ALTERATION IN THE ACTIVITIES OF ANTIOXIDATIVE ENZYMES ON THE LEAVES OF MAIZE (ZEA MAYS) PLANTED IN CRUDE OIL CONTAMINATED SOIL

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BENIN CITY.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN BIOCHEMISTRY.

DECEMBER, 2022

CERTIFICATION

This is to certify that this project titled, **ALTERATION IN THE ACTIVITIES OF ANTIOXIDATIVE ENZYMES ON THE LEAVES OF MAIZE (ZEA MAYS) PLANTED IN CRUDE OIL CONTAMINATED SOIL** was carried out and written by **DJEVWEDJE HOSANNA RUKEVWE** with matriculation number **LSC1605047** of Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin city, Edo State.

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DEDICATION

This work is dedicated to God Almighty who has been my source of strength in time of weakness, my provider in time of need and it was through his mercy that I was able to complete this project and my undergraduate program in the University of Benin, Benin City.

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ABSTRACT

This study investigated the alterations in the activities of antioxidative enzymes on the leaves of maize (zea mays) planted in crude oil contaminated soil. Maize was grown on soil contaminated with 5ml, 10ml and 15ml of crude oil. The experiment lasted for 4 weeks. Data obtained were analysed using ANOVA and least significant difference (LSD) at $p<0.05$. The number of leaves, leaf area and leaf weight were determined at the start of every assay, the percentage germination was evaluated at the end of every week. Results showed that the mean leaf area and percentage germination of maize plant (zea mays) decreased with increasing level of contamination with crude oil. Antioxidants like superoxide dismutase (SOD), catalase, malonyldialdehyde (MDA), glutathioneperoxidase (GPx) in zea mays differed significantly ($P<0.05$) at 5ml levels from those planted in 10ml and 15ml levels of the crude oil contaminated soils. The reductions in these growth characteristics measured were crude oil concentration dependent and elevated activity of antioxidative enzymes can assist as important components of antioxidative defense mechanism against oxidative damage. The results of this study could be beneficial in the understanding of the role of defense system beside these parameters, also significant reduction in lipase activity of the plant was recorded and yellowish leaf coloration due to reduction in chlorophyll content were observed among the plants grown in the crude oil contaminated soils. This study thus revealed that crude oil had pronounced effects on the germination and growth of maize plant. This means that maize plant should not be grown near or on soil contaminated with crude oil.

CHAPTER ONE

1.0 INTRODUCTION

As one of the cheapest sources of food energy, maize plays a major role in meeting the rising consumption of both food and animal feed in developing countries (FAO, 2002). FAO (2002) maintained that each part of maize including the stalk, leaves, silk, cob and kernels has a commercial value, the kernel being the most useful; hence, Cunard (1971) stated that maize is a universal crop species. Maize is grown in every important agricultural area of the world (Russell and Halluauer, 1980) including the southern part of Nigeria where oil industrial activities are predominant (Agbogidi *et al.*, 2005).

Environmental pollution from oil activities in a major oil producing country as Nigeria is inevitable (Agbogidi and Eshegbeyi, 2006). Considering the large quantities of oil reportedly lost to agricultural lands (Ogri, 2001), it has become necessary to investigate the effects of oil spillage on agricultural lands and the crops grown in them. The objective of this study was to evaluate the effects of crude oil levels on the growth of maize.

Various studies have reported the adverse effect of petroleum products on plants ranging from reduced germination of seeds, reduced survival of plants to reduced yield of plants (Akinola *et al*., 2004; Andrade *et al.,* 2004). Most of the reports on the effects of petroleum products on plants have focused on crude oil (Siddiqui and Adams, 2002; Inoni *et al*., 2006) which get to the environment through accidental spillage. However, through the activities of automobile, generator, other machines, and servicing engineers (mechanics) crude oil is discharged to the environment indiscriminately. The oil and gas industry is the major source of revenue for most countries with millions of barrels of crude oil in their reserve. This is because on a yearly bases billions of tons of crude oil are exported and processed into refined products such as fuel oils, diesel fuel, kerosene and aviation liquefied petroleum gas (Olajire, 2014; Ngene *et al*., 2016). Technological and Anthropogenic activities that are applied in crude oil exploration includes refining and transportation for distribution to producers and consumers. Besides the useful products produced from the industry, some negative impacts are associated with the exploration process and the major consequence is environmental pollution through drilling cutting, drilling effluents, gas flaring and Oil spillage (Olajire, 2014; Ngene *et al*., 2016). Oil spillage is the most common source of environmental pollution and it is caused by accidental leakages and bursting of old pipelines, blow out wells, sabotage and transportation (Khosravi *et al*., 2013; Agbonifo, 2016). Apart from the physical impact on the ecosystem, the underlying effects of crude oil on Lliving organism results from its toxic components.

This has caused detrimental effects to the abiotic and biotic environment components. The physiochemical components of the soil influences plant nutrients and fertility and changes in these properties are indicatives of soil pollution. Oil spillage impacts the soil physiochemical properties by causing limitations in their optimal availability and a good number of research have demonstrated these changes (Abii & Nwosu, 2009; Bello and Anobeme, 2015). Crude oil, which is hydrocarbon containing complex mixture can be classified into four classes and they include saturates, aromatics, asphaltenes and resins. The most toxic component are the aromatics and they include polycyclic aromatics (PAHs) and alkyl aromatics like Benzene, toluene, ethyl benzene and xylenes (Ite *et al*., 2013). It also contains non-hydrocarbon components like sulfur, nitrogen, oxygen and trace amount of nickel, copper, vanadium and iron (Sivansankar, 2008).

Crude oil here refers to a naturally occurring petroleum product composed of hydrocarbon deposits and other organic materials. A type of fossil fuel, crude oil is refined to produce usable products including gasoline, diesel, and various other forms of petrochemicals. It is a nonrenewable resource, which means that it can't be replaced naturally at the rate we consume it and is, therefore, a limited resource.

Crude oil is typically obtained through drilling, where it is usually found alongside other resources, such as natural gas (which is lighter and therefore sits above the crude oil) and saline water (which is denser and sinks below).

After its extraction, crude oil is refined and processed into a variety of forms, such as gasoline, kerosene, and asphalt, for sale to consumers.

Although it is often called "black gold," crude oil has a range of viscosity and can vary in color from black to yellow depending on its hydrocarbon composition. Distillation, the process by which oil is heated and separated into different components, is the first stage in refining.

Although fossil fuels like coal have been harvested for centuries, crude oil was first discovered and developed during the Industrial Revolution, and its industrial uses were developed in the 19th century. Newly invented machines revolutionized the way we do work, and they depended on these resources to run.

1.01 AIMS AND OBJECTIVES

a) To study the effect of different concentrations of crude oil on the activity of some selected antioxidative enzymes; superoxide dismutase, catalase, glutathione peroxidase and lipid peroxidation, in the leaves of maize (*Zea mays*).
b) To study the effect of different concentrations of crude oil on the activity of a selected hydrolytic enzyme; lipase, in the leaves of maize (*Zea mays*).
c) To study the the effect of different concentrations of crude oil land spent engine on the total cholorophyll content in the leaves of maize (*Zea mays*).
d) To study the effect of the different concentrations of crude oil on the total protein concentration in the leaves of maize (*Zea mays*).
e) For vegetative studies: possible effect of crude oil on selected physiological properties; leaf number, leaf area and leaf weight, in maize (*Zea mays*).

1.1 LITERATURE REVIEW

1.1.1 THE BIOLOGY OF *Zea mays*

The word *Zea mays* comes from two languages. *Zea* comes from ancient Greek and is a generic name for cereal and grains. Some scientists believe that *Zea* stands for "sustaining life". *mays* comes from the language Taino, meaning "life giver". Maize (UK) or corn (USA) (*Zea mays*. L) is the world's third leading cereal crop, after wheat and rice. It probably originated from Central America, specifically Mexico. Maize belongs to the family Poaceae and is a tall annual herb with an extensive fibrous root system. It is a cross-pollinating specie with female and male flowers at separate places on the plant (Parle and Dhamija, 2013). Maize provides nutrients for human and animals; and serves as a basic raw material for the production of starch, oil, protein, alchoholic beverages, food sweetener and more recently fuel. Corn is considered a staple food in many parts of the world. Traditional Nigerian dishes prepared from maize include pap, 'tuwo', 'donkunnu', 'masa and wainna', 'cous-cous', 'gwate', 'akple', 'ukejuka', 'nakia', 'dambu alubosa', 'abari', 'egbo', 'donkwa', popcorn, 'ajepasi', 'kokoro', 'elekute', cooked or boiled maize, roasted maize, e.t.c (Abdulrahaman and Kolawole, 2006).

In traditional medicine, maize is used for relieving diarrhea, dysentery, urinary tract disorders, prostatitis, lithiasis, angina, hypertension and tumor. The plant is pharmacologically exploited for hypoglycemic anti-inflammatory, antioxidant and diuretic properties (Hu and Deng, 2011). Other uses of maize include the production of Cornstarch, Corn syrup and Kitty litter. The green plant has been used in the dairy and beef industries, as fodder.

BOTANICAL DESCRIPTION

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae

Subfamily: Panicoideae

Tribe: Andropogoneae

Genus: *Zea*

Species: *Zea mays*

Climate and soil requirement: Maize is basically a sunny crop, so it requires warm and moist climate. Annual rainfall of 60cm is required, throughout its growing stage. It cannot

withstand frost at any stage. Prolonged cloudy period is harmful for its growth. It needs full sunlight for its accelerated photosynthetic activity. It needs fertile soil and thrives best in deep loamy soil along with abundant moisture. The ideal soil for maize should be rich in organic matter and well-drained. Soil pH should be in the range of 7.5 to 8.5 for good crop growth.

Botanical features: Maize is a tall, determine annual plant varying in height from 1 to 4 metres. The plant produces large, narrow, opposing leaves, borne alternately along the length of solid stem. Maize is a monoecious plant that is, the sexes are partitioned into separate pistillate (ear), the female flower and staminate (tassel), the male flower. The main shoot terminates in a staminate tassel. Maize is generally protandrous meaning male matures earlier than the female flower. Normally, maize plants have three types of roots;

a. Seminal roots- persist for long period,
b. Adventitious roots, fibrous roots developing from the lower nodes of the stem below ground level which are the effective and active roots of the plant and
c. Brace or prop roots, produced by lower two nodes.

The roots grow very rapidly and almost equally outwards and downwards. Favourable soils may allow maize root growth up to 60cm laterally and in depth (Parle and Dhamija, 2013).

1.1.2 PHYTOCONSTITUENTS OF *Zea mays.*

Zea mays contains cytokine zeatin, a biologically active purine derivative, flavonoids, alkaloids, allantoin, saponins, volatile oil (about 0.2%), mucilage, vitamins C, E and K, minerals especially potassium, starch, sugar, fat, maizenic acid, gluten, dextrin, glucose, cellulose, silica, phosphates of lime and magnesia. It also contains isoquercitin, chrysanthemium, cyanogenetic material, 6-methoxybenzoazoline, dicarboxylic acids such as oxalic acid, polysaccharide, essential fatty aids anthocyanin, glycosides and a triterpene cyclosadol. Maize kernel contains carbohydrates 66.2%, protein 11.1%, fat 3.6%, minerals 1.5% and fibres 2.7% (Parle and Dhamija, 2013).

1.1.3 CRUDE OIL

Crude oil, liquid petroleum that is found accumulated in various porous rock formations in Earth's crust and is extracted for burning as fuel or for processing into chemical products.

1.1.4 CHEMICAL AND PHYSICAL PROPERTIES

Crude oil is a mixture of comparatively volatile liquid hydrocarbons (compounds composed mainly of hydrogen and carbon), though it also contains some nitrogen, sulfur, and oxygen. Those elements form a large variety of complex molecular structures, some of which cannot be readily identified. Regardless of variations, however, almost all crude oil ranges from 82 to 87 percent carbon by weight and 12 to 15 percent hydrogen by weight.

Crude oils are customarily characterized by the type of hydrocarbon compound that is most prevalent in them: paraffins, naphthenes, and aromatics. Paraffins are the most common hydrocarbons in crude oil; certain liquid paraffins are the major constituents of gasoline (petrol) and are therefore highly valued. Naphthenes are an important part of all liquid refinery products, but they also form some of the heavy asphalt like residues of refinery processes. Aromatics generally constitute only a small percentage of most crude. The most common aromatic in crude oil is benzene, a popular building block in the petrochemical industry.

Because crude oil is a mixture of such widely varying constituents and proportions, its physical properties also vary widely. In appearance, for instance, it ranges from colorless

to black. Possibly the most important physical property is specific gravity (i.e., the ratio of the weight of equal volumes of a crude oil and pure water at standard conditions). In laboratory measurement of specific gravity, it is customary to assign pure water a measurement of 1; substances lighter than water, such as crude oil, would receive measurements less than 1. The petroleum industry, however, uses the American Petroleum Institute (API) gravity scale, in which pure water has been arbitrarily assigned an API gravity of 10°. Liquids lighter than water, such as oil, have API gravities numerically greater than 10.

1.1.5 EXTRACTING AND PROCESSING

Crude oil occurs underground, at various pressures depending on depth. It can contain considerable natural gas, kept in solution by the pressure. In addition, water often flows into an oil well along with liquid crude and gas. All these fluids are collected by surface equipment for separation. Clean crude oil is sent to storage at near atmospheric pressure, usually aboveground in cylindrical steel tanks that may be as large as 30 metres (100 feet) in diameter and 10 metres (33 feet) tall. Often crude oil must be transported from widely distributed production sites to treatment plants and refineries. Overland movement is largely through pipelines. Crude from more isolated wells is collected in tank trucks and taken to pipeline terminals; there is also some transport in specially constructed railroad cars. Overseas transport is conducted in specially designed tanker ships. Tanker capacities vary from less than 100,000 barrels to more than 3,000,000 barrels.

The primary destination of crude oil is a refinery. There any combination of three basic functions is carried out: (1) separating the many types of hydrocarbon present in crude oils into fractions of more closely related properties, (2) chemically converting the separated hydrocarbons into more desirable reaction products, and (3) purifying the products of unwanted elements and compounds. The main process for separating the hydrocarbon components of crude oil is fractional distillation. Crude oil fractions separated by distillation are passed on for subsequent processing into numerous products, ranging from gasoline and diesel fuel to heating oil to asphalt. The proportions of products that may be obtained by distillation of five typical crude oils, ranging from heavy Venezuelan Boscan to the light Bass Strait oil produced in Australia.

1.1.6 TRANSFORMATION OF LUBRICATING OIL DURING USE

The chemical composition of lubricating oil is transformed during the motor operation by tracking the polymers, decomposition of the organometallic compounds, oxidation and nitration because of its subjection to a high mechanic strains and high temperature (Severinski, 1997). The oil also accumulates other contaminants like petrol, antifreeze and

insoluble particles from the petrol and motor wear, metal oxides and products of combustion and water.

The major differences between the unused and used lubricating oil is the presence of heavy metals, used lubricating oil contains high concentration of heavy metals and the concentration increases with time of motor operation, and also depends on the type of fuel as well as the mechanical status of the engine.

The polyaromatic hydrocarbons (PAH) are also present in higher concentration in used engine oil than new oil (Hoffman *et al*., 1982). The PAH are dangerous to life as a result of their mutagenic and carcinogenic effects.

1.1.7 HEAVY METAL TOXICITY

Heavy metals are defined as that group of elements that have specific weights higher than about $5g/cm^3$ .A number of them (Co, Fe, Mn, Mo, Ni, Zn, Cu) are essential micronutrients and are required for normal growth and take part in redox reactions, electron transfers and other important metabolic processes in plants. Metals which are considered nonessential (Pb, Cd, Cr, Hg etc.) are potentially highly toxic for plants (Rai *et al*.,2004). Large areas of land are contaminated with heavy metals (the main group of inorganic contaminants) resulting from urban activities, agricultural practices and industry (Clemens, 2001). Excessive concentrations of trace elements (Cd, Co, Cr, Hg, Mn, Ni, Pb, and Zn) are toxic and lead to growth inhibition, decrease in biomass and death of the plant (Zenk,1996).

Heavy metals inhibit physiological processes such as respiration, photosynthesis, cell elongation, plant-water relationship, N-metabolism and mineral nutrition (Zornoza *et al*.,2002).

Some external mechanisms that limit the uptake of metals by roots can help plants tolerate a certain amount of toxic metal in soil. One of them is the formation of non-toxic metal-ligand chelates in rhizosphere involving organic acids and other substances exuded from roots. Heavy metals tolerance is enhanced by the action of mycorrhizae (Khan *et al*.,2000). Metals can be transported via an apoplastic system and immobilized in cell

walls. Toxic metals become a threat to plants mainly when they reach the cytosol of the cell. Therefore, the ability of root cells to control the transport of heavy metals via membranes determines their tolerance by plants. They can be immediately complexed, inactivated and transformed into a physiologically tolerable form via action of phytochelatins and sequestered in cell vacuoles (Zenk, 1996). In many cases plants resistant to heavy metal stress have lower nutritional requirements and specific mineral (Cadmium, Potassium, and Phosphorus) and water economies to cope with this stress. According to the chemical and physical properties of heavy metals we can divide their harmful action into:

a) Generation of ROS (reactive oxygen species) by auto-oxidation and fenton reaction.
b) Blocking of essential functional groups to biomolecules: proteins (by the inactivation of the SH-groups in enzymes active centers) and polynucleotides (Mithofer *et al*.,2004).
c) Substitution of essential metal ions by other incorrect ones (Rai *et al*.,2004).

The increasing levels of metals into the environment drastically affect plant growth and metabolism, ultimately, leading to severe losses in crop yields (Mishra and Dubey, 2005). One of the consequences of the presence of the toxic metals within the plants tissues in the formation of ROS, which can be initiated directly or indirectly by the metals and, consequently, leading to oxidative damage to different cell constituents (Gallago *et al*.,2002); Sharma and Dubey, 2007). Under metal stress condition, net photosynthesis (Phn) decreases due to damage to photosynthetic metabolism, including phorosynthetic electron transporter (Phet). For example, copper has been shown to negatively affect components of both the light reactions (e.g PSII, thylakoid membrane structure, and chlorophyll content) and $C0_2$-fixation reactions (Vinit-Dunand *et al*., 2002). These alterations in photosynthetic metabolism lead to overproduction due to metals (cadmium and zinc) in *Nicotiana tabacum* L. cv. Bright Yellow 2 (TBY-2) cells in suspension cultures showed properties comparable to the elicitor-induced oxidative burst in other plants (Zrobek-Sokolnik *et al*., 2009). Redox-active metls, such as iron, copper, and chromium, undergo redox cycling producing ROS, whereas redox-inactive metals, such as

lead, cadmium, mercury, and others, deplete cells major antioxidants, particularly thiol-containing antioxidants and enzymes (Srivastava and Dubey,2011). If metal-induced production of ROS is not adequately counterbalanced by cellular antioxidants, oxidative damage of lipids, proteins, and nucleic acid ensue (Dat *et al*.,2000). The increased activity of the antioxidant enzymes in metal stressed plants appears to serve as an important component of antioxidant defense mechanism of plants to combat metal induced oxidative injury (Shah *et al*.,2001). Responses of metal exposure to plants vary depending on plant species, tissues, stages of development, type of metal and its concentration. One of the key responses including triggering of a series of defense mechanisms which involve enzymatic and non-enzymatic components (Maheshwary and dubey.,2009) Various groups of workers have reported increased activities of antioxidant enzymes like guaiacol peroxidase (GPX), superoxide dismutase (SOD), monodehydroascorbate dehydrogenase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) as well as nonenzymatic antioxidantsin metal-treated plants and suggested involvement of antioxidant defense system in the adaptive response to metal ions (Verma and Dubey, 2003). However, results suggest that activation of antioxidant enzymes in response to oxidative stress induced by metals is not enough to confer tolerance to metal accumulation. Cooperative study of antioxidative response of two maize lines differing in Aluminium tolerance suggested that better protection of the Aluminium tolerant maize root from AI-induced oxidative damage results, at least partially, from the increased activity of their antioxidative system. After 24hrs of aluminium exposure, a gradual increase in the membrane lipid peroxidation in Aluminium-stressed root of the susceptible maizeline was accompanied by decreased activities of the antioxidant enzymes superoxide dismutase and peroxidase were found in AI-treated roots of the tolerant maize line, in which the level of membrane lipid peroxidation remained almost unchanged (Giannakoula *et al*.,2010).

1.1.8 GENERATION OF REACTIVE OXYGEN SPECIES.

An unavoidable consequence of aerobic metabolism is production of reactive oxygen species (ROS). ROS include free radicals such as superoxide anion (O_2-), hydroxyl radical (*OH), as well as non-radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and so forth. Stepwise reduction of molecular oxygen (O_2) by high-energy exposure or electron-transfer reactions leads to the production of the highly reactive ROS. In plants, reactive oxygen species are always formed by the inevitable leakage of electrons onto O_2 from the electron transport activities of the chloroplasts, mitochondria, and plasma membrane or as a bye-product of various metabolic pathways localized in different cellular compartments (Henyo *et al*., 2011). Environmental stresses such as drought, salinity, chilling, metal toxicity and UV-B radiation as well as pathogens attack to enhanced generation of reactive oxygen species in plants due to disruption of cellular homeostasis (Srivastava and Dubey, 2011). Scavenging or detoxification of excess reactive oxygen species is achieved by an efficient antioxidative system comprising of the nonenzymic as well as enzymic antioxidants. The enzymic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), enzymes of ascorbate-glutathione (AsA-GSH) cycle such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Noctor and Foyer, 1998).

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1 MATERIALS

2.1.1 Maize (*Zea mays*): This was purchased from Uselu market, Benin City, Edo state.

2.1.2 Crude oil: This was obtained from an oil company in Warri, Delta state.

2.2 CHEMICAL REAGENTS

- Hydrochloric acid
- Sulphuric acid
- Acetone
- Pyrogallol
- Biuret's reagent
- Ethylene diamine tetra acetic acid (EDTA)
- Hydrogen peroxide
- Phosphate buffer
- Sodium hydroxide
- TCA-TBA-HCL

2.3 APPARATUS

- pH meter

- Visible spectrophotometer – 721G visible spectrophotometer

- Electric centrifuge – 80-2 Techmel and Techmel USA

- Water bath

- Cereamic homogenizing mortar and pestle

- Pipettes

- Beaker

2.4 SEED VIABILITY TEST: Maize seeds (*Zea mays L.*) were put in a bucket of water. Submerged seeds were collected and planted while those that remained afloat were discarded.

2.5 SOIL COLLECTION: The soil was collected from a portion of land beyond the faculty of pharmacy, University of Benin.

2.6 SOIL TREATMENT: 20 bags each containing 1.5kg of soil were used. They were divided into four groups labeled 1 to 4. Each group contains 5 bags. Group 1 was untreated and served as control. Group 2 was treated with 5ml Crude oil. Group 3 was treated with 10ml Crude oil. Group 4 was treated with 15ml Crude oil.

2.7 PRELIMINARY SOIL ANALYSIS: Before treating the soil with Crude Oil, the water retention capacity was determined by means of the perculation method.

2.8 PLANTING OF SEEDS: 6 seeds of maize (*Zea mays* L.) were planted in each bag.

2.9 SAMPLE COLLECTION: The plants were harvested and the leaves were cut after two weeks, they were weighed and the homogenized using mortar and pestle and 10ml of distilled water. The homogenized samples were placed in sample bottles and then centrifuged at 4000g for 15 minutes. The supernatant was then used for different enzyme assays. For the determination of total chlorophyll content, homogenization was carried out using 10ml of acetone and the homogenized samples were centrifuged at 3000g for 10minutes, the supernatant obtained was then used for the assay.

2.10 FREQUENCY OF SAMPLE COLLECTION: Samples for each of the variables to be tested were collected in multiples of threes to enable calculation of mean and standard error of mean at the end of the experiments. Leaves were collected at weeks 2,3 and 4.

2.11 ASSAY PROCEDURES

DETERMINATION OF CATALASE ACTIVITY

Principle

This is based on the method of (Cohen *et al*, 1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2), after the addition of the material containing the enzyme.

Catalase catalyzes the reaction: $2H_2O_2 \longrightarrow 2H_2 + O_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is

measured by reacting it with excess potassium permanganate ($KMNO_4$) and then measuring the residual $KMNO_4$ spectrophotometrically at 480nm.

Assay Procedures

Plant homogenate (0.5mL) was placed in ice-cold test tubes, the blank contained 0.5mL distilled water. Cold phosphate-buffered H_2O_2 (30mM, 5mL) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1mL of 6M $H_2O_2SO_4$. The tubes were mixed thoroughly by inversion after which 7mL of 0.01M $KMNO_4$ was added. Absorbance was read at 480nm within 3 minutes.

DETERMINATION OF GLUTATHIONE PEROXIDASE ACTIVITY

Glutathione peroxidase (GPx) activity was measured according to the method described by Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting to a deep brown coloration, which is read at 430nm.

Procedure

To a plant homogenate (0.2mL), 5mL of phosphate-buffered H_2O_2 and 1.5mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430nm.

DETERMINATION OF TOTAL SERUM PROTEIN

Principle

Cupric ions, in an alkaline medium, interact with the peptide bonds of protein resulting in the formation of a violet colored complex which is then read at 540nm in a spectrophotometer.

Procedure

Plant sample and standard (2.5mL) were pipetted into their respective test tubes. Biuret reagent (2.5mL) was added to each tube. The blank tube contained 2.5mL of Biuret reagent solution in each were mixed thoroughly and allowed to incubate for 10minutes at 37˚C. The absorbance was read at 540nm against reagent blank.

DETERMINATION OF CHLOROPHYLL CONTENT

Procedure

In this method, the leaves (0.5g) were ground in 10mL 80% acetone in the dark and centrifuged at 3000g for 5 minutes. The absorbance of the recovered supernatant was read at 645 and 663nm.

PEROXIDASE ACTIVITY ASSAY

Procedure

2.10mL of deionized water, 0.32mL of potassium phosphate buffer, 0.16mL of 0.50% (w/w). H_2O_2 and 0.32ml pyrogallol was added to sample tubes and the blank. Each tube was mixed by inversion and allowed to equilibrate to 20˚C. The content of the blank tube was poured into the cuvette, it was monitored at 420nm until constant using a spectrophotometer. 0.10mL of potassium phosphate buffer was added. It was mixed immediately by inversion, the absorbance at 0sec and 20sec was recorded. Same was done

to each sample test tubes but in this case, 0.10mL of individual extract was taken. Increase in absorbance was taken for approximately 3 minutes.

LIPID PEROXIDATION ASSAY

Principle

Malondialdehyde which is formed from the breakdown of polyunsaturated fatty acids serve as a convenient index for the determination of the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with the thiobarbituric acid to give a red specie absorbing at 530nm˚

Procedure

1.0ml of extract and 2.0ml of TCA-TBA-HCL reagent was placed in a sample bottle, 3.0mL of TCA-TBA-HCL reagent was also placed in a sample bottled labelled blank. The tubes were heated for 15min in boiling water (100˚C). After cooling, it was centrifuged to give a flocculent precipitate of 1000g for 10mins. Absorbance of the supernatant of the sample was read at 535nm against the blank.

DETERMINATION OF LIPASE ACTIVITY

Procedure

A 250mL Erienmeyer flask containing 2mL of 0.1M phosphorous buffer, 1mL of olive oil and 1mL of crude enzyme extract was incubated at 40˚C for 30min. The reaction was stopped by the addition of 5mL of ethanol and then titrated against 0.1M NaOH using phenolphthalene as an indicator. Appearance of pale pink color indicated the end point.

DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Procedure

This method is well described by Mccord and Fridovich (1969) and can be applied for determination of antioxidant activity of a sample. It is estimated in the plant homogenate prepared. To 50µL of the plant homogenate, 75mM of Tris-HCL buffer (pH 8.2), 30mM EDTA and 2mM of pyrogaloll are added. An increase in absorbance is recorded at 420nm for 3 min by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of autoxidation of pyrogallol as determined by change in absorbance/min at 420nm. The activity of SOD is expressed as units/mg protein.

CHAPTER THREE

3.0 RESULTS

Table 3.1: Effect of Crude Oil on superoxide dismutase activity in the leaves of maize (*Zea mays*).

SUPER OXIDE DISMUTASE ACTIVITY (unit/mg)

	WEEK 2 ($\times 10^{-5}$)	WEEK 3 ($\times 10^{-5}$)	WEEK 4 ($\times 10^{-5}$)
CONTROL	1.52 ± .08	3.33 ± .64	2.22 ± .64
5ml	4.60 ± 2.45	2.94 ± .68	.78 ± .42
10ml	1.14 ± .32	19.83 ± 12.49	3.68 ± .99[a]
15ml	5.52 ± 3.17	7.02 ± .64	2.41 ± .60

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- There was a significant increase in the SOD activity in the 10mL contaminant group when compared to the 5ml contaminant group.
- At week 2 and week 3, there was a significant increase in superoxide dismutase activity in the contaminant groups.

Table 3.2: Effect of Crude Oil on total protein concentration in the leaves of maize (*Zea mays*).

TOTAL PROTEIN CONCENTRATION (g/dl)

	WEEK 2	WEEK 3	WEEK 4
CONTROL	4.09 ± .15	3.31 ± .25	3.22 ± .09
5ml	3.24 ± .45	6.88 ± . 10*	5.12 ± .75
10ml	4.26 ± .64	8.08 ± 1.55*	6.55 ± 1.25
15ml	2.68 ± .84	23.66 ± .37*[ab]	6.44 ± .75

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 3, there was a significant increase in the total protein concentration of the Contaminant groups as contamination increased.

Table 3.3: Effect of Crude Oil on catalase activity in the leaves of maize (*Zea mays*).

CATALASE ACTIVITY (unit/mg)

	WEEK 2 ($\times 10^{-3}$)	WEEK 3 ($\times 10^{-3}$)	WEEK 4 ($\times 10^{-3}$)
CONTROL	6.88 ± .00	6.88 ± .00	3.21 ± 6.06
5ml	3.23 ± 1.30*	1.49 ± .13*	1.06 ± .28*
10ml	2.15 ± .99*	10.13 ± 5.31*	3.56 ± 2.38*
15ml	2.92 ± .92*	6.51 ± 1.74*	3.33 ± .76*

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 2, week 3 and week 4, there was a significant reduction in the catalase activity of the contaminant groups as contamination increased.

Table 3.4: Effect of Crude Oil on total chlorophyll content in the leaves of maize (*Zea mays*).

TOTAL CHLOROPHYLL CONTENT (unit/mg)

	WEEK 2 ($\times 10^{-6}$)	WEEK 3 ($\times 10^{-6}$)	WEEK 4 ($\times 10^{-6}$)
CONTROL	95.33 ± 1.98*	84.49 ± .66	85.66 ± 1.07
5ml	53.50 ± 1.90*	68.97 ± 29.43	10.00 ± .15*
10ml	56.43 ± .78*	98.87 ± .48	10.03 ± .27*
15ml	55.23 ± .24*	66.57 ± 28.40	9.74 ± .31*

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 2 and week 4, there was a significant reduction in the total chlorophyll content of the contaminant groups as contamination increased.

Table 3.5: Effect of Crude Oil on lipid peroxidation in the leaves of maize (*Zea mays*).

LIPID PEROXIDATION (unit/mg)

	WEEK 2 ($\times 10^{-6}$)	WEEK 3 ($\times 10^{-6}$)	WEEK 4 ($\times 10^{-6}$)
CONTROL	.27 ± .01	.03 ± .00	.03 ± .00
5ml	2.08 ± .56	3.06 ± .34	2.59 ± .34
10ml	1.92 ± .46	20.80 ± 13.32	8.40 ± 5.57
15ml	4.08 ± 1.66*	12.82 ± 3.65	4.74 ± .66

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- There was a significant increase in the lipid peroxidation activity in the 15mL contaminant group when compared to the 10ml contaminant group.

Table 3.6: Effect of Crude Oil on lipase activity in the leaves of maize (*Zea mays*).

	WEEK 2	WEEK 3	WEEK 4
CONTROL	.20 ± .00	.43 ± .03	.37 ± .03
5ml	.87 ± .02*	.75 ± .00*	1.22 ± .10
10ml	.72 ± .12*	.88 ± 04*[ab]	1.12 ± .03
15ml	.73 ± .06*	.63 ± .04*[ab]	6.35 ± 2.65*[ab]

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 2, there was a significant increase in the lipase activity of the contaminant groups.
- At week 3 and week 4 there was a significant increase 5ml,10ml and 15ml when compared with the control.

Table 3.7: Effect of Crude Oil on glutathione peroxidase activity in the leaves of maize (*Zea mays*).

GLUTATHIONE PEROXIDASE (unit/mg)

	WEEK 2 ($\times 10^{-3}$)	WEEK 3 ($\times 10^{-3}$)	WEEK 4 ($\times 10^{-3}$)
CONTROL	1.54 ± .12	.42 ± .01	.10 ± .02
5ml	1.68 ± .45	1.44 ± .10	3.18 ± 1.82
10ml	2.15 ± 1.11	18.16 ± 9.08*[a]	4.95 ± 2.05*
15ml	2.15 ± 1.11	5.83 ± 1.27	6.19 ± .68*

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 3 there was a significant increase in the glutathione peroxidase activity in the 10ml group when compared with the 5ml and control.
- At week 4, there was a significant increase in the 10ml and 15ml group when compared with the control.

Table 3.8: Effect of Crude Oil on the leaf number of maize (*Zea mays* L.).

LEAF NUMBER

	WEEK 2	WEEK 3	WEEK 4
CONTROL	4.00 ± .00	3.67 ± .33	4.00 ± .00
5ml	3.33 ± .33	6.33 ± .33	6.00 ± .00*
10ml	4.67 ± .33[a]	5.33 ± 1.45	5.00 ± 1.15
15ml	3.33 ± .33[b]	4.33 ± .33	4.33 ± .33

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 2 and week 4, there was a significant reduction in the number of leaves of the contaminant groups as contamination increased.

Table 3.9: Effect of Crude Oil on the leaf area of maize (*Zea mays* L.).

LEAF AREA (cm^2)

	WEEK 2	WEEK 3	WEEK 4
CONTROL	23.6 4 ± 3.86	17.71 ± 3.67	31.02 ± 2.46
5ml	22.53 ± 4.12	70.70 ±5.59*	73.80 ± 2.55*
10ml	28.17 ±10.04	60.57 ± 30.76	52.53 ± 19.02
15ml	13.93 ±.56	30.27 ± 5.11	34.20 ± 11.31

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- At week 3 and week 4, there was a significant reduction in the leaf area of the contaminant group when compared to the control.

Table 3.10: Effect of Crude Oil on leaf weight of maize (*Zea mays* L.).

LEAF WEIGHT (mg)

	WEEK 2 ($\times 10^3$)	WEEK 3 ($\times 10^3$)	WEEK 4 ($\times 10^3$)
CONTROL	1.25 ± .12	.683 ± .14	.94 ± .04
5ml	.69 ± .14	4.54 ± .43	4.80 ± .62
10ml	1.54 ± .77	5.43 ± 4.49	6.15 ± 4.44
15ml	.47 ± .04	1.30 ± .29	1.71 ± .22

All values are expressed as mean ± SEM

* Significance difference as compared with control group ($p < 0.05$)

a. Significance difference as compared with 5ml group ($p < 0.05$)

b. Significance difference as compared with 10ml group ($p < 0.05$)

- There was no significant changes in the leaf weight.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The high plant mortality in four weeks old maize was probably due to the contact of the crude oil with a large percentage of the photosynthetic leaf surfaces of the vegetation and the penetration of the oil into the plant tissue. This finding corresponds with the report of Klingman (1961) that oil applied to roots can move up to the leaves and oil applied to leaves can move down into roots. The observed yellowing of leaves resulted in loss of photosynthetic ability and general physiological weakening of the plants causing the three weeks old maize plants from all the varieties tested to succumb. Cessation of growth or stunting, wilting and withering may all be attributed to one or a combination of these stress conditions. Agbogidi and Eshegbeyi (2006) noted that symptoms of oil pollution in soil were typical of extreme nutrient deficiency in plants while McKee (1995) reported that nutrient deficiency symptoms could be directly proportional to water uptake. Also the reduced leaf areas of the plants due to the addition of the crude oil can aggravate the photosynthesis level in the plant with resultant poor performance of the plant. All these can lead to low yield of the plant and low availability of food.

The reduction of the plant growth observed in this study could be due to reduction of mineral element with increasing oil concentration in the soil reported by Odjegba and Atebe (2007). This could have occurred as a result of reduced availability of mineral elements because according to Clarkson and Hanson (1980), plant nutrition is based not only on the presence of mineral elements in the soil but their availability. Another possible cause of the effects of crude oil on the maize plant observed in this study could be due to either the increased acidity in the soil or reduction in the catalase activity reported by Achuba and Peretiemo-Clark (2007). Such increase soil acidity can affect the microbial distribution in the soil reducing their activities in the rhizosphere. The reduction of the catalase activity can affect the optimal soil conditions required for plant growth hence the reduction of plant growth observed in this study. The high level of toxic heavy metals and polycyclic aromatic hydrocarbon which has been reported to be present in crude oil can also account for the growth inhibition observed in this study. The reduction of the chlorophyll content of the plant could be due to the interference of the oil on the ability of the plant to absorb some of the mineral nutrients. Minerals like magnesium, iron, boron, and manganese are essential for chlorophyll synthesis (Campbell, 1996; Taylor *et al*., 1997; Kent, 2000). Such interference and the reduced rate of photosynthesis which accompanies reduction of chlorophyll can lead to plant death and stunted growth. Also the reduced leaf areas of the plants due to the addition of the crude oil can aggravate the photosynthesis level in the plant with resultant poor performance of the plant. All these

can lead to low yield of the plant and low availability of food. The lower performance of the plants treated with crude oil at the first week of growth indicates that the plant has less resistant to pollution by spent at tender age than when it grows older.

4.2 CONCLUSION

In conclusion, from the results obtained in this study, the effect of crude oil pollutant results in stunted growth of leaves and shouldn't be exposed to plants at their tender stages. The exposure of crude oil should be curbed in the environment and proper measures should be taken in the production and storage of these pollutants so as not to reduce the yield of crops in farmlands.

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APPENDIX I

Preparation of 0.005M HCL solution

This was prepared by diluting 5ml of 0.1M HCL solution to 1000ml with distilled water.

Preparation of 0.10M HCL solution

This was prepared by diluting 1ml of commercial concentrated HCL to 120ml with distilled water.

Preparation of 0.01M KMnO4 solution

Dissolve 1.584 of KMnO4 in 250ml of distilled water and make it up to 1 litre.

Preparation of 6M H2SO4 solution

31.96ml of concentrated H2SO4 is added to 50ml of H2O and made up to 1000ml.

Preparation of 30mM H2O2 solution

Dilute 3.4ml of 30% of hydrogen peroxide solution to 100ml using phosphate buffer (pH 7.4)

Preparation of Phosphate buffer

Dissolve 0.13g of Na_2HPO_4 , 0.019g of NaH_2PO_4 and 0.8g of NaCl in about 80ml distilled water. pH is adjusted to 7.4 with 0.1M NaOH and make up to 100ml with distelled.

Preparation of 0.1M NaOH solution

Dissolve 0.4 g of NaOH pellets in 1000ml of distilled water.

Preparation of TCA-TBA-HCL reagent (stock)

Dissolve 15g of trichloroacetic acid, 0.375g of thiobarbituric acid in 100ml of 0.25N hydrochloric acid. The solution may be mildly heated to assist in the dissolution of thiobarbituric acid.

Preparation of 0.25N HCL

25ml of hydrochloric acid was dissolved in 1000ml of distilled water.

Preparation of 0.50% (w/w) H_2O_2 solution

Prepare 50ml in deionized using hydrogen peroxide, 30% (w/w) solution, Sigma Prod. No. H-1009.

Preparation of 5% (w/v) Pyrogallol solution

Prepare 10ml in deionized water using Pyrogallol, Sigma Prod. No. P-0381.

APPENDIX II

ASSAY CALCULATIONS

1. Total cbhlorophyll content = ((20.2(OD645) + 8.02 (OD663)) ÷ (D × 1000 × w) × v

Where, OD = optical density

D = distance travelled by the light path

W = weight of the leaf

V = volume of the extract taken

2. Super oxide dismutase activity ;

% Inhibition = ((O.Dmin 2 – O.Dmin 1) + (O.Dmin 3 – O.Dmin 2)) ÷ 2

Enzyme activity = % inhibition ÷ (50 × Y)

Where Y = mg of protein in the volume of the sample.

O.D = optical density

3. Catalase activity ;

Enzyme activity = (O.D/min × Vt × 1000) ÷ (M × L × V × Y)

Where, O.D = optical density

Vt = total volume of reaction mixture

M = molar extinction coefficient of H_2O_2 = 43.6M-1 cm-1

L = light path = 1.0cm

V = volume of sample homogenate used

Y = mg of protein in tissue used

4. Glutathione peroxidase activity;

$$\text{Enzyme activity} = (\text{O.D/min} \times \text{Vt}) \div (\text{E} \times \text{Vs} \times \text{Y})$$

Where. O.D = optical density

Vt = Total volume of reaction mixture

E = Molar extinction coefficient (12/M/cm)

Vs = Volume of sample

Y = mg of protein used

5. Determination of total serum protein = (Atest ÷ Astandard) × concentration of standard

6. Malondialdehyde concentration = $(\text{O.D} \times \text{V} \times 1000) \div (\text{A} \times \text{v} \times \text{L} \times \text{Y})$

Where, O.D = optical density

V = Total volume of reaction mixture

A = molar estimation coefficient of product = 1.56 × 105 m-1 cm-1

L = light path = 1cm

Y = weight (mg) of tissue in the volume of sample used.