EARTHWORM ECOLOGY, HEAVY METALS AND TOTAL PETROLEUM HYDROCARBON ASSESSMENT OF AN OIL SPILL SITE IN AGAYE, LAGOS STATE, NIGERIA.

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A THESIS IN THE DEPARTMENT OF ZOOLOGY

SUBMITTED TO THE FACULTY OF SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD

OF

DOCTOR OF PHILOSOPHY

Of the

UNIVERSITY OF IBADAN, IBADAN

AUGUST, 2014

ABSTRACT

Agaye has experienced frequent spills of premium motor spirit due to pipeline vandalization. Over time,the spills have contaminated the water sources and farmlands, and with the attendant inferno, destroyed the soil biota. There is dearth of scientific information on effect of the spills in this area. This study investigated the abundance and heavy metals level of earthworm, and determined the physico-chemical parameters of the groundwater, surface-water and soil in Agaye. Twenty topsoil samples (0.5m x 0.5m x 0.2m) were randomly collected monthly within the epicenter of the spill and 500 meters away from the spill between June 2007 and April 2009 for earthworm analysis. The earthworms were handpicked, identified with standard keys and counted. Cadmium, Copper, Nickel, Zinc, Manganese and Lead concentrations in tissue of the two most abundant earthworm species were determined using atomic absorption spectrophotometry. Seven existing water sources (GW_1 to GW_5 for groundwater; SW_1 and SW_2 for surface-water) were sampled along the transect of spill. Soil samples (S_1-S_5) were collected around same loci of the groundwater sites. GW_6 and S_6 served as control, being 500m away from spill. Samples collected every two months were analysed for pH, Total Petroleum Hydrocarbon (TPH) and the heavy metals according to APHA, 1995. Earthworm diversity and abundance were analysed using Shannon Weiner (HS) and Mann-Whitney respectively while data on physicochemical parameters were analysed with ANOVA at p=0.05. In the first 12 months, *Lybiodrilus violaceous* was the only earthworm encountered within the epicenter while *L. violaceous* and *Dichogaster modigliani* (H = 0.3) were found 500m away. In the last 11 months, *L. violaceous, D. modigliani, Ephyriodrilus afroccidentalis* and *Heliodrilus lagosensis* were encountered in both sites (H = 0.3, at the epicenter; H = 0.9, 500 m away). The abundance of earthworm 500m away (204 earthworms/m^2) was significantly higher than within the epicenter (45 earthworms/m^2) in the first 12 months but not significantly different in the last 11 months. The concentration (µg/g) of Lead, Cadmium, Copper, Zinc and Manganese in *L. violaceous* was 0.4±0.02, 0.2±0.003, 0.1±0.003, 5.5±0.02 and 3.7±0.002 respectively; Nickel was not detected. Only zinc (6.7±0.4) and Cadmium (0.06±0.002) were detected in *D. modigliani.* Cadmium (0.0–0.1 mg/L) and Nickel (0.1-1.6 mg/L) levels in GW_1 to GW_5 were higher than control (GW_6) and NESREA drinking water limits. Cadmium, Copper and Nickel levels (mg/L) in SW_1 and SW_2, (ranged 0.0-0.1, 0.07-0.7 and 1.5-2.8 respectively) were higher than NESREA permissible limit. Mean concentrations of TPH were significantly higher in surface-water (3.3±0.5 mg/L) than groundwater (1.3±0.6 mg/L) while pH was significantly lower in groundwater than surface-

water. Mean pH at S_1-S_5 ranged from 5.3±0.04 to 6.5±0.02. Soil TPH, cadmium and copper reduced significantly during the last 11 months. Soil TPH level was significantly higher than control soil. The increase in number and species of earthworm in the last 11 months indicated possible remediation of the environment. The high concentrations of heavy metals in the earthworms suggest possible roles in bioaccumulation. The higher levels of heavy metals and total petroleum hydrocarbon in surface and groundwater indicated that they are unsafe for drinking.

Keywords: Oil spillage, Heavy metals, Total petroleum hydrocarbon, Earthworm abundance.

Word Count: 498

CERTIFICATION

I certify that this work was carried out by OGUNLAJA, Aemere of the Department of Zoology, University of Ibadan Under my supervision.

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DEDICATION

This research work is dedicated to my husband and children, Mr O.O. Ogunlaja, Simi, Rotimi and Wemimo.

ACKNOWLEDGEMENT

I am grateful to my supervisor, Dr. (Mrs.) Olajumoke A. Morenikeji for her counsel, encouragement and support during this research. I am also thankful to Prof. S.O. Owa for his support through the course of this work and his relentless effort to see that the work comes to a fruitful completion. I sincerely thank members of Staff of the Zoology Department, University of Ibadan for their advice and support. I specially thank Prof. Hassan for his fatherly role during the course of this work.

I appreciate my children, Simi, Rotimi, Wemimo and Tina for their understanding and support throughout the period of this study. during which I might not have been there for them fully. I am very much indebted to my treasured husband, Mr. O.O. Ogunlaja who was always ready to stand in for me in the family whenever the need arose.

My appreciation goes to the entire staff of Biology and Chemistry Department, Redeemer's University, Mowe, Ogun State, especially Mr Popoola, Mr. Sojinu, Mr. Akanbi and Mr. Adebayo for their technical support. My appreciation also goes to my colleagues and friends for their selfless, useful and helpful contribution towards the successful completion of this research work. I also thank my friends, so many to mention that stood by me emotionally and otherwise when I needed them.

To my family- The Aigbologas, especially my Father and Mother, I am very thankful to you. I am grateful also to my in-laws, the Ogunlajas and everyone who in one way or the other contributed to my well being- it is impossible to mention all.

Finally I want to thank God for this wonderful opportunity.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Environmental Pollution

Pollution continues to be an issue of concern to mankind. It can be traced to the emergence of the human race and his ignorant use of toxic materials like cadmium used in building materials and glazing pigment more than 5000 years ago and the use of mercury to alleviate teething pains and as treatment for syphilis by the Romans between 1300 and late 1800 (Lars, 2003). The awareness of pollution escalated overtime with increasing human population, increase in industrialization and consequently adverse health effect linked with pollution became evident. However, exposure to pollutants remarkably increased with a steep increase in heavy metal production by the 19th century onward for more than 100 years, (Nriagu, 1996).

Naturally, the conditions of the environment, both biotic and abiotic, mediate to support man and other living organisms but one major stress on the environment remains the uncompromising result of pollution, (Rapport *et al.*, 1985). The presence of substances, be it chemicals, gases or metals is not the problem by itself but the presence of such things in wrong places, amounts and time. This results in changes in the environment that could be acute or chronic, affecting organisms, at their cellular, molecular, organ level or even future generation by mutation (Lundberg and Moberg, 2003; Somers *et al.,* 2004). Such substances which when introduced into the environment cause pollution are pollutants.

The word pollution comes from a Latin word "pollure" which means to "defile" or render "unclean" (Osibanjo, 1986). Pollution can be defined as the presence, in the environment of significant amount of unnatural substances or abnormally high concentration of natural constituents at a level that causes undesirable effects, (Johnson *et al.,* 1997). Pollutants are actually "resources out of place", being too much in a system and thus constituting an insult or stress to that` system, (Kormondy, 1976). Pollutants could act as stimulants, terminating or initiating biological processes. The interference of pollutants in the environment results in environmental degradation. Environmental degradation is the process through which

an ecosystem's capacity to support a constant quality of life is reduced (Johnson *et al*., 1997). Pollutants alter the dynamics and development of an ecosystem (Sattirn, 1981). Pollutants are from various sources including agricultural, industrial and arthropogenic. Pollution from fossil fuels is one of the major environmental challenges in the 21st century (Orebiyi and Awomeso, 2008) this is as a result of increasing energy demand; the most common fossil fuel in use is petroleum.

1.2 Petroleum sector in Nigeria

The word petroleum is derived from the two Latin words *petra* which means rock and *oleum* which means oil (Groysman, 2014). Crude oil is also referred to as black gold, (Watts, 2004). It appears black, dark brown, yellowish, or even greenish liquid and it is found in formations in the earth. It is a substance, generally liquid, occurring naturally in the earth and composed mainly of mixtures of chemical compounds of carbon and hydrogen with or without other nonmetallic elements such as sulphur, oxygen and nitrogen usually formed by a complex and incompletely understood series of chemical reactions from organic material laid down in previous geological eras (Wallington, *et al*., 2006). Based on their various sources, crude oils vary widely in both physical and chemical properties (Steffens *et al*., 2009).

The petroleum sector plays vital roles in the energy and economy of virtually every country in the world. In Nigeria, the petroleum industry is the mainstay of the economy contributing largely to the nation's GDP, and 90% of foreign exchange (Ogunleye, 2008). The activities of the petroleum sector ranging from exploration, exploitation, refining to distribution of their products, leave behind trails of pollution of various degrees in the environment. Over the last few years, particularly in the last decade; resource exploitation, environmental pollution, mode and means of appropriation of the revenue derived from oil, mode of information dissemination as regards risk associated with the oil industries' activities, have generated intense social conflict in the Niger – Delta (NDES, 1997). It has resulted to frequent vandalization of oil installations, overall breakdown of economic activities and kidnapping of expatriates and indigenes associated with the oil industry. Niger Delta remains the most impacted region by oil spillages (Ordinioha and Brisibe, 2013). However, the issues of vandalization of oil installations have been rampant in recent

times and have not been restricted to the Niger – Delta region alone. A number of fire blasts have been reported in various parts of the country resulting either from activities of vandals, construction workers or as a result of leakage from obsolete pipes used for distributing petroleum products around the nation. In the last 10 years, 16,083 pipeline breaks had been recorded; 398 (2.4%) were due to ruptures and 15, 685 (97.5%) of the breaks were due to the activities of vandals (Ogbeni, 2012). A report by a newspaper outfit USTODAY (2006), focused on fires resulting from explosions at oil pipelines in Nigeria from the year 1998 to 2003; this they reported often caused large numbers of casualties. A total of 12 incidents were recorded, and more than 1,000 people were reported killed by such occurrences (an average of about 83 deaths per incident). Several tons of petroleum products, especially gasoline are usually spilled in such vandalized sites and this result into changes in the environment which has deleterious effects on organisms. More importantly, the environment remains polluted due to contamination of the soil and water, the alteration of diversity and population of soil fauna and flora. The polluted environment continues to cause health hazards long after the incident is forgotten.

The environmental problems in Nigeria surrounding the extraction of oil and the distribution of oil products are extensive. Pollution from oil production and distribution causes soil erosion, groundwater and marine area contamination, air pollution and severe health problems for the indigenous communities surrounding oil production and other such communities with incidences of vandalization (UNEP, 2006).

A major geographical area exposed to oil spills in Nigeria is the Niger-Delta area. The Niger Delta located in the central part of southern Nigeria is a 40,000 km^2-70,000km^2 sedimentary basin widely considered to be of global economical significance because of the vast mineral deposits, especially crude oil and gas. The Niger - Delta in Nigeria is a notable region for oil and gas exploration and production in the world, it is the largest delta in Africa and third largest in the world (HRW, 1999). It is the most affected area in terms of oil spillage in Nigeria. Vigorous upstream and downstream activities in this region have led to an enormous environmental contamination over the years. The environmental problems of the Niger delta are complex, interconnected and caused by many factors (SNAR, 2005).

Although environmental degradation also arises from natural sources, the most common environmental problems in the region results from oil spills, gas flaring, construction of oil facilities, pollution from industries and dredging of canals (Oil for Nothing, 1999).

Aside the Niger Delta communities, several communities have experienced oil spill due to vandalized pipelines in recent times, most of these spills are left without clean up measures.

1.3 Remediation of petroleum contaminated sites

There are various works focused on the remediation of petroleum polluted sites and some common methods of clean–up are used. These methods include engineering (excavation, transportation and incineration of the contaminated soi ; *ex-situ)*), pump-and–treat, soil vapour extraction, sparging and chemical washes *(in-situ)* (Cunningham *et al*, 1995) and more environmentally friendly bioremediation. Bioremediation mostly involve use of micro- organisms like bacteria, fungi and protozoa to degrade contaminants into less toxic or non-toxic compounds (Pierzynski *et al*, 1994).

Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, biostimulation and bioaugmentation. With natural attenuation, remediation occurs without human intervention other than monitoring. Biostimulation is the use of indigenous microbial populations to remediate contaminated soils and involves adding nutrients and other substances to soil in order to catalyze natural attenuation processes. Bioaugmentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes it employs genetically altered microorganisms (Biobasics, 2006).

The use of plants in remediation is referred to as phytoremediation while the use of animals in remediation is referred to as zooremediation (Cunningham *et al*, 1995). Although the uses of various plants for remediation are well reported, fewer reports are available for the use of animals. Phytoremediation involves exploiting plant's natural ability to contain, degrade, or remove toxic chemicals and pollutants from soil or water. It can be used to clean up metals, pesticides, solvents, explosives,

crude oil, and contaminants that may leak from landfill sites. Several plants such as sunflower, ragweed, cabbage and geranium, as well as other less known species are known phytoremediants. The plants are often used in combination with other traditional technologies for cleaning up contaminated sites because of the phytoremediation's limitations. Zooremediation mostly involves biostimulation process; the animals' actions improve the environmental conditions of the site and thereafter influence the activities of microorganisms. This is a less researched area; however some investigations involving earthworms and other invertebrates indicates that animals play a role in enhancing the activities of microorganism and hence could be exploited for bioremediation (Zachery and Reid, 2008b; Sinha, *et al*, 2010). Earthworm based technology has proved commercial potentials as the role of earthworms in the conversion of organic materials and improvement of soil has been observed and appreciated (Biobasic, 2006; Zachery and Reid, 2008a).

1.4 Earthworms

Earthworms are detritus feeders occupying a notable position of producer in the terrestrial food chain. They are hardy organisms capable of surviving highly toxic environment, for instance, it was the only survival soil fauna after the 1976 Seveso chemical plant explosion in Italy (Satchell, 1983).

Earthworms belong to the class annelida, Annelids have cylindrical body which is segmented both outside and inside. They are invertebrate organisms found in marine, freshwater, as well as brackish and arboreal environments, seashore and terrestrial habitats; they may be pelagic, surface dwelling, or benthic, burrowers or tube dwellers, mobile or sessile. There are approximately 4500 identified species of worms in the world, about 2500 are earthworm species and more than five hundred species of earthworms have been identified in India (Kale and Karmegam, 2009). Lavelle, 1978, had reported the earthworm density for western Africa as 0.1–4.0 million worm/ha and in European soils as 2.5–9.5 million worms/ha. Owa *et al*., 2002 reported the earthworm density in their study of earthworm density and diversity across ecological zones Nigeria as 0.85 million worms/ha. He also reported the species of earthworms in the ecological zones as *Ephyriodrilus afroccidentalis, Eudrilus eugeniae, Hyperiodrilus africanus, Parapoly-toreutus obiensis,*

Eminoscolex steindach-neri, and *Libyodrilus mekoensis* in same report. The ecological zone secondary forest had the highest diversity of earthworms in Nigeria. The life span of earthworms range between 3 to 7 years depending on the species and the environmental factors; they are known to produce 300-400 young earthworms during their life cycle (Hand, 1988). The average temperature range suitable for their survival is 20^0C – 25^0C while their optimum moisture ranges between 60-75%.

Earthworms are either earthmovers or composters. Earthmovers tend to be solitary species which tunnel through the earth, aerating, decompacting and mixing soil strata and thus making surface nutrients available to plant roots at lower levels. (Kale and Kaomagma, 2009). Earthworms are key organisms in (environmental) toxicology; it was identified as a model organism for assessing the effects of chemicals on terrestrial saprotrophic invertebrates. (OECD, 1984).

1.5 Research rationale

Oil spillages have far reaching implications on the socio-economic well being of the people. The numbers of petroleum contaminated sites are on the increase in Nigeria, most of such sites are not assessed to determine the extents of pollution hence are neglected even if they need clean-up. The Nigerian government and oil companies are either slow or insensitive. The Agaye community, in Lagos state Nigeria is one of such sites that have experienced repeated oil spill that resulted to inferno as reported in the Punch, 29th Dec, 2006. No baseline records are available on the physicochemical parameters of soil and water in this area. Investigation to estimate the extent of contamination and researches geared towards remediation using locally available materials and environmentally friendly measure for site reclamation is therefore necessary.

Based on the foregoing premise, this study involved empirical estimation of the extent of contamination in Agaye soil and water. It also involved the estimation of the earthworm species diversity and abundance after the petroleum spill and determining the role of the most abundant and most tolerant indigenous earthworm species in bioremediation of the soil.

1.6 Aim and Objectives

This study aimed at determining the levels of petroleum contaminants in the soil and water in the oil spill area of Agaye and the impact of the spill on the population of earthworms.

The specific objectives were therefore to evaluate the;

i. physiochemical status of surface and underground water.
ii. physicochemical status of the soil within the oil spill area in Agaye.
iii. abundance and species diversity of the earthworm population in the study area
iv. accumulation of heavy metals by the indigenous earthworms.
v. role of the indigenous earthworms in the remediation of the soil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pollution

Man's health, his physical and psychological development are intimately associated with the natural conditions of the environment but pollution remains a major stress on the environment and causes changes which are deleterious to life. Pollution according to GESAMP (1991) is "any alteration of the chemical, physical or biological quality of the environment which results in any unacceptable depreciation which adversely and unreasonably affects its subsequent beneficial uses". Environmental pollution refers to any unwanted change in the quality of the earth that is caused by the introduction, either by natural events or human activities, of substances in quantity and duration, which harm the health of living organisms or damage materials.

Man's continuous advance in science and technology has caused severe environmental changes which are very difficult to evaluate and fully comprehend. Some of these activities result in the interference in nature which cause imbalance and displacement of ecosystem's linkages and relationships and may markedly reduce the system's life supporting capabilities. Pollution could result in chronic damage to water, air or soil.

The atmosphere comprises of a complex mixture of gases and it provides the gases required for life's vital biochemical process. It acts both as an insulating blanket, maintaining life-supportive temperature range on earth and as a shield, reducing or blocking radiation from space that would otherwise be lethal to most life-forms. (Umolu and Aemere,1999). Pollution is occurs as a result of increasing human population, increasing industrialization with accompanying concentration of population into conurbation, (Adeyemo, 2003). The resultant effects range from direct or indirect impact on the health of living organism, acid rain falls (which affects the soil and water pH), perforation of the ozone layer and the resultant global warming which produces conditions threatening the survival of living organisms. Air pollution due to the release of isocyanate from the union carbide plant had been reported at Bhopal, India which led to the death of over 2,000 people in 1984 (Naik, *et al*, 1986) . The atmosphere habours a lot of pollutants such as metals, pesticides,

asbestos, radioactive materials, in particulate form and most reach the aquatic system as fall out (FAO/SIDA 1977; GESAMP, 1991).

Water bodies, especially surface water are prone to pollution as they serve as repositories of run-offs from their surrounding environment. Apart from direct damage due to contact with and ingestion of water polluted by pathogens and other toxic substances, incalculable damage is done to life in water bodies. Basically, the hydrological conditions of a water body are very vital when assessing its productivity and other characteristics (Adebisi, 1981). In water bodies, important elements among others include nitrogen, phosphorus and oxygen. Nitrogen and phosphorus occur normally in low concentrations which are almost entirely consumed by routine plant physiological activity; excess amounts of these elements remain in the environment as pollutants. Furthermore, the survival of many fish species requires 3-4mg of dissolved oxygen per litre of water (Hamilton and James, 1976), with limited amount, the survival of fishes is jeopardized and putrefaction phenomena becomes encouraged.

Industrial and agricultural activities are major sources of pollutants. Industries are varied and diversified and so the nature of their operations and products. Thus different pollutants could be generated by any one industry depending on the level of industrial technology in use and the control measures adopted the significant impact of the different source types vary from one country to another, (Osibanjo, 1986), hence it is difficult to estimate the quantity of pollution a particular source would contribute. Agricultural sources contribute through run-off, from cultivated land, fertilizers and pesticides used on farm lands. It also contributes a lot of organic waste, although its contribution to pollution is a small fraction of the total pollutants getting into the aquatic habitats. Agricultural sources discharge over a geographical area, thus restriction of most of its pollutants is difficult (Osibanjo, 1986). Natural radioactivity and bush burning are other sources of pollution which results in radioactive pollution (Asamoah, 2013) and contribution of organic wastes respectively while weathering of rocks erodes constituent minerals, these finally reach the aquatic system through under-ground water or runoffs. Other pollutants common in water are heavy metals (like lead and mercury) and phenols. They are known to be absorbed and concentrated in the tissue of aquatic organisms thus

entering into the human food chain resulting in bioaccumulation and bioconcentration. Soil provides the base for virtually all human activities, settlements and it sustains the existence of plants and animals in general. Soils have large holding capacity for pollutants and acts as a long-term sink and major repository of contaminants in the environment (Wild and Jones, 1995). Plants absorb contaminants and serves as a route to the food chain and some contaminants percolate into ground water systems and eventually to surface water.

Petroleum occurs in nature and normally seeps into the environment (Stones and Seager, 1979). The exploitation, production and marketing of crude oil have very remarkable effect resulting in extensive damage to aquatic life, impairment of recreational value of beaches, losses in fisheries and rejection of aquatic food resources by the public and changes in water quality (Umolu and Aemere, 2000). Etkins, 2001 described "oil spill" as events where oil is discharged accidentally, due to neglect or intentionally for a short period but neither slow oil leaks for a long period nor operational discharge.

2.2 Petroleum

Petroleum is generally described as a complex mixture of hydrocarbons (having molecules of only carbon and hydrogen, mostly alkanes) and non hydrocarbons molecules such as nitrogen, sulphur, and oxygen. The elemental compositions and appearance of petroleum vary greatly in various crude oil types. Petroleum is usually found in porous rock formations in the upper strata in some areas of the earth's crust (Timmis *et al*., 1998).

There are two schools of thought regarding the origin of petroleum: a western school suggesting that its origin is biogenic (biotic) resulting from the decay of organic biological matter and stored in sedimentary basins near the earth's surface and an Ukrainian – Russian school proposing that the origin is abiogenic (abiotic) with inorganic origin deep within the earth's crust dating back to the creation of the earth. The issue of the origin of petroleum remains a controversy between the two schools of thought, however, that of biogenic formation remains the most widely accepted theory. Its formation involves a slow breakdown process, known as diagenesis, which produces a range of hydrocarbons and hydrocarbon complexes

significantly altered from the structure found in the original biomass (Aigeson, 1996). The process involves the physical, chemical or biological alteration of sediments into sedimentary rocks at relatively high temperatures and pressures that can result in changes to the rock's original mineralogy and texture (Marfil *et al.*, 1998).

Petroleum hydrocarbons are organic compounds which are known to arrange in varying structural configurations. Crude oil hydrocarbons are divided into two families, *aliphatics* (fatty) and *aromatics* (fragrant). Crude oil and refined petroleum products contain four major groups of hydrocarbons: alkanes, olefins, alicyclics, and aromatics.

2.2.1 Constituents of petroleum

The main elements of petroleum are combined to form a complex mixture of organic compounds that range in molecular weight from 16 (methane; CH_4) to several thousands. A wide range of metals are also found in trace amounts in crude oil. All metals through the atomic number 42 (molybdenum) have been found, with the exception of Rubidium and Niobium; a few heavier elements also have been detected. Nickel and Vanadium are the most important, because they are present in all crudes, usually at concentrations far higher than any other metal (Ali and Abass, 2006). Organic compounds containing sulfur, nitrogen and oxygen may be encountered at significant concentrations in crude oil and in some heavier fuels such as No.6 fuel oil.

Some other components of petroleum apart from hydrocarbons can be grouped under asphaltenes and resins, polar and porphyrin constituents. Asphaltenes and resins makes up a large fraction of crude oils and heavy fuel oils, making those oils very dense and viscous. Asphaltenes are substances in petroleum that are insoluble in solvents of low molecular weight such as pentane or hexane and they are solids at normal temperatures. Oils that have high asphaltene contents are very viscous, with high pour point and are generally nonvolatile in nature. The porphyrins, asphaltene, and resin compounds are considered the residual oil, or residuum. During the weathering process, this fraction is the last to degrade, and its persistence over years is known. Other constituents include porphyrins, which are complex large cyclic

carbon structures derived from chlorophyll and characterized by the ability to contain a central metal atom (trace metals are commonly found within these compounds).

2.2.2 Petroleum production

The processes involved in the petroleum industry include exploration, drilling, extraction, refining and transportation / distribution. Oil exploration is the act of searching for crude petroleum in the ground, either onshore or offshore. As the search has become increasingly difficult, the industry has moved to more sophisticated detection techniques (Surdam, 1997). Airplanes and satellites make remote sensing possible, using a combination of photography, radar, infrared imagery, microwave frequency receivers and other technologies to identify possible production areas and to predict the likelihood of significant reserves (Beaumont and Foster, 1992).

Once oil and gas is found, development begins with the drilling of 10 to 30 wells per platform. Since more wells are drilled during development than during exploration, a larger volume of drilling mud and cuttings is discharged in this process. Once the drilling unit used in development is removed, extraction of hydrocarbons from the underground formation begins (Menzie, 1983).

Offshore oil platforms produce a wide variety of liquid, solid and gaseous wastes, some discharged directly into the ocean. Onshore oil production operations produce quantities of cuttings and mud, ranging from 60,000 to 300,000 gallons per day, while offshore oil platforms use nearly 400,000 gallons of water per day, released directly into the ocean. Lined pits for disposal are sometimes used in association with land rigs, but mud, drill cuttings, and other materials are often discharged into the ground (Guidotti, 1995). Some extraction techniques require the use of sub-surface explosives, or ‘torpedoing’ to breach certain geologic features. Oil and gas exploration and drilling are the most hazardous sectors of the oil industry

When extraction is completed, crude oil is transported to an oil refinery where complex hydrocarbon compounds are separated, converted, and treated, becoming useable fuel sources. The process of refining oil manufactures nearly 2,500 useful products (Gennaro *et al.*, 2000) but the major end product of oil is gasoline, followed

by diesel fuel, jet fuel, fuel oils, kerosene, lubricating oils, and asphalt used for road paving (Oduntan, 2000).

Commercial petroleum hydrocarbons are produced through distillation of crude oil. In general, the lighter fractions represent gasoline-range material. The intermediate or middle distilled fractions represent feedback for diesel, jet fuels, and light heating oils. The residuum in this process serves as heavy fuel oils or other non-fuel products (Nyer and Skladany, 1989; Potter, 1992). Table 2.1 indicates some of the major commercial products associated with different distillation fractions. Gasoline, diesel, and fuel oils are the most common petroleum products contaminating soils and groundwater because of their widespread usage, (Block, 1991).

Robust technology has emerged to move (transport) extracted crude oil from point of extraction to oil tanks from where it is further transported through pipes to refineries. This technology involves the use of ships and pipelines to transport extracted oil. Refined products also need to be moved to myriad distribution points which are made possible by the immense networks of pipelines. Pipelines are highly pressured conduits for the transfer of large volume of oil, varying in width and carrying capacity. Pipelines are able to function 24 hours in a day, under any weather conditions, hence they are preferred. They are prone to corrosion and burst relatively frequently due to faulty equipments, human error and intervention. These often lead to spills and fires posing serious threats to neighbouring populations and surrounding environments. The life span of a pipeline is acknowledged to be 15 years (Borasin *et al*., 2002).

2.2.3 Major petroleum products

Gasoline is a mixture of volatile hydrocarbons suitable for use in internal combustion engines. The major chemical components of gasoline are branched chain paraffins (branches chain alkanes), cycloparaffins (cycloalkanes), and aromatics. The composition of gasoline may vary depending upon the origin of the crude oil, differences in processing and the incorporation of various additives to improve performance. Common additives include: metals such as alkyl lead;

Table 2.1 Petroleum Distillation Products

Fraction	Distillation Temperature °C	Carbon Number
Gas	Below 20	**C-1 to C-4**
Petroleum ether	20 to 60	**C-5 to C-6**
Ligroin (light Naphtha)	60 to 100	**C-6 and C-7**
Natural Gasoline	40 to 205	**C-5 to C-10 and Cycloalkanes**
Kerosene	175 to 325	**C-12 to C-18 and Aromatics**
Gas Oil	Above 275	**C-12 and higher**
Lubricating Oil	Non-volatile liquids	**Probably long chains attached to cyclic compounds**
Asphalt or Petroleum Coke	Non-volatile solids	**Polycyclic structures**

Adapted from Morrison and Boyd (1973).

oxygenates such as ethanol, methanol, methyl tertiary butyl ether (MTBE), tertiary butyl alcohol (TBA), tertiary amyl methyl ether (TAME), and ethyl tertiary butyl ether (ETBE); additional aromatic hydrocarbons such as benzene, toluene and xylene; and others including ethylene dibromide (EDB), ethylene dichloride (EDC), and methyl cyclopentadienyl manganese tricarbonyl (MMT) (Caprino and Togna, 1998). These varieties of additives are meant to improve engine performance. Methyl tertiary-butyl ether (MTBE) is found in many drinking water wells, but mostly at levels below that known to cause human health effects.

Diesel is Number 2 Fuel Oil, composed primarily of unbranched paraffins (straight chain alkanes) with a flash point between 110 and 190 ^{o}F (43 and 88^{o}C). Diesel is largely comprised of simple un-branched n-alkanes, with only around 4% of polyaromatic compounds (Heath *et al* 1993). Fuel oils are generally chemical mixtures having flash points greater than 100 ^{o}F (38^{o}C). Fuel oils can be distilled fractions of petroleum, residuum from refinery operations, crude petroleum, or a mixture of two or more of these materials.

Petroleum is also the raw material for many other chemical products, including solvent, fertilizers, pesticides, and plastics. Most of the petroleum extracted is processed as fuels, including gasoline, diesel, jet, heating, and other fuel oils, and liquefied petroleum gas; the other is converted into other materials such as plastic.

2.3 Petroleum pollution and its ecological effects

The pollution problems caused by oil industries are extensive ensuing from exploitation, production and marketing of crude oil. These have very remarkable effect including extensive damage to aquatic life, impairment of recreational value of beaches, losses in fisheries and rejection of aquatic food resources by the public and changes in water quality (Umolu and Aemere, 2000). Dating back to World War II, the use of petroleum products became common and there was a shift from the use of coal for the generation of energy in most economy (Onwurah, *et al.*, 2007). The volume of crude oil or petroleum products that is used today cannot be compared to all other chemicals of environmental and health concerns. Due to the number of facilities, individuals involved, processes and the various ways the products are stored and handled; environmental pollution and contamination associated with

petroleum is potentially widespread. Petroleum occurs in nature and normally seeps into the environment (Stones and Seager, 1979). Etkins, 2001 described "oil spill" as events where oil is discharged accidentally, due to neglect or intentionally for a short period but not slow oil leaks for neither long periods nor operational discharge.

In the year 2003, worldwide crude oil production volumes surpassed 82.3 million barrels per day and this volume is estimated to increase to 94.3 barrels per day in 2010 and 101.6 barrels per day in 2015 (US DOE/EIA, 2006). Improper management and disposal has often led to environmental pollution, particularly of the soil and groundwater systems due to the chemical complexity of petroleum.

A lot of petroleum pollutants are introduced by accidental discharge of oils from loaded vessels on seas, other common routes include:

I Flow-line / pipeline leakages and rupture.

II Corrosion of flow-lines or pipelines

III Over pressure of pipelines

IV Valve failure

V Hose failure

VI Collision and grounding of tankers.

During exploration and production activities, accidental spills occurs e.g. blowout from off-shore, oil drilling and production. Spills can also occur during transportation and supply operation. Over 25 million barrels of oil spill reported worldwide since 1980, mostly in small quantities (<7 tonnes) occurring frequently from bunkering, routine discharge and loading of tankers, and larger spills (< 700 tonnes) occurring 84% of the time due to accidental causes (ITOPF, 2006). The international oil and gas pipelines running through several million kilometers on land and water bodies serve as potential points of spills as these pipelines are subject to wearing overtime (Beller, *et al.* 1996). Oil spill with worldwide attraction include the Torrey Canyon spill in 1967; wherein hundreds of kilometers of the southern England coasts and the Brittany region of France were polluted by oily mousse, this resulted in the fouling of organisms with petroleum residuum. Ecological damages also emanated from the clean-up chemicals used resulted in the death of 30, 000 seabirds (Harvey, 1997). Consequently, this affected their population size for several years after the incidence. The aftermath effect of the detergents and dispersant used for this clean-up however brought to limelight the need

for judicial consent as regards choice of clean-up materials as well as the amount to be applied. One of the largest oil spills in the 1970's was the Amoco Cadiz oil spill in 1978. There was an estimated 223, 000 tonnes of oil spilled with the number of birds mortality as 300, 000 (Godson *et al*, 2009). The *Exxon Valdez* accident which occurred in North American waters in 1989 recorded an estimated 37,000 tonnes of oil spilled and an estimated 350,000 seabirds were reported dead (Short, *et al.* 2002; Oil Spill Intelligence Report, 1999). A number of other oil spills of large sizes, caused little or no environmental damage and did not impact coastlines because they occurred several kilometers offshore (ITOPF, 2012). However, the claim of no environmental damage might be untrue as this assertion is likely due to difficulty in the evaluation of environmental impact such distances offshore.

Earlier reports from 1993 to 2002 indicated no correlation between the size of an oil spill and the number of estimated seabird mortalities however findings from Tan *et al.*, 2010 indicated that oil spills <50,000 tonnes, had strong correlation between oil spill size and estimated bird mortality but not with larger spills (100, 000-225, 000 tonnes). The 2010, BP oil spill in the gulf of Mexico is one recent spills which attracted international interest, during this spill, > 200 million gallons of oil poured into the Gulf of Mexico followed by 1.8 million gallons of dispersants used for clean-up (Repanich, 2010)

Petroleum refineries are major sources of hazardous and toxic air pollutants such as BTEX (benzene, toluene, ethylbenzene, and xylene) compounds. They are also a major source of air pollutants like particulate matter (PM), nitrogen oxides (NOx), carbon monoxide (CO), hydrogen sulfide (H_2S), and sulfur dioxide (SO_2). Refineries also release less toxic hydrocarbons such as natural gas (methane) and other light volatile fuels and oils (Anderson *et al*., 1998; Epstein and Selber, 2002). Oil production contributes to air pollution in form of flaring, burning of natural gas extracted along with crude oil. Worldwide gas flaring contributes 35 million tons of carbondioxide and 12 million tons of methane contributing greatly to global greenhouse effect. Gas flares release smoke into the atmosphere which contributes to rising amount of acid rain (Moffat and Linden, 1995). 75% of natural gas i.e. by-product of oil extraction has been flared in Nigeria covering the surrounding with black soot. For Saudi Arabia ; 20%, Iran 19%, Mexico 5%, Britain 4.3%, Algeria

4%, former Soviet union 1.55, U.S. 0.6%, Nertherland 0% (Epstein and Stilber, 2002).

Contamination of soils from the refining processes is generally a less significant problem when compared to contamination of air and water. Post production practices may have led to spills on the refinery properties that now need to be cleaned up. Natural bacteria that may use the petroleum products as food are often effective at cleaning up petroleum spills and leaks compared to many other pollutants. Many residuals are produced during the refining processes and some of them are recycled through other stages in the process. Other residuals are collected and disposed of in landfills, or they may be recovered by other facilities. Soil contamination including some hazardous wastes, spent catalysts or coke dust, tank bottoms, and sludges from the treatment processes can occur from leaks as well as accidents or spills on or off site during the transport process. Many refineries go to great lengths to treat or filter petroleum waste in order to prevent environmental damage. Water used in refining process must be treated to remove traces of heavy metals, noxious chemicals, solvents and residual aromatic hydrocarbons before this water can be released into disposal wells or waterways (Epstein and Selber, 2002). Oil refineries also contribute other forms of pollution like thermal pollution of water body which disrupts surrounding marine ecosystems.

Generally, when petroleum products are released into the environment, changes occur that significantly affect their potential effects. Physical, chemical, and biological processes affect the location and concentration of hydrocarbons at any particular site. The ultimate environmental exposure to petroleum products is determined by how the product changes with use, by the nature of the release, and the hydrocarbon's environmental fate.

2.4 Health effects of petroleum pollution

Some of the chemicals released during petroleum contamination are known or suspected cancer-causing agents, responsible for developmental and reproductive problems. They may also aggravate certain respiratory conditions such as childhood asthma. Along with the possible health effects from exposure to these chemicals, these chemicals may cause worry and fear among residents of surrounding

communities. Air emissions can come from a number of sources within a petroleum refinery including: equipment leaks (from valves or other devices); high-temperature combustion processes in the actual burning of fuels for electricity generation; the heating of steam and process fluids; and the transfer of products. Many thousands of pounds of these pollutants are typically emitted into the environment over the course of a year through normal emissions, fugitive releases, accidental releases, or plant upsets

The primary component of concern in the BTEX complex is benzene, which has been classified as a known human carcinogen by the U.S. Environmental Protection Agency (USDHHS, 1993).

2.4.1 Benzene

Acute benzene exposure causes central nervous system depression, irritation to the eyes and respiratory tract while continued exposure may cause euphoria, nausea, staggering gait, and coma. Inhalation of lower concentrations causes vertigo, drowsiness, headache, and nausea. Chronic exposures to benzene induce well-recognized hematotoxicity, especially bone marrow suppression. Benzene has an odor threshold in water of 2.0 ppm, and a taste threshold of 0.4-4.5 ppm. The EPA maximum contamination level (MCL) of benzene in drinking water supply is 5.0ppb. EPA recommends a short-term (10 days) advisory level for benzene in water at 200 ppb for children. The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) in the workplace for benzene is 1.0ppm (OSHA, 2010). Benzene is the most water soluble fraction of the BTEX complex with solubility of 1780 mg/L (Irwin, 1997) it is usually found to be in the highest concentration in petroleum contaminated water.

2.4.2 Toluene

Toluene is usually found in gasoline, paints, paint thinners, adhesives, fingernail polish, and other petroleum-based products. It has an odor threshold of 2.14 ppm in air. Acute adverse health effects from exposure to toluene include headache, confusion, and memory loss, depending on the concentration, duration, and route of exposure. Brief exposure to a concentration of 100 ppm causes central

nervous system dysfunction. Exposures to 500 ppm to 800 ppm cause progressively increasing headache, drowsiness, nausea, fatigue, weakness, and confusion. Toluene also may interact with some common medicines like aspirin and acetaminophen to affect hearing (Irwin, 1997). Ingestion of toluene-contaminated drinking water may temporarily affect the kidneys. In most cases, the kidneys will return to normal after the exposure stops. The EPA MCL for toluene in drinking water is 1.0 ppm. The EPA Health Advisory for "toluene in drinking water for children is 20 ppm for 1 day and 2.0 ppm for 10 days" (EPA, 1997). The OSHA PEL for toluene in the workplace is 200 ppm. Studies in workers and animals exposed to toluene indicate that toluene has not been shown to cause cancer (OSHA, 2010)

2.4.3 Ethylbenzene

Ethylbenzene is a colorless liquid that smells like gasoline, with an odor threshold of 2.0 ppm in air. It occurs naturally in coal tar and petroleum, and it is found in paints, inks, carpet glues, varnishes, and insecticides. Gasoline contains about 2% ethylbenzene. Ethylbenzene is an irritant of the skin and mucous membranes. At high concentrations, it causes narcosis in animals. Humans briefly exposed to 1,000 ppm experienced eye irritation, but tolerance develope rapidly, exposure at 2,000 ppm caused lacrimation, nasal irritation, and vertigo. Ethylbenzene is also an irritant to the skin and mucous membranes and a high concentration can possess narcotic properties (Fishbein, 1985).

Exposure at 5,000 ppm produced intolerable irritation of the eyes and nose (ACGIH 1991) while sleepiness, headache, and mild irritation of the eyes and respiratory tract were reported from chroni exposure of 100 ppm (Hathaway et al. 1991). Studies to date indicate that ethylbenzene is neither carcinogenic nor teratogenic in humans (NTP-TR, 1999, USEPA, 1991). The EPA MCL for ethylbenzene is 0.7 ppm. The EPA Health Advisory for ethylbenzene in drinking water is 30 ppm (1 day - child) and 3.0 ppm (10 days - child), and a lifetime advisory for adults of 0.7 ppm. The OSHA PEL for ethylbenzene in the workplace is 100 ppm (OSHA, 2010)

2.4.4 Xylene

Xylene is a colorless liquid with a sweet odor. Xylene has an odor threshold of about 2.2 ppm in water. Xylene exists in three forms: meta-xylene, ortho-xylene, and para-xylene. Chemical industries produce xylene from petroleum, and it is found naturally in petroleum and coal tar. Xylene vapor is an irritant of the eyes, mucous membranes, and skin; at high concentrations it causes narcosis. Air levels of 60 ppm to 350 ppm have produced giddiness, anorexia, and vomiting. Volunteers exposed to 460 ppm for 15 minutes had a slight tearing and lightheadedness (Chen, *et al.*, 1994). A level of 230 ppm was not considered to be objectionable to most of these same volunteers. Exposure of pregnant women to high levels of xylene may be teratogenic and cause harmful effects to the fetus (Donald *et al.*, 1991). Studies of unborn animals indicate that high concentrations of xylene may cause an increase in the number of deaths, decreased weight, skeletal changes, and delayed skeletal development (USDHHS) Data from animal studies indicate that xylene is not carcinogenic. The EPA MCL for total xylenes in drinking water is 10.0 ppm. The 1-day, 10-day, and longterm EPA Health Advisory for children exposed to xylene is 40 ppm. The Health Advisory for long-term exposure in adults is 100 ppm. The OSHA PEL for xylene in the workplace is 100 ppm.

2.4.5 Metals

Metals are constituents of petroleum found in the porphrin complex of crude oil (Barwise, 1990) although at low concentrations, some of these metals are of health importance. Lead, manganese, copper, cadmium, zinc, chromium, nickel and vanadium are some of these metals. Among these metals, nickel and vanadium are known parameters diagnostic for petroleum type (Barwise, 1990 and Filby, 1994).

Lead is used as an additive in gasoline; it is an antiknock agent for automobile engines and it improves the octane quality of the product. The use of lead as antiknock agents dates back to 1921 when it was discovered by Midley (Mc Grayne, 2001, Kovarik and Charles, 1994, Steinberg, 2009) but its use became popular in the 1970's. This led to the increased levels of lead found in urban areas. Although, the use of leaded fuel has been banned in most developed countries, it is still in use in

some undeveloped countries. This is because other alternative antiknock agents are more expensive than lead. Other major sources include natural weathering and painting rinds, (WHO, 1993). The ban in its use as antiknock agent was due to numerous health implications associated to lead.

20 to 30% of human lead poisoning is attributed to water sources majorly when leaded pipes are used in old building and as coatings in some ceramic plates and cups; other sources include food and air (Brown and Margolis, 2012). Lead levels in surface water and ground water are relatively low. Health implications from lead poisoning include impaired physical and brain development especially in infants and children below seven years of age (Reagan and Silbergeld, 1989), resulting in low attention span and learning rate indicated by a low IQ, 10µg/dl increase in blood lead level can be sufficient to cause this effect (WHO, 1993). In adults, high levels of lead could result in high blood pressure and kidney complications. The absorption of lead in the body depends on the route of the exposure and the part of the body absorbing the metal, however, 50% of lead is absorbed in children and pregnant women but only 15% is absorbed in adult. Other symptoms of lead poisoning include fatigue, insomnia, retarded development of feotus, hearing and vision impairment and decreased sperm count. The WHO limit of lead in drinking water is 0.01mg/L (WHO, 2007) while the NESREA limit is 0.2mg/L (NESREA, 2010)

Manganese is a common metal found abundantly in nature, it is an essential element in all species and necessary for human survival (Jakubovis and Jenkinson, 2001). The metal is needed in trace amount as it is a major constituent of some enzymes and bones. It is also needed in the functioning of the immune system, respiration, digestion and reproduction. Elevated amount of the metal can be toxic and is usually ingested as food (tea and herbs), manganese poisoning otherwise called manganism can develope into parkinson disease at later age (Spiegel-Ciobanu and McMillan, 2007). The symptoms include hallucination, forgetfulness, bronchitis and nerve damage. Chronic manganese poisoning can affect the central nevourse system. Outbreaks of manganese toxicity reported in Japan and Greece resulted from drinking well water with 1.8 – 14 mg/l of manganese (Kawamaru *et al.*, 1941 and Kondakis *et al.*, 1989). Clinical cases associated with chronic ingestion of as low as approximately 1.2ppm of manganese have

also been reported (Aschner, *et al.*, 2005). In the US, <0.05ppm is the recommended limit for drinking water while the WHO health-based value for manganese is of 0.4 mg/l while the acceptability threshold is 0.1 mg/l and NESREA drinking limits 0.2mg/L .

Nickel is a hard silvery- white metal which occurs more commonly as iron-nickel molten core. Nickel is easily absorbed by organic matter hence is found in relatively high amount in coal and crude oil. Soil nickel content could vary from 0.2-450 ppm depending on the type of soils. It is naturally found in some plants (beans and tea). In nature it could be found combining with sulphur and arsenic. This metal can be bioaccumulated by plants like vegetables. Nickel is grouped as a carcinogenic element and exposure to high levels can cause dizziness, birth defect, heart disorder, respiratory irritant and chronic exposure could result in dermatitis. Nickel is known to bind to soil particles hence becoming immobile but in acidic soils it becomes mobile and can be rinsed into ground water. Nickel could be toxic and antangonistic to microorganisms but they can develop resistance.

Cases of cancer around refineries are associated with nickel; it is also reported to affect the nervous system and the kidney (Gosselin, 1984). Athough, exposure to animals showed no carcinogenicity (Hathaway *et al.*, 1991), positive mutagenicity was demonstrated in invitro test but not invivo. Acute exposure is known to cause nickel itch which is expressed in itching and swellings especially on moist skin (Hathaway *et al.*, 1991). Nickel does not accumulate in fish or small soil fauna. Exposure of nickel to feotus is possible from mother to child and through breast feeding; higher concentrations of nickel have been found in processed baby milk of cow origin. Levels of nickel is usually low or not detectable in surface water like lakes and rivers but accidental exposure of nickel of upto 250ppm from contaminated water was reported to have caused stomach ache, increased protein in kidney and increased red blood cells. Chronic exposure led to bronchitis and reduced liver function in workers of refineries. WHO limit for drinking water is 0.07mg/L while the NESREA limit is 0.1mg/L

Vanadium is and element usually found combined with a wide range of minerals or it can be found in various oxidation state (V^{+2}, V^{+3}, V^{+4} and V^{+5}). It is

also found in carbon rich deposit like coal, tar sand and oil shale hence its diagnostic role in oil spills (Fish and Komlenic, 1984), forest fires are also known sources of vanadium (Nriagu, 1990). It is used in producing alloys, although the ores of vanadium is known but it is not normally mined. The compounds of vanadium are highly soluble mostly in its monomeric forms hence it can easily be distributed by water. Vanadium is also found abundantly is soils and it can be accumulated by plants; the level of accumulation is an indication of its concentration in soil. It concentration in soil ranges from 0.04 - 220µg/L based on the type of soil.

Vanadium is an important component of some enzyme like the vanadium dehydrogenase in nitrogen fixing bacteria. There are no serious health implication associated with exposure to vanadium however severe eye, nose and throat irritation were observed in workers exposed to vanadium peroxide dust. Intake of food with vanadium is the commonest form of ingestion by humans. Heavy exposure to vanadium peroxide could result in bronchitis and pneumonia. Acute effects of vanadium pentoxide exposure include irritation of lungs, throats, eyes and nasal cavities Lees (1980). The particular symptoms expressed depend on the oxidation state of the vanadium exposed to individuals. Organisms like algae, plants and invertebrates bioaccumulate vanadium. Laboratory test on animals indicated impact on the reproductive system of male, lung cancer, anaemia, accumulation of the metal in the placenta, and DNA changes (WHO, 2006) however there are no proven evidence of being carcinogenic.

Zinc occurs naturally as ore or stable isotope in the environment. Increased levels of zinc are as a result of release from industries during mining and processing. Zinc in high levels seep into ground water. Plants and fishes are known to absorb high levels of zinc. In the environment, high levels of zinc are found along side with cadmium and lead. Zinc is an essential element needed in both human and animals as it is play roles in the bone formation and in the functioning of enzymes like DNA and RNA polymerase. Lack of zinc can result in dermatitis, reduced reproductive capacity and retarded growth rate, increased feotal malformation (Cotran *et al.*, 1989), impacts on carcinogenicity (Fong *et al.*, 1978).

Exposure is majorly through food although in small amount, beverages in metal cans and water which flows through zinc coated pipes are also sources of ingestion. A little amount is usually absorbed through skin. Chronic effect of ingestion of zinc could result in aneamia, central nervous system disorder while acute effect could include stomach cramp, nausea and vomiting. Exposure of zinc in the atmosphere had caused death in different incidences (Evans, 1945, Milike, *et al.*, 1963 and Hjortso, *et al.*, 1988). It had also caused "metal fume fever" which is symptomized by impaired pulmonary function (Malo *et al.*, 1990) and increased leucocytes (Blanc *et al.*, 1991), nausea, abdominal discomfort and decreased number of red blood cells.

Copper is a reddish metal that occurs naturally in the environment. It is a widely used metal in the industry and in agriculture. Increasing amount of copper is recorded in the environment with increased human activities like mining, phosphate ferlizer production and use of fossil fuel. Wind blown dust, forest fires and decaying vegetation are known natural sources (Davies and Bennett, 1985). Copper binds with water sediments and soil particles, it is water soluble. Increased level of copper in drinking water could be attributed to the copper fittings in some plumbing systems; food, drinking water and atmospheric exposure are also known. Chronic effects include eye and mouth irritation, headaches, stomach aches, dizziness and aggravation of Wilson's disease. High levels of copper could cause decline in intelligence of adolescents, liver and kidney damage or even death acutely.

Copper attaches to organic matter and minerals but hardly enters ground water, it is however fully mobile in surface water. Bioaccumulation in plants and animals occur affectingthe diversity of plants in soils with very high levels. Copper interrupts the activities of microorganisms and earthworms. Direct atmospheric exposure in males indicates toxicity to sperm; low motility counts (Battersby, *et al.*, 1982). Examinations of CuSO,-poisoned animals showed signs of acute toxicity in the spleen liver and kidney (Clayto and Clayton, 1981), other symptoms of copper poisoning are shown by animals even at very low levels of its concentration like in sheep grazed on field with high copper contamination. Although the human body has a mechanism for maintaining the level of copper (Rutherford and Bird, 2004), this mechanism is not well developed in

children below 1 yr of age. Reproductive dysfunction, delayed growth and decreased litter have been observed in experimental animals exposed to copper (ATDSR, 2004).

Cadmium is found naturally in the earth crust, sometimes in combination with zinc. Natural contamination is by weathering, forest fires and volcano. Human activities like manufacturing can also release cadmium. It is not mined but it is a by-product from zinc extraction (ZnS) when cadmium sulphide is released. Cadmium can be ingested through food like liver and mushroom. Smoking of cigarette also increases cadmium level in the system. Inhalation of high concentration can result in severe damage of lungs. When absorbed, cadmium is hardly excreted through the kidney instead it destroys its filtering mechanisms. Other effects include diarrhea, bone fracture, nervous system and DNA damage and cancer development (ATSDR (2004). Cadmium flow from wastes to streams and end up in soils or air, artificial phosphate fertilizer plants also release cadmium. Earthworms and soil fauna are susceptible to cadmium poisoning causing their death at certain level however, bioaccumulation occur in aquatic ecosystem. High blood pressure, liver disease and brain damage are some of the symptoms of cadmium poisoning.

The effects of individual constituents of petroleum are well documented and humans can be affected by oil spills from damage to surrounding plants and animals, and perhaps by direct contamination. One Scottish study found an increase nausea, headache, throat irritation and itchy eyes in local populations following spills, but long-term effects of this mixture are unknown (Borasin *et al.,* 2002). Rigorous clinical studies are needed to assess the direct effects of oil spills on human beings

2.5 Oil Production in Nigeria

The first obvious indication of oil resources in Nigeria dates back to 1908 with the appearance of oil at Araromi, in the present Ondo State. A German company, Nigeria Bitumen Corporation started this pioneering effort that was short-lived as a result of the outbreak of the 1914-1918 First World War. Another exploratory activity took off in 1937 by an Anglo-Dutch consortium that served as a forerunner of the present-day Shell D'Arcy. The exploratory activity started in 1937 after Shell D'Arcy had been awarded the sole concession rights that covered the whole territory of Nigeria. The company operated under the Mineral Oil Ordinance of No. 17 of

1914 and its amendments of 1925 and 1950 which allowed only companies registered in Britain or any of its protectorates the rights to prospect for oil in Nigeria and further provided that the principal officers of such companies must be British subjects. The 1939-45 Second World War interrupted the exploratory activities of Shell D'Arcy.

The Shell BP undertook the preliminary geological reconnaissance and intensified its geographical surveys from the 1946- 1951. It drilled its first wildcat well in 1951, which later dried up. Shell BP discovered its first commercial crude oil in the country in 1956 at Oloibiri in the present Rivers State (SNAR, 2005). That discovery ushered Nigeria into the international oil arena. Two years later (1958) Shell started oil exportation from Oloibiri field at a rate of 5,100 barrels per day. In order to increase the pace of oil exploration and to ensure that the country was not dependent on one oil company or nation, Shell's sole concession right over the country was reviewed and exploration rights were granted to companies of other nationalities. Oil companies like Mobil, Gulf, Agip, Safrap (ELF), Tenneco and Amoseas (Texaco/Chevron) were allowed to join the explorers for oil in the onshore and offshore areas of Nigeria. Having dug its first well in 1956, Royal Dutch/Shell now controls over half of Nigeria's oil production. When Nigeria gained independence in 1960, oil production had been established in the country and it was exporting over 170,000 barrels per day (bpd). It was Gulf Oil Company that first struck offshore oil on the Okan structure of Bendel State in 1964 being granted both offshore and onshore licenses. With these commercial discoveries in petroleum products, the socio-economic and political development of Nigeria began to crystallize as well as its internal dynamic ethnicity (NDES, 1997).

2.5.1 Nigeria oil refineries

Once extracted, crude oil is transported to oil tanks from where it is further transported through pipes to refineries where it is refined to various useful products. Nigeria's total installed refining capacity is 445,000 barrels per day of crude oil, as at 1990 to date. The three refineries in Nigeria are Kaduna refinery (KRPC), Port-Harcourt refinery (PRPC) and Warri refinery (WRPC).

The Port-Harcourt refinery has 60,000 barrels per day processing capacity and was built by Shell-BP in 1964. It was taken over by the Nigerian government in 1977 and became Nigeria's first refinery. The newest refinery having a processing capacity of 150,000 barrels per day which was initially designed as an export refinery has its location at the coastal village of Eleme, near the old Port Harcourt refinery. It is the most modern of Nigeria refineries and was commissioned in 1991 (Nigerian crude oil and Gas, 2011). The Warri refinery has a processing capacity of 125,000 barrels per day of crude. It has an adjoining petrochemical plant with production capacity for carbon black. It was built in 1978, with an initial capacity for 100,000 bbl. per day, however it was de-bottlenecked in 1991 to increase processing capacity to 125, 000 bbl/day. The Kaduna refinery was built and commissioned in 1988 with processing capacity of 150,000 barrels per day. It has adjoining petrochemical plant which can produce asphalt, benzene and heavy paraffin base oils, used in the manufacture of vehicular lubricants and oils.

The distribution of petroleum products conventionally involves pumping products through a crisscross of pipeline networks around the nation serviced by about twenty-one oil depots and the major terminals; Bonny, Qua Iboe, Brass, Forcados, Escravos, Pennington and Warri (NDES, 1997). With the completion of the pipeline interlink project of 1994/95 (Pipeline Phase III), the length of the nations products distribution pipeline linking 20 storage complex increased to about 4950km (Adubi, 1995). The complex is classified into 5 basic systems referred to as systems 2A, 2B, 2C, 2D and 2E. The Atlas Cove depot (a marine receipt terminal serving as a distribution and pump station for local and overseas refined products in Nigeria) marks the origin of the system 2B pipeline network of Petroleum Products Marketing Company Ltd. (PPMC) and is the largest petroleum products depot in Nigeria. It has a holding capacity of 114,300m^3 [Petrol – 48,000m^3, Dual Purpose Kerosene, DPK – 34,000m^3 and Diesel (Automobile Gas Oil, AGO) – 32,300m^3] (Adubi, 1995). Nigeria is the largest oil producer in Africa and the tenth largest in the world. The mainstay of Nigeria economy is the petroleum sector, contributing about 90% of the nation's foreign exchange earnings and about 25% of the Gross Domestic products. In 2006, its contribution to foreign exchange earnings reduced by 25% due the activities of the militant youths of the Niger –Delta (Leahy, 2006). A significant

proportion of the Nation's oil is produced onshore and is subsequently transported by pipelines, although recently oil production has witnessed increased activities in the offshore. The estimated national oil reserve is put at 35.2 billion barrel and average production of between 2.5 million barrel and 3.0 million barrel per day (bbl/d) (WAE, 2010)

2.5.2 Nigeria Oil spills and their ecological impact

Although the quantity of oil drilling in Nigeria is small compared to that done in some other producing countries, lack of regulatory bodies and dependence on oil for income have led to sub-standard production operations. Oil pollution from normal operations including spills, accidents, leaks and waste discharges have caused significant ecological damage to the Niger - Delta. Vigorous upstream and downstream activities in this region have led to enormous environmental contamination over the years. The environmental problems of the Niger delta are complex, interconnected and caused by many factors (SNAR, 2005). Although environmental degradation also arises from natural sources, the most common environmental problems in the region results from oil spills, gas flaring, construction of oil facilities, pollution from industries and dredging of canals. (Oil for Nothing, 1999)

Shell Oil alone reported 130 spills in 1997; attributing 53 to equipment failure, 23 to human error and 54 to sabotage by those frustrated with the government and oil industry (NDES, 1997). According to Moffat and Linden (1995), at least 2300 cubic meters of oil from at least 300 spills contaminate the Niger Delta region annually. This is the "official" number reported. The actual amount of oil spilled annually "may be 10 times higher" (Moffat and Linden, 1995). In Nigeria, reports from 1976 to 2001 showed that there had been 6,817 oil spills with approximately three million barrels of oil lost; an average of 273 oil spills and 115,000 barrels/year spilled in the Niger Delta alone (UNDP, 2006). However, Shell reported a total of 284,000 barrels of oil spilled and about 28,000 barrels year between 1990 and 2007 (SNAR, 2008). In a related report by IUCN/CEESP (2006), 9 to 13 million barrels of oil are claimed to be spilled into the Niger Delta ecosystem over the past 50 years. The discrepancies in the reports on the amount of oil spilled by oil companies operating in Nigeria and

world bodies like UNDP are indications that there are underestimated evaluations of oil wrecks caused by the operations of oil companies. It therefore calls for caution to out rightly depend on their reports of such spills and more international / national bodies and individual researchers with no affiliation to oil companies should be encouraged to investigate oil spills and there impacts when such occurs. Some notable oil spills recorded in Nigeria include Bomu 11 oil well blowout,1970, GOCON's Escravos spill, 1978 (300, 000 barrel), Forcados Terminal Spillage, 1980 (580, 000 barrels), Oyakama pipelines spill (1980), Texaco Funiwa 5 blow out in 1980 (400, 000 barrels), Abudu Pipeline Spill, 1982 (18,818 barrels), Ikata Pipeline Spill (1984), Okoma Pipeline Spillage (1985) and Oshika Pipeline Spill (1993), the massive Oloibiri Well 14 oil spill (2004), and very recently, Bodo oil spills (August 2008 and February 2009) and K. Dere spill (April 2009), (Steiner, 2008). The 1980, Texaco Funiwa 5 blowout was acclaimed as the worst oil spills in the 1980's with an estimated 200,000 barrels of oil spilled into the Atlantic Ocean and a damaged 340 hectares of mangrove (Nwilo and Badejo, 2005). Table 2.2 shows the total number of spill and volume of spilled between 1995 and 2013 in Nigeria by shell alone (SNAR, 2005).

Other forms of pollution identified to be contributed by oil refineries include thermal pollution of water bodies. In Nigeria, oil pollution appears to be the major pollution problem especially in the oil rich areas where river sources and coastal waters are most polluted with petroleum wastes (Ibanga, 1978). Niger Delta remains the most impacted region by oil spillages and has resulted to, amidst others, frequent vandalization of oil installations. Recently, the issue of pipeline vandalization has caused a wide spread environmental alarm all over the country as the occurrences of vandalization is not restricted to the oil producing area alone. Incidents of Oil pipeline explosion Disaster in Nigeria between 1998 and 2008 is presented in tab 2.3. A number of petroleum contaminated sites requiring cleanup in Nigeria is on the increase, therefore more research into the use of inexpensive, easily accessible and environmentally friendly measures to reclaim these areas becomes necessary.

Table 2.2 Oil spill data: SPDC 1995-2013

	Number of spills	Volume in barrels (bbl)
1995	235	31,000
1996	326	39,000
1997	240	80,000
1998	248	50,000
1999	320	20,000
2000	330	30,000
2001	302	76,000
2002	262	19,980
2003	221	9,916
2004	236	8,317
2005	224	11,921
2006	170	20,000
2007	320	32,000
2008	215	100,000
2009	190	104,000
2010	175	28,000
2011	210	16,000
2012	190	26,000
2013	200	20,000
Total	**4614**	**722,134**

Source: SNAR, 2005; Shell spill incident data, 2013

Table 2.3 Incidents of Oil pipeline explosion Disaster in Nigeria (1998-2008)

DATE	LOCATION/STATE	DEATH TOLL	OBSERVED CONSEQUENCES
May 16, 2008	Ijegun / Lagos	Undetermined	Many people were injured
Dec. 2006	Ije-Ododo/ Lagos	≤ 1	Environmental pollution, damage to farmland
May 30, 2005	Akinfo/Oyo	≤ 1	34 persons were injured, 15 died after eleven days
Sept 16, 2004	Ijegun/Lagos	≤ 60	Air and water pollution
July 30, 2004	Agbani/ Enugu	≤ 7	Several people injured, environmental pollution
June 19, 2003	Ovim in Abia state	≤ 125	Dozens of people injured, damage to farmlands
November 5, 2001	Umudike, in Imo.	≤ 3	≥17 people injured
July 16, 2000	Oviri court / Delta	≤ 300	Dozens of people injured, environmental pollution
July 10, 2000	Adeje, Delta state	≤ 150	Environmental pollution, damage to farmlands
June 20, 2000	Okuedjeba, Delta State	Undetermined	Dozens of people injured, environmental pollution
March 14, 2000	Umugbede , in Abia state	≤ 50	Environmental pollution, damage to farmlands
February 7, 2000	Ogwe, Abia state	≤ 15	Environmental pollution, damage to farmlands
June 8, 1999	Akute-Odo , Ogun	≤ 15	Damage to farmlands, pollution (air and water)
October 18, 1998	Jesse, in Delta State	≤ 1000	Environmental pollution, damage to farmlands, dozens of people injured

Source: Adapted from Okoli *et al.*, 2013

2.6 Remediation techniques for oil spills

Remediation of petroleum polluted sites is not new and the methods used include; engineering, pump-and–treat, soil vapours extraction, sparging, chemical washes etc. Most of these remediation technologies have been used and are effective but they are known to be generally expensive and require professionals. However, bioremediation is a cheaper and a more environmentally friendly approach to resolving the problems of contaminated soils. Bioremediation is one of nature's prudent ways to purify the polluted environment and that degraded by the anthropogenic activities. Although, the term "bioremediation" may be recent, the process is not a new one, (Hoff, (1993; Atlas, 1995a). Its origin relates to the origin of life when the first organism was stressed by certain compounds and it evolved the process to convert such compounds into less harmful forms by adopting certain detoxifying mechanisms in order to overcome the stress.

Bioremediation is a useful method for soil remediation, if pollutant concentrations are moderate and non-biological techniques are not economical. Microbiologists have studied the process since the 1940s. However, bioremediation became known to a broader public in the U.S. only in the late 1980s as a technology for cleanup of shorelines contaminated with spilled oil. The *Exxon Valdez* oil spill in 1989 in Prince William Sound, Alaska was the catalyst for this attention. In the years since 1989, bioremediation has become a technology that is discussed, applied, and considered in many different circumstances (Hoff, 1993), the history of bioremediation in spill response can be divided into three development periods according to Hoff, 1993: the 'courtship' period until 1989, the 'honeymoon' period from 1989 until 1991, and the 'establishment' period since 1992.

Biodegradation is a large component of oil weathering and is a natural process whereby bacteria or other microorganisms alter and break down organic molecules into other substances, eventually producing fatty acids and carbon dioxide (Hoff, 1993). Bioremediation is the acceleration of biodegradation by adding exogenous microbial populations, stimulating indigenous populations or by manipulating

contaminated media using techniques like aeration and temperature control or addition of nutrients (Hoff, 1993; Atlas, 1995b; Swannell *et al*., 1996).

Many microorganisms possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes, others aromatics, and others both paraffinic and aromatic hydrocarbons. Often the normal alkanes in the range C_{10} to C_{26} are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by many marine microorganisms. More complex structures are more resistant to biodegradation, meaning that fewer microorganisms can degrade those structures and the rates of biodegradation are lower than biodegradation rates of the simpler hydrocarbon structures found in petroleum. The greater the complexity of the hydrocarbon structure, i.e., the higher the number of branched methyl substituent or condensed aromatic rings, the slower the rates of degradation (Atlas, 1995a).

PAHs in contaminated soils can be treated with bioremediation. The oxidation of PAH involves oxygenases (monooxygenases and dioxygenases). Fungi complete the process by adding an oxygen to the substrate PAH to form arene oxides and then enzymatically adding water to form trans-dihydrodiols and phenols. Bacteria mainly use dioxygenases, adding two oxygens to the substrate and the further oxidizing it to dihydrodiols and dihydroxy products. Ring oxidation is the rate limiting step in the reaction, and subsequent reactions occur fairly quickly, yielding the typical metabolic intermediate Catechol found in Lignin degradation as well as Gentisic and Protocatechuic Acids (Philip *et al.*, 2005).

Intermediate metabolites degrade further through ortho and meta ring cleavage to produce succinic, fumaric, pyruvic, and acetic acids and acetyl-CoA, which are shunted into major metabolic and anabolic pathways. The byproducts of these reactions are carbon dioxide and water. The breakdown of PAHs can be accomplished by microorganisms that use PAH as their energy and carbon source, and also by other microbes through a process termed "co-metabolism." Co-metabolism refers to the degradation of two compounds, one of these compounds the microbe obtains energy from, while the other is degraded "unintentionally." In such cases, the microbe directs it enzymes at the primary substrate, but these enzymes are

also capable of degrading the pollutant. Co-metabolism has been shown to be an important phenomenon in the bioremediation of larger aromatic chains (Philip and Atlas, 2005).

The biodegradation of petroleum in the environment is carried out largely by diverse bacterial populations. The hydrocarbon-biodegrading populations of the marine environment for example are widely distributed in the world's oceans; surveys of marine bacteria indicate that hydrocarbon-degrading microorganisms are ubiquitously distributed in the marine environment (Prince and Atlas, 2005). Generally, in pristine environments, the hydrocarbon-degrading bacteria comprise < 1% of the total bacterial population. These bacteria presumably utilize hydrocarbons that are naturally produced by plants, algae, and other living organisms. They also utilize other substrates, such as carbohydrates and proteins.

When an environment is contaminated with petroleum, the numbers of hydrocarbon-degrading microorganisms are known to increase rapidly. Particularly, in marine environments contaminated with hydrocarbons, there is an increase in the proportion of bacterial populations with plasmids containing genes for hydrocarbon utilization. The proportions of hydrocarbon-degrading bacterial populations in hydrocarbon-contaminated marine environments often exceed 10% of the total bacterial population (Atlas, 1995a; Prince and Atlas, 2005)

2.7 Bioremediation techniques

There are several bioremediation techniques; the underlying idea is to accelerate the rates of *natural* hydrocarbon biodegradation by overcoming the rate-limiting factors. Several techniques can lead to the results striven for. Indigenous populations of microbial bacteria can be stimulated through the addition of nutrients or other materials. Exogenous microbial populations can be introduced in the contaminated environment (bioaugmentation). If necessary, genetically altered bacteria can be used. Once the bacteria are chosen, the engineer must carefully meet their nutritional needs by choosing the correct mix of fertilizer (Irwin, 1997). Furthermore, the contaminated media can be manipulated by, for example, aeration

or temperature control. Two of these concepts involved include: seeding with microbial cultures and environmental modification.

The success of microorganisms in biodegradation depends upon a wide array of variables and conditions, which often limit effective bioremediation and might include oxygen and nutrient availability, pH, C:N ratio, presence, number and activity of organic contaminant degrading microorganisms, enzyme induction, temperature, toxic levels of contaminants, presence of co-contaminants (determining added toxic effects or preferential degradation), and presence of terminal electron acceptors (Atlas, 1995b; Boopathy, 2000 and Romantschuck *et al*, 2000).

Some bioremediation techniques include composting, bioaugmentation, phytoremediation and zooremediation. Composting was originally applicable for organic waste conversion into mulch and soil conditioner. It is now being applied to the hazardous waste treatment. Composting involves conversion of waste to less complex and relatively more stable material, significant decrease of water content and reduction of mass of the residue. Materials which are amenable to the bioremediation composting process include sewage sludge, soils contaminated with diesel fuel or other petro-products, wastes from brewing etc. Bulking agents like fibrous plant material, wood chips, bark, are added to increase porosity so as to improve aeration (Zachery and Reid, 2008a)

Bioaugmentation is the enhancement of decontamination in the media or waste-biodegradation by seeding of competent microflora and supplemented with desired level of nutrients. Faster decontamination has been achieved successfully by stimulation of existing microbial populations or augmentation with adapted strains. Thus, augmentation refers to establishing suitable conditions for bioremediation by means of adding nutrients for growth promotion, addition of terminal electron acceptor (oxygen or nitrate), moisture level adjustment or raising the temperature (Lloyd *et al.*, 2005)

Phytoremediation is a cost-effective, simple and sustainable beneficiary technique to remove pollutants from the environmental components - air, water or soil, using plants. It is a nondestructive and cost-effective technology employed to clean up contaminated soils. Phytoremdiation has been used on target contaminants

like metals, metalloids, petroleum hydrocarbons, pesticides, explosive or toxic gases, chlorinated solvents and a range of industrial by-products. Commercially viable phytoremediation systems for clean-up of shallow aquifers and water-borne contaminant are now well in practice. (Biobasic, 2006)

Zooremediation involves the decontamination of environment through the activities of animals. Animals used for this purpose include the range of different arthropods, fishes, other filter feeders in aquatic systems and earthworms in solid organic waste management systems. It has been demonstrated that many aquatic animals were successfully used for water pollution treatment but it has not been encouraged owing to significant ecological safety reasons. However, earthworm based technology has proved commercial potentials as the role of earthworms in the conversion of organic materials and improvement of soil has been observed and appreciated (Biobasic, 2006; Zachery and Reid, 2008a)

2.8 Earthworms and their ecological importance

Earthworms are either earthmovers or composters. Earthmovers tend to be solitary species which tunnel through the earth, aerating, decompacting and mixing soil strata and thus making surface nutrients available to plant roots at lower levels. Composters live en masse in organic matter on the soil surface, where they consume bacteria present in dead vegetation, animals and manure, turning it into humus. Common worm species with market value include *Eisenia fetida, Lumbricus rubellus, Lumbricus hortensis, Lumbricus terristris, Eudrilus engeniae, Eisenia andrei, and Perionyx excavatus.* Some of these species and other species share similar common names even while their sizes, appearances, natural habitats, feeding and breeding habits, temperature requirements and behaviors are quite different. (Kale and Karmegam, 2010).

Earthworms are key organisms in (environmental) toxicology; Interest in earthworm ecotoxicology can be traced back to the inception, ring-testing and international standardisation of the acute earthworm toxicity test (OECD, 1984). Acute toxicity test was designed to be included in the risk assessment framework for newly registered chemicals and pesticides and the earthworm *Eisenia fetida* was

identified as a model organism for assessing the effects of chemicals on terrestrial saprotrophic invertebrates.

On the other hand, earthworms are hardy organisms being capable of surviving highly toxic environment like being the only survival soil fauna after the 1976 Seveso chemical plant explosion in Italy (Satchell, 1983)

2.8.1 Earthworm use for remediation and ecotoxicity

Charles Darwin referred to earthworms as "unheralded soldiers of mankind"; this was one of the earliest references of the wonder works of earthworms. Their role as waste managers is well documented by vermiculture scientists but recently, research in the use of earthworms is tending towards its role in remediation of soil pollutants, development of medicine, as feeds in fisheries and dairy including its use as raw materials in rubber, lubricant, soap and detergent industries (Sinha, *et al.*, 2010).

Earthworms can be employed in bioremediation strategies to promote biodegradation of organic contaminants. This is because of their biological, chemical and physical activities ranging from burrowing , production of casts both surface and below ground, its internal gut and processes, its surface in contact with the soil, other biological, chemical and physical interactions, in addition to the associated soil microorganisms (Brown and Doube, 2004). Earthworms aerate and bioturbate soils and improve their nutritional status and fertility, which are variables known to limit bioremediation. Earthworms also retard the binding of organic contaminants to soils, release previously soil-bound contaminants for subsequent degradation, and promote and disperse organic contaminant degrading microorganisms (Zachery and Reid, 2008a).

In any bioremediation strategy, it is often necessary to ensure appropriate moisture, oxygen and nutrient levels, while ensuring that they can be homogenously dissipated, especially if, for example, dealing with deeper soils, compacted soils or soils rich in clay. Although techniques exist which is in use in the optimization of these variables, they could be time consuming, labour intensive and expensive methods. There might be a relatively low input, low technological tool available to

undertake this work. As earthworms move throughout the soil environment, their resulting burrows act as input points of, and preferential pathways for, water and particle movement (Kretzshmar, 1984; Shipitalo and Le Bayon, 2004 and Dominguez, 2004), and nutrient flow and aeration (Dominguez, 1998; Zachery and Reid, 2008b).

Earthworms are useful in degradation of agrochemicals (Eijsackers *et al*, 2001), petroleum and crude oil hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs) and other compounds by increasing hydrocarbon availability and remobilizing dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) bound residues (Verma and Pillai, 1991), however, such findings were in conflict with those of (Bolan and Baskaran, 1996) who investigated the effect of earthworm (*Lumbricus rubellus* and *Allolobophora calignosa*) casts upon the sorption and movement of ^{14}C-atrazine, ^{14}C-2,4-dichlorophenoxy acetic acid (2,4-D) and ^{14}C-metsulforon methyl. They stated that the casts absorbed higher amounts of herbicides than the source soil due to the higher levels of organic carbon and fine size fractions, present due to earthworm grinding actions and selective feeding. Although both theories are plausible, they emphasize both the differences in compound behaviour, experimental set-up or earthworm species and the wide variability between the effects of earthworm mechanics upon compound fate and subsequent earthworm casts upon compound fate (Philip and Atlas, 2005; Zachery and Reid, 2008).

Ecotoxicity test with earthworm can be used for artificial soil acute toxicity test, in screening chemical toxicity. These include not only classical toxicity studies but also field soil assessment, remediation evaluation and evaluations of bioavailability (Spurgeon *et al.* 2004; Edwards and Bohlen 1996). Several factors are of importance when using earthworm for ecotoxicity test; Weight loss during acute tests is a validation criterion to be considered. If more than 15-20% weight loss occurred in the control, it would indicate the test was not valid because the earthworms were unhealthy or the substrate not suitable for the test. Observation of significantly more weight loss than this in treatments should be considered as an indication of sublethal effects (OECD, 1984). For studies specifically measuring weight loss, it is

recommended to ensure an even weight distribution at the outset between treatments. It is also important to measure chemical availability during toxicity tests. This included not only total chemical in the soil, but also potentially bioavailable and body residue levels (Spurgeon *et al.* 2004).

Studies using earthworms in ecotoxicity of petroleum pollution are reported; most indicates its use as toxicity assays in monitoring bioremediation of petroleum sites (Knoke, *et al*, 1999; Lors *et al*, 2009). Knoke *et al*, (1999) used five bioassays to measure toxicity during bioremediation of a soil contaminated with pentachlorophenol (PCP; 335 ppm), polycyclic aromatic hydrocarbons (PAHs; 1225 ppm) and petroleum hydrocarbons (19125 ppm). Different bioremediation treatments were tested in soil microcosms including amendment with phosphorus and/or PCP-degrading Pseudomonas sp. UG30, either as free cells or encapsulated in κ-carrageenan. Soil toxicity was monitored using the solid-phase Microtox test, SOS-chromotest, lettuce seed germination, earthworm survival and sheep red blood cell (RBC); the trend indicated earthworm survival LC_{50} values varying with each treatment with results showing toxicity trends in a contaminated soil during bioremediation differ according to the assay used.

A determination of the limits and extent of hydrocarbon biodegradation using earthworm and plant toxicity and waste leachability of crude oil-containing soils was carried out by Salanitro, *et al.*, 1997. Three oils (heavy, medium, and light of API gravity 14, 30, and 55, respectively) were mixed into silty loamy soils containing low (0.3%) or high (4.7%) organic carbon at 4000−27 000 mg/kg TPH. Most oily soils were initially toxic to earthworms in which few animals survived in 14-day bioassays. In a solid phase Microtox test, most oily soils had EC50 values that were ≤50%. Seed germination and plant growth (21-day test, wheat and oat but not corn) were also significantly reduced (0−25% of controls) in untreated soils containing the medium and light crude oils but not the heavy oil. Bioremediated soils were neither toxic to earthworms, inhibitory in the Microtox assay, nor inhibited seed germination after 5 (high organic soil) or 10−12 (low organic soil) months of treatment. Data suggested that the remaining petroleum compounds may be bound or unavailable in that they are not (a) biodegraded further, (b) toxic to soil-dwelling species (earthworms and plants), and (c) susceptible to leaching and subsequent impact to

groundwater. These findings provide a basis for a framework in which petroleum hydrocarbon-containing soils can be evaluated by ecological assessment methods such as biodegradability, ecotoxicity and leaching potential of regulated substances.

Lors *et al*, 2009 studied the performance of a biological treatment of a PAH-contaminated soil with respect to its physicochemical and ecotoxicological properties. After six months, the biological treatment led to a significant reduction of 2- and 3-ring PAHs and to a lesser extent to 4-ring PAHs. As a consequence a significant decrease of the acute ecotoxicity was observed passing from highly ecotoxic before treatment to non-ecotoxic according to *Lactuca sativa* seedling and growth inhibition test and *Eisenia fetida* mortality test. This could be related to the bioavailability of PAHs. Indeed, tests performed on aqueous leachates of the soil showed a strong decrease of 2- and 3-ring PAHs correlated with a significant reduction of acute and chronic ecotoxicity responses. The biological treatment led to the mutagenicity reduction and the genotoxicity disappearance in the leachate. Thus, bioassays are complementary to chemical analyses to evaluate the efficiency of a bioremediation process and to evaluate the bioavailability of the organic pollutants as the total concentration of a contaminant is not the only criterion to consider. The comparison of the ecotoxic responses allowed us to underline the best sensitivity of the earthworm, Microtox, Alga and Ames bioassays among the tested set. These bioassays could thus be good candidates to build a toxicity evaluation procedure for PAHs contaminated/ remediated soils.

Generally, earthworms bioaccumulate and biodegrade several organic and inorganic chemicals like tetra chorodibenzo-p-dioxin released during the Seveso explosion as recorded by Satchell, 1983, PAHs (Ireland, 1983; Contreras-Ramos et al, 2006), Endocrine distrupting chemicals (EDCs) from sewage e.g. bisphenol –A (Markman et al, 2007). They are also known to tolerate 1.5% crude oil (Hanna and Weaver, 2002).

2.8.2 Earthworms and heavy metals

Earthworms were found to have high potential for the accumulation of some heavy metals like cadmium, mercury, lead, manganese, calcium, iron and zinc in polluted soils, (Hendriks *et al*., 1995) but are also known to disappear at low levels of

soil Cu and Ni (Klok *et al.*, 2000). *Lumbricus terristis*, *L. rubellus* and *D. rubida* are known to accumulate high levels of lead and cadmium in their tissues (Ireland, 1983). Cadmium levels up to 100mg/kg was found in tissues of earthworms; *L. terrestis* could bioaccumulate 90-180mg/g of lead while *L. rubellus* and *D. rubida* assorbed 2600mg/g and 7600mg/g of lead. Zinc, Manganese and iron are reported to be excreted through the calciferous gland of earthworms (Ireland, 1983 and Hartenstein *et al.*, 1980). *L .rubellus* degraded soil spiked with PAHs (100µg/kg of phananthrene and fluoranthrene) after 56 days (Contreras-Ramos et al, 2006) recording 86% removal of phenanthrene 100% (Ma *et al.*, 1995). *E.fetida* had reduced oil contents when compared with control. Earthworms can mineralize asphaltens and decontaminate hydrocarbon polluted soils (Ceccanti *et al.*, 2006; Martin-Gil *et al.*, 2007 and Tomoko *et al.*, 2005). Sinha, 2010 had shown that sludge (brittle and black) was transformed to homogenous and porous and light texture and over 80% free of cadmium and lead after a twelve weeks experiment.

However, earthworms are considered useful for assessing heavy metal pollution in soils (Menzie *et al.*, 1983). Though some metals like copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) are essential as micronutrients for life processes in plants and microorganisms, others like Cd, Cr and Pb are with unknown physiological activity, but are detrimental beyond certain limits (Bruins *et al.*, 2000).

2.8.3 Action of earthworms in remediation

Earthworms passively absorbs dissolved fraction through its body wall and ingest soil through its mouth followed by intestinal uptake during which they either biotransform or biodegrade contaminants. Some metals bind to metallothioniens (a protein found in earthworms), their chloragogen cells are suspected to accumulate heavy metals by their guts and then immobilize them in their small spheroidal chloragosomes and debris vesicles which the cell contains. Other substances degraded by earthworms in this manner include phthalate and flouranthene in soils. Generally, vermiremediation would cost $500 - $1000 per hectare while mechanical evacuation is estimated to cost $10, 000 – 15, 000 (Sinha, *et al.*, 2010). Although there are several work indicating the use of earthworms for ecotoxicity or remediation, reports on the use of African earthworm species for remediation is

however limited and not readily available. However, *Eudrilus eugeniae* an earthworm species found in Nigeria, was not significantly effective in the remediation of spent engine oil (Ameh, *et al.*, 2012) but caused a drop in total petroleum hydrocarbon content in soils from mechanic workshop after a 35 days exposure (Ameh, *et al.*, 2013). On the other hand, there was reduction in population of *Eudrilus eugeniae* in petroleum polluted soils and dump site (Oboh *et al.*, 2007).

2.9 Physicochemical parameters

Several physiochemical analyses are important to be carried out when investigating portability of water samples; some of such include; hydrogen ion (H^+) concentration (pH), total dissolved solutes (TDS), conductivity, total hardness, sulphate, calcium hardness, nitrate, phosphate, heavy metals including cadmium, copper, zinc, lead, nickel, vanadium and manganese and organic parameters; Total Petroleum Hydrocarbon (TPH), Total organic carbon (TOC) and Total organic matter (TOM). Petroleum spills have negative impact on the environment and in Nigeria such events are common placed in the Niger-Delta hence more reports of investigations on oil spills are focused on this area.

Benka-Coker and Ekundayo, 1995 had reported the average total hydrocarbon in Ekeremor soil after an oil spill to range between 0.8 to 12.4 ppm while studies by Okop and Ekpo, 2012 on Ikot-Abassi soil reported 9 – 289 mg/kg. An assessment of soils from oil spill site in Mese, Ondo state showed that the average pH, moisture content and organic carbon were 6.7, 78.65 % and 2.76 % respectively compared with soils from a site in Bonny, River state having average pH, moisture content and organic carbon to be 4.6, 61.51% and 4.23% respectively. (Folake, 2013). Nickel and vanadium assessment of oil polluted soil from Ogbodo-Isiokpo (Rivers state) had shown Ni levels ranging from 0.15 to 1.65 mg/kg and V levels ranging from 0.19 to 0.70 mg/kg (Osuji and Adesanya, 2005). TPH levels recorded for both surface and groundwater within the vicinity of NNPC oil depot in Apata, Ibadan ranged from 20.34±1.79 to 27.40 ±5.32 and 2.67±0.80 to 13.03±2.21mg/l respectively. Heavy metals like Pb, Cd, Zn, Ni, Cu and Cr in surface water and groundwater were reportedly higher than the WHO permissible limit and drinking water limits (Adewuyi amd Olowu, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was carried out in Agaye settlement in Ije –ododo community which is located in Iba (LSDC) Ojo local government area of Lagos State (Lat. 06^{o} 29'N and Long. 03^{o} 15' E) (Fig. 3.1). Lagos is a coastal city bounded by the Atlantic Ocean in the south. FAO, 2009, described Lagos to be ecologically located partly in the swampy mangrove and partly in the delta swamp area and climatically under the Equatorial Climate which extends from the coast to about 150km inland. Rainfall is between 1500 and 3000mm per annum, with an average temperature range of 17–24°C and relative humidity ranging between 60–90%. It has two seasons, the wet season March to October, and dry season November to March having bi–modal rainfall maxima annually. Lagos majorly has the mangrove forest and coastal vegetation interspersed with numerous creeks and lagoons. The mangrove swamp is noted for the mangrove species of trees; *Rhizophora*, dominated by *Rhizophora racemosa* spp (occupying an estimated 99% of the entire mangrove area) and less frequently *Conocarpus erectus* and *Laguncularia racemosa* (white mangrove) (Garnier 1967; Iloeje 1980).

Agaye falls under the swampy mangrove area lying north of the Lagos Badagry expressway and west of Festac town. The major surface water body is a wetland which suffices for a river with flowing water current towards the South during rainy season and dries up during dry season becoming a typical wetland with sparsely distributed patches of land. The surface water lies southwards of the settlement extending to the Lagos – Badagry expressway and Festac town, the water body remains inaccessible major parts of the year. The only accessibility used to be through a constructed footbridge usually used by fishermen to access the inland of the water body; however, the footbridge is presently dilapidated. The other portion of Agaye settlement is land of flat topography with swampy terrain; the settlement was sparsely occupied by residential building at the time of the study. The remaining land was either bare or used for subsistence farming of staple crops like maize and cassava. The predominant farming practice involved the use of organic manure

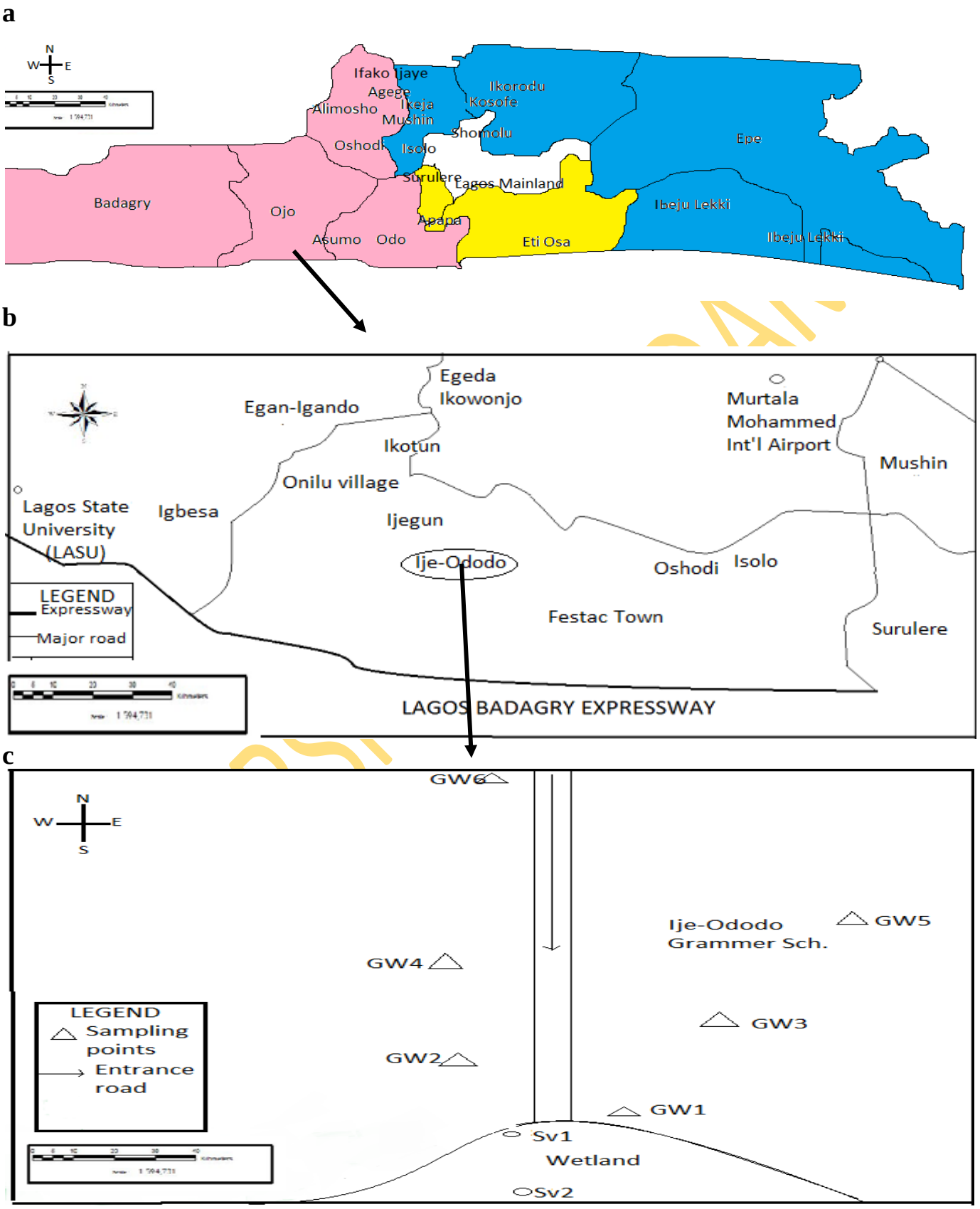


Fig. 3.1 Map of Lagos state (a), indicating Ije-Ododo (b) and highlighting sampling points (c)

(chicken droppings) and crop rotation system. High pressure oil pipelines owned and monitored by the Nigeria National Petroleum Corporation (NNPC) are commonly found within the area and extending towards the inaccessible parts of the wetland. The study site was chosen because of the repeated oil spills in the area and its attendant health effect as reported in the Punch, a Nigerian newspaper of 29th Dec, 2006 (Plates 3.1 - 3. 4) (Okoli *et al.*, 2013). The petroleum product suspected to be spilled was Premium Motor Spirit (PMS). Subsequent oil spill had been reported to have occurred in Agaye by 2012 and 2013. There is no baseline record of physicochemical parameters of soil and water in this area. The contamination in this case was the accidental discharge of petroleum oil resulting from leaking oil pipelines lying towards the inaccessible wetland. This lasted for some weeks (eye witness account) and resulted in an inferno (engulfing a great portion of the wetland and 30 – 100m of land) that burned for days and could only be put out by the intervention of sophisticated fire fighting approach (use of helicopters).

For this study, the area was delineated based on impact of the spill and inferno; sampling points were located 100m within the spill while the control was chosen from an area 500m away from the spill, this was to ensure soil type similarities because of the earthworm population study. There was an oil spill and fire outbreak that occurred in May, 2008 at Ijegun (an adjacent community located south of Agaye) about 1km away from the study site during the period of the study.

Plate 3.1 Resultant inferno in Agaye settlement, Iba LSDC, Ojo LGA, Lagos state after Oil Spill (Source: Punch Newspaper, 26th December, 2006)

*Picture showing onlookers behind the community secondary school premises as they watch the fire out break ensued in Agaye settlement after the oil spill

Plate 3.2 Water body in Agaye after oil spill and inferno

(Source: Field Survey, 2007)

* Wetland covered with widespread oil films two months after the oil spill, an indication of the impact on the water body.

Plate 3.3 Agaye vegetation after oil spill and inferno

(Source: Field Survey, 2007)

*The Raparian tree community out rightly destroyed by the inferno indicating the impact on the vegetation of Agaye community.

Plate 3.4 A farmland after oil spill and inferno

(Source: Field Survey, 2007)

*** A farmland within the area of inferno indicating the negative economic impact of the incident**

3.2 Sample collection and analysis - Quality assurance and quality control

All containers (plastic and glass ware) were soaked in 1M HNO_3 overnight (Onianwa, 2001) and washed with teepol, a laboratory detergent rinsed with tap water and finally with deionised water. Samples for organic analysis were collected using glassware while that for trace metals and other parameters were collected in plastic containers. Parameters such as temperature and pH were determined on the field. Wavelengths setting of the spectrometers used were done daily by the standard instrumental procedure and other equipments used were always calibrated against reference standards. Blank analyses were also carried out.

3.2.1 Water and Soil sampling

Water samples, surface water (SW1 and SW2), groundwater including well water and borehole (GW1 – GW5) were collected once every two months. Surface water were collected 0 and 20m into the water body (from land) while ground water were collected from randomly selected sampling points 20, 40, 60, 80 and 100m away from water body, along transect of the spill. Well water was also collected 500m away from the sampling pont (GW6) served as control (Fig. 3.5). Sampling was done for 2 years between June, 2007 and April, 2009. Triplicate water samples were collected in glass bottles for hydrocarbon analysis while other water samples were collected in 5L plastic kegs for heavy metals and other physicochemical analysis carried out. Samples collected were then labeled as designated in Table 3.1. Triplicate soil samples were collected 0 - 20cm deep from eight locations around same loci of the groundwater sites (S1-S5; S6 as control) and Sv1 and Sv2 (sampled close to Sv1 and Sv2) using soil auger and packed into small polythene bags and labelled according to the locations (Table 3.2). These were taken to the laboratory for further treatment.

Table 3.1 Sampling points, designation, description and GPS locations

Sample designation	Description	GPS Location
GW_1 / S_1	Well 1	N 06° 29. 40' E003° 15. 23'
GW_2 /S_2	Well 2	N 06° 29. 41' E003° 15.20'
GW_3 /S_3	Well 3	N 06° 29.42' E003° 15. 24'
GW_4 /S_4	School Well	E003° 15.19' N 06° 29. 44'
GW_5/S_5	School Borehole water	N 06° 29. 43' E003° 15. 25'
GW_6/S_6	Control well	N 06° 30. 00' E003° 15.25'
SW_1/SV_1	Wet land 1	N 06° 29. 092' E003° 15. 297'
SW2/SV_2	Wet land 2	N 06° 29. 110' E003° 15. 302'

3.3 Physicochemical analyses

3.3.1 Water analysis

Collected water samples were analyzed and parameters such as pH, conductivity, and temperature were determined in-situ. All other samples were packed in ice inside a cooler and transported to the laboratory within six hours of collection for further analysis or preservation. 2ml of Hcl was added to 1L of samples for heavy metal analysis and kept in the refrigerator at $4^{O}C$ until the time of analysis.

3.3.1.1 Heavy metals

Heavy metals were determined by digesting a known volume of water sample with analytical grade HNO_3. The digested sample was filtered into a 50 ml standard flask, made up to the mark with deionized water and stored in a nitric acid pre-washed polyethylene bottle in the refrigerator prior to chemical analysis. The water extracts were analyzed for metals (Pb, Mn, Zn, Ni, Cd, Cr, V and Cu) by atomic absorption spectrometer (Schimazo model 2380). Triplicate samples were analyzed and the mean values recorded (APHA, 1998).

3.3.1.2 pH

The pH of water samples was determined by a pH meter (Hand held Hawna pH meter, Hi-8424). The pH meter was calibrated using a three point calibration method. The water sample was stirred vigorously using a clean glass stirring rod and 50 ml of the sample was poured into the glass beaker using the watch glass as a cover. The sample was allowed to stand to allow for temperature stabilization. Stirring was occasionally done while waiting. The pH meter was standardized by means of standard solutions with known pH. The pH meter electrode was immersed into each water sample and allowed to stabilize in the sample before reading was made. After each reading the electrode was rinsed well with distilled water, and then dabbed lightly with tissues to remove any film formed on the electrode.

3.3.1.3 Temperature

Temperature was measured using portable calibrated mercury in glass thermometer (EPA, 1998) at the collection point. Temperature measurement was made by taking a portion of the water sample (about 1litre) and immersing the thermometer into it for a sufficient period of time (till the reading stabilized).

3.3.1.4 Total Dissolved Solid (TDS)

The difference in the weights of Total Solids (W_1) and Total Suspended Solids (W_2) expressed in the same units gives Total Dissolved Solids (TDS). Fifty millimeter (50ml) of a well-mixed sample was measured into a pre-weighed dish and evaporated to dryness at 103 ^{o}C on a steam bath. The evaporated sample was dried in an oven for about an hour at 103-105 ^{o} C and cooled in a desiccator and recorded for constant weight (W_1). Another vigorously shaken 50ml of the sample was filtered into a pre-weighed glass fibre filter disk fitted to suction pump, and washed successively with distilled water. The filter was carefully removed from the filtration apparatus and dried for an hour at 103-105 ^{o} C in an oven, cooled in dessicator and weighed for constant weight (W_2).

Total Dissolved Solids (mg/L) = (W_1-W_2) (1000) / Sample volume (ml)

(APHA, 1992)

3.3.1.5 Total Hardness (TH)

50ml of a well-mixed sample was put into a conical flask using a pipette, to which 1ml of ammonia /ammonium chloride buffer of pH 10 and 2-3 drops of Eriochrome black -T indicator were added. The mixture was titrated against standard 0.01M EDTA until the wine red colour of the solution turned pale blue as the end point (APHA / AWWA/ WEF, 1995).

Total hardness (mg/L) = (T) (1000) / V

Where, T = Volume of titrant

V = Volume of sample

3.3.1.6 Conductivity

Conductivity was measured with a conductivity meter (Accumet Basic AB30) calibrated with potassium chloride solution. The electrode of the conductivity meter was dipped into the sample, and the readings were noted at stable value.

3.3.1.7 Chloride (Cl^-) and Alkalinity

Chloride (Cl^-) was determined by Mohr's titration mothod. 20 ml of sample was placed in a conical flask and pH adjusted to 6 - 8 with small amount of (0.1 M) calcium carbonate solution. One millilitre of potassium chromate solution prepared by dissolving 50 g of potassium chromate in 1L of distilled water was added and the solution was titrated with (0.0141 M) silver nitrate solution with constant stirring.

Alkalinity (Aci-Base titration) was determined by titrating known volumes of water sample with 0.10M HCl. One hundred milliliters (100ml) of water sample was poured into a 250ml Erlenmeyer flask followed by addition 3-5 drops of methyl orange. The burette to be used was rinsed twice with the acid solution for titration (0.1M HCl); this was only done before the first titration. The burette was then filled with the acid and the initial volume recorded. The sample was titrated with the 0.1M HCl to the endpoint (orange to red), and the final volume recorded. The alkalinity of the sample in ppm (mg/l) was calculated by using the equation below:

Alkalinity = [(ml of HCl) (Molarity of HCl) (100.0g/mol)] / [(2) (ml of sample)]

3.3.1.8 Sulphates

One hundred millimeters (100ml) of the sample was filtered into a Nessler's tube containing 5ml of conditioning reagent. About 0.2g of barium chloride crystals was added with continued stirring. A working standard was prepared by taking 1ml of the standard H_2SO_4, 5ml of conditioning reagent and made up to 100ml, to give 100 NTU. The turbidity developed by the sample and the standards were measured using a UV Spectophotometer.

3.3.1.9 Phosphate

To 50ml of the filtered sample, 4ml of ammonium molybdate reagent and about 4-5 drops of stannous chloride reagent was added. After about 10 min to 12 min, the colour that developed was measured using UV spectrophotometer at 690nm and calibration curve was prepared. A blank sample was also prepared. The value of phosphate was obtained by comparing absorbance of sample with the standard curve and expressed as mg/L (APHA 1992, APHA/AWWA, WEF, 1995)

3.3.1.10 Nitrate

Nitrate was determined by uv-spectrophotometric method. 50ml of the sample was pipetted into a porcelain dish and evaporated to dryness on a hot water bath. 2ml of phenol disulphonic acid was added to dissolve the residue by constant stirring with a glass rod. Concentrated solution of sodium hydroxide and distilled water was added with constant stirring to make it alkaline. This was filtered into a Nessler's tube and made up to 50ml with distilled water. The absorbance was read at 410nm using a spectrophotometer after the development of colour. The value of nitrate was found by comparing absorbance of sample with the standard curve and expressed in mg/L. (APHA/AWWA, WEF, 1995)

3.3.1.11 Total petroleum hydrocarbon

Oil and grease was first determined by partition – gravimetrc method. Sample levels were marked on sample bottle at the water meniscus (to later determine sample volume). Sample was acidified with 1:1 HCl to pH 2 by adding 5 mL in 1 L sample. Sample was transferred to a separatory funnel and sample bottle was carefully rinsed with 30 mL 100% *n*-hexane and solvent washings were added to separatory funnel. The mixture was shaken vigorously for 2 min and layers were left separate. Aqueous layer was drained with small amount of organic layer into original sample container. Solvent layer was drained through a funnel containing a filter paper and 10 g Na_2SO_4, both of which have been solvent-rinsed, into a clean, distilling flask. Solvent layer with estimated >5mg emulsion, including draining emulsion and solvent layers were drained into a glass

centrifuge tube and centrifuge for 5 min at approximately 2400 rpm. Centrifuged material was then transferred to an appropriate separatory funnel and solvent layer was drained through a funnel with a filter paper and 10 g Na_2SO_4, both of which have been prerinsed, into a clean, distilling flask.

Aqueous layers were recombined with any remaining emulsion or solids in separatory funnel. With clear solvent, centrifuging process is not necessary. Extraction was done twice more with 30 mL solvent each time, but sample containers were first rinsed with each solvent portion. Centrifugation step was repeated if emulsion persisted in subsequent extraction steps. Extracts were combined in distilling flask, and a final rinsing of filter and Na_2SO_4 with an additional 10 to 20 mL solvent were included in flask. Solvent was then distilled in distillation flask with a distillation adapter equipped with a drip tip in a water bath at 85°C and solvent was collected in an ice-bath-cooled receiver. When visible solvent condensation stopped, flask was removed from water bath. Flasks were dried on cover water bath still on at 85°C, for 15 min. Air was drawn through flask using a suction pump for the final 1 min. Distillate was cooled in desiccator for at least 30 min and weighed.

Oil/grease in mg/L was calculated by: $\frac{A-B \times 1000}{\text{mL Sample}}$

TPH was then determined by redissolving the extracted oil and grease in 100 mL *n*-hexane. To 100 mL solvent, 3.0 g silica gel/100 mg total oil and grease was added, up to a total of 30.0 g silica gel (1000 mg total oil and grease). Container was stoppered and stirred on a magnetic stirrer for 5 min and solution was filtered through filter paper pre-moistened with solvent, silica gel and filter paper were washed with 10 mL solvent and combined with filtrate. Solvent was then distilled in distillation flask with a distillation adapter equipped with a drip tip in a water bath at 85°C and solvent was collected in an ice-bath-cooled receiver. When visible solvent condensation stopped, flask was removed from water bath. Flasks were dried on cover water bath still on at 85°C, for 15 min. Air was drawn through flask using a suction pump for the final 1 min. Distillate was cooled in desiccator for at least 30 min and weighed.

TPH in mg/L was calculated by: $\frac{A-B \times 1000}{\text{mL Sample}}$

Where A =Total gain in weight of distilling flask and

B = less calculated residue from solvent blank

3.3.2 Soil analysis

Soil samples (0-20cm deep) were collected in triplicates from eight locations around same loci of groundwater sites using soil auger. Samples were prepared based on the analysis to be done and the methods are described below;

3.3.2.1 Heavy Metal Analysis

The soil samples were air–dried, crushed and passed through a 2mm sieve. A portion (1g) of the soil sample was digested in a 2 M nitric acid. The heavy metals determined in the analysis were Cr, Cd, Pd, Zn, Cu, Mn, Ni and V. The concentrations of the metals in the digested soil sample solutions were determined with atomic absorption spectrometer (Schimazo model 2380) within three weeks. Each sample was analyzed in triplicate and the average of the results reported. Actual concentration was calculated with the formular below:

$$\text{AAS reading} = \frac{\text{Weight of sample in mg}}{\text{Volume in liters}}$$

3.3.2.2 pH

pH measurement was performed using Accumet pH Model 15 meter (Fisher Scientific). The pH meter was calibrated with standard buffer solutions of pH-4, pH-7 and pH-10. Between sample analyses, the electrode was rinsed with distilled water and gently blot-dried with tissue. Constant stirring of sample was performed to quickly reach a steady potential. Soil pH was measured using EPA Standard method No. 9045C (USEPA, 1987). It involved adding 20±0.1g of soil to 20 ml of distilled water and continuously stirring for 5 minutes. After allowing the soil suspension to stand for about 1 hour, most of the suspended clay was allowed to settle out from the suspension then pH measurement was taken on the aqueous phase. The glass electrode was immersed just deep enough into the clear supernatant solution to establish good electrical contact. All results are reported as "soil pH measured in water at 25^{o}C,

which is the temperature at which pH meter is calibrated. Three replicates per sample were done.

3.3.2.3 Conductivity

A 1:5, soil to water suspension ratio was prepared by weighing 10 g air-dried soil (<2 mm) into a bottle. 50 ml deionised water was added and the resulting solution mechanically shake at 15 rpm for 1 h to dissolve soluble salts. The conductivity meter was calibrated using the KCl reference solution to obtain the cell constant.

Before usage the cell was rinsed thoroughly with deionised water before the measurement of the electrical conductivity of the 0.01M KCl at the same temperature as the soil suspensions was done. The conductivity cell was then rinsed with the soil suspension before refilling the conductivity cell without disturbing the settled soil. The value indicated on the conductivity meter was recorded. The cell was rinsed with deionised water in-between sample analysis (Rayment and Higginson, 1992).

3.3.2.4 Sulphates

Ten grams (10 g) air-dried, sieved soil was placed into a 50 ml Erlenmeyer flask. A twenty five millimeters (25 ml) of monocalcium phosphate extracting solution was added and solution shaken at 200 rpm for 30 minutes. 0.25 g of charcoal was then added and shaken for an additional 3 minutes. The resulting solution was filtered through sulphate-free filter paper (Whatman No. 42) (Schulte and Elk, 1988). Ten millimeters (10 ml) of the filtrate was pipetted into a 50 ml Erlenmeyer flask, and 1 ml of acid "seed" solution added. The Erlenmeyer flask and solution were gently swirled before adding 0.5 g of $BaCl_2.2H_2O$ crystals.

The mixture was left to stand for one minute, and then placed on magnetic stirrer until the crystals were dissolved. A UV spectrophotometer was used to read the transmittance at a wavelength of 420 nm for samples and standard solutions. A plot of percent transmittance reading vs. concentration for standard curve was obtained from which sample concentration was determined (APHA, 1985).

mg SO^{4-} S/kg of soil = mg S /L x 0.025L / 0.010 kg soil = mg S/L x 2.5

Where S = Sample

3.3.2.5 Phosphate

Nearly all methods of determining Phosphate in soil involve extraction into a liquid phase. Deionized water is a commonly used extractant for Phosphate analysis (Potter, 1992). Soil samples were extracted by a deionized water extraction method with a soil: extractant ratio of 1:10. Samples were shaken in 50 ml conical tubes for 60 minutes at 180rpm on an orbital shaker. The tubes were centrifuged for 10 minutes at 2500rpm. After centrifugation, extracts were decanted and filtered. Filtered extracts were stored at room temperature for analysis.

Soil samples were analyzed by ascorbic acid colorimetry (Murphy and Riley 1962). To prepared samples, 4.0 ml Reagent B and 19.0 ml deionized water was added to 2.0 ml of each extract. Standards consisting of 5.0 ml of each standard Phosphate solution (0.1 ppm to 1.0 ppm P), 4.0 ml Reagent B, and 16.0 ml deionized water and a 0.0 ppm P standard consisting of 4.0 ml Reagent B and 21.0 ml deionized water were also prepared. Samples were allowed 30 minutes for colour development. The absorbance of the samples and standard solutions at 882 nm was measured with a Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

Reagent A: Mixture of 6g Ammonium molybdate, 0.146g Antimony potassium tartrate and 72ml Sulphuric acid all brought to 1 litre with deionized water.

Reagent B: Mixture of 1.584g/ l ascorbic acid and 300ml reagent A

3.3.2.6 Nitrate

Five grams (5 g) of air-dried, ground and sieved (2 mm) soil was placed into a 125 ml Erlenmeyer flask. 50 ml of 0.01 M $CaSO_4$ was then added. The solution was shaken for 15 minutes on a reciprocating shaker at 200 rpm. The resulting soil suspension was then filtered using Whatman No. 2 filter paper to provide a clear filtrate without contributing measurable amounts of NO_3^-–N to the filtrate. The extract was then measured colorimetrically using Cd reduction method (Keeney and Nelson, 1982).

3.3.2.7 Potassium

The filter of the flame photometer was set at 766.5 nm (marked for Potassium, K) and the flame was adjusted for blue colour. The scale was set to zero and maximum

using the highest standard value. A standard curve of different concentration was prepared by feeding the standard solutions. The sample was filtered using filter paper and filterate was fed into the flame photometer. The concentration was found as direct reading (Hussain *et al*., 2000).

3.3.2.8 Sodium

The filter of the flame photometer was set to 589nm (marked for Sodium, Na). By feeding distilled water the scale is set to zero and maximum using the standard of highest value. A standard curve between concentration and emission was prepared by feeding the standard solutions. The sample was filtered through filter paper and fed into the flame photometer and the concentration was found by direct readings (Hussain *et al*., 2000).

3.3.2.9 Calcium

5ml aliquot of soil extract was pipetted into 50ml white porcelain dish and diluted with distilled water to a volume of approximately 25ml. 2ml of 4 M NaOH and 2-3 mg of cal red indicator was then added. The resulting solution was titrated slowly with 0.01 M EDTA until a sky-blue end point was obtained. A blank titration was also done using 5ml distilled water.

$$\frac{1000 \text{ X (Vol. EDTA used for soil extract – Vol. EDTA for blank) X N of EDTA soln.}}{\text{Vol. sample taken (aliquot)}}$$

3.3.2.10 Total Organic Carbon and Total Organic Matter (TOC and TOM)

One gram (1g) of soil samples were crushed to pass through 2mm sieve after which they were weighed in duplicate and transferred to 250 cm^3 Erlenmeyer flasks. Exactly 10 cm^3 of 1 M potassium dichromate ($K_2Cr_2O_7$) was pipetted into each flask and swirled gently to disperse the soil followed by addition of 20 cm^3 of concentrated, sulphuric acid. The flask was swirled gently until soil and reagents were thoroughly mixed. The mixture was then allowed to stand for 30 minutes on a glass plate to allow for the oxidation of potassium dichromate to chromic acid. Distilled water (100 cm^3) was added followed by addition of 3-4 drops of ferroin indicator, after which the

mixture was titrated with 0.5M ferrous sulphate solution ($(NH_4)_2SO_4Fe$). A blank titration (without 1 g of soil) was similarly carried out. The % organic carbon is given by the following equation:

$$\%TOC = \frac{M_1e_1\ K_2Cr_2O_7 - M_2e_2FeSO_4 \times 0.0031 \times 100 \times F}{\text{Sample Weight (air dried soil)}}$$

F = Correction factor (1.33)
M_1 = mole of $K_2Cr_2O_7$
e_1 = volume of $K_2Cr_2O_7$
M_2 = mole of $FeSO_4$
e_2 = volume of $FeSO_4$

% Organic matter in soil = % Organic carbon x 1.729 (Bamgbose *et al*, 2000).

3.3.2.11 Total Extractable petroleum Hydrocarbon (TPH)

One gram (1.0 g) of each soil sample was put into a 500 mL volumetric flask and to this was added 200 mL of xylene. The xylene/soil mixture was shaken vigorously for five minutes and filtered into 400 mL cylinder. The volumetric flask and solid materials were rinsed properly with 500 mL xylene and filtered again into the cylinder. Total petroleum hydrocarbon content (TPH) in the xylene/hydrocarbon mixture was thereafter determined by photometric method using Electrophotometer at a wavelength of 425 nm. TPH was estimated from a calibration curve, obtained by measuring absorbance of a standard prepared in 2.5, 5.0, 10.0, 20.0, 25.0 and 30 (Osuji and Adesanya, 2005)

3.4 Field study on earthworm distribution and abundance

This investigation was carried out through two years (April, 2007 – March, 2009). Based on nearness to point of pollution and impact of inferno, samples were collected 100 m within epicenter of spill and 500 m away from spill. Twenty topsoil samples (0.5mx0.5mx0.2m) were collected randomly from the epicenter of the spill and 500 meters away from the spill for earthworms. Earthworms were collected by hand sorting method into plastic bottles containing Formoacetoalcohol (FAA), (Owa *et al*, 2004). It was ensured that whole earthworms including adults and juveniles earthworms were collected (ISO Protocol, 2005) and were then taken to laboratory for onward enumeration and identification. Sampling was done monthly through seasons

so as to monitor the effect of seasonal variation on earthworm abundance and distribution.

Earthworms for each quadrat throw were first sorted out into species and based on maturity (adults and juveniles), they were then enumerated and recorded. The earthworms were identified following the original descriptions (Beddard 1891; Rosa 1896, Sims 1971) with the assistance of the earthworm taxonomist, Prof. S.O. Owa of Olabisi Onabanjo University, Ago-Iwoye.

3.5 Determination of levels of contaminants in earthworm species

The concentration of metals in the abundant earthworm species, were carried out. Samples of the earthworm species were collected and kept in the freezer at 4^{o}C prior to heavy metal analyses. Heavy metals (Mn, Cu, Zn, Pb, Cd, Cr, V and Ni) concentrations were determined using atomic absorption spectrophotometry. 3g of thawed earthworm samples were weighed and digested with 2ml concentrated nitric acid and heated to dryness on a hotplate. The digest was redissolved in 1ml concentrated nitric acid after which it was made up to 50ml with distilled water. Heavy metal analysis was done with atomic absorption spectrophotometer (Schimazo model 2380), (Bamgbose *et al*, 2000).

3.6 Sensitivity test

3.6.1 Test animal and soil preparation

Similar indigenous earthworm species in Agaye soil were collected by hand picking from a garden soil in Ring road, Ibadan. The earthworms were collected from the same site in order to reduce variability in biotype. Earthworms were brought into the laboratory in plastic buckets containing soil collected from the same garden soil and supplemented with half-boiled, ground water-leaf (*Talinium triangulare*) (Fafioye and Owa, 2000) and then moistened with distilled water. They were left to acclimatize for 7 days. Subsequently, earthworm were fed with leaflets of lettuce *Nymphea lotus*, 3g per 10 worms every 4days throughout the period of all studies carried out (Otitoloju, 2005). Adult earthworms were picked and used for the sensitivity tests in order to determine the earthworms' tolerance to the contaminated soil.

Soil preparation was done according the method adopted by Otitoloju (2005) with slight modifications. Loamy soil sourced from the same garden was air-dried, ground and sifted through a 0.30mm (mesh size) screen so as to standardize the grain size. One kilogram (1kg) portion of the prepared soil moistened with 5ml of distilled water was prepared and put into Plastic vessel with size 20cm in diameter (base) by 35cm height and 35cm in diameter (top), the bottom was perforated while the top was covered with mesh and made to stay with elastic bands. This soil was used as control and designated NS. 1kg of fresh soil from Agaye community was also prepared as described above and designated CS.

A survival and avoidance test for the two most abundant earthworms in soil sample from Agaye was carried out in October, 2008 and repeated in October, 2009.

3.6.2 Fourty – eight hours (48h) Avoidance Test

A two chambered test system was used (Schaefer, 2001). 1 kg of contaminated soil (CS) from Agaye settlement was applied to one chamber of the system and another 1 kg of non-contaminated soil (NS) was then applied to the second chamber of each system; the two chambers were entirely separated so as to prevent mixing up of both soil types. The separator was then removed thereby creating a groove in between both soil types; this was done to allow free movement of earthworms between the two chambers. Ten earthworms each were added to the groove of each set-up. Each chamber was covered with mesh and made to stay with the aid of elastic band and left for 48hrs. Prior to counting, the separator was put back in place (in groove), each soil type removed separately and the number of earthworms in each chamber was counted after 48hrs and recorded.

3.6.3 Seven days Survival Test

Ten earthworms were applied to each set – up described above (i.e. CS and NS in a plastic bucket) and this was done in triplicate. Set-up was arranged in a randomized block design. The vessels were thereafter covered with meshes which were kept in place by elastic band. The number of surviving earthworms in both soils was observed and recorded 1day, 2days, 5days and 7-day period.

Based on the result from the sensitivity test, an ex-situ bioremediation was done in 2009.

3.7 Experimental set-up for bioremediation study

The bioremediation study was carried out to monitor the TPH and heavy metals (Pb, Mn, Zn, Ni, Cd, Cr, V and Cu) levels in the soil over a 12 weeks period. It was carried out to test the effectiveness of the most abundant indigenous earthworm in remediation. The experiment was set up according to the description by Hickman and Reid (2008) with slight modifications. Contaminated soil from Agaye (CS) and non-contaminated soil samples (NS) were collected, macerated and sieved with mesh of size 5mm and mixed thoroughly. The soils were distributed into pots (30cm diameter x 45cm height) in triplicates and arranged in the plot design (Table 3.3 and Fig. 3.2).

Plastic piping (5mm diameter) was inserted into the soil and three holes were drilled in the base of the pots, this was to aerate the treatments for 15mins every 24hrs. Further manual stirring of set up was not done so as to ensure aeration to be potentially due to the presence of earthworm activities (burrowing and bioturbation). Before soils were applied into pots, washed gravel 3cm thick were laid in the base of each pot (to promote uniform air dissipation). The gravel was then covered with plastic mosquito net cut to size that allowed air to percolate up from the base and prevent the migration of earthworms into gravel (Zachery and Reid, 2008a).

3.7.1 Earthworm application

Soil was rehydrated and manually mixed into the plastic pots. Mass of contaminated soil (70% maximum water holding content - WHC) was kept constant at 2kg wet weight/vessel. Adult earthworms were left to acclimatize for one week and allowed to depurate for 24h prior to use. Five earthworms per kg of material were added to each of the required treatments and quadruplicates. The earthworms were applied and the surfaces of the vessels were covered with plastic mesh. Water was added periodically to maintain levels at 70% maximum WHC. The following were monitored and data collected over the period. Heavy metals (Pb, Mn, Zn, Ni, Cd, Cr, V and Cu) and Total Petroleum Hydrocarbon (TPH) were analysed as scheduled in Table 3.4.

Table 3.3: Experimental set-up for bioremediation study

Row 1	Row 2	Row 3
CS+E	CS	NS
NS	CS+E	CS
CS	NS	CS+E

Key: Contaminated Soil (CS), Earthworm (E) , Non-contamianted Soil and Earthworm (NSE)and Control (NS)

Fig.3.2 Picture of **experimental set-up for bioremediation study**

Table 3.4: A modified Small-Scale Bioremediation Sampling Plan

Sampling period	Analysis done
Pretreatment Sampling	**TPH [1] and Heavy metals[2]**
after 6 weeks	**TPH and Heavy metals**
End of Season – 12 weeks	**TPH and Heavy metals**

Source: New York State Dept of Environment Conservation Protocol (1996)

(1) TPH analysis was performed using gravimetric method.

(2) Indicator compounds analyzed were Cr, Cd, Pd, Zn, Cu, Mn, Ni and V using Atomic Absorption Spectrophotometer (AAS)

* The recommended protocol for bioremediation study requires carrying out microbial counts and Nutrient analyses (N,P and K). However, it was carried out in this study

3.8 Statistical Analysis

Earthworm diversity and abundance were analysed using Shannon Weiner (H) and Mann-Whitney respectively while data on physicochemical parameters were subjected to correlation, student t-test and ANOVA at $p \leq 0.05$ using spss version 16.0. Results were presented in means and standard deviation.

CHAPTER FOUR

4.0 RESULTS

4.1 Heavy metal concentrations in water samples

The monthly mean concentration of V, Ni, Mn, Cu, Zn, Pb, Cd and Cr in ground water samples (GW1 – GW5) between June, 2007 and April, 2009 is shown in Fig. 4.1. Chromium, cadmium, and lead showed reduction over the period of study whereas zinc, copper, manganese, nickel and vanadium increased over the period of study. The result showed that Zinc concentration ranged from 0.54–0.99mg/ and Ni concentrations ranged from 0.16-1.59mg/l. The concentrations of Chromium, Cadmium and Lead were lowest having concentrations < 0.2mg/l.

The mean concentrations of V, Ni, Mn, Cu, Zn, Pb, Cd and Cr in surface water samples (SW_1 and SW_2) showed that concentrations of most heavy metals were highest in the months of June and August (rainy season) and lowest between December and February (dry season). Manganese, Copper and Zinc had maximum concentrations of 9mg/l, 6.2mg/l and 6.1mg/l respectively while Lead, Cadmium and Chromium showed the lowest concentrations of <1mg/l (Fig. 4.2).

The mean concentrations of heavy metals in all water samples compared with WHO/NESREA standards are shown in Table 4.1. Cd, Pb, and Ni of well water samples had concentrations higher than the WHO/NESREA recommended limit for drinking water; also Cd, Cu and Ni of surface water sample were higher than the WHO/NESREA permissible limit for surface water. Mean concentrations of heavy metals (V, Ni, Mn, Cu, Zn, Pb, Cd and Cr) concentration in water samples from each sampling point are shown in Table 4.2.

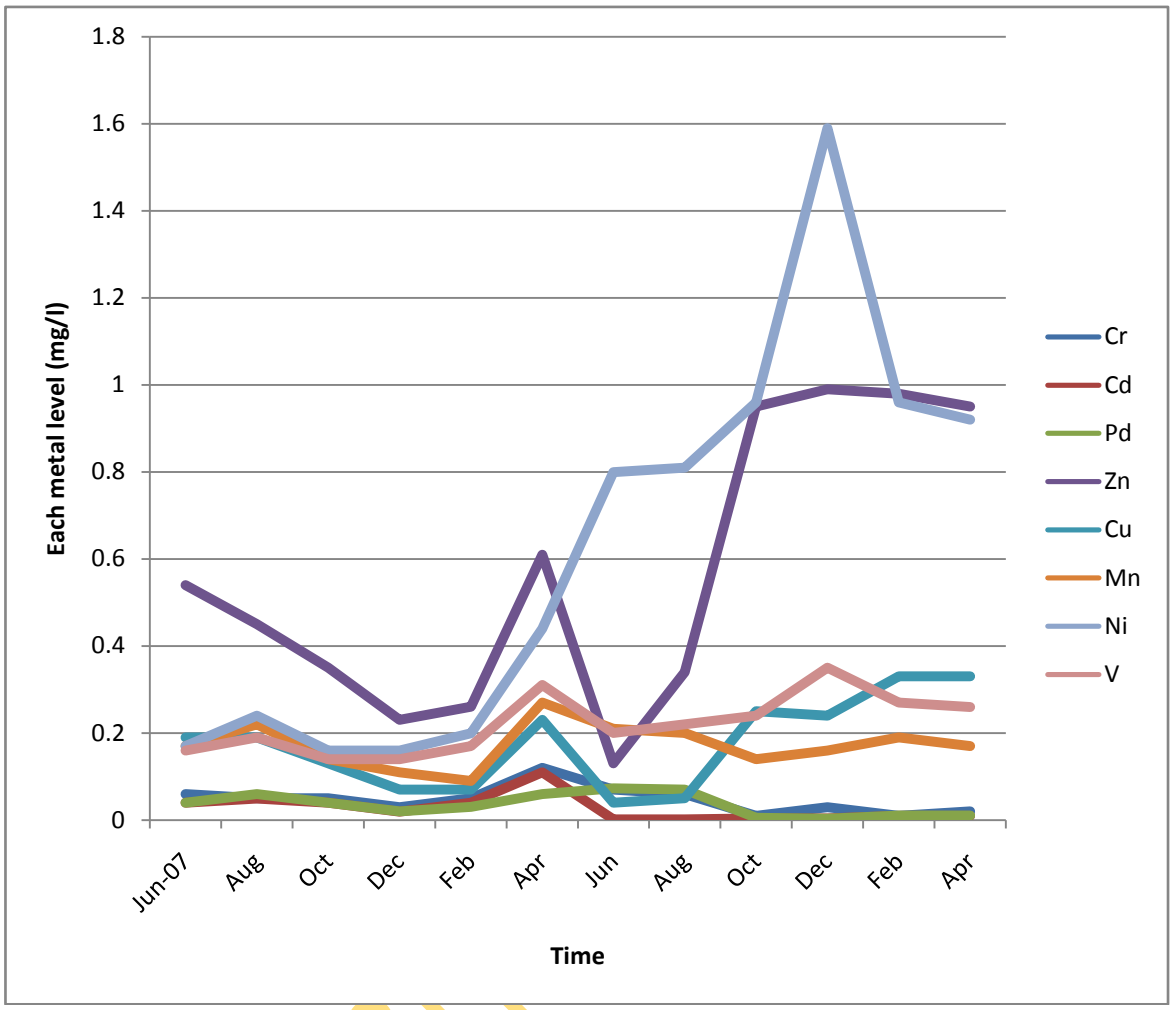


Fig 4.1 Temporal variation of the heavy metal profile for ground water (GW1 and GW5)

Keys:
Cd=Cadmium
Pb=Lead
Zn=Zinc
Cu=Copper
Mn=Manganese
Ni=Nickel
V=Vanadium

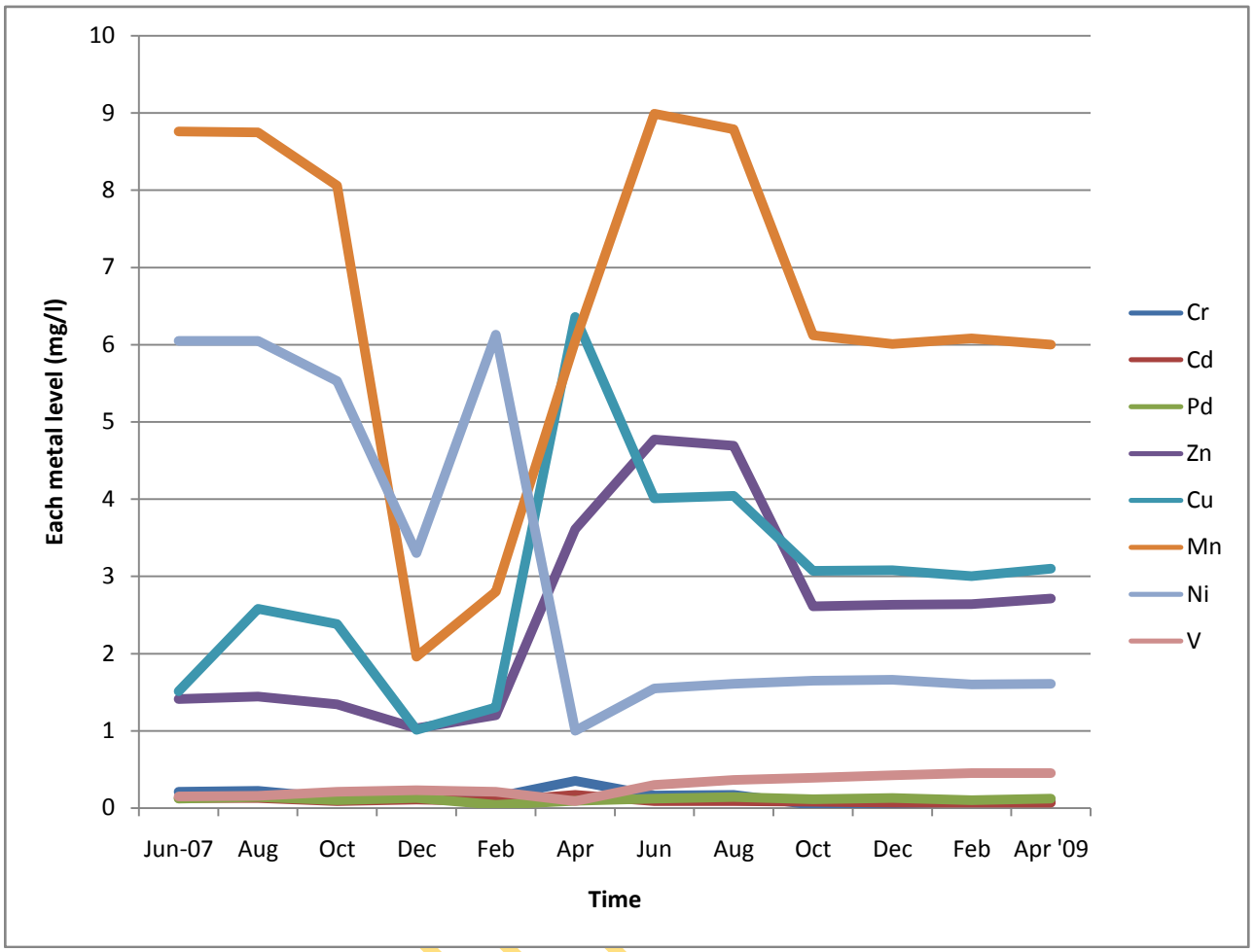


Fig. 4.2 Temporal variation of the heavy metal profile for surface water (SW1 and SW2)

Keys:
Cd=Cadmium
Pb=Lead
Zn=Zinc
Cu=Copper
Mn=Manganese
Ni=Nickel
V=Vanadium

Table 4.1: Mean concentrations of heavy metals in water samples compared with WHO/NESREA standards

Metals	Well water	Well water Ctrl ± SD (mg/l)	WHO/NESREA drinking/ standard (mg/l)	SW ± SD (mg/l)	WHO/NESREA Surface water standard (mg/l)
Cr	0.04±0.03	0.04±0.03	0.05/0.5	0.04 ± 0.03	-/ 0.5
Cd	0.05±0.04	0.03 ± 0.03	0.003/0.001	0.03 ± 0.03	<0.0004 /0.005
Pb	0.04±0.04	0.03 ± 0.03	0.01/0.2	0.05 ± 0.05	- / 0.1
Zn	0.79±0.67	0.59 ± 0.41	3.0/3.0	0.88 ± 1.03	<10 µg/litre / 0.02
Cu	0.22±0.17	0.17 ± 0.08	1.00 /1.00	0.23 ± 0.24	0.036/ 0.001
Mn	0.19±0.07	0.19 ± 0.07	0.4/0.2	0.15 ± 0.07	- / 40
Ni	0.98±0.81	0.26 ± 0.24	0.01/0.1	2.18 ± 0.44	<0.1
V	0.22±0.13	0.12 ± 0.08	-	0.26 ± 0.05	-

Keys:
Cd=Cadmium
Pb=Lead
Zn=Zinc
Cu=Copper
Mn=Manganese
Ni=Nickel
V=Vanadium

Table 4.2: Monthly mean concentrations of heavy metals in water samples from each sampling points

CODE	Cr (mg/l)	Cd (mg/l)	Pd (mg/l)	Zn (mg/l)	Cu (mg/l)	Mn (mg/l)	Ni (mg/l)	V (mg/l)
GW1	0.04±0.03	0.03±0.04	0.03±0.03	0.67±0.36	0.21±0.14	0.18±0.07	0.62±0.36	0.20±0.04
GW2	0.03±0.02	0.02±0.04	0.02±0.01	0.65±0.39	0.21±0.11	0.16±0.07	0.51±0.39	0.17±0.07
GW3	0.05±0.05	0.03±0.03	0.03±0.03	0.51±0.26	0.17±0.11	0.18±0.07	0.29±0.14	0.22±0.22
GW4	0.02±0.01	0.03±0.01	0.06±0.06	0.17±0.08	0.13±0.06	0.20±0.15	0.13±0.24	0.18±0.03
GW5	0.05±0.02	0.03±0.03	0.04±0.03	0.54±0.34	0.15±0.09	0.17±0.02	0.83±0.62	0.25±0.09
GW6	0.04±0.03	0.03±0.03	0.03±0.03	0.59±0.41	0.17±0.08	0.19±0.07	0.26±0.24	0.12±0.05
SW1	0.04±0.03	0.04±0.01	0.05±0.03	0.30±0.10	0.10±0.02	0.11±0.33	1.81±0.23	0.26±0.04
SW2	0.04±0.03	0.03±0.03	0.05±0.06	1.08±1.13	0.28±0.26	0.17±0.07	2.30±0.43	0.26±0.05
WHO/NESREA drinking standard	0.05/0.5	0.003/0.001	0.01/0.2	3.0/3.0	1.00 /1.00	0.4/0.2	0.01/0.1	-
WHO/NESREA Surface water standard	-/ 0.5	<0.0004 /0.005	-/ 0.1	<10µg/ litre / 0.02	0.036/ 0.001	- / 40	<0.1	-

Keys:

GW1 = Well 1 GW2 = Well 2 GW3 = Well 3 GW4 = Well in school premises GW5 = Borehole in school premises GW6=Control SW1=Surface water 1 SW2= Surface water2

4.2 Concentration of sulphate, nitrate, phosphate and TPH in water samples over study period

The mean concentrations of sulphate, nitrate, phosphate, pH, temperature, TDS, TH, alkalinity, conductivity and chlorine are shown in table 4.3 while the monthly variation of sulphate, nitrate and phosphate in water samples are presented in fig. 4.3. The Mean concentrations of sulphate, nitrate, phosphate in water samples compared with WHO/NESREA standard are shown in Table 4.4. All concentrations for well water samples fell within the WHO/NESREA standard for drinking water. The means of physicochemical parameters (pH, Temperature, Total Dissolved Solids, Total hardness, Alkalinity, Conductivity and Chlorine) in water samples compared with WHO/NESREA standards are presented in Table 4.5. In well water samples, pH fell below the WHO/NESREA recommended limit while temperature was above the WHO/NESREA limit for drinking water; all other parameters were within the recommended WHO/NESREA limit for drinking water.

Table 4.3 Monthly mean concentrations of sulphate, nitrate, phosphate, pH, Temperature, Total Hardness, Alkalinity and Conductivity in water samples

Stations	**Sulphate (mg/l)**	**Nitrate (mg/l)**	**Phosphate (mg/l)**	**pH**	**Temperature (0^c)**	**TDS (mg/l)**	**TH (mg/l)**	**Alkalinity**	**Conductivity**
GW1	0.11±0.04	0.11±0.04	0.1±0.05	5.71±0.35	26.85±1.33	241.37±388.20	118.96±68.80	13.37±6.35	32.41±18.30
GW2	0.09±0.03	0.13±0.05	0.1±0.05	5.98±0.9	26.95±1.67	274.15±403.37	137.05±75.25	10.94±5.78	43.09±36.21
GW3	0.11±0.05	0.09±0.04	0.12±0.04	6.45±0.29	27.06±1.37	218.61±91.11	132.01±85.93	15.12±10.37	39.74±13.39
GW4	0.05±0.01	0.04±0.01	0.07±0.09	5.46±0.38	25.63±0.95	86.27±49.78	91.25±86.23	3.37±2.20	57.91±22.38
GW5	0.1±0.05	0.1±0.06	0.14±0.09	5.81±0.6	26.71±1.42	251.53±466.86	115.71±86.25	9.93±7.27	56.89±20.63
CW6	0.16±0.04	0.18±0.06	0.09±0.04	6.10±0.69	27.09±1.63	256.92±249.30	133.97±103.01	6.96±5.77	17.49±3.89
SW1	0.06±0.02	0.03±0.01	0.07±0.02	5.43±0.24	26.50±1.00	61.86±47.51	109.70±47.87	3.07±2.51	90.56±112.98
SW2	0.11±0.04	0.08±0.05	0.11±0.05	5.23±0.43	26.47±2.00	158.92±168.92	127.93±54.36	4.19±3.16	36.33±19.59

Keys:

GW1 = Well 1 GW2 = Well 2 GW3 = Well 3 GW4 = Well in school premises GW5 = Borehole in school premises GW6=Control SW1=Surface water 1 SW2= Surface water2

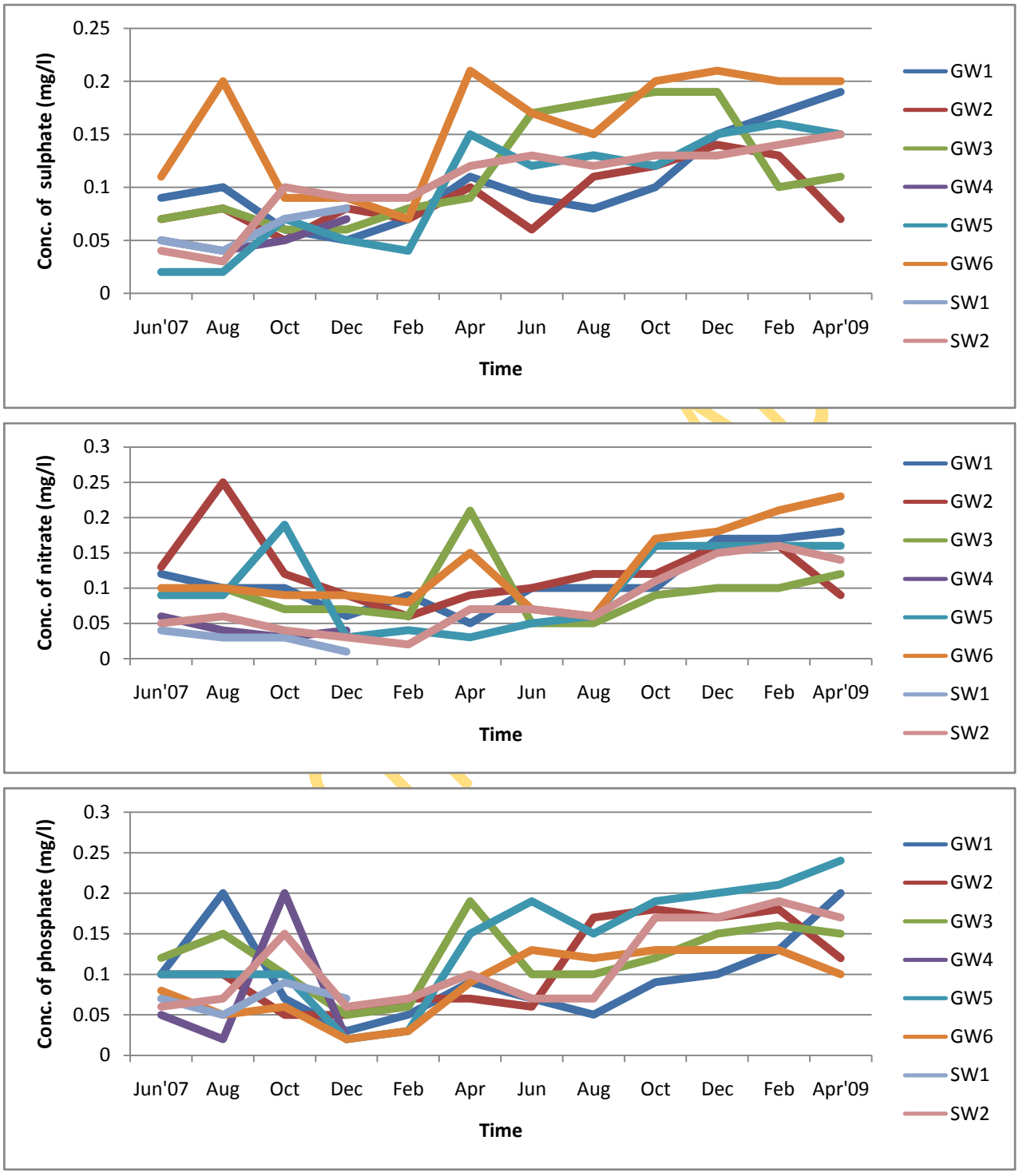


Fig. 4.3 Mean monthly variation of sulphate, nitrate and phosphate in water samples

Keys for sampling points:

GW1 = Well 1, GW2 = Well 2 GW3 = Well 3
GW4 = Well in school premises GW6=Control SW1 = Wet land 1
SW2= Wetland

Table 4.4 Mean concentrations of sulphate, nitrate, phosphate in water samples compared with WHO/NESREA standard

Parameter	Well water	Well water Ctrl ± SD	WHO/NESREA drinking water standard (mg/L)	Wet Land ± SD
SO_4^- (mg/l)	0.13± 0.04	0.16 ± 0.05	250 /200	0.09 ±0.04
NO_3^- (mg/l)	0.11± 0.05	0.13 ± 0.06	20/45	0.07 ±0.005
PO_4^- (mg/l)	0.13±0.05	0.09 ± 0.09	<5/-	0.10 ±0.005
TPH (mg/l)	1.34±0.64	0.64±0.26	- /-	3.30±0.54

Keys: SO_4^- = Sulphate,
NO_3^- = Nitrate,
PO_4^- = Phosphate and
TPH =Total petroleum hydrocarbon

Table 4.5 Mean concentrations of other physicochemical parameters in water samples compared with WHO/NESREA standards

Parameter	Well water	Well water (Ctrl)	WHO/NESREA drinking water standard	Wet land
pH	5.88±0.63	6.10 ± 0.69	6.50-9.50	5.28 ± 1.78
Temp (^{0}C)	27.1±1.76	27.09 ± 1.63	25.00	26.48 ± 1.78
TDS (mg/l)	195±157	256.43 ± 249.30	500	134.65 ±152. 51
TH (mg/l)	141±83.5	133.97 ± 103.01	-	123.38 ± 51.88
Alk.	11.2±7.94	6.96 ± 5.77	-	3.89 ± 3.08
Cond. (ohms)	58.1± 80.86	42.78 ± 48.56	-	56.90 ± 73.23
Cl^- (mg/l)	33.4±14.6	17.49 ± 3.89	<250	49.89 ± 58.50

Key: Temp= Temperature
TDS= Total dissolved solute
TH= Total Hardness
Cond= Conductivity
Cl = Chlorine
Alk= Alkalinity

4.3 Heavy metal concentration in soil samples

The mean concentrations of heavy metals (V, Ni, Mn, Cu, Zn, Pb, Cd and Cr) in soils from sampling points are shown in Fig 4.4 Manganese had the highest concentration recorded during the rainy season. The mean concentrations of heavy metals in soils from wetland showed that most were highest in month of August for both years of study and lowest between December and February. Manganese and Nickel recorded the highest concentration with a maximum concentration of 8.99 mg/l and 6.05 mg/l respectively while Chromium, Cadmium and Lead, showed the lowest concentration of 0.35 mg/l, 0.17 mg/l and 0.14 mg/l recorded during this study (Fig 4.5). The monthly heavy metal (V, Ni, Mn, Cu, Zn, Pb, Cd and Cr) levels in soils for each sampling point are shown in Table 4.6. The mean concentrations of heavy metals in soil samples compared with control soil are shown in Table 4.7. All heavy metal concentrations except Cr were lower than the control sample.

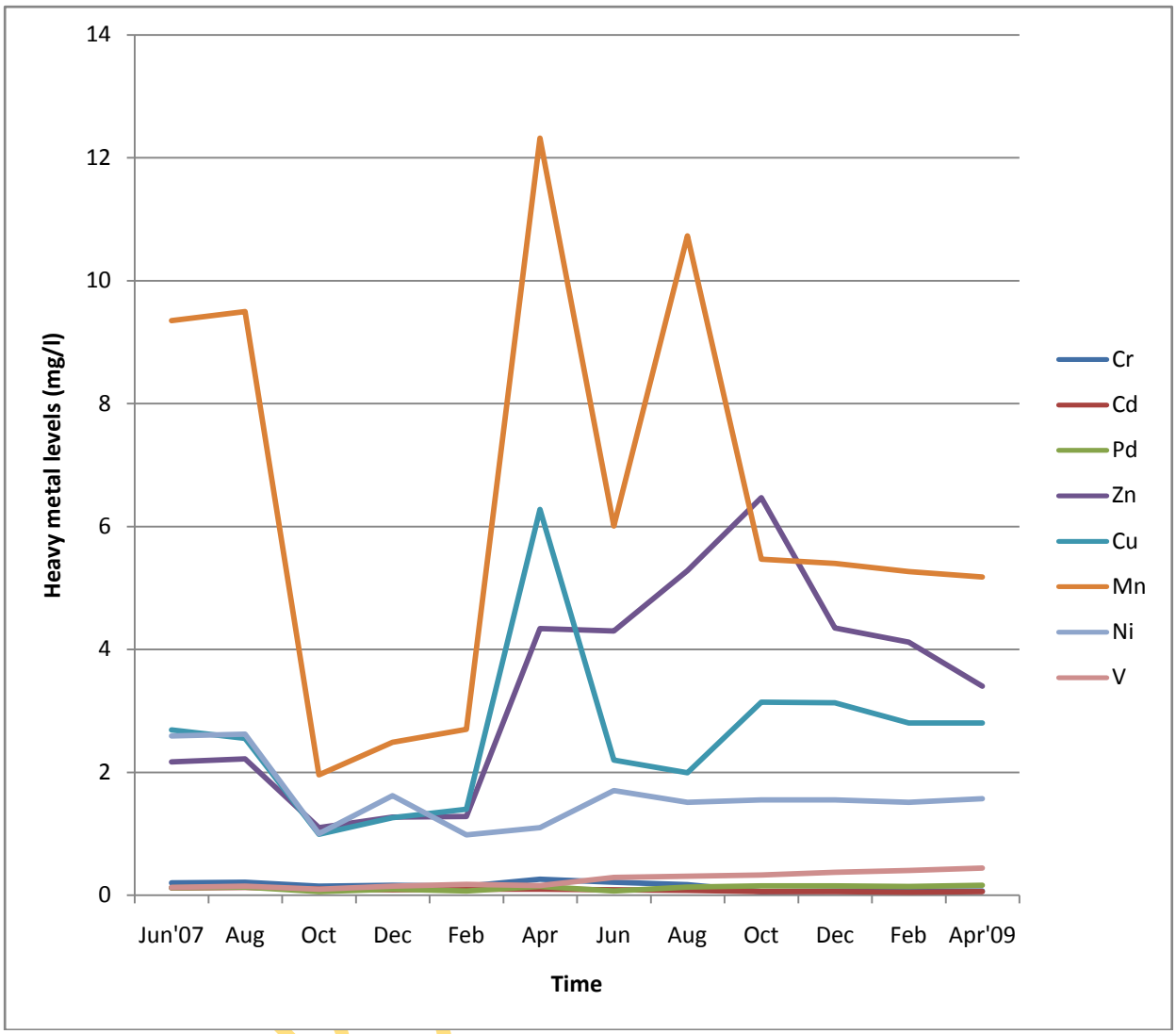


Fig.4.4 Mean concentrations of heavy metals in soil samples between June 2007 and April 2009.

Keys:
Cd=Cadmium
Pb=Lead
Zn=Zinc
Cu=Copper
Mn=Manganese
Ni=Nickel
V=Vanadium

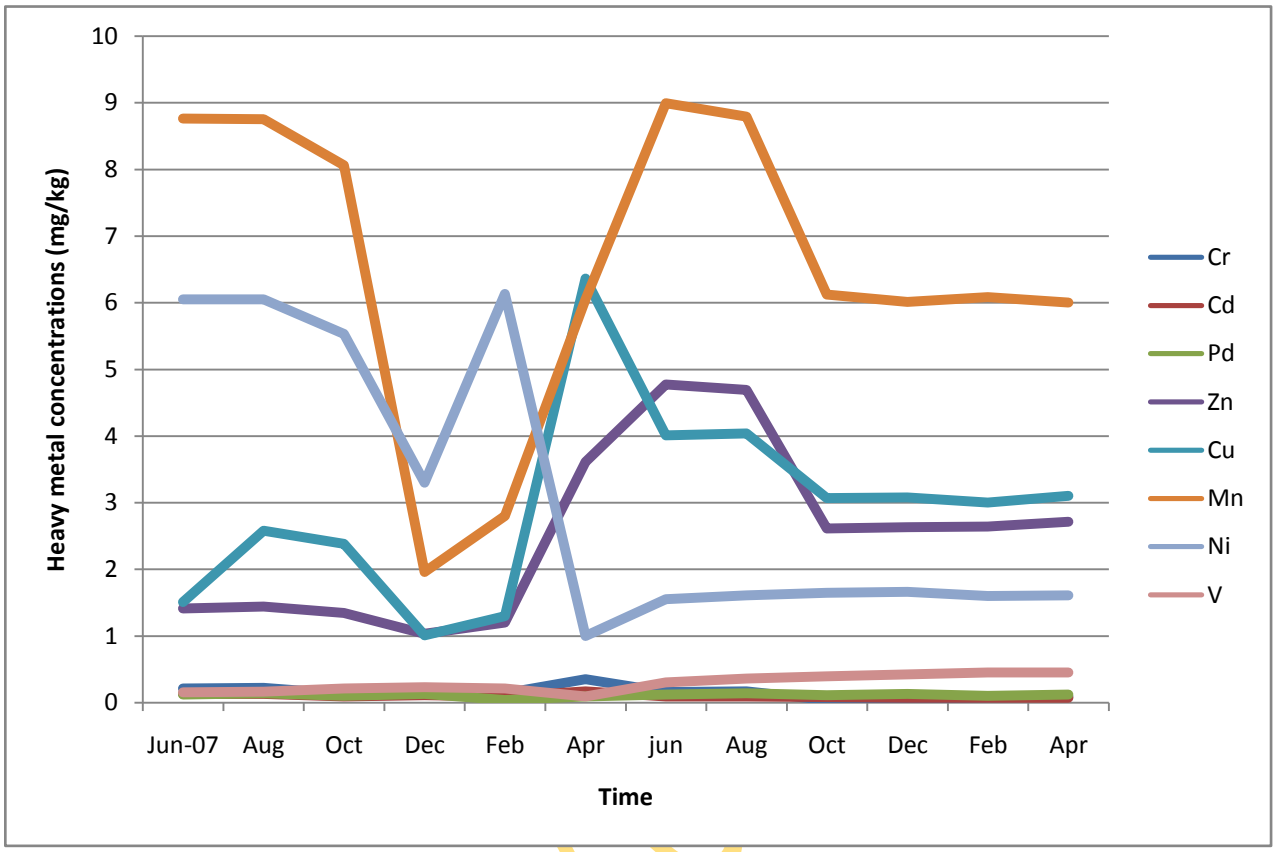


Fig.4.5 Mean concentrations of heavy metal in soils from wetland between June 2007 and April 2009.

Keys:
Cd=Cadmium
Pb=Lead
Zn=Zinc
Cu=Copper
Mn=Manganese
Ni=Nickel
V=Vanadium

Tab. 4.6 Mean concentrations of heavy metals in soil samples from each sampling points

Stations	Cr (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	V (mg/kg)
S1	0.15±0.06	0.08±0.04	0.1±0.04	5.25±4.83	2.81±1.69	4.29±2.02	1.53±0.43	0.26±0.17
S2	0.15±0.09	0.07±0.04	0.13±0.02	3.70±1.66	2.69±2.83	10.07±4.64	1.18±0.1	0.24±0.08
S3	0.15±0.1	0.1±0.04	0.15±0.07	2.70±1.50	2.81±1.44	8.18±4.30	0.92±0.43	0.22±0.10
S4	0.13±0.6	0.1±0.05	0.12±0.02	2.73±1.41	2.84±1.53	7.68±4.06	1.42±0.35	0.27±0.14
S5	0.14±0.07	0.08±0.03	0.14±0.04	2.85±1.71	2.20±1.52	5.64±2.91	3.11±2.54	0.26±0.11
S6	0.14±0.14	0.12±0.05	0.12±0.06	2.70±1.36	1.76±2.03	6.36±4.13	2.74v2.37	0.24±0.06
Sv1	0.13±0.08	0.08±0.04	0.11±0.04	2.90±1.45	1.95±1.69	5.44±2.29	2.18±1.90	0.29±0.17
Sv2	0.16±0.12	0.1±0.05	0.1±0.05	2.66±1.35	2.08±1.80	8.16±3.20	1.91±1.70	0.25±0.1

Keys for sampling points:

S1= Soil sampled within loci of well 1
S2= Soil sampled within loci of well 2
S3 = Soil sampled within loci of well 3
S4 =Soil sampled within loci of well in school
S5=Soil sampled within loci of bor hole
S6 =Soil sampled within loci of well 6
Sv1 = Soil sampled within loci of wetland 1
Sv2 =Soil sampled within loci of wetland 2

Keys for metals:

Cd=Cadmium,
Pb=Lead,
Zn=Zinc,
Cu=Copper,
Mn=Manganese,
Ni=Nickel
V=Vanadium

Table 4.7: Mean concentrations of heavy metals and TPH in soil samples compared with control (June 2007 – April 2009)

Metals	Soil samples (S1-S5)	Wet land samples (SW_1 and SW_2)	Control samples (S6)	P value	Significance
Cr	0.14±0.07	1.4±0.08	0.14±0.08	0.73	>0.05
Cd	0.09±0.04	0.1±0.04	0.1±0.45	0.25	>0.05
Pb	0.13±0.04	0.11±0.05	0.14±0.08	0.4	>0.05
Zn	3.45±2.69	2.80±1.38	2.66±1.35	0.35	>0.05
Cu	2.68±1.83	1.86±1.83	2.08±1.8	0.15	>0.05
Mn	7.2±4.14	5.9±3.3	8.16±3.2	0.21	>0.05
Ni	1.61±1.33	2.46±2.11	1.91±1.7	0.1	>0.05
V	0.25±0.12	0.26±0.13	0.25±0.1	0.91	>0.05
TPH	1.33±0.5	2.96±0.56	0.67±0.31	0.00	<0.05

4.4 Other physicochemical parameters in soil samples

Table 4.8 shows the mean concentrations of the mean concentrations of sulphate, nitrate, phosphate, pH, Potassium, Sodium, Calcium, T0C, TOM and TPH in soil samples, the mean TPH level ranged between 1.17 – 2.03 mg/g, TOC ranged between 0.95 – 1.36 mg/g and TOM ranged between 1.64 - 2.34 mg/g.

4.5 Soil / Weather conditions of the study area

Soil temperature ranged from 21.3^{O}C – 28.5^{O}C while the atmospheric temperature ranged from 23 ^{O}C – 33 ^{O}C. The soil moisture content at 15cm – 20cm depth ranged between 15.0 – 65.0 percent in the study area during the study period (Table 4.9). The soil temperature, soil moisture and rainfall showed seasonal fluctuations.

Rainfall measurement used in this study was adapted from Nigeria meteorological agency (NIMET) weather records. There were two major seasons; the rainy season, with the heaviest rainfall from April to July which continued in October and November. There was a brief relatively dry spell in August and September and a longer dry season from December to March. The highest and lowest rainfall of 442.7mm and 0.8mm were recorded during July 2008 and Jan, 2008 respectively in the study area (Table 4.10).

Table 4.8: Monthly mean concentrations of Total Organic Carbon, Total Organic Matter, Total Petroleum Hydrocarbon, sulphate, nitrate and phosphate$^-$ in soil samples (June, 2007 – Apr 2009)

	SO_4- (mg/kg)	NO_3- (mg/kg)	PO_4- (mg/kg)	TOC (mg/kg)	TOM (mg/kg)	TPH (mg/kg)
Jun	0.12±0.05	0.15±0.03	0.12±0.03	1.36±0.75	2.34±1.29	2.03±1.2
Aug	0.14±0.05	0.15±0.03	0.11±0.05	1.24±0.61	2.15±1.05	1.98±1.15
Oct	0.12±0.04	0.1±0.04	0.12±0.09	1.16±0.59	1.99±1.03	1.92±1.08
Dec	0.08±0.02	0.08±0.03	0.06±0.03	1.13±0.53	1.94±0.95	1.87±1.06
Feb	0.09±0.05	0.08±0.04	0.07±0.04	0.97±0.43	1.68±0.75	1.69±0.97
Apr	0.12±0.04	0.1±0.02	0.1±0.04	0.95±0.43	1.63±0.75	1.61±0.91
Jun	0.23±0,02	0.21±0.05	0.59±0.13	2.25±0.67	3.87±1.15	1.59±0.9
Aug	0.2±0.06	0.19±0.07	0.32±0.12	2.05±0.77	3.53±1.32	1.46±0.82
Oct	0.23±0.05	0.21±0.09	0.19±0.01	1.87±0.45	3.23±0.77	1.42±0.84
Dec	0.25±0.07	0.2±0.07	0.21±0.04	1.33±0.4	2.29±0.7	1.31±0.83
Feb	0.2±0.13	0.14±0.11	0.07±0.06	1.01±0.21	1.74±0.37	1.24±0.81
Apr	0.21±0.13	0.14±0.11	0.07±0.05	0.95±0.24	1.64±0.41	1.17±0.79

TOC = Total Organic Carbon,

TOM = Total Organic Matter

TPH = Total Petroleum Hydrocarbon

Table 4.9: The mean concentrations of sulphate, nitrate, phosphate, pH, Potassium, Sodium, Calcium, T0C, TOM and THC in soil samples

Stations	**Sulphate (mg/kg)**	**Nitrate (mg/kg)**	**Phosphate (mg/kg)**	**pH**	**Potassium (mg/kg)**	**Sodium (mg/kg)**	**Calcium (mg/kg)**	**TOC (mg/kg)**	**TOM (mg/kg)**	**THC (mg/kg)**
S1	0.14±0.07	0.1±0.05	0.14±0.14	6.05±0.32	0.09±0.01	0.04±0.01	2.10±0.16	1.01±0.29	1.76±0.49	1.11±0.19
S2	0.21±0.11	0.17±0.06	0.18±0.05	6.70±0.58	0.09±0.01	0.04±0.01	2.14±0.08	1.02±0.20	1.77±0.35	1.20±0.18
S3	0.16±0.1	0.18±0.1	0.16±0.05	6.30±0.56	0.09±0.01	0.04±0.01	2.14±0.09	1.15±0.49	1.99±0.84	0.89±0.23
S4	0.17±0.05	0.11±0.05	0.17±0.05	5.52±0.38	0.09±0.01	0.04±0.01	2.31±0.18	1.40±0.71	2.45±1.28	2.08±0.32
S5	0.18±0.05	0.15±0.04	0.08±0.02	6.08±0.67	0.09±0.01	0.07±0.09	2.24±0.12	1.43±0.60	2.64±0.99	1.38±0.42
S6	0.13±0.05	0.08±0.02	0.18±0.05	6.01±0.70	0.08±0.02	0.04±0.01	2.24±0.18	1.77±0.76	3.05±1.31	3.03±0.40
Sv1	0.15±9.11	0.08±0.02	0.12±0.03	5.71±0.57	0.09±0.02	0.04±0.01	2.20±0.17	1.37±0.77	2.20±1.32	2.90±0.70
Sv2	0.17±0.08	0.09±0.03	0.2±0.06	5.82±0.46	0.08±0.02	0.05±0.01	2.07±0.13	1.64±0.77	2.82±1.32	0.67±0.31

Keys:

S1 =Soil sample around GW1 S2 = Soil sample around GW2 S3 = Soil sample around GW3 S4 = Soil sample around GW4 S5 = Soil sample around GW5 Sv1= Soil sample around GW6 Sv2= Soil sample around SW2

Table 4.10: Monthly average rainfalls, temperatures and Relative humidity in Lagos area

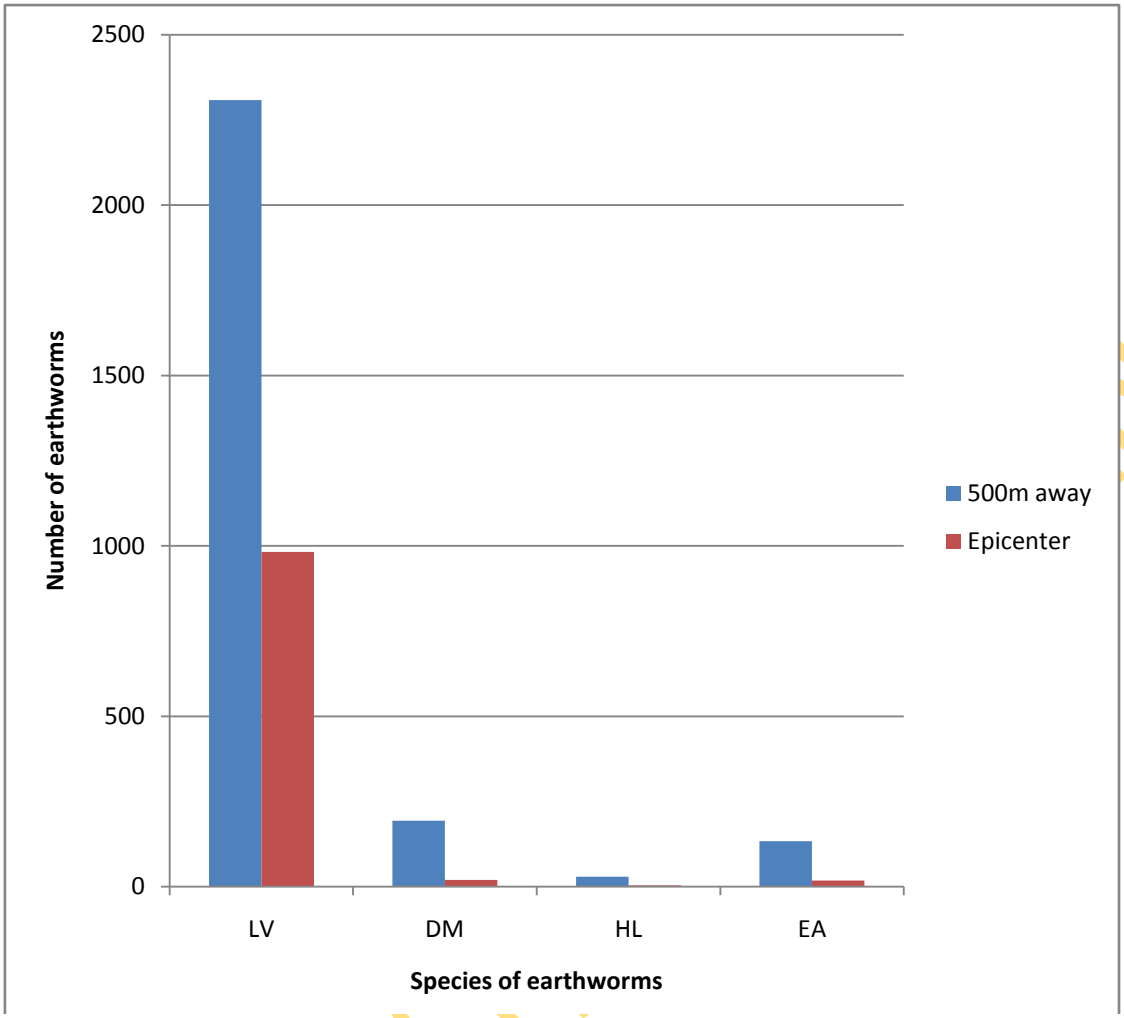
Months	Rainfall in area (mm)	Ave. air temp (^{o}C)	Relative Humidity
June '07	367.7	25.9	90
July '07	228	25.7	88
Aug '07	287.9	25.4	88
Sept '07	156.7	25.8	89
Oct '07	120.3	26.3	87
Nov '07	118.3	27.3	85
Dec '07	5.4	27.4	83
Jan '08	0.8	26.6	66
Feb '08	3.3	28.6	74
March '08	69.6	28.5	81
April ,08	96.8	28.5	81
May '08	230	27.3	86
June '08	365	26.2	89
July '08	442.7	25.5	91
Aug '08	134.3	25.6	88
Sept '08	226.8	26	89
Oct '08	98.8	26..6	88
Nov '08	98.9	27.8	85
Dec '08	49	27.8	82
Jan '09	1.6	27.9	79
Feb '09	16.3	28.7	81
March '09	33.9	29.0	81
April ,09	115.5	28.0	83

Adapted from Nigerian meteorological agency (NIMET), 2014

4.6 Earthworm abundance and distribution

The species of earthworms encountered during the study period were *Lybiodrillus violaceous, Dichogaster modiglanin, Ephyriodrilus afroccidentalis* and *Heliodrillus lagosensis*. Fig 4.6 shows the total earthworm species in epicenter and 500m away from spill. *L. violaceous* was the most abundant followed by *D. modigliani* within epicenter and 500m away from the spill. The monthly abundance of earthworm species and their weights within epicenter and 500m away is presented in table 4.11-4.14, *E. afroccidentalis* and *H. lagosensis* were only encountered in the second year of study.

Mann-Wittney analysis comparing population size of earthworm species in the epicentre and 500m away from the spill determined in the first year indicated that there was a significant difference in size between the two sites ($P= 0.07$: $P < 0.1$). However, in the second year there was no significant difference between the two sites ($P= 0.229$; $P > 0.1$). *L.violaceous* was identified to be the most abundant earthworm spp found within Agaye community. The Shannon-Wiener diversity Index showed that in the first year, $H = 0.2813$; while in the second year, $H = 0.7315$. This indicated that there was more earthworm species diversity in the second year compared to the first year in the study area.



Keys: L.V= *Lybiodrillus violaceous*, D.G= *Dichogaster modiglanin*,
E.A= *Ephyriodrillus afroccidentalis*, H.L= *Hiliodrillus lagosensis*

Fig. 4.6 Total earthworm species in epicenter and 500m away from spill

Table 4.11 Monthly number of earthworm species found in the epicentre of spill

Months	LV	DS	HL	EA	TOTAL No.
Jun'07	18	0	0	0	18
July'07	29	0	0	0	29
Aug'07	33	0	0	0	33
Sept'07	48	0	0	0	48
Oct'07	43	0	0	0	43
Nov'07	27	0	0	0	27
Dec'07	5	0	0	0	5
Jan'08	*	*	*	*	*
Feb'08	*	*	*	*	*
Mar'08	*	*	*	*	*
Apr'08	16	2	0	3	21
May'08	21	3	0	2	25
Jun'08	68	2	0	0	70
July'08	98	1	0	3	102
Aug'08	122	3	0	1	130
Sept'08	157	3	1	2	163
Oct'08	178	5	2	4	189
Nov'08	57	1	1	3	62
Dec'08	23	0	0	0	23
Jan'09	8	0	0	0	8
Feb'09	6	0	0	0	6
Apr'09	11	0	0	0	11

Keys: L.V= *Lybiodrillus violaceous,* D.G= *Dichogaster modigliani,*
E.A= *Ephyriodrillus afroccidentalis,* H.L= *Hiliodrillus lagosensis*
* = Not done

Table 4.12: Weight of earthworm species found in the epicentre of spill

Months	LV (g)	DS (g)	HL (g)	EA (g)	TOTAL WGT (g)
Jun'07	3.32	0	0	0	3.32
July'07	23.89	0	0	0	23.89
Aug'07	11.91	0	0	0	11.91
Sept'07	43.03	0	0	0	43.03
Oct'07	46.8	0	0	0	46.8
Nov'07	22.17	0	0	0	0
Dec'07	1.38	0	0	0	0
Jan'08	*	*	*	*	*
Feb'08	*	*	*	*	*
Mar'08	*	*	*	*	*
Apr'08	2.84	0.4	0	0.39	3.63
May'08	3.18	0.37	0	0.24	3.79
Jun'08	50.09	0.4	0	0	50.89
July'08	73.13	0.15	0	0.43	73.71
Aug'08	99.09	0.34	0	0.18	99.94
Sept'08	124.25	0.41	0.36	0.13	124.97
Oct'08	138.78	0.94	0.77	0.31	139.68
Nov'08	43.89	0.12	0.29	0.27	44.57
Dec'08	3.93	0	0	0	3.93
Jan'09	1.41	0	0	0	1.41
Feb'09	1.28	0	0	0	1.28
Apr'09	2.06	0	0	0	2.06

Keys: L.V= *Lybiodrillus violaceous*, D.G= *Dichogaster modigliani*,
E.A= *Ephyriodrillus afroccidentalis*, H.L= *Hiliodrillus lagosensis*
✳ = Not done

Table 4.13: Number of earthworm species found 500m away from the spill

Months	LV	DS	HL	EA	TOTAL
Jun'07	65	11	0	0	76
July'07	120	9	0	0	129
Aug'07	186	8	0	0	194
Sept'07	198	10	0	0	208
Oct'07	211	14	0	0	303
Nov'07	28	9	0	0	37
Dec'07	5	2	0	0	7
Jan'08	*	*	*	*	*
Feb'08	*	*	*	*	*
May'08	*	*	*	*	*
Apr'08	19	5	2	3	29
May'08	89	8	0	19	126
Jun'08	169	15	5	24	219
July'08	204	19	3	20	320
Aug'08	262	21	0	22	388
Sept'08	308	20	7	18	353
Oct'08	362	23	10	22	417
Nov'08	*	*	*	*	*
Dec'08	13	3	2	5	23
Jan'09	6	0	0	0	6
Feb'09	5	2	0	0	7
Apr'09	16	5	0	0	21

Keys: L.V= *Lybiodrillus violaceous*, D.G= *Dichogaster modigliani*,
E.A= *Ephyriodrillus afroccidentalis*, H.L= *Hiliodrillus lagosensis*
* = Not done

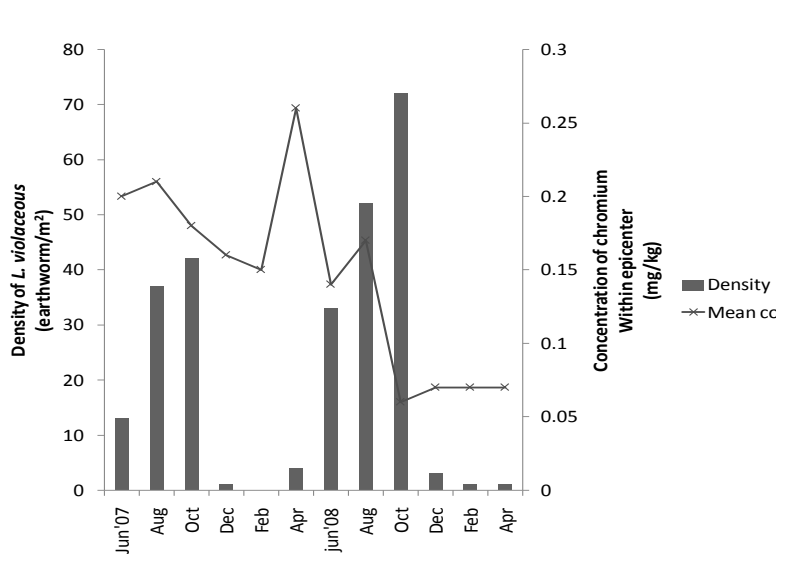
Table 4.14: Weight of earthworm species found 500m away from the spill

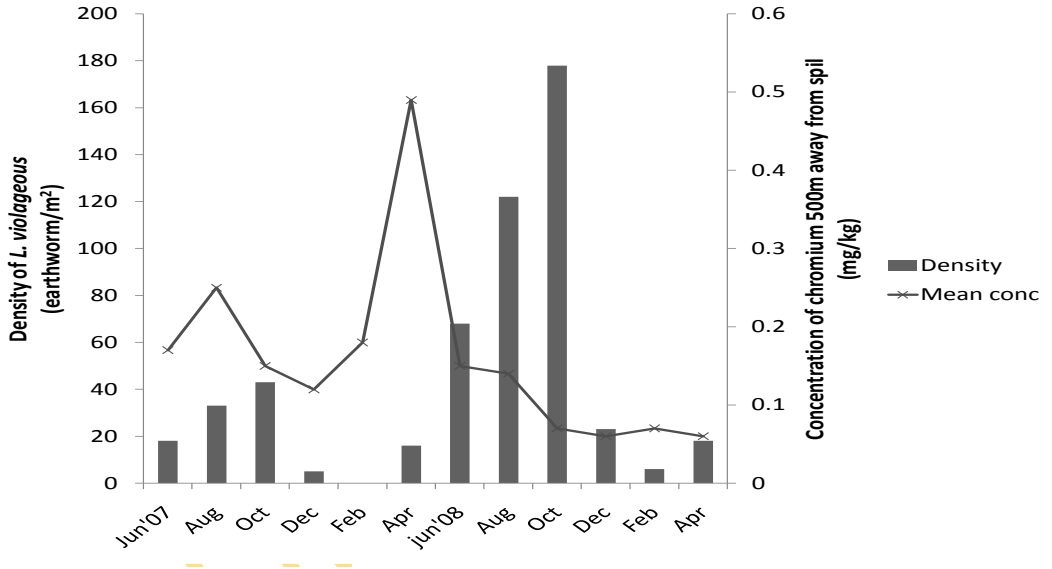
Months	LV (g)	DS (g)	HL (g)	EA (g)	TOTAL WGT (g)
Jun'07	18.99	0.31	0	0	19.3
July'07	29.78	0.24	0	0	30.02
Aug'07	41.75	0.19	0	0	41.94
Sept'07	42.88	0.28	0	0	43.16
Oct'07	45.62	0.82	0	0	46.44
Nov'07	9.01	0.29	0	0	9.3
Dec'07	1.09	0.08	0	0	1.17
Jan'08	*	*	*	*	*
Feb'08	*	*	*	*	*
May'08	*	*	*	*	*
Apr'08	3.77	0.15	0.05	0.16	4.13
May'08	21.22	0.32	0	1.97	24.31
Jun'08	34.2	1.44	0.17	2.27	37.93
July'08	43.25	1.89	0.09	2.01	51.24
Aug'08	290.27	2.15	0	2.43	304.02
Sept'08	311.15	2.08	0.21	1.86	315.3
Oct'08	337.81	2.79	6	10.16	350.58
Nov'08	*	*	*	*	*
Dec'08	2.17	0.09	0.03	0.25	2.54
Jan'09	1.08	0	0	0	1.08
Feb'09	1.02	0.05	0	0	1.07
Apr'09	3.29	0.14	**0**	0	3.43

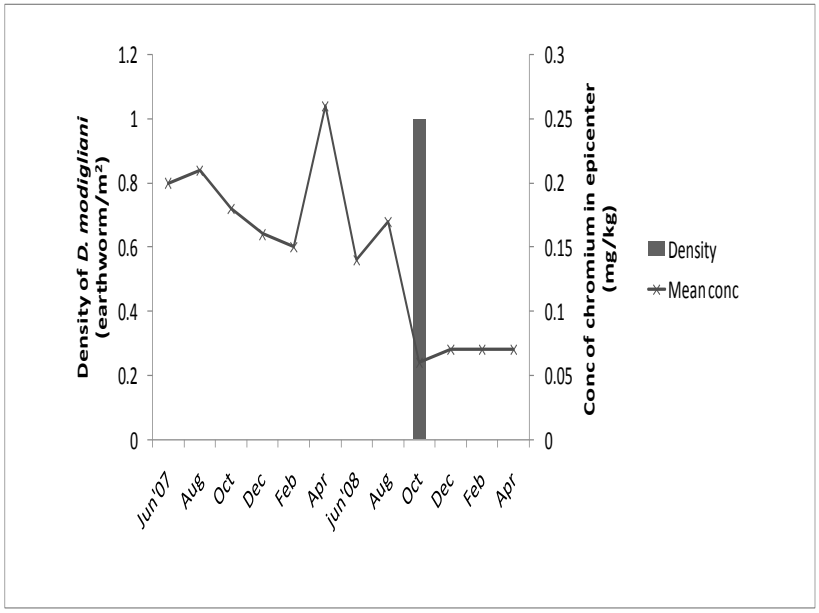
Keys: L.V= *Lybiodrillus violaceous*, D.G= *Dichogaster modigliani*,
E.A= *Ephyriodrillus afroccidentalis*, H.L= *Hiliodrillus lagosensis*
* = Not done

The densities of earthworm species in comparison with concentration of metals in the epicenter and 500 m away are shown in Fig. 4.7 – 4. 14. The trend shows that the heavy metal concentrations had no major influence on the fluctuations in earthworm species abundance. A comparison of earthworm species abundance between the epicentre of oil spill and 500m away from the spill in the first and second year are illustrated in Fig. 4.15 - 4.18.

The mean concentrations of metals in the two most abundant species (*L.violaceous and D. modiglanin*) are presented in Table 4.15. The mean concentrations of Cu, Pb and Cd for *L.violaceous* (mg/g) were Pb (0.41±0.02) > Cd (0.17±0.003) >Cu (*0*.13±0.003) while that for *D.modiglanin* were Cd (0.06±0.002) = Cu (0.06±0.44). Cr, Ni and V were not detected in both species







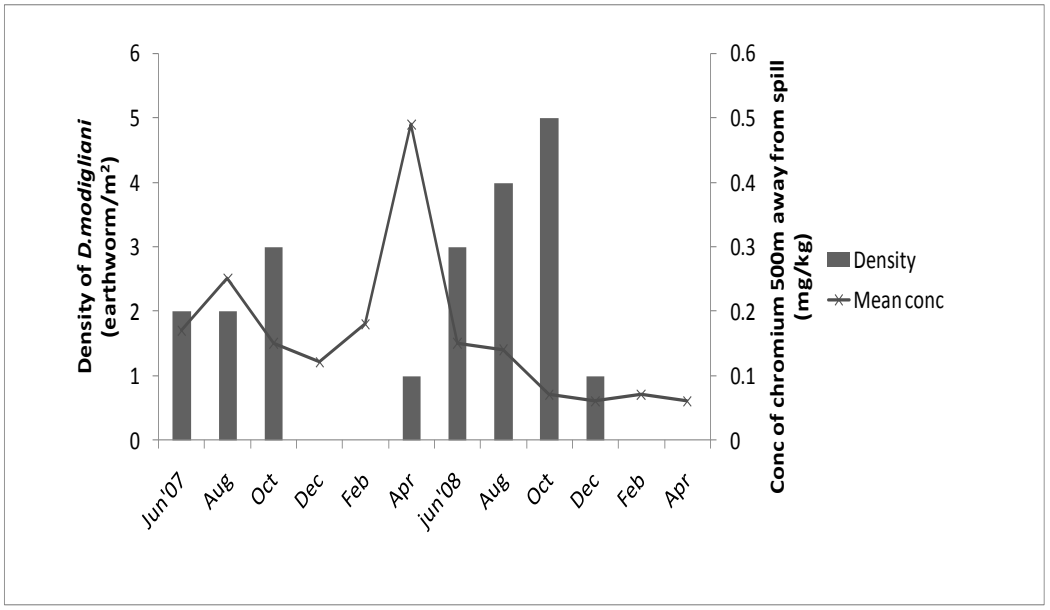


Fig. 4.7 Comparison of soil chromium concentrations and population densities of *L. violaceous* and *D.modigliani* (epicenter and 500m away from spill)

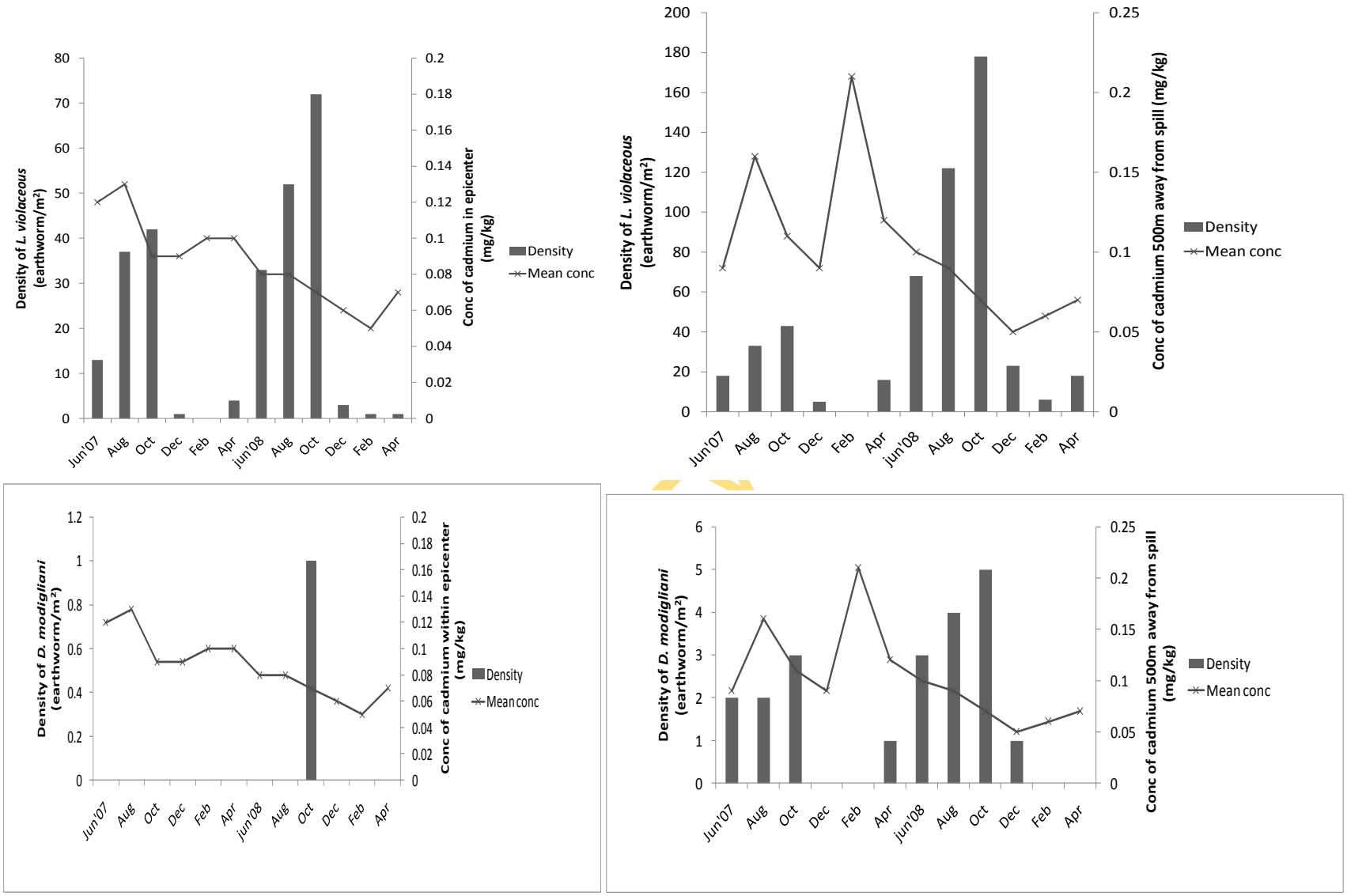


Fig. 4.8 Comparison of soil cadmium concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)

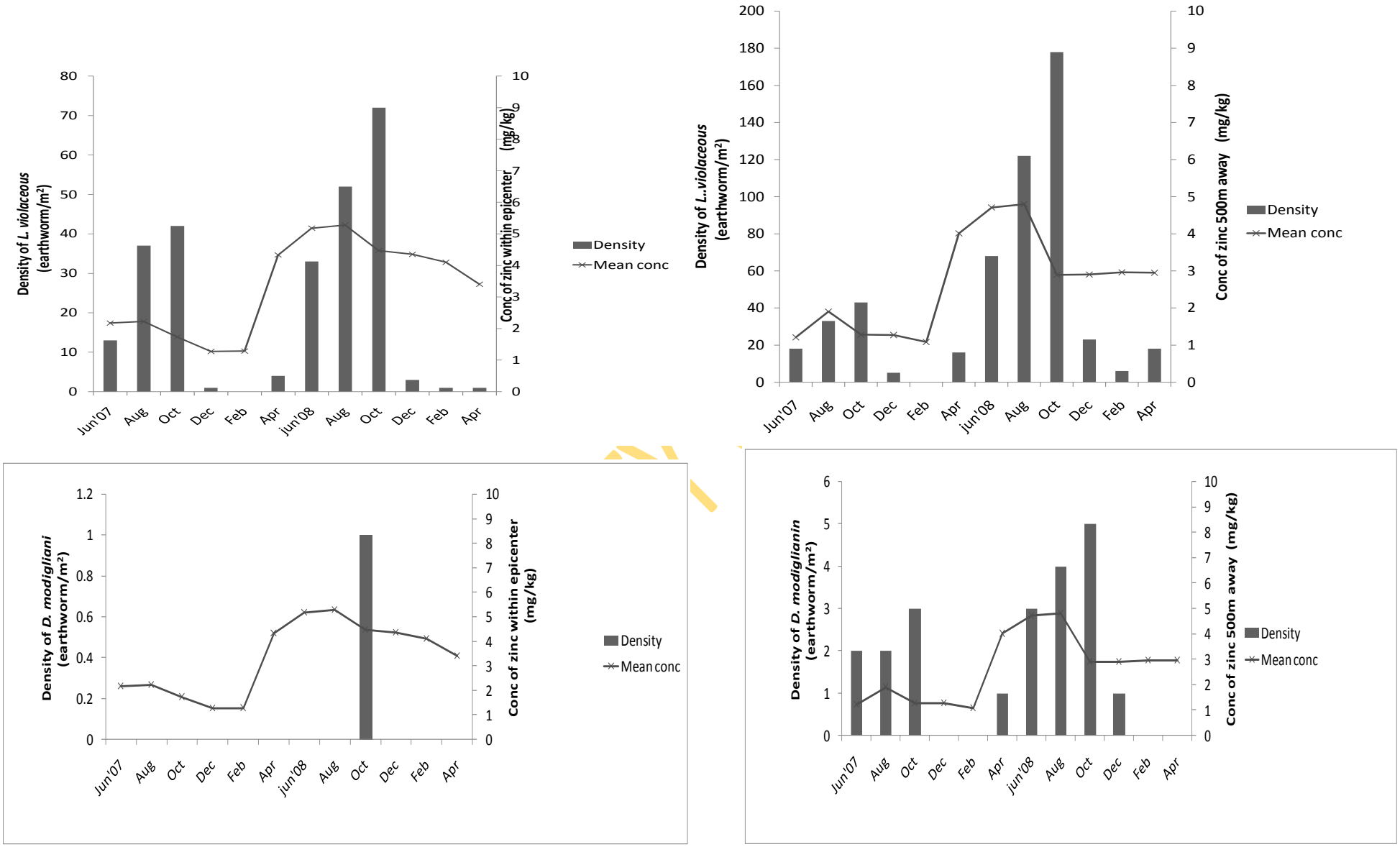


Fig. 4.9 Comparison of soil zinc concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)

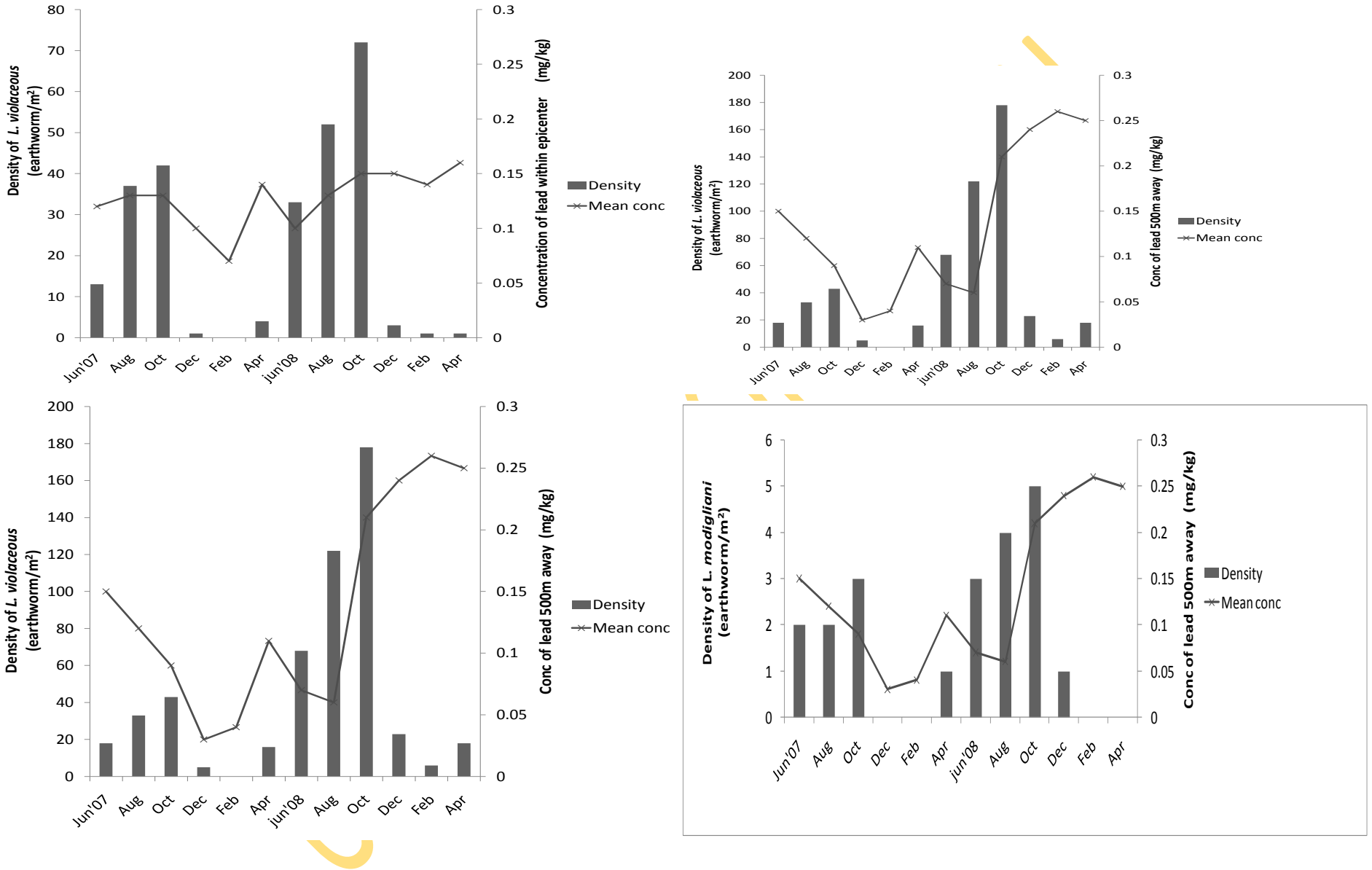


Fig. 4.10 Comparisons of soil lead concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)

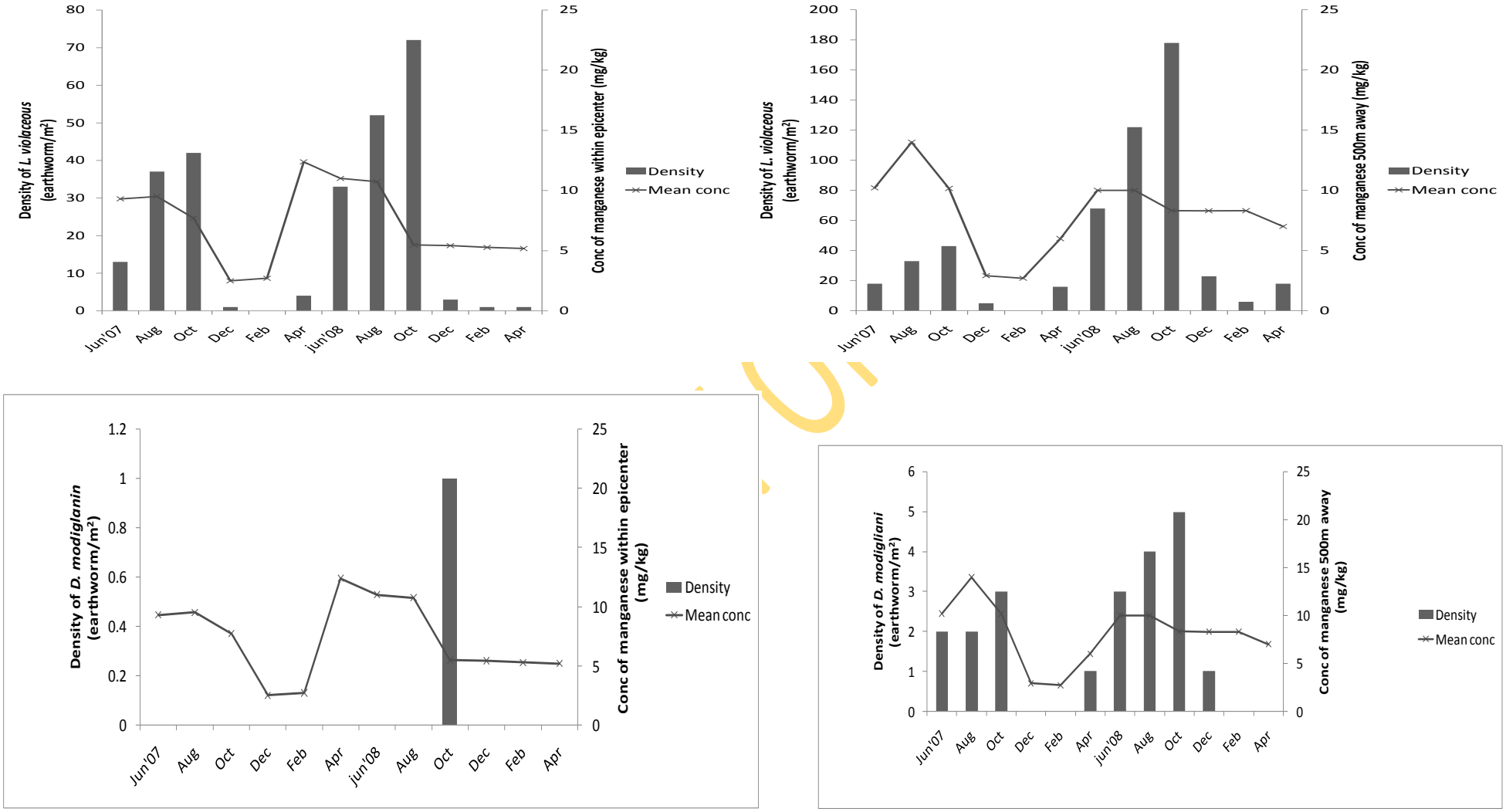
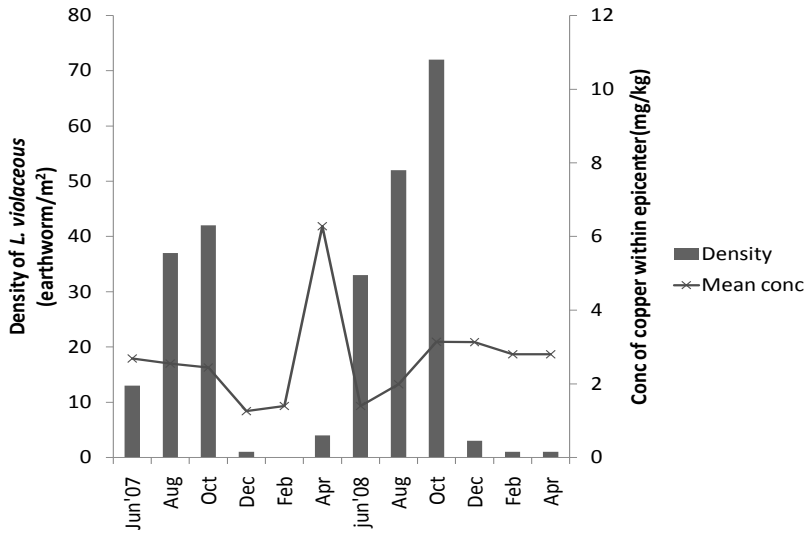
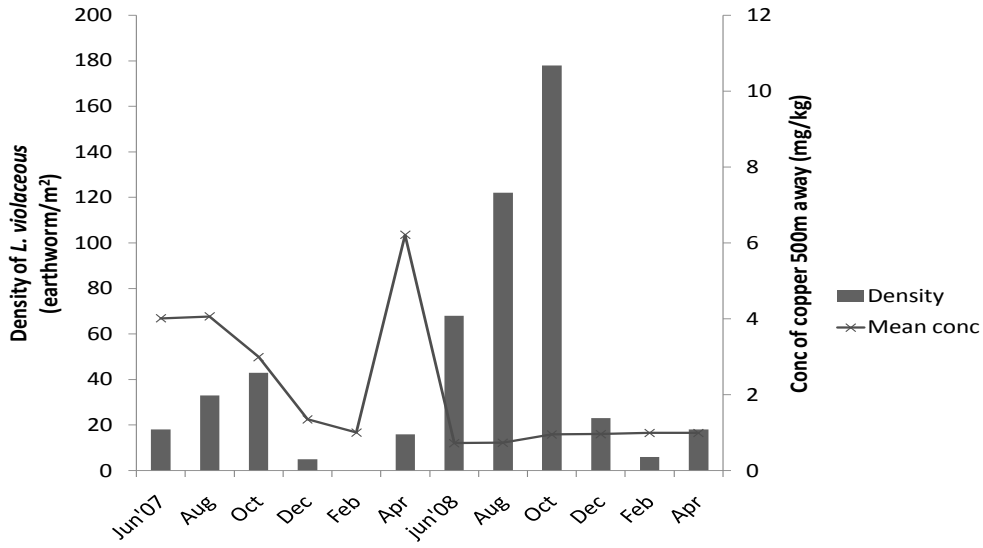
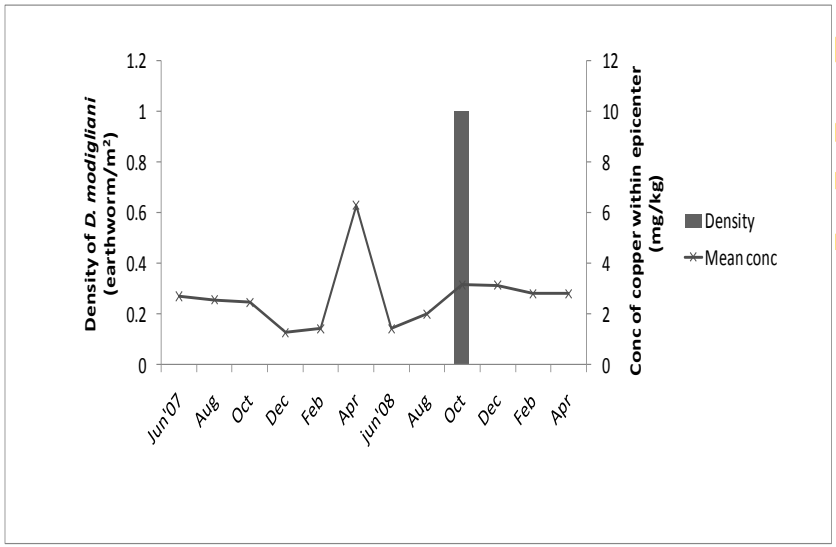


Fig. 4.11 Comparison of soil manganese concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)







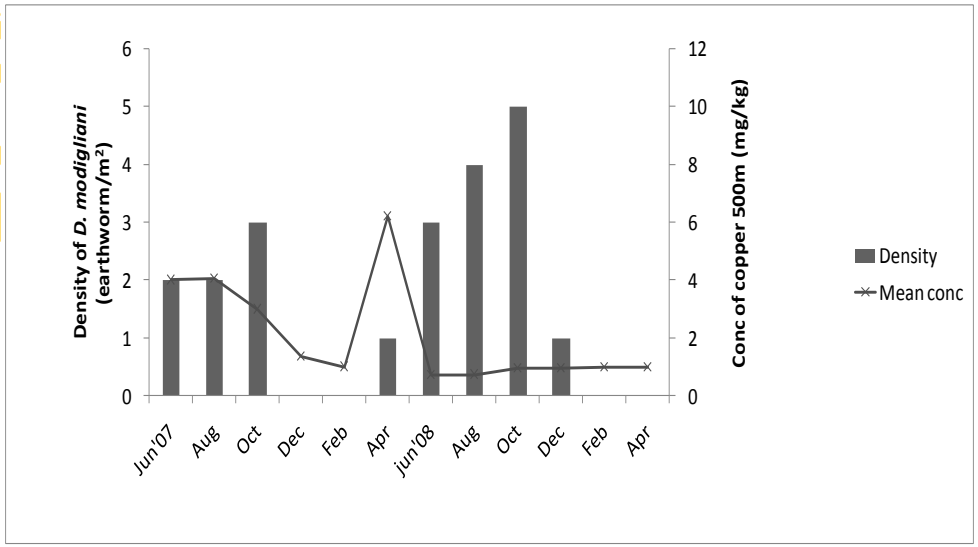


Fig. 4. 12 Comparison of soil copper concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)

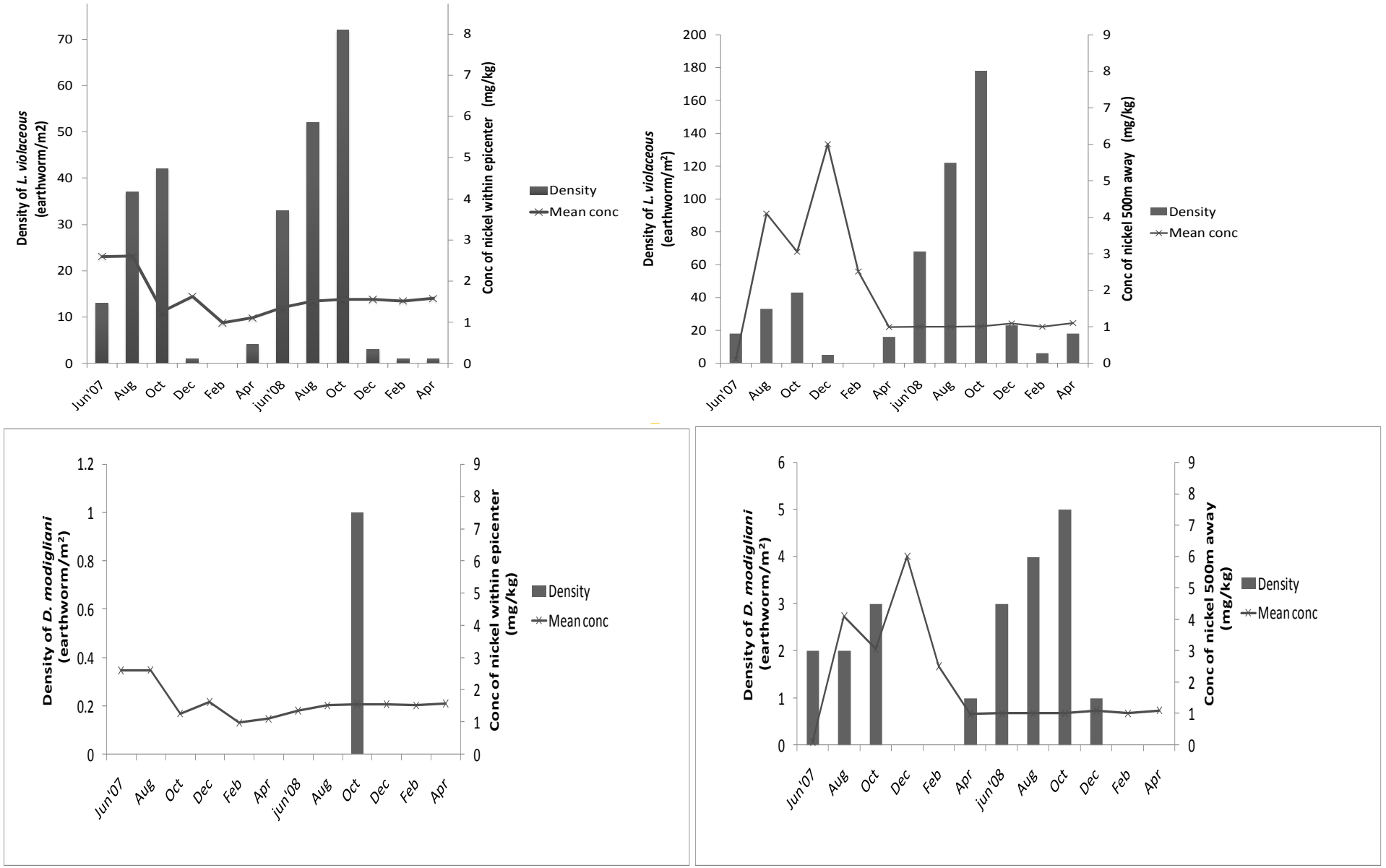
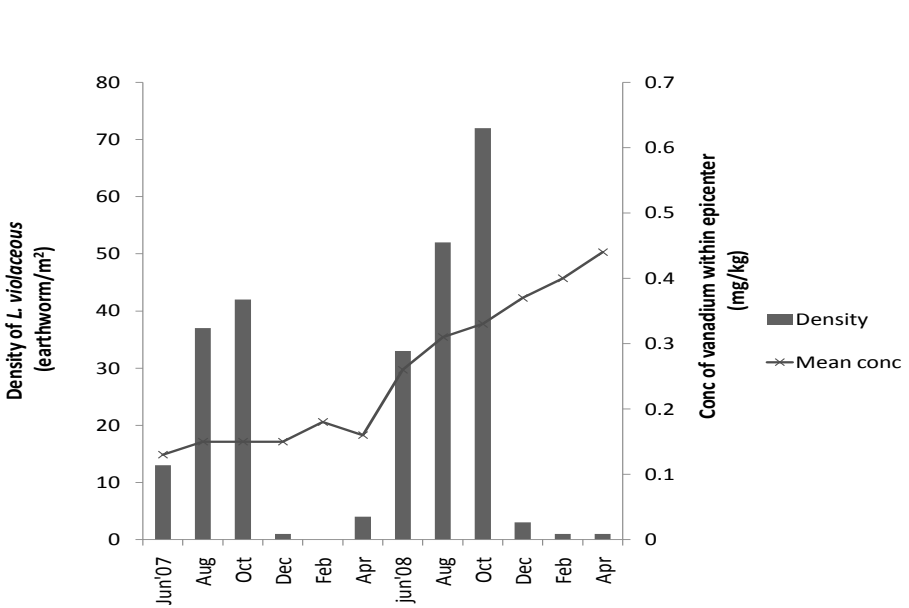
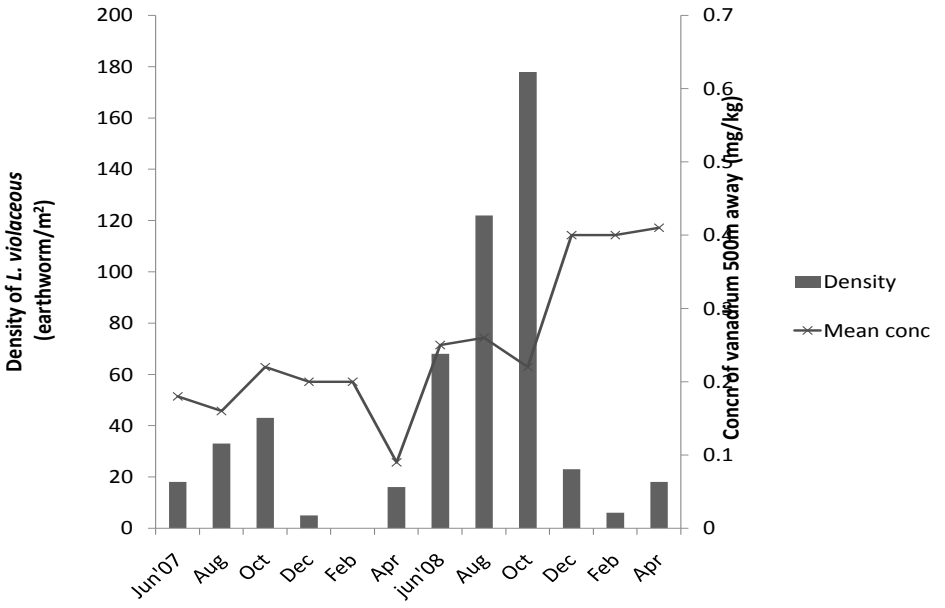
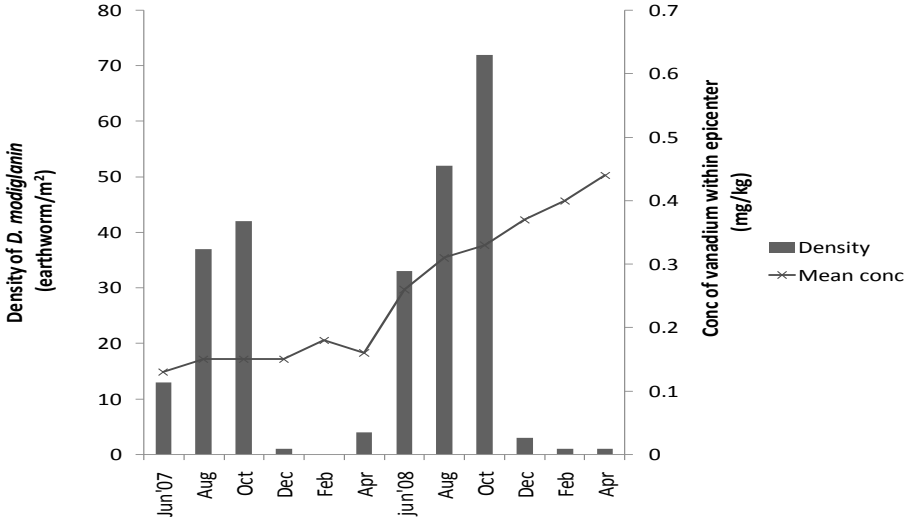


Fig. 4.13 Comparison of soil nickel concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)







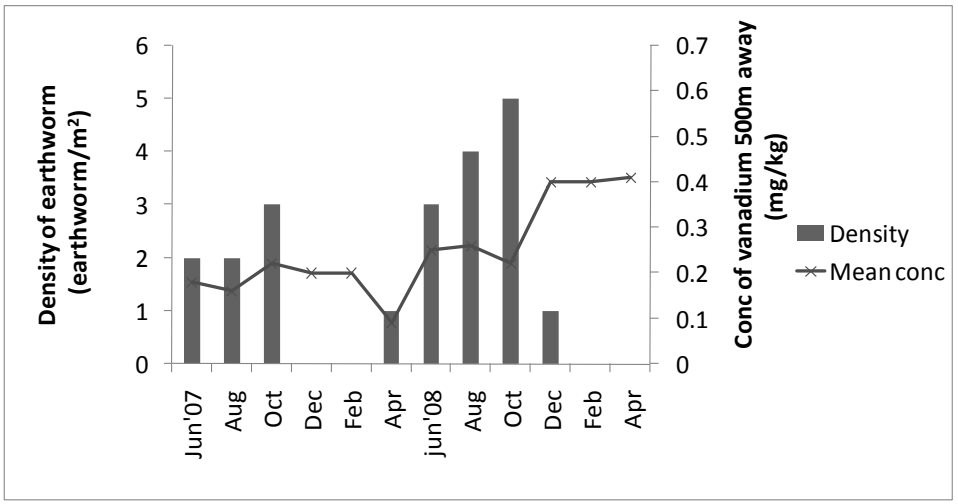


Fig. 4. 14 Comparison of soil vanadium concentrations and population densities of *L. violaceous* and *D. modigliani* (epicenter and 500m away from spill)

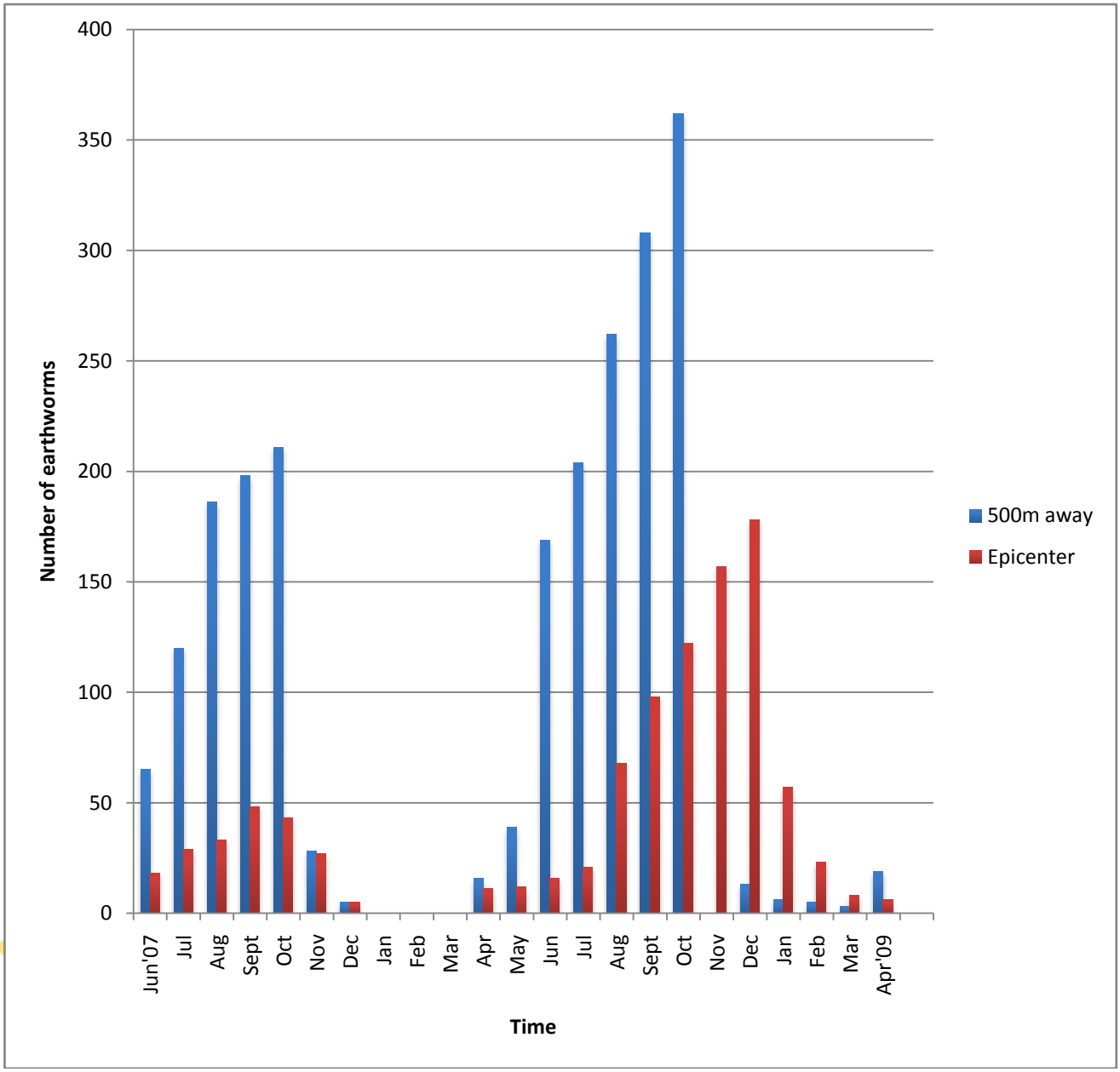


Fig.4.15: Comparison of *L.violaceous* abundance in the epicentre of oil spill and 500m away from the spill

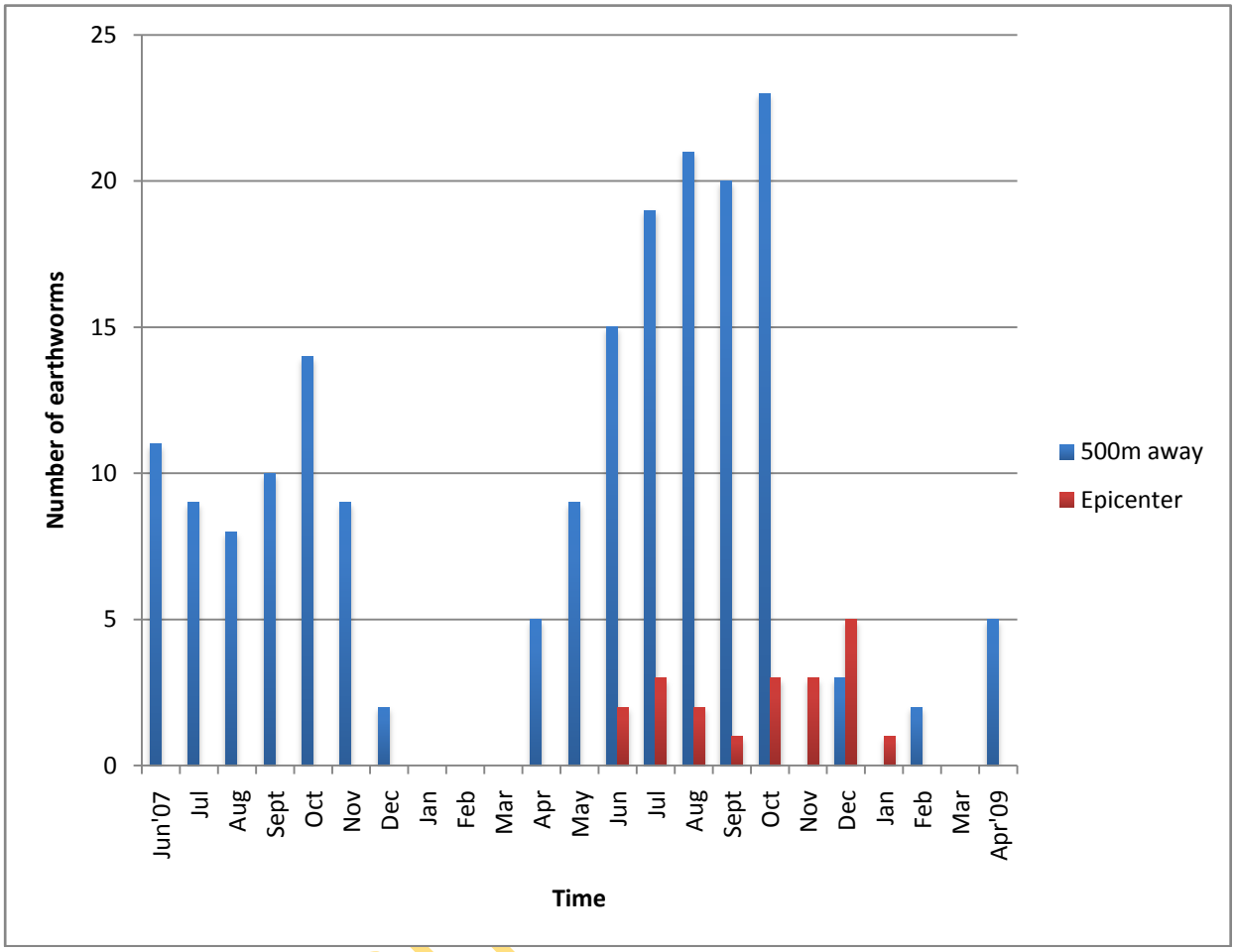


Fig.4.16: Comparison of *D. modigliani* abundance in the epicentre of oil spill and 500m away from the spill.

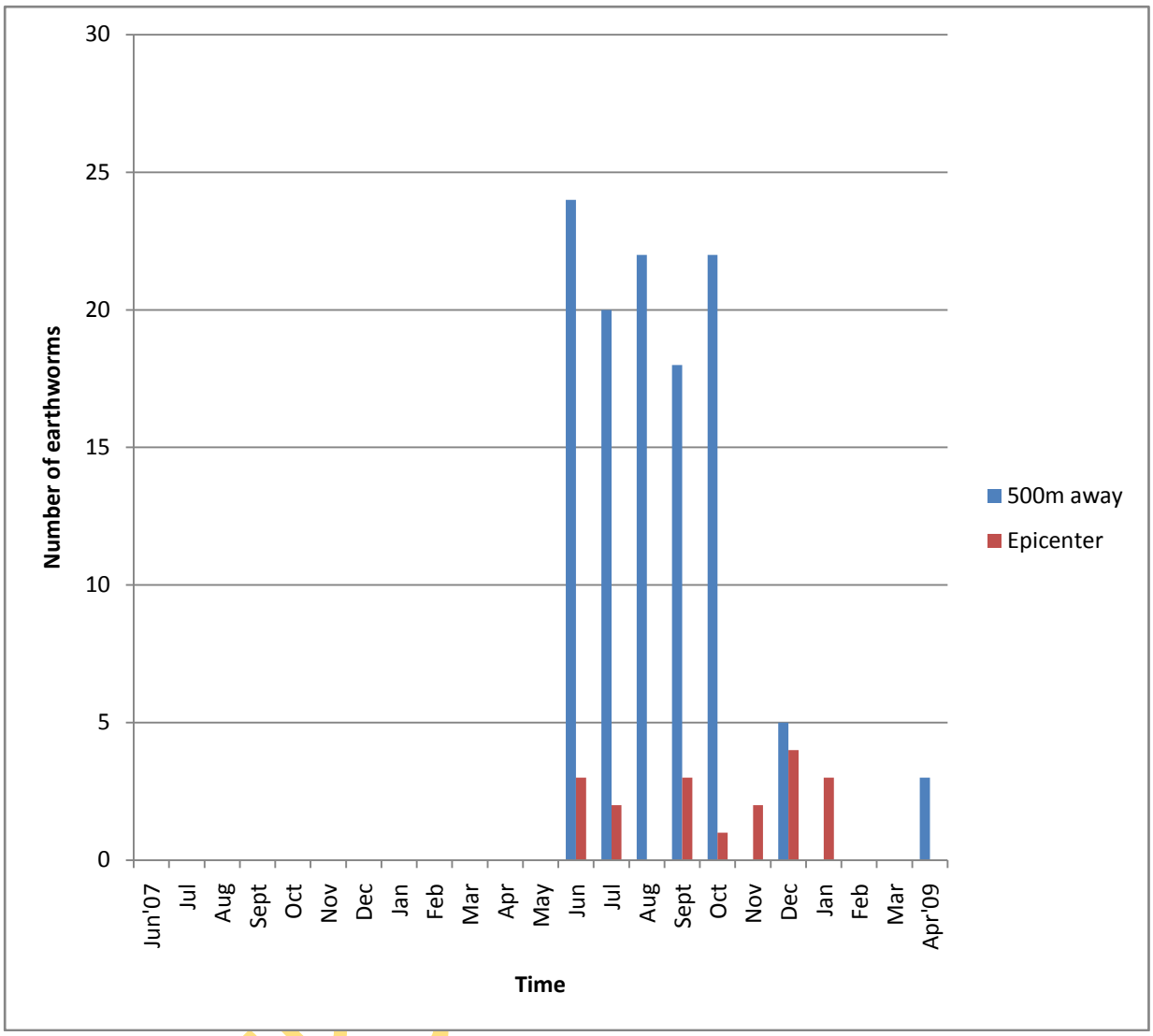


Fig.4.17 Comparison of *E.afroccidentalis* abundance in the epicentre of oil spill and 500m away from the spill.

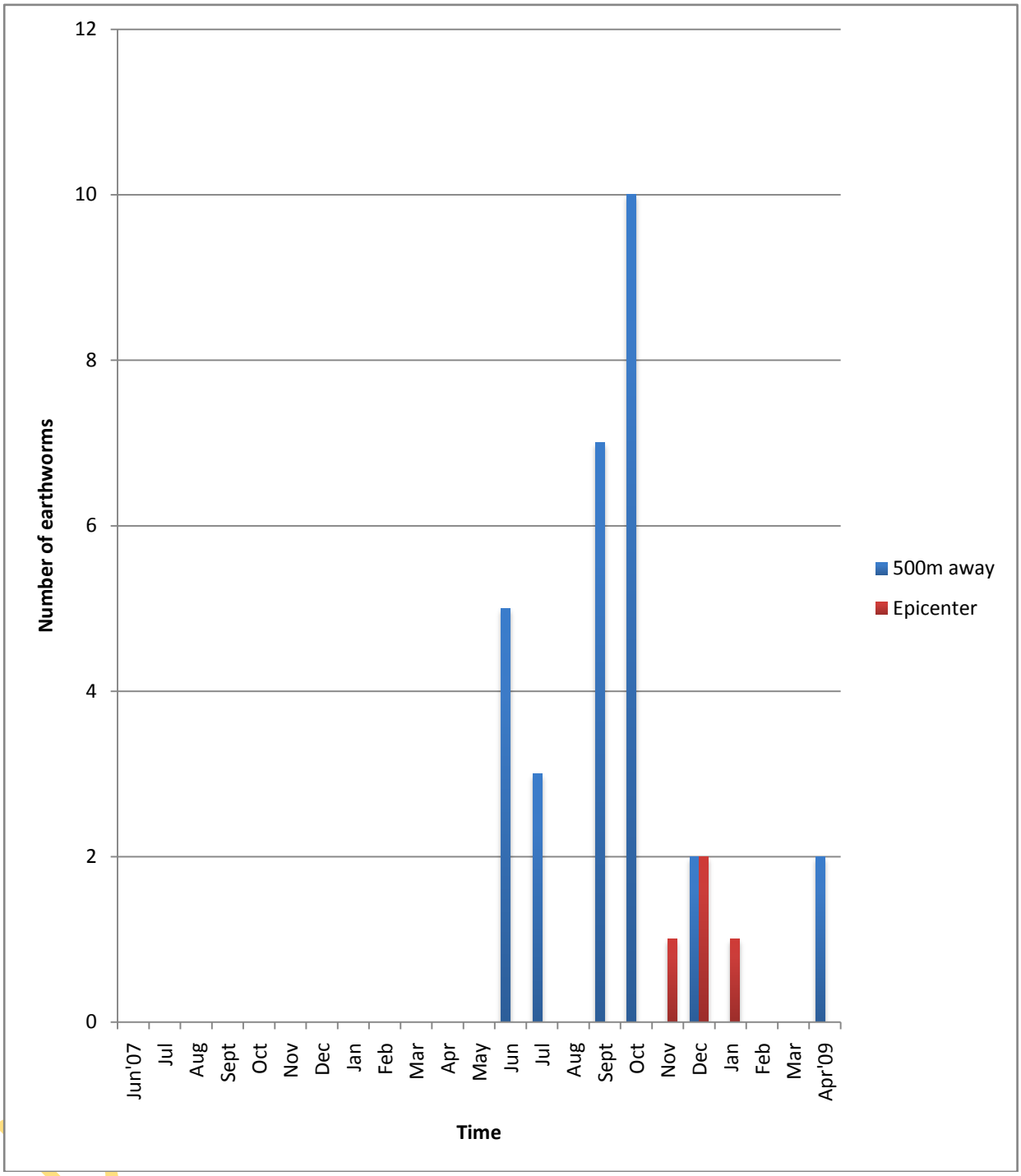


Fig.4.18: Comparison of *H.lagosensis* abundance the epicentre of oil spill and 500m away from the spill

Table 4. 15: Heavy metal accumulation in *L.violaceous* and *D.modiglani* species

Metals measured in earthworm spp	Epicenter Metal Conc (mg/g) (Mean±SD)	500m away Metal Conc (mg/g) (Mean±SD)
Mn_1	ND	ND
Mn_2	3.66±0.04	1.37±0.06
Cu_1	ND	ND
Cu_2	0.13±0.003	0.09±0.02
Zn_1	6.7±0.4	3.76±1.06
Zn_2	5.46±0.03	2.03±0.8
Pb_1	ND	ND
Pb_2	ND	ND
Cd_1	0.06±0.002	0.05±0.02
Cd_2	0.17±0.003	0.06±0.02
Cr_1	ND	ND
Cr_2	ND	ND
Ni_1	ND	ND
Ni_2	ND	ND
V_1	ND	ND
V_2	ND	ND

1 = *D.modigliani*

2= *L.violaceous*

4. 7 Correlation of physicochemical parameters with earthworm abundance

The statistical correlation of earthworm abundance in the epicenter and 500m away were done with (i) heavy metal concentration which includes Chromium (Cr), Cadmium (Cd), Magnese (Mn), Nickel (Ni), Lead (Pd), Zinc (Zn) and Vanadium (V) and Other parameters including Sulphate (SO_4), Nitrate (NO_3), pH level, temperature, Total organic carbon (TOC) and Total organic matter (TOM) (Table 4.16 and 4.17) and (iii) climatic parameters like rainfall (RF) and soil temperature (ST) (Fig.4.19 and 4.20).

Within the epicenter, concentration of zinc in soil alone showed a strong positive colleration with the abundance of both earthworm species encountered, (0.77 and 0.65 for *D.modiglanin* and *L.violaceous* respectively) while concentrations of chromium, cadmium, copper and nickel showed weak negative correlation (-0.31, -0.45, -0.23 and -0.1) with *L.violaceous* species. Also, pH and concentration of Total Petroleum Hydrocarbon (TPH) showed weak negative correlation with both earthworm species while concentrations of nitrate (NO_3), Total Organic Carbon (TOC) and Total Organic Matter (TOM) showed a strong positive correlation with both earthworm species (Table 4.16). The concentrations of chromium and cadmium 500m away from the spill, showed weak negative correlation with the abundance of individual earthworm species encountered, also the concentration of nickel showed a weak negative correlation with the abundance of *E.afroccidentalis* and *H.lagosensis* alone. The concentration of zinc showed positive correlation with *E.afroccidentalis* and *H.lagosensis* and not *D.modiglanin* and *L.violaceous* (Table 4.17)

Soil temperature had a negative correlation with all earthworm species whereas rainfall and soil moisture had positive correlations with earthworm abundance within the epicenter and 500m away from spill. (Fig 4.19 and Fig 4.20).

REPLACE WITH CORRELATION

Table 4.16: Correlation of earthworm species with heavy metal concentrations and other parameters within the epicenter of oil spill

Earthworm species	Cr	Cd	Pb	Zn	Cu	Mn	Ni	V
D.modiglanin	0.003	-0.29	0.04	0.77	0.10	0.35	0.09	0.09
L.violaceous	-0.31	-0.45	0.17	0.65	-0.23	0.24	-0.1	0.28

Table 4.17: Correlation of earthworm species with heavy metal concentrations and other parameters 500m away from oil spill

Earthworm species	SO_4	NO_3	PO_4	PH	K	Na	Ca	TOC	TOM	TPH
D.modiglanin	0.38	0.55	0.43	-0.09	0.48	0.76	0.56	0.67	0.67	-0.25
L.violaceous	0.51	0.66	0.41	-0.27	0.36	0.78	0.35	0.75	0.75	-0.31

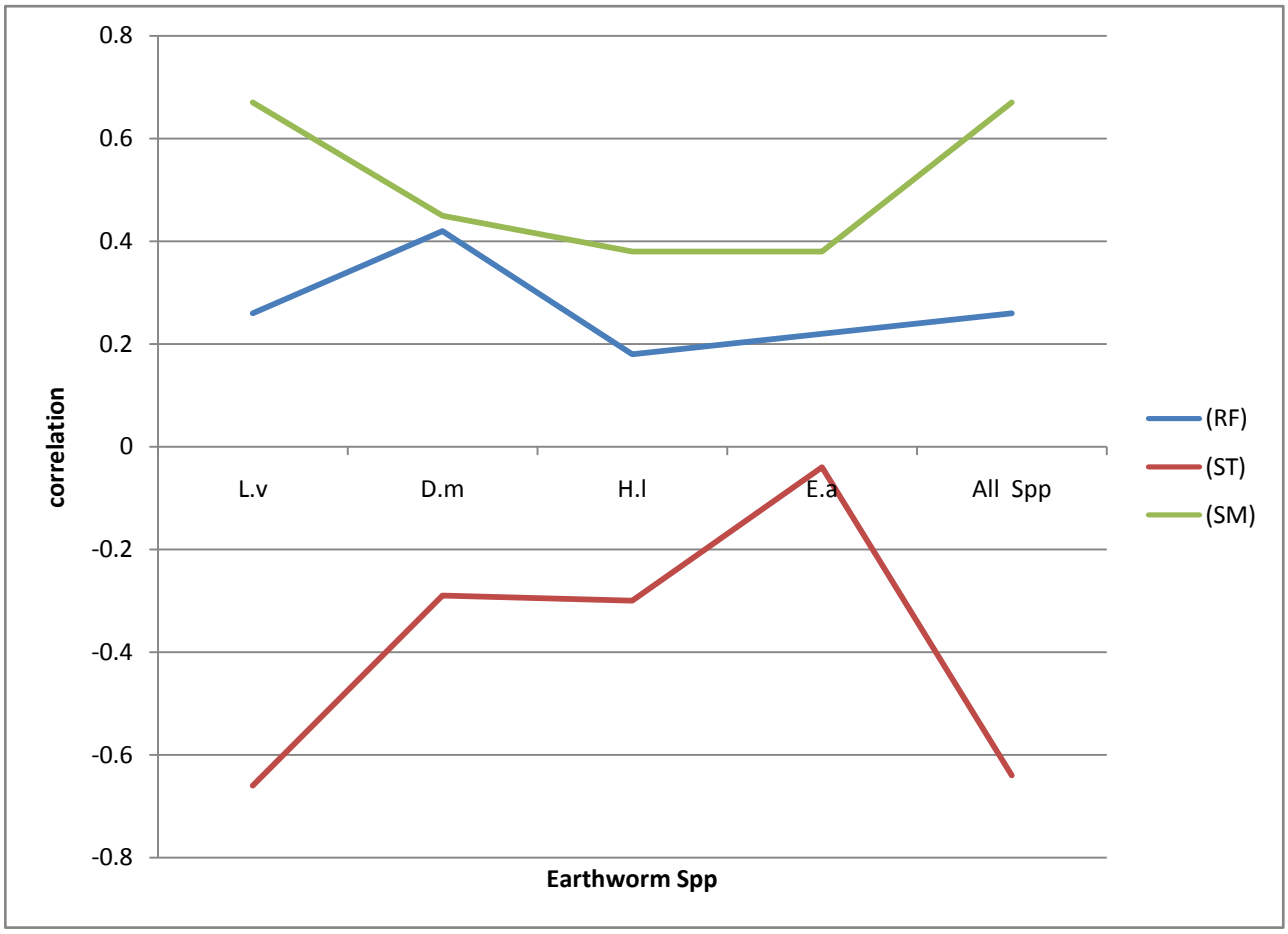


Fig 4.19: Correlation of climatic parameters with earthworm species abundance within the epicenter of oil spill

Key:
L.v = *L. violaceous*
D.m = *D. modiglianin*
H.l = *H. lagosiensis*
E.a = *E. afroccidentalis*
RF= Rainfall
ST= Soil temoerature
SM= Soil moisture

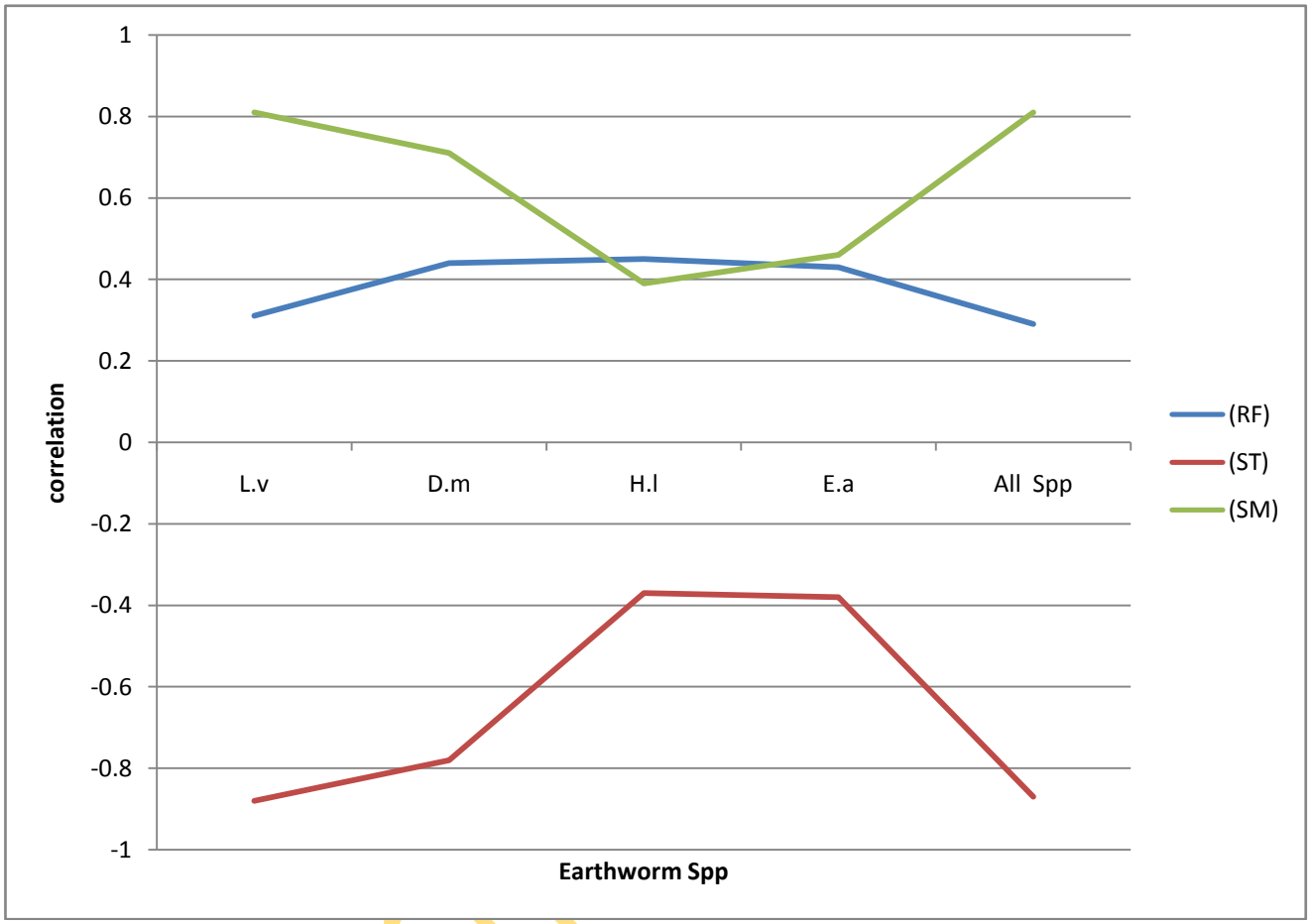


Fig. 4.20: Correlation of climatic parameters with earthworm species abundance 500m away from oil spill

Key:
L.v = *L. violaceous*
D.m = *D. modiglianin*
H.l = *H. lagosiensis*
E.a = *E. afroccidentalis*
RF= Rainfall
ST= Soil temoerature
SM= Soil moisture

4.8 Earthworm toxicity tests

The result indicated that both earthworm species showed low percentage survival (20% and 10% for *L.violaceous and D.modiglanin* respectively) compared to that in the control soil. However, in the subsequent year, *L.violaceous* showed a higher percentage survival of 70% compared to *D.modiglanin* which showed a lower percentage survival of 30% (Table 4.18).

The result of avoidance test indicated that there was high percentage migration of both earthworm species into the control soil whereas, by the subsequent year, there was lower percentage migration of *L.violaceous* species but not *D.modiglanin* into the control soil (Table 4.19).

4.9 Ex-situ bioremediation study

During the ex-situ bioremediation study, TPH and Heavy metal concentrations were monitored and the mean concentrations and t-test results for various soil types against control (non- contaminated soil) are shown in Table 4.20 – 4.27. T-test results indicated a significant reduction of the concentrations of TPH and all heavy metals except copper in the earthworm treated soil (CSE) during the period of study ($P>0.05$) unlike the t-test results of other soil type (CS) which showed no significant reduction ($P<0.05$).

Table 4.18: Percentage means of surviving earthworm species

Earthworm species	Oct, 2008		Oct, 2009	
	CSE (%)	NSE (%)	CSE (%)	NSE (%)
L.violaceous	20	90	70	80
D.modiglanin	10	70	30	90

CSE = Contaminated soil and earthworm
NSE = Non-contaminated soil and earthworm

Table 4.19 Percentage means of migrated earthworm species

Earthworm species	Oct, 2008		Oct, 2009	
	CSE (%)	NSE (%)	CSE (%)	NSE (%)
L.violaceous	30	70	60	40
D.modiglanin	0	100	20	80

CSE = Contaminated soil and earthworm
NSE = Non-contaminated soil and earthworm

Table 4.20: Mean concentrations of Total Hydrocarbon in soil samples for bioremediation study

	NS (ex-situ)	**CS** (ex-situ)	**CSE (ex-situ)**
Pre-treatment	0.41	2.65	2.37
6 weeks	0.39	2.35	1
12 weeks	0.4	2.3	0.85
***P-value**	-	0.0013	0.0851
Sign.	-	P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil
Key: CS = Contaminated Soil, NS=Non-contaminated soil (Control), CSE=Contaminated Soil with earthworm

Table 4.21: Mean concentrations of Copper in soil samples for bioremediation study

	NS (ex-situ)	**CS** (ex-situ)	**CSE (ex-situ)**
Pre-treatment	0.32	0.77	0.76
6 weeks	0.3	0.72	0.63
12 weeks	0.31	0.67	0.59
***P-value**		0.0020	0.0088
Sign.		P<0.05	P<0.05

*t-test (P-value) on concentrations of other soil types against control soil

Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

Table 4.22: Mean concentrations of Lead in soil samples for bioremediation study

	NS (ex-situ)	CS (ex-situ)	CSE (ex-situ)
Pre-treatment	0.08	0.17	0.16
6 weeks	0.07	0.16	0.09
12 weeks	0.06	0.16	0.06
***P-value**		0.0006	0.1499
Sign.		P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil

Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

Table 4.23: Mean concentrations of Vanadium in soil for bioremediation study

	NS (ex -situ)	CS (ex-situ)	CSE (ex-situ)
Pre-treatment	0.18	0.59	0.57
6 weeks	0.13	0.57	0.5
12 weeks	0.16	0.43	0
***P-value**		0.0095	0.1911
Sign.		P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil

Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

Table 4.24: Mean concentration of zinc in soil samples for bioremediation study

	NS (ex-situ)	CS (ex-situ)	CSE (ex-situ)
Pre-treatment	0.26	0.42	0.46
6 weeks	0.21	0.51	0.35
12 weeks	0.29	0.47	0.11
***P-value**		0.019	0.3477
Sign.		P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil
Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

Table 4.25: Mean concentrations of manganese in soil samples for bioremediation study

	NS (ex-situ)	**CS (ex-situ)**	**CSE (ex-situ)**
Pre-treatment	1.82	8.38	8.54
6 weeks	1.93	6.62	4.81
12 weeks	1.63	0.47	0
***P-value**		0.1424	0.1928
Sign.		P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil
Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

Table 4.26: Mean concentrations of Cadmium in soil samples for bioremediation study

	NS (ex-situ)	CS (ex-situ)	CSE (ex-situ)
Pre-treatment	0	0.02	0.02
6 weeks	0	0.03	0
12 weeks	0	0.01	0
***P-value**		0.0370	0.2113
Sign.		P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil
Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

Table 4.27: Mean concentrations of Chromium in soil samples for bioremediation study

	NS	CS	CSE
Pre-treatment	0	0.08	0.08
6 weeks	0	0.06	0.02
12 weeks	0	0.07	0
***P-value**		0.0033	0.1499
Sign.		P<0.05	P>0.05

*t-test (P-value) on concentrations of other soil types against control soil
Key: CS = Contaminated Soil, NS= Non-contaminated soil (Control)
CSE=Contaminated Soil with earthworm

CHAPTER FIVE

5.0 DISCUSSION

5.1 Water quality analysis

This study shows that mean concentrations of Lead (Pb) in most water sampled and analysed during the study period were above the WHO and NESREA drinking water limit of 0.01mg/L and 0.2mg/L respectively. The elevated concentration might be due to the inferno which produced fumes with deposits seeping into the ground water. In addition, earlier investigations show that much of the gasoline available in developing countries are still heavily leaded (Borasin *et al*., 2002). The school borehole water (GW_5) had one of the highest concentrations of Lead (0.15mg/l in August, 2007) and it is a major source of water for drinking by the school community, however this dried up during the course of the study. The elevated concentration of Lead (Pb) in borehole water could have arisen from metal plumbing systems containing Lead in pipes, solder, fittings or the service connections as reported by WHO and EPA (WHO 1993; WHO 2003. A likely consequence of high Lead level is bioaccumulation which could eventually result in Lead poisoning of the populace.

A decrease of IQ in children is reported to be associated with an increase in blood Lead (>30mg/L) and tooth Lead. It is also reported that prenatal exposure to Lead may have early effects on mental development that do not persist after the age of 4 years (Schwartz, 1994, McMichael *et al*., 1994). Other possible consequences may include fatigue, insomnia, retarded development of foetus, hearing and vision impairment and decreased sperm count. A case of a deafened student was reported during this study; although there was no study to prove that the cause of the deafening was Lead poisoning.

The mean cadmium concentrations were higher than the WHO and NESREA drinking water limit of 0.003mg/l for all the well water samples analysed with highest value of 0.1mg/L recorded in the month of April, 2008. The values later dropped below these standards and then fluctuated between 0.001mg/L and 0.006mg/L from June, 2008 to April, 2009. The relatively high concentration of cadmium in water could generally be due to other natural contaminations like weathering, forest fires and volcano; in this case, the inferno could have contributed to the high levels of

cadmium. Cadmium is known to cause high blood pressure, liver disease and brain damage (ATSDR, 1999)

Nickel (Ni) concentrations were higher than WHO drinking water limit of 0.02mg/L in all the water samples investigated. The highest concentration of Nickel (Ni), 2.85mg/L in December, 2008 was obtained in the surface water samples (SW_1). This is indicative of hydrocarbon contamination due to the oil spill. Nickel is a micronutrient but an excessive level of the metal in the soil might be toxic to some soil fauna, like earthworms, which are adjuncts to the micro-flora in organic matter decomposition and may also reduce heterotrophic activity of the micro-flora (Osuji, 2002). The increase in Nickel level recorded in water samples (GW and SW) especially in the second year is not fully understood, however, it could be somewhat connected with the enormous Ijegun (adjacent settlement, about 1 km away) oil spill which occurred at this period.

Zinc (Zn), copper (Cu) and manganese (Mn) concentrations were all within WHO drinking water limits of 3.00mg/l, 1.00mg/l and 0.4mg/l respectively for all the water sampled within the two years of investigation. The highest levels of Zinc (Zn), copper (Cu) and Manganese (Mn) obtained were 2.65mg/l (GW1, December 2008), 0.67mg/l (GW2, February 2009) and 0.35mg/l (GW6 April 2008) respectively.

Petrol has a high penetrating ability due to its very low boiling point and volatility even at room temperature than the higher boiling point of oils like diesel. However, the latter has more chronic effect and persists longer in the environment causing harmful effects than light oils which may be evaporated before fatal damages are caused. From results obtained, especially close to the point of spills, it is revealed that the %TOC and %TOM of soil samples were relatively low as compared with those sampled away from the point of spills. Some heavy metal loads observed in soils from this study could be attributed to the low soil pH. According to Emongor (2007), low pH 5.5-5.9, is the optimal range within which heavy metals catalyse. Organic-Pb will form complexes and become more soluble and bioavailable in the soil. Chromium in some forms may be cationic and therefore adsorb onto clay particles, organic matter, metal hydroxide, and other negatively charged particles or adsorb to oxide and clay particles. Compared to water medium, the concentration of Cr ions in soil solution

may have been lower given the soil solution is a heterogenous mixture of ions which may interfere with the availability and uptake of Cr ions (Sivakumar and Subbhuraam, 2005). Metals like chromium may pose public health risks such as dermatitis especially when in direct contact with the skin while Zinc and Nickel are known to replace enzyme polymerase in DNA formation thereby leading to mutation.

Total petroleum hydrocarbon (TPH) ranged from 0.05mg/kg to 6.32mg/kg in soil providing evidence of hydrocarbon contamination at the study site. Usually, such hydrocarbon ranges deplete available oxygen and reduce gaseous diffusion in surface and subsurface soils, thereby stressing the organisms trapped beneath, some of which eventually die of asphyxiation. Such hydrocarbon levels may also discourage plant growth, thereby reducing the population density and species diversity of plant cover and other vegetations found in the affected area. The fire incidence that followed the spills had consumed a major amount of petroleum hydrocarbon at the point of spill, hence the relatively low value of petroleum hydrocarbon. The results obtained especially at the point of spills presuppose that there are still contaminable percolates of the spilled-oil at depths of the polluted site sampled. In soils, petroleum hydrocarbon creates conditions which lead to availability of heavy metals toxic to plants and the soil remains unsuitable until the crude oil is degraded to a tolerable level.

The fumes from the resultant inferno contain complex mixture of very toxic gases like toluene which is known to synergistically act with acetaminophens causing negative impact on the central nervous system (Irwin, 1997; Zhen *et al.,* 1994). This also, could have been the cause the deafening of a school girl; however, the claim was not investigated by this study.

5.2 Earthworm abundance, distribution and densities

The low diversity of earthworm species is an indication of the impact of the spill on fauna in Agaye soil. The species *E. afroccidentalis* and *H. lagosensis*, were most impacted by the spill as they were only encountered in the second year and the area 500m away from the spill. The soil within 100m of the spill was the most affected from the spill and inferno as there was low abundance of earthworm species and there was a significant difference between the abundance of earthworm species during the

period immediately after the spill but a marked improvement by the second year. Osuji and Adesanya (2005) had reported the impact of hydrocarbon contaminant on nematode and earthworms.

The marked recovery of the earthworm population size and increased diversity of earthworm species observed in the soil by the second year of the study could be attributed to natural attenuation and enhanced activities of the earthworms. Earthworm species have been shown to improve remediation of petroleum contaminated soils (Zachery and Reid, 2008a; Nana – Osei Mainoo *et al*, 2008). The improvement of soil structure and texture being enhanced by earthworm is reported to be factors contributing to increasing microbial load and action.

Greatest percentage of earthworm biomass was contributed by *L.violaceous*, followed by *D.modiglanin.* This investigation showed that biomasses of *H.lagosensis* and *E. afroccidentalis* were insignificant to the total biomass of earthworms. The species *L.violaceous* was identified to be the most abundant earthworm spp found within Agaye community, this earthworm species showed tolerance to the spill as indicated by its occurrence throughout the period of study. This also confirms the report of Segun and Owa (2003) that *L.violaceous* is commonly found in south western Nigeria.

5.3 Effect of physicochemical / climatic parameters on earthworm abundance

Although there is information on the general distribution pattern of earthworms in the SW zone of Nigeria as reported by Owa and Olojo, 2003; Segun and Owa (2003), there is limited information on the influence of oil spills on earthworm distribution patterns.

The earthworms were more abundant during the raining seasons because certain physiological activities of earthworms such as cutaneous respiration and excretion of nitrogenous ammonia and urea need moist environment. This in turn, is essential for the maintenance of their life processes (Kale and Karmegam, 2010) hence; there was a relative increase in abundance during the rainy season with higher moisture content.

Soil moisture plays a major role in earthworm abundance and diversity as observed by Edwards and Bohlen, 1996 ; Nana – Osei and Mainoo *et al.*, (2008). Earthworms are sometimes more abundant in areas with higher soil organic carbon

content as reported by Hendrix *et al*., (1992), Poier and Richter (1992); Nuutinen *et al*., (2001). Whalen, 2004 and Rossi *et al.* (2006) however disagreed with this view. The climatic parameters, (soil temperature, soil moisture and rainfall) showed seasonal fluctuations and earthworm abundance and biomass throughout the year. Earthworm activity and populations are determined essentially by the moisture content of the soil as observed in this study.

Water constitutes 75–90 percent of the body weight of earthworms. So the prevention of water loss is a major factor for their survival. They apparently lack a mechanism to maintain constant internal water content, so that their water content is influenced greatly by the water potential of the soil, which directly depends on the adequate availability of soil moisture. The temperature and moisture are usually inversely related and higher surface temperature and dry soils are limiting factors to earthworms than low surface temperature and water logged soils.

Soil temperatures also play an important role in the maintenance of earthworm population in an ecosystem and available information from this study also indicates the negative correlation of soil temperature to earthworm population. According to Radha and Ntachimuthu, (2010); temperature also largely affects activity of earthworms in temperate regions. However, tropical species are known to tolerate higher temperatures to an extent (max 30^{O}C); this could be because in tropical regions the temperature fluctuations are minimal when compared to temperate regions.

5.4 Correlation between some physicochemical parameters and earthworm abundance

In this present study only a lower percentage of the earthworm population fluctuations can be explained by the physicochemical effect and higher percentage by climatic parameters. It is presumed that the remaining may depend on other environmental factors. The correlation analysis technique may be used to quantify and rationalize the effects of physicochemical parameters on the earthworm population. However, no single factor is likely to be solely responsible for the distribution of earthworms, but rather the interaction of several factors provides suitable soil conditions for the existence of earthworm populations. Radha and Ntachimuthu (2010)

also reported a similar trend in their review on Indian indigenous earthworm distribution pattern.

The results from this investigation indicates a negative impact of the spill on the earthworm community and since earthworms play a vital role in maintaining the soil structure and texture, the impact of this spill may have affected the fertility and productivity for agricultural use.

5.5 Bioremediation

This study showed the accumulation of some heavy metals by *L. violaceous* and *D. modiglanin* and also indicated a possible positive role of *L.violaceous* in remediating heavy metals (Cr, Cd, Pb, Zn, Ni and V) and TPH except copper in the soil. Similar research by Parra *et al*, (2010) revealed that with the use of *Eisenia fetida*, there was efficiency of remediating arsenic and mercury in approximately two weeks. They recommended that *Eisenia fetida* be used to process hazardous solid and liquid wastes with high metal content. Earthworms have been reported to stimulate the degradation of petroleum hydrocarbons in soil according to reviews reported by McCosh and Getliff (2004); Zachary and Reid (2008). Zachary and Reid (2008), Kale and Karmegam (2010) and Sinha, 2010 had also reported that earthworms play a role in remediation. The effectiveness of earthworms in remediation they reported owes to the fact that earthworms' digestion pattern (microflora of their gut enhances biodegradation), turbation and borrowing activities have been shown to improve conditions suitable for microbial action thereby enhancing biodegradation. Also they absorb metals thereby bioaccumilating them into their tissues. Furthermore, Kreis *et al*., (1987) and Vandecasteele, *et al.*, (2004) reported the transfer of pollutants towards other higher trophic levels, Earthworms constitute the largest terrestrial faunal biomass and they occupy a key position.

CHAPTER SIX

6.0 CONCLUSION

This study recorded high levels of lead, cadmium and chromium found in the water and soil of the study area. The earthworm species, *L.violaceous* was the most tolerant earthworm species to petroleum pollution than other indigenous earthworm species found in the study site. The study also showed that *L.violaceous* was effective in remediating heavy metal in the soil. The recovery of a petroleum polluted site was successful monitored with the use of tropical earthworms as bioindicator and the results of physicochemical analysis and earthworm ecology provide a data base for further research of the study area.

One serious environmental problem the spillage caused is the leakage of oil into the ground water. The extent of the penetration of spill was inadequately estimated as only one borehole existed during the period of study, all wells sampled in all the houses were shallow. It is therefore necessary to monitor more ground water (boreholes) for oil and heavy metal contents. This investigation has also shown the study area have high level of TPH. There was significant difference in the measured concentration of TPH between the years of study.

There are many oil spill sites and several loss of number and diversity of fauna and flora in ecosystems. There is need to develop cheaper remediation techniques especially for third world countries like Nigeria where oil spills are common placed and response by government or oil companies are quite slow. Since the earthworm species, *L.violaceous* have shown potentials in remediation of oil contaminants, more research can be done to ascertain its efficacy, its breeding and culturing and exploiting its use as a bioremediant.

Scientists are therefore saddled with the responsibility to search for solutions that address oil independence and security, protect the global environment and stimulate global economy. Although, new technologies to improve energy efficiency and generate energy and minimizing pollution are being researched, use of fossil fuel could be significantly decreased if advanced vehicle technologies, such as electric-hybrid and hydrogen-fuel cells, are widely deployed. Renewable energy resources, such as biomass, geothermal, solar, tidal and wind, are already abundant and located

throughout the United States. In Nigeria, distributed generation of renewable energy sources is a way to diversify energy supply hence disbursing the locations of energy generation. Interdisciplinary approach of environmental problem-solving through combination of disciplines like biotechnology, microbiology, genetic engineering on the sphere of ecological practices has given rise to promising research and application of bioremediation tools.

Generally, Oil companies should be mandated to play parts in working with N.N.PC on remedial intervention in order to achieve the target values in oil spilled areas like Agaye community. It is also necessary to develop modified oil pipelines with components which will make them more difficult to tamper with. N.NP.C should foster the cooperation of indigenes, local and federal governments in the protection of oil installations. It is specifically recommended that the populace in Agaye community be educated on the dangers of using their well water and other water sources for domestic purposes. Government should construct standard and deep bore hole water supply as an alternative water source for the populace. N.N.P.C should replace obsolete pipes and town planners should ensure that construction of buildings should be as far as the stipulated perimeter distance from petroleum pipelines.

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APPENDICES

I: HEAVY METALS CONCENTRATION OF WATER SOURCES IN IJE-ODODO

Heavy metal concentration for water samples, June 2007

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.07	0.03	0.03	0.52	0.19	0.20	0.30	0.20
W2	0.04	0.02	0.02	0.61	0.18	0.21	0.10	0.15
W3	0.09	0.05	0.05	0.73	0.21	0.15	0.12	0.10
W4	0.10	0.03	0.02	0.69	0.15	0.19	0.09	0.09
Rv	0.02	0.03	0.03	0.36	0.10	0.12	1.95	0.25
Rv1	0.01	0.04	0.02	0.43	0.14	0.13	2.05	0.20
Tsch	0.02	0.03	0.02	0.26	0.15	0.13	0.10	0.20
Wsch	0.06	0.05	0.06	0.60	0.20	0.15	0.25	0.15

Heavy metal concentration for water samples, Aug 2007

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.06	0.03	0.03	0.50	0.22	0.21	0.35	0.21
W2	0.04	0.04	0.02	0.60	0.20	0.22	0.20	0.20
W3	0.08	0.06	0.06	0.60	0.30	0.10	0.22	0.20
W4	0.09	0.03	0.02	0.60	0.20	0.20	0.08	0.08
Rv	0.04	0.03	0.04	0.26	0.12	0.15	2.00	0.21
Rv1	0.01	0.05	0.03	0.52	0.15	0.15	2.10	0.30
Tsch	0.02	0.03	0.15	0.05	0.20	0.40	0.15	0.15
Wsch	0.05	0.07	0.05	0.50	0.01	0.15	0.30	0.20

Heavy metal concentration for water samples, Oct 2007

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.03	0.06	0.05	0.47	0.12	0.15	0.20	0.18
W2	0.02	0.01	0.03	0.31	0.13	0.17	0.10	0.14
W3	0.06	0.02	0.07	0.36	0.15	0.13	0.10	0.10
W4	0.05	0.03	0.01	0.52	0.20	0.17	0.05	0.07
Rv	0.01	0.03	0.03	0.39	0.10	0.10	1.80	0.28
Rv1	0.05	0.06	0.02	0.37	0.09	0.05	2.01	0.30
Tsch	0.03	0.05	0.02	0.19	0.10	0.06	0.13	0.15
Wsch	0.09	0.04	0.03	0.43	0.15	0.21	0.28	0.15

Heavy metal concentration for water samples, Dec 2007

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.03	0.02	0.02	0.27	0.04	0.06	0.19	0.10
W2	0.04	0.01	0.01	0.25	0.09	0.10	0.12	0.12
W3	0.04	0.03	0.02	0.30	0.05	0.07	0.15	0.09
W4	0.03	0.06	0.05	0.19	0.08	0.06	0.01	0.02
Rv	0.07	0.05	0.10	0.18	0.07	0.07	1.50	0.30
Rv2	0.06	0.05	0.15	0.21	0.09	0.10	1.55	0.29
Tsch	0.01	0.02	0.03	0.19	0.06	0.19	0.15	0.21
Wsch	0.05	0.01	0.02	0.14	0.11	0.15	0.21	0.19

Heavy metal concentration for water samples, Feb 2008

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.03	0.05	0.05	0.30	0.07	0.06	0.20	0.20
W2	0.04	0.02	0.03	0.27	0.10	0.05	0.15	0.18
W3	0.06	0.05	0.03	0.25	0.05	0.05	0.18	0.10
W4	0.05	0.07	0.06	0.01	0.09	0.06	0.01	0.04
Wsch	0.06	0.04	0.02	0.20	0.07	0.18	0.28	0.20
Rv	0.05	0.05	0.18	0.22	0.08	0.06	1.80	0.32

Heavy metal concentration for water samples, April 2008

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.08	0.12	0.05	0.67	0.27	0.31	0.50	0.16
W2	0.09	0.13	0.07	0.72	0.25	0.3	0.40	0.01
W3	0.17	0.1	0.08	0.6	0.21	0.29	0.55	0.19
W4	0.07	0.1	0.11	0.59	0.24	0.35	0.15	0.09
Wsch	0.09	0.1	0.05	0.45	0.19	0.19	0.30	0.15
Rv	0.1	0.11	0.06	0.62	0.13	0.25	2.00	0.91

Heavy metal concentration for water samples, Jun 2008

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.09	0.001	0.07	0.10	0.05	0.21	0.90	0.20
W3	0.05	0.002	0.06	0.14	0.01	0.25	0.50	0.15
W4	0.05	0.001	0.03	0.10	0.06	0.21	0.20	0.10
Wsch	0.06	0.003	0.09	0.15	0.05	0.16	1.00	0.25
Rv	0.05	0.001	0.07	0.12	0.07	0.22	2.50	0.20

Heavy metal concentration for water samples, Aug 2008

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.08	0.001	0.08	0.95	0.06	0.22	0.95	0.21
W2	0.05	0.001	0.08	0.09	0.04	0.22	0.90	0.20
W3	0.05	0.001	0.04	0.12	0.04	0.21	0.30	0.15
W4	0.04	0.001	0.04	0.12	0.05	0.21	0.21	0.12
Wsch	0.07	0.004	0.09	0.18	0.06	0.16	1.10	0.30
Rv	0.05	0.002	0.08	0.10	0.07	0.24	2.53	0.21

Heavy metal concentration for water samples, Oct 2008

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.005	0.001	0.007	1.05	0.29	0.15	0.98	0.21
W2	0.009	0.003	0.010	1.05	0.32	0.10	0.90	0.22
W3	0.007	0.006	0.001	0.73	0.19	0.17	0.35	0.19
W4	0.008	0.005	0.002	1.11	0.22	0.18	0.55	0.19
Wsch	0.020	0.006	0.002	0.96	0.21	0.14	1.60	0.32
Rv	0.007	0.002	0.005	2.60	0.53	0.19	2.70	0.22

Heavy metal concentration for water samples, Dec 2008

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.004	0.001	0.008	1.07	0.30	0.15	0.90	0.22
W2	0.009	0.002	0.020	1.09	0.35	0.12	0.92	0.22

W3	0.008	0.002	0.002	0.83	0.20	0.25	0.36	0.23
W4	0.007	0.003	0.002	1.15	0.23	0.20	0.58	0.21
Wsch	0.030	0.004	0.003	0.99	0.24	0.16	1.59	0.35
Rv	0.010	0.002	0.005	2.65	0.65	0.22	2.85	0.25

Heavy metal concentration for water samples, Water Feb 2009

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.004	0.002	0.009	1.08	0.40	0.20	0.99	0.25
W2	0.010	0.002	0.020	1.10	0.37	0.13	0.90	0.23
W3	0.010	0.001	0.002	0.80	0.30	0.24	0.35	0.23
W4	0.008	0.003	0.002	1.00	0.27	0.20	0.59	0.23
Wsch	0.030	0.005	0.003	0.95	0.25	0.17	1.60	0.37
Rv	0.030	0.004	0.007	2.60	0.67	0.24	2.80	0.28

Heavy metal concentration for water samples, Apr 2009

CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
W1	0.003	0.001	0.009	1.1	0.45	0.19	0.98	0.20
W2	0.009	0.001	0.010	1.1	0.30	0.10	0.91	0.24
W3	0.010	0.001	0.001	0.7	0.31	0.23	0.30	0.20
W4	0.006	0.002	0.001	1.0	0.25	0.21	0.60	0.24
Wsch	0.040	0.004	0.003	0.9	0.24	0.17	1.50	0.38
Rv	0.040	0.003	0.005	2.5	0.65	0.14	2.70	0.30

II: HEAVY METALS CONCENTRATION OF SOIL SAMPLES IN IJE-ODODO

Soil Jun 2007								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.21	0.15	0.14	2.91	1.99	5.99	2.14	0.10
S2	0.20	0.12	0.12	3.01	5.26	13.05	1.20	0.18
S3	0.19	0.10	0.12	1.96	3.29	12.01	0.50	0.12
S4	0.17	0.09	0.15	1.21	4.01	10.21	0.10	0.18
Sv2	0.23	0.17	0.13	1.83	2.01	14.02	4.05	0.15
Ssch	0.19	0.07	0.10	1.01	2.32	3.30	0.45	0.12
Sf	0.21	0.15	0.12	1.96	1.90	12.19	1.05	0.10
Sv1	0.19	0.09	0.10	0.99	1.00	3.50	8.05	0.15
Soil Aug 2007								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.22	0.13	0.16	2.95	1.95	6.01	2.20	0.12
S2	0.20	0.13	0.15	3.20	5.20	13.5	1.31	0.20
S3	0.20	0.11	0.11	1.98	2.50	11.99	0.51	0.14
Sv2	0.25	0.16	0.12	1.90	4.06	14.00	4.10	0.16
S4	0.18	0.09	0.10	1.25	2.05	0.24	0.11	0.17
Ssch	0.19	0.06	0.10	1.02	2.35	4.01	0.46	0.15
Sf	0.22	0.16	0.10	1.99	2.01	12.50	1.10	0.12
Sv1	0.19	0.10	0.15	0.98	1.09	3.51	8.00	0.16
Soil Oct 2007								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.15	0.12	0.09	1.46	1.21	3.21	1.05	0.12
S2	0.19	0.10	0.10	2.71	4.20	9.95	1.00	0.16
S3	0.32	0.06	0.17	1.71	3.00	10.99	0.55	0.19
Sv2	0.15	0.11	0.09	1.28	2.99	10.16	3.05	0.22
Sv1	0.10	0.07	0.10	1.39	1.76	5.96	8.00	0.20
Ssch	0.05	0.12	0.05	1.19	1.86	2.76	0.42	0.11
Sf	0.07	0.10	0.15	1.20	1.46	7.19	1.50	0.10
Soil Dec 2007								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.15	0.10	0.06	1.10	0.99	1.96	1.00	0.10
S2	0.19	0.09	0.10	1.02	1.09	4.32	1.00	0.15
S3	0.10	0.19	0.05	1.92	1.28	3.27	0.50	0.10
S4	0.12	0.09	0.03	1.27	1.35	2.91	6.00	0.20
Sv2	0.17	0.20	0.05	1.05	1.01	2.71	2.50	0.20
Ssch	0.11	0.06	0.07	1.65	0.96	2.91	0.42	0.20
Sf	0.18	0.08	0.10	1.31	1.92	1.69	1.50	0.15
Ssv1	0.16	0.01	0.20	1.00	1.00	1.20	4.10	0.25

Soil Feb 2008								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.15	0.10	0.04	1.00	0.95	1.90	1.01	0.11
S2	0.17	0.09	0.12	1.04	1.00	4.40	1.10	0.17
S3	0.13	0.18	0.04	1.95	1.20	3.31	0.51	0.13
Sv2	0.18	0.21	0.04	1.08	1.00	2.70	2.51	0.20
S4	0.14	0.09	0.04	1.20	1.30	2.80	0.13	0.21
Ssch	0.12	0.06	0.06	1.66	0.96	2.89	0.43	0.21
Sf	0.15	0.01	0.11	1.10	1.93	1.22	0.55	0.28
Soil Apr 2008								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.22	0.10	0.11	4.56	2.54	8.11	1.01	0.20
S2	0.34	0.05	0.13	4.92	9.74	19.21	1.20	0.16
S3	0.30	0.05	0.21	3.07	5.61	10.21	0.60	0.10
S4	0.49	0.12	0.11	4.01	6.21	5.99	0.99	0.09
Sv2	0.32	0.06	0.16	3.71	8.01	6.15	2.00	0.20
Ssch	0.35	0.17	0.09	3.61	6.36	6.05	1.00	0.09
Sf	0.13	0.19	0.11	5.01	7.01	14.09	1.50	0.19
Soil Jun 2008								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.20	0.09	0.07	4.28	2.00	6.02	1.50	0.26
S3	0.10	0.08	0.15	6.21	1.08	14.61	1.20	0.29
Ssch	0.12	0.06	0.10	5.36	0.96	13.08	1.00	0.20
S4	0.15	0.10	0.07	4.71	0.72	10.01	1.00	0.25
Sv2	0.14	0.12	0.05	3.92	0.65	9.26	4.00	0.35
Sf	0.16	0.09	0.12	4.77	4.01	8.99	1.55	0.30
Soil Aug 2008								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.21	0.09	0.07	4.30	2.20	6.01	1.70	0.29
S2	0.10	0.07	0.15	6.20	1.09	13.49	1.20	0.30
S3	0.21	0.07	0.16	6.20	1.09	14.60	1.30	0.30
S4	0.14	0.09	0.06	4.80	0.73	10.00	1.00	0.26
Ssch	0.13	0.05	0.09	5.36	0.98	13.10	1.20	0.21
Sv2	0.16	0.13	0.07	3.56	0.66	9.20	4.25	0.36
Sf	0.17	0.09	0.14	4.69	4.04	8.79	1.61	0.36
Soil Oct 2008								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.08	0.04	0.16	18.27	5.43	3.01	1.73	0.30
S2	0.06	0.05	0.12	4.03	0.96	7.29	1.21	0.31
S3	0.07	0.09	0.19	2.11	3.92	4.71	1.36	0.32
S4	0.07	0.07	0.21	2.89	0.95	8.32	1.01	0.22
Ssch	0.07	0.10	0.17	3.61	0.79	6.99	1.24	0.25
Sv2	0.09	0.05	0.15	4.20	0.96	7.03	4.32	0.38
Sf	0.04	0.08	0.11	2.61	3.07	6.12	1.65	0.39

Soil Dec 2008								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.09	0.03	0.14	9.20	5.40	3.00	1.70	0.40
S2	0.08	0.04	0.11	4.01	0.99	7.30	1.20	0.32
S3	0.05	0.08	0.21	2.01	3.90	4.75	1.39	0.33
S4	0.06	0.05	0.24	2.90	0.96	8.30	1.09	0.40
Ssch	0.06	0.13	0.17	3.02	0.78	7.01	1.20	0.29
Sv2	0.06	0.06	0.14	4.10	0.99	6.02	4.30	0.40
Sf	0.06	0.07	0.13	2.63	3.08	6.01	1.66	0.42
Soil Feb 2009								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.08	0.02	0.10	8.10	4.50	3.10	1.60	0.50
S2	0.06	0.02	0.12	4.03	0.89	7.00	1.21	0.33
S3	0.05	0.08	0.22	2.02	3.50	4.60	1.40	0.33
S4	0.07	0.06	0.26	2.96	0.99	8.31	1.00	0.40
Ssch	0.08	0.16	0.19	3.02	0.87	4.01	1.21	0.30
Sv2	0.07	0.06	0.16	4.00	0.98	6.00	4.31	0.55
Sf	0.08	0.07	0.10	2.64	3.00	6.08	1.60	0.45
Soil Apr 2009								
CODE	Cr	Cd	Pd	Zn	Cu	Mn	Ni	V
S1	0.09	0.03	0.10	4.90	4.55	3.10	1.70	0.60
S2	0.05	0.03	0.16	4.00	0.80	6.75	1.30	0.35
S3	0.05	0.09	0.25	2.01	3.43	4.67	1.45	0.36
S4	0.06	0.07	0.25	2.95	0.99	7.00	1.10	0.41
Ssch	0.05	0.15	0.19	3.00	0.91	4.03	1.20	0.31
Sv2	0.05	0.08	0.17	3.95	0.99	6.10	4.30	0.60
Sf	0.09	0.07	0.12	2.71	3.10	6.00	1.61	0.45

Metals	Soil samples									
	S_1		S_2		S_3		S_4		S_5	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Cr	0.06 –0.22	0.15±0.60	0.05 - 0.34	0.15±0.09	0.05 – 0.32	0.15±0.1	0.04 – 0.22	0.13±0.06	0.06 – 0.31	0.14±0.07
Cd	0.02 - 0.15	0.08±0.04	0.02 – 0.13	0.07±0.04	0.05 – 0.19	0.1±0.04	0.01 – 0.19	0.1±0.05	0.01 – 0.11	0.08±0.03
Pd	0.04-0.25	0.11±0.06	0.01-0.16	0.13±0.02	0.04-0.25	0.15±0.07	0.09-0.15	0.12±0.02	0.06-0.20	0.14±0.04
Zn	1.00-18.27	5.08±4.86	1.02-6.21	3.7±1.66	1.71-6.21	2.69±1.49	1.10-5.01	2.73±1.41	0.98-5.34	2.85±1.71
Cu	0.95-5.43	2.67±1.69	0.80-9.74	2.69±2.83	0.96-5.61	2.81±1.44	1.46-7.01	2.84±1.53	1.00-6.50	2.20±1.52
Mn	1.90-8.11	4.49±2.07	4.32-19.21	10.07±4.65	3.27-14.60	8.18±4.30	1.22-14.09	7.68±4.06	1.20-10.74	5.64±2.91
Ni	1.00-2.20	1.49±0.43	1.00-1.31	1.18±0.1	0.50-1.45	0.92±0.43	0.55-1.45	1.42±0.34	1.19-8.05	3.11±2.53
V	0.10-0.60	0.27±0.17	0.20-0.40	0.24±0.08	0.10-0.40	0.22±0.10	0.10-0.40	0.27±0.14	0.20-0.40	0.26±0.11
SO_4	0.03-0.23	0.14±0.07	0.08-0.40	0.21±0.11	0.05-0.33	0.16±0.1	0.07-0.32	0.17±0.08	0.08-0.25	0.18±0.05
NO_3	0.03-0.20	0.1±0.05	0.10-0.31	0.17±0.07	0.05-0.36	0.18±0.10	0.05-0.21	0.11±0.05	0.08-0.21	0.15±0.43
PO_4	0.00-0.50	0.14±0.13	0.10-0.70	0.21±0.18	0.10-0.6	0.17±0.16	0.00-0.60	0.15±0.17	0.10-0.30	0.14±0.08
pH	5.80-7.10	6.4±0.37	5.80-7.80	6.71±0.58	5.40-7.50	6.30±0.56	5.00-6.30	5.52±0.38	4.90-7.00	6.08±0.67
K	0.10-0.10	0.09±0.01	0.10-0.10	0.09±0.01	0.10-0.10	0.09±0.01	0.10-0.10	0.09±0.01	0.10-0.10	0.09±0.01
Na	0.03-0.05	0.04±0.01	0.02-0.06	0.04±0.01	0.03-0.05	0.04±0.01	0.03-0.06	0.04±0.01	0.03-0.33	0.04±0.09
Ca	2.00-2.00	2.08±0.16	2.00-2.00	2.14±0.08	2.00-2.00	2.14±0.09	2.00-3.00	2.31±0.18	2.00-2.00	2.24±0.12
TOM	1.34-3.10	1.72±0.48	1.34-2.50	1.77±0.35	1.34-4.50	2.00±0.84	1.05-4.62	2.45±1.28	1.63-4.48	2.65±1.0
TOC	0.78-1.80	1.00±0.28	0.76-1.45	1.02±0.2	0.78-2.61	1.46±0.7	0.61-2.68	1.43±0.6	0.80-2.60	1.71±0.75
THC	0.29-1.34	1.05±0.29	0.92-1.42	1.20±0.18	0.52-1.15	0.89±0.23	1.56-2.67	2.08±0.32	0.92-1.83	1.38±0.42

III: Mean soil concentration of heavy metals and other parameters

Issues with V, PO4, pH, K and Ca

IV: Mean water concentration of heavy metals and other parameters

Metals	Water samples									
	GW_1		GW_2		GW_3		GW_4		GW_5	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Cr	0.00-0.09	0.4±0.03	0.01-0.09	0.03±0.02	0.01-0.17	0.05±0.05	0.01-0.09	0.04±0.02	0.05-0.09	0.06±
Cd	0.00-0.12	0.03±0.04	0.00-0.13	0.02±0.04	0.00-0.1	0.03±0.03	0.00-0.1	0.03±0.03	0.01-0.07	0.04±
Pd	0.01-0.08	0.03±0.03	0.01-0.08	0.03±0.02	0.00-0.08	0.03±0.03	0.00-0.15	0.04±0.05	0.02-0.06	0.04±
Zn	0.0-1.10	0.67±0.36	0.09-1.10	0.65±0.39	0.12-0.83	0.5±0.26	0.05-0.99	0.48±0.38	0.14-0.60	0.42±
Cu	0.04-0.45	0.21±0.14	0.04-0.37	0.21±0.12	0.01-0.31	0.17±0.11	0.05-0.25	0.16±0.08	0.01-0.20	0.12±
Mn	0.10-0.30	0.18±0.07	0.00-0.30	0.16±0.07	0.00-0.30	0.18±0.08	0.10-0.40	0.18±0.08	0.20-0.20	0.17±
Ni	0.20-1.00	0.62±0.36	0.10-0.90	0.51±0.39	0.10-0.60	0.29±0.14	0.10-1.60	0.76±0.69	0.20-0.30	0.26±
V	0.10-0.20	0.20±0.04	0.00-0.20	0.17±0.07	0.10-0.90	0.22±0.22	0.20-0.40	0.25±0.09	0.20-0.20	0.17±
SO_4	0.05-0.19	0.11±0.04	0.05-0.14	0.09±0.03	0.06-0.19	0.12±0.05	0.04-0.16	0.10±0.05	0.02-0.07	0.04±
NO_3	0.05-0.18	0.11±0.04	0.06-0.25	0.13±0.05	0.05-0.21	0.09±0.04	0.03-0.16	0.08±0.06	0.03-0.19	0.10±
PO_4	0.00-0.20	0.1±0.05	0.00-0.20	0.11±0.05	0.00-0.20	0.12±0.04	0.00-0.20	0.14±0.09	0.00-0.10	0.08±
pH	5.0-6.13	5.7±0.35	3.47-6.80	6.0±0.9	35.96-6.89	6.45±0.29	5.10-6.89	5.73±0.54	5.06-5.60	5.43±
Temp	26.00-30.0	26.85±1.33	26.00-32.0	26.95±1.67	26.00-31.0	27.06±1.37	25.00-31.0	26.59±1.63	26.0-26.0	26.12
TH	40-216	118.96±68.80	46-240	137.05±75.25	40-270		14-220			
Alk										
Cond										
Cl										
THC										

Issues with PO4, pH, Alk, Cond, Cl and THC

V: Table showing the means and ANOVA of metals (S_1-S_5) in soils for the two years

Metals	Mean value		Sign.
	YR 1	YR2	
Cr	0.19±0.08	0.09±0.05	0.00
Cd	0.11±0.05	0.07±0.03	0.00
Pb	0.11±0.04	0.14±0.05	0.00
Zn	2.01±1.1	4.39±2.5	0.00
Cu	2.78±2.13	2.00±1.41	0.04
Mn	6.67±4.58	7.32±2.98	0.42
Ni	1.95±2.09	1.77±0.99	0.6
V	0.16±0.04	0.35±0.09	0.00
TPH	1.85±1.02	1.47±0.79	0.04

VI: Table showing the means and ANOVA of metals in soil samples, (S_1-S_5) and control soil (S_6) during the study period

Metals	Soil samples (S1-S5)	Wet land samples	Control samples (S6)	Sign
Cr	0.14±0.74	1.4±0.08	0.14±0.08	0.73
Cd	0.09±0.04	0.1±0.04	0.1±0.45	0.25
Pb	0.13±0.04	0.11±0.05	0.14±0.08	0.4
Zn	3.45±2.69	2.80±1.38	2.66±1.35	0.35
Cu	2.68±1.83	1.86±1.83	2.08±1.8	0.15
Mn	7.2±4.14	5.9±3.3	8.16±3.2	0.21
Ni	1.61±1.33	2.46±2.11	1.91±1.7	0.1
V	0.25±0.12	0.26±0.13	0.25±0.1	0.91
TPH	1.33±0.5	2.96±0.56	0.67±0.31	0.00

VII: Table showing the means and ANOVA of metals, TPH and pH in surface water (SW_1-SW_2), ground water (GW_1-GW_5) and control ground water (GW_6)

Metals	Ground water (GW1-GW5)	Ground water control (GW6)	Surface water (SW1-SW2)	Sign.
Cr	0.04±0.03	0.04±0.03	0.04±0.03	0.87
Cd	0.03±0.03	0.03±0.03	0.03±0.03	0.85
Pb	0.03±0.03	0.03±0.03	0.05±0.05	0.17
Zn	0.57±0.34	0.6±0.41	0.88±1.03	0.15
Cu	0.18±0.11	0.17±0.08	0.23±0.24	0.4
Mn	0.17±0.07	0.19±0.07	0.15±0.07	0.43
Ni	0.05±0.44	0.26±0.24	2.18±0.09	0.00
V	0.21V0.12	0.12±0.08	0.26±0.05	0.01
pH	5.82±1.04	6.10±0.68	5.28±0.4	0.04
TPH	1.34±0.64	3.31±0.54	0.64±0.26	0.00