PHYSICOCHEMICAL AND BIOCHEMICAL CHARACTERISATION OF ASPHALT PLANT DUMPSITE LOCATED AT OBINZE, IMO STATE, NIGERIA

BY

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CERTIFICATION

This is to certify that the thesis titled "**PHYSICOCHEMICAL AND BIOCHEMICAL CHARACTERISATION OF ASPHALT PLANT DUMPSITE**" was carried out by ALIGWEKWE, IKECHUKWU ANTHONY, B Tech (FUTO) with Reg No: 20124763168, of the Department of Biochemistry, Federal University of Technology, Owerri, Imo state, Nigeria.

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DEDICATION

This work is dedicated to my mother Mrs E. A Aligwekwe, my sisters Christiana Ahuchaogu, Stella Aligwekwe and Norah Chukwu, my cousins, uncles especially Engineer Emmanuel Ojoko Nwaiwu and aunties for their love, support and contributions to my success in life.

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ABSTRACT

Some physicochemical properties, levels of heavy metals, polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans, enzyme assay, microbial population of soil samples from depths of 0, 15 and 30 cm around asphalt dump site and the control site about 5 km uphill away from the asphalt dump site both located at Obinze, Owerri, Imo State, Nigeria, were analysed. The phytochemical and proximate analysis of two plant samples *Sida acuta* and *Sporobulus pyramidalis* found in the area were also analysed. These analyses were carried out in order to assess the effects of the dumps on the soils and plant samples with a view to establish the pollution or contamination status of the soil and the plants as a result of anthropogenic input. There was slight alteration in the physicochemical properties of the soil, the mean pH values of the soil samples from the dump site were 5.43 ± 0.49^a, 5.30 ± 0.18^a, 6.46 ± 0.03^c while pH values from control soil samples were 6.06 ± 0.05^b, 5.94 ± 0.07^b, 7.83 ± 0.03^d at depths of 0, 15 and 30 cm respectively. The level of most heavy metals was higher in the test soil compared to the control with manganese recording the highest concentration followed by aluminium. There was significant difference ($p < 0.05$) in the level of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans in the soil from dump site compared to the control for example PAH recorded 86.73 ± 1.73, 49.49 ± 2.79, 63.37 ± 2.60 at the dump site while the control site recorded 0.16 ± 0.02, 0.14 ± 0.10, 0.05 ± 0.00 at depths of 0, 15, and 30 cm respectively. There was also slight alteration in the enzyme activity, the result of acid phosphatase in the test soil was 3.62 ± 0.76 compared to result from control which was 0.73 ± 0.01. The microbial population in the control soil sample were higher than that in the test soil. There were significant differences in the phytochemical and proximate results of the plant samples from test soil compared to the control. The overall results show the test soil is polluted and constitutes a major health risk to the local population as a result of the asphalt dump.

Keywords: Petroleum, hydrocarbons, asphalt, phytochemical, pollution

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Soil can be defined as the top layer of the earth upon which plants grow. It is a mixture of minerals, organic matter, gases, liquids and myriad organisms that together support plant life. Soils are formed by the decomposition of rock and organic matter over many years. Soil consists of a solid phase (minerals and organic matter) as well as a porous phase that holds gases and water (Voroney, 2006; Danoff-Burg, 2014). Soil is the end product of the influence of the climate, relief (elevation, orientation and slope of terrain), biotic activities (organisms), and parent materials (original minerals) interacting over time (Gilluly, 1975).

Soil is a living-dynamic, non-renewable resource and its conditions influence food production, environmental efficiency and global balance (Dick, 1997). Soil formation or pedogenesis is the combined effect of physical, chemical, biological and anthropogenic processes working on soil parent material. Soil is said to be formed when organic matter has accumulated and colloids are washed downward, leaving deposits of clay, humus, iron oxide, carbonate, and gypsum, producing a distinct layer called the B horizon (Bishop *et al.*, 2002). These constituents are moved from one level to another by water and animal activity, and as a result, horizons or layers form in the soil profile.

Soil particles are the result of weathering of rocks and organic decomposition. Weathering is achieved by mechanical (physical) and chemical means. Mechanical weathering disintegrate rocks into small particles by temperature changes, frost action, rainfall, running water, wind, ice, abrasion, and other physical phenomena (Cheng and Jack, 2008). These cause rock disintegration by breaking, grinding, crushing, and so on. The effect of temperature change is especially important. Rocks subjected to large temperature variations expand and contract like other materials, possibly causing structural deterioration and eventual breakdown of rock material. When temperatures drop below the freezing point, water trapped in rock crevices freeze, expands, and can thereby break rock apart (Cheng and Jack, 2008). Smaller particles produced by mechanical weathering maintain the same chemical composition as the original rock.

Chemical weathering causes chemical decomposition of rock, which can drastically change its physical and chemical characteristics. This type of weathering results from reactions of rock minerals with oxygen, water, acids, salts, and so on. It may include such processes as oxidation, solution (strictly speaking, solution is a physical process), carbonation, leaching, and hydrolysis. These cause chemical weathering actions that can (i) increase the volume of material, thereby causing subsequent material breakdown; (ii) dissolve parts of rock matter, yielding voids that make remaining matter more susceptible to breaking; and (iii) react with the cementing material, thereby loosening particles. The type of soil produced by rock weathering is largely dependent on rock type (Cheng and Jack, 2008).

Soil properties vary from place to place with differences in bedrock composition, climate and other factors. This includes; soil texture, soil structure, moisture, density, porosity, consistency, temperature, colour, soil pH (acidity) and reactivity (Hannah *et al.*, 2009).

The soil texture is determined by the relative proportions of sand, silt and clay in the soil. The addition of organic matter, water, gases and time causes the soil of a certain texture to develop into a larger soil structure. At that point, a soil can be said to be developed, and can be described further in terms of colour, porosity, consistency and reaction (Six *et al.*, 2000).

Soil pH is a measure of the hydrogen ion (acid-forming) soil reactivity, and is in turn a function of the soil materials, precipitation level, and plant root behaviour. Soil pH strongly affects the availability of nutrients.

Soil performs four major functions such as;

i. It is a medium for plant growth. This is as a result of the fact that soil is a recycling system for nutrients and organic wastes thereby providing plants with the basic requirements for growth. They offer plants physical support, air, water, temperature, nutrients and protection from toxins as they can effectively remove impurities, kill disease agents and degrade contaminants.

ii. It is a means of water storage, supply and purification. Via ventilation provided by a network of soil pores, soil also absorb and hold rain water making it readily available for plant uptake.

iii. It is a modifier of the atmosphere of earth (via methods such as natural cycles which include the nitrogen cycle, water cycle, carbon cycle etc.). For instance, the carbon content stored in soil is eventually returned to the atmosphere through the process of respiration, which is carried out by heterotrophic organisms that feed upon the carbonaceous materials in the soil.

iv. It is a habitat for organisms that participate in the decomposition of organic matter and creation of habitat for other organisms. Soil has a vast range of available niches and habitats; it contains most of the earth's genetic diversity. For instance, a gram of soil can contain billions of organisms belonging to thousands of species (Donahue *et al.*, 1977).

Certain chemical elements occur naturally in soil as components of minerals, yet may be toxic at some concentrations when they exceed levels recommended for the health of humans, animals or plants (Hannah *et al.*, 2009). Other potentially harmful substances may end up in soils through human activities via which contaminants are released to the soil thereby causing soil pollution. Soil pollution may be more likely if the site has any of the following contaminants; lead paint, high traffic, use of fertilizers or pesticides, industrial or commercial activity, petroleum product spills, automobile or machine repair, junk vehicles, heavy metals, furniture refinishing, fires, landfills or garbage dumps. These together with past land use and current activities on the site affect the soil properties (Hannah *et al.*, 2009).

Studies such as that of Abii and Nwosu (2009); Henry and Heinke (2005), suggest that the most common source of soil pollution is crude oil spill and heavy metal contamination. Plants germinate, develop and grow in soil medium where water, air and nutrient resources supply plants for healthy growth for productive and profitable agriculture. Frequent crude-oil spillage on agricultural soils, and the consequent fouling effect on all forms of life, renders the soil (especially the biologically active surface layer) toxic and unproductive. The oil reduces the soil's fertility such that most of the essential nutrients are no longer available for plant and crop utilization (Abii and Nwosu, 2009). The enormity of toxicity by oil spillage on crop performance is exemplified in mangrove vegetation, which has been dying off (Henry and Heinke, 2005). Spilled crude-oil which is denser than water reduces and restricts permeability: organic hydrocarbons which fill the soil pores expel water and air, thus depriving the plant roots the much needed water and air. Heavy metals on the other hand are not biodegradable and are accumulated in living organisms when released into the environment. Although trace quantities of heavy metals are essential to animals and plants growth, they are of considerable environmental concern due to their toxicity and cumulative behaviour (Perkins, 1974).

Another source of soil pollution results from the indiscriminate dumping of asphalt and its' residues on the soil. This renders the soil un-fit for agricultural and other purposes. Asphalt used on roads is supposedly used in a manner which binds most of the harmful compounds together as part of a tough, cement-like solid; the main hazard associated with asphalt is from the polycyclic aromatic hydrocarbons (PAHs) and alkyl PAHs in asphalt that can move into the ecosystem from the breakdown of asphalt. Hazards include inhalation of compounds in heated or fresh asphalt as well as ingestion of PAHs entering the food chain as the result of breakdown of asphalt (Roy *et al.*, 1997). Ross *et al.* (1999) reported significant concentrations of PAHs and hydrocarbons were recorded in soil beneath asphalt cover. The site had not received any significant inputs from internal combustion vehicles and hence the contaminants would appear to have originated through leaching from the asphalt surface. Asphalt is considered a potential carcinogen due to the fact that it typically may contain many carcinogenic compounds, including many PAHs (Roy *et al.*, 1997).

1.2 Justification for the Study

Asphalt cement is a chemically complex material derived from crude petroleum which has been used for many years to pave thousands of miles of highways because of its availability, convenience, relatively low cost and long history of use. Due to this fact, much has not been said about asphalt being a potential toxic agent. Therefore, this study will investigate the potential toxicity arising from the disposal of asphalt wastes on the soil.

1.3 Aim of Study

The aim of this study was to determine the physicochemical and biochemical characteristic of asphalt plant dumpsite located at Obinze, Imo State, Nigeria on the soil.

1.4 Objectives of the Study

The following were the objectives of the study:

I. To determine the physiochemical properties of soil around asphalt plant dump site.
II. To determine the concentration of heavy metals, polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and dioxin-like compounds in the soil around asphalt plant dump site.
III. To determine the microbial population in the soil around asphalt plant dump site.
IV. To assay the activities of enzymes in the soil around asphalt plant dump site.
V. To determine the phytochemical and proximate composition of plants found at the asphalt dump site.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil Contamination

Soil contamination is the presence of unwanted substances on or in the soil that can cause adverse effects to the soil. Soil pollution on the other hand can be defined as a physical, biological or chemical change that makes the soil harmful and undesirable for life and agricultural purposes. It is the unfavourable alteration of the soil, wholly or largely as a by-product of man's actions. Soil pollution on the other hand can be seen as the introduction of contaminants and pollutants into the soil that can cause adverse change.

2.2 Sources of Soil Contamination

The substances that cause soil contamination include the following:

2.2.1 Lead paint

Lead is a heavy metal and one of the contents of paints manufactured before 1978. As lead paint ages and peels off or intentionally removed through activities such as stripping, scraping or sandblasting, lead can make its way into the soil surrounding homes or other buildings. The concentrations of lead in soil are usually highest right near a building, and tend to decrease with distance away from the contamination source (Hannah *et al.*, 2009).

Lead is one of the few heavy metals that have influenced the world both for good and for bad. Its extensive use by the Romans made lead to be popularly referred to as a Roman Metal (Nriagu, 2009). A number of studies have been conducted on lead use in consumer products such as premium motor spirit (PMS), paints, toils and plastics. The overwhelming evidence of the high lead concentrations in these consumer products as well as the attendant publicity, has successfully led to the total phase-out of lead in petrol (Kumar and Gottesfeld, 2008; Clark *et al.*, 2009; Adebamowo *et al.*, 2007). The extent of that global attention that effected lead phase out from petrol has so far not been extended to lead use in paint (Montgomery and Mathee, 2005).

Table 1: Lead Concentration (ppm) in New Enamel House paints in Selected Countries of Three Continents – Developing Countries.

Country	Continent	No. of Paint Samples	Average lead concentration (ppm)
Egypt	Africa	20	26,200
Nigeria	"	25	15750
Seychelles	"	28	24,880
China	Asia	64	15,070
India	"	72	29,660
Indonesia	"	11	14770
Malaysia	"	72	24,510
Singapore	"	41	6,988
Thailand	"	18	19410
Ecuador	South America	10	31,960
Peru	"	10	11,550
Total / Average		371	20,070

Source: Clark *et al.*, 2009.

Other sources of lead poisoning include mining and smelting activities and lead-battery recycling, leaded gasoline, traditional medicines, toys and discarded electronic devices (Babajide, 2011). In many developing countries, attention is more often given to disaster caused by lead exposures from smelters and battery-recycling operations than from consumer products.

In the most recent human disaster episode in Nigeria, over 300 persons died in some local government areas of Zamfara State and Niger State, as a result of poisonous lead gas emanating from mineral mining sites in those area (The Nigerian Guardian, June, 2010; WHO, 2010). The global body (WHO) stated that a random sample of 56 children under the age of five from the affected villages of Abare and Tunga-guru showed that more than 90% of them had lead poisoning, with the vast majority requiring urgent treatment. The report stated that death occurred predominantly in children under 5 years. Lead concentrations in soils greater than 10000 µg/g were found around habitations in those villages (CNN, 2010). Similar incident occurred in a battery recycling plant in the city of Thiaroye SurMer, 2008. Some 17 deaths were reported and scores of resident poisoned. In China, 15000 people from 10 different villages were relocated from Jiyuan in central Henan Province to another location after about 1000 children living around China’s largest smelter plant died (Reuters, 2009; AFP, 2009; Watts, 2009). World Health Organization, has identified lead in paint as one of the 20 risk factors contributing to global burden of disease with 40 % of children having blood lead levels greater than 5µg/dl of which 97 % are from developing countries (WHO, 2010). Subsequently, the United Nation Environment Program (UNEP), rising from the World summit on sustainable development through its Strategic Approach to International Chemical management (ICCM) and International Forum for Chemical Safety (IFCS) has placed their plan of implementation on the total phase out of the sale and manufacture of Paint-containing lead (SAICM/ICCM/INF/38, 2008).

The acceptable and holistic practical approach necessary for preventing lead poisoning in children and adult is the identification and reduction of all possible sources of lead exposure; paint inclusive (Meyer *et al.*, 2003). Markowitz (2000) stated that many countries had placed regulations on lead limit in paint. Only a few reports on lead use in paint from Africa have so far been documented. In Johannesburg South Africa, Montgomery and Mathee (2005), observed that despite the voluntary agreement to limit lead used in paint increasingly high lead concentrations continued to be used. The study found that, one sample from one of the newest suburbs contained lead concentrations as high as 29000 µg/g. The average concentration in the newest suburb rose to an all-time high of 5940 µg/g. Since 2008, a maximum limit of 600 µg/g had been put in place by the Department of Housing South Africa. In Nigeria Adebamowo *et al.* (2007) analysed 25 samples of enamel paint which consisted of 5 different colours from 5 different manufacturers and observed that the lead concentrations exceeded 600 µg/g. The reported mean and median respectively were 14500 µg/g and 15800 µg/g which varied by colour with yellow colour registering the highest. A report has also been carried out in Tanzania on the lead levels paints. The report showed that of the 26 paint samples, 20 were enamel paint. All enamel paints had concentrations greater than 90 µg/g while 19 of the enamel samples exceeded 600 µg/g, which also exceeded the voluntary lead limit of 450 µg/g in Tanzania. In all, 77 % exceeded 90 µg/g. Senegal study showed that 86 % of enamel paint sample had concentration greater than 90 µg/g while 76 % of the enamel paints were higher than 600 µg/g, an average of 4108.2 µg/g (Kumar, 2009). Presently, USEPA allows a maximum lead limit of 90 µg/g. This was sequel to the import and recall of millions of lead-based paint contaminated toys from China (LawLib, 2010). This effort and the awareness already created is yielding positive result as the percentage of housing containing lead-based paint is on the decrease (Wilson *et al.*, 2006; Jacobs *et al.*, 2002; Clark *et al.*, 2009). In India, Kumar and Gottesfeld (2008) observed that the 38 samples of new latex paint sampled contained lead concentrations lower than the national limit of 600 µg/g. In the 31 samples of enamel paint analysed, over 83 % of the enamel paint brands were very high in their lead content. The mean lead concentration of the enamel paint was about 26000 µg/g. The results of research carried out by Ahamefuna *et al.* (2014), show that lead (Pb) concentration of paints produced in Nigeria exceeded the standard limit of Pb in paint in most developed and developing countries of the world. The new US limit of Pb in paint is 90 µg/g i.e. 90 ppm. China, South Africa and India have a limit of 600 µg/g Pb concentration in paint. Out of the 59 samples analysed, it was observed that 83 % exceeded 90 µg/g Pb concentrations. Occupational Knowledge International (2014) reported titanium dioxide and zinc oxide as substitutes for lead in paint.

2.2.2 Pesticides/Fertilizers

One of the major farm inputs used in agriculture is the addition of fertilizers and pesticides to soil for adequate growth of crops and to increase yield as these fertilizers supply both micro and macro nutrients to the soil. Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate nitrogen, phosphorus and potassium for crop growth. These substances are necessary in agriculture but also have their side effects. Pesticides such as insecticides, herbicides, fungicides, rodenticides and other kind of poisons contaminate the soil as they leave behind their chemical residues especially at a pesticide mixing area. Several common pesticides used in agriculture and horticulture contained substantial concentration of metals (Hannah *et al.,* 2009).

Lead arsenate was used in fruit orchards to control parasitic insects. Arsenic containing compounds were also used extensively to control cattle ticks and pests in banana in New Zealand and Australia. Timbers have been preserved with formulations of copper, chromium and arsenic (CCA) and there are now many derelict sites where soil concentrations of these elements greatly exceed background concentration and such contamination can cause problems when sites are redeveloped for other purposes (McLaughlin *et al.*, 2000). Fertilizers on the other hand pose their own threat in the following ways; the use of fertilizers based on waste materials particularly sewage bio solid or fly ash, may result in addition of heavy metals such as copper, zinc, cadmium and lead to the soil. They also add PBTs (Persistent, bio accumulative, toxic chemicals) to soils. Fertilizers made from cement kiln dust may also contain heavy metals and dioxins. Phosphate fertilizers are known to contain some cadmium and manures are sometimes relatively high in copper or zinc (Hannah *et al.*, 2009). Addition of certain phosphatic fertilizers inadvertently adds cadmium and lead and other potentially toxic elements to the soil including fluorine, mercury and lead (Raven *et al.*, 1998) which after continued fertilizer application may significantly increase their content in soil (Raymond *et al.*, 2011).

2.2.3 Industrial activities such as metal mining/milling

The soil contaminant from this group depends entirely on the type of industry or commercial activity occurring in the area or that has occurred in the past. The level of contamination depends on factors such as the closeness of the soil to the site of activity and how long the activity has been taken place in the area e.g. asphalt plant (Hannah *et al.*, 2009). Mining and milling of metal ores coupled with industries have bequeathed many countries, the legacy of wide distribution of metal contamination in soil (Raymond *et al.*, 2011).

2.2.4 High traffic, automobile/machine repair and spoilt vehicle storage area

Soil located along high ways and lots of traffic is usually contaminated by heavy metals such as lead from gasoline; they are also contaminated with gas such as carbon monoxide from incomplete combustion of fuel (Hannah *et al.*, 2009). Polycyclic aromatic hydrocarbons (PAHs) are chemicals associated with the incomplete combustion of fossil fuels and with coal tars and asphalt. The levels of PAHs and some other chemicals may also be higher in high traffic areas as compared to other areas. The lowest levels of contamination would be expected in the areas of the property farthest away from traffic. Automobile or machine repair activities may result in accidental spills or intentional dumping of chemicals into residential or community soils. Many possible contaminants could be associated with these activities, including petroleum products, PAHs (particularly from motor oil), solvents like trichloroethylene (TCE), used tires and rubber products, metals (used engine oil may contain chromium, lead, molybdenum, or nickel from engine wear), or used batteries (which may release lead or mercury). Spoilt vehicles may also be a source of these chemicals or other contaminants, depending on their condition and how and where they are stored (Hannah *et al.*, 2009).

2.2.5 Treated lumber and furniture refinishing

Arsenic, in the form of chromated copper arsenate (CCA), has been used in wood preservatives to make pressure-treated lumber. Some of the arsenic in CCA-treated wood can move from the wood to nearby soil. Some chemical strippers used in furniture refinishing contain methylene chloride and other solvents, including toluene and methanol. These substances can contaminate the soil and groundwater if handled improperly during commercial operations or projects (Hannah *et al.*, 2009).

2.2.6 Landfills/garbage dumps

Different soil contaminants can leach from landfills or other garbage disposal sites, including petroleum products, solvents, pesticides, lead and other heavy metals. The chemicals that may be present in soils near locations used for waste disposal (currently or in the past) will depend on the specific conditions of a particular site, and on what types of materials were disposed of at that site (Hannah *et al.*, 2009).

2.2.7 Petroleum spills and fire

Petroleum leaks or spills from gas stations, oil pipelines, fuel tanks, or other activities can result in elevated levels of contaminants such as benzene, toluene, and xylene in the soil.

Some of these chemicals (especially volatiles) are unlikely to remain in the surface soil where they would be taken up by plants or be in direct contact with humans, unless the spill was very recent or large. Sometimes this results in fire outbreak. The intentional or accidental burning of materials can produce and release PAHs, dioxins or other chemicals into soils, depending on what was burned and how long ago. Burning yard wastes, such as tree branches, is much less likely to release harmful contaminants than intentional or accidental fires that burn garbage, buildings or their contents, or other synthetic substances (Hannah *et al.*, 2009).

2.2.8 Bio solids and manures

The application of numerous biosolids (e.g. livestock manures, composts and municipal sewage sludge) to land inadvertently leads to the accumulation of heavy metals such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Ti, etc. in soil (Basta *et al.*, 2005). In the pig and poultry industry, the Cu and Zn added to diet as growth promoters and As contained in poultry health products may also have potential to cause metal contamination of the soil if repeatedly applied to the soil (Sumner, 2000; Chanley and Oliver, 1996). Studies on some New Zealand soils treated with biosolids have shown increased concentration of Cd, Ni and Zn in drainage leachates (Keller *et al.*, 2002; McLaren *et al.*, 2004).

2.2.9 Wastewater

Application of municipal and industrial wastewater and effluents to land dates back 400 years and now is a common practise in the world (Reed *et al.*, 1995). Worldwide, it is estimated that 20 million hectares of arable land are irrigated with wastewater. In several Asian/African cities, studies suggest that agriculture based on wastewater irrigation accounts for 50 % of the vegetable supply to urban areas (Bjuhr, 2007). Farmers are not bothered about environmental benefits/hazards but primarily interested in maximizing their yields and profits. Although the metal concentration in waste water effluents are usually relatively low, long term irrigation of land with such can eventually result in heavy metal accumulation in the soil (Raymond *et al.*, 2011).

2.3 Major Soil Pollutants

In addition to the contaminants listed above, there are major pollutants which cause danger to the soil, plants and other living organisms that comes in contact with the soil. These pollutants include; heavy metals, polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and dioxin-like compounds.

2.3.1 Heavy metals

Environmental pollution as a result of man's increasing activities such as burning of fossil fuels and automobile exhaust emission has increased considerably in the past century due mainly to significant increases in economic activities and industrialization. Burning of fossil fuels and petroleum industry activities have been identified as primary sources of atmospheric metallic burden leading to environmental pollution. Several studies have shown that heavy metals such as lead, cadmium, nickel, manganese and chromium amongst others are responsible for certain diseases (Hughes, 1996). Many authors have reported high levels of heavy metal ions in the soil, rivers and groundwater in different areas of Nigeria (Ibeto and Okoye, 2010). Soil may become contaminated by accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine failings, disposal of high metal wastes, leaded gasoline/paint, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals and atmospheric deposition (Khan *et al.*, 2008; Zhang *et al.*, 2010). Soils are the major sink for heavy metals released into the environment by anthropogenic activities and unlike organic contaminants which are oxidized to CO_2 by microbial action, most metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.*, 2006) and their total concentration in soils persist for a long time after their introduction (Adriano, 2003).

In general, heavy metals are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects (Hughes, 1996). Heavy metals can directly influence behaviour by impairing mental and neurological function, influencing neurotransmitter production and utilization, and altering numerous metabolic processes. Systems in which toxic metal elements can induce impairment and dysfunction include the blood and cardiovascular, eliminative pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive and urinary that have lethal effects on man and animals. These diseases include abdominal pain, chronic bronchitis, kidney disease, pulmonary oedema (accumulation of fluid in the lungs), cancer of the lung and nasal sinus ulcers, convulsions, liver damage and even death (Hughes, 1996).

Soil being an important component of terrestrial ecosystem supports plant growth and biogeochemical cycling of nutrients, these wastes end up interacting with the soil system thereby changing the physical and chemical properties. Akubugwo *et al.* (2010) reported that soil heavy metal contamination results in the uptake of metals by plants causing accumulation of these metals in plant tissues, hampered crop quality and phytotoxicity. Vousta *et al.* (1996) demonstrated that high level of heavy metals in soil could indicate similar concentration in plants by accumulation at high concentration causing serious risk to human health when consumed. Fu *et al.* (2008) reported that accumulation of heavy metals in crops grown in metal-polluted soil may easily cause damage effect on human health through food chain. Excessive concentration of these metals in food is associated with aetiology of a number of diseases (WHO, 1992; 1995). Some heavy metals like As, Cd and Pb have been reported to have no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (Nolan, 2003). Due to the non-biodegradable and persistent nature, heavy metals are accumulated in vital organs in the human body such as the kidneys, bones and liver and are associated with numerous serious health disorders (Duruibe *et al.*, 2007). The table below shows the regulatory limits of some heavy metals.

Table 2: The heavy metal concentration of soils with regulatory limits.

Heavy Metals	Maximum Concentration in soil mg/kg (ppm)
As	75
Cd	85
Cr	3000
Cu	4300
Pb	420
Hg	840
Mo	57
Ni	75
Se	100
Zn	7500

Source: U. S. EPA, 1993/1996.

Some of these heavy metals and their effects are highlighted below:

2.3.1.1 Lead

Lead is a naturally occurring metal that has been used in many industrial activities and therefore many occupations may involve exposure to it such as auto-mechanic, painting, printing, welding etc. In the atmosphere, lead exists primarily in the form of $PbSO_4$ and $PbCO_3$. Lead in paints and automobile exhausts are still recognized for its toxicity (Hughes, 1996). Episodes of poisoning from occasional causes such as imperfectly glazed ceramics (Matte *et al.*, 1994), the use of medicines which may contain as much as 60 % lead available from Asian healers and cosmetic preparations, may affect any age group and cases may present as acute emergencies (Bayly *et al.*, 1995). The main source of adult exposure is food, air inhalation accounts for 30 % and water of 10 % (John *et al.*, 1991).

Lead is a ubiquitous environmental toxin capable of causing numerous acute and chronic circulatory, neurological, haematological, gastrointestinal, reproductive and immunological pathologies (Nemsadze, 2009). The mechanism of lead induced toxicity is not fully understood. The prime targets to lead toxicity are heme synthesis enzymes, thiol-containing antioxidants and enzymes (superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, glutathione peroxidase and antioxidant molecules like GSH). Low blood lead levels are sufficient to inhibit the activity of these enzymes and induce generation of reactive oxygen species and intensification of oxidative stress. Oxidative stress plays an important role in pathogenesis of lead-induced toxicity. The primary target of lead toxicity is the central nervous system and there is different cellular, intracellular and molecular mechanism of lead neurotoxicity such as induction of oxidative stress, intensification of apoptosis of neurocites, interfering with Ca^{2+} dependent enzyme like nitric oxide synthase (Nemsadze, 2009). Lead exposure also leads to subsequent development of hypertension and cardiovascular disease. The vascular endothelium is regarded as the main target organ for toxic effect of lead. Lead affects the vasoactive function of endothelium through the increased production of reactive oxygen species, inactivation of endogenous nitric oxide and down regulation of soluble guanylatecyclase by reactive oxygen species leading to limiting nitric oxide availability, impairing nitric oxide signalling (Nemsadze, 2009). Lead exerts numerous adverse mechanism of toxicity. It has a high affinity for sulfhydryl groups. It is therefore particularly toxic to multiple enzyme systems. Many of lead's toxic effects also result from its inhibition of cellular function requiring calcium. Lead binds to calcium-activated proteins with much higher (105 times affinity than calcium). For instance Pb^{2+} and Ca^{2+} compete at the plasma membrane for transport systems, which affect their entry or exit. It thereby disturbs intracellular Ca^{2+} homeostasis (Christopher, 2015).

2.3.1.2 Cadmium

The principal form of cadmium in air is cadmium oxide, although some cadmium salts, such as cadmium chloride, can enter the air, especially during incineration. Environmental discharge of cadmium due to the use of petroleum products, combustion of fossil fuels (petroleum and coal) and municipal refuge contribute to airborne cadmium pollution (De Rosa *et al.*, 2003) and possibly introduce high concentrations of this potential reproductive toxicant into the environment. This may be particularly true for Nigeria where refuse are burnt without control. In addition, humans may be unwittingly exposed to cadmium via contaminated food or paper (Wu *et al.*, 1995) cosmetics and herbal folk remedies (Lockitch, 1993). All these factors put Nigerian population at high risk of cadmium toxicity (Okoye, 1994). The greatest potential for above average exposure of the general population to cadmium is from smoking which may double the exposure of a typical individual. Smokers with additional exposure are at highest risk (Elinder, 1985). Soil distribution of urban waste and sludge is also responsible for significant increase in cadmium content of most food crops (WHO, 1996). People, who have cadmium-containing plumbing, consume contaminated drinking water or ingest grains or vegetables grown in soils treated with municipal sludge or phosphate fertilizer may have increased cadmium exposure (Elinder, 1985).

2.3.1.3 Nickel

Exposure to nickel can be as a result of breathing air, drinking water or smoking tobacco containing nickel. It can also be as a result of skin contact with soil, water, metals containing or coated with nickel as well as jewellery plated with nickel or made from nickel alloys (Gerbeding, 2005b). Exposure of an unborn child to nickel is through the transfer of nickel from the mother's blood to foetal blood. Likewise, nursing infants are exposed to nickel through the mother to breast milk (Gerbeding, 2005b). However, the concentration of nickel in breast milk is either similar or less than the concentration of nickel in infant formulas and cow's milk. Children may also be exposed to nickel by eating soil. Normally, the exact form of nickel one is exposed to is not known. It could be in form of nickel sulphate, nickel oxide, nickel silicate, iron-nickel oxides, nickel subsulfide or metallic nickel (Gerbeding, 2005b).

2.3.1.4 Manganese

Manganese in the environment is in the form of their oxides or carbonates e.g. MnO_2, $MnCO_3$ etc. Populations living in the vicinity of ferromanganese or iron and steel manufacturing facilities, coal-fired power plants, or hazardous waste sites are exposed to elevated manganese particulate matter in air or water, although this exposure is likely to be much lower than in the workplace (Koplan, 2000a). Manganese is eliminated from the body primarily through the bile. Interruption of the manufacture or flow of bile can impair the body's ability to clear manganese. Several studies have shown that adults and children as well as experimental animals with cholestatic liver disorders have increased manganese levels in their blood and brain and are at risk from potentially increased exposure to manganese due to their decreased homeostatic control of the compound (Devenyi *et al.*, 1994).

Persons who live close to high density traffic areas, automotive workers, and taxi drivers may be exposed to higher concentrations of manganese arising from the combustion of methylcyclopentadienyl manganese tricarbonyl (MMT) (Koplan, 2000a). Methylcyclopentadienyl manganese tricarbonyl is actually a fuel additive developed in the 1950s to increase the octane level of gasoline and thus improve the antiknock properties of the fuel. Farmers, people employed as pesticide sprayers, home gardeners etc. may also be exposed to higher concentrations of these pesticides than the general public. People who ingest fruits and vegetables that have been treated with these pesticides and that contain higher-than-usual residues of the compounds (due to incomplete washing or over-application) may be exposed to increased concentrations of the pesticides (Koplan, 2000a).

2.3.1.5 Chromium

Blue prints, primer paints, household chemicals and cleaners, cements, diesel engines utilizing anti-corrosive agents, upholstery dyes, leather tanning processes, welding fumes, battery, rubber, dye, candles, printers and matches are occupational and environmental sources of chromium (Koplan, 2000b). People may also be exposed to higher levels of chromium if they use tobacco products, since tobacco contains chromium. Workers in industries that use chromium are one segment of the population that is especially at high risk to chromium exposure. Occupational exposure from chromate production, stainless steel welding, chromium plating, and ferrochrome and chrome pigment production is especially significant since the exposure from these industries is to chromium (VI) (EPA, 1984a). Persons using contaminated water for showering and bathing activities may also be exposed via inhalation to potentially high levels of chromium (VI) in airborne aerosols. Elevated levels of chromium in blood, serum, urine, and other tissues and organs have also been observed in patients with cobalt-chromium knee and hip arthroplasts (Koplan, 2000b). Chromium in the environment can exist in many forms e.g. chromium trioxide, potassium dichromate, sodium dichromate, potassium chromate, sodium chromate or ammonium dichromate etc.

The major sources of heavy metal pollution in urban areas of Africa are anthropogenic while contamination from natural sources predominates in rural areas. Anthropogenic sources of pollution include those associated with fossil fuel i.e. the non-renewable energy resources of coal, petroleum or natural gas (or any fuel derived from them) combustion, mining and metal processing (Nriagu, 1996). Fossil fuel consumption in Nigeria has risen ten-fold in the last two decades and consumption by urban households' accounts for a large percentage, a trend which is expected to continue in the future. Pollution problems associated with incidents of oil spills around automobile repair workshop resulting in metal contamination have been the subjects of many reports (Onianwa *et al.*, 2001). Lead, cadmium, nickel, manganese and chromium are associated with automobile related pollution. They are often used as minor additives to gasoline and various auto-lubrication and are released during combustion and spillage (Lytle *et al.*, 1995). It is estimated that 8.5 million kg of nickel are emitted into the atmosphere from natural sources such as windblown dust and vegetation each year. Five times that quantity is estimated to come from anthropogenic sources (Nriagu and Pacyna, 1988) and the burning of residual and fuel oil is responsible for 62 % of anthropogenic emissions. Chromium is released into the atmosphere mainly by anthropogenic stationary point sources including industrial, commercial and residential fuel combustion via the combustion of natural gas, oil, and coal. It has been estimated that emissions from the metal industry ranged from 35 % to 86 % of the total chromium and emissions from fuel combustion ranged from 11 % to 65 % of the total chromium. The main sources of manganese release to the air are industrial emissions, combustion of fossil fuels and re-entrainment of manganese-containing soils (EPA, 1987). High concentration of cadmium is released by human activities such as mining smelting operations and fossil fuel combustion.

Coal, wood and oil combustion can all contribute cadmium to the atmosphere. It has been suggested that coal and oil used in classical thermal power plants are responsible for 50 % of the total cadmium emitted to the atmosphere (Thornton, 1992). Anthropogenic sources of lead also include the mining and smelting of ore, manufacture of lead-containing products, combustion of coal and oil most notably leaded gasoline that may still be used in some countries including Nigeria. The use of leaded petrol has been banned in many parts of the world. Currently only some African countries and some Eastern European and Asian nations still use leaded petrol. In order to achieve a cleaner environment and improve public health, 5 West African countries Benin, Chad, Niger, Nigeria and Togo set a deadline of 2004 to phase out distribution of leaded petrol from their territories (Abiodun, 2002). Lead (Pb) is added to fuel as a cost-effective way of increasing the octane number and providing a measure of protection from valve seat wear, which occurs when metal to metal contact of the valve and seat faces cause premature wear (Segui, 2005). The presence of trace metals and non-metals in the crude oil and refined petroleum products is destructive, especially in a refining process (Tijjani *et al.*, 2012). Several toxic metals, including arsenic, cadmium, lead, zinc, antimony, and their compounds, are associated with fine particulate matter in ambient air and are known to be emitted during the combustion of fuel in electric power plants, engine of vehicles, furnaces and fire places (Atiku *et al.*, 2011). Anthropogenic sources of Pb account for its most common sources in the environment largely because it has been widely used throughout history and remain persistent in the environment. These sources also are the most prevalent sources of human Pb exposure, including exposure of the general population and occupational (U.S. Environmental Protection Agency, 1986). A variety of sources and activities contribute to air emissions of Pb, including mobile, area, and stationary sources. In the recent past, the major source of Pb-containing air emissions was the consumption of leaded gasoline in motor vehicles. In 1984 over 89 % of an estimated total of 39,000 tons of

Pb emitted was attributed to gasoline consumption (U.S. Environmental Protection Agency, 1986).

It is important to note that land is the ultimate repository for lead, and lead released to air and water ultimately is deposited in soil or sediment. For example, lead released to the air from leaded gasoline or in stack gas from smelters and power plants will settle on soil, sediment, foliage or other surfaces (Gerbeding, 2005a).

Lead in the form of tetra-ethyl lead $Pb(C_2H_5)_4$ is the most common additive to petrol to raise its octane number. Upon combustion in the petrol engine, the organic lead is oxidized to lead oxide according to the following reaction:

$$2Pb\,(C_2H_5)_4 + 27O_2 \longrightarrow 2PbO + 16CO_2 + 20H_2O$$

The lead oxide (PbO) formed, reacts with the halogen carriers (the co-additives) to form particles of lead halides- $PbCl_2$, PbBrCl, $PbBr_2$- which escape into the air through the vehicle exhaust pipes. By this, about 80% of lead in petrol escapes through the exhaust pipe as particles while 15-30 % of this amount is air borne. Human beings, animal and vegetation are the ultimate recipients of the particulate (Ademoroti, 1996).

Atmospheric lead emissions in Nigeria have been estimated to be 2800 metric tonnes per year with most (90 %) derived from automobile tail pipe (Nriagu *et al.*, 1997). In 2011, atmospheric lead emission in Nigeria was estimated to be 1.28 metric tonnes per year. This can be attributed to the fact that lead concentrations ranged from 0.491-1.903 mg/L for gasoline which is below the Department of Petroleum Resources (DPR) Nigeria limit of 3.37 mg/L, emissions from diesel was 2.301-10.97 mg/L (no lead limit was specified by DPR for the lead level of diesel fuel) (Jimoda *et al.*, 2014). Therefore, the percentage lead contributions from gasoline and diesel fuel consumption were minimal due to various fuel lead-level regulations that have been established over the years (Jimoda *et al.*, 2014).

2.3.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a family of organic compounds with a molecular structure consisting of two or more fused aromatic (benzene) rings in linear, angular or cluster arrangements (Sander and Wise, 1997). By definition they contain carbon and hydrogen atoms although nitrogen, sulphur, chlorine and oxygen may readily substitute into the benzene ring to form heterocyclic aromatic compounds, commonly grouped with the PAHs. The PAH family includes 660 substances indexed by the National Institute of Standards and Technology (Sander and Wise, 1997).

Approximately 30 to 50 of them commonly occur in the environment (Grimmer, 1983; US DHHS, 1995). PAHs are formed as a result of incomplete combustion of organic matter through the condensation of ethylenic radicals in the gas phase to form the larger polycyclic compounds (Lane, 1989; Strosher, 1996).

Polycyclic aromatic hydrocarbons may be subdivided into two groups based on whether they exist in the gas or solid (particle) phase. The phase state of a given PAH is determined by its vapour pressure and ambient temperature. Compounds with vapour pressures in excess of $1x10^{-5}$ kPa occur predominantly in gas phase, while those with vapour pressures below $1x10^{-9}$ kPa exist exclusively in the particle phase (Bacci *et al.*, 1990).

Another important characteristic of PAHs is their water solubility, which can be described using the octanol-water partitioning coefficient (K_{ow}). Low molecular weight, volatile PAHs with less than four rings are water soluble with a low affinity for particle adsorption (log $K_{ow} < 5$). PAHs with high molecular weights are generally water insoluble and have a strong affinity for adsorption onto particle surfaces that are suspended in air and water (log $K_{ow} > 5$). For example, solubility in distilled water at 25°C varies from 31,690 ppb for the two-ringed naphthalene (log K_{ow} – 3.29) to 1.8 ppb for the four-ringed chrysene (log K_{ow}– 5.86) (May, 1980).

Since PAHs possess lipophilic properties, they tend to associate with organic matter containing fatty acids and waxes such as plant membranes and cuticles. Pollutant metabolites and breakdown products are occasionally more convenient to measure than the pollutant itself.

The metabolic degradation of PAHs by pure cultures of a variety of bacteria, fungi and primitive algae has been documented and the biochemical pathways have been elucidated (Varanasi, 1989; Cerniglia, 1992).

Polycyclic aromatic hydrocarbons, considered as xenobiotics because of their physico-chemical nature (Connell, 1990), are expected to be metabolized in a similar fashion as other xenobiotics. There are three main steps involved in the metabolism of xenobiotics (Martinoia *et al.*, 1993). In the first step compounds are reduced or oxidized. PAH toxicity may be enhanced through these processes. This phenomenon is referred to as bio-activation. In the second step PAHs are further metabolized through conjugation with amino acids, oligopeptides or hexoses. This step of degradation of xenobiotics was confirmed to occur under exposure to airborne PAH in four species of woody plants. Nakajima *et al.* (1996), quantitatively measured synthesis of β-*O*-glucoside and β –*O*glucuronide conjugates of 1-hydroxypyrene in leaves of cherry, maple, ginko and camphor plants growing at two sites near busy highways within the Tokyo metropolitan area. There were large differences in the amounts of conjugates and conjugate/pyrene ratio among species. These differences may be caused by variations in conjugation activity and enzymatic hydroxylation activity for pyrene. The authors concluded that the amounts of atmospheric PAHs scavenged by vegetation were much larger than estimates based on measurements of intact compounds in plant tissues. The ability of plants to metabolize pyrene has also been demonstrated by Huckelhoven *et al.* (1997). They found that in wheat about 90 % of applied pyrene was transformed into a complex mixture of carbohydrate conjugates while 1-hydroxypyrene methyl ether was the main metabolite accumulated in foxglove. The third step in the metabolism of xenobiotics involves the excretion of conjugated compounds from the cytoplasm. This is done by glutathione S-conjugate transporters, which transport toxins to the vacuole. Although conjugated PAHs might also be sequestered to vacuoles in a similar fashion, no experimental data is available (Jan *et al.*, 2000).

They occur normally in nature primarily as a result of forest fire; however anthropogenic combustion activities and refining processes of fossil fuels are principal sources of these compounds in ambient air. Concentrations of PAHs in air, water and soils have constantly increased over the last 100 years, particularly in industrialized areas (Jones *et al.*, 1989). In their purest form, PAHs are solid and range in appearance from colourless to white or pale yellow-green. PAHs are created when products like coal, oil, gas, and garbage are burned but the burning process is not complete (USEPA, 2008). Natural sources of emissions such as prairie and forest fires or volcanic eruptions contribute to the global background of PAHs in the atmosphere. Anthropogenic activities such as automobile and furnace exhausts, coal and oil-fired power plants, gasification/liquefaction of fossil fuels, coke and asphalt production, waste incinerators, aluminium smelting and gas and oil flare operations are the primary sources of air borne forms of these compounds. These sources result in emissions of PAHs into all environmental compartments, including soil, vegetation, aquatic bodies and their sediments. Oil spill is another major source of PAH emission into aquatic bodies and soil. (Jan *et al.,* 2000). It should be noted that some PAHs are listed individually on EPA's priority Chemical list. They are:

I. Acenaphthene, 83-32-9

II. Acenaphtylene, 208-96-8

III. Anthracene, 120-12-7

IV. Benzo(g,h,i)perylene, 191-24-2

V. Fluorene, 86-73-7
VI. Phenanthrene, 85-01-8
VII. Pyrene, 129-00-0 (USEPA, 2008).

PAHs are a concern because they are persistent. Because they do not burn very easily, they can stay in the environment for long periods of time. Individual PAHs vary in behaviour. Some can turn into a vapour in the air very easily. Most do not break down easily in the water.

One of the most common ways PAHs can enter the body is through breathing contaminated air, eating food or drinking water contaminated with PAHs and skin contacts with PAH-contaminated soil or products like heavy oils, coal tar, roofing tar, or creosote. Creosote is an oily liquid found in coal tar and is used to preserve wood. Once in your body, PAHs can spread and target fat tissues. Target organs include the kidneys and liver. However, PAHs will leave your body through urine and faeces in a matter of days.

PAHs exist naturally in the environment and are man-made; you can be exposed in a number of ways. Fumes from vehicle exhaust, coal, coal tar, asphalt, wildfires, agricultural burning and hazardous waste sites are all sources of exposure. You could be exposed to PAHs by breathing cigarette and tobacco smoke, eating foods grown in contaminated soil, or by eating meat or other food that you grilled. Grilling and charring food actually increases the amount of PAHs in the food. If you work in a plant that makes coal tar, asphalt and aluminum, or that burns trash, you can be exposed to PAHs. You can also be exposed if you work in a facility that uses petroleum or coal, or where wood, corn, and oil are burned (USEPA, 2008).

A number of PAHs have caused tumours in laboratory animals that were exposed to PAHs through their food, from breathing contaminated air, and when it was applied to their skin. When pregnant mice ate high doses of a PAH (benzo[a]pyrene) they experienced reproductive problems. In addition, the offspring of the pregnant mice showed birth defects and a decrease in their body weight. Other effects include damage to the skin, body fluids, and the immune system. However, these effects have not been seen in humans. There is no information available from studies on humans to tell what effects can result from being exposed to individual PAHs at certain levels. However, breathing PAHs and skin contact seem to be associated with cancer in humans (USEPA, 2008).

High occupational exposure to PAHs occurs in several industries and occupations, some of which includes aluminium production, coal gasification, coke production, iron and steel foundries, tar distillation, shale oil extraction, wood impregnation, roofing, road paving (with asphalt), carbon black production, carbon electrode production etc. Workers exposed to diesel engine exhaust in the transport industry and in related occupation are exposed to PAHs and nitro-PAHs (Boffelta *et al.*, 1997). Heavy exposure to PAHs entails a substantial risk of lung, skin and bladder cancer, which is not likely to be due to other carcinogenic exposure present in the same industries. The lung seems to be the major target organ of PAH carcinogenicity. Increased risk of skin cancer follows high dermal exposure. An increase in bladder cancer risk is found mainly in industries with high exposure to PAHs from coal tars and pitches (Boffelta *et al.*, 1997).

Ross *et al.* (1999) observed that significant concentrations of PAHs and hydrocarbons were recorded in soil beneath an asphalt cover. The site had not received any significant input from internal combustion vehicles. Therefore, a significant amount of PAH can enter the environment (soil) through leaching from asphalt surfaces. This implies that some of the road side PAH contamination probably results from this pathway in addition to deposition from exhaust fumes. Recycling of asphalt has been promoted by rapid increase in both the use and price of petroleum-based bitumen. Malin *et al.* (2004) discovered that large quantities of semi-volatile organic compounds (such as naphthalene, butylated hydroxyl toluene (BHT) and dibutyl phthalate (DBP) and PAHs were found in leachates from reclaimed asphalt pavement. The major leaching mechanism indicated was dissolution of organic contaminants from the surface of asphalt gravels.

PAH are mainly produced by combustion processes and consists of a number of toxic compounds. While the concentration of individual PAHs in soil produced by natural processes (e.g. vegetation fire, volcanic exhalations) are estimated to be around 1-10 µg/kg, recently measured lowest concentration are frequently 10 times higher. Organic horizons of forest soils and urban soils may even reach individual PAH concentrations of several 100 µg/kg.

PAHs accumulate in C-rich topsoil, in the stem foot area, at aggregate surfaces and in fine-textured particle fractions, particularly the silt fraction. PAHs are mainly associated with soil organic matter (SOM) and soot-like carbon. Although the water solubility of PAHs is low, they are encountered in the subsoil suggesting that they are transported in association with dissolved oxygen matter (DOM). The uptake therefore of PAHs by plants is small as most PAHs detected in plant tissue are from atmospheric deposition (Wolfgang, 2000). The N-heterocyclic derivatives of PAHs (NPAHs such as phenanthridine, acridine, benzo[h]quinoline etc.) are widespread concomitantly with their parent analogues and have been detected in air, water, sediments and soil (Pakova *et al.*, 2006).

In their study, Pakova *et al.* (2006) showed that PAHs and NPAHs are toxic to plants. This study evaluated toxic effects of 3 homocyclic aromatic hydrocarbons (PAHs- phenanthrene, anthracene, fluorene) and their seven N-heterocyclic derivatives on higher terrestrial plants *Sinapis alba*, *Triticum aestivum* and *Phaseolus vulgaris*. Germinability, morphological endpoints, parameters of detoxification and antioxidant components of plant metabolism as well as lipid peroxidation were studied in acute phytotoxicity test. The phytotoxicity of NPAHs was generally more pronounced than the effects of parent PAHs. These chemicals inhibited germination/growth of plants, induced activities of detoxification and antioxidant enzymes (glutathione reductase, glutathione peroxidase and glutathione-s-transferase) at nano-molar to low micro-molar concentrations and also increased lipid peroxidation (Pakova *et al.*, 2006).

2.3.3 Total Petroleum Hydrocarbons

Total petroleum hydrocarbons (TPH) are defined as carbon chains in the range of C6 through C35. Total petroleum hydrocarbon is a term used for any mixture of hydrocarbons that are found in crude oil. There are several hundreds of these compounds but not all occur in any one sample (DEQ, 2012). Crude oil is used to make petroleum products that can contaminate the environment. Due to the fact that there are many different chemicals in crude oil and other petroleum products, it is not practical to measure each one separately. However, it is useful to measure the total amount of TPH at a site. The chemicals that occur in TPH include hexane, benzene, toluene, xylenes, naphthalene, fluorine, other constituents of gasoline, of jet fuels and of mineral oils and other petroleum products (DEQ, 2012). Evaluations for overall TPH are common and generally accepted. Some hydrocarbon mixtures may also contain priority pollutants including volatile organic compounds (VOCs), semi-volatile compounds (SVOCs), and metals, each of which have their own specific toxicity information. Other TPH mixtures do not contain significant levels of priority pollutants.

There are three ranges of TPH which are monitored at various levels depending on the state and site. Department of environmental quality (DEQ) Oklahoma defines three ranges of TPH which are; gasoline range (GRO) >C6-C10, diesel range (DRO) >C11-C28 and lube oil range >C28-C35 (DEQ, 2012). Total petroleum hydrocarbons are also the sum of volatile petroleum hydrocarbon (VPH) and extractable petroleum hydrocarbon (EPH). Volatile petroleum hydrocarbons are also known as petrol range organics (PRO) and includes hydrocarbons from C2-C5. Extractable petroleum hydrocarbons are also known as diesel range organics (DRO) and includes hydrocarbons from C6-C40 (DEQ, 2012).

2.3.4 Dioxin and Dioxin-Like Compounds (Furans)

Dioxins and dioxin-like compounds are chlorinated organic pollutants formed as trace amounts of undesired impurities in the manufacture of other chemicals such as chlorinated phenols and their derivatives, chlorinated diphenyl ethers, and polychlorinated biphenyls (PCB) (WHO, 1988) and combustion of chlorine containing materials under some conditions. These compounds are also referred to as unintentionally produced persistent organic pollutants (UPOP). Dioxins and furans are chlorinated, planar tricyclic aromatic hydrocarbons representing a class of compounds that includes 210 possible congeners. The group dioxins comprise 75 polychlorinated dibenzo-p-dioxin (PCDD) congeners and 135 polychlorinated dibenzofuran (PCDF) congeners. There are no known technical uses for PCDD and PCDF (WHO, 1988). PCDD/F congeners are named according to the position of the chlorine atoms on the molecule. Each possible position on the aromatic cycle is designated by a number from one to nine. The basic structure and numbering of each chemical class are shown in figure below.

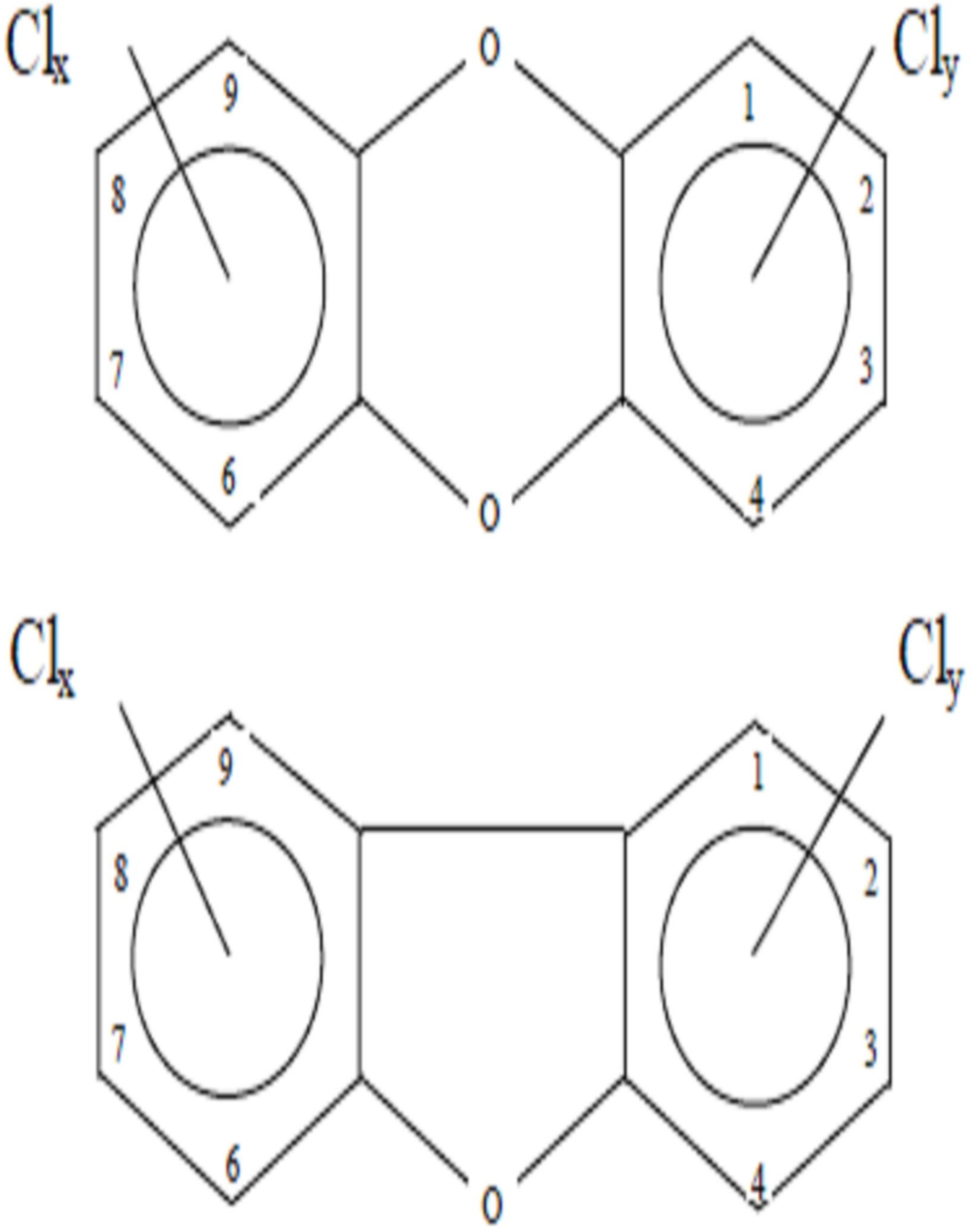

Figure 1: Chemical structure of PCDDs and PCDFs. 2,3,7,8-Substituted congeners have chlorine atoms in at least the four lateral positions, numbered 2,3,7, and 8. Source: CCME, 2002

Some PCB also has dioxin-like properties and are included as part of dioxin and dioxin-like compounds. Polychlorinated biphenyls (PCBs) are a class of organic compounds with 1 to 10 chlorine atoms attached to the biphenyl molecule. There are 209 possible PCB congeners although only 130 were found in commercial PCB mixtures. Van den Berg *et al.,* (2006) identified 29 dioxins and dioxin-like compounds of environmental concern based on similar toxicological profiles. These include seven PCDD, ten PCDF and twelve co-planar “dioxin-like” PCB.

Dioxins and dioxin-like compounds are toxic substances because they are persistent and bioaccumulative and are present in the environment primarily due to human activity. Dioxin and furan congeners with chlorine substitution in the 2, 3, 7, and 8 positions are believed to elicit their toxicity, at least in vertebrate species, via binding to a cellular protein called the aryl hydrocarbon *(Ah)* receptor (CCME, 2002). This mode of action is commonly referred to as “dioxin-like” toxicity. Of these congeners, only 7 out of the possible 75 PCDD congeners and 10 out of the 135 PCDF congeners are thought to have “dioxin-like” toxicity. However, the degree of response, or potency, differs depending on the congener. The congeners 2, 3, 7, 8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD; $C_{12}H_4C_{l4}O_2$) and 1, 2, 3, 7, 8 –PCDD ($C_{12}H_3C_{l5}O_2$) are considered the most toxic of all the dioxin and furan congeners, although the former is the most studied congener of all the dioxins and furans (CCME, 2002).

Each congener has unique physico-chemical properties. Although these properties vary, PCDD/PCDFs usually exhibit low solubility in water, very low vapour pressure, and high octanol/water partition coefficients (IARC, 1997; ATSDR, 1998). These properties determine their fate and behaviour in the environment. In particular, they tend to bind strongly to soils.

Whilst these substances have similar toxicological profiles, they have differing toxicological potencies. Dioxin and furan congeners are rarely encountered individually in the environment, and are generally present as mixtures of several congeners. In order to compare the toxicity of samples with different congener profiles, toxic equivalency factors (TEFs) have been developed that standardize “dioxin-like” substances to a toxicologically equivalent amount of 2,3,7,8-TCDD, the most toxic congener. Toxic equivalents of 2,3,7,8-TCDD are usually expressed as TEQ (Toxic Equivalent). The sum of the resultant TEQ for each substance yields a single concentration for the TEQ of the mixture. The following TEQ systems have been used historically:

I. The International TEQ (I-TEQ) developed largely by the United States Environmental Protection Agency (US EPA) in 1990.

II. The WHO modified the I-TEQ in 1998 by incorporating “dioxin-like” PCBs, this was known as the WHO98 TEQ.

III. In 2005 the WHO 98 TEQ system was updated to WHO 05 TEQ (Van den Berg *et al.,* 2006).

The major causes of soil contamination by dioxin and dioxin-like compounds are from accidental or incidental spillages in the manufacture, transport, storage and use of various chlorinated compounds and past disposal of these compounds to unregulated landfill. Land uses associated with waste disposal, pulp and paper mills, chemical manufacturing and dry cleaning industry may have resulted in soil contamination by these compounds. Other industrial sources of dioxin and dioxin-like compounds such as thermal or combustion sources and reservoir sources such as sludge may be less significant as contaminant sources for soil. Dioxins and dioxins-like compounds are also released into the atmosphere from forest fires and volcanoes (Environment Canada, 2001). Principal source activities which result in the release of PCDD/PCDFs to the environment include: waste incineration, chemical manufacturing, petroleum refining, wood burning, metallurgical processes, fuel combustion, and electric power generation. An inventory of anthropogenic releases of PCDD/PCDFs in Canada has been conducted by Environment Canada and the

Federal/Provincial Task Force on dioxins and furans (Environment Canada, 2001). From 1990 to 1997, a substantial reduction in the release of PCDD/PCDFs was observed in Canada, mainly as a result of the adoption and implementation of more stringent regulations for pulp and paper effluents. The inventory includes details on the potential release of PCDD/PCDFs directly to soil; for example by land application of municipal or industrial sludge, or by the use of pesticides that could contain trace amounts of PCDD/PCDFs.

The potential releases of PCDD/PCDFs to the environment from municipal or pulp and paper sludge are expected to be minimal because sludge contain low concentrations of these compounds and are dispersed on land at low application rates. With regard to pesticides, the Pest Management Regulatory Agency has estimated that the annual release of PCDD/PCDFs to soil is 1 g TEQ/year (Environment Canada, 2001). However, for both sludge and pesticide application, repeated applications to the same area could result in an accumulation of PCDD/PCDFs in the soil, since these compounds are persistent.

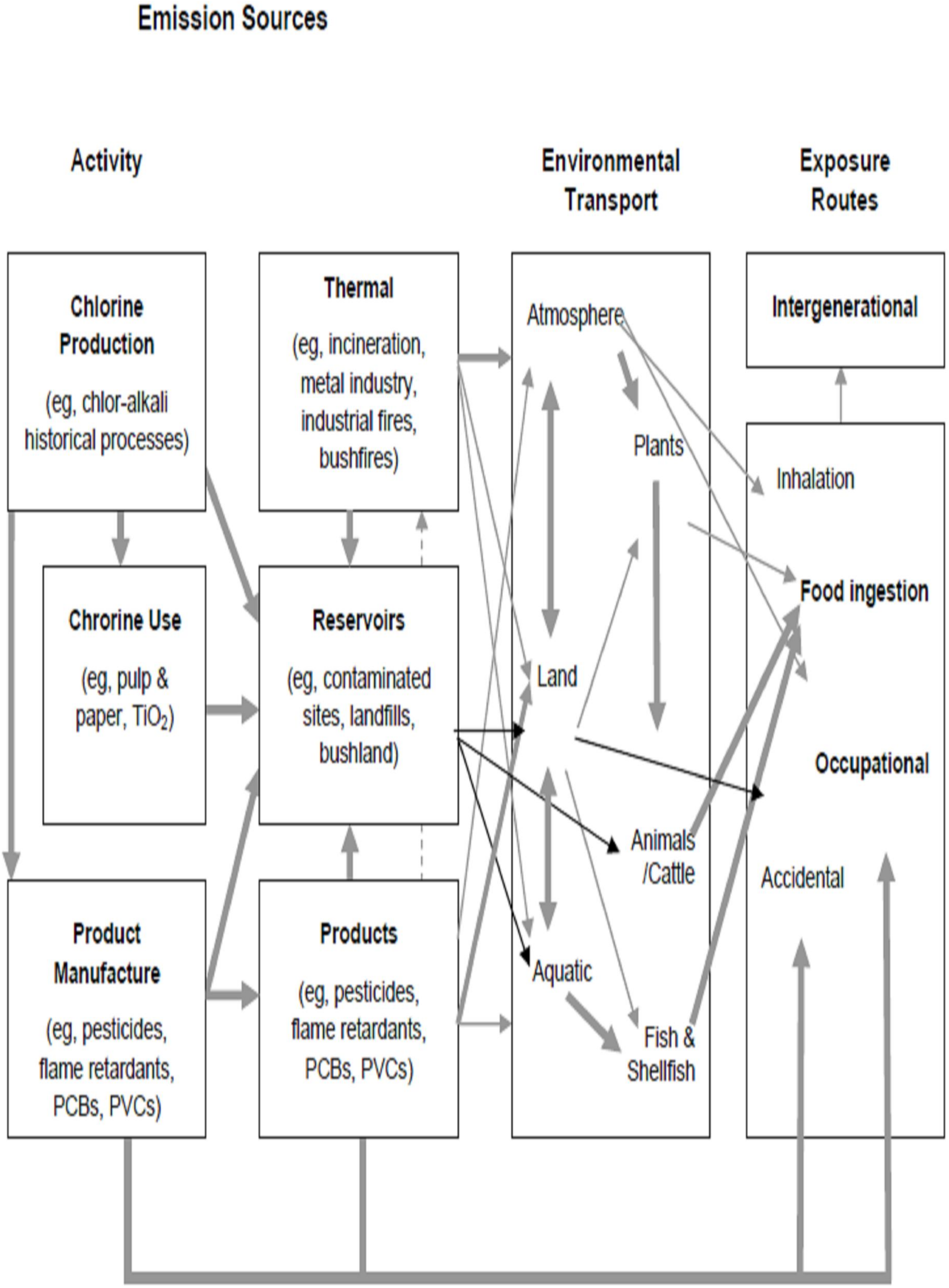

Figure 2: Sources, reservoirs, environmental transport and major human exposure pathways of PCDD/PCDFs. Source: Weber *et al.*, 2008

Modes of exposure to PCDD and PCDF include dietary intake of fish, meat and dairy products. Other exposure pathways include inhalation, inadvertent ingestion of soil, and absorption through the skin contacting air, soil or water.

Dioxins and furans are persistent in the environment. Photo degradation appears to be the only environmentally significant transformation, occurring mainly on non-sorbed species in the gaseous phase or at the soil-air and water-air interfaces. Hydrolysis, however, does not seem to occur (USEPA, 1994).Once released into the atmosphere, PCDD/Fs tend to adsorb onto particulates, and are removed from the atmosphere by photo degradation and dry and wet deposition. Half-lives of PCDD/Fs in the atmosphere vary from hours to days, depending on the congener, with lower chlorinated dioxins and furans being the main product. In general, the rate of photolysis increases as the degree of chlorination increases (USEPA, 1994).

Once deposited on soil, there can be an initial loss of PCDD/Fs by photo degradation and/or volatilisation, with the extent of these processes dependent on several factors, including the exposure to sunlight, climatic conditions, and the form under which the PCDD/Fs were deposited (i.e., airborne particulates versus sludge). Photo degradation is limited to the soil surface, and is not a significant process below the first few millimetres (USEPA, 1994). However, studies with octachlorinated dibenzo-*p*-dioxin have demonstrated that photo degradation acts through reductive dechlorination of the molecule, with chlorine preferentially removed from the 1, 4, 6, and 9 positions. This results in an increase in the concentration of 2,3,7,8-substituted congeners in relation to other congeners in the soil (Miller *et al*, 1989; Tysklind *et al.*, 1992; McLachlan *et al.*, 1996), which may result in increased toxicity. In soil, PCDD/Fs exhibit a low potential for leaching (McLachlan *et al.*, 1996) or volatilisation (USEPA, 1994). As a result, in-place burial and erosion of soil to water bodies appears to be the predominant fate of PCDD/Fs adsorbed into soil (USEPA, 1994). Concentrations in soils may remain significant for long periods when surface runoff to water bodies and wind erosion are limited. PCDD/Fs tend to be resistant to degradation in the soil, with half-lives reported of greater than 10 years (McLachlan *et al.*, 1996).

PCDD/Fs tend to bioaccumulate and/or bioconcentrate in living organisms. Bioconcentration refers to the direct uptake of compounds from water whereas bioaccumulation involves biological uptake of the contaminant from all compartments, including water, food and sediment (Branson *et al.*, 1985; Muir *et al.*, 1992). Bioconcentration is measured by calculating a bioconcentration factor (BCF), which is the ratio of the concentration of a substance in the organisms on a lipid basis to the concentration in water. Substances with BCFs in fish greater than 5000 are considered bioaccumulative according to the Federal Toxic Substance Management Policy (Environment Canada, 1997). BCFs for PCDD/Fs have been reported for a number of aquatic organisms. For example, BCFs ranged from 2 710 for 1,2,3,4,6,7,8-HpCDD in fat head minnow to 5 100 000 for 2,3,7,8-TCDD in medaka *(Oryzia slatipes)* (Muir *et al.*,1985; Schmieder *et al.*, 1995; Environment Canada, 2000).

A few studies have demonstrated that dioxins and furans can also accumulate in terrestrial organisms. Bioconcentration factors for octachlorinated dibenzo-*p*-dioxin in carrots ranged from 0.07-0.99 (Schroll and Scheunert, 1993). Studies have reported BAFs of 65-80 for TCDD in earthworms (Nash *et al.*, 1980; Heida *et al.*, 1986), and BAFs of less than 0.1 to 12 for 2,3,7,8-TCDD and a variety of furans in earthworms (Heida *et al.,* 1986). For mammals, BAFs range from 0.05-40 for rats, cattle and monkeys administered 2,3,7,8-TCDD (Kobica *et al.*, 1978; Kenaga,1980; Jensen *et al.*, 1981; Geyer *et al.*, 1986; Parker *et al.*, 1980; Firestone *et al.*, 1979; Fries 1996). These BAFs indicate that generally the concentration of PCDD/Fs in terrestrial organisms is below, or only slightly elevated above, the concentration in the soil. Heida *et al.* (1986), suggested, however, that the ability of PCDD/Fs to bioaccumulate in the terrestrial ecosystem may be dependent on the soil matrix.

Several studies have found increased rates of cancer in people who have had many years of exposure to dioxins in their workplace (CCME, 2002). Dioxins have also been linked to cancer in many experiments in laboratory animals. However, the amount of exposure in these studies was significantly higher than would occur from soils. Based on data from animal studies, there is some concern that exposure to lower levels of dioxins over long periods (or higher levels at sensitive times) might affect reproduction or development. Dioxins may also have harmful effects on the liver, peripheral nerves, and the immune system (CCME, 2002).

2.4 Are Contaminants Biologically Available?

The bioavailable portion is the amount of a substance that can cause direct effects on plants, animals or humans because it can be taken up by their bodies. Usually, not all of a contaminant found in soil is biologically available. The bioavailability of a contaminant depends on many characteristics of the soil and of the site. The conditions of the site affect how tightly the contaminant is held by soil particles and its solubility (how much of it will dissolve in water). Greater solubility usually means that more of the contaminant is bioavailable, but this also means that the contaminant is more likely to leach out of the soil. Certain chemicals show an “aging effect” and can become less bioavailable the longer they remain in soils (Hannah *et al.*, 2009). The bioavailable portion of a contaminant may be only a small fraction of the total amount. Changes in site conditions, such as soil acidity or organic matter content, can change the bioavailability of a contaminant. The most effective way to estimate the bioavailability of a contaminant is via bioassay tests to measure the uptake of contaminants by plants or soil organisms. The disadvantage of this method is that bioassay takes time and is very expensive (Hannah *et al.*, 2009).

2.5 What Happens to Contaminants in the Soil?

Once contaminants are in soils, where they go and how quickly they travel depends on many factors. Some organic (carbon-based) contaminants can undergo chemical changes or degrade into products that may be more or less toxic than the original compound. Chemical elements (such as metals) cannot break down, but their characteristics may change so that they can be more or less easily taken up by plants or animals. Different contaminants vary in their tendency to:

I. End up in water held in the soil or in the underlying groundwater (by leaching through the soil);
II. Volatilize (evaporate) into the air; or
III. Bind tightly to the soil.

The characteristics of the soil also affect the fate of contaminants and whether they can be readily taken up by plants or animals. Site management and land use (such as gardening practices) can affect some soil characteristics thereby affecting the contaminants (Hannah *et al.*, 2009).

2.6 Methods by Which Soil Contaminants are distributed in the Soil

The distribution of contaminants or pollutants in the soil can take place in the following ways; for instance, the amount of contaminants in the soils of an industrially-contaminated site may vary depending on the activities conducted on the site. The movement of air and water will also affect how soil contaminants move throughout a site (Hannah *et al.*, 2009). Chemicals may be carried by winds and deposited on the surface of soils; tilling can then mix these surface deposits into the soil. The movement of groundwater or surface water may also affect how these contaminants spread from the source. Many pesticides and soil amendments used for agricultural, industrial, or commercial activities may be found in residential soils.

This could happen if former industrial or agricultural lands are later used for residential properties, and contaminants remain in the soil (Hannah *et al.*, 2009).

2.7 Soil Microorganisms

The biological activity in soil is largely concentrated in the top soil, the depth of which may vary from a few to 30 cm. In the topsoil, the biological components occupy a tiny fraction (<0.5 %) of the total soil volume and make up less than 10 % of the total organic matter in the soil. These biological components consist mainly of soil organisms, especially microorganisms (Shonkor and Ajit, 2011).

A large number of bacteria in the soil exist, but because of their small size, they have a smaller biomass. Actinomycetes are a factor of 10 times smaller in number but are larger in size so they are similar in biomass to bacteria. Fungus population numbers are smaller but they dominate the soil biomass when the soil is not disturbed. Bacteria, actinomycetes, and protozoa are hardy and can tolerate more soil disturbance than fungal populations so they dominate in tilled soils while fungal and nematode populations tend to dominate in untilled or no-till soils. Soils contain about 8 to 15 tons of bacteria, fungi, protozoa, nematodes, earthworms, and arthropods (James and Rafiq, 2010). Despite their small volume in soil, microorganisms are key players in the cycling of nitrogen, sulphur, and phosphorus, and the decomposition of organic residues. Thereby they affect nutrient and carbon cycling on a global scale (Pankhurst *et al.,* 1997). That is, the energy input into the soil ecosystems is derived from the microbial decomposition of dead plant and animal organic matter (Shonkor and Ajit, 2011).

Organic matter decomposition serves two functions for the microorganisms, providing energy for growth and supplying carbon for the formation of new cells. Soil organic matter (SOM) is composed of the “living” (microorganisms), the “dead” (fresh residues), and the “very dead” (humus) fractions. The “very dead” or humus is the long-term SOM fraction that is thousands of years old and is resistant to decomposition. Soil organic matter has two components called the active (35 %) and the passive (65 %) SOM. Active SOM is composed of the “living” and “dead” fresh plant or animal material which is food for microbes and is composed of easily digested sugars and proteins. The passive SOM is resistant to decomposition by microbes and is higher in lignin (James and Rafiq, 2010). Microbes need regular supplies of active SOM in the soil to survive in the soil. Long-term no-tilled soils have significantly greater levels of microbes, more active carbon, more SOM, and more stored carbon than conventional tilled soils. A majority of the microbes in the soil exist under starvation conditions and thus they tend to be in a dormant state, especially in tilled soils. Dead plant residues and plant nutrients become food for the microbes in the soil. Soil organic matter (SOM) is basically all the organic substances (anything with carbon) in the soil, both living and dead. SOM includes plants, blue green algae, microorganisms (bacteria, fungi, protozoa, nematodes, beetles, springtails, etc.) and the fresh and decomposing organic matter from plants, animals, and microorganisms (James and Rafiq, 2010). The organic residues are, in this way, converted to biomass or mineralized to CO_2, H_2O, mineral nitrogen, phosphorus, and other nutrients (Bloem *et al.*, 1997). Microorganisms are further associated with the transformation and degradation of waste materials and synthetic organic compounds (Torstensson *et al.*, 1998).

In addition to the effect on nutrient cycling, microorganisms also affect the physical properties of the soil. Production of extra-cellular polysaccharides and other cellular debris by microorganisms help in maintaining soil structure as well as soil health. Thereby, they also affect water holding capacity, infiltration rate, crusting, erodibility, and susceptibility to compaction (Elliott *et al.*, 1996). They are also the major sources of soil enzymes.

Microorganisms possess the ability to give an integrated measure of soil health, an aspect that cannot be obtained with physical/chemical measures and/or analyses of diversity of higher organisms. Microorganisms respond quickly to changes; hence they rapidly adapt to environmental conditions, and thus they can be used for soil health assessment, and changes in microbial populations and activities may therefore function as an excellent indicator of change in soil health (Kennedy and Papendick, 1995; Pankhurst *et al.*, 1995).Microorganisms also respond quickly to environmental stress compared to higher organisms, as they have intimate relations with their surroundings due to their high surface to volume ratio. In some instances, changes in microbial populations or activity can precede detectable changes in the soil's physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation (Pankhurst *et al.*, 1995). The impact of some chemicals on soil health is dependent on microbial activities. For example, the concentration of heavy metals in soil will not change over small time periods, but their bioavailability may. In this way, soil microbes are acting as important indicators of soil health.

2.8 Soil Enzymes

Enzymes are the vital activators in life processes, likewise in the soil they are known to play a substantial role in maintaining soil health and its environment. The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell-associated or free enzymes. A unique balance of chemical, physical, and biological (including microbial, especially enzyme activities) components contribute to maintaining soil health. Evaluation of soil health therefore requires indicators of all these components. Healthy soils are essential for the integrity of terrestrial ecosystems to remain intact or to recover from disturbances, such as drought, climate change, pest infestation, pollution, and human exploitation including agriculture (Ellert *et al.*, 1997). Deterioration of soil, and thereby soil health, is of concern for human, animal, and plant health because air, groundwater, and surface water consumed by humans, can be adversely affected by mismanaged and contaminated soil (Singer and Ewing, 2000). As soil is the part of the terrestrial environment and supports all terrestrial life forms, protection of soil is therefore of high priority and a thorough understanding of soil enzymes activities is a critical factor in assuring that soil remains healthy.

An enzyme is a biological catalyst which alters the rate of a biochemical reaction but which itself is unaffected by the reaction. Soil enzymes are a group of enzymes whose usual inhabitants are the soil and are continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility, and soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al.,* 1991). They are important in catalysing several vital reactions necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling, hence playing an important role in agriculture (Dick *et al.*, 1994; Dick, 1997). All soils contain a group of enzymes that determine soil metabolic processes (McLaren, 1975) which, in turn, depend on its physical, chemical, microbiological, and biochemical properties. The enzyme activities in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content, composition, and activity of its living organisms and intensity of biological processes. In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for microorganisms (Kiss *et al.*, 1978). These enzymes may include amylase, arylsulphatases, β-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease, and urease released from plants, animals (Kanfer *et al.*, 1974), organic compounds, and microorganisms (James *et al.*, 1991; Richmond, 1991; Shawale and Sadana, 1981) and soils (Gupta *et al.*, 1993; Ganeshamurthy *et al.,* 1995). Sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animals. Soil enzymes are important due to the fact that; they release nutrients into the soil by means of organic matter degradation, they are necessary in the identification of soils and identification of microbial activity and they are sensitive indicators of ecological change. Therefore, soil enzymes can be seen as bioindicators of soil health and they carry out these functions most effectively at an optimum temperature and pH. A bioindicator is defined as an organism, part of an organism, the product of an organism (e.g., enzyme), collection of organisms or biological process which can be used to obtain information on the quality of all or part of the environment (Killham, 2002).

2.8.1 Soil enzymes and the role they play in maintaining soil health

Soil health is the net result of on-going conservation and degradation processes, depending highly on the biological component of the soil ecosystem, and influences plant health, environmental health, food safety, and quality (Halvorson *et al.*, 1997; Parr *et al.*, 1992). The term soil health is used to assess the ability of a soil to sustain plant and animal productivity and diversity, maintain or enhance water and air quality, support human health and habitation. The following are some of the soil enzymes and the role they play in maintaining soil health.

2.8.1.1 Amylase

The starch hydrolyzing enzyme amylase (Ross, 1976) is known to be constituted by α-amylase and β-amylase (Thoma *et al.*, 1971). The α-amylases are synthesized by plants, animals, and microorganisms, whereas, β-amylase is synthesized mainly by plants (Thoma *et al.*, 1971). This enzyme is widely distributed in plants and soils so it plays a significant role in the breakdown of starch, which converts starch like substrates to glucose and/or oligosaccharides and β-amylase, which converts starch to maltose (Thoma *et al.*, 1971). Studies have, however, indicated that the roles and activities of α-amylase and β-amylase enzymes may be influenced by different factors ranging from cultural practices, type of vegetation, environment and soil types (Ross, 1975).

2.8.1.2 Arylsulphatases

As a result of the fact that certain proportions of sulphur in different soil profiles are bound into organic compounds which are indirectly available to plants, the arylsulphatases are typically widespread in nature (Dodgson *et al.,* 1982) as well as in soils (Gupta *et al.,* 1993; Ganeshamurthy *et al.*, 1995). They are responsible for the hydrolysis of sulphate esters in the soil (Kertesz and Mirleau, 2004) and are secreted by bacteria into the external environment as a response to sulphur limitation (McGill and Colle, 1981). Its occurrence in different soil systems is often correlated with microbial biomass and rate of S immobilization (Klose and Tabatabai, 1999; Vong *et al.*, 2003). This enzyme has a role in the hydrolysis of aromatic sulphate esters ($R–O–SO_3$-) to phenols (R–OH) and sulfate, or sulfatesulfur (SO_4^{-2} or SO_4–S), (Tabatabai, 1994a, b).

2.8.1.3 *β*-Glucosidase

Glucosidase is a common and predominant enzyme in soils (Eivazi and Tabatabai, 1988; Tabatabai, 1994a, b). It is named according to the type of bond that it hydrolyses. This enzyme plays an important role in soils because it is involved in catalysing the hydrolysis and biodegradation of various β-glucosidase present in plant debris decomposing in the ecosystem (Ajwa and Tabatabai, 1994; Martinez and Tabatabai, 1997). Its final product is glucose, an important C energy source of life to microbes in the soil (Esen, 1993; Tabatabai, 1994), according to the following equation:

β-glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilize the soil organic matter, and can be used to detect management effect on soils (Bandick and Dick, 1999; Ndiaye *et al.*, 2000). This has greatly facilitated its adoption for soil quality testing (Bandick and Dick, 1999). β-

Glucosidase enzyme is very sensitive to changes in pH, and soil management practices (Acosta-Martı´nez and Tabatabai, 2000; Madejo´n *et al.*, 2001). Acosta-Martı´nez and Tabatabai, 2000 reported β-glucosidase as sensitive to pH changes. This property can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification in situations involving activities of this enzyme.

2.8.1.4 Cellulases

Cellulose is the most abundant organic compound in the biosphere, comprising almost 50 % of the biomass synthesized by photosynthetic fixation of CO_2 (Eriksson *et al.*, 1990). Growth and survival of microorganisms important in most agricultural soils depends on the carbon source contained in the cellulose occurring in the soils (Deng and Tabatabai, 1994). However, for carbon to be released as an energy source for use by the microorganisms, cellulose in plant debris has to be degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulases enzymes (White, 1982). Cellulases are a group of enzymes that catalyze the degradation of cellulose, polysaccharides built up of β-1,4 linked glucose units (Deng and Tabatabai, 1994).The cellulolytic enzyme consists of at least three enzymes (Joachim and Patrick, 2008). They include: endo-1,4-β-glucanase which attacks the cellulose chains at random, exo-1,4-β-glucanase which removes glucose or cellobiose from the non-reducing end of the cellulose chains, and β-D-glucosidase which hydrolyses cellobiose and other water soluble cellodextrins to glucose. It has been reported that cellulases in soils are derived mainly from plant debris incorporated into the soil, and that a limited amount may also originate from fungi and bacteria in soils (Richmond, 1991). Studies have shown that activities of cellulases in agricultural soils are affected by several factors. These include temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon (Deng and Tabatabai, 1994), quality of organic matter/plant debris and soil mineral elements (Sinsabaugh and Linkins, 1989; Deng and Tabatabai, 1994) and the trace elements from fungicides (Deng and Tabatabai, 1994; Arinze and Yubedee, 2000). Srinivasulu and Rangaswamy, (2006) reported a significantly more stimulatory effect of cellulases in black soil than red soil. Demonstrating the effects of increasing concentrations of fungicides on cellulases activities, Arinze and Yubedee (2000) showed that fungicides benlate, calixin and captan inhibited cellulase activity in *Fusarium monoliforme* isolates. Captatol inhibited the cellulose activity in the sandy loam soil, and chlorothalonil showed a clear reduction in cellulase activity under flooded or non-flooded conditions (Joachim and Patrick, 2008). Several mechanisms have been proposed in the degradation of cellulose by cellulases. For instance, chitin in the presence of cellulose induces the synthesis of chitinase and other cell wall lytic enzymes which promote the release of the intramural β-glucosidase into the medium. All these findings suggest that the activities of cellulases can be used to give preliminary indication of some of the physical and chemical properties of soil, thus, easing agricultural soil management strategies (Joachim and Patrick, 2008).

2.8.1.5 Chitinase

Chitinase or chitinolytic enzymes are key enzymes responsible for the degradation and hydrolysis of chitin (polyb-1-4-(2-ncetamido-2-deoxy)-D-glucoside). They are also considered as the major structural component of many fungal cell walls that use the hyperparasitism mechanisms against pests/pathogen attack (Chet, 1987). These biological agents also reduce disease-producing agents by using other mechanisms such as antibiosis or competition mechanisms (Park, 1960). This agriculturally important enzyme is produced or released by various organisms including plants and microorganisms (Deshpande, 1986). Its presence in different forms in the ecosystem has demonstrated its effectiveness in the control of soil-borne diseases such as *Sclerotium rolfsii* and *Rhizoctonia solani* in beans and cotton, respectively (Ordentlich *et al.*, 1988; Shapira *et al.*, 1989). One of the mechanisms proposed involves lytic enzymes chitinase that cause the degradation of cell walls of pathogenic fungi (Ordentlich *et al.*, 1988; Chet *et al.*, 1990; Singh *et al.*, 1999).

2.8.1.6 Dehydrogenase

The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978). This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are the part of respiration pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions (Glinski and Stepniewski, 1985; Kandeler, 1996). Since these processes are the part of respiration pathways of soil microorganisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility as well as soil health. A study by Brzezinska *et al.* (1998), suggested that soil water content and temperature influence dehydrogenase activity indirectly by affecting the soil redox status. After flooding the soil, the oxygen present is rapidly exhausted so that a shift of the activity from aerobic to anaerobic microorganisms takes place. Such redox transformations are closely connected with respiration activity of soil microorganisms. They may serve as indicators of the microbiological redox systems in soils and can be considered a possible measure of microbial oxidative activity (Tabatabai, 1982; Trevors, 1984). Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil (Reddy and Faza, 1989; Wilke, 1991; Frank and Malkomes, 1993), as well as a direct measure of soil microbial activity (Trevors, 1984; Garcia and Herna´ndez, 1997). It can also indicate the type and significance of pollution in soils. For example, dehydrogenase enzyme is high in soils polluted with pulp and paper mill effluents (McCarthy *et al.*, 1994) but low in soils polluted with fly ash (Pitchel and Hayes, 1990). Similarly, higher activities of dehydrogenases have been reported at low doses of pesticides, and lower activities of the enzyme at higher doses of pesticides (Baruah and Mishra, 1986).

2.8.1.7 Phosphatases

Phosphatases play critical roles in phosphate (P) cycles (Speir and Ross, 1978) as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzyme plays a key role in the soil system (Eivazi and Tabatabai, 1977; Dick *et al.*, 2000). For example, when there is a signal indicating P deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Versaw and Harrison, 2002). Understanding the dynamics of enzyme activities in these systems is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth, later on, where soil health is concerned.

2.8.1.8 Proteases

Proteases in the soil play a significant role in nitrogen (N) mineralization (Ladd and Jackson, 1982), an important process regulating the amount of plant available N and plant growth. This enzyme in the soil is generally associated with inorganic and organic colloids (Burns, 1982; Nannipieri *et al.*, 1996). The amount of this extra cellular enzyme activity may be indicative not only of the biological capacity of soil for the enzymatic conversion of the substrate, which is independent of the extent of microbial activity, but might also have an important role in the ecology of microorganisms in the ecosystem (Burns, 1982).

2.8.1.9 Urease

Urease enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH (Byrnes and Amberger, 1989). This, in turn, results in a rapid N loss to the atmosphere through NH_3 volatilization (Simpson *et al.*, 1984; Simpson and Freney, 1988). Due to this role, urease activities in soils have received a lot of attention since it was first reported by Rotini, (1935), a process considered vital in the regulation of N supply to plants after urea fertilization. Soil urease originates mainly from plants (Polacco, 1977) and microorganisms found as both intra- and extra-cellular enzymes (Burns, 1986; Mobley and Hausinger, 1989). On the other hand, urease extracted from plants or microorganisms is rapidly degraded in soil by proteolytic enzymes (Pettit *et al.*, 1976; Zantua and Bremner, 1977). This suggests that a significant fraction of ureolytic activity in the soil is carried out by extracellular urease, which is stabilized by immobilization on organic and mineral soil colloids. The enzyme urease has been widely used to evaluate changes in soil quality related to management, since its activity increases with organic fertilization and decreases with soil tillage (Saviozzi *et al.*, 2001). This enzyme, mostly the cases are an extra-cellular enzyme representing up to 63 % of total activity in the soil (Martinez-Salgado *et al.*, 2010). It has been shown that its activity depends on microbial community, physical, and chemical properties of soil (Corstanje *et al.*, 2007), and its stability is affected by several factors: organo-mineral complexes and humic substances make them resistant to denaturing agents such as heat and proteolytic attack (Makoi and Ndakidemi, 2008). Urease activity is used as a soil biological indicator because it is influenced by soil factors. These include cropping history, organic matter content of the soil, soil depth, soil amendments, heavy metals, and environmental factors such as temperatures (Tabatabai, 1977; Yang *et al.*, 2006). For example, studies have shown that urease was very sensitive to toxic concentrations of heavy metals (Yang *et al.*, 2006). Generally, urease activity increases with increasing temperature. It is suggested that higher temperatures increase the activity coefficient of this enzyme. Therefore, it is recommended that urea be applied at times of the day when temperatures are low.

Table 3: Types of soil enzymes and their role in the soil.

Soil enzyme	**Enzyme reaction**	**Indicator of Microbial Activity**
Dehydrogenase	Electron transport system	C-cycling
β- glucosidase	Cellobiose hydrolysis	C-cycling
Cellulose	Cellulose hydrolysis	C-cycling
Phenol oxidase	Lignin hydrolysis	C-cycling
Urease	Urea hydrolysis	N-cycling
Amylase	N-mineralization	N-cycling
Phosphatase	Release of PO_4^-	P-cycling
Arylsulphtase	Release of SO_4^-	S-cycling
Soil enzymes	Hydrolysis	General organic matter degradative enzyme activities

Source: Shonkor and Ajit, 2011.

2.9 Asphalt

Asphalt cement is chemically complex material derived from crude petroleum which has been used for many years to pave thousands of miles of highways though the exact composition of which depends upon the individual source. Asphalts are bituminous materials which occur naturally or are derived from non-destructive separation of petroleum fractions; typically, this is achieved through fractional distillation or solvent de-asphalting (Roy *et al.*, 1997). Asphaltic concrete or more commonly known as simply hot mix asphalt (HMA) contains three main materials. The first is course aggregate such as crushed rocks and small stones. The second is fine aggregate such as sand or the dust washed off of crushed stones. These first two are generally obtained from local sources, quarries or gravel pits, and are mineralogically similar to the rock surrounding the future roadway. The third and most important material is the binder, bitumen. The mixture proportion of these three main components affects roadway strength and durability. The requirements for both of these are based on location, climate, and usage of the roadway which varies city to city and state to state. While there may be some variation in the mixture, typically the course aggregates and sand make up approximately 95 % of the mix and the binder makes up the remaining 5 % by mass (Andrew *et al.*, 2010).

2.9.1 Production Process of Asphalt

Bitumen, the binder, also known simply as asphalt, is the residual portion left over from the refining process of crude petroleum. The refining process separates the bitumen from lighter oils and fuel oils which include: kerosene, diesel oil, butanes and the components to gasoline (Andrew *et al.*, 2010). This is shown in the figure below. The bitumen is composed of long chained hydrocarbons found in the crude oil, which have very high vapourization temperatures and remain after the smaller, lighter molecules have been, refined away

(Speight, 2006). The types and content of the long chained hydrocarbons found in bitumen varies batch by batch because the crude oil, from which bitumen is derived, varies by depth and content of the different oil fields around the world (Asphalt Institute, 1990). At room temperature the bitumen binder is solid because of its high viscosity and must be heated to a temperature of approximately 150°C before it becomes liquid and properly workable (Andrew *et al.*, 2010).

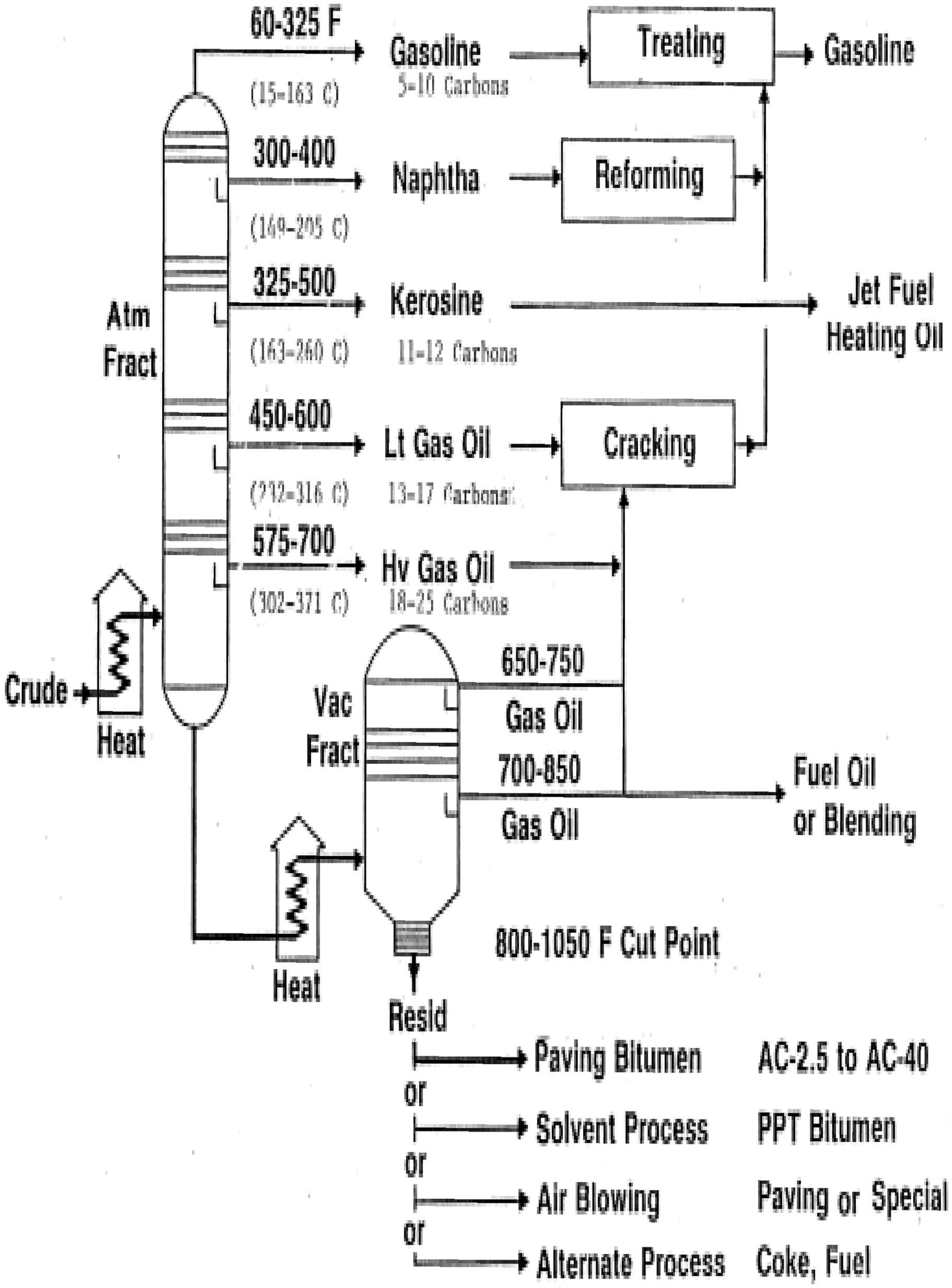

Figure 3: Fractional distillation of crude oil. Source: Corbete, 1984.

There are two types of hot mix asphalt used in the construction of roadways: virgin asphalt and reclaimed asphalt pavement (ASTM, 1980). Virgin asphalt is comprised of aggregates, either recycled from other projects or freshly quarried, as well as bitumen binder directly from the refining process. Reclaimed asphalt pavement (RAP) is pavement which has been removed from the roadway and typically reprocessed. Reclaimed asphalt pavement contains both asphalt binder and aggregates. The RAP is generated during reconstruction and repaving when layers of the road surface are milled off or the full depth of asphalt pavement is removed. After this collection process the RAP is brought to a central facility to be processed before it can be reused as pavement. This process involves crushing, filtering and optionally mixing in an additive to change its properties such as binder, aggregate or various rejuvenating and softening compounds (ASTM, 1980).

2.9.2 Chemical Composition of Asphalt

Asphalt is composed mostly of carbon (82.87 %), and hydrogen with small amounts of nitrogen (usually < 1.0 %), oxygen (< 1.1 %) and sulphur (usually less than 7 %) plus small amounts of vanadium, nickel or iron. There are also a very large number of different kinds of molecules-aliphatic, alicyclic and aromatic molecules as well as molecules in which all are combined. Aromatic molecules are a concern because certain kinds of polynuclear aromatic compounds (PNA's) are known to be carcinogenic (Joan, 1994).

Asphalt consists of an aggregate (from a local source) and a bitumen binder (composed of long chain hydrocarbon). Virgin asphalt contains only aggregates and binder. So its constituents of concern come only from the binder. Reclaimed asphalt pavement however contains pollutants, deposited on the road surface during use, and possibly rejuvenating agents in addition to aggregates and binder. Thereby constituents of concern in RAP could also come from these two additional sources which are not involved in the creation of new hot mix asphalt (Andrew *et al.*, 2010).

Pollutants and concentrations on the road surface are linked to traffic and usage. Sources include vehicle exhaust, tire wear, accidents, lubricating oils, and deicing operations, among others (Mangiani, 2003)**.** These will contain various sized hydrocarbons, metals, and salts. Hydrocarbons, specifically polycyclic aromatic hydrocarbons, are present in exhausts, fuels, and oils. Copper is present in brake linings, cadmium in tires, and zinc in roadway barriers and tires. Sodium, calcium, and chlorine, as salts, are found in winter time deicing compounds as well as zinc and cadmium but not to the same extent. The rejuvenating agents added to reclaimed asphalt mixes are usually comprised of simple paraffins. These are "soft" asphalts and small, monocyclic hydrocarbons and are used to increase viscosity. These materials are typically found in most asphalt binders. The purpose of these rejuvenating agents is to restore the binder compounds which are leached out of asphalt by time and water (Andrew *et al.*, 2010).

Tests of reclaimed asphalt pavement would account for both pollutants deposited on the surface of roadways as well as any pollutants still remaining in asphalt. One such series of tests was performed in Florida. The tests were performed in leaching columns using deionized water and other leaching agents intended to mimic the most severe natural precipitation cases, such as an acid solution to mimic acid rain. These experiments concluded that the highest concentration leachate coming off of the asphalt was the polycyclic aromatic hydrocarbon (PAH) Anthracene at 2100 μg/L. Other important leached constituents were Benzo[a]pyrene at 0.2 μg/L, Pyrene at 210 μg/L, and Benz[a]anthracene at 4 μg/L (Brantley, 1999).

Polycyclic aromatic hydrocarbons found in roadway runoff and first flush storm water collection analysis include the four constituents above as well as many others. In varying concentrations these include; Benzo(ghi)perylene, Chrysene, Coronene, Dibenz(ah)anthracene, Fluoranthene, Fluorene, Indeno(cd)pyrene, Naphtalene , Phenanthrene and Pyrene (Mangiani, 2003; Krein *et al*., 2000).

2.9.3 Ecological and health concern of constituents of asphalt

Asphalt has been used for many years to pave thousands of miles of highways because of its availability, convenience, relatively low cost and its long history of use. Asphalt is widely used and widely recycled therefore asphalt pavement and its effects on soil/water quality should be better understood to protect human health and the health of the ecosystems surrounding roadways.

The majority of products deposited into the environment by the asphalt paving process are thought to be products of pyrolysis (a form of incineration that decomposes organic materials by heat in the presence of oxygen) of tiny branches of asphaltenes. Asphaltenes are large, bulky hydrocarbons with wildly variable and highly branched structures that make the asphalt composition resist cracking by lying between long-branch bituminous hydrocarbons. This pyrolysis generates a wide variety of hydrocarbons of variable size and stability which includes benzo[a]pyrene, pyrene and anthracene. Benzo[a]pyrene, pyrene, and anthracene are highly toxic and easily detected with HPLC (Andrew *et al*., 2010).

Benzo[a]pyrene, pyrene, anthracene, and other similar pyrolysis products are not water soluble. Their only point of entry into ecological systems as well as storm water systems is generally thought to be by sorbing from fine particulate matter in the immediate area of the location of the pyrolysis. This model is substantiated by the discovery of PAH accumulation on silty river beds and other such locations (Sutton, 2009).

The principle of biological magnification or bioaccumulation states that the concentration of substances in ecology can increase across the higher ends of the food chain because many deleterious compounds (such as PAHs) cannot be broken down by metabolic processes.

This can unbalance an entire ecosystem: toxic products can affect predators higher up in the food chain (Sutton, 2009) by inducing cancer or even death. Groundwater contaminated with PAHs has the potential to affect human populations by this mechanism via accumulation of toxic products in livestock and plants.

Polycyclic aromatic hydrocarbons have the potential to act as enzymatic inhibitors with the capability of disrupting a number of important biological processes, such as tumour suppression and programmed cell death. This is as a result of the fact that polycyclic aromatic hydrocarbons have the tendency to behave as endocrine-disruptors. This tendency is due to the fact that many hormones, cell signallers, and steroid derivatives have similar, bulky multi-ringed structures (Andrew *et al.*, 2010).

A general concern is growing regarding the presence of benzo[a]pyrene (BaP) in groundwater. Benzo[a]pyrene is one of the only PAHs recognized by the International Agency for Research on Cancer as a definite carcinogen. Benzo[a]pyrene appears to enter the environment mainly through combustion of fuels, burning of coal, and the laying of asphalt. This contaminant has a strongly cytotoxic effect in concentrations over $0.25x10^{-6}$ g due to its metabolic products (Tarantini, 2009).

Asphalt is considered a potential carcinogen due to the fact it typically may contain many carcinogenic compounds, including many PAHs. Modes of exposure include; inhalation of compounds in heated or fresh asphalt as well as ingestion of PAHs entering the food chain as the result of breakdown of asphalt. Finished waters from various treatment sites are transported to consumers through a variety of pipelines. Polycyclic aromatic hydrocarbons leach from the tar or asphalt linings of these pipes, resulting in increased concentrations of these compounds in water reaching consumers (Roy *et al.*, 1997).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area is New Idea Asphalt plant site located at Obinze. Obinze is a village in South Eastern Nigeria, located near the city of Owerri. Its geographical coordinates are $5^{o}25'0''$ North, $6^{o}58'0''$ East. Obinze comprises of five clans, Umuagam, Obokwu, Umuekpu, Umueje and Umuezoroche.

3.2 Materials

3.2.1 Soil samples

The soil samples were taken at 0 – 5 cm for top soil, 15 – 20 cm for middle soil and 30 – 35 cm for bottom soil from the polluted area which is new idea asphalt plant dump site at Obinze, Owerri West Local Government area, in Imo State and from the unpolluted area, about 5 km uphill away from the asphalt plant dump site (FUTO front gate). The soil samples were air dried, debris and plant materials were carefully removed, sieved through a 2 mm sieve and stored in well labelled sampling containers which were taken to the laboratory and stored in the refrigerator prior to use for analysis.

3.2.2 Plant samples

Fresh leaves of *Sida acuta* and *Sporobulus pyramidalis* were collected following the WHO (2003) guidelines, from the dump site and the control site. The plant samples were identified and authenticated by Mr Francis Iwunze of the Department of Forestry and Wildlife, School of Agriculture Technology, FUTO. The leaves were washed, air dried, ground into powder using Corona manual hand grinder and stored in well labelled sampling containers prior to use for analysis.

3.3 Methods

3.3.1 Physiochemical analysis of soil

3.3.1.1 pH determination

Introduction:

pH was carried out according to method described by APHA (1998).

Principle:

The principle employed is the determination of the negative logarithm of the hydrogen ion concentration to base 10 in solutions.

Procedure:

Ten percent (10 % w/v) suspension of the sample was prepared in distilled water and mixed thoroughly. The pH meter electrode was rinsed with distilled water and then immersed into the sample. The pH meter was turned on and then the pH was determined.

3.3.1.2 Determination of electrical conductivity

Introduction:

Electrical conductivity was carried out according to APHA 2510B guideline Model DDS-307 (APHA, 1998).

Principle:

The principle employed is the determination of the magnitude of the total concentration of ionic solutes in solution and also the degree of dissociation.

Procedure:

Ten grams (10 g) of sample was dissolved in 100 ml of distilled water. The conductivity cell was rinsed with at least three portions of the sample and then immersed in sufficient volume of the sample. The conductivity meter was turned on and the conductivity of the sample recorded.

3.3.1.3 Determination of specific gravity

Introduction:

Specific gravity was carried out according to method described by APHA (1998).

Principle:

The principle employed is the comparison of the weight of sample to the weight of equal volume of water (APHA, 1998).

Procedure:

A 50 ml pycnometer bottle was thoroughly washed with detergent, water and petroleum ether then dried and weighed. It was filled with water and weighed, then dried and filled with soil sample and weighed.

Calculation: Specific gravity = $\frac{\text{Weight of soil sample}}{\text{Weight of water.}}$

3.3.1.4 Determination of % nitrogen

Introduction:

The micro-kjeldahl method for protein determination was employed for protein determination of the soil as described by APHA (1998).

Principle:

This is based on three principles;

Digestion; $RNH_2 + 2H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2 + H_2O$

Distillation; $(NH_4)_2SO_4 + 2NaOH \longrightarrow NH_3 + H_2O + Na_2SO_4$

Absorption; $3NH_3 + H_3 + H_3BO_3 \longrightarrow (NH_4)_3BO_3$

Titration; $(NH_4)_3BO_3 + HCl \longrightarrow H_3BO_3 + 3NH_4Cl$

Procedure:

A quantity of 0.5 g of sample was weighed and added into the micro-kjeldahl flask. One kjeldahl catalyst tablet and 10 ml of conc. H_2SO_4 was added to it. These were set in the appropriate hole of the digestion block heaters in a fume cupboard. The digestion was left on for 4 hours after which a clear colourless solution was left in the tube. The digest was carefully transferred into 100 ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the volume of the flask made up to the mark with distilled water. 5 ml portion of the digest was then pipetted to kjeldahl apparatus and 5 ml of 40% w/v NaOH was added. The mixture was then steam distilled and the liberated ammonia collected into a 50 ml conical flask containing 10 ml of 2% boric acid plus mixed indicator (bromo cresol green and methyl orange) solution. The green colour solution was then titrated against 0.01N HCl solution. At the end point, the green colour turns to wine colour which indicates that all the nitrogen is trapped as ammonium chloride. The percentage nitrogen is then calculated.

Calculation: %N = Titer value x atomic mass of nitrogen x normality of HCl used x 4

3.3.1.5 Determination of nitrate

Introduction:

Nitrate was determined using spectrophotometric method as described by APHA (1998).

Principle:

Nitrate reacts with phenoldisulphonic acid and produces a nitrate derivative, which in alkaline solution develops yellow colour due to rearrangement of its structure. The intensity of the colour produced is directly proportional to the concentration of nitrates present in the sample.

Procedure:

Fifty millilitres (50 ml) of digest was used for the experiment. The sample was filtered and was pipetted into a porcelain dish and evaporated to dryness on a hot water bath. Then, 2 ml 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 stirring to make it alkaline. This was filtered into a Nessler's tube and made up to 50 ml with distilled water. The absorbance was read at 410 nm using a spectrophotometer after the development of colour. Standard nitrate solution was prepared by preparing 10mg/l of standard potassium nitrate. Nitrate concentration was then calculated as follows:

Calculation:

$$\frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Conc of Std}$$

3.3.1.6 Particle size determination

Introduction:

Particle size was determined by method described by Laker and Dupreez (1982).

Principle:

Particle size analysis is the standard laboratory procedure for the determination of the particle size distribution of a soil. Soil consists of an assembly of ultimate soil particles of various shapes and sizes. The objective of a particle size analysis is to group these particles into separate ranges of sizes and so determine the relative proportion by weight of each size range. The method employs sieving and sedimentation of a soil/water/dispersant suspension to separate the particles. The sedimentation technique is based on an application of stokes law to a soil/water suspension and periodic measurement of the density of the suspension.

Sample preparation:

The sample was passed through the 2 mm sieves making sure that no aggregates are retained on the sieve.

Sample pretreatment:

Fifty gram (50 g) of air dried soil sample (< 2 mm) was weighed and added into a shaking bottle. This was filled to the half mark with hot tap water and agitated for 10 mins. This was allowed to settle until the supernatant is clear. The clear liquid was siphoned off and refilled with deionised water. This was repeated five times.

Dispersion and Shaking:

Two hundred millilitres (200 ml) of deionised water and 20 ml of 25 % sodium hexametaphosphate was added to the above sample. This was placed with the bottle on an end-over-end shaker and shaken for 16 hrs (overnight) at 15 rpm.

Sand measurement:

The contents of the cylinder were passed through the 0.20 mm sieve and washed free of all fine particles. Those particles retained on the sieve were the coarse sand fraction. The coarse sand was transferred from the sieve into a pre-weighed, numbered weighing tin. This was placed in a drying oven between 105°C and 110°C. When dried, it was cooled in a desiccator and weighed.

Calculation: Sand % =

$$\frac{\text{Oven dry sand mass}}{\text{Original sample mass}} \text{ X } 100\ \%$$

The settled silt fraction was then dried in the beaker at 105°C to constant weight. The soil sand % and silt % were calculated based on their fraction of the original sample mass.

Calculation: Silt % =

$$\frac{\text{Oven dry silt mass}}{\text{Original sample mass}} \text{ X } 100\ \%$$

The clay % was determined by calculating the difference of 100 % minus the sum of the sand % and silt %.

Calculation: Clay % = 100 % – (sand % + silt %).

3.3.1.7 Determination of heavy metals in soil

Introduction:

This was done using atomic absorption spectrophotometric method as described by Adrian (1998).

Principle:

Atomic absorption spectrophotometer's working principle is based on the sample being aspirated into the flame and atomized when the atomic absorption spectrophotometer's light beam is directed through the flame into the monochromator, and onto the desiccator that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Procedure:

Sample digestion; Twenty millilitre (20 ml) of the acid mixture (650 ml conc HNO_3, 80 ml perchloric acid, 20 ml conc H_2SO_4) was added to 2 g of the dried sample in a digestion flask. This was heated until a clear digest was obtained. The digest was then diluted with water to the 250 ml mark. Appropriate dilutions were then made for each element. The digested sample was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250 ml volume. The sample was aspirated into the oxidising air-acetylene flame or nitrous oxide acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1 % absorption was observed.

3.3.2 Determination of polycyclic aromatic hydrocarbons (PAHs) in soil

Introduction:

This was carried out according to method from Shell Petroleum laboratory manual SPDC series 15 (1998).

Procedure:

A quantity of 250 ml of sample was measured by means of a 500 ml measuring cylinder to a 1000 ml beaker. Then, 2 ml of 30 % H_2O_2 was added and transferred to a 500 ml separating funnel. About 100 ml of pentane was added and stoppered for shaking for 10 mins to extract all the PAHS. Then, 50 ml of isopropyl alcohol was added to the mixture in the separating funnel stoppered and shaken to cause a separation of the pentane layer. This extraction was repeated twice and all pentane extracts were combined in the second 500 ml separating funnel and later transferred to a 50 ml volumetric Flask which was made up to mark with pentane. Working standards solution of range 10-50 µg/ml anthracene were prepared from 100 µg/ml anthracene stock solution and treated like sample above, pentane was used as blank. The absorbance of sample as well as standard working solutions were read on a Cecil 2843 spectrophotometer at a wavelength of 650 nm. PAH as anthracene in mg/l was calculated using the formula below;

Calculation:

$$\frac{\text{Absorbance x Gradient Factor x 1000}}{\text{Volume of sample taken}}$$

3.3.3 Determination of total petroleum hydrocarbon (TPH) in soil

Introduction:

This was carried out according to method from Shell Petroleum laboratory manual SPDC series 15 (1998).

Procedure:

A quantity of 20 ml of sample was measured into a 250 ml beaker and 100 ml of carbonteterachloride was added and stirred with a Gallenkamp Magnetic stirrer to mix properly. The mixture was transferred to a 250 ml separating funnel into and shaken vigorously until all materials dissolved. The mixture was allowed to stand out and carbonteterachloride (CCl_4) phase was carefully decanted into a clean 100 ml conical flask. Anhydrous Na_2SO_4 was added to remove all traces of water that may still have been present in the mixture. The resultant clear solution was analysed on a UV Spectrophotometer using crude oil of working standard range 0-20 ppm at a wavelength of 420 nm.

Calculation: TPH (mg/l) =

$$\frac{\text{Absorbance of sample x slope x dilution factor}}{1000}$$

3.3.4 Determination of polychlorinated dibenzo-p-dioxins (PCDD) in soil

Introduction:

This was carried out according to method from Shell Petroleum laboratory manual SPDC series 15 (1998).

Procedure:

A quantity of 200 ml of sample was measured into a 500 ml beaker by means of 250 ml measuring cylinder. Then, 100 ml of methylene chloride was added and the mixture was transferred to a 500 ml separating funnel, stoppered and agitated for 20 min to separate the organic layer containing the PCDD. This extraction process was repeated twice and all combined extracts were put in a 100 ml volumetric flask and made up to mark with methylene chloride. Working standard solutions of range 5-25 μg/ml of PCDD were prepared from stock 100 μg/ml PCDD and treated similarly like sample. The Absorbance of sample as well as standard was taken on a cecil uv spectrophotometer model 2843 at a wavelength of 515 nm.

Calculation: PCDD mg/l = $$\frac{\text{Absorbance x Gradient factor x 1000}}{\text{Volume of sample taken}}$$

3.3.5 Determination of polychlorinated dibenzofurans (PCDF) in soil

Introduction:

This was carried out according to method from Shell Petroleum laboratory manual SPDC series 15 (1998).

Procedure:

Two hundred and fifty millilitres (250 ml) of sample was measured with 500 ml measuring cylinder into a 500 ml beaker. Two hundred milliliters (200 ml) of butanol-acetone mixture was added to extract all the PCDF. Then, 2 ml of 45 % sodium sulphite was added and the whole mixture was transferred to 500 ml separating to separate all the PCDF. This was repeated thrice and the combined extracts were transferred to a 100 ml volumetric flask and made up to mark with butanol-acetone solution. Working standards of PCDF of range 5 – 50 μg/ml of PCDF were prepared from stock 100 μg/ml PCDF and treated similarly like sample. The absorbance of sample as well as standard against blank was taken on a cecil model 2843 UV spectrophotometer at a wavelength 418 nm.

Calculation:PCDF mg/l = $$\frac{\text{Absorbance x Gradient factor x 1000}}{\text{Volume of sample taken.}}$$

3.3.6 Assay of Soil Enzyme Activity

3.3.6.1 Assay of soil alkaline phosphatase activity

Introduction:

Koch mar *et al.,* (1976) method for assaying alkaline phosphatase was used.

Principle:

The alkaline phosphatase acts upon the amino methylpropanol (AMP) buffered sodium thymolphthaleinmonophosphate. The addition of an alkaline reagent stops enzymes activity and simultaneously develops a blue chromogen which was measured photometrically.

Procedure:

Test- tubes were set-up and labelled blank, standard and sample respectively. 0.5 ml standard solution (thymolphthaline in n- propanol 0.5 mM/l) was added to the standard tube only, followed by addition of 1.0 ml of water to blank tube, and 0.95 ml of sample was added to sample tube only. 0.5 ml substrate (sodium thymolphthaleinmonophosphatase), was added to the 3 tubes and allowed to stand for 15 mins. Then, 2.5 ml colour developer (0.1 M NaOH, 0.1 M sodium carbonate) was added to the 3 test tubes and absorbance was read at 590 nm.

Calculation: Alkaline phosphatase =

$$\frac{A_T – A_B \text{ x Concentration of std}}{A_S – A_B}$$

Where A_T is absorbance of sample, A_B is absorbance of blank, A_S is absorbance of Standard.

3.3.6.2 Assay of soil acid phosphatase activity

Introduction:

The acid phosphatase activity was assayed using methods described by Andersch, and Szczypiinski, (1947), and Fishman *et al.*, (1953).

Principle:

Acid phosphatase act on soil samples amended with p- nitrophenol and inorganic phosphate, and its activity is a measure of the p-nitrophenol released.

Procedure:

Test tubes labelled blank and sample were set-up. 1.0 ml of p-nitrophenylphosphate was added to both tubes and then incubated for 10 mins, at 37°C at 30 sec interval. 1.0 ml of enzyme extract was added to only the sample tube, followed by incubation for another 45 mins at 37°C. Then at 30 sec interval, 10 ml of the dilute NaOH was added to both tubes, 0.05 ml of sample was pipetted into a test tube and 0.5 ml of reagent was pipetted into a test tube it was mixed properly and absorbance was taken at 405 nm against blank.

Calculation: Acid phosphatase = $\frac{A_T - A_B \text{ x TV x DF}}{\text{Time x 18.3 x V}}$

Where A_T is absorbance of sample, A_B is absorbance of blank, TV is total volume in tubes, DF is dilution factor, 18.3 is millimolar extinction coefficient of p-nitrophenol and V is volume of enzyme.

3.3.6.3 Assay of soil dehydrogenase activity

Introduction:

Soil dehydrogenase activity was assayed using method as described by Tabatabai (1982).

Principle:

Dehydrogenases convert 2,3,5-triphenyltetrazolium chloride to formazan. The absorbance of formazan was read spectrophotometrically at 485nm.

Procedure:

Sieved soil (1.0 g) was placed in test tubes, mixed with 1.0 ml of 3 % aqueous (w/v) 2, 3, 5-triphenyltetrazolium chloride and stirred with a glass rod. After 96 hr of incubation (27°C), 10 ml of ethanol was added to each test tube and the suspension was vortexed for 30 sec. The tubes were then incubated for 1 hr to allow suspended soil to settle. The resulting supernatant (5 ml) was carefully transferred to clean test tubes using Pasteur pipettes. Absorbance was read spectrophotometrically at 485 nm.

3.3.6.4 Assay of soil lipase activity

Introduction:

Lipase activity was assayed as described by Ugochukwu *et al.,* (2008).

Procedure:

A quantity of 20 ml of each soil microcosm treatment cells of soil sample was introduced into a mortar and pestle and ground gently for 5 min, to release the extracellular microbial enzyme in a liquid medium. The whole solution was then transferred into a glass-separating funnel and allowed to stand for 30 min at room temperature. A quantity of 3 ml each of the supernatant was used as source of crude enzyme solution. Lipase activity was measured by titrating the fatty acid released with 0.1 M NaOH using 0.1 % alcoholic phenolphthalein as indicator. Fresh palm oil was used as the source of glyceride. The oil (0.5 ml) was taken in a glass stopper Erlenmeger flask and 10 ml of acetate buffer (0.107 M) and 1.0 ml of hexane were added. The contents were vortexed for 5 min (for thorough mixing). Then 1.0 ml of the enzyme solution was added with vigorous shaking. The set up was allowed to stand for 20 min for hydrolysis to take place with continuous shaking on a rotary shaker at 30 ± 1°C. At the end of the hydrolysis, 20 ml of ethyl alcohol was added. The liberated fatty acid was titrated against 0.1 M NaOH. The determination of the blank was also carried out with the only difference that the enzyme solution was added after 20 min. The activity was expressed in arbitrary units–one arbitrary unit of enzyme activity corresponds to 1 ml of 0.1 M NaOH required to neutralize the fatty acid liberated during the incubation period of 20 min at 30°C. Appropriate controls were always run to compensate for spontaneous hydrolysis and the inherent acidity of the enzyme preparation. Zero time control titres were subtracted from each of the determinations and the averages of duplicate analyses were reported.

3.3.7 Microbial studies of soil samples

3.3.7.1 Preparation of media and diluents

Potato Dextrose agar, Bushnelli Has agar, Nutrient agar and Certified agar was prepared according to the manufacturer’s specification. Nutrient agar was used in the isolation of heterotrophic bacteria while certified agar, Bushnelli Has and Potato Dextrose agar were used to isolate *Pseudomonas aeruginosa*, hydrocarbon degrading bacteria and fungi respectively from the samples.

Physiological saline used as diluent was prepared by dissolving 9.8 g of sodium chloride in 1000 ml of distilled water and dispensed in 90 ml portions. Both diluent and media were sterilized in an autoclave at 121^{o}C for 15 mins.

3.3.7.2 Preparation of samples and inoculation

Ten grams (10 g) of soil samples were dispersed in 90 ml of sterile physiological saline. Ten-fold dilution method was used by transferring 1.0 ml from each tube until the required dilution was obtained.10 ml of water samples were dispersed in 90 ml of sterile physiological saline. Ten-fold dilution method was used by transferring 1.0 ml from each tube until the required dilution was obtained.

Aliquot portion (0.1 ml) of appropriate dilution was inoculated into the pre-sterilized and surface dried medium. Inocula were spread evenly to ensure uniform and countable colonies. Plates were incubated at ambient temperature for 48 hrs for heterotrophic bacteria.

3.3.7.3 Determination of microbial population

Colony counts obtained on the media were expressed as colony forming units per gram (cfu/g) and (cfu/ml) to obtain total population. That is number of colonies multiplied by the dilution factor. For instance: 4.3 X 10^8 cfu/g.

3.3.7.4 Characterization and identification of microbial isolates

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manual. The identities of the isolates were cross-matched with reference to standard manuals for identification of bacteria. Microscopic characterization includes gram staining, spore staining and motility test. Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to few biochemical tests described by Cheesbrough (2000) and Beishir (1987). Biochemical characterization includes catalase test, coagulase test, oxidase test. Other tests include sugar fermentation/oxidation, hydrogen sulphide production test, urease test, imvic test and indole test.

3.3.7.4.1 Gram staining test

Introduction:

The gram staining technique used for the bacterial isolates was described by Cheesbrough (2000).

Principle:

The principle is based on the ability of the bacterial isolates to retain the colour of the crystal violet as the main stain or safranin as a counter stain.

Procedure:

A smear of the isolates was made on a grease free slide with a drop of water and allowed to dry. The smear was fixed by mild heating with the Bunsen flame. The fixed smear was flooded with crystal violet and allowed to stand for 30 secs. The crystal violet was rinsed off with water. Lugo's iodine was added and allowed to stand for 30 secs. This was washed with water and decolourized with ethanol and washed again with water. The Lugo's iodine forms a complex with the organism and the primary stain such that if the organism is gram + ve, the primary stain will be retained even when washed off with ethanol. This was counter stained with Safranin for 10 secs and rinsed with water. The wet slide was air dried. A drop of oil immersion was added on the slide and viewed using X100 objective lens of the microscope.

3.3.7.4.2 Spore staining test

Introduction:

The spore staining test described by Cheesbrough (2000), was used to confirm the presence of spores when indicated in the gram stain.

Principle:

The principle is based on the ability of the spores to retain the colour of the malachite green as the main stain or safranin as a counter stain.

Procedure:

Isolates were fixed on a slide and flooded with 5 % malachite green. This was steamed for 3 mins without allowing boiling, dried and cooled. It was then rinsed off with water and stained with Safranin for 30 secs. This was rinsed with water, dried with blotting paper and viewed under the microscope using oil immersion lens. The positive spores showed green while the negative cells were stained pink.

3.3.7.4.3 Motility test

Introduction:

This test described by Cheesbrough (2000), was used to determine the motility of bacteria isolated. The test was carried out on a semi-solid agar medium.

Principle:

The principle is based on the ability of motile bacteria to swim and give a diffuse spreading growth on the semi-solid agar medium.

Procedure:

The medium was dispensed into test tube, sterilized and allowed to set in an upright position. It was then inoculated using an inoculation needle by stabbing it into the medium in the test tube. This was incubated at 37^{o}C for 24 hours. Diffuse growth from the straight line of inoculation was recorded as positive result.

3.3.7.4.4 Catalase assay

Introduction:

The catalase activity in most cytochrome containing anaerobic bacteria was assayed using methods described by Cheesbrough (2000). Catalase has one of the highest turnover numbers of all enzymes such that one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second.

Principle:

The principle was based on the ability of the bacterial isolates to produce the enzyme catalase which reduces the hydrogen peroxide to water and oxygen.

Procedure:

Catalase activity was assayed by adding the substrate H_2O_2 to an appropriately incubated (24 hours) cryptic soy agar slant culture. Organisms which produced the enzyme breakdown the hydrogen peroxide and the resulting oxygen production produces bubbles in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to breakdown peroxide into O_2 and water and are catalase negative.

3.3.7.4.5 Coagulase assay

Introduction:

The coagulase activity in the microorganisms was assayed using methods described by Cheesbrough (2000).

Principle:

Coagulase is the enzyme that clots blood plasma by a mechanism that is normal clotting. The coagulase assay identifies whether an organism produces this enzyme and clots the plasma component of blood. The only significant disease causing bacteria of humans that produce coagulase are *Staphylococcus aureus.* Thus, this enzyme is a good indicator of *Staphylococcus aureus.*

Procedure:

In the test, the sample was added to rabbit plasma and held at 37^oC for a specified period of time, about 24 hour. Formation of clot within four hour is indicated as a positive result and indicative of a virulent *Staphylococcus aureus* strain. The absence of coagulation after 24 hour of incubation is a negative result indicative of an avirulent strain.

3.3.7.4.6 Oxidase assay

Introduction:

This test described by Cheesbrough (2000), was used to assay for oxidase in gram negative bacteria. Oxidase test is an important differential procedure that should be performed on all gram negative bacteria for their rapid identification.

Principle:

The test depends on the ability of certain bacteria to produce idophenol blue from the oxidation of dimethylphenylenediamine and naphtol. This method uses N,N-dimethyl-phenylenediamine oxalate in which all *staphylococci* are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram negative bacteria), the N,N-dimethylphenylenediamine oxalate and naphtol react to indophenols blue. *Pseudomonas aeruginosa* is an oxidase positive organism.

Procedure:

A portion of a colony from the test organism was smeared by the inoculating needle onto an oxidase disk. The oxidase test is positive if the cell mass turns dark purple in 5-10 seconds.

3.3.7.4.7 Sugar fermentation/oxidation

Introduction:

The sugar fermentation/oxidation test was determined using methods described by Cheesbrough (2000).

Principle:

This test is used to differentiate between bacteria groups that oxidize carbohydrate such as members of enterobacteriaceae.

Procedure:

About 1.0 ml of 10 % glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes containing 9.0 ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1 % of each sugar. The tubes were stab inoculated in duplicates while two uninoculated tubes serve as control. Vaseline was used to cover one set of the duplicate tubes, one control to discourage oxidative utilization of sugar. All tubes were incubated at 37^{o}C for 48 hour. After incubation, they were observed for acid production in the culture. Yellow colouration indicates acid production in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction.

3.3.7.4.8 Hydrogen sulphide production (H_2S) test

Introduction:

This test described by Cheesbrough (2000), was used to determine the hydrogen sulphide production by the bacteria isolates.

Principle:

The principle is based on the ability of the microbes to reduce sulphur containing compounds to sulphides during the process of metabolism.

Procedure:

The test isolates were aseptically inoculated into a tube containing triple sugar iron agar started by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37^{o}C for 72 hour and was examined daily. Black precipitation and yellow colouration was checked for. Black precipitate indicates H_2S production and yellow colouration for sucrose, lactose and glucose fermentation.

3.3.7.4.9 Urease assay

Introduction:

This test described by Cheesbrough (2000), was used to assay for urease activity in the bacteria isolates.

Principle:

The principle is based on the ability of the bacterial isolates to metabolise the urea agar base and produce a pink colour.

Procedure:

Urease agar slant in McCartney bottle was inoculated with the isolate at 30^{o}C for 6 hours and then overnight. A pink colour in the medium indicated a positive result.

3.3.7.4.10 Imvic test

Introduction:

The imvic test was determined using methods described by Cheesbrough (2000).

Principle:

This test consists of four different test, they are indole production, methyl-red test, Voges Proskaeur test and citrate utilization test. This test is specifically designed to determine the physiological properties of microorganism. They are especially useful in the differentiation of gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter klebsiella* group.

Indole test:

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole. The bacteria isolates were inoculated into the medium and incubated at 37^{o}C for 48 hr. At the end of incubation period, 3 drops of Kovac's reagent (consisting of isoamyl alcohol, para-Dimethylaminobenzaldehyde, concentrated HCl) was added and then shaken. A red colour ring at the interface of the medium denotes a positive result.

Methyl-red (MR) test

Methyl red test was performed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red positive organisms produce a red colouration when five drops of methyl-red indicator is added into 48 hour old MR-VP broth culture.

Voges-proskauer(VP) test

The Voges-proskauer test demonstrates the ability of organisms to metabolise glucose and produce a substrate. Some organisms metabolise glucose to produce pyruvic acid which is further broken down to yield butane-diol and acetyl-methyl carbinol.

Methyl red and Voges-proskauer test must be considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, which are MR+/VP- or MR-/VP+.

3.3.8 Physico-chemical Analysis of Plants

3.3.8.1 Qualitative phytochemical screening

Phytochemical analysis was carried out on the powdered leaves of *Sida acuta* and *Sporobulus pyrimidalis* from dump site and control site using standard procedures as described by Trease and Evans (2002) and Harborne (1973). The decoction (extract) of the plant samples were prepared by suspending about 40 g of the powdered samples in 200 ml of distilled water and leaving it overnight at room temperature. This was filtered to obtain the liquid portion. This was used for qualitative determination of saponin, tannin, flavonoid, alkaloid, oxalate and phenol.

3.3.8.1.1 Test for saponins (frothing test)

One millilitre (1.0 ml) of the filtrate was diluted with distilled water in ratio 1:3, shaken vigorously and observed on standing for 1 min for stable froth.

3.3.8.1.2 Test for tannin

One millilitre (1.0 ml) of 1 % ferric chloride was added to 1.0 ml of extract and observed for green precipitate.

3.3.8.1.3 Test for flavonoids

One millilitre (1.0 ml) of 2 M sodium hydroxide was added to 2.0 ml of extract and observed for yellow colouration.

3.3.8.1.4 Test for alkaloids

One millilitre (1.0 ml) of sample was shaken with 5.0 ml of 2 % HCl on a steam bath and filtered when cool. Two drops of Wagner's reagent was added to 1.0 ml of filtrate and observed for reddish-brown precipitate.

3.3.8.1.5 Test for oxalate

Drops of ethanoic acid glacial was added to 3.0 ml of the extract and observed for greenish black colouration.

3.3.8.1.6 Test for phenols

One millilitre (1.0 ml) of water and few drops of 5 % sodium hydroxide was added to 1.0 ml of extract and observed for orange colouration.

3.3.8.2 Quantitative determination for phytochemicals

3.3.8.2.1 Determination of saponins

Introduction:

This was done using the method described by Obadoni and Ochuko (2001).

Procedure:

Two grams (2.0 g) of the powered sample was placed in a 250 ml beaker and a 100 ml of 20 % v/v of ethanol was added into the beaker. The beaker and its contents were placed on a hot water bath set at 55^oC for 4 hour with occasional stirring. The mixture was filtered and re-extracted with a fresh 100 ml of 20 % v/v ethanol solution. Both extracts were combined and the volume was reduced to about 50 ml on a water bath set at 90^oC and later cooled. The mixture was then extracted with 20 ml of diethyl ether in a separating funnel. The ethereal layer was discarded and 50 ml of n-butanol was added into the lower layer. The mixture was shaken and allowed to separate. The organic layer was washed twice with 10 ml of 5 % w/v sodium chloride in a separating funnel. The twice washed organic layer was poured into a pre-weighed beaker and evaporated to dryness on a boiling water bath. The beaker was cooled and weighed again.

Calculation: % Saponin = $\frac{W_3 - W_2}{W_1} \times 100$

Where W_3 is weight of beaker and sample after evaporation, W_2 is weight of beaker alone and W_1 is the weight of the sample.

3.3.8.2.2 Determination of tannin

Introduction:

This was done using the method described by Van-Burden and Robinson (1981).

Procedure:

To start, 0.5 g of the powdered sample was placed in a 250 ml beaker and 50 ml of distilled water was added. This was shaken on a rotator shaker for 1hour at room temperature and filtered into a volumetric flask. Afterward, 5.0 ml each of the filtrate, tannic acid solution and distilled water were pipetted into three 50 ml volumetric flask respectively. The three solutions were left to stand at 20^{o}C for 1.5 hr after which they were respectively made up to 50 ml mark with distilled water. The absorbance of the different solutions was read at 760 nm using the spectrophotometer.

Calculation: Tannin mg/ml = $\frac{(X-Z)}{(Y-Z)}$

Where X is absorbance of extract, Y is absorbance of tannic acid and Z is absorbance of distilled water.

3.3.8.2.3 Determination of flavonoids

Introduction:

The method of Obadoni and Ochuko (2001) was used in the determination of flavonoids.

Procedure:

Two grams (2 g) of the powdered sample of the plant was added into a 250 ml beaker and 100 ml of 80 % v/v methanol solution was added. The mixture was stirred on a magnetic stirrer for a minimum of 3 hr and filtered using Whatman No. 42 filter paper. The residue was re-extracted with a fresh 100 ml of 80 % v/v methanol and filtered. The combined extracts were poured into a pre-weighed beaker and evaporated to dryness in a boiling water-bath. The beaker was cooled and weighed again.

Calculation: % Flavonoid = $\frac{W_3 – W_2}{W_1}$ X 100

Where W_3 is weight of beaker and flavonoid, W_2 is weight of beaker alone and W_1 is weight of sample.

3.3.8.2.4 Determination of alkaloids:

Introduction:

The method used was alkaline gravimetric method, as described by Harborne (1973).

Procedure:

Two grams (2.0 g) of powdered sample of the plant was added into a 250 ml beaker and 100 ml of 20 % v/v ethanoic acid solution was also added into the beaker. This was covered and allowed to stand for 3 hr with stirring on a magnetic stirrer. The mixture was filtered and the residue was re-extracted with a fresh 100 ml of 20 % v/v ethanoic acid solution. Both extracts were combined in a beaker and concentrated on a water bath to about one-quarter of the original volume. This was cooled and concentrated ammonium hydroxide was added to it in drops till complete precipitation of the alkaloids has occurred. The resulting mixture was left overnight and filtered using a pre-weighed filter paper. The filter paper and contents were dried in an oven set at $70^{o}C$ for at least 6 hr. This filter paper was later cooled to room temperature in a desiccator and then weighed.

Calculation: % Alkaloids =

$$\frac{W_3 - W_2}{W_1} \times 100$$

Where W_3 is the weight of the filter paper and alkaloid after drying, W_2 is weight of filter paper, and W_1 is weight of sample.

3.3.8.3 Proximate Analysis of Plant Samples

Proximate analysis of a substance is the determination of the major components of the substance which includes moisture, lipids (fats), ash (minerals), protein, carbohydrates and fibre. The proximate analysis of the leaves of *Sida acuta* and *Sporobulus pyramidalis* was carried out using the standard procedure as described by A.O.A.C (1970).

3.3.8.3.1 Determination of moisture content

Procedure:

A crucible was thoroughly washed and dried in the oven at 80°C for 10 min, cooled in a desiccator for 15 min and weighed. 2.0 g of the sample was added into the crucible and placed in an oven at 120°C for 2.5 hr and cooled in a desiccator for 1 hr and weighed again.

Calculations: $$\%\ \text{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 is weight of crucible, W_2 is weight of crucible and sample before drying and W_3 is weight of crucible and sample after drying.

3.3.8.3.2 Determination of ash content

Procedure:

Two grams (2.0 g) of the powdered sample was added to thoroughly washed, dried and weighed crucible. The crucible and sample were placed into a pre-heated muffle furnace at 550°C for 2 hr until white / light grey ash results. The crucible was brought out, cooled and weighed again.

Calculations: $$\%\ \text{Ash} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 is weight of crucible, W_2 is weight of crucible and sample before furnace and W_3 is weight of crucible and sample after furnace.

3.3.8.3.3 Determination of fibre content

Procedure:

Two grams (2.0 g) of sample was transferred to a boiling 200 ml of 1.25 % H_2SO_4 in a 1000 ml beaker placed on a heating device of crude fibre for 30 min. During this time, a funnel was prepared with muslin cloth and boiling water poured into the funnel to keep the cloth hot. After 30 min, the acid mixture was poured into the funnel and filtered for 10 min. The filtrate was further washed with boiling water to free it from acid and then returned to 200 ml of 1.25 % NaOH in 1000 ml beaker and boiled for another 30 min. This is filtered through the muslin cloth on Buchner funnel and washed with boiling water, 1 % HCl and with boiling water again to free it from acid. The filtrate was further washed twice with alcohol and thrice with hexane and drained. This was transferred to a pre-weighed crucible, dried in an oven at 60^oC and ignited in a muffle furnace at 600^oC for at least 2 hr, cooled in a desiccator and weighed again.

Calculations: % Fibre = $\frac{W_2 - W_3}{W_1}$ X 100

Where W_1 is weight of sample, W_2 is weight of crucible and sample before furnace and W_3 is weight of crucible and sample after furnace.

3.3.8.3.4 Determination of protein content

Procedure:

Few boiling regulators (beads) were placed in the Kjeldahl flask, followed by the addition of 15 g of potassium sulphate and 0.5 g of copper sulphate to the Kjeldahl. Then, 2.0 mg of the sample was weighed out in a grease proof paper, wrapped and transferred to the Kjeldahl flask, followed by addition of 25 ml sulphuric acid and mixed by gentle swirling. The

Kjeldahl flask was placed on the heating device of Kjeldahl apparatus at an angle of 40° from the vertical in the fume hood and heated gently until foaming had ceased. The content in the Kjeldahl flask was digested by boiling vigorously until the solution was clear and a light blue-green colour obtained, cooled to room temperature, diluted with 50 ml of deionized water transferred to 100 ml volumetric flask and made up to mark with deionized water. After the digestion, 10 ml of 2 % boric acid solution and 2 drops of phenolphthalein were placed in the receiving flask under the condenser of distillation apparatus so that the outlet of the adapter of the delivery tube extended below the surface of the boric acid solution. Then 10 ml of the digest was transferred to the distillation flask followed by the addition of 35 ml of 40 % NaOH and then attached immediately to the splash head of the distillation apparatus. The mixture was distilled until 30 ml of the distillate was collected and the conical flask lowered before the distillate collection was terminated. The distillate collected was titrated against 0.1 N HCl and the titre value recorded. The blank experiment was set up with all the material in the procedure except the sample and the titre value was also recorded.

Calculation: $$\%\ \text{Protein} = \frac{(V2 - V1)\ \text{X}\ N\ \text{X}\ 0.01401\ \text{X}\ 6.25\ \text{X}\ 100}{W}$$

Where V2 is the volume (ml) of HCl solution required for sample test, V1 is the volume (ml) of HCl solution required for blank test, W is the weight (g) of the sample, N is the normality of the acid (HCl), 0.01401 is the Kjeldahl constant while 6.25 is the protein constant.

3.3.8.3.5 Determination of fat content

Procedure:

This was determined by soxhlet extraction, 250 ml of petroleum spirit was poured into a pre-weighed round bottom flask and placed on a heating mantle. Then 2.0 g of the sample was weighed on a Watchman No 2 filter paper, wrapped and placed in the inner tube of the soxhlet apparatus. The apparatus was fitted to a pre-weighed round bottom flask containing 250 ml of petroleum spirit and to a reflux condenser. The petroleum spirit was boiled gently until it vapourized and condensed into the inner tube of the soxhlet apparatus. When it reached the top of the tube, it siphoned over into the round-bottom flask to removed portion of the oil which had been extracted. This procedure was repeated until complete extraction had been achieved. After extraction, the petroleum spirit was redistilled, collected and stored. The filter paper was removed, transferred to an oven to dry then cooled in a desiccator and weighed.

Calculation: $$\% \text{ Fat} = \frac{W_2-W_3}{W_2-W_1} \times 100$$

Where W_1 is weight of filter paper, W_2 is weight of filter paper and sample before extraction and W_3 is weight of filter paper and sample after extraction.

Statistical analysis:

Data obtained were presented as simple percentages and some were analysed by one-way ANOVA at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

Table 4.1 shows the physicochemical properties of the soil samples from the dump site and control site. The values are mean of triplicate determination ± standard deviation of the physiochemical properties of the soil from depths of 0 cm, 15 cm and 30 cm respectively both from the dump site and the control site which is about 5 km away from the dump site. Values in the same row with different superscripts are statistically significant ($p < 0.05$). The table shows that there is a significant difference ($p < 0.05$) between values obtained from soil samples. For instance, the table shows that the result of chlorides and nitrates were less in the soil samples from the dump site compared to the soil samples from the control also, the pH and conductivity of the soil samples from the dump site were lower compared to that from the control site. On the other hand, the result obtained from hardness, exchangeable acidity, % clay, % base saturation and carbonate were higher at the dump site compared to the result obtained from soil samples from the control site.

Table 4.1a: Physicochemical properties of the soil samples from the dump site and control site.

	SOIL SAMPLES					
PARAMETERS	Dump Site 0 cm	Control Site 0 cm	Dump Site 15 cm	Control Site 15 cm	Dump Site 30 cm	Control Site 30 cm
Chloride (mg/kg)	37.50 ± 0.50^{b}	41.00 ± 1.00^{c}	33.00 ± 1.00^{a}	33.00 ± 0.00^{a}	37.50 ± 0.50^{b}	41.00 ± 1.00^{c}
Hardness (mg/kg)	66.00 ± 2.00^{b}	47.00 ± 1.00^{a}	124.00 ± 4.00^{e}	91.00 ± 1.00^{c}	105.00 ± 5.00^{d}	63.00 ± 1.00^{b}
Exchangeable acidity (cmol/kg)	28.60 ± 1.40^{c}	22.80 ± 0.40^{b}	14.80 ± 0.40^{a}	23.00 ± 1.00^{b}	13.50 ± 0.70^{a}	23.00 ± 1.00^{b}
% Nitrogen	0.048 ± 0.008^{a}	0.116 ± 0.004^{b}	0.116 ± 0.004^{b}	0.116 ± 0.004^{b}	0.112 ± 0.001^{b}	0.112 ± 0.000^{b}
% Organic matter	25.24 ± 0.98^{e}	7.45 ± 0.37^{d}	0.94 ± 0.03^{a}	2.65 ± 0.23^{b}	1.50 ± 0.09^{a}	4.16 ± 0.05^{c}
% Sand	55.88 ± 0.57^{a}	71.00 ± 1.00^{b}	74.26 ± 1.04^{c}	71.06 ± 0.79^{b}	74.15 ± 0.86^{c}	71.41 ± 1.00^{b}
% Silt	14.61 ± 1.13^{a}	25.83 ± 0.49^{c}	20.90 ± 0.45^{b}	25.80 ± 0.69^{c}	19.82 ± 0.59^{b}	25.23 ± 0.89^{c}

Result in rows with different letters are significantly different ($p < 0.05$).

Table 4.1 b: Physicochemical properties of the soil samples from the dump site and control site.

	SOIL SAMPLES					
PARAMETERS	Dump Site 0 cm	Control Site 0 cm	Dump Site 15 cm	Control Site 15 cm	Dump Site 30 cm	Control Site 30 cm
% Clay	29.52 ± 0.56^{c}	3.16 ± 0.50^{a}	4.83 ± 1.49^{ab}	3.13 ± 1.48^{a}	6.03 ± 0.26^{b}	3.36 ± 1.90^{a}
pH	5.43 ± 0.49^{a}	6.06 ± 0.05^{b}	5.30 ± 0.18^{a}	5.94 ± 0.07^{b}	6.46 ± 0.03^{c}	7.83 ± 0.03^{d}
Conductivity (µs/cm)	14.44 ± 0.43^{b}	17.27 ± 0.16^{d}	12.44 ± 0.34^{a}	16.15 ± 0.14^{c}	21.23 ± 1.11^{e}	12.59 ± 0.15^{a}
Specific Gravity	1.15 ± 0.01^{ab}	1.13 ± 0.01^{a}	1.17 ± 0.00^{b}	1.16 ± 0.02^{b}	1.16 ± 0.02^{b}	1.16 ± 0.02^{b}
Nitrate (mg/kg)	1.17 ± 0.01^{a}	3.58 ± 0.30^{d}	1.36 ± 0.08^{a}	2.88 ± 0.12^{b}	3.22 ± 0.05^{c}	4.88 ± 0.04^{e}
Carbonate (mg/kg)	637.00 ± 3.00^{f}	49.99 ± 1.00^{a}	124.00 ± 4.00^{e}	91.00 ± 1.00^{c}	102.50 ± 2.50^{d}	55.00 ± 1.00^{b}
CEC (cmol/kg)	0.26 ± 0.00^{e}	0.06 ± 0.00^{a}	0.16 ± 0.00^{d}	0.42 ± 0.01^{f}	0.12 ± 0.00^{b}	0.15 ± 0.00^{c}
% Base Saturation	49.51 ± 0.50^{c}	23.27 ± 0.26^{a}	75.37 ± 0.57^{e}	27.32 ± 0.44^{b}	69.89 ± 0.33^{d}	87.03 ± 0.19^{f}

Result in rows with different letters are significantly different ($p < 0.05$).

Table 4.1 c: Physicochemical properties of the soil samples from the dump site and control site.

	SOIL SAMPLES					
PARAMETERS	Dump Site 0 cm	Control Site 0 cm	Dump Site 15 cm	Control Site 15 cm	Dump Site 30 cm	Control Site 30 cm
Total Base (cmol/kg)	0.28 ± 0.00^{d}	0.07 ± 0.00^{a}	0.16 ± 0.00^{c}	0.43 ± 0.00^{e}	0.13 ± 0.01^{b}	0.14 ± 0.00^{b}
Calcium (cmol/kg)	0.049 ± 0.00^{d}	0.012 ± 0.00^{a}	0.053 ± 0.00^{e}	0.042 ± 0.00^{c}	0.050 ± 0.00^{d}	0.025 ± 0.00^{b}
Magnesium (cmol/kg)	0.08 ± 0.00^{d}	0.00 ± 0.00^{a}	0.07 ± 0.00^{c}	0.07 ± 0.00^{c}	0.03 ± 0.00^{b}	0.07 ± 0.00^{c}
Potassium (cmol/kg)	0.129 ± 0.00^{d}	0.039 ± 0.00^{c}	0.039 ± 0.00^{c}	0.309 ± 0.00^{e}	0.036 ± 0.00^{b}	0.015 ± 0.00^{a}
Sodium (cmol/kg)	0.022 ± 0.00^{c}	0.017 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.022 ± 0.00^{c}	0.022 ± 0.00^{c}

Result in rows with different letters are significantly different ($p < 0.05$).

Table 4.2 shows the heavy metal concentration of the soil samples from the dump site and control site. The values are mean of triplicate determination ± standard deviation of the heavy metal concentration of the soil from depths of 0 cm, 15 cm and 30 cm respectively both from the dump site and the control site. This result shows that natural soil contains traces of metals. The concentration of nickel, lead, cadmium, chromium, manganese, cobalt, iron, selenium, copper, zinc and silver were higher at the dump site compared to the control site. The result also shows that of all the heavy metals detected, manganese had highest concentrations in the dump site and control site while the concentration of mercury and molybdenum in the dump site were lower than that in the control site. Values in the same row with different superscripts are statistically significant ($p < 0.05$).

Table 4.2a: Heavy metal concentrations in the soil samples from dump site and control site.

	SOIL SAMPLES					
PARAMETERS (mg/kg)	Dump Site 0 cm	Control Site 0 cm	Dump Site 15 cm	Control Site 15 cm	Dump Site 30 cm	Control Site 30 cm
Nickel	0.56 ± 0.09^{a}	0.49 ± 0.05^{a}	1.99 ± 0.05^{d}	0.80 ± 0.02^{b}	3.16 ± 0.06^{e}	1.16 ± 0.07^{c}
Lead	1.48 ± 0.20^{d}	0.00 ± 0.00^{a}	0.67 ± 0.18^{c}	0.23 ± 0.05^{b}	0.52 ± 0.06^{c}	0.49 ± 0.03^{c}
Cadmium	0.19 ± 0.02^{d}	0.02 ± 0.00^{b}	0.12 ± 0.00^{c}	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}	0.00 ± 0.00^{a}
Chromium	1.21 ± 0.03^{b}	0.58 ± 0.04^{a}	4.05 ± 0.13^{d}	3.50 ± 0.18^{c}	6.68 ± 0.15^{f}	4.36 ± 0.33^{e}
Manganese	380.69 ± 1.53^{c}	125.44 ± 1.22^{a}	883.53 ± 1.87^{f}	443.02 ± 2.82^{d}	559.65 ± 4.65^{e}	166.79 ± 2.79^{b}
Cobalt	5.27 ± 0.26^{c}	0.06 ± 0.00^{a}	3.74 ± 0.27^{b}	0.09 ± 0.01^{a}	0.31 ± 0.01^{a}	0.14 ± 0.00^{a}

Result in rows with different letters are significantly different ($p < 0.05$).

Table 4.2 b: Heavy metal concentrations in the soil samples from dump site and control site.

	SOIL SAMPLES					
PARAMETERS (mg/kg)	Dump Site 0 cm	Control Site 0 cm	Dump Site 15 cm	Control Site 15 cm	Dump Site 30 cm	Control Site 30 cm
Iron	21.19 ± 0.97^{ab}	20.84 ± 0.18^{a}	23.82 ± 0.62^{e}	22.45 ± 0.22^{cd}	21.87 ± 0.13^{bc}	22.80 ± 0.06^{d}
Mercury	0.16 ± 0.00^{a}	0.85 ± 0.07^{d}	0.28 ± 0.02^{b}	0.79 ± 0.05^{d}	0.17 ± 0.02^{a}	0.47 ± 0.02^{c}
Molybdenum	5.13 ± 0.12^{b}	6.39 ± 0.31^{c}	2.66 ± 0.11^{a}	7.11 ± 0.08^{d}	15.59 ± 0.41^{f}	13.37 ± 0.35^{e}
Selenium	8.33 ± 0.12^{c}	7.19 ± 0.17^{b}	12.52 ± 0.49^{e}	9.15 ± 0.12^{d}	3.33 ± 0.15^{a}	9.35 ± 0.10^{d}
Copper	3.08 ± 0.07^{d}	0.18 ± 0.05^{a}	1.60 ± 0.09^{c}	9.15 ± 0.10^{e}	0.47 ± 0.03^{b}	12.12 ± 0.09^{f}
Zinc	0.79 ± 0.05^{a}	0.07 ± 0.01^{a}	0.37 ± 0.04^{a}	51.04 ± 50.96^{b}	11.50 ± 0.52^{a}	0.49 ± 0.05^{a}
Silver	0.17 ± 0.01^{b}	0.13 ± 0.01^{a}	0.18 ± 0.01^{b}	0.17 ± 0.00^{b}	0.18 ± 0.00^{b}	0.18 ± 0.00^{b}

Result in rows with different letters are significantly different ($p < 0.05$).

Table 4.3 shows the results of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans in the soil samples studied. The values are mean of triplicate determination ± standard deviation. From the result, polycyclic aromatic hydrocarbons recorded 86.73 ± 1.73, 63.37 ± 2.60 and 49.49 ± 2.79 at the dump site and 0.16 ± 0.02, 0.05 ± 0.00 and 0.14 ± 0.10 at the control site at depths of 0 cm, 15 cm and 30 cm respectively while total petroleum hydrocarbon recorded 245.10 ± 6.42, 235.30 ± 5.15 and 223.10 ± 2.52 at the dump site and 28.40 ± 3.56, 0.16 ± 0.02 and 0.12 ± 0.02 at the control site at depths of 0 cm, 15 cm and 30 cm respectively. The values obtained for dioxins are 37.25 ± 2.35, 18.98 ± 1.05 and 25.28 ± 3.29 at the dump site and 9.59 ± 0.66, 0.14 ± 0.02 and 0.36 ± 0.04 at the control site at depths of 0 cm, 15 cm and 30 cm respectively while the values obtained for furans are 45.67 ± 3.06, 20.78 ± 1.92 and 28.64 ± 1.84 at the dump site and 11.29 ± 1.49, 0.22 ± 0.03 and 0.49 ± 0.03 at the control site at depths of 0 cm, 15 cm and 30 cm respectively. This shows that there is significance difference ($p < 0.05$) between the soil samples; this implies that there was an increase in the concentration of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans in the samples from the dump site compared to the control site samples. This increase in concentration implies that the study area is polluted compared to the control site. The result also shows that among the four pollutants, total petroleum hydrocarbon had the highest concentration with dioxins recording the least concentration at the dump site.

Table 4.3: Results of polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans in the soil samples studied.

	SOIL SAMPLES					
PARAMETERS (mg/kg)	Dump Site 0 cm	Control Site 0 cm	Dump Site15 cm	Control Site 15 cm	Dump Site 30 cm	Control Site 30 cm
PAH	86.73 ± 1.73	0.16 ± 0.02	49.49 ± 2.79	0.14 ± 0.10	63.37 ± 2.60	0.05 ± 0.00
TPH	245.10 ± 6.42	28.40 ± 3.56	223.10 ± 2.52	0.12 ± 0.02	235.30 ± 5.15	0.16 ± 0.02
PCDDS	37.25 ± 2.35	9.59 ± 0.66	18.98 ± 1.05	0.36 ± 0.04	25.28 ± 3.29	0.14 ± 0.02
PCDFS	45.67 ± 3.06	11.29 ± 1.49	20.78 ± 1.92	0.49 ± 0.03	28.64 ± 1.84	0.22 ± 0.03

Figure 4.3 shows an increase in the activity of acid phosphatase in the test soil compared to the control soil as the value obtained from the test soil is 3.62 ± 0.76 compared to the value obtained from the control soil sample which is 0.73 ± 0.01.

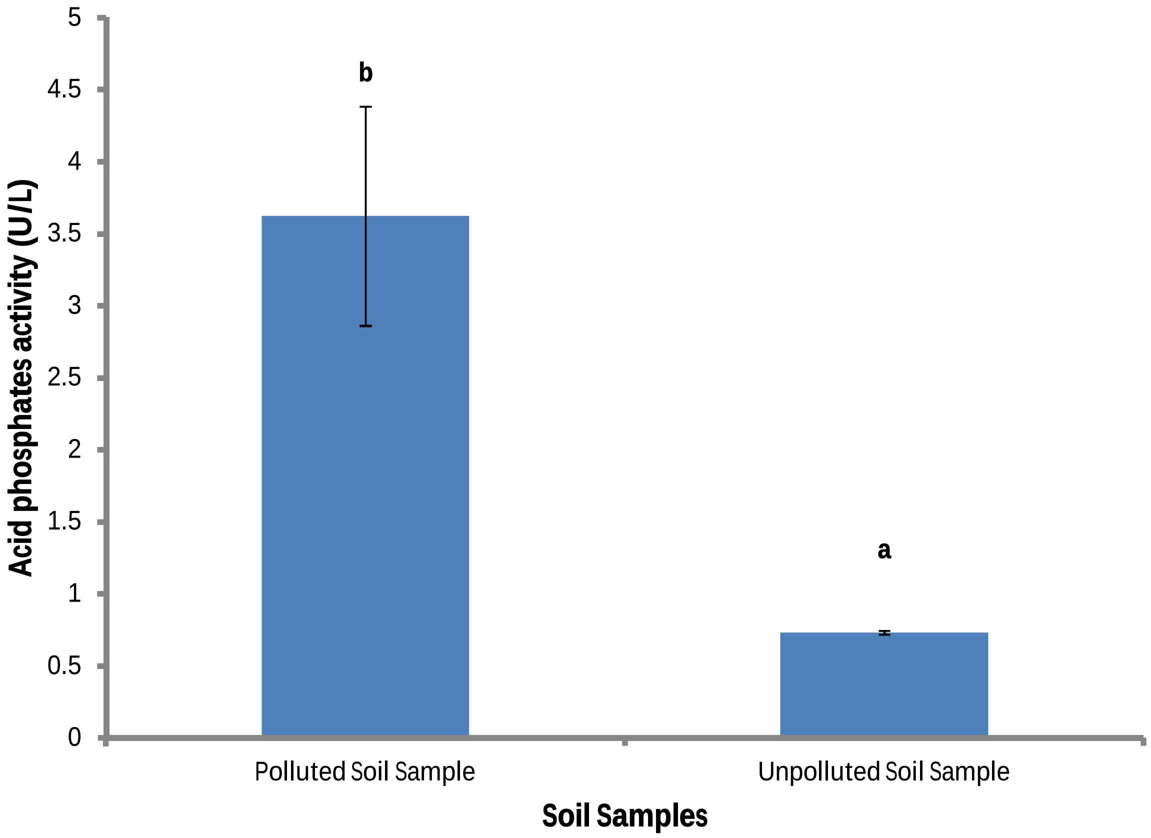

Figure 4.3: Effect of industrial pollution on the activities of acid phosphatase enzyme. Bars with different letters are significantly different ($p < 0.05$).

Figure 4.4 shows a decrease in the activity of alkaline phosphatase in the test soil sample compared to the control unpolluted soil sample as the test soil recorded 23.47 ± 9.16 while the control soil sample recorded 32.13 ± 11.60.

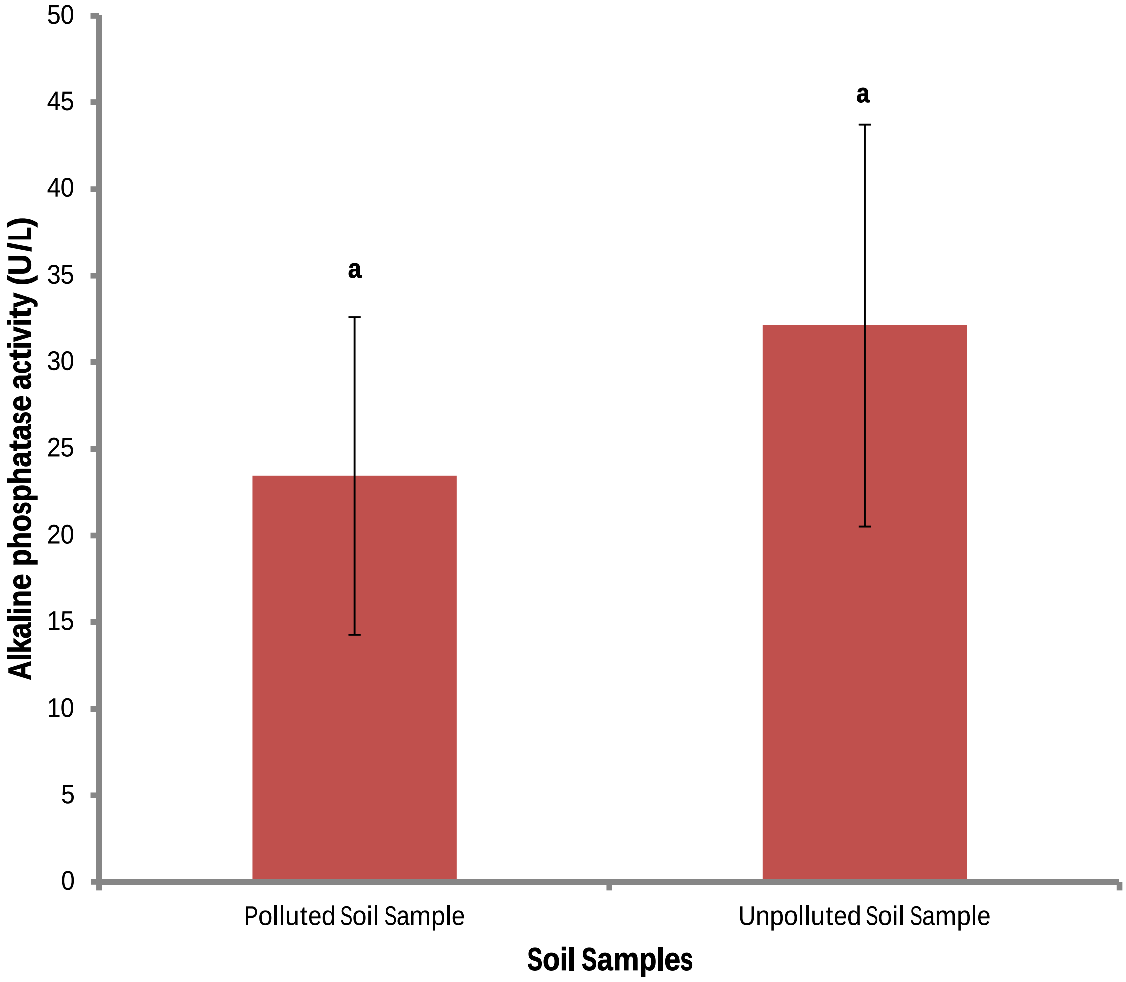

Figure 4.4: Effect of industrial pollution on the activity of alkaline phosphatase enzyme. Bars with different letters are significantly different ($p < 0.05$).

Figure 4.5 shows a decrease in the activity of lipase in the test soil sample compared to the control unpolluted soil sample as the test soil recorded 6.77 ± 0.45 while the control soil sample recorded 9.47 ± 3.41.

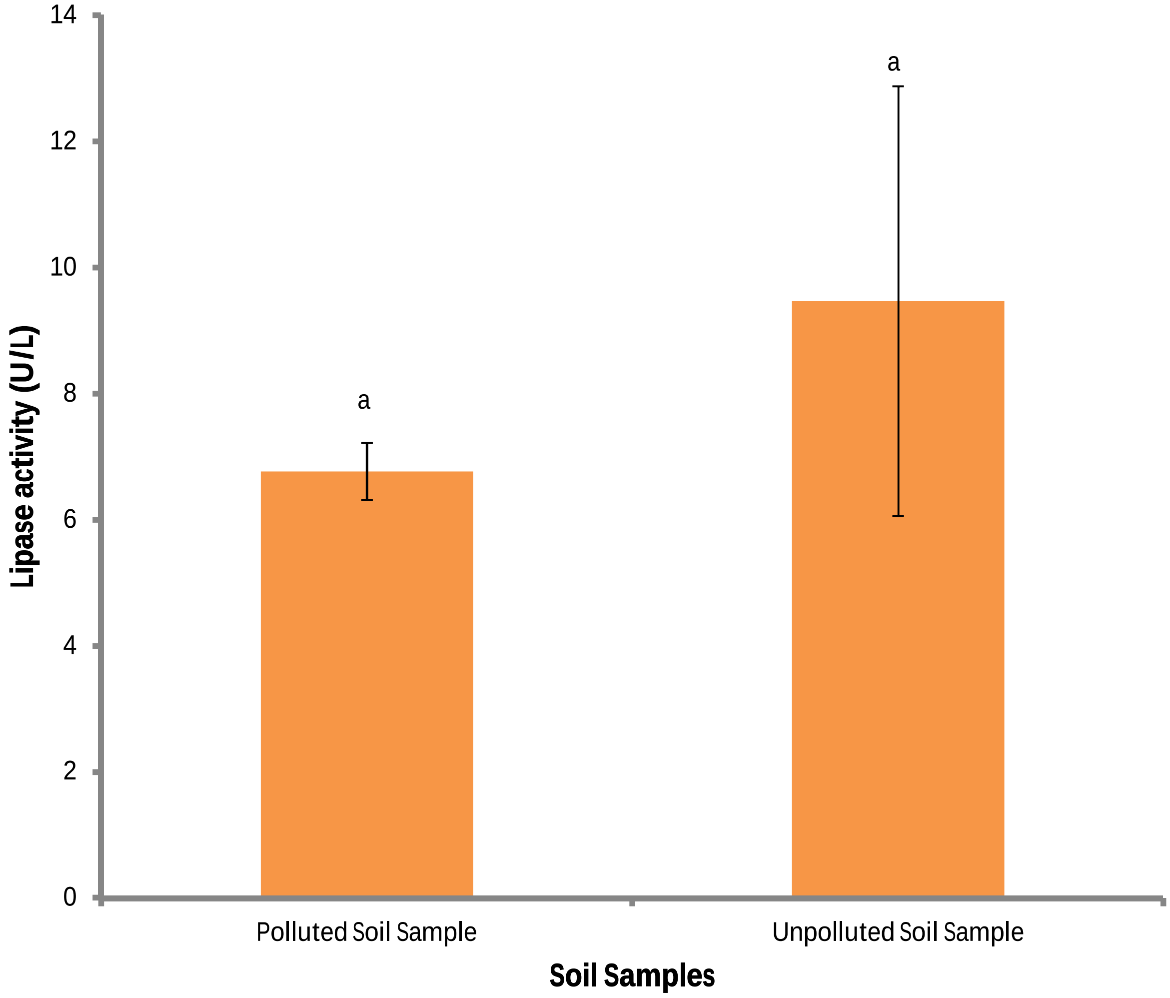

Figure 4.5: Effect of industrial pollution on the activity of Lipase enzyme. Bars with different letters are significantly different ($p < 0.05$).

Table 4.4 shows the total heterotrophic counts and colonial characteristics of fungal isolates on potato dextrose agar. The result implies that microbial population exists in both the test soil and the control soil samples. The test soil recorded 4.0 X 10^6, 5.0 X 10^6 and 2.0 X 10^6 fungal colonies at depth 0 cm, 15 cm and 30 cm respectively while the control soil sample recorded 6.0 X 10^6, 7.0 X 10^6 and 6.0 X 10^6 fungal colonies at depth 0 cm, 15 cm and 30 cm respectively. This implies that the control soil sample contains a higher population of fungal colonies compared to the test soil sample at the various depths.

Table 4.4: Total Heterotrophic counts and colonial characteristics of Fungal isolates on potato dextrose agar.

Sample points	Total colony count	Colonial characteristics	Microscopic characteristics	Identity of isolates
0 cm depth (test)	4.0 X 10^6	Short white filamentous hyphae	Non septate hyphae. Sporogiospores enclosed in a round sporangium, with septate sporangiophore	*Mucor sp*
15 cm depth (test)	5.0 X 10^6	Black spores attached at the apex of short white hyphae	Septate hyphae. Conidia globosed and attached to vesicle via sterigma	*Aspergillus sp*
		Tall white filamentous hyphae bearing orange spores	Hyphae are non septate. Spores enclosed in a sporangium	*Rhizopus nigricans*
				Saccharomyces ellipsoideus
30 cm depth (test)	2.0 X 10^6	Dirty green powdery spores surrounded by short white hyphae at the periphery	Hyphae are septate. Conidia arranged like mop head	*Penicillium notatum*
0 cm depth (control)	6.0 X 10^6	Dirty green powdery spores surrounded by short white hyphae at the periphery	Hyphae are septate. Conidia arranged like mop head	*Penicillium notatum*
				Saccharomyces cerevisiae
		Short white filamentous hyphae	Non septate hyphae. Sporogiospores enclosed in a round sporangium, with septate sporangiophore	*Mucor sp*
15 cm depth (control)	7.0 X 10^6			*Saccharomyces ellipsoideus*
		Short white filamentous hyphae	Non septate hyphae. Sporogiospores enclosed in a round sporangium, with septate sporangiophore	*Mucor sp*
30 cm depth (control)	6.0 X 10^6	Dirty green powdery spores surrounded by short white hyphae at the periphery	Hyphae are septate. Conidia arranged like mop head	*Penicillium notatum*
				Saccharomyces cerevisiae
		Short white filamentous hyphae	Non septate hyphae. Sporogiospores enclosed in a round sporangium, with septate sporangiophore	*Mucor sp*

Table 4.5 shows the total heterotrophic counts and colonial characteristics of bacterial isolates on nutrient agar. Despite the fact that no growth was recorded at depths of 0 cm and 15 cm of the control soil samples, the results therefore shows that the control soil sample has a higher population of bacterial colonies compared to the test soil sample. The test soil sample recorded the following population 1.8 X 10^9, 2.7 X 10^9 and 6.0 X 10^8 at depth of 0 cm, 5.0 X 10^8, 2.7 X 10^9 and 7.0 X 10^8 at depth of 15 cm and 4.0 X 10^8, 2.24 X 10^{10} and 4.0 X 10^8 at depth of 30 cm while the control soil recorded 3.0 X 10^9 and 2.1 X 10^{10} at depth of 15 cm.

Table 4.5: Total Heterotrophic counts and colonial characteristics of Bacterial isolates on nutrient agar.

Sample points	**Total count**	**Size (mm)**	**Shape**	**Colour**	**Elevation**	**Edge**	**Surface appearance**
0 cm depth (test)	$1.8 X 10^{9}$	1-3	R	Cream	High convex	Entire	Moist and shiny
	$2.7 X 10^{9}$	2-5	IR	Cream	Flat	Serrated	Dull and dry
	$6.0 X 10^{8}$	1	R	Orange	Low convex	Entire	Moist and shiny
15 cm depth (test)	$5.0 X 10^{8}$	3-5	IR	Cream	Raised	Serrated	Dull and dry, mucoid and slimy
	$2.7 X 10^{9}$	3	IR	Cream	Flat	Serrated	Dull and dry
	$7.0 X 10^{8}$	2	R	Cream	Low convex	Entire	Moist and shiny
30 cm depth (test)	$4.0 X 10^{8}$	1	R	Orange	Low convex	Entire	Moist and shiny
	$4.0 X 10^{8}$	3-5	IR	Cream	Flat	Serrated	Dull and dry
	$2.24 X 10^{10}$	1	R	Golden yellow	Low convex	Entire	Moist and shiny
0 cm depth (control)	NO GROWTH						
15 cm depth (control)	$3.0 X 10^{9}$	1-2	R	Cream	Flat	Entire	Dull and dry
	$2.1 X 10^{10}$	1	R	Cream	Low convex	Entire	Moist and shiny
30 cm depth (control)	NO GROWTH						

Table 4.6 shows the total counts and colonial characteristics of bacteria on Bushnelli Haas Agar. The results shows that microbial population exists in both the test and control soil samples but the bacterial population in the test soil sample is less compared to that in the control soil sample. The test soil recorded 1.0 X 10^5, 2.0 X 10^4 and 3.0 X 10^4 at depth of 0 cm while control soil recorded 4.0 X 10^4, 3.0 X 10^4, 3.0 X 10^4 and 3.9 X 10^5 at depth of 0 cm. The test soil recorded 3.0 X 10^4, 1.0 X 10^4 and 1.0 X 10^4 at depth of 15 cm while the control soil sample recorded 4.0 X 10^4 and 4.0 X 10^4 at depth of 15 cm. At depth of 30 cm, the test soil recorded 3.0 X 10^4, 1.0 X 10^4 and 6.0 X 10^4 while the control soil recorded 5.0 X 10^4 and 2.0 X 10^4.

Table 4.6: Total counts and colonial characteristics of Bacteria on Bushnelli Haas Agar.

Sample points	**Total count**	**Size (mm)**	**Shape**	**Colour**	**Elevation**	**Edge**	**Surface appearance**
0 cm depth (test)	1.0 X 10^5	1	R	Golden yellow	Low convex	Entire	Moist and shiny
	2.0 X 10^4	2-3	R	Yellow	Low convex	Entire	Moist and shiny
	3.0 X 10^4	1	R	Cream	Flat	Serrated	Dull and dry
15 cm depth (test)	3.0 X 10^4	1	R	Golden yellow	Low convex	Entire	Moist and shiny
	1.0 X 10^4	1	R	Yellow	Low convex	Entire	Moist and shiny
	1.0 X 10^4	1	IR	Cream	Flat	Serrated	Dull and dry
30 cm depth (test)	3.0 X 10^4	1	R	Golden yellow	Low convex	Entire	Moist and shiny
	1.0 X 10^4	1	R	Orange	Low convex	Entire	Moist and shiny
	6.0 X 10^4	1-3	IR	Cream	Flat	Serrated	Dull and dry
0 cm depth (control)	4.0 X 10^4	2	R	White	Flat	Serrated	Dull and dry
	3.0 X 10^4	1	R	Orange	Low convex	Entire	Dull and dry
	3.9 X 10^5	1	R	Cream	Flat	Entire	Dull and dry
	3.0 X 10^4	1	R	Cream	Low convex	Entire	Moist and shiny
15 cm depth (control)	4.0 X 10^4	1	R	Cream	Low convex	Entire	Moist and shiny
	4.0 X 10^4	2	IR	Cream	Flat	Serrated	Dull and dry
30 cm depth (control)	5.0 X 10^4	1-3	IR	Cream	Flat	Entire	Dull and dry
	2.0 X 10^4	1	R	White	Flat	Serrated	Dull and dry

Table 4.7 shows the biochemical and carbohydrate fermentation test of bacterial isolates on nutrient agar medium.

Key: Cat = Catalase, Caog = Coagulase, Oxi = Oxidase, In = Indole, Mr = Methyl red, Vp = Vogesproskaeur, Ure = Urease production, NO_3 = Nitrate production, H_2S = Hydrogen sulphide production, Glu = Glucose, Suc = Sucrose, Mal = Maltose, Lac = Lactose, Mann = Mannitol, Fru = Fructose, Xyl = Xylose, +R = Gram positive rod, -R = Gram negative rod, +S = Gram positive spherical.

Key: *E. faecalis = Enterococcus faecalis, B. sp = Bacillus species, M. roseus = Micrococcus roseus, B. subtilis = Bacillus subtilis, B. meagterium = Bacillus megaterium, S. aureus = Staphylococcus aureus, B. cereus = Bacillus cereus, C. sp = Corynebacterium sp.*

Table 4.7a: Biochemical and carbohydrate fermentation test of bacterial isolates on nutrient agar medium from test site.

Colony code	Test site 0 cm X	Test site 0 cm Y	Test site 0 cm Z	Test site 15 cm X	Test site 15 cm Y	Test site 15 cm Z	Test site 30 cm X	Test site 30 cm Y	Test site 30 cm Z
Cat	-	+	+	+	+	-	+	+	+
Oxi	-	-	-	-	-	-	-	-	-
Coag	-	-	-	-	-	-	-	-	+
In	-	-	-	-	-	-	-	-	-
MR	+	-	+	-	-	+	+	-	-
VP	-	+	-	+	+	-	-	+	+
Cit	+	+	+	+	+	+	+	+	-
H_2S	-	-	-	-	-	-	-	-	+
NO_3	+	+	+	+	+	+	+	+	+
Ure	-	-	-	-	-	-	-	-	+
Glu	+	+	+	+	+	+	+	+	+
Suc	+	-	+	-	-	+	+	-	+
Lac	+	-	-	-	-	+	-	-	+
Fru	+	-	-	-	-	+	-	-	+
Mal	+	-	-	-	+	+	-	-	+
Mann	+	-	-	+	+	+	-	-	+
Xyl	-	-	-	+	-	-	-	-	-
Gram reaction	+S	+R	+S	+R	+R	+S	+S	+R	+S
Identity of isolates	*E. faecalis*	*B. sp*	*M. roseus*	*B. subtilis*	*B. meagterium*	*E. faecalis*	*M. roseus*	*B. sp*	*S. aureus*

Table 4.7 b: Biochemical and carbohydrate fermentation test of bacterial isolates on nutrient agar medium from control site.

Colony code	Control site 0 cm X	Control site 0 cm Y	Control site 0 cm Z	Control site 15 cm X	Control site 15 cm Y	Control site 15 cm Z	Control site 30 cm X	Control site 30 cm Y	Control site 30 cm Z
Cat	+	+	-	+	+	-	+	+	+
Oxi	-	-	-	-	-	-	-	-	-
Coag	-	-	-	-	+	-	-	-	-
In	-	-	-	-	-	-	-	-	-
MR	-	-	+	-	-	+	-	-	+
VP	+	+	-	+	+	-	+	+	-
Cit	+	+	+	+	-	+	+	+	+
H_2S	-	-	-	-	+	-	-	-	-
NO_3	+	+	+	+	+	+	+	+	-
Ure	-	-	-	-	+	-	-	-	-
Glu	+	+	+	+	+	+	+	+	+
Suc	-	-	+	-	+	+	-	-	-
Lac	-	-	+	-	+	+	-	-	-
Fru	-	-	+	-	+	+	-	-	-
Mal	-	-	+	-	+	+	-	+	+
Mann	+	-	+	-	+	+	+	+	-
Xyl	+	-	-	-	-	-	+	-	-
Gram reaction	+R	+R	+S	+R	+S	+S	+R	+R	+R
Identity of isolates	*B. subtilis*	*B. cereus*	*E. faecalis*	*B. sp*	*S. aureus*	*E. faecalis*	*B. subtilis*	*B. megaterium*	*C. sp*

Table 4.8 and table 4.9 shows the result for the qualitative and quantitative phytochemical screening of *Sida acuta* and *Sporobulus pyramidalis* leaves from dump site and the control. The qualitative phytochemical screening reveals the presence of saponin, tannin, flavonoid, oxalate, alkaloid and phenol in both leaf samples from the dump and control sites. The quantitative phytochemical screening reveals *Sida acuta* leaf from test site contains higher quantity of saponin (7.00 ± 0.53), tannin (1.75 ± 0.35), flavonoid (5.06 ± 1.01), alkaloid (5.43 ± 0.49) and lower quantity of oxalate (0.01 ± 0.01) and phenol (0.36 ± 0.09) compared to the leaf from control site which recorded saponin (5.40 ± 0.41), tannin (1.46 ± 0.20), flavonoid (4.57 ± 0.99), alkaloid (4.80 ±1.03), oxalate (0.03 ± 0.00) and phenol (0.80 ± 0.19). *Sporobulus pyramidalis* leaf from the dump site contains higher quantity of saponin (5.59 ± 1.03), flavonoid (4.87 ± 1.02), alkaloid (4.38 ± 0.53) and lower quantity of tannin (0.14 ± 0.05) compared to the leaf sample from the control site which recorded saponin (5.30 ± 0.32), flavonoid (3.34 ± 0.62), alkaloid (3.50 ± 0.03), and tannin (2.66 ± 0.04).

Table 4.8: Result for the qualitative phytochemical screening of *Sida acuta* and *Sporobulus pyramidalis* leaves from test site and the control.

TEST/ SAMPLES	SAPONIN	TANNIN	FLAVONOID	OXALATE	REDUCING SUGAR	ALKALOID	STEROID	PHENOL
***Sidaacuta* Polluted**	+	+	+	+	**ND**	+	**ND**	+
***Sidaacuta* Control**	+	+	+	+	**ND**	+	**ND**	+
***Sporobulus pyramidalis* Polluted**	+	+	+	+	**ND**	+	**ND**	+
***Sporobulus pyramidalis* Control**	+	+	+	+	**ND**	+	**ND**	+

Key: **+** stands for present, **-** stands for absent while **ND** stands for not detected.

Table 4.9: Result for the quantitative phytochemical screening of *Sida acuta* and *Sporobulus pyramidalis* leaves from test site and the control.

TEST/ SAMPLES	**% SAPONIN**	**% TANNIN**	**% FLAVONOID**	**% OXALATE**	**% ALKALOID**	**% PHENOL**
***Sida acuta* Polluted**	7.00 ± 0.53^{b}	1.75 ± 0.35^{b}	5.06 ± 1.01^{a}	0.01 ± 0.01^{a}	5.43 ± 0.49^{b}	0.36 ± 0.09^{b}
***Sida acuta* Control**	5.40 ± 0.41^{a}	1.46 ± 0.20^{b}	4.57 ± 0.99^{a}	0.03 ± 0.00^{b}	4.80 ± 1.03^{b}	0.80 ± 0.19^{c}
***Sporobulus pyramidalis* Polluted**	5.59 ± 1.03^{a}	0.14 ± 0.05^{a}	4.87 ± 1.02^{a}	0.01 ± 0.01^{a}	4.38 ± 0.53^{ab}	0.10 ± 0.01^{a}
***Sporobulus pyramidalis* Control**	5.30 ± 0.32^{a}	2.66 ± 0.04^{c}	3.34 ± 0.62^{a}	0.01 ± 0.01^{a}	3.50 ± 0.03^{a}	0.09 ± 0.02^{a}

Results in columns with different letters are significantly different ($p<0.05$).

Table 4.10 shows the result for the proximate analysis of *Sida acuta* and *Sporobulus pyramidalis* leaves from dump site and the control. *Sida acuta* from the dump site recorded a higher concentration of carbohydrate (47.61 ± 2.00), fiber (10.80 ± 0.96) and ash (8.33 ± 1.03) and lower concentration of protein (15.84 ± 1.00), fat (1.64 ± 0.30) and moisture (15.78 ± 1.03) compared to the leaf samples from the control site which recorded carbohydrate (44.53 ± 0.70), fiber (9.70 ± 0.14), ash (7.83 ± 0.08), protein (18.15 ± 1.00), fat (1.71 ± 0.19) and moisture (17.78 ± 0.13). *Sporobulus pyramidalis* from the dump site recorded a higher concentration of protein (9.45 ± 1.00), fat (3.49 ± 1.00), fiber (12.99 ± 0.12), moisture (14.51 ± 0.06), and ash (6.62 ± 0.29) and a lower concentration of carbohydrate (54.27 ± 3.00) compared to the leaf samples from the control site which recorded the following results protein (6.92 ± 0.02), fat (1.46 ± 0.10), fiber (12.51 ± 1.05), moisture (13.49 ± 1.05), ash (4.95 ± 0.96) and carbohydrate (59.69 ± 9.84).

The proximate analysis of the plant samples reveals that the plants both the samples from the dump site and samples from the control contained high amount of carbohydrate and low amount of fat. The result also shows that *Sporobulus pyramidalis* leaves have higher carbohydrate content than the leaves of *Sida acuta.*

Table 4.10: Result for the proximate analysis of *Sida acuta* and *Sporobulus pyramidalis* leaves from dump site and the control.

TEST/ SAMPLES	**% PROTEIN**	**% FAT**	**% CARBOHYDRATE**	**% FIBER**	**% MOISTURE**	**% ASH**
Sida acuta **Polluted**	15.84 ± 1.00^{c}	1.64 ± 0.30^{a}	47.61 ± 2.00^{a}	10.80 ± 0.96^{a}	15.78 ± 1.03^{b}	8.33 ± 1.03^{c}
Sida acuta **Control**	18.15 ± 1.00^{d}	1.71 ± 0.19^{a}	44.53 ± 0.70^{a}	9.70 ± 0.14^{a}	17.78 ± 0.13^{c}	7.83 ± 0.08^{bc}
Sporobulus pyramidalis **Polluted**	9.45 ± 1.00^{b}	3.49 ± 1.00^{b}	54.27 ± 3.00^{ab}	12.99 ± 0.12^{b}	14.51 ± 0.06^{ab}	6.62 ± 0.29^{b}
Sporobulus pyramidalis **Control**	6.92 ± 0.02^{a}	1.46 ± 0.10^{a}	59.69 ± 9.84^{b}	12.51 ± 1.05^{b}	13.49 ± 1.05^{a}	4.95 ± 0.96^{a}

Results in columns with different letters are significantly different ($p<0.05$).

4.2 DISCUSSION

There was slight alteration ($p < 0.05$) in the physicochemical properties of the soil as a result of the pollution. The mean chloride values of test site soil samples were low compared to that of the control soil samples at 0 cm, 15 cm and 30 cm depth respectively. This implies that the control soil samples contained more soluble chloride salts necessary for plant growth than the test site.

Hardness values at 0 cm, 15 cm and 30 cm depth showed that soil samples from the test site have higher hardness than the control. This may be due to the asphalt debris and other metal pollution on the soil. According to Horn *et al.* (1995), soil hardness or compaction can be caused as a result of higher bulk density and homogenised soil. Soil hardness or compaction enhances harmful physical, chemical and biological processes which, in the context of inappropriate soil management, lead to soil degradation. Soil compaction is shown to result in changes in soil properties which control the emission of greenhouse gases, the runoff of water and pollutants into surface waters, and the movement of nitrate and pesticides into ground waters. Soil compaction will also affect the amounts of fertiliser and energy used in crop production, which may have additional adverse environmental consequences (Soane and Van Ouwerkerk, 1995). As a result of increased bulk density and homogenisation, soil hardness can also cause decreased aeration and increased penetration resistance, which results in impeded root development. Reduced water permeability may result in soil erosion, with serious negative effects on the environment. Hardened or compacted soil may also contribute to global atmospheric warming due to increased emission of CO_2, CH_4 and N_2O from such soils. Anthropogenic changes in soil structure and soil functions remain constant for extended periods of time and efforts to restore deteriorated soil structure very often fail because of excessive loosening and homogenisation, cultivation of too wet soil or, afterwards, illadapted soil management practices, resulting in even worse soil properties (Horn *et al.*, 1995).

There was significance ($p < 0.05$) difference in the exchangeable acidity between the test site soil samples and control soil samples. The test site soil sample recorded a higher level of exchangeable acidity at the depth of 0 cm and this decreased as the depth increased compared to the control. This increase in exchangeable acidity at depth of 0 cm for test site soil was also recorded by Akubugwo *et al*. (2009), and may be due to the presence of free metal ions on the soil surface resulting from the asphalt dump and other hydrocarbon pollutants.

Organic matter values shows that the soil samples from test site recorded high organic matter content at depth of 0 cm which reduced as the depth increased with respect to the control soil. This may be due to presence of asphalt debris and that of dead plants which accumulate on the soil surface. The result of carbonate also shows that the test soil samples recorded a higher level of carbonate compared to the control. This may be due to the high level of carbonaceous materials which leaked from the asphalt dumps into the soil. Increase in organic matter and carbonate of polluted soils was also recorded by Osuji and Onojake. (2006), who attributed this to the metabolic processes that facilitates agronomical addition of organic carbon from petroleum hydrocarbon pollutants by reducing the carbon mineralizing capacity of the soil microflora.

There was significance ($p < 0.05$) difference in the particle size distribution. Soil samples from the test site recorded higher values of sand and clay and a lower value for silt when compared to the values obtained from soil samples from the control site. This proportion show that the soils from the test site were coarse and as such have low supply of nutrients and moisture unlike fine textured soils from the control site that have sufficient water holding capacity, good aeration and high supply of nutrients. The clay also makes the soil to be water logged easily which also leaches out soil nutrients.

The mean pH values indicate that the soil samples from the asphalt test site had lower pH compared to the control soil samples. This shows that the test soil samples were acidic and that the acidity decreased with increase in depth. The increase in acidity of soil samples associated with petroleum hydrocarbon pollution was also reported by Nwaogu and Onyeze. (2010) and Akubugwo *et al.* (2009). Soil pH and other soil properties are especially important in soil processes responsible for solubility of heavy metals in soil and their transportation (Matthews – Amune and Kakukulu, 2012). This implies that pH has a great effect on solute concentration and absorption in the soil. At high pH, metals tend to form metal mineral phosphates and carbonates which are insoluble while at low pH they tend to be found as free ionic species or as soluble organometals and are more bioavailable (Rensing and Maier, 2003; Hoffman, 2007; Egbeda *et al*., 2015). High soil acidity creates chemical and biological conditions which may be harmful to plants and soil microorganisms. This observation agrees with the reports of Onwerendu *et al.* (2007), who reported that heavy metals from automobile service centres decreased soil pH thus increasing the soil acidity. Therefore, the low pH values of soils from test site can be as a result of the heavy metals and other pollutants which have accumulated on the soil surface. Since at low pH, metals are more soluble and more bioavailable in the soil solution, the range of pH values obtained in this study therefore favour plant uptake of heavy metal and hence toxicity problems may arise. Nwaogu and Onyeze. (2010), reported that the acidity of the polluted area can cause a shift in normal metabolism of living things within an ecosystem.

The result of electrical conductivity measurement in the test soil sample was significantly ($p < 0.05$) lower than the control. This may be due to the increase in the concentration of some soluble salts in the control soil samples. It may also be as a result of the fact that some of the soluble salts or trace metal ions may have leached out of the soil from test site.

The mean value of nitrate shows that nitrate level in the test soils was lower than that of the control. This could be as a result of the activities of nitrogen-fixing bacteria and other microbes associated with decomposition of organic matters, which might be inactivated in the polluted area (Ezeigbo *et al*, 2013). This implies that the pollution prevented easy circulation of nitrates into the soil which is necessary for growth of plants and microorganisms.

There was higher concentration of exchangeable cations (Ca^+, Mg^+, K^+ and Na^+) on the test soils at depth of 0 cm when compared to the control. This observation agrees with result obtained by Akubugwo *et al*. (2009), and this can be attributed to the presence of pollution caused by the asphalt dump.

There was significant ($p < 0.05$) difference in the % base saturation between the test soils and the control as the test soils recorded higher % base saturation on depths of 0 cm and 15 cm compared to the control while the concentration of total base was also higher in the test soil sample at depth of 0 cm when compared to the control soil sample. This observation agrees with result obtained by Akubugwo *et al*. (2009), and this can be attributed to the presence of pollution caused by the asphalt dump.

There was no significant ($p < 0.05$) difference in the mean values of nitrogen, specific gravity, CEC obtained for all the polluted soil samples relative to the control.

The heavy metal results show that the test and control soil samples contain numerous metals. All the heavy metals studied i.e. nickel, lead, cadmium, cobalt, chromium, manganese, iron, selenium, copper, zinc and silver in the test soil were significantly ($p < 0.05$) higher than that in the control soil site except for mercury and molybdenum. This can be attributed to the asphalt dump at the test site. The presence of these heavy metals in the test soil poses great danger to the soil and the ecosystem. For instance, exposure to intake of large amount of nickel from plants, grown on nickel rich soil leads to higher chances of developing cancer of the lungs, nose, larynx and prostrate as well as respiratory failures, birth defects and heart disorders (Lentech, 2009) also, exposure to Chromium, a carcinogen causes cancer of respiratory organs (Langard, 1980) and so on.

The high concentration of these metals in the test sites is in agreement with results of Sr. Prema *et al*. (2015) which stated that 15 metals, namely, As, Ba, Co, Cr, Cu, Pb, Sr, Zn, Al, Fe, Mn, Mg, Mo, Ti, and P were found to be higher in polluted soils than that of natural soils. The United States Environmental Protection Agency (U.S. EPA) included 13 metals in their pollutants list: Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Ti, and Zn. The results are also consistent with the information given by several workers who accounted for the environmentally important metals As, Cd, Cr, Co, Hg, Pb, Mn, Ni, Se, and Zn and the less well-known but environmentally important elements Sb, Ba, Au, Mo, Ag, Th, Sn, Cu, U, and V (Brian, 2013). In this study of metals in test site and control site, soil samples from test site showed higher concentrations than the soil sample from control site and all heavy metals fall below the regulatory limits of the heavy metals applied to soils by the U.S. EPA, 1996. These results showed high anthropogenic input levels for these metals. These high concentrations observed may not be unconnected with the large asphalt deposits on the soils.

Results of Table 4.3 shows there was a significant difference ($p < 0.05$) in the concentration of the polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans in the dump site soil sample compared to the control. The dump site soil sample had a higher concentration of these contaminants compared to the control soil sample.

The increase in soil concentration of PAH may be due to the test site being located at an industrial area. This is in agreement with research of Wcislo. (1998) who reported that soil contamination with PAHs increased considerably in industrial and urban areas. PAH concentrations in soils effected by emission from anthropogenic sources are up to hundreds times higher than concentrations found in reference areas (Wcislo, 1998). The PAH content from the result showed a decrease with increasing soil depth, this agrees with results of

Siewniak, (1975) who reported that PAH mainly decreases with soil depth, but they can also be found at the depths up to 2 m, such as for example, phenanthrene (Siewniak, 1975), and their quantity is narrowly related to biodegradation, which is in turn conditioned by external factors. The results also show that the concentrations of total petroleum hydrocarbons, dioxins and furans also reduced with increase in depth. The high concentration of these contaminants in the test site may be due to the asphalt dump which leached many of these contaminants into the soil and also be as a result of anthropogenic sources.

Enzyme assay of the soil samples studied revealed that sub lethal concentrations of the asphalt pollution altered the soil acid phosphatase, alkaline phosphatase, and lipase activities as shown in the results of figures 4.3, 4.4, and 4.5 above.

The mean activity of acid phosphatase in the test soil was significantly higher than the activity in the control. This may be as a result of the acidic nature of the test soil which therefore increased the production of the enzyme by the microorganisms present in the soil. The alkaline phosphatase activity and lipase activity of the soil samples show a significant ($p < 0.05$) decrease in activity in the test soil sample compared to the control soil sample. There was no significant difference in the dehydrogenase activity of the soil as both test and control soil samples did not record any presence of dehydrogenase enzyme.

Enzyme activity is a soil property that is chemical in nature but has a direct biological origin. This activity arises from the presence of many types of enzymes that are present in the soil, and within soil microorganisms. From an assortment of enzymes present and active in soil, phosphatases are interesting groups of enzymes that catalyze the hydrolysis of phosphate from organic monoester linkages (Dimitri and Maria, 2008). Phosphates released from such phosphatase action are very important to the plants and microorganisms that depend on soil for their phosphorus requirements.

The decrease in alkaline phosphatase and lipase activity could be an alteration in the enzyme activity. Alterations in the enzyme activity could be highly connected to the pollution as a result of the asphalt dump which released contaminants such as heavy metals which reduced the microbial population and also their ability to secrete these enzymes as necessary. This is in agreement with report of Liu *et al*. (2007) who found altered activity of soil enzymes with the presence of heavy metals in the soil. When soil enzyme activities are negatively affected by pollution, be it from petroleum hydrocarbons, heavy metals, effluents from cassava, palm oil or pharmaceutical, the physicochemical properties of the soil are affected which ultimately decreases its productive potential (Osuji and Nwoye, 2007).

There was a significant difference ($p < 0.05$) in the microbial population recorded in the test and control soil samples. There was higher population of fungal isolates on potato dextrose agar (Table 4.4) and higher population of bacterial isolates on Bushnellis Haas agar (Table 4.6) in the control soil compared to the test soil while the bacterial isolates on nutrient agar was higher in the test soil compared to the control soil (Table 4.5). This change in microbial population may be due to the contaminants released by the asphalt waste which deposited heavy metals, polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans into the soil. These contaminants are toxic to the microbes and therefore reduce the microbial population and microbial processes and activities in the test soil. Giller *et al*. (1997) reported that an increasing body of evidence suggests that microorganisms are far more sensitive to heavy metal stress than soil animals or plants growing on the same soil. Studies have shown that long-term heavy metal contamination of soils has harmful effects on soil microbial activity, especially microbial respiration (Doelman and Haanstra, 1984). Heavy metals have been known to disrupt ecosystem structure and functioning for a long time. In the multicellular organisms, heavy metals target specific organs and pathways (Fulladosa *et al*, 2005a; Fulladosa *et al*, 2005b), resulting in disruption of definitive metabolic functions. By the same token, one can expect that some microbial pathways would be more sensitive to metal than others, resulting in selective inhibition as metal concentration increases (Allen *et al*, 1995, Fulladosa *et al*, 2005a). Selective inhibition of specific pathways would result in decline of both numbers and diversity of organisms relying on those pathways. Aside from long-term metal-mediated changes in soil enzyme activities, many reports have shown large reductions in microbial activity due to short-term exposure to toxic metals (Hemida *et al*, 1997). Bacterial activity, measured by thymidine incorporation technique, had been shown to be very sensitive to metal pollution both under laboratory (Diaz-Ravina and Baath, 1996a; Diaz-Ravina and Baath, 1996b) and field conditions. Moreover, habitats that have high levels of metal contamination show lower numbers of microbes than uncontaminated habitats (Kandeler *et al*, 2000). All metal levels produced at least some change in the community. Invariably, such change resulted in decrease of genetic diversity of the community compared to the control.

The qualitative phytochemical screening reveals the presence of saponin, tannin, flavonoid, oxalate, alkaloid and phenol in both *Sida acuta* and *Sporobulus pyramidalis* leaf samples from the dump site and control sites. The presence of these phytochemicals in the leaf samples of *Sida acuta* is in agreement with results of Oboh and Onwukaeme. (2007) and Rami *et al.* (2014) who also detected these phytochemicals in the powdered leaves of *Sidaacuta* and also in high quantities. The quantitative phytochemical screening reveals *Sida acuta* leaves from the dump site contains higher quantity of saponin, tannin, flavonoid, alkaloid and lower quantity of oxalate and phenol compared to the leaves from the control site. *Sporobulus pyramidalis* leaves from the dump site contain higher quantity of saponin, flavonoid, alkaloid and lower quantity of tannin compared to the leaves from the control site. The increase in the concentration of these phytochemicals in the leaf samples from the dump site compared to the leaf samples from the control site may be as a result of the fact that these phytochemicals are also used as defence mechanism of the plants against conditions they cannot tolerate, conditions such as the pollution by the asphalt dump and other anthropogenic contaminants. The results are at par with the results of Daniel and Daniang. (2011), where many phytochemicals found in plants are either the products of plant metabolism or synthesized for defence purposes. This is also in agreement with the work of Sr. Prema *et al*. (2015) who indicated that there is a positive correlation existing between heavy metal concentration and phytochemical concentrations as increase in heavy metal pollution leads to increase in phytochemical concentration.

The result of the proximate analysis shows there was significant difference in the proximate result of *Sida acuta* and *Sporobulus pyramidalis* leaf samples from the dump site and control site. This can be as a result of the chemical changes which occurred in the plants due to the contamination caused by the asphalt dump which altered the plants metabolism. Chemical changes in plants associated with contaminated environment have been established by an array of scientific investigation. These include heavy metal accumulation in lower plants (Ruhling *et al*., 1987); reduction in chlorophyll, protein and carotenoids (Agrawal, 1992) and increase in total amino acids (Schmeink and Wild, 1990).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion:

The results obtained from the physicochemical and biochemical characterization of asphalt plant dump site reveals that the environment has been imparted slightly by the presence of asphalt in the soil. The study herein successfully relates the presence of toxic heavy metals, polycyclic aromatic hydrocarbons, total petroleum hydrocarbons, dioxins and furans in the soil as a result of the asphalt dump. These contaminants therefore altered the physicochemical properties of the soil as it made the soil slightly acidic, increased the soil hardness etc. These contaminants also altered the soil enzyme activity and microbial population. It also increased the defence mechanism of the plants studied as shown in the results of phytochemical and proximate analysis of the plants. These contaminants pose a great threat to the soil as they make the soil not fit for agriculture. They also pose a threat in the environment at large as they are highly toxic and are strong carcinogens. This therefore implies that the asphalt dump represents a potential source of these contaminants to the environment.

5.2 Recommendation:

It is therefore recommended that there should be alternative means of disposing the asphalt wastes or better still recycle them and put them into further useful means in other to prevent the accumulation of these contaminants into the soil. Further research should also be carried out to determine the concentration of these heavy metals and other toxic contaminants in the plants and animals present at the dump site.

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APPENDIX I

Apparatus

pH meter, beaker, weighing balance, spatula, conductivity meter, 50ml pycnometer, kjeldahl flask, kjeldahl apparatus, fume cupboard, volumetric flask, conical flask, porcelain dish, pipette, Nessler's tube, spectrophotometer, glass rod, Soil (ASTM 152H hydrometer preferred), uniform set of sedimentation cylinders with internal depth of 340±20mm and capacity of 11iter. 0.20, 2.00 and 4.75mm sieves, internal timer, end-over-end shaker rotating at 15rpm, FS 240 varian atomic absorption spectrophotometer, nitrous oxide oxidant gas, acetylene gas, air oxidant gas, measuring cylinder, separating funnel, pipetting device, test tubes/ rack, timer, centrifuge, heating bath/block, slide, bunsen flame and microscope, test tube, inoculation needle, incubator and autoclave, water bath, rotator shaker, volumetric flask, pipette, oven, desiccator, crucible, muffle furnace , funnel, fume hood, condenser, distillation apparatus, distillation flask and conical flask, round bottom flask, heating mantle, soxhlet apparatus and reflux condenser.

APPENDIX II

Reagents

Reagents for determination of % nitrogen:

Concentrated H_2SO_4, 40% w/v NaOH, 2% boric acid, 0.01N HCl solution, bromo cresol green and methyl orange.

Reagents for determination of nitrate:

Phenol disulphonic acid, concentrated NaOH and standard potassium nitrate.

Reagents for determination of particle size:

25 % sodium hexametaphosphate (calgon). Dissolve 250 g of sodium hexametaphosphate in 900 ml warm deionised water. When cool, add sufficient sodium carbonate to bring to pH 8 or 9 and dilute to 1liter with deionised water.

Reagents for determination of heavy metals in soil:

1000ppm K standard solutions, 1000ppm Pb standard solutions, 1000ppm As standard solutions, 1000ppm Al standard solutions, 1000ppm Ag standard solutions, 1000ppm Co standard solutions, 1000ppm Na standard solutions, 1000ppm Mg standard solutions, 1000ppm Ca standard solutions, 1000ppm Mn standard solutions, 1000ppm Zn standard solutions, 1000ppm Fe standard solutions, 1000ppm Cu standard solutions.

Reagents for determination of polycyclic aromatic hydrocarbons:

30% H_2O_2, pentane, isopropyl alcohol and 10-50µg/ml anthracene standard.

Reagents for determination of total petroleum hydrocarbon:

Carbontetrachloride, anhydrous Na_2SO_4, crude oil working standard.

Reagents for determination of polychlorinated dibenzo-p-dioxins in soil:

Methylene chloride and 5-25 µg/ml of PCDD working standard.

Reagents for determination of polychlorinated dibenzofurans in soil:

Butanol-acetone, 45% sodium sulphite and 5-50μg/ml PCDF working standard.

Reagents for assay of soil alkaline phosphatase activity:

Alkaline phosphate substrate: 3.6mM, sodium thymolph-thalein monophosphate in 0.2M 2-amino-2-methyl-1-propanol buffer. Magnesium chloride 1.0mM wetting agent inactive ingredients preservatives, pH 10.2 ± 0.1.Alkaline phosphatase colour developer: 0.1M sodium hydroxide, 0.1M sodium carbonate.Alkaline phosphatase standard: thymolphthaline in n- propanol 0.5mM/l. equivalent to 50u/l enzyme activity when used according to the alkaline phosphatase procedure.

Reagents for assay of soil acid phosphatase activity:

Citrate buffer (pH 4.8), substrate (p- nitrophenylphosphate), sodium hydroxide, sodium tartrate.

Reagents for dehydrogenase assay:

2,3,5-triphenyltetrazolium chloride and ethanol.

Reagents for lipase assay:

0.1M NaOH, 0.1% alcoholic phenolphthalein, 0.107M acetate buffer, hexane and ethyl alcohol

Reagents for Gram staining test:

Crystal violet, Lugo's iodine, ethanol, safraninn and oil immersion.

Reagents for spore staining test:

5% malachite green, safraninn and oil immersion.

Reagents for sugar fermentation/oxidation:

Hugh and Leifson's medium.

Reagents for hydrogen sulphide production test:

Triple sugar iron agar.

Reagents for determination of saponins:

20% ethanol, diethyl ether, n-butanol and 5% NaCl.

Reagents for determination of flavonoids:

80% methanol.

Reagents for determination of alkaloids:

20% ethanoic acid and concentrated ammonia hydroxide.

Reagents for determination of % fibre:

1.25% $H_2SO_{4,}$ 1.25% NaOH, 1% HCl, alcohol and hexane.

Reagents for determination of % protein:

Potassium sulphate , copper sulphate, sulphuric acid, 2% boric acid solution, phenolphthalein, 40% NaOH, and 0.1N HCl.

Reagents for determination of % fat:

Petroleum spirit

APPENDIX III

Asphalt plant dump site, Obinze, Imo State:

APPENDIX IV

Plant samples at the dump site