants was done every morning with 50ml of water measured with the measuring cylinder throughout the period of the experiment. Over watering was avoided as much as possible. Emergence of the young seedlings of the plant occurred between the third and fourth day after planting. The data collected based on the performance of *Zea mays* L. in the presence of soil polluted with spent engine oil and unused engine oil accurately taken within the period of seven weeks were: Germination, Plant height; Number of leaves; leaf length; Leaf breadth. Data collected were subjected to analysis of variance (ANOVA).

3. RESULTS

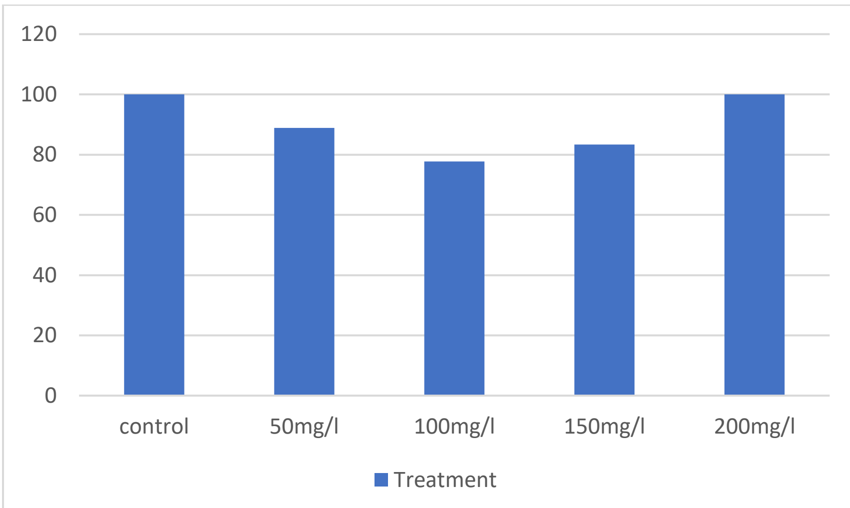

Fig 1: Effects of Spent Engine Oil on the Percentage Germination of *Zea mays* L.

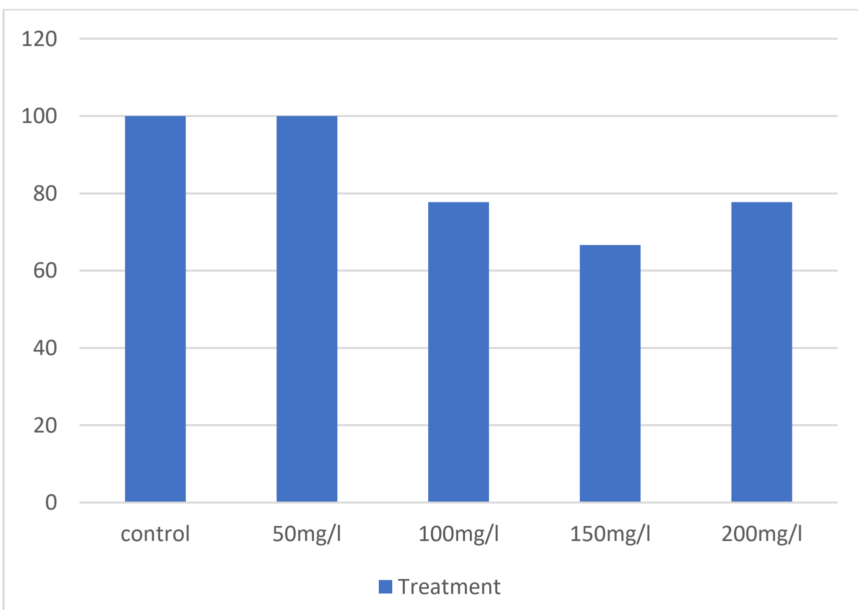

Fig 2: Effects of Unused Engine Oil on the Percentage Germination of *Zea mays* L.

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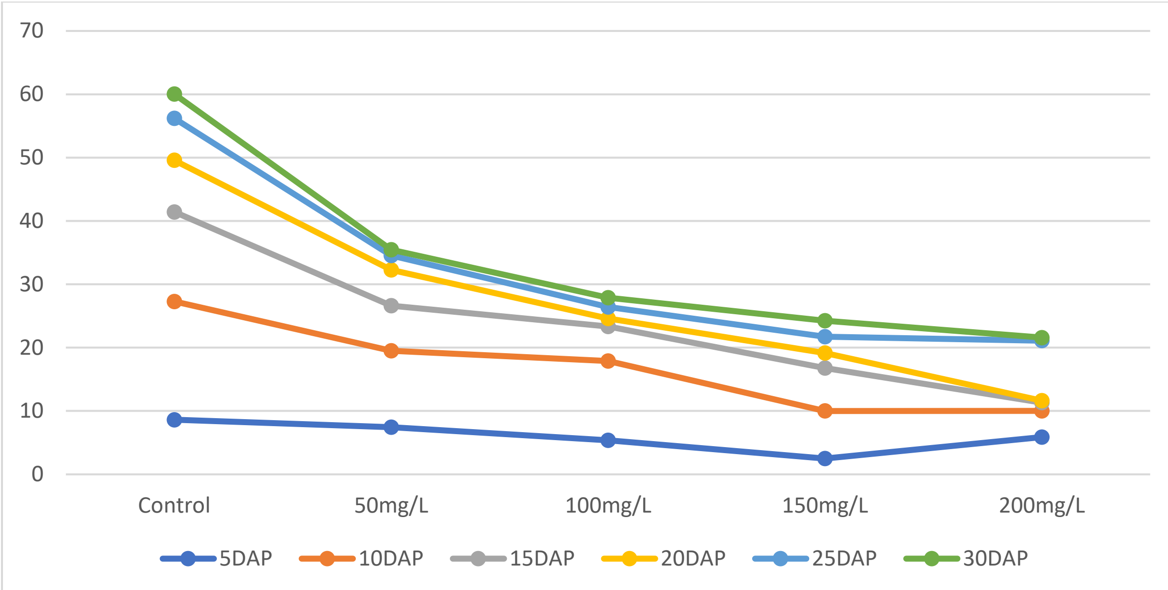
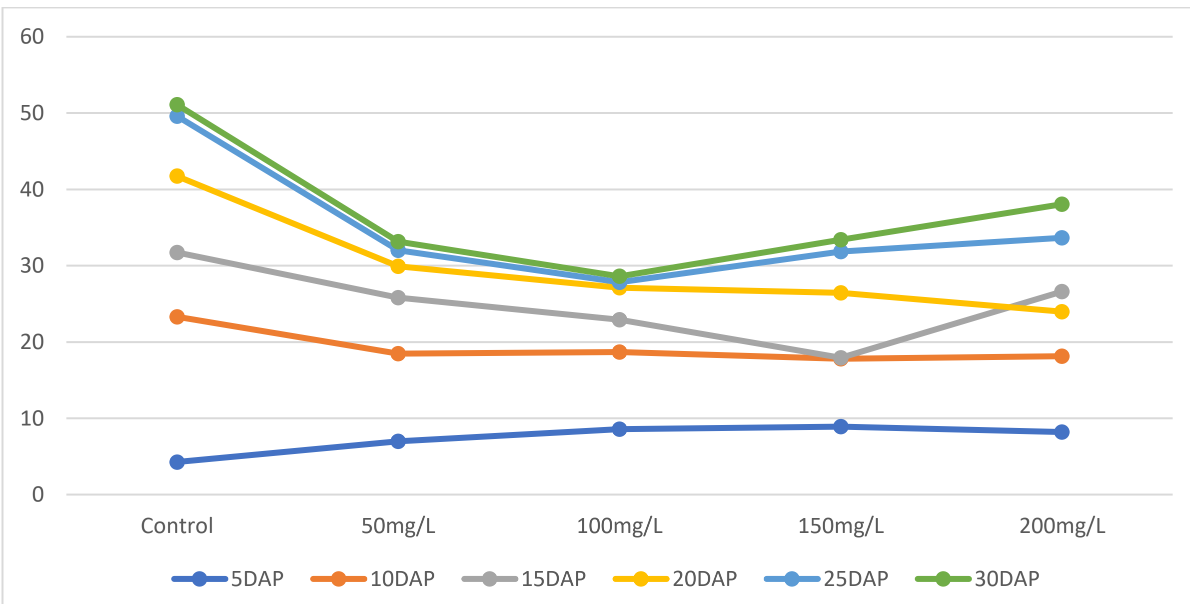


Height in cm
DAP: days after planting

Fig 3: Effects of Spent Engine Oil on Plant Height of *Zea mays* L.



Height in cm
DAP: days after planting

Fig 4: Effects of Unused Engine Oil on Plant Height of *Zea mays* L.

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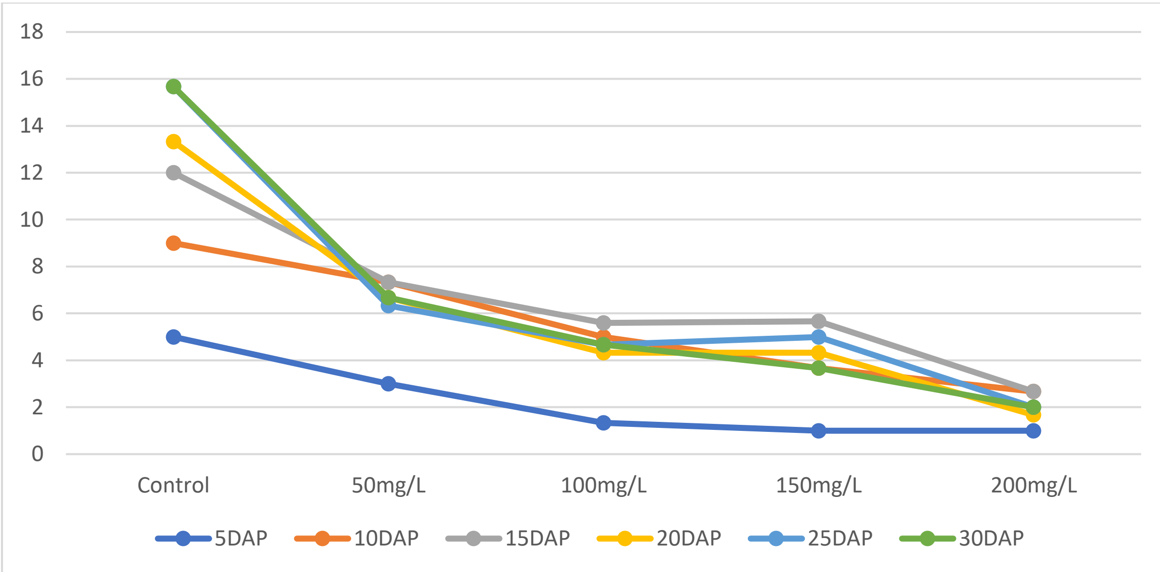
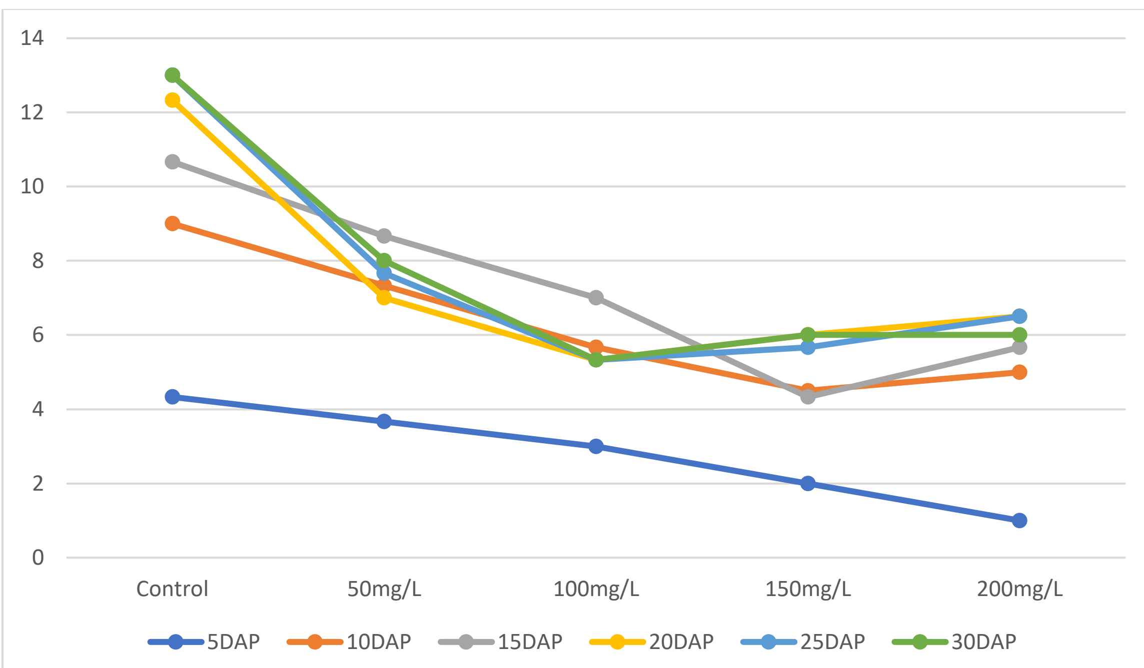


Height in cm

DAP: days after planting

Fig 5: Effects of Spent Engine Oil on the Number of Leaves of *Zea mays* L.



Height in cm

DAP: days after planting

Fig 6: Effects of Unused Engine Oil on the Number of Leaves of *Zea mays* L.

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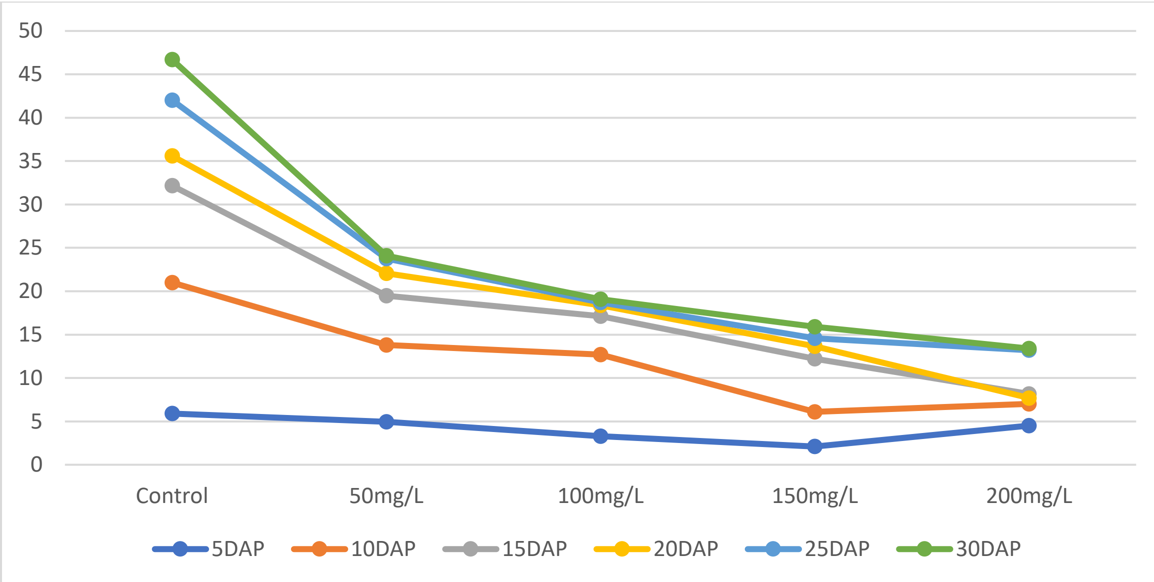
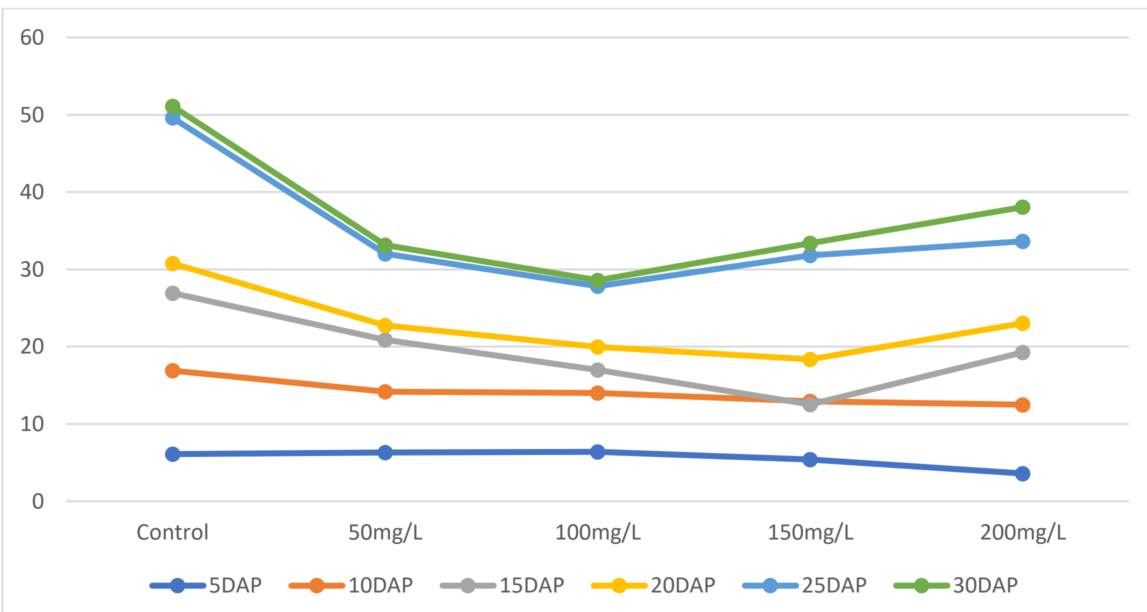


Height in cm

DAP: days after planting

Fig 7: Effects of Spent Engine Oil on the Leaf Length of *Zea mays* L.



Height in cm

DAP: days after planting

Fig 8: Effects of Unused Engine Oil on the Leaf Length of *Zea mays* L.

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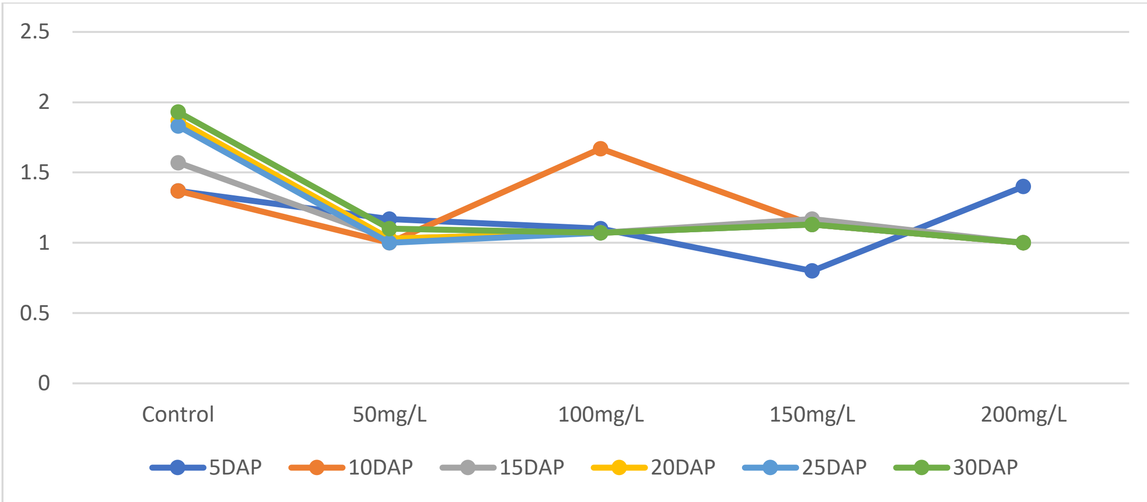
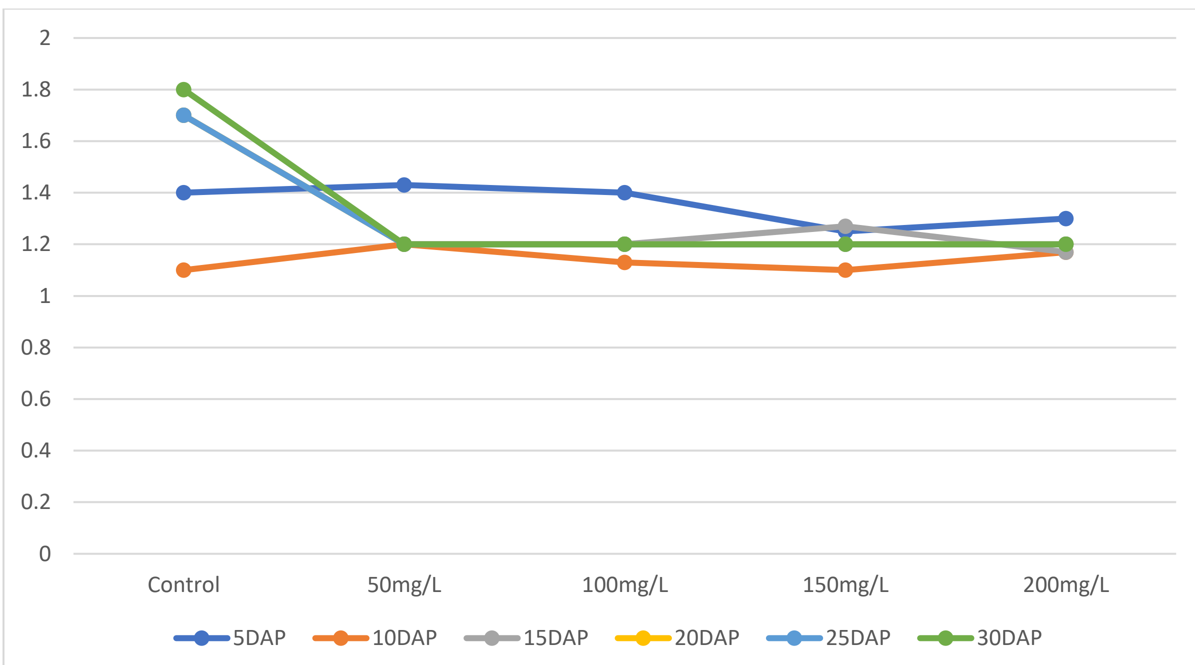


Height in cm

DAP: days after planting

Fig 9: Effects of Spent Engine Oil on the Leaf Breadth of *Zea mays* L.



Height in cm

DAP: days after planting

Fig 10: Effects of Unused Engine Oil on the Leaf Breadth of *Zea mays* L.

4. DISCUSSION

The maize plant grown in soils polluted with either of the petroleum derived products had generally poorer growth than those grown in soils not polluted with either of the products. This is in line with previous research findings (Odjegba and Sadiq, 2002). Some of the reasons cited for the impacts of oil pollution on soil and plants, such as interruption of water and nutrient uptake (Njoku, 2008) and depletion of soil nitrogen and phosphorus content, can be ascribed to this (Baran *et al*., 2002).

The biological, chemical, and physical aspects of soil have all been known to be altered by oil contamination. For instance, Merkl *et al*., (2005) reported that because oil is hydrophobic, it modifies soil moisture conditions and can result in a non-homogeneous distribution of water in the soil. In addition, Terge, (1984) suggested that oil contamination leads to poor wetness and aeration of

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soil. This leads to water and air deficiency in such soil (Njoku *et al*., 2008) and low availability of water to plants. Oil contamination also reduces the soil fertility by causing immobilization of nutrients by microbes (Agbogidi *et al*., 2007). Such immobilization of nutrients leads to difficulty in the uptake of nutrients in oil contaminated soil.

Germination

From Figure 1 and 2 above, the control experiment germinated better than those polluted with spent and unused engine oil. Statistical analysis revealed that there was a significant difference in the percentage of seeds that germinated from the soils treated with spent and unused engine oil ($p<0.05$).

Plant height

Figure 3 revealed that the height of plants grown in used engine oil-polluted soil was statistically significant ($p<0.05$), while there was no statistically significant difference in the height of plants grown in unused engine oil-polluted soil ($p>0.05$) (Figure 4). This could be due to the fact that crops respond to pollutants differently (Adenipekun *et al*., 2006). Unfavorable soil conditions, including insufficient aeration as a result of a decrease in air filled pore spaces (Atuanya, 1987), effects on soil microbes, presence of toxic oil components/herbicidal properties of the soil (Siddiqui and Adams, 2002), reduced biochemical activities, and the presence of heavy metals, could all contribute to the plant's reduced height (Agbogidi and Egbuchua, 2010). The reduced height of *Zea mays* L. due to high level of spent engine oil could also be attributed to deficiency in availability of nutrients needed to maintain physiological processes involved in plant growth. These findings support the findings of Ogbuehi and Ezeibekwe (2010), who found that motor oil depletes available nutrients required for crop growth, particularly in the apical areas. These findings agree with those of Molina *et al*., (2005), who found similar results and speculated that the negative effect could be as a result of the impermeability effect of petroleum hydrocarbons, or the immobilization of nutrients, particularly nitrogen, or the inhibitory effect of some polycyclic aromatic compounds. The retardation in the height of *Zea mays* L. results in its scarcity for man's consumption. Also, its consumption could lead to drastic adverse effects in health of man.

Number of Leaves

From figure 5 and 6, it was observed that there were statistically significant effects at ($p<0.05$) on the number of leaves for plants polluted with both spent and unused engine oil. There were fluctuations in relation to concentration and duration of both spent and unused engine oil. This may be linked to the increased organic matter present in the sand-loam soil which might have induced increased production of leaves in the maize plant. This was in line with the discovery of Fernandes and Henriques, (1991) who discovered that low concentrations of some heavy metals are essential micro-nutrients for plants species, but may cause metabolic disorders at high concentrations and growth inhibition in most plants. Agbogidi *et al*. (2007) also mentioned that a small amount of hydrocarbon in substrates can improve growth media and growth characteristics indirectly. A lower number of leaves were reported at other times when the engine oil concentration was at its highest. This is consistent with Jung's (2008) findings, which show that when pollution levels rise, the number of leaves drops. This could be attributed to a decrease in available macro and micro components required for leaf development. It could also be due to the presence of heavy metals and polycyclic aromatic compounds in spent and unused engine oil, which could induce plant tissue distortion. Wang *et al*. also reported this (2000).

Leaf length

The length of plants grown in soils polluted with used engine oil was significantly different ($p<0.05$) (see figure 7) Plants were found to be dehydrated, indicating a lack of water as a result of an increase in soil toxic levels, as greater levels of soil contamination continued to reduce leaf length. Odu (1981) confirmed similar findings, claiming that the negative effects of oil in soil are caused by particular conditions that make nutrients for plant growth unavailable to plants. The length of plants growing in soil polluted with unused engine oil did not differ significantly ($p>0.05$) (see figure 8).

Leaf breadth

The leaf breadth of the plants grown in both spent and unused engine oil was statistically significant at ($p<0.05$). The observed reduction in leaf breadth at greater levels of oil application (figures 9 and 10) might be attributable to the fact that motor oil application to soil caused conditions that inhibited the plants' water supply. Water stress in *Nicotiana tabacum* inhibits leaf development mostly through a reduction in cell size rather than cell number, according to Cutler *et al*., 1987. The reduction and depression in the leaf breadth of maize plants grown in greater levels of oil most likely resulted in a reduction in photosynthesis due to the reduced leaf breadth of leaves. The reduced leaf breadth following engine oil application to soil may also imply leaf

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shrinkage and Baker (1970) ascribed leaf shrinkage to a reduction or delay in cell expansion after applying oil to the soil. Differential changes in the rate of leaf growth could be linked with anatomical and morphological changes caused by the oil (Agbogidi *et al*., 2007).

Yellowing of leaves

Yellowness of leaves with little patches of light spot coloration was observed on the plants in the soil mixed with 100, 150 and 200 mg/l of spent engine oil. Yellow coloration with grey patches at the middle of the leaf and around the blades was observed on the plants in the soil mixed with 50, 100, 150 and 200 mg/l of unused engine oil. Oil pollution has been observed to promote nutrient immobilization, resulting in the unavailability of some important nutrients while hazardous nutrients are more readily available (BenkaCoker and Ekundayo, 1997, Agbogidi and Ejemete, 2005). This observation agrees with Opeolu and Fadina's (2000) findings that oil contamination causes leaf yellowing. The oil could be interfering with the plant's capacity to absorb some of the mineral elements, resulting in a decrease in chlorophyll levels. Chlorophyll production requires minerals such as magnesium, iron, boron, and manganese (Kent, 2000). Plant death and stunted growth can result from such interference and the lowered rate of photosynthesis that comes with diminished chlorophyll.

Shedding of leaves

During the fourth week of the research, leaf shedding was noticed on the plants at random and on a regular basis on both spent and unused engine oil. The wilting and leaf fall in the treatment groups could be because of the inability of the seedlings to absorb water because they were watered in the same manner as the control experiment (Agbogidi and Eruotor, 2012). This could have resulted from the soil's change due to the presence of oil. This observation concurs with Opeolu, (2000), who stated that modest oil pollution induced leaf yellowing and dropping shortly after planting, whereas excessive oil pollution caused full leaf loss.

5. CONCLUSION

This research has demonstrated that soil contamination with spent and unused engine oil adversely affected the tissues of *Zea mays* L. due to heavy metals that they contained. It is therefore concluded that both spent and unused engine oil as low as 50mg/l is capable of becoming destructive to *Zea mays* L. growth, the soil components and its microorganisms, human and animal health and to the environment in general.

Author Contribution

The two authors Akpan E.N. and Edem, C. A. have made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data of this research.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Adenipekun, C. O., Oyetunji, O. J. and Kassim, L. S. Effect of spent engine oil on the growth parameters and chlorophyll content of *Cocchorus olitorus Linn*. *Environmentalist*, 2008: 28:446-450.
2. Agbogidi, O. M. and Egbuchua, C. O. Heavy metal concentrations of soil contaminated with spent engine oil in Asaba, Delta State. *Agronomica Nigeriana*, 2010:9(1):1-6.
3. Agbogidi, O. M. and Ejemeta, O. R. An assessment of the effect of crude oil pollution on soil properties, germination and growth of *Gambaya albida L*.). *UniswaRes.J.Agric.SciTechnology*, 2005: 8(2):148-155.
4. Agbogidi, O. M. and Eruotor, P. G. Morphological Changes due to spent engine oil` contamination and its heavy metal components of Jatropha curcas. In: Baby, S. and Sandhu, P.S. eds. Proceedings of the International Conference on Bioscience, Biotechnology and Health Sciences ICBBHs'2012

organized by *Planetary Science Centre Research* December 14 and 15, 2012 in Singapore, 2012: Pp.88-93.

5. Agbogidi, O. M. Screening six cultivars of cowpea (Vigna unguiculuta L.) for adaptation to soil contaminated with spent engine oil. *Academic Arena*, 2010: *2(4)*:33-40.
6. Agbogidi, O. M., Eruotor, P. G., Akparobi, S. O. and Nnaji, G. U. Effect of crude oil contaminated soil on the mineral elements of maize (*Zea mays L.*). *Journal of Agronomy*, 2007:6(1):188-193.
7. ATSDR (Agency for Toxic Substances and Disease Registry). Toxicology profile for used mineral based crank case Oil. Department of Health and Human Services, *Public Health Service Press*, Atlanta, GA, USA. 1997:81-97.
8. Atuanya, E. J. Effect of oil pollution on physical and chemical properties of oil, a case study of waste oil contaminated Delta Soil in Bendel State, Nigeria. *Journal of Applied Science*, 1987:55:155-176.
9. Ayoola, S. O.and Akaeze, C. O. Genotoxic evaluation toxicity of spent engine oil on Claria sgariepinus. *Research Journal of Environmental Toxicology*, 2012:6:133-141.
10. Baker, J. M. The effects of oils on plants. *Environmental Pollution*, 1970:1:27-44.
11. Baran, S., Bielinska, E. J. and Wojeikowska Kapusta, A. The formation of enzymatic activity of soil contaminated by petroleum products. *Acta Agrophiscica*, 2002:70:9-19.
12. Benka-Coker, M. O. and Ekundayo, J. A. Effects of an oil spill on soil physico-chemical properties of a spill site in the Niger Delta Area of Nigeria. *Environmental Monitoring and Assessment*, 1995:36(2).93-104.
13. Cutler, J. M., Rains, O. W. and Loomis, R. S. The importance of cell size in the water relations of plant. *Physiol. Plants*, 1987: 40:255-260.
14. DeJong, E. The effect of a crude oil spill on cereals. *Environ. Pollut. Ser. A: Ecol. Biol*., 1980:22:187-196.
15. Dominguez-Rosado, R .E. and Pitchel, J. Phytoremediation of soil contaminated with used motor oil. Enhanced microbial activities from laboratory and growth chamber studies. *Environmentalist*, 2004:251-264.
16. Evdokimov, A. Y. and Fooks, I. G. *Ecological problems of rational use of lubricating materials*. Neftekhimia, Moscow, 1989:64pp.
17. Fernandes, J. C. and Henriques, F. S. Biochemical, physiological, and structural effects of excess copper in plants. *Bot. Rev*., 1991:57:246--273
18. Jung, M. C. Heavy metal contamination in soils and factors affecting metal uptake by plants in the vicinity of a Korean Cu-Wmine, Sensors *Sci. Tot .Environ*, 2008:8:2413-2423.
19. Kent, M. *Advanced Biology*, Oxford University Press, UK, 2000: pp623.
20. Meinz, V. Used oil characterization study. Washington state department of ecological solid and hazardous waste programme. Hazardous waste section Olympia, Washington.1999:98:504-871.
21. Merkl, N., Schutze-Kraft, R. and Infante, C. Assessment of tropical grasses and legumes for phytoremediation of petroleum contaminated soils.*Water, Air and Soil Pollution*, 2005:165(1-4):195-209.
22. Njoku, K. L. Evaluation of *Glycine max* and *Lycopersicon esculentum* in the Remediation of Crude oil Polluted soil. Ph.D Thesis submitted to the School of Postgraduate Studies, University of Lagos, 2008: pp200.
23. Njoku, K. L., Akinola, M. O. and Oboh, B. O. Germination,Survival and growth of accession of *Glycine max L.*(Merril) (Soybean) and *Lycopersicon esculentum* L. (Tomato) in crude oil polluted soil. *Research Journal of Environmental Toxicology*, 2008:2(2):77-84.
24. Odjegba, V. J. and Sadiq, C. A. Effect of Spent Oil on the growth parameters, chlorophyll and proteins levels of *Amaranthus hybridus, L. The Environmentalist*, 2002:22:23-28.
25. Odu, C. T. I. *Degradation and weathering of crude oil under tropical conditions*. In proceedings of seminar on the petroleum industry and the Nigerian environment. Fed. Ministry of Works and Housing and NNPC, warri, 1981:84-91pp.
26. Ogbeide, S. O. An investigation into the recycling of spent engine oil. *Journal of Engineering Science and Technology*, 2011:3(1):32-35.
27. Ogbuehi, H. C. and Ezeibkwe, I. O. Growth performance of cassava variety NR8082 in crude oil polluted soil in Imo State. *International Science Research Journal*, 2010:2:90-94.
28. Opeolu, B. O. and Fadina, O. O. *Sustainable Agriculture-Women and Pesticides.* Proceedings of Gender and Science Technology Association, African Regional Conference, Abuja. 2000:pp288-289.
29. Osinakachukwu ON, Peter UC. Crude oil remediation in selected soil environment of Niger Delta Area of Nigeria. *Discovery*, 2020, 56(289), 16-25
30. Sanni, A.I. Microbiology and physioco-chemical changes during fermentation of masa production. *African Journal of Microbiology Research*, 2013:7(34):435-462.
31. Siddiqui, S. and Adams, W. A. The fate of diesel hydrocarbons in soils and their effects on the germination of perennial grass. *Environ. Toxicol*., 2002:17(1):49-62.
32. Smith, M. J., Flowers, T. H., Duncan, H. J. and Alder, J. Effects of polycyclicaromatic Hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAHs residues. *Environmental Pollution*, 2006:141:519-525.
33. Sztompka, M. Biodegradation of engine oil in soil. *Acta Microbiologica Polonica*, 1999:48(1):185-196.
34. Terge, K. *Oil and Petroleum Journal*, 1984:2:25-30.
35. Udonne, J. D. A comparative study of recycling of used

lubricating oil using distillation, acid and activated charcoal with clay method. *Journal of Petroleum and Gas Engineering*, 2011:2(2):12-19.

36. Wang, J., Jia, C. R., Wong, C. K. and Wong, P. K. Characterization of polycyclic aromatic hydrocarbons created in lubricating aromatic hydrocarbons created in lubricating oil. *Water, Air and Soil Pollution*, 2000120:381–396.
37. Wyszokowska, S. and Kucharski, S. Biochemical properties of soil contaminated by petrol. *Polish Journal of Environmental Studies*, 2000:9(6):476-485.

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