CYTOTOXIC AND GENOTOXIC EFFECTS OF CRUDE OIL POLLUTED AGRICULTURAL SOIL ON SELECTED PLANTS DURING RHIZOREMEDIATION.

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CERTIFICATION

This is to certify that this project work; **Cytotoxic and genotoxic effects of crude oil polluted agricultural soil on selected plants during rhizoremediation study** was carried out by Mercy Ngozi Madubuike (20184141698) of the Department of Biotechnology, Federal University of Technology Owerri, Imo State.

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DEDICATION

I dedicate this work to God almighty who made everything possible for me to see the completion of this project work, may His name alone be praised.

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TABLE OF CONTENT

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF PLATES

ABSTRACT

Oil spills devastate farmlands, destroying agricultural crops that affect humans through the food chain. Assays for this study were conducted between two soil groups polluted with 100ml crude oil (A - soil polluted before planting [PB] and B - soil polluted two weeks after planting [PA]) within a period of 28 days from their pollution time. The impact of oil spill on three edible plants (*Allium cepa*, *Telfairia occidentalis and Zea mays*) were evaluated through plant growth measurement, total chlorophyll test, tissue sectioning of leaves and alkaline comet assay. Results obtained showed a decrease in plant height, leaf area and leaf number for all the polluted plants compared with their controls. However, plants grown on "soil polluted before planting" experienced delayed emergence and retarded growth but survived better than plants grown on "soil polluted two weeks after planting". The comet frequency values obtained were statistically lower ($P<0.05$) in all the control samples for *T. occidentalis* (UC) = 8.00 ± 3.61^{bc}, *Allium cepa* (OC) = 11.67 ± 3.06^{b} and *Zea mays* (MC) = 4.67 ± 2.08^{c}. However, higher but nonsignificant comet values were recorded amongst the polluted soils for *T. occidentalis* polluted before planting (UPB) = 46.33 ± 2.08^{a}, *T. occidentalis* polluted after planting (UPA) = 50.00 ± 0.00^{a}, *Allium cepa* polluted before planting (OPB) = 49.67 ± 0.58^{a}, *Allium cepa* polluted after planting (OPA) = 48.67 ± 1.53^{a}, *Zea mays* polluted before planting (MPB) = 49.33 ± 1.16^{a} and *Zea mays* polluted after planting (MPA) = 50.00 ± 0.00^{a}). The photomicrographs revealed alterations and anomalies in leaf structures for both polluted groups as compared to their control. A notable decline in total chlorophyll contents occurred amongst the plants grown on "soil polluted two weeks after planting" when compared to plants grown on "soil polluted before planting". Therefore, this study deduced an elevated cytotoxic and genotoxic effects of crude oil in all the polluted plants, except for mild anomalies observed in the leaves of *T. occidentalis* grown on "soil polluted before planting" (UPB); which also showed no physical signs of crude oil pollution based on the non-observance of leaf yellowing, deformity or defoliation.

Key words: genotoxicity, cytotoxicity, comets, hydrocarbons, phytoremediation, pollution

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Crude oil consists of tens of thousands of hydrocarbons (aliphatics and aromatics) and non-hydrocarbons (sulfur, nitrogen, oxygen and various trace metals) (Singh, Kumari & Mishra, 2012). Its physical and chemical compositions depend to a large extent on the origin of the oil, the hydrocarbon content, and the ratios in which they are mixed. Also, the variation in the number of hydrogen and carbon atoms and their arrangement pattern determines the volatility of the oil (FiriAppah, Okujagu & Bassey, 2014). It is broadly used in the industries, companies, homes, communities and other institutions as transport fuels and primary energy source for daily human activities (Turner & Renegar, 2017). However, the extensive distribution and overexploitation of crude oil gives rise to pollution through oil spillage and extraction activities, tanker accidents, pipeline vandalism, leakage from underground storage tanks, industrial activities from oil refinery sites and oil well blowouts (Ogbulie, Nwigwe, Okpokwasili & Iwuala, 2011). Oftentimes, pollution of the soil and marine bodies with crude oil occurs in municipal areas and some rural communities where petroleum and natural gas are obtained (Nwakanma, Ikegwu & Osaigbovo, 2018). Recent study have shown that the Niger Delta region of Nigeria produces above 90% of the nation's oil exports since 1981 and has recorded innumerable cases of distressed ecosystem due to oil exploration and exploitation activities (Adati, Mohamad & Fadhilah, 2012),.

Globally, crude oil pollution on land and water bodies are of major concern due to their direct or indirect negative impacts on the environment and health of humans and other living organisms; solely caused by the high level of toxicity, mutagenic and carcinogenic properties of crude oil (Mahjoubi, Cappello, Souissi, Jaouani & Cherif, 2018). Crude oil contaminated

agricultural soil affects plants hostilely by limiting the availability of water and essential nutrients (such as oxygen, nitrogen and phosphorus) required for plant growth and survival, due to the hydrophobic properties of oil (Ogbo, Zibigha & Odogu, 2009). However, some plants through rhizoremediation can stabilize oil contaminated sites by stimulating the growth and activities of degrading microbes in the rhizosphere, exuding phenolic compounds from their roots and converting petroleum hydrocarbons into less toxic substances like carbon dioxide, water, methane and fatty acids (Ogbulie, Nwanebu & Nwachukwu, 2014).

Yuniati (2018), reported that crude oil degradation depends on the nature and number of hydrocarbons present in the oil, its toxicity level and bioavailability of the contaminant, the microbial population of the contaminated site, environmental parameters, and the duration of pollution. Despite the existence of both physicochemical and biological methods of oil spill clean-up, the use of the latter is widely recommended due to their reduced cost, low maintenance, naturally efficient, sustainable, and high aesthetic nature (Fodelianakis, Antonio, Mapelli, Magagnini, Nikolopoulou, Marasco, Barbato, Tsiola, Tsikopoulou, Giaccaglia, Mahjoubi, Jaouani, Amer, Hussien, Al-Horani, Benzha, Blaghen, Malkawi, Abdel-Fattah, Cherif, Daffonchio & Kalogerakis, 2015). Bioremediation is an eco-friendly method employed to detoxify oil contaminated sites through biodegradation carried out mainly by bacteria and fungi; these microbes have enzymatic systems that aid oil degradation, and they utilize different hydrocarbons as carbon and energy sources. Yuniati (2018) also stated that based on location, bioremediation can take place in situ (treatment at the pollution site) or ex situ (treatment outside the pollution site). Bioaugmentation and biostimulation are the two major approaches to bioremediation. Bioaugmentation involves the introduction of natural, foreign or genetically engineered pollutant-degrading microorganisms into a polluted environment to supplement the existing microbial population in accelerating degradation while biostimulation constitutes the alteration of environmental conditions by adding nutrients, growth supplements or coenzymes

and providing prerequisite environmental conditions in order to induce native microbial activity (Hassanshahian, Emtiazi, Caruso & Cappello, 2014). However, Mahjoubi *et al.* (2018) stated that autochthonous bioaugmentation constitutes the inclusion of indigenous microorganisms that are adaptive to the polluted sites due to favourable environmental conditions whereas; allochthonous or exogenous microorganisms which originate from different sites are unable to fit to the indigenous microbes found within the polluted sites as the environment may not be suitable for their growth and biochemical activities. Studies carried out by Liu, Whang, Yang & Cheng (2008) and Couto, Monteiro & Vasconcelos (2010) reported more effective bioremediation of petroleum contaminated sites attainable through isolation and reintroduction of hydrocarbon degrading bacteria (bioaugmentation), unlike other remediation methods (biostimulation and bioattenuation) applied on same sites.

1.2 STATEMENT OF THE PROBLEM

The ecosystem consists of intertwined organisms (plants, animals, humans and microbes) and the elements (air, water, light and soil) relating with each other. It is a life-supporting system and the release of any form of pollutant into it will spontaneously trigger lethal effects to its biological, physico-chemical, and socio-economic components. Pollution caused by crude oil exploration, refining and transportation activities has critically raised environmental and health concerns, due to the escape of toxic substances into the ecosystem. These chemical substances produce varying effects on the mitosis, germination and development of plants. Extensively, this could give rise to terminal health conditions (respiratory disorders, coronary heart disease, cancers, kidney, and liver malfunctions or even death) in humans because of the bioaccumulation of toxic substances from plants to humans by virtue of our food chain.

Nigeria being a major oil-producing country has predominant oil polluted sites especially in the Niger Delta regions and other oil-producing states, Imo State inclusive. Oil spills destroy

farmlands and living organisms in the soil with detrimental impact on agricultural crops, thereby affecting the health of humans and other animals that consume the crops from these regions. In this regard, there is an increase on the nation's food insecurity and death rates, thus drastically affecting the country's economy and population respectively. Furthermore, the hydrophobic nature of crude oil easily injures or kills plants by inducing stomata closure, chloroplasts damage, tissue infiltration, gaseous exchange obstruction, vascular bundles damage, inefficient water, and nutrient uptake as well as disruption of photosynthesis. At high concentration, most genotoxic substances disintegrate nucleic acids and chromosomal DNA of living organisms thereby hindering certain cellular processes in them. However, cytogenetic tests (such as chromosome aberration test, comet assay, micronucleus assay) are usually conducted to detect the potential mutagenic hazards caused by toxic substances to the cells and genomes of humans and other living organisms.

To solve these problems, many scientists around the globe have worked tirelessly towards achieving environmental protection and sustainability, but there is still need for additional information on the level of mutation and toxicity triggered by crude oil at the genetic, cellular, tissue levels as well as the vegetative structures of some edible plants grown on agricultural soil harbouring autochthonous microorganisms. Hence, the quest to fill this gap forms the crux of this research study.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to assay the cytotoxic and genotoxic effects of crude oil on selected plants grown on polluted soil during rhizoremediation study.

The specific objectives of the study include to:

i. Determine the physicochemical properties of the agricultural soil before and after crude oil pollution.

ii. Analyse the proximate component of the test plants prior and after crude oil pollution.

iii. Evaluate the morphological growth characteristics of selected plants during treatment.

iv. Ascertain cytotoxic effect of crude oil pollution on test plants' cells and tissues.

v. Determine the genotoxic effect of crude oil pollution on chromosomal DNA of test plants.

1.4 SIGNIFICANCE OF THE STUDY

This study will enable scientists and the public to clearly identify the potential hazards of crude oil; to gain insight into the extent of damage done by crude oil to the genetic, cellular, tissue and morphological structures of the selected food crops in the presence of autochthonous microorganisms within the rhizosphere. It can serve as a guide to understand how this genotoxic substance extrapolates to humans through the food chain and to assess the phytoremediation potentials of the test plants. This information will be pivotal to environmentalists, agriculturists, biotechnologists, economists, statisticians, government, policy makers, pharmacists and other medical practitioners in the detection and development of strategies to tackle the health, environmental and socio-economic risks posed by crude-oil and its related pollutants to the populace, ecosystem, and economy of the nation.

1.5 JUSTIFICATION OF THE STUDY

The terrestrial environment hosts a myriad of persistent genotoxins ranging from heavy metals (lead, mercury, nickel, copper, arsenic, zinc, cadmium, chromium among many others) to

petroleum hydrocarbons (crude oil, diesel, gasoline, creosote, and polycyclic aromatic hydrocarbons), which indirectly stretch into the food chain jeopardizing the biotic community and aesthetic nature of the ecosystem (Dar, Yousuf & Balkhi, 2016). Crude oil spill on soil alters its structure and texture, making it dysfunctional for plant propagation. FiriAppah *et al.* (2014) reported that the exposure of a germinated *Hibiscus esculentus* (okra crop) to 100mls of crude oil and above resulted to stunted growth and development while no germination occurred at the same concentration of crude oil on its seeds. Besides, crude oil has acute and chronic impacts on human health as most of its components are carcinogens, mutagens or teratogens that cause harm even at low concentration (Ramirez, Arevalo, Sotomayor & Bailon-Moscoso, 2017). Therefore, the frequent oil spills in Nigeria pose colossal concern to the government and public prompting their actions towards protecting and sustaining the environment and its inhabitants. This justifies the continuous attempts by indefatigable scientists to investigate novel, quick, precise, and safe methods for ecological risks assessment using bioassays.

Over the years, biomarkers have been used to easily detect and measure insidious forms of environmental pollution. The noble capabilities of plants to easily grow and adapt to environmental stress and their major role in the food chain, qualify them as great biomarkers or bio indicators during genotoxicological studies. An observed genotoxic effect on plant-based assays implies serious health risks to humans and other organisms that feed on contaminated plants in the long-term (Ramirez *et al.*, 2017). Furthermore, plants are preferred to animals as bioassays due to their economic, simple, high-sensitivity and less-sacrificial efficacies (Mustapha, Njoku, Adesuyi & Jolaoso, 2019). *Zea mays* (a cereal known as maize), *Telfaria occidentalis* (a vegetable known as fluted pumpkin) and *Allium cepa* (a vegetable commonly known as onion) selected as test plants for this study are some Nigerian staple crops that are highly tolerant to a variety of soil, tropical weather conditions and environmental stress. So,

they are mostly available during greater time of the year and are commonly consumed as local foods all over the country, making them appropriate for this study.

Comet assay remains a gold standard in cytogenetic evaluation due to its reproducible ability, high sensitivity to detect low level damages, flexibility to use, ease of application, economic, rapid and reliable nature.

1.6 SCOPE OF THE STUDY

This study focused on the use of some selected edible plants such as *Zea mays*, *Telfairia occidentalis* and *Allium cepa* grown on agricultural soil harbouring autochthonous bacteria, to determine the cytotoxic and genotoxic effects of crude oil and the rhizoremediation efficiency of the selected plants. Analysis of the physico-chemical properties of the agricultural soil and proximate composition of test plants before and after crude oil pollution including the total hydrocarbon content of polluted soil and plant samples were carried out. Evaluation of the test plants performance on polluted soil was equally carried out by measuring their height, leaf number, leaf area and examination of their genetic, cellular and tissue structures using molecular and cytogenetic techniques such as comet assay, total chlorophyll content test and tissue sectioning of leaves for crystals.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CLASSES OF PETROLEUM HYDROCARBONS

Crude oil is found underground in its unrefined state but are drilled and refined for human utilization. It is a complex mixture of ten thousand of hydrocarbons (saturated and unsaturated) and non-hydrocarbons such as sulphur, nitrogen, oxygen, and various trace metals (Mahjoubi *et al.*, 2018). The variation in the number of hydrocarbons and their arrangement model give rise to different types of crude oil, ranging from light or denser crude to light yellow or dark brown/black crude (Singh *et al*., 2012; FiriAppah *et al*., 2014). Petroleum hydrocarbons are commonly grouped into three classes, they include, aliphatic hydrocarbons, aromatic hydrocarbons and heterocyclic compounds (Mahjoubi *et al.*, 2018).

2.1.1 ALIPHATIC HYDROCARBONS

This is a class of organic compounds in which the carbon and hydrogen atoms are arranged in linear, branched, or cyclic forms. They consist of the saturated alkanes (n-heptadecane, paraffin, cyclopentane and pristane) and unsaturated alkenes and alkynes. In crude oil, the alkanes are found in copious supply and are easily degraded (Mahjoubi *et al.*, 2018).

2.1.2 AROMATIC HYDROCARBONS

This group have a closed ring of alternate single and double bonds with delocalized electrons. They include monocyclic aromatic hydrocarbons (benzene, toluene and xylene) and polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, fluorene, chrysene, triphenylene, quinone, pyrene, phenanthrene, acenaphthene and anthracene. PAHs are environmental pollutants that are highly persistent, recalcitrant to degradation, carcinogenic and mutagenic (Mustapha *et al*., 2019).

2.1.3 HETEROCYCLIC COMPOUNDS

Heterocyclic compounds have one or more atoms (nitrogen, sulphur, and oxygen) other than carbon in at least one of their rings. Examples are xanthene, quinolines, dibenzothiophenes, among others. This group is the most recalcitrant for degradation (Mahjoubi *et al.*, 2018).

2.2 CRUDE OIL POLLUTION – OVERVIEW OF GLOBAL PERSPECTIVE

Oil spillage being a major source of environmental pollution is described as the unplanned release of crude oil and its derivatives into the soil, aquatic bodies, and atmosphere during operational activities (drilling, production, exploitation, transportation and storage) or through deliberate acts of vandalism such as pipeline destruction, sabotage and theft (Ilyushina, 2020). The ever-increasing demands of crude oil as primary energy source for daily human activities and extensive industrial development birthed crude oil pollution; as most documented global oil spill incidents such as Mobil Nigeria 1998 oil spill, Project Deep 2000 Spill in Norway, Prestige 2002 oil spill in Spain, Deepwater Horizon 2010 spill in Gulf of Mexico, Yellow River 2010 oil spill in China, Willowton 2019 oil spill in South Africa, as well as the most recent Russian Norilsk diesel fuel spill and Canadian Trans Mountain oil spill both in 2020 accrue from oil well blowouts, tanker accidents, underground tank leakages, pipeline vandalism, oil drilling and refining activities, natural seeps, industrial and urban effluent, among others (Abii & Nwosu, 2009; Ogbulie *et al.*, 2011; Ilyushina, 2020). Oftentimes, pollution of the soil and marine bodies with crude oil occurs in municipal areas and some rural communities where petroleum and natural gas are obtained (Nwakanma *et al.*, 2018).

2.2.1 OIL SPILLS IN NIGERIA

Certainly, Nigeria being one of the major oil-producing countries in Africa has recorded numerous oil spills in the past few decades, especially in its Niger Delta region and other oil-producing States. However, Adati *et al.* (2012) reported that the Niger Delta region produces

above 90% of the nation's oil exports since 1981 and is profoundly endowed with rich vegetation and habitats harbouring millions of multi-ethnic groups, variety of terrestrial and aquatic animals, as well as diverse mineral resources. Yet, this region has recorded innumerable cases of distressed ecosystem due to oil exploration and exploitation activities. In most cases, oil spills in Nigeria are orchestrated by host communities through pipeline vandalism, theft and sabotage while erroneous logistics and mismanagement of pipelines by the government and oil companies also contribute to oil leakages and seepages (Osuagwu & Olaifa, 2018). Table 2.1 shows some recent oil spill incident in Nigeria as documented by two prominent oil companies in the country (Shell Nigeria and Nigerian Agip Oil Company-NAOC), spills were measured in oil barrels (bbls).

Table 2.1: Oil spill incident in Nigeria (May, 2020).

Date Reported	Incident Site	Estimated Spill Volume (bbls)	Terrain	Cause	Owner
May 01, 2020	28” Nkpoku-Bomu pipeline at B-Dere	16	Land	Sabotage	Shell
May 02, 2020	28” Nkpoku-Bomu pipeline at Elelenwo	60	Land	Sabotage	Shell
May 03, 2020	14” Okordia-Rumuekpe pipeline at Edeoha	8	Land	Sabotage	Shell
May 03, 2020	16” Egbema-Assa pipeline at Etekwuru	63	Land	Sabotage	Shell
May 04, 2020	8” Nkali-Imo River pipeline at Odagwa	1	Land	Sabotage	Shell
May 05, 2020	4” imo River Well 55S Flowline at Igiriukwu	1	Land	Sabotage	Shell
May 08, 2020	3” AdibawaWelĺ 6T Flowline Flange at Edagberi Betterland	0.7	Land	Operational	Shell
May 09, 2020	4” Bonny Well 10S Flowline Riser at Oloma	0.1	Swamp	Operational	Shell

May 10, 2020	10” Oshie/Ogoda pipeline, Ahoada west	98	Land	Hacksaw cut	NAOC
May 12, 2020	10” Clough Creek/Tebidaba pipeline, Southern Ijaw	78	Creek	Oil theft	NAOC
May 13, 2020	10” Clough Creek/Tebidaba pipeline, Southern Ijaw	30	Creek	Corrosion	NAOC
May 13, 2020	10” Clough Creek/Tebidaba pipeline, Southern Ijaw	5	Creek	Oil theft	NAOC
May 15, 2020	18” Tebidaba/Brass pipeline, Southern Ijaw	30	Creek	Corrosion	NAOC
May 18, 2020	24” Ogoda/Brass pipeline, Brass	4	Creek	Corrosion	NAOC
May 27, 2020	20” Kolocreek-Rumuekpe pipeline at Aminigboko	3	Land	Sabotage	Shell
May 28, 2020	4” Kanbo Well 5L Flowline at Tunu	0.03	Swamp	Sabotage	Shell
May 30, 2020	12” ImoRiver 2-Ogale pipeline at Komkom	2	Land	Sabotage	Shell

Source: Shell Nigeria (2020); Akinpelu (2021)

2.3 THE PERILS OF CRUDE OIL SPILL

Undoubtedly, oil industries have immensely contributed to the economic growth of their host countries but have caused serious pollution problems and ecological hazards in the long run (Adati *et al*., 2012). The deleterious effects of oil spill are portrayed mostly in the environmental structure and human health as pinpointed below.

2.3.1 CRUDE OIL IMPACT ON SOIL

It is general knowledge that the soil is a mixture of sand and organic matter which serve as a natural medium for plant growth and survival of other soil-dependent living organisms, ranging from soil micro and macro-organisms to humans. Crude oil spill on soil alters its structure and texture, making it dysfunctional for plant propagation (Erhenhi & Ikhajiagbe, 2012).

This is expressed by impaired water supply due to the hydrophobic effect of crude oil on soil particles, binding them into water impenetrable clumps; and inadequate soil aeration caused by crude oil displacement of air spaces in the soil, thereby limiting dispersion of oxygen and other essential soil nutrients as well as impeding microbial activities as shown in several studies (Abii & Nwosu, 2009; Ogbo *et al.*, 2009; Agbogidi, 2011; Erhenhi & Ikhajiagbe, 2012 & FiriAppah *et al.*, 2014). Countless occurrence of crude oil spills in Nigerian oil-producing States especially the Niger-Delta regions, give rise to serious deterioration of farmlands, vegetative cover and mangrove habitats situated in these areas. As crude oil contamination triggers accumulation of toxic ionic metals such as aluminium and manganese in the soil, water logging, limited oxygen diffusion, unavailability of soil nutrients, altered soil pH and even suffocation of microbes and other soil organisms (Onwurah, Ogugua, Onyike, Ochonogor, & Otitoju, 2007). Consequently, oil spillage has undesirable effect on ground water, soil fertility, plant growth and food supply.

2.3.2 CRUDE OIL IMPACT ON PLANTS

Crude oil exhibits herbicidal effects on direct contact with plants as well as prevents germination and development of plants. Its negative impact on plants is depicted by their poor yield and retarded growth features such as reduced leaf area, leaf number, plant height, stem girth, plant biomass, further to root length, resulting from impaired absorption of essential nutrients by plant roots and limited water supply (Osuagwu, Okigbo, Ekpo, Chukwurah, &

Agbor, 2013). The quantity of crude oil and its duration in the soil or on plants (if sprayed) influence the rate of toxicity in plants, as no germination occur at high concentration of crude oil while stunted growth and reduced performance happen at low concentration (Agbogidi, 2011; FiriAppah *et al.*, 2014). All these were attributed to the ability of the volatile oil to penetrate the seed integument or through plant cuticles, giving rise to suffocation of embryo in seeds or coalescence of cells at different tissues due to oxygen deprivation. A lot of experimental studies have been conducted to reveal the effects of petroleum contaminants on the genetic and morphological structures of some plants. For instance, Njoku, Akinola, & Oshodin (2011), Komolafe, Akinola & Agbolade (2015) and Iheme, Akinola & Njoku, (2017) in their respective works, reported chromosomal anomalies (sticky and vagrant chromosomes) in *Sorghum bicolor* at different phases of mitosis and higher percentage of chromosomal aberrations in the plant because of petroleum toxicity. Ogbo *et al*. (2009) also reported that crude oil caused a notable decrease in the biomass, height, and leaf area of *Paspalum scrobiculatum*. In air potato, Osuagwu *et al*. (2013) observed significant reduction of plant growth, yield, and leaf chlorophyll content after crude oil pollution; hence the reduced leaf area, number and chlorophyll content interrupt its photosynthetic activity. Morphological evaluation of *Telfairia occidentalis* showed restrained seed emergence in a pre-existing crude oil polluted soil and eventually leaf chlorosis as well as plant death in soil polluted three weeks after planting (Erhenhi & Ikhajiagbe, 2012). Research findings by Okonwu, Amakiri, Etukudo, Osim & Mofunanya, (2010) presented the fact that crude oil application on the foliage and rhizosphere of *Zea mays* resulted to stunted growth, lowered chlorophyll content, defoliation, damaged tissues, and necrosis. However, some plant species survive crude oil toxicity as was observed in *Arachis hypogaea* which showed better tolerance of crude oil due to the endophytic bacteria it harbours (Iheme *et al*., 2017). Ogbo *et al*. (2009) highlighted an apparent tolerance to high concentration of crude oil in a weed at varying plant density, ascribable to its

phytoremediation potential. Remarkably, the adverse effect of crude oil in plants is attributable to its hydrophobic nature which induce stomata closure, damaged chloroplasts, tissue infiltration, obstructed gaseous exchange, distorted vascular bundles, inefficient water and nutrient uptake as well as disrupted photosynthesis (FiriAppah *et al.*, 2014).

2.3.3 CRUDE OIL IMPACT ON AQUATIC ENVIRONMENT

Oil spillage in water bodies is usually uncontainable as crude oil form oil slick which may spread to the whole ecosystem, endangering aquatic organisms and reducing the aesthetic nature of the habitat. It has been established that oil spills account for diminished oxygen content in water through massive accumulation of toxic minerals, leading to smothering and reduction of diverse marine population with indirect negative impact on the trophic level. In addition, crude oil when exposed to ultraviolet rays cause advanced toxicity in aquatic community by generating polycyclic aromatic hydrocarbons such as quinone which are bioaccumulative (Onwurah *et al.*, 2007). To this effect, prolonged oil spill on water bodies declines fish productivity as demonstrated by Ekpenyong & Udeme (2015) in a study, where a high density of toxic petroleum hydrocarbons was observed to accumulate in the tissues of different fish species because of crude oil contamination. Osuagwu & Olaifa (2018) also affirmed that the continuous rise in oil spillage in Niger Delta region of Nigeria have unfavourable impact on fish production. Also, spilled oil infiltrates the fur and plumage of marine mammals and birds respectively, impairing their insulating effect and the birds' flying ability thus exposing them to unstable temperatures and predatory attacks. Conclusively, damaged mangrove swamp, dead fish, poisoned sea birds and animals are serious threats directly posed by crude oil to the marine ecosystem.

2.3.4 CRUDE OIL IMPACT ON HUMAN HEALTH

The exposure of most animals and humans to crude oil happen directly or indirectly through dermal contact, ingestion of oil polluted food and water, further to inhalation of vapour emitted by spilled oil. Generally, crude oil spills have acute and chronic impacts on human health as most of its components are carcinogens, mutagens or teratogens that cause harm even at low concentration. Toxic hydrocarbons and heavy metals contained in petroleum accumulate in the internal organs of humans and other animals, altering their gene pool thereby causing serious health deformities such as cancers, infertility, respiratory problems, conjunctivitis, renal failure, liver dysfunction, epidermolysis, neurological effects, skin irritations and many others (Ordinioha & Brisibe, 2013; Ramirez *et al.*, 2017). High rate of genotoxicity is constantly observed in inhabitants of oil-contaminated zones (Webb, Coomes, Mergler & Ross, 2016) on oil spill clean-up workers at Peruvian Amazon four months after the incident, with twice as much mercury detected in their urine when compared to that of non-clean-up workers in the area. Besides, oil spillage engenders poor air quality by releasing toxic particulates which when inhaled penetrates human lungs causing adverse health effects. Hazardous fire outbreaks also emanate from some oil spill scenes claiming human lives. Obviously, an undeniable correlation exists between environmental contamination and human health, due to the possible genotoxic transfer from polluted plants and animals to humans by virtue of our food chain and inhalation of toxic chemicals from contaminated air.

2.3.5 CRUDE OIL IMPACT ON THE ECONOMY

On a general note, oil spillage has numerous negative economic impacts. These include prolonged restriction of fishery and agricultural activities in affected regions, which may conceivably lead to a vast decline in the economy via reduction of fish and food production (Osuagwu & Olaifa, 2018). Furthermore, short-term, or long-term interruption of land and sea

travel is inevitably employed as post-spill protection measure, thereby causing a plummet in import-export activities. Crude oil pollution instigates the destruction of the environmental structure with its flora and fauna (Webb *et al.,* 2016) prompting the contaminated areas to lose their aesthetic value and drastically reduces revenues realized from tourism. Finally, a huge sum of money is usually required to remedy the pollution and health-deteriorating menaces caused by crude oil spills, as the adverse effects of untreated spills stretch to the populace accounting for their death and ineffectiveness in the labour force (Ramirez *et al.,* 2017).

2.4 BIODEGRADATION OF CRUDE OIL

Globally, the health and environmental threats posed by crude oil spills are imputable to its complex mixture of highly persistent, recalcitrant aromatic hydrocarbons and heterocyclic compounds which are difficult to degrade. Hence, several physical, chemical, and biological methods have been employed over the years to clean up crude oil from our environment (Mahjoubi *et al.,* 2018). Yet, biological method remains the most preferred due to its natural, sustainable, cost-effective, and eco-friendly nature (Fodelianakis *et al.,* 2015). Biodegradation of crude oil is mainly executed by microbes (bacteria and fungi), which enzymatically utilize different hydrocarbons as carbon and energy sources. Hence, these microbes play a major role in converting toxic pollutants such as petroleum, heavy metals, dyes, pesticides, ectcetra into non-toxic organic compounds like carbon dioxide and water (Yuniati, 2018). Additionally, in a review paper, Abatenh, Gizaw, Tsegaye & Wassie (2017) made a list of a plethora of crude oil degradrading microbes that exist in nature. They include: *Aspergillus niger, Candida glabrata Candida krusei, Saccharomyces cerevisiae, Bacillus brevis, Pseudomonas aeruginosa KH6, Bacillus licheniformis, Bacillus sphaericus, Pseudomonas cepacia, Bacillus cereus, Bacillus coagulans, Citrobacter koseri, Serratia ficaria* and many others.

2.4.1 FACTORS AFFECTING BIODEGRADATION OF CRUDE OIL

To a large extent, successful biodegradation of crude oil depends on nature and number of hydrocarbons present in the oil, its toxicity level, the microbial population of the contaminated site, bioavailability of contaminants, temperature, pH, moisture content and the duration of pollution (Yuniati, 2018). However, Gkorezis, Daghio, Franzetti, Van Hamme, Sillen & Vangronsveld (2016) summarised these factors into three main groups under listed below:

2.4.1.1 HYDROCARBON FEATURES

The biodegradation rate of crude oil is influenced by its varying hydrocarbon composition, their arrangement pattern, bioavailability, oil volatility, solubility, concentration, hydrophobicity and toxicity level. Bioavailability is a major determinant factor of biodegradation, as it signifies the amount of contaminant that readily penetrates microbial cell membrane. Exemplarily, PAHs exhibit low bioavailability due to their reduced aqueous solubility. However, biosurfactants secreted by microbes and plants limit substrate-microbial surface tension and increase contaminant bioavailability to degrading microbes (Gkorezis *et al.,* 2016; Mahjoubi *et al.,* 2018).

Yuniati (2018) reported that crude oil degradation depends on the nature and number of hydrocarbons present in the oil, its toxicity level and bioavailability of the contaminant, the microbial population of the contaminated site, environmental parameters and the duration of pollution. Despite the existence of both physicochemical and biological methods of oil spill clean-up, the use of the latter is widely recommended due to their reduced cost, low maintenance, naturally efficient, sustainable and high aesthetic nature (Fodelianakis *et al.,* 2015).

2.4.1.2 MICROBIAL PROPERTIES

Generally, the microbial population of contaminated site as well as their diverse genetic make-up, gene regulation and modification, enzymatic capabilities, synergistic and antagonistic interactions, nutrient uptake, microbial adhesion, toxicity tolerance, biosurfactant secretion and cell surface hydrophobicity; are all microbial properties utilized during oil degradation (Abatenh *et al.*, 2017).

Bacteria, fungi and yeast are primary biodegraders of hydrocarbons, but bacteria are the common active degraders. A host of indigenous microorganisms can degrade hydrocarbons at different habitats including land, water, air and living systems. Bioavailability of hydrocarbons determines the efficiency of microbes in biodegradation. These biodegraders oxidize toxic substances to produce harmless products.

2.4.1.3 ENVIRONMENTAL PARAMETERS

The environmental conditions of the polluted site (temperature, pH, moisture content, oxygen concentration, nutrient availability, metal ion content, quantity of pollutant, site characterization and duration of pollution) greatly affect the rate of interaction between microorganisms and the pollutant. For instance, temperature directly influences the physicochemical properties of pollutants as well as the growth and metabolic activities of microbes; as biodegradation rate decreases with decreasing temperature (Yuniati, 2018). Therefore, the harmonization of all these environmental parameters at optimal levels and balanced proportions may give rise to an effective biodegradation of crude oil.

2.5 BASIC BIOREMEDIATION TECHNIQUES

Bioremediation is a green, safe and cheap means of detoxifying contaminated sites through biodegradation carried out mainly by microbes. Some basic bioremediation techniques are bioattenuation, biostimulation, bioaugmentation, bioventing and biopiles. Though,

Hassanshahian *et al.* (2014) highlighted biostimulation and bioaugmentation as two major approaches to enhancing bioremediation.

2.5.1 BIOATTENUATION

Bioattenuation or Natural Attenuation (NA) is a form of bioremediation by which native microbes naturally annihilate toxic compounds from the environment, using several physical (dilution, dispersion, diffusion, volatilization, sorption and elution), chemical (ion exchange, radioactive decay and abiotic transformation) and biological (aerobic and anaerobic biodegradation, plant and animal uptake) processes. NA is also referred to as intrinsic bioremediation or biotransformation (Ogbulie, Duru & Nwanebu, 2015; Abatenh *et al.*, 2017). However, bioattenuation is a slower remediation process which can be improved with either biostimulation or bioaugmentation.

2.5.2 BIOSTIMULATION

Biostimulation constitutes the alteration of environmental conditions by adding nutrients, growth supplements or coenzymes and providing prerequisite environmental conditions like temperature, pH and oxygen in order to induce native microbial activity; thus improving their growth, enzymatic actions and biodegradable abilities (Hassanshahian *et al.*, 2014). Nonetheless, pollutants in minute quantity could trigger the release of biodegradable enzymes from biodegraders present in pollution site.

2.5.3 BIOAUGMENTATION

Bioaugmentation involves the introduction of natural, foreign or genetically engineered pollutant-degrading microorganisms into a polluted environment to supplement the existing microbial population in accelerating degradation. These microbes could be isolated from the contaminated site (autochthonous or native strains), separately cultured from another polluted site (allochthonous or foreign strains) or be genetically engineered in the laboratory. As

engineered microbes break down toxic substances faster than natural species and ecologically outwit them (Abatenh *et al.,* 2017). However, Mahjoubi *et al.* (2018) stated that autochthonous bioaugmentation constitutes the inclusion of indigenous microorganisms that are adaptive to the polluted sites due to favourable environmental conditions whereas; allochthonous or exogenous microorganisms which originate from different sites are unable to fit to the indigenous microbes found within the polluted sites as the environment may not be suitable for their growth and biochemical activities. Studies carried out by Liu *et al.* (2008) and Couto *et al.* (2010) reported more effective bioremediation of petroleum contaminated sites, attainable through isolation and reintroduction of hydrocarbon degrading bacteria (bioaugmentation) unlike other remediation methods (biostimulation and bioattenuation) applied on same sites. Moreover, an effective bioaugmentation depends on proper management of indigenous microbial consortia and environmental conditions as proven in a research work conducted by Fodelianakis *et al.* (2015) which depicted that under ideal environmental conditions, autochthonous microbiome at a polluted site will probably perform better than any allochthonous consortium in biodegradation of crude oil.

2.5.4 BIOVENTING

Azubuike, Chikere & Okpokwasili (2016) defined bioventing as a controlled supply of airflow (oxygen) to unsaturated zone in polluted soil, to promote microbial growth and metabolic activities needed for effective bioremediation. This technique also requires nutrient amendments. Besides, experiments carried out by many researchers (Ho¨hener & Ponsin, 2014; Agarry & Latinwo, 2015) have confirmed the relevance of bioventing in treating petroleum contaminated sites.

2.5.5 BIOPILES

Biopiles also known as bioheaps, biocells, biomounds or compost piles constitute the heaping of excavated polluted soil above the surface of the ground as well as application of nutrient amendments and aeration to enhance bioremediation. Biopiles serve as an effective tool in remediating polluted sites in extremely cold regions (Azubuike *et al.*, 2016).

2.6 PHYTOREMEDIATION

Phytoremediation is the employment of plants and their related microorganisms in treating polluted surroundings through the absorption, conversion, degradation and detoxification of organic and inorganic pollutants. It is a safe, clean, sustainable, cheap and eco-friendly approach to bioremediation (Wuana & Okieimen, 2010). The synergistic interaction between plants and microbes are executed by these bacterial groups: endophytic bacteria (located within plants tissues and endowed with pathogenic or predatory-protective abilities), rhizospheric bacteria (located in the root area and exhibit mutualistic association with host plants) and phyllospheric bacteria (located in the aerial region of plants and possess the ability to degrade gaseous pollutants). Generally, plant-microbe relationship is characterized by the ability of the host plants to harbour and supply nutrients to microbes, which in return promote their metabolic and degradative activities. Most plants use in phytoremediation are selected based on their high-competitive capabilities, fast and easy growth, high tolerance to toxic substances, secretion of hydrocarbon-degrading enzymes and efficient root system (Gkorezis *et al.*, 2016). Depending on the nature of pollutants, different mechanisms applicable in phytoremediation are phytostabilization, phytovolatilization, phytoextraction or phytosequestration and rhizodegradation or rhizoremediation. Inorganic pollutants like heavy metals and radioactive compounds are mainly eradicated through phytoextraction (the ability of plants to absorb pollutants, segregate and store them within their biomass where they can be recovered through

phytomining) whereas organic pollutants like hydrocarbons and chlorinated compounds are especially removed through phytovolatilization (the use of plants to convert volatile toxic compounds into gases released into the atmosphere), phytostabilization (plants' ability to reduce the mobility, solubility and bioavailability of soil contaminants through minimized wind transport, erosion and leaching) and rhizodegradation - the release of root exudates to stimulate rhizospheric bacteria in biodegradation of contaminants (Wuana & Okieimen, 2010; Azubuike *et al.,* 2016). Furthermore, phytoremediation potential of plants grown on polluted environment can be optimized by the addition of autochthonous or allochthonous bacteria (bioaugmentation) or by biostimulation.

2.6.1 RHIZOREMEDIATION

Rhizoremediation is a form of phytoremediation which involves biodegradation of organic pollutants through combined mutualism between plant roots and microbes found within the root area. It is a relevant alternative for remediation of petroleum hydrocarbon-contaminated soils due to its safe, effective, economic, sustainable and environmental-friendly qualities. Ogbulie *et al.* (2014) stated that plants through rhizoremediation can stabilize oil contaminated sites by stimulating the growth and activities of degrading microbes in the rhizosphere, exuding phenolic compounds from their roots and converting petroleum hydrocarbons into less toxic substances like carbon dioxide, water, methane and fatty acids. Proper selection of plant-microbe pair, organic and inorganic soil amendments (such as fertilizer, compost, biochar, surfactants, nutrients) and agronomic practice can improve rhizoremediation performance (Hussain *et al*., 2018). The natural existence of innumerable hydrocarbon-degrading organisms reasonably vindicate the introduction of isolates or consortium of indigenous or exogenous microorganisms, as mitigation tools to improve the rate of pollutant degradation using their metabolic capabilities.

Besides, the concentration of inoculum and successive inoculation of these degraders to the root zone can lead to optimum plant growth, pollutant degradation and microbial overflow; since the rhizosphere is commonly considered the flash point for horizontal gene transfer where root exudates stimulate the actions of microbial degraders especially bacteria, the principal degraders of organic pollutants (Correa-Garcıa, Pande, Seguin, St-Arnaud & Yergeau, 2018). Hence, the degrading-ability of bacteria in oil contaminated soil is enhanced by the presence of plants as stated by Gkorezis *et al.* (2016). Plants can be favourably influenced directly or indirectly by the ability of bacteria to construe severe modifications in their well-being via hormone synthesis, repression of ethylene production and the mobilization of unavailable nutrients. For instance, Asghar, Rafique, Khan & Zahir (2017) highlighted a notable degradation of petroleum hydrocarbon and an increase in height, weight and root length of *Zea mays* grown on crude oil contaminated soil inoculated with *Bacillus subtilis*. This strain altered the rhizobiota and enhanced crude oil degradation through synergistic interactions with the plant roots.

2.7 THE CONCEPT OF GENOTOXICOLOGY

Genotoxicology is the methodical evaluation of the impact of genotoxins released into the ecosystem on the genome of an organism. The term “genotoxins” denote substances (physical or chemical) that stimulate alterations in the genetic system of organisms at sub-toxic concentration. The effects of genotoxins on living organisms include chromosomal fragmentation and translocation, chromatid deletions, neoplasm and even cell death. These alterations may occur in the somatic or germ cells of living organisms and failure of the cells to undergo successful DNA repair or apoptosis might lead to mutagenesis (Mohamed, Sabita, Rajendra & Raman, 2017). Thus, not all genotoxins are mutagenic but all mutagens are genotoxic. The terrestrial environment hosts a myriad of persistent genotoxins ranging from heavy metals (lead, mercury, nickel, copper, arsenic, zinc, cadmium, chromium among many

others) to petroleum hydrocarbons (crude oil, diesel, gasoline, creosote and polycyclic aromatic hydrocarbons), which indirectly stretch into the food chain jeopardizing the biotic community and aesthetic nature of the ecosystem (Dar *et al.,* 2016). At high concentration, most genotoxic substances disintegrate nucleic acids and hinder certain cellular processes as they may act as carcinogens (causing cancers), mutagens (causing mutations) or teratogens (causing birth defects). Exemplerily, FiriAppah *et al.* (2014) reported that the exposure of a germinated *Hibiscus esculentus* (okra crop) to 100mls of crude oil and above resulted to stunted growth and development while no germination occurred at the same concentration of crude oil on its seeds. Research findings by (Ajah, Osuji & Anoliefo, 2019) also supported the fact that polluted food sources have direct link to the increased rates of terminal illnesses like cancer, diabetes, liver and kidney failures in Nigeria.

2.8 GENOTOXICITY TESTING METHODS

Genotoxicity testing is usually conducted with the intention of detecting potential mutagenic hazards caused by genotoxic substances to humans and other living organisms, as well as highlighting their mode of action both *in vivo* and *in vitro* (Mohamed *et al.,* 2017). Under listed below are some genotoxicity testing methods.

2.8.1 CHROMOSOME ABERRATION TEST

Chromosome aberration (CA) denotes any irregularity or abnormality of chromosome distribution, number, structure or arrangement. The use of chromosome aberrations as indicators for monitoring the effects of genotoxic compounds and radiation both *in vivo* (within living organisms) and *in vitro* (outside living organisms) has been in existence. As Dar *et al.* (2016) mentioned how some previous studies utilized CA test as a genotoxicity evaluation tool on various animals (especially rodents and fishes), plants (such as *Allium cepa*) and insects (like *Drosophilia*). Hence, CA test is employed to evaluate genotoxins potential to stimulate

structural aberrations (clastogenicity) or numerical aberrations (aneuploidy), cell division anomalies, chromatid bridges, nuclear injuries and other anti-mitotic activities in living cells (Ajah *et al.,* 2019). The precise method for conducting CA test in both plant and animal cells include cell exposure to genotoxic substance, followed by cell treatment with colchicine or colcimide capable of arresting cells at metaphase and then, appropriate staining prior to microscopic analysis to visualize aberration as described in an article by Dar *et al.* (2016). CA test can also serve as complementary test to bacterial reverse mutation assay (Ames test). Undoubtedly, *Allium cepa* assay has proven over the years to be a sensitive, quick, cost-effective and reliable bio-monitoring tool for detecting genotoxic and anti-mitotic chemicals, effluents, substances, drugs as well as herbal concoctions and their impacts on eukaryotic systems (Firbas & Amon, 2013; Soodan, Katnoria & Nagpal, 2014; Onwamah *et al.,* 2014 & Timothy, Idu, Olorunfemi & Ovuakporie-Uvo, 2014).

2.8.2 MICRONUCLEUS ASSAY

This is among the most sensitive, simple, reliable and popular genotoxic assessment tool use *in vitro* for screening carcinogenic chemicals or substances and *in vivo* for detecting high frequency of micronuclei in bone marrow or erythrocytes of mammals treated with genotoxic compounds. Micronuclei (MN) are small nuclei formed during anaphase from lagging acentric chromosomes or chromatid fragments because of cell repair dysfunction, chromosome aberrations and mitotic malfunctions. Different types of micronucleus assays exist based on the procedures and cells used. They include Cytokinesis-block micronucleus assay, Mammalian erythrocyte micronucleus assay, Buccal micronucleus assay and micronucleus assay in other cell types (Sommer, Buraczewska & Kruszewski, 2020). However, the main limitation to the use of micronucleus assays is in their inability to evaluate various forms of chromosome aberrations as well as fluctuations caused by mitotic rate and degree of cell death, thereby giving rise to biased results.

2.8.3 COMET ASSAY

Comet assay also known as single cell gel electrophoresis (SCGE) assay is a simple, sensitive, economic and standard technique used for probing DNA damage and measuring breakages in single and double DNA strands located in eukaryotic cells (Gichner, Patkova, Szakova & Demnerova, 2004). This genotoxicity assay can be performed *in vivo* or *in vitro* where the individual cells exposed to genotoxic agents such as ultraviolet rays, radioactive elements and organic or inorganic compounds; are enclosed in a low-melting-point agarose suspension prior to cell lysis with highly concentrated salt solution and detergent which disentangle and dissolve protein, RNA and cellular membranes. Then, the suspended lysed cells are electrophoresed in neutral or high pH conditions to reveal the pattern DNA migrates through the gel matrix, as shown in the form of a comet during fluorescence microscopy (Azqueta, Gutzkow, Brunborg & Collins, 2011; Pourrut, Pinelli, Mendiola, Silvestre & Douay, 2015). The level of DNA damage can be scored manually or automatically using software imaging. Numerous literature have portrayed the relevance of comet assay in monitoring DNA damages in plants and animals undergoing stress and also in assessing eukaryotic cell development pathways (Azqueta *et al.,* 2011; Pourrut *et al.,* 2015; Lanier, Manier, Cuny & Deram, 2015; Santos, Pourrut & Ferreira de Oliveira, 2015 & Dar *et al.,* 2016).

2.9 THE ROLE OF PLANT BIOASSAYS IN GENOTOXICOLOGICAL STUDIES

Plants play an efficient role in the production and circulation of oxygen and organic compounds in the ecosystem. They are preferred over other bioassays used for environmental monitoring due to their high sensitivity, straightforward, low cost, and easy handling abilities (Aksoy, 2017). Exposure of plants to mutagens or carcinogens usually triggers morphological, anatomical, cytological, and genotoxic changes on them which directly or indirectly are displayed through inhibited growth and development of different plant parts, chromosomal abnormalities at various mitotic phases, DNA breakages and damages as well as tissue

enlargement or shrinkage. Many scientists (Onwuamah *et al.*, 2014; Pourrut *et al.*, 2015; Liu, Liu, Zhang & Liu, 2017; Iheme *et al.,* 2017 & Nwakanma *et al.,* 2018) have examined toxicity in plants with the aid of some standard molecular and cytogenetic methods as shown in Table 2.2. Although, the choice of any of these techniques depend basically on the nature of test plants and type of pollutants being analyzed. Nevertheless, prolonged experiment due to plants' life span and the variations in plant and animal biochemical systems denote few limitations existing with the use of plant bioassays (Aksoy, 2017).

However, their advantages outweigh the aforementioned limitations especially as they have been widely accepted as excellent indicators of carcinogens, mutagens and teratogens in the environment.

Table 2.2: List of some toxicological studies with plant bioassays

Test Plants	Pollutants	Techniques	Sources
Vernonia amygdalina	Spent engine oil (SEO)	Chromosome aberration and mitotic index	Nwakanma *et al.*, 2018
Vicia faba L.	Wastewater from semi-coking plant	Micronucleus assay	Liu *et al.*, 2017
Arachis hypogaea and Sorghum bicolor	Crude oil	Chromosome aberration and mitotic index	Iheme *et al.*, 2017
Vicia faba, Trifolium repens, Lolium perenne and Miscanthus x giganteus	Ethyl Methanesulfonate (EMS)	Comet assay	Pourrut *et al.*, 2015
Allium cepa	Antiretroviral drugs zidovudine (ZDV) and nevirapine (NVP)	*Allium cepa* root tip assay	Onwuamah *et al.*, 2014
Zea mays	Crude oil	Chromosome aberration test	Udebuani, Otitoju, Abara, Eze & Duru, 2017

2.10 A BRIEF PROFILE OF THE SELECTED TEST PLANTS

2.10.1 MAIZE PLANT (*Zea mays*)

In most African countries including Nigeria, maize also known as corn is a prevalent cereal crop vastly consumed after sorghum and millet in the grain family. It is a monocotyledonous plant that belongs to the family, Poaceae. Several species of this cereal exist in the form of popcorn, sweet corn, pod corn, flint corn, dent corn and flour corn. Wuana & Okieimen (2010), reported that a typical mature maize plant grows up to 2 to 3 m tall, having 16 to 22 alternate leaves that are each (5 to 10 cm broad and 50 to 100 cm long) with adaptive roots (adventitious and brace) required to prevent lodging. They form cobs or ears of about 15 to 39 cm long and tassels at the plant tip for pollination. It strives mostly in fertile, well-drained soil with neutral pH and requires intermittent sunlight and rain for its ideal growth. Excessive rainfall could harm the crop due to its susceptibility to water logging. Recently, the giant strides attained by Nigerian researchers and agriculturists in plant breeding, tissue culture and genetic engineering led to an undeniable increase in maize production and its utilization in food and feed industries. Maize plant has proven its relevance in phytoremediation through its phytoextraction capability in heavy metal contaminated soils (Azubuike *et al.,* 2016).

However, some research findings have revealed the plant's inability to strive well in crude oil polluted soils (Agbogidi *et al.,* 2007; Okonwu *et al.,* 2010 & Udebuani *et al.*, 2017). Hence, it is necessary to use maize plant as an assay system to monitor genotoxic substances in the environment with an attempt to eradicate possible risks within associated food chains.

2.10.2 FLUTED PUMPKIN PLANT (*Telfairia occidentalis*)

Fluted pumpkin is a dicotyledonous vegetable crop dominant in West and Central Africa. It is a drought-resistant, dioeciously perennial that belongs to the Cucurbitaceae family and is usually grown trellised due to its creeping nature. It is propagated using the seeds, but the leaves

can be harvested at least a month after cultivation, while the fluted or furrowed fruits take 5 months to mature and weighs 5 to 20 kg with about 30 to 100 seeds per fruit (Ajayi, Berjak, Kioko, Dulloo & Vodouhe, 2006). Also, pumpkin seeds produce about 45% of edible oil. In Nigeria, fluted pumpkin is widely cultivated and distributed by the southerners with varying native names such as "ugu" (Igbo-Nigeria), "eweroko" or "aworoko" (Yoruba-Nigeria) and "ikong-ubong" (Efik/Ibibio-Nigeria). The leaves, shoots and seeds of the plant are highly nutritious and medicinal as they can be administered to anaemic patients (Eseyin, Sattar & Rathore, 2014). In an experiment, Elfiky, Elelaimy, Hassan, Ibrahim & Elsayad (2012) portrayed a possible protective and curative effects of pumpkin seed oil against cytotoxicity and genotoxicity of an anti-cancer drug, azathioprine. Furthermore, Erhenhi & Ikhajiagbe (2012) reported that the survival of fluted pumpkin on crude oil contaminated soil is dependent on the nature and level of pollution while Iyagba & Offor (2013) and Akpan & Usuah (2014) from their research findings recommended fluted pumpkin as a potential phytoremediator in oil and heavy metal contaminated soils. So, there is need to further investigate the genotoxic effect of crude oil on this plant using molecular and cytogenetic techniques.

2.10.3 ONION PLANT (*Allium cepa*)

Onion is a monocotyledonous, biennial plant of the Amaryllidaceae family, having a fan of hollow, cylindrical green leaves. Onions contain high percentage of water, minerals and oil, low proportions of protein, carbohydrates, dietary fibre, and negligible fat (Dini, Tenore & Dini, 2008). The bulbs are edible but exude a volatile liquid that irritate the eyes. Onions require well-drained, fertile soil to grow and are propagated using seeds or sets (partially grown bulbs). The plants are shallow-rooted and thus very little quantity of water and moderate sunlight is needed for its survival after germination. Onion plant serves as a reliable bioassay used for detecting DNA damages, chromosomal aberrations, alterations during mitosis and presence of micronuclei in meristem cells, especially after exposure to toxic compounds in the environment

(Nefic, Musanovic, Metovic & Kurteshi, 2013; Timothy *et al.,* 2014). The possession of large chromosomes with stable but less chromosome number (2n = 16), microscopically visible mitotic phases, sensitive root tips, diverse chromosome morphology, easily separated bulb epidermis and stable karyotype are qualities that make onion a good candidate for eco-toxicological screening (Firbas & Amon, 2013; Onwuamah *et al.,* 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF STUDY AREA

The study was conducted at Federal University of Technology, Owerri (FUTO) Imo State which is one of the oil rich states in South-eastern Nigeria. It is located between latitude 5°20'N and 5°25'N and longitude 7°00'E and 7°05'E, with a humid tropical climate and an average rainfall of about 2500mm pouring heavily within April and August. The land topography of the area is flat with typical rainforest vegetation.

3.2 SAMPLE COLLECTION

Soil samples were collected randomly using sterilized soil auger within the depth of 20cm from the Teaching and Research farm, School of Agriculture and Agricultural Technology (SAAT) Research farm FUTO, as the site has no history of crude oil contamination. The samples were air-dried and sieved using a 2mm mesh sieve. Then, 5kg soil each was weighed into perforated sterile seed bags and a quantity taken to the laboratory for analysis within 1 hour of collection. The crude oil used for this research is bonny light which was collected with sterile containers from Akiri in Oguta, Imo State, Nigeria.

3.3 PROCUREMENT OF SEEDS AND BULBS

The test plants used for this study are two monocotyledonous crops (maize - *Zea mays* and onion – *Allium cepa*) and a dicotyledonous crop (fluted pumpkin - *Telfairia occidentalis*). The maize seeds and onions were purchased from Ekeonunwa market in Owerri while the pumpkin seeds were bought from Afor-Enyiogugu market in Mbaise, all in Imo State.

3.4 EXPOSURE TO CRUDE OIL

The experiment was carried out in two (2) different groups as adopted from the work of FiriAppah *et al.* (2014) and shown in Table 3.1. Group A was polluted before planting using same quantity (100ml) of crude oil and the soil (5kg) mixed to homogeneity using a spatula while Group B was polluted 2 weeks after planting (2 WAP) using 100ml of crude oil together with 50ml of sterile water poured evenly to the soil and around the plants (ring application) as described by Agbogidi, Eruotor & Akparobi (2007). Unpolluted group served as control with zero amount of crude oil. The same procedure was repeated for all test plants in triplicates and the seed bags were labelled properly, making a total of 27 seed bags to be arranged in a completely randomized design.

Table 3.1 Experimental Design

	Treatment		
Group	**Maize**	**Fluted Pumpkin**	**Onion**
Unpolluted (Control)	0ml Crude Oil/5kg Soil		
A (Before Planting)	100ml Crude oil/5kg soil		
B (2 Weeks After Planting)	100ml Crude oil/5kg soil		

3.5 SOIL ANALYSIS

Soil analysis was carried out on the polluted and unpolluted soil samples at zero day of pollution to determine the physicochemical parameters present in the soil including total hydrocarbon contents (THC). The following physicochemical parameters were analysed (soil pH, soil texture, organic carbon, organic matter, soil minerals, exchangeable acids, and bases).

Analyses for these parameters were done following standard methods (APHA, 1995). Physicochemical analysis was repeated for all experimental groups at the end of the 28 days period based on their pollution time.

3.5.1 DETERMINATION OF SOIL TEXTURE

Soil texture was determined by relative percentage of clay, silt and sand using the hydrometer method. 50g of the air-dried soil was taken in a 500 ml beaker and treated with 55 ml of 6 % H_2O_2. The beaker was covered with a watch glass and placed on a water bath at about 70°C until it becomes cool. Then, the above process was repeated three times while placed on the water bath again to remove excess H_2O_2. About 400 ml of distilled water and 100 ml of calgon (sodium hexametaphosphate) solution were added into it and the suspension stirred with an electric stirrer for about 10 minutes, then transferred into a settling cylinder and the volume made up to one litre with distilled water. The mixture was shaken vigorously back and forth for 1 minute by placing a rubber stopper over the mouth of the cylinder and time recorded immediately. Then, hydrometer was inserted into the suspension and the first hydrometer reading taken after 4 minutes, after which the hydrometer was removed and washed with distilled water. Also, the temperature of the suspension was recorded as well. The hydrometer was calibrated at 19.45°C. Then, the suspension was allowed to remain undisturbed for two hours and hydrometer reading was taken again by reinserting the hydrometer into the suspension. The sand, silt and clay percentage were then calculated from the following expressions:

$$\text{Sand\%} = 100 - P_4$$

$$\text{Silt\%} = P_4 - P_{120}$$

$$\text{Clay\%} = P_{120}$$

Where,

$$P_4 = \frac{(R_4 \pm r) \times 100}{W}$$

$$P_{120} = \frac{(R_{120} \pm r) \times 100}{W}$$

and R_4 = hydrometer reading at 4 min, R_{120} = hydrometer reading at 120 min

r = temperature correction = ± (t - 19.45) x 0.2

W = oven dry weight of soil sample

t = temperature in °C at the time of measurement.

If the working temperature is more than 19.45°C, the temperature correction, r, is positive and if it is less than 19.45°C, r is negative.

3.5.2 DETERMINATION OF SOIL PH

Soil pH was determined using digital pH meter in 1:2 soil/water suspension using standard buffers for calibration. About 20g of air dried soil sample was taken in a beaker and 40 ml of water was added to it and the mixture stirred with glass rod for 10 minutes after which it was allowed to stand for 30 minutes. Then, the electrode of the pH meter was inserted into the supernatant to take the pH reading.

3.5.3 DETERMINATION OF MOISTURE CONTENT

Soil moisture content was analysed by oven drying method. About 10g of soil sample was taken and oven dried at 105°C for 24 hours. The dry weight of the sample was taken till it showed constant weight and then the percentage moisture content in each soil sample was calculated using the following formula shown:

$$\text{Moisture Content (MC) (\%)} = \frac{(W_2 - W_1)}{100} \times 100$$

Where,

W_1 = weight of soil before oven drying (g)

W_2 = weight of soil after oven drying (g)

3.5.4 DETERMINATION OF ORGANIC CARBON

The organic carbon (%) of the soil samples was determined by titrimetric method. Here, lg of the soil sample was taken in a 500 ml conical flask. About 10 ml of IN potassium dichromate solution was added and shaken. Also, 20 ml of concentrated sulphuric acid (containing 1.25 % H_2SO_4) was added and the flask was swirled 3 times. Then, it was allowed to stand for 30 minutes, and 200 ml of distilled water was poured into the flask to dilute the suspension while 10 ml of 85 % H_3PO_4 and 1 ml of diphenylamine indicator were added and the solution titrated with 0.5 N ferrous ammonium sulphate till the colour flashed from violet through blue to bright green. The volume of the ferrous ammonium sulphate solution was noted. A blank titration without soil was carried out in a similar manner and the calculation done as follows:

Weight of the sample = W (10 g)

Volume of 0.5 N $Fe(NH_4)_2(S0_4)_2$ solution used = B ml for the blank titration

Volume of 0.5 N $Fe(NH_4)_2(S0_4)_2$ solution used = S ml for the sample titration.

Volume of the IN $K_2Cr_2O_7$ used for oxidation of C = 0.5 x (B-S) ml

[1 ml of IN $K_2Cr_2O_7$ (=l meq) = 0.003 g of org. C]

% of org. C in the soil (uncorrected) = 0.5 x (B-S) x IN x 0.003 x (100/W) = Q

% of Org. C in the soil (corrected) = Q x 1.3 = R

3.5.5 DETERMINATION OF AVAILABLE NITROGEN

The available nitrogen was estimated by alkaline permanganate method. In a distillation apparatus, about 20 g of the soil sample was added to 10ml of 0.35% potassium permanganate, 10ml of 2.5% sodium hydroxide and 10 ml of distilled water. Then, 0.02 N sulphuric acid was poured through a 25 ml pipette together with 2 drops of methyl red indicator into a 250 ml beaker. The solution formed was titrated against 0.02N potassium hydroxide till the pink colour changes into light yellow. The percentage of nitrogen present in the given soil sample was calculated from the titre value of 0.02N sulphuric acid consumed by ammonia.

3.5.6 DETERMINATION OF AVAILABLE POTASSIUM

Out of the total non-exchangeable form of potassium (fixed K) present in soil samples, there is small amount of potassium held in exchangeable form (available K). The available potassium in soil samples was estimated by flame photometric method. Here, about 25 ml of 1N ammonium acetate was added to a 150 ml conical flask containing 5g of air-dried soil sample and was shaken for 5 minutes mechanically after which it was filtered instantly through a dry grade-1 filter paper. Then, 25 ml of distilled water was added to 5ml of the filtrate. The diluted extract was atomized to a flame photometer to note the quantity of potassium in soil samples (mg/L).

3.5.7 DETERMINATION OF AVAILABLE PHOSPHORUS

About 10g of air-dried soil sample was taken in a 500 ml conical flask and 200 ml of 0.002N H_2SO_4 was added. The suspension was shaken for about half an hour and filtered through Whatman No. 50 filter paper to get a clear solution. Then, 2 ml of ammonium molybdate solution and 5 drops of stannous chloride reagent was added to 50 ml of the extract to develop a blue colour. The intensity of the blue colour was measured using spectrophotometer at 660 nm. A standard curve was prepared with potassium hydrogen orthophosphate solutions in the

range of 0.0 to 10 mg dm-3 following the same procedure. Then, the available phosphorus was calculated by:

$$P\ mg/kg = \frac{mg\ P/dm3\ in\ soil\ extract}{S \times v} \times V$$

Where,

V = total volume of the soil extract prepared (200 ml)

S = weight of soil taken in gram

v = volume of the aliquot taken for analysis (50ml)

3.5.8 DETERMINATION OF EXCHANGEABLE BASES

About 30ml of 1N NH_4OAc was added into 5g of air-dried soil, and this was shaken on a mechanical shaker for 2 hours. The suspension was centrifuged at 2000rpm for 10mins, the clear supernatant was carefully decanted into a 100ml volumetric flask. Another 30ml of NH_4OAc solution was added and shaken for 30mins, centrifuged and the supernatant was transferred into the same volumetric flask. The solution was made up to mark by adding more NH_4OAc solution. Then, Na was determined using a flame photometer while Mg and Ca was determined on an atomic absorption spectrophotometer.

3.5.9 DETERMINATION OF EXCHANGEABLE ACIDS

About 5g of air-dried soil was weighed and transferred into a 50ml centrifuge tube. Then, 30ml of 1N potassium Chloride was added to each centrifuge tube and covered tightly with a rubber stopper. The centrifuge tube and its contents were shaken intermittently for one hour using a silent shake. Thereafter, it centrifuged at 2000rpm for 15minutes. Then a clear supernatant was carefully transferred into a volumetric flask and titrated with 1N HCl using 3 drops of

Bromocresolgren indicator. The colour change was from initial deep yellow to green. After that, exchangeable acidity was calculated using the formula:

$$\text{Exchangeable acidity (Meq/100g)} = (T - B) \times E \times \frac{100}{A} \times \frac{N}{W}$$

Where:

T = ml of NaOH sample

E = Extract volume

A = Aliquot volume taken

W = weight of soil used

N = Normality of base

3.5.10 DETERMINATION OF TOTAL HYDROCARBON CONTENT (THC) IN POLLUTED SOIL

The total hydrocarbon content (THC) in the polluted soil samples was determined at 2 weeks intervals for each polluted group using a modified method described by Akpan and Usuah (2014). About 10g of soil samples was measured into a 50 ml flask and the hydrocarbon content in oil polluted soil was extracted using 10 ml of n-hexane. The mixture was shaken vigorously on a magnetic stirrer for 30 minutes and allowed to stand for 10 minutes until the n-hexane extract separate the oil completely from the soil sample. The solution was then filtered using a Whatman filter paper and the filtrate diluted by taking 1 ml of the extract into 50 ml of n-hexane. The absorbance of this solution was measured in spectrophotometer at a wavelength of 480nm using n-hexane as blank. Total hydrocarbon content was then expressed in mg/kg.

3.6 PLANT GROWTH EXPERIMENT

Three viable seeds of each test plant (maize, onion and fluted pumpkin) were planted in each of the labelled perforated bag and kept under a shade away from excessive sunshine and rainfall

while being watered every 48 hours. The growth characteristics including plant height, number of leaves and leaf area were calculated for each experimental group on fortnight basis for a period of 28 days after exposure to crude oil (Odjegba & Sadiq, 2002; Ogbulie *et al*., 2014; Udebuani *et al.*, 2017).

3.6.1 Plant Height (cm)

Plant height for each test plant, was measured using a meter rule. Measurement was from the soil surface to the tip of the plant for *Allium cepa* and *Telfaria occidentalis* but for *Zea mays*, it was from the soil surface to the highest point of the arch of the uppermost leaf.

3.6.2 Number of leaves

The number of leaves on each test plant was counted on fortnight basis for each plant stand and the average number of leaves were calculated.

3.6.3 Leaf Area (cm^3)

The leaf area for each test plant was measured by multiplying the length and the width of the leaf except for maize leaf that the area was multiplied with the co-factor 0.75 as described by Udebuani *et al.* (2017).

3.7 DETERMINATION OF PROXIMATE COMPONENTS OF TEST PLANTS

The ground samples of the test plants were examined for moisture content, crude protein, crude fibre, crude fat, ash content and carbohydrate using the standard methods described by (AOAC, 1990). This analysis was carried out for all experimental groups at the end of the 28 days period based on pollution time.

3.7.1 DETERMINATION OF MOISTURE CONTENT

This was carried out by gravimetric method. About 5g of the sample was weighed into a previously weighed moisture can and dried in an oven at 105°C for 3 hours. Then, it was cooled in a desiccator, weighed, and returned to the oven for further drying. The drying, cooling and weighing was repeated at hourly interval until a constant weight is obtained. The weight of moisture lost was calculated by this expression:

$$\text{Moisture Content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where,

W_1 = weight of empty moisture can

W_2 = weight of empty can + sample before drying

W_3 = weight of can + sample dried to constant weight

3.7.2 DETERMINATION OF PROTEIN

Here, the Kjeldahl method was used. The total nitrogen was determined and multiplied with factor 6.25 to obtain protein content. Sample (0.5g) was mixed with 10ml of concentrated H_2SO_4 in a digestion flask, together with a tablet of selenium catalysts before heating under a fume cupboard till a clear solution is obtained. The digest was diluted to 100ml in a volumetric flask and 10ml of the digest was mixed with equal volume of 45% NaOH solution in Kjeldahl distillation apparatus. Then, the mixture was distilled into 10ml of 40% boric acid containing 3 drops of mixed indicator (bromo cressol green/ methyl red). A total of 50 ml distillate was collected and titrated against 0.02N EDTA from green to deep red end point. A reagent blank was also digested, distilled and titrated. Thus, the nitrogen/ protein content was calculated using this formula:

1 ml of 1N H_2SO_4 = 14mg

Protein (%) = N_2 (%) × 6.25

$$N2\ (\%) = \frac{100}{W} \times \frac{Nx14}{1000} \times \frac{Vt}{Va} \times T.B$$

Where,

W = weight of sample (0.5g)

N = normality of to grant (0.02N H2SO4)

Vt = total digest volume (100 ml)

Va = volume of digest analysed (10 ml)

T = sample titre value

B = blank titre value

3.7.3 DETERMINATION OF TOTAL ASH CONTENT

This was done by the furnace incineration gravimetric method. The processed sample (5g) was measured into an already weighed porcelain crucible and allowed to burn to ashes in a muffle furnace at 550°C, cooled in desiccator, weighed, and calculated thus:

$$Ash\ (\%) = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_1 = weight of empty crucible

W_2 = weight of crucible + ash

3.7.4 DETERMINATION OF CRUDE FIBRE

About 5g ground sample was boiled in 150ml of 1.25% H_2SO_4 solution for 30 minutes under reflux and the boiled sample was washed in several portions of hot water using a two-fold cloth to trap the particles. After which the boiling was repeated in 150ml of NaOH for another 30 minutes under same condition. After washing severally with hot water, the sample was allowed to drain dry before being transferred quantitatively to a weighed crucible where it was oven-dried at 105°C to obtain a constant weight. Thereafter, it was transferred to a muffle furnace where it was burnt to ashes. The calculation was done thus:

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_2 = weight of crucible + sample after washing, boiling and drying

W_3 = weight of crucible + sample of ash

3.7.5 DETERMINATION OF CRUDE FAT

This was determined by solvent extraction gravimetric method. Here, 5g of sample was wrapped in a Whatman filter paper and put in a thimble, which was put in a sohxlet reflux flask and mounted into a weighted extraction flask containing 200 ml of petroleum ether. The upper of the reflux flask was connected to a water condenser. The petroleum ether was heated, boiled, vaporized and condensed into the filled reflux flask. Soon, the sample in the thimble was covered with solvent till the reflux flask fills up and siphons over, carrying its oil extract down to the boiling flask. This process was allowed to repeat severally for 4 hours before the defatted sample was removed, the solvent was recovered, and the oil extract left in the flask. The flask containing the oil extract was dried in the oven at 60°C for 30 minutes to remove any residual solvent, cooled in desiccator, weighed, and then analysed thus:

$$\text{Fat (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_1 = weight (g) of empty extraction flask

W_2 = weight of flask + oil (fat) extract

3.7.6 DETERMINATION OF CARBOHYDRATE

Carbohydrate was determined commonly by weight difference.

Carbohydrate = [100 – (% weight of fibre + % weight of ash + % weight of fat + % weight of protein)].

3.8 DETERMINATION OF CHLOROPHYLL CONTENT IN TEST PLANTS

Chlorophyll content of test plants was estimated at 14 days intervals for each experimental group through spectrophotometry of leaf samples prepared by 80% acetone extraction as described by Liang *et al.* (2017). The leaves were incubated at room temperature in a 1.5-mL tube with 1mL 80% acetone solution for at least 24 hours and were centrifuged for 5 minutes. The absorbance of the supernatant was measured at wavelengths 646 and 663 nm (A646 and A663) using a double-beam spectrophotometer. Samples having absorbance greater than one was diluted by half with 80% acetone and re-evaluated. Chlorophyll concentration was estimated with the following equation:

$$\text{Chlorophyll a } (\mu g/mL) = -1.93A646 + 11.93A663$$

$$\text{Chlorophyll b } (\mu g/mL) = 20.36A646 - 5.50A663$$

$$\text{Total chlorophyll } (\mu g/mL) = 6.43A663 + 18.43A646$$

3.9 DETERMINATION OF TOTAL HYDROCARBON CONTENT (THC) IN POLLUTED TEST PLANTS

This was determined using UV-vis spectrophotometric method as described by Numbere (2019). The fresh leaves of the polluted test plants were oven dried at 60°C for 24 hours to get rid of the moisture. The dried samples were crushed and 2g of each was weighed into a glass beaker and 2ml of n-hexane added. The samples were homogenized with the use of a glass rod by stirring for about 30 minutes. Then, the samples were filtered through a glass funnel packed with cotton wool, silica gel and anhydrous sodium sulphate. After the filtration, 10ml of the filtered organic extract was transferred into 10ml sample covet and analysed with spectrophotometer at (wavelength 610nm) using n-hexane as blank. Total hydrocarbon content was expressed in mg/kg.

3.10 ANATOMICAL ANALYSIS TO DETERMINE CRUDE OIL TOXICITY ON TEST PLANTS

Sectioning of leaves of test plants was done at the end of the experiment according to the procedures outlined by (Ilodibia *et al*., 2015; Moghanm *et al.,* 2020) with some modifications. The samples (0.5 cm length) were dipped in formalin-acetic-alcohol solution (2:1:1v/v) for 1 week, in order to impede autolysis and fix the cells. After which, they were rinsed with water and dehydrated by passing through ascending series of ethanol (60%, 70%, 80% till absolute concentration) for 30 minutes each. Then, the samples were further subjected to two changes of chloroform for 30 minutes each and embedded in molten paraffin wax at 50°C for another 30 minutes. Subsequently, a sledge microtome was used to section the leaves into slices of 5 micrometres thick before staining with a primary dye; safranin and counter-staining with methylene blue. Canada balsam was used to mount the specimens on slides. Each slide was

carefully covered with a 22mm x 22mm coverslip. Hence, the mounted specimens were observed under a light microscope and photomicrographs were taken.

3.11 GENOTOXIC EVALUATION OF TEST PLANTS

3.11.1 DETERMINATION OF DNA DAMAGE USING ALKALINE COMET ASSAY

The standard protocol was performed according to (Gichner *et al.,* 2004) with some modifications as described by AL-Huqail & Abdelhaliem (2015) and Pourrut *et al.* (2015). All operations were conducted under inactinic red light to avoid light-induced damage. All the slides for the experiment were soaked in absolute ethanol for one week and cleaned with cotton wool before the day of experiment. After 4 weeks of exposure, the plant leaves in each experimental group were harvested and carefully rinsed thrice with water prior to nuclei extraction. Then, the excised organs (150mg) were placed in a 60-mm petri dish kept on ice and spread with 1.5mL of cold 400mM Tris-HCl buffer, pH 7.5. The studied organs were vigorously chopped into a large number of smaller fragments using a fresh razor blade. The plate was kept tilted on ice for about 10 minutes and the released nuclei in the solution was properly collected by filtering through a 20 µm nylon cloth, while the cell debris were discarded. Then, the sterilized glass slides were dipped into a container having 1% normal melting point agarose and left to dry overnight. About 50µl of 1% low melting point agarose [prepared with PBS and stained with 40 µl of ethidium bromide (20 µg/mL)] was mixed with 50µl of the nuclear suspension and then 100µl of the mixture was placed on the pre-coated slides and covered with cover slips. The slides were placed on ice for 5 minutes to ensure proper gelling. Afterwards, the coverslips were removed, and the slides placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (200mM EDTA and 10N NaOH, pH > 13). The nuclei were incubated for 20 minutes to allow the DNA to unwind prior to electrophoresis at 0.72 V/cm (75V, 300 mA) for 10 minutes at 4°C. Then, the slides were neutralized with 3 washes of 400mM Tris-HCl buffer, pH 7.5 for 5 minutes each

and another drop of Trivegen silver staining was added before covering with slips. Lastly, the extent of DNA damage for 50 randomly chosen cells in each slide was analysed and manually assessed by visual scoring using a light microscope. Overall damage score was calculated with this formula:

(Average cells in class 0 x 0) + (Average cells in class 1 x 1) + (Average cells in class 2 x 2) + (Average cells in class 3 x 3) + (Average cells in class 4 x 4)

3.12 STATISTICAL ANALYSIS

All data samples from each experimental group in triplicates were analysed statistically using Microsoft Excel 2013 and Minitab 2017 one-way analysis of variance (ANOVA). The means were separated with Dunnett's multiple and Tukey's pair wise tests. Results were presented in mean ± standard deviation (SD).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 PHYSICOCHEMICAL PROPERTIES OF CRUDE OIL POLLUTED AND UNPOLLUTED SOIL.

The physicochemical properties of the unpolluted and polluted soil samples (UPS and PS) analysed on the zero day of crude oil application are shown in Table 4.1a. The pH value (7.86), organic carbon (OC = 1.31%), organic matter (OM = 2.25%), calcium (Ca = 3.72cmol/kg), magnesium (Mg = 0.950 cmol/kg), sodium (Na = 0.147cmol/kg) and (silt = 3.38%) contents were significantly higher at ($P<0.05$) in the treated soil sample than the untreated sample (pH 6.25, OC 0.31%, OM 0.45%, Ca 2.83 cmol/kg, Mg 0.750 cmol/kg, Na 0.138 cmol/kg and silt 2.37% respectively). However, the values for nitrogen, phosphorous, potassium, exchangeable acids, sand, and clay increased in the untreated soil sample compared to the treated sample. No significant differences were recorded at (5% probability level) for sodium (UPS 0.138 ± 0.003^{a}, PS 0.147 ± 0.009^{a}) and clay (UPS 3.25 ± 0.06^{a}, PS 3.19 ± 0.02^{a}) respectively.

The result of soil physicochemical analysis obtained after 28 days of pollution, for group A - polluted before planting are presented in Table 4.1b, with soil pH ranges of 6.17 to 6.37 for the control samples and 6.90 to 7.42 for the polluted samples. Crude oil contamination significantly ($P<0.05$) increased the mean values of organic carbon, organic matter, calcium, magnesium, sodium, nitrogen, clay and silt in the treated soil samples of each plant (maize – *Zea mays*, onion – *Allium cepa* and fluted pumpkin – *Telfairia occidentalis*) used in our study; except for maize's polluted soil sample that showed significant ($P<0.05$) decrease in sodium content compared to its unpolluted sample. Also, the nitrogen and magnesium contents of the oil contaminated soil samples harbouring grown fluted pumpkin were insignificantly different

from their control groups. Nevertheless, the mean values of exchangeable acids, potassium, sand and phosphorous recorded in the control samples were higher than the polluted groups.

More observations from this study, revealed soil pH ranges of 6.25 to 6.58 for the control groups and 6.85 to 7.43 for the treated groups after 28 days of crude oil exposure as shown in Table 4.1c for group B - polluted 2 weeks after planting (2 WAP). Organic matter, organic carbon, nitrogen, calcium, magnesium, sodium, clay, and silt contents statistically increased in the polluted samples compared to the unpolluted samples while the mean values of potassium, phosphorus, sand, and exchangeable acids increased in the unpolluted soil samples. Notwithstanding, the polluted soil exchangeable acids values showed no significant difference with their controls.

4.1.2 TOTAL HYDROCARBON CONTENT OF CRUDE OIL POLLUTED AND UNPOLLUTED SOIL.

Findings from this research as expressed in Table 4.2, indicated a huge statistical difference ($P<0.05$) between the total hydrocarbon content values of the unpolluted and polluted soil subjected to this analysis on day zero (UPS = 96.38; PS = 1082.80). However, results obtained on day 14 showed significant difference between the unpolluted soil (UPS) and all the polluted soils (UPB, UPA, OPB, OPA, MPB, MPA and PS) while no significant difference was recorded amongst all the polluted soil. All polluted soils were significantly different from the unpolluted soil but, UPB soil had reduced hydrocarbon content value (381.00) when compared to the other polluted samples (PS = 883.14, UPA = 789.51, OPB = 695.90, OPA = 670.29, MPB = 820.66, MPA = 845.30). No significant difference was observed amongst PS, UPA, MPB and MPA. Also, OPB and OPA soils were insignificantly different from each other. Noticeably, the planted polluted soils (UPB, UPA, OPB, OPA, MPB and MPA) consistently showed a decline in their THC levels compared to the non-planted polluted soil (PS).

Table 4.1a: Physicochemical Properties of Polluted and Unpolluted Soils at Day Zero

TREATMENT (ml)	UPS (00)	PS (100)
pH	6.25±0.20[a]	7.86±0.05
OC (%)	0.31±0.02[a]	1.31±0.01
OM (%)	0.45±0.10[a]	2.25±0.04
N (%)	0.149±0.006[a]	0.126±0.008
P (ppm P/g)	6.85±0.07[a]	3.51±0.03
Ca (cmol/kg)	2.83±0.09[a]	3.72±0.04
Mg (cmol/kg)	0.750±0.062[a]	0.950±0.036
EA (cmol/kg)	0.333±0.004[a]	0.282±0.027
Na (cmol/kg)	0.138±0.003[a]	0.147±0.009[a]
K (cmol/kg)	0.022±0.003[a]	0.003±0.001
Sand (%)	94.51±0.02[a]	93.53±0.02
Clay (%)	3.25±0.06[a]	3.19±0.02[a]
Silt (%)	2.37±0.03[a]	3.38±0.02

Legend: UPS = Unpolluted soil, PS = Polluted soil. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level mean across the rows at ($p \leq 0.05$)

Table 4.1b: Physicochemical Properties of Soil Samples Polluted before Planting at Day 28

A – SOIL POLLUTED BEFORE PLANTING						
TREATMENT (ml)	**UC (00)**	**UPB (100)**	**OC (00)**	**OPB (100)**	**MC (00)**	**MPB (100)**
pH	6.20±0.14[a]	6.99±0.18	6.37±0.18[a]	7.42±0.06	6.17±0.07[a]	6.90±0.12
OC (%)	0.40±0.01[a]	1.19±0.01	0.38±0.00[a]	1.15±0.24	0.32±0.01[a]	1.02±0.01
OM (%)	0.69±0.00[a]	2.02±0.00	0.65±0.01[a]	1.64±0.11	0.60±0.04[a]	1.77±0.02
N (%)	0.053±0.005[a]	0.060±0.002[a]	0.117±0.008[a]	0.140±0.003	0.063±0.007[a]	0.122±0.005
P (ppm P/g)	5.17±0.08[a]	4.72±0.05	5.91±0.31[a]	5.00±0.05	5.20±0.08[a]	4.75±0.09
Ca (cmol/kg)	1.50±0.10[a]	2.17±0.15	1.43±0.06[a]	2.07±0.25	1.50±0.20[a]	2.30±0.20
Mg (cmol/kg)	0.630±0.027[a]	0.657±0.021[a]	0.617±0.023[a]	0.700±0.027	0.620±0.036[a]	0.887±0.025
EA (cmol/kg)	0.306±0.004[a]	0.288±0.004	0.272±0.011[a]	0.237±0.014	0.230±0.019[a]	0.189±0.024
Na (cmol/kg)	0.130±0.002[a]	0.148±0.004	0.141±0.004[a]	0.160±0.015	0.134±0.006[a]	0.121±0.006
K (cmol/kg)	0.005±0.002[a]	0.002±0.001	0.013±0.004[a]	0.004±0.002	0.007±0.001[a]	0.003±0.001
Sand (%)	93.44±0.17[a]	92.50±0.03	92.87±0.17[a]	92.21±0.07	92.68±0.07[a]	91.85±0.46
Clay (%)	4.23±0.15[a]	4.53±0.06	5.02±0.02[a]	5.23±0.04	4.91±0.12[a]	5.11±0.04
Silt (%)	2.32±0.04[a]	2.62±0.06	2.35±0.07[a]	2.45±0.10[a]	2.32±0.04[a]	2.61±0.04

Legend: MC = Maize Control, MPB = Maize Polluted before Planting, OC = Onion Control, OPB = Onion Polluted before Planting, UC = Fluted pumpkin Control, UPB = Fluted pumpkin Polluted before Planting. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the rows of each plant group at ($p \leq 0.05$)

Table 4.1c: Physicochemical Properties of Soil Polluted 2 Weeks after Planting at Day 28

B – SOIL POLLUTED 2 WEEKS AFTER PLANTING						
TREATMENT (ml)	**UC (00)**	**UPA (100)**	**OC (00)**	**OPA (100)**	**MC (00)**	**MPA (100)**
pH	6.35±0.09[a]	7.43±0.09	6.58±0.12[a]	7.25±0.11	6.25±0.13[a]	6.85±0.11
OC (%)	0.35±0.03[a]	1.43±0.01	0.35±0.01[a]	1.01±0.17	0.31±0.01[a]	0.95±0.18
OM (%)	0.68±0.02[a]	1.71±0.65	0.60±0.06[a]	1.61±0.00	0.59±0.02[a]	1.39±0.03
N (%)	0.062±0.003[a]	0.087±0.004	0.107±0.005[a]	0.125±0.002	0.056±0.006[a]	0.111±0.005
P (ppm P/g)	5.14±0.05[a]	4.51±0.03	6.08±0.04[a]	4.97±0.08	5.63±0.12[a]	4.63±0.08
Ca (cmol/kg)	1.13±0.06[a]	2.30±0.10	1.30±0.27[a]	2.20±0.20	1.27±0.15[a]	1.90±0.20
Mg (cmol/kg)	0.513±0.025[a]	0.717±0.035	0.580±0.027[a]	0.810±0.027	0.593±0.038[a]	0.880±0.036
EA (cmol/kg)	0.299±0.004[a]	0.293±0.006[a]	0.261±0.020[a]	0.237±0.013[a]	0.220±0.013[a]	0.199±0.006[a]
Na (cmol/kg)	0.119±0.004[a]	0.140±0.005	0.131±0.006[a]	0.145±0.004	0.127±0.003[a]	0.142±0.005
K (cmol/kg)	0.005±0.001[a]	0.002±0.001	0.007±0.001[a]	0.002±0.002	0.008±0.001[a]	0.005±0.001
Sand (%)	93.27±0.13[a]	92.31±0.19	92.83±0.23[a]	92.01±0.23	92.57±0.05[a]	91.94±0.18
Clay (%)	4.07±0.06[a]	4.60±0.10	4.96±0.08[a]	5.18±0.04	4.84±0.06[a]	5.18±0.03
Silt (%)	2.27±0.02[a]	2.49±0.04	2.26±0.05[a]	2.55±0.09	2.29±0.07[a]	2.64±0.04

Legend: MC = Maize Control, MPA = Maize Polluted 2 Weeks after Planting, OC = Onion Control, OPA = Onion Polluted 2 Weeks after Planting, UC = Fluted pumpkin Control, UPA =Fluted pumpkin Polluted 2 Weeks after Planting. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the rows of each plant group at ($p \leq 0.05$).

Table 4.2: Total Hydrocarbon Content (THC) for Polluted and Unpolluted Soils at Day Zero

SOIL	TOTAL HYDROCARBON CONTENT OF SOIL (mg/kg) DAY 0	DAY 14	DAY 28
UPS	96.38±0.85[b]	77.21±0.84[c]	57.21±0.99[e]
PS	1082.80±15.02[a]	963.44±1.66[a]	883.14±11.12[a]
UPB	nil	726.10±131.70[b]	381.00±80.00[d]
UPA	nil	836.39±6.91[ab]	789.51±0.93[ab]
OPB	nil	738.50±64.30[b]	695.90±81.80[bc]
OPA	nil	717.67±0.45[b]	670.29±1.42[c]
MPB	nil	891.23±1.16[a]	820.66±1.75[a]
MPA	nil	913.00±21.80[a]	845.30±20.60[a]

Legend: UPS = Unpolluted soil, PS = Polluted soil, MPB = Maize Polluted before Planting, MPA = Maize Polluted 2 Weeks after Planting, OPB = Onion Polluted before Planting, OPA = Onion Polluted 2 Weeks after Planting, UPB = Fluted pumpkin Polluted before Planting. UPA =Fluted pumpkin Polluted 2 Weeks after Planting. Values are mean ±standard deviation from triplicates. Means that do not share a letter across the columns are significantly different at ($p \leq 0.05$).

4.1.3 GROWTH PARAMETERS OF TEST PLANTS EXPOSED TO CRUDE OIL POLLUTED AND UNPOLLUTED SOILS

The plant height, leaf area and leaf number as clearly depicted in Tables 4.3, 4.4 and 4.5 respectively; where the plant growth parameters were measured at 2 weeks intervals for both experimental groups (A - polluted before planting and B - polluted 2 weeks after planting), beginning from their pollution time till 28 days after each group's exposure to crude oil. Results obtained showed statistical ($P<0.05$) decrease in plant height, leaf area and leaf number for both polluted groups as compared to their control plants at day 14 and day 28 respectively; with the exception of UPB that showed no significant decrease ($P<0.05$) in their values for plant height at both day 14 and 28, leaf area at day 14 only and leaf number at days 14 and 28 respectively. The control plants portrayed abundant growth than the polluted plants. Though, UPB portrayed mildly flourishing growth when compared to its control.

4.1.4 PROXIMATE COMPOSITION OF TEST PLANTS EXPOSED AND UNEXPOSED TO CRUDE OIL SOILS.

The proximate compositions of crude oil polluted and unpolluted test plants used for this research is shown in Table 4.6a and Table 4.6b for experimental groups A and B. It was observed from the control samples, that onion leaf recorded the highest percentage of moisture (86.99%) than maize (71.87%) and fluted pumpkin (66.91%) leaves. However, fluted pumpkin leaf had the highest rates of crude protein (4.35%), ash content (5.39%), fat (1.86%) and crude fibre (3.61%) compared to onion and maize leaves. Then, the biggest value of carbohydrate was obtained in maize (21.42%), followed by fluted pumpkin (17.88%) while onion recorded the least (3.40%). Furthermore, the results obtained from group A disclosed that the proximate values for all polluted test plants were significantly ($P<0.05$) lower than that of the unpolluted plants, but UPB showed no significant decrease in moisture content (65.97 ± 0.50^{a}) and crude protein content (4.19 ± 0.06^{a}) compared to UC (MC 66.91 ± 1.57^{a} and P 4.35 ± 0.04^{a}). Precisely,

group B recorded significant differences at 5% probability level in all the polluted plants when compared with the control plants.

Table 4.3: Plant Height of Plants in Groups A and B

A - POLLUTED BEFORE PLANTING			B - POLLUTED 2 WEEKS AFTER PLANTING		
TREATMENT (ml)	**DAY 14**	**DAY 28**	**TREATMENT (ml)**	**DAY 14**	**DAY 28**
UC (00)	38.60±8.14[a]	53.47±13.66[a]	UC (00)	53.47±13.66[a]	68.00±15.37[a]
UPB (100)	22.27±16.57[a]	31.23±13.32[a]	UPA (100)	12.40±4.76	0.00±0.00
OC (00)	33.50±3.10[a]	42.57±4.76[a]	OC (00)	42.57±4.76[a]	50.70±4.63[a]
OPB (100)	10.23±8.90	18.20±14.26	OPA (100)	17.67±4.74	10.77±2.40
MC (00)	23.57±4.70[a]	31.77±1.66[a]	MC (00)	31.77±1.66[a]	43.17±1.47[a]
MPB (100)	7.40±2.01	15.00±2.59	MPA (100)	3.23±5.60	2.00±3.46

Legend: MC = Maize Control, MPB = Maize Polluted before Planting, MPA = Maize Polluted 2 Weeks after Planting, OC = Onion Control, OPB = Onion Polluted before Planting, OPA = Onion Polluted 2 Weeks after Planting, UC = Fluted pumpkin Control, UPB = Fluted pumpkin Polluted before Planting. UPA =Fluted pumpkin Polluted 2 Weeks after Planting. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the columns of each plant group at ($p \leq 0.05$).

Table 4.4: Leaf Area of Plants in Groups A and B

A - POLLUTED BEFORE PLANTING			B - POLLUTED 2 WEEKS AFTER PLANTING		
TREATMENT (ml)	**DAY 14**	**DAY 28**	**TREATMENT (ml)**	**DAY 14**	**DAY 28**
UC (00)	42.40±5.85[a]	96.30±8.66[a]	UC (00)	96.30±8.66[a]	112.30±7.63[a]
UPB (100)	24.53±13.87[a]	35.80±10.91	UPA (100)	15.47±3.15	0.00±0.00
OC (00)	31.57±2.76[a]	42.93±7.43[a]	OC (00)	42.93±7.43[a]	50.40±4.12[a]
OPB (100)	11.07±9.69	15.90±9.75	OPA (100)	13.50±1.80	11.20±1.57
MC (00)	72.57±6.12[a]	100.30±12.95[a]	MC (00)	100.30±12.95[a]	124.50±3.97[a]
MPB (100)	20.30±2.44	36.60±8.12	MPA (100)	7.70±13.34	5.00±8.66

Legend: MC = Maize Control, MPB = Maize Polluted before Planting, MPA = Maize Polluted 2 Weeks after Planting, OC = Onion Control, OPB = Onion Polluted before Planting, OPA = Onion Polluted 2 Weeks after Planting, UC = Fluted pumpkin Control, UPB = Fluted pumpkin Polluted before Planting. UPA =Fluted pumpkin Polluted 2 Weeks after Planting. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the columns of each plant group at ($p \leq 0.05$).

Table 4.5: Leaf Number of Plants in Groups A and B

A - POLLUTED BEFORE PLANTING			B - POLLUTED 2 WEEKS AFTER PLANTING		
TREATMENT (ml)	**DAY 14**	**DAY 28**	**TREATMENT (ml)**	**DAY 14**	**DAY 28**
UC (00)	10.00±1.73[a]	13.67±3.21[a]	UC (00)	13.67±3.21[a]	16.00±3.61[a]
UPB (100)	6.67±2.08[a]	8.33±3.06[a]	UPA (100)	4.67±0.58	0.00±0.00
OC (00)	22.67±1.53[a]	24.00±1.73[a]	OC (00)	24.00±1.73[a]	25.00±1.73[a]
OPB (100)	7.00±6.24	11.33±6.66	OPA (100)	12.00±3.46	4.67±0.58
MC (00)	5.00±0.00[a]	6.00±0.00[a]	MC (00)	6.00±0.00[a]	7.67±0.58[a]
MPB (100)	2.00±0.00	3.33±0.58	MPA (100)	1.67±2.89	0.67±1.16

Legend: MC = Maize Control, MPB = Maize Polluted before Planting, MPA = Maize Polluted 2 Weeks after Planting, OC = Onion Control, OPB = Onion Polluted before Planting, OPA = Onion Polluted 2 Weeks after Planting, UC = Fluted pumpkin Control, UPB = Fluted pumpkin Polluted before Planting. UPA =Fluted pumpkin Polluted 2 Weeks after Planting. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the columns of each plant group at ($p \leq 0.05$).

Table 4.6a: Proximate Composition of Test Plants Polluted before Planting at Day 28

A - POLLUTED BEFORE PLANTING						
TREATMENT (ml)	**MCT (%)**	**AC (%)**	**P(%)**	**FB(%)**	**FT(%)**	**C(%)**
UC (00)	66.91±1.57[a]	5.39±0.41[a]	4.35±0.04[a]	3.61±0.02[a]	1.86±0.03[a]	17.88±1.99[a]
UPB (100)	65.97±0.50[a]	4.43±0.22	4.19±0.06[a]	3.31±0.09	1.07±0.04	11.89±0.58
OC (00)	86.99±0.08[a]	2.67±0.04[a]	2.87±0.09[a]	2.59±0.04[a]	1.47±0.07[a]	3.40±0.08[a]
OPB (100)	70.22±0.24	1.26±0.23	0.57±0.05	1.40±0.08	0.96±0.14	0.52±0.11
MC (00)	71.87±0.82[a]	1.09±0.04[a]	1.76±0.09[a]	2.43±0.02[a]	1.42±0.14[a]	21.42±0.63[a]
MPB (100)	57.07±0.39	0.65±0.09	0.59±0.12	1.76±0.16	0.64±0.13	9.66±0.05

Legend: MC = Maize Control, MPB = Maize Polluted before Planting, OC = Onion Control, OPB = Onion Polluted before Planting, UC = Fluted pumpkin Control, UPB = Fluted pumpkin Polluted before Planting, MCT = Moisture Content, AC = Ash Content, P = crude Protein, FB = Crude Fibre, FT = Crude Fat, C = Carbohydrate. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the columns of each plant group at ($p \leq 0.05$)

Table 4.6b: Proximate Composition of Test Plants Polluted 2 Weeks after Planting at Day 28

B - POLLUTED 2 WEEKS AFTER PLANTING						
TREATMENT (ml)	**MCT (%)**	**AC (%)**	**P(%)**	**FB(%)**	**FT(%)**	**C(%)**
UC (00)	67.02±0.18[a]	5.52±0.37[a]	4.31±0.04[a]	3.90±0.05[a]	1.87±0.10[a]	17.36±0.55[a]
UPA (100)	20.87±0.13	2.87±0.07	0.76±0.07	2.27±0.04	0.79±0.06	6.17±0.27
OC (00)	86.38±0.25[a]	2.87±0.11[a]	2.92±0.05[a]	2.81±0.06[a]	1.52±0.07[a]	3.50±0.03[a]
OPA (100)	51.99±0.34	1.54±0.11	0.78±0.09	1.60±0.03	0.42±0.12	0.48±0.07
MC (00)	72.00±0.60[a]	0.97±0.10[a]	1.73±0.02[a]	2.60±0.03[a]	1.28±0.06[a]	21.42±0.59[a]
MPA (100)	34.94±0.20	0.49±0.04	0.28±0.03	1.50±0.04	0.35±0.05	6.10±0.03

Legend: MC = Maize Control, MPA = Maize Polluted 2 Weeks after Planting, OC = Onion Control, OPA = Onion Polluted 2 Weeks after Planting, UC = Fluted pumpkin Control, UPA =Fluted pumpkin Polluted 2 Weeks after Planting, MCT = Moisture Content, AC = Ash Content, P = crude Protein, FB = Crude Fibre, FT = Crude Fat, C = Carbohydrate. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the columns of each plant group at ($p \leq 0.05$

4.1.5 TOTAL CHLOROPHYLL CONTENT OF LEAVES OF TEST PLANTS EXPOSED AND UNEXPOSED TO CRUDE OIL SOIL.

Total chlorophyll content of plants in group A and group B was determined at day 14 and day 28 as shown in Table 4.7. Results obtained showed higher chlorophyll values for UC, UPB, OC, OPB and MC at day 28 compared to their values at day 14. Meanwhile, the total chlorophyll content values for MPB had no significant difference at days 14 and 28 respectively. For the control samples, UC had the highest total chlorophyll value, followed by MC and finally OC whereas the total chlorophyll values for the polluted samples were highest in UPB, then MPB and least in OPB. However, statistical differences were recorded between OC and OPB as well as MC and MPB at both day 14 and 28 but, no significant difference was observed between UC and UPB at days 14 and 28 respectively. All the control samples had higher chlorophyll values than their polluted counterparts on both days. Significant differences occurred between UC and UPA, OC and OPA as well as MC and MPA even though; total chlorophyll content values were not detected for UPA and MPA at day 28.

4.1.6 TOTAL HYDROCARBON CONTENT OF LEAVES OF TEST PLANTS EXPOSED AND UNEXPOSED TO CRUDE OIL SOIL.

As shown in Table 4.8, UPB had the highest total hydrocarbon content value of 14.47 mg/kg and 36.73 mg/kg for days 14 and 28, while MPB had the least value of 5.38 mg/kg at day 14 and MPA, the least total hydrocarbon content value of 7.76 mg/kg at day 28. However, there were no significant differences recorded in OPB, OPA, MPB and MPA total hydrocarbon content values at days 14 and 28. Similarly, the UPB and UPA total hydrocarbon content values were insignificantly different at day 14 but showed statistical difference ($P<0.05$) at day 28. Also, the THC values of UPB and UPA were significantly higher than the THC values of OPB, OPA, MPB and MPA at days 14 and 28 respectively.

Table 4.7: Total Chlorophyll Content of Plants in Group A at Day 14 and Day 28.

TOTAL CHLOROPHYLL CONTENT OF LEAVES (mg/100g)					
POLLUTED BEFORE PLANTING			**POLLUTED 2 WEEKS AFTER PLANTING**		
TREATMENT (ml)	**DAY 14**	**DAY 28**	**TREATMENT (ml)**	**DAY 14**	**DAY 28**
UC (00)	123.57±0.09[a]	164.02±0.02[a]	UC (00)	164.02±0.02[a]	189.17±0.24[a]
UPB (100)	97.40±44.8[a]	163.95±0.06[a]	UPA (100)	34.98±2.24	ND
OC (00)	57.96±0.41[a]	61.31±0.53[a]	OC (00)	61.31±0.53[a]	64.86±0.12[a]
OPB (100)	8.69±7.54	25.20±1.05	OPA (100)	24.88±0.37	13.07±0.06
MC (00)	101.07±0.19[a]	109.86±1.11[a]	MC (00)	109.86±1.11[a]	115.35±0.10[a]
MPB (100)	50.18±0.04	50.66±0.24	MPA (100)	5.63±9.75	ND

Legend: MC = Maize Control, MPB = Maize Polluted before Planting, MPA = Maize Polluted 2 Weeks after Planting, OC = Onion Control, OPB = Onion Polluted before Planting, OPA = Onion Polluted 2 Weeks after Planting, UC = Fluted pumpkin Control, UPB = Fluted pumpkin Polluted before Planting. UPA =Fluted pumpkin Polluted 2 Weeks after Planting, ND = Not Detected. Values are mean ±standard deviation from triplicates. Means not labeled with the letter A are significantly different from the control level means across the columns of each plant group at ($p \leq 0.05$).

Table 4.8: Total Hydrocarbon Content (THC) for Polluted Test Plants at Day 14 and 28.

TOTAL HYDROCARBON CONTENT OF LEAVES (mg/kg)		
PLANT	**DAY 14**	**DAY 28**
UPB	14.47±3.50[a]	36.73±2.75[a]
UPA	13.90±0.18[ab]	19.80±0.39[b]
OPB	5.41±4.69[c]	13.04±3.48[c]
OPA	7.65±0.19[bc]	10.62±0.23[cd]
MPB	5.38±0.86[c]	9.81±0.32[cd]
MPA	5.79±0.33[c]	7.76±0.42[d]

Legend: MPB = Maize Polluted before Planting, MPA = Maize Polluted 2 Weeks after Planting, OPB = Onion Polluted before Planting, OPA = Onion Polluted 2 Weeks after Planting, UPB = Fluted pumpkin Polluted before Planting, UPA = Fluted pumpkin Polluted 2 Weeks after Planting. Values are mean ±standard deviation from triplicates. Means that do not share a letter across the columns are significantly different at ($p \leq 0.05$).

4.1.7 ANATOMICAL EVALUATION OF THE LEAVES OF TEST PLANTS EXPOSED AND UNEXPOSED TO CRUDE OIL SOILS.

The cross-sectional leaves of the crude oil treated and untreated test plants (maize, onion and fluted pumpkin) were represented as photomicrographs in Plates 4.1, 4.2 and 4.3. Plate 4.1 compared the leaf structures of contaminated samples (maize polluted before planting - MPB and maize polluted 2 WAP - MPA) with an uncontaminated sample (maize control - MC). Observations from MC revealed the upper and lower epidermis of the leaf, the stoma, vascular bundles (xylem and phloem) with their vessels encircled by chloroplast-filled bundle sheath cells and layers of mesophyll cells. MPB showed enlarged vascular bundles and coalesced mesophyll cells while MPA exhibited total cell coalescence as well.

The sectioned leaves of onion polluted before planting (OPB) and onion polluted 2 WAP (OPA) were presented in Plate 4.2, together with the unpolluted plant (onion control - OC). The photomicrograph for OC showed the leaf epidermis, stoma, vascular bundle and parenchyma in normal condition whereas OPB revealed distorted cells. Then, the vascular bundles and leaf epidermis of OPA were shrivelled.

Fluted pumpkin leaves exposed to crude oil (fluted pumpkin polluted before planting – UPB and fluted pumpkin polluted 2 WAP - UPA) were displayed in Plate 4.3 with the untreated fluted pumpkin leaves (UC). Healthy forms of spongy parenchyma, vascular bundles and epidermis were observed in UC but, UPB unveiled enlarged vascular bundles and slightly enlarged spongy cells. However, deformed spongy parenchyma and shrivelled vascular bundles were discovered in the photomicrograph for UPA.

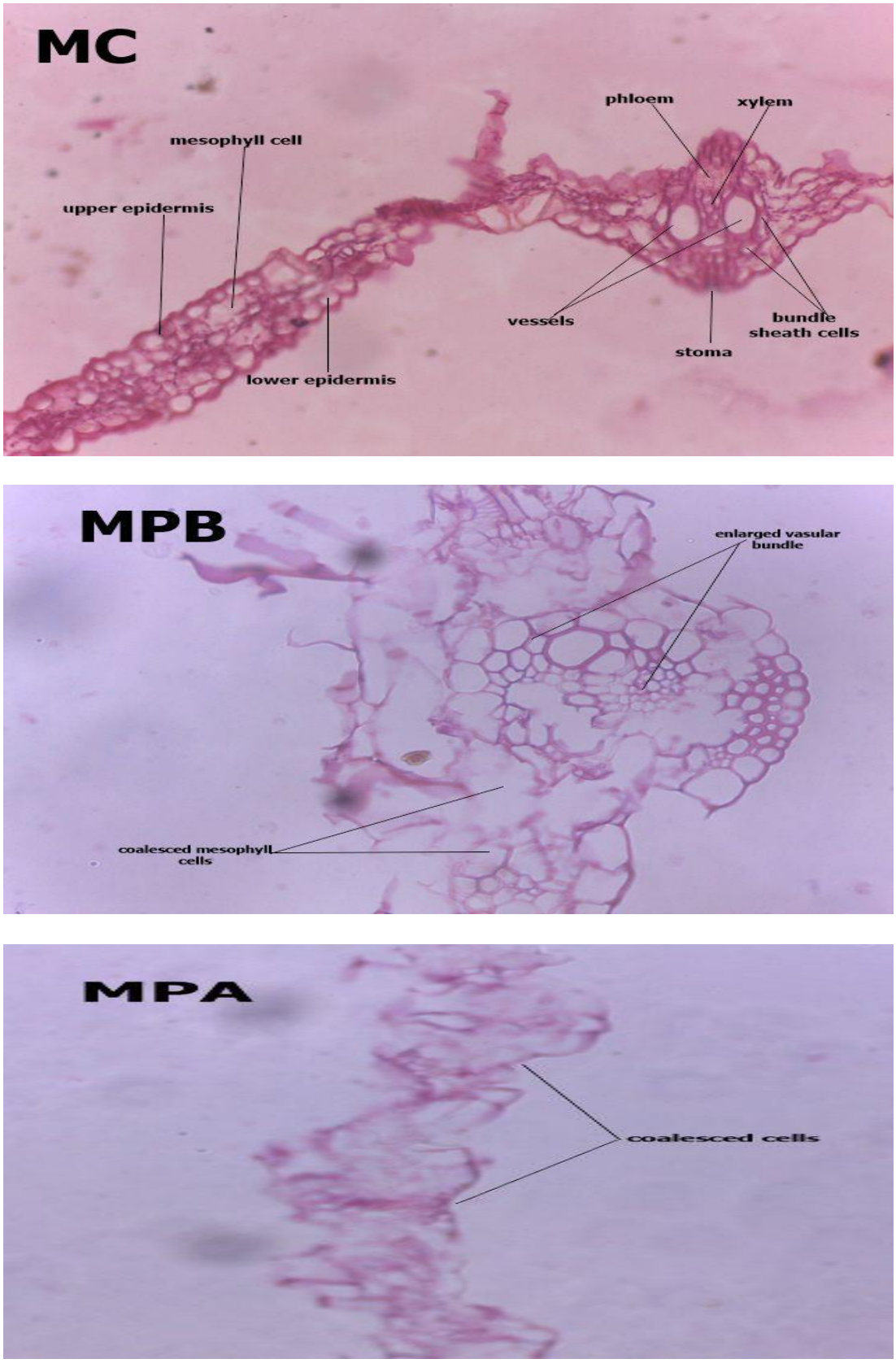


Plate 4.1: Photomicrographs of Polluted and Unpolluted Maize Leaves
LEGEND: MC – Maize Control (20x), MPB – Maize Polluted before Planting (40x), MPA – Maize Polluted 2 WAP (20x).

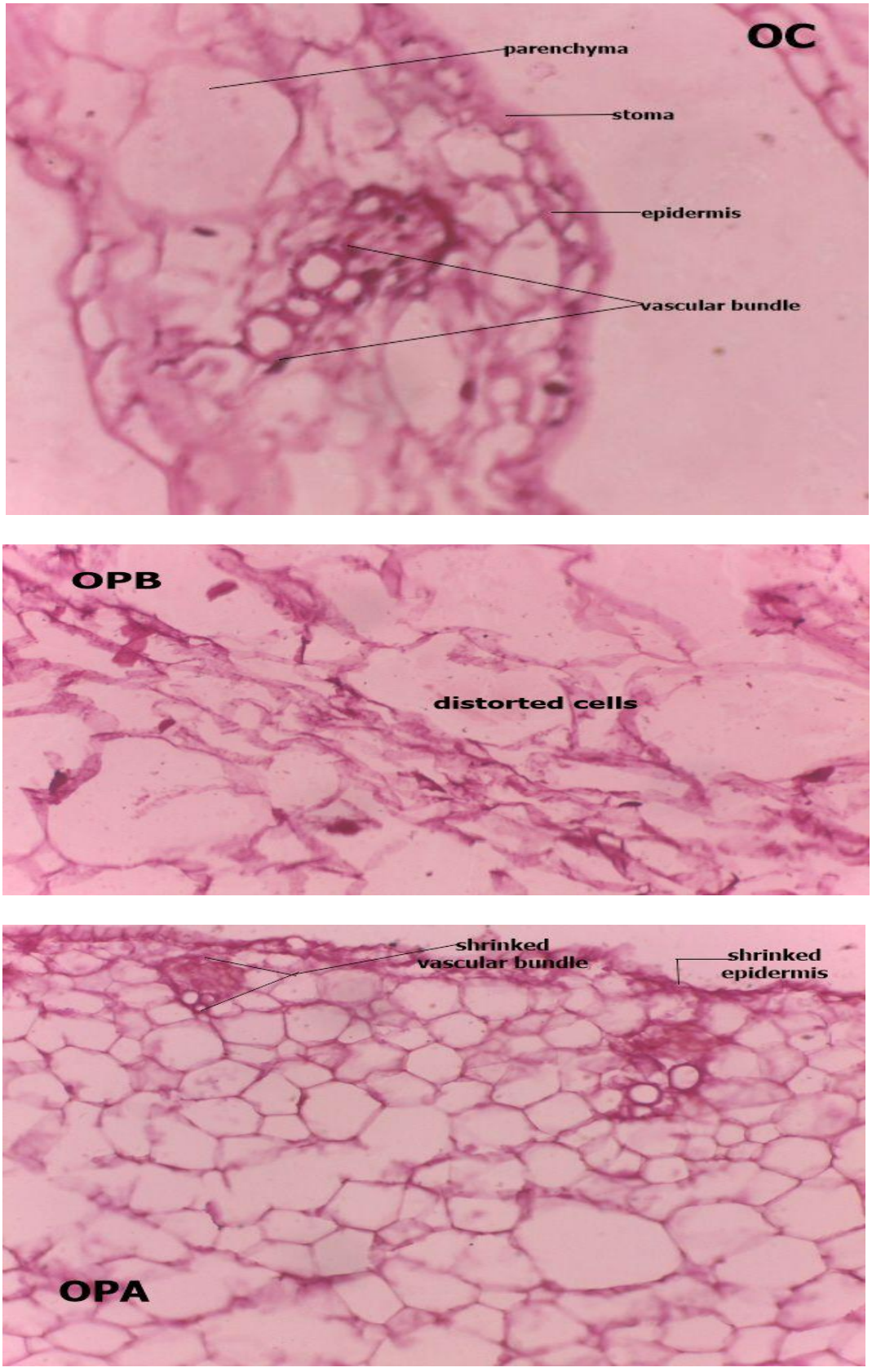
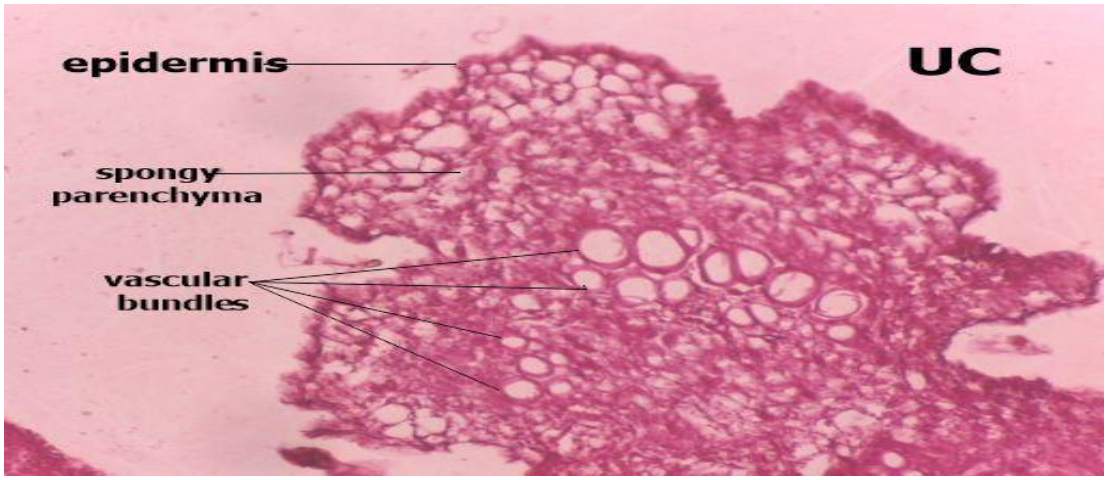
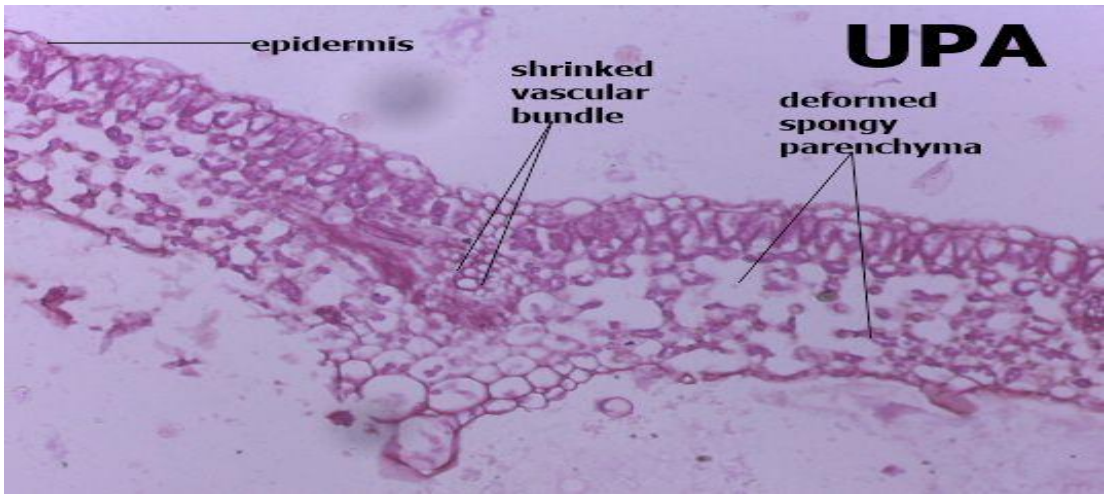


Plate 4.2: Photomicrographs of Polluted and Unpolluted Onion Leaves
LEGEND: OC – Onion Control (20x), OPB – Onion Polluted before Planting (20x), OPA – Onion Polluted 2 WAP (20x).





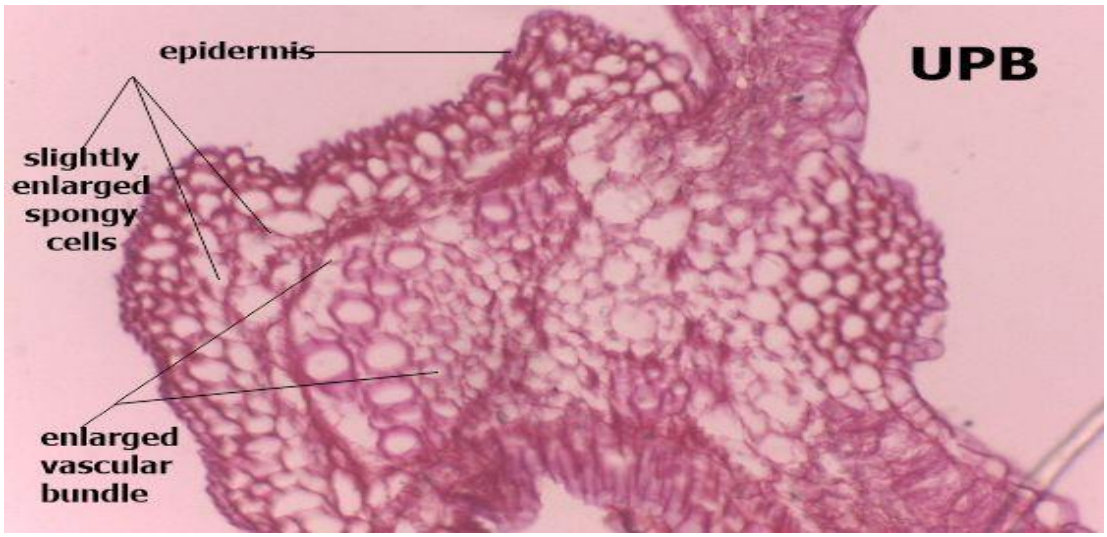


Plate 4.3: Photomicrographs of Polluted and Unpolluted Fluted Pumpkin Leaves
LEGEND: UC – Fluted pumpkin Control (20x), UPB – Fluted pumpkin Polluted before Planting (20x), UPA – Fluted pumpkin Polluted 2 WAP (20x)

4.1.8 DETERMINATION OF DNA DAMAGE IN THE LEAVES OF TEST PLANTS EXPOSED TO CRUDE OIL POLLUTED AND UNPOLLUTED SOIL.

Comet assay results for this study were expressed in Figures 4.9a, 4.9b, 4.9c, 4.9d, 4.9e, 4.9f and 4.9g as comet frequencies and damage scores through proper visual scoring classification (class 0 = no damage, class 1 = little damage, class 2 = medium damage, class 3 = extensive damage and class 4 = extreme damage) for each leaf sample analysed at the end of the experiment. The length of the comet tail, indicated the extent of DNA damage, as longer comet tails showed extreme and extensive damages while shorter comet tails indicated medium damage but, mild or no tails showed no DNA damage. The comet frequency values obtained were statistically lower ($P<0.05$) in all the control soils (UC = 8.00 ± 3.61^{bc}, OC = 11.67 ± 3.06^{b} and MC = 4.67 ± 2.08^{c}), when compared to the higher but nonsignificant comet values recorded in the polluted samples (UPB = 46.33 ± 2.08^{a}, UPA = 50.00 ± 0.00^{a}, OPB = 49.67 ± 0.58^{a}, OPA = 48.67 ± 1.53^{a}, MPB = 49.33 ± 1.16^{a}, MPA = 50.00 ± 0.00^{a}). The damage scores ascertained were in the range of 9.33±4.93 for UC to 188.33±2.08 for MPA.

In addition, various levels of DNA damages were revealed in the photomicrographs shown in Plate 4.4, Plate 4.5 and Plate 4.6 for each test plant. Extreme genetic damages occurred in MPA, UPA, MPB and OPB while OPA had extensive damages. Furthermore, medium DNA damages were observed in UPB whereas, mild and zero damages were shown in the leaves of all the controls.

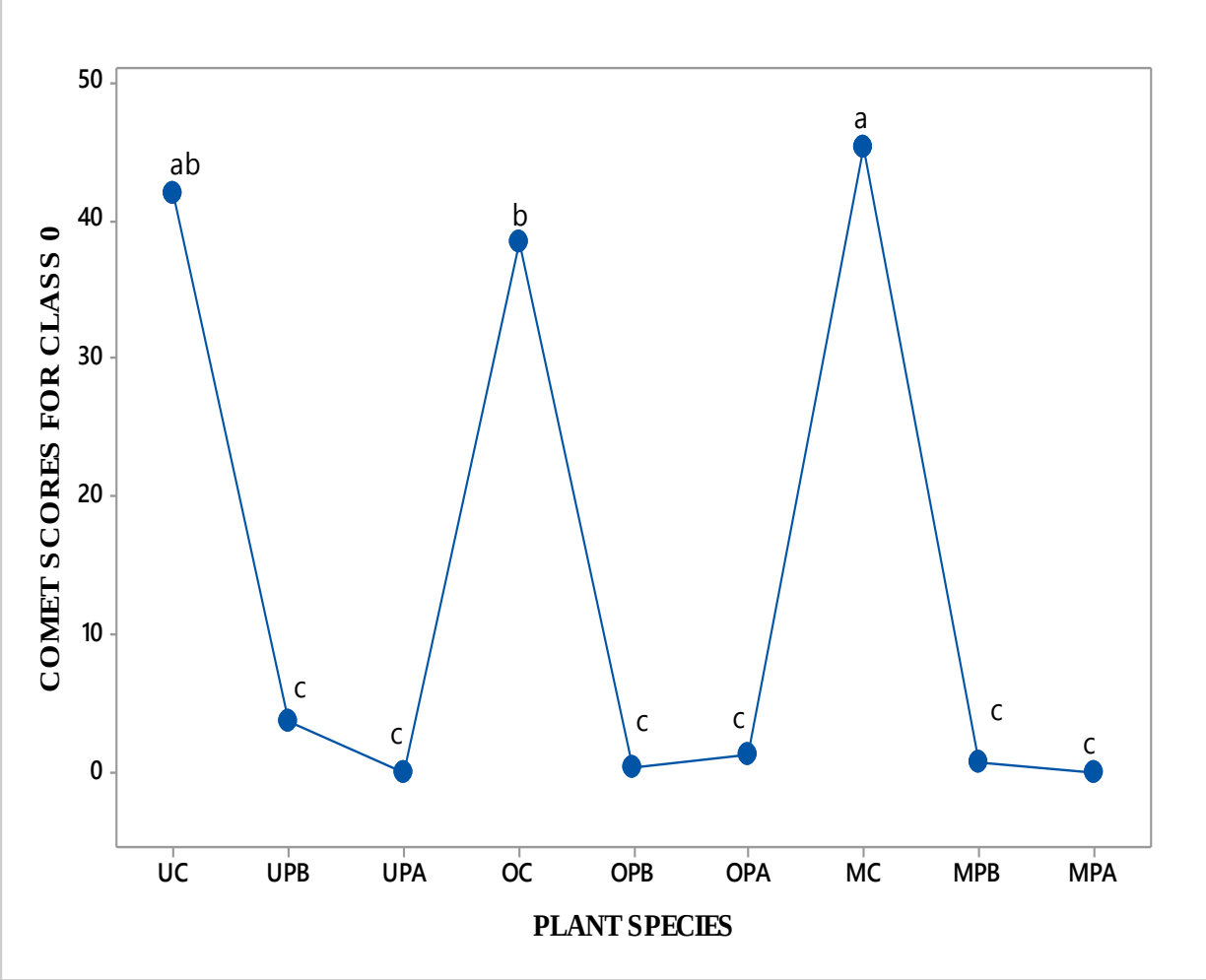


Figure 4.1a: Class Zero (0) Comet Frequency for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

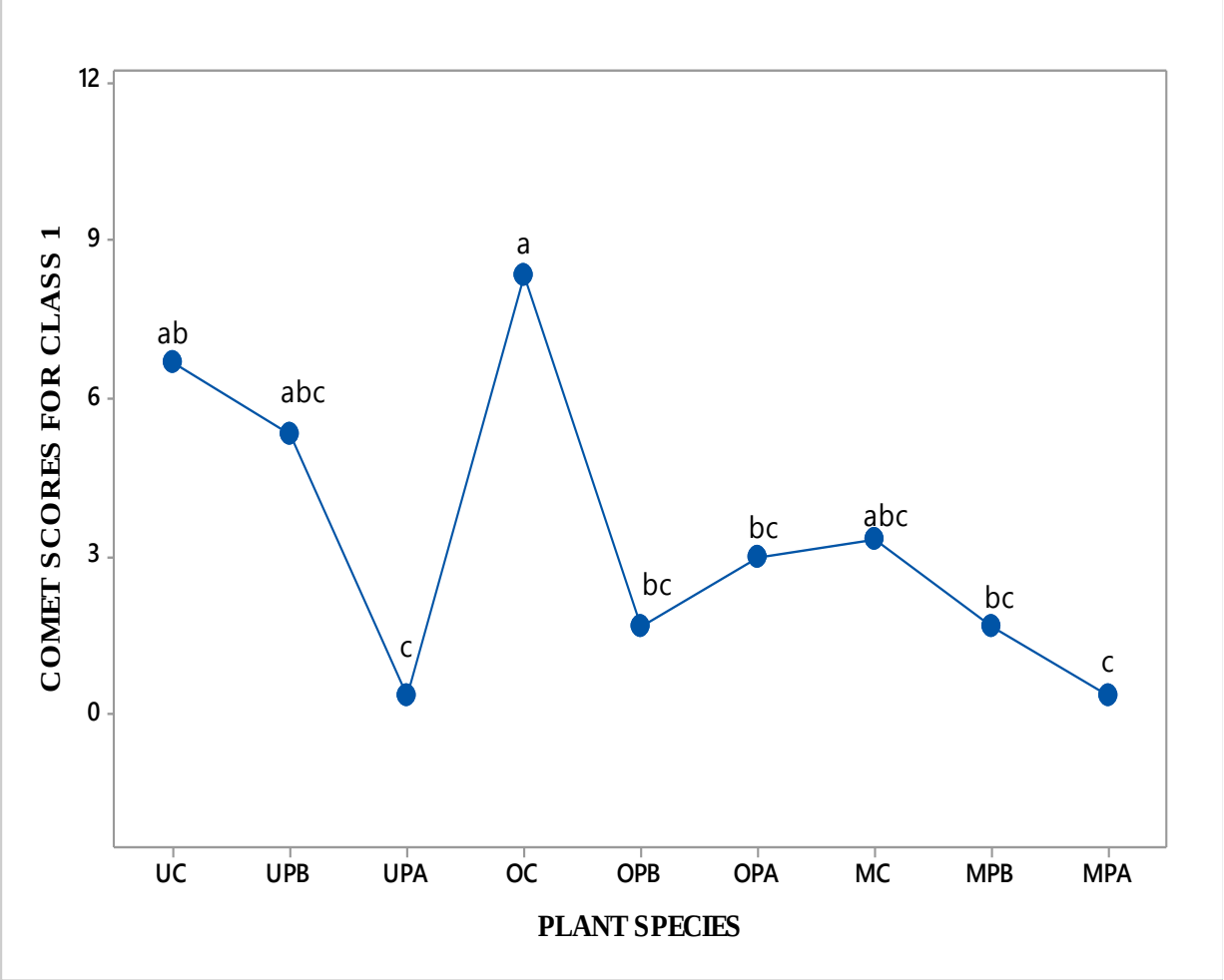


Figure 4.1b: Class One (1) Comet Frequency for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

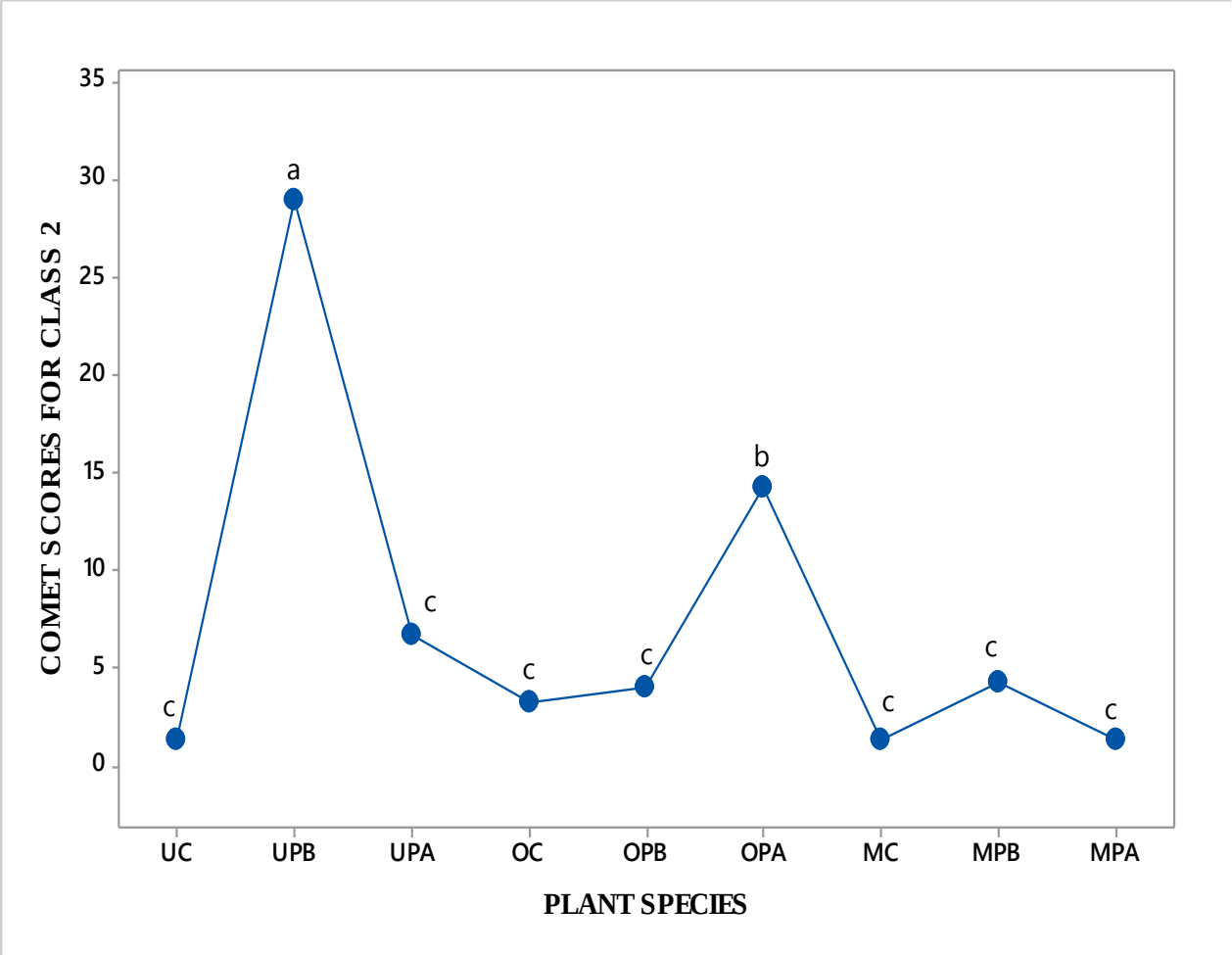


Figure 4.1c: Class Two (2) Comet Frequency for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

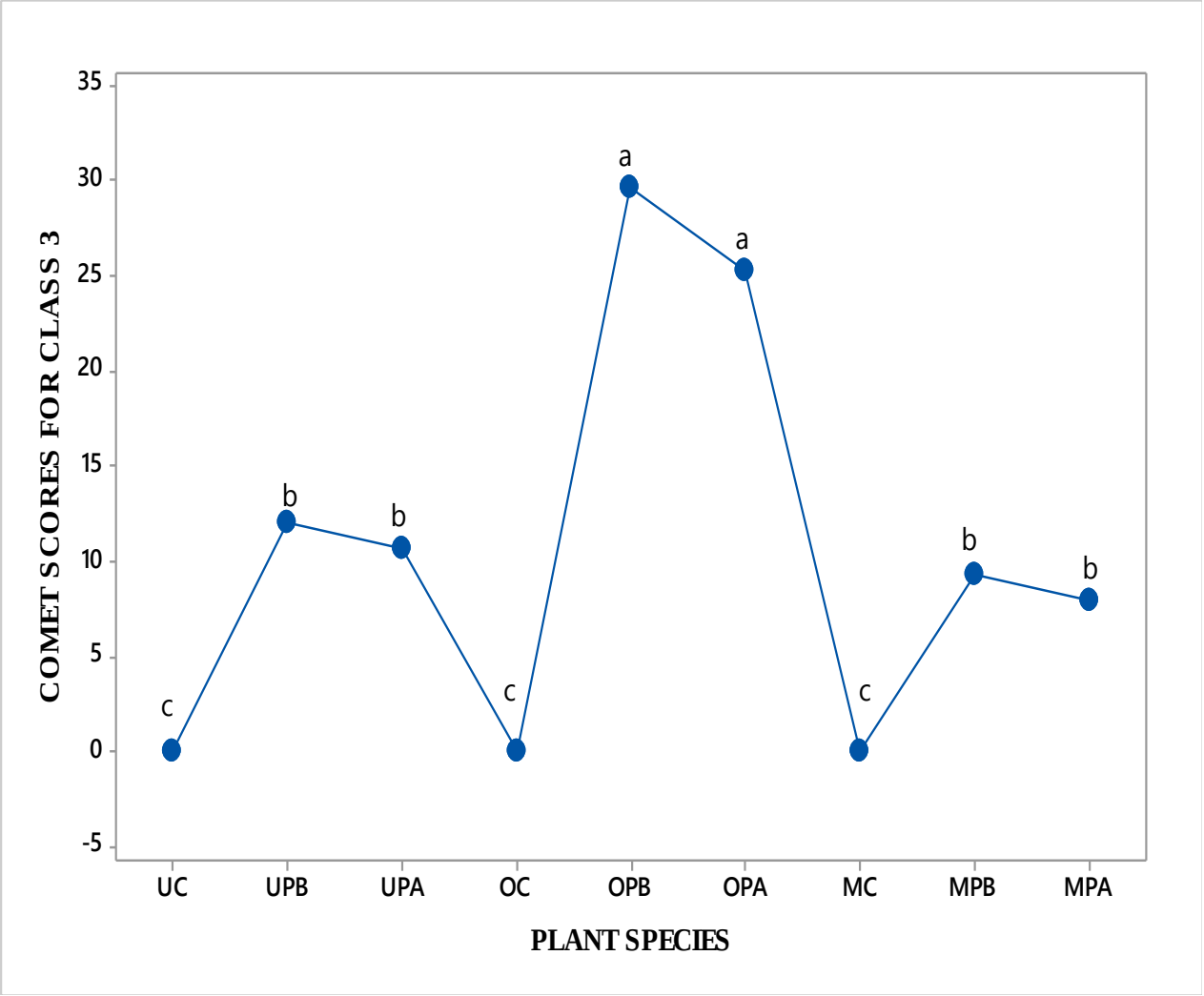


Figure 4.1d: Class Three (3) Comet Frequency for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

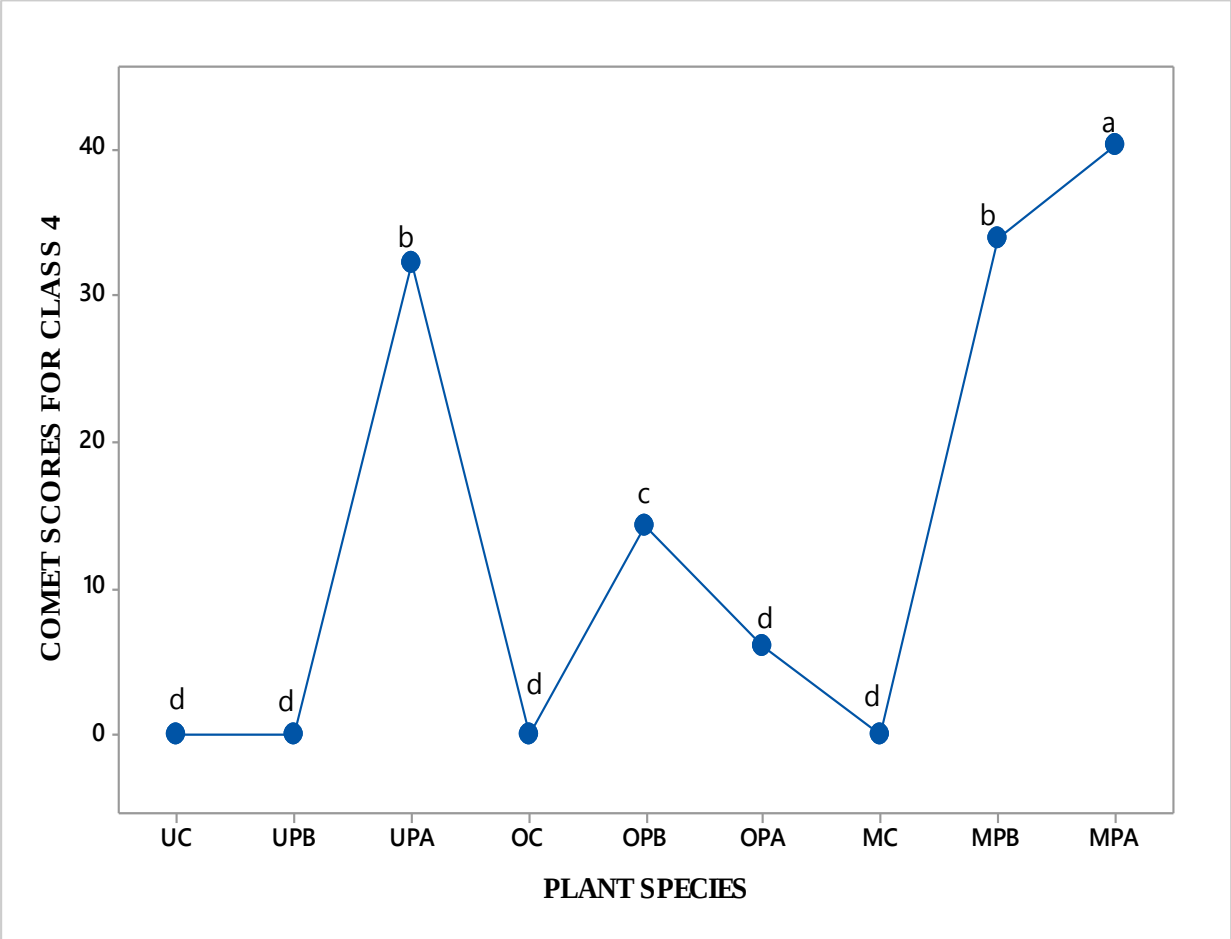


Figure 4.1e: Class Four (4) Comet Frequency for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

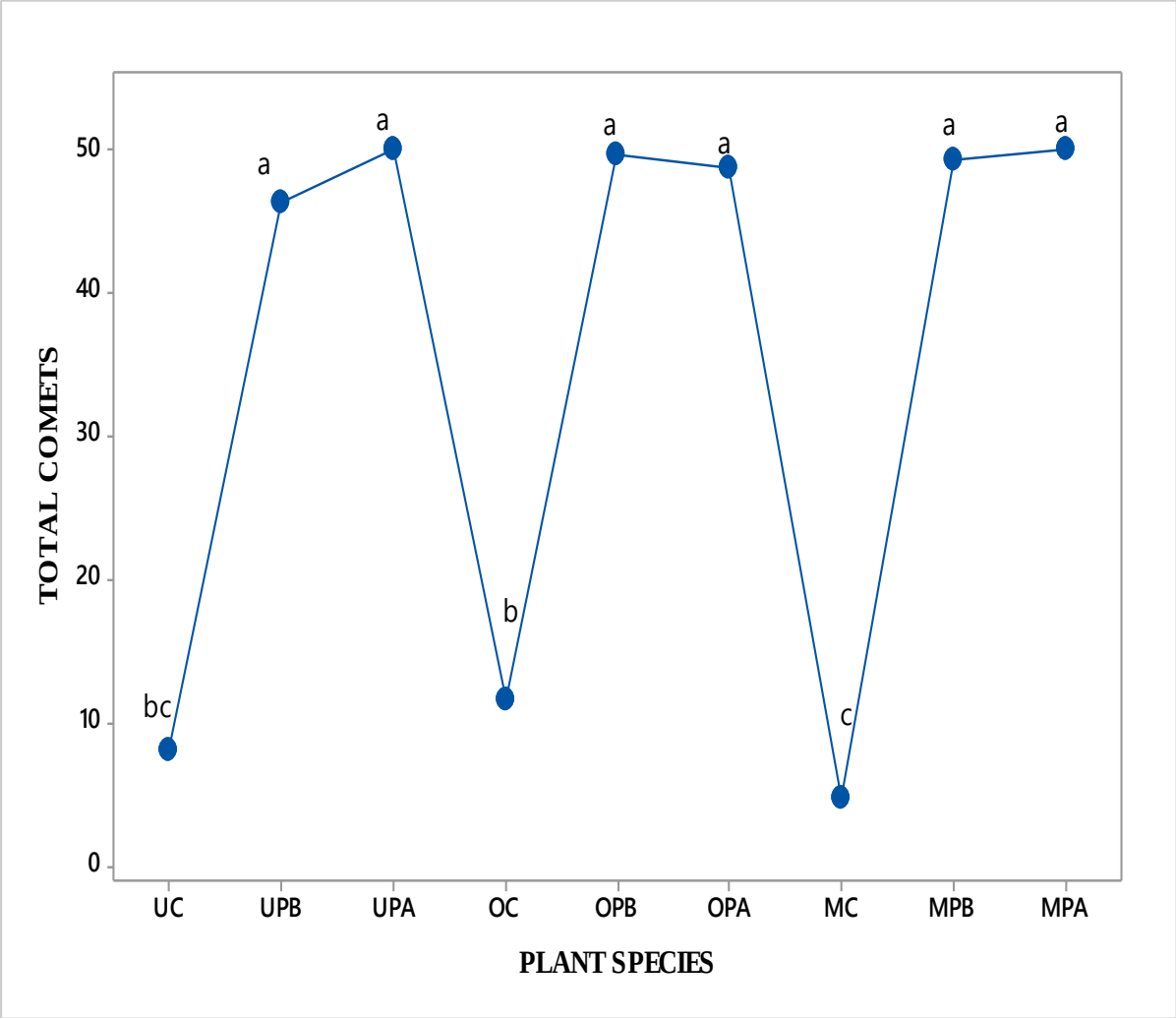


Figure 4.1f: Total Comet Scores for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

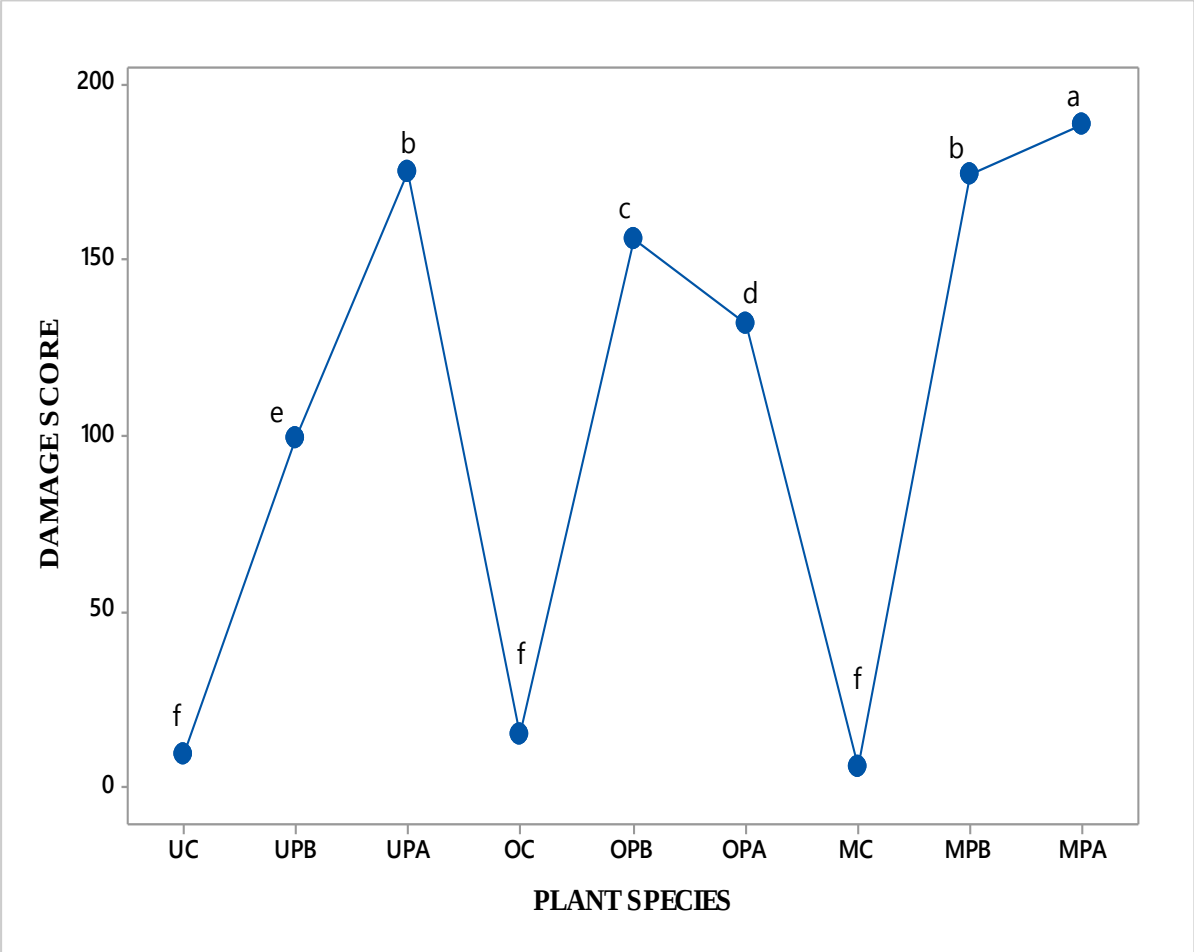


Figure 4.1g: Total Number of Damaged Comets Recorded for Polluted and Unpolluted Test Plants

Legend: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

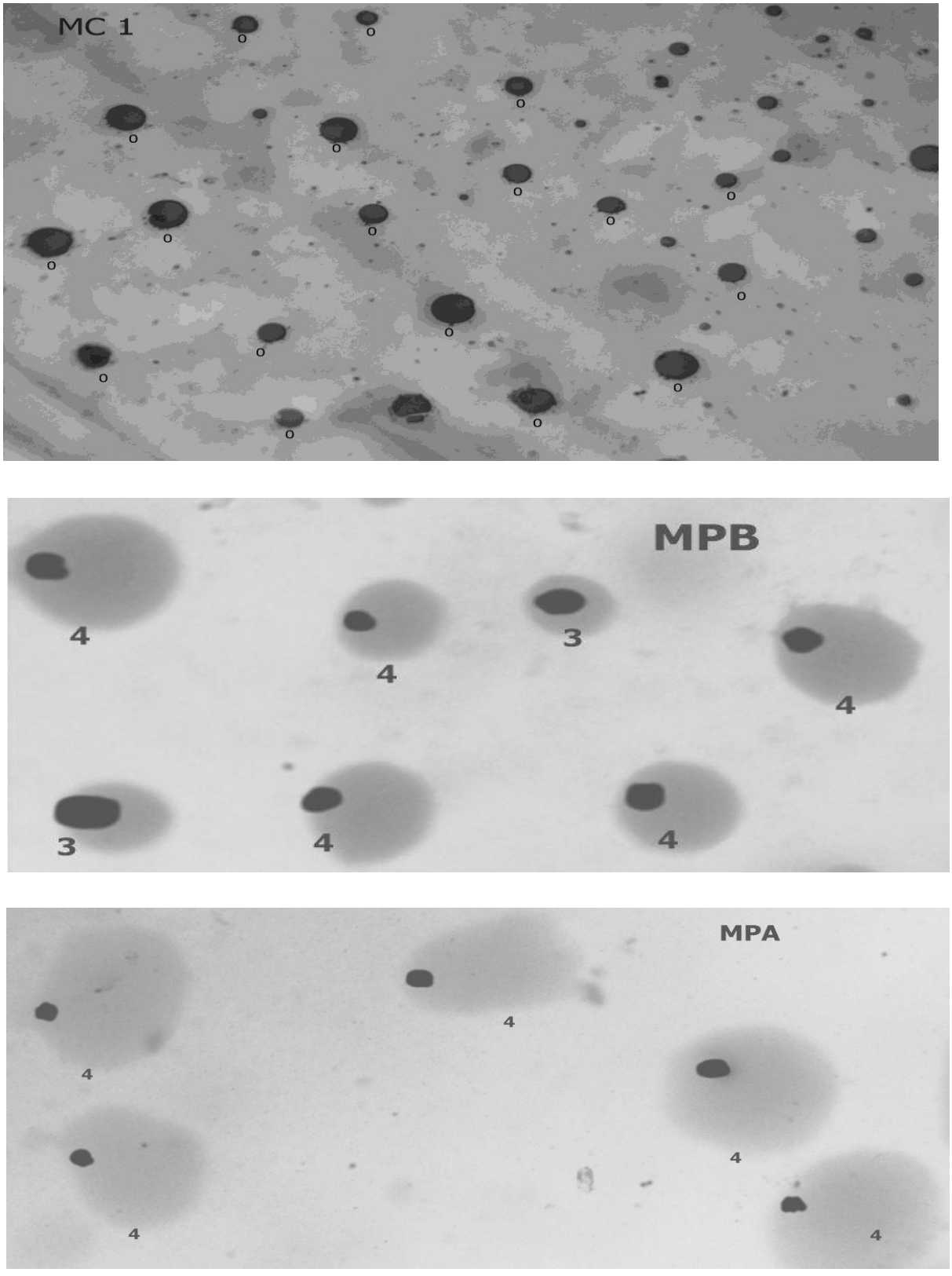


Plate 4.4: Photomicrographs of MC, MPB and MPA Showing Some Classified Comets

LEGEND: Class 0 = no tail (no damage), class 1 = short tail (little damage), class 2 = medium tail (medium damage, class 3 = long tail (extensive damage) and class 4 = very long tail (extreme damage). MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP.

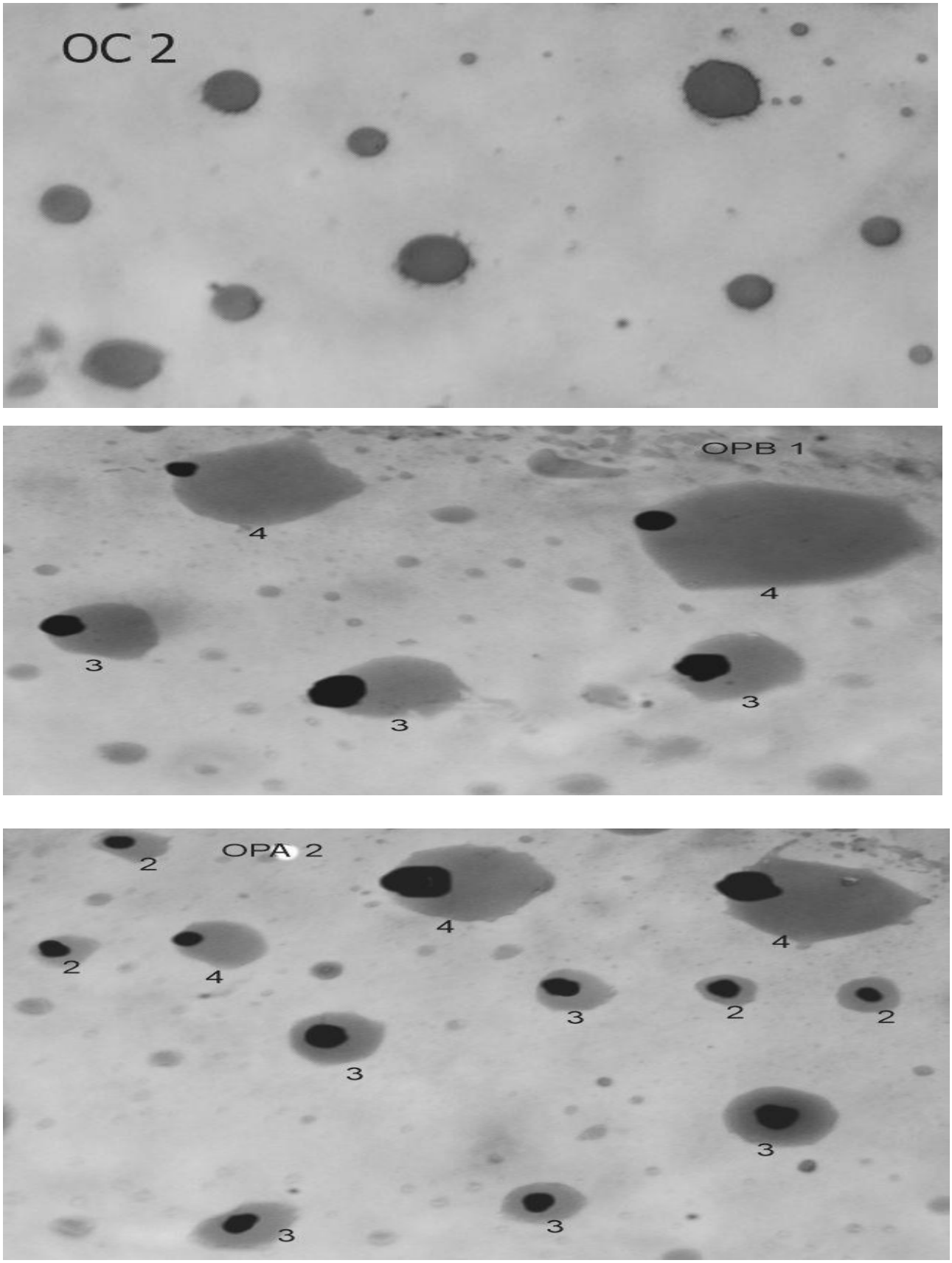


Plate 4.5: Photomicrographs of OC, OPB and OPA Showing Some Classified Comets.
LEGEND: Class 0 = no tail (no damage), class 1 = short tail (little damage), class 2 = medium tail (medium damage, class 3 = long tail (extensive damage) and class 4 = very long tail (extreme damage). OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP.

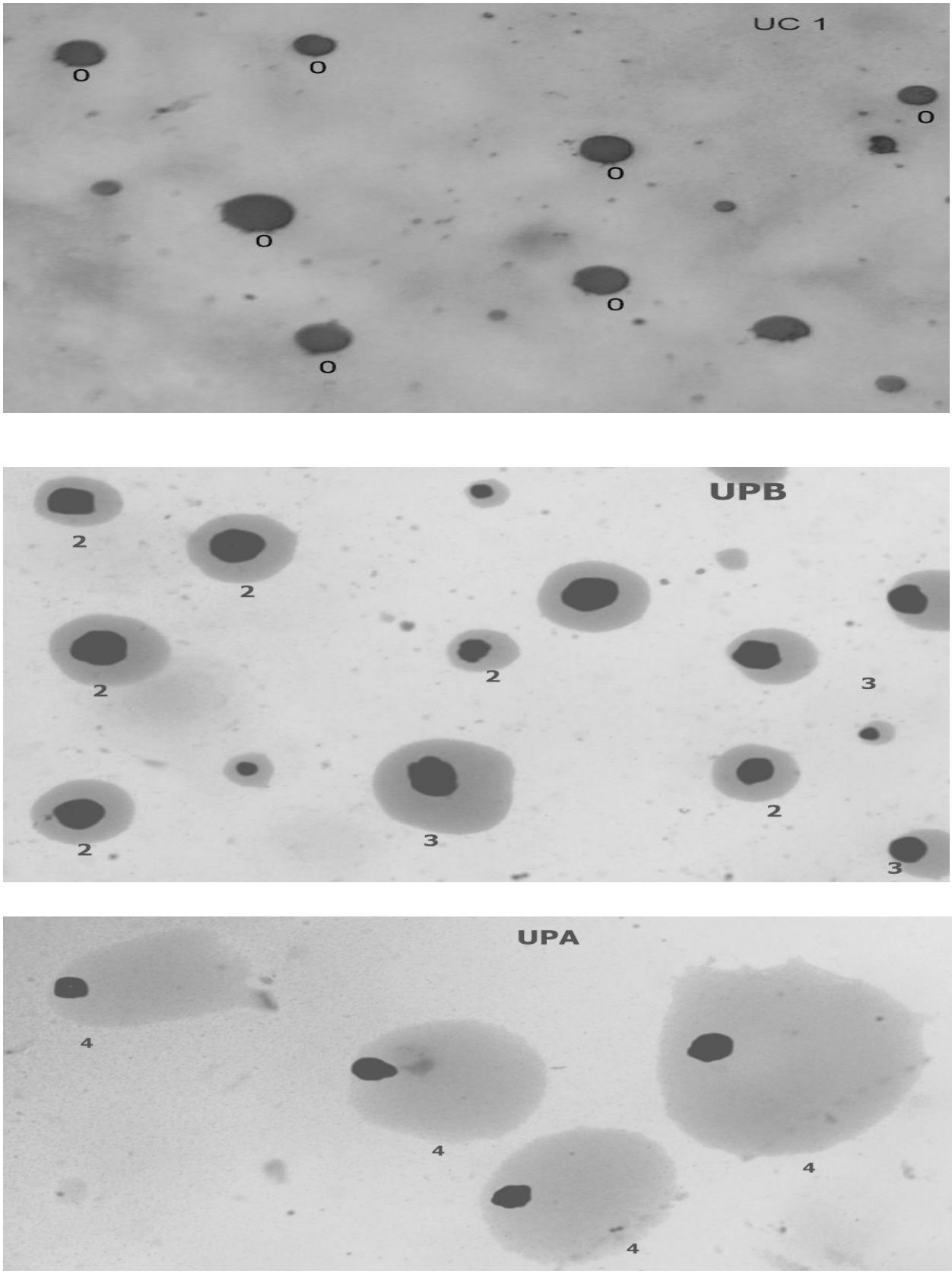


Plate 4.6: Photomicrographs of UC, UPB and UPA Showing Some Classified Comets

LEGEND: Class 0 = no tail (no damage), class 1 = short tail (little damage), class 2 = medium tail (medium damage, class 3 = long tail (extensive damage) and class 4 = very long tail (extreme damage). UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP.

4.2 DISCUSSION

4.2.1 PHYSICOCHEMICAL PROPERTIES OF CRUDE OIL POLLUTED AND UNPOLLUTED SOILS.

In this study, the physical and chemical parameters were assessed to determine the status of both polluted and unpolluted soils in all the experimental groups. Results obtained expressed noticeably higher pH values in all the polluted soil samples than the control soils, and this is ascribed to the ionic imbalance caused by the accumulation of exchangeable bases in oil contaminated soils. Also, soil pH had a considerable effect on nutrients efficiency and availability to plants and microorganisms. For instance, optimal potassium and phosphorus uptake occur mostly between pH 6.0 and 7.5. This strongly aligns with our research findings on the reduced levels of potassium and phosphorus recorded in the polluted groups than the control groups. Results obtained in this study is in tandem with the reports of Ezeigbo *et al.* (2013), Wang *et al*. (2013) and Uquetan *et al.* (2017), which stated that crude oil when spilled may alkalinize the soil.

However, the increase in organic carbon, organic matter and nitrogen observed in all polluted soils suggested the hydrocarbon nature of crude oil while the significant and insignificant decrease in soil exchangeable acid values for groups A and B could be attributed to the rise in soil alkalinity. It was observed that the textural class (sand, silt, and clay) of all the treated soils were altered due to the hydrophobic effect of crude oil on soil particles. This was demonstrated by the higher clay contents and lower sand contents obtained in all the polluted groups, which conversely led to impaired water supply, formation of impenetrable clumps that may cause water logging and displacement of air spaces in the soil, thereby limiting dispersion of oxygen and other essential soil nutrients as well as impeding microbial activities as shown in several studies (Abii & Nwosu, 2009; Ogbo *et al.*, 2009; Agbogidi, 2011; Erhenhi & Ikhajiagbe, 2012, FiriAppah *et al.*, 2014 & Ajah *et al.*, 2019). Nevertheless, results from (Odiyi *et al.*, 2020) and

(Adesina, Akinnfesi, Akinyemi & Adesina, 2022) contradicts our findings that crude oil affects soil texture.

4.2.2 TOTAL HYDROCARBON CONTENT OF CRUDE OIL POLLUTED AND UNPOLLUTED SOILS.

The study showed that the values for total hydrocarbon content of soil increased with crude oil pollution. This is justified by the significantly higher THC values obtained in the polluted soils when compared to the unpolluted sample. The work done by Ekemube, Emeka, Nweke, Atta & Opurum (2022) affirmed our report, as the soil THC levels were directly proportional to the volumes of crude oil applied to the soil. High THC levels in polluted soils negatively affect soil textural class, aeration, soil nutrients and water flow, which equally impede plant growth and activities of soil macro and microorganisms.

It was observed that OPB and MPB showed significant reduction in THC values when compared with OPA, MPA and UPA and this could possibly be caused by the adaptive abilities of the plants as influenced by the mode of crude oil application. A soil clean-up operation had however, been initiated as evidenced by the reduced soil THC levels. Hence, the lowest THC value obtained in UPB soil sample signifies that the highest soil remediation attempt was attained by fluted pumpkin 'polluted before planting'. The lower THC values recorded for all the planted polluted soil samples in this study strongly asserted the fact that biodegradation of petroleum hydrocarbon occurs more in agricultural soil than in non-agricultural soil, probably due to phytoremediation capability of some plants. Edwin-Wosu & Albert (2010), conducted a comparative study, where a particular legume, *Leucaena leucocephala* accumulated higher levels of hydrocarbon from crude oil polluted soil than the other legume, *Bauhinia monandra.*

4.2.3 EFFECT OF CRUDE OIL ON PLANT GROWTH PERFORMANCE

Results on growth performance of test plants showed luxuriant growth of the control plants but statistical ($P<0.05$) decrease in plant height, leaf area and leaf number for both polluted groups as compared to their controls. All these can possibly be attributed to the ability of the volatile oil to penetrate into the seed integument or through plant cuticles, giving rise to suffocation of embryo in seeds or coalescence of cells at different tissues due to oxygen deprivation. Obute, Ekeke & Izuka (2016), reported in their work that refined petroleum products caused varying anomalies in mitosis, germination, and development of *Allium cepa*. Ogbo *et al*. (2009), reported crude oil caused a notable decrease in the biomass, height, and leaf area of *Paspalum scrobiculatum*. Osuagwu *et al*. (2013) also observed significant reduction of plant growth, yield, and leaf chlorophyll content in air potato after crude oil pollution; hence the reduced leaf area, number and chlorophyll content interrupted its photosynthetic activity. However, no significant decrease ($P<0.05$) was observed in plant height, leaf area and leaf number for *T. occidentalis* in group A and this might probably be due to its phytoremediation capability, as was observed in *Arachis hypogaea* which showed better tolerance of crude oil due to the endophytic bacteria it harbours (Iheme *et al*., 2017).

Based on the morphological evaluation of all the polluted plants, onion and maize plants in group A had delayed seed emergence for about a week and experienced retarded growth but later survived better than the plants in group B, which exhibited high level of leaf wilting, slimy shoots, chlorosis, necrosis and even death recorded at day 28 for maize plants. This confirmed the results on morphological evaluation of *T. occidentalis* which showed restrained seed emergence in a pre-existing crude oil polluted soil and eventually leaf chlorosis, as well as plant death in soil polluted three weeks after planting (3 WAP) as described by Erhenhi & Ikhajiagbe (2012). It was observed that the mode of crude oil application and its duration in the soil or on plants influenced the rate of toxicity in our study groups.

4.2.4 PROXIMATE COMPOSITION OF LEAVES OF TEST PLANTS EXPOSED TO CRUDE OIL POLLUTED AND UNPOLLUTED SOILS.

In the proximate compositions of crude oil polluted and unpolluted test plants, the rich nutritive contents of all the unpolluted test plants were indicated and could strongly be traced to the optimum distribution and assimilation of soil minerals, oxygen, and water within the plant tissues. However, the highest moisture content shown in onion control suggested its low shelf life (Dini *et al*., 2008) whereas the maximum values of crude protein, ash content, crude fat and crude fibre attained in untreated fluted pumpkin leaves affirmed their highly nutritious and medicinal properties (Eseyin *et al*., 2014). Subsequently, the biggest value of carbohydrate obtained in maize control is in accordance with the work of Oas & Adams (2022).

Crude oil adversely affected the proximate compositions of MPB, MPA, OPB and OPA while UPA experienced apoptosis. Our result is in line with the scientific work done by Ifemeje & Egbuna, (2016), who reported that crude oil spill negatively affected the nutritional composition of cassava. Nevertheless, the proximate composition of UPB was mildly reduced when compared to UC. In addition, the results of the study conducted by Ekpo, Asuquo & Offiong (2014) revealed that diesel oil negatively affected the proximate compositions and mineral elements of scent leaves (*Occimum gratissimum*) and this was in line with our research findings. The lower proximate values obtained in both polluted groups could have resulted from ineffective water and nutrients uptake by the plant roots and bridged photosynthesis due to deformed structures developed during plants' physiological response to stress.

However, Iyagba & Offor (2013) and Akpan & Usuah (2014) from their experiments recommended fluted pumpkin as a potential phytoremediator. Hence, the phytoremediation capability of UPB was further proven by its exceptional ability to retain moisture and nutrients after exposure to crude oil; especially as insignificant differences were determined in the crude

protein and moisture contents of the contaminated test plant compared to the control plant in this study.

4.2.5 TOTAL CHLOROPHYLL CONTENT OF TEST PLANTS EXPOSED TO CRUDE OIL POLLUTED AND UNPOLLUTED SOIL.

The significant reduction in total chlorophyll contents as seen in all the polluted test plants, strongly indicate damages in the plants' cells and tissues likely caused by nutrient unavailability because of crude oil spill. This corresponds with the report from Olubodun & Eriyamremu (2015) on reduced total chlorophyll, glucose and starch contents of maize polluted with varying crude oil fractions. The hydrophobic nature of crude oil easily injures or kills plants by inducing stomata closure, chloroplasts damage, tissue infiltration, gaseous exchange obstruction, vascular bundles damage, inefficient water, and nutrient uptake as well as disruption of photosynthesis. In addition, Odiyi *et al*. (2020) stated a decline in leaf chlorophyll content of maize plant which adversely affected its yield.

Harzadous effects of petroleum products on the chlorophyll contents of *Barbula lambarenensis* was also reported by Fatoba, Olorunmaiye & Ogunlade (2010). A study conducted at the amazon rain forest of Ecuador highlighted that decreasing chlorophyll contents in petroleum polluted plants indicate stress-response even at family-specific levels (Arellano, Tansey, Balzter & Tellkamp, 2017). Also, results from the experiment carried out by Baruah, Saikia, Baruah & Deka (2014) demonstrated that chlorophyll content of Cyperus brevifolius reduced as the crude oil concentration increased.

The stress-adaptive ability of UPB was portrayed in this study due to its exceptional increase in total chlorophyll content on both day 14 and 28. However, UPA and MPA were mostly affected at day 28 and exhibited chlorosis, necrosis and even death.

4.2.6 TOTAL HYDROCARBON CONTENT OF TEST PLANTS EXPOSED AND UNEXPOSED TO CRUDE OIL SOIL.

Based on the varying THC levels observed in the polluted test plants, it can be connoted that bioaccumulation of hydrocarbons depends on the plant's phytoremediation capability and the nature of toxicity. The massive accumulation of crude oil by UPB and its survival till the end of the experiment showed the high capability of fluted pumpkin to remediate oil polluted soil. Our result is in tandem with the report of Akpokodje & Uguru (2019) which showed the ability of *T. occidentalis* to degrade soil THC from 964.35mg/kg to 82.67mg/kg, even far better than *Abelmoschus esculentus* which lowered soil THC from 964.35mg/kg to 104mg/kg at 14 weeks interval. Still in support of this research finding, Idisi & Uguru (2020) stated that, the ability of guinea grass to grow and accumulate hydrocarbon in an oil site; qualifies it as a potential phytoremediator. Orji-Oraemesi & Njoku (2022), recommended the use of cowpea plant to remediate crude oil polluted soil within a short time frame. Nevertheless, UPA struggled greatly to survive with the accumulated THC level but eventually died at the end of the experiment and this might be attributed to the post-planting crude oil treatment.

The lower THC values observed in MPB, MPA, OPB and OPA could possibly explain that maize and onion plants strived poorly in crude oil polluted soil, as the plants exhibited stunted growth, leaf wilting, chlorosis, necrosis and even death. This agrees with the work of Adesina & Adelasoye (2014), where poor performance of maize and cowpea grown on crude oil polluted agricultural soil was reported. However, on some occasions, they survive in spent-engine oil contaminated soil through phytoextraction of heavy metals. With the aid of organo-mineral fertilizer (OMF), *Zea mays* and *Panicum coloratum* strived well on hydrocarbon polluted soil and even extracted heavy metals from the soil (Anukwa, Onuoha, Nkang & Nkereuwem, 2021).

4.2.7 ANATOMICAL STRUCTURES OF TEST PLANTS EXPOSED AND UNEXPOSED TO CRUDE OIL POLLUTED SOIL.

Alterations and anomalies in leaf structures were mostly discovered in the polluted plants (MPB, MPA, OPB, OPA, UPB and UPA) but the control plants (MC, OC and UC) had well-arranged and healthy leaf structures. The results clearly demonstrated coalesced mesophyll cells in MPB and MPA, distorted cells in OPB and deformed spongy parenchyma in UPA. These defects resulted from the bioaccumulation of crude oil in plant cells, which hindered efficient photosynthesis and oxygen diffusion in the treated plants; thereby causing suffocation, stunted growth and necrosis in them (Moghanm *et al*., 2020). On the other hand, an experiment conducted by (Oluwanisola & Abdulrahaman, 2018) expressed the negative influence that spent engine oil had on the internal structures of okra plant.

Maize and onion are monocotyledonous plants whose leaves are linear shaped with stomata located on both upper and lower epidermis as shown in the photomicrographs labelled MC and OC whereas, fluted pumpkin (UC) is a dicotyledonous plant with broader leaves whose stomata are located on either side of the epidermis. Conversely, the shrivelled epidermis seen in OPA confirmed leaf wilting and inadequate gaseous exchange caused by the hydrophobic and air impenetrable nature of crude oil polluted soil. Komolafe *et al*. (2015) reported in their work that petrol and spent oil, at higher concentration, negatively impacted the epidermal layer and stomata of guinea corn leaf.

The vascular bundles (xylem - for water and dissolved minerals dispersal and phloem - for sugars transportation) found in both monocot and dicot leaves are surrounded with bundle sheaths which aid in photosynthesis. Therefore, the shrivelled vascular bundles noticed in OPA and UPA may have led to chlorosis and plant death. Similar research was made by (Baruah *et al*., 2014) about compressed vascular bundles obtained in *Cyperus brevifolius* planted in soil

polluted with 50,000 ppm crude oil. However, the enlarged vascular bundles and spongy cells shown in MPB and UPB could be traced to the plant's adaptive response to poor supply of water, nutrients and oxygen, which to an extent reduced their growth performance when compared to the control plants. Results from this study is in line with the reports of (Punwong, Juprasong & Traiperm, 2017; Olaranont, Stewart & Traiperm, 2018) which demonstrated the negative impacts of crude oil on the physiological and anatomical parameters of coastal plants (*Terminalia catappa* L. and *Ischaemum muticum* L.) respectively.

4.2.8 GENOTOXIC EFFECT OF CRUDE OIL IN THE LEAVES OF TEST PLANTS.

In the present study, different levels of DNA damages were noticed in all the leaves of the treated test plants. However, UPB having recorded mild and medium damages, exhibited similar phenotypic behaviour with the untreated plant (UC). This observed trait could be attributed to the plant's high level of stress tolerance or DNA repair ability, usually triggered by unfavourable environmental factors. Comet assays are used mostly to detect single and double stranded DNA breaks but, single strands are less severe and easier to repair (Gontijo *et al.*, 2003; Porrut *et al*., 2015; Wesam, 2018; Tasneem & Yasmeen, 2018).

Literature have proven that plants possess diverse genetic repair mechanisms ranging from mismatch repair to base and nucleotide excision repair (BER and NER), which help them to acclimatize better in polluted environment (Mostafa *et al.*, 2021). Moreover, we observed that same species of plant (UPA) could not survive post-germination crude oil spill. This correlates with the findings from Jordan which demonstrated that variations in levels of DNA damage occurred in leaves with respect to their different locations (Wesam, 2018). Our work was in agreement with FiriAppah *et al.* (2014), which stated that the exposure of a germinated *Hibiscus esculentus* (okra crop) to 100mls of crude oil and above resulted to stunted growth,

disintegration of nucleic acids and bridge of certain cellular processes whereas, no germination occurred at the same concentration of crude oil on its seeds.

The extreme genotoxic effects observed in MPA, MPB, OPB and UPA; as well as the extensive DNA damage noticed in OPA could strongly be traced to the build-up of reactive oxygen species (ROS) in cells of polluted plants due to severe damage done by crude oil.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTIONS TO KNOWLEDGE

5.1 CONCLUSION

Our study established that the bioaccumulation of hydrocarbons is proportional to the plants phytoremediation capbility and the method of pollution. Also, *T. occidentalis* in group A (UPB) recorded mild as well as medium damages, and exhibited similar phenotypic behaviour with the untreated plants. Hence, the different levels of tissue damages, DNA breakages and the varying THC levels observed in the leaves of crops used for this study, implies serious health risks to humans and other organisms that feed on contaminated plants in the long run.

5.2 RECOMMENDATIONS

We recommend the following:

i. *T. occidentalis* should be employed as a bio monitoring agent in phytoremediation studies.
ii. Further research should be conducted to isolate, characterize and identify autochthonous microorganisms in crude oil polluted agricultural soil via pre-planting and post-planting treatments.
iii. To determine microorganisms with hydrocarbon-degrading genes resident in *Telfairia occidentalis* and around its rhizosphere.
iv. Based on the toxicity of crude oil to plants as observed in our study, we suggest that agriculturists, environmentalists, conservationists and the government continually develop eco-friendly strategies and enforce policies to curb pollution.
v. Soil-plant-animal feeding pathways should be continuously monitored in all the oil-producing states in Nigeria due to the level of THC, cytotoxic and genotoxic effects

observed in UPB and other polluted plant samples; in order to prevent future exposure of the dwellers in these regions to hydrocarbons.

5.3 CONTRIBUTIONS TO KNOWLEDGE

1. This study showed that *Telfairia occidentalis, Zea mays* and *Allium cepa* grown on agricultural soil after oil spill incidents can survive better compared to when these plants experience crude oil spillage two (2) weeks after their growth.
2. This study further showed that *T. occidentalis* strived well in crude oil polluted soil than *Allium cepa* and *Zea mays.*
3. *T. occidentalis* has shown obvious evidence of possession of resistant traits to crude oil pollution.
4. Finally, anything that can alter the anatomy and DNA of plants can as well affect the anatomy and DNA of other living organisms.

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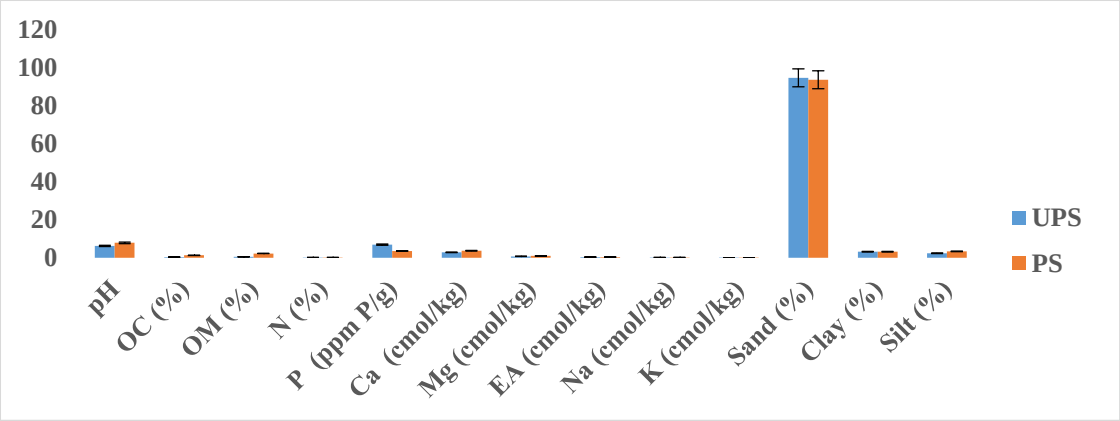
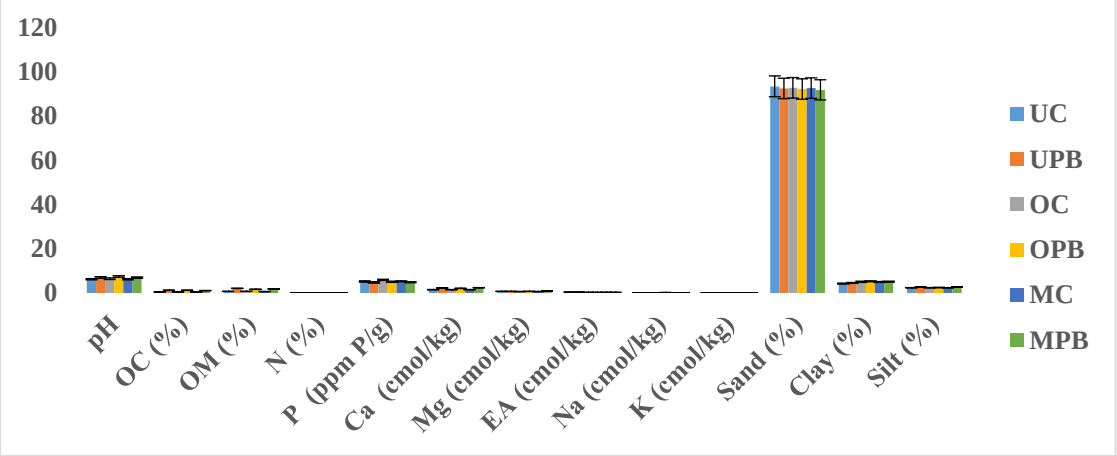
APPENDICES

Appendix I: Physicochemical Properties of polluted and unpolluted soil samples at Day zero



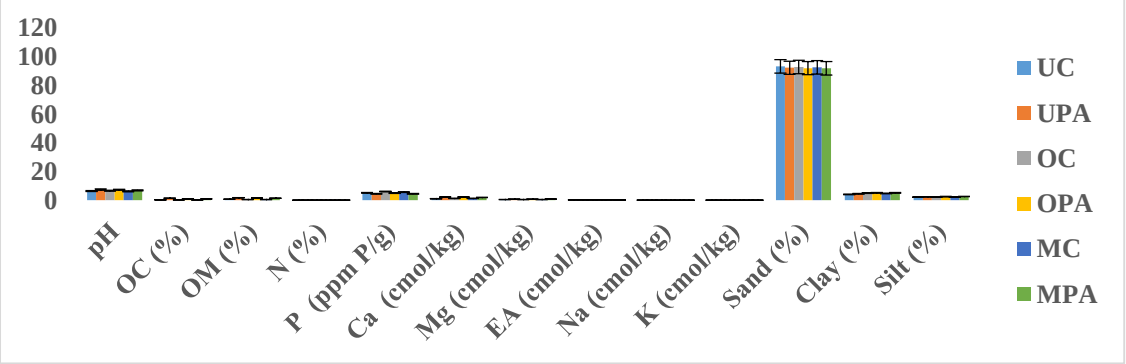
Legend: UPS = Unpolluted soil, PS = Polluted soil.

Appendix II: Physicochemical properties of soil samples polluted before planting at Day 28



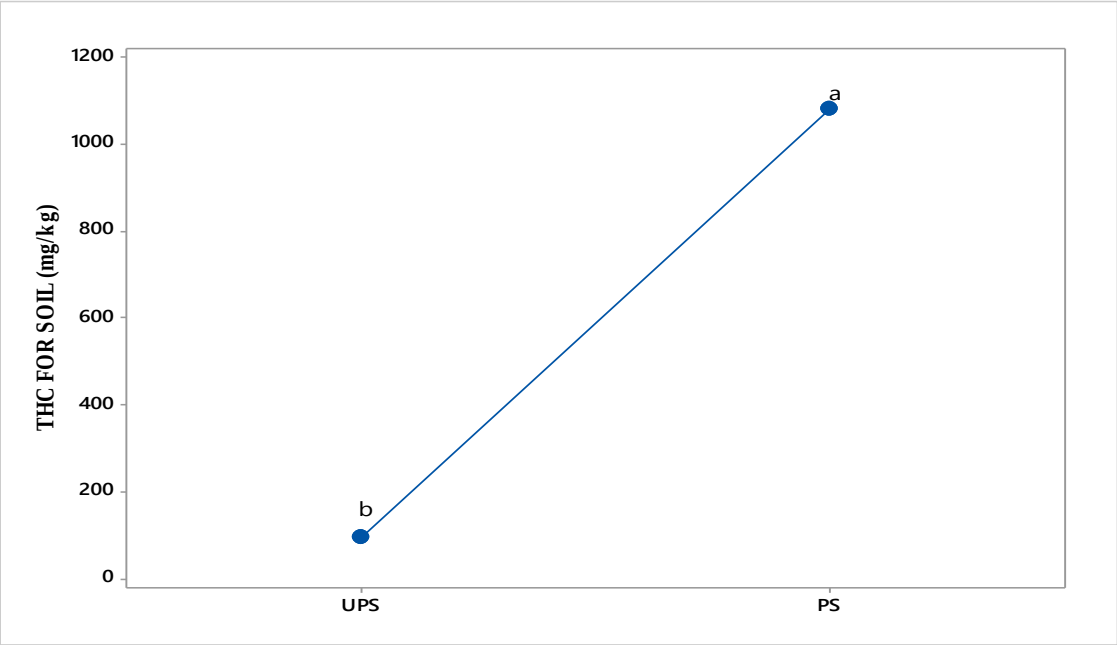
Legend: MC – Maize Control, MPB – Maize Polluted before Planting, OC – Onion Control, OPB – Onion Polluted before Planting, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting.

Appendix III: Physicochemical properties of soil samples polluted 2 WAP at Day 28



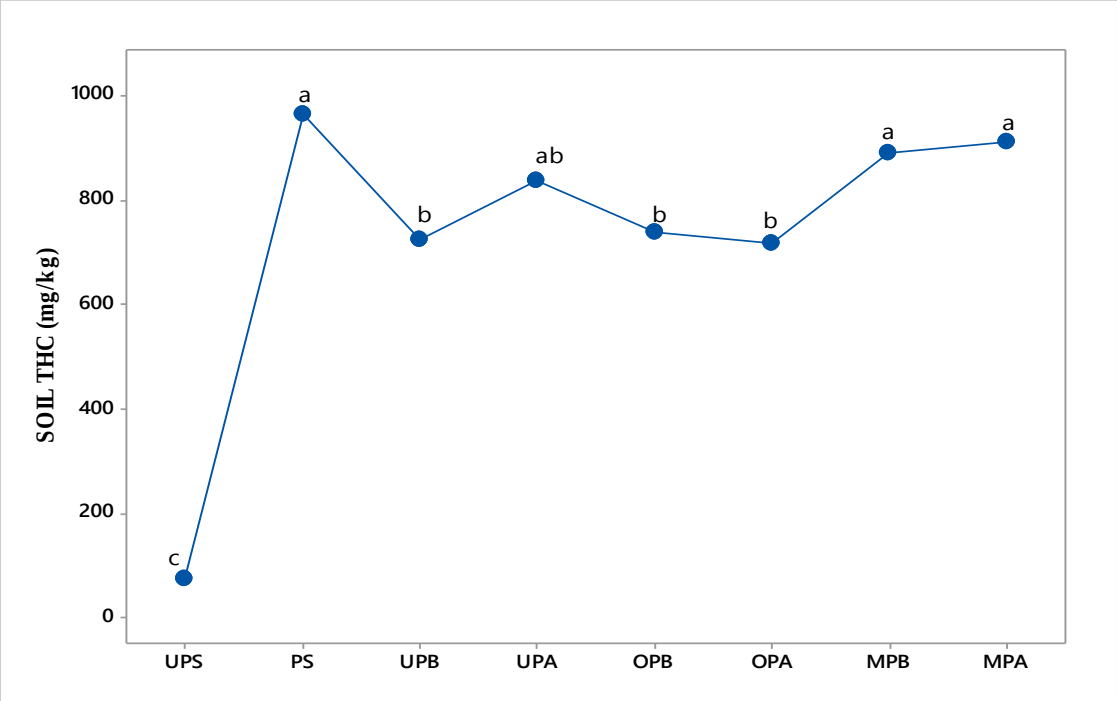
Legend: MC – Maize Control, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPA – Fluted pumpkin Polluted 2 WAP.

Appendix IV: Total Hydrocarbon Content (THC) for polluted and unpolluted soil samples at Day zero



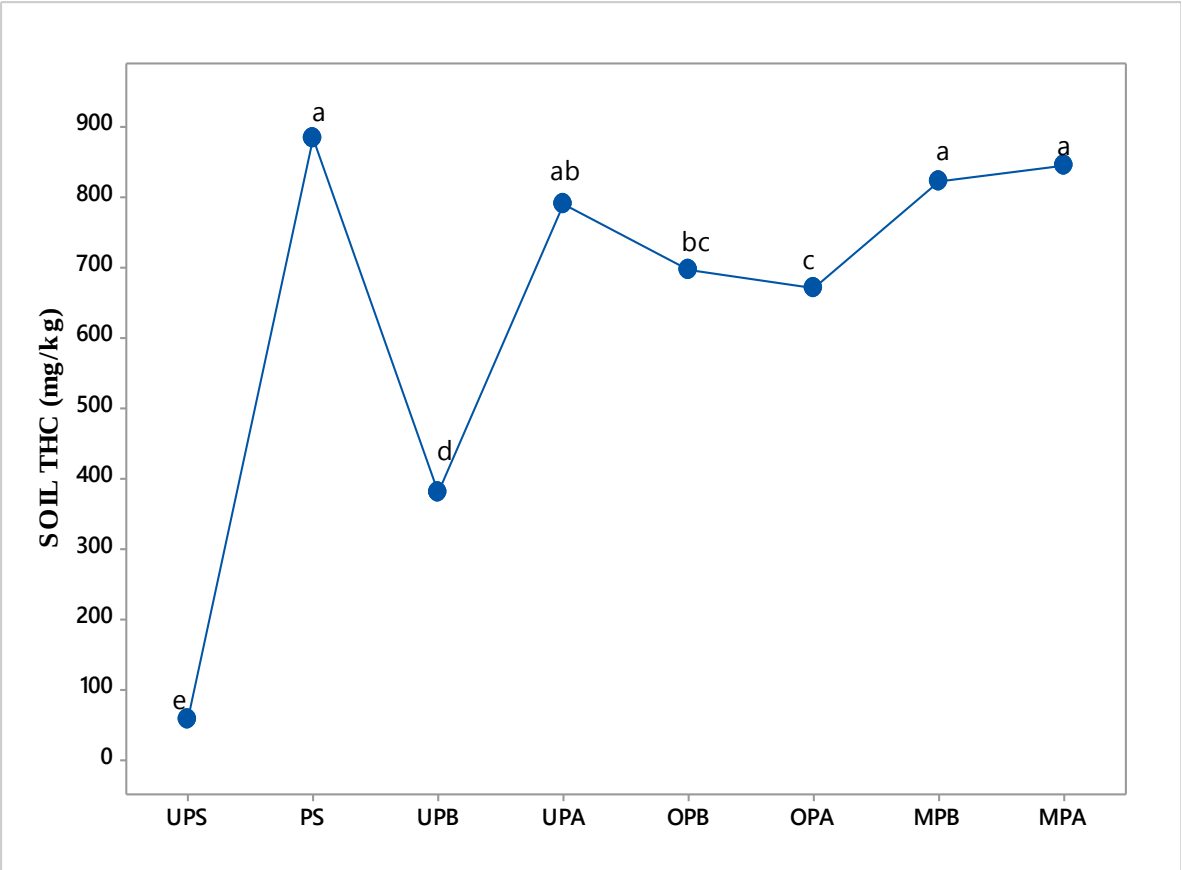
Legend: UPS = Unpolluted soil, PS = Polluted soil. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

Appendix V: Total Hydrocarbon Content (THC) for polluted and unpolluted soil samples at Day 14



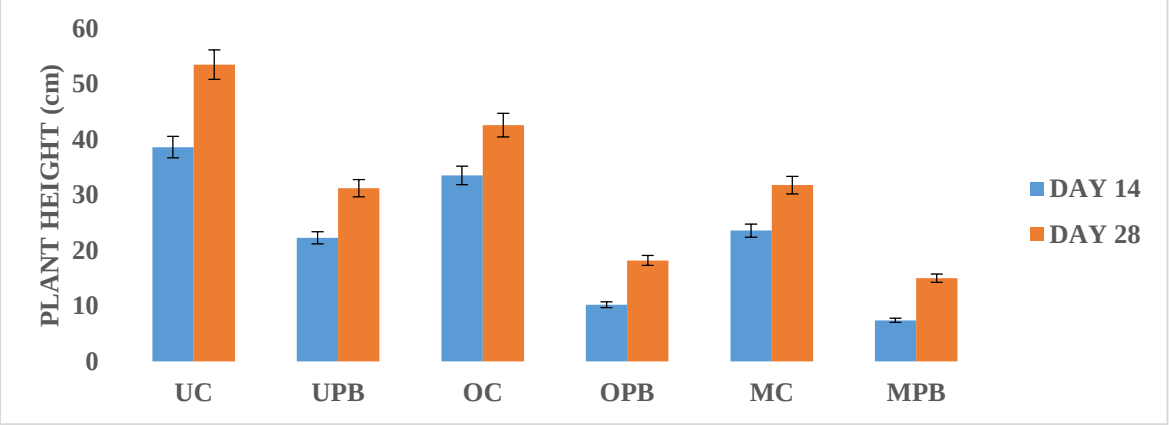
Legend: UPS = Unpolluted soil, PS = Polluted soil. Other polluted soil samples from MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UPB –Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

Appendix VI: Total Hydrocarbon Content (THC) for polluted and unpolluted soil samples at Day 28



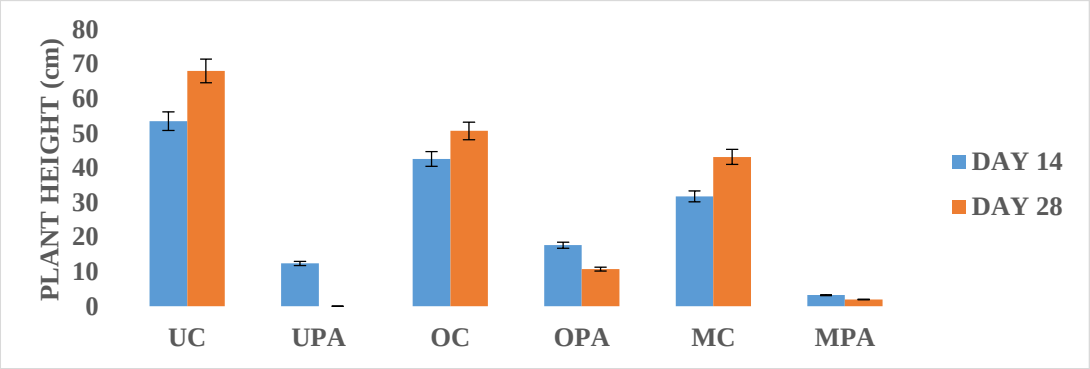
Legend: UPS = Unpolluted soil, PS = Polluted soil. Other polluted soil samples from MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

Appendix VII: Plant Height of plants in group A at Day 14 and Day 28



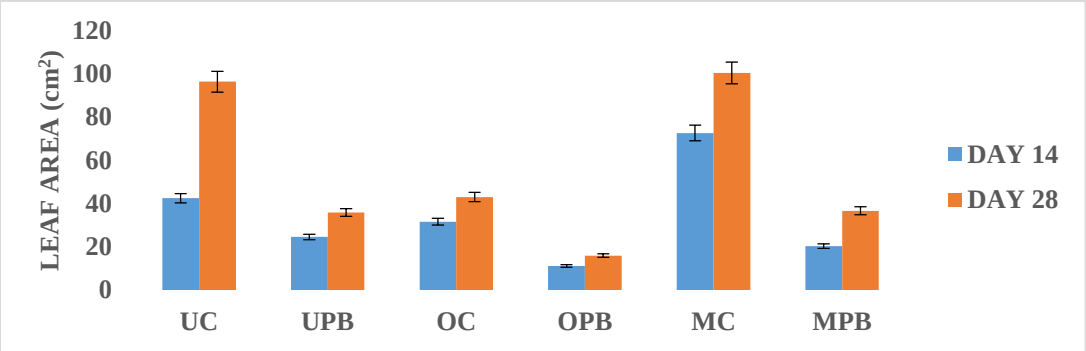
Legend: MC – Maize Control, MPB – Maize Polluted before Planting, OC – Onion Control, OPB – Onion Polluted before Planting, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting.

Appendix VIII: Plant Height of plants in group B at Day 14 and Day 28



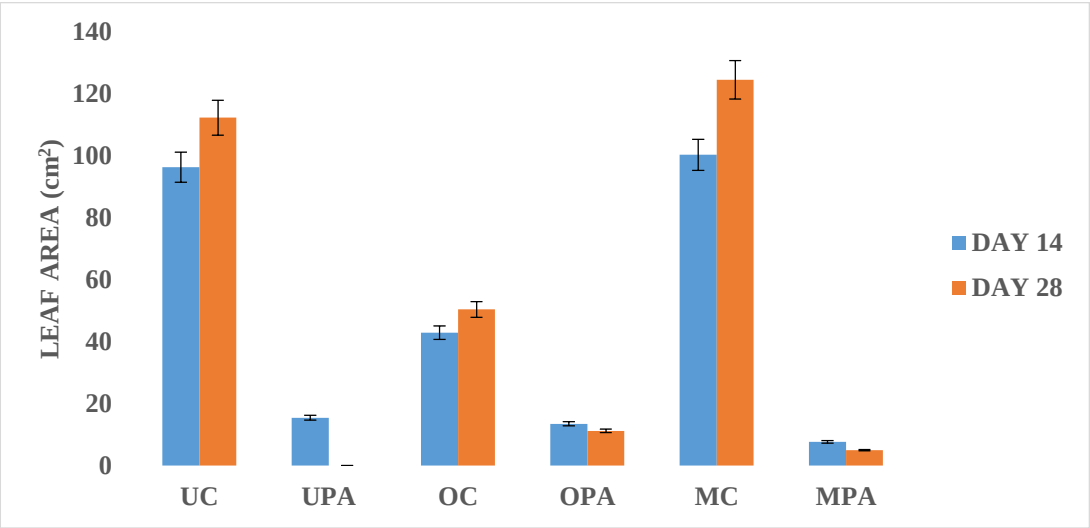
Legend: MC – Maize Control, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPA – Fluted pumpkin Polluted 2 WAP.

Appendix IX: Leaf Area of plants in group A at Day 14 and Day 28



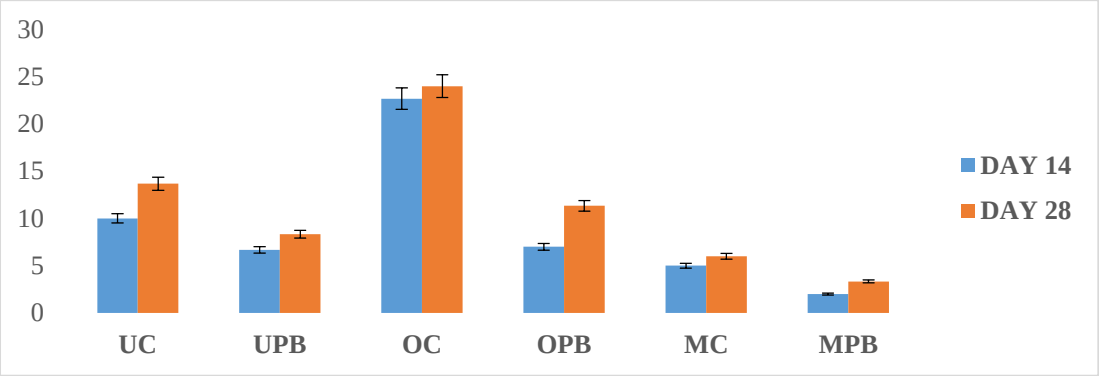
Legend: MC – Maize Control, MPB – Maize Polluted before Planting, OC – Onion Control, OPB – Onion Polluted before Planting, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting.

Appendix X: Leaf Area of plants in group B at Day 14 and Day 28



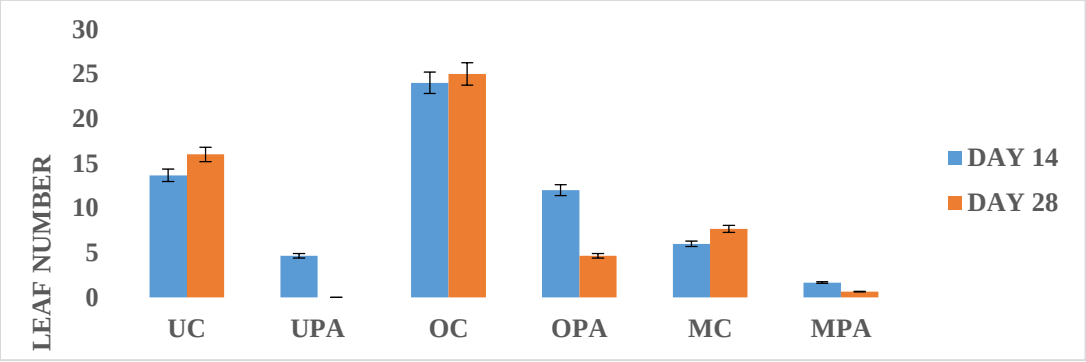
Legend: MC – Maize Control, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPA – Fluted pumpkin Polluted 2 WAP.

Appendix XI: Leaf Number of plants in group A at Day 14 and Day 28



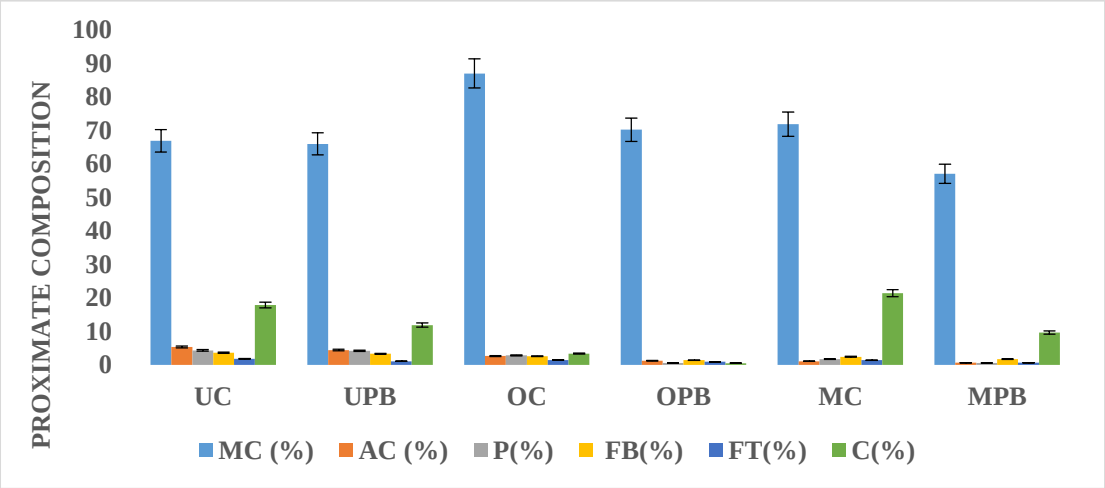
Legend: MC – Maize Control, MPB – Maize Polluted before Planting, OC – Onion Control, OPB – Onion Polluted before Planting, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting.

Appendix XII: Leaf Number of plants in group B at Day 14 and Day 28 respectively



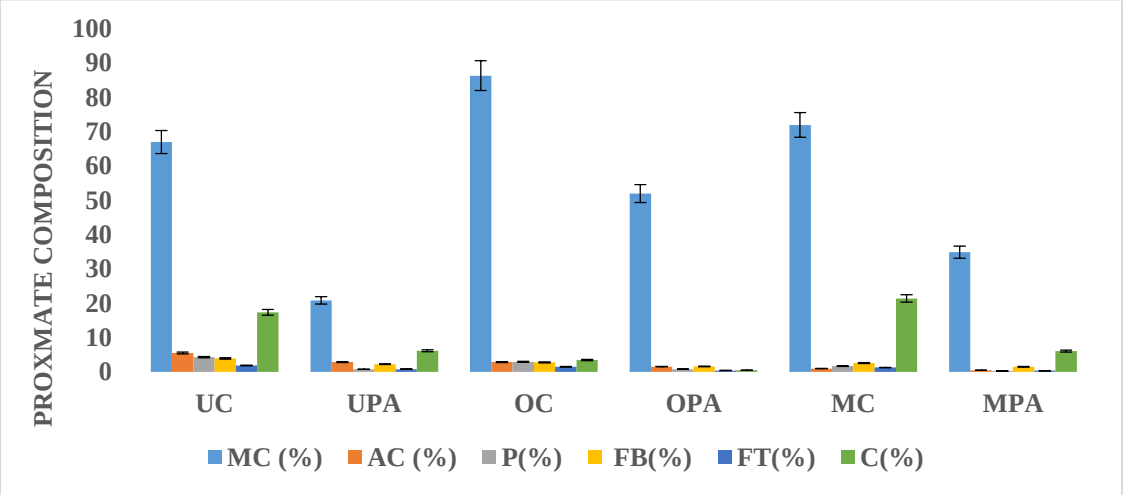
Legend: MC – Maize Control, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPA – Fluted pumpkin Polluted 2 WAP

Appendix XIII: Proximate Composition of plants in group A at Day 28



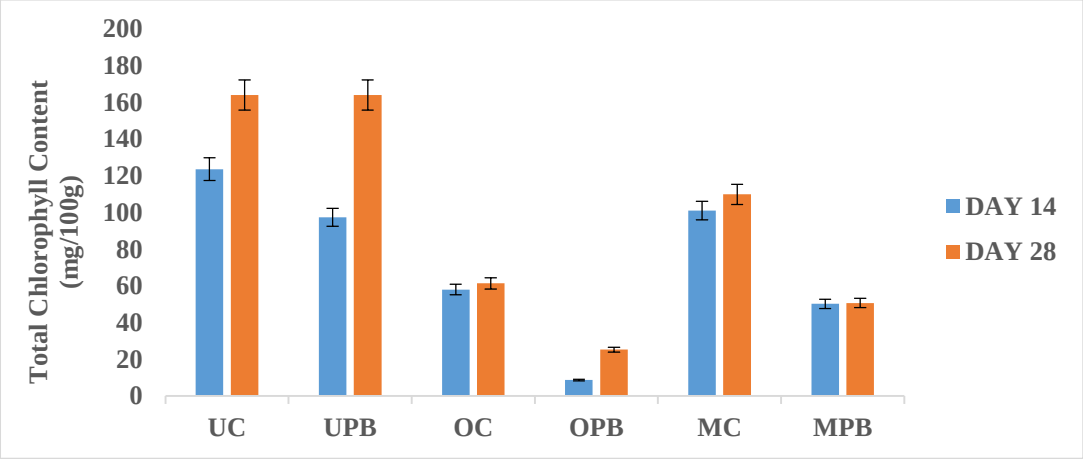
Legend: MC = Moisture Content, AC = Ash Content, P = crude Protein, FB = Crude Fibre, FT = Crude Fat, C = Carbohydrate. UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, OC – Onion Control, OPB – Onion Polluted before Planting, MC – Maize Control, MPB – Maize Polluted before Planting.

Appendix XIV: Proximate Composition of plants in group B at Day 28



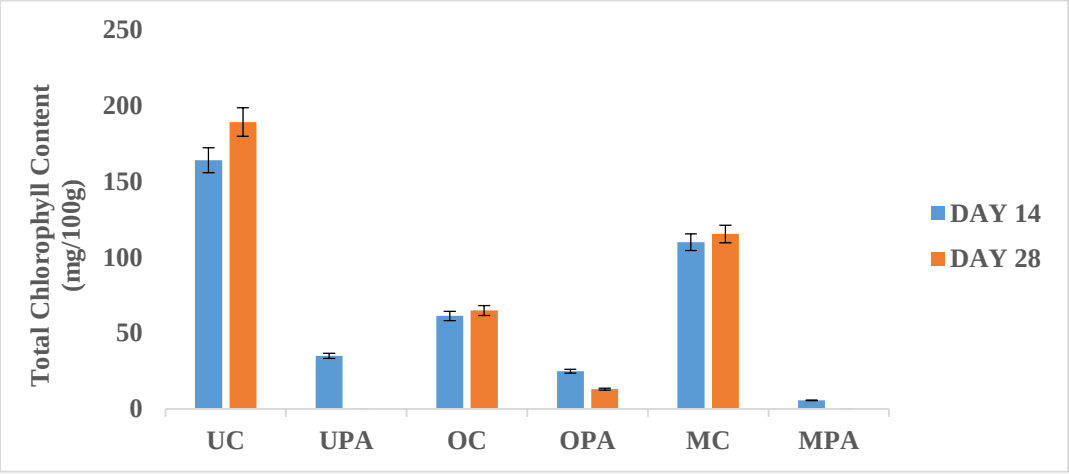
Legend: MC = Moisture Content, AC = Ash Content, P = crude Protein, FB = Crude Fibre, FT = Crude Fat, C = Carbohydrate. UC – Fluted pumpkin Control, UPA – Fluted pumpkin Polluted 2 WAP, OC – Onion Control, OPA – Onion Polluted 2 WAP, MC – Maize Control, MPA – Maize Polluted 2 WAP.

Appendix XV: Total Chlorophyll content of plants in group A at Day 14 and Day 28



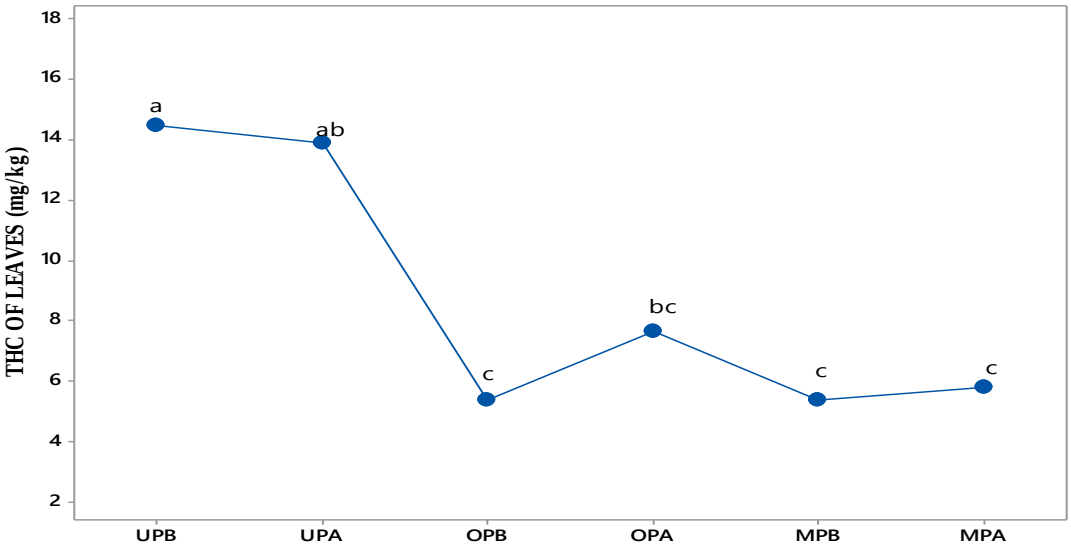
Legend: MC – Maize Control, MPB – Maize Polluted before Planting, OC – Onion Control, OPB – Onion Polluted before Planting, UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting.

Appendix XVI: Total Chlorophyll content of plants in group B at Day 14 and Day 28



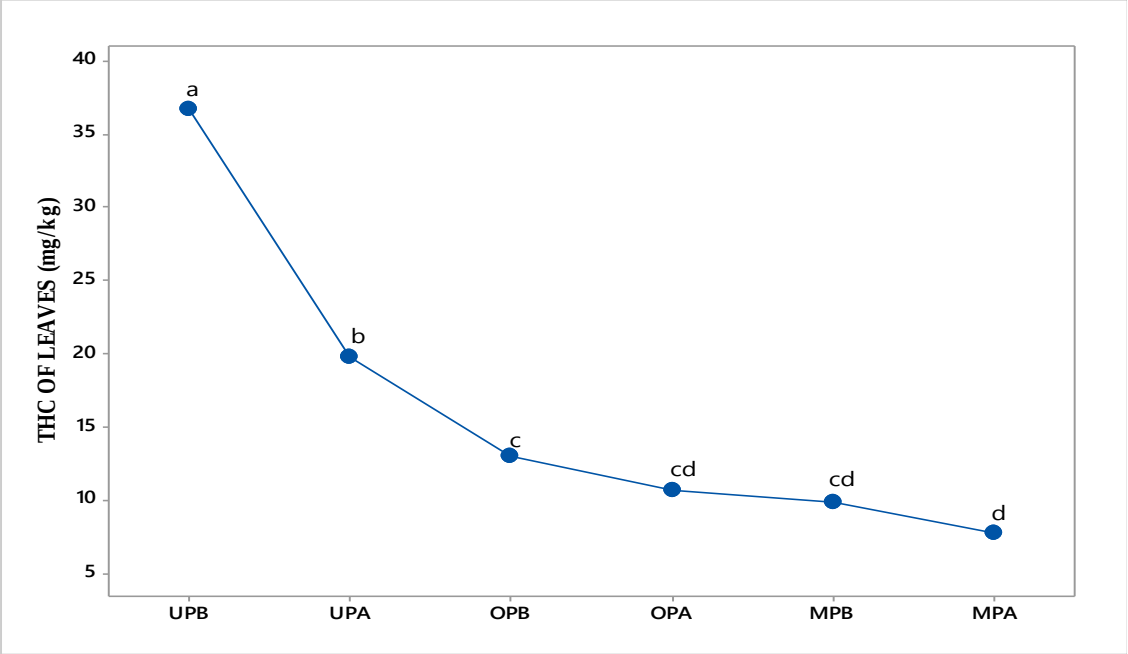
Legend: MC – Maize Control, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPA – Onion Polluted 2 WAP, UC – Fluted pumpkin Control, UPA – Fluted pumpkin Polluted 2 WAP

Appendix XVII: Total Hydrocarbon Content (THC) for polluted test plants at Day 14



Legend: MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

Appendix XVIII: Total Hydrocarbon Content (THC) for polluted test plants at Day 28



Legend: MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter are significantly different at ($p \leq 0.05$)

Appendix XIX: Comet frequency and damage score of test plants

SAMPLE	NA	COMETS	CLASSES					SCORE
			0	1	2	3	4	
UC	150	8.00±3.61^{bc}	42.00±3.61^{ab}	6.67±2.52^{ab}	1.33±1.53^{c}	0.00±0.00^{c}	0.00±0.00^{d}	9.33±4.93^{f}
UPB	150	46.33±2.08^{a}	3.67±2.08^{c}	5.33±2.08^{abc}	29.00±5.29^{a}	12.00±2.00^{b}	0.00±0.00^{d}	99.33±5.13^{e}
UPA	150	50.00±0.00^{a}	0.00±0.00^{c}	0.33±0.58^{c}	6.67±1.53^{c}	10.67±1.53^{b}	32.33±3.21^{b}	175.00±5.29^{b}
OC	150	11.67±3.06^{b}	38.33±3.06^{b}	8.33±3.51^{a}	3.33±0.58^{c}	0.00±0.00^{c}	0.00±0.00^{d}	15.00±2.65^{f}
OPB	150	49.67±0.58^{a}	0.33±0.58^{c}	1.67±0.58^{bc}	4.00±1.00^{c}	29.67±3.06^{a}	14.33±3.06^{c}	156.00±5.20^{c}
OPA	150	48.67±1.53^{a}	1.33±1.53^{c}	3.00±1.00^{bc}	14.33±1.53^{b}	25.33±4.51^{a}	6.00±3.00^{d}	131.67±2.52^{d}
MC	150	4.67±2.08^{c}	45.33±2.08^{a}	3.33±1.53^{abc}	1.33±0.58^{c}	0.00±0.00^{c}	0.00±0.00^{d}	6.00±2.65^{f}
MPB	150	49.33±1.16^{a}	0.67±1.16^{c}	1.67±0.58^{bc}	4.33±1.16^{c}	9.33±3.06^{b}	34.00±2.65^{b}	174.33±4.73^{b}
MPA	150	50.00±0.00^{a}	0.00±0.00^{c}	0.33±0.58^{c}	1.33±0.58^{c}	8.00±2.65^{b}	40.33±2.52^{a}	188.33±2.08^{a}

Legend: NA – total nucleoids analysed**,** MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP, OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP, UC – Ugu Control, UPB – Ugu Polluted before Planting, UPA – Ugu Polluted 2 WAP. Values are mean ±standard deviation from triplicates. Means that do not share a letter across the columns are significantly different at ($p \leq 0.05$)

MC

MPB

MPA

Appendix X: Polluted and Unpolluted Maize plants

LEGEND: MC – Maize Control, MPB – Maize Polluted before Planting, MPA – Maize Polluted 2 WAP

OC **OPB**

OPA

Appendix XI: Polluted and Unpolluted Onion plants at the end of their experimental time

LEGEND: OC – Onion Control, OPB – Onion Polluted before Planting, OPA – Onion Polluted 2 WAP

UC **UPB**

UPA

Appendix XII: Polluted and Unpolluted Ugu plants at the end of their experimental time

LEGEND: UC – Fluted pumpkin Control, UPB – Fluted pumpkin Polluted before Planting, UPA – Fluted pumpkin Polluted 2 WAP