EFFECTS OF OIL CONTAMINATED SOIL ON THE GROWTH OF PLANTS (*Zea mays*)

BY

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SEPTEMBER, 2023

CERTIFICATION

This is to certify that this research titled "**effects of oil contaminated soil on the growth of plants (*Zea mays*)**" was carried out by "**David Chisom Williams**" and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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MR. M. J. EMEJELU **Project supervisor**	**Date**

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PROF. A. A. ENUNEKU **Head of Department**	**Date**

DECLARATION

I "Oisamuede Bright Izebere" declare that "**effects of oil contaminated soil on the growth of plants (*Zea mays*)**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

Davide Chisom Williams

Date

DEDICATION

This project is dedicated to GOD Almighty, the giver of life, strength and wisdom.

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ABSTRACT

Presence of oil in the soil – plant microenvironment influences normal chemistry of soil wherein the release of nutrient and uptake and also the quantity of water is reduced. The present study assessed the effect of oil contaminated soil on the growth and development of maize (*Zea mays*) over a period of 38 days. Result of the study revealed that oil contaminated soil affect the growth of maize in varying degree. The growth in height, root length and leaf area was evaluated using soil contaminated with oil at 25%, 50%, 75% 100% concentration and a control sample. Result showed that there was gradual decrease in height of maize with increasing concentration and the control sample recorded the greatest height. At 10 to 38 days after planting (DAP) lowest growth in height was observed in 100% sample. Also, there was a steady decrease in length of root as the concentration increases while the control sample had the greatest length throughout the experiment. The leaf area of maize gradually decreases as the concentration increases. The control sample also had the greatest leaf area. Lowest growth in leaf area at 10 to 38 days after planting (DAP) was observed in the 100% concentration. This shows that soil contamination with oil affects plants growth and development. It is recommended that care should be taken during oil exploration, exploitation, processing, storage and distribution to avoid contamination of soil by oil which will affect crops leading food shortage. Furthermore, remediation should be carried out on soils that have been previously contaminated.

CHAPTER ONE

INTRODUCTION

1.1 **Background of the study**.

The discovery of oil has revolutionized the modern world and has led to significant advancements in technology, transportation, and energy production. However, oil exploration, drilling, and transportation activities have led to the release of large amounts of oil into the environment, resulting in oil pollution.

Oil contamination of soil is a significant environmental issue that affects both the environment and human health. The release of oil into the environment through exploration, drilling, transportation, and accidental spills has led to soil contamination, which alters soil properties and affects plant growth and survival. The growth and survival of plants in oil-contaminated soils is a critical area of research, as plants play a crucial role in the ecosystem and the maintenance of ecological balance.

Early studies on the effects of oil contamination on plants showed that oil pollution affects plant growth and development, resulting in reduced biomass, stunted growth, and reduced yield (Dekker et al., 2000). The effects of oil on plant growth and development depend on several factors, including the type of oil, the soil type, the plant species, and the level of oil contamination. In recent years, there has been an increasing interest in the study of the growth and survival of plants in oil-contaminated soils, driven by the need to develop sustainable remediation strategies and restore contaminated sites. Oil contamination of soil occurs through accidental spills, leakages from storage tanks, and oil exploration, production, and transportation activities. Oil contamination of soil can have significant impacts on the environment, including the loss of biodiversity, the degradation of soil quality, and the contamination of groundwater and surface water (Ghosh and Singh, 2005). Oil contamination affects soil properties by altering soil texture, reducing water-holding capacity, and inhibiting nutrient availability. The toxicity of oil to plants depends on the chemical composition of the oil, with polycyclic aromatic hydrocarbons (PAHs) being the most toxic components (Nwaichi et al., 2016). PAHs are carcinogenic and mutagenic, and they pose a significant risk to human health.

The impact of oil contamination on plant growth and development has been widely studied, and several mechanisms of plant tolerance to oil pollution have been identified. Plants have developed several mechanisms to tolerate oil pollution, including the breakdown of hydrocarbons by plant enzymes, the excretion of toxic compounds, and the formation of symbiotic relationships with soil microbes (Nwaichi et al., 2016). The understanding of plant response to oil contamination is critical for the development of sustainable remediation strategies and the restoration of contaminated sites.

Aim and objectives of Study

The aim of the study is to investigate the effects of oil contamination on plant growth and survival, identify the mechanisms underlying plant tolerance and resistance to oil pollution, and explore the potential of plants for phytoremediation of contaminated soils.

The objectives include:

- To investigate the effects of different levels of oil contamination on plant growth and survival.
- To identify plant species thatare tolerant and resistant to oil contamination and explore their mechanisms of tolerance.

- To examine the potential of phytoremediation for the remediation of oil-contaminated soils.
- To determine the effects of soil properties such as texture, pH, and nutrient levels on plant growth and survival in oil-contaminated.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sentinels in Soil

The term "soil sentinel organisms" encompasses a diverse group of organisms that inhabit the soil, ranging from bacteria and fungi to nematodes, earthworms, and arthropods. These organisms are not merely passive inhabitants but active participants in the soil ecosystem, influencing its physical, chemical, and biological properties. Their presence, abundance, and activities are sensitive indicators of changes occurring within the soil environment. One of the most fundamental groups of soil sentinel organisms is bacteria (He *et al.,* 2019). These microorganisms are the workhorses of the soil, responsible for decomposing organic matter and cycling nutrients essential for plant growth. Healthy soils are characterized by a diverse and abundant bacterial community, as they help maintain soil structure, pH, and nutrient availability. Changes in bacterial diversity and activity can indicate shifts in soil health, such as pollution, contamination, or changes in land use (Sargsyan *et al.,* 2019).

Fungi, another essential component of soil sentinel organisms, play a complementary role to bacteria. They form symbiotic relationships with plants, known as mycorrhizae, facilitating nutrient uptake by plants and enhancing their resilience to stress. In addition, saprophytic fungi contribute to organic matter decomposition, making nutrients available to other organisms. Monitoring fungal diversity and the presence of mycorrhizal associations can provide valuable insights into soil vitality and plant health (Cofone *et al.,* 2020). Nematodes, tiny, thread-like worms, represent another key group of soil sentinel organisms. These microscopic creatures are found in nearly every soil habitat and occupy various trophic levels in the soil food web. Nematodes are sensitive indicators of soil quality, as their population structure reflects the balance of predation, competition, and resource availability within the soil ecosystem. Their abundance and diversity can reveal information about soil disturbance, contamination, or shifts in nutrient cycling (Zhu *et al.,* 2020).

Earthworms, often celebrated as "ecosystem engineers," are sentinel organisms that have a profound impact on soil structure and fertility. They burrow through the soil, creating channels that improve aeration and water infiltration. Earthworms also ingest organic matter, transforming it into nutrient-rich castings that enrich the soil. Their presence or absence can signify the health and functionality of soil ecosystems, especially in agricultural and disturbed landscapes (Cofone *et al.,* 2020). Arthropods, which include insects, spiders, and other invertebrates, constitute a diverse and dynamic group of soil sentinel organisms. They contribute to various ecological functions, such as decomposition, nutrient cycling, and pest control. Certain soil-dwelling insects, like ants and ground beetles, serve as indicators of soil disturbance and habitat quality. Studying arthropod communities can provide valuable information about the overall health and sustainability of terrestrial ecosystems (He *et al.,* 2019).

Beyond individual organism groups, soil sentinel organisms are integral to broader soil ecosystem services. These services encompass soil fertility, carbon sequestration, water purification, and erosion control, all of which are vital for sustaining agriculture, forestry, and natural habitats. As such, the health and resilience of these soil ecosystems depend on the well-being of their sentinel organisms. Soil sentinel organisms are also critical tools in assessing the impacts of human activities on the environment (Sargsyan *et al.,* 2019). For instance, changes in land use, such as urbanization or agriculture expansion, can dramatically alter soil ecosystems. Monitoring sentinel organisms in such areas helps identify potential ecological disruptions and informs land-use planning and conservation strategies. Additionally, these organisms can indicate the presence of contaminants like heavy metals or pesticides, guiding remediation efforts (Zhu *et al.,* 2020).

2.2 Bioassays with plants

Bioassays with plants are vital tools in environmental science and toxicology, serving to assess the impact of various substances, such as pollutants, chemicals, or environmental stressors, on plant health and the surrounding ecosystem. These experiments are ecologically relevant as plants play a fundamental role in terrestrial ecosystems and can serve as sensitive indicators of environmental changes. Plant-based bioassays find applications in several key areas. By exposing plants to contaminants like heavy metals, pesticides, or organic pollutants, researchers can observe changes in growth, morphology, and biochemical responses (Mkandawire *et al.,* 2014). This helps in assessing the health of an environment and detecting pollution. Certain plants, known as hyperaccumulators, can accumulate high levels of metals in their tissues. Bioassays assist in identifying these species and evaluating their effectiveness in cleaning up contaminated sites, contributing to phytoremediation efforts. Plant growth and root development are closely tied to soil health. Bioassays help gauge soil fertility, nutrient availability, and the impact of soil pollutants, aiding in sustainable land management and agriculture (Khan *et al.,* 2019).

As climate patterns shift, bioassays with plants provide insights into how these changes affect plant growth, distribution, and phenology. They serve as indicators of ecosystem responses to climate change. These assays determine the availability of essential nutrients and pollutants in soil. They help in understanding how readily plants can access nutrients or encounter harmful substances, informing agricultural practices and environmental management (Lutterbeck *et al.,* 2015). The methodology of plant-based bioassays involves selecting suitable plant species, exposing them to the stressor of interest (e.g., in soil or water), monitoring their growth and development, and conducting biochemical analyses to assess molecular responses. Data analysis, often using statistical tools, enables researchers to draw conclusions about the stressor's effects. Challenges in plant-based bioassays include variability in plant responses, the complexity of environmental factors, and the need for standardized protocols. However, ongoing advancements in molecular biology and technology are improving the precision and applicability of these assays (Pihneiro *et al.,* 2015).

Plant growth is inhibited by both the nature of the hydrocarbons and the hydrocarbon concentration present. The hydrophobic properties of hydrocarbons limit the ability of plants to absorb water and nutrients from the soil. Low molecular weight hydrocarbons can be more detrimental because they are highly bioavailable to plants. Shorter chain compounds with lower boiling points (150 - 275 °C) are rather more toxic. Plant uptake of low molecular weight hydrocarbons may result in blockage of stomata and intercellular spaces, leading to a reduced plant transpiration rate along with cell membrane damage. Plant growth is also suppressed by petroleum contamination at high concentrations (Khan *et al.,* 2018). Wheat and maize are used as model bio-indicators in assays to evaluate total petroleum hydrocarbon toxicity in the soil. They are often used due to their worldwide availability, higher germination efficiency, ability to grow in a wide range of soil types and environment. Due to variation in plant morphology (e.g., roots), physiology (e.g., root exudates), and microbial interactions in the rhizosphere, not all plant species tolerate the same level of contamination (Sharonova and Breus, 2012).

Analysis of seed germination and the rate of root growths are among the most important factors used in assessing terrestrial ecotoxicology. The growth of roots has been shown to be a more sensitive biomarker than the germination of seeds Species of economic plants have been used to assess contamination of soils by crude oil and these include *Lactuca sativa* (lettuce), *Lepidium sativum* (cress), *Zea mays* (maize) and *Triticum vulgare* (wheat) (Paton *et al.,* 2005). Some other plant species are used in the genotoxic and mutagenic evaluation of contaminated soils and include *Allium cepa, Tradescantia* and *Vicia faba.* Genetic biomarkers used include leaves, pollen and endosperm. Different methods of exposure are used in bioassays and the most commonly used are direct contact, use of aqueous and organic extracts. The direct contact method is considered to be the most effective (Souza *et al.,* 2011).

2.3 Other bioassays

The Microtox test is a common bioassays of acute toxicity which is used to assess petroleum hydrocarbon-contaminated soils. The test works on the principle of measuring the inhibition of bioluminescence produced by *Vibrio fischeri* when in stressed conditions. The method is used for its high sensitivity, speed and the possibility of application in real-time monitoring (Paton *et al.,* 2005). The Ames test is another bioassay which is used to detect genotoxicity of petroleum hydrocarbons using *Salmonella typhimurium.* The method is important in cases of contamination with polycyclic aromatic hydrocarbons which, though originally inert, may undergo metabolism and lead to formation of electrophilic derivatives which bind to nucleophilic centres of DNA, form adducts and induce frameshift mutations, deletions, strand breaks and other chromosomal abnormalities. The chromotest and umu-test have been developed as alternatives to the Ames test.

The earthworm species *E. fetida*, has been commonly used for acute and subchronic toxicity testing of contaminated soil due to their widespread availability, ease of growth under laboratory conditions, possible transfer of contaminants from soil through mucous membranes in the organism's exterior, and the organism's direct ingestion of soil particles while feeding on organic matter in the soil (Lionetto *et al.,* 2012). Earthworms have been shown to be as much as 14 times more sensitive to contamination than some bacteria and up to 77 times more sensitive than plants to petroleum contamination. The use of potworms may also offer similar benefits. These direct exposures of hydrocarbon cause oxidative stress and damage to immune response of these soil animals. Thus it is likely that if these organisms are not affected, other important soil organisms equally vulnerable (such as nematodes) or even less vulnerable (such as ants and beetles), will also not be affected (Khan *et al.,* 2018).

2.4 Petroleum in the Soil

Petroleum hydrocarbons are utilized as fuels all over the world. Crude oil, diesel, gasoline, heavy oil, and kerosene are the most regularly utilized petroleum products. Petroleum and its derivatives enter the soil through the following routes: accidental spills caused by crude oil leaks in oil pipes, accidents at gas stations and those involving petroleum transportation tankers, and improper discharge of industrial solid residues and used oils from mechanic shops and automobile repair shops (Salanitro, 2000).

Petroleum is made up of several complex hydrocarbon mixes that may be classified into three main groups: saturated hydrocarbons, aromatic hydrocarbons, and non-hydrocarbon chemicals (Wang *et al*., 2017). Saturated hydrocarbons contain biodegradable molecular architectures composed of carbon-to-carbon and carbon-to-hydrogen bonds. Because they have low boiling temperatures, they can evaporate from polluted media via volatilization and photosynthesis. Aromatic hydrocarbons have more complicated structures. They are made up of benzene rings, which have higher boiling temperatures, making removal from environmental media more challenging. Polycyclic aromatic hydrocarbons (PAHs - the most prevalent) are examples of this type of hydrocarbons, as are fluoranthene, phenanthrene, naphthalene, acenaphthylene, anthracene, benzo[a]anthracene, diphenyl[a,h]anthracene, and [1,2,3-cd]pyrene (Wang *et al*., 2017).

The group with the most carbon atoms is non-hydrocarbon compounds. Their features include water insolubility and extremely high boiling and fusion temperatures. It has been discovered that non-hydrocarbon molecules include trace quantities of heavy metals such as nickel and vanadium. They are exceedingly difficult to remove from environmental media and are known to be mutagenic and hazardous to the environment (Chiara *et al*., 2009).

2.5 Impacts of Petroleum in the Soil

2.5.1 Effects on physicochemical properties of soil

Petroleum hydrocarbons are rapidly absorbed and adsorbed in the surfaces of soil particles due to their low densities, high viscosities, and poor capacity for emulsification, resulting in negative impacts on the porosity and permeability of the contaminated soil. Petroleum and its derivatives are high in carbon but low in nitrogen compounds; as a result, they can alter the structure and content of organic matter, the C/N and C/P balances, and the salinity, pH, and conductivity of soils. The heavy metals in petroleum, together with the salt concentration, harm the soil ecology (Li *et al.,* 2009; Wang *et al.,* 2017)

2.5.2 Effects on soil microorganisms

The microbial community and populations are an essential component of the soil that is greatly influenced by oil pollution or contamination. It is difficult, if not impossible, to discover a well-developed population of microorganisms that can survive oil stress or biodegrade petroleum in uncontaminated soils. However, when petroleum or its derivatives are introduced into soil, these microbes generate enzyme-systems that allow them to adapt to changes in their environment via the establishment of symbiotic or synergistic effects (Chiara *et al*., 2009). According to research, petroleum hydrocarbon contamination of soil can cause changes in the soil microbial population, microbial community structure composition, and soil enzyme systems (Wang *et al*., 2017).

2.5.3 Adverse effects on crops

The presence of petroleum hydrocarbons in soil prevents agricultural plants from growing normally. This can manifest as a decline in germination rate, fertility, or the induction of diminished resistance to pests and diseases. Petroleum and its derivatives can react with the inorganic phosphorus and nitrogen in the soil. This decreases the quantity of accessible nutrients due to nitrification and phosphoric acid removal, resulting in lower crop uptake of these nutrients (Liao *et al*., 2015).

2.5.4 Pollution of water and air

Petroleum hydrocarbons are easily evaporated and enter the atmosphere due to their low molecular weights and boiling temperatures. They can reach surface water by runoff from polluted soils, and groundwater via leaching and downward vertical movement. All of these media wind up in the food chain, where they bioaccumulate before entering the human body via eating, cutaneous contact, or respiratory routes. These substances have been shown to have a variety of deleterious effects on human health, including carcinogenicity, teratogenicity, mutagenicity, toxicity to organs such as the liver and kidneys, and so on. Petroleum components that are insoluble in water float and create thin coatings on the surface. This produces particle and organic matter agglomeration and has a negative impact on oxygen transport between air and water. Heavier fractions accumulate in sediments at the bottom of the water body affecting bottom-feeding fish and other organisms (Ahmed and Fakhruddin, 2018).

2.4 Empirical literature review

Oyedeji *et al.* (2012) studied the effect of crude oil contaminated soil on the germination and growth performance of *Abelmoschus esculentus* L. Moench – a vegetable crop widely grown in Nigeria. The experiment was carried out in the screen house, under a controlled environmental condition. The girth, height and seedling emergence percentage were assessed to ascertain the growth performance of the crop in soil contaminated with crude oil. The result of the study showed that soil contaminated with crude oil affects the growth performance of *Abelmonschus esculentus L. Abelmonschus esculentus* grown in oil contaminated soil started germination after 6 days of sowing in comparison to control soil were germination started in 4 days of sowing. There was 100% emergence in uncontaminated soil (control). There was steady decrease in co-efficient of velocity and emergence as the concentration of oil increased above 1ml. stunted growth was observed in plants in contaminated soil accompanied with yellow leaves, some leaves dropped while some plant died in the 4th week of sowing. Height and girth of plant were also affected by the concentration of crude oil in the soil polluted with oil. The study proved that crude oil introduction into agricultural soils severely and adversely affects agronomic development and growth of plant (*Abelmonschus esculentus*) and also decreased the girth and height of the plant which consequently affects adversely the plant yield. Therefore, agricultural soil contamination with crude oil should be avoided and awareness of the public should be raised concerning the deleterious effects of crude oil pollution in the environment. Furthermore, remediation should be carried out on agricultural soils that have been grossly and previously contaminated with crude oil exploration and exploitation.

Ekpo, *et al.* (2012) In Calabar, Nigeria, scientists looked into how crude oil affected the germination and growth of the Glycine max plant. Each polythene bag used for planting was 60 x 25 x 15 cm in size, with 5 kilogram of top soil (0–15 cm depth) gathered from the biological science experimental farm. Each crude oil concentration required the usage of five polythene bags, for a total of 25 bags. Various crude oil concentrations (20ml, 40ml, 60ml, and 80ml) were added to soil samples that were placed in polythene bags in order to treat the soil. Each soil sample enclosed in 5 polythene bags received a different concentration of crude oil, which was applied before being well mixed with a hand trowel. Three soybean (Glycine max) seeds were inserted into each of the soil sample treated with varying concentrations of crude oil. The outcome showed that pollution from crude oil considerably slows (p 0.05) growth of the soybean plant when pollution levels are higher than when pollution levels are lower. This suggests that the more crude oil there is or is concentrated in the soil, the more of an impact it would have on plant growth and germination soybean crop.

Han *et al.* (2016) studied the effects of crude oil contaminated soil on photosynthesis of *Amorpha fruticosa* seedlings. From April through September, a pot experiment was carried out to observe the dynamic response of photosynthesis of *Amorpha fruticosa* seedlings to various amounts of petroleum-contaminated soils. The results demonstrated that throughout the three specified sampling periods of July 31 (early), August 30 (mid-term), and September 29 (late), the photosynthetic rates, stomatal conductance, and transpiration rate of seedlings considerably decreased in soil contaminated with petroleum at a concentration of 5–20 g kg^{-1}. However, the intercellular CO_2 concentration greatly decreased in the 20 g kg^{-1} contaminated soil during the early sampling time and the 20 g kg^{-1} contaminated soil during the late sampling period, while it significantly increased in the 10 g kg^{-1} contaminated soil. During the early sampling period, in soil polluted with 20 g kg^{-1}, seedlings' leaf relative water content significantly rose, however during the late sampling period, in soil contaminated with 15-20 g kg^{-1}, it significantly decreased. In the three sampling periods in the polluted soil, the amounts of chlorophyll a, chlorophyll b, and total chlorophyll in the seedlings exhibited a dramatic drop. *A. fruticosa* was tolerant of petroleum-contaminated soil and may be useful for the phytoremediation of petroleum-contaminated sites in northern Shaanxi, China, when taking into account the negative effects of petroleum on photosynthesis, growth performance, and remediation effect on petroleum of seedlings.

Emerhi and David-Sagoro (2017) carried out the effect of crude oil on the germination and growth performance of *Moringa oleifera* Lam. In a three-times-repeated experiment, top-soil samples weighing 1 kg each were thoroughly mixed with 0, 4, 8, and 16 ml of crude oil to test the effects of different crude oil contamination levels on *Moringa oleifera's* adaptability, germination, and growth. Each pot included three *Moringa oleifera* seeds, which were observed over 12 weeks. The outcomes demonstrated that crude oil in soil had a significant impact on *M. oleifera* seedling performance, with the effect being oil concentration dependant. With germination percentages ranging from 20.00% to 73.33%, germination began on the fourth day after sowing (DAS) and was completed between the fourteenth and fifteenth days. With a germination rate of 73.33% in week 1, the control group (0 ml/kg of soil) had the greatest germination rate. Plant heights were 2.12 cm for the control group, 1.25 cm, and 0.79 cm for the 4 and 16 ml/kg of soil groups, respectively, at the eighth week after planting (WAP). While the control group had the highest mean leaf number (6.57), and plants in soil contaminated at 16ml/kg had the lowest (3.47), some seedlings died at higher oil concentration levels of 12ml and 16ml/kg of soil. The concentration of crude oil had a significant impact on the mean height (cm) of the seedlings in soils contaminated with 8 and 16ml of oil per kilogram of soil. According to the study, crude oil in the soil considerably decreased the plant height, number of leaves, and leaf girth of *M. oleifera* seedlings. It was determined that even though *Moringa oleifera* seedlings could withstand small amounts of crude oil, increasing levels of oil contamination in the study had a significant negative impact on their performance.

Odiyi *et al.* (2020) studied the effect of crude oil contamination on maize. The goal of the research was to determine the impact of crude oil pollution on the morphology, growth, and heavy metal content of maize (*Zea mays* L.), by using standard field experimental techniques to analyze heavy metals and chosen physicochemical characteristics in soil samples and plant sections. The study's findings demonstrate that crude oil pollution had a negative impact on all of the growth characteristics of *Zea mays* planted in the polluted soil. The highest mean height for the 50 ml, 100 ml, and 150 ml treatments was 33.54 cm, followed by 31.34 cm, and 27.44 cm, respectively. The control's highest mean height was 87.58 cm. As the amount of crude oil increased, the pH of the soil rose. The amount of crude petroleum increased, which resulted in a decrease in the chlorophyll content of *Zea mays* leaves. Among the plant parts tested, the roots had the highest concentrations of chromium (Cr) (0.22 ppm), nickel (0.46 ppm), lead (0.06 ppm), and cadmium (0.02 ppm), whereas the highest copper (Cu) value of 0.28 ppm (150 ml) was found in the leaves. The content of heavy metals considerably increased (P 0.05) along with the volume of crude oil pollution. This study has demonstrated that the growth, yield, and leaf chlorophyll of maize plants are negatively impacted by soil that has been contaminated with crude oil. This could result in shortages and safety concerns in conjunction with rising heavy metal concentrations; consumption of corn in locations where contamination from crude oil is a problem.

Ismail *et al.* (2021) studied the effect of soil contaminated with crude oil on Cowpea. They opined that soil contamination with petroleum hydrocarbon is still a major issue affecting food security and soil health in areas were oil exploration is performed. The study assessed the impact of bonny light crude oil on Cowpea (*Vigna unguiculata* L. Walp) with the idea of evaluating the toxicity on the growth and performance of plant. To obtain contamination levels of 0.0%, 2.5%, 5.0%, 7.5%, and 10.0% v/w, pure soil samples were taken in various pots and contaminated with crude oil. Cowpea seeds that were viable were sown, and for a 12-week period, the emergence and subsequent growth were observed. Results revealed that the crude oil delayed the emergence of sprouts by two days at a rate of 96.7%, 80.0%, 50.0%, and 96.7%, respectively, and prolonged the time it took for seeds to germinate. Shoots, roots, and leaves of the plants were all longer in the control soil than they were in the polluted soil.

According to a phytotoxicity research, the plants' shoots, roots, and leaves were all much shorter than the control by about 50%. The effects on relative plant weights, chlorophyll content, and leaf count were most severe in plants growing in greater crude oil concentrations, where fewer or no leaves were seen at the conclusion of the tests. In contrast to the control where flowers, fruits, and seeds were produced, no yield parameter was seen in any of the plants cultivated in polluted soil. The results showed that hydrocarbon contamination significantly slowed the growth rate of *V. unguiculata* in a concentration-dependent manner. It also illustrated the threat to food security that is already there, particularly in border basins where oil exploration activities are about to begin. As a result, there is a need to develop and implement efficient strategies that reduce or eliminate oil spills during oil exploration activities.

Balogun *et al.* (2022) studied the effect of crude oil pollution of soil on the vegetative growth of plantain (*Musa paradisiaca*). They opined that the growth of plant is impacted by climatic, genetic and edaphic factors. Soil pollution may affect biota adversely by limiting the survival and growth of plants. Hence, the study was carried out to evaluate the potential effect of petroleum contaminated soil on the growth of two varieties (French and False Horn plantain) of *Musa paradisiaca.* Suckers of plantain were grown on agricultural soil and crude oil was then added to the soil. Growth of plantain suckers were evaluated periodically in terms of total leaf area, height and girth of stem. It was observed that the height of plant (*Musa paradisiaca*) was reduced enormously in the two variety cultivated in crude oil polluted soil compared to the controls cultivated on soil that is unpolluted. Also, the girth of stem was decreased insignificantly in cultivars grown in polluted soil. There was increase in total leaf area for French plantain grown on soil contaminated with crude oil than in control. However, in False Horn plantain total leaf area was greater in control than those grown in polluted soil. There was no uniformity with time in the rate of increase in stem girth, height and leaf area for the two cultivars. French plantain survived the stress better caused by soil pollution than the False Horn plantain. However, both varieties grew appreciably in crude oil polluted soil when compared with the controls. Therefore, they may be important in remediating crude oil polluted soils.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Sample Collection

3.1.1 Soil Collection

The soil used in the experiment was collected from Oredo flow station , Ologbo Benin city, Edo state around the gas flaring sites. The soil was randomly collected from depths of 0-15cm within 200m radius from the flare stack at four different points 50 m apart in triplicate and was pooled together as a composite sample .The soil was air dried, plant residues, stones and other debris excluded, and passed through 2 mm sieve.

3.1.2 Study design

The study employed a randomized complete block design involving five sets of plastic pots. The pots were arranged in three replicates and were properly labeled as Control, 25%, 50%, 75% and 100%. Soil (sandy soil made up of 89.4% sand, 8.6% silt, and 2.0% clay particles; pH 6.8 ± 0.60; 27°C) weighed and placed into the plastic pots.

3.1.3 Collection of Seeds for the study

The seeds were purchased from Edo state Agricultural Development Programme (EDO ADP).They seeds were *Phaseolus vulgaris* (beans)

3.1.4 Selection and Storage of seeds

The seed were purchased in large number from B.A.D.P Benin City and were selected (eliminating unhealthy looking , scarred or infected seeds) thereby obtaining fairly homogenous seed populations which were stored in tightly closed bottles placed inside large dessicators. The dessicators were kept on the desk cupboard in the laboratory. The seed samples were obtained from this homogenous population for experiments.

3.1.5 Seed Viabilty Test

All seeds used during these study were tested for viability at the start of the experiments. Floating method (Carolyn, 2019) was used in these test. This involved taking seeds and placing them in a container of water .let the seeds to sit for 15minutes. If the seeds sink, they are still viable; if they float, discard, because they probably will not sprout.

3.1.6 Germination test procedure

To ensure that viable seeds were exposed to essential conditions necessary for germination, selected seeds were presoaked in distilled water six hours prior to planting water was blotted out of the seeds before planting to prevent excessive soaking, this process is essential as first sign of germination to ensure uniform germination of the seeds.

3.1.7 Plant germination and root elongation inhibition

Plants were cultivated with 1210 mg/kg TPH contaminated soil. The experiment was carried out in a natural climatic condition , the pots contained 4kg of soil for each sample .The germination rate was measured after 10 days of planting with four days intervals successively. Plant growth experiment at different TPH concentrations was carried out based on toxicity according to procedures developed in EPA guidelines (EPA 712-C-96-154 ,1996; EPA 712-C-96-153 ,1996).. Beans were used. The contaminated soil was set to four concentrations: 100%, 75%, 50%, 25%, by diluting 1210 mg/kgTPH contaminated soil with soil used as control in weight/weight ratio. Root lengths were measured at the end of the experiment.

3.1.8 Water holding

The soil was weighed and put in a bowl with holes underneath it. The soil samples were then watered to saturation point and allowed to drain. The water holding capacity was then calculated below as wet mass – dry mass of soil.

3.1.9 Soil pH and electrical conductivity

Twenty grammes (20 g) of fine soil were weighed and placed in a container and 50 ml of distilled water added. The suspension was mixed for 30 mins and allowed to settle. Electrical conductivity and pH of the solution were then measured using a pH meter (model 215) and conductivity meter. The pH meters were first standardized using a buffer solution.

3.1.10 Analysis of Total Petroleum hydrocarbon (TPH)

The oil content was estimated using the method of USEPA (1986). For the extraction of hydrocarbon, one gram of soil sample was delivered into 10ml chloroform in an extraction flask. The mixture was shaken vigorously for 2 minutes and allowed to stand for the soil particle to settle. The oil was extracted and determined by the absorbances of the extract at 420nm in an SP 6 Pyeunican spectrophotometer. A standard curve of the absorbance of different known concentration of equal amount of crude oil in the extract was first drawn after taken reading from the spectrophotometer. The standard curve was used to estimate the oil concentration after multiplying by an appropriate dilution factor.

3.2 Statistical analysis

Data from the laboratory analyses were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS 20.0) and Microsoft Excel 2013 . The descriptive statistics were conducted. Means were separated using Duncan Multiple Range Test at 5% confidence limit.

CHAPTER FOUR

4.0 RESULT

4.1 Maize Height

Table 4.1 shows the mean height in response to exposure of different concentration of the contaminated soil, it was observed that at 10days after planting there was a highly significant difference $p<0.01$ between the mean heights of maize planted on the control soil with that of the stock soil which was designated as the soil with 100% concentration, this same trend was also observed at day 38 after planting. At day 38 after planting there were no significant difference between the mean height of maize on the control soil and the mean height of maize on the soil that is 25% contaminated. However there was a significant difference between the mean height in the control soil and the mean height of the remaining soils with various level of contamination.

Table 4.1: Maize Height

Conc.	Control	25	50	75	100	
	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	p-value
10 DAP	1.97 ^{a} ±0.06 (1.90-2.00)	1.60 ^{b} ±0.10 (1.50-1.70)	1.07 ^{c} ±0.06 (1.00-1.10)	1.10 ^{c} ±0.10 (1.00-1.20)	0.53 ^{d} ±0.06 (0.50-0.60)	p<0.01
14 DAP	4.20 ^{a} ±0.10 (4.10-4.30)	3.03 ^{b} ±0.06 (3.00-3.10)	2.53 ^{c} ±0.06 (2.50-2.60)	2.23 ^{d} ±0.06 (2.20-2.30)	2.10 ^{d} ±0.10 (2.00-2.20)	p<0.01
18 DAP	4.43 ^{a} ±0.06 (4.40-4.50)	3.47 ^{a} ±0.06 (3.40-3.50)	3.10 ^{b} ±0.10 (3.00-3.20)	2.67 ^{c} ±0.06 (2.60-2.70)	2.50 ^{d} ±0.10 (2.40-2.60)	p<0.01
22 DAP	4.90 ^{a} ±0.10 (4.80-5.00)	3.87 ^{b} ±0.15 (3.70-4.00)	3.40 ^{c} ±0.10 (3.30.3.50)	3.13 ^{d} ±0.06 (3.10-3.20)	3.03 ^{d} ±0.06 (3.00-3.10)	p<0.01
26 DAP	5.47 ^{a} ±0.06 (5.40-5.50)	4.53 ^{b} ±0.06 (4.50-4.60)	3.87 ^{c} ±0.15 (3.70-4.00)	3.77 ^{c} ±0.06 (3.70-3.80)	3.53 ^{d} ±0.06 (3.50-3.60)	p<0.01
30 DAP	6.50 ^{a} ±0.00 (6.50-6.50)	6.03 ^{b} ±0.06 (6.00-6.10)	5.77 ^{b} ±0.06 (5.70-5.80)	5.30 ^{c} ±0.35 (4.90-5.50)	5.03 ^{c} ±0.06 (5.00-5.10)	p<0.01
34 DAP	9.13 ^{a} ±0.15 (9.00-9.30)	8.53 ^{b} ±0.06 (8.50-8.60)	8.10 ^{c} ±0.10 (8.00-8.20)	7.53 ^{d} ±0.06 (7.50-7.60)	7.07 ^{e} ±0.06 (7.00-7.10)	p<0.01
38 DAP	11.60 ^{a} ±0.10 911.50-11.70)	11.47 ^{a} ±0.06 (11.40-11.50)	11.17 ^{b} ±0.15 (11.00-1.30)	10.17 ^{c} ±0.06 (10.10-10.20)	9.13 ^{d} ±0.15 (9.00-9.30)	p<0.01

Table 4.2: Maize Root Length

Conc.	Control	25	50	75	100	
	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	$\bar{X}$±SD (Min-Max)	p-value
38 DAP	42.33 ^{a} ±2.08 (40.00-44.00)	39.40 ^{b} ±0.17 (39.20-39.50)	18.00 ^{c} ±0.00 (18.00-18.00)	17.00 ^{cd} ±1.00 (16.00-18.00)	15.33 ^{d} ±0.58 (15.00-16.00)	p<0.01

4.2 Maize Root Length

Table 4.2 shows the mean root length which is also a criteria for assessing germnation/growth and survival of plant in an ecological risk studies. The mean root length in the control soil was significantly different from that which was grown in all the various level of contamination used in the study. For instance the root length from the control ranged from 40.00-44.00 whereas the root length from the 100% contaminated soil ranged from 15.00-16.00. It was also observed that root length at the 25% contamination rate was also different from that in the 50% sample. However ,it was noted that the mean root length at the 50% contamination has some shared similarity with the mean root length in the 75% soil sample but completely different from that of 100% sample.

4.3 Effect of concentration of Gas Flared Contaminated Soil Concentrations on leaf areas of maize.

The effect of concentration of pollutants on leaf area of maize were analyzed and it was observed that as from 10 days after planting (DAP) to 38 days after planting (DAP) leaf area of maize were all significant ($p<0.05$) compared with the control. It can be inferred that significantly influenced the leaf area of maize even as the days after planting progressed. It can be inferred that concentration significantly influenced the leaf area of maize even as the days after planting progressed.

Table 4.3: Effect of concentration of Gas Flared Contaminated Soil Concentrations on leaf areas of maize

CONC. (%)	10 DAP	14 DAP	18 DAP	22 DAP	26 DAP	30 DAP	34 DAP	38 DAP
Leaf areas of maize(cm^2)								
100	0.15±0.01	0.37±0.01	3.15±0.01	6.02±0.02	11.25±0.01	16.24.1±0.04	24.15±0.01	29.37±0.06
75	0.15±0.01	0.75±0.01	3.67±0.01	6.11±0.02	12.22±0.04	17.02±0.02	25.17±0.06	31.17±0.06
50	0.15±0.01	1.13±0.01	4.17±0.06	7.17±0.06	12.75±0.06	18.87±0.06	26.25±0.01	33.57±0.06
25	0.15±0.01	1.5±0.01	4.73±0.01	7.77±0.06	13.47±0.06	19.77±0.06	27.27±0.06	36.24±0.01
control	0.15±0.01	1.87±0.01	5.25±0.01	8.37±0.06	14.25±0.01	20.67±0.06	28.35±0.01	43.43±0.02
P-values	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CHAPTER FIVE

5.0 DISCUSSION

Pollution of soil with oil has been a serious challenge over the past years. Soil supplies the required nutrients for the growth and development of plant. These nutrients are available for uptake by plant through solubilisation of these nutrients into the liquid phase. The process of nutrient release for usage by plant is essential. Hence, disruption or disturbance of this process through contamination of soil by oil or other pollutants negatively affects plant growth and development. The availability of oil in the soil – plant microenvironment influences normal chemistry of soil wherein the release of nutrient and uptake and also the quantity of water is reduced (Nwoko *et al.,* 2007). The present study assessed the effect of oil contaminated soil on the growth and development of maize (*Zea mays* L.) over a period of 32 days. Result of the study revealed that oil contaminated soil affect the growth of beans in varying degree.

5.1 Growth as influenced by the concentration of oil contaminated soil

5.1.1 Maize height

Result of the experiment presented in table 4.1 shows that concentration of oil affected maize height. In 10 days after planting (DAP) the mean value of maize height was 1.97, 1.60, 1.07, 1.10 and 0.53 for control, 25%, 50%, 75% and 100% concentration respectively. There was a steady decrease in the height of beans plant as the concentration increases. The control sample which was not contaminated with oil had the greatest height. At 14 days after planting the mean value of maize height was 4.20, 3.03, 2.53, 2.23 and 2.10 for control, 25%, 50%, 75% and 100% concentration respectively. The height of maize plant in the control sample was higher in every other sample and the least growth in height was observed in the 100%

concentration sample. Nwoko *et al.* (2007) and Ekpo *et al.* (2012) reported a similar result wherein under 14 days after planting, there was a gradual decrease in the height of beans and soybean plant respectively as the concentration of oil increased in soil and drastic height reduction was observed in the sample which had the highest concentration of oil. At 18 days after planting the mean value of maize height was 4.43, 3.47, 3.10, 2.67 and 2.50 for control, 25%, 50%, 75% and 100% concentration respectively. At 22 days after planting, the mean value for maize height was 4.90, 3.87, 3.40, 3.13 and 3.03 for control, 25%, 50%, 75% and 100% concentration respectively. The highest growth in the height of maize was recorded in the control sample while the least growth was observed in 100% concentration. Okonokhua *et al.* (2007) also had similar result of maize height reduction as the concentration increase. At 26 days after planting, the mean value of maize height was 5.47, 4.53, 3.87, 3.77 and 3.53 for control, 25%, 50%, 75% and 100% concentration respectively. At 30 days after planting, the mean value was 6.50, 6.03, 5.77, 5.30 and 5.05 for control, 25%, 50%, 75% and 100% concentration respectively. At 34 days after planting, the mean value of maize height was 9.13, 8.53, 8.10, 7.53 and 7.07 for control, 25%, 50%, 75% and 100% concentration respectively. while at 38 days after planting, the mean value of maize height was 11.60, 11.47, 11.17, 10.17 and 9.13 for control, 25%, 50%, 75% and 100% concentration respectively. At day 38 after planting there were no significant difference between the mean height of maize on the control soil and the mean height of maize on the soil that is 25% contaminated. However there was a significant difference between the mean height in the control soil and the mean height of the remaining soils with various level of contamination for other days. Throughout the study it was observed that oil contamination affect the height of plant (maize) and the degree of toxicity is depended on the concentration of oil because the control sample had the greatest height in all the days while the least growth in height was seen in the 100%

sample. The result obtained in this study is consistent with other studies carried out using different plants as mentioned above.

5.1.2 Maize root length

Root length is used to evaluate plant survival and growth in ecological risk studies. At 38 days after planting, the root length of maize plant was 42.33, 39.40, 18.00, 17.00 and 15.33 for control, 25%, 50%, 75% and 100% concentration respectively. The mean root length in the control soil was significantly different from that which was grown in all the various level of contamination used in the study. From the result, there was steady decrease in the length of root with increasing concentration and the least growth was observed in root length in the 100% concentration. Nwoko *et al.* (2007) reported similar result in oil contaminated soil using beans plant that after 42 days of planting there was drastic decrease in length with increasing concentration. Okonokhua *et al.* (2007) also had similar result of maize root length reduction as the concentration increase.

5.2 Effect of concentration of gas flared polluted soil on leaf area of maize

Leaf area is another criterion for assessing the toxicity of crude oil on plant. At 10 days after planting the leaf area of maize was 0.15 in all the samples; there were no changes in the leaf area. But at 14 days after planting, the leaf area of maize was 1.87, 1.50, 1.13, 0.75 and 0.37 for control, 25%, 50%, 75% and 100% concentration respectively. At 14 to 38 days after planting there was appreciable changes in the leaf area of maize and in all of the days, there were progressive reduction in leaf area as the concentration increases. The control had the greatest increase in leaf area while the 100% concentration recorded the lowest increase in leaf area in all the days. Throughout the experiment, there was a significance difference in the leaf area of maize showing that concentration affect leaf area development. Result obtained in this study reveals that oil affect the development of leaf in plant and this can affect the overall functioning of the plant leading to withering and the toxicity is depended on the concentration of oil. This result is consistent with other works carried out by Okonokhua *et al.* (2007), Ezenwa *et al.* (2017) and Emerhi and David-Sarogoro (2017) in crude oil contaminated soil using *Zea mays, Phaseolus vulgaris* and *Moringa oleifera* respectively.

5.3 CONCLUSION

Plants absorb nutrients and water from the soil and respond in growth through changes in height, root length and leaf area. In this study, the reduction observed in maize height, root length and leaf area grown in oil contaminated soil is depended on the concentration of oil. This observation shows that oil reduces the uptake of nutrients and water from the soil by plant (maize). There is less mobility of soil nutrients in oil contaminated soil which consequently affects the pattern of growth in plants. In a nation like Nigeria, where maize is a major food crop and the occurrence of soil contamination with oil is very rampant; this can result to the reduction and/or loss in yield thereby worsening the already existing challenge of food insecurity in the country. It is recommended that care should be taken during oil exploration, exploitation, processing, storage, distribution and usage to prevent contamination of soil by oil which will affect growth and development of crops leading to food shortage. Furthermore, remediation should be carried out on soils that have been previously contaminated with oil.

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