UNIVERSITY OF ZULULAND

INFLUENCE OF DIESEL SPILLAGE ON THE PRODUCTIVITY OF *Ipomoea batatas* AND *Lactuca sativa*

BY

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SUPERVISOR: DR. G. E. ZHARARE

A RESEARCH DISSERTATION PRESENTED TO THE DEPARTMENT OF AGRICULTURE, UNIVERSITY OF ZULULAND, KWADLANGEZWA, SOUTH AFRICA, FOR THE AWARD OF A M.Sc. AGRICULTURE (AGRONOMY)

OCTOBER, 2013

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OCTOBER, 2013

Statement of Original Authorship

This is to acknowledge that the work contained in this thesis has not been previously submitted to meet the requirements for an award at this or any other higher education institution. Furthermore, the thesis contains no material previously published or written by another person except where due reference is made.

Kayode FATOKUN

Dedication

This work is dedicated to the memory of the following people who played significant roles in my life, but who are now resting in the beyond.

Iya mi, Madam Ruth Jolayemi

My mother, Cecilia Abeni

My maternal grandfather, Chief Michael Oyedokun Babarinsa

Acknowledgements

I thank the Almighty God who gave me the strength and kept me in good health throughout the period of this study. I also thank the members of my family who for common interest, I 'abandoned' at home to study abroad. Special thanks to the immediate past Executive Dean, Faculty of Science and Agriculture Prof. G. Ori and my Head of Department, Prof. N. Kunene for their support and encouragement in the course of my study. I am very grateful to my supervisor, a thorough scientist, and a man from whom I have learnt a lot Dr. G.E. Zharare, for supervising this research and for his contributions at various stages of this work. My profound gratitude goes to Dr. F.B. Lewu, for sponsoring the greater part of this research with his personal generated fund and for his useful advice, in the course of this project. My sincere gratitude to the Departmental staff and staff of the teaching and research farm of the Department of Agriculture, University of Zululand, of special note are, Dr. Corlien Van Jaarsveld, Dr. S. Mavengahama, Mr. S. Hlophe, Ms. E. Maupa and Mrs R. Pakathi for their assistance at various times. This acknowledgement will be incomplete without mentioning the following friends/colleagues: Bafowethu Mavule and Portia Sibiya. I thank them for being my friends.

Ethical Consideration and Safety Caution

Diesel, like other petroleum products is a contaminant when spilt into the environment. Hence, to ensure that the diesel used did not spill on the ground the following precautions were taken to ensure non contamination of the areas used for the research:

- Plastic sheets were spread on the floor of the green house during the process of mixing the soil with diesel, to prevent contaminating the soil beneath with diesel.
- Plastic lids were placed under pots used in the experiments, to receive oil that leached out of the pots.

Diesel, like other Volatile Aliphatic Hydrocarbons from petroleum products, are suspected carcinogens (Krahl *et al.*, 2002), hence the following safety cautions were observed.

- In the course of mixing the soil with diesel and other activities performed in the green house; ventilated hood, respirators and latex gloves were worn to avoid inhalation and skin contact.
- Inside the laboratory, all handling of soil samples, diesel and other chemicals used for analyses were done wearing special latex gloves, goggles and masks.

When the experiment was concluded, the soil in the pots, the plastic containers, and all plant residues were burnt at a safe distance of about 50 meters from the green house. This was done to remove diesel from the soil so that the environment would not be contaminated with diesel. The soil left was subsequently disposed of in a landfill.

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Abstract

The effects of diesel contamination in soil on the germination, growth and dry matter partitioning in *Lactuca sativa* (crisp head variety) and *Ipomoea batatas* (dagga variety) were studied at two diesel concentration ranges 0-30 and 0-6 ml/kg soil. The first experiment tested the effects of diesel concentration and age of diesel contamination in soil on the germination of *L. sativa* and *I. batatas.* The second experiment investigated the effects of diesel contamination in soil on the growth and dry matter partitioning in *I. batatas* and *L. sativa.*

The effects of diesel concentration in soil contamination on the germination of *L. sativa* and *I. batatas* varied with the diesel concentration and the age of diesel contamination in the soil. Diesel inhibited the germination of *L. sativa and I. batatas* in a concentration dependent manner, showing increased inhibition with increasing concentration of diesel contamination in the soil. Also, the critical concentration of diesel for toxicity on the germination of *L. sativa* and *I. batatas* increased with the aging of diesel contamination in soil. However, the influence of diesel contamination in soil on the germination of the two species diminished with the age of diesel contamination in soil, suggesting possible reduction in diesel toxicity over time. The germination of *L. sativa* was more affected by diesel contamination in soil than that of *I. batatas.*

The result of experiment two indicated that, after 14 weeks of growth, the plant height, number of leaves, stem lengths, root lengths and the leaves chlorophyll content of *L. sativa* and *I. batata*, were highly negatively correlated with diesel concentration in soil contamination. The effects of diesel contamination in the soil on dry matter partitioning of the two species also varied with diesel concentration in soil contamination. At low diesel concentration in soil contamination in soil contamination (0-6 ml/kg soil), allocation of dry matter to the shoot system was favoured resulting in high shoot: root ratio of 4.54 and

12.91 for *L. sativa* and *I. batatas* respectively. However, at diesel concentration in soil contamination 0-30 ml/kg, allocation of dry matter to the root was favoured, an indication of the effort of *L. sativa* and *I. batatas* to survive the phytotoxic effects of diesel hydrocarbons in the soil. The effects of diesel contamination in soil on the germination and growth of *Ipomoea batatas* were more pronounced on the germination and growth of *Lactuca sativa.*

It can be concluded that, the phytotoxicity of diesel contamination in soil on the germination and growth of *L. sativa* is markedly stronger than the phytotoxity of diesel on the germination and growth of *I. batatas.*

INTRODUCTION

One of the most serious environmental/soil pollution problems confronting the world is the spillage of crude oil and other petroleum products, which include diesel, petrol, kerosene, asphalt and black oil (Leffler, 1985; USEPA, 2000). Oil spillages occur in the course of extracting, transporting, storage, distribution and marketing of petroleum products (PPMC, 2006). Sources of oil spillage include; equipment failure, damage to oil pipelines, ruptured loading hoses, oil well blow out, and accidental oil loss during loading of oil tankers, sabotage, grounding and collisions (Anderson, 2005; Etikerentse, 1985; ITOPF, 2011). Globally, approximately 5.71 million tonnes of oil have been spilled into the environment as a result of spillages from oil tankers between 1970 and 2010 (ITOPF, 2011). It is generally known that even in the best of oil field practices oil spillage and the resultant pollution cannot be completely eliminated (Etikerentse, 1985). Hence, pollution is part of the price that must be paid for the developments associated with the petroleum industry (Etikerentse, 1985). Oil pollution problems have largely been associated with impacts on sea and coastal land environments. This is because spillages from tanker ships are large and they receive a lot of attention from the public and the media. Little attention is given to 'minor' spillages of petroleum products among which is diesel on inland environments such as agricultural land which are far more complicated, unpredictable and constitute the vast majority of oil spills in many countries (USEPA, 2000; Fingas, 2001; ITOPF, 2011). Among petroleum products, diesel spills are by far the largest, because it is used in many sectors, which include; transport and non-transport/commercial uses (SAPIA, 2007).

Diesel spillage on cultivated land and potential agricultural land is a common occurrence in many countries because most of the machineries used on agricultural lands use diesel as a source of power. With the ever increasing world population, which currently stands at

over seven billion (UNFPA, 2011), increased use of machinery on agricultural land is inevitable to meet the rising world's food demand (FAO, 2011). This will lead to more incidences of diesel spillages on agricultural land. Hence, with the certainty that the incidence of diesel spillage on agricultural land will increase in the years to come; it is very reasonable that efforts be made to study the effects of diesel on soil and crop productivity, and to determine options for ameliorating the effects. Hence, this study was conducted.

The study sought to answer the following questions;

i. What are the effects of different diesel concentration in soil contamination on the germination of lettuce and sweet potato?
ii. What are the effects of age of diesel contamination in soil on the germination of lettuce and sweet potato?
iii. What are the effects of diesel contamination in soil on the vegetative growth of lettuce and sweet potato?
iv. What are the effects of diesel contamination in soil on mineral nutrients in the leaves of lettuce and sweet potato?

To answer these questions, chemical properties of diesel and of soil before and after diesel contamination were examined, and so were the effects of diesel concentrations in soil contamination on germination and growth of lettuce and sweet potato. The former is a leaf vegetable that is established from seed, and the latter is a root crop that is established from vegetative cuttings. The experiments were done under controlled environments.

NOTE: The references for this introduction are included in the references for chapter 5.

CHAPTER 1

LITERATURE REVIEW

1.1 WHAT IS DIESEL?

Diesel oil otherwise called diesel, is in general any liquid fuel used to power compression-ignition engines, which can be tractors, generators or transport vehicles such as buses, trucks, trains and boats (Walker *et al*., 1978). The word diesel was derived from the family name of a German inventor Rudolf Diesel who in 1892 invented the compression-ignition engine, popularly called diesel engine (Sandhyarani, 2012). Originally, diesel was a specific fractional distillate of petroleum fuel oil (Freudenrich, 2000). Now, in addition to diesel derived from petroleum distillate, there are other sources of diesel such as; algae, plants and animal fats. Based on the raw materials, such types of products are known as, biodiesel, biomass to liquid (BTL) or gas to liquid (GTL) diesel. Diesel from these alternative sources are increasingly being developed and adopted. To be more precise and to distinguish these types, petroleum/crude oil-derived diesel is increasingly called petroleum diesel or petrodiesel (Dabelstein *et al.,* 2007; Sandhyarani, 2012).

1.2 COMPOSITION OF PETRO DIESEL

Petrodiesel (from here on referred to as diesel) is a petroleum product (Wang *et al*., 2004) that is produced from fractional distillation of crude oil between 200 °C (392 °F) and 350 °C (662 °F) at atmospheric pressure. It is a complex mixture of carbon chains that typically contain between 8 and 21 carbon atoms per molecule (Czes & Radolf, 2005; Collins, 2007) such as low molecular weight alkanes and polycyclic aromatic hydrocarbons (PAHs) (Walker *et al*., 1978; Adam & Duncan, 2002). The saturated hydrocarbons (75 %) are primarily paraffin which include; *n*, *iso* and cyclo paraffins and the aromatic hydrocarbons (25 %) includes naphthalenes and alkyl benzenes (Ogbo, 2009). Diesel also contains sulphur, nitrogen and oxygen in low concentrations as well as metals such as; copper, nickel, lead, sodium, calcium and uranium (Posthuma, 1970). The

average chemical formula for common diesel is $C_{12}H_{23}$, ranging approximately from $C_{10}H_{20}$ to $C_{15}H_{28}$.

1.3 DIESEL SPILLAGE

As mentioned previously, diesel spills are by far the largest of all products from crude oil, because it is used in many sectors, which include; transport, and non-transport/ commercial use (SAPIA, 2007). Diesel is widely used for engines of cars, generators, industrial trucks and most agricultural machineries (Walker *et al*., 1978; SAPIA, 2007). The consumption of diesel has continued to grow world-wide (Figure 1.1). The increased usage of diesel has led to an increase in accidental spillages of diesel, and consequently environmental pollution, including agricultural soil contamination (Hill & Moxey, 1960; Danbo, 1989). Agricultural soil contamination by diesel occurs through; leakages from storage containers, refuelling of farm machineries, wrecks of oil tankers and through improper disposal by mechanics when cleaning diesel tankers (Hill & Moxey, 1960; Alkio *et al*., 2005). Diesel spills have been implicated in the destruction of forests, farmlands, aquatic and terrestrial ecosystems (Alkio *et al*., 2005).

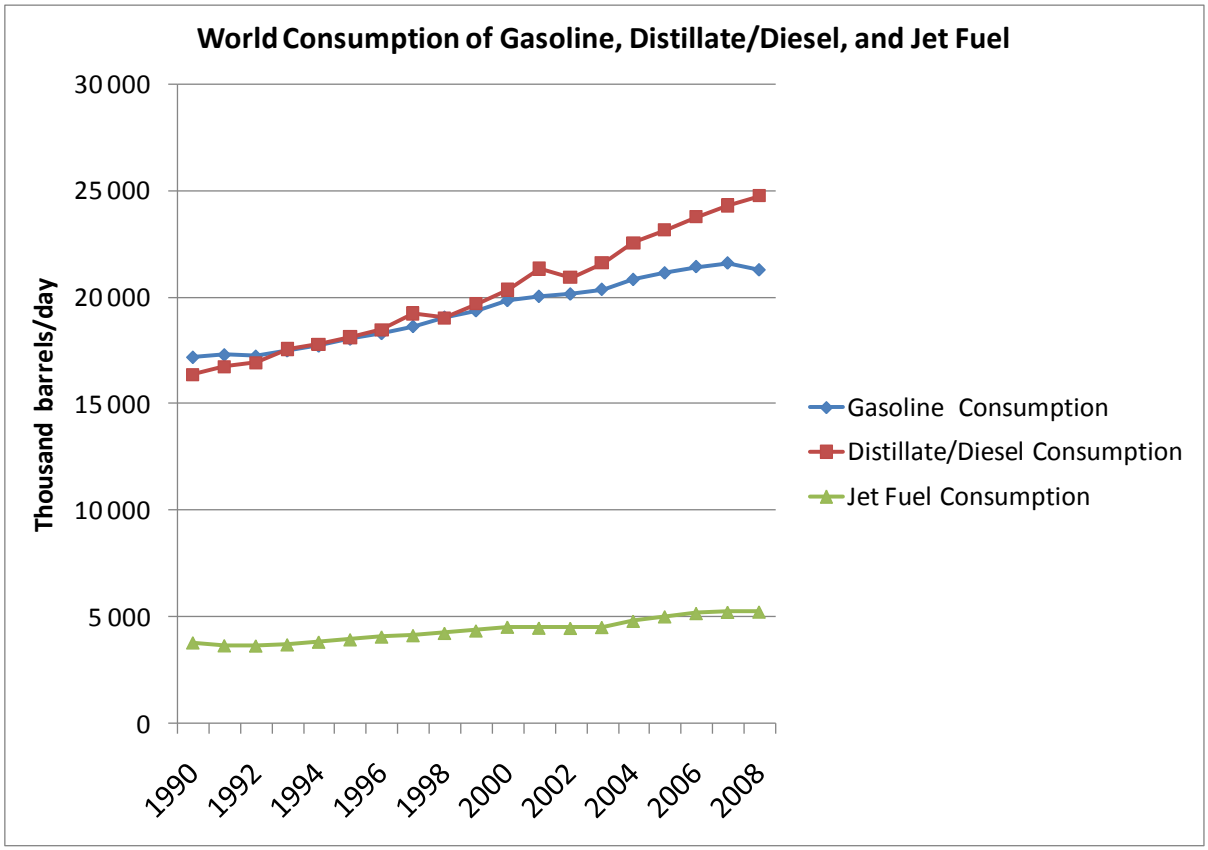

Figure 1.1 World consumption of selected petroleum products
SOURCE: EIA, 2009

Incidences of diesel spillage on agricultural and potential agricultural land abound. These include; the spillage of an estimated 676 barrels (30,000 gallons) of #2 diesel into a soy bean field in DeKalb County, Indiana, USA. The spillage exposed the lower 7 miles of the creek to diesel contamination in September, 1993 when a pipeline owned by NORCO, Inc. ruptured (USDI, 1997). Briggs, (2008) reported that after diesel theft at Huntley Gate Farm, Whalley Road, Samlesbury, the raiders left the tank's tap running, causing about ten thousand litres of diesel to spill onto the farm land. In 1993, the tank barge "OCEAN 255" and the tank barge "B-155" collided with the freighter "BALSA 37" just south of Mullet Key near the entrance of Tampa Bay, Florida, USA. Approximately 362,000 gallons of Jet 'A' diesel and gasoline were discharged into lower Tampa Bay (Tampa Bay, 1993). The diesel was later moved to the sea shore impacting a total of 240,864 square feet 5.53 acres of agricultural land (Tampa Bay, 1993). Diesel spillage may also occur directly on agricultural land under cultivation or those to be used for raising crops. Even when diesel

spillage does not occur directly on agricultural land, it is eventually moved by erosion onto agricultural land.

South Africa is especially vulnerable to diesel spills due to the high volume of diesel and other petroleum products transported around the country's coasts by ship from the Middle East to Europe and the Americas (EIA, 2000). This is in addition to spillages that do occur due to internal production, transportation, storage and usage of petroleum products. Also, if South Africa enters into oil extraction off the coast of Cape Town, the vulnerability of South Africa to spillages of diesel and other petroleum products will increase. Which will result in increased pollution and associated effects on the environment as is the case in all other oil producing countries (Fingas, 2001, Anderson, 2005; Etikerentse, 1985; ITOPF, 2011).

1.4 EFFECT OF DIESEL SPILLAGE ON SEED GERMINATION

Germination in plants is the resumption of growth of a mature seed's embryo into a seedling under the right growing conditions. The conditions include; water, oxygen, suitable temperature, and there must be no inhibitory substances present (Taiz & Zeiger, 2010). Germination also includes the re-growth of the vegetative part of a plant into an entire new plant. Germination and seedling establishment are the most vulnerable stages in the life cycle of plants (Vange *et al.,* 2004). Seed germination and seedling growth are sensitive to soil contamination/pollution by toxic substances (Asli & Houshmandfai, 2011; Ogbo, 2009). The most notable effect of soil contamination on seed germination and seedling growth is reduced elongation of the primary root (Ogbo, 2009). Thus, seedling growth is often used as an indicator of plants' ability to survive environmental pollution, including that of hydrocarbon soil contamination (Njoku *et al.,* 2009). Although considered short term, the use of seed germination in acute toxicity tests of soil contaminants has received considerable attention over the years (Millioli *et al.,* 2009).

Seed germination has been shown to decrease significantly in soils contaminated with heavy metals and other pollutants (Millioli *et al.*, 2009).

Plants that are sensitive to soil contaminants are often used as bio-indicators of soil contamination. Among the species recommended by USEPA and FDA as bio-indicators are; radish (*Raphanus sativus*); carrot (*Daucus carota*); rice (*Oryza sativa*); turnip (*Brassica rapa*); soybean (*Glycine max*); oats (*Avena sativa*); cabbage (*Brassica campestri*); corn (*Zea mays*); tomato (*Lycopersicon esculentum*); bean (*Phaseolus aureus; Phaseulus vulgaris*); onion (*Allium cepa*); sorghum (*Sorghum bicolor*), and lettuce (*Lactuca sativa*) (Fletcher, 1991).

1.5 EFFECTS OF DIESEL CONTAMINATION IN SOIL ON CROP PRODUCTIVITY

Diesel contamination in soil affects plant growth in many ways and nearly all stages of plant growth are affected. However, the effects of diesel spillage on the soil and crops may be difficult to quantify (Gelpke, 2011). The injury to plant as a result of diesel contamination may be immediate, occurring during the initial growing season or could be cumulative over a prolonged time (Gelpke, 2011).

Diesel contaminated soil has been reported to adversely affect plant growth in a number of species that have been so far investigated; e.g. *Solanum melongena* (Akujobi *et al.,* 2011), *Arachis hypogaea*, *Vigna unguiculata*, *Sorghum bicolor* and *Zea mays* (Ogbo, 2009). The higher the level of soil contamination with diesel, the worse the detrimental effects of diesel on plants growth (Akujobi *et al*., 2011). Diesel has also been reported to adversely affect crops even at low soil contamination concentration. The heavy metals in diesel have been reported to cause yield depression in maize. For example, as little as 4 ppm of Mn has been reported to cause yield depression in maize (Black, 1957).

The ability among different vegetation to recover after diesel spillage is an interaction of several factors. These factors include; the resistance of the species to the direct toxic effect of diesel, temperature, microbial activities and the moisture condition of the soil. High moisture content in the soil has been reported to accelerate recovery of diesel polluted sites (Holt, 1987; Wyszokowski & Ziolkowska, 2008).

1.6 EFFECTS OF DIESEL ON SOIL CHEMICAL FERTILITY

Soil chemical fertility which include the macro nutrients (such as nitrogen, phosphorus and potassium), trace element (manganese, zinc, copper, iron, molybdenum, boron, chloride, and cobalt), cation exchange capacity, electrical conductivity and soil pH are affected by diesel soil contamination (Obire & Nwabueta, 2002).

1.6.1 Effects of Diesel on Soil Macro Nutrients

Diesel contamination in soil generally causes deficiency in essential plant nutrients (Wyszkowski *et al*., 2004). For example, decreased plant available forms of calcium, phosphorus, potassium and nitrogen (Dimitrow & Markow, 2000; Baran *et al*., 2002; Obire & Nwabueta, 2002; Wyszokowski & Ziolkowska, 2008; Bayram *et al*., 2009), which may adversely affect plant growth (Akujobi, 2011).

Diesel contains low amount of nitrogen, hence the C/N ratio of soil contaminated with diesel is altered in a way that leads to nitrogen immobilization (Peña *et al.,* 2007). This is when microorganisms assimilate soil nutrients; tie them up in the organic form and temporarily making such nutrients unavailable to plants until the death of the micro-organisms (Aubert *et al.,* 2005). The organically bound nutrients are released and made available to plants only after the microorganisms die. For example, contamination of soil with petroleum hydrocarbon was reported to have stimulated immobilization of N derived

from mineralization of organic N, leading to reduced nitrification (Deni & Penninckx, 1999).

1.6.2 Effects of Diesel on Soil pH

The report of the effects of diesel contamination on the pH of soil has been diverse. While, some authors have reported that soil pH tends to shift to neutral values after hydrocarbon addition in both acidic and alkaline soils (Vanloocke *et al.*, 1975). For example, Obire & Nwabueta, (2002) reported that the pH of a sandy loam soil increased from 5.9 to 6.6 when it was contaminated with diesel. However, Peña *et al*. (2007) have reported that soil contamination with diesel does not alter the pH of soil when measured in KCl or may slightly decrease when measured in H_2O. The optimum pH is in the range 6.0 to 6.8 for most plants although some prefer acid or alkaline conditions (Obire & Nwabueta, 2002).

1.6.3 Diesel and Heavy Metals Accumulation in Soil

Diesel contains heavy metals (Wang, 2004). Therefore, diesel contamination may cause an increase in heavy metals in soil (Singh & Mishra, 1987). The metals cobalt, copper, iron, manganese, molybdenum, nickel and zinc, also called micro nutrients in agriculture are classified as heavy metals because of their density which is greater than 5g/ml in their standard states (Lambers *et al.*, 1998). Although essential for plants, micronutrients are toxic to plants at high concentrations (Lambers *et al.*, 1998). The other heavy metals uranium, mercury, silver and gold are toxic to plants at even lower concentrations (Lambers *et al*., 1998). Singh & Mishra (1987) reported that the contamination of soil with petroleum hydrocarbon lead to an increase in the iron, zinc, copper, manganese, lead, chromium, and nickel contents of the soil. The accumulation of heavy metals in soil has been found to retard seedling growth in rice and corn (Singh & Mishra, 1987) and radish (Khan & Frankland, 1983). Epstein (1972) reported that lead and cadmium prevent

mineral uptake in plants by antagonistic reactions. The antagonistic effects of lead and cadmium on mineral uptake in plants have been reported to negatively affect physiological activities of plants. Such physiological activities that may be affected include; photosynthesis, gaseous exchange, and nutrient absorption (Epstein, 1972). The negative effects on the physiological activities of plants could cause reductions in plant growth, dry matter accumulation and yield (Ogri, 1998; Wyszkowski *et al.*, 2004). The toxic effects of heavy metals on mineral uptake are significant at high concentration of diesel (Benson & Ebong, 2005). The toxic effects of heavy metals at lower concentrations have also been reported, for example manganese has been reported to be capable of causing yield depression in crops at 4 ppm (FMANR, 1990).

1.7 SOIL PARTICLE SIZE AND TOXICITY OF DIESEL

Soil particle size has been proven to influence the toxicity of diesel on plant. The less volatile fraction of diesel, which is about 80-90% of diesel hydrocarbons, causes significant deterioration in soil physical properties such as colour (Luthy *et al*., 1997; Atkinson & Arey, 2003). Generally, metals accumulate in the clay fraction of the soil because clay particles have a large number of ionic binding sites due to high surface areas (Epstein, 1972). Hence, diesel soil contamination is more detrimental in clay soil when compared with sandy soil. Metals can also be bound to the organic matter content of the soil, and this is gradually released during the process of mineralization (Epstein, 1972). Diesel soil pollution is capable of affecting soil physical condition, which is one of the variables that influence crop production (Hardy *et al*., 2011). McGrath (1992) reported that the addition of diesel to soil with different permeabilities/porousities to diesel in a pot experiment depressed the growth of grass in the sequence: peat<silty clay<loam=clay loam=sandy loam<loamy sand.

1.8 EFFECT OF DIESEL ON SOIL MICROBIAL ACTIVITY

It is generally known that microbial population in soil is adversely affected by diesel soil contamination (Zucconi *et al.*, 1981; Young *et al.*, 1992; Richard & Vogel, 1999). It has been reported that microbial population increases after moderate application of diesel (Linkins *et al.*, 1972). The increase in soil microbial population after moderate application of diesel is attributable to the fact that diesel contamination provides a source of carbon for soil microorganisms (Frankenberger, 1989). However, not all soil microorganisms can use the carbon in diesel (Aislabie *et al.*, 2004). Thus, diesel hydrocarbons cause changes in the structure of the microbial community (Niewolak & Koziello, 1998; Megharaj *et al*., 2000) in addition to a general decrease in the diversity and number of microorganisms (Lindstrom *et al*., 1999).

1.9 DEGRADATION OF DIESEL IN SOIL

Spilt diesel has been reported severally as soil contaminant (Black, 1957; Ben *et al.*, 1996; Cunningham & Philip, 2000; Fatima *et al*., 2004; Bento *et al.*, 2005; Adedokun & Ataga, 2007). The aromatic hydrocarbons of diesel, in particular the polycyclic aromatic hydrocarbons (PAH), are poorly mobile in soil (Galas *et al*., 1997). They can have long-term adverse effects on soil, plants or ground water (Sparrow & Sparrow, 1988; Racine, 1994; Wyszkowska *et al*., 2004).

When petroleum products are accidentally released to the environment, they are immediately subjected to a wide variety of degradation processes. The degradation processes include, evaporation, dissolution, dispersion, emulsification, and adsorption on suspended materials, microbial degradation or photo oxidation (Brookes & Verstraete, 1989; Wang *et al*., 2004; Onuoha *et al.*, 2011). Degradation of diesel mainly depends on the soil biological and biochemical properties (diversity, number and activity of

microorganisms present and the activity of intra and extracellular enzymes) (Joergensen *et al*., 1995; Wyszkowski & Wyszkowska, 2005; Peña *et al.,* 2007). Shortly after a spill, volatilisation is the main origin of the decrease in diesel contamination by aliphatic hydrocarbons in agricultural soils. Serrano *et al.* (2007) reported that evaporation caused a dramatic decrease in diesel concentrations in the soil within 18 days after the spill.

Physical degradation of diesel hydrocarbons in soil is influenced by the prevailing climatic conditions, such as abundant rain and high temperature (Serrano *et al.,* 2007). However, diesel soil contamination may remain persistent in the environment for a long period. Serrano *et al*. (2007) reported that, 3 months after a diesel spill, the soil biological activity failed to return to its pre-contamination condition, probably because of the persistence of the less volatile fraction of diesel. After diesel contamination in soil, the majority of aliphatic hydrocarbons remain in the surface layer (0-10 cm) of the soil (Serrano *et al.,* 2007). Therefore, soil remediation can probably be improved by tilling or adding water, which facilitates evaporation of these compounds (Hejazi & Husain, 2004). Biodegradation; which is the removal of diesel by microbial activities, is the main source of removal of the more persistence fraction of diesel (Hejazi & Husain, 2004). Hence, remediation of diesel contaminated soil can be expedited by using appropriate microorganisms. In addition, an optimal soil pH for microbial activities, moisture and temperature level for biodegradation are required to increase enzymatic activities involved in diesel biodegradation (Hejazi & Husain, 2004; Serrano *et al.*, 2007).

Some microorganisms can utilise the hydrocarbons in diesel. Hence, one of the best approaches to resolve the issue of soil contamination with diesel is to make use of microorganisms (Fatima *et al.,* 2004). Such microorganisms have been found to be effective in reducing and transforming petroleum hydrocarbon to less toxic compound (Millioli, 2009). Diesel biodegradation in soil could be promoted by the stimulation of the

indigenous microorganisms by introducing nutrients and oxygen into the soil through a process called bio stimulation (Seklemora *et al.,* 2001). Biodegradation can also be promoted by the inoculation of an enriched microbial consortium into the soil; the process is called bio augmentation (Richard & Vogel, 1999; Barathi & Vasudevan, 2001).

There is a range of remediation techniques available for contaminated sites. However, *in situ* bioremediation and natural attenuation are considered two of the most environmentally friendly options (Serrano *et al.,* 2008). Example of *in situ* bioremediation include; stimulation and augmentation of the activity of indigenous microorganisms by supplying oxidants and nutrients in various compounds). *In situ* bioremediation and natural attenuation preserve the soil structure and require little energy input (Serrano *et al.,* 2008). Many microorganisms can use complex pathways to degrade diesel hydrocarbons (Dibble & Bartha, 1979; Young *et al.,* 1992). Hence, when diesel contamination occurs in the presence of microorganisms capable of degrading them, and under environmental conditions (temperature, humidity, nutrient content, etc.) that are suitable for optimum microbial activities, the contamination can be reduced naturally, without any external intervention (Peña *et al.,* 2007). Processes such as hydrolysis and photo decomposition can also lead to degradation of diesel (Young *et al.,* 1992). Bioremediation techniques are often used when accelerated degradation of contaminants is required (Margesin & Schinner, 1999). The techniques consist of optimizing soil conditions to increase microbial metabolic activity and or adding microorganisms that are capable of degrading a particular contaminant (Peña *et al.,* 2007).

Phytoremediation uses plants and their associated microorganisms to restore or recover the degraded areas. Recent studies have shown that an increase in the degradation of diesel hydrocarbons occurs in the soils inhabited by plants that are tolerant of diesel (Muratov *et*

al., 2003; Molina-Barahona *et al*., 2004; Barruti *et al*., 2011). Plants with high biomass production are better for phytoremediation (Lambers *et al.,* 1998). Njoku *et al.* (2009) reported that some toxic substances in diesel spill may evaporate quickly, therefore, plant, animal, and human exposure to the most toxic substances are reduced with time, and are usually limited to the initial spill area. However, it must be noted that the growth of crops in diesel contaminated soil, even though it affects the physico-chemistry of the soil leading to reduction in the phyto-toxic effects, may not necessarily lead to a reduction in the concentration of the hydrocarbon in the soil (Njoku *et al.,* 2009).

1.10 SCOPE OF THESIS

Although the effect of diesel on plant growth have been investigated in a number of species, e.g. *Madicagolu pulina, Brassica napus* (Adam & Duncan, 2002) and *A. hypogaea, V. unguiculata, S. bicolor, Z. mays* (Ogbo, 2009), there has not been any work that compares the effects of diesel on seed germination versus vegetative germination of crops that are established from vegetative cuttings. Also, none of the work so far done has attempted to establish the critical concentration of diesel in the soil for toxicity to plants. This study makes a comparison of the effects of diesel contamination in soil on germination and growth of lettuce (*L. sativa*) and sweet potato (*I. batatas*). Sweet potato is generally established through cuttings. Hence, the germination of the cuttings is an important process that determines the establishment of the crop. Lettuce is one of the crops recommended by USEPA and FDA as bio indicators of soil pollution because of its sensitivity to soil pollutants (Fletcher, 1991).

Diesel is considered to be the most toxic component of artificial refinery mixtures (Buikeima *et al*., 1981). It is potentially more destructive to plants than crude oil (Lawson *et al.,* 1972; McCrown *et al*., 1972 & Walker *et al*., 1978). It has drastic effects on

vegetation, causing reduction in plant productivity, and up to 100 % mortality in some instances (Nyman *et al.*, 1999; Njoku *et al.*, 2009). Soil contamination with diesel, has been found to reduce seed germination and root length of peanut, cowpea, sorghum and corn (Ogbo, 2009), and accumulation of heavy metals in the tubers of cassava (Amatya, 2002), but no work or at best very little work has been reported on the effects of diesel spillage on the productivity of *L. sativa* and *I. batatas*. Hence, *L. sativa* (a leafy vegetable) and *I. batatas* (a root crop) were used as test crops in this study.

The aim of this research was to examine and compare the effects of diesel spillage on soil in relation to the production of *I .batatas* and *L. sativa.*

The general objectives of this study were:

i. To determine the effects of diesel spillage on biological and chemical fertility of soil.
ii. To determine the effects of diesel spillage on the germination and growth of sweet potato and lettuce.
iii. To compare the effects of diesel contamination in soil on the germination and growth of lettuce and sweet potato.

The hypotheses that were tested in this study were:

i. Diesel contamination in soil has adverse effects on biological and chemical fertility of soil.
ii. Diesel contamination in soil affects the germination and growth of sweet potato and lettuce to different extents.

The null hypotheses are:

i. Diesel contamination in soil does not have any effect on biological and chemical fertility of soil.
ii. The effects of diesel contamination in soil on the germination of lettuce and sweet potato are not different.

The specific objectives are presented in details for each experiment in the introductions of the pertinent chapters. It must also be noted that diesel as being used in this research is petroleum diesel because there are other minor types of diesel.

1.11 REFERENCES

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CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

Two experiments were conducted to investigate the effects of diesel contamination in soil on lettuce and sweet potato. The first experiment investigated the effect of diesel contamination in soil on the germination of lettuce and sweet potato. The second experiment tested the effect of diesel contamination in soil on the growth and dry matter partitioning in lettuce and sweet potato. The two experiments involved; simulation of diesel contamination in soil, diesel chemical analyses, and the analysis of soil physical and chemical properties. This chapter contains the detailed descriptions of the general procedures relating to simulation of diesel contamination, diesel analyses, soil analyses and leaves analyses. Some methods which have been adequately included in the relevant chapters have been omitted from this chapter to avoid repetitions.

2.2 SIMULATION OF DIESEL CONTAMINATION IN SOIL

2.2.1 Study Area and Materials Sourcing

The study was conducted under controlled environment at the University of Zululand (28° 51′ 06″ S; 31° 51′ 08″ E; altitude 102 m) in the KwaZulu Natal province of South Africa. Agriculturally productive soil was collected from the university's farm, air dried, homogenized using a spade, and sieved through a grid wire mesh to remove stones.

The diesel used for soil contamination treatments was obtained from Zulu Fuel at Empangeni, in KwaZulu Natal Province of South Africa.

2.2.2 Simulation of Diesel Contamination in Soil

The simulation of diesel contamination was performed in 20 kg of potted soil for each diesel concentration treatment. Uniformity of soil contamination with diesel was achieved by thoroughly mixing the soil with the diesel manually using a spade.

2.3 DIESEL CHEMICAL ANALYSIS

2.3.1 Extraction of Total Petroleum Hydrocarbon (TPH)

Into a beaker was weighed 10 g of diesel and 0.1 ml of squalene (internal Standard) was added. Added to the solution was 30 ml of hexane: dichloromethane (3:1) mixture and the mixture were transferred into a separating funnel. The funnel was shaken intermittently, while the funnel stopper was removed to release pressure; this was done five times, and then left to settle. The entire organic component in the diesel sample by now had moved into the solvent mixture and this was collected from the funnel.

A column was prepared using activated silica gel (80–120 mesh) chromatographic grade. The column was dry packed using n-hexane. The extract from the funnel was run into the silica gel column and the biogenic hydrocarbons were adsorbed while only the petro genic hydrocarbons passed through the column. Nitrogen gas was passed over the eluate to reduce the volume and, therefore, concentrate the eluate. The eluate collected was then divided into two parts: One part was run directly on the gas chromatograph (GC) to determine TPH, while the other half was passed through an alumina column to collect the aromatic hydrocarbons (Winefordner & Speight, 2005).

2.3.2 Extraction of Polynuclear Aromatic Hydrocarbons (PAH)

An alumina column was prepared by using activated alumina (80–120 mesh) chromatographic grade. The column was dry packed using hexane. The TPH eluate was poured into the column and first eluted with 20 mls of hexane. This was to remove all aliphatic components of the TPH which comes out with the hexane. Then, 30 ml of hexane: dichloromethane (3:1) mixture was added to the column to elute the aromatic hydrocarbons component of the eluate which was then run on the gas chromatograph (Pavlova *et al.*, 2004).

2.3.3 Gas Chromatograph Analysis (GC)

A 1.5 µl of sample of the diesel was analysed for TPH by a GC (PH5890) equipped with a flame ionisation detector. A 200 cm glass column packed with 3 % OV 101 Chromosorb WHP on 80 – 110 mesh was used to resolve the components. The column temperature was between 100-250 °C. Nitrogen was used as carrier gas with a flow rate of 50 ml/min. Hydrogen and air flow rates were 40 ml/min and 300 ml/min, respectively. Injector port and detector temperature were 250 °C and 320 °C, respectively. Squalane (one of DPR's recommended Internal Standard) (DPR, 1991) was used for quantification.

2.3.4 Heavy Metals Determination in Diesel

Into a platinum crucible was placed 1.0 g of diesel and ashed in a furnace at a temperature of 800 °C for 2 hours. The crucible was allowed to cool down. The residue in the crucible was dissolved in dilute hydrochloric acid and the solution was transferred to a 100 ml volumetric flask and made up to mark. The solution was then run on a Buck 210 Atomic Absorption Spectrophotometer (Buck Scientific, Inc, Norwalk CT., 2011) to determine the concentration of iron (Fe), zinc (Zn), chromium (Cr) , lead (Pb), copper (Cu), cadmium (Cd), mercury (Hg) and vanadium (V).

2.4 ANALYSES OF SOIL CHEMICAL AND MICROBIAL PROPERTIES

2.4.1 Soil Chemical Analyses

2.4.1.1 Determination of soil pH

Twenty grams of air-dried soil which had been sieved with a 2 mm sieve was weighed into 50 ml beaker and 20 ml distilled water was added. The content was stirred vigorously for 15 seconds using a glass rod and allowed to stand for 30 minutes. The pH reading was taken by inserting the electrode of a pH meter in the slurry (Mendham *et al.,* 2000).

2.4.1.2 Determination of soil nitrogen

Kjedahl method was used to determine soil total nitrogen (Bremner, 2009).

2.4.1.2.1 Digestion

One gram of soil was put inside a 100 ml calibrated digestion tube, and 5 g of catalyst mixture and 15 ml concentrated sulphuric acid were then added and the mixture swirled carefully. The mixture was left in the tube overnight. The next day, the mixture was heated in a block digester to about 370 °C until it became clear. Heating was continued for about 3 hours, and the tubes taken out of the block-digester. The tubes were allowed to cool to room temperature after which deionised water was added to the clear solution to bring it to volume. Each batch of soil samples for digestion contained at least one sample blank (no soil) and one reference standard.

2.4.1.2.2 Distillation and titration

Ten millilitres of the clear solution/digest was transferred to a 100 ml distillation flask to which10 ml of 10 N sodium hydroxide solution was added. The flask was immediately attached to a distillation unit with a clamp. The distillation was done for about 4 minutes and the distillate was captured in a flask. Titration of the excess acid with standard sodium hydroxide solution to yellow endpoint (colour change from red to orange to yellow) was then performed.

2.4.1.3 Determination of mineral nutrients for plants in soil

The soil sample was prepared using the method of Allen *et al.* (1974) as described earlier. Into a 250 ml reaction vessel was weighed 3 g of the soil sample. The sample was moistened with 1ml of water and 21 ml of hydrochloric acid (HCl) was added. A quantity of 7 ml of nitric acid was added drop by drop to avoid foaming. The mixture was made to stand for about 16 hours at room temperature to allow for slow oxidation of the organic matter. The temperature of the reaction mixture was raised slowly until reflux condition

was reached and the temperature maintained for 2 hours. The mixture was then allowed to cool. This is to enable any insoluble residues to settle out of the suspension. The relatively sediment free supernatant was filtered through a #1 whatman filter paper. The filtrate was collected in a 100 ml volumetric flask. The insoluble residue at the bottom of the centrifuge tube was washed with 0.5 mol/l of nitric acid. The elements in the extract were determined by flame absorption spectrophotometer.

2.4.2 Soil Particle Size Analysis

Forty grams of sodium polymetaphosphate [$(NaPO_3)_{13}$], and 10 g of sodium carbonate (Na_2CO_3) was dissolved in DI water, and the solution was brought to 1 litre volume with DI water to form the dispersing solution. Soil samples were air dried and passed through a 2 mm sieve. For each sample, 40 g of the soil was weighed into a 600 ml beaker and 60 ml of the dispersing solution was added. The beaker was covered with a watch glass and kept overnight. The following day, the suspension in the beaker was transferred into a soil stirring cup and filled to three-quarters with deionised water. The suspension was stirred for 3 minutes on a stirrer and then transferred into a 1-l calibrated cylinder (hydrometer jar), and made up to volume with deionised water (Bouyoucos, 1962).

Dispersing solution (60 ml) was transferred into 1-l hydrometer jar and made to volume with water. It was thoroughly mixed and a hydrometer was inserted to take the reading ($R_{b)}$.

R_b = First hydrometer reading (used to determine the value for blank). The blank was used to calibrate the hydrometer. The amount of sand, silt and clay were determined thus:

2.4.2.1 Determination of silt and clay

Amyl alcohol (10 ml) was added to the suspension in the hydrometer jar and with the aid of a paddle the suspension was mixed. The paddle was then withdrawn carefully and the hydrometer was inserted to take the reading - $R_{sc.}$

% (silt + clay) = ($R_{sc.}$-R_b) X 100/oven dry soil (g)

R_{sc}= Second hydrometer reading (for the determination of the value of silt + clay).

R_b = First hydrometer reading (used to determine the value of blank).

2.4.2.2 Determination of clay

The suspension in the hydrometer jar was mixed with a paddle. The paddle was then withdrawn with care. The hydrometer was then inserted after 4 hours, and the third hydrometer reading (R_c) was taken.

% clay = Rc - R_bX 100 /oven dry soil (g)

% silt = [% (silt + clay] – [% clay]

R_c= Third hydrometer reading.

R_b = First hydrometer reading (used to determine the value of blank).

2.4.2.3 Determination of sand

The suspension was poured through a 50 μm sieve after taking readings required for clay and silt. Deionised water was passed through the sieve until water passing the sieve was clear. The sand was then transferred from the sieve to a 50 ml beaker of known weight and allowed to settle, after which the excess water was decanted. The sand was oven dried for about 12 hours at 105 °C and cooled in desiccators and weighed.

% sand = Sand weight X 100 /oven dry soil (g).

2.4.3 Determination of Soil Microbial Biomass

Rapid chloroform fumigation-extraction method was used to determine microbial biomass (Witt *et al*., 2000).

2.4.3.1 Preparation of fumigated soil sample

Into a glass beaker was weighed 20 g moist soil. It was then placed in vacuum desiccators with a small quantity of water in the bottom to prevent drying of soil samples during

fumigation. Another beaker containing 50 ml ethanol-free chloroform and few boiling chips were also placed in the desiccators. The desiccators were then evacuated and the chloroform was allowed to boil vigorously for 2 minutes. The boiling treatment was repeated twice within 12 hours. The vacuum valve of the desiccators was closed and left for 48 hrs from period of first fumigation. After this period (48 hours), the vacuum was opened for 5 minutes to remove the chloroform.

2.4.3.2 Preparation of non-fumigated soil sample (control)

Into a beaker was weighed 20 g of moist soil. The beaker was placed in vacuum desiccators with a small quantity of water in the bottom to prevent drying of soil samples during fumigation.

2.4.3.3 Extraction of Carbon for controls and fumigated samples

Into a beaker was weighed 5 g of soil (3 replicates). Twenty millilitres of 0.5 M potassium sulphate ($K_2SO_{4)}$ was then added and shaken on an end-over shaker for 1 hour. The mixture was then centrifuged at 1200 rpm for 5 min and the extract filtered.

2.4.3.4 Analysis of carbon in the fumigated and non-fumigated samples

Two blanks were prepared (one was digested with the sample and the other was not digested) using 8 ml K_2SO_4 in blanks. Into digester tubes were pipetted 8 ml of filtered extract and 10 ml of 0.05 M potassium dichromate ($K_2Cr_2O_7$) was added and shaken, after which10 ml of sulphuric acid was added and shaken. The tubes were then placed in a block digester which was preheated to 150 °C in a fume cupboard with extractor fan running. The digestion was done for 30 minutes and the tubes were removed and allowed to cool for 15 minutes. The timing (30 minutes) was measured from the time of boiling. The content of the tube was transferred to a 100 ml erlenmeyer flask using sufficient deionised water (to 100 ml mark).The diluted digest was allowed to cool to room

temperature and 0.3 ml of diphenylalamine indicator was added. Titration was done with 0.1 M ferrous ammonium sulphate (the end point was purple green). The same was done for the blanks.

2.5 LEAF TISSUE ANALYSIS

2.5.1 Leaf Sample Preparation

Youngest fully matured leaf (YFML) or most recently matured leaves were used for tissue chemical analysis of lettuce and sweet potato. The YFML were oven dried at 65° until constant weight. The leaves were ground to pass a 1.0 mm screen. After grinding, the samples were thoroughly homogenized, placed in securely sealed sample bottles, labelled and stored in a refrigerator maintained at 4 $^{\circ}$C (Jones *et al.*, 1991). The brush and vacuum systems were used to clean the grinding apparatus after grinding each sample. Representative sub samples were taken and wet digested for analysis.

2.5.2 Accelerated Wet Digestion of Leaf Samples

For each sample, a quantity of 0.5 g of the dried plant material was placed in a 50 ml erlenmeyer flask to which was added 10 ml of concentrated nitric acid. The mixture was digested in a microwave oven for 30 minutes at 30 % power (210 watts). The sides of the erlenmeyer flasks were flushed with 30 % hydrogen peroxide (H_2O_2) after which the sample was digested for 5 minutes at 60 % power (390 watts).While the sample was still warm, deionised water was used to fill the flask to 50 ml volume and the digest well shaken. The digest was filtered through a #1 whatman filter paper. An aliquot of 10 ml was transferred to a centrifuge tube and analysed with Inductively Coupled Plasma Atomic Emission Spectrometer (ICPAES).

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CHAPTER 3

PHYTOTOXICITY OF DIESEL CONTAMINATION IN SOIL ON THE GERMINATION OF *LACTUCA SATIVA* AND *IPOMOEA BATATAS*

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Abstract

The phytotoxic effects of diesel contamination in soil on the germination of *Lactuca sativa* and *Ipomoea batatas* were investigated and compared at two diesel contamination concentration ranges, 0-6 ml/kg soil and 0-30 ml/kg soil in a germination chamber. Diesel contamination in soil was simulated and soil samples were taken from the contaminated soil at; 1, 5, 10, 15, 25, 50, 75 and 100 days before planting. The result indicated that the effect of diesel contamination in soil on the germination of both species varied with the diesel concentration and the age of contamination. In both species, diesel inhibited germination in a concentration dependent manner showing increased inhibition with increasing concentration of diesel contamination. Also, the influence of diesel contamination in soil diminished with the age of contamination, suggesting possible reduction in diesel toxicity over time. However, the germination of lettuce ($r^2 = 0.941$) was more negatively correlated with diesel contamination in soil than that of sweet potato ($r^2 = 0.638$), while, the critical concentrations of diesel for toxicity in relation to seed germination of *Lactuca sativa* were lower than that of *Ipomoea batatas,* indicating that the germination *of Ipomoea batatas* is less sensitive to diesel soil contamination than that of *Lactuca sativa.*

Keywords: diesel, germination, *Lactuca sativa, Ipomoea batatas,* critical concentrations, phytotoxicity, simulation, soil contamination.

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3.1 INTRODUCTION

One of the most serious environmental/soil pollution problems confronting the world is oil spillage (USEPA, 2000). Oil pollution problems have largely been associated with impacts on sea and coastal land environments of large oil spillages from tanker ships. This is because such spillages receive a lot of attention from the public and the media (ITOPF, 2011). Little attention is given to 'minor' spillages of petroleum products among which are diesel on inland environments such as agricultural land. Inland pollution from petroleum products are far more complicated, unpredictable and constitute the vast majority of oil spills in many countries (Fingas, 2001). Diesel spills are by far the largest of all products from crude oil (SAPIA, 2007). This is because it is used in many sectors, which include transport and non-transport/commercial uses. The pressure to feed the ever growing world population will lead to increased use of machinery on the farms. The increased use of machinery is expected to lead to more spillages of diesel on the farms and the associated effects on cultivated crops.

Among the methods used to test for phytotoxicity of hydrocarbons contaminated soil is seed germination (Marwood *et al*., 1998; Asli & Houshmandfar, 2011). This is because seed germination is the most sensitive stage of plant growth to environmental pollution, including heavy metals pollution (Millioli *et al.,* 2009). Hence, seed germination is often used as an indicator of plant's ability to survive hydrocarbon pollution (Njoku, 2009). Examples of such plants include; tomato, rice and turnip (Fletcher, 1991). The inhibition of germination in oil polluted soil (Bona *et al.,* 2010) has been attributed to the volatile fraction of oil (Serrano *et al.,* 2007) that penetrates the seed coat and kills the embryo (Ogbo, 2009). In addition, inhibition of germination in oil contaminated soil arises from oil sheen coatings on seeds that prevent water and air entry into the seed (Baker, 1971).

Unfavourable soil conditions such as surface crust and increase in soil temperature, which are related to soil contamination by petroleum products, have also been reported to hinder seed germination (Njoku *et al.,* 2009). There are several reports on the adverse effects of diesel on seed germination, for example, reduction in seed germination of peanut, corn, cowpea and sorghum (Ogbo, 2009), safflower and corn (Asli & Houshmandfar, 2011). Ogbo (2009) and Asli & Houshmandfar, (2011), further reported that the reductions in germination as a result of soil pollution with diesel were dependent on level of diesel concentration in soil and the crop species.

Among the crops that are sensitive to soil pollutants, *Lactuca sativa* (lettuce) has been recommended by USEPA and FDA for use as a bio-indicator of soil pollution (Fletcher, 1991). However, despite the recommendation of lettuce for acute toxicity test by USEPA and FDA and the fact that diesel is the most toxic and the most likely among petroleum products to be spilt on agricultural land, no work has been done on the effect of diesel soil contamination on the germination of lettuce. Also there has been no attempt to compare the effects of diesel contamination in soil between the germination of seed and vegetative propagated crops. The objective of this study was to compare the effects of diesel concentration in soil contamination and age of the contamination on seed germination of *Lactuca sativa* and vegetative germination of *Ipomoea batatas*. Three hypotheses were tested:

i) Diesel is toxic on the germination of *Lactuca sativa* and *Ipomoea batatas.*

ii) The toxic effects of diesel are positively correlated with diesel concentrations and negatively correlated with the age of contamination.

iii) Seed germination of *Lactuca sativa* and the vegetative germination of *Ipomoea batatas* differ in sensitivity to diesel contamination.

3.2 MATERIALS AND METHODS

3.2.1 Study Area and Materials Sourcing

The study was conducted under controlled environment at the University of Zululand (28° 51′ 06″ S; 31° 51′ 08″ E; altitude 102 m) in the KwaZulu Natal province of South Africa. Diesel used was sourced and analysed as described in section 2.2.

3.2.2 Diesel Contamination in Soil Treatments

The simulation of diesel contamination in soil was done as described in section 2.2.2. There were two sets of incremental diesel concentrations treatments that were tested at two separate concentration ranges. At diesel concentration 0-30 ml/kg soil, the diesel contamination treatments were: 0, 5, 10, 15, 20, 25 and 30 ml/kg soil. At diesel concentration range 0-6 ml/kg soil, the diesel concentration levels tested were: 0, 1, 2, 3, 4, 5 and 6 ml/kg.

3.2.3 Soil Physical and Chemical Analyses

A composite sample was taken for chemical analyses from the potted soil before and after diesel contamination treatments from each of the treatment replicates. The hydrometer method (Bouyoucos, 1962) was used to determine the relative amounts of soil separates (sand, silt and clay), and the soil textural class was determined based on the USDA-FAO soil textural triangle (FAO, 1990). The samples were tested for pH using the method of Bates (1954). Total carbon and nitrogen were analysed by the Automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation USA, Michigan, USA; Matejovic, 1996) using vanadium pentoxide as catalyst. Phosphorus, potassium, zinc, copper, and manganese were extracted using Ambic-2 extracting solution (0.25 M ammonium carbonate (NH_4CO_3) + 0.01M disodium ethylene diamine tetra acetate

(NaEDTA) + 0.01M ammonium fluoride (NH4F) + 0.05 g L^{-1} Superfloc). From the extract; potassium, zinc, copper and manganese were determined by atomic absorption and phosphorus was determined using a modification of the Murphey & Riley (1962) molybdenum blue procedure (Hunter, 1974). Acidity, calcium and magnesium were extracted with potassium chloride solution. Calcium and magnesium were determined by atomic absorption. Rapid chloroform fumigation-extraction method was used to determine microbial biomass (Witt *et al.*, 2000).

3.2.4 Lettuce Seed Germination

A quantity of fresh lettuce seeds were placed in a beaker containing distilled water to test for viability, using the floatation method (Bell *et al.*, 1993). Seeds that floated were considered non-viable, and were discarded. Only the seeds (full and healthy) that sank to the bottom of the beaker were used for further experimentation.

Thirty grams of soil were drawn at 1, 5, 10, 15, 25, 50, 75, and 100 days, after the application of diesel from each of the three replicates of the simulated diesel contamination treatments in both 0-6 ml/kg soil and 0-30 ml/kg soil diesel concentration ranges and placed in petri dishes. The soil was moistened with distilled water after which ten seeds of lettuce were placed on top of the moist soil and incubated in a seed germination chamber for two weeks at 28 °C in light (Serrano *et al.*, 2007). The effects of diesel contamination concentrations and age of diesel contamination on the germination of lettuce were investigated at; 100 (D100), 75 (D75), 50 (D50), 25 (D25), 15 (D15), 10 (D10), 5 (D5) and 1 (D1) days before planting. Germinated seeds were counted at 14 days after seeding. Seeds that had not germinated at 14 days after the start of incubation were considered not germinable.

3.2.5 Vegetative Germination of Sweet potato

Three hundred grams of soil from each diesel contamination treatment levels were drawn at 1, 5, 10, 15, 25, 50, 75, and 100 days, after the application of diesel from each of the three replicates and placed in 350 ml Styrofoam cups. The soil was moistened with distilled water. Five cuttings of sweet potato were then planted in the soil in each of the Styrofoam dishes, each cutting bearing 3 buds. The cuttings were planted in such a way that two of the buds were buried in the soil and one bud was above the soil. The cuttings were incubated in a germination chamber for two weeks at 28 °C in light (Serrano *et al.*, 2007).

The cuttings were examined for germination at 14 days after planting. Those that failed to germinate after 14 days were considered as not germinated (Amadi, 1992). The effects of diesel concentration in soil on the germination sweet potato were investigated at the following ages of diesel soil contamination: 100 (D100), 75 (D75), 50 (D50), 25 (D25), 15 (D15), 10 (D10), 5 (D5) and 1 (D1) days. Seed germination was expressed as a percentage

3.2.6 Statistical Analysis

The germination data were first subjected to ANOVA procedure using GenStat Release 12.1 (PC/Windows Vista) (VSN International Ltd., 2009). Means of the treatments were separated by least significant difference at 5 % level ($LSD_{0.05}$). However, because the treatments involved manipulating a gradient of quantitative levels (incremental diesel concentration), the data was also subjected to correlation and regression analyses. Thus, mathematical functions expressing correlations and regression relationships between diesel contamination concentration in soil and the germination of lettuce and sweet potato were obtained using the curve fitting programme of Table Curve 2D v5.01.01 (Systat Software Inc., San Jose, CA, USA, 2002). From these regression relationships between

diesel concentration and germination of lettuce and sweet potato were interpolated the critical diesel concentration for toxicity for each crop.

3.3 RESULTS

3.3.1 Diesel and Soil Analysis

The polynuclear aromatic hydrocarbon and the BTEX represented 0.37 % and 0.0021 % of the total hydrocarbon in the diesel, respectively. The PAH varied widely, ranging from 1775.53 ppm in phenanthrene to 1.05 ppm in dibenzo(a,h) anthracene (Table 3.1). The BTEX in the diesel was mainly the xylene isomers representing about 58.29 % of the total BTEX in the diesel used (Table 3.1). The contents of the heavy metals in the diesel were generally low, ranging from 7.18 ppm for V to 2.33 ppm for Fe (Table 3.2).

The changes in the macro and heavy metals did not vary to a great extent and also did not follow a definite pattern. Though some significant changes could be seen in the values of same nutrient, all the nutrients, OC and pH were within the optimum range required for the growth of lettuce and sweet potato (Table 3.3). The soil used for the experiment was sandy loam containing 18 % clay, 8 % silt and 74 % coarse silt and sand.

Table 3.1 Hydrocarbon composition of the diesel used

HYDROCARBON	HYDROCARBON COMPONENTS	CONCENTRATION (ppm)	TOTAL (ppm)
POLYNUCLEAR AROMATIC HYDROCARBONS (PAH)	Naphthalene	1438.9156	3,693.6762
	Acenaphthylene	52.9216	
	Acenaphthene	77.1736	
	Fluorene	243.5930	
	Phenanthrene	1775.5283	
	Anthracene	24.4193	
	Fluoranthene	13.6358	
	Pyrene	15.9533	
	Benzo(a) anthracene	12.6010	
	Chrysene	30.4386	
	Benzo(b)fluoranthene	3.0563	
	Benzo(k)fluoranthene	2.0229	
	Benzo(a) pyrene	2.6208	
	Indeno{1,2,3-cd}pyrene	3.8619	
	Dibenzo(a,h)anthracene	1.0537	
	Benzo(g,h,i)perylene	3.0448	
BTEX	Benzene	2.8691	20.3460
	Toluene	3.4002	
	Ethyl Benzene	2.2181	
	P-Xylene(C8H10)	2.0107	
	M-Xylene(C8H10)	5.9796	
	0-Xylene(C8H10)	3.8686	
ALIPHATIC HYDROCARBONS AND OTHERS			984530.9680
TOTAL PETROLEUM HYDROCARBON (TPH)			9.88245×10^5

Table 3.2 Concentrations of some heavy metals in the diesel used

HEAVY METALS (ppm)	CONCENTRATION (ppm)
Fe	2.3318
Zn	1.9371
Cr	0.3637
Pb	2.6427
Cu	0.5211
Cd	0.0013
Hg	0.0003
V	7.1834
Ni	2.1526
Ba	0.4266

Table 3.3 Soil chemical analyses after diesel treatment at 0-30ml/kg soil contamination concentration range

ml diesel/kg soil	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	pH	Zn ppm	Mn ppm	Cu ppm	OC %	N %
0	350.00	143.33	1646.00	261.33	6.26	87.03	3.00	15.80	3.37	0.24
5	333.33	221.67	1670.33	255.00	6.40	79.80	2.33	13.33	2.87	0.22
10	330.00	236.67	1586.33	248.00	6.19	86.47	2.67	14.53	3.97	0.27
15	330.00	233.00	1549.33	258.67	6.08	87.77	2.67	14.93	3.83	0.29
20	353.33	236.67	1506.33	241.67	6.17	90.70	3.00	15.53	4.20	0.29
25	353.33	274.00	1499.00	245.67	6.15	94.37	3.33	15.87	5.60	0.40
30	396.67	249.67	1531.67	236.67	6.20	83.03	4.00	16.00	5.27	0.41
mean	**349.52**	**227.86**	**1569.86**	**249.57**	**6.21**	**87.02**	**3.00**	**15.14**	**4.16**	**0.30**
$Lsd_{0.05}$	**40.95**	**39.30**	**129.40**	**30.90**	**0.20**	**8.76**	**0.76**	**2.35**	**0.91**	**0.08**
CC/SR	**110-365**	**120-500**	**800-1800**	**219-438**	**5.5 - 7.0**	**30-150**	**20-30**	**20-30**	**≥0.20**	**≥0.20**

CC/SR = Critical concentration/sufficiency range, OC = organic carbon. Soil properties *with similar superscripts along the same column are not significantly different (p<0.05). ppm = parts per million = mg/kg = µg/g; 10 000 ppm = 1 percent*

3.3.2 Main Effects of Diesel Contamination in Soil in the Concentration Range 0-30 ml/kg of soil on the Germination of Lettuce and Sweet Potato

All lettuce seeds and sweet potato cuttings planted in the uncontaminated soil germinated. In the diesel contaminated soil, germination decreased progressively with each increase in the concentration of diesel contamination in the soil (Figure 3.1). In lettuce, there was a steep decline of 20 % in germination with the addition of 5 ml of diesel/kg of soil followed by smaller decreases in germination at diesel concentrations > 5 ml/kg soil (Figure 3.1). By contrast, sweet potato was less sensitive to small additions of diesel in the soil and only showed a marked decline in germination when the diesel concentration exceeded 20 ml/kg soil compared to the control. In both plant species, germination was highly negatively correlated with the concentration of diesel contamination in the soil. The relationships of diesel concentrations in the soil and the germination of lettuce and sweet potato were described by quadratic equations (Figure 3.1). The adverse effect of diesel was stronger on the germination of lettuce seed when compared to adverse effect of diesel on the germination of sweet potato cuttings. Diesel reduced the germination of lettuce to 48.33 % in 5 ml/kg soil and 25 % at 30 ml/kg soil diesel contamination concentration treatment compared with the control. However, in sweet potato, diesel reduced the germination of sweet potato to 99.5 % in the 5 ml/kg soil diesel treatment and 60 % in the 30 ml/kg soil treatment, which are the least and highest diesel concentration treatments investigated in the study (Figure 3.1).

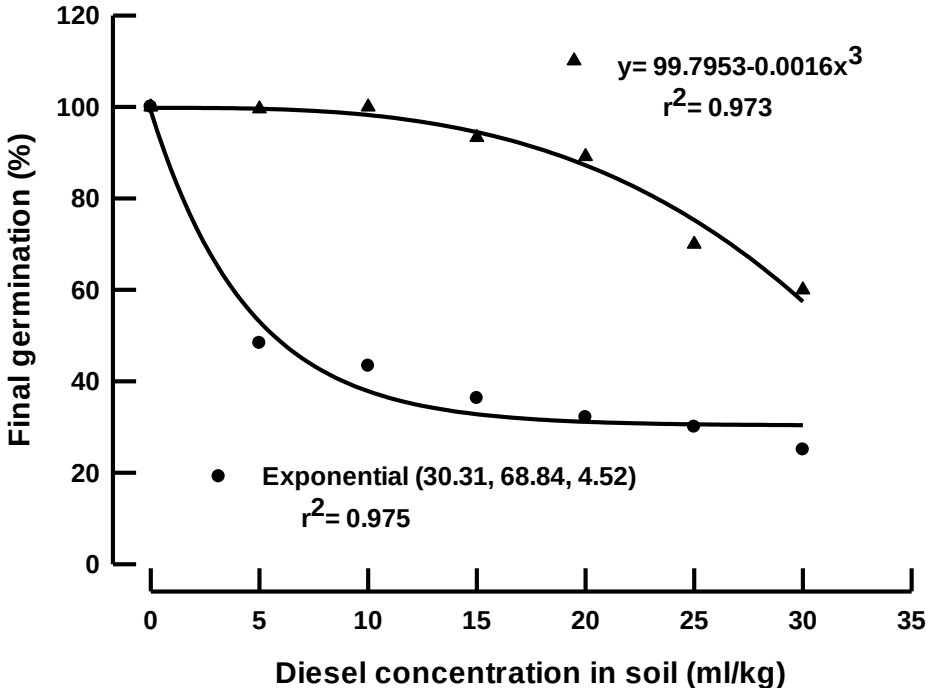

Figure 3.1 The main effects of diesel contamination in soil in the 0-30 ml/kg concentration range on germination of Lettuce (●) and sweet potato (▲)

3.3.3 Main Effects of Age of Diesel Contamination in Soil in the 0-30 ml/kg Soil Concentration Range

There were marked differences in the response of lettuce and sweet potato to age of diesel contamination in soil. The differences were large in fresh contamination and diminished with age of soil contamination (Figure 3.2). Lettuce was more sensitive to age of diesel contamination in soil when compared with sweet potato. The germination of lettuce was still less than 60 % in 80 days old contamination. By contrast, sweet potato showed less sensitivity to age of diesel contamination in soil, as the germination was greater than 70 % in cuttings planted in the 1-day old contamination in soil (Figure 3.2). Thus, the mean germination of lettuce varied within a large range from 14.29 in 1 day old diesel contamination in soil to 89.52 % in 100 day old diesel contamination in soil (Figure 3.2). By contrast that of sweet potato varied within a narrower range from 89.52 % in 1 day old

diesel contamination in soil to 100 % in 100-day old diesel contamination in soil (Figure 3.2).

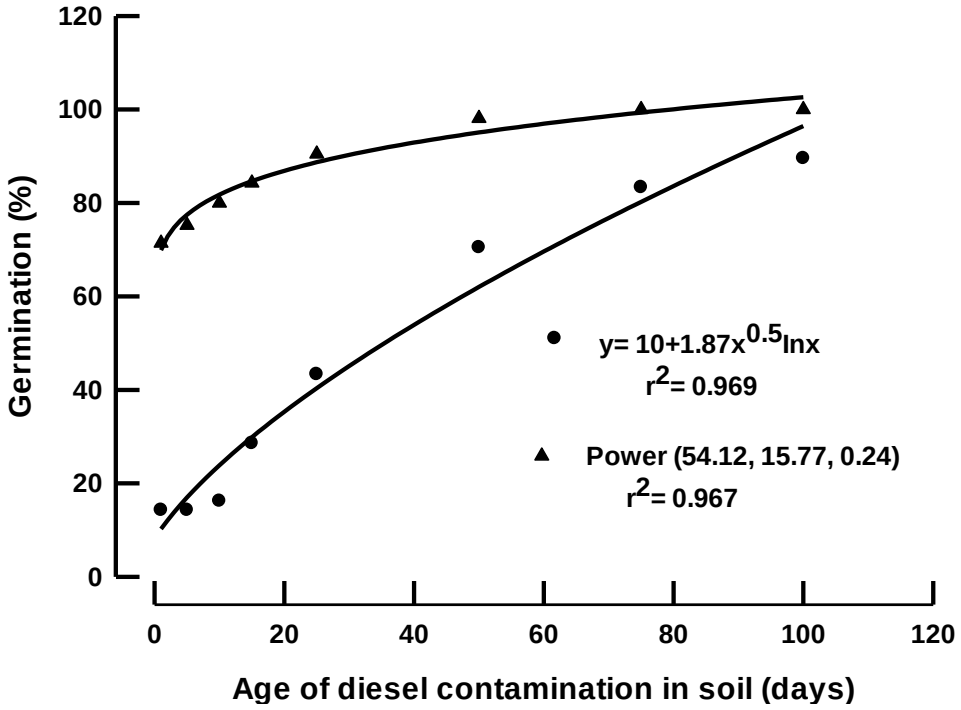

Figure 3.2 Main effects of age of diesel contamination in soil on the germination of lettuce (●) and sweet potato (▲) in the 0-30 ml diesel/kg soil contamination range

3.3.4 Interaction of Diesel Concentration in the Range 0-30 ml/kg and Age of Diesel Contamination in Soil on Seed Germination of Lettuce and Vegetative Germination of Sweet Potato

Generally, an increase in percentage germination as the age of diesel contamination in soil increased was observed in both lettuce (Table 3.4) and sweet potato (Table 3.5). In lettuce, the 5 ml diesel/kg soil treatment achieved 100 % germination at D ≥ 50 days of diesel contamination (Table 3.4). In the rest of the treatments containing > 5 ml/kg diesel, although germination increased with aging of the contamination, the germination % at the termination of the experiment (100 days) was still below 100 %. It ranged from 96.67 % at 10ml to 70 % at 30 ml diesel/kg soil. However, in sweet potato, at D > 50days 100 % germination was achieved in all treatments (Table 3.5). The effect of diesel on the

germination of sweet potato was greatest on the cuttings planted on the day the contamination with diesel took place (D1). Compared to D1, a significant ($p < 0.05$) difference was observed in the germination of sweet potato planted from D25 to D100.

Table 3.4 Interactions of diesel concentration in the 0-30ml/kg soil contamination concentration range and age of contamination on the germination of lettuce seeds

Age of contamination (D) day	Diesel concentration in soil (ml diesel/kg soil)							Lsd (5%)	[y]Mean Germination (%)
	0	5	10	15	20	25	30		
1	100.00^{a}	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	-	**14.29**
5	100.00^{a}	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	-	**14.29**
10	100.00^{a}	3.33^{a} *	10.0^{b} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	0.00^{a} *	7.64	**16.19**
15	100.00^{a}	36.67^{b} *	20.0^{c} *	16.67^{b} *	13.33^{b} *	10.0^{b} *	3.33^{a} *	13.78	**28.57**
25	100.00^{a}	46.67^{c} *	40.0^{d} *	36.67^{c} *	30.00^{c} *	30.0^{c} *	20.0^{b} *	12.67	**43.33**
50	100.00^{a}	100.00^{d}	83.33^{e}	63.33^{d} *	50.00^{d} *	46.6^{d} *	50.0^{c} *	18.72	**70.48**
75	100.00^{a}	100.00^{d}	96.67^{f}	83.33^{e} *	76.67^{e} *	70.0^{e} *	56.6^{d} *	15.29	**83.33**
100	100.00^{a}	100.00^{d}	96.67^{f}	90.00^{f}	86.67^{f}	83.3^{f} *	70.0^{e} *	14.80	**89.52**
[x]Mean Germination (%)	**100.00**	**48.33**	**43.33**	**36.25**	**32.08**	**30.00**	**25.00**	-	**45.00**

** Means along the same row (D) are significantly different from the control ($p<0.05$). Figures along the same column with same letters are not significantly different ($p<0.05$) [x]Main effect of diesel concentrations [y]Main effect of age (D) of contamination*

Table 3.5 Interactions of diesel concentration in the 0-30ml/kg soil contamination concentration range and age of contamination on the vegetative germination of sweet potato cuttings

Age of diesel contamination (D) day	Diesel concentration in soil (ml diesel/kg soil)							Lsd (5%)	[y]Mean Germination (%)
	0	5	10	15	20	25	30		
1	100.0^{a}	100.0^{a}	100.00^{a}	80.00^{a}*	*66.67^{a}	33.3^{a}*	20.00^{a}*	17.09	**89.52**
5	100.0^{a}	100.0^{a}	100.00^{a}	80.00^{a}*	*73.33^{a}	46.6^{b}*	26.67^{a}*	13.24	**75.24**
10	100.0^{a}	100.0^{a}	100.00^{a}	93.33^{b}	86.67^{b}	53.3^{bc}*	26.67^{a}*	15.29	**80.00**
15	100.0^{a}	96.67^{a}	100.00^{a}	93.33^{b}	86.67^{b}	60.0^{c}*	53.33^{b}*	13.78	**84.29**
25	100.0^{a}	100.0^{a}	100.00^{a}	100.00^{b}	100.00^{c}	73.3^{d}*	60.00^{b}*	76.43	**90.48**
50	100.0^{a}	100.0^{a}	100.00^{a}	100.00^{b}	100.00^{c}	93.33^{e}	93.33^{c}	11.46	**98.10**
75	100.0^{a}	100.0^{a}	100.00^{a}	100.00^{b}	100.00^{c}	100.0^{e}	100.0^{c}	-	**100.00**
100	100.0^{a}	100.0^{a}	100.00^{a}	100.00^{b}	100.00^{c}	100.0^{e}	100.0^{c}	-	**100.00**
[x]Mean Germination (%)	**100.00**	**99.58**	**100.00**	**93.33**	**89.17**	**70.00**	**60.00**	-	**87.44**

** Means along the same row (D) significantly different from the control ($p<0.05$). Figures along the same column with same letters are not significantly different ($p<0.05$) [x] Main effect of diesel concentrations [y]Main effect of age (D) of contamination*

3.3.5 Main effects of Diesel Contamination in Soil in the 0-6 ml/kg soil Contamination Concentration Range on the Germination of Lettuce and Sweet Potato

The germination of lettuce and sweet potato correlated negatively with the level of diesel contamination in soil (Figures 3.3), but the effects were not significantly different from the control at most diesel treatments. Although lettuce germination showed more sensitivity to diesel contamination in soil than sweet potato at 0-6 ml/kg diesel concentration range, the differences in germination was not as pronounced as it was in the case of the 0-30 ml diesel/kg soil treatments when compared with the control (Figure 3.3). Diesel contamination in soil reduced germination of lettuce by 11.67 % and 30.83 % in the 1 ml/kg soil and 6 ml/kg soil treatment respectively. In sweet potato; diesel did not reduce the germination of sweet potato at 1 ml/kg soil, and at 6 ml/kg soil, only 4.17 % reduction occurred.

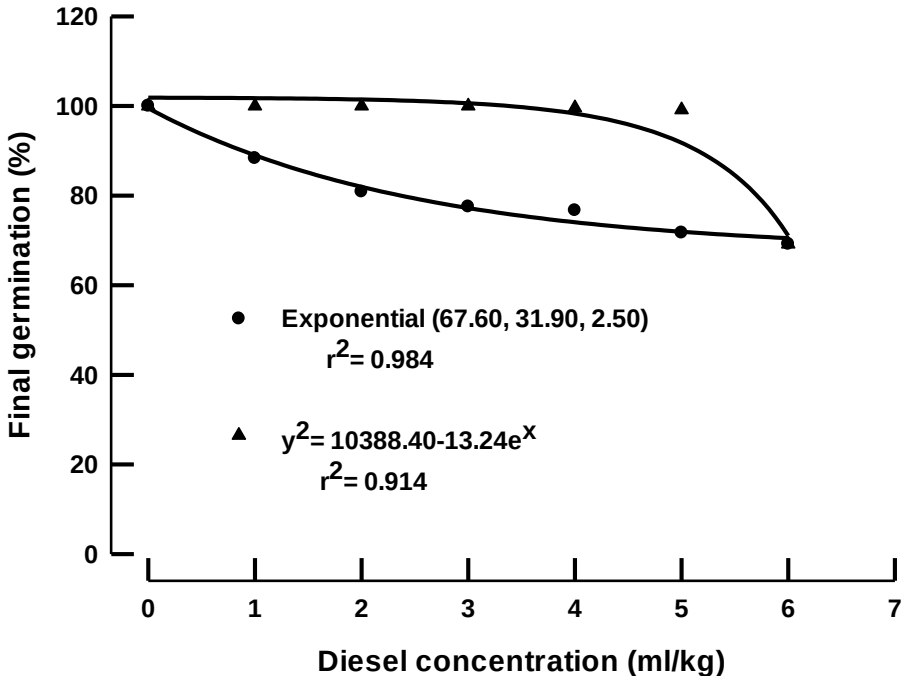

Figure 3.3 Germination responses of Lettuce (●) and sweet potato (▲) to diesel contamination in soil in the 0-6 ml/kg soil contamination concentrations range

3.3.6 Main Effects of Age of Diesel Contamination in Soil in the 0-6 ml/kg soil Contamination Concentration Range on the Germination of Lettuce and Sweet Potato

The mean germination of lettuce and that of sweet potato in the 0-6 ml/kg soil diesel contamination concentration range correlated positively with the age of diesel contamination in the soil (Figure 3.4). However, some differences were noticed in the way the germination of the two crops responded to the age of diesel contamination (Figure 3.4). In the 0D treatment, mean seed germination of lettuce was 21.9 %. It required the contamination to age by 50 days for the mean seed germination of lettuce to be restored to 100 % (Figure 3.4). By contrast, the vegetative germination sweet potato was slightly depressed (3.33 %) compared to seed germination of lettuce (78.1 %) at 0D. Furthermore, the germination of sweet potato was 100 % when the contamination had aged by 15 days compared with 50 days for lettuce (Figure 3.4).

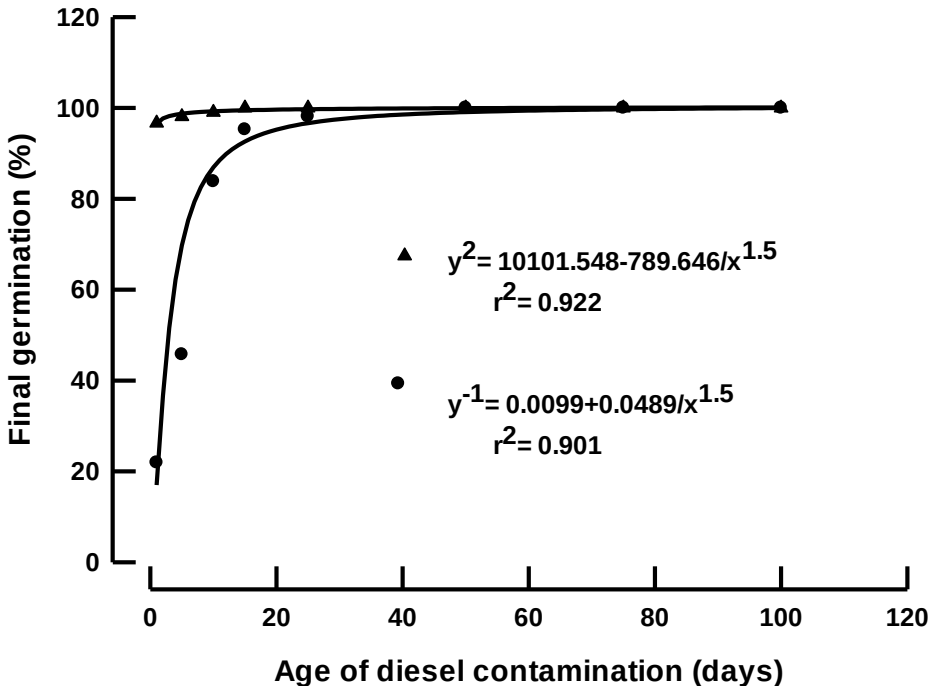

Figure 3.4 Germination responses of Lettuce (●) and sweet potato (▲) to age of diesel contamination in soil in the 0-6 ml/kg concentration range

3.3.7 Interactions of Diesel Concentration in 0-6 ml/kg soil Contamination Range and Age of Diesel Contamination in Soil on the Seed Germination of Lettuce and Vegetative Germination of Sweet Potato

Diesel contamination in soil had only very little effect on the germination of sweet potato. The little effect of diesel contamination in soil on the germination of sweet potato occurred only in fresh contamination, occurring only at D ≤ 5days and at concentration 6 ml diesel/kg soil (Table not shown). At diesel concentration in soil contamination 4 and 5 ml/kg soil, all sweet potato cuttings germinated, but various level of decline in seed germination of lettuce occurred. While, all sweet potato cuttings grown in the 6 ml diesel/kg of diesel contamination treatment germinated at D≥15 days, lettuce showed greater sensitivity to diesel contamination in soil, as 100 % germination did not occur until the age of diesel contamination was ≥ 50D (Table 3.6). Generally, in both crops, germination increased as the age of diesel contamination in soil increased. Also, germination declined as the concentration of diesel contamination in the soil increased.

Table 3.6 Interactions of diesel concentration in the 0-6 ml/kg soil contamination range and age of contamination on the germination of lettuce.

Age of contamination (D) day	Diesel concentration in soil (ml diesel/kg soil)							Lsd (5%)	[y] Mean Germination (%)
	0	1	2	3	4	5	6		
1	100.00[a]	26.67[a] *	26.67[a] *	0.00[a] *	0.00[a]*	0.00a*	0.00[a]*	11.46	**21.90**
5	100.00[a]	80.00[b]	46.67[b] *	46.67[b] *	33.33[b]*	13.33[b]*	0.00[a]*	7.64	**45.71**
10	100.00[a]	100.00[c]	73.33[c]	73.33[c]	80.00[c]	86.67[c]	73.33[b]*	7.64	**83.81**
15	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	86.67[c]*	80.00[c]*	-	**95.24**
25	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	86.67[c]*	100.0[d]	-	**98.10**
50	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	100.0[d]	100.0[d]	-	**100.00**
75	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	100.0[d]	100.0[d]	-	**100.00**
100	100.00[a]	100.00[c]	100.00[d]	100.00[d]	100.0[d]	100.0[d]	100.0[d]	-	**100.00**
[x] Mean Germination (%)	**100.00**	**88.33**	**80.83**	**77.50**	**76.67**	**71.67**	**95.83**	-	**80.60**

** Means along the same row (D) significantly different from the control (p<0.05). Figures along the same column with same letters are not significantly different (p<0.05) [x] Main effect of diesel concentrations [y] Main effect of age (D) of contamination*

3.3.8 Critical Diesel Concentrations for Toxicity in Relation to Germination for the Pooled Data for Diesel Contamination in Soil in 0-6 and 0-30 ml/kg soil Contamination Concentration Ranges

The pooled data for germination for both diesel contamination in soil ranges 0-30 and 0-6 ml/kg soil showed that there was a strong correlation between germination and diesel concentrations in soil that were described by quadratic equations (Figure 3.5). The critical concentrations (the amount of diesel in the soil required to reduce germination by 10 %) were interpolated from these regression relationships (Figure 3.5). It was determined from the extrapolations of this regression relationship that the critical diesel concentration for toxicity in relation to seed germination of lettuce was 1.32 ml/kg soil compared with 15.5 ml/kg soil for vegetative germination of sweet potato.

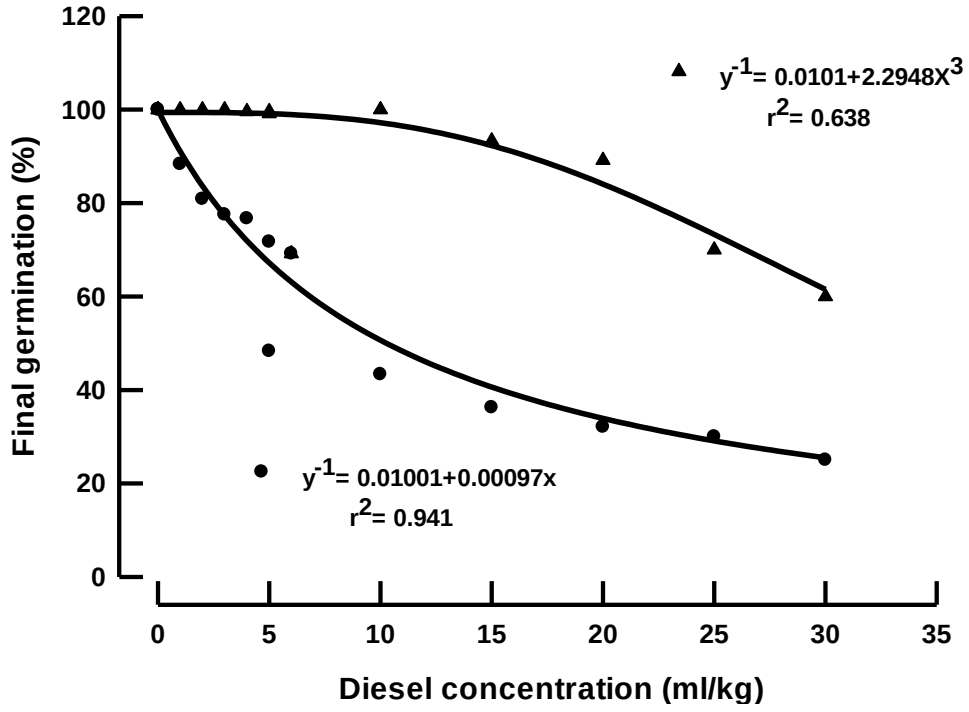

Figure 3.5 The main effects of diesel contamination concentration in soil on the germination of lettuce (●) and sweet potato (▲) for the pooled data in the 0-6 and 0-30 ml/kg diesel concentration ranges

The critical concentration for toxicity for each diesel treatment level as interpolated from the combined regression of 0-6 ml/kg soil and 0-30 ml/kg soil show that the critical concentration of diesel for toxicity increased as the age of diesel contamination in the soil increased (Table 3.7). In lettuce, the critical concentrations increased from 0.16 ml/kg soil (1D) to 17.74 ml/kg soil (100D) when the study was terminated. In the case of sweet potato the critical concentration of diesel for toxicity increased from 10.83 ml/kg soil at 1D to 21.6 ml/kg soil 25D. As the age of diesel soil contamination became ≥ 50D, the diesel soil contamination did not reduce the vegetative germination of sweet potato by up to 10 %, hence the lack of critical concentration of diesel for toxicity.

Table 3.7 Critical Concentrations of diesel for toxicity in relation to germination of lettuce and sweet potato

Contamination age (days after soil contamination)	Critical diesel concentrations associated with 10 % reduction in germination (ml diesel /kg of soil)	
	Lettuce	Sweet potato
1	0.16	10.83
5	0.37	12.05
10	1.49	14.07
15	2.80	16.73
25	4.09	21.6
50	8.87	-
75	12.96	-
100	17.74	-

3.4 DISCUSSIONS

3.4.1 Composition of Diesel Used in this Study

The analysis of the diesel used in the present study indicated that the composition of the diesel in terms of monocyclic hydrocarbons (BTEX), polycyclic aromatic hydrocarbons (PAH) and heavy metals is different from those of diesel analysed by Neff *et al.*, 2000;

Simonato *et al*., 2008; Ricardo *et al*., 2010. For example; in this study, the diesel presented high concentrations of PAHs (3,693.68 ppm) and low concentrations of BTEX (20.35 ppm), when compared with the values (38.61 ppm and 2815.16 ppm for PAHs and BTEX, respectively) for the diesel analysed by Ricardo *et al.* (2010). However, the BTEX in both studies were mainly the xylene isomers, which constituted 58 % in this study and 62.95 % in the study of Ricardo *et al*., 2010. The differences in the chemical composition of diesel may be linked to the source of crude oil or to the distillation plant.

3.4.2 Effects of Diesel Contamination in Soil on Seed Germination of Lettuce and Vegetative Germination of Sweet Potato Cuttings

All the lettuce seeds and potato cuttings planted in the uncontaminated soil in this study germinated. Hence, the various levels of decline in the germination of lettuce and sweet potato planted in diesel contaminated soil are attributable to the effects of diesel on the germination of both species. Generally, the germination of both species reduced as the concentration of diesel in soil increased. A number of authors have reported a negative correlation between the concentration of diesel contamination in soil and the germination of some crops, for example; *Vigna unguiculata* (Njoku *et al*., 2009); *S. terebinthifolius* (Bona *et al*., 2010). The age of diesel contamination also affected the germination of both species leading to strong positive correlation between the age of contamination and the germination of lettuce at both 0-6 ml/kg soil (Figure 3.2) and 0-30 ml/kg soil (Figure 3.4) diesel concentration ranges. The strong positive correlations between the age of diesel contamination and the germination of lettuce seeds and sweet potato cuttings in this study clearly indicated that the longer the time between the contamination and planting, the higher was the germination of both species. Several authors have also reported increased germination of some crops with increase in age of diesel contamination e.g. cowpea, maize (Ogbo, 2009); *S. terebinthifolius* (Bona, 2010). However, the germination of both

lettuce and sweet potato were markedly different, with the germination of lettuce being more sensitive to diesel contamination in soil than that of sweet potato. The critical concentrations of diesel for toxicity at all ages of diesel contamination for sweet potato were higher than that of lettuce (Table 3.7). There are no data in the literature on critical concentration of diesel for toxicity on lettuce and sweet potato germination nor any other plant to compare with the data obtained in the present study. The marked differences observed in the response of lettuce and sweet potato to diesel soil contamination is an indication of differences in the sensitivity of the germination of the two species to diesel soil contamination. A number of authors have reported differences in the sensitivity of different crops to diesel contamination e.g. some gramineous, herbaceous and leguminous plants (Adam & Duncan, 2002); *Cochorus olitorius*, *Hordeum spontaneum*, *Triticum aestivum* and *Atriplex halimus* (Saadoun & Al-Ghazawi, 2010); safflower and corn (Asli & Houshmandfar, 2011). Reasons for the more sensitivity of lettuce when compared with sweet potato may be attributed to the fact that sweet potato was propagated from cuttings. Cuttings are matured plants which are already hardened by forces of nature. In contrast, lettuce was germinated from seeds. Seed germination is known to be vulnerable to inhibitory substances (Taiz & Zeiger, 2010). Lettuce seeds are small; hence they could easily be completely covered with diesel sheen. The covering of lettuce seed could be a barrier to the entry of water and oxygen into the seeds. In sweet potato, only the part of the cuttings in the soil could be covered with diesel with the other parts above the soil having access to oxygen. Water and oxygen are required for germination (Taiz & Zeiger, 2010). Also, the penetration of diesel into lettuce seed could kill the embryo of lettuce seeds. In contrast, Sweet potato cuttings do not germinate through an embryo and germination could occur through any of the buds present on the cuttings. Hence, sweet potato may not be as vulnerable as lettuce to diesel soil contamination.

3.4.3 Causes Diesel Toxicity

The diesel and soil chemical analysis were done to determine the causes of diesel toxicity on seed germination; that is, was it heavy metal toxicities, nutrient deficiencies or direct effects of hydrocarbons. The causes of diesel toxicity to plants have generally been blamed on toxic heavy metal content of diesel (Baran *et al*., 2002; Millioli *et al.*, 2009), induction of N, Ca, K and P deficiencies by diesel (Wyszokowski & Ziolkowska, 2008; Njoku *et al.*, 2009; Akujobi, 2011). In this study, the concentrations of heavy metals in diesel were generally low, ranging from 7.18 ppm for V to 2.33 ppm for Fe (Table 3.2). Also in diesel polluted soil, the heavy metal in soil did not follow a definite pattern (Table 3.3). Furthermore, none of the heavy metals analysed were at concentrations that could be considered toxic to plants.

Although there were significant correlations between the concentration of diesel soil contamination and the concentrations of N, P, K, Mg and Ca in soil, all these nutrients and the soil pH were within the optimum range required for the growth of lettuce and sweet potato over the entire range of diesel concentration tested (Table 3.3). Thus, heavy metal toxicity and mineral nutrient deficiencies were not involved in the toxic effects of diesel contamination in soil on seed germination of lettuce and vegetative germination of sweet potato. This indirectly confirms that the toxic effect of diesel soil contamination was via the hydrocarbons in the diesel. The improved germination of the crops with aging of the diesel soil contamination also corroborates this hypothesis, since the toxic hydrocarbons can be lost from the soil through volatilization or degradation (Muratova *et al.*, 2003; Molina-Barahona *et al*., 2004; Barruti *et al*., 2011; Onuoha *et al.*, 2011). If the diesel toxicity on germination were due to heavy metal pollution, the aging of diesel pollution would have no consequence on germination. Similarly, no improvement in seed germination would occur with aging of pollution if the toxic effect of diesel were via

mineral nutrient deficiencies. Thus, it was deduced that the diesel affected the germination of lettuce seeds and sweet potato cuttings through its hydrocarbon components. The exact mechanism by which the diesel's hydrocarbon affected seed germination of lettuce and vegetative germination of sweet potato could not be determined from this study. Whatever the mechanism, the vegetative germination of sweet potato was less sensitive to the hydrocarbons. The practical implication of this study is that, since sweet potato exhibited better germination at higher concentration of diesel, it could be used for phytoremediation of diesel contaminated soils. The results indicate a wider possibility for utilizing agricultural species like sweet potato that are tolerant of diesel in remediation of soil contaminated with diesel, and sensitive species like lettuce to monitor the level of diesel contaminated soil. This study highlights that vegetative germination is also adversely affected by diesel soil contamination, but it is less sensitive than seed germination.

3.5 CONCLUSIONS

All the lettuce seeds and potato cuttings sown in the uncontaminated soil in this study germinated, but various levels of decline in germination were observed in lettuce seeds and potato cuttings sown in diesel contaminated soil depending on diesel concentration in the soil. It could be concluded from the result of this study that diesel contamination affects the germination of *Lactuca sativa* and *Ipomoea batatas* in a concentration dependent manner, the higher the concentration of diesel the lower the germination of lettuce seeds and sweet potato cuttings. The phytotoxic effect of diesel on the germination of lettuce and sweet potato decreases as the age of diesel contamination increases. The germination of lettuce was more negatively correlated with diesel concentration than the germination of sweet potato. Lastly, the critical concentration of diesel for toxicity on the germination of sweet potato was higher than that of lettuce. Hence, it could be concluded

that the germination of lettuce seed is more sensitive to diesel contamination in soil than the vegetative germination of sweet potato cuttings.

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CHAPTER 4

INFLUENCE OF DIESEL CONTAMINATION IN SOIL ON THE GROWTH AND DRY MATTER PARTITIONING OF *LACTUCA SATIVA* AND *IPOMOEA BATATA*

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Abstract

The effects of diesel contamination in soil on the growth and dry matter partitioning in *Lactuca sativa* (lettuce) and *Ipomoea batatas* (sweet potato) were studied in a greenhouse pot experiment at two concentration ranges (0-30 ml/kg soil and 0-6 ml/kg soil). After fourteen weeks of growth in the diesel treatments, the chlorophyll content of the leaves, the dry matter of the roots, stems and leaves as well as the mineral nutrient concentrations in the youngest fully expanded leaves were determined. Whole plant biomass in the two species was negatively correlated with increasing diesel concentrations, and so were plant heights, number of leaves, root lengths and the leaf chlorophyll. The critical concentrations of diesel associated with 10 % decrease in plant growth were 0.33 ml/kg soil for lettuce and 1.50 ml/kg soil for sweet potato. Thus, the growth of lettuce was more sensitive than that for sweet potato in diesel contaminated soil. The pattern of dry matter partitioning between the plant parts varied with the concentration of diesel contamination in the soil in a similar manner in the two test plant species. In the 0-6 ml/kg soil diesel concentration range, allocation of dry matter to the shoot system was favoured resulting in high shoot: root ratios of 4.54 and 12.91 for lettuc*e* and sweet potato respectively. However, in the 0-30 ml/kg soil diesel concentration range, allocation of dry matter to the root was favoured at diesel concentrations $\geq$ 10 ml/kg soil which may have been an adaptive mechanism in which the root system was used for storage in addition to increasing the capacity for foraging for mineral nutrients and water. Although lettuce accumulated more metals in its tissue than sweet potato, the tissue mineral nutrients in both species did not vary to a great extent. The plant tissue analyses indicated that heavy metal toxicity and nutrient deficiencies were not involved in the adverse effects of diesel on the growth of both species. Hence, the cause of mortality and poor growth of sweet potato and lettuce grown in diesel contaminated soil may therefore have been direct toxicity of diesel hydrocarbons.

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Keywords: diesel: Biomass; *Ipomoea batatas; Lactuca sativa;* dry matter partitioning; critical concentration; soil contamination; mortality; hydrocarbons.

4.1 INTRODUCTION

Petroleum diesel is a yellow viscous liquid blended/obtained from fractional distillation of crude oil (Wang *et al.,* 2004). It is mainly a complex mixture of saturated and unsaturated hydrocarbons with an average chemical formula of $C_{12}H_{23}$, ranging approximately from $C_{10}H_{20}$ to $C_{15}H_{28}$ (Ogbo, 2009). The non-hydrocarbon components of diesel include; sulphur, nitrogen and phosphorus. Diesel contains heavy metals such as iron, lead, manganese vanadium and nickel (Kaczmarek *et al.,* 1981; Adam & Duncan, 1999). Several elements including arsenic, calcium, cobalt, chlorine, chromium, copper, fluorine, potassium and sodium have also been reported in diesel (Kaczmarek *et al.*, 1981; Adam & Duncan, 1999).

Diesel is considered to be the most phytotoxic product from crude oil (Buikeima *et al*., 1981). The phytotoxicity of diesel is dependent mostly on its aromatic hydrocarbons and to a lesser extent on the aliphatic hydrocarbons (Alkio *et al*., 2005). The monocyclic aromatic hydrocarbons (BTEX) cause acute injury to plants, while polycyclic aromatic hydrocarbons (PAH) cause chronic injury (Graef & Winter, 1968; Alkio *et al*., 2005). The aliphatic hydrocarbons are very volatile and less toxic to plants (Alkio *et al*., 2005). When diesel is spilt on land, the toxicity of diesel hydrocarbons decrease as the contamination ages, because of evaporation and degradation processes such as microbial activities (Brunnock *et al*., 1968; Mackay *et al.,* 1972). Since diesel also contains heavy metals (Wang, 2004); diesel soil contamination may cause an increase in heavy metals content of the soil (Singh & Mishra, 1987). Although some of the heavy metals such as manganese, copper, iron, zinc are essential for plants growth; they are phytotoxic at high concentrations (Lambers *et al.,* 1998). The other heavy metals; uranium, mercury, silver and gold are toxic to plants even at very low concentrations (Singh & Mishra, 1987; Lambers *et al*., 1998).

Diesel contamination in soil affects a number of physiological processes in plants, which include; reduction in transpiration due to physical interference of water transport in plants (Baker, 1971), reduced or increased respiration (Baker, 1971) and reduction in photosynthesis (Baker, 1971; Malallah *et al.,* 1998). Also diesel soil contamination inhibits translocation of water and plant nutrients (Baker, 1971), which leads to decrease in growth and biomass production (Brandt *et al*., 2006; Daniel-Kalio & Pepple, 2006; Adenipekun *et al.,* 2008). The injury to plants by diesel in soil may be immediate, occurring during the initial growing season or could be cumulative, occurring after the initial growing season (Gelpke, 2011). Apart from direct hydrocarbon and heavy metals toxicity as a result of diesel contamination in soil, the adverse effect of diesel on crops could also be linked to formation of other toxic compounds in the soil through some chemical reactions in soil and photo-oxidation processes (Baran *et al*., 2002; Wyszokowski & Ziolkowska, 2008; Bayram *et al*., 2009; Akujobi *et al*., 2011).

The consumption of diesel has continued to grow world-wide because it is widely used for engines of cars, generators, industrial trucks and most agricultural machineries (Walker *et al*., 1978). The increased usage of diesel has led to an increase in accidental spillages of diesel, and consequently, environmental pollution (Hill & Moxey, 1960; Dambo, 1993). Diesel contamination of agricultural farms occurs through; leakages from storage containers, refuelling of farm machineries, wrecks of oil tankers and through improper disposal by mechanics when cleaning diesel tankers (Hill & Moxey, 1960). Diesel spillage on cultivated land and potential agricultural land is a common occurrence in many countries because most agricultural machineries use diesel as a source of power. With the ever increasing world population, which currently stands at seven billion (UNFPA, 2011), the use of machinery on agricultural land is inevitable to meet the rising world's food demand (FAO, 2011). The

increased use of machinery will lead to more incidences of diesel spillages on agricultural land. Hence, with the certainty that the incidence of diesel spillage on agricultural land will increase in the years to come; it is therefore very reasonable that efforts be made to study the effects of diesel spillages on soil and crop productivity, and to determine options for ameliorating the effects. Hence this study was conducted.

The toxic effects to plants of diesel contamination in soil has been investigated for a number of species, including: melon (*Solanum melongena)* (Akujobi *et al.,* 2011), groundnut (*Arachis hypogaea),* cowpea (*Vigna unguiculata),* sorghum (*Sorghum bicolor)* and maize (*Zea mays)* (Ogbo, 2009). However, all the reports in the literature have involved plants that get established from seeds. Thus, it is currently not known how plants that are established from cuttings and other vegetative propagules compare with those that are established from seeds in tolerating diesel toxicity. The present study was thus designed to compare the effects of diesel soil contamination on the establishment and vegetative growth of lettuce grown from seed and sweet potato grown from cuttings. Two hypotheses were formulated:

i. Soil contamination with diesel negatively affects the establishment and growth of lettuce and sweet potato.
ii. The vegetative growth of lettuce established from seeds and that of sweet potato established from cuttings differ in their sensitivity to diesel toxicity.

4.2 MATERIALS AND METHODS

4.2.1 Diesel Contamination in Soil Treatments

The study was conducted at the University of Zululand in Kwa-Zulu Natal province of South Africa. Agriculturally productive soil was collected from the university's farm. Diesel was obtained from Zulu Oil at Empangeni. Two concentration ranges of diesel contamination were tested. The experiment was first conducted at 0-30 ml diesel/kg soil contamination range. The reductive effect of diesel at contamination in the soil on the growth of both species was very severe in the 0-30 ml diesel/kg soil contamination range treatment. Hence, the plants were then grown in 0-6 ml diesel/kg soil concentration range treatments. This was done to facilitate accurate determination of the critical concentration for toxicity at which diesel contamination in soil is associated with 10 % reduction in the growth of lettuce and sweet potato.

In the first range of the diesel concentration treatments, the treatment intervals were 0, 5, 10, 15, 20, 25 and 30 ml diesel/kg of soil [Average temperature (AT) = 22.65 °C, Average humidity (AH) = 73.25 %]. In the second concentration range treatments, the intervals were; 0, 1, 2, 3, 4, 5, and 6 ml diesel/kg of soil [AT = 19.15 °C, AH = 73.25 %]. The simulation of diesel contamination was done as described in section 2.2.2 in 20 litre pots in three replicates for each diesel treatment. The pots in each case were laid in a completely randomised design (CRD).

4.2.2 Diesel and Soil Analyses

The chemical composition of diesel and the soil used in the study were analysed as described in section 3.2.3.

4.2.3 Lettuce Plant Management

Seedlings were raised in a rainproof nursery. The seeds were planted singly per compartment in a germination tray containing 200 compartments. The growth medium used was hygromix. After 4 weeks of growth, vigorous seedlings of equal size were transplanted to the diesel contaminated soil in pots at two seedlings per pot. The pots were irrigated as at when necessary.

4.2.4 Sweet Potato Plant Management

Healthy vines of *Ipomoea batatas cv.* Dagga were obtained from the University of Zululand farm. Each cutting was about 250 mm long and had 5 buds. Two cuttings were planted per pot. The cuttings were planted with three buds below the soil and two buds above soil level. The pots were arranged 30 cm within row and the rows were one meter apart. The pots were kept moist throughout the period of the experiment. The plants were grown for 14 weeks.

4.2.5 Plant Measurements

Plant height for lettuce was measured weekly with a measuring tape. For sweet potato, the length of the longest vine was measured from the soil level to the terminal bud and was considered as the plant height. The mortality of the plants in each replication of the treatments was determined weekly by counting the number of plants that died. At 14 WAP, ten leaves were randomly selected per plant per treatment replication and their chlorophyll content measured with Chlorophyll meter CCM-200 (Opti Sciences, 8 Winn Avenue, Hudson, USA, 2000). The measurements were taken at the centre of each leaf.

After 14 weeks of growth, the lettuce and sweet potato plants were carefully pulled out of the soil to avoid damage to the roots. The soil was washed off from the roots, and the plants separated into leaves, stems and roots. The lengths of the roots and stems were measured.

The plants parts were oven dried at 65 $^{\circ}$C until constant weight, after which their dry weights were determined.

4.2.6 Chemical Analysis of Leaf Tissue

Youngest fully matured leaves (YFML) were collected at harvest, for tissue chemical analysis of lettuce and sweet potato. The YFML were oven dried at 65^{o} until constant weight. The leaves were ground to pass through a 0.84 mm sieve. After grinding, representative sub samples (5 g) were taken and wet digested in 1.0 M hydrochloric acid (HCl). The digest was filtered through a #1 Whatman filter paper. An aliquot of 10 ml was transferred to a centrifuge tube and analysed with ICP atomic emission spectrometer to determine P, K, Ca, Mg, Na, Cu, Mn and Zn concentrations. Total carbon and N were analysed by the Automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation, Michigan, USA; Matejovic, 1996). (NB: Full descriptions of the procedures are in Chapter 2).

4.2.7 Statistical Analyses

The data on plant growth, microbial activity, soil chemical and leaf tissue analyses were first subjected to ANOVA procedure using GenStat Release 12.1 (PC/Windows Vista) (VSN International Ltd., 2009). Missing plot technique was used in treatments where mortalities were recorded. Means of the treatments were separated by least significant difference at 5 % level ($LSD_{0.05}$). However, because the treatments involved manipulating a gradient of quantitative levels (incremental diesel concentration), the data were also subjected to correlation and regression analyses. Thus, mathematical functions expressing correlations and regression relationships between diesel contamination concentrations and the biomass of the plants and plant parts were obtained using the curve fitting programme of Table Curve 2D v5.01.01 (Systat Software Inc., San Jose, CA, USA, 2002). This facilitated the

determination of critical levels of diesel concentration for toxicity in soil pollution at which plant growth was reduced from maximum by 10 % for each treatment range (The average of the values calculated at 0-6 ml/kg soil and 0-30 ml/kg soil diesel concentration treatments was then calculated and reported as critical diesel concentration for toxicity - CDC). The value of the concentrations of mineral nutrients at the critical concentrations of diesel for toxicity was also determined with the curve fitting programme of Table Curve for each diesel treatment range (The average of the values calculated at 0-6 ml/kg soil and 0-30 ml/kg soil diesel concentration treatments was then calculated and reported as critical mineral nutrients concentration in the leaves of the test crops - CMNC). The same programme was also used to determine relationships between concentrations of diesel contamination and plant height, mortality, shoot biomass, root biomass and nutritional contents of leaves.

4.3 RESULTS

4.3.1 Effect of Diesel on Soil Physical, Chemical and Biological Properties

The results of diesel analysis and those of soil with and without the diesel treatments have been presented in Chapter 3.

4.3.2 Plant Mortality

All the seedlings of lettuce and sweet potato cuttings grown in the uncontaminated soil survived to maturity at both concentration ranges. In the 0-30 ml/kg soil diesel concentration treatments, there were strong positive correlation between diesel contamination concentrations in the soil and the mortality of both species (Figure 4.1). Differences were however observed in the number of plants that died and the period it took from planting to the time of death of the plants (Figure 4.1). The least diesel contamination treatment in which mortality occurred was 15 ml diesel/kg soil in both species. However, the mortality of lettuce occurred between weeks 4 and 6 after seedlings were transplanted to the diesel

treatments (Table 4.1). Whilst in sweet potato, the mortality occurred earlier, occurring at 2 and 3 weeks after planting of the cuttings in the 0-30 ml/kg soil diesel concentration range treatments (Table 4.2). In the 0-6 ml/kg soil diesel concentration range treatments, all lettuce and sweet potato plants survived to maturity.

Table 4.1 Effects of diesel contamination in soil on the mortality of lettuce in the 0-30 ml diesel/kg soil concentration range treatments

WAP	Diesel contamination concentration in soil (ml diesel/kg soil)							TOTAL MORTALITY
	0	5	10	15	20	25	30	
1	0	0	0	0	0	0	0	**0**
2	0	0	0	0	0	0	0	**0**
3	0	0	0	0	0	0	0	**0**
4	0	0	0	0	0	2	2	**4**
5	0	0	0	0	2	0	0	**2**
6	0	0	0	1	0	0	0	**1**
TOTAL MORTALITY	**0**	**0**	**0**	**1**	**2**	**2**	**2**	**7**

WAP: Weeks after planting NOTE: No mortality occurred at WAP> 6

Table 4.2 Effects of diesel contamination in soil on the mortality of sweet potato in the 0-30 ml diesel /kg soil concentration range treatments

WAP	Diesel contamination concentration in soil (ml diesel/kg soil)							MEAN MORTALITY
	0	5	10	15	20	25	30	
1	0	0	0	0	0	0	0	**0**
2	0	0	0	0	0	2	2	**4**
3	0	0	0	1	1	0	0	**2**
TOTAL MORTALITY	**0**	**0**	**0**	**1**	**1**	**2**	**2**	**6**

WAP: Weeks after planting NOTE: No mortality occurred at WAP> 3

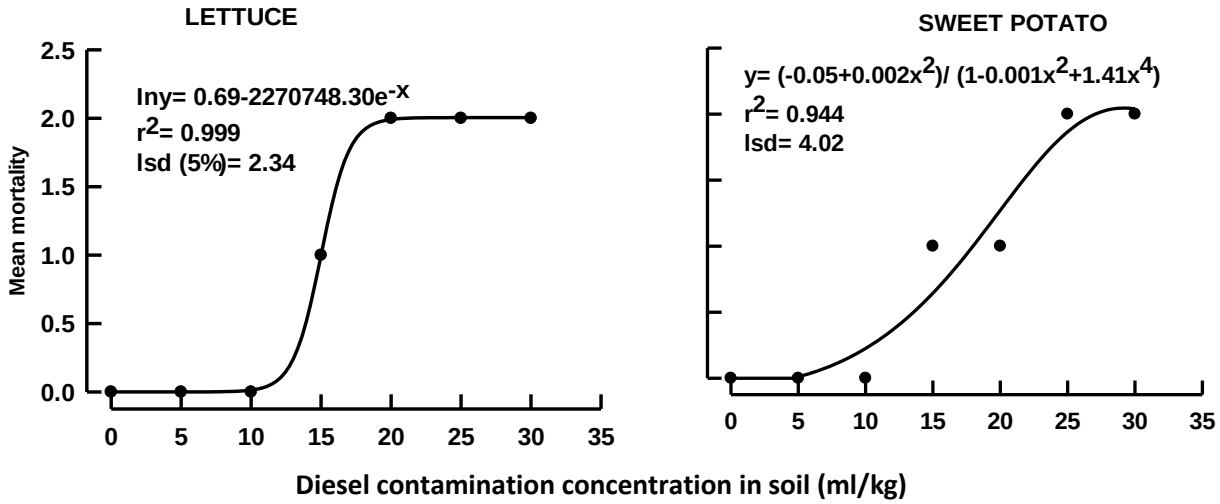

Figure 4.1 The relationship between diesel contamination concentrations in soil and the mean mortality of lettuce and sweet potato.

4.3.3 Effect of Diesel Soil Contamination on Some Growth Parameters

4.3.3.1 Plant height of lettuce and vine length of sweet potato at 14 WAP

In the 0-30 ml/kg soil diesel concentration range treatments, the height of lettuce and the length of sweet potato vines declined progressively as the concentrations of diesel treatment in the soil increased (Figure 4.2 A and B). Also, in both species, the first increment of 5 ml of diesel/kg soil had the largest impact, whereas additional 5 ml increments had smaller additional increments on growth. Differences were however noted in the extent of the responses of the two crops. In sweet potato, the reduction in the longest vine (plant height) caused by 5 ml diesel per kg soil, the least contamination concentration investigated, was 44.94 % whereas in lettuce, it was 49.73 %.

In the 0-6 ml/kg soil diesel concentration range, although there was high correlation between the level of diesel contamination in the soil and the height of both species, there were no significant ($p<0.05$) differences between plant height at most of the adjacent treatments in lettuce and sweet potato (Figure 4.2 C and D).

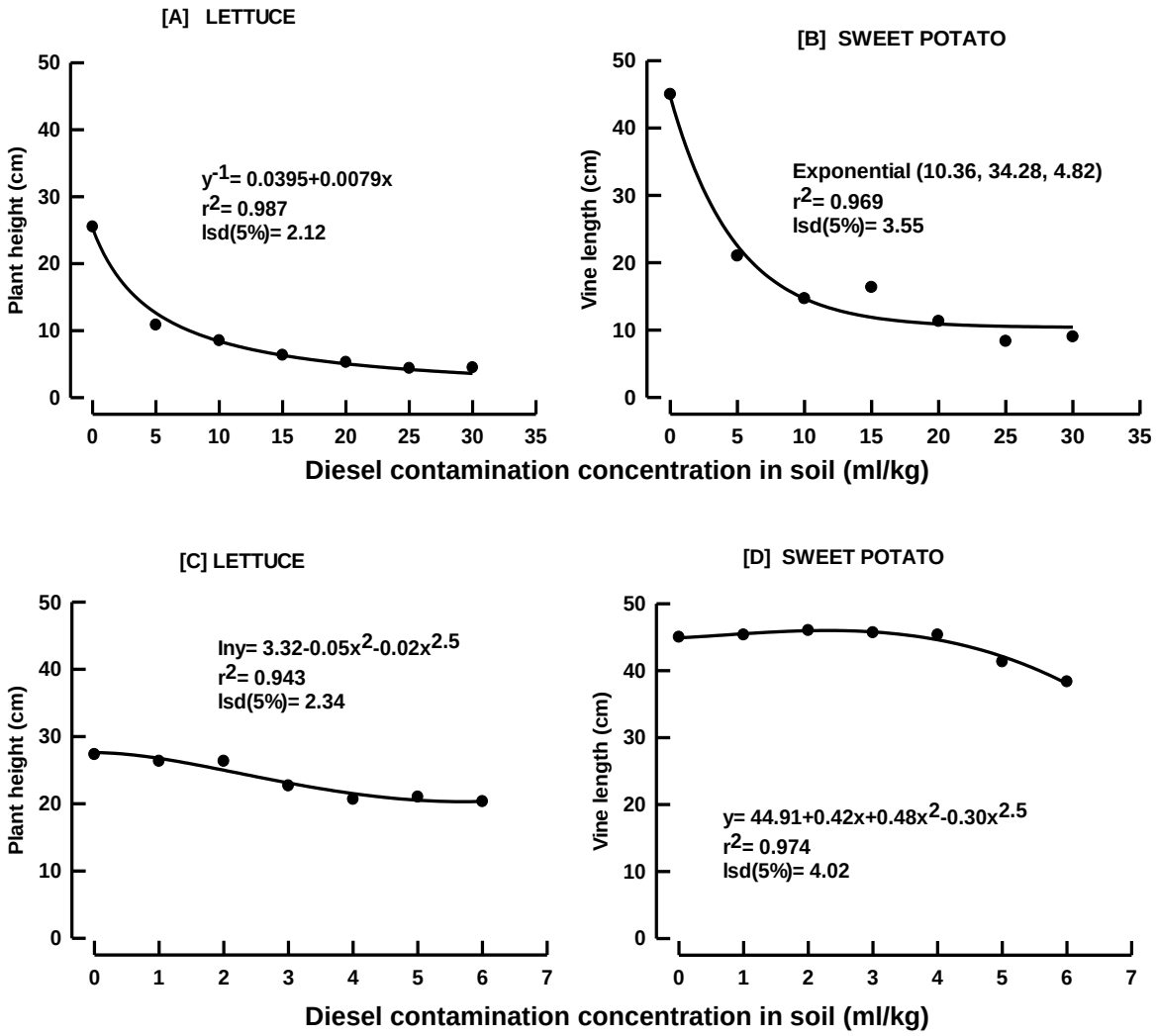

Figure 4.2 Mean lettuce plant height and sweet potato vine length at harvest (14 WAP) in the 0-30 ml/kg soil [A, B] and 0-6 ml/kg soil [D, C] diesel concentration range treatments

4.3.3.2 Effect of diesel contamination in soil on some growth parameters at harvest other than plant biomass

In the 0-6 ml/kg soil diesel concentration range treatments, there were no significant differences between most treatments and the control (Tables 4.3). However, diesel contamination in soil significantly ($p<0.05$) affected the chlorophyll content, root length and number of leaves in lettuce and sweet potato in the 0-30 ml diesel/kg soil concentration range treatments (Table 4.4). A regression analysis performed on the data also indicated high negative correlation between the level of diesel in soil and the leaf chlorophyll content and number of leaves of both species in the 0-30 ml diesel/kg soil concentration range (Table

4.4). However, in the 0-6 ml diesel/kg soil concentration range treatments, the leaf chlorophyll content, root length, and number of leaves of sweet potato was highly negatively correlated with the level of diesel concentrations in soil (Table 4.3). In lettuces, it was only the leaf chlorophyll content that was highly negatively correlated with the level of diesel contamination concentration in soil.

Table 4.3 Effects of diesel contamination in soil on chlorophyll content, root length and number of leaves of lettuce and sweet potato at 14 WAP for 0-6ml/kg diesel concentration range

Diesel concentration (ml/kg soil)	LETTUCE			SWEET POTATO		
	Chlorophyl content (CCI)	Root length (cm)	Number of leaves	Chlorophyl content (CCI)	Root length (cm)	Number of leaves
0	10.87	14.67	28.00	46.00	31.67	87.00
L	9.63	12.67	28.00	42.00	32.00	80.30
2	8.93	9.33	21.67	36.33	32.67	85.30
3	8.87	10	21.00	30.00	33.00	85.70
4	9.73	13.33	25.00	35.00	32.33	76.00
5	8.33	12.67	21.33	29.30	18.33	66.70
6	7.97	11	19.00	29.67	15.33	67.00
MEAN	9.19	11.95	23.43	36.90	27.90	78.30
$LSD_{0.05}$	1.75	2.86	5.07	7.83	4.10	19.78
r^2	0.768	0.653	0.686	0.855	0.871	0.819

r^2 = regression coefficient

Table 4.4 Effects of diesel contamination in soil on chlorophyll content, root length and number of leaves of lettuce and sweet potato at 14 WAP for 0-30 ml/kg diesel concentration range in soil

Diesel concentration (ml/kg soil)	LETTUCE			SWEET POTATO		
	Chlorophyl content (CCI)	Root length (cm)	Number of leaves*	Chlorophyl content (CCI)	Root length (cm)	Number of leaves*
0	12.53	19.17	36.33	46.00	12.70	96.00
5	8.67	11.33	22.33	36.30	11.30	13.30
10	7.63	11.00	11.33	30.30	10.80	11.30
15	7.50	7.83	7.67	29.00	7.50	11.30
20	7.30	7.00	6.67	26.30	16.20	12.70
25	6.60	6.67	6.33	22.30	9.00	7.00
30	6.17	5.00	6.00	21.00	10.00	5.70
MEAN	8.06	9.71	13.81	30.20	11.10	22.50
$LSD_{0.05}$	1.043	4.407	4.953	9.13	8.49	18.82
r^2	0.994	0.981	0.970	0.992	0.224	0.994

r^2 = regression coefficient

4.3.4 Biomass Response of Lettuce and Sweet Potato to Diesel Contamination in Soil

4.3.4.1 Effect of diesel contamination in soil on dry matter of lettuce and sweet potato at harvest

In the 0-30 ml/kg soil diesel concentration range treatments, diesel contamination in soil significantly ($p<0.05$) affected the leaf weight, stem weight and root weight of lettuce (Table 4.5) and sweet potato (Table 4.6) in a concentration dependent manner. In both species, strong negative correlations were obtained between the level of diesel concentration in soil and the leaf weight, stem weight and root weight of lettuce (Table 4.5) and sweet potato (Table 4.6). While the leaf weight of lettuce plants grown in soil contaminated with 5 ml diesel/kg soil (the least diesel soil contamination treatment investigated in the 0-30 ml diesel/kg soil concentration range treatments) declined by 71.39 % when compared with the control, that of sweet potato declined by 90.95 %. There was also a significant decline of 81.35 % in the root weight of lettuce grown in soil contaminated with 5 ml diesel/kg soil when compared with the control. In sweet potato, a decline of 94.46 % occurred. In the 0-30

ml diesel/kg soil concentration range treatments, there were 85.46 and 85.95 % decline in the stem weight of lettuce and sweet potato grown in soil contaminated with 5 ml diesel/kg soil respectively.

In the 0-6 ml/kg soil diesel contamination in soil concentration range treatments, there was negative correlation between the leaf weight, stem weight and root weight of lettuce (Table 4.5) and sweet potato (Table 4.6), however, there was no significant difference in the root, stem and leaf weights of both species at most of the diesel contamination treatments levels investigated when compared with the control. The least diesel contamination treatment that caused significant difference between the control and lettuce plants was 2 ml/kg soil (root and leaf dry weights) and 3 ml/kg soil (stem weight). However, in sweet potato, it was 3 ml (root weight), 4 ml/kg soil (stem weight) and 5 ml/kg (leaf weight).

Table 4.5 Effect of diesel contamination in soil on the dry matter and shoot: root ratio of lettuce leaf, stem and root at 14 WAP

Diesel (ml/kg)	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Whole plant dry weight (g)	Shoot/root ratio
0	15.23	8.50	4.50	23.73	28.23	5.36
1	13.03	6.67	4.33	19.7	24.03	4.53
2	6.67	5.00	2.67	11.67	14.33	4.44
3	10	2.83	3.17	12.83	16	4.06
4	9.67	4.83	3.00	14.5	17.5	4.81
5	10	4.67	3.33	14.67	18	4.44
6	8	3.50	2.83	11.5	14.33	4.11
MEAN	10.37	5.14	3.40	15.51	18.92	4.54
$LSD_{0.05}$	4.62	1.92	1.13	6.01	6.76	1.47
0	21.67	5.02	5.20	26.68	31.88	5.21
5	6.2	0.73	0.97	6.93	7.9	8.09
10	1.42	0.38	0.633	1.8	2.43	2.83
15	0.77	0.33	0.47	1.1	1.57	2.61
20	0.45	0.20	0.30	0.65	0.95	2.39
25	0.5	0.27	0.40	0.77	1.17	1.94
30	0.37	0.25	0.18	0.62	0.8	3.36
MEAN	4.48	1.03	1.16	5.51	6.67	3.78
$LSD_{0.05}$	2.84	0.84	0.43	3.52	3.24	4.13

Table 4.6 Effect of diesel contamination in soil on the dry matter and shoot: root ratio of sweet potato leaf, stem and root at 14 WAP

Diesel (ml/kg)	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Whole plant dry weight (g)	Shoot/root ratio
0	18.33	4.60	2.43	22.93	25.37	9.82
1	18.00	4.07	2.37	22.07	24.43	9.48
2	17.37	4.10	2.10	21.47	23.57	10.25
3	16.13	4.37	1.73	20.50	22.23	12.05
4	16.53	2.97	1.97	19.50	21.47	10.18
5	13.43	3.13	0.97	16.57	17.53	18.24
6	13.47	2.40	0.80	15.87	16.67	20.35
MEAN	16.18	3.66	1.77	19.84	21.61	12.91
$LSD_{0.05}$	3.92	0.81	0.63	4.02	4.12	6.06
0	15.47	4.77	24.00	20.23	44.20	0.84
5	1.40	0.67	1.33	2.07	3.40	1.56
10	0.77	0.57	1.27	1.33	2.60	1.05
15	1.30	1.13	1.97	2.43	4.40	1.23
20	1.07	1.00	2.13	2.07	4.20	0.97
25	0.80	0.73	1.67	1.53	3.20	0.92
30	0.60	0.50	2.15	1.10	3.30	0.51
MEAN	3.06	1.34	4.93	4.40	9.30	0.89
$LSD_{0.05}$	2.86	1.35	7.88	3.67	10.01	0.91

A regression analysis performed on the data obtained at the two concentration ranges of diesel contamination in soil indicated that, a strong regression relationship occurred between the concentrations of diesel contamination in soil and the relative dry matter in lettuce and sweet potato. The average critical concentration (cc) of diesel that was associated with 10 % reduction of maximum biomass as determined from the regression relationships were 0.33 ml and 1.50 ml for lettuce (Figure 4.3) and sweet potato (Figure 4.4) respectively.

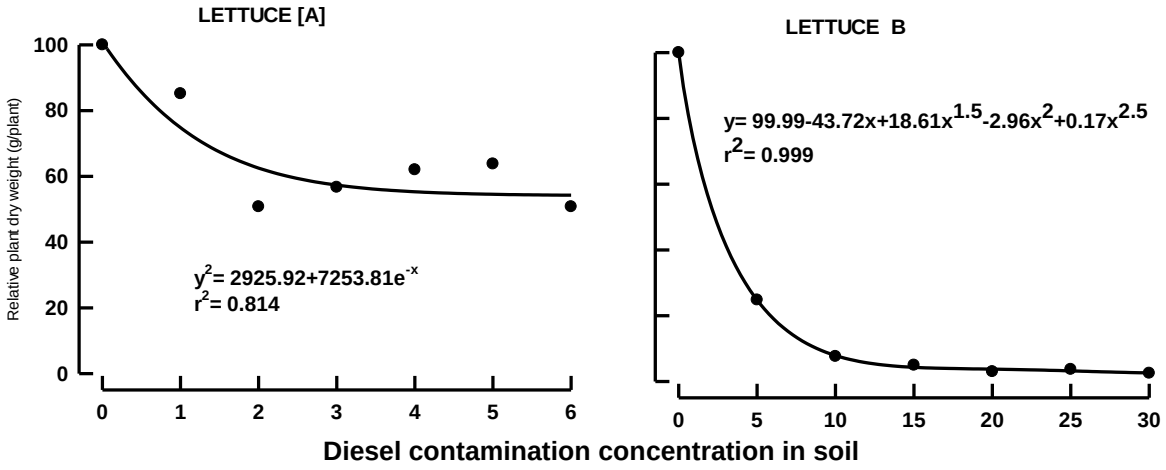

Figure 4.3 Effect of diesel contamination in soil on relative whole plant weight of lettuce for the 0-6 ml/kg (A) and 0-30 ml/kg (B) diesel contamination concentration range treatments at 14 WAP

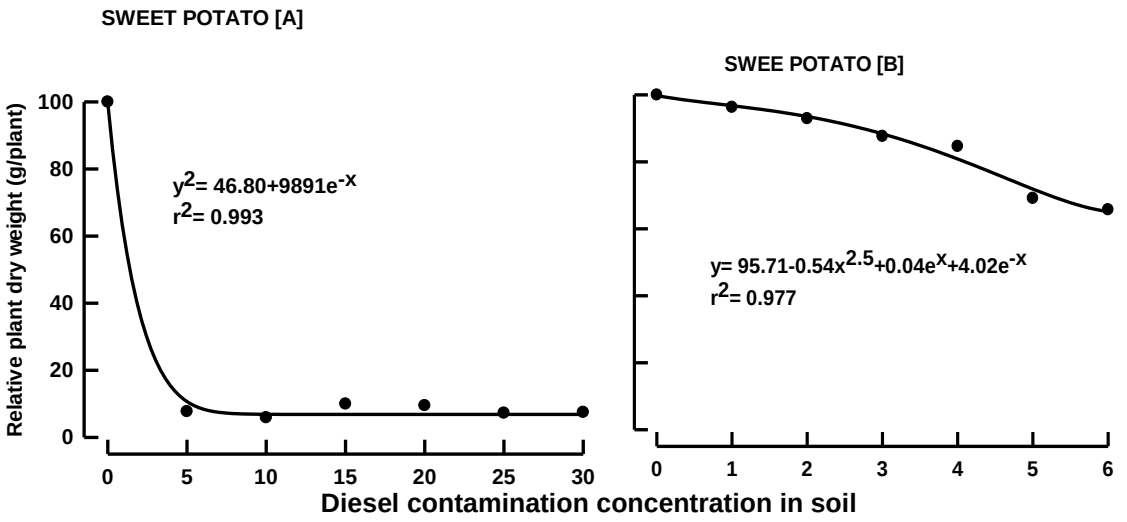

Figure 4.4 Effect of diesel contamination in soil on relative whole plant dry weight of sweet potato for the 0-6 ml diesel /kg soil (A) and 0-30 ml diesel/kg soil (B) contamination concentration range at 14 WAP

4.3.3.2 Effects of diesel contamination in soil on the shoot: root ratio of lettuce and sweet potato

Generally, the shoot: root ratio of lettuce varied less than that of sweet potato in response to increasing diesel contamination in soil. Also, the shoot: root of both species varied more in the 0-30 ml diesel/kg soil contamination range treatments when compared with that of the 0-6 ml diesel/kg soil concentration range treatments (Figure 4.5). Furthermore, the response of the shoot: root ratio to diesel soil contamination differed in the two plant species. The root to

shoot ratio of lettuce was not significantly affected by diesel contamination at the 0–6 ml /kg soil contamination concentration range. However, in sweet potato, significant ($p<0.05$) changes occurred as diesel contamination in soil concentration increased. Regression performed on the data obtained in the two diesel concentration range treatments indicated a strong correlation between the level of diesel soil contamination and the shoot: root ratio in both species (Figure 4.5).

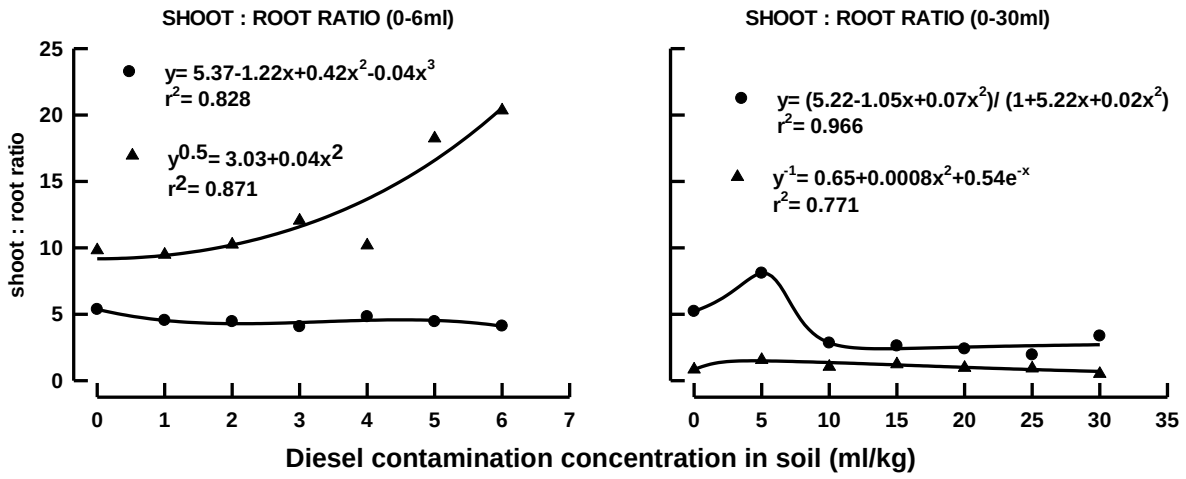

Figure 4.5 The relationship between shoot : root ratio and diesel contamination concentration in soil for Lettuce (●) and sweet potato (▲) at 0-6 ml/kg soil and 0-30 ml/kg soil diesel contamination range treatments

4.3.5 Effect of Diesel Contamination in Soil on Dry Matter Partitioning in Lettuce and Sweet Potato at 14 WAP

Dry matter partitioning of lettuce and sweet potato was influenced by diesel contamination in soil (Table 4.7). In the uncontaminated soil, both lettuce and sweet potato allocated more dry matter to the shoot than the root with the exception of sweet potato at 0-30 ml/kg soil diesel contamination range treatment. Generally, both lettuce and sweet potato plants grown in diesel contamination in soil ≤ 6 ml /kg soil allocated more dry matter to the shoot when compared to the root. However, at diesel concentrations > 6 ml/kg soil (sweet potato) and at

diesel concentration ≥10 ml/kg soil (lettuce), allocation of dry matter to the root improved resulting in lower shoot: root ratio (Figure 4.5). The allocations of dry matter to the roots are more pronounced in sweet potato when compared with lettuce (Table 4.7). It must be noted that the leaves constituted the greater percentage of the shoot in both species (Table 4.7).

Table 4.7 Effect of diesel concentration in soil on the dry matter (DM) partitioning of lettuce and sweet potato leaf, stem and root

DIESEL (ml/kg)	LETTUCE				SWEET POTATO			
	% DM leaf	% DM stem	% DM root	% DM shoot	% DM leaf	% DM stem	% DM root	% DM shoot
0	53.50	30.39	16.13	83.87	71.93	18.34	9.73	90.27
1	54.20	27.71	18.08	81.92	73.56	16.83	9.61	90.39
2	46.60	34.78	18.58	81.42	73.57	17.49	8.95	91.05
3	62.40	17.86	19.78	80.22	72.46	19.69	7.85	92.15
4	56.00	26.77	17.24	82.76	77.04	13.78	9.19	90.81
5	54.40	26.53	19.12	80.88	76.32	18.06	5.62	94.38
6	55.70	24.36	19.91	80.09	80.96	14.28	4.76	95.24
MEAN	**54.70**	**26.91**	**18.41**	**81.59**	**75.12**	**16.92**	**7.96**	**92.04**
$LSD_{0.05}$	**8.52**	**5.72**	**4.49**	**4.49**	**5.50**	**4.31**	**2.74**	**2.74**
0	67.80	15.60	16.60	83.40	36.50	11.30	52.20	47.80
5	76.70	9.70	13.60	86.40	41.10	17.70	41.20	58.80
10	57.80	15.90	26.20	73.80	32.10	19.00	48.90	51.10
15	48.50	22.10	29.40	70.60	30.20	22.30	47.50	52.50
20	48.00	20.70	31.30	68.70	25.80	23.70	50.60	49.40
25	44.30	21.70	34.00	66.00	22.70	18.90	58.50	41.50
30	43.30	29.10	27.60	72.40	21.00	17.50	61.40	38.60
MEAN	**55.20**	**19.30**	**25.50**	**74.50**	**29.90**	**18.60**	**51.50**	**48.50**
$LSD_{0.05}$	**21.41**	**11.54**	**14.09**	**14.09**	**17.59**	**13.93**	**22.16**	**22.16**

4.3.6 Effects of Diesel Contamination in Soil on the Mineral Composition of Lettuce and Sweet Potato Leaves

Diesel contamination in soil affected the composition of the mineral nutrients in the leaves of lettuce and sweet potato in a concentration dependent manner. However, there were marked differences in the mineral nutrient contents of the two species. Lettuce (Table 4.8) accumulated more macro nutrients and heavy metals than sweet potato (Table 4.9). In the 0-

30 ml/kg soil diesel contamination concentration range, the mineral nutrients, N, P, K, Ca, Mg and S, in the leaves varied within a narrow range. Although there were wide variations in the concentrations of Al, Zn, Fe and Cu in the leaves, most of the variations did not form a definite pattern in relation to diesel contamination concentration in soil. The concentration of Al in the leaves of lettuce was within the toxic range at most of the diesel concentrations (Table 4.8). In sweet potato, only in the 20 and 30 ml/kg soil diesel concentration treatments were the levels of Al in the leaves higher than the lower limit expected to cause toxicity (Table 4.9). In the 0-6 ml/kg soil diesel contamination concentration range, none of the nutrients varied to a great extent in both lettuce (Table 4.10) and sweet potato (Table 4.11) leaves with the exception Zn. At critical diesel concentration for toxicity, none of the nutrients was in toxic amounts in the leaves of both lettuce (Table 4.12) and sweet potato (Table 4.13).

Table 4.8 Concentrations of nutrients in the dry leaves of lettuce grown in the 0-30 ml diesel /kg soill concentration range

ml diesel/kg soil	S	N	Ca	Mg	K	Na	Zn	Cu	Mn	Fe	P	Al
	%	%	%	%	%	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	%	mg/Kg
0	0.16	1.64	0.85	0.22	5.19	3164.20	80.00	5.80	27.00	510.00	0.45	762.00
5	0.25	1.82	1.48	0.31	5.89	5817.20	102.00	6.00	48.00	323.00	0.51	467.00
10	0.24	2.01	1.51	0.43	6.94	9800.00	152.00	9.90	76.00	562.00	0.52	862.00
15	0.19	1.71	1.31	0.45	6.26	7997.40	145.00	10.30	69.00	713.00	0.49	1137.00
20	0.17	1.48	1.35	0.51	4.92	8608.40	131.00	10.30	100.00	835.00	0.42	1246.00
25	0.16	1.42	1.26	0.49	4.67	8181.70	128.00	9.70	101.00	798.00	0.41	1242.00
30	0.18	1.38	1.27	0.52	4.48	6857.20	112.00	9.20	85.00	740.00	0.44	1015.00
Mean	0.19	1.64	1.29	0.42	5.48	7203.71	121.43	8.74	72.43	640.00	0.46	961.57
ONCRL	>0.3[b]	2.5-4.0[b]	1.0-2.0[a]	0.3-0.7[b]	2.5-8.0[a]	-	30-100[b]	5-30[b]	50-100[b]	50-500[b]	0.4-0.6[b]	>500

ONCRL Optimum nutrient concentration range in lettuce leaves according to sources. (a) Hochmuth *et al.,* (2012) (b) *http://biocyclopedia.com/*
ppm = parts per million = mg/kg = µg/g; 10 000 ppm = 1 per cent.

Table 4.9 Concentration of nutrients in the dry leaves of sweet potato grown in the 0-30 ml/kg soil concentration range

ml diesel/kg soil	S	N	Ca	Mg	K	Na	Zn	Cu	Mn	Fe	P	Al
	%	%	%	%	%	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	%	mg/Kg
0	0.36	2.75	2.33	0.54	5.79	1023.30	44.00	6.10	23.00	292.00	0.48	349.00
5	0.26	2.13	1.58	0.55	4.43	1567.00	39.00	7.00	96.00	427.00	0.50	518.00
10	0.24	1.77	1.56	0.57	3.60	2467.00	49.00	5.30	58.00	391.00	0.42	522.00
15	0.16	1.45	1.12	0.47	2.37	1029.80	19.00	3.60	41.00	380.00	0.32	517.00
20	0.18	1.55	1.34	0.57	2.64	1613.30	39.00	6.10	39.00	922.00	0.39	1561.00
25	0.21	1.77	1.33	0.62	2.78	1217.70	41.00	6.40	41.00	349.00	0.60	347.00
30	0.28	2.09	1.74	0.62	2.90	2412.00	48.00	14.30	55.00	1661.00	0.36	2781.00
Mean	0.28	1.93	1.57	0.56	3.50	1618.60	40.00	7.00	50.00	632.00	0.44	942.14
ONCRSP	0.2-0.6[b]	2.8-3.5[b]	0.8-1.6[a]	0.25-0.5[b]	2.0-4.0[b]	-	25-50[a]	5-30[b]	40-400[b]	50-500[b]	0.2-0.5[b]	>500[b]

ONCRSP = Optimum nutrient concentration range in sweet potato according to sources (a) Hochmuth *et al.*, (2012) and (b) *http://biocyclopedia.com/*

Table 4.10 Concentrations of nutrients in the dry leaves of lettuce grown in the 0-6 ml/kg soil concentration range

ml diesel/kg soil	S %	N %	Ca mg/Kg	Mg mg/Kg	K mg/Kg	Na mg/Kg	Zn ppm	Cu ppm	Mn ppm	Fe ppm	P mg/Kg	Al mg/Kg
0	0.34	4.73	1.42	0.57	9.96	11431.62	89.83	6.44	30.17	126.84	0.76	154.24
1	0.36	4.39	1.42	0.57	13.03	14357.36	132.48	7.26	38.38	189.12	0.84	164.01
2	0.37	4.79	1.20	0.59	12.34	12776.35	133.07	9.05	46.91	167.93	0.80	229.27
3	0.37	4.34	1.17	0.53	12.63	13291.39	145.40	11.93	39.52	138.29	0.86	154.46
4	0.37	3.67	1.29	0.52	11.90	12713.49	126.52	13.38	48.35	154.28	0.84	160.54
5	0.34	3.26	1.29	0.54	12.87	12197.62	113.13	11.54	59.77	124.86	0.83	143.62
6	0.35	4.19	1.16	0.52	11.99	13131.03	128.92	9.34	50.29	131.38	0.94	156.96
mean	0.36	4.20	1.28	0.55	12.10	12842.69	124.19	9.85	44.77	147.53	0.84	166.16
ONCRL	>0.3[b]	2.5-4.0[b]	1.0-2.0[a]	0.3-0.7[b]	2.5-8.0[a]	-	30-100[b]	5-30[b]	50-100[b]	50-500[b]	0.4-0.6[b]	>500

ONCRL Optimum nutrient concentration range in lettuce leaves according to sources. (a) Hochmuth *et al.,* (2012) (b) *http://biocyclopedia.com/*

Table 4.11 Concentration of nutrients in dry leaves of sweet potato grown in the 0-6ml/kg soil concentration range

ml diesel/kg soil	S %	N %	Ca mg/Kg	Mg mg/Kg	K mg/Kg	Na mg/Kg	Zn ppm	Cu ppm	Mn ppm	Fe ppm	P mg/Kg	Al mg/Kg
0	0.52	5.34	1.62	0.81	5.94	689.38	38.88	4.98	43.64	224.29	0.38	327.22
1	0.61	5.24	1.53	0.82	6.47	709.31	46.63	6.08	53.40	290.84	0.43	453.91
2	0.52	4.31	1.51	0.77	6.46	855.61	44.12	6.18	57.68	326.83	0.39	536.26
3	0.57	4.56	1.38	0.76	6.61	877.39	41.53	7.01	71.51	223.21	0.39	311.55
4	0.56	4.39	1.44	0.77	6.65	709.45	37.56	5.91	79.32	244.93	0.39	352.09
5	0.54	4.37	1.43	0.76	7.04	741.92	40.93	7.21	77.64	350.65	0.41	536.85
6	0.52	4.51	1.56	0.83	6.57	781.79	40.12	8.39	73.49	230.41	0.40	298.76
mean	0.55	4.68	1.49	0.79	6.54	766.41	41.39	6.54	65.24	270.17	.040	402.37
ONCRSP	0.2-0.6[b]	2.8-3.5[b]	0.8-1.6[a]	0.25-0.5[b]	2.0-4.0[b]	-	25-50[a]	5-30[b]	40-400[b]	50-500[b]	0.2-0.5[b]	>500[b]

ONCRSP = Optimum nutrient concentration range in sweet potato according to sources (a) Hochmuth *et al.*, (2012) and (b) *http://biocyclopedia.com/*

Table 4.12 Concentrations of mineral nutrients in the leaves of lettuce at critical diesel concentrations for toxicity

Mineral nutrients		Nutrient concentration in dry leaves at critical diesel concentration for toxicity		
Nutrients	**Unit**	**0-30 ml diesel/kg soil concentration range**	**0-6 range ml diesel/kg soil concentration range**	**Average**
S	%	0.19	0.35	0.27
N	%	1.64	4.70	3.17
P	mg/Kg	0.45	0.82	0.64
K	mg/Kg	6.18	10.40	8.29
Ca	mg/Kg	1.25	1.37	1.31
Mg	mg/Kg	2.25	0.58	1.41
Na	mg/Kg	980.00	750.00	865.00
Al	mg/Kg	270.00	230.00	250.00
Zn	ppm	83.00	5.60	44.30
Cu	ppm	5.92	5.24	5.58
Fe	ppm	305.00	120.00	212.50
Mn	ppm	42.00	28.00	35.00

Table 4.13 Concentrations of mineral nutrients in the leaves of sweet potato at critical diesel concentrations for toxicity

Mineral nutrients		Nutrient concentration in dry leaves at critical diesel concentration for toxicity		
Nutrients	**Unit**	**0-30 ml diesel/kg soil concentration range**	**0-6 range ml diesel/kg soil concentration range**	**Average**
S	%	0.34	0.58	0.46
N	%	2.57	4.56	3.56
P	mg/Kg	0.46	0.39	0.43
K	mg/Kg	5.85	6.57	6.21
Ca	mg/Kg	2.01	1.57	1.79
Mg	mg/Kg	0.54	0.79	0.66
Na	mg/Kg	1000.00	800.00	900.00
Al	mg/Kg	375.00	330.00	352.50
Zn	ppm	45.00	44.00	44.50
Cu	ppm	6.00	5.50	5.75
Fe	ppm	450.00	230.00	340.00
Mn	ppm	46.00	62.00	54.00

4.4 DISCUSSION

4.4.1 Effect of Diesel Contamination in Soil on the Growth of Lettuce and Sweet Potato

Diesel spills on agricultural land have generally been reported to cause mortality and reduction in plant growth (Wyszkowski & Wyszkowska, 2005; Nwaogu *et al*., 2008; Bona *et*

al., 2011). In the current study, high amounts of diesel in the 0-30 ml/kg soil contamination concentration range treatment severely reduced plant growth in both species. The reduction in growth was accompanied by reduction in chlorophyll content and leaf area (Table 4.3), which probably contributed to the reduction in the growth of lettuce and sweet potato as a result of reduced photosynthesis. The progressive decrease in leaf chlorophyll with increasing concentration of diesel contamination in soil was as noted with lettuce and sweet potato has also been reported for *Solanum melongena* (Akujobi *et al*., 2011), cereals (Seklemora *et al*., 2001).

Lettuce was highly sensitive to diesel contamination in soil than sweet potato. As little as 0.33 ml of diesel/kg of soil was enough to cause a 10 % reduction in plant biomass in lettuce, whereas 4.5-fold in this amount was required to cause a 10 % reduction in plant biomass of sweet potato. The higher sensitivity of lettuce than sweet potato was probably due to the fact that lettuce was propagated from seedlings, which had little carbohydrate reserves. Unlike sweet potato cuttings, this obviously contained a higher amount of carbohydrate reserves. Hence, the sweet potato was able to make more growth in diesel contaminated soil. A number of researchers have also reported negative correlation between the level of petroleum hydrocarbon contamination in soil and the biomass of some crops. They include Kuhn *et al*., 1998 (tomato); Brandt *et al.*, 2006 (vetiver); Daniel-Kalio & Pepple, 2006 (*Commelina benghalensis* L.); Adenipekun *et al*., 2008 (*Corchorus olitorius Linn*) and Bona *et al.*, 2010 (*Schinus terebinthifolius*).

4.4.2 Causes of Diesel Toxicity on the Growth and Mortality of Lettuce and Sweet Potato

Previous studies have attributed the causes of plants mortality and reduced growth largely to deficiencies of S, N, P, K, Ca or Mg (Dimitrow & Markow, 2000; Wyszokowski & Ziolkowska, 2008; Bayram *et al*. 2009). This is probably because of the symptoms associated

with plants grown in petroleum contaminated soil (also observed in this study), which include wilting, stunted growth and leaf chlorosis (Watts *et al.*, 1982; Baran *et al*., 2002). The result of plant tissue analyses indicated that the mineral nutrients in the leaves of lettuce (Table 4.8) and sweet potato (Table 4.9) did not vary to a great extent, and most of the mineral nutrients except N were within the optimum range for the normal functioning of both test plant species. Leaf N levels were marginal in both lettuce and sweet potato plants, but could not be attributed to diesel pollution since it was also marginally deficient in the control plants. Hence, N deficiency could not have been the cause of mortalities and poor growth of lettuce and sweet potato grown in diesel contaminated soil in this study.

Heavy metal toxicity to plants has also been associated with petroleum diesel contamination for example, *Zea mays* (Ogbo, 2009) and *Phaseolus vulgaris* L. (Aade-Ademilua & Mbamalu, 2008). Although the heavy metal Fe accumulated to toxic level in the leaves of sweet potato at 20 and 30 ml diesel/kg soil, its accumulation could not be held responsible for reduced plant growth because the growth of sweet potato was also severely reduced at other concentration levels other than 20 and 30 ml (Table 4.9). In lettuce Zn and Fe accumulated in the leaves to toxic levels in plants grown in soil contaminated with ≥10 and 15 ml diesel/kg soil, respectively (Table 4.8). Nonetheless, at critical diesel concentration in the soil for toxicity, the concentration of these two nutrients interpolated from the relationship between relative plant biomass and Zn or Fe concentration were below the toxic levels. Thus again, nutrient toxicity could not account for the decline in plant growth, at least at critical diesel concentration for toxicity for lettuce (Table 4.12) and sweet potato (Table 4.13). The cause of mortality and poor growth of sweet potato and lettuce grown in diesel contaminated soil may therefore have been direct toxicity of diesel hydrocarbons. A further proof that diesel hydrocarbon was involved could be seen in the time the mortality of the two species occurred. The mortalities in both species occurred at relatively early stages of growth of the crops. No mortality occurred after 6 and 3 WAP for lettuce and sweet potato, respectively.

The non-mortality at later stages of growth of the two species may be due to reduction in diesel hydrocarbons in the soil since the hydrocarbons are subject to removal from the soil through evaporation (Hejazi & Husain, 2004; Serrano *et al.*, 2007), and biodegradation (Dibble & Bartha, 1979; Young *et al.*, 1992; Onuoha *et al.*, 2011).

The practical implication of this study is that it is reasonable to recommend lettuce for evaluating soil contamination with diesel, because lettuce is more sensitive to diesel soil contamination than sweet potato. Hence, this study confirms the recommendation of lettuce by USEPA for acute toxicity tests of hydrocarbon polluted soil (Fletcher, 1991). Since sweet potato is more tolerant of diesel soil contamination, it can be recommended for phytoremediation of diesel contaminated soil. The downside to the use of sweet potato is that it is not a nitrogen fixer, and hence N fertilizer application will always be necessary. Another disadvantage of using sweet potato for phytoremediation purpose is that, the biomass of sweet potato is relatively small when compared with plants like *Glycine max* that have been recommended for phytoremediation of crude oil contaminated soil (Lambers *et al.*, 1998; Njoku *et al*., 2009). However, the storage root of sweet potato may be an advantage. It has been reported that plants with large root biomass are good for phytoremediation of crude oil contaminated soil (Issoufi, 2005). The case for using sweet potato for phytoremediation of diesel contaminated soil may be strengthened by the ability of the storage roots to absorb substantial quantity of diesel hydrocarbons and heavy metals from the soil (not measured in this study). In addition to the advantage associated with the storage root system in sweet potato, the process of harvesting sweet potato, which involves substantial digging of the soil will enhance natural attenuation of diesel contaminated soil (Serrano *et al.*, 2007). Sweet potato can also serve dual purposes of being an economic crop while being used for soil remediation purposes, because the harvested tubers could be used to feed large ruminants (subject to the level of the contamination of the tuber). It is, therefore, recommended that

further research should be conducted to confirm the suitability of sweet potato for phytoremediation of diesel contaminated soil.

4.4.3 Effects of Diesel Contamination in Soil on the Dry Matter partitioning of Lettuce and Sweet Potato

Diesel contamination in soil affected the dry matter partitioning in both lettuce and sweet potato. Sweet potato partitioned more dry matter to the root. Diesel contamination in soil has been reported to cause unfavourable soil condition for plant growth, which include; water stress, accumulation of heavy metals and oxygen deficiency (Ogbo, 2009). Bloom *et al*. (1985); Zharare & Scogings, (2011) reported that plants change their pattern of allocation of DM in response to environmental stress. In this study, the allocation of more dry matter to the root at concentration ≥10 ml/kg soil (lettuce) and in the 0-30 ml/kg soil diesel concentration range (sweet potato) occurred due to unfavourable environment created in the soil by diesel. The allocation of more dry matter to the root by both species may have arisen as an adaptive mechanism in which the root system is used for storage in addition to increasing the capacity for foraging for mineral nutrients and water. It has been reported that plants can fully exploit their environment through storage rather than changing its allocation to some pattern that would be inappropriate for the normal growth of the plant when the environment is unfavourable (Bloom *et al*., 1985). Sweet potato may not have found this adaptive system difficult since it is by nature a root crop.

4.5 CONCLUSIONS

Diesel contamination in soil was phtytotoxic on the growth of lettuce and sweet potato. The phytotoxicity of diesel on the growth of lettuce and sweet potato was concentration dependent. Diesel soil contamination concentration ≥15 ml/kg was associated with mortality of some lettuce and sweet potato plants. Lettuce was more sensitive to diesel contamination than sweet potato. For example, lettuce recorded higher mortality than sweet potato. Also, the

critical concentration of diesel contamination in soil for toxicity in lettuce (0.33 ml diesel/kg soil) was also much lower than that for sweet potato (1.50 ml diesel/kg soil). Diesel contamination in soil also affected the dry matter partitioning in both species. At lower diesel concentration, allocation of dry matter to the shoot was favoured. The allocation of dry matter to the root by lettuce and sweet potato at high diesel contamination may be an an attemptto overcome phytotoxic effects of diesel hydrocarbons.

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CHAPTER 5

GENERAL DISCUSSIONS, RECOMMENDATIONS AND CONCLUSION

5.1 DIESEL, SOIL AND LEAF ANALYSES

Crude oil is derived from plant and other organic materials (Cheng *et al.,* 2013). Hence, diesel which is one of the products derived from crude oil is of organic matter origin.It is therefore reasonable to assume that the organic materials that formed the crude oil varied from place to place in terms of species and in mineral composition (Cheng *et al.,* 2013). The latter is influenced by the plants' ability to accumulate mineral nutrients and the chemical composition of the soil in which the plants grew. Hence, diesel from different places is also expected to vary in hydrocarbon and mineral compositions. Indeed, the chemical composition of the diesel used in this study was different from that reported by Ricardo *et al.* (2010). Thus, reflecting differences in the composition of crude oil mined from different places. For example, in this study, the diesel presented high concentrations of PAHs (3,693.68 ppm) and low concentrations of BTEX (20.35 ppm), when compared with the values (38.61 ppm and 2815.16 ppm for PAHs and BTEX, respectively) for the diesel analysed by Ricardo *et al.* (2010). Also, samples of diesel were taken from 25 regions in the United States indicated variation in composition (Harold, 2011). For example, the aromatic hydrocarbons of the samples varied between 15.47 % and 38.18 %. Similarly, Selco *et al.* (2011) found differences in PAH content between two samples of diesel obtained from different areas. It is, therefore, highly probable that differences in composition of diesel may be traceable to the source of the parent material (crude oil).

When diesel is spilt, the chemical composition of the soil is altered. It has severally been reported that diesel contamination in soil affect mineral nutrients and heavy metals in the soil (Agbogidi *et al*., 2007; Njoku *et al.,* 2009). The result of soil analyses (Table 3.3) indicated in the present study that the concentration of diesel contamination in soil had little impact on soil chemical properties, because all the soil chemical nutrients (macro nutrients and heavy metals) analysed (N, P, K, Ca, Mg, P, K, Cu, Zn, Mn and pH) were within the optimal range for lettuce and sweet potato (Table 3.3). Although Zn, Fe and Al concentrations in plant tissue exceeded the optimal threshold for plant growth, toxicity from these heavy metals was discounted because at critical diesel concentration for toxicity, none of the heavy metals was at toxic level for lettuce (Table 4.12) and sweet potato (Table 4.13).

Having discounted the contribution of heavy metals in diesel and nutrient deficiency in the phytotoxic effects of diesel on the germination and growth of lettuce, it is reasonable to ascribe the toxicity of diesel on plant growth to the hydrocarbon component of the diesel. Thus, remedial action in spills must be directed at removal of the hydrocarbon component of the diesel from the soil as soon as possible.

It has been reported that the phytotoxicity of diesel is dependent mostly on its aromatic hydrocarbons and to a lesser extent on the aliphatic hydrocarbons (Alkio *et al*., 2005). While the monocyclic aromatic hydrocarbons (BTEX) cause acute injury to plants, the polycyclic aromatic hydrocarbons (PAH) cause chronic injury (Graef & Winter, 1968; Alkio *et al*., 2005). Alkio *et al*. (2005) has argued that the aliphatic components of diesel hydrocarbons are less toxic on plants since they are very volatile and therefore quickly diminish from the soil. In the light of the foregoing discussion, it is therefore important to assess the chemical composition of the diesel after a spill to estimate the expected impact to plants and persistence of the diesel effects. Also, for forensic purposes, the analyses will help to

establish the source of diesel or crude oil if a data base is maintained on chemical composition of crude oil or petroleum products from different sources. It is important to have such data base considering the fact that diesel is the most toxic of all products from crude oil and the most likely to be spilt on farms because most of the machineries used on farms are powered by diesel (FAO 2011). It would greatly help to strategize rehabilitation of diesel polluted land if it can be established, which of the hydrocarbon component is particularly toxic to plants.

5.2 SENSITIVITY OF LETTUCE AND SWEET POTATO TO DIESEL SOIL CONTAMINATION

The 'critical level' of an input in the soil in relation to plant growth is a concept usually used in soil fertility and plant nutrition to relate to the adequacy or toxicity level of an essential nutrient (Plank & Kissel, 2008). In this study, this concept was used to relate the toxicity of diesel in the soil to the germination and vegetative growth of lettuce and sweet potato, and provided a means for comparing the sensitivity of the two crops to diesel toxicity. In this case, diesel concentration in the soil associated with a 10 % reduction in germination or vegetative growth due to diesel toxicity was successfully used to compare the sensitivity of lettuce and sweet potato to diesel toxicity.

The critical concentration of diesel for toxicity on the germination of lettuce increased from 0.16 ml (1D) to 17.74 (100D) when the study was terminated (Table 3.3). In the case of sweet potato the critical concentration of diesel for toxicity increased from 10.83 at 1D to 21.6 at 25D (Table 3.3). As the age of diesel pollution became ≥ 50D, the diesel soil contamination did not reduce the vegetative germination of sweet potato by up to 10 %, hence the lack of critical concentration of diesel for toxicity. It was determined in this study that the critical

diesel concentration for toxicity in relation to seed germination of lettuce was 1.32 ml compared with 15.5 ml for vegetative germination of sweet potato. Hence, the germination of lettuce was highly sensitive to diesel pollution than sweet potato. Also, it was shown that as little as 0.33 ml of diesel/kg of soil was enough to cause a 10 % reduction in lettuce plant biomass, whereas 4.3-fold in this amount was required to cause a 10 % reduction in plant biomass of sweet potato. These data showed that both the germination and the vegetative growth of sweet potato were less sensitive to diesel soil contamination than those for lettuce.

Reasons for the more sensitivity of lettuce when compared with sweet potato may be attributed to the fact that sweet potato was propagated from cuttings. Germination of plants, irrespective of the type of propagule, is supported by food materials stored in the propagule. Generally, the bigger the storage, the better the germination of the propagule is able to withstand unfavourable conditions such as shading, drought, and toxic environments (Eckstein *et al*., 2012). Cuttings are matured parts of plants which are already hardened by forces of nature and have more carbohydrate reserve when compared with seedling emerging from seeds as was the case for lettuce in this study. Seeds germination and seedling growth are known to be vulnerable to inhibitory substances in the soil (Taiz & Zeiger, 2010; Bona *et al.*, 2010). Lettuce seeds are small, hence they could easily be completely covered with diesel sheen, which could pose a barrier to the entry of water and oxygen into the seeds. In sweet potato, only the part of the cuttings in the soil could be covered with diesel with the other parts above the soil having access to oxygen. Water and oxygen are required for germination (Taiz & Zeiger, 2010). Also the penetration of diesel into lettuce seed could kill the embryo of lettuce seeds. In contrast, sweet potato cuttings do not germinate through an embryo and germination could occur through any of the buds present on the cuttings. Hence,

sweet potato germination from cuttings may not be as vulnerable as lettuce seed germination to diesel contamination in soil.

The significance or practical implication of these results is that, since sweet potato exhibited better germination and growth at higher concentration of diesel when compared with lettuce, it could be used for phytoremediation of diesel contaminated soils. The use of sweet potato for phytoremediation may also be enhanced by its storage root system that may take up diesel heavy metals from the soil (not measured in this study). The results indicate a wider possibility for utilizing agricultural species like sweet potato with good germinability and good growth in diesel contaminated soil in the remediation of diesel soil contamination. Species with sensitive germination and growth like lettuce could be used to monitor the level of diesel pollution in soil. The strength of using sweet potato for phytoremediation of diesel contaminated soil may lie in the ability of the storage roots to absorb substantial quantity of diesel hydrocarbons and heavy metals from the soil (not measured in this study). This is in view of the fact that sweet potato produces relatively low shoot biomass, when compared with other plants like *Glycine max* that have been successfully evaluated for remediation of hydrocarbon contaminated soil (Njoku *et al.,* 2009). While the relatively low shoot biomass may be a disadvantage, the storage root may be a serious advantage when considering sweet potato for phytoremediation purposes. It has been reported that living plant root growth and distribution in diesel-contaminated soil play an important role in the effectiveness of phytoremediation (Hou, 2000). Issoufi *et al.,* (2005) reported that plants with large root biomass are good for phytoremediation of crude oil contaminated soil.

It is recommended that further researches should be conducted to confirm the suitability of sweet potato for phytoremediation of diesel contaminated soil. Future study involving sweet

potato should include the effect on the accumulation of heavy metals in the tuber of the crop. The effects of diesel spillages on various types of soil and the productivity of different crops should be studied. Options for ameliorating the effects of diesel soil contamination on crops and the soil should also be explored further. Since phytoremediation is likely to reduce the operation cost for treating soil contaminated with diesel and other petroleum products, it could be economically feasible to link land farming and phytoremediation as a treatment strategy for diesel contaminated soil.

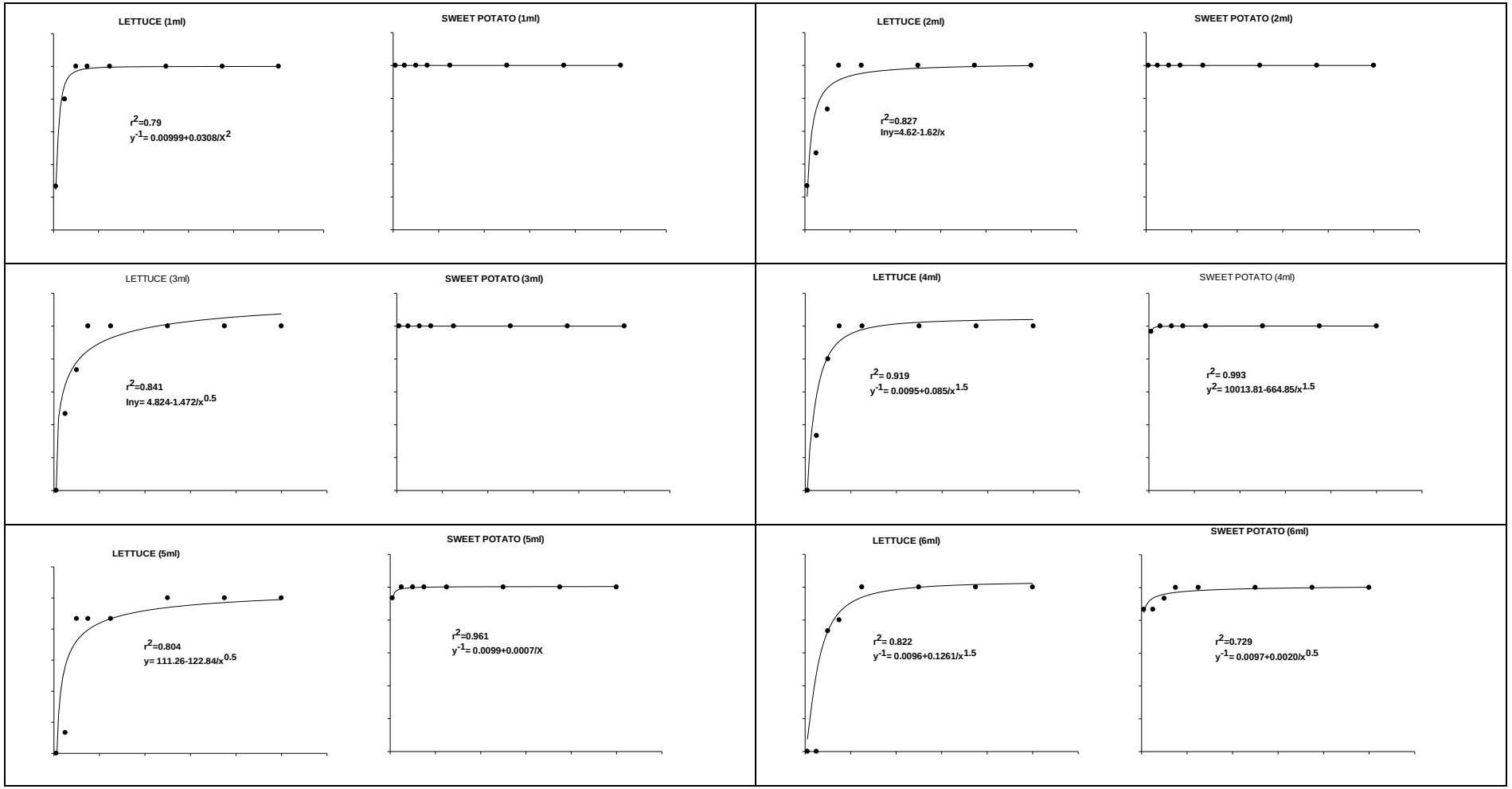
5.3 CONCLUSIONS

This study indicated that diesel contamination affects soil chemical fertility, germination and growth of lettuce and sweet potato. The effects were dependent on the concentration of diesel contamination in the soil and the crop species. Lettuce was shown to be more sensitive to diesel soil contamination than sweet potato in both the germination and growth experiments. The effects of diesel soil contamination reduced with the aging of the contaminant. The response of lettuce and sweet potato to diesel contamination indicated that lettuce could be used as an indicator of soil contamination with diesel and sweet potato could be used to monitor diesel contaminated sites. Hence, data from this study could be used for environmental impact assessment and rehabilitation of studies.

APPENDICES

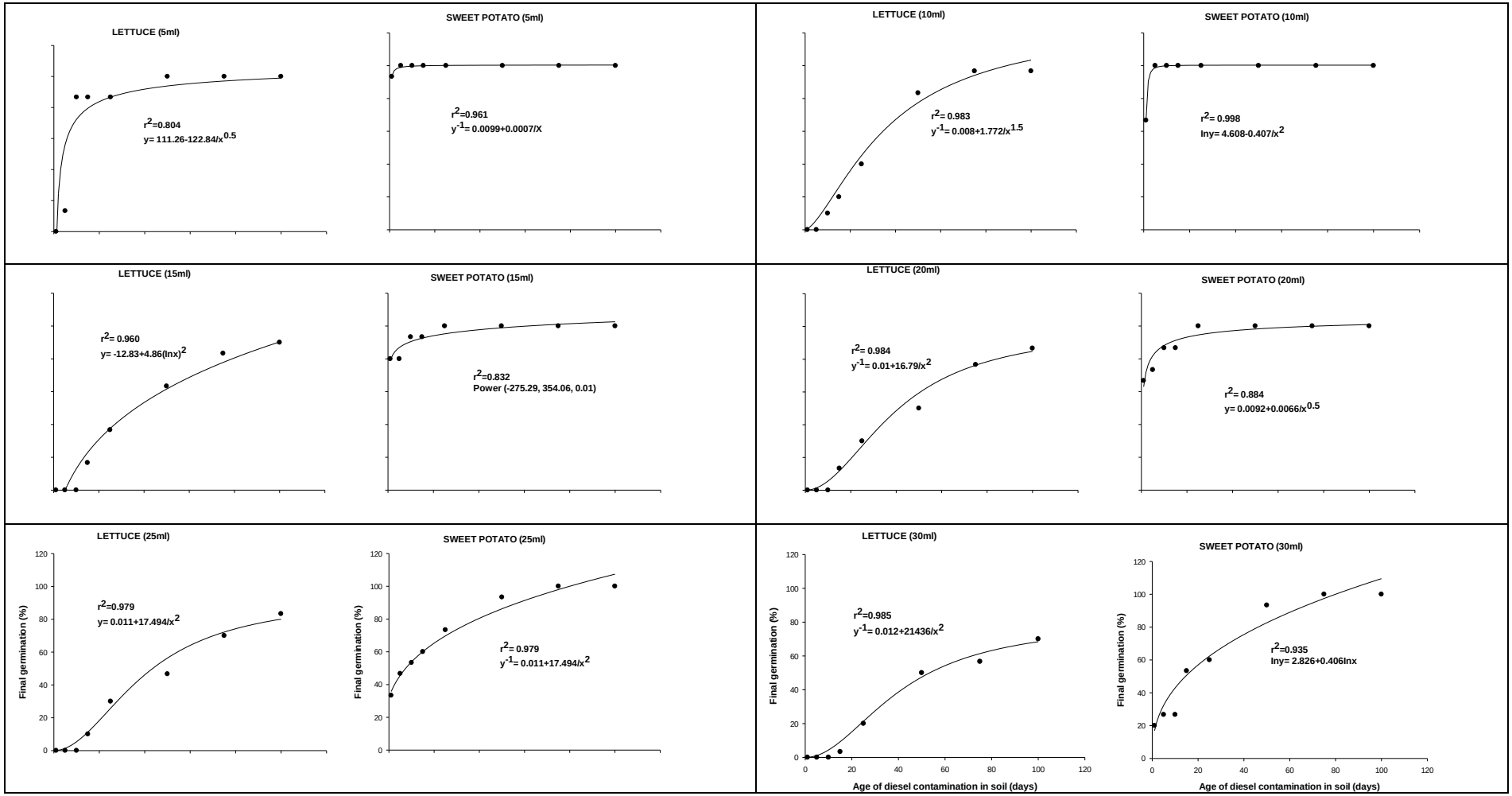
APPENDIX 1 GERMINATION RESPONSES OF LETTUCE AND SWEET POTATO AT 0-6 ml DIESEL CONTAMINATION RANGE

Appendix 1.1 Germination responses of lettuce and sweet potato to age of contamination at 0-6 ml diesel contamination concentration *[The figure shows the relationship between germination and age of contamination at each level of diesel contamination concentration/crop indicated on the curves]*



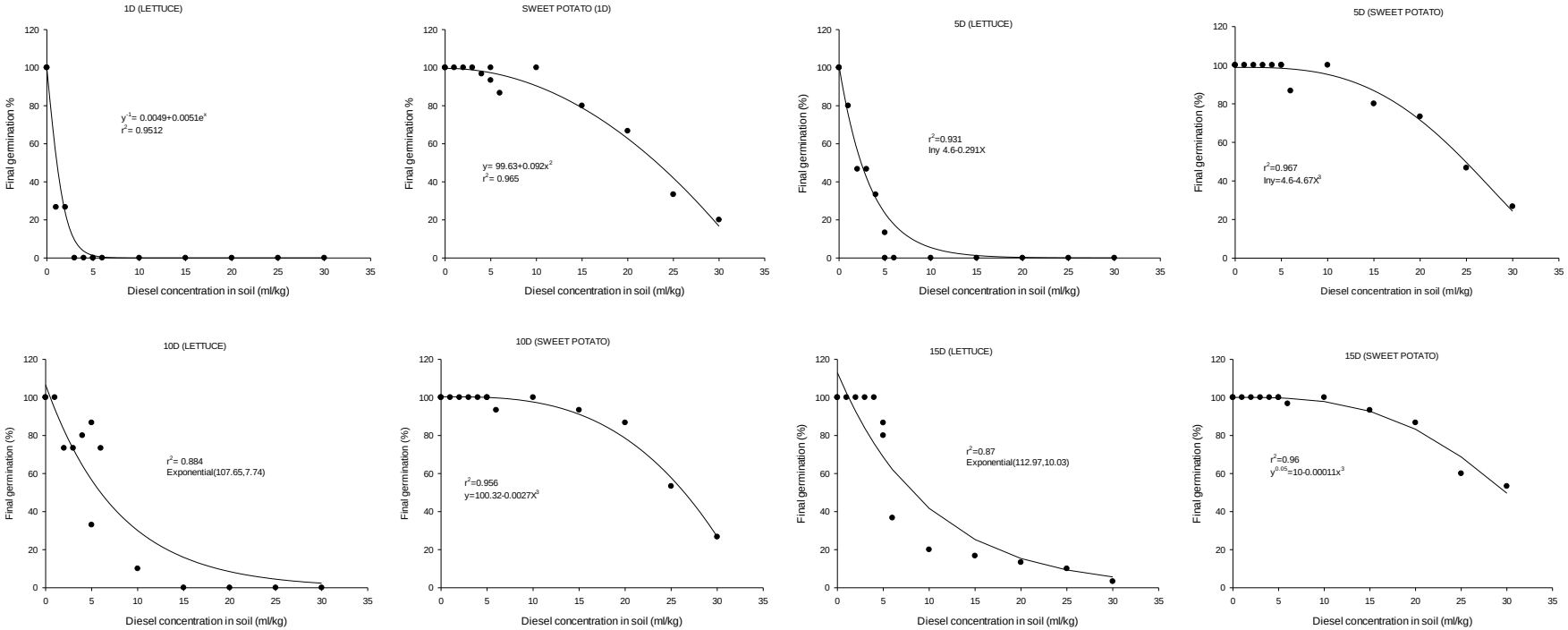
Appendix 1.2 Germination responses of lettuce and sweet potato to age of contamination at 0-30 ml diesel contamination concentration range

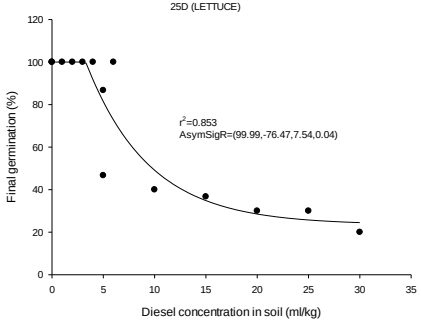
[The figure shows the relationship between germination and age of contamination at each level of diesel contamination concentration/crop indicated on the curves]

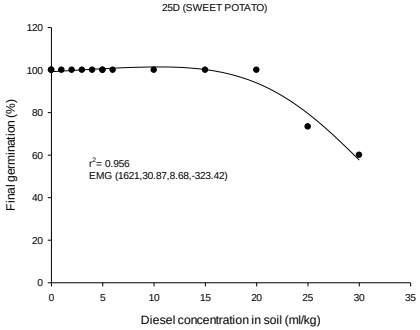


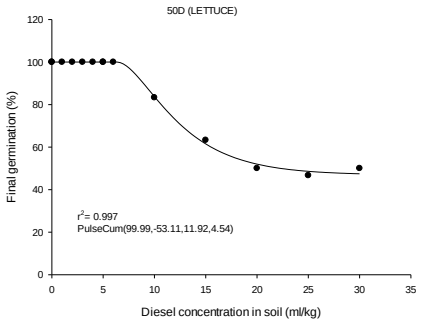
Appendix 1.3 Germination responses of lettuce and sweet potato to diesel contamination concentration range

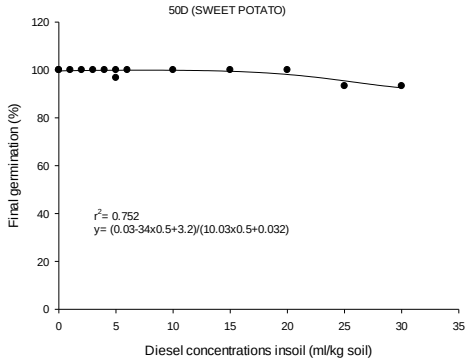
NOTE: The figure shows the combined regression of both 0-30 and 0-6 ml diesel contamination concentration ranges. The combined regression show the relationship of diesel concentration treatment at the particular age of contamination/crop indicated. It was used to determine the critical concentrations of diesel and the toxicity index of diesel on the germination of lettuce and sweet potato

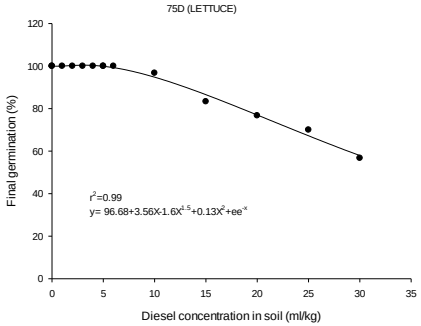


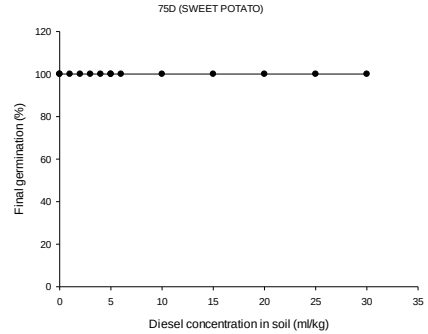

25D (LETTUCE)
Final germination (%)
Diesel concentration in soil (ml/kg)
r²=0.853
AsymSigR=(99.99,-76.47,7.54,0.04)

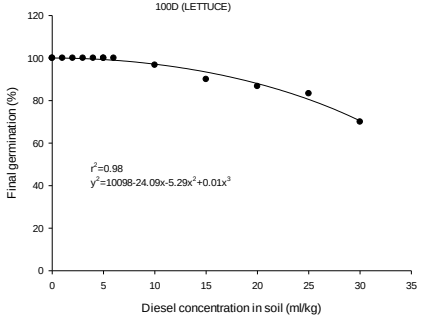

25D (SWEET POTATO)
Final germination (%)
Diesel concentration in soil (ml/kg)
r²= 0.956
EMG (1621,30.87,8.68,-323.42)

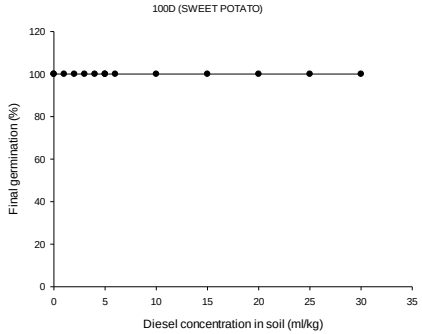

50D (LETTUCE)
Final germination (%)
Diesel concentration in soil (ml/kg)
r²= 0.997
PulseCum(99.99,-53.11,11.92,4.54)


50D (SWEET POTATO)
Final germination (%)
Diesel concentrations insoil (ml/kg soil)
r²= 0.752
y= (0.03-34x0.5+3.2)/(10.03x0.5+0.032)


75D (LETTUCE)
Final germination (%)
Diesel concentration in soil (ml/kg)
r²=0.99
y= 96.68+3.56X-1.6X^1.5+0.13X²+ee^-x


75D (SWEET POTATO)
Final germination (%)
Diesel concentration in soil (ml/kg)


100D (LETTUCE)
Final germination (%)
Diesel concentration in soil (ml/kg)
r²=0.98
y²=10098-24.09x-5.29x²+0.01x³


100D (SWEET POTATO)
Final germination (%)
Diesel concentration in soil (ml/kg)

APPENDIX 2 ETHICS CLEARANCE CERTIFICATE

UNIVERSITY RESEARCH ETHICS COMMITTEE
(Reg No: UZREC 171110-30)

UNIVERSITY OF ZULULAND
Website: http://www.unizulu.ac.za

Private Bag X1001
KwaDlangezwa 3886

Tel: 035 902 6887
Fax: 035 902 6635
Email: MangeleS@unizulu.ac.za

ETHICAL CLEARANCE CERTIFICATE

Certificate Number	**UZREC 171110-030 PGM 2013/49**								
Project Title	**Influence of diesel spillage on the productivity of *Ipomoea batatas* and *Lactuca sativa***								
Principal Researcher/ Investigator	K Fatokun								
Supervisor and Co-supervisor	Dr. DE Zharare				Dr. FB Lewu				
Department	Agriculture								
Nature of Project	Honours/4th Year		Master's	x	Doctoral		Departmental		

The University of Zululand's Research Ethics Committee (UZREC) hereby gives ethical approval in respect of the undertakings contained in the above-mentioned project proposal and the documents listed on page 2 of this Certificate. Special conditions, if any, are also listed on page 2.

The Researcher may therefore commence with the research as from the date of this Certificate, using the reference number indicated above, but may not conduct any data collection using research instruments that are yet to be approved.

Please note that the UZREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the documents that were presented to the UZREC
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UZREC in the prescribe format, where applicable, annually and at the end of the project, in respect of ethical compliance.

The table below indicates which documents the UZREC considered in granting this Certificate and which documents, if any, still require ethical clearance. (Please note that this is not a closed list and should new instruments be developed, these may also require approval.)

Documents	Considered	To be submitted	Not required
Faculty Research Ethics Committee recommendation	X		
Animal Research Ethics Committee recommendation			X
Health Research Ethics Committee recommendation			X
Ethical clearance application form	X		
Project registration proposal	X		
Informed consent from participants			X
Informed consent from parent/guardian			X
Permission for access to sites/information/participants			X
Permission to use documents/copyright clearance			X
Data collection/survey instrument/questionnaire			X
Data collection instrument in appropriate language		Only if necessary	
Other data collection instruments		Only if used	

Special conditions: Documents marked "To be submitted" must be presented for ethical clearance before any data collection can commence.

The UZREC retains the right to

- Withdraw or amend this Certificate if
    - Any unethical principles or practices are revealed or suspected
    - Relevant information has been withheld or misrepresented
    - Regulatory changes of whatsoever nature so require
    - The conditions contained in this Certificate have not been adhered to
- Request access to any information or data at any time during the course or after completion of the project

The UZREC wishes the researcher well in conducting the research.

Professor Rob Midgley
Deputy Vice-Chancellor, Research and Innovation
Chairperson: University Research Ethics Committee
27 May 2013

CHAIRPERSON
UNIVERSITY OF ZULULAND RESEARCH
ETHICS COMMITTEE (UZREC)
REG NO: UZREC 171110-30

27 -05- 2013

RESEARCH & INNOVATION OFFICE

APPENDIX 3 DIESEL CHROMATOGRAM

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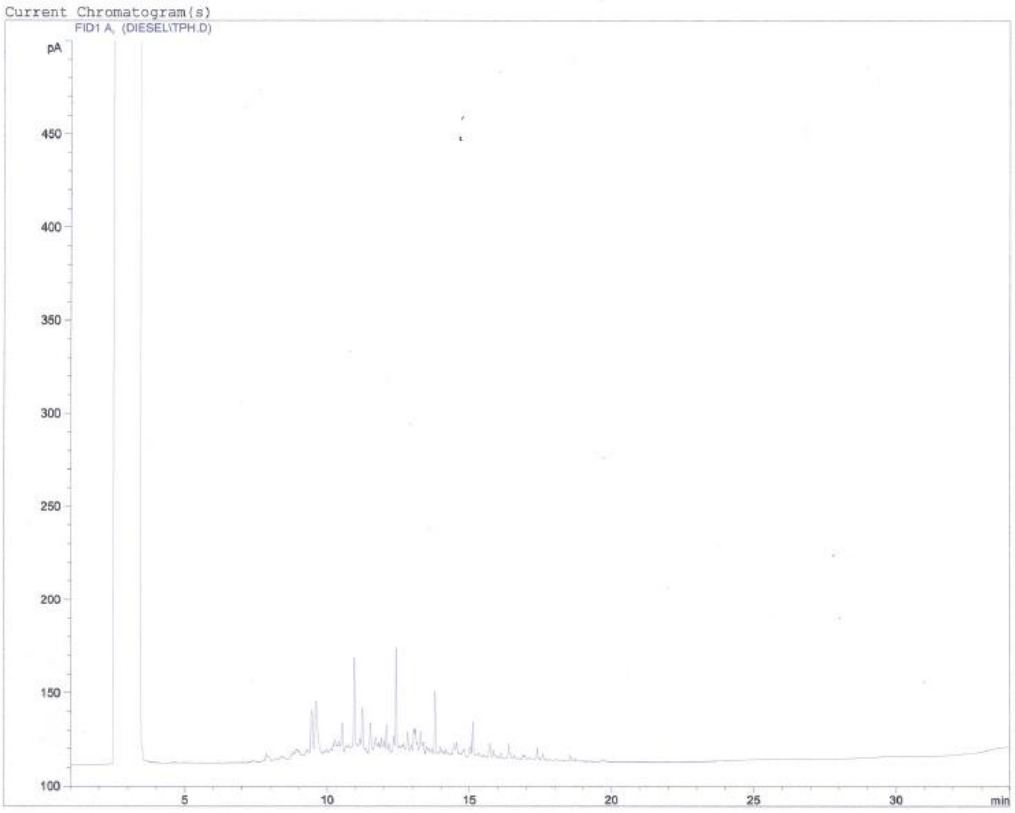

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Page 1 of 1

Analysis Method : C:\HPCHEM\1\METHODS\TPH-AGO.M
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TOTAL PETROLEUM HYDROCARBON

External Standard Report

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Multiplier : 0.3000
Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/Kg]	Grp	Name
9.978	VV	78.28115	1.68919e4	3.96695e5	1	HC
11.502	VV	119.55389	3571.42857	1.28093e5	1	HC
12.947	VV	45.69773	1.16822e4	1.60156e5	1	HC
13.966	VV	66.38828	9541.98473	1.90043e5	1	HC
17.807	VV	9.34615	3.90625e4	1.09525e5	1	HC
18.986	VV	1.93558	3623.18841	2103.89249	1	HC
20.757	BV	9.55934e-1	5681.81818	1629.43283	1	HC

Totals : 9.88245e5

Results obtained with enhanced integrator!
Group summary :

Group ID	Use	Area [pA*s]	Amount [mg/Kg]	Group Name
1		322.15872	9.88245e5	TPH

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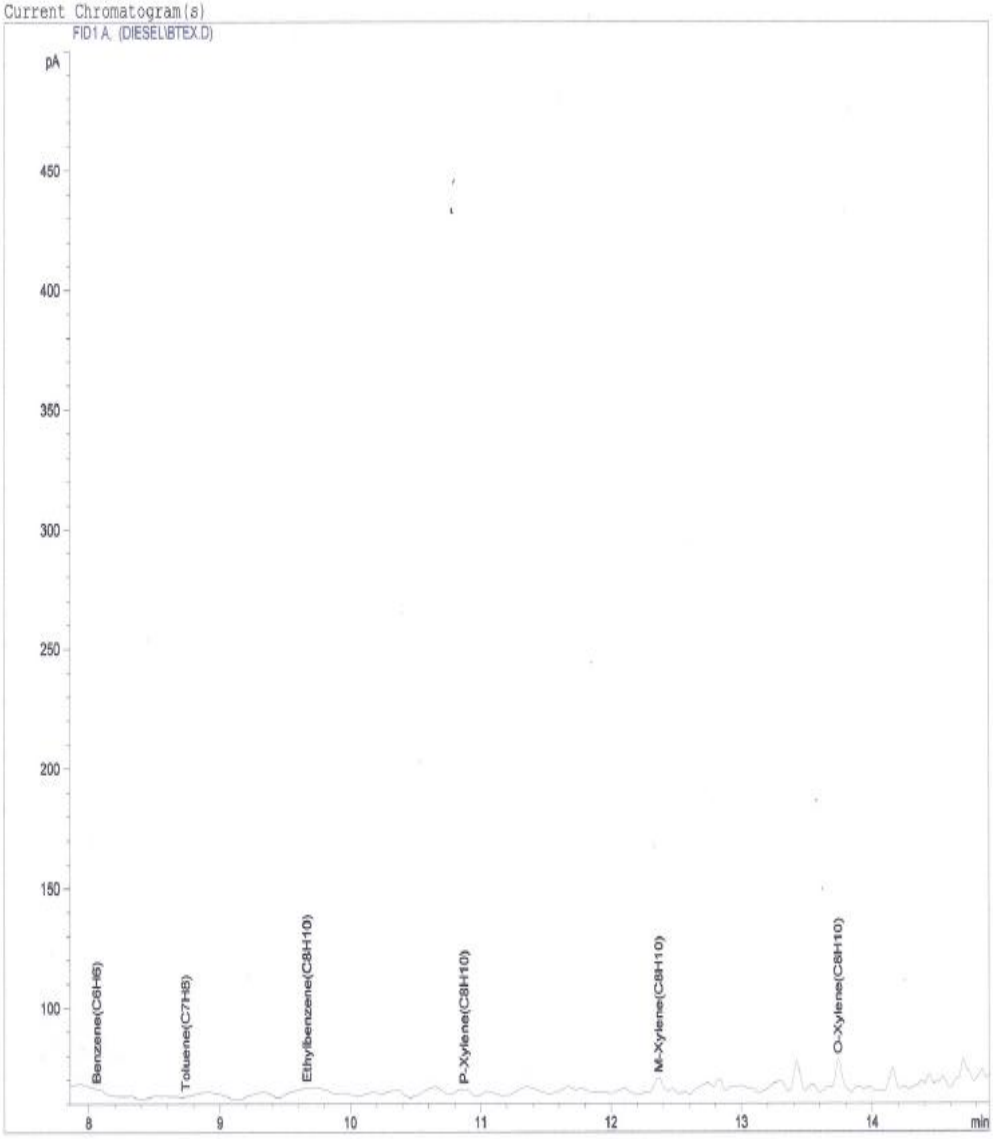
Current Chromatogram(s)



Instrument 1 2/16/2012 8:50:20 AM

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Method of analysis of BTEX

External Standard Report

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Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/Kg]	Grp	Name
8.061	VV	17.21473	1.66667e-1	2.86912	1	Benzene(C6H6)
8.740	VV	1.36010	2.50000	3.40025	1	Toluene(C7H8)
9.669	VV	44.36245	5.00000e-2	2.21812	1	Ethylbenzene(C8H10)
10.875	VV	14.09493	1.42653e-1	2.01069	1	P-Xylene(C8H10)
12.365	VV	47.83672	1.25000e-1	5.97959	1	M-Xylene(C8H10)
13.741	VV	81.08644	4.77099e-2	3.86863	1	O-Xylene(C8H10)

Totals : 20.34640

Results obtained with enhanced integrator!
Group summary :

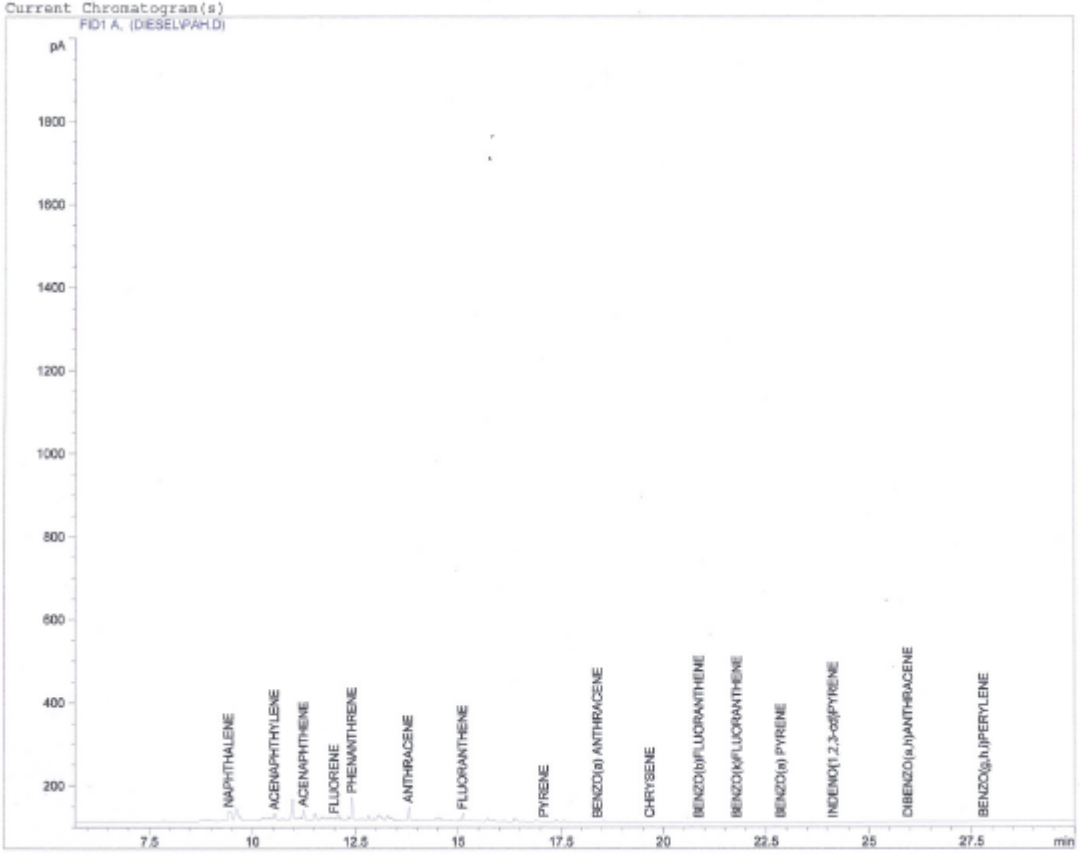
Group ID	Use	Area [pA*s]	Amount [mg/Kg]	Group Name
1		205.95538	20.34640	BTEX

*** End of Report ***

Print of Window 38: Current Chromatogram(s)

Current Chromatogram(s)

FID1 A, (DIESEL\PAH.D)



Instrument 1 2/16/2012 8:23:53 AM

Page 1 of 1

Last changed : 2/16/2012 8:23:26 AM
POLYAROMATIC HYDROCARBON ANALYSIS

External Standard Report

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Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/Kg]	Grp	Name
9.427	VV	161.73412	8.89680	1438.91562	1	NAPHTHALENE
10.531	VV	108.38354	4.88281e-1	52.92165	1	ACENAPHTHYLENE
11.230	VV	130.26900	5.92417e-1	77.17358	1	ACENAPHTHENE
11.988	VV	46.86729	5.19751	243.59301	1	FLUORENE
12.408	VV	199.21428	8.91266	1775.52833	1	PHENANTHRENE
13.791	VV	128.49423	1.90042e-1	24.41928	1	ANTHRACENE
15.108	VV	74.77854	1.82349e-1	13.63577	1	FLUORANTHENE
17.082	VV	17.51669	9.10747e-1	15.95327	1	PYRENE
18.385	VV	3.88363	3.24465	12.60100	1	BENZO(a) ANTHRACENE
19.665	VV	8.32191	3.65764	30.43860	1	CHRYSENE
20.858	VV	3.34966	9.12409e-1	3.05626	1	BENZO(b)FLUORANTHENE
21.781	VV	5.22723	3.86997e-1	2.02292	1	BENZO(k)FLUORANTHENE
22.853	VV	2.18578	1.19904	2.62084	1	BENZO(a) PYRENE
24.117	VV	7.46284	5.17491e-2	3.86195e-1	1	INDENO(1,2,3-cd)PYRENE
25.955	VV	18.10059	5.82140e-3	1.05371e-1	1	DIBENZO(a,h)ANTHRACENE
27.789	VV	18.62558	1.63479e-2	3.04489e-1	1	BENZO(g,h,i)PERYLENE
Totals :				3693.67617		

Results obtained with enhanced integrator!
Group summary :

Group ID	Use	Area [pA*s]	Amount [mg/Kg]	Group Name
1		934.41490	3693.67617	PAH TOTAL