A Study of the Feasibility of Integrated-Composting as a Method for the Remediation of Hydrocarbon-Contaminated Soil Under Intense Rainfall Conditions

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School of Civil Engineering and Surveying

DECLARATION

I declare that whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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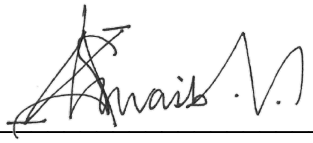
Signature: 

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ABSTRACT

The current growth-surge in global population has led to an increase in the demand for crude oil and its constituent products to support and improve the day-to-day running of our lives. However, this has been accompanied with pollution due to frequent hydrocarbon spills that have become a great cause for concern in the world today. A significant number of these occurrences are a direct consequence of anthropogenic activities. These spilled hydrocarbons have not only caused environmental degradation but also adversely affected human and animal well-being.

This study assesses the suitability and potential use of integrated-composting as a method for remediating hydrocarbon-contaminated soils. Animal manures (cow and chicken) and white-rot fungi (*Pleurotus ostreatus*) were utilised as the bacteria and fungi dominated inocula during the integrated-composting process. The effectiveness of the remedial method was examined by monitoring the changes in the concentration of hydrocarbon contamination, microbial population, and physicochemical properties. An examination into the application of this method under intense rainfall conditions was also investigated with a view to determining if the leachate released would possess high pollutant levels. In a bid to establish the suitability of the treated soil and leachate generated, contaminant concentrations in both matrixes were measured against existing soil and water quality guideline limits for agricultural purposes. The study consisted of two phases. The first phase involved laboratory-based bench-scale dynamic respirometric tests (DR-4) over a 24-day period, carried out as a screening protocol to establish the most effective amendment combination for the treatment of the contaminated soil. The second phase was a pilot study that used 100 L reactors fitted with external air supply with leachate collection component following the rainfall simulation tests.

The results of the study showed that integrated-composting was successful in the breakdown of hydrocarbon contaminants. Total petroleum hydrocarbons (TPHs) saw reductions of up to 90%. However, it was noted that Low (LMW) and medium (MMW) molecular weight polycyclic aromatic hydrocarbons (PAHs) were considerably degraded compared to high molecular weight (HMW) PAHs over the test period. The treatment with animal manures only and fungi only showed the most potential while the manure-fungi combination treatment showed a comparatively lower rate of contaminant degradation. It was also established that a number of physicochemical parameters influenced the rate of degradation. Finally, the results indicated that the contaminant levels in leachate were within permissible threshold ranges and thus deemed suitable and safe for agrarian uses.

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CHAPTER 1

1.0 INTRODUCTION

The turn of the 20th century has seen a rapid rise in global population and energy consumption resulting in an increased demand for crude oil and its by-products to improve and sustain the quality of our everyday lives (Chakrabaty *et al*., 1988). However, the processes involved in its exploration, production, and storage often present drawbacks in the form of accidental spills, pipeline leakages due to sabotage, careless disposal and mismanagement of some of its hazardous and toxic by-products (National Research Council, 1983; Okop, 2010). It is important to note that the vast quantity of crude oil spilt is inadvertently released into the soil and waterbodies consequently leading to severe adverse effects on marine and terrestrial lives, human health, and the environment (Wang & Fingas, 2005). These frequent spills have left a legacy of contaminated sites across developing and industrialised nations involved in oil production (Semple *et al.*, 2001). The presence of these industrially-derived pollutants poses a threat to human health and the integrity of the broader environment (Huang *et al*., 2004). Soil and water contamination is a global concern and can be considered a significant barrier to sustainable development (Sayara, 2010). Today, environmentalists and scientists are continually researching and seeking new ways to alleviate the contamination's detrimental effects on the environment.

Several oil-producing regions worldwide have experienced widespread cases of environmental pollution at particular periods as a result of the refining, transportation, and storage of crude oil (John *et al*., 2011). Despite the sustained and continually growing interest in environmental remediation, divisions in opinions on potential ways forward are still heavily debated. Thus, the various programs and policies initiated by the government and multinational bodies alike have consistently failed to reach an adequate basis for conciliation of interests and remediation of the environmental issues caused by oil pollution (Chokor, 2004). Most current initiatives are driven mainly by the economic benefits to the government and multinational companies with minor ecological consideration (Chokor & Odemerho, 1994). Taking the case of the Niger Delta region in Nigeria for instance, the option of engaging rural communities who are most affected by these oil spills about potential strategies of mitigating the degradation within their environment has not been explored. Narayan & Petesch (2002) state that minimal attention is given to the ordinary people of these affected communities who rely heavily on soil and surface water to support their everyday lives. Kadafa & Ayuba (2012) describe the region as a diverse ecosystem, which comprises of freshwater and mangrove swamps, rainforests, and is the largest wetland in Africa and amongst the most important marine ecosystems in the world.

However, the high rate of oil pollution has meant this area is now an ecological wasteland, affecting the indigenous people and resulting in poverty and displacement.

These problems bring to the fore a need to seek alternative and effective remediation techniques that are efficient, economical, and easily deployable in a wide range of physical settings to treat contamination (Catallo & Portier, 1992). In the past, hydrocarbon pollution in the soil has often been treated with physical and chemical processes proven to be expensive (Semple *et al*., 2001). According to Brown *et al.* (1986), traditional methods of treating soil and water have heavily relied upon removal or containment. Lee & Ward (1985) pointed out that these methods are partially effective 50% of the time and completely effective 16% of the time. Many of these treatment schemes are not usually wholly adequate or offer permanent solutions but create additional uncontrolled hazardous wastes (Riser-Roberts, 1998). In a bid to address these issues, several techniques have been developed, including bioremediation, which is of particular interest due to the possibility of re-use of the contaminated soil after treatment (Mancera-López *et al*., 2008). The pursuit of an environmentally sustainable approach towards contamination in the environment has made the ever-changing field of bioremediation a preferred option for restoring the polluted environment (Ekperusi & Aigbodion, 2015). Government agencies, corporations, and the public alike appear to appreciate the need to reduce the volume and toxicity of waste by developing safe, effective, and economical alternatives for its treatment (Nicholas, 1987).

Bioremediation is a process where the biodegradation capacities of indigenous microbial populations are enhanced by nutrient addition and or cultured microorganisms (Adesodun *et al*., 2008; Onakughotor & Aguele, 2014). Exploiting the biochemical abilities of microorganisms is one of the most prominent strategies of biological treatment, as these organisms have the unique ability to interact both physically and chemically with a diverse array of artificial or naturally occurring compounds (Head, 1998). Although the use of composting strategies in the bioremediation of organic pollutants has existed for some time, there is a paucity of information concerning its effects on the remediation of weathered hydrocarbon pollutants in soils.

Against this background, the current study assesses the suitability of composting as a remediation method for weathered hydrocarbon contaminated soils. It also seeks to understand the potential transferability and application of this technique in oil-polluted regions experiencing high rates of rainfall, as well as establish the degree of contaminant transfer during the process.

1.1 Rationale

The exploration and production of oil worldwide has led to the release of hydrocarbons and their associated pollutants into soils and water bodies (UNEP, 2011). The effects of hydrocarbon pollution have resulted in environmental degradation, resulting in a substantial decline of biodiversity, adverse impact on public health and disruption of life support systems of the local inhabitants (Ekperusi & Aigbodion, 2015). These soils are often left untreated, thereby being weathered or as in, most cases set alight. On this premise, the weathered hydrocarbon contaminated soil in Horsea Lagoon was investigated as it compares to some of the most polluted sites that can be found today.

These hydrocarbon-contaminated sites are characteristically vast in size. They often occur in remote locations where the removal and transportation of the contaminated soils for ex-situ treatment can be costly. Therefore, it is of utmost importance that these factors are carefully considered when looking at possible remediation techniques. Composting is a viable remediation alternative due to its low operational complexity, minimal running cost, and effectiveness in reducing/removing contaminants from different soils under varying conditions. On average, composting is amongst the most economical methods for the detoxification of soils contaminated with organic pollutants (Diaz, 2003). When comparing the total economic cost for the clean-up process of large sites, the savings associated with bioremediation (composting) versus chemical- or physical-based remediation technologies give composting an overwhelming monetary advantage (Table 1.1).

Composting has become recognised as an efficient and cost-effective method for the remediation of contaminated soils. According to Diaz (2003), over the last few decades, the application of composting has been widespread, hence its overall utilisation in the remediation of weathered hydrocarbon contaminated soils which represent a fundamental part of this research.

Table 1.1: Total costs of remediation options (Diaz, 2003)**.**

Remediation Method	**Total Estimated Cost[a] in millions (US$)**
Vacuum extraction	2.5
Compost-based	**3.6**
Solidification	7.3
Thermal desorption	11.4
Offsite landfill	10.8
Onsite incineration	18.9
[a] costs based on a 1-acre (4000 m^2) site. Values are an average for various biodegradable contaminants such as fuels, lubricants, PAHs and TPHs.	

Bio-augmentation of soil (introduction of microorganisms to contaminated soils) and bio-stimulation of soil (modifying contaminated soils to enhance the growth of intrinsic microorganisms) are fundamental approaches for soil remediation. While positive motives exist for both methods holding great potentials in successfully being applied as composting bioremediation strategies, a significant number of studies have investigated the use of bacteria or fungi singly, with very few examining the combined use of microorganisms from both kingdoms. There is limited evidence showing the effectiveness of the combined application of bacteria and fungi dominated material on the degradation of weathered hydrocarbon contaminated soils at relatively large scales (mesocosm trials) as well as field-scale applications. In addition to this, there is a lack of research on the effectiveness of the process under high-intensity rainfall and the fate of leachate run-off from the system.

Typically, fungal rates of degradation are comparatively slower than bacteria, but fungi can reach and degrade hydrocarbon compounds that are physically and chemically beyond the range of bacterial degradation (Loick et al., 2009). Therefore, fungi could play an ecologically significant role as they can hydroxylate a wider variety of hydrocarbons. Their subsequent polar intermediates can be mineralised by bacteria or detoxified to innocuous components (Loicke, 2008).

The use of white-rot fungi (*Pleurotus ostreatus*) and animals wastes (cow and chicken manures) and understanding the interaction between both during the biodegradation of weathered hydrocarbons will lead to a better process-based understanding of the competitive and symbiotic relations of these oil-degrading organisms. Thus, providing information to determine the suitability and feasibility of using integrated composting as a technique to remediate weathered hydrocarbon contamination.

1.2 Hypothesis

The overall hypotheses of this research are that:

- The combined use of bacterial and fungal dominated inocula promotes the breakdown of TPHs and PAHs in weathered hydrocarbon-contaminated soils.
- Intense rainfall during the remediation process leads to the formation of leachate that will contain contaminants.
- The leachate generated from the process, if any, can be used for livestock feeding and agricultural irrigation without requiring further treatment, provided that the concentration levels of the contaminants are within guideline limits.

1.3 Aims

In addressing the hypothesis, the aims of this study were the following:

- To establish the effectiveness and feasibility of integrated composting as a remediation technique for weathered hydrocarbon-contaminated soils under intense rainfall conditions to enable its use for agriculture purposes.
- To determine whether the leachate derived from this process is within the permissible limit for agricultural irrigation and livestock feeding.

1.4 Objectives

- To identify the effects of various animal manures (cattle and chicken) with different nutrient characteristics on the biodegradation of TPHs and PAHs in weathered hydrocarbon-contaminated soil.
- To examine the effectiveness of white-rot fungi (*Pleurotus ostreatus)* on the biodegradation of TPHs and PAHs in weathered hydrocarbon contaminated soil.
- To assess if there are complementary effects of animal manure and fungi on the biodegradation of TPHs and PAHs in weathered hydrocarbon-contaminated soil.

- To investigate the influence of physicochemical parameters on the degradation of TPHs in weathered hydrocarbon contaminated soil.
- To establish the presence of microbial consortia capable of surviving in weathered hydrocarbon contaminated soil.
- To monitor TPH and PAH degradation under high-intensity rainfall.
- To evaluate TPH and PAH concentration levels within the soil and leachate; ascertain its conformity to regulatory standard limits, and recommend its suitability for the proposed use.

1.5 Thesis Outline

The subsequent parts of this thesis are as follows:

Chapter 2 – *Literature Review*

This chapter provides a critical review of existing studies in the remediation of hydrocarbon pollution in soils and the roles of bacteria and fungi in the integrated composting process. The findings will be discussed in the context of the current research gaps identified.

Chapter 3 – *Materials and Methods*

In this section, all materials and methods used for this project are described. It encompasses the general experimental setup and statistical analysis procedures.

Chapter 4 – *Microcosm Study*

This chapter investigated the effect of the various animal wastes and fungi inoculated substrate on the hydrocarbon breakdown in the contaminated sample. This primarily involved the use of respirometric tests over a 30-day period. During the process, germination index and changes in overall hydrocarbon contents were monitored. These initial tests served as a screening protocol and the most promising results conducted in a larger scale pilot study.

Chapter 5 – *Mesocosm Study*

In this chapter, the three most effective treatments from the microcosm study were conducted using a 100 L composting bioreactor system with forced aeration. A high-intensity rainfall was simulated to determine the efficiency of the process under such

conditions and the degree of pollutant run-off. The hydrocarbon concentration, microbiological loading, and physicochemical parameters were monitored.

Chapter 6 – *Conclusion and Further work*

This chapter collates, assimilates and summarises the findings of the various tests conducted and gives an overall evaluation of the suitability of the process as a hydrocarbon remediation method. It also identifies future areas of research within the scope of bioremediation.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Petroleum Hydrocarbons

Hydrocarbons are amongst the simplest organic compounds as they consist of two major elements; hydrogen and carbon. These compounds are classified into two main groups known as aliphatic (no aromatic rings) and aromatic (benzene rings) hydrocarbons that are most prevalent in the environment (Figure 2.1) (Ababio, 2001). Hydrocarbons are naturally occurring compounds formed by the decay of organic substances trapped within sedimentary rocks that have undergone high temperatures and pressures. In nature, these compounds can be found in liquid form (crude oil), gaseous form (natural gas), and even solids (tars and asphalts) (Lyons, 1996).

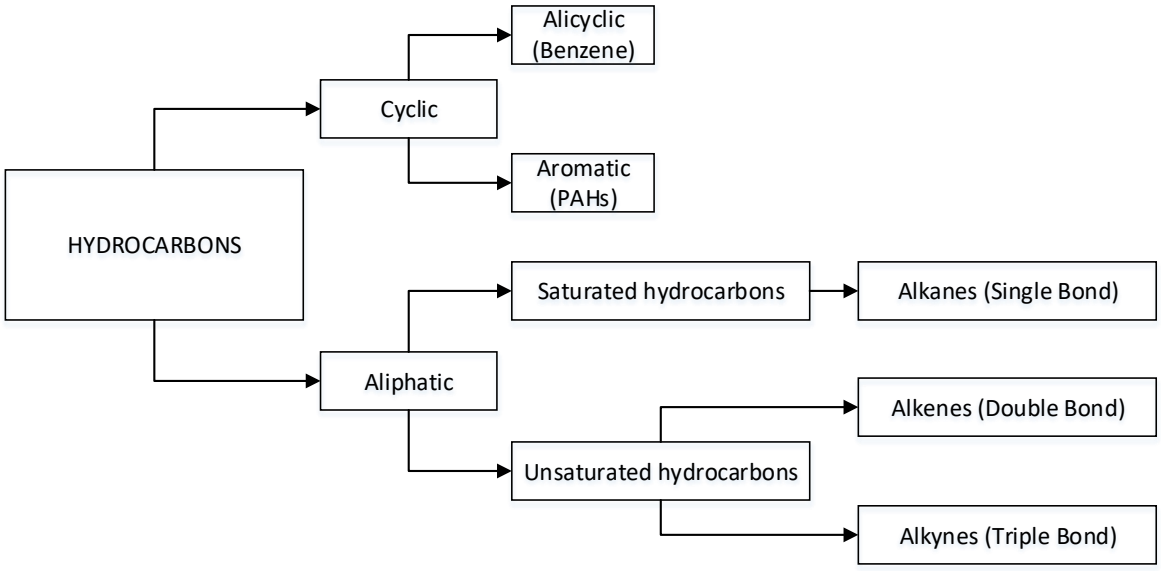


Figure 2.1: Categorisation of petroleum hydrocarbons

Several methods have been devised to understand better the various components of hydrocarbons, which include their carbon number (e.g. C_6 – C_{10} for Gasoline Range Organics (GRO) and C_{10} – C_{40} for Diesel Range Organics (DRO)) or by chemical group, e.g. Polycyclic Aromatic Hydrocarbons (PAHs). Most studies often refer to a combination of GRO and DRO as Total Petroleum Hydrocarbons (TPHs).

2.1.1 Crude Oil

Today, crude oil is one of the most important sources of energy in the world today, used as a raw material in a vast number of industries, including the refinery-petrochemical sector. Here, crude oil is refined through several technological processes into commonly use everyday consumer products such as gasoline, paraffin oils, asphalt, lubricants, domestic fuel oil, and polymers (Wolicka & Borkowski, 2012).

It is an extraordinarily complex and variable mixture of organic compounds which consist primarily of hydrocarbons in addition to heterocyclic compounds that contain elements such as sulphur, nitrogen and oxygen, all of which constitute less than 3% (v/v) (Saadoun & Al-Ghzawi, 2005). There are also trace constituents comprising less than 1% (v/v), including phosphorus and heavy metals such as vanadium and nickel. According to Prince (2014), up to 75% of crude oil consists of mainly organic compounds and approximately 10 - 15% inorganics, including aliphatic and aromatic hydrocarbons, resins and asphaltenes. The non-hydrocarbon constituents include sulphur compounds (0.01 – 8%), which occur mainly as hydrogen sulphide (H_2S), disulphides, thiophenes, benzothiophenes and naphthothiophenes. Oxygen compounds such as phenols, carboxylic acids, and nitrogen compounds such as pyrroles, carbazoles, indoles are also present (Prince, 2014). Swaine (2000) points out that besides porphyrins, trace elements occur as soaps (particularly as compounds of Zn, Ti, Ca, Mg), as well as metal-organic bonds (V, Cu, Ni, Fe) with a ranging concentration of 1200 mg/kg to 1500 mg/kg. Ezeonu *et al*. (2012) mention that the composition of crude oil may vary with the location, age of oil field, depth within oil wells and this is exemplified in (Table 2.1) which shows the different physicochemical properties of various crude oil samples.

Table 2.1: Physico-chemical properties of some Crude oils (Odebumni *et al*., 2002)

Parameter	**Bonny Light**	**Bonny Medium**	**Forcados Blend**	**Qua Iboe**
Specific gravity @ 60/60 °F	0.8576	0.8845	0.8808	0.8706
API gravity @ 60/60 °F	33.4	29.3	39.1	30.9
Kinematic viscosity (cSt) @ 100 °F	7.16	7.01	7.00	9.86
Sulphur content (*w/w* %)	0.06	0.16	0.11	0.04

Pour point (°F)	85	5	25	35
Total acid content (mg KOH/g)	-	-	0.33	-
Water content (v/v %)	-	-	0.08	-

2.1.2 Polycyclic Aromatic Hydrocarbons (PAHs)

These are a group of hydrocarbons that consist of multiple interconnected (benzene) rings which in nature are by-products of incomplete combustion of carbon-containing fuels (Freeman & Cattell, 1990). These compounds are extensive globally and most prevalent in areas with high industrial activities (Cerniglia, 1992). They have been described as persistent pollutants and are known to be present in air, soil, surface and groundwater, and road run-off (Lim *et al*., 1999; Márquez-Rocha *et al*., 2000; Martínez *et al*., 2003; Roinas *et al*., 2014). Kanaly & Harayama (2000) point out that due to their natural and anthropogenic sources, PAHs are found in every region of the world even in remote areas such as Antarctica where there are minimal human activities and impacts. It is important to note that their chemical properties are partly dependent upon their molecular size (number of aromatic rings) in addition to their molecular topology (e.g. linear- anthracene; angular- dibenzo(a,h)anthracene; or clustered- pyrene) (Figure 2.2) (Kanaly & Harayama, 2000).

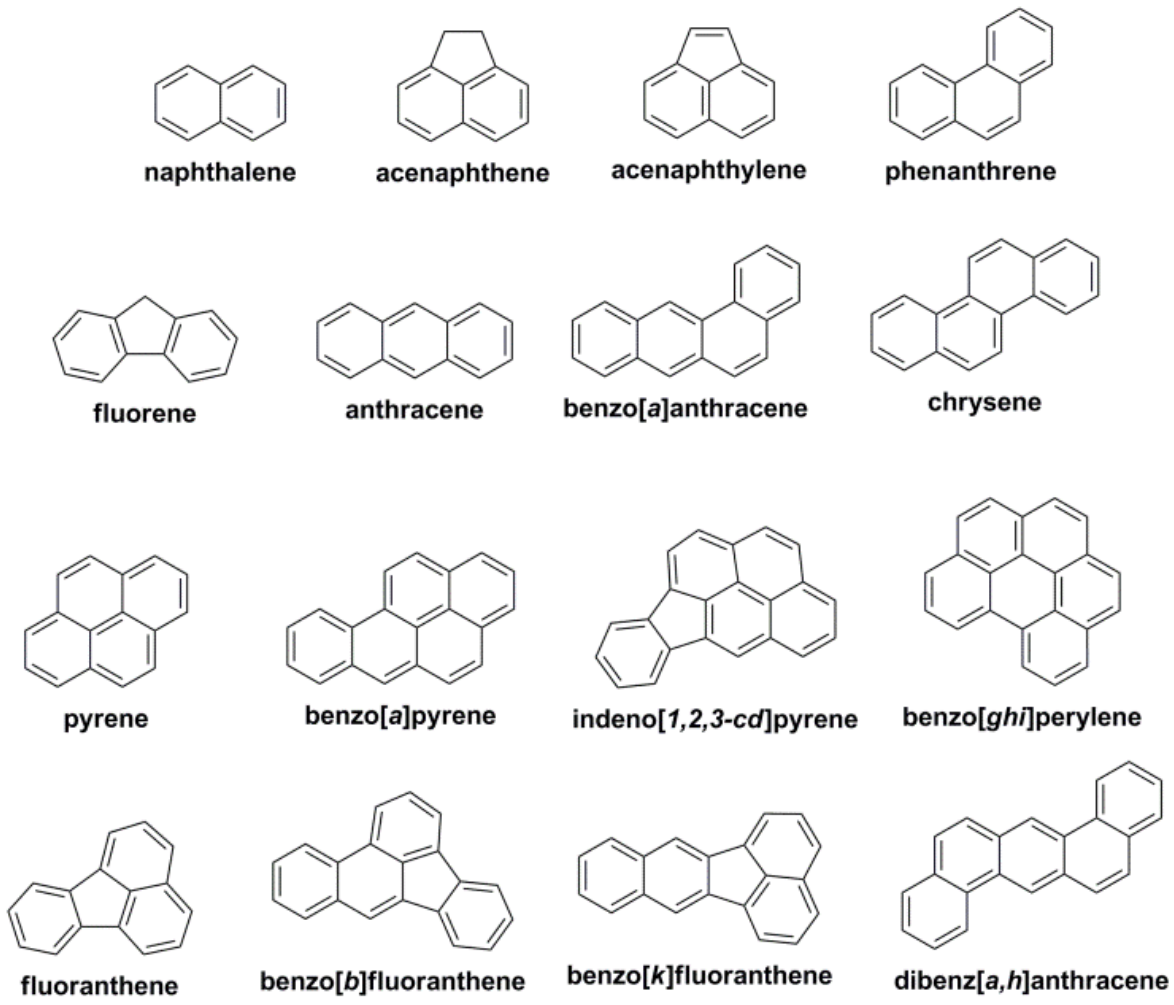


Figure 2.2: 2-Dimensional structure of 16 priority PAHs identified by the U.S. EPA

Its persistence in the environment is attributed to an increase in the molecular size and angularity which effectively results in an associated elevation in hydrophobicity and electrochemical stability (Kanaly & Harayama, 2000). The physicochemical properties of these organic pollutants are influenced mainly by their aqueous solubility (Sw) and octanol-water coefficient (Kow) (Table 2.2). Interestingly, the relationship between these key variables is inversely proportional (CCME, 2008).

Table 2.2: Selected properties of the 16-Priority PAHs (CCME, 2008)

No	PAHS	Molecular Weight	Vapour Pressure (Pa) at 25 °C	Rings	Aqueous Solubility (mg/L) at 25 °C	Octanol-Water Coefficient (Log K_{OW})	Melting point (°C)/ Boiling point (°C)
1	Naphthalene	128	11.87	2	31.7	3.60	80/218
2	Acenepthylene	152	3.86	3	16.1	3.90 to 4.10	92/265
3	Acenapthene	154	0.50	3	3.90	3.92	96/279
4	Fluorene	166	0.432	3	1.90	4.18	116/293
5	Phenanthrene	178	9.07E-2	3	1.15	4.46, 4.55	101/340
6	Anthracene	178	3.40E-3	3	4.3E-2 to 7.5E-2	4.45,4.55	216/340
7	Fluoranthene	202	1.08E-3	4	2.60E-1	4.95	111/-
8	Pyrene	202	5.67E-4	4	1.35	4.88 to 5.18	149/360
9	*Benzo(a)anthracene**	228	2.05E-5	4	9.40E-3	5.70	158/400
10	*Chrysene**	228	1.04E-6	4	2.0E-3 to 6.3E-3	5.70	255/448
11	*Benzo(e)acephenthrylene** *or Benzo(b)fluoranthene**	252	1.07E-5	4	1.50E-3	6.20	167/
12	*Benzo(k)fluoranthene**	252	1.28E-8	4	8.00E-4	6.20	217/480
13	*Benzo(a)pyrene**	252	6.52E-7	5	1.60E-3	5.97, 6.11	179/496
14	*Indeno(1,2,3-c,d)pyrene**	276	1.87E-8	6	2.20E-5	6.60	163/-
15	*Dibenzo(a,h)anthracene**	278	2.80E-9	4	2.49E-3	6.50, 6.69	262/-
16	*Benzo(ghi)pyrelene**	276	1.33E-8	6	2.60E-5	6.70	222/-

*The U.S. EPA has classified PAHs in italics as possible human carcinogens

There is an inverse relationship between the octanol-water partition coefficient (K_{OW}) and aqueous solubility expressed in (Equation 2.1) (Wick *et al*., 2011).

$$\mathbf{K_{OW}} = \frac{\mathbf{amount\ of\ chemical\ in\ octanol\ (mgL^{-1})}}{\mathbf{amount\ of\ organic\ chemical\ in\ water\ (mgL^{-1})}} \quad \textbf{(Equation 2.1)}$$

The octanol-water coefficient is often expressed as log K_{OW}. Naphthalene has a K_{OW} of 3.60, while benzo(ghi)pyrelene has a K_{OW} of 6.70. In this case, naphthalene is said to be more soluble than benzo(ghi)pyrelene.

The vapour pressure is the point at which PAHs in the solid-state transform by sublimation into the gaseous state or deposition back to a solid-state (Wick *et al*., 2011). The higher the vapour pressure at normal temperatures (25 °C), the more volatile the compounds. Thus, Mackay & Calllot (1998) point out that naphthalene with a vapour pressure of (11.87 Pa) is more volatile and would readily evaporate more rapidly than dibenzo(a,h)anthracene with a vapour pressure of (2.80E-9 Pa) at normal temperatures.

2.2 Sources of Hydrocarbon Pollution

The Chinese first discovered crude oil as far back as the pre-Christian times (Ezeonu *et al*., 2012). However, the modern-day oil industry has its roots in Romania and a well sunk in Pennsylvania by Colonel E. A. Drake in 1859 (Alloway & Ayres, 1993). Although, it was only after the advent of the industrial revolution that the demand for crude oil and its constituents experienced a surge and as a consequence of its production, there was an increase in the number of pollutants released into the environment (Smith, 2011). Most environmental pollutants originate from a source and employ various routes to infiltrate the environment. Numerous studies have reported that oil contaminants are usually introduced into the environment either naturally/biogenically or anthropogenically (Sayara, 2010). Contributions from natural or biogenic sources to environmental pollution include; emissions from plants and trees, natural forests fires, biological decays, release from volcanic eruptions, anaerobic processes in bogs and marshes (Richard, 1995). On the other hand, anthropogenic sources stem from human activities which include; petroleum spills, leakages and during transport and storage, natural gas flares and explosions, combustion/ burning of fossil fuels, e.g. power stations, nuclear waste disposal, industrial/ domestic chemicals usage, e.g. cleaning fluids, used motor oils (Mueller *et al*., 1996).

2.3 Impacts of Hydrocarbon Spills

2.3.1 Human Impacts

According to Ezeonu *et al*. (2012), there is a direct correlation between environmental health and human health; describing the former as the assessment of the health of individual organisms with a direct correlation of observable changes in the environment. Crude oil pollution has been linked to being the causative effect of many diagnosed diseases. Chindah (1998) and Sawyer & McCarty (1978) mention that petroleum hydrocarbons have been found to have carcinogenic, mutagenic, and teratogenic effects on humans. This is corroborated by Short & Heintz (1997) who state that hydrocarbon pollutants can affect genetic integrity by mutagenesis and impairment of reproductive capacity. Verdin *et al*. (2004) also mention that the acutely toxic effects of PAHs have led to the U.S. EPA classifying some of these as priority pollutants. The toxic components often exert effects by inhibiting protein synthesis, nerve synapse function, and disruption in the membrane transport system and damage to the plasma membrane (Prescott *et al*., 1996). Ana *et al.* (2009) analysed hospital data and records from Eleme and Ahaoda General Hospitals. They revealed the presence of common morbidity cases including disorders in the respiratory system, skin rashes, gastrointestinal tract disorders, rheumatism, and poisoning. Volatile components of crude oil after spills have been documented to cause aggravation of asthma, bronchitis and accelerated deterioration of the lungs (Ologunorisa, 2001). According to a report by the United Nations Environment Programme (2011), most oil spills occur on land and often reach water bodies via weathering agents (Figure 2.3).

Figure 2.3: The cumulative impact of artisanal refining on land and water in the Ogoni area of the Niger Delta (UNEP, 2011)**.**

2.3.1.1 Human Exposure Pathways

Human exposure to organic compounds is not to individual compounds but a mixture of these compounds in either occupational or environmental situations (WHO, 2003). The primary route of exposure to organic pollutants is breathing direct ambient air, ingesting food that may have been contaminated, and dermal contact in both occupational and non-occupational settings (Abdel-Shafy & Mansour, 2016). This is as illustrated in Figure 2.4. For some communities that agriculture plays a crucial role in their lives as a source of feeding and a means to boost the local economy by generating income for harvests sold. However, farmers/ individuals involved in this practice are often left exposed to the harsh effects of these pollutants, which have detrimental health effects thereby hampering their ability to work effectively and be productive (Osuji & Uwakwe, 2006). Crude oil can enter water via direct spills that initially occur on land and subsequently get into water bodies through the effects of rain, wind, surface or sub-surface flow (O'Reilly *et al*., 2001).

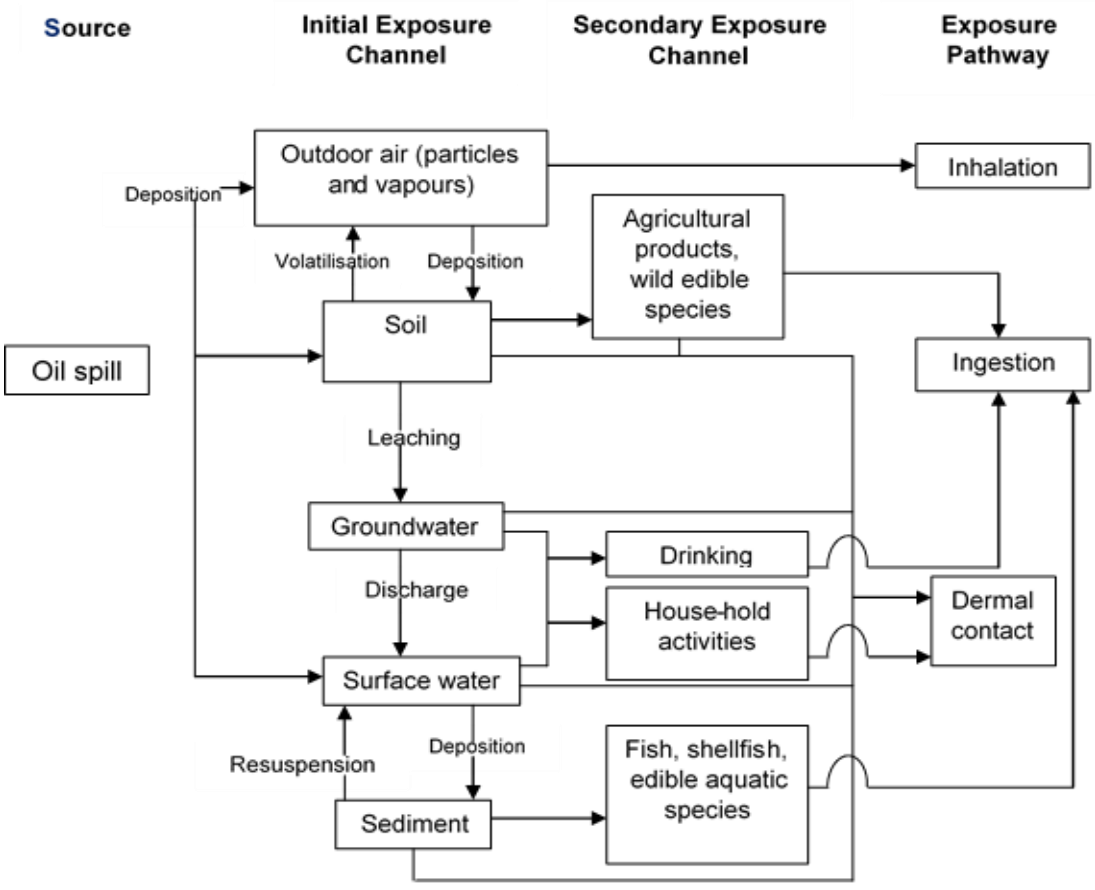


Figure 2.4: Conceptual framework of direct human exposure to oil spills by the author

Hydrocarbons are found in mangrove floors, and intertidal shores and are usually inundated by flooding and tend to end up consumed by aquatic life, which is then consumed by humans leading to severe health complications *(Osuji et al*., 2010). UNEP (2011) reports

that members of small fishing communities within these coastal regions risk exposure if they drink, bathe with, or collect shellfish from the polluted water bodies around their area.

2.3.2 Environmental Impacts

2.3.2.1 Soil

Cerniglia (1992) reports that point sources may originate from hydrocarbon spills and various industrial processes such as gasification during coke production and crude oil refining represent the most significant causes of the release of pollutants to the environment. A substantial amount of crude oil spilt often results in the damage of soil properties particularly as it causes anaerobiosis which depletes soil oxygen (Amadi *et al*., 1993). A study by Osuji & Ukale (2005) suggests that the surface film of the oil reduces gaseous diffusion into the soil and increases the stress on surface and subterranean organisms. This accords with earlier observations by Odu *et al.* (1985) who reported that contamination of soil by crude oil led to the depression of microbial density and reduced bacterial activities in cases of relatively light contamination. Such light concentrations of contamination can render soil dysfunctional even months after clean-up activities have been carried out making crop cultivation practically impossible (Osuji *et al.*, 2004). Baker (1976) suggests that beyond 3% concentration, oil is increasingly deleterious to soil biota and plant growth. This corroborates results presented by John *et al.* (2011) which indicate that the increased percentage of oil pollution from 0.5 to 2% resulted in the decreased amount of primary macro-nutrients (nitrogen and phosphorus) by 50% leading to poor crop growth. NDES (1999) reports that crude oil spills have adverse effects on physicochemical properties such as pH, temperature, moisture content, and electrical conductivity, critical variables that affect nutrient availability. Earlier experiments by Manahan (1994) showed that an acidic pH range of 3.1 – 3.8 has implications on nutrient availability in hydrocarbon-polluted soils. Osuji & Ukale (2005) point out that pH range may also affect the solubility of minerals and allow toxic elements and compounds to become mobile and available for plant uptake. These acidic soil often possess higher levels of aluminium and manganese which are generally harmful to most plants (Ayotamuno & Kogbara, 2007). Akomeo (1981) observed that moisture content decreased considerably in polluted soils due to the migration of heavier hydrocarbon components downwards under the effects of gravity. Further stating that, during this process, oil occupies interstices resulting in reduced moisture content.

2.3.2.2 Water

The pollution of waters, i.e. streams and rivers by oil and its derivatives, is a significant challenge facing oil-producing regions today (Olajire *et al.*, 2005). In aquatic environments, water usually floats on the water surfaces, where it is dispersed to shorelines by wind and wave actions, thus affecting the soil around (Osuji *et al*., 2010). These organic pollutants rapidly adhere to particles found in the water column which subsequently become sources of contamination of water upon release via sediment suspension (Abu & Dike, 2008). According to O'Reilly *et al*. (2001), the persistence of hydrocarbons can have both physical and chemical effects on water even in small quantities; it prevents oxygen transfer in the water column, thus affecting the aquatic organism's support systems.

2.3.2.3 Aquatic and Terrestrial Wildlife

The severity of the damage is primarily dependent on the type of hydrocarbon; quantity spilt, temperature, and season. In 2010, the US Fish and Wildlife Service described the presence of dissolved or emulsified oil in the water column as a contaminant that affects plankton, algae, fish eggs and invertebrate larvae (U.S FWS, 2010). Earlier studies by Diaz-Pifferrer (1962) on the Argea Prima crude oil spill in the southern shore of Puerto Rico revealed that the hydrocarbon incursion caused a high mortality rate of shallow and shore-dwelling organisms and extensive damage to the mangrove swamp. Physical contact with oil destroys the insulation properties of fur and feathers, resulting in detrimental effects on birds and other fur-bearing animals. A UNEP (2011) report points out that heavily oiled birds lose their ability to fly, as well as their buoyancy, causing drowning. In efforts to clean themselves, these birds tend to ingest oil pollutants, leading to deaths (IPIECA, 2000).

2.3.2.4 Flora and Fauna

The mode in which petroleum hydrocarbons affects plants is generally complex; it involves direct contact with the toxic pollutant and indirect contact, usually mediated by the interactions of petroleum hydrocarbon with abiotic and microbial components of soil assimilated by plants (Osuji *et al*., 2010). Osuji *et al*. (2004) carried out a field survey of the surface and subterranean flora and fauna and revealed that crude oil pollution was responsible for reducing plant and animal species. Kinako (1981) observed that the lower seral stages of a Nigerian rainforest community were more adversely affected by crude oil than perennial crops, while herbaceous plants were also susceptible to damage. As subsistence farming is the predominant occupation of members of the local communities in this region, it is not only vital for the local economy but a source of food (Osuji & Ozioma,

2007). Chokor (2004) also points out that tuberous crops are the major crops cultivated by farmers in the region, including yams, cassava, plantain, and pepper. Odu *et al.* (1985) earlier suggested that these crops are the most negatively affected by oil spills as the minimal growth of these crops were observed even after six months after the spill occurred. Great losses in biodiversity have been witnessed in the oil-rich Niger Delta region due to acid rain which is a consequence of gas flaring activities (Kadafa & Ayuba, 2012). Uyigue & Agho (2007) suggest that the prevalence of grasses and shrubs in certain areas within the region may indicate losses in natural forests due to acid rain. Adding that, the concentration of acid rainwater appears to be considerably higher in the region and decreases as one moves further away. The vast quantities of heat generated during gas flaring kill nearby vegetation, destroys mangrove swamps and marshes, suppress the growth of some flowering plants, and diminish agricultural productivity (Mba, 2000). Previous studies in the area have demonstrated a direct relationship between gas flaring and the decline in agricultural productivity (Adeyemo, 2002; Salau, 1993), as shown in (Table 2.3).

Table 2.3: Impact of gas flaring on agricultural output in the Niger-Delta region of Nigeria (Adeyemo, 2002; Salau, 1993)

Distance of farmland from flaring site	**Loss in yield of crops (%)**
200 meters	100
600 meters	45
1 kilometer	10

2.3.3 Hydrocarbon Pollution in the Oil-producing regions

The discovery of oil in many areas has been accompanied by enormous financial gain for several countries. However, its production and exploration have posed adverse effects, especially on indigenous/local communities that live near areas where these activities are prominent; unfortunately making it a sad case of “a gift within a curse”.

Most spills that occur in the oil-producing areas for example the Niger Delta region of Nigeria are mainly a result of anthropogenic causes. Osuji & Uwakwe (2006) have stated that oil spills in this area mostly emanate from pipeline ruptures and oil well blowouts (Figure 2.5).

These are a direct consequence of intentional damage to these facilities often referred to as sabotage, which is the primary cause of crude oil spills in the region.

Figure 2.5: Typical blown-out wellhead in the oil-producing Niger Delta region, Nigeria (Okop, 2010)

This is consistent with the findings of Ndimele (2010) who points out that the primary cause of oil spills is pipeline vandalisation by saboteurs (individuals or groups). These individuals or groups seek attention from the government to correct the economic marginalisation and ecological disaster occasioned by many years of crude oil exploration and exploitation in their areas. A report published by Shell Petroleum Development Company (SPDC), a principal stakeholder in oil production within Nigeria seems to corroborate earlier findings indicating that a majority of the spills observed in the oil-producing Niger Delta region of the country are the result of third-party interference, mainly sabotage, theft of equipment, leaks caused by thieves drilling into pipelines or opening wellheads to steal it. On average, such incidents have accounted for more than 75% (Figure 2.6) of the oil spill incidences in SPDC run facilities within the Niger Delta region (SPDC, 2011). The Department of Petroleum Resources (DPR) concedes that a vast number of oil spill incidences are traceable to equipment failure due to vandalism. Accidental releases by vessels carrying the product, oil blowouts from flow stations are also mentioned causes of oil pollution in the area (Nwilo & Badejo, 2005).

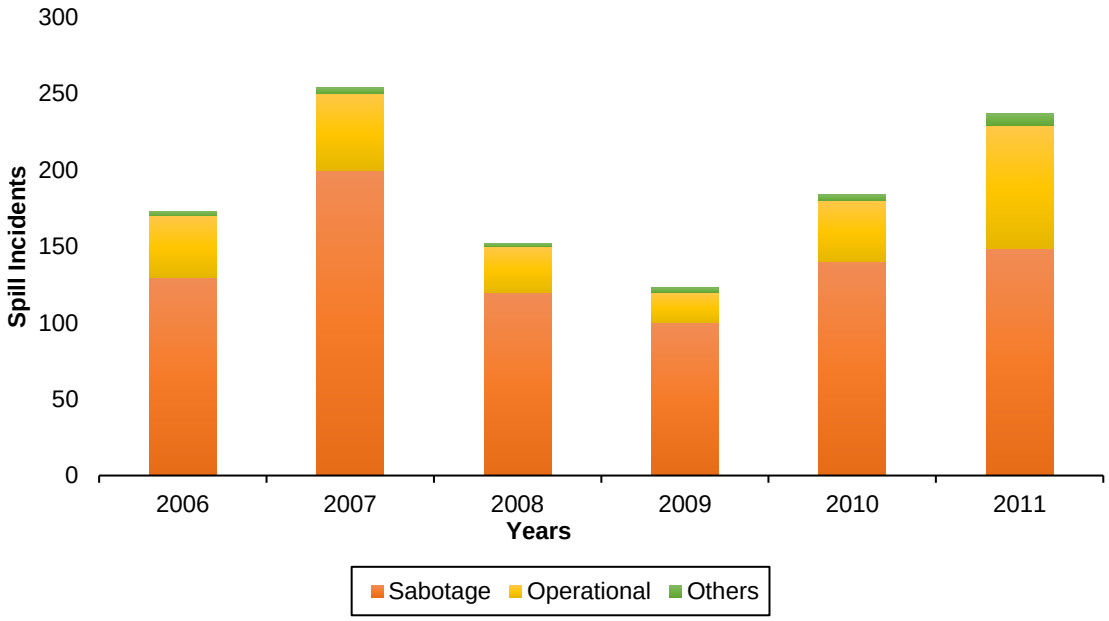


Figure 2.6: Oil Spills in the Niger Delta region of Nigeria between 2006 - 2011 and their respective causes (SPDC, 2011)

Kadafa & Ayuba (2012) report that between 1976 and 2001 over 3 million barrels of oil were lost via 6,817 spill incidences; of which over 70% of the spilt oil was released into the environment.

2.4 Environmental Fate of Hydrocarbons

Semple *et al*. (2001) have previously stated that the soil acts as a prominent sink for organic contaminants in the environment and the prolonged contact with the soil means they are less bioavailable and extractable (Reid *et al*., 2000). Crude oil contains high molecular weight compounds that make it heavier, and as a result, it tends to bind to soil particles and remains trapped within the organic phase, making its departure from the soil difficult (Ezeonu *et al*., 2012). Cerniglia (1992) and Hatzinger & Alexander (1995) describe this occurrence as weathering, of which the extent of its effects is hugely dependent on the characteristics of the pollutants and the soil properties. Elsewhere, Wang & Stout (2007) identify weathering as the physical, chemical, and biological processes undergone by organic pollutants released into the environment. Some of these processes are listed in (Table 2.4) and illustrated in (Figure 2.7). According to Serrano *et al.* (2008), weathering can alter the state chemical fingerprint of spilt or discharged hydrocarbons to the point it no longer matches its original form. Within the initial period of oil spills occurring, the rate of non-biological degradation is often very rapid (Alloway, 1997), with photo-decomposition transpiring within this time (Fingas, 2001). This can be attributed to the high toxicity of these

organic pollutants, which inhibit development and prevent any form of metabolic activity within the microbial population at the early stages of the spill. However, Das & Chandran (2011) suggest that after a so-called “epoch of stress”, the microorganisms within the population slowly begin to adapt to the pollutants. The biodegraded aliphatic hydrocarbons are used as a source of carbon and energy with complete degradation brought about under aerobic conditions.

Table 2.4: Processes that enhance the transportation and transformation of organic contaminants (Riser-Roberts, 1998)

Processes	**Transport and Transformation route**
Physical	Advection, dispersion and volatilization, adsorption and ion-cation exchange, sedimentation, diffusion
Chemical	Ionization, hydrolysis, oxidation-reduction and complexation
Biological	Bioaccumulation and biodegradation

It is widely accepted that adsorption is one of the most common factors controlling the weathering process. Crude oil is highly viscous with low volatility. Its hydrophobicity contributes to its rate of adsorption and as such, ions and molecules of the oil adhere to the solids often resulting in a higher concentration on the soil surface over the concentration present in the soil moisture in some cases (Riser-Roberts, 1998). Several compounds, particularly those as hydrophobic as oil, adsorb onto the organic matter fraction of soils rather than the clay constituents, and the extent is directly related to the octanol-water coefficient (K_{OC}) (Beaudin *et al.*,1999). Atlas (1995) suggests that organic matter in soils generally has high surface areas and exchange properties ideal for the adsorption of organic compounds. Some authors explain a preferential accumulation of organic chemicals on the hydrophobic soil particles (hydrophobic bonding) and are most likely related to van der Waal forces. Another critical factor is the diffusion of these compounds into the soil pore spaces soil and within the soil organic matter (Loick *et al*., 2009). As compound uptake by microorganisms is far more extensive from liquids than from absorbed states, it is proposed that pollutant mass transfer dictates bioavailability (Semple *et al*., 2003). Table 2.5 further elaborates on the processes involved in the transfer, degradation and sequestration in the environment.

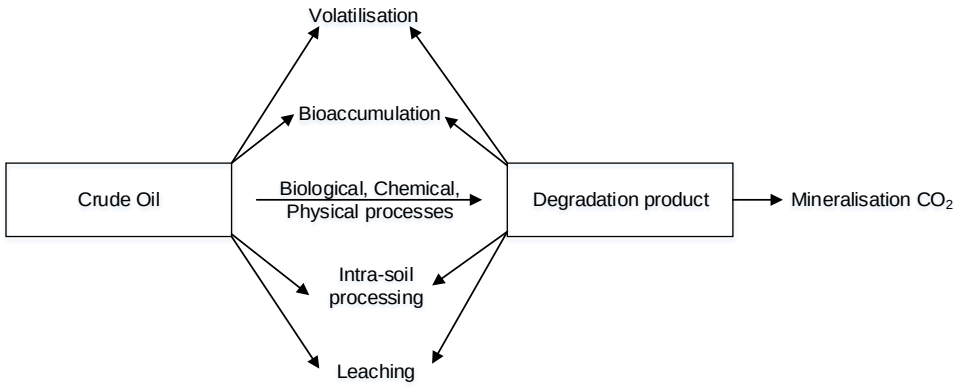


Figure 2.7: Summary of the environmental fate of hydrocarbons in soil (Semple *et al*., 2001)

According to CCME (2008), organic contaminants can be divided into three major categories in terms of their environmental fate tendencies: compounds that are volatile and quickly released from the soil to the atmosphere; compounds that are rapidly mineralised (metabolised) by microbes and exhibit little persistence; and persistent compounds, being strongly adsorbed to the soil (organic matrix).

Table 2.5: Movement and fate of organic pollutants such as PAHs in the environment (Pierzynski *et al*., 2000)**.**

Process	**Consequence**	**Factors**
Transfer (*Processes that relocate PAHs without altering their structure*)		
Volatilisation	PAH losses due to evaporation from soil, plant, or aquatic ecosystems.	Vapour pressure, wind speed, temperature.
Absorption	PAH uptake by plant roots or animal ingestion.	Cell membrane transport, contact time, susceptibility, plant species.
Leaching	PAH translocation laterally or downward through soils.	Water content, macro-pores, soil texture, clay and organic matter content, rainfall intensity, irrigation.
Erosion	PAH movement by water or wind action.	Rainfall, wind speed, size of clay and organic matter particles with adsorbed PAHs on them.
Degradation (*Processes that alter PAH structure*)		
Biological	PAHs degradation by microorganisms, biodegradation, and cometabolism.	Environmental factors (pH, moisture, temperature, oxygen), nutrients, organic matter, PAH bioavailability, microbial presence, molecular weight.
Chemical	PAH alterations by chemical processes, e.g. photochemical (i.e. UV light) and redox reactions.	High and low pH, the structure of PAH, intensity and duration of sunlight, exposure to sunlight, and the same factors for microbial degradation.
Sequestration (*Processes that relocate PAHs into long-term storage without altering structure*)		
Adsorption	PAH removal from bioavailable pools through interaction with soils and sediments.	Clay and organic matter content, clay type, moisture.
Diffusion	PAH diffusion into soil micro-pores where it is unavailable for microbial degradation.	Hydrophobic nature of micro-pores and PAHs.

However, in aquatic environments, the highest concentration of PAHs will be adsorbed to the suspended particulate matter, and very minimal quantities will be dissolved in the water due to their relatively low solubility (Roinas *et al*., 2014). Potential sedimentation of the particulate matter will occur over some time, which may result in the entrapment and preservation of PAHs, turning the soils into their main environmental sink (Roinas *et al*., 2014). There is usually a possibility of accumulation and this is when the build-up rate is faster than the removal processes such as biodegradation. According to the U.S. EPA (2003), the behaviour of PAHs is hugely influenced by their physicochemical properties, Low Molecular Weight (LMW) PAHs being more susceptible than High Molecular Weight (HMW) PAHs to various degradation/weathering processes. Furthermore, LMW-PAHs tend to be hydrophilic, whereas HMW-PAHs are known to be hydrophobic (Wild & Jones, 1995). In the absence of dissolved organic matter, Guggenberger *et al.* (1996) point out that HMW-PAHs are not capable of environmental transport in aqueous solutions.

2.4.1 Hydrocarbon Weathering Mechanisms

2.4.1.1 Volatilisation

The process of volatilisation involves the loss of hydrocarbons from the surfaces in the vapour phase, indicating it requires the vaporisation and movement of chemicals from a surface into the atmosphere above the surface (Jury *et al*., 1990). It is worth noting that the reduction of hydrocarbons is not solely down to mineralisation as they can also be lost due to volatilisation (Loicke, 2008). The rate of PAH volatilisation is influenced by their molecular weight, water movement, and prevailing weather conditions (Slooff *et al*., 1989). Hartzell (1989) states that for volatilisation to take place, organic compounds must navigate through a complex structure of solid particles and void spaces to the soil surface. Riser-Roberts (1998) mentions that a thorough understanding of the mechanisms involved in pollutant transformation through various matrixes is vital for predicting its volatilisation. Thus, it is important to note a few of these mechanisms; diffusion through the vapour and aqueous phases, the flow of water-soluble pollutants to the surface due to capillary action, and evaporation of water from the soil surface (Riser-Roberts, 1998).

According to Jury *et al*. (1990), the rate of volatilisation is a complex function of varying properties of hydrocarbon pollutants and the surrounding environment in which it is released. For most organics in soil systems, the key factors affecting their volatilisation include contaminant vapour pressure; contaminant concentration; Henry's Law constant;

soil/chemical adsorption reaction; contaminant solubility in water; contaminant solubility in organic matter; soil temperature, water content, organic content, porosity and bulk density (Johnson *et al*., 2003; Yates *et al*., 2002). However, Jury *et al*. (1990) identify vapour pressure as the vital attribute affecting contaminant volatilisation, while the critical environmental factors affecting mobility are the various partition coefficients for the different soil/water/air conditions within the soil system.

Results from an air-soil exchange experiment of semi-volatile organic compounds (PAHs and Polychlorinated biphenyls) revealed that significant losses of these compounds in spiked soil were observed. Interestingly, losses in acenaphthene and fluorene were so substantial that similar levels were found in un-spiked soils (Cousins & Jones, 1998). Similarly, Park *et al*. (1990) found that volatilisation accounted for an estimated 30% of the total loss of naphthalene, about 20% of 1-methylnaphthalene and only 0.1% of HMW PAHs.

Contrary to the previous studies, Guerin (2000) found no losses of LMW PAHs through volatilisation. Kirchmann & Ewnetu (1998) during a 20-week composting experiment of oil-contaminated soil using horse manure showed no significant losses of PAHs due to volatilisation.

2.4.1.2 Photolysis

Biodegradation of oil-polluted sites may be enhanced by photochemical reaction (Sims & Bass, 1984). Photolysis reactions are oxidative and as such stimulate microbial degradation through the oxidation of complex structures (Gohre & Miller, 1986). These types of reactions are often limited to the surface of the soil or surface treatment of groundwater, although, coupled with soil mixing, it may help treat relatively recalcitrant pollutants (Riser-Roberts, 1998). Photolysis can be as a result of direct light absorbed by a hydrocarbon (direct) or due to reactions mitigated by energy-transferring sensitiser molecule (sensitised photo-oxidation) (Sims & Bass, 1984). These photo-sensitizers generally enhance the process of photolysis, dissolution of organic matter and nitrates, and are considered to be very crucial in the water column (Sanches *et al*., 2011). Interestingly, Fasnacht & Blough (2003) showed that dissolved organic matter essentially does not affect the photo-fate of PAHs.

2.4.1.3 Sorption and Sedimentation

Solid particles can act by adsorption, which is the retention of solutes in solution by the surface of the particle, or by absorption, which is the retention of the solutes within the mass of the solid rather than on the surfaces (Alexander, 1999). The term *sorption* refers to both

processes. Sorption is perhaps one of the principal factors affecting the bioavailability of organic pollutants, including their toxic and recalcitrant fractions in the environment (Sims, 1990). Solid organic matter plays a significant role in the adsorption of hydrocarbon pollutants (mainly hydrophobic organic compounds), clay minerals, and amorphous minerals (e.g. iron hydroxides) (Kan & Tomson, 1990). According to Carroquino & Alexander (1998), organic matter may slow down the biodegradation of bound PAHs that are otherwise readily metabolised. PAHs such as phenanthrene are less degradable when adsorbed to organic constituents than in liquid matrix. The mineralisation of pyrene is hindered by fulvic acids, partially due to possible sorption of the compound making it less bioavailable (Grosser *et al*., 1994).

An array of compounds, hydrophobic ones, sorb to the organic fractions of soils and soils (Alexander, 1999). This includes a vast number of PAHs and other non-polar contaminants, which are adsorbed primarily by the natural organic matter, rather than the clay constituents. The extent of the retention is directly dependent on the octanol-water coefficients (Riser-Roberts, 1998). Adsorbed PAHs present in suspended solids of aquatic environments often undergo sedimentation, which is primarily one of their weathering mechanisms (Cerniglia, 1992). Tansel *et al*. (2011) point out that heavier fractions of hydrocarbons gradually sink and undergo sedimentation and that the sinking of these fractions occurs by adhesion of particles to soil particles and/or organic matter. In particular, shallow waters are usually laden with suspended solids providing favourable conditions for sedimentation. Organic pollutants on shorelines as is the case in the Niger Delta, are often washed back into rivers after which sedimentation is likely to occur. Illegal oil refineries in the Niger Delta often tend to burn off oils once spilt; the residues can be quite dense and therefore sink (ITOPF[2], 2011; ITOPF[3], 2010). Payne *et al*. (1988) consider sediments to be the final environmental sink for PAHs after sedimentation.

2.4.1.4 Diffusion and Dispersion

The rate of hydrocarbon pollutant movement through soil, water, and air is directly proportional to the concentration of the toxicant and its diffusion coefficient (Lapworth *et al*., 2012). Diffusion results from random molecular motion due to the thermal kinetic energy of the solute (Riser-Roberts,1998). All contaminants that have dissolved disperse as they move with groundwater because of molecular diffusion and dispersion. This causes a net flux of the soluble fraction of the pollutant to move from a zone of high concentration to one of lower concentration (Mackay *et al*., 1985). According to Gilot *et al.* (1997), the coefficient of diffusion is a proportionality constant between the molar flux and the concentration gradient which is a function of temperature and molecular weight. The authors' further state

that, in locations where the advective flux is low (sedimentary rock and aquitards), diffusion is often the dominant transport mechanism. Due to this movement, concentrations are reduced with increasing distance from the contaminant source. Riser-Roberts (1998) points out that the coefficient of diffusion in liquids is less in porous medium than in pure liquid because a collision with the solids of groundwater medium hinders diffusion. Mechanical dispersion also referred to as hydrodynamic dispersion occurs as a result different flow paths water particles take in a geological medium (Ram *et al*., 1993). The authors indicate that this is due to: fluids moving faster through the centre of the pores than along the edges due to less friction; fluids travelling shorter pathways and/or splitting or branching to the sides; fluids travelling faster through larger than smaller pores. Other flow paths may be slower because of their close proximity to the grain boundaries, thus being exposed to more friction in the pore throat, slowing down water particles (Leahy & Colwell, 1990). These varying flow paths of the water particles cause mechanical dispersion, mechanical mixing and dilution of the solute within the bulk movement of groundwater.

Mechanical dispersion is numerically described as the product of advective groundwater velocity and dispersivity. The dispersivity is a characteristic property of the geological medium and differs in value for each spatial component. When dispersion occurs in the direction of flow, it is referred to as longitudinal dispersion and when it is perpendicular to the flow, it is called transverse dispersion both in a horizontal and vertical plane to flow (Farrington, 2014). Lapworth *et al*. (2012) draw our attention to the fact that the dispersion of hydrocarbons in the direction of flow is much greater than in the direction transverse to the flow. In the water column, petroleum-based oils exhibit weathering profiles due to natural dispersion, photo-oxidation, and evaporation, mainly in the mixing layer near the surface and emulsification (ITOPF[1], 2011). Owing to weathering, light fractions swiftly disappear by volatilisation and solubilisation in the ocean mixing layer. However, heavy fractions of crude oil possess less soluble and volatile properties; thus can persist in water and sediments for extended periods after more volatile fractions such as benzene, toluene, ethylbenzene, and xylene (BTEX) have disappeared (ITOPF[1], 2011).

2.4.1.5 Advection

According to Mackay *et al*. (1985), advection is the most prominent factor in the migration of dissolved organic contaminants in a process by which the bulk motion of flowing groundwater transports them. In most cases, groundwater flows from subsurface regions where the water level is high to those that are low in response to a hydraulic gradient is a process referred to as advection. Under natural gradient conditions, flow velocities are said to be between 10 and 100 m/year (Riser-Roberts, 1998).

2.4.1.6 Biodegradation

Biodegradation is a process that involves the breakdown of organic compounds in nature over time by the activities of microorganisms such as bacteria, actinomycetes, and fungi (Sims & Bass, 1984). Alexander (1980) describes it as an array of diverse microbial processes that occur in natural ecosystems, such as mineralisation, cometabolism, and detoxication. Degradation of organic pollutants in soil and aquifers are generally influenced by various environmental constraints such as temperature, pH, dissolved oxygen, salinity, inorganic nutrients (e.g. phosphorus and nitrogen) and molecular factors (number and structure of PAH rings). The organisms that appear in most soil systems (indigenous microbial populations) appear to be the central agents involved in the metabolism of the chemicals in soil and water (Riser-Roberts, 1998). These microorganisms involved in the degradation process often derive energy by using the organic pollutants as a carbon source, increasing their biomass (Lee & Ward, 1985). Roinas *et al.* (2014) point out that severe hydrocarbon pollution can result in anoxic and acidic conditions, which can significantly lower the rate of bacterial degradation of some organic pollutants leading to increased residence time. Although, Delaune *et al.* (1981) suggest that the residence time of some organic pollutants in the soil is not indefinite, as individual constituents can potentially be metabolised anaerobically. The authors go on to state that it occurs at a much slower rate than in aerobic conditions. Regardless of the medium (soil or water), HMW-PAHs often take more extended periods to biodegrade than the LMW-PAHs. According to Herbes & Schwall (1978), the biodegradation half-lives of sediment-bound naphthalene a LMW-PAH ranged from 0.3 to 129 days while that of benzo(a)pyrene a HMW-PAH ranged from 0.3 to 58 years. However, in water, half-lives of these PAHs are considerably shorter. CCME (2008) reports that the half-life of naphthalene in water ranged between 0.5 to 20 days and for benzo(a)pyrene ranged from 0.6 to 5.2 years under aerobic conditions, adding that many of the PAHs in water volatilise. Table 2.6 shows half-lives of PAHs in soil and a selected few in water. Several authors describe weathering as an important mechanism of biodegradation due to the fact that it breaks down organic contaminants particularly water-soluble hydrocarbons and does not present significant problems with respect to the generation of hazardous by-products (Lee & Ward, 1985; Riser-Roberts, 1998).

Table 2.6: The half-lives of selected PAHs in soil and water (Paraíba *et al*., 2011) [a](Irwin, 1997), [b](Khan, 1980), [c](Spectrum Laboratories, 2010)

No	PAHS	Half-life in Soil at 25 °C (Days)	Half-life in Water at 25 °C (Days)
1	Naphthalene	48	-
2	Acenaphthylene	60	-
3	Acenaphthene	102	-
4	Fluorene	60	-
5	Phenanthrene	200	-
6	Anthracene	460	0.75[a]
7	Fluoranthene	440	2.96[b]
8	Pyrene	1870	70.83[b]
9	Benzo(a)anthracene	670	-
10	Chrysene	990	686.37[c]
11	Benzo(b)fluoranthene	610	-
12	Benzo(k)fluoranthene	2140	-
13	Benzo(a)pyrene	530	-
14	Indeno(1,2,3-c,d)pyrene	730	-
15	Dibenzo(a,h)anthracene	940	-
16	Benzo(ghi)pyrelene	650	-

However, Alexander (1999) is cautious about this suggestion and points out that it is possible through microbial transformations (activation) to convert a non-toxic parent compound into a toxic one. This is corroborated by an earlier study in which Lee *et al.* (1987) suggest that some partial degradation products might be more toxic than the parent compound. Thus, Mackay *et al*. (1985) insist that, though biodegradation is a feasible option for treating hazardous organic pollutants, there is no assurance of its conversion into simple or even less hazardous products.

2.5 Soil and Water Quality Guidelines

Guidelines are usually intended to protect human health, sustain and enhance the quality of the environment, social and economic well-being, and overall intrinsic value. The guidelines specified in this project are generic numerical concentrations obtained from several sources, narrative statements that specify levels of substance toxicity and other potential stressors that may be found in the environment. These have been established using the best available scientific information, below which risks to humans or animals are expected to be negligible.

In terms of soil assessment, the Canadian Soil Quality Guidelines (CSQGs) was primarily used to prescribe limits for hydrocarbon concentration in soils. The guidelines provide

concentrations of contaminants in the soil at or below which no appreciable risks to human health are expected. Based on this protocol, both the environmental and human health soil quality guidelines for various land-use scenarios (agricultural, residential, commercial, and industrial) are developed. Here, the lowest value generated by both approaches (human health-based and environmentally based derivation) for the various land uses has been incorporated into the guidelines to establish suitable limits (CCME, 2008).

The DEC categorises the degree of soil contamination according to Ecological Investigation Levels (EILs) and Health Investigation Levels (HILs). Kalf *et al*. (1997) also describe Maximum Permissible Concentration (MPC) of contaminants in soils as reported by the Netherlands Environmental Quality Objectives for PAHs which is referenced in the Canadian soil quality guidelines (CCME, 2008). The Massachusetts Department of Environmental Protection promulgates Upper Concentration Limits (UCLs) for contaminants in soil and groundwater.

EIL: The Ecological Investigation Level is the concentration of a substance above, which further appropriate investigation and the evaluation of risks to the environment or environmental values will be required. These levels are used in the context of an initial screening assessment to determine whether the concentration of substances in the soil at specific sites potentially pose a risk to the environment or relevant environmental values. If these levels are exceeded, the appropriate next step is to investigate further if the levels present are likely to pose an actual risk in the site-specific setting. The additional investigation may take the form of comparative assessment with background concentrations and/or consideration of site-specific factors that may affect contaminant availability. Once this step is concluded, possible remediation methods may be considered.

HIL: The Health Investigation Level is the concentration of a substance above which further appropriate investigation and assessment of risks to human health may be required. These levels are primarily established on the health-based soil investigation levels presented in the National Environment Protection Measure (NEPM) (NEPC, 1999) which were developed through the enHealth Council in 2001.

UCL: The Upper Concentration Limit is the concentration of contaminants, which, if exceeded under specified conditions, indicate the potential for significant risk or harm to public welfare and the environment under future conditions. This assessment is published by the Massachusetts State Department of Environmental Protection in the Massachusetts Contingency Plan (MCP) as “Method 3”. These guidelines use the existing standards of the US EPA, UCLs in groundwater and soil, quantitative estimates of cancer and non-cancer

health risk, and quantitative and qualitative evaluations of risk to public welfare and the environment. It stipulates that once an evaluation of the feasibility of reducing the pollutant concentration to levels below the applicable soil UCLs; a suitable comprehensive remedial alternative be selected as a potential solution that would keep contaminant concentration below the UCL.

ISQG: This is the Interim Sediment Quality Guidelines adopted by the Department of Environment and Conservation of Western Australia. The guideline contains the ISGQ-Low concentration (trigger value) and ISGQ-High concentration. The trigger value is the threshold concentration; and below this concentration, the frequency of adverse effects is expected to be very low. The ISGQ-High concentration is intended to represent a concentration above which adverse biological effects are expected to occur more frequently.

MPC: This is the Maximum Permissible Concentration which are limits provided in the Netherlands Environmental Quality. This guideline is not just for human health protection but also ecological protection. This assessment method was developed in light of the minimal data available in the literature. Thus, these MPCs are calculated from the available soil ecotoxicity data for anthracene, benzo(a)anthracene, and benzo(a)pyrene.

Where water is assessed, the most appropriate assessment level depends on the beneficial use of the groundwater or surface water resource itself. Soils are hydrologically linked to both surface and groundwater systems, and a major concern with soil contamination is that it can lead to water contamination (DEC, 2010). Thus, the model used to calculate the soil quality guidelines for the protection of potable water considers four key processes: portioning from soil to leachate; transport of leachate from the base of contamination to the water table; mixing of leachate and groundwater; and groundwater transport down the gradient to the receptor. The Department of Health assessment level for domestic non-potable groundwater and surface water use is deemed an appropriate method to establish suitable guideline limits. The domestic water for non-potable use is primarily water that is not intended for direct human consumption but can be used for other domestic purposes such as vehicle washing, toilet flushing, garden irrigation etc. (DoH, 2006).

The guidelines adopted for the soil and water limits in this project have been presented in Table 2.7; also, the individual limits prescribed by the various institutions are explained in more detail.

Table 2.7: Soil assessment levels with limits adopted by various institutions compiled by the author.

SOIL							SEDIMENTS			WATER
PARAMETER	**EIL[1] (mg/kg)**	**HIL[2] (mg/kg)**				**UCL[3] (mg/kg)**	**ISQG-Low[4] (mg/kg) (trigger value)**	**ISQG-High[5] (mg/kg)**	**MPC[6] (mg/kg)**	**Domestic non-potable groundwater use[7] (mg/L)**
		A	**D**	**E**	**F**		-	-	-	
TPH		-	-	-	-	10,000	-	-	-	50
C_6-C_9	100	-	-	-	-	5,000	-	-	-	-
C_{10}-C_{11}	500	-	-	-	-	20,000	-	-	-	-
C_{15}-C_{18}	1000	-	-	-	-	20,000	-	-	-	-
>C_{16}-C_{35} (aromatics)	-	90	360	180	450	5,000	-	-	-	-
>C_{35} (aliphatics)	-	56,000	224,000	122,000	280,000	10,000	-	-	-	-
TOTAL PAHs	-	20	80	40	100	-	4,000	45,000	-	100
Napthalene	5	60	-	-	190	10,000	0.016	2.1	0.14	100
Acenepthylene	-	-	-	-	-	10,000	0.044	0.64	-	100
Acenapthene	-	-	-	-	-	144	0.016	0.50	-	100
Fluorene	-	-	-	-	-	10,000	-	-	-	0.4
Phenanathrene	10	-	-	-	-	10,000	0.24	1.5	0.51	100
Anthracene	10	17,200	-	-	170,000	10,000	0.085	1.1	0.12	0.6
Fluoranthene	10	2,300	-	-	22,000	10,000	0.6	5.1	2.6	2
Pyrene	10	1,700	-	-	17,000	10,000	0.67	2.6	-	0.6

B(a)anthracene	-	-	-	-	-	3,000	0.26	1.6	0.36	10
Chrysene	-	-	-	-	-	10,000	0.38	2.8	10.7	0.7
B(b)fluoranthe	-	-	-	-	-	3,000	-	-		4
B(k)fluoranthe	-	-	-	-	-	10,000	-	-	2.4	1
B(a)pyrene	1	1	4	2	5	300	0.43	1.6	2.7	0.0001
Id(1,2,3-cd)pyrene	-	-	-	-	-	3,000	-	-	-	1
Dibe(a,h)anthra	-	-	-	-	-	300	0.063	0.26	-	0.4
B(ghi)pyrelene	-	-	-	-	-	10,000	-	-	7.5	0.5
HMW PAHs	-	-	-	-	-	-	1700	9600		
METALS/METALLOIDS										
Arsenic	20	100	400	200	500	-	2	70	-	0.07
Aluminium	-	-	-	-	-	-	-	-	-	2
Barium	300	15,000	-	-	190,000	-	-	-	-	7
Cadmium	3	20	80	40	100	-	1.5	10	-	0.02
Chromium (iii/vi)	400/1	120,000/100	480,000/400	240,000/200	600,000/500	-	-/80	-/370	-	-/0.5
Cobalt	50	100	400	200	500	-	-	-	-	-
Copper	100	1,000	4,000	2,000	5,000	-	65	270	-	20
Iron	-	-	-	-	-	-	-	-	-	3
Lead	600	300	1,200	600	1,500	-	50	220	-	0.1
Manganese	500	1,500	6,000	3,000	7,500	-	-	-	-	5

Mercury	1	15	60	30	75	-	0.15	1	-	0.01
Molybdenum	40	390	-	-	5,100	-	-	-	-	0.5
Nickel	60	600	2,400	600	3,000	-	21	52	-	0.2
Tin	50	47,000	-	-	610,00	-	-	-	-	-
Vanadium	50	550	-	-	7,200	-	-	-	-	40
Zinc	200	7,000	28,00	14,00	35,000	-	200	410	-	30
OTHER INORGANICS										
Boron	-	3,000	12,000	6,000	15,000	-	-	-	-	40
Phosphorus	2,000	-	-	-	-	-	-	-	-	-
Sulphur	600	-	-	-	-	-	-	-	-	-
Ammonia	-	-	-	-	-	-	-	-	-	5
Chloride	-	-	-	-	-	-	-	-	-	2500
Nitrate	-	-	-	-	-	-	-	-	-	500
KEY TO SOURCES	Dutch'B'	VIC EPA	USEPA RLA	DoH	ANZECC 'B'	NEPM	MCP	CCME	NL	

Dutch'B- (Indicative value for further investigation) from (Moen *et al*., 1985)

VIC EPA- Victorian Environment Protection Authority (VIC EPA, 1990)

USEPA RLA- United States Environment Protection Agency Regional Screening Levels (US EPA, 2009)

DoH- Department of Health (DoH, 2006)

ANZECC 'B'- Australian and New Zealand Guidelines for the Assessment and Management of Contaminated Sites (ANZECC & NHMRC, 1992)

NEPM- National Environment Protection Measure (NEPC, 1999)

MCP- Massachusetts Contingency Plan (Department of Environmental Protection, 2014)

NL- Netherlands Environmental Quality (Kalf *et al.*, 1997)

CCME- Canadian Council of Ministers of the Environment soil quality guidelines (CCME, 2008)

Irrigated agriculture is dependent on the adequate supply of usable quality water. Conceptually, water quality refers to water supply characteristics that will influence its suitability for a specific use, i.e. how the quality meets the user's need. The Food and Agriculture Organisation (FAO) indicates that in evaluating irrigation water, the emphasis is

placed on the water's physicochemical properties, and only rarely are other factors considered important (Ayres & Wescot, 1994). Thus, the suitability of water for irrigation is determined not only by the quantity of salt present but by the kind of salt. This is because cropping problems are said to develop with high salt contents, and as a result, unique management practices may be required to maintain acceptable crop yields. The FAO guidelines for water quality for irrigation presented in Tables 2.8 and 2.9 function as a field guide for evaluating the suitability of water for irrigation and livestock feeding. These guideline values identify the potential problems with the water being used as it relates to the salinity, specific toxicity and other miscellaneous effects.

Table 2.8: Permissible limits for toxic compounds Irrigation and Livestock water by Food and Agriculture Organisation (FAO) (Ayres & Wescot, 1994); [1](Hibbs & Thilstead, 1983); [2](National Academy of Sciences, 1972); [3](Beede, 2018).

PARAMETER	IRRIGATION WATER	LIVESTOCK WATER
Chloride (Cl^-) (mg/L)	0 – 1065	<789[1]
Sulphate (SO_4^{--}) (mg/L)	0 – 960	<2000[3]
Nitrate-Nitrogen (NO_3-N) (mg/L)	0 – 10	90[2]
Ammonium-Nitrogen (NH_4-N) (mg/L)	0 – 5	None proposed
Phosphate-Phosphorus (PO_4-P) (mg/L)	0 – 2	< 10[3]
Electrical Conductivity (µ∫/cm)	700 - 3000	Refer to Table 2.10
pH	6.0 – 8.5	6.0 – 8.5
PAHs (µg/L)		
Naphthalene	IDA	320
Acenepthylene	IDA	530
Acenapthene	IDA	95
Fluorene	IDA	66
Phenanthrene	IDA	32
Anthracene	IDA	36
Fluoranthene	IDA	12
Pyrene	IDA	17
B(a)anthracene	IDA	3.8

Chrysene	IDA	3.5
B(b)fluoranthene	IDA	1.1
B(a)pyrene	IDA	1.6
Dibe(a,h)anthracene	IDA	0.6

*IDA- Insufficient Data Available

Table 2.9: Recommended maximum concentration of metals in water used for farm Irrigation and Livestock water (National Academy of Sciences, 1972) adopted by the Food and Agriculture Organisation (FAO).

Element	Irrigation (mg/L)	Livestock (mg/L)	Effects on plants
Al (aluminium)	5.0	5.0	It can cause non-productivity in acid soils (pH < 5.5), but more alkaline soils at pH > 7.0 will precipitate the ion and eliminate any toxicity.
As (arsenic)	0.10	0.2	Toxicity to plants varies widely, ranging from 12 mg/L for Sudan grass to less than 0.05 mg/L for rice.
Be (beryllium)	0.10	0.1	Toxicity to plants varies widely, ranging from 5 mg/L for kale to 0.5 mg/L for bush beans.
Cd (cadmium)	0.1	0.05	Toxic to beans, beets and turnips at concentrations as low as 0.1 mg/L in nutrient solutions. Conservative limits recommended due to its potential for accumulation in plants and soils to concentrations that may be harmful to humans.
Co (cobalt)	0.05	1.0	Toxic to tomato plants at 0.1 mg/L in a nutrient solution. It tends to be inactivated by neutral and alkaline soils.
Cr (chromium)	0.10	1.0	Not generally recognized as an essential growth element. Conservative limits recommended due to a lack of knowledge on its toxicity to plants.
Cu (copper)	0.20	0.5	Toxic to many plants at 0.1 to 1.0 mg/L in nutrient solutions.
Fe (iron)	5.0	-	Not toxic to plants in aerated soils but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum. Overhead sprinkling may result in unsightly deposits on plants, equipment and buildings.

Li (lithium)	2.5	-	Tolerated by most crops up to 5 mg/l; mobile in soil. Toxic to citrus at low concentrations (<0.075 mg/l). Acts similarly to boron.
Magnesium (Mg)	-	250-500	-
Mn (manganese)	0.20	0.05	Toxic to several crops at a few-tenths to a few mg/L, but usually only in acid soils.
Mo (molybdenum)	0.01	-	Not toxic to plants at normal concentrations in soil and water. It can be harmful to livestock if forage is grown in soils with high concentrations of available molybdenum.
Ni (nickel)	0.20	-	Toxic to several plants at 0.5 mg/L to 1.0 mg/L; reduced toxicity at neutral or alkaline pH.
Pb (lead)	5.0	0.1	Can inhibit plant cell growth at very high concentrations.
Se (selenium)	0.02	0.05	Toxic to plants at concentrations as low as 0.025 mg/L and poisonous to livestock if forage is grown in soils with relatively high levels of added selenium. An essential element to animals but in very low concentrations.
V (vanadium)	0.10	0.1	Toxic to plants at relatively low concentrations.
Zn (zinc)	2.0	24.0	Toxic to plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine-textured or organic soils.

These guideline values are trigger values below which there should be minimal risk of adverse effects. However, further investigation is required if these concentration values are significantly exceeded.

Irrigation canals frequently serve as sources for livestock drinking water but other sources, including inadequate quality supplies, are often used. In industrially active areas such as the Niger Delta region of Nigeria, livestock commonly uses poor or marginal quality water from sources such as small wells, streams or waterholes (Kuruk, 2004). Seasonal changes affect salinity levels from these water sources. During hot and dry periods, natural salinity increases due to evaporation and salt content are generally elevated (Ayres & Wescot, 1994). Considering the adverse effect posed to animal health by highly saline water or water containing toxic elements, the National Academy of Sciences (1972) established that from a salinity standpoint, livestock water with an electrical conductivity of less than (5000 µS/cm) under most circumstances (Table 2.10). However, the FAO recognises that physiological upset might occur with water near this limit, but there will be little chance of economic losses

or death. Therefore, complementing these guidelines with sound judgment should provide a suitable framework for making decisions.

Table 2.10: Water quality guideline for livestock water by Food and Agriculture Organisation (FAO) (Ayres & Wescot, 1994).

Water Salinity (EC) (µ∫/cm)	Rating	Remarks
<1500	Excellent	Usable for all classes of livestock and poultry.
1500 – 5000	Very Satisfactory	Usable for all classes of livestock and poultry. May cause temporary diarrhoea in livestock not accustomed to such water; watery droppings in poultry.
5000 – 8000	Satisfactory for Livestock	May cause temporary diarrhoea or be refused at first by animals not accustomed to such water.
	Unfit for Poultry	Often causes watery faeces, increased mortality and decreased growth, especially in turkeys.
8000 – 11000	Limited Use for Livestock	Usable with reasonable safety for dairy and beef cattle, sheep, swine and horses. Avoid use for pregnant or lactating animals.
	Unfit for Poultry	Not acceptable for poultry.
11000 – 16000	Very Limited Use	Unfit for poultry and probably unsuitable for swine. Considerable risk in using for pregnant or lactating cows, horses or sheep, or the young of these species. In general, its use should be avoided although older ruminants, horses, poultry and swine may subsist on waters such as these under certain conditions.
>16000	Not Recommended	Risks with such highly saline water are so significant that it cannot be recommended for use under any conditions.

2.6 Remediation of Hydrocarbon Pollution

Strategies used to remediate hydrocarbon spills depend on several variables: pollution concentration, applicability, cost, time, and operational complexity. In socio-economically challenged areas such as the Niger Delta region of Nigeria, cost and operational complexity

pose massive constraint for the local fishing and farming population due to low income and high illiteracy.

A majority of physical and chemical techniques such as solidification/stabilisation, encapsulation, soil washing, thermal treatment, air stripping and sparging, usually require multiple stages for soil remediation to be complete. More often than not these processes involve excavation and ex-situ clean-up processes which are very expensive (Riser-Roberts, 1998). Henner *et al*. (1997) point out that, although highly effective, thermal desorption is exceptionally costly due to the multiple stages involved in the process, i.e. soil excavation, transportation, and heat treatment off-site.

These remediation methods are not only expensive but usually have by-products. Incineration, for instance, can generate incomplete combustion products and residual ash that may need to be disposed of as hazardous waste (Riser-Roberts, 1998). Even with the high remediation efficiency of soil washing, a key drawback is the production of residual sludge formed in the extraction process, which requires additional specialist handling due to toxicity and the operational complexity involved in using this equipment (Rulkens & Assink, 1984).

2.6.1 Natural Attenuation

This is the decrease in contaminant concentrations in the environment through weathering and similar natural processes (Ezeonu *et al*., 2012). Prince (2014) explained that as the age of an oil spill increases, there would be greater opportunity for the oil to weather and for its constituents to attenuate to the environment. Thus indicating that the more weathering takes place, the less biodegradation occurs. Shell Petroleum Development Company (2011), a major industry stakeholder in oil operations within the Niger Delta region revealed that the main method the company has employed for land remediation is through natural attenuation. However, this has generally been deemed insufficient not only by members of affect communities but also by the Nigerian government (Abu & Dike, 2008; Osuji *et al*., 2006).

2.6.2 Physical/ Chemical Treatment Methods

2.6.2.1 Thermal Treatment

This method is described by Fox *et al*. (1991) as a non-incineration technology for treating soils contaminated with organic pollutants. In this process, contaminated soil is heated under an inert atmosphere to increase the vapour pressure of the organic contaminants,

thus separating this pollutant from the soil matrix in the form of expelled gases (Wilbourn *et al*., 1994). These treatments can be on-site or off-site and are based on the thermal removal of oxidisable organic pollutants with lower boiling points (Boehm, 1992). According to Velazquez & Noland (1993), a mobile thermal processor which uses low-temperature was used to remediate soils contaminated with Volatile Organic Compounds (VOCs). Here, the contaminated soil was heated in an indirect heat exchanger at 450 °C. However, Jensen & Miller (1994) cite the requirement for the soil to be heated at temperatures over 600 °C for successful thermal treatment of petroleum hydrocarbon contaminated soils. Temperature, residence time volatility, and purge gas velocity are the main parameters affecting the effective desorption of contaminated soil, with higher temperatures and longer residence times resulting in higher removal efficiency. For up to 98% removal of LMW-PAHs at 20-minute residence time, temperatures a minimum of 150 °C while removing HMW-PAHs would require a minimum of 250 °C (Chern & Bozelli, 1994). Higher temperature treatments such as incineration, pyrolysis, and vitrification technologies are generally not considered for remediating petroleum hydrocarbon contaminated soils as they are highly complex and costly (Ram *et al*., 1993; Riser-Roberts, 1998).

2.6.2.2 Soil Washing

Soil washing is an ex-situ process involving water use (usually with added reagents or solvents) or solvent to remove hazardous contaminants from excavated soils. Soil washing is performed above ground in a reactor and is both cost and time effective compared to in-situ flushing systems (Lyman *et al*., 1990). The concept of soil washing is based on the principle that contaminants tend to adhere to fine-grained soils (silt and clays) which generally bind with coarse-grained soils. Therefore, soil washing aims at separating the contaminated fines and wastewater from the cleaned coarse-grained soil (sand and gravels) (Hyman & Dupont, 2001). Riser-Roberts (1998) identifies soil washing as a more effective system than soil flushing because it overcomes problems such as low hydraulic conductivity, channelling, and contamination of underlying aquifers, often associated with the latter. The process of soil washing significantly reduces the volume of contaminated soil at the site, often making it a pre-treatment step for a different remediation technique (USEPA, 1985). According to Sharma & Reddy (2004), the process can remove a range of contaminants, both organic and inorganic, from the soil at the same time. A study by Iturbe *et al*. (2004) engaged soil washing as a treatment for petroleum hydrocarbon contaminated soils and results showed that after six weeks, TPH concentration was reduced to 1,407 mg/kg from an initial concentration of 55,156 mg/kg indicating a removal efficiency of 98% was achieved. Sharma & Reddy (2004) point out that a key drawback to this remediation

technique is that the high levels of wastewater generated from the process often contain chemicals additives that require specialised treatments that are generally complex and expensive.

2.6.2.3 Soil Solidification/ Stabilisation

This approach incorporates chemical stabilisation processes to treat excavated soils (Ram *et al*., 1993). Most stabilisation techniques for remediating organic pollutants in a soil matrix use pozzolanic materials (Portland cement, fly ash, kiln dust) as the main ingredient (McDowell, 1990). However, Riser-Roberts (1998) insists that this process is not suitable for moderate to high levels of hydrocarbons. The increase in volume and need for pozzolanic materials can be reduced or even avoided by the siallon process for microencapsulation of hydrocarbons, which uses two water-based products, and emulsifier (specifically chosen for varying soil types and hydrocarbons), and a reactive silicate. It has successfully been used in the remediation of sites contaminated with gasoline, diesel, crude oil, and coal tars (Riser-Roberts, 1998).

2.6.2.4 Air Stripping

The air stripping involves injecting air into the soil, forced through injection wells and pulled out of extraction well (Peng *et al*., 2011). As it flows through the soil, volatile compounds are stripped off into the air stream, while the contaminated air is vented to an emission control system or the atmosphere depending on the concentration levels of the contaminants. These gaseous organic pollutants can be removed from the air in a vapour-phase carbon adsorption system or by fume incineration (Riser-Roberts, 1998). As the success of the process heavily relies upon the relatively unrestricted and uniform flow of air through the soil, clay soils or densely packed soils with high water table are not suitable for the process. Mehrotra *et al.* (1996) point out that the interphase pollutant transport from the sorbed to the vapour phase plays a principal role in influencing the effectiveness of the process. A key disadvantage of this process is its applicability as it is limited to volatile compounds, low groundwater table, and loose sandy soil formation (Riser-Roberts, 1998).

2.6.2.5 Soil Venting

This is also referred to as Soil Vapour Extraction (SVE) and may be carried out actively or passively (Lyman *et al*., 1990). According to Hyman & Dupont (2001), the passive process consists of perforated pipes embedded into the contaminated area, providing an outlet for gases in the subsurface. While in the active process, it uses an induced pressure gradient to move vapours through the soil. Here, a vacuum is applied to the subsurface to volatilise

and remove the organic contaminants. This vacuum may be applied through vertical extraction wells (low water tables) or horizontal extraction systems (high water tables) using a vacuum blower. The resulting pressure gradient forces the soil gas to migrate through soil pores toward the vapour extraction wells (Lyman *et al*., 1990). Positive pressure injection rate and the use of plastic sheeting on the ground may avoid short-circuiting the airflow, thus enhancing the removal rate. VOCs are volatilised and transported out of the subsurface by the migrating soil gas. The removed vapours may require treatment before discharge to the atmosphere (Riser-Roberts, 1998).

2.6.2.6 Vitrification

This approach employs soil-melting technology using electric current passed between electrodes positioned in the ground (Lendvy, 1992). Inorganic contaminants are encapsulated and immobilised by heating the soil at high temperatures (1500 to 2000 °C) (Johns & Nyer, 1996). Organic pollutants and other naturally occurring organic compounds are volatilised and subsequently removed by vapour extraction or pyrolysed, resulting in gases ejected.

2.6.3 Biological Methods (Bioremediation)

This is a process where organic pollutants are biologically broken down under controlled conditions into an innocuous state, or to levels below the limits established by regulatory authorities (Cairney, 1992). Biodegradation is a function of bioremediation. It is the biological transformation of complex compounds into smaller molecules with less toxicity (usually water and carbon dioxide, which is then termed as mineralisation) (Wick *et al*., 2011). Thus, Olson *et al.* (2003) define bioremediation as the application of biodegradation to decrease pollutant concentrations.

The two major forms of bioremediation are the microbiological approach (bioaugmentation) and microbial ecology approach (biostimulation) with the former involving the supplication of pre-conditioned microorganisms to biodegrade specific target compounds while the latter involves altering the environment of the indigenous organisms to optimise their capacity to biodegrade contaminants (Piotrowski, 1991). This is consistent with the idea of Wick *et al*. (2011) that biostimulation techniques are employed to improve the soil microbial habitat, and bioaugmentation strategies manipulate the microbial population structure to make it more capable of degrading organic pollutants. Several key factors influence the effectiveness of the biodegradation of organic contaminants. Some of which include: limited supply of nutrient for microbial activities or carbon sources; non-optimal abiotic conditions

of (temperature, oxygen concentration, pH, salts, and toxins); low microbial biomass capable of degrading organic compounds; low contaminant bioavailability to degrading organisms; and physicochemical properties of the pollutants (Alexander, 1999; Harmsen *et al*., 2007; Olson *et al*., 2003; Straube *et al*., 2003). The revisions and adjustments made to the limitations mentioned above are the basis for the successful conduct of the bioremediation process (Wick *et al*., 2011).

Several studies have shown biostimulation and bioaugmentation are very promising alternatives for the remediation of hydrocarbon contamination in soils. A study by Liebeg & Cutright (1999) applied varying combinations and levels of macro- and micronutrients to enhance bioremediation of an aged gas manufacturing plant soil (pH 7.5, 3.5% organic carbon, PAH concentration 620 mg/kg) via biostimulation and/or bioaugmentation. The best overall combination was a low level of macronutrients with phosphorus as the dominant macronutrient, combined with high levels of micronutrients. In augmentation with *Achromobacter* sp. and *Mycobacterium* sp., bioactivity was observed to be at its highest when micronutrient levels were increased, and no macronutrient was added. Bioaugmentation, however, did not significantly enhance PAH breakdown. Straube *et al*. (2003) carried out a microcosm study to evaluate the effects of biostimulation with nitrate fertiliser and bioaugmentation using bio-surfactant producing *Pseudomonas aeruginosa* strain on Superfund site soils with high levels of PAH concentrations (up to 1300 mg/kg). It was concluded that the combination of biostimulation and bioaugmentation significantly elevated PAH degradation and benzo(a)pyrene equivalent toxicity reduction. Similar studies have combined biostimulation, and bioaugmentation with soil tillage and the results have shown a decrease in PAH concentration levels seven times more than the control after 16 months (86 to 12 %). However, according to Alexander (1999), successful field-scale applications of bioaugmentation are limited as the most favourable results reported have been applied in confined systems where conditions are controlled to aid the growth of added microbes. Opinions on the effectiveness of bioaugmentation differ amongst certain researchers as Lendvay *et al*. (2003) and Silva *et al*. (2004) conclude that it has a substantial positive influence on the bioremediation of contaminated soil, while Bouchez *et al*. (2000) report that the procedure generally failed to improve biodegradation. Perhaps, an insight into this failure can be understood through earlier observations by Fantroussi *et al*. (1999) who suggest that bioaugmentation was more successful in sterilised soil (one not found in a field setting) due to predation by nematodes and competition with indigenous microorganisms for resources. It is broadly accepted that a vast array of microorganisms are required for the comprehensive bioremediation of oil pollution. The rate of

biodegradation relies on the type of hydrocarbons present, the environmental conditions, and the native microbial population (Balba *et al*., 1998).

According to Antizar-Ladislao *et al*. (2004), the initial reports reviewing several approaches using composting as a bioremediation technique to treat contaminated materials emerged in the 1980s. In 1993, Wilson & Jones appraised several bioremediation technologies such as in-situ, ex-situ, and bioreactor treatments in the breakdown of organic pollutants. The researchers concluded that in-situ treatments were not as effective as ex-situ treatments when it came to removing HMW hydrocarbon compounds from the soil within acceptable periods. The authors also state that the inefficiency of the ex-situ treatment was because of the low aqueous solubility of the contaminants and strong absorption to the soil as well as temperature and soil type restrictions. It is suggested that ex-situ treatments, i.e. bioreactors are usually favourable as conditions necessary for the degradation of pollutants can be more easily controlled and thus enhanced (Loick *et al*., 2009). Generally, in-situ methods of remediation are significantly able to degrade LMW compounds, i.e. medium distillate fuels relatively very well compared to HMW compounds which may still be degraded but take a considerably longer period (Song *et al*., 1990; Wilson & Jones, 1993).

A range of techniques is adopted while conducting field-scale biostimulation and bioaugmentation. Solid-phase (landfarming, phytoremediation, bio-surfactants, and composting) and slurry-phase (bioreactors) treatments can be implemented depending on the volume of material remediated.

A major study using contaminated soil from Kuwaiti oil lakes by Balba *et al*. (1998) evaluated the efficiency of various bioremediation methods (landfarming, windrow composting piles, and static bioventing piles) was assessed. Inorganic fertilisers mixed with compost were supplemented to the contaminated soils to stimulate microbial growth and metabolic activities. After 365 days, all treatments showed a reduction in oil concentration. The overall loss in alkanes was between 82 - 91 % compared to the 16 - 26 % loss in the untreated controls. The total TPH losses for the treatments were between 64 - 83 %, while the control showed a 13 - 20 % loss.

2.6.3.1 Landfarming

This technology is a commonly used and inexpensive remedial method applied to removing organic contaminants from the soil through leaching and volatilisation (Harmsen *et al.*, 2007). However, by the 1970s, landfarming techniques and ideas changed with increased regulations and the change in mindset to focus more on the biological removal of pollutants

(Eweis *et al*., 1998). Riser-Roberts (1998) describes it as the controlled application of waste materials to the soil for immobilisation or degradation or transformation by the resident microflora. Maintaining oxygen is usually crucial in this technique, and it is achieved by spreading the polluted soil evenly over an impermeable layer and treated using basic agricultural practices, i.e. tillage (Wick *et al*., 2011). Biodegradation allows landfarming to function both as a treatment mechanism and a disposal process (Huddleston *et al*., 1986). Such disposal can be useful, if application rates and scheduling do not result in conditions that allow unwanted constituents or by-products of degradation to runoff or leach through the soil, and provided that no materials accumulate to toxic levels in the soil (Arora *et al*., 1982). The process of landfarming has proved to be an alternative to incineration when energy conservation is considered (Arora *et al*., 1982). The optimum C:N:P ratio for degrading hazardous wastes is reportedly between 100:10:1 (Straube *et al*., 2003) and 300:10:1 (Eweis *et al*., 1998). A major limitation is its massive reliance on weather conditions and the severe potential for contaminant movement from the treatment area, leading to damaging effects on groundwater (Wilson & Jones, 1993). Also, this method cannot degrade the heavy components of petroleum oils. Mueller *et al*., (1996) report that naphthalene, alkanes, and other light-weight compounds with half-lives less than 30 days were rapidly degraded, while refractory compounds as creosote and bunker oil accumulated in the soil.

2.6.3.2 Bioreactors

Here, contaminated soils are treated in bioreactors by extraction and biodegradation (Castaldi, 1994). This treatment can be implemented by continually mixing contaminated soil with liquid (Alexander, 1999). Here, sediment is put into bioreactors in small batches, although continuous flow operations are possible. For the treatment, the slurry is mixed with nutrients and microbial cultures then aerated. Once the sediment settles, and the water is transferred to a treatment plant, the sediment is returned to a contained area (Eweis *et al*., 1998). The process is similar to activated sludge as it provides for aeration and mixing. It also allows for the addition of nutrients, oxygen, surfactants and microorganisms, and can incorporate a means of capturing volatile organic compounds (VOCs) generated (Riser-Roberts, 1998). De Jong & Verstrate (1993) reported a 70% removal of PAHs from a soil initially polluted with up to 3000 mg/kg using a bioreactor. An investigation by Kuyukina *et al.* (2003) showed that soils heavily contaminated with crude oil (200g TPH/kg) could be treated using pilot bioreactors reaching 1 to 2 g TPH/kg in approximately six weeks of treatment. However, the use of bioreactors is complex, which, in turn, increases treatment costs compared to most bioremediation techniques (Cookson, 1995). Despite these

shortcomings, Castaldi (2003) argues that bioreactors are still cheaper than incineration, solvent extraction and thermal desorption in many cases. In contrast, Wick *et al*. (2011) point out that bioreactors are commercially available but are nonetheless very expensive.

2.6.3.3 Phytoremediation

Phytoremediation is a remedial technique that utilises natural plants to reclaim contaminated soil or groundwater primarily through enhancing microbial activity within their rhizospheres while degrading organic pollutants by metabolic processes (Salt *et al*., 1995; White & Newman, 2011). Yang (2008) describes it as a plant-microbe remediation system that degrades, contains, or renders harmless organic and inorganic contaminants in soil or water. Plants can moderate the geochemical environment in the rhizosphere through various mechanisms, providing ideal conditions for the bacteria and fungi to grow and degrade organic contaminants (Susarla *et al.*, 2002). These mechanisms are briefly explained in Table 2.11. The rhizosphere is the most important region during the phytoremediation process. The small volume of soil immediately surrounding the roots has the highest concentration of root exudates, CO_2 pressures, and microbial activity (Hutchinson *et al*., 2003). According to Harms (1996), the principal stimulator of pollutant degradation is by the microbial population within the rhizosphere with several studies indicating the potential uptake and metabolism of PAHs. This uptake is dictated by the molecular configuration and size and the capability of specific plant species. Root morphology is essential for successful phytoremediation. Some important root morphological properties include length, surface area and mass, depth of penetration, quantity and composition of dead roots and exudates, root hairs, and bacterial and fungal associations (Hutchinson *et al*., 2003). In annual plants, 30 - 60 % of new fixed photosynthetic carbon is transferred to the roots of which 40 - 90 % is transferred directly into the rhizosphere (Olson *et al*., 2007). In perennial plants, a similar range of photosynthetic carbon, 30 - 70 % is transferred to the soil, of which 25 - 80 % is transferred from the roots to the soil (Olson *et al*., 2007). This supply of carbon from plants to the soil stimulates microbial communities and enhances the degradation of hydrocarbon pollutants (Chen *et al.*, 2003; Robinson *et al.*, 2003; Xu *et al*., 2006). Most organic contaminants with a low water solubility (high log K_{OW}) tend not to be transported within a plant. However, those with lower log K_{OW} thus higher water solubility are likely to be transported within a plant (Burken & Schnoor, 1998).

Table 2.11: Mechanisms for phytoremediation (Susarla *et al*., 2002)

Mechanism	**Process**
Phytoextraction and Phytoaccumulation	Phytoextraction involves the deletion of contaminants via the uptake by plants tissues while Phytoaccumulation occurs when absorbed contaminants of Phytoextraction are not degraded swiftly or completely, hence resulting in accumulation by the plant.
Phytodegradation or Phytotransformation	Conversion of chemicals to less toxic metabolites by enzymes or coenzymes.
Phytopumping	The process of removing or minimising migration of contaminants mostly applied to remove groundwater pollutants. Plants act as organic “pumps” to extract large quantities of contaminated water underground as part of evapotranspiration, in addition to potential uptake.
Phytostabilization	Reduction of contaminant migration by using the ability of plants roots to alter soil properties (e.g. pH, moisture content), occurs through the secretion of root exudates in plant roots.
Rhizosphere Effect	Secretion of photosynthate in root exudates to support the growth and metabolic activities of the diverse microbial population within the rhizosphere.

Phytoremediation is a very lengthy process (1 – 3 years), usually limited by phytotoxic contaminants and as a result, is generally applied as a follow-up (secondary) treatment in soils contaminated with residual levels of pollutants (Braddick *et al*., 2002; Cunningham *et al*., 1995). The overall success of the phytoremediation process depends on environmental conditions such as the presence of adequate oxygen supply, water and nutrients, and edaphic factors like pH and electrical conductivity, soil type, temperature, and weathering (Cunningham *et al*., 1995).

2.6.3.4 Bio-Surfactants

The oil-water interfacial tension and mass transfer of contaminant compounds to the aqueous phase in the soil solution are some of the most significant factors influencing biodegradation of crude oil in soils (Setti *et al*., 1995; Volkering *et al*., 1992). Surfactants are surface-active agents that lower the surface tension of liquids (Alexander, 1999). Emulsifiers improve stability by reducing surface or interfacial tension, most emulsifying agents are surfactants, but not all surfactants are emulsifying agents (Becher, 1965). Thus, most microbial emulsifying agents are surfactants. Surfactants may be described as synthetic or natural, with synthetic surfactants made by humans via chemical processes and available commercially. Natural surfactants are produced by microorganisms and are fatty acids and ester compounds that are by-products of the biological degradation of organic materials (Mao *et al*., 2015). Both the natural and synthetic surfactants enhance wetting, solubilisation, and emulsification of various organic chemicals (U.S. EPA, 2001). During wetting, surfactants decrease the interfacial tension between the aqueous and solid phases (soil), allowing the water to preferentially wet the soil, thereby displacing the contaminants (U.S. EPA, 1993).

In most cases, synthetic surfactants might have detrimental effects on the permeability of the microbial cell membrane, which would interfere with the capacity of microorganisms to biodegrade organic pollutants (Sandbacka *et al*., 2000). This accords with earlier suggestions of Chakrabaty *et al*. (1988) who state that bio-surfactants are generally much less toxic than chemical surfactants, often as effective, and more readily biodegradable. Microorganisms produce Bio-surfactants during growth on insoluble organic substrates to increase their solubility (Falatko & Novak, 1992). Bio-surfactants are synthesised and excreted into the environment by soil bacteria such as *Pseudomonas*, *Rhodococcus*, and *Arthrobacter* (Maier & Soberón-Chávez, 2000). This is consistent with Nitschke et al. (2005), who found that *Pseudomonas aeruginosa* produces and excretes a bio-surfactant, which is a glycosylated, anionic, and amphipathic known as rhamnolipids. According to Wilson & Jones (1993) using microorganisms that produce their bio-surfactants could lower treatment costs. These bio-surfactants tend to have lower toxicities and are effective at wider temperature, pH, and electrical conductivity ranges (Bordas *et al.*, 2005). Surfactants enhance contaminant solubility and emulsification, or dispersion of an insoluble organic phase within the aqueous phase, of the contaminant (U.S. EPA, 1993). These surfactants often accumulate at gas/liquid, liquid/liquid, and solid/liquid interfaces and they are particularly effective in stimulating the mobilisation of organic compounds of relatively low water solubility and high lipid solubility (high K_{OW} values) (JRB Associates Inc, 1984). Van

Ginkel (1996) describes surfactants as amphiphilic (i.e. possess hydrophobic or lipophilic, hydrophilic or water-soluble) properties. They are organic molecules, which can be either cationic, anionic, or non-ionic (JRB Associates Inc, 1984). At or above a certain concentration level referred to as critical micelle concentration, the hydrophobic parts of the surfactants will tend to associate together to form a micelle (an aggregate of surfactant molecules dispersed in a liquid colloid) with a hydrophobic core (Santharam *et al*., 1997). Surfactants solubilise hydrophobic contaminants by partitioning them into the hydrophobic core of the micelle. If the concentration of surfactant exceeds the critical micelle point, the solubility of hydrophobic compounds can increase by order of magnitude over normal aqueous solubility (Gao *et al*., 2007; Mulligan *et al*., 2001). This critical micelle concentration of a specific surfactant is dependent on temperature, ionic strength, and surfactant chemistry (Santharam *et al*., 1997). However, other researchers studied the rhamnolipid produced by *P. aeruginosa* did not enhance biodegradation of 13 EPA priority PAHs in creosote-contaminated soil (Deschênes *et al*., 1996). HMW-PAHs, surfactants presence seemed harmful to the biodegradation process.

2.7 Composting as a Bioremediation Technology

Composting is an aerobic process that is principally dependent on the actions of microorganisms to degrade organic materials, leading to thermogenesis and the production of organic and inorganic compounds (Semple *et al*., 2001). Similarly, Haug (1993) defines composting as the biological decomposition and stabilisation of organic materials under the condition that enhances the furtherance of thermophilic temperatures due to biologically produced heat. Thus, composting allows for the biological decomposition of organic components of waste materials under controlled conditions (Golueke, 1972). According to Wilson & Jones (1993), it can be used to treat highly contaminated materials in a process that involves the activity of a succession of mesophilic and thermophilic microorganisms. Usually, the soil is mixed with an organic bulking agent (e.g. straw or wood chips). Moisture, nutrient, and pH levels can be controlled with aeration achieved by forced air or pile turning. It has been described as an alternative to landfarming and landfilling as it not only combines several positive attributes of incineration and landfarming but also minimises their drawbacks (Savage *et al*., 1985). A key characteristic of composting is said to be the metabolically generated heat trapped within the compost matrix, leading to overall temperature elevations (Williams *et al*., 1992). Traditionally, the practice of composting is intended to reduce the volume and water content of vegetable wastes, to destroy pathogens, and to remove odour-producing compounds (Henner *et al*., 1997). Today, the technology is now applied for handling polluted soil or sediments in two major ways: (i)

composting of polluted soils for efficient degradation and (ii) addition of composted materials.

2.7.1 Types of Composting

According to Savage *et al*. (1985), composting is divided into three major categories: turned windrow systems, static-pile systems, and in-vessel (reactor) systems. These systems were initially designed and developed to stabilise sewage waste, thus relying primarily on aerobic microbial activity rather than anaerobiosis which often leads to the formation of H_2S and SO_2 (Miller *et al*., 1991). Furthermore, aerobic composting gives a greater degree of decomposition of compounds even though there are microsites of anaerobic activities (Wick *et al*., 2011). Hence, these composting technologies utilise bulking agents (straw, bark chips, chopped sugar beets), which generally increases the porosity, and therefore, aerobicity of the medium under treatment and decreases the moisture content (Semple *et al*., 2001). Inorganic nutrients, as well as nitrogen and phosphorus, may be added.

Windrow composting typically consists of placing the mixture of raw materials in long, narrow piles known as windrows. Typically, compost mixture is placed on an impermeable layer with this system to prevent leaching of contaminants (Epstein, 1997). The cross-section of the pile must be such that high temperatures are maintained on the interior of the pile with minimal heat losses on the outer parts of the pile (Wick *et al*., 2011). Their dimensions are generally 3 – 6 m in width and 3 – 4 m in height. The bulk density of the material composted is an important variable that determines the size of the windrow and the equipment suitable for turning (Notton *et al*., 2008). In general, windrows aerate primarily by natural or passive air movement, i.e. convection and gaseous diffusion, although this is significantly affected by particle size and porosity of materials constituting the windrow (Ali, 2007). Although numerous studies suggest they are usually turned and agitated regularly using mechanical mixing equipment (a front-end loader or windrow turner), this enhances the level of aeration.

Additionally, turning and mixing reduces the potential for pockets of anaerobic decomposition in areas that are too moist or too rich in the nitrogenous substrate (McClintock, 2004). In cases where the windrows are very large and very challenging to mix, pipes can be installed to increase the aeration within the pile. To get effective aeration into a windrow, it is essential to determine the porosity of the material (NRAES, 1992). Eweis *et al*. (1998) report that windrows usually covered with polyethene (high density) HDPE material or woodchips in outside environments to prevent leaching from the pile during rainfall events.

Static-piles composting consists primarily of a stacked pile of contaminated soil left to decompose naturally via biological degradation (Wright, 2002). Here, the substrates can be mixed with a bulking agent, such as woodchips, which provides structural stability to the material and maintains air voids without routine agitation (Haug, 1993). Hence, no agitation or mixing/turning is required during the compost cycle. According to Wick *et al*. (2011), the compost material placed on an impermeable platform with perforated pipes is connected to a blower system. These piles are aerated at either fixed or variable rates depending on the microbial activity. Forced aeration is usually introduced at a controlled rate to aid aerobic decomposition and maintain temperature (Tyrrel *et al*., 2001). Modifications such as the placement of water pipe system over the pile and covering the outer surface with a flexible non-porous material help reduce natural convection of air through the waste material and assist in maintaining elevated temperatures and adequate moisture content (Epstein, 1997). As a result, there is a higher degree of process control and an increased rate of decomposition.

In-vessel composting has been adopted to cover a wide range of composting systems, from enclosed halls to tunnels and containers. These closed systems contain mixing devices through drum rotation or mixing instruments within the tank (Eweis *et al*., 1998). In-vessel systems control and accelerate decomposition by creating ideal conditions for the microbes by bringing the compost mixes up to the optimum temperature as quickly as possible (Ali, 2007). A common feature of in-vessel composting is that composted material e.g. contaminated soil is contained in an enclosure to allow a higher degree of process control, such as forced aeration rather than aeration via mechanical turning only (Edwards *et al.*, 1998). According to Edwards *et al*. (1998), these composting systems can be categorised broadly into five types based on their design and structure, namely: agitated bays, silos or vertical composting units (VCU), container systems, enclosed halls, and rotary composting systems.

Silos/ Vertical Composting Units (VCUs) are of two types: dynamic flow and plug flow. The dynamic VCUs operate in batches and agitate waste with paddles or augers. Due to the high mixing rate of this system, it is usually suitable when managing wet waste provided dry amendments such as woodchips be added. On the other hand, waste is stacked in a plug flow system to form a tower that descends under its weight as the composted material is removed from the base (Notton *et al*., 2008). These are usually used for drier wastes as they avoid dangers present in the compaction of wet wastes.

Enclosed hall systems materials are composted on the floor of the hall and are usually contained in one long bed. The whole composting process tends to occur in the same hall,

with a large bucket wheel used to turn and move the material through the system (Ali, 2007). Aeration is achieved with either positive or negative pressure. In the case of the former system, air from the hall area is forced through compost and subsequently recirculated within the hall and exhausted to a bio-filter. In contrast, the latter system sucks the air through the compost and passes it to a bio-filter limiting the air movement directly into the environment (Notton *et al*., 2008).

Rotary composting systems involve composting in a slowly rotating drum usually at a rate of approximately four turns per hour. The external diameter of the drum is lagged for heat insulation. In practice, these systems are generally a component of the complete process that entails sorting, shredding, treatment (reactor), screening and reloading. It can be a continuous or batch system, and aeration can be either forced or natural (Ali, 2007). Some of the vessels are inclined at an angle to achieve better mixing and to avoid uneven pocket that could interfere with the aeration process. Typical residence time is three days, assuming temperature regime requirement has been reached, after which the material is unloaded and stockpiled for up to three weeks for stabilisation using either windrows or static piles. Generally, the rotary system used a pre-treatment process, where additional maturation and screening are required (Ali, 2007).

2.7.2 Key Parameters affecting Composting

The optimisation of key parameters is paramount if successful composting is to be achieved. According to Savage *et al*. (1985), temperature, aeration (oxygen levels), moisture content, pH, and nutrient availability should be monitored and altered if necessary to ensure the best results during the process.

Temperature changes in composting are often due to a series of events. The temperature evolution during the composting is facilitated by aerobic conditions as a significant quantity of heat is released by aerobic decomposition (Sayara, 2010). Alexander (1999) suggests that temperature ranges between 50 °C to 60 °C are most favourable for the process and its associated microorganisms. Fogarty & Tuovinen (1991) have categorised the composting process into four primary stages with respect to temperature: mesophilic (20 °C to 45 °C), thermophilic (45 °C to 85 °C), cooling and maturation. With the temperature changes come related changes in the structure of the microbial communities. As the rate of microbial activity increases, there is an increase in temperature resulting in a decrease in mesophiles and increased thermophiles (Haug, 1993). At these thermophilic temperatures, most microbial decomposition and biomass formation occur (Fogarty & Tuovinen, 1991). The cooling phase is usually due to a decrease in microbial activity (particularly

thermophiles) as most of the utilisable carbon has been eradicated, thus increasing mesophilic microorganisms (Fogarty & Tuovinen, 1991).

A study by Strom (1985) on the effects of temperature on the microbial diversity in composting revealed that temperature above 60 °C had a marked detrimental impact on the process. In contrast, Droffner *et al.* (1995) report that compost sampled at temperatures above 60 °C had the presence of bacteria strains that were classed as mesophiles, suggesting the ability of these bacteria to survive and reproduce even at elevated temperatures. This is further supported by Beaudin *et al*. (1999) who concluded that the length of the thermophilic phase of composting correlated with the amount of hydrocarbon degradation, suggesting the presence of microorganisms at high temperatures. However, Chung *et al*. (2000) indicate that the contaminant concentration primarily influences the maintenance of thermophilic temperatures.

A feasibility study was undertaken by Matteau & Ramsay (1997) to understand the effects of thermophilic and mesophilic temperatures during the composting of leaves and alfalfa to biodegrade toluene. Both conditions recorded an average degradation of toluene at a rate of 104 g/m^3 per hour. The results highlight the influence of temperature during the biodegradation process.

During the composting of lagoon sewage sludge mixed with straw over a 180-day period, the fate of PAHs was undertaken by (Amir *et al*., 2005) using temperature as an indicator of microbial activities. It was found that, while the stabilisation phase of the composting process took place, significant microbial degradation of LMW PAHs was achieved because of the increasing presence of thermophilic microbial populations.

It is quite apparent that temperature is crucial in regulating microbial activities within the process of composting, but it also affects the behaviour of organic contaminants (PAHs). The solubility of PAHs increases with elevated temperatures resulting in higher bioavailability (Loicke, 2008).

The ***Oxygen levels*** determine the rate and extent of the destruction of the waste since this is primarily an aerobic process (Savage *et al*., 1985). Composting can occur in about half the normal concentration in air, i.e. as low as 10% (Notton *et al*., 2008), although some researchers suggest levels as little as 5% can be sufficient. This accords to earlier work by Metacalf and Eddy (2002) which indicates composting process can be inhibited at oxygen levels below 10 %, and as such alternative air supply must be provided to ensure the process is efficient. The chemical and physical makeup of the waste determines the quantity

and rates of aeration required. Insufficient aeration leads to anaerobiosis, which can result in the formation of methane and the generation of odorous gases and contaminated leachate, which may pollute groundwater (Diaz, 2003).

Composting most organic pollutants requires approximately two parts of oxygen to completely metabolise one part of the organic compound (Riser-Roberts, 1998). In the case of dissolved oxygen, the U.S. EPA (2001) suggests this should be maintained above critical consideration for the advancement of aerobic activity, which ranges from between 0.2 to 0.5 mg/L. The flow of oxygen into the composting system is controlled by its concentration in the carrier and the permeability of the geological material to that carrier (Wilson & Jones, 1993).

Furthermore, Edwards *et al*. (1998) point out that the composting materials' fragment size usually determines the free air space in the mass and, therefore, dictates the availability of air and flow patterns. Airflow is inhibited when particle sizes are very small; thus, the best particle size to promote sufficient aeration is between 3 to 50 mm in diameter, depending on the material properties, i.e. bulk density (Edwards *et al*., 1998). Generally, the more oxygen, the faster the process of composting (Zhou & Crawford, 1995).

Several authors have pointed out aeration as an essential component when trying to optimise the efficiency of composting; it influences both microbial growth and gas emissions (Guo *et al*., 2012; Jiang *et al*., 2011; Zang *et al*., 2016). According to Gao *et al*. (2010) and Hu *et al*. (2012), the rate of aeration in a composting system significantly affects substrate degradation due to altering the microbial makeup of the matrix and inducing temperature variation during the process. In light of this, Rasapoor *et al.* (2009) found that low to medium rates of aeration often led to an elevation in total nitrogen levels, a steep decline in C/N ratio, as well as induce prolonged periods of the thermophilic phase. On the other hand, higher aeration rates were shown to increase the electrical conductivity value of the compost.

Moisture content severely inhibits bacterial activity once the level drops below 40% (Savage *et al*., 1985). Mears *et al*. (1975) indicated that compost's thermal conductivity and specific heat capacity are linearly proportional to its moisture content. Thus, several researchers suggest that the maximum level of moisture content is a function of the physical structure of the wastes being composted and the ratio of air to water in the soil (Riser-Roberts, 1998).

According to the results of a study by Edwards *et al*. (1998) moisture levels below 45% will inhibit microbial growth. Although this differs from earlier findings by Jerris & Regan (1973) who suggest moisture contents below 20% will have that effect, both studies are however consistent in that, moisture content levels of above 70% will not only inhibit the flow of air but may also create anaerobic conditions which could significantly decrease microbial activities. Savage *et al*. (1985) point out that a significant problem in composting hazardous wastes is their high moisture contents and amorphous structure, hence the need to add a bulking agent that will provide sufficient porosity. These bulking agents are absorbent, resist compaction, degrade slowly, and can easily be recovered from the composted wastes and, subsequently, recycled.

Nutrients must be available in the adequate quantities and proportions necessary to meet the nutrient demands of the microbial population required for active composting, some of which are presented in (Table 2.12). In some cases, microorganisms tend to use carbon for energy and nitrogen for protein synthesis. Carbon is known to be the most ubiquitous element in most living cells and the basis of all functional biological molecules, comprising approximately 50% of the mass of microbial cells (Loicke, 2008). On average, microbes require a C:N ratio of about 25:1 but the optimum ratio for composting is 30:1 (dry weight basis) (Edwards *et al*., 1998). This ratio is often a determinant of the rate at which the microorganisms break down the organic wastes. Nitrogen becomes limiting with higher carbon, although in excess creates ammonia, producing pungent odours (Ali, 2007). Nitrogen is needed as a crucial component of proteins, nucleic acid and other cellular constituents. Although nitrogen (N_2) is ubiquitous in the atmosphere, i.e. circa 79%, it is still unavailable for use by most organisms. For the nitrogen to be used by microorganisms is essential for it to be in the form of ammonia (NH_3), ammonium (NH_4) or nitrate (NO_3) ions (Loicke, 2008).

Table 2.12: Nutrients required by Microorganisms during Composting (Alexander, 1977)

Energy source	Organic compounds Inorganic compounds Sunlight
Electron acceptor	O_2 Organic compounds NO_3, NO_2, N_2O, SO_4, CO_2
Carbon source	CO_2, HCO_3 Organic compounds
Minerals	C, N, P, K, Mg, S, Fe, Ca, Cu, Mn, Zn, Co, Mo
Growth factors[a] (i) Amino acids	Alanine, aspartic acid, glutamic acid, etc.
(ii) Vitamins	Thiamine, biotin, riboflavin, lipoic acid, B_{12}, pyridoxine, *p*-aminobenzoic acid, etc.
(iii) Others	Purine bases, pyrimidine bases, choline, peptides, inositol, etc.
[a] where growth proceeds in the absence of growth factors, the compounds are presumably synthesised by the organisms.	

According to Stentiford & Lasaridi (2000), most organic materials do not have an ideal C:N ratio. As a result, it is necessary to alter this ratio to accelerate the composting process. Composting complex oily sludge in soil occurs most rapidly when nitrogen is added to reduce the C:N ratio to 9:1 (Brown *et al*., 1983).

For the rapid degradation of petrochemical sludge, nitrogen, phosphorus, and potassium are added at a rate of 124:1 (C:NPK). The optimal ratios may be different for different soils (Zhou & Crawford, 1995). The addition of key nutrients, nitrogen, phosphorus, and potassium, can be supplied via the addition of inorganic fertilisers (JRB Associates Inc, 1984). These nutrients can stimulate the degradation of saturated hydrocarbons or aromatic

hydrocarbons during composting depending on the preference of the microbial population found within the matrix (Zhou & Crawford, 1995). However, nutrients increase the total biomass available for degradation of all compounds and reduce the difference noticed. Sims & Bass (1984) point out that nutrients may be present in contaminating wastes, but these may be in forms that may not be readily available or in the amounts required for composting. Thus supplementation may be needed.

2.7.3 Integrated Composting of Hydrocarbon Contaminated Soil

The application of composting utilising various organic amendments as a form of bioremediation has proven to be successful in ameliorating soils polluted with hydrocarbon contaminants. A broad range of the ordinary and uncommon environmental pollutants that can be degraded during the composting process are summarised in Table 2.13. Mature green waste compost, animal wastes, and fungi inoculated materials can be added to soils polluted with these contaminants for remedial purposes. These additions can sustain the diverse microbial populations present during the composting process; such as bacteria and lignin-degrading fungi which all possess the potential to degrade an array of organic contaminants (Semple *et al*., 2001). Furthermore, these amendments can act as an ameliorant capable of altering pH, soil structure, moisture content, and acting as a nutrient source. The properties mentioned above improve the environment within the contaminated soil for indigenous or newly supplemented microorganisms to perform degradative activities. However, there is a degree of scepticism involved with the application of compost for bioremediation. This concern stems from mixing non-contaminated with contaminated materials, as this could lead to much higher quantities of polluted material if the attempted bioremediation method employed proves to be unsuccessful. This possible pitfall can be avoided by undertaking essential research and subsequent bench-scale study followed by pilot-scale testing before field application.

Numerous investigations on the biodegradation of hydrocarbon contamination in soils using composting were conducted, and these are summarised in Appendix 1 (Table 8.1). Most of these pollutants have been studied extensively in experiments ranging from bench (laboratory) scale to large pilot studies. Diaz (2003) suggests that when contaminants are degraded completely, the disposal of the excess volume poses no threat or significant cause for concern. However, stating that a substantially larger volume of the polluted material will need to be additionally treated or disposed of if the degradation is incomplete. With this in mind, Saber (1995) draws our attention to how this dilemma can be avoided following a gradualist approach from bench-scale to pilot-scale and subsequent full-scale field projects, to ensure reliable and sufficient degradation of contaminants can be achieved.

Thus, this project adopts a similar approach with laboratory-based screening tests initially carried out followed by a pilot-scale trial.

Table 2.13: Contaminants that can be degraded during the composting process (Diaz, 2003)

General Contaminants	Examples of Causes
Total petroleum hydrocarbons (TPHs)	Gasoline, jet fuel, diesel, lubricants, grease, crude oil
Polycyclic aromatic hydrocarbons (PAHs)	Incomplete combustion, coal gasification wastes, refinery wastes, wood preservatives
Pesticides	Herbicides and insecticides
Explosives	Trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-s-triazine (RDX), nitrocellulose

Composting bioremediation strategy relies on mixing the primary ingredients of composting with the polluted sediment, wherein as the compost matures, the pollutants are degraded by the active microflora within the mixture (Semple *et al*., 2001). Given this, Liu & Cole (1996) suggest that increasing the total volume of materials is less of a problem when mature compost is added to the contaminated soil since a mixture of 40% (by weight) compost and 60% contaminated soils provided good degradation of several pesticides. Hence, in this research, mature green waste (MGW) compost was added to the weathered hydrocarbon soils on a 1:1 ratio (by weight). It is important to note that most research reviewing the use of composting approach to bioremediation has primarily focused on the operational conditions rather than on physical, chemical, and biological mechanisms that underpin bioremediation and composting technologies (Antizar-Ladislao *et al*., 2004).

In three separate studies by Antizar-Ladislao *et al*. (2005[1]; 2005[2], 2006), the degradation of aged coal-tar contaminated soil using in-vessel composting conditions was examined. In the initial test, the polluted soil was composted with green waste. In the second test, microbial activity was inhibited with $HgCl_2$ within the soil-green waste mix to evaluate abiotic losses, while the last test was a control where only the soil was incubated. In subsequent investigations by Antizar-Ladislao *et al*. (2006), the effects of varying incubation temperatures, soil:green waste ratios and moisture contents were evaluated. It was suggested that the optimal temperature for enhancing degradation rates be 38 °C,

soil:green waste ratio of 0.8:1, and a moisture content of 60%—these conditions provided for removing up to 77% of the total PAHs over a 98-day period.

According to Loicke (2008), the biodegradation of PAHs via composting at 38 °C resulted in the greater removal of these contaminants from the soil compared to the temperature profile simulating a natural composting process. That is, 38 °C for two weeks, 55 °C for another two weeks, 70 °C for a week and 38 °C for the last two weeks, therefore, suggesting that mesophilic temperatures are preferred for the removal of PAHs. Civilini (1994) carried out a similar composting study using municipal wastes and fertiliser to remediate creosote-contaminated soil. A degradation rate of up to 98.63% for HMW PAHs was achieved after 15 days of incubation at 45 °C.

Breitung *et al*. (1996) further investigated the remediation of TNT-contaminated soil using two different composting regimes. The first composting system was aerated from the commencement of incubation, while the second system was allowed to be anaerobic before being aerated subsequently. The fully aerated system showed a rapid decline in extractable TNT, i.e. 92%. In the combined aerobic/anaerobic system, TNT was practically metastasised into mono- and di- aminonitrotoluene intermediates under the initial anoxic conditions in the anaerobic phase, which subsequently transformed to putatively less toxic acetylated metabolites or were removed entirely.

2.7.3.1 Use of Inoculants/Amendments during Composting

Hydrocarbon contaminated soils often possess TPH concentrations of approximately 5,000 to 2000 mg/kg. Several authors suggest that compost can remediate the material in about two weeks to two months instead of six months or more needed for typical landfarming operations (Diaz, 2003). In a study, Persson *et al*. (1995) compared the effectiveness of composting amended with additives (maple leaves at 35 % *v/v* and alfalfa at 20 % *v/v*) and landfarming of highly weathered hydrocarbon contaminated soils. The authors indicated that only 30 % of contaminants were degraded after 180 days during the landfarming test. In contrast, however, over 50 % degradation was achieved by composting within the same time.

During a composting experiment of a PAH (anthracene) contaminated soil, Kästner *et al*. (1995) added mature compost to the soil. The result showed that the supplementation of the mature compost significantly aided the breakdown of anthracene during the composting process. The addition of compost increased the mineralisation of anthracene from 43 to 67%, while the non-recoverable fraction adsorbed to soil decreased from 45 to 21%.

Two studies (Hupe *et al*., 1996; Stegmann *et al*., 1991) documented the effects of mature compost on the degradation of TPH contaminated soil using laboratory compost reactors. The studies reported that the best results were achieved by mixing mature 6-month old compost with the contaminated soil. The average estimated degradation rate of 375 mg/kg per day observed in the studies was much higher than the value of 40 mg/kg per day reported by (Atlas, 1991). Similarly, Laine & Jørgensen (1997) investigated bench-scale composting of chlorophenol-contaminated soils utilising various inoculants: mushroom straw compost remediated soil and indigenous soil microflora. Throughout the process, up to 90% of chlorophenols was mineralised in all the composting systems. With these results, a pilot-scale was undertaken using windrow systems and the different inoculants. It was found that 80% of chlorophenols were removed, reaching acceptable levels of less than 10 mg/kg from an initial concentration of 44 mg/kg after a 60-day period.

Bhatt *et al*. (2002) worked on decontaminating anthracene polluted soil using white-rot fungi (*P. ostreatus*) induced biodegradation. They reported that fungal degradation resulted in the reduction of 80% of anthracene compared to the 44% observed in the control. In a separate experiment by Okparanma *et al*. (2011), spent white-rot fungi (*P. ostreatus*) substrate was used to remediate oil-based drill-cuttings. After 56 days of composting, results showed residual PAH levels decreasing by 14%, with overall degradation rates increase of 86%.

The use of swine manure at varying ratios (12.5%, 25%, 50%) as additives in the bench-scale composting treatment of PAH spiked soil at a concentration of 100 mg/kg was investigated by Wong *et al*. (2002). The presence of the manure increased the population of both thermophiles and mesophiles as well as PAH-degrading bacteria. This accords with findings by Diaz (2003) which indicates that decomposed horse manure was used to maintain mesophilic (25 to 35 °C) composting conditions. In addition, Wong *et al*. (2002) also suggest that in the early stages of the composting process, swine manure increased the quantities of soluble carbon, ammonia nitrogen, and soluble phosphorus. Upon completion of the process, the 25% manure ratio was highlighted as the most effective in degradation of PAHs as over 90% was removed.

A similar experiment was undertaken by Adesodun & Mbagwu (2008) to evaluate the applicability of organic wastes from animal droppings (cow manure, chicken manure, pig wastes) as composting additives for soils spiked with waste lubricating oil (WLO). After the first year, the pig waste stimulated the highest TPH losses for soils spiked with 5000 mg/kg (0.5 % WLO) and 50,000 mg/kg (5 % WLO) concentration. The authors also highlight that at the third month up to 14.6% net reduction in TPH was observed with the addition of poultry manure for high-range contamination, i.e.100,000 mg/kg (10 % WLO); whereas

within the same period, the other animal wastes (cow and pig) were more effective for soils with less oil contamination, i.e. 10,000 mg/kg. The poultry manure was noticeably most effective in remediating mid-range contaminant concentration 25,000 mg/kg (2.5 % WLO) at the initial stages, although poultry manure addition was most effective additive by the end of the second year irrespective of total loading. Comparatively, cow manure showed lower degradation levels despite oil concentration levels. Overall, even with the differential performance of these amendments, their addition stimulated the degradation of contaminants present.

2.7.3.2 Augmentation and Stimulation of Biodegradation during Composting

Some authors suggest that the addition of nutrients significantly enhance the biodegradation of organic pollutants during composting. Also, in some cases, nutrient augmentation stimulates biodegradation more than when microbial products are added under aerobic conditions, i.e. with a continuous supply of oxygen (O_2) (Lee *et al.*, 2003; Venosa *et al*., 1996). According to Bamforth & Singleton (2005), one way to enhance the successful bioremediation of hydrocarbon contamination in soils is through the addition of surfactants. These surface-active molecules are said to possess hydrophobic and hydrophilic properties that can reduce surface and interfacial tension of the microorganisms in their growth medium, to the soil. Hence, the authors point out that it provides a “bridge” between the hydrophobic hydrocarbon compounds and the hydrophilic microbial cells. Surfactants can reduce the hydrophobicity of organic pollutants and form stable emulsions, thereby increasing their solubility, elevating the bioavailability in the environment (Cameotra & Bollag, 2003).

The addition of non-ionic surfactants glycoside and alcohol ethoxylate was found to enhance the degradation of coal tar contaminated soil, where benzo(a)pyrene and pyrene were detected to be heavily recalcitrant without the addition of surfactants (Madsen & Kristensen, 1997). Furthermore, the addition of phenanthrene-utilising bacteria in combination with surfactants was found to mineralise the phenanthrene in a freshly contaminated soil, although, Madsen & Kristensen (1997) suggest that a more diverse inoculum would be required to obtain a full breakdown of phenanthrene.

Following an investigation to establish the effects of nitrogen sources and concentrations on the degradation rate of PAH in diesel contaminated limited nutrient soil at two C:N ratios. Brook *et al.* (2001) found the highest degradation rates for ammonium sulphate to be at 25 °C and a C:N ratio of 20:1. This led to the authors developing a degradation rate correlation as a function of nitrate and ammonium concentrations. This function suggested that the

presence of excessive nitrate inhibited the active degradation of contaminants. This corroborates the ideas of Fernando & Aust (1994) who indicate that fungal peroxidase enzymes are formed during secondary metabolism as a consequence of limited nutrient, i.e. nitrogen which is said to activate the production of ligninolytic enzymes and thus contaminant degradation.

It should be noted though that, some other authors suggest that some pollutant degrading organisms may require higher concentrations of nutrients. For example, Collins & Dobson (1995); Kaal *et al*. (1995) and Levin *et al.* (2016) all suggest that high concentrations of carbon or nitrogen stimulate the production of peroxidase enzymes in some various species of white-rot fungi, and can therefore positively enhance the degradation rates of organic pollutants within a medium. Thus, Hatakka (1994) concludes after a study that lignin-degrading enzymes in *Pleurotus ostreatus* can function effectively in mixtures with high nitrogen concentration.

While composting oil sludge waste using chopped barley straw, heat-treated peat moss, and peat moss enriched with nutrients and oil-degrading microbes. Milne *et al*. (1998) report that after a 30-day period, the enriched peat moss was found to have reduced TPH concentrations by 55%, coupled with high CO_2 production, suggesting high microbial respiratory activities. In comparison, only a 25% decrease noted for the other amendments. Overall, this study and others alike point out that composting coupled with nutrient and bioaugmentation is a suitable approach for the successful remediation of hydrocarbon contamination (Asgari *et al*., 2017; Saxena & Prakash, 2015). Although, Viñas *et al*. (2005) argue that nutrient addition is more effective in pre-adapted hydrocarbon contaminated soils and report that during the treatment of creosote-contaminated soil, benzo(a)anthracene and chrysene showed higher rates of degradation with no nutrients added. This implies that moisture adjustment and aeration had greater influences on the degradation of the pollutants. Thus, concluding that the addition of neither bio-surfactants, bio-augmentation, nor the addition of nutrients led to significant changes in PAH and TPH biodegradation.

In keeping with similar studies, Grotenhuis *et al*. (1999) while investigating the ability of *Bjerkandera,* a type of white-rot fungi to degrade PAHs in soils, found that the rate of degradation was higher when bioavailability of PAHs increased as surfactants were added. This was opposed to the minimal increase observed when the peroxidase activity was enhanced and aeration optimised. Similarly, Wong *et al.* (2004) monitored the effect of surfactants on the degradation of PAHs under thermophilic conditions and reported that degradation is significantly improved when surfactants produced by *Pseudomonas aeruginosa* were added. These results are echoed by Jacques *et al*. (2005) who state that

anthracene was effectively degraded by surfactants produced by *P. aeruginosa* strain (*P. citronellolis*).

2.7.4 Microorganisms present in the Composting of Hydrocarbons

Microorganisms are the principal intermediaries responsible for the recycling of carbon in nature. In a variety of ecosystems, many indigenous microbial communities possess catabolic abilities capable of extensive biodegradation of hydrocarbons, provided the environmental conditions are favourable (Antizar-Ladislao *et al*., 2004; Atlas, 1991). Atlas (1991) and Song *et al*. (1990) suggest that most soils even those found in marine and freshwater environments except for highly acidic ones contain microorganisms capable of degrading oil products.

A diverse range of microorganisms such as cyanobacteria, filamentous fungi, and yeasts are widely distributed in natural ecosystems (Cerniglia, 1997). According to Loicke (2008), the ability of microorganisms to biodegrade hydrocarbon contaminants in the environment is a complex one and is dependent on several factors. These include; the nature and concentration of the pollutant; the environmental conditions; the variety and proportion of the microbial population; and, the ability of these organisms to carry out metabolic activities based on the prevailing soil conditions, i.e. temperature, aeration, moisture content etc. Thus, Atlas (1984) suggests that the population levels of hydrocarbon utilisers and their proportions within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons. It is worth mentioning that within unpolluted ecosystems, hydrocarbon utilisers usually make up less than 0.1% of the microbial composition; in oil-contaminated ecosystems, they can constitute up to 100% of the viable microorganisms. This difference appears to quantitatively reflect the degree or extent of exposure of an ecosystem to hydrocarbon pollutants (Atlas, 1995). This accords with earlier observations of Leahy & Colwell (1990), which point out that previously, microbial populations exposed to hydrocarbons typically exhibit higher rates of biodegradation rates than populations with no history of contact with hydrocarbon contamination.

An organism's overall effect on a complex substrate is limited by its capacity to attack only certain substances or accumulate intermediates that it cannot further degrade (Bausum & Taylor, 1986). Usually, bacteria assume a more dominant role within marine ecosystems with fungi being more prevalent in terrestrial and freshwater environments. These two groups of microorganisms are the key functionaries for hydrocarbon degradation (Leahy & Colwell, 1990). An extensive breakdown of hydrocarbon contaminants is broadly achieved by mixed microbial populations, rather than single microbial species (Atlas, 1981). A mixture

of bacteria, yeasts, and fungi are said to provide about twice as much degradation of a blend of hydrocarbon substrates as do bacterial and fungal strains individually (Farrington, 2014). According to Cerniglia (1992), microorganisms in the environment may act independently or in combination to metabolise hydrocarbons.

2.8 Hydrocarbon Degradation Pathways

A vast array of microorganisms can degrade hydrocarbon compounds such as PAHs, which is observed to be most effective under aerobic conditions (Das & Chandran, 2011; Kanaly & Harayama, 2000). Bacteria and fungi via specific enzymatic systems can mediate the degradation of hydrocarbon compounds, and Figure 2.8 shows the pathways and the initial disintegration of these PAHs by oxygenases.

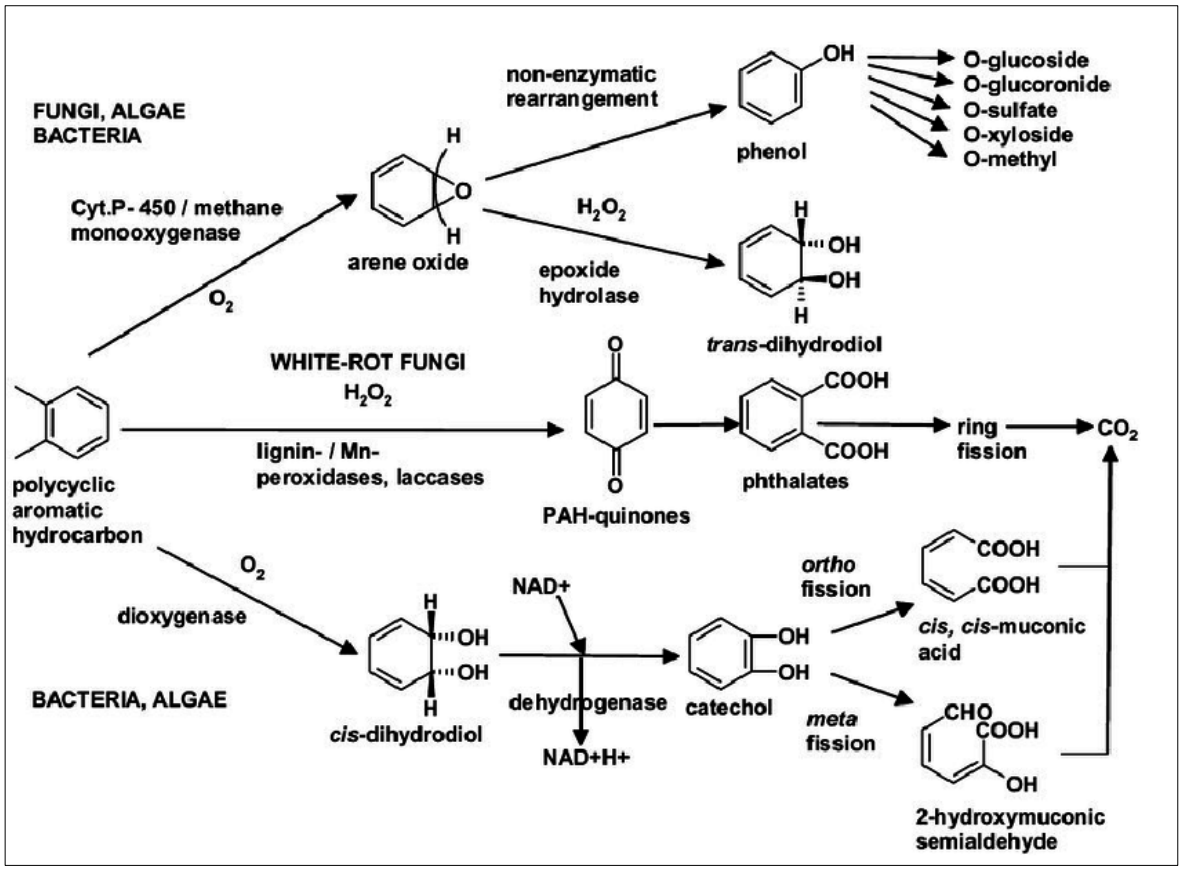


Figure 2.8: Pathways of microbial degradation of PAHs (Cerniglia, 1992)

The initial intracellular attack of organic contaminants is an oxidative process. The activation and the incorporation of oxygen are the crucial enzymatic reaction catalysed by oxygenases and peroxidases (Das & Chandran, 2011). Peripheral degradation pathways convert organic pollutants sequentially into intermediates of the central intermediary metabolism, e.g. the tricarboxylic acid cycle. Biosynthesis of cell biomass also occurs from the central

precursor metabolites, and sugars required for these processes and growth are synthesised by gluconeogenesis (Das & Chandran, 2011). The quantities of extractable PAHs can be reduced by microorganism degradation and mineralisation, adsorption to soil particles; and, volatilisation where these compounds evaporate into the atmosphere (Loicke, 2008). Several authors (Annweiler *et al*., 2000; Muller *et al*., 1998) suggest that metabolites produced as a consequence of thermophilic and mesophilic conditions contrasted, indicating different degradation pathways. A number of the microorganisms (bacteria and fungi) present in the degradation process have been presented in Tables 8.2 and 8.3 (see Appendix 2).

2.8.1 Bacterial Degradation Pathways of Hydrocarbons

The initial steps in the aerobic catabolism of PAHs molecules by bacteria are oxidation to a dihydrodiol by a multi-component enzyme system incorporating both atoms of molecular oxygen into the PAH nucleus (Gibson *et al*., 1975), as shown in Figure 2.8 (Section 2.10). Upon completing the oxidation process, the dehydrated intermediates may be processed through either an *ortho*- or a *meta*- cleavage type of pathway resulting in the formation of intermediates which are further converted to intermediates of the tricarboxylic acid (TCA) cycle (Kanaly & Harayama, 2000). Dioxygenases catalysed oxidation of arenes generally occur in aerobic bacterial systems to yield vicinal *cis*-dihydrodiols as early bioproducts by a multicomponent enzyme system (Peng *et al*., 2008). According to Peng *et al*. (2008), dioxygenases responsible for forming *cis*-dihydrodiols from arenes of PAH substrates appear to be the most ubiquitous in bacteria.

Bacteria can use organic pollutants like carbon and energy sources; here, these pollutants are oxidised for energy with the remainder used as building blocks for cellular synthesis (Knox *et al*., 1986). Henner *et al*. (1997) also suggest oxygenase production by bacteria can be increased using biostimulants such as salicylic acid, a known inducer of naphthalene dioxygenase. Fermentation, aerobic respiration, and anaerobic respiration are listed as the three methods by which heterotrophic bacteria can obtain energy. According to Kaufman & Plimmer (1972), bacteria possess a higher degree of degrading hydrocarbon compounds that are water-soluble and not strongly adsorbed. Glick (2010) corroborates this, suggesting that some bacteria make relatively insoluble PAHs more bioavailable before their degradation. This occurs through the formation of a bacterial biofilm directly on the surface of some crystal-like PAHs. However, the author points out that this mechanism is likely to exist for only a limited number of bacteria that contain hydrophobic external surfaces would be dependent upon the (limited) concentration of PAHs in the bulk liquid. Riser-Roberts (1998) notes that some bacteria are autotrophic and can derive energy from light absorption

via photosynthesis or respiration of inorganic electron donors (lithotrophs). Usually, oxygen is supplied to the soil through diffusion and thus if oxygen demand exceeds the supply, then there is a potential for the soil to be anaerobic. The level of molecular oxygen present within a matrix plays a pivotal role in the rate of biodegradation which occurs via the *ortho* pathway, a much more immediate and efficient metabolic pathway than anaerobic reactions (Cerniglia, 1997).

An investigation by Shimura *et al*. (1999) showed the existence of two independent degradation pathways for *Bacillus* sp. strain, as biphenyl grown cells of strains JF8 that degraded p-chlorobiphenyl barely degraded naphthalene, while naphthalene grown cells did not degrade p-chlorobiphenyl. This can be explained by an investigation by Mrozik *et al.* (2003) that showed several enzymes responsible for hydrocarbon degradation were found encoded chromosomally or either on plasmids. According to Kobayashi & Rittmann (1982), *Pseudomonas* species appear to be the most ubiquitous and the most adaptable to a vast array of contaminants, while *Corynebacterium* species may be major agents for decomposing heterocyclic compounds and hydrocarbons in contaminated environments. A wide range of bacteria has been identified and reported in several studies some of which are seen in Table 2.14.

Table 2.14: Bacteria capable of degrading hydrocarbon compounds

Hydrocarbons Degrading Bacteria	**References**
Achromobacter, Acidovorax, Acinetobacter, Aeromonas, Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Beijerinckia, Burkholderia, Comamonas, Corynebacterium, Flavobacterium, Microbacterium, Micrococcus, Moraxella, Mycobacterium, Neptunomonas, Nocardia, Paenibacillus, Porphyrobacter, Pseudomonas, Raistonia, Rhodococcus, Sphingomonas, Streptomyces, Vibrio, Xanthomonas, Afipia, Janthinobacterium, Leptothrix, Massilia, Methylobacterium, Rhizobium, Sinorhizobium, Thiobacillus	(Atlas, 1981; Bodour *et al*., 2003; Bossert & Bartha, 1984; Leahy & Colwell, 1990; Sorkhoh *et al*., 1993)

While most biodegradation studies often focus on the activities of bacteria under aerobic conditions, Fulghum (1977) draws our attention to the ability of some bacteria to thrive under anaerobic conditions. The author states that these anaerobic bacteria cannot synthesise or

thrive in the oxygen-linked respiratory chain. This would suggest anaerobic bacteria are not tolerant of oxygen and are inhibited or potentially killed by oxygen or oxidised components of media in which they are present.

2.8.2 Fungal Degradation Pathways of Hydrocarbons

Fungi are eukaryotic microorganisms that rely on heterotrophic metabolism due to their lack of photosynthetic structures. They may be unicellular or filamentous (yeast-like or amoeboid) or in combination to form large structures, i.e. Plasmodium or fruit bodies (mushrooms) (Solanas *et al*., 1984; Varjani, 2017). Some fungi are aquatic; however, most are terrestrial, and these inhabit soil or dead plant matter, thus playing a crucial role in the mineralisation of organic carbon (Madigan *et al*., 2008). A diverse array of fungi are said to possess the ability to degrade a wide range of hydrocarbon compounds (Table 2.15). Most filamentous fungi are reported to be aerobic, and yeasts are facultatively anaerobic. Lignin is described as a complex, recalcitrant, biogenic, irregular, nonhydrolysable, and environmentally persistent wood polymer of phenol propane units (Martínez *et al*., 2005). Due to the random nature of lignin, its degradation is non-specific; organisms with the capability of degrading lignin may also degrade recalcitrant PAHs (Bugg *et al*., 2011). Crawford & Crawford (1980) opine that fungal degradation of lignin by lignin-peroxidases occurs at high rates only with nutrient limitation and it requires cellulose and glucose as a primary growth substrate. PAHs possess relatively similar structures to lignin and some white-rot fungi such as *Pleurotus ostreatus, Bjerkandera sp.* are widely recognised to degrade lignocellulose as well as have the potential to oxidise PAHs via distinct extracellular enzymes (Grotenhuis *et al*., 1999), some of which include: lignin peroxidases, manganese peroxidases, laccases, and epoxide hydrolase (Harayama *et al*., 1999). During the degradation of hydrocarbons, Cerniglia (1997) points out that ligninolytic fungi cometabolise PAHs to form *trans*-dihydrodiols, phenols, and quinones with arene oxides (epoxides) as intermediates as seen in Figure 2.8 (Section 2.10). Hatakka (1994) draws our attention to the fact that not all white-rot fungi can produce enzymes, as different fungal strains have different ligninolytic systems that distinguish them from each other. Thus attributing the slower degradation rates of *P*. ostreatus compared to other fungi, to the possible selective degradation ability of this organism that is often classified as a moderate lignin degrader. The effectiveness of extracellular peroxidases to oxidise complex PAHs further indicates the ability of white-rot fungi to efficiently degrade recalcitrant compounds (Bishnoi *et al*., 2008; Field *et al*., 1992; Pozdnyakova, 2012).

Table 2.15: Fungi capable of degrading hydrocarbon compounds

Hydrocarbons Degrading Fungi	References
Agrocybe, Bjerkandera, Coriolopsis, Crinipellis, Flammulina, Kuehneromyces, Laetiporus, Marasmiellus, Naematoloma, Phanerochaete, Pleurotus, Ramaria, Rhizoctonia, Rhodotorula, Trametes, Trichosporon, Candida, Chrysosporium, Fusarium, Neurospora, Penicillium, Saccharomyces, Trichoderma	(Alexopoulos *et al*., 1996; Swann & Hibbett, 2003)

According to Gibson *et al*. (1975), fungi metabolise hydrocarbons in a manner similar to mammalian systems, i.e. via a monooxygenase-catalysed reaction. Adding that it is highly likely that a cytochrome P450 dependent reaction may be responsible for the initial oxygenation of hydrocarbons by these microorganisms.

In the degradation of PAHs filamentous fungi hydroxylations is used as a prelude to detoxification rather than catabolism and assimilation (Biache *et al*., 2017; Fuchs *et al*., 2011). The authors indicate that these organisms do no degrade aromatic hydrocarbons as nutrients but rather detoxify them. Cerniglia & Yang (1984) point out that fungi can form glucuronide and sulphate conjugates of phenolic PAHs, which may be necessary for detoxifying and eliminating PAHs. *Phanerochaete chyrysosporium*, a type of Basidiomycete (white-rot fungi) has been known to have superior lignin-degrading abilities (Higuchi, 2004). Here, the fungus destroys the lignin matrix to access the hemicellulose and cellulose and generate a carbon-centred free radical enzyme system that allows it to catalyse numerous nonspecific cleavage reactions of the lignin macrostructure (Field *et al*., 1992). According to Henner *et al*. (1997), white-rot fungi oxidise some PAHs by destabilising their benzene rings using non-specific enzymes, lignin peroxidase and Mn-dependent peroxide enzymes. These enzymes produce highly reactive intermediates which are then oxidised to produce quinones.

The earliest and most significant advances in the utilisation of fungi in bioremediation came following a significant study by Bumpus *et al*. (1985) that found white-rot fungi Basidiomycete (*Phaenerochaete chrysoporium*) was able to moderately breakdown benzo(a)pyrene to CO_2. Similar studies carried out subsequently corroborated initial reports, indicating the metabolisation of an array of PAHs by *P. chrysoporium* under

ligninolytic and non-ligninolytic conditions to CO_2 (Haemmerli *et al*., 1986; Reddy, 1995; Sanglard *et al*., 1986).

2.8.3 Other Life Forms involved in Hydrocarbon Degradation

The role of soil macrofaunae such as insects, protozoa, earthworms, and slugs, in the decomposition of organic contaminants is predominantly indirect. It is minor compared with microorganisms but still essential (Parr et al., 1983). Due to their inability to produce enzymes for pollutant degradation, Riser-Roberts (1998) suggests that their main mode of degradation is mechanical. Most soil macrofaunae carry microorganisms within their guts which may secrete necessary enzymes for substrate degradation (Parr *et al*., 1983). Earthworms particularly play a prominent role in the degradation of contaminants as their movement promotes aeration and transfer nutrients to deeper soil profiles, where these stimulate microbial growth and decomposition (Parr *et al*., 1983). Also, they have been found to bio-accumulate certain metals such as zinc, iron, cadmium, and zinc in oily wastes, although, Loehr *et al*. (1985) opine that they do not bio-accumulate hydrocarbons. In general, Hornick *et al*. (1983) conclude that macro-fauna has an essential role in waste decomposition, and the addition of materials that toxic to these organisms can alter the rate of decomposition.

2.9 Summary of Review

From the review, it is fundamental to appreciate the difference between compost and composting at the outset. Composting is the process by which compost is produced, i.e. the maturation of materials such as manure, straw, and green waste. On the other hand, Compost is the resultant product of composting, except for horticultural potting composts (Semple *et al*., 2001). Thus, composting as a bioremediation strategy relies on the addition of compost's primary ingredients to the contaminated soil, wherein the presence of the compost matures in the presence of the contaminated soil thereby aiding biodegradation. In composting, compost can be added before or after its maturation.

Most of the studies examined in this review assessed different techniques of biodegrading hydrocarbon contaminated materials under a wide range of conditions. Composting was particularly of interest; hence, its effectiveness in hydrocarbon degradation was appraised. Due to the disparities in optimal conditions during composting of contaminated materials, it is significantly challenging to adopt an idyllic unified system for the operation of this remediation technology.

Therefore, emphasis must be laid on developing a standardised procedure for thoroughly assessing the influence of varying conditions by understanding the characteristics of the contaminated material and the type of pollutants and the indigenous microbial populations. This is expected to lead to the development of a suitable protocol to methodically remediate contamination in soils. The process by which this technique is adopted should be cost-effective and very efficient to ensure that either complete degradation of the contaminants or at least biotransformation to more innocuous compounds is achieved.

A majority of the composting bioremediation feasibility studies undertaken have traditionally focused mainly on single pollutants as it allows the elucidation of the fate processes for specific compounds. However, it will be useful to assess the effectiveness of this method on a vast array of a combination of compounds that may be recalcitrant in some cases, thereby making them a significant concern due to their toxicity and carcinogenicity (U.S. EPA, 2001).

A key observation in the several investigations reviewed indicate that despite suggestions of a synergistic association between microorganisms, the most common practice in bioremediation involves the use of bacteria or fungi singly or separately, with a limited number of studies exploring the integrated use of microbial consortia of both kingdoms as bio-additives during composting bioremediation of hydrocarbon contamination in soils. It is quite apparent that both organisms possess positive attributes and shortcomings; hence their integrated use may compensate for each of their deficiencies. Typically, bacterial rates of hydrocarbon degradation are comparatively higher than fungal rates of degradation, often described as slow and inefficient. Be that as it may, the ecological role of fungi cannot be overstated as they can hydroxylate a wide variety of hydrocarbon compounds, and these polar intermediates can be mineralised by bacteria or detoxified into innocuous compounds (In der Wiesche *et al*., 2003). An advantage of fungi over bacteria is that fungal mycelium can grow and spread within a contaminated solid matrix to degrade hydrocarbon compounds (Cerniglia, 1997). The extracellular enzymes released by fungi can degrade HMW PAHs, whereas bacteria mainly possess intracellular enzymes that are often limited to the degradation of LMW PAHs (Grotenhuis *et al*., 1999). However, an essential characteristic that makes bacteria very viable for bioremediation is their ability to use hydrocarbons as a sole source of carbon and energy, thus mineralising these pollutants more rapidly than fungi (which do not utilise hydrocarbon compounds as their sole source of carbon) (Cerniglia, 1992).

The advantages and limitations of using organisms from different groups as amendments in composting bioremediation of contaminated soils have been investigated. It is apparent

that most studies focus on the application of bacteria or fungi bio-additives singly during composting despite reports of a synergistic relationship existing between both organisms during contaminant degradation.

The following conclusions were reached based on the findings from the review, and these have been enumerated below:

1. There is a lack of investigations that utilise the 'integrated-composting approach' where both groups of microorganisms (bacteria and fungi) are exploited in the remediation of hydrocarbon contaminated soils; while focusing on harnessing their biodegradative capacities through the provision of a suitable environment.
2. Only a handful of studies have analysed and discussed more than one contaminant, as most seem to either monitor TPHs or specific PAHs only.
3. So far, there have been no studies undertaken to assess the effectiveness of composting under high-intensity rainfall, i.e. conditions found in tropical regions of the world.
4. There is yet to be any composting remediation study investigating the transfer of contaminants via leaching from soil to water.

On account of the listed research gaps, this study will seek to address these issues through robust experimental procedures and rigorous data analysis.

CHAPTER 3

3.0 MATERIALS AND METHODS

Unless otherwise stated, all chemicals used for analytical procedures were of analytical grade from Fisher Scientific Ltd, UK or Sigma-Aldrich Company Ltd, UK. The PAH standards were purchased from Qmx Laboratories, UK with the Liquid Chromatography columns (Silica and Sodium-sulphate) purchased from Agilent Technologies Ltd, UK.

3.1 Research Strategy

This research focuses on using animal wastes (cattle and chicken manures) and white-rot fungi (*Pleurotus ostreatus*) inoculated material as additional nutrient sources for the stimulation of biodegradation of polluted soils. For a greater understanding of the effects of these additives, the Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAH) contents in the soil and leachate will be monitored at various time intervals over the experimental duration. Due to the cocktail of pollutants present in weathered hydrocarbons, measuring TPH levels provides a more precise quantification of the overall contaminants that have been degraded. PAHs, on the other hand, are of particular concern due to their toxicity with the Environmental Protection Agency (EPA) reporting these compounds as carcinogenic and mutagenic (Verdin *et al*., 2004). Physicochemical parameters will also be monitored, as these tend to affect the efficiency of the composting process.

In a bid to ensure scientific rigour and data robustness, the conduct of the microcosm study will involve the use of three replicates of each treatment combination. This is especially necessary with reproducibility being an issue often confronted during the conduct of such respirometric experiments. These replicates will show potential variation so that statistical tests can be applied to evaluate the efficacy of the various amendments in treating the contaminated soil. However, the limited availability of the contaminated soil, as well as some of the amendments, will mean that the use of replicates in the mesocosm study will not be feasible. This is primarily due to the substantial quantity of materials required to be sufficient to complement the greater size of the reactors with a 100L capacity. To address this shortfall, samples will be taken in triplicates from each reactor during this phase of the study. Despite these anticipated constraints, the data collected will be sufficient to make sound and informed decisions on composting as a strategy for remediating hydrocarbon contaminated soils using various animal wastes and fungi-inoculated substrates.

The research strategy consists of a two-phase plan that will address the objectives as shown in the complete study outline illustrated in Figure 3.1. It is worthy to note that some researchers suggest that laboratory-scale composting tests may not always provide results similar in either extent or time scale to the results obtained in large-scale trials or even field-scale projects. An investigation by Kaplan & Kaplan (1982) using laboratory reactors in composting trinitrotoluene (TNT) showed no mineralisation and reduced degradation of the contaminant overall. Although, the formation of ^{14}C-labelled reduction products including various aminonitrotoluenes were detected. Whereas in a composting pilot study carried out by Williams *et al*. (1992), results showed significant degradation of TNT as it was reduced from 11,190 to 50 mg/kg. Based on this paradigm, Diaz (2003) points out that even if only partial degradation under laboratory test conditions is observed, then there is sufficient justification for conducting larger-scale pilot studies as is the case with this research project.

3.1.1 Phase 1

This initial microcosm study will be conducted using a modified dynamic respiration index test (DR-4) over a 24-day period. This test mimics the core of a compost heap while the process is active. It serves as an initial screening protocol to identify the degradative effects of different animal-derived manures and white-rot fungi on the weathered hydrocarbon contaminated soil. The contaminated sample will be treated under aerobic conditions and high temperature. The respiration rates (CO_2 produced) during the process, seed cress germination index test and overall changes in hydrocarbon concentration will be monitored and used as an indicator for degradation. Results of the investigated treatment combinations shall be compared, and the most successful manure and fungal treatments are to be carried forward to the mesocosm study. While several parameters will be monitored, emphasis is placed on the reduction of hydrocarbon concentration at the end of the treatment cycle.

3.1.2 Phase 2

This will consist of a mesocosm study of the three most effective treatment combinations from the first phase. A lagged 100 L composting bioreactor system with forced aeration will be used to treat the weathered hydrocarbon contaminated soil. During this trial, the effect of high-intensity rainfall will also be simulated to determine the contaminant load that could runoff from the process. The leachate and the soil sample shall be monitored for TPH and PAH concentration levels, nutrients, heavy metals, and other physicochemical parameters at 2-week intervals. The quantification of hydrocarbon tolerant microorganisms will be carried out on the soil sample monthly over the course of the project.

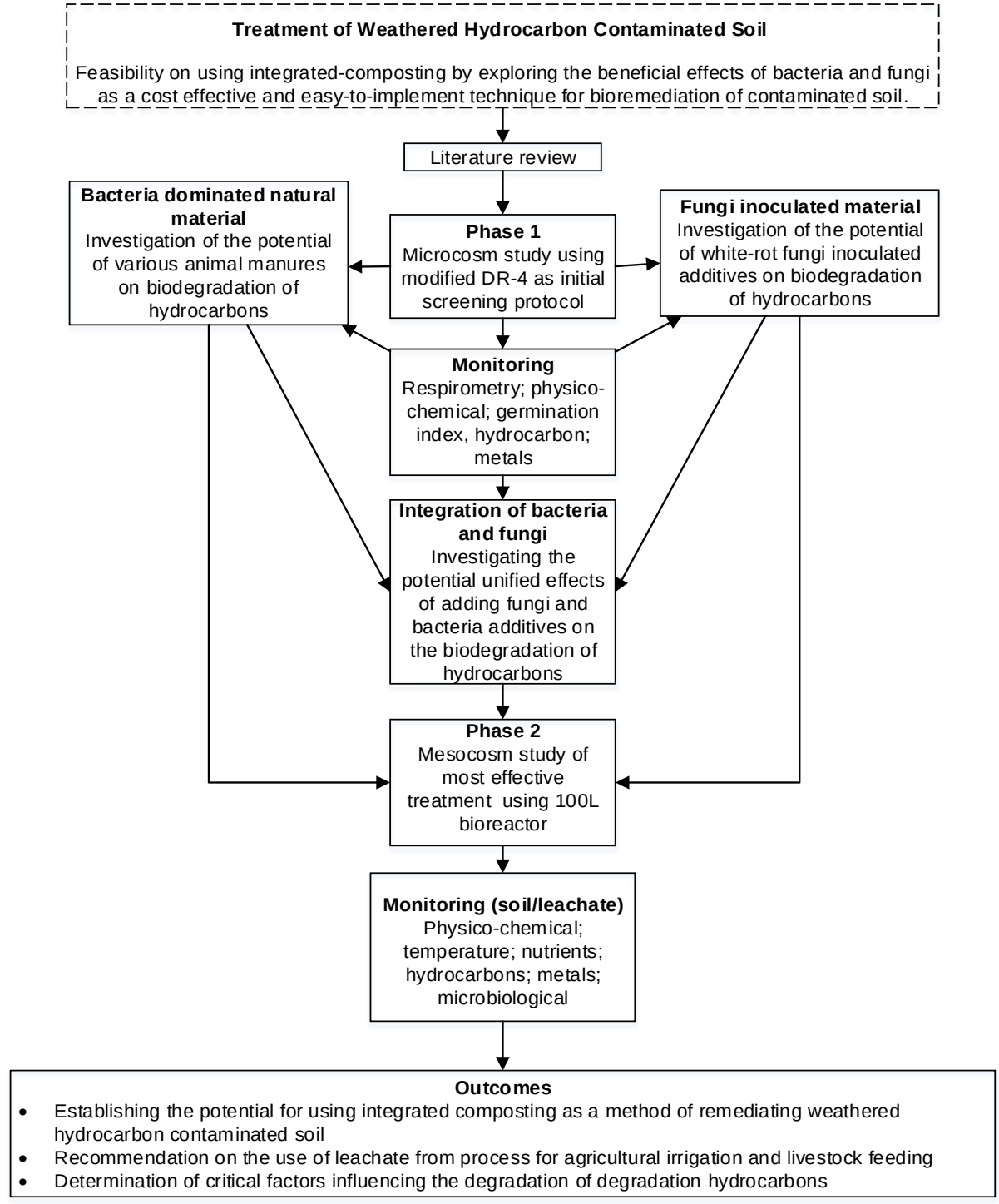


Figure 3.1: Complete study outline indicating the various phases and experiments performed.

Further details on all the tests and monitoring procedures are given below.

3.2 Composting Materials

3.2.1 Contaminated Soil

The weathered hydrocarbon-contaminated soil under investigation was collected from an old Royal Navy fire-training facility (FTF) at Horsea Lagoon in April 2007 for use in an earlier phytoremediation study. The facility has been decommissioned but has left a legacy of excessive hydrocarbon contamination. The shallow lagoon has an area of 75 m by 25 m and is situated at Horsea Island, North East of Portsmouth Harbour, UK with Grid Reference SU 635 044. The soil is made up of primarily sand (58 – 85 %) with silt (10 – 38 %) and clay content (5 – 9 %) (Pinchin, 2012). A major investigation carried out prior by the Environmental Science Group (ESG) of the UK MOD reported the soil as a black, fine-grained material with the underlying soil structure comprising alluvium with the chalk fragments found sporadically. The identified contaminants of concern are the Total Petroleum Hydrocarbons (TPHs) in particular the Diesel Range Organics (DRO) and Oil Range Organics (ORO) and then Polycyclic Aromatic Hydrocarbons (PAHs). Initial tests on the samples determined levels of DRO to be approximately up to 615,000 mg/kg dry weight (Pinchin, 2012).

3.2.2 Amendments

Manures from animals with different digestive systems to give contrasting organic and nutrient content and physicochemical and microbial properties were used. Cattle manure was collected from Heath Farm, a local dairy farm in Petersfield, UK with Grid Reference SU 757 224 and chicken manure provided by Elle Hubbard of the Milland free-range Chicken Farm Petersfield, UK).

White-rot fungi have been known to be effective in the bioremediation of organic contaminants in soil (Loick *et al.*, 2009). Oyster mushroom (*Pleurotus ostreatus*) growing kits were purchased from MushroomBox™ UK. The kits were prepared as instructed on the box and distilled water was added at regular intervals to keep the substrate/spawn mixture damp. In 14 to 21 days once mycelium growth had occurred through the substrate, it was ready for use (Figure 3.2).

Figure 3.2: *Pleurotus ostreatus* mycelium ridden substrate

Mature Green Waste (MGW) compost screened to 10 mm was used as a bulking agent. It was provided by Fieldfare Limited, UK, a composting facility in Osier Dell Manor Farm, Hayling Island with Grid Reference SU 721 007. All necessary tubing fitted to the air pumps, non-return valves, and air stones were purchased from Arundel Aviaries and Fisheries Limited UK.

3.2.3 Sampling Strategy

3.2.3.1 Microcosm Study

20 g of soil sample was taken from each of the three replicates of a treatment combination at the start and end of every trial period. The collected sample was manually mixed to ensure homogeneity is achieved. On completion, the batch was sequestered into sub-samples for individual testing to commence. Samples for hydrocarbon testing were wrapped in aluminium foil and stored at -5°C before extraction and further analysis. See Table 3.1 for further details.

Table 3.1: Sequence for sample collection during Microcosm study

	Test	Quantity used	Time taken (days)	Number of samples taken
Microcosm Study	TPH	1-1.5 g	0, 24	1 per replicate
	PAH	1-1.5 g	0, 24	1 per replicate
	Moisture/Organic Contents	2-5 g	0, 24	1 per replicate
	pH/EC	2 g	0, 24	1 per replicate
	CO_2 (titration)	25 mL	0,1,2,4,7,10,14,17,21,24	1 per replicate
	Metals	1 g	0, 24	1 per replicate
	Germination Index	1 g	0, 24	1 per replicate

3.2.3.2 Mesocosm Study

Destructive sampling of the soil mixture in triplicates was carried out for each reactor every fortnight. To ensure a high degree of homogeneity was achieved, each reactor content was thoroughly mixed manually before removing 50 g of the solid samples from each vessel at random sampling points. Core samples were collected via a modified 100 mL syringe (BD Plastipak, UK) inserted into the soil matrix to obtain core samples. The 50 g portion of the sample collected was further homogenised before sub-sampling for the various tests. To ensure no biological degradation of hydrocarbons in the soil occurred after sampling, samples requiring further analysis were frozen to suppress microbial activity.

The leachate samples were collected in amber glass bottles, in triplicates, and immediately tested for physicochemical parameters. However, as the samples were not immediately analysed for hydrocarbons, the pH was adjusted to 2 the addition of 6M HCl to inhibit biological activity, before being stored at 4°C. Samples were extracted within seven days, and analysis was completed within 40 days. See Table 3.2 for further details. The leachate generated was not accumulated rather than discarded as this is indicative of whether the collected leachate meets water quality guidelines.

Table 3.2: Sequence for sample collection during Mesocosm study

	Test	Quantity used	Time taken (Weeks)	Number of samples taken
Mesocosm Study	TPH	1-1.5 g	0,3,5,7,9,11,13,15,17,20	3 points per reactor
	PAH	1-1.5 g	0,3,5,7,9,11,13,15,17,20	3 points per reactor
	MC/OM	2-5 g	0,3,5,7,9,11,13,15,17,20	3 points per reactor
	pH/EC	2 g	0,3,5,7,9,11,13,15,17,20	3 points per reactor
	Nutrients	2 g	0,3,5,7,9,11,13,15,17,20	3 points per reactor
	Metals	1 g	0,3,5,7,9,11,13,15,17,20	3 points per reactor
	Microbial	1 g	0,4,8,12,16	3 points per reactor
	LEACHATE			
	TPH	100 mL	0,3,5,7,9,11,13,15,17,20	1
	PAH	100 mL	0,3,5,7,9,11,13,15,17,20	1
	pH/EC	20 mL	0,3,5,7,9,11,13,15,17,20	1
	COD	10 mL	0,3,5,7,9,11,13,15,17,20	1
	Metals	25 mL	0,3,5,7,9,11,13,15,17,20	1

3.3 Microcosm Study

This initial laboratory-scale study was employed as a screening tool in order to determine the most effective treatment combinations for hydrocarbon degradation within the contaminated sample. Upon completion, three of the best treatments were established and taken forward to the second phase of the project (mesocosm study) using 100 L composting reactor systems. This was determined based on the hydrocarbon concentration remaining following the 24-day experimental process. It should be noted that the respirometric results showed the presence of aerobic microbial activities that are often associated with hydrocarbon degradation in these tests.

3.3.1 Dynamic Respiration Index Test (DR4)

This is a solid-state aerobic biodegradation test method devised by the WRc Group, UK, to measure the potential biodegradability of contaminated soil samples by monitoring the production of carbon dioxide (CO_2) and/ or oxygen (O_2) consumed over a four-day period. The method is based on the standard test method for measuring organic waste biodegradation (ASTM D5975-96). Metabolic activities of the microbial population are often directly related to respiration. Microorganisms respire at elevated rates in the presence of large amounts of bioavailable organic matter while slower respiration rates indicate a scarcity of these materials (Barrena Gómez *et al.*, 2006). Thus, in composting, respiration is an essential parameter for determining the compost's stability and maturity. These respirometric assays are affected by parameters such as temperature, humidity, and incubation conditions. Results from these protocols are generally expressed as respiration indices as either CO_2 produced, O_2 uptake or release of heat. Godley *et al.* (2005) suggest that the time scale of this test is insufficient to obtain a meaningful decomposition of the contaminated organic material. Against this background, a modified version of this test was conducted over a 24-day period previously reported by Sayara et al. (2011) to be sufficient to achieve significant degradation of hydrocarbons.

3.3.2 Treatment Combinations

In order to assess the effectiveness of each treatment, 100 g of the weathered hydrocarbon-contaminated soil was mixed with 100 g of MGW that functioned as the feedstock and bulking agent at a 1:1 ratio (*w*/*w*) for all the treatments. An additional 60 g of the various amendments, i.e. animal wastes and fungi inoculated substrate to give a ratio of 1:0.3 (*w*/*w*). Details of these ratios are shown in Table 3.3. The sample was prepared as one bulk entity and then split into three separate batches of 260 g to represent one replicate of each set of treatments. To enhance aeration, straw was introduced to the mix.

Table 3.3: Treatments and Ratios for microcosm study.

Mixtures	*Mix Ratio
(Contaminated Soil + Mature Green Waste) + Cattle manure	1:0.3
(Contaminated Soil + Mature Green Waste) + Chicken manure	1:0.3
(Contaminated Soil + Mature Green Waste) + Fungi substrate	1:0.3
(Contaminated Soil + Mature Green Waste) + Cattle manure + Chicken manure + Fungi substrate	1 : 0.1 : 0.1: 0.1
(Contaminated Soil + Mature Green Waste) + Cattle manure + Fungi substrate	1: 0.15 : 0.15
(Contaminated Soil + Mature Green Waste) + Chicken manure + Fungi substrate	1: 0.15 : 0.15
(Contaminated Soil + Mature Green Waste) + Cattle manure + Chicken manure	1: 0.15 : 0.15
Control 1: Contaminated Soil	-
Control 2: Mature Green Waste	-
Control 3: Cattle manure	-
Control 4: Chicken manure	-
Control 5: Fungi substrate	-
	-

*All ratios are taken on a dry weight basis

3.3.3 Experimental Set-up

The apparatus consisted of a reactor vessel which was a 2.5 L wide-necked powder jar (Fisher Scientific, UK), a humidifier (conical flask containing distilled water), and two alkaline traps (conical flasks containing 1 molar sodium hydroxide) for the collection of CO_2 evolved from the biodegradation process, as can be seen in the schematic illustration (Figure 3.3).

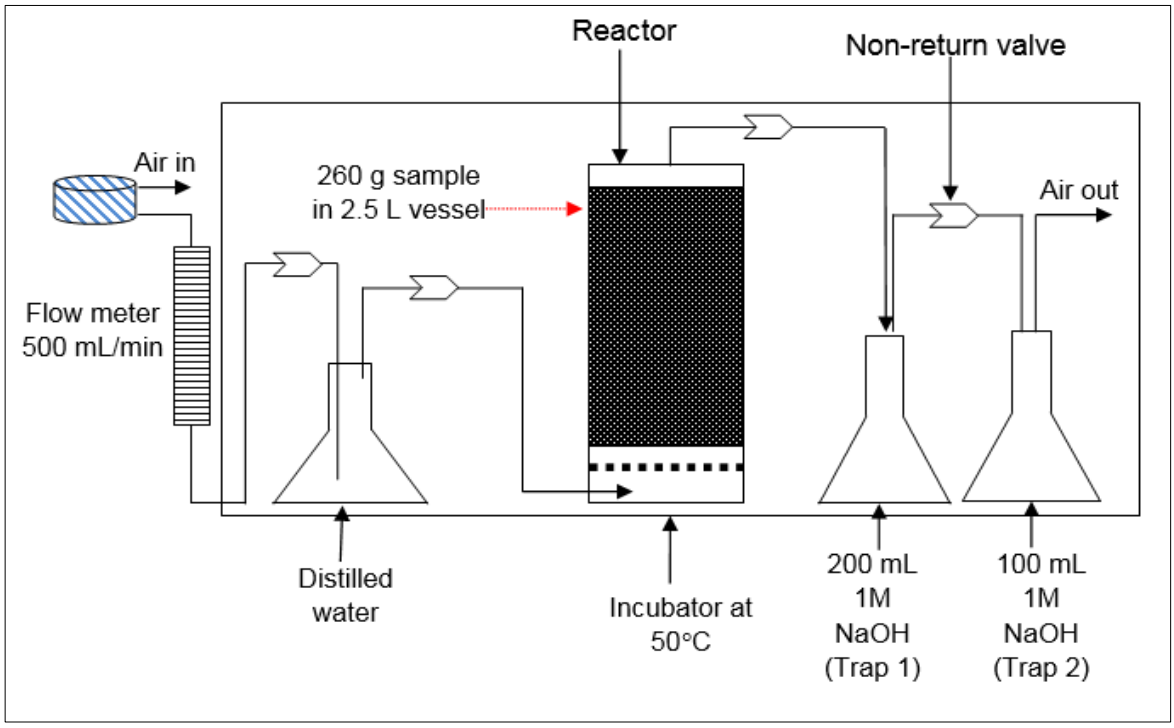


Figure 3.3: Schematic representation of the experimental set-up for one reactor vessel.

A 4 mm air tube was threaded through the bottom of the perforated floor which was connected to an adjustable 600 l/hr 4 outlets, 4 mm output Hailea Air Pump with non-return valves and (Omega Series FL-2000-FL-2069 flow meters) at a rate of 500 mL/min to provide sufficient air supply required for aerobic degradation to take place. The entire set-up was kept in a temperature-controlled incubator (Figure 3.4).

The contaminated soil sample was mixed with an equal amount (by dry weight) of mature green waste (MGW) compost, which supplements the fermentative micro-organisms needed to biodegrade the test material under controlled conditions (Turrell *et al*., 2009). Once the moisture content of the sample was adjusted to 40 ± 5 %, it was then placed into a vessel which was subsequently transferred into a temperature-controlled incubator for 24 days at 50°C to simulate the representative mesophilic and thermophilic stages (Antizar-Ladislao *et al*., 2006), under aerobic conditions using forced aeration.

Figure 3.4: Experimental set-up of the microcosm experiment showing the incubator and aeration system. The image inset shows the rectangular perforated plastic floor supported by a semi-rigid ring on which sample mix is placed within the reactor vessel.

Over the 24-day test period, the microorganisms present within the mixture aerobically decompose some of the biodegradable organic pollutants present, consequently producing CO_2 measured at specific time intervals. Control vessels containing just MGW, an appropriate quality control substrate, were used to determine any carbon dioxide present in the air supply.

3.3.4 Monitoring CO_2 Evolution

Soil CO_2 respiration is a widely used and simple measure of biological activity in the soil as it is directly correlated with aerobic respiration. Here, the microbial activity measured by CO_2 respiration is a function of substrate availability, which is related to the quantity or quality of organic carbon and nitrogen (Haney *et al.,* 2008). For many years, CO_2 respiration from soils has been used as an indicator to quantify the impact of microbial activities on various treatment and management inputs (Haney *et al*., 2008). Other authors opine that it can be challenging to distinguish between aerobically produced CO_2 from that produced anaerobically; since these methods assume the CO_2/O_2 ratio is always 1 (Barrena Gómez

et al., 2006). The authors' further state that it can also vary depending on the oxidation degree of the organic carbon. On the basis, Lasaridi & Stentiford (1998) argue that it does not give a reasonable estimate of the respiration index of a material. Conversely, it has been indicated that if the assay is carried out under controlled aerobic conditions, all CO_2 will be produced under aerobic conditions (ADAS, 2003).

3.3.4.1 Titration Analysis

Acid-base titration was used to measure CO_2 production over time. This quantitative chemical analysis method is utilised when determining the unknown concentration of an identified analyte through the neutralisation reaction (Equation 3.1) between an acid and a soluble base (Widmer, 2006).

$$\mathbf{HCl + NaOH \rightarrow NaCl + H_2O} \qquad \textbf{(Equation 3.1)}$$

Carbon dioxide is an acidic gas and reacts with alkaline sodium hydroxide producing sodium carbonate (Equation 3.2). This results in the retention of the CO_2 from the air stream into the NaOH.

$$\mathbf{2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O} \qquad \textbf{(Equation 3.2)}$$

This reaction part-neutralises the NaOH, and therefore titration of the remaining hydroxide with 1 molar hydrochloric acid (HCl) allows an estimation of the quantity of CO_2 captured by the trap (Turrell *et al*., 2009).

For this experiment, the alkali traps were titrated and changed on each of the first four days and subsequently at three-day intervals until the 24th day of the test. Before commencing the titration, the burette was filled with 25 mL of 1M HCl with the liquid set at the zero graduation mark. Following this, 25 mL of alkali was collected from the trap, and 10 mL of 0.5 molar barium chloride ($BaCl_2$) was added, causing carbonate formation because of the absorption of CO_2 precipitated as insoluble barium carbonate (Equation 3.3). The intensity of the cloudy white precipitate formed indicated the quantity of CO_2 present within the solution. Barium chloride is used because it separates the reacted NaOH from the unreacted NaOH through precipitation (Turrell *et al*., 2009).

$$\mathbf{Na_2CO_3 + BaCl_2 \rightarrow BaCO_3 + 2NaCl} \qquad \textbf{(Equation 3.3)}$$

After that, three drops of 0.5 wt. % in ethanol: water (1:1) phenolphthalein indicator solution was added. This turns the solution pink, which is indicative of the occurrence of alkali. Finally, the solution was titrated with 25 mL of standardised 1M HCl solution until the endpoint was reached, i.e. once neutralisation occurred. This was observed through the

sudden disappearance of the pink colour known as the equivalent point, leaving a transparent solution. At this point, the titre value, which is the volume of acid used, was recorded. The same procedure was carried out for the second CO_2 trap.

3.3.4.2 Calculation

The volume of alkali solution within each NaOH trap (primary and secondary) on sampling days was measured to quantify the possible loss of the alkali solution through evaporation or increase due to water condensation from the humidifier. These volumes were adjusted to reflect any changes in the initial volumes of NaOH in each trap as shown in Equations 3.4 and 3.5. The initial volumes of NaOH in the primary and secondary traps were 200 mL and 100mL respectively.

$$\mathbf{A1'} = \mathbf{A1} \times \mathbf{V1/Volume}\ \boldsymbol{of\ NaOH\ in\ primary\ trap\ at\ start} \quad \textbf{(Equation 3.4)}$$

Where:

$A1'$= Adjusted titration volume for the primary trap

$A1$ = Volume of acid used for the titration of the primary trap

$V1$ = Volume of alkali solution left in primary trap before sampling

$$\mathbf{A2'} = \mathbf{A2} \times \mathbf{V2/Volume}\ \boldsymbol{of\ NaOH\ in\ secondary\ trap\ at\ start} \quad \textbf{(Equation 3.5)}$$

$A2'$ = Adjusted titration volume for the secondary trap

$A2$ = Volume of acid used for the titration of the secondary trap

$V2$ = Volume of alkali solution left in secondary trap before sampling

The quantity of CO_2 collected in each trap was expressed in terms of carbon. Considering 22 mg of CO_2 contains 6 mg of carbon, the results are given in (mg CO_2-C). For full rigour, 25 mL of fresh 1M NaOH solution was titrated against fresh 1M HCl (both fresh from stock). The resulting value, B mL of acid was exactly 25 mL.

(B – A1') x 6 x V1 / 25 (mg CO_2-C)

Where:

B = Volume of acid needed to titrate blank NaOH.

The calculations are similar for the second trap, so the mass of CO_2 collected in both traps is expressed as

$$\mathbf{6 * [(B – A1') x V1 / 25 + (B – A2') x V2 / 25]}$$

The cumulative 24-day respiration was determined by the collective sum of the daily measurements of CO_2 production values given in (mg CO_2-C). Each of these values was divided by the mass of dry solids of the test sample within the reactor to obtain the 24-day respiration in (mg CO_2-C/kg dry solids).

Finally, the mg CO_2-C/kg per dry solids value was divided by the mass fraction of volatile solids in the material to obtain the cumulative 24-day respiration expressed in mg CO_2-C/kg volatile solids.

3.3.5 Germination Index

The seed germination and root length test were carried out on the soil-water mixture extracts obtained via mechanically shaking the sample at a solid to distilled water ratio of 1:10 (*w*/*v*, dry weight basis) for 15 minutes with a whirl mixer within a sterile test tube. Approximately 5 mL of each extract was transferred into a sterilised petri dish lined with Whatman No.1 filter paper. Twenty cress seeds (*Lepidium sativum* L.) were placed evenly on a filter paper and kept at room temperature and normal light for a 5-day period (Figure 3.5).

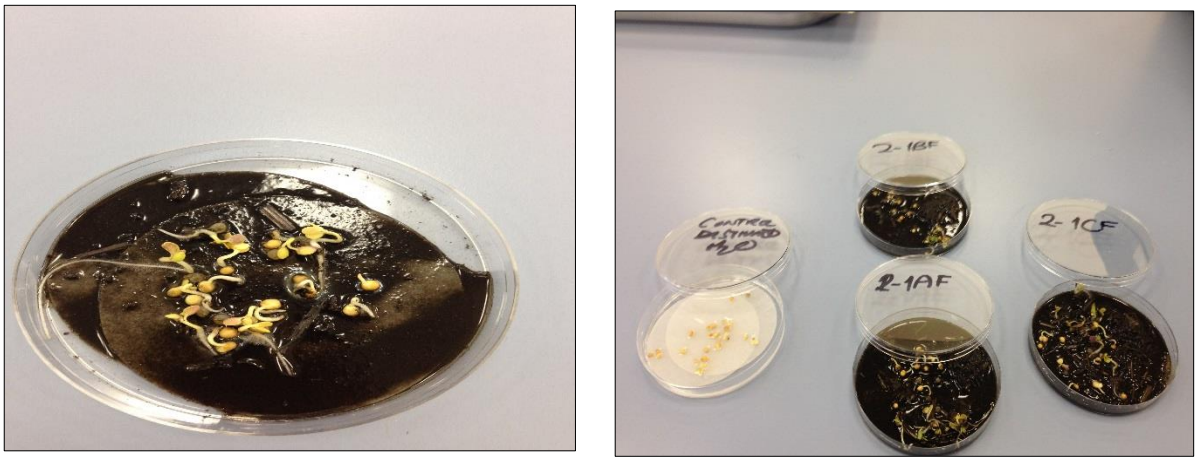


Figure 3.5: Seed cress germination test on petri dishes using contaminated soil liquid extract and a distilled water as control.

Triplicates were analysed for each microcosm sample. The treatments were evaluated by enumerating the number of germinated seeds and measuring the length of the roots. The responses were calculated using a germination index (GI) as described by Zucconi *et al*. (1981) (Equation 3.6).

$$\text{Germination Index (\%)} = \frac{\text{Seed germination (\%) x root length of treatment}}{\text{Seed germination (\%) x root length of control}} \text{ x } 100 \quad \textbf{(Equation 3.6)}$$

3.4 Mesocosm Study

Once the initial trials were concluded and the three most effective treatment combinations selected, the pilot test (microcosm study) used for screening the treatment efficiency of amendment alternatives was scaled up by 100 fold, reconstructed, and conducted using large-scale reactor systems. This phase of the project had a total duration of 150 days (5 months). It provided information on the feasibility of applying this remedial method on hydrocarbon-contaminated soils on a field-scale. A rainfall simulation test was used to mimic conditions similar to those found in tropical regions during this study. This will assess the effectiveness of the treatment in such conditions and provide a better understanding of hydrocarbon pollutant transfer due to leaching from soils onto the surface and groundwater sources. Evaluating the levels of hydrocarbon concentration in these water sources will inform end-users of its suitability for agricultural irrigation and livestock feeding.

3.4.1 Treatment Combinations

The high variability in the microcosm study results meant the degradation of TPHs was analysed on a mass removal basis, while the breakdown of PAHs was evaluated using an exponential rate. Based on these findings, the most effective treatments were utilised. Table 3.4 provides details of each combination.

Table 3.4: Treatments and Ratios for mesocosm study.

Treatment	Cont. Soil (kg)	MGW (kg)	Chicken manure (kg)	Cattle manure (kg)	Fungi (kg)
(Cont.Soil + MGW) + Cattle man. + Chicken man.	10	10	3	3	-
Cont.Soil + MGW) + Fungi substr.	10	10	-	-	6
(Cont.Soil + MGW) + Cattle man. +Chicken man. + Fungi substr.	10	10	2	2	2
Cont.- Contaminated; MGW- Mature Green Waste; Man.- Manure; Substr.- Substrate					

3.4.2 Batch Reactor Systems

The composting process was conducted using three identical closed reactor systems with forced aeration via an external air supply as illustrated in the schematic diagram (Figure 3.6). The reactors measured 1.25 m x 0.35 m x 0.32 m (H x W x D) with a 100 L capacity.

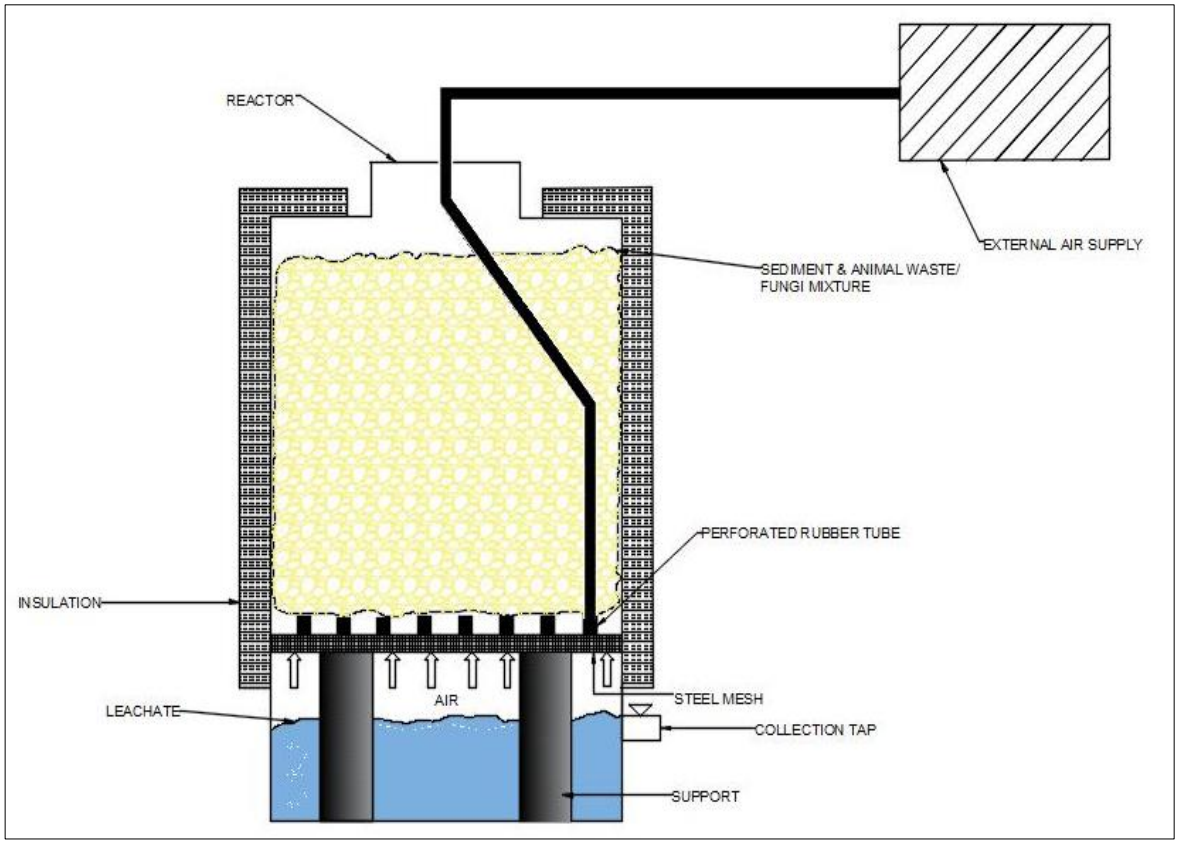


Figure 3.6: Schematic representation of a single reactor system.

The external air supply was pumped into the reactors via (Ponddpro Airlab EV80) high-performance air pumps. Each reactor was wrapped with earth wool, a thermal insulation material of 100 mm thickness, 0.040 W/mK thermal conductivity, and 2.50 m^2K/W thermal resistance (Knauf Insulation, UK) for additional insulation (Figure 3.7).

Figure 3.7: Experimental set-up of the mesocosm test showing the external air supply and reactors wrapped with the insulation material.

A stainless steel woven wire mesh with a nominal aperture of 5.15 mm was installed inside the reactor 25 cm from the bottom, supported by 90 mm diameter plastic pipes as shown in (Figure 3.8). Leachate recovered at the bottom of the section was sampled through the opening provided by the collection tap.

Figure 3.8: Perforated tubing for air supply.

Before commencing the test, all constituents used were weighed separately. The weathered hydrocarbon-contaminated soil was manually mixed with the MGW thoroughly until a high

degree of homogeneity was sufficiently achieved. Following this, the amendments were added and mixed comprehensively to maximise the distribution of the hydrocarbons within the sample. The 26 kg of the homogenised mix was placed onto a steel-wired mesh that securely holds perforated tubes for aeration. The circular configuration and the transverse points of perforation on the tubes ensured a uniform distribution of air all through the sample as shown in Figure 3.8.

Temperature data loggers (iButton® DS1920) were used to monitor temperature during the process (Figure 3.9) were planted within the sample and set to record readings six-hourly. In order to introduce oxygen and encourage biodegradation, the sample was turned every three days during the first two months of the process and later turned once a week. Irrigation was implemented to simulate rainfall and to maintain the moisture content. This was carried out twice weekly.

Figure 3.9: iButtons used for temperature data logging

3.4.3 Rainfall Simulation

In seeking to understand the effectiveness of composting under high-intensity rainfall conditions and pollutant transfer due to leaching, a simulation of the process was conducted based on a volume adjusted rainfall intensity for all the reactors. Existing precipitation data for tropical climates with similar rainfall conditions (Figure3.10) was used to quantify the amount of water applied to the samples in the reactors.

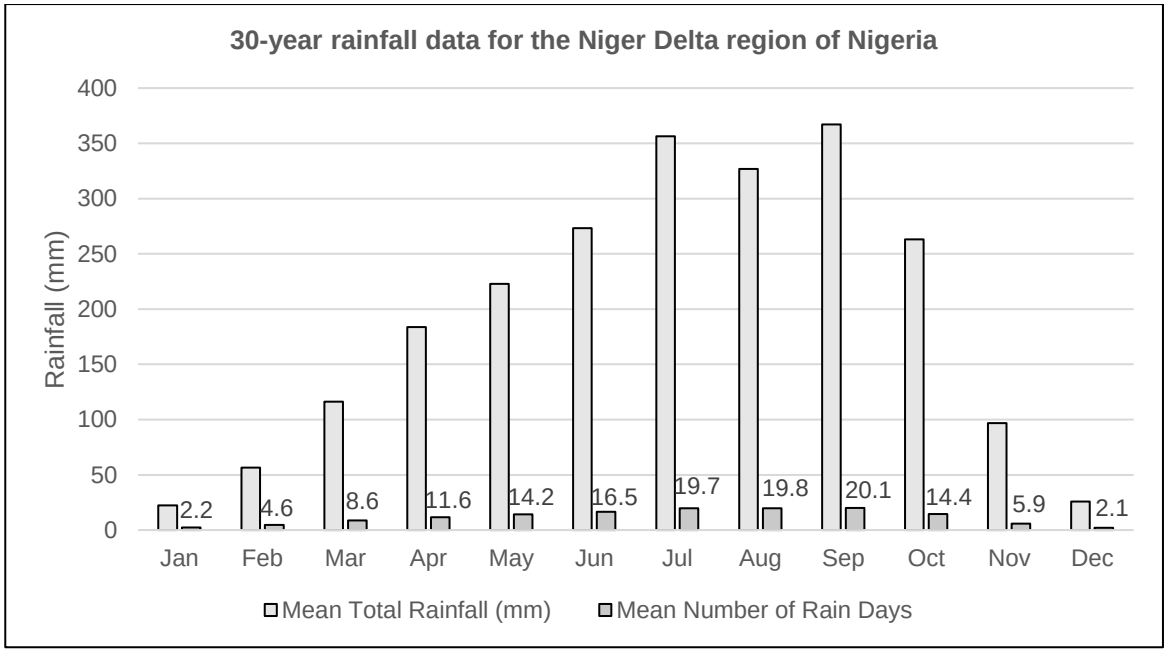


Figure 3.10: Graph illustrating 30-year rainfall data for the tropical climatic conditions experienced in the Niger Delta region of Nigeria that was used to represent rainfall on composting reactors used for this study (World Meteorological Organisation, 2014)**.**

Calculation

Area of reactor = 4375 cm^2

Rain frequency= $\frac{\textbf{Total number of days a year}}{\textbf{Total mean number of rainy days/year}} = \frac{365}{139.7} = \boldsymbol{2.6\ days}$ **(Equation 3.7)**

From Equation 3.7, it rains every 2.6 days. This was the interval used for water added to the compost reactors.

From the rainfall data in Figure 3.10, the average annual rainfall in the region per month is 192.575 mm. Equation 3.8 shows the average rainfall per day.

$$\frac{\textbf{Mean total annual rainfall per month}}{\textbf{Average number of days in a month}} = \frac{192.575}{30} = \boldsymbol{6.42\ mm/rainy\ day} \quad \textbf{(Equation 3.8)}$$

Converting 6.42mm into cm gives 0.642cm

$$\text{Volume of water added on compost area} = 0.642\ \text{cm x}\ 4375\ \text{cm}^2 = 2808.75\ cm^3 \quad \textbf{(Equation 3.9)}$$

The amount of water added to the compost reactor was measured using a graduated cylinder and manually transferred into a perforated container placed over the reactor. This was done to mimic similar conditions of rainfall. Each rain event took approximately 15 minutes with 2808.75 mL of water applied. This ensured excessive saturation of the sample

at the top of the reactor was avoided. This process was conducted at 2.6-day intervals over the experimental duration.

3.4.4 Microcosm Study

The optimal aeration required for laboratory-scale composting reactors have been well documented for this experimental phase, the external air supply implemented for this phase of the study was 0.5 L/min. In a similar experiment by Lu *et al*. (2001), results showed that the composting process using an aeration rate between 0.42 to 0.86 L/min performed significantly better compared to 1.72 to 3.44 L/min. In a pilot-scale in-vessel composting using forced aeration at a rate of 0.19 L/min – 0.38 L/min, Kim *et al*. (2008) found that the highest CO_2 and lowest O_2 concentrations corresponded to elevated temperatures, thus demonstrating that the air supply was sufficient enough to aerobically degrade the substrates. While composting food waste with leaves and garden soil through a perforated plate, other authors used a flow rate of 0.3 L/min during the process. This flow rate was found to provide sufficient air and aided to maintain appropriate temperatures (Yu & Huang, 2009). Evidence from these previous composting experiments generally suggests an aeration rate in the range of 0.5 L/min – 1.0 L/min. On this premise that 0.5 L/min was used as the rate of air supply for this phase of the study. According to Li *et al*. (2013), this type and rate of this aeration yielded better results in experiments compared to natural ventilation since the oxygen concentration within the compost reactors could be adequately controlled via the adjustment of the air supply rates.

3.4.5 Mesocosm Study

A significant reason for supplying air into a compost system is to deliver fresh oxygen to stimulate microbial activities. The average value of O_2:CO_2 ratio for bacterial and fungal degradation is 0.872 and 0.892 respectively as reported by Notton *et al*. (2008). These are expressed on a molar basis and given an overall average of 0.882. Therefore, to determine the volumetric flow rate of air per cubic metre of compost per second required to provide a stoichiometric supply of oxygen to the system, Equation 3.10 developed by Notton *et al*. (2008) was used.

$$\mathbf{V_{air}} = \frac{\boldsymbol{\rho_{compost}}\ \mathbf{X}\ (\mathbf{1}-\mathbf{MC})\ \mathbf{VS}\ \mathbf{X}\ \mathbf{F}\ \mathbf{X}\ \mathbf{M_{O_2}}}{\mathbf{1000}\ \mathbf{X}\ \mathbf{86400}\ \mathbf{X}\ \mathbf{M_{CO_2}}\ \mathbf{X}\ \mathbf{0.882}\ \mathbf{X}\ \mathbf{0.23}\ \mathbf{X}\ \boldsymbol{\rho_{air}}} \qquad \textbf{(Equation 3.10)}$$

Where:

$P_{compost}$ is the density of the compost in kgm^{-3}; MC is the moisture content on a wet basis; VS is the volatile solids content on a dry basis; F is the composting rate in $gCO_2\ kgVS^{-1}day^{-1}$; MO_2 is the molar mass of oxygen (32); 1000 converts of grams into kilograms; 86400 converts days into seconds; MCO_2 is the molar of CO_2 (44), 0.882 is average molar O_2:CO_2; 0.23 is the proportion of air that is oxygen by mass, and ρ_{air} is the density of air in kgm^{-3}

It is essential to note that Equation 3.10 gives a stoichiometric value for aeration and thus assumes that all of the oxygen in the supply air is used by the composting process. Considering an average moisture content of 40% on a wet basis and organic matter content of 50% on a wet basis at room temperature, Equation 3.11 is a function of compost density and the composting rate. Based on ranges of compost densities and composting rates, the air requirements were calculated and varied between 70 L/min to 77 L/min. As such, air pumps with an 85 L/min air capacity were deemed sufficient and adjusted to the required air supply needed to aerate the composted matrix.

3.5 Physico-Chemical Analysis

The growth and survival conditions necessary for microorganisms and the behaviour of contaminants (e.g. solubility and availability) are affected by physicochemical parameters (Loicke, 2008). The measurements of these characteristics were used to assess the progress of the composting process.

3.5.1 Moisture Content

This was determined according to procedures described in (BS EN 13040:2000). A suitable quantity of sample, i.e. 3-5 g was placed into a pre-weighed tin container using a four-figure balance (Sartorius basic), after which samples were placed in an oven (Gallenkamp Hotbox Oven) and allowed to dry for 24 hours at 103°C ± 2° C. Upon removal; samples were left to cool in the desiccator before being reweighed. The moisture content is expressed as a percentage by mass as shown in Equation 3.11.

$$\textbf{Dry mass}\ (\%) = \frac{\textbf{Dry sample mass}\ (\textbf{g})}{\textbf{Inital sample mass}\ (\textbf{g})} \times \mathbf{100} \qquad \textbf{(Equation 3.11)}$$

3.5.2 Organic Matter and Ash Content

This was carried out following the procedures described in (BS EN 13039:2011). Approximately 2 g of oven-dried sample was placed into a dried weighed crucible and then placed into a muffle furnace (Carbolite CMF 12/7) at 450°C for six hours. Afterwards, it was

transferred into a desiccator to cool at room temperature and then subsequently weighed. The ash content (ash %) was calculated using Equation 3.12.

$$\textbf{Ash}\ (\%) = \frac{\textbf{Sample weight after furnace (g)}}{\textbf{Oven dry weight (g)}} \times \mathbf{100} \qquad \textbf{(Equation 3.12)}$$

The total organic matter is material lost during ignition and is calculated as shown in Equation 3.13.

$$\textbf{Organic matter}\ (\%) = \mathbf{100} - \textbf{Ash}\ \% \qquad \textbf{(Equation 3.13)}$$

3.5.3 pH and Electrical Conductivity

The pH and Electrical Conductivity (EC) were determined on aliquots of 2 g sample in 10 mL distilled water (*w*:*v*) for the soils and 10 mL on the leachate samples. pH and EC tests were conducted in accordance with (BS EN 13037:2011) and (BS EN 13038:2011) respectively.

The pH is the measure of hydrogen ion (H^+) concentration in an aqueous solution as defined as the logarithm of the reciprocal hydrogen ion concentration as shown in Equation 3.14 (Patnaik, 2010). This logarithmic scale ranges from 1 – 6 for acidic levels, 7 for neutral, and 8 – 14 for alkaline levels.

$$\mathbf{pH} = -\mathbf{log_{10}}[\mathbf{H^+}] \qquad \textbf{(Equation 3.14)}$$

It was determined using a pH meter (Jenway 3305) which was calibrated using pH 4 and 7 buffer solution. The soil was mixed with distilled water before the electrode was immersed into the mixture and once the machine drift ceased, the pH was recorded.

Electrical Conductivity (EC) is a numerical expression of the ability of an aqueous solution to conduct electrical current. It depends on the presence of ions, their total concentration, mobility, and temperature; hence it indicates the salt and nutrient content of a mixture (Yateem *et al*., 1998). It is valuable for evaluating the effect of water loss, which results in a more concentrated solution, thus a higher conductivity. The conductivity meter (Mettler Toledo FE30) was calibrated with a Whatman conductivity standard solution of 1413 µS at 25 °C before the probe was inserted into the soil distilled water mixture. The recorded values were in units of µS/cm.

3.5.4 Chemical Oxygen Demand (COD)

The COD is a valuable measure of water quality and is often used to determine the concentration of organic pollutants in water. It works on the principle of the complete

oxidisation of organic matter in water to CO_2 with a potent oxidising agent under acidic conditions. In this study, the Palintest COD method was used. Aliquots of 2 mL used were diluted at an appropriate level before being transferred into the reagent tube (50 – 2000 mg/L) containing 84% sulphuric acid, mercury sulphate and chromium trioxide. The test tubes were inverted gently to mix the contents then placed into a preheated (150°C) COD Tubetests Heater (Palintest, UK) for two hours. Subsequently, the samples were allowed to cool at room temperature and then placed into an Automatic Wavelength Selection Photometer (Palintest, UK) to be read. Lastly, the photometer readings were multiplied by the dilution factor, and the results expressed as milligrams of oxygen consumed per litre of the sample (mg/L O_2). Importantly, the sample used was not filtered; thus, the total COD was obtained.

3.5.5 Nutrient Analysis

Ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), and chloride (Cl^-) concentrations for the soil and leachate were determined using the Palintest system. The intensity of the colour produced is proportional to the concentration nutrient being determined and was measured using the Palintest photometer.

For the soils: 2 g of the soil sample was thoroughly homogenised with 10 mL of distilled water in a test tube using a mechanical stirrer. The mixture was subsequently filtered through a glass fibre filter paper (Whatman GF/C) under vacuum. The aliquot was then analysed for nutrients.

For leachate: 10 mL of the leachate sample was collected. Once filtered through a glass fibre filter paper (Whatman GF/C) under vacuum, it was analysed for nutrients.

3.5.5.1 Ammonium

The test was based on an indophenol method. Here ammonia reacts with alkaline salicylate in the presence of chlorine to form a blue-green indophenol complex (Palintest Limited, 2016).

3.5.5.2 Nitrate

In this method, nitrate is first reduced to nitrite, and the resulting nitrite is then determined by a diazonium reaction. The nitrite from the reduction is determined by a reaction between the sulphanilic acid in the presence of N-(1-naphthyl)-ethylene diamine to form a reddish dye (Palintest Limited, 2016).

3.5.5.3 Phosphate

The test was based on the vanadomolybdate method. In this test, phosphates react with ammonium molybdate in the presence of ammonium vanadate to form a yellow phosphovanadomolybdate (Palintest Limited, 2016).

3.5.5.4 Chloride

This test was based on an iodine release method. Chlorine reacts with potassium iodide in an acid solution to release iodine which is brown (Palintest Limited, 2016).

3.5.6 Metals

Sites polluted with organic compounds often contain metals, such as arsenic, mercury, lead and zinc (Roane & Kellogg, 1996). Approximately 50% of sites have such inorganic pollutants, which may persist in the soil after biodegradation of organic contaminants or which may hinder the activities of native organisms or introduced organic compound degraders (Diels *et al*., 1991). Heavy metals in composts are a cause for adverse effects on animal and human health, transmitted through the food chain from the soil, groundwater, and plants (Hseu, 2004).

3.5.6.1 Metals in Soils and Leachates

During the microcosm and mesocosm studies, the total concentrations of the elements were determined by digesting oven-dried samples using a combination of concentrated acids as described in (BS EN13652: 2001). A stock solution of aqua-regia was used for this digestion and was prepared by mixing three parts concentrated hydrochloric acid (37% (w/w) HCl) and one part concentrated nitric acid (70% (w/w) HNO_3). A representative mass (1 ± 0.001 g) of the dried ground test sample was weighed and placed into an acid-washed beaker before adding 10 mL of the stock solution. This was transferred onto a hotplate at 85°C for two hours with water intermittently added to prevent the sample from drying out. Once a suitable dissolution of solids was achieved, the mixture was passed through sterile and endotoxin-free 0.2 µm puradisc filter media (Whatman). The resultant 25 mL solution was acidified with 250 µL HCl, and a blank solution with 25 mL of acidified distilled water was prepared and stored before further analysis was carried out. It should be noted that aqua-regia provides information on total elements rather than bioavailable.

The leachate samples were separated using 0.47 µm Whatman cellulose nitrate filters and stored in 25 mL vials. No pre-digestion was required, and samples were preserved with 250 µL of HCl.

3.5.6.2 Inductive Coupled Plasma-Mass Spectrometer (ICP-MS) Analysis

This method combines an ICP with a quadrupole mass spectrometer. High energy ICP generates singly charged ions from the atoms of the elements present in the sample. These ions are directed to the mass spectrometer, separated, and measured according to their mass-to-charge ratio (Patnaik, 2010).

An Agilent 7500ce ICP-MS with Enhanced Octopole Reaction System (ORS) using argon gas with a flow rate of 0.8 – 1.3 L/s was used for this study. The helium collision mode was used with an inert gas to minimise interference (Agilent Technologies, 2004). The method of analysis was semi-quantitative with parameters being measured each time. The instrumental parameters applied to attain optimal plasma conditions used are as follows:

RF Power: 1500 W; Sample depth: 8 mm; Carrier gas: 0.85 L/min; Makeup gas: 0.2 L/min; Spray chamber temperature: 2°C; Extract 1: 0 V; Extract 2: -160 V; Omega bias: -24 V; Omega lens: -0.6 V; Cell entrance: -30 V; QP focus: -11V; Cell exit: -44V; Octopole bias: -18 V; QP bias: -14.5 V; Cell gas flow: 4.5 mL/min helium.

Before analysis, calibration was performed using a tuning solution of 10 ppb of lithium, yttrium, cerium, titanium, cobalt, and 0.5% HCl. Standards were also prepared by diluting a custom-made multi-element solution (all elements at 100 mg/L) (Qmx Laboratory Ltd). The concentration ranges for the standard was 0.1, 0.5, 1, 2, 10 mg/L and values exceeding the range on the ICP further diluted. For quality control, a 10 mg/L solution was prepared using a different multi-metal mix. All the samples were calibrated against these standards. The entire analytical process was conducted in the ISO 9001:2008 accredited laboratory of the Environmental Chemistry Analysis Laboratory (ECAL) of the University of Portsmouth.

3.6 Hydrocarbon Analysis

The two principal categories of pollutants monitored and analysed in this study are the Total Petroleum Hydrocarbons (TPHs) and Polycyclic Aromatic Hydrocarbons (PAHs). The TPHs describe a broad array of several hundred chemical compounds that originally stem from crude oil (Adesodun & Mbagwu, 2008). The weathered hydrocarbon that has contaminated the soil under study contains these compounds. Thus, USDHHS (1999) defined TPH as the measurable amount of petroleum-based hydrocarbons in environmental media. Many

regulatory agencies often use TPH as an indicator as it provides a “one number” value of TPH in any environmental matrix being monitored. It is important to note that this value does not provide information on the composition (i.e. the individual constituents of the hydrocarbon mixture). The removal of 18 PAHs classified as priority pollutants by the U.S Environmental Protection Agency (US EPA) namely Naphthalene, 2-methyl Naphthalene, 1-methyl Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benz[b]fluoranthene, Benz[k]fluoranthene, Benzo[a]pyrene, Ideno[1,2,3,-c,d]pyrene, Benzo[g,h,I]perylene, was investigated. However, it should be noted that Acenaphthylene and Dibenz[a,ah]anthracene concentrations were below the detection limit through the course of the experiment in the samples tested; thus, removing the remaining 16 listed were considered. As well as analysing individual PAHs, total PAHs are reported as the sum of these compounds. For a general analysis of changes in contamination concentrations, PAHs have been classified into three groups according to their respective molecular weights:

- Low Molecular weight (LMW) PAHs, which are 2- and 3- ring PAHs

- Medium molecular weight (MMW) PAHs, which are 4-ring PAHs

- High molecular weight (HMW) PAHs, which are 5- and 6- ring PAHs

To better understand their degradative behaviours, the TPHs, were further divided into Diesel Range Organics (DRO: C_8 – C_{22}) and Engine Range Organics (ERO: C_{22} – C_{40}) (Complete Laboratory Solutions, 2011). According to Foxhoven (2009), in the 1990s, this method was created as a vast number of analytical results generated by several laboratories needed to be standardised for better understanding hence its application in this study.

Due to the heterogeneity of contamination within the soil matrix despite best efforts to ensure the soil was thoroughly mixed, an entirely homogenous distribution could not be guaranteed, especially while handling large quantities. Hence, to account for the initial differences in concentrations across the treatments, TPH and PAH concentrations were compared by referring to the changes in the percentage remaining in the reactor relative to the start as shown in Equation 3.12.

$$\textbf{Percentage remaining}\ (\%) = (\mathbf{Conc_t/Conc_0})\ \mathbf{X\ 100} \qquad \textbf{(Equation 3.15)}$$

Where $Conc_t$ is the concentration of hydrocarbon at sampling time, t and $Conc_0$ is the concentration of the hydrocarbon at the start of the experiment.

The biodegradation rate was evaluated using the decay curves estimated by the first-order kinetic model (Equation 3.13) as described by Yeung *et al.* (1997).

$$\mathbf{y} = \mathbf{A}e^{-\mathbf{kt}} \qquad \textbf{(Equation 3.16)}$$

Where *y* is the residual hydrocarbon content in the sample (mg/kg), *A* is the initial hydrocarbon content in the sample (mg/kg), *k* is the biodegradation rate constant per day (d^{-1}), and *t* is time (days).

The organic matter is often decomposed during the composting process, resulting in weight losses due to CO_2 and other gaseous emissions. Taking into account, the possible weight loss in the soil/manure/chopped straw mix may cause a distortion of the result when not considered during analysis (i.e. when expressing amounts on per weight basis). The data measured from the extracted hydrocarbons were corrected by referring to the ash content, thus recording the results in ng per g of ash (ng/g). Using the ash content is preferred as it is considered the most chemically stable parameter during the degradation process (Amir *et al*., 2005).

While several studies have used a variety of methods to assess hydrocarbon reduction within different contaminated matrixes, this research investigates these using three main approaches where applicable, namely:

- "**Percentage change**": Quantities of hydrocarbons measured relative to initial concentration. This compensates for initial variations in concentration.
- "**Rates of degradation**": Using a first-order rate of decay model and half-life to estimate treatment efficiency.
- "**Concentration**": Quantities of hydrocarbons per kg dry weight to determine the actual concentrations and the effect of concentrations on hydrocarbon removal.

3.6.1 Leachate Extraction

To carry out the extraction for the TPHs hexane was the solvent used as prescribed in EPA method 1164 revision A (1999) while for the PAHs dichloromethane (DCM) was the solvent used as specified in EPA method 550.1 (1990) (using application note 54 from Supelco (Sigma Aldrich) for C18 discs). For the extraction process, the following steps were taken:

- Cleaning of all glass constituents of the apparatus with acetone;

- Placement of C18 empore disc on the filtration apparatus;

- Disc washing phase: 10 mL of hexane (TPH extraction) or DCM (PAH extraction) was added with vacuum pump turned on for two until the disc was dry;

- Disc conditioning phase: 10 mL of methanol was added, and then a small amount was vacuumed through with the remainder allowed to soak the disc for one minute. The remaining methanol was drawn through under vacuum while leaving just enough liquid to cover the disc surface to prevent complete drying;

- 30 mL of distilled water was added under vacuum, and most was sucked through the filter leaving enough water to cover the disc;

- Sample filtration phase: 100 mL of sample was added with the vacuum on and once the sample was sucked through; the disc was allowed to dry under vacuum but for no more than five minutes. Once completed, collection vials were inserted into the solid-phase water extraction unit;

- Extraction phase: 10 mL of hexane (TPH extraction) or DCM (PAH extraction) was added to the sample flask to rinse out. Then this was added to the filter assembly to rinse down the sides. The vacuum was turned on briefly to allow a few drops through. After two minutes, the vacuum was turned on to draw the remaining solvent through (into the collection vial);

- The previous step was repeated again; and

The collection vial was removed on completion, wrapped in aluminium foil and stored in cool dark conditions until the next stage of the process.

The extract was then passed through a 1 g anhydrous sodium sulphate (Na_2SO_4) cartridge (Bond Elut) to eradicate any residual water that may be present. Nonane (50 µL) was added to prevent losses of TPHs and PAHs, and the samples (PAH extraction) were concentrated down to 1 mL at 40°C in a stream of nitrogen gas (N_2) before injection into the GC-MS.

3.6.2 Soil Extraction

The extraction of the TPHs and PAHs in the soils was undertaken using an Accelerated Solvent Extractor (ASE 200 Dionex®). The ASE is preferred to other extractions techniques such as sonication and soxhlet. The US EPA recommends this extraction method as it is rapid, efficient, and requires considerably fewer volumes of solvents (Wick *et al*., 2011). A known weight (1 – 1.5 g) of soil samples were mixed with an equal volume of diatomaceous earth (DE) Hydromatrix (Varian, UK), which serves as a drying agent to eradicate any moisture in the sample. Following this, samples were loaded into a steel extraction cell

(Dionex, UK) interposed between acid-washed sand (VWR, UK) with a disposable cellulose filter (Dionex UK) at the bottom, middle and top of the loaded cells (Figure 3.11).

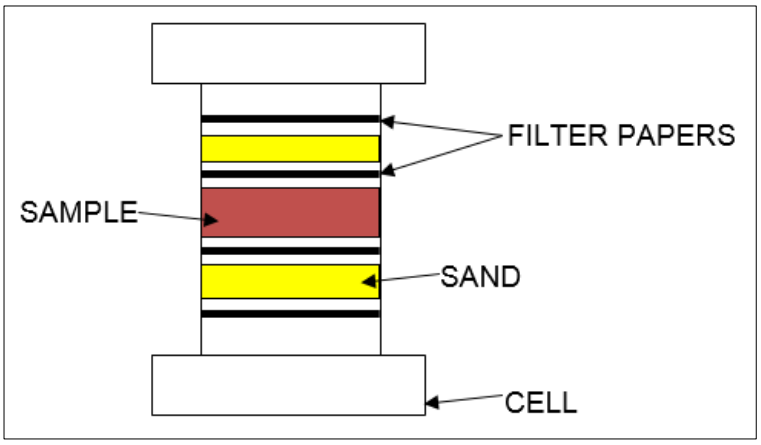


Figure 3.11: ASE extraction cell configuration.

Once the preparations were complete, the loaded cells were transferred on to the auto-sampler carousel of the ASE (Figure 3.12). Table 3.5 provides the ASE operating conditions used for sample extraction.

Figure 3.12: Accelerated Solvent Extractor (ASE 200) apparatus.

Table 3.5: ASE operating conditions.

Conditions	TPH	PAH
Solvent	1:1 (Acetone:Hexane) (*v/v*)	1:1 (Acetone:DCM) (*v/v*)
Temperature	200°C	100°C
Pressure	10.342 MPa	10.342 MPa
Heat-up time	9 minutes	5 minutes
Static time	5 minutes	5 minutes
Flush volume	60 %	60 %
Purge time	60 seconds	60 seconds
Cycles	1	1
Dionex methods	Application note 324 (Dionex 2011a)	Application note 313 (Dionex 2011b)

The extracted solvent was initially made up to a known quantity with hexane before going through a solid phase extraction (SPE) process. The samples (TPHs and PAHs) were passed through a Bond Elut anhydrous $NaSO_4$ 1 g cartridge (Agilent Technology, UK) to remove any residual water before 50 µL of nonane was added to prevent any TPH and PAH losses.

The clean-up stages for both TPHs and PAHs were based on US EPA Methods 3611B revision 2 1996 and 36030C revision 3 1996 respectively. A 2 mL aliquot of the TPH was dispensed into a silica column (LRC Si 500 g Bond Elut) conditioned previously with 3 mL of hexane. Hexane (4 mL) and dichloromethane (4 mL) was used to elute the sample in the silica column with the resultant 8 mL of the sample containing aliphatics, and aromatics constituents were collected in a vial and subsequently made up to 10 mL. From this eluent, 1mL was transferred into a GC-MS vial for analysis.

For the PAHs, the samples were transferred onto a heating block (Techni Dri-Block DB.3 Sample Concentrator) and blown down to 1 mL with nitrogen (N_2) gas. The concentrated 1

mL aliquot was transferred into a silica column (LRC Si 500 g Bond Elut) conditioned previously with 3 mL of hexane. The sample was then eluted with 4 mL of (3:2 v/v) of hexane:dichloromethane. The eluent was also blown down to 1 mL with N_2. Subsequently, the concentrated 1 mL sample containing the PAHs was transferred into a GC-MS vial for analysis.

3.6.3 GC-MS Analysis

For the identification and analysis of contaminants within the samples, a Varian 430/210 GC-MS was used for TPHs, and an Agilent 6890/5973 GC-MS was used for the PAHs. The operational conditions applied to the analysis are detailed below:

Ion trap conditions: Trap: 220ºC; Manifold: 80ºC; Transfer-line: 300ºC; Split/split-less ratio: 1:50; Injection volume of sample: 1 µL.

Column conditions (TPHs): Injector temperature: 280ºC; Initial temperature at 50^0C for 1.5 minutes and increased at a rate of 15^0C per minute until it reaches 300^0C where it is held for 10 minutes.

Column conditions (PAHs): Injector temperature at 250°C; Initial temperature of 60°C held for 1 minute then increased to 150°C at a rate of 30°C per minute; and then increased to 186°C at a rate of 6°C per minute, and finally ramped up to 280°C at a rate of 4°C per minute and held for 20 minutes.

To obtain the calibration curves for the numerical evaluation of the TPHs, diesel fuel at concentrations between 100 – 1500 µg/mL was used. The retention time for the DRO used from the GC-MS output traces was between 5.5 to 17 minutes. A baseline integration of the area under the curve (Figure 3.13) was computed between the retention times to cover the DRO carbon range (C_8 to C_{22}).

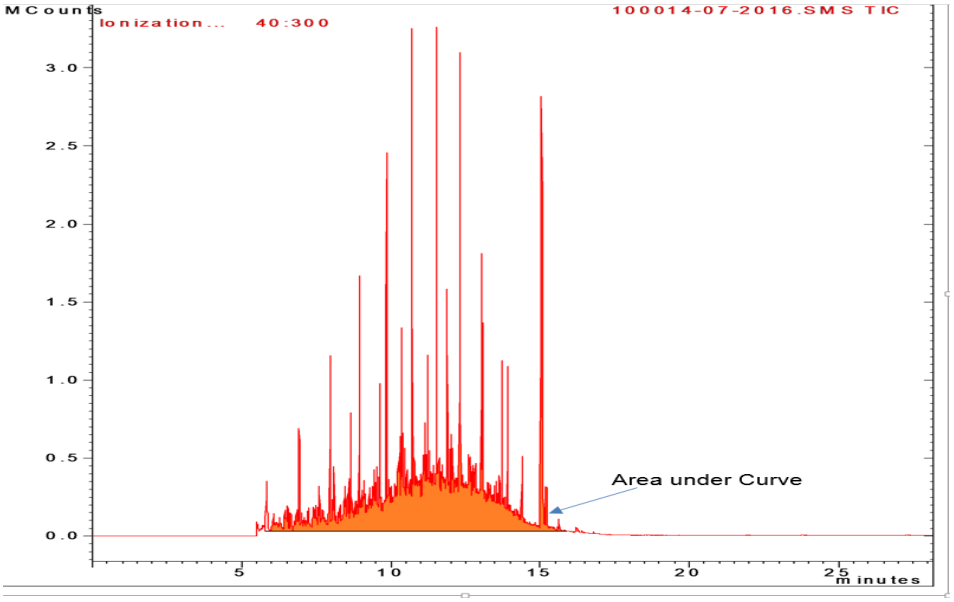


Figure 3.13: GC-MS chromatogram showing area under the curve for DRO.

A linear regression equation was obtained by plotting the area under the curve against diesel calibration standard concentrations in (µg/mL) as shown in Figure 3.14. This, in turn, was used to convert the area under the curve into µg/mL of TPH. The resultant value was converted to obtain the concentration per dry weight of the soil sample:

Dry weight of sample extracted in ASE cell = (wet weight * % dry mass)/100

TPH (µg/mL)/ Dry weight of sample extracted = mg/g

mg/g *1000 = µg/g

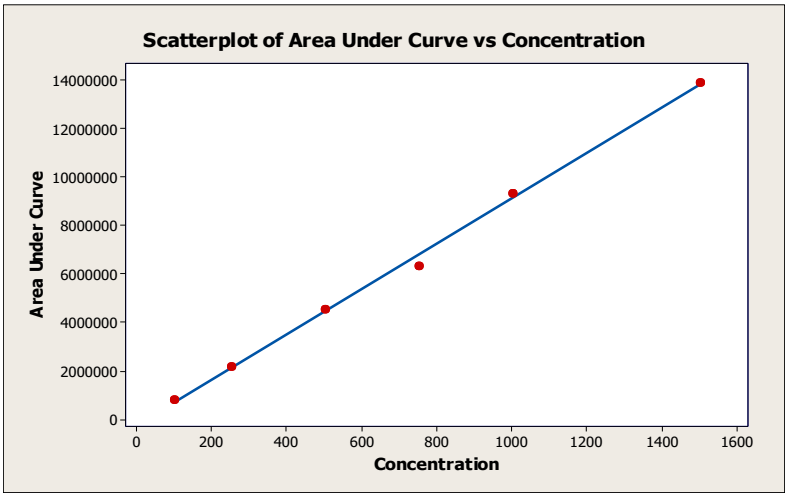


Figure 3.14: Plot of linear regression (Y= C + MX; Area = -235399 + 9367 * Conc)

The PAH peaks were integrated and calibrated using PAH 14 mix (Qmx Laboratories, UK). This contained the 16 US EPA priority PAHs and the additional methylnaphthalene compounds identified. The PAH peaks were confirmed using their identifier ion numbers and retention times (Table 3.6).

Table 3.6: PAHs retention times and ion numbers.

NO	PAHs	Ion Number	Retention time (min)
1	Napthalene	128	5.27
2	2-methylnapthalene	142	6.19
3	1-methylnapthalene	141	6.34
4	Acenapthylene	152	7.84
5	Acenapthene	153	8.19
6	Fluorene	165	9.53
7	Phenanthrene	178	12.56
8	Anthracene	178	12.76
9	Fluoranthene	202	17.67
10	Pyrene	202	18.73
11	Benzo[a]anthrancene	228	25.18
12	Chrysene	228	25.37
13	Benzo[b] flouranthene	252	30.89
14	Benzo[k]flouranthene	252	31.03
15	Benzo[a]pyrene	252	32.49
16	Ideno(1,2,3-c,d)pyrene	276	38.56

17	Dibenzo[a,h]anthracene	278	38.82
18	Benzo[g,h,i] pyrelene	276	40.33

The standards for the PAHs were made up at concentrations between 100 – 1500 ng/mL. Each PAH peak (Figure 3.15) which represents an individual compound, was calculated by baseline integration with a calibration curve derived by linear regression using data analysis station MSD Chemstation D.02.00.275. For the soil samples, a conversion was done to have results per gram of dry weight (ng/g). However, in some instances, the PAH concentrations were evaluated and analysed as total PAHs rather than individual ones.

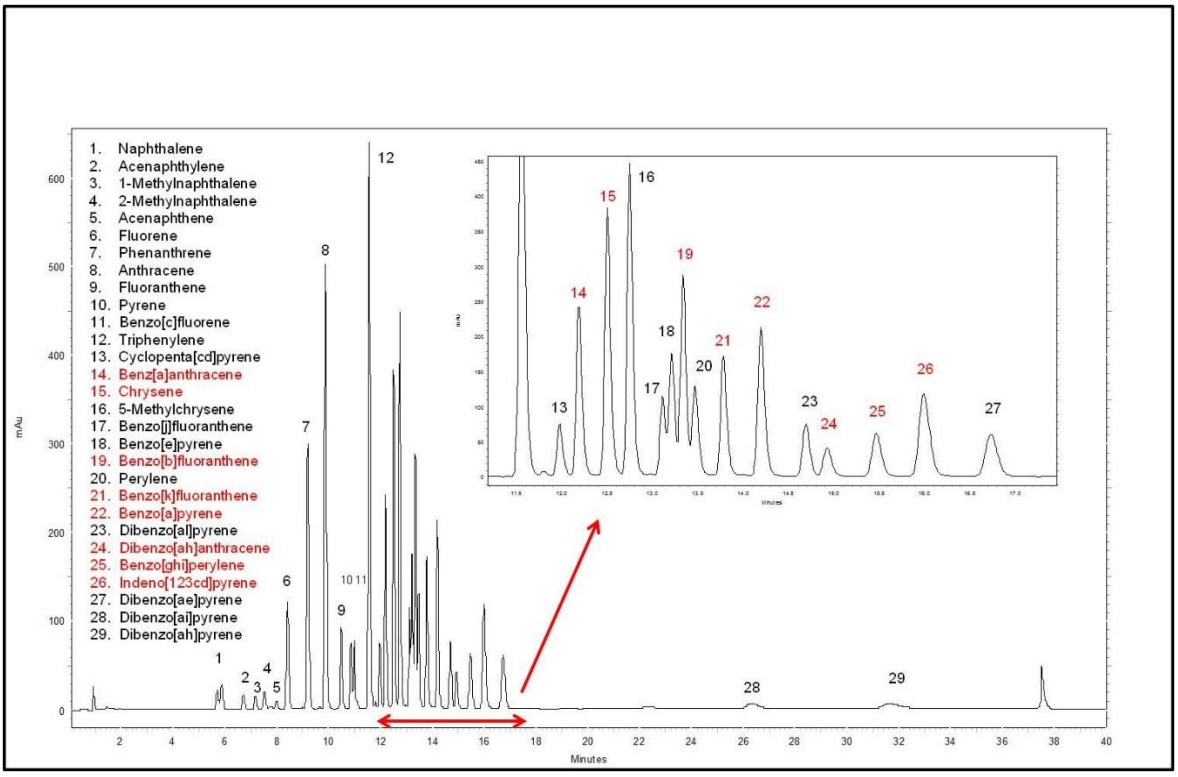


Figure 3.15: GC-MS Chromatogram showing peaks representing individual PAHs.

3.7 Microbial Analysis

3.7.1 Plate Counts

This technique is primarily used on the premise that each viable organism will produce a colony when grown on an agar plate (Michael, 2004). The hydrocarbon tolerant bacteria (HTB) and hydrocarbon tolerant fungi (HTF) were determined and human pathogens

(indicator species), i.e. *Salmonella* spp and *Escherichia coli* were also tested to conform to compost quality requirements described in PAS100:2002 specification for composted materials (BSI, 2002). The soil sample (1 g) was suspended in 9 mL of phosphate buffer saline (PBS) (Fisher Scientific, UK) solution and tenfold serial dilutions of the soil samples from 10^{-1} to 10^{-5}. Aliquots of 0.1 mL were pipetted onto a petri dish containing solidified agar. A sterilised spreader was used to evenly distribute the sample on the agar. Once complete, the Petri dishes were placed in the incubator at the required temperatures for specific time durations (Table 3.7). The number of viable microorganisms in the sample was calculated from colonies formed (Figure 3.16) and the volume of inoculum and the dilution factor expressed in colony-forming units (CFU/g).

Table 3.7: Incubation times, temperatures and microorganisms cultured.

Microorganism	Incubation temperature (°C)	Incubation time (days)	Colony Description
HTB	28°C	7	Round; bright orange, fuchsia, translucent
HTF	28°C	3	Convex, fuzzy green, filamentous, white opalescent (yeasts)
E.coli and Salmonella	44°C	1	blue/ colourless, mucoid

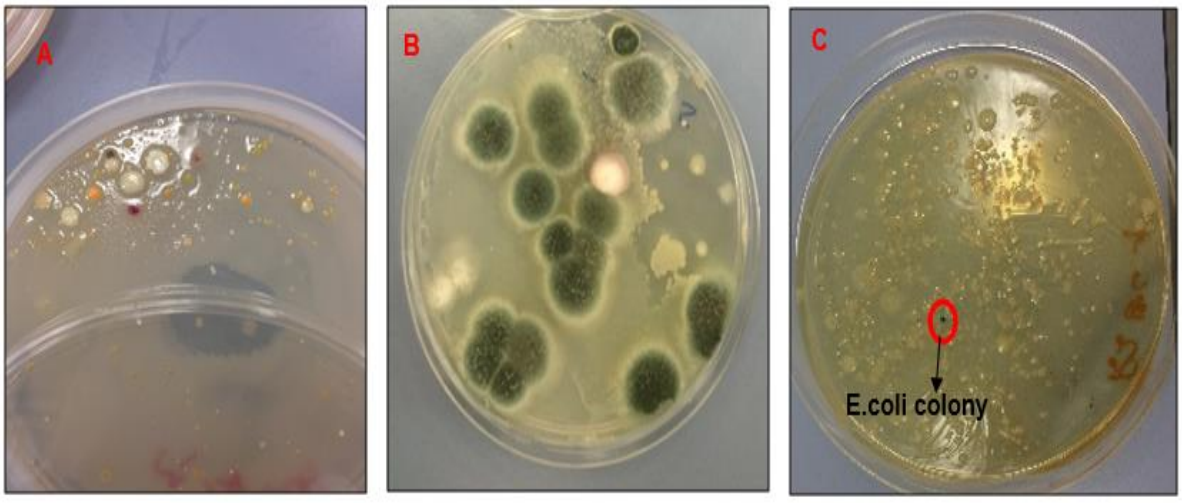


Figure 3.16: A) Bacteria colony B) Fungi colony C) Salmonella colonies with a single E.coli colony.

3.7.1.1 Hydrocarbon Tolerant Bacteria (HTB)

This was determined using the method prescribed by (Kirk, *et al.*, 2005). Samples were plated on Bushnell Haas broth (Sigma Aldrich, UK) containing 1 g monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), 1 g ammonium nitrate (NH_4NO_3), 0.2 g magnesium sulphate ($MgSO_4.7H_2O$), 0.05 g ferric chloride ($FeCl_3$), 0.02 g calcium chloride ($CaCl_2.H_2O$). This broth (3.27 g) was mixed with 15 g of bacteriological agar (Acros Organics, UK) and suspended in 1000 mL of distilled water before being autoclaved at 121°C for 15 minutes at 0.103 MPa. Once removed and transferred to cool in the water bath at 50°C, 10 mL of diesel was filtered through 0.45 µm Whatman filters into the prepared agar broth. The mix was finally amended with 1 mL of nystatin (Fisher Scientific, UK) to inhibit fungal growth before being poured into the sterile Petri dishes and allowed to solidify.

3.7.1.2 Hydrocarbon Tolerant Fungi (HTF)

This was carried out according to methods used by (Ekhaise & Nkwelle, 2011). Potato dextrose agar (Oxoid, UK) containing 4 g potato starch extract, 20 g dextrose, 15 g agar. The agar was prepared by suspending 39 g in 1000 mL of distilled water. At this point, the mixture was put through the same process as can be seen in Section 3.7.1.1 with the key variation being the addition of 1 mL of chloramphenicol (Acros Organics, UK) to suppress bacterial growth before being poured into sterile Petri dishes and allowed to solidify. Nystatin was not added to the agar broth.

3.7.1.3 E.coli and Salmonella

The enumeration of these microorganisms was carried out on specialised agar HiCrome ™ E.coli Agar B (Sigma Aldrich, UK). It contains 20 g casein enzymic hydrolysate, 1.5 g bile salts mixture, 0.075 g x-glucuronide, 15 g agar. To prepare this agar, 36.6 g was suspended in 1000 mL of distilled water, sterilised by autoclaving at 121°C for 15 minutes at 0.103 MPa. It was allowed to cool at 50°C before being transferred into sterile Petri dishes.

3.8 Statistical Analysis

All data collected was systematically collated using Microsoft Excel before subsequent statistical analysis that was performed using Minitab Software version 17.3.1. To gain full understanding and appreciation for the data, a normality test was run. Here the Anderson-Darling normal probability plots were used to assess if the data were normally distributed or not. In cases where data failed the normality test, a logarithmic transformation was adopted in a bid to improve the data distribution. For data that was found to not be normal

even after this conversion, non-parametric tests were used for their analysis. In light of the aforementioned, a statistical hypothesis test for significant differences between two groups otherwise known, as a *t*-test was not used, as the test is more effective between normally distributed groups by supporting a null hypothesis. Therefore, to identify the significant differences between variables of the treatments, i.e. at the start, during and at the end of the trials, the Kruskal-Wallis test was performed. It offers a non-parametric alternative to the one-way analysis of variance (ANOVA). Although, in particular cases, the data was tested using one-way ANOVA which also provided further insight into differences in treatments. The association between variables within each treatment was tested using Spearman's correlation. This test evaluates the monotonic relationship between two continuous or ordinal variables. In this monotonic relationships, variables tend to change together but not necessarily at a constant rate (Minitab Inc, 2016).

Tukey-Kramer was used to check significant differences. The method compares possible pairs of level means for the specified factors. It produces intervals for the mean differences which have the simultaneous confidence level specified. The multiple comparison method used to construct the family of confidence intervals from which the grouping table is generated and each grouping information table compares levels of one factor, or combine levels of multiple factors (Minitab Inc, 2016).

Fisher's LSD method was also used to check for significant differences. This method compares possible pairs of level means, or compare each mean to the mean of a control group. It uses the individual confidence level you specify (Minitab Inc, 2016).

A regression analysis was also carried out to investigate and model the relationship between the nominal variable (response) and one or more measurement variables (predictors). This analysis was used to suggest which independent variables significantly affect the dependent variable (hydrocarbon removal); the best regression model to describe the relationship between them was selected.

CHAPTER 4

4.0 RESULTS AND DISCUSSION (MICROCOSM STUDY)

This chapter presents the results and discusses the first phase of this project (microcosm study). It primarily consisted of 2.5 L reactors used for a laboratory-based bench-scale study of a modified dynamic respiration index test as a screening protocol to establish the efficiency of animal wastes and fungi inoculated materials used (singly and in combination) during the degradation of TPHs and PAHs. According to Atlas (1995), laboratory experiments must be conducted to assess the improvement of pollutant degradation under controlled conditions; these experiments establish the credibility of the particular bioremediation process. In these respirometric trials, the CO_2 evolution was monitored and measured to indicate biological activities within the composting reactors. The physicochemical parameters, metal concentration as well as an ecotoxicity bioassay using the germination index of *Lepidium sativum* (cress seed) at day 0 (before treatment) and day 24 (after treatment) was assessed. From the results obtained, the three most effective treatment combinations were recommended and, thus, transferred to a pilot-scale study using 100 L reactors for further investigation; the results were used to establish the suitability and field-scale applicability of the treatment method.

4.1 Dynamic Respiration Index and Monitoring CO_2 Evolution

Composting is an aerobic process during which microorganisms convert putrescible organic matter into CO_2, H_2O and a host of metastable compounds. Usually, the final compost produced is expected to be a stable, sanitised and humus-like material (Barrena Gómez *et al*., 2006). A compost is described to have reached maturity based on its fitness for the desired end-use, while its stability is the extent to which readily biodegradable matter within it has decomposed (Brewer & Sullivan, 2003). The maturity of compost is often associated with its plant-growth potential and the levels of phytotoxic compounds such as NH_3 or short-chain organics present within it (Brewer & Sullivan, 2003; Iannotti *et al*., 1993). On the other hand, compost is regarded as unstable if it contains a high proportion of biodegradable matter that may sustain microbial activities. Stability is an important parameter as it can be used to assess and evaluate the efficiency of various composting systems (Lasaridi & Stentiford, 1998). On that premise, respiration can be considered a general measure to evaluate microbial activity. Thus, for this phase of the project, respirometry in the form of the CO_2 respiration rates for the different treatments has been used to monitor microbial activity and establish the degree of stability of the composted sample.

4.1.1 Respiration Index

A series of modified dynamic respiration index trials (DR-4) were conducted to assess the impact of different amendments on the composting rate within a 24-day experimental period. The outcome of these tests are summarised in Figures 4.1 and 4.2 and it can be observed that the addition of the animal wastes stimulated the rate of aerobic degradation activities. As temperature measurements proved challenging to collect, there was no substantial evidence to determine the occurrence of composting, but it has increased the rate of aerobic activities in the systems as evidenced by the off-gas.

Therefore, the hypothesis is that the rate of composting in these reactors can be manipulated by amendment with different animal wastes and substrates. While monitoring these amendments, it was found that the addition of specific animal wastes and fungi-inoculated substrates gave significant increases in the intensity of the activity measured by CO_2 evolution. These results provide valuable information on whether the rates of aerobic activities are indicative of pollutant removal.

The RIs for all the treatments showed a similar behaviour within the temperature-controlled incubator the tests were performed. An initial peak in activity was detected by day 10 for all the treatments after which, a gradual and continuous decline was observed. This incident may be attributed to the presence of easily biodegradable amendments. However, this was not the case with the control treatments, which all exhibited, as expected, low RIs throughout the testing process. It is assumed that the increase in CO_2 release rate in all the treatment reactors is proportional to the activities of microorganisms during the process. This rate was significantly different amongst all treatments (Kruskal-Wallis H (12) = 154.69, p = < 0.001). Further analysis indicated the mean ranks for both treatment 3 (M = 318.6; Z = 6.22) and treatment 4 (M = 294.6; Z = 5.01) differed significantly from the overall mean rank of 195.5 for all other treatments and also, these treatments also possessed the highest absolute Z-value in comparison to the remaining treatments.

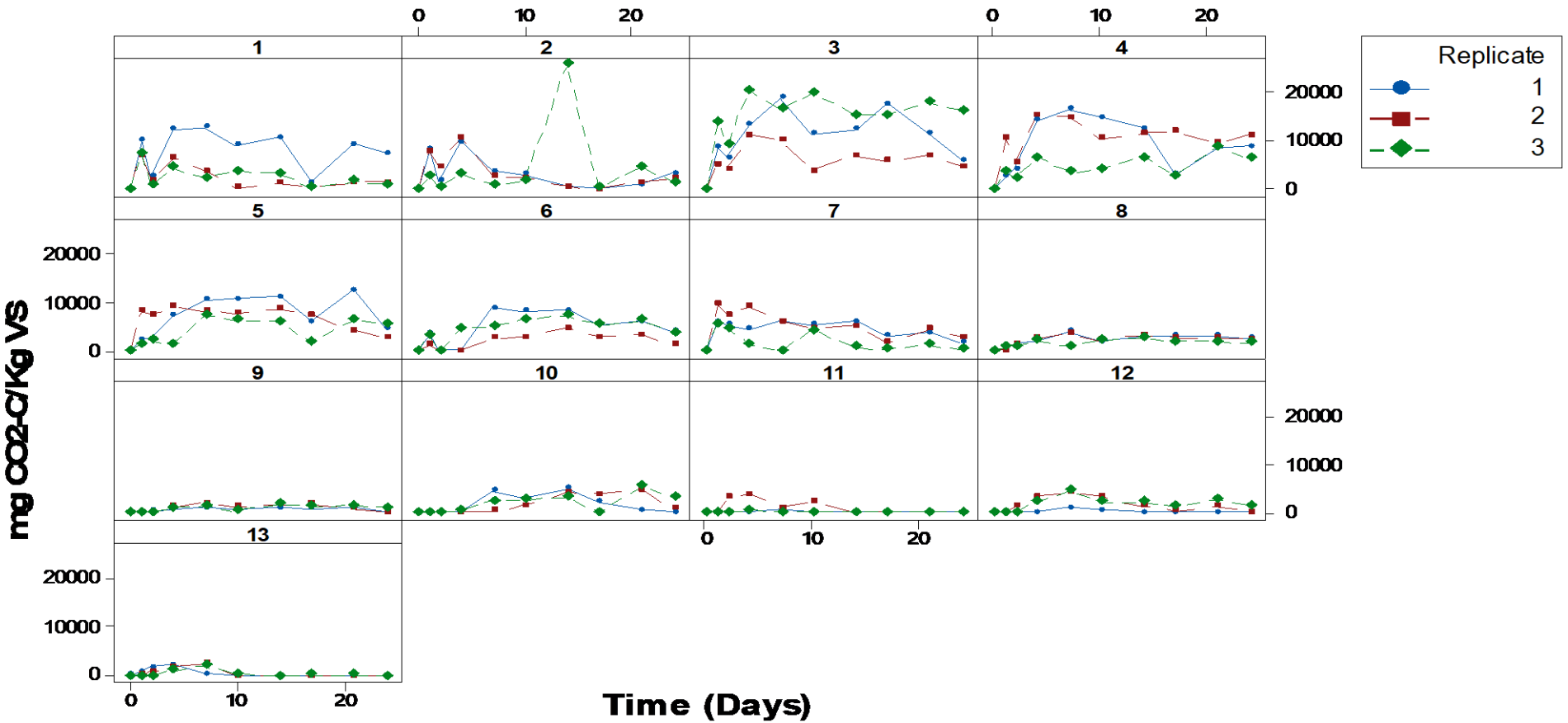
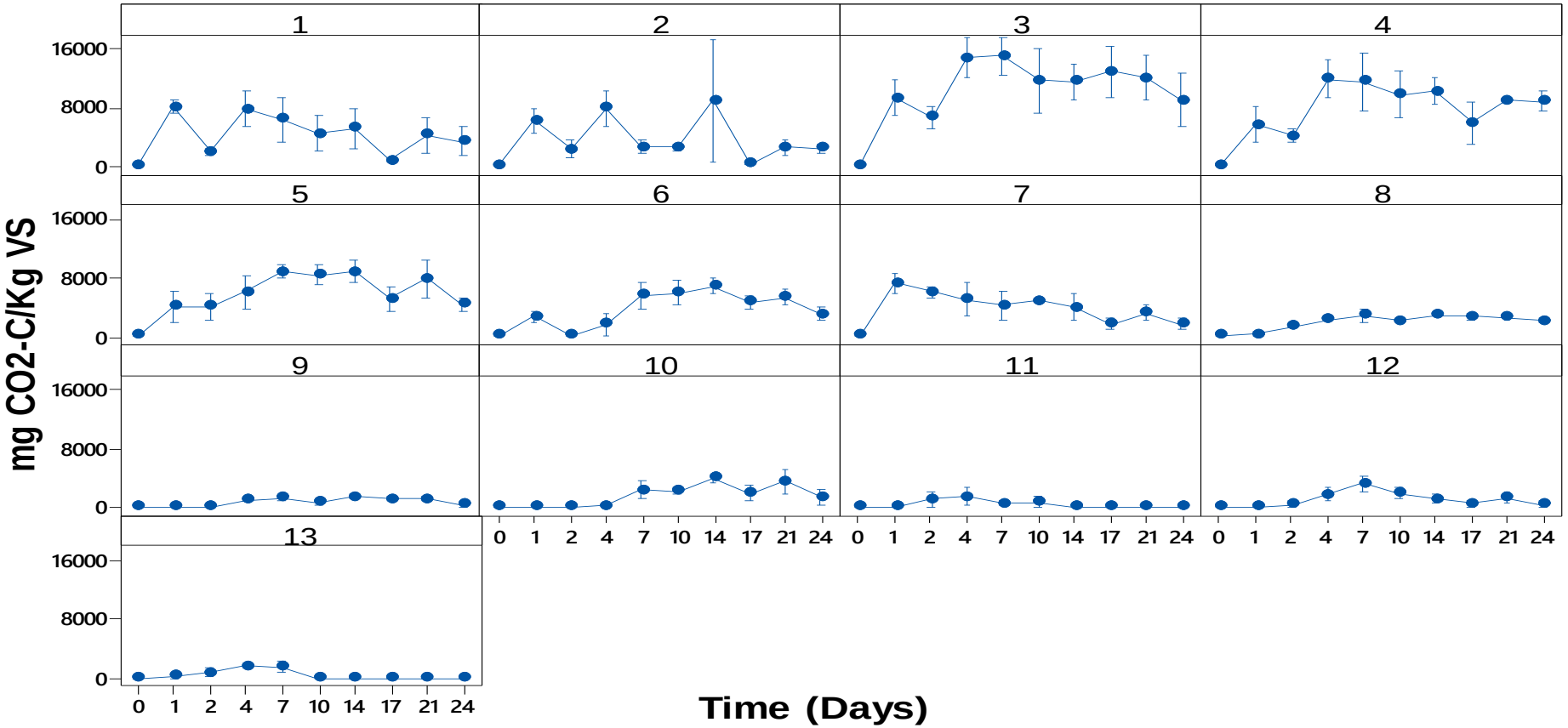


Figure 4.1: Respiration Index at 50 °C for all treatments and controls during the 24-day experimental period. The influence of added waste can be seen to stimulate respiration in the treatments, thus showing the rates of aerobic biodegradation



1: SOIL + MGW; 3: SOIL + MGW + CHI; 4: SOIL + MGW + COW; 5: SOIL + MGW + CHI + COW; 6: SOIL + MGW + FUN
7:SOIL + MGW + COW + CHI + FUN; 8: SOIL + MGW + COW + FUN; 9:SOIL + MGW + CHI + FUN
CONTROLS- 2: MGW; 10: SOIL; 11: CHI; 12: COW; 13: FUN

Figure 4.2: Mean respiration index of treatments and controls at 50 °C during 24-day experiment period. Error bars represent standard error of the mean ±S.*E*.

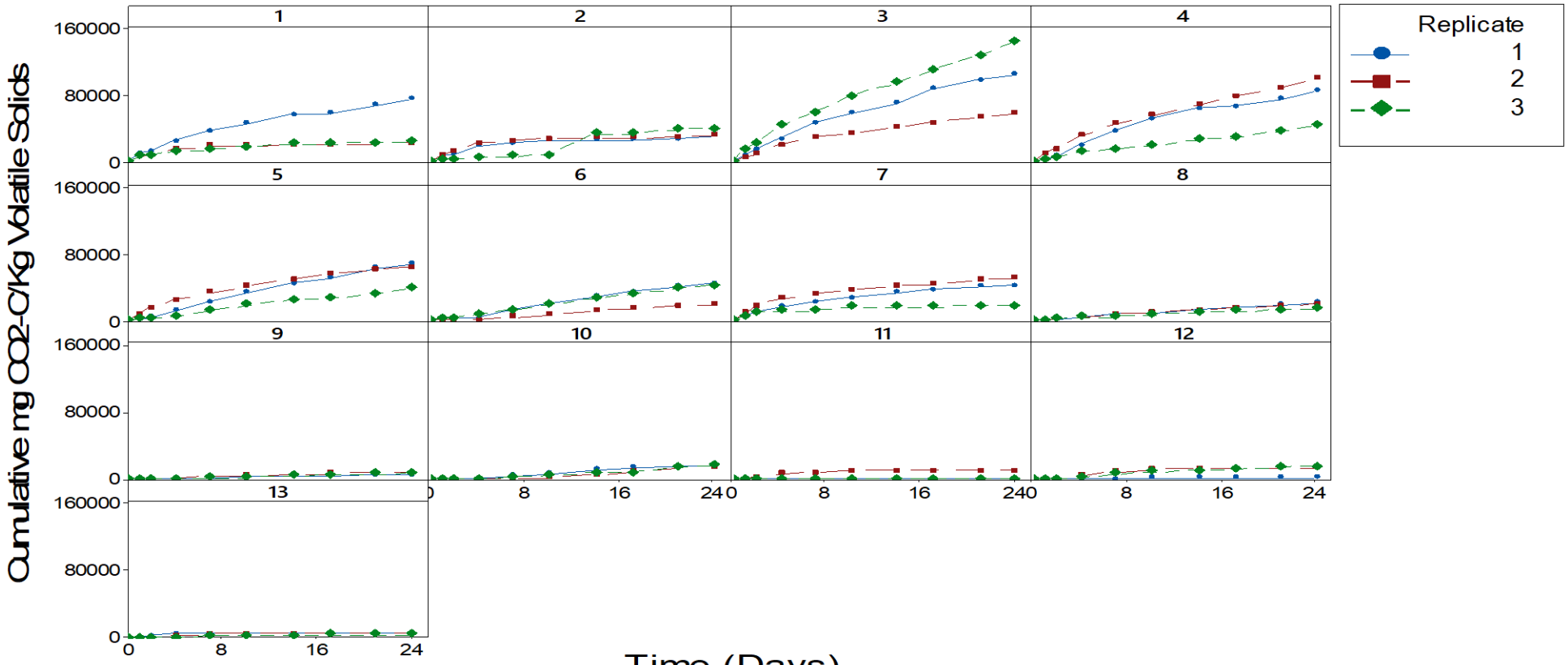
In both cases, animal manures (cow and chicken) were applied singly, respectively. Thus, the degree of fluctuation and variability seen between the replicates of these treatments, i.e. 3 and 4 may be due to the consistent change occurring within the highly biodegradable fractions of the animal manures. Conversely, treatments 5 (soil, MGW, cattle, and chicken), 6 (fungi substrate only), and 7 (soil, MGW, cattle & chicken manures, and fungi) exhibited similar RI patterns. These treatments had an average peak RI of 8125.86 mg CO_2-C Kg^{-1} VS day^{-1} followed by a decline that saw the RI drop below 1500 mg CO_2-C Kg^{-1} VS day^{-1} even though the temperature incubator where the composting reactors were placed was maintained at 50 °C. According to Barrena *et al*. (2005) and Ruggieri *et al*. (2008), this trend often demonstrates a typical composting pattern observed using laboratory-scale reactors. Considering thermophilic temperatures were maintained and microbial activity within these treatments still seemed to descend after the tenth day of the process, the possible occurrence of the maturation phase of the compost material cannot be ruled out. However, this is not conclusive as the temperature was kept at the thermophilic range throughout the process. Several studies suggest that the maturation phase of the composting process occurs at mesophilic temperatures within the range of 35 °C to 38 °C (Ponsá *et al*.,2010). Treatments 8 (soil, MGW, cattle manure, and fungi substrate) and 9 (soil, MGW, chicken manure and fungi substrate) also displayed a very similar trend in terms of possessing significantly lower RIs, with no noticeable rise in the daily CO_2 release rate recorded. A possible reason for this could be the inability of the microbial populations within these substrates to co-exist. Perhaps adding an extra substrate into the matrixes may have a stimulatory effect or possibly alter the properties of the compost mix, consequently furnishing an enabling environment for microbial activities to be fully established.

It is worthy to note that after reaching maximum values, most RIs showed a marked decline during the last two weeks of the process. This decline may imply that increased biological activity witnessed in most treatments is due to the availability of readily biodegradable organic matter consumed by microorganisms. After the organic matter pool was exhausted, the RIs mainly remained constant, with a marginally decreasing tendency. Barrena *et a*l. (2006) explain that this occurrence is common because less-easily biodegradable organic matter generally requires lower oxygen consumption. This might be the case in this study as the oxygen levels were maintained at a constant rate throughout the composting period. Interestingly, as the process drew to an end, treatment 3, 4, 5, 6, and 7 continued to show constant RIs albeit at much lower levels, which possibly infers that basal respiration was indeed maintained throughout the entire composting period.

The RIs of the treatments generally suggest the composition of each treatment plays an essential role in the degree of microbial activity observed. As shown in Figure 4.1, treatments 8 and 9 had the combination of each animal manure with fungi separately, i.e. the soil, MGW, cattle & fungi; and the soil, MGW, chicken & fungi yielded very low RIs. Surprisingly, the RI of treatment 6 (fungi substrate only) was defined by a comparatively higher level of CO2 release. It is possible that the microbial community within the fungi substrate was active, without the presence of secondary microorganisms introduced via the addition of the animal manures. The effective dilution of bacterial numbers could explain the difference in respiration rates observed between the animal waste only treatments (3 and 4) and the single manure with fungal treatments (8 and 9) by the inclusion of fungal substrate (i.e. the single manure treatment had twice as much manure substrate, thus presumably twice as many bacteria added).

4.1.2 Cumulative CO_2 Evolution

The stability and degree of microbial activity in each treatment were assessed by evaluating the cumulative CO_2 respiration rates for the different samples as shown in Figure 4.3. Each panel of the graph shows the cumulative evolution of CO_2 after the 24-day test period. This information is a valuable indicator of compost stability and the cumulative CO2 released by the microbial population present within the treatments, reflecting any change that could occur in the composted materials (Ponsá *et al*., 2010). The results are based on a mixture of the contaminated soil samples with the various animal wastes and fungi inoculated substrate. Several researchers describe these assessment criteria as logically sound based upon well-understood scientific principles. Although the results from these experiments provide an adequate assessment of biological activities in the treatments, it has proven somewhat challenging in some cases to obtain similar results on replicates of the same sample. This is often attributed to the high variability in oxygen uptake by the decomposition microorganisms; the degree of sample homogeneity and experimental conditions; the microbial population balance and the tractable nature of the biological material available (Collier,2005).



1: SOIL + MGW; 3: SOIL + MGW + CHI; 4: SOIL + MGW + COW; 5: SOIL + MGW + CHI + COW; 6: SOIL + MGW + FUN
7:SOIL + MGW + COW + CHI + FUN; 8: SOIL + MGW + COW + FUN; 9:SOIL + MGW + CHI + FUN
CONTROLS- 2: MGW; 10: SOIL; 11: CHI; 12: COW; 13: FUN

Figure 4.3: The Cumulative CO2 produced over the 24-day test period of each treatment combination in triplicates. This shows the stability and degree of microbial activity in each treatment were assessed by evaluating the cumulative CO_2 respiration rates for the different samples Individual lines represent each replicate of the treatment.

However, some prominent trends can be observed from the results. In particular, the higher cumulative respiration rates over the 24-day testing period recorded when the contaminated sample was mixed with the various amendments compared to the control treatments all showed meagre cumulative CO_2 release rates except for MGW. This would indicate the presence of aerobic activity. Although the CO_2 production rates seemed reasonably consistent for the actual treatments, the initial analysis showed that there is a significant difference (one-way ANOVA F (7, 232) = 14.27, $p < 0.001$). Post-hoc comparison test result presented in Table 4.1 reveals how treatments differ with the means that do not share a letter being significantly different (Tukey-Kramer, $p < 0.05$). It is quite apparent that treatments 3 and 4 showed considerably higher cumulative respiratory rates. However, a significant cause for concern in these results is the degree of variability and dispersion between the respective replicates within each of these treatments.

Table 4.1: Tukey-Kramer post hoc test at 95% confidence of the average CO_2 production rates. Means that do not share a letter are significantly different.

Treatment	N	Mean	Standard Deviation	Tukey-Kramer
3- Soil, MGW, Chicken	30	50360	40304	A
4- Soil, MGW, Cow	30	36579	30407	A B
5- Soil, MGW, Cow, Chicken	30	27274	22183	B C
1- Soil, MGW	30	23003	19472	B C D
7- Soil, MGW, Cow, Chicken, Fungi	30	22352	15229	B C D
6- Soil, MGW, Fungi	30	15116	14492	C D E
8- Soil, MGW, Cow, Fungi	30	8203	6796	D E
9- Soil, MGW, Chicken Fungi	30	3056	2803	E

A common highlight in treatments 3 and 4 is that individual animal wastes are applied singly to the contaminated sample, i.e. Soil, MGW, and cattle manure and Soil, MGW, and chicken manure, respectively, have the highest increases. The prominent variability seen between all replicates of both treatments may be attributed to the collection source of the animal wastes. It is probable that the animal wastes may have been exposed to cross-contamination with other material while in the field before collection, thereby altering the properties of the waste samples. Thus, the inadvertent introduction of these materials via

the animal wastes may have interfered with the sample matrix being composted, leading to the high variability observed. Furthermore, it is likely that the high variability accompanied with the elevated cumulative CO_2 respiration rates as demonstrated by these treatments might be due to the presence of slowly biodegradable matter contained in the animal wastes, which is likely to cause constant biological activity.

In terms of the treatment compositions, the highest cumulative CO_2 level was observed for treatment 3 (Soil, MGW, and Chicken manure) with a mean rate of 143,823 mg CO_2-C Kg^{-1} VS after 24 days. At the same time, the least was recorded in treatment 9 (Soil, MGW, Chicken, and Fungi) with a mean rate of 3056 mg CO_2-C Kg^{-1} VS after the same period. On the other hand, the control treatments were all characterised by negligible cumulative CO_2 production at the end of the testing period. Based on the low respiratory rates observed in the control treatments, it is logical to assume that MGW and the animal wastes stimulate the intrinsic microbial population within the contaminated soil sample as evidenced by the elevated aerobic activity. Thus, the significance of MGW (bulking agent) and the feedstocks, i.e. the different animal wastes and fungi substrate used in these tests cannot be overstated. The mix and heterogeneity of the MGW may generally be the reason for the higher rates and variable responses observed. Reasoning from this fact, Ros *et al*. (2006) maintain that the presence of bulking agents during composting yields high microbial activity, implying that the carbon compounds incorporated with bulking agents, accompanied by a higher porosity and oxygen availability, stimulated the microbial population within a composted matrix.

Interestingly, the results further indicate the emergence of a noticeable trend for treatments containing the fungi inoculated substrate. Here, the presence of the fungi substrate seemed to have a moderating effect on microbial activities resulting in a more balanced and steadier; although relatively modest respiration rate in comparison to treatments with solely animal wastes as amendments. The reason for this is not apparent, but it may have something to the dilution of the animal waste or the high lignin content of the fungi substrate material which makes its biodegradability difficult (Komilis, 2006). It is worthy to note that the final cumulative CO_2 rate at the end of the 24-day test period was determined in terms of the mass of CO_2 released per mass of the initial volatile solids by assuming that the bulking agent used (MGW compost) was not significantly degraded. In essence, this value was obtained by the numerical integration of the respiration index values at particular sampling times. Ponsá *et al*. (2010) state that if the total process time is considered, then the values obtained can be a useful indicator for determining the efficiency of the composting process and a measure of the stability of the final compost product. Thus, Ponsá *et al*. (2010)

suggest that cumulative CO_2 production rates greater than 25,000 mg CO_2-C Kg^{-1} VS can be used to denote high levels of organic matter degradation and, for values below 2000 mg CO_2-C Kg^{-1} VS indicate low respiratory activities. The author further states that the use of the cumulative CO_2 produced as an index to predict compost stability ought to be linked to the effectiveness of the degradation of organic matter during the composting process and the extent to which composting occurs.

The stability of compost is a crucial parameter when establishing its fitness for use; and respirometry can be a useful indicator of this, as it refers to the aerobic biological activity of the material at different temperatures. The composting reactors were placed in temperature-controlled incubators at 50 °C which mimics the core of a compost pile during the very active stages, commonly described as the sanitisation phase (USDA, 2000). Thus, the cumulative respiration rate was determined at this temperature, considering it is indicative of the real process activity at operating conditions. Given that an increase in microbial activity is associated with the release of CO_2 and consumption of O_2, the respiration rates at 50 °C has been used to define the class of compost based on the prescribed guidelines of the United States Department of Agriculture. On this basis, by the end of the 24-day process, the average cumulative CO_2 of the replicates in each of these treatments 1, 5, 6, and 7 as seen in Figure 4.3 were between 34,000 mg CO_2-C Kg^{-1} VS and 41,000 mg CO_2-C Kg^{-1} VS. This indicates a respiration rate that is medium to high and primarily fresh material (Brinton *et al*., 1995; Korner *et al*., 2003). According to the USDA (2000), the compost is immature with an 'active composting' status. This is expected, as the contaminated samples, animal wastes, as well as the fungi substrate, are likely to have active microbial communities present within them.

Moreover, it supports the premise in which these tests are performed, suggesting that the active phase of the composting process usually occurs at thermophilic temperatures of above 45 °C. According to Kuo *et al*. (2004), a low respiration rate may not necessarily be a good indicator of stability or exemption from phytotoxicity. This is precisely the case when microbial activity is inhibited by high metal concentration within the hydrocarbon contaminant (Kuo *et al*., 2004). In the same vein, Wu *et al*. (2000) found that compost samples from a composting facility exhibited phytotoxicity despite the low CO_2 evolution rates seen. Some of these disparities positively reinforce the importance of carrying out further composting experiments at a pilot-scale before full industrial-scale implementation. This not only addresses the limitations of laboratory-scale reactors but also takes cognisance of the process efficiency under real weather conditions.

As these experiments were conducted under thermophilic temperatures (50 °C), it can be suggested that the RI obtained was established under similar operating conditions of an actual composting process (i.e. thermophilic temperature range) and the existing microorganisms within the compost matrix were active, as reflected in the RI values at the initial stages of the process. For this reason, Ponsá *et al*. (2010) suggest that the RI at the sanitisation stage can be used for monitoring and assessing the biological activity of the composting process. However, the authors state that for the RI to be used to assess microbial activities, it should be determined at sanitisation temperature (thermophilic), usually above 45 °C. RI determination at 37 °C (mesophilic temperature) should be solely utilised as a stability parameter in the maturation phase of the composting process.

Barrena *et al*. (2006) point out that there is no single respirometric method that can be used to monitor the process and the determination of the stability of a compost sample. The authors' further state that the most appropriate method chosen for these characterisations will depend on the end-use of the assay. The RIs are very useful in monitoring biological activity during the composting process, while the cumulative CO_2 can predict the effectiveness of the organic matter degradation and the extent at which composting occurs. Therefore, bearing in mind that cumulative CO_2 respiration is a function of the daily RI, it is only rational that both variables are applied when assessing the performance process and establishing key properties of the resultant by-product.

4.2 Assessment of Hydrocarbon Contamination levels

The TPHs and PAHs were the key hydrocarbon parameters monitored to assess and evaluate the efficiency of the treatments during the experimental composting trials. Due to the heterogeneity of the samples despite best efforts in ensuring a significant degree of homogeneity was achieved, the hydrocarbon parameters were observed to have varying starting points in terms of the contaminant concentration as shown in Figure 4.4a.

Furthermore, logistical constraints meant only two samples were taken from the reactors, i.e. at the start and end of the treatment period. With all of these considered, the TPHs were analysed based on a mass removal basis (Figure 4.4b) while the PAHs were analysed using their exponential rate constants (Figure 4.7). Literature generally suggests a first-order rate of decay occurs during the breakdown of PAHs. Thus, an adjustment to the data was carried out to compensate for the observed variabilities, a standardisation of the initial concentrations of the monitored HMW PAHs was conducted by fitting an exponential. This displayed the rate of degradation of HMW PAHs over time while considering the difference

in starting concentrations. The varying initial and end concentrations of the PAHs monitored are shown in Figure 4.6.

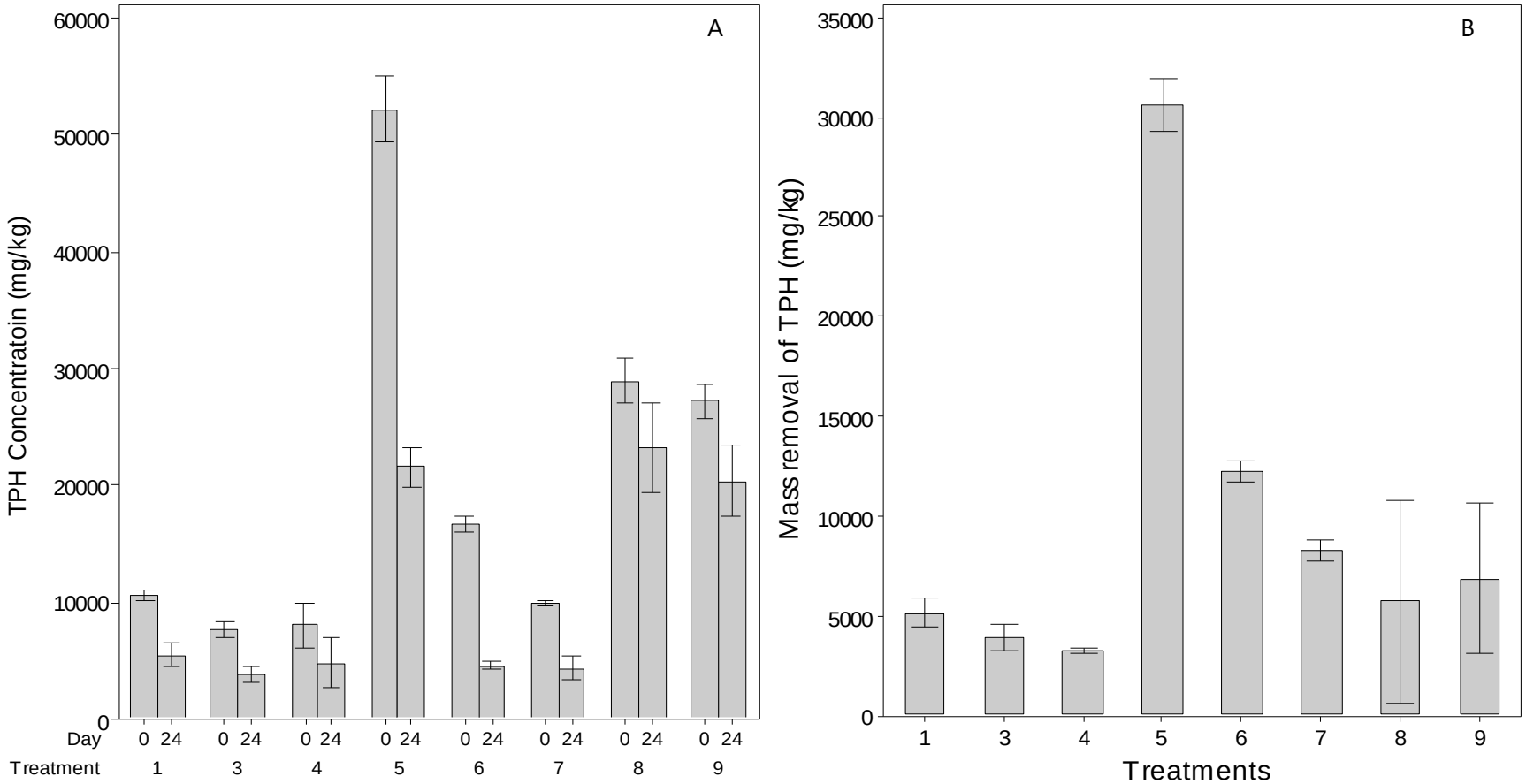
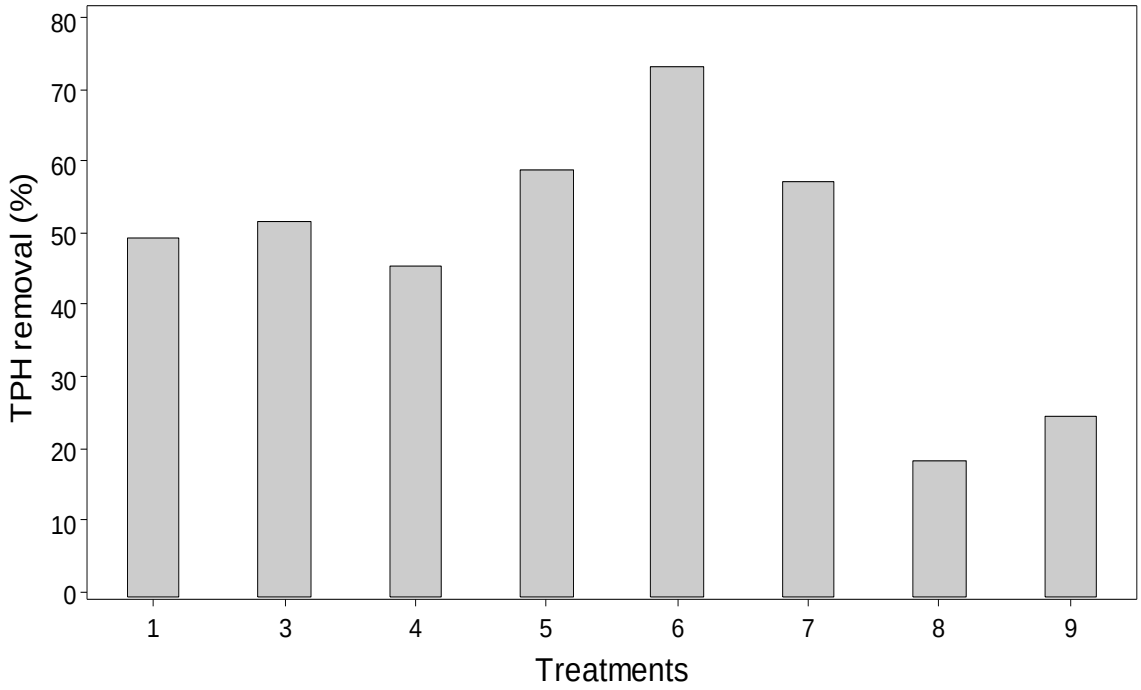


Figure 4.4: Graph showing (A) High variability in the samples and changes between the initial and final TPH concentrations and (B) Mass removal of TPHs for each treatment combination. Error bars represent mean ±S.E.

The total mass removal of TPHs was evaluated by calculating the difference in concentration values of the corresponding start (day 0) and the end (day 24) for each treatment. Treatments 5, 6, and 7 were seen to have the highest mass removals ranging between 8,261 mg/kg and 30,694 mg/kg based on mean values. However, large variations in treatments 8 and 9 make it possible that these treatments could be just as effective as treatments 6 and 7. It is important to note that the initial concentrations are highly variable and therefore, the percentage removal was also used to determine the most effective treatment. It was observed that a majority of treatments displayed over 40% TPH removal, treatment 6 recorded the highest removal (73.2%) followed by treatments 5 and 7 which had removals of 58.9% and 56.9% respectively (Figure 4.5).



1: SOIL + MGW; 3: SOIL + MGW + CHI; 4: SOIL + MGW + COW; 5: SOIL + MGW + CHI + COW; 6: SOIL + MGW + FUN

7:SOIL + MGW + COW + CHI + FUN; 8: SOIL + MGW + COW + FUN; 9:SOIL + MGW + CHI + FUN

Figure 4.5: Graph illustrating the percentage removal of TPH after the 24-day experimental process. The figure shows treatment 6 has the highest percentage removal at 73.2% and followed by treatments 5 and 7 which had removal percentage removal rates of 58.9% and 56.9% respectively.

Statistical results indicate there to be a significant difference between the percentage removal of TPHs in the treatments (one-way ANOVA F (7, 23) = 3.23, $p < 0.05$) however, post hoc test showed that the percentage removals did not differ significantly except for treatment 6 which had significantly higher removal compared to treatment 8 (Tukey-Kramer, $p < 0.05$) as shown in Table 4.2.

Table 4.2: Tukey-Kramer post hoc test at 95% confidence showing the difference in percentage TPH removals between the treatments. Treatments 6 and 8 were seen to differ significantly while others did not. Means that do not share a letter are significantly different.

Treatment	N	Mean	Standard Deviation	Tukey-Kramer
6- Soil, MGW, Fungi	3	73.21	2.56	A
5- Soil, MGW, Cow, Chicken	3	58.87	2.17	A B
7- Soil, MGW, Cow, Chicken, Fungi	3	56.97	16.56	A B
3- Soil, MGW, Chicken	3	51.58	12.55	A B
1- Soil, MGW	3	49.35	14.39	A B
4- Soil, MGW, Cow	3	45.5	18.3	A B
9- Soil, MGW, Chicken Fungi	3	24.5	23.3	A B
8- Soil, MGW, Cow, Fungi	3	18.2	30.2	B

There was a significant difference between the mean cumulative initial and end concentrations of all treatments (one-way ANOVA F (1, 46) = 6.64, p = 0.013). The total mean initial concentration was (M = 20142, SD = 14928) while the end was (M = 10962, SD = 95031). Post hoc test revealed treatment 5 had a significantly higher TPH concentration at the start (Fisher LSD $p < 0.05$) compared to the remaining treatments as seen in Table 4.3. These results suggest high variability in the samples within the various treatments.

Table 4.3: Fisher's LSD post hoc test at 95% confidence showing the difference in initial TPH concentrations between the treatments. Treatment 5 had a significantly high initial TPH concentration. Although, initial concentrations for treatments 1, 7, 4, and 3 did not differ significantly. Means that do not share a letter are significantly different.

Treatment	N	Mean	Standard Deviation	Fisher's LSD
5- Soil, MGW, Cow, Chicken	3	52224	4975	A
8-Soil, MGW, Cow, Fungi	3	28943	3479	B
9- Soil, MGW, Chicken Fungi	3	27211	2576	B
6- Soil, MGW, Fungi	3	16704	1145	C
1- Soil, MGW	3	10567	728	D
7- Soil, MGW, Cow, Chicken, Fungi	3	9853	394	D
4- Soil, MGW, Cow	3	7976	3281	D
3- Soil, MGW, Chicken	3	7654	1229	D

The large variations in the mass removal of TPHs in treatments 8 and 9 as shown by the error bars in Figure 4.4b are likely due to errors during sampling or sample heterogeneity. Despite there being an overall reduction in TPH concentrations across all the treatments by the end of the process, it is possible that the remaining hydrocarbons present are the weathered residuals that may be bound to the surface of the soils. The volatile organics and less recalcitrant fractions of the TPH may have undergone possible microbial degradation during the 24-day composting process, and it is likely that heavier fractions are remaining. Perhaps, if the tests were conducted for longer periods, further mass removals may be recorded to include these residual fractions. According to Pezeshki *et al*. (2000), the heavier fractions of TPHs in soils strongly adhere to soil particles and are only slightly and slowly moveable within the matrix and the introduction of regular sample turning could enhance contaminant movement thereby making it available for microbial degradation.

The U.S. Environmental Protection Agency has described PAHs as priority pollutants that occur in the environment due to natural or anthropogenic activities (Johnsen *et al*., 2005; Mackay *et al*., 2006). The very deleterious characteristics of these compounds, i.e. toxicity

and carcinogenicity mean their remediation is critically required. On that basis, high molecular weight (HMW) PAHs were prioritised in this phase of the study. Here, the treatments with the highest exponential rates of decay were deemed most effective in the breakdown of these contaminants. Bearing in mind, the initial phase of this study functioned as a screening protocol to assess the different efficiencies of the array of amendments; it was observed that treatments 5, 6, and 7 displayed the most potential in terms of degrading high molecular weight (HMW) PAHs based on their exponential rate constants (ERC) (Figure 4.7). The five HMW PAHs examined were: Benzo(e)acephenthrylene; benzo(k)fluoranthene; benzo(a)pyrene; indol(1,2,3-cd)pyrene; and, benzo(ghi)pyrelene.

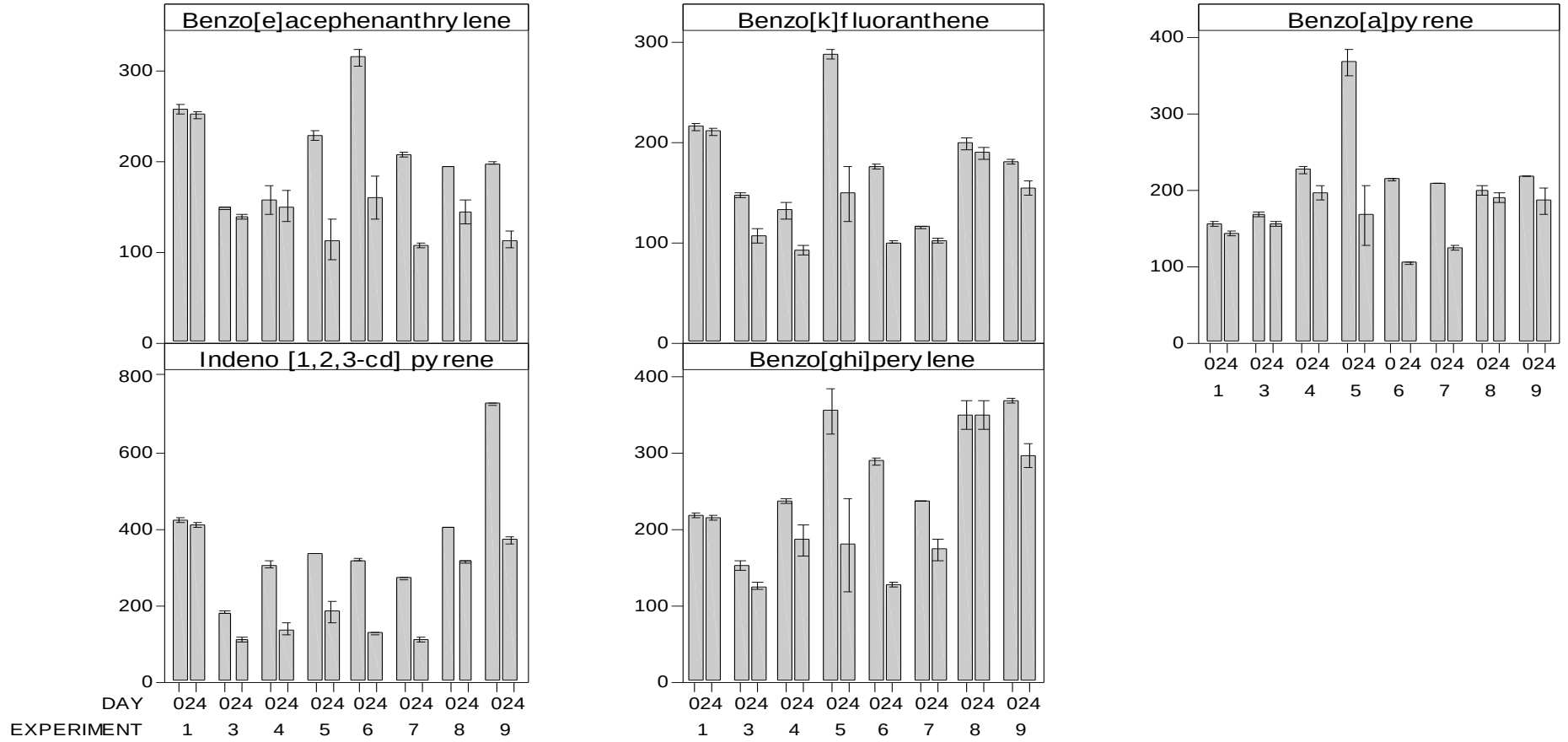
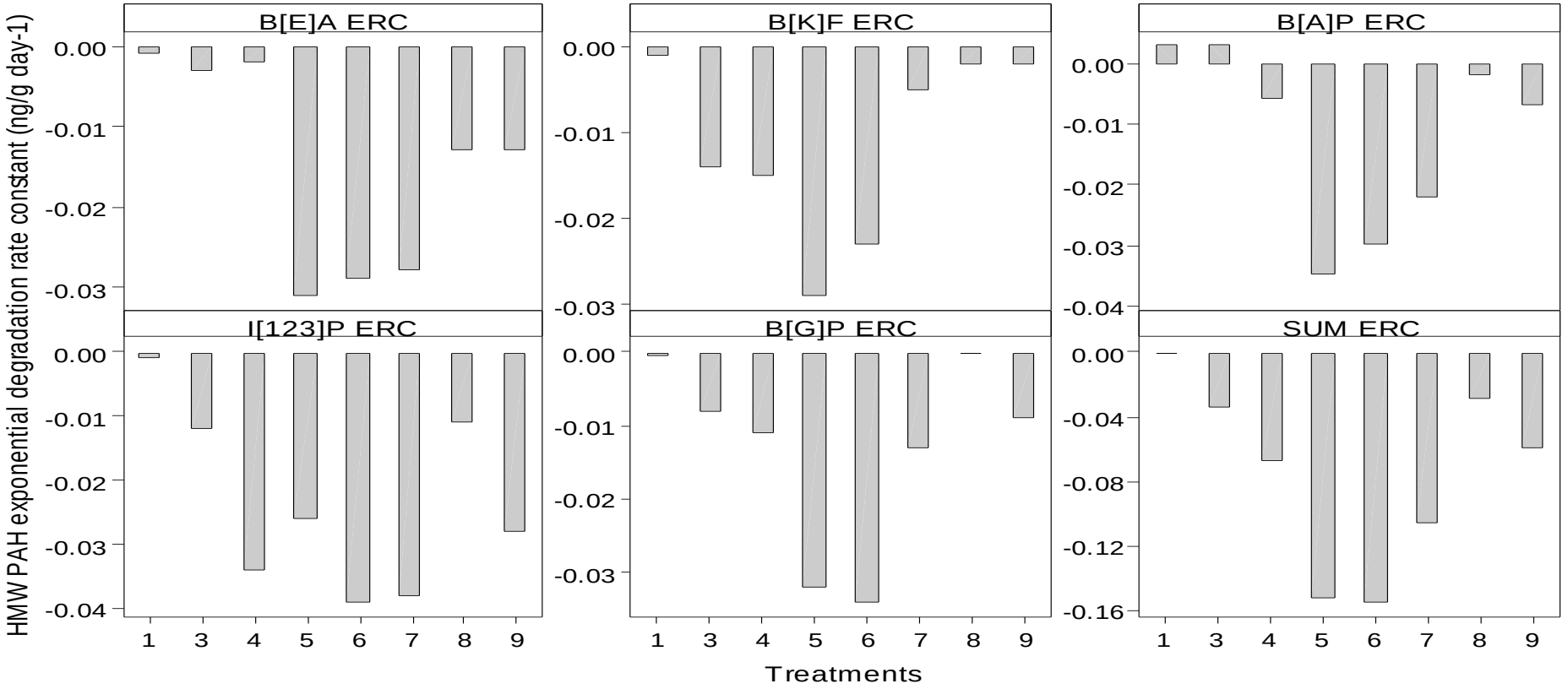


Figure 4.6: Graph showing the initial and end concentrations of HMW PAHs. There was notable variability in the concentrations for samples of the different treatments. Error bars represent mean ±S.E.



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7:SOIL + MGW + COW + CHI + FUN; 8: SOIL + MGW + COW + FUN; 9:SOIL + MGW + CHI + FUN

Figure 4.7: Graph illustrating the individual and total exponential rates constants of HMW PAHs by treatments. Treatments 5, 6, and 7 displayed the most potential for the breakdown of HMW PAHs.

It is important to note that the HMW PAHs monitored possess similar octanol-water partition coefficient constants and ranged between log K_{ow} 6.11 to 6.70. Thus, an assumption can be made that these compounds have similar aqueous solubility. The observed rate constants for treatments 5 and 6 were quite similar in terms of their orders of magnitude for most of the PAH compounds being assessed. However, a reduced rate constant by treatment 7 was particularly noticed in benzo(k)fluoranthene and benzo(ghi)pyrelene therby signifying the very recalcitrant and complex nature of these compounds thus making it harder to breakdown by microbial populations within the matrix. The lower ERC in treatment 7 compared to treatments 5 and 6 may also be attributed to sample heterogeneity observed in the varying initial concentrations, suggesting that a higher concentration of HMW PAHs were present during the sampling of treatment 7 and this can inhibit microbial activity due to elevated toxicity resulting in lower ERCs. Perhaps, the presence of more organic matter could explain the lower ERC observed in treatment 7. According to Riser-Roberts (1998) the availability of these compounds for biodegradation is considerably restricted by very low solubility which impacts bioavailability. This could mean their hydrophobicity is the reason they have a high tendency to sorb onto organic matter, thus making it less bioavailable and harder to breakdown, resulting in lower ERCs. Hamaker (1972) points out that the persistence of a compound is higher as the initial concentration increases and this may account for the reduced ERCs witnessed by some treatments in specific PAHs. Also, the lower ERCs can be explained by the limited availability of active sites (Hance & McKone, 1971) or by the toxic effect on microorganisms or enzyme inhibition (Riva *et al*., 2011). However, it is plausible that higher ERCs observed are because with PAHs, there is an increasing trend of the initial rate of degradation as the initial concentration increases (Sims *et al*., 1983). As expected, statistical analysis shows that a significant difference exists between the exponential rate constants for the individual treatments (Kruskal-Wallis H (7) = 46.29, $p < 0.010$).

The hydrocarbon concentration is likely to have an effect on the respiration rates owing to possible toxicity effect. This is due to the fact that treatments have different hydrocarbon concentration despite best efforts to achieve high level of homogeneity. It is important to note that because one-third of each of the treatments apart from the controls was the soil, the hydrocarbon contamination in the treatments differed and hence the respiration rates observed. All the treatments contain the same proportion of soil but the controls do not.

4.3 Changes in Metal Concentration

Areas that have been exposed to hydrocarbon contamination tend to contain heavy metals such as arsenic, mercury, zinc, and lead (Roane & Kellogg, 1996). Although, Khuhawar *et*

al. (2012) point out that the predominant metals in crude oil are mostly nickel, vanadium, copper, chromium, cadmium, and cobalt. In up to 50% of polluted sites, inorganic contaminants are said to persist in the remediated soil even after biodegradation, thus Diels *et al*. (1991) state that these metals may inhibit the activities of native microorganisms as well as the introduced organic compound degraders. Concentrations of heavy metals were recorded at the start and end of the laboratory composting trials. The samples of individual treatments had varying concentrations at the different time points they were measured (Figures 4.8, 4.9, and 4.1).

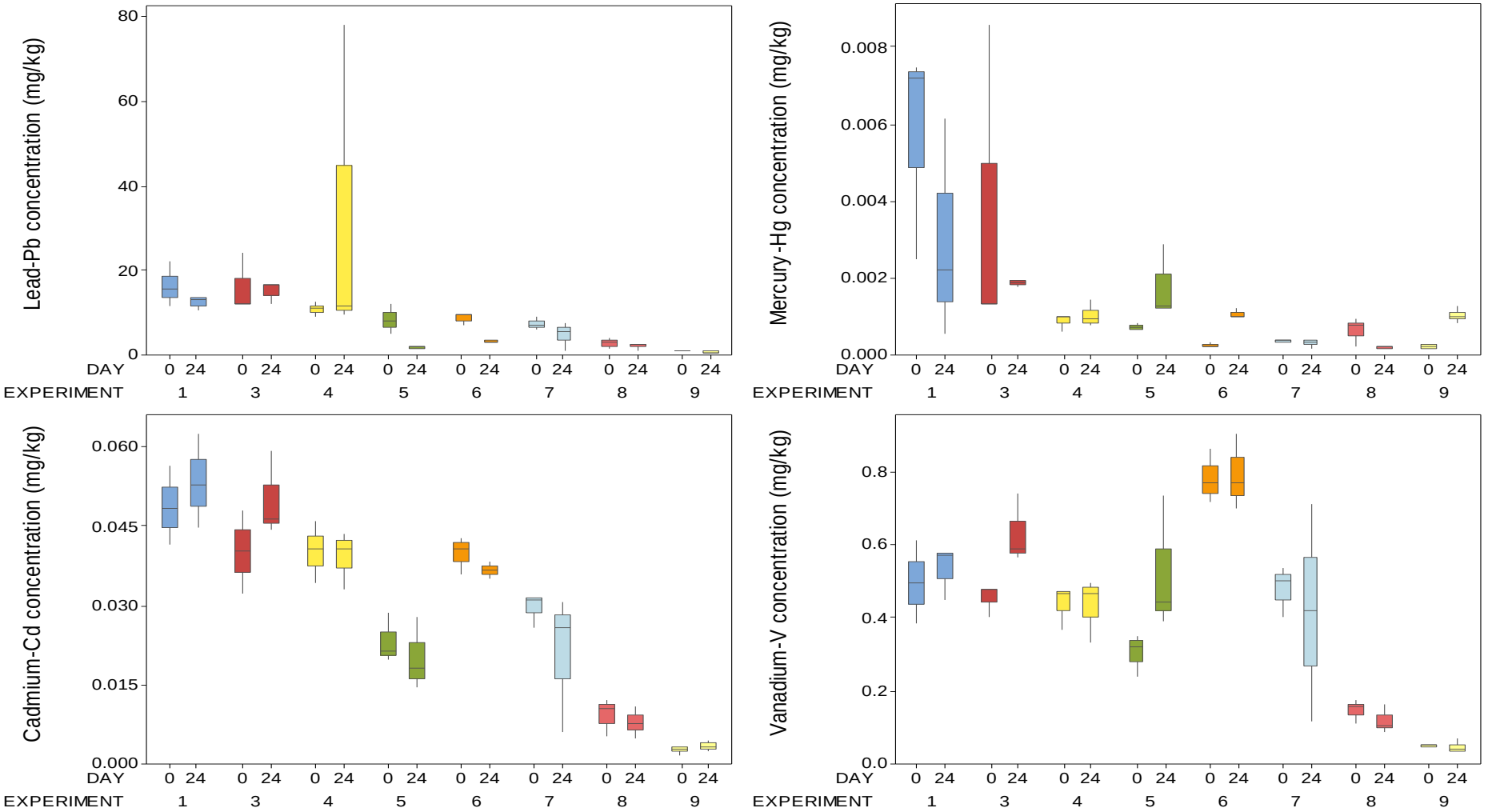


Figure 4.8: initial and final (after 24 days) metal concentrations for lead, mercury, cadmium, and vanadium. The samples of individual treatments had varying concentrations at the different time points they were measured. 1- Soil, MGW; 3- Soil, MGW, Chicken; 4- Soil, MGW, Cow; 5- Soil, MGW, Cow, Chicken; 6- Soil, MGW, Fungi; 7- Soil, MGW, Cow, Chicken, Fungi; 8- Soil, MGW, Cow, Fungi; 9- Soil, MGW, Chicken Fungi.

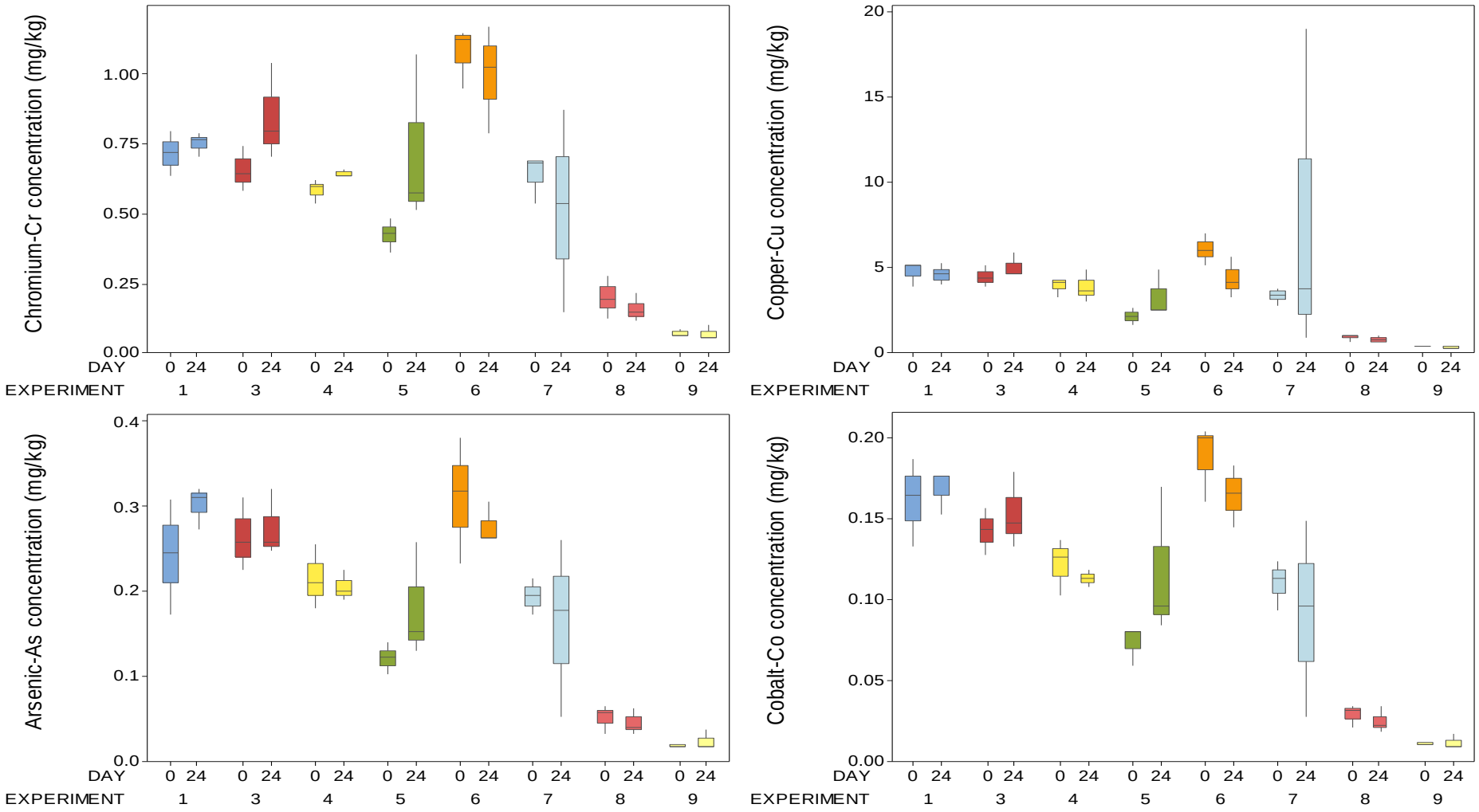


Figure 4.9: initial and final (after 24 days) metal concentrations for chromium, copper, arsenic, and cobalt. The samples of individual treatments had varying concentrations at the different time points they were measured. 1- Soil, MGW; 3- Soil, MGW, Chicken; 4- Soil, MGW, Cow; 5- Soil, MGW, Cow, Chicken; 6- Soil, MGW, Fungi; 7- Soil, MGW, Cow, Chicken, Fungi; 8- Soil, MGW, Cow, Fungi; 9- Soil, MGW, Chicken Fungi.

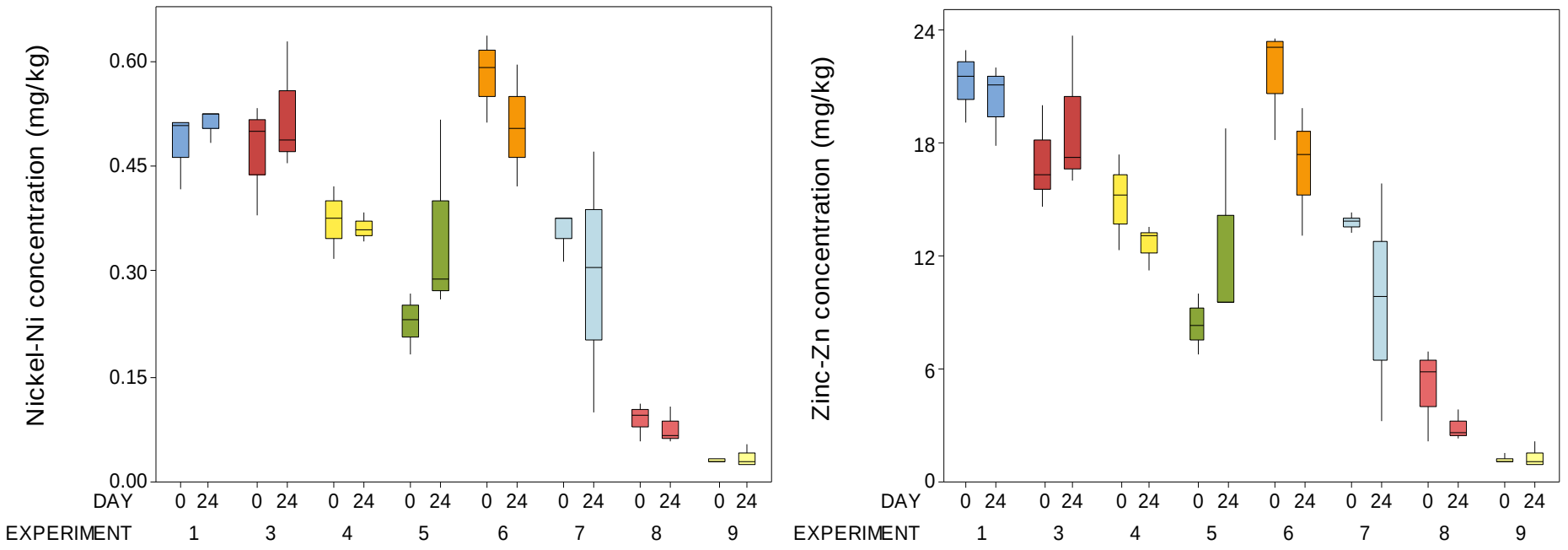


Figure 4.10: initial and final (after 24 days) metal concentrations for nickel and zinc. The samples of individual treatments had varying concentrations at the different time points they were measured. 1- Soil, MGW; 3- Soil, MGW, Chicken; 4- Soil, MGW, Cow; 5- Soil, MGW, Cow, Chicken; 6- Soil, MGW, Fungi; 7- Soil, MGW, Cow, Chicken, Fungi; 8- Soil, MGW, Cow, Fungi; 9- Soil, MGW, Chicken Fungi.

The metals monitored have been identified in PAS100 as potentially toxic elements (PTEs), and upper limits have been established for these contaminants (BSI, 2011). Test indicated a significant difference in the mean cumulative initial concentration of individual metals (one-way ANOVA F (24, 575) = 46.04, $p < 0.001$) and also the mean cumulative end concentrations (one-way ANOVA F (24, 575) = 32.49, $p < 0.001$) across all the treatments. However, post hoc tests (Tukey-Kramer $p < 0.05$) for both initial and final concentrations suggest these differences to be a result of higher concentrations specific metals (Al, Ca, Fe, Cl) compared to the other metals detected as shown seen in Table 4.4.

Table 4.4: Tukey-Kramer post hoc test at 95% confidence of the cumulative mean concentrations of metals at the initial and end points of the experiments. Means that do not share a letter are significantly different

Initial Concentration				Final concentration		
Metals	**N**	**Mean**	**Grouping**	**Metals**	**Mean**	**Grouping**
Al	24	1078	A	Al	1215	A
Ca	24	1052	A	Ca	1029	A
Fe	24	535.7	B	Fe	577.3	B
Cl	24	486.1	B	Mg	518	B
Mg	24	310.6	B C	Cl	420.0	B C
S	24	218.6	C D	S	387.8	B C
K	24	178.7	C D	P	179.1	C D
P	24	175.3	C D	K	159.1	C D
Si	24	50.09	D	Si	68.7	D
Na	24	28.94	D	Na	34.04	D
Zn	24	12.95	D	Zn	11.95	D
Pb	24	9.12	D	Pb	9.36	D
Mn	24	5.420	D	Mn	5.378	D
Cu	24	3.272	D	Cu	3.806	D
Cr	24	0.5477	D	Cr	0.5914	D
B	24	0.4573	D	B	0.4883	D
V	24	0.3936	D	V	0.4366	D
Ni	24	0.3272	D	Ni	0.3341	D
As	24	0.1784	D	As	0.1849	D
Sn	24	0.1130	D	Co	0.1058	D
Co	24	0.1049	D	Sn	0.1007	D
Br	24	0.0760	D	Mo	0.07294	D
Mo	24	0.07015	D	Br	0.04209	D
Cd	24	0.02918	D	Cd	0.02895	D
Hg	24	0.001588	D	Hg	0.001309	D

Zinc had a mean overall reduction of up to 1mg/kg with an average global initial concentration (SE±) of 12.95 mg/kg (±1.49) to an end concentration of 11.95 mg/kg (±1.49). Differences between initial and final concentrations of other metals ranged between ±0.001 to ±0.5. Lead (Pb) was found to be highest in treatment 4 with a mean total of (M = 22.10, ±SE = 11.20). Concentrations for mercury (Hg), cadmium (Cd), arsenic (As), nickel (Ni),

cobalt (Co), chromium (Cr), and vanadium (V) were all generally found to be low (less than 1mg/kg). For zinc (Zn) and copper (Cu), slightly higher concentrations were found although still within the ambit of acceptability, ranging between 0.40 to 20.77 mg/kg. Osuji *et al*. (2006) draw attention to the fact that different locations have trace amounts of metals even in natural soils regardless of whether the environment is disturbed or undisturbed. Perhaps, this could explain the presence of some metals that are mainly found in regions severely polluted with crude oil. Across most treatments, the metal concentrations seemed to have decreased by day 24, although some metals were seen to have increased over the same period. The most striking of these increments was seen in the lead (Pb) concentration of treatment four that went from 10.96 to 33.20 mg/kg and this may simply be attributed to the sample heterogeneity or sampling experimental error. It should be noted that all the other treatments monitored showed a reduction in lead concentration by the end of the experimental period. For treatment 5, zinc concentration was higher at the end of the 24-day period, i.e. from 8.44 to 12.69 mg/kg. Conversely, in treatment 7 there was a reduction in zinc with a corresponding increase in copper. Interestingly, nickel (Ni) and vanadium (V) have also shown similar increases in their concentration in over 60% of the treatments. These differences are likely due to the variability in metal concentrations arising from the sampling methods. It is also possible that the weathering undergone by the hydrocarbon-polluted sample may be responsible. No notable changes were recorded for all the metal concentrations in treatments eight and nine, as they remained more or less the same. The overall fluctuations seen in the results suggest that the samples under examination may require longer composting process time to be stable. Epstein *et al*. (1992) state that it is important that composts are allowed to achieve maturation because over time the solubility of heavy metals decreases thereby reducing their bioavailability in the environment. The authors indicate that these metals become bound to humic compounds, metal oxides, and phosphates within the matured composted matrix.

4.4 Changes in Physico-Chemical Properties

4.4.1 pH and Electrical Conductivity

Observed variations in the pH of the soils supplemented with the different organic amendments (cattle and chicken manures) were expected and may be ascribed to the differences in pH status of these amendments. The initial pH levels generally started off marginally alkaline then tended to shift towards a more neutral pH by the end of the composting process (Figure 4.11). This is evident as the overall mean pH at day 0 was (M = 7.76, ±*SE* = 0.14) while that at day 24 was (*M* = 7.08, ±*SE*= 0.15). This trend may be due to the formation of organic acids formed during the initial composting process (Petric *et al*.,

2012). Despite the general decline of pH values for all the treatments by day 24, they remained within relatively neutral pH. However, treatment 1 appears to have drifted past the neutral pH towards the acidic zone. The pH of the cow manure only treatment was found to differ significantly with most of the treatments (one-way ANOVA F (7, 40) = 4.63, p = 0.001) with the exception of treatments 5 and 8 which also had cow manure present. This is further described in the post-hoc comparison test result presented in Table 4.5 that shows treatments differ with the means that do not share a letter being significantly different (Tukey-Kramer, $p < 0.05$). The presence of the cattle manure seems to influence pH of the treatments. The decrease in pH is often expected during composting of organic wastes because acids are formed when readily available carbon which is often given off as CO_2 goes through a metabolism process. This subsequent decrease seen by day-24 may be attributed to the degradation of animal wastes which may have resulted in the release of acidic intermediate and final products that can cause a decline in pH (Atagana, 2008).

According to Liu *et al*. (2011), changes in pH during the thermophilic phase can be attributed to the production of ammonia associated with protein degradation in the samples and the degradation of organic acids. Osuji *et al.* (2006) opine that that the supply of constant aeration may influence the pH via the enhancement of microbial mediated oxidation of organic acids that are formed. In the case of the current study similar operating conditions were used as the reactors were incubated in temperatures within thermophilic range and under constant supply of oxygen.

Table 4.5: Tukey-Kramer post hoc test for average pH of the samples at 95% confidence. This shows pH across the treatments did not differ significantly. Means that do not share a letter are significantly different.

Treatment	N	Mean	Standard Deviation	Tukey-Kramer
4- Soil, MGW, Cow	6	8.582	0.813	A
5- Soil, MGW, Cow, Chicken	6	7.658	0.799	A B
8- Soil, MGW, Cow, Fungi	6	7.625	0.560	A B
9- Soil, MGW, Chicken Fungi	6	7.272	0.280	B
7- Soil, MGW, Cow, Chicken, Fungi	6	7.202	0.463	B
3- Soil, MGW, Chicken	6	7.167	0.280	B
6- Soil, MGW, Fungi	6	7.115	0.396	B
1- Soil, MGW	6	6.757	0.995	B

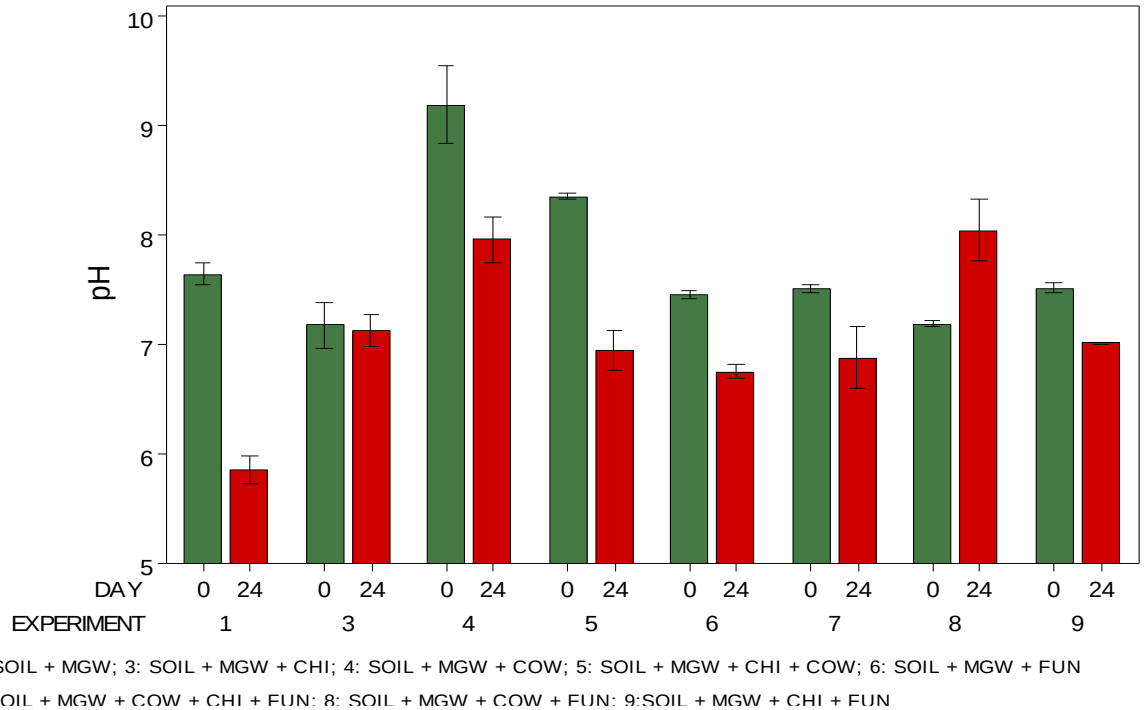


Figure 4.11: Graph illustrating the pH levels at the initial and end-points of each treatment. The initial pH levels generally started off marginally alkaline then tended to shift towards a more neutral pH by the end of the composting process. Error bars represent mean ±S.E.

Generally, acidification occurred as expected during the composting process in all the treatments except treatment 8 (cow and fungi). This acidification is likely a result from the decomposition of the animal wastes and other organic matter that form organic acids. These conditions have been reported to be favourable for the stimulation of microbial communities within the matrix. Acidification may have occurred due to the aerobic conditions within the systems, which was aided by the addition of an external air supply. If anaerobic conditions prevailed, these organic acids might accumulate rather than break down, thus aerating the mixture prevents such. In the case of treatment 8, it is possible that there was variability during sampling or insufficient oxygen supply through the composted matrix, thus lowering the degree of oxygen transfer through the sample and resulting in the alkaline pH observed at the end of the process.

The changes between day 0 and 24 in the electrical conductivity (EC) of the treatment mixtures in the reactors are shown in Figure 4.12. The EC varied amongst the different reactors during the process, with the most notable decrease seen in treatment 4. The final EC values across the reactors ranged from between 838 to 3373 µS/cm. However, the general trend in the EC values showed only minimal changes between the initial and final values for each of the composting experiments. It was observed that there were significant

differences between the mean EC values of the treatments at the start (one-way ANOVA *F* (7, 16) = 4.22, p = 0.008) and at the end of the experiments (one-way ANOVA *F* (7, 16) = 3.81, p = 0.013). Tukey-Kramer post hoc test ($p < 0.05$) show treatment 4 had a higher EC value initially compared to the other treatments and the lowest EC by the end of the experiment as show in Table 4.6.

Table 4.6: Tukey-Kramer post hoc test for average EC values of the samples at the start and end of the experimental period at 95% confidence. Electrical Conductivity value for treatment 4 significantly higher than the other treatments at the start but was significantly lower by the end of the experiment. Means that do not share a letter are significantly different.

Initial EC				Final EC		
Treatment	**N**	**Mean**	**Tukey-Kramer**	**Treatment**	**Mean**	**Tukey-Kramer**
4	3	3373	A	5	2263	A
5	3	2060	A B	1	1950.3	A B
9	3	1806	B	6	1894	A B
3	3	1745	B	9	1758	A B
6	3	1653.3	B	3	1537	A B
8	3	1477	B	7	1429.0	A B
7	3	1457.7	B	8	1146	A B
1	3	1450.7	B	4	838	B
1- Soil, MGW; 3- Soil, MGW, Chicken; 4- Soil, MGW, Cow; 5- Soil, MGW, Cow, Chicken; 6- Soil, MGW, Fungi; 7- Soil, MGW, Cow, Chicken, Fungi; 8- Soil, MGW, Cow, Fungi; 9- Soil, MGW, Chicken Fungi						

It is possible that the significant decrease in the EC values recorded between the days 0 and 24 of treatment 4 is likely due to sample heterogeneity. However, it may be that this treatment did not have much breakdown of organic matter which often produces mineral salts that increase EC values. On the other hand, the slight variations in EC shown at the end of the composting process for the remaining treatments may be associated with ions that may have been influenced by changes in moisture content. According to Huang *et al*. (2004), since the EC reflects the measure of salinity of the compost, it is highly likely to be increased by the presence of salts and conductivity ions. Although organic compounds like hydrocarbons are said to be very poor conductors of electricity, their presence is expected

to cause an overall alteration in EC values when compared to natural uncontaminated soils (Osuji & Ukale, 2005). It is important to note that the mean final EC values (M = 1602; ±*SE* = 114) are above 1000 µS/cm prescribed by the U.S. Department of Agriculture for crop production. The end-use of the compost is likely to determine the suitability of its EC. As this product is most likely to be used at a subsistence scale, there should not be expectations of severe detriments associated with its utilisation. It will be useful to conduct a larger experiment with more representative environmental conditions as the results may reveal otherwise with respect to the final EC values.

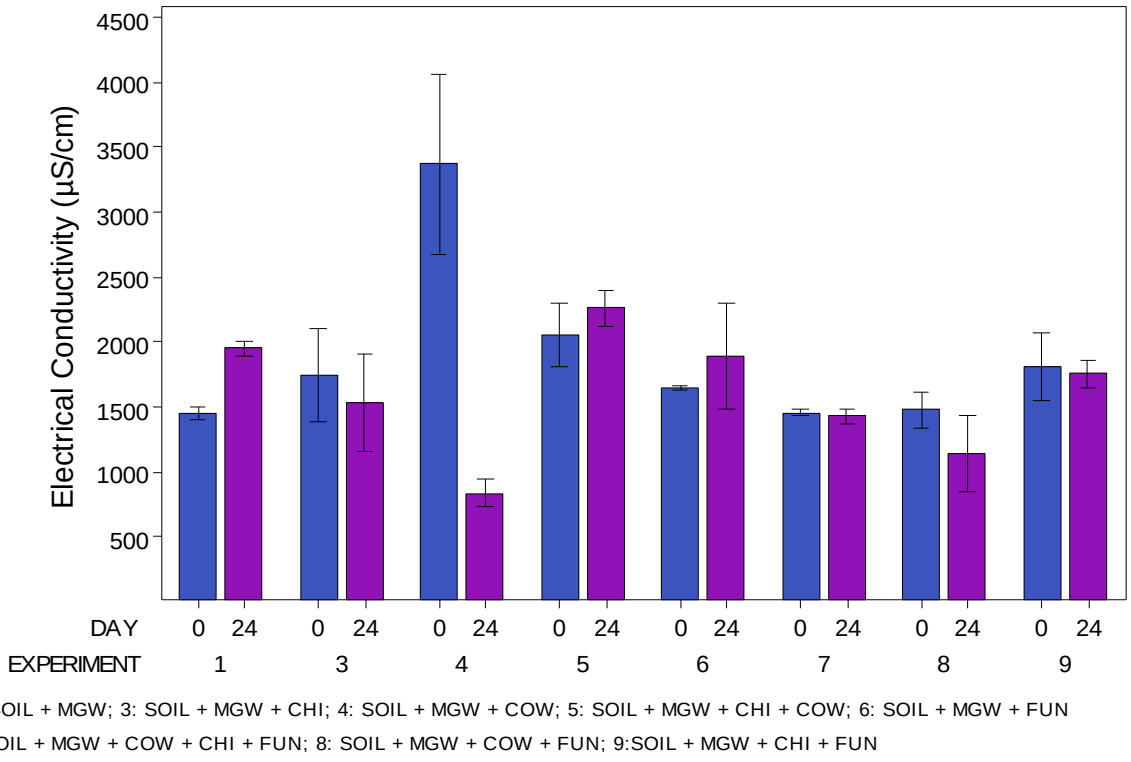


Figure 4.12: Graph illustrating EC levels at the initial and end-points of each treatment. EC varied amongst the different reactors during the process, with the most notable decrease seen in treatment 4. The final EC values across the reactors ranged from between 838 to 3373 µS/cm. Error bars represent mean ±S.E.

4.4.2 Moisture and Organic Matter Contents

The moisture content plays a crucial role in composting because it influences several key properties: microbial activity, available air-space in the pores, oxygen, and temperature transfer (Haug, 1993). Similarly, de Bertoldi (1983) states that the optimal moisture content in composting varies and is principally dependent on the various physical properties, including the particle size of the matrix. The moisture content varied significantly between treatment one and all other treatments (one-way ANOVA F (7, 40) = 4.80, p = 0.001) as it had overall average moisture content through the experiments. At day 0, the moisture ranged between 20.34% and 48.68% while by day 24, it ranged between 31.02% and

83.66%. This discrepancy is due to the sample heterogeneity and the methodology of the test that consisted of the intermittent addition of water to the system to prevent the drying out of the samples that were placed in a temperature-controlled incubator at 50 °C. Given this circumstance, the moisture seemed to decline due to the combination of the thermophilic temperature conditions and the constant aeration. In a bid to avoid excessive drying, water was applied, and the inlet air was humidified. It should also be noted that moisture losses during the process could be considered an index of the decomposition rate because the heat generation that accompanied decomposition may have driven vaporisation (Liao *et al*., 1997). This addition of water via spraying at various times during the process meant the moisture content recorded at the start of the process (M = 32.77%; ±*SE* = 1.97) was lower than that at the end (*M* = 47.34%; ±*SE* = 4.02). The global average moisture content (*M* = 40.06%; ±*SE* = 2.46) between day 0 and 24 for all treatments is deemed sufficient for the composting process to occur (Haug, 1993). However, Iqbal *et al*. (2010) suggest 50% to 70% to be the most favourable moisture content level for biodegrading a wide variety of compost mixtures. Other authors generally indicate that the optimum moisture content depends on the specific physicochemical properties and biological characteristics of the material being composted (Huet *et al*., 2012; Kumar *et al*., 2010).

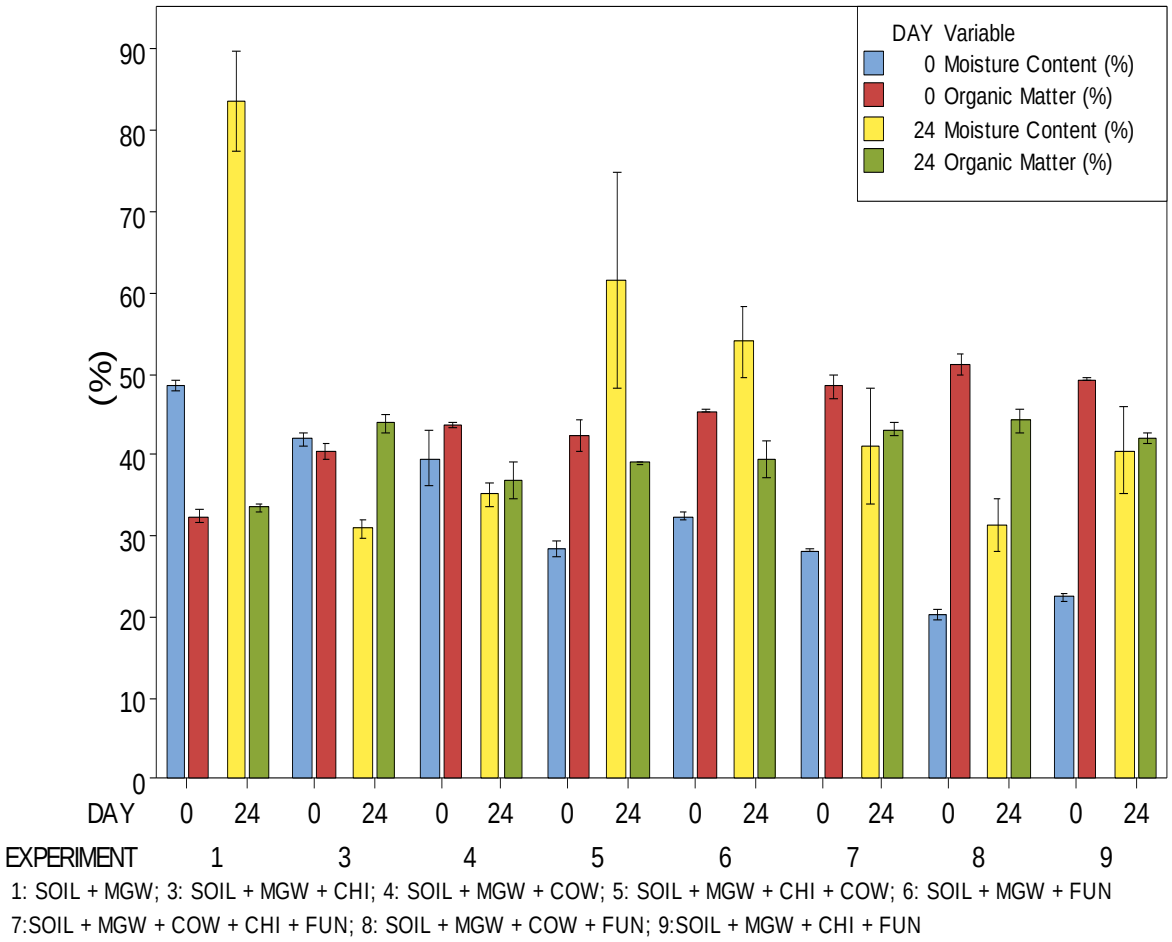


Figure 4.13: Graph illustrating moisture and organic matter contents at the initial and end-points of each treatment. Error bars represent mean ±S.E. The blue and yellow bars represent day 0 and 24 of the moisture content respectively. The red and green bars represent the day 0 and 24 of the organic matter content respectively.

The organic matter (OM) tended to decrease by day 24 for most treatments except for treatments 1 and 3 that had very negligible increases possibly due to sample heterogeneity. It is possible that the presence of non-degradable organic matter from the animal manure or bulking agent may cause a degree of variation in the results.

The average OM content across the treatments at day 0 was 44.18%; decreasing to 40.29% by day 24. The most plausible reason perhaps might be due to metabolic activities by microorganisms stimulated by changes in the physicochemical properties and the introduction of oxygen into the system. In literature, however, up to 50 % of organic matter is said to be lost due to the presence of readily mineralisable organic nitrogen compounds. Therefore, the 4 % organic matter loss does indicate some degree of composting to have occurred, although not at levels observed in full-scale processes. It may also be that the 24-day experimental period was not sufficient to see a significant reduction in OM levels as the complete composting cycle can be up to 6 months. The OM content has remarkable implications on mineralisation because carbon-mineralising capacity is directly related to

the OM content of the soil and the release of CO_2 is proportional to the OM content (Sims, 1990). This leads to a reduction in oxygen levels and subsequently hinders microbial metabolism. The effect of weathering cannot be overlooked, as it is said to enhance the redistribution of the toxic fractions of the hydrocarbons that will reduce activities of certain soil microbes (Osuji & Ukale, 2005).

After composting, the mineralisation of OM is primarily attributable to the degradation of readily degradable compounds, which microorganisms use as carbon and nitrogen sources. Barrington *et al*. (2002) state that microorganisms convert up to 70% carbon to CO2 during the degradation of organic compounds and utilise about 30 to 40% into their bodies as cellular components. The rate at which organic matter decreases is usually an overall indicator of composting (Petric *et al*., 2012).

It is generally imperative that the organic matter content of compost is monitored, especially if its intended end-use is for crop cultivation and other agro-allied activities. Therefore, understanding the influence of this parameter may be the difference between productive crop yield and poor crop yield as it were. Several authors suggest that high organic matter inhibits plant growth since not enough microbial bacteria can break down the organic matter into simpler fractions that can be assimilated by plants (Ogboghodo *et al*., 2003; Roy *et al*., 2003). With this being said, Ogboghodo *et al*. (2003) report that crude oil spilt on soils increased the total organic matter by up to 2.6%, a result of which showed a high level of inhibition in the germination and growth of *Zea mays* (maize).

4.5 Germination Index

The emergence of cress seeds after 5 days was used to determine the effectiveness of the different amendments used for remediating the contaminated samples during the laboratory-scale composting trials. The method proposed by Zucconi *et al*. (1981) was used in this study to measure germination index (GI) before the treatment was initiated and after it was concluded. It has been described as an integrated biological indicator, thus widely regarded as the most sensitive parameter used to assess the level of compost toxicity and maturity. In order to determine the GI, the combined measure of relative seed germination and relative root elongation of cress seed (*Lepidium sativum* L.) was calculated. A GI of 50% as prescribed by Zucconi *et al*. (1981) was used in this study as an indicator of phytotoxin-free compost. From the graph in Figure 4.14, the general trend observed suggests only a marginal average increase of 9.8 % in the germination indexes of all the treatments after the 24-day experiment. This trend is backed by results from the analysis, indicating there to be no significant difference (one-way ANOVA F (1, 46) = 4.19, p = 0.046)

between the pre- and post-treatment GIs of the samples. However, the post-hoc test would suggest a significant difference between the day 0 and 24 of treatments 4, 5 and 9. These treatments showed increases in the GIs of 32%, 17% and 15.7% respectively at day 24.

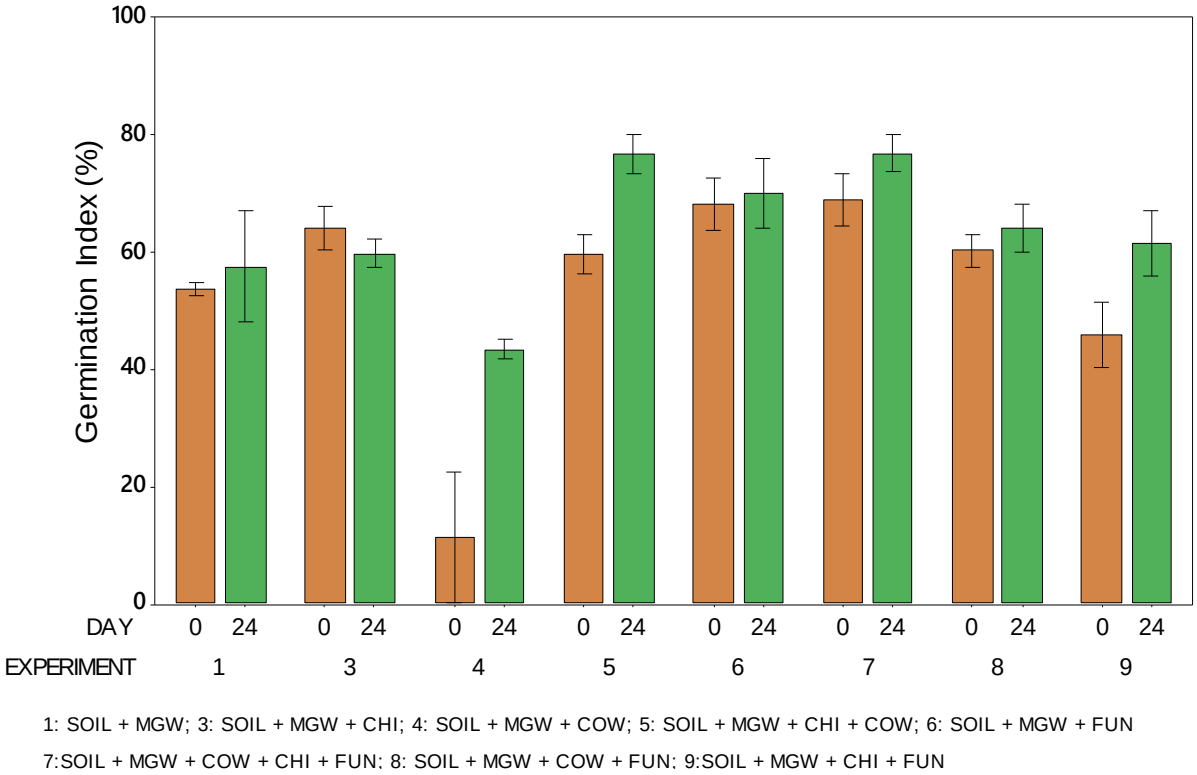


Figure 4.14: Graph illustrating the germination index of seed cress at the initial and end-points of each treatment. The general trend observed suggests only a marginal average increase of 9.8 % in the germination indexes of all the treatments after the 24-day experiment. Error bars represent mean ±S.E.

It should be noted that there was an insignificant decrease in the GI of treatment 3 (chicken manure only). This may be attributed to the release of toxic levels of low molecular weight short-chain volatile fatty acids, primarily acetic acid and ammonia, which is a major compound present within chicken manure (Wong, 1985). The results mostly suggest the absence of phytotoxic compounds in the compost. Interpretation of treatment 4 must be done with caution as there is a considerable degree of variability in the initial sample as evidenced by the large error bar. According to Roletto *et al*. (1985), a GI of 50 – 70% signifies a low level of phytotoxic substances and compost maturity. The GI demonstrates the cumulative potential effects of all chemical factors that may harm the plants even though this may not be related to humification. The Pearson's correlation showed that the electrical conductivity (EC) was moderately negatively correlated to the GI (r = -0.446, p = 0.001) suggesting its possible influence on the GI. This seems to be consistent with earlier research by Huang *et al*. (2004) who found that high EC values in the resulting compost were detrimental to seed germination and it has to be reduced to levels that would not exert inhibition on plant growth. As such, the authors state that multi-indicators should be

recommended for evaluating compost maturity as opposed to single chemical indicators. In light of this, it is interesting to note that TPH concentration was found not to have significant inhibitory effects on seed germination (r = 0.138, p = 0.350). Salanitro *et al*. (1997) found that untreated soils contaminated with heavy and medium crude oils significantly enhanced the growth of corn seeds by 40 – 70% over control seeds grown in oil-free soils. Other authors suggest that crop yield increased by 50% in fields contaminated with TPH concentration of up to 7,500 mg/kg; however, concentrations greater than 25,000 mg/kg were found to affect nodule formation and growth (Baker, 1970).

Interestingly, the results in this study suggest HMW PAHs seem to have very low inhibitory effects on GI though not significant (r = -0.258, p = 0.076). This would indicate that the more recalcitrant fractions of petroleum hydrocarbons or similar metabolites might be affecting plant growth. This accords with observations by Raymond *et al*. (1967) who indicate that although TPH concentrations of up to 25,000 mg/kg were significantly degraded after six months, the germination of seedlings was still restricted, meaning residual hydrocarbons or their metabolites were phytotoxic.

4.6 Summary

The chapter compared the effectiveness of different animal manures and fungi inoculated materials on the remediation of hydrocarbon-contaminated soils by monitoring the TPH removals and PAHs assessing the exponential rate constants of the HMW PAHs. The treatments selected for the microcosm study showed the best rates of hydrocarbon degradation based on the exponential rate constants of the HMW PAHs monitored.

The amendments were found to change the rate of composting, as shown through the aerobic activities witnessed during the dynamic respiration index tests. However, there was no direct correlation between the rate of composting and the hydrocarbon removals. The RIs showed that the composting rates could be manipulated by adding various materials, which was observed in the first 10 days.

Cattle manure only and chicken only, treatments 3 and 4 respectively stimulated high activity rates and correspondingly gave moderate rates of TPH removal. However, the best rates of TPH removal and HMW PAH degradation were seen in treatments 5, 6, and 7 despite lower respiration activities. This indicates that high rates of composting may not necessarily enhance the degradation of hydrocarbons.

The fungi only treatment showed an ability to break down hydrocarbons in the absence of high levels of organic carbon introduced via the animal wastes. Therefore, a direct mechanism could be seen for the fungi; thus, treatments 5, 6, and 7 have been selected for further study, having displayed the most effective pollutant removal rates.

As a means of assessing the phytotoxicity, the results of GI test showed a relative improvement in the germination levels of cress seed post soil treatment.

CHAPTER 5

5.0 RESULTS AND DISCUSSION (MESOCOSM STUDY)

This chapter presents results and discusses the second phase of this project (mesocosm study) by investigating the influences of microbial communities present in animal manures and fungi additives on the degradation of TPHs and PAHs. Also, understand the role of temperature and various physicochemical parameters as contributors in the successful use of an integrated composting low-tech alternative to reduce hydrocarbon contamination in soils. A rainfall simulation was carried out to understand the movement of hydrocarbon contamination from solid to liquid medium and to establish the efficiency of this remediation technique under intense rainfall conditions.

5.1 Changes in Hydrocarbon Content

5.1.1 Changes in Total Petroleum Hydrocarbon

The changes in TPH concentration levels varied amongst the treatments and decreased progressively throughout the composting process. Assessment of the efficiency of individual treatment combinations applied to the contaminated soil was evaluated by the “percentage concentration remaining” (Figure 5.1). By the end of the experiment, notable overall reductions were observed across treatments (Table 5.1).

Table 5.1: Percentage reduction in TPH content in each treatment by week 20.

TREATMENT	TPH REDUCTION (%)
Cow + Chicken	96.0
Fungi	87.3
Cow + Chicken + Fungi	95.4

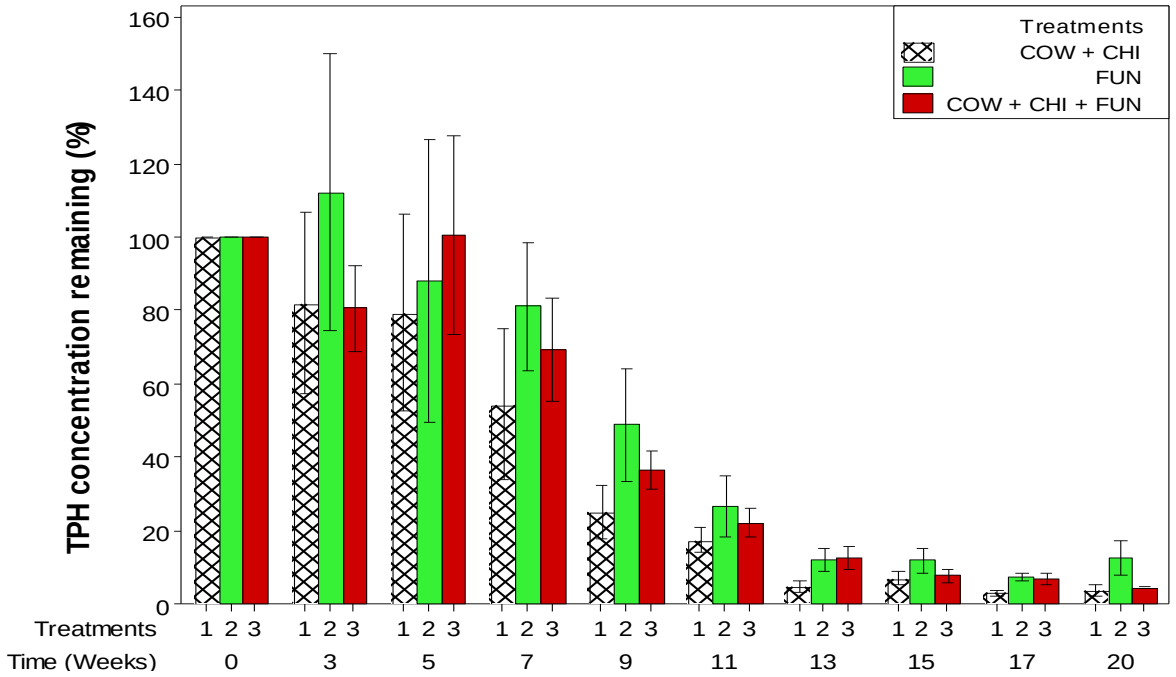


Figure 5.1: Mean percentage of TPHs remaining across the different treatments after the 20-week experimental period. The overall TPH remaining in all the treatments reduced over time. Error Bars represent ±S.E. (n = 3).

There was a significant reduction of the TPH concentration across the three treatments over time (one-way ANOVA F (9, 23) = 25.08, $p < 0.001$) although post hoc test Tukey-Kramer ($p < 0.05$) shows that despite reductions in the TPH concentrations by week three across the treatments (Table 5.2), the most significant decline was observed from the 9th to the 20th week.

Table 5.2: Tukey-Kramer post hoc test for the percentage mean TPH remaining of the samples through the experimental period at 95% confidence. A significant average decrease was observed at week nine across all the treatments. Means that do not share a letter are significantly different.

Time (Weeks)	N	Mean TPH remaining (%)	Tukey-Kramer
0	9	100.0	A
3	9	91.4	A
5	9	89.3	A
7	9	68.23	A B
9	9	36.84	B C
11	9	22.27	C
13	9	9.92	C

15	9	9.09	C
20	9	7.11	C
17	9	5.841	C

Although, it should be noted that reduction across all treatments can be clearly delineated from the fifth week as seen in Figure 5.1. The degradation pattern followed a typical first-order degradation. A pattern is often reported by most authors independent of the starting concentrations. Thus, the degradation of the TPHs by the different treatments was determined using a first-order kinetic model. Table 5.3 compares the different TPH biodegradation rates of each treatment combination.

Table 5.3: Parameters for the first-order biodegradation of TPHs in the treated sample during the 20-week process. The displayed r^2 values indicate the best fit for the first-order decay model applied.

TREATMENT	FIRST-ORDER RATE CONSTANT, k (DAY^{-1})	COEFFICIENT OF DETERMINATION (r^2)
Cow + Chicken	0.206	0.86
Fungi	0.154	0.82
Cow + Chicken + Fungi	0.180	0.90

The first-order kinetics estimation is commonly used to assess the biodegradation of organic compounds because of simple application and high prediction accuracy (Namkoong *et al*., 2002; Venosa *et al*., 1996). These kinetic rate constants demonstrate the efficiency and rapidity of the various treatment combinations in this study.

The highest rates of TPH degradation are recorded in treatments containing animal manures. Treatments containing only animal manures possessed a marginally higher rate constant compared to the combined treatment of animal manure with fungi, in turn marginally higher than fungi alone. The three treatments under assessment exhibited biodegradation kinetic rates, ranging from 0.154 day^{-1} to 0.206 day^{-1}. The coefficient of determination (r^2) value showed that treatment 3 (cow, chicken and fungi) was a better fit for the first-order rate decay model applied. This was followed by treatment 1 (cow and

chicken) and treatment 2 (fungi only) with r^2 values of 0.86 and 0.82 respectively. The fungi treatment had a comparatively lower rate constant and Hatakka (1994) reports that *P. ostreatus* generally exhibited slower degradation rates of hydrocarbons possibly due to its selective degradation ability resulting in its classification as a moderate lignin degrader. Cerniglia (1997) points out that bacteria can use other hydrocarbons as their sole source of carbon and energy and mineralise hydrocarbons more rapidly than fungi (which do not utilise hydrocarbons as their sole source of carbon and energy). This suggests that for fungi to be more efficient in breaking down hydrocarbons, they must be complemented with additional carbon sources which can be in the form of animal manures. The effect of different white-rot fungi on the degradation of coal-tar contaminated soil during a microcosm study by Canet *et al*. (2001) found that the highest degradation of hydrocarbons was achieved when no fungi were added to the soil. Implying that when fungi are not introduced, native microorganisms colonised the straw added as organic substrate thus degrading the hydrocarbons co-metabolically, whereas with the introduction of fungi, the colonisation of the straw by natural microflora was inhibited by the fungi previously inoculated to the straw. It seems possible that *P ostreatus* species of white-rot fungi will have difficulty in breaking down weathered hydrocarbons as these often contain heavier and more recalcitrant compounds present in them. Another plausible reason for the lower degradation rate seen in the fungi only treatment of the current study compared to the animal manure treatments is reported by Eggen & Sveum (1999) who treated weathered creosote-contaminated soil and found that in temperatures as low as 8 °C, fungal inoculation had the highest biodegradation efficiency when no organic manure was added. It may be the case therefore that the mesophilic temperatures recorded in the fungi only treatment may not have provided favourable conditions for fungal degradation of the hydrocarbon contaminants to take place effectively

Although hydrocarbon degraders have a significant presence and diversity in contaminated soils, Nie *et al*. (2009) state that the relationships between bacterial communities and TPH concentration differs amongst various studies. LaMontagne et al. (2004) found that overall bacterial diversity and abundance reduce with increasing soil hydrocarbon content.

The Subset regression used showed the various factors that influenced the TPH concentrations in each treatment. These factors were subsequently fit into a regression model to describe their relationship with TPH concentration (Table 5.4).

Table 5.4: Subset and multiple regression comparing factors influencing TPH concentration.

Treatments	r^2	C_p	S	MC	OM	EC	NO_3^-	PO_4^{3-}	Cl^-	Temp
Cow + Chicken	86.5	3.0	70860	0.017			0.073	0.001*	0.001*	0.300
Fungi	88.1	6.5	54764	0.737	0.001			0.001*	0.001*	0.001*
Cow + Chicken + Fungi	85.8	2.0	43443		0.050	0.001*	0.015		0.001	0.044

Asterisks mark different levels of significance: *p < 0.001; C_p, Mallows' statistic; S, Error standard deviation; r^2, coefficient of determination

The regression equation for each of the treatments is estimated as:

$$\boldsymbol{TPH_{Co+Ch}} = -235959 + 16326\,(MC) - 8618\,(NO_3) + 5691\,(PO_4) - 286.8\,(Cl^-) - 11822\,(℃) \quad \textbf{(Equation 5.1)}$$

$$\boldsymbol{TPH_{Fun}} = \mathbf{9}10262 + 2010\,(MC) + 20199\,(OM) + 14781\,(PO_4) - 290.8\,(Cl^-) - 90920\,(℃) \quad \textbf{(Equation 5.2)}$$

$$\boldsymbol{TPH_{Co+Ch+F}} = -39542 + 6966\,(OM) + 268.3\,(EC) + 6971\,(NO_3) - 135.1\,(Cl^-) - 18545\,(℃) \quad \textbf{(Equation 5.3)}$$

The regression models in Equations 5.1, 5.2, and 5.3 show the main limiting factors concerning TPH concentration levels in the individual treatments. The models suggest there to be shared variations between the parameters of the treatments. Temperature and chloride are common variables that influence the rate of hydrocarbon breakdown as shown in the regression equations. It could be possible that in the treatments containing animal wastes, increases in these variables will enhance the rate of TPH degradation. In contrast, in the fungi only treatment, the reverse seems to be the case. This could possibly be due to the different nutrient, and temperature requirements of microbial populations in animal wastes and fungi inoculated substrate. The treatments containing animal wastes are expected to have more organic matter compared to the fungi only treatment and it may be suggested from Equations 5.1 and 5.3 that an increase in the OM of the fungi only treatment and its reduction in the fungi and animal waste treatment could improve their biodegradation rates respectively. Animal manures are likely to get saturated due to their water retention capacity, and this level of saturation is not often favourable for biodegradation. This may explain the reduced moisture requirement for enhanced degradation seen in Equation 5.1. Unsurprisingly, the fungi only treatment would likely benefit from organic matter and moisture content increase for higher effectiveness. From these observations, OM may be a surrogate for moisture content as it could probably be wetter at the start of the process,

which could subsequently improve the degradation rates. It seems probable that the combination of fungi and animal waste constitutes a matrix that is balanced in its phosphate requirements as opposed to when these treatments and separate, as shown in Equations 5.1 and 5.3.

The overall predictions of each treatment's model generally indicate the influences of nutrient availability, moisture content and organic matter on TPH reduction. A closer look at Equation 5.3 would suggest that the combination of bacteria and fungi substrate requires less nitrate for efficient degradation to occur. This contrasts with Equation 5.1, which suggests an increase in nitrate would provide better conditions for TPH degradation when only animal manures are used. Hydrocarbon spills adversely affect soil ecology, fertility and physicochemical properties. It increases toxins, such as zinc and iron in the soil (Udo & Fayemi, 1975), resulting in reduced amounts of nutrients.

At the end of the experimental process, an assessment of the individual efficiencies of treatment combinations on the polluted soil sample was evaluated further by examining the various end concentrations of TPH (Figure 5.2). It is important to note that there was a prominent degree of variation of over 200000 mg/kg for the initial sample concentrations between treatments, as shown in week 0. This can possibly result from sample heterogeneity despite best efforts to prevent such via thorough mixing of samples before being transferred to the different treatment reactors. Ensuring the sample was as homogenous as possible was crucial in the study.

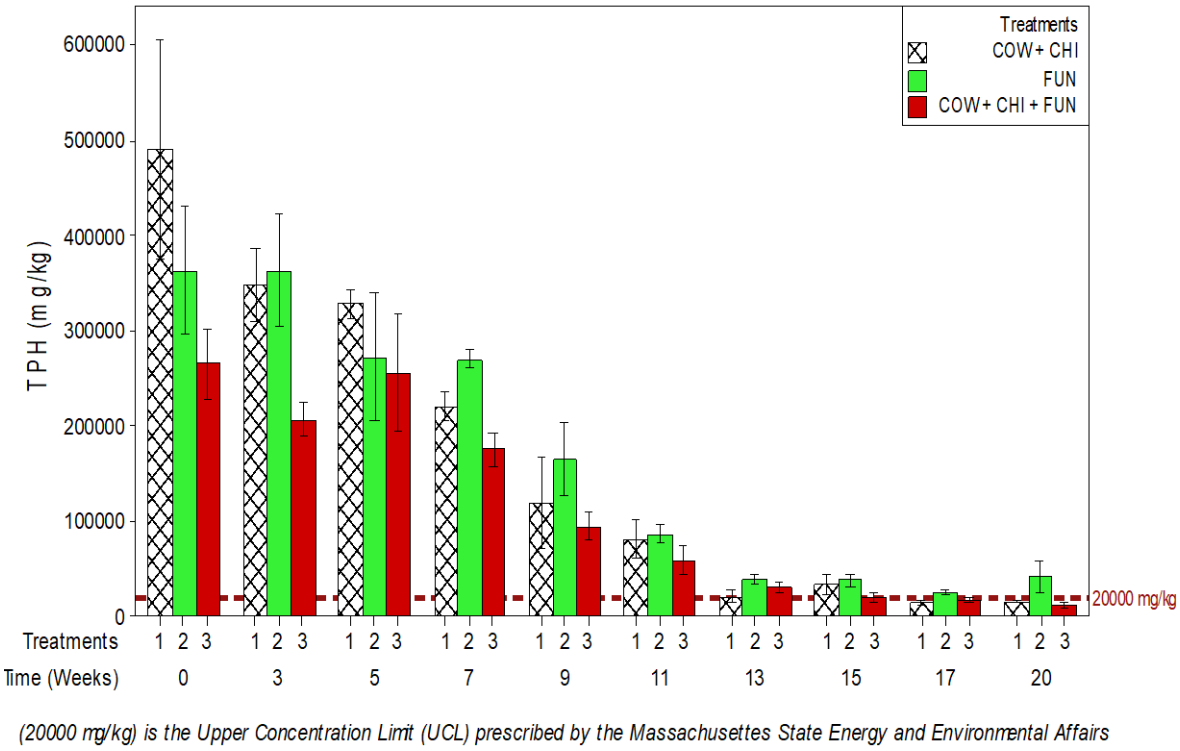


Figure 5.2: Total TPH concentrations over the 20-week experimental period. Error Bars represent ±S.E. (n = 3).

The steady decline in the residual TPH concentrations is quite apparent, as it appears that one-twentieth of the initial concentrations were present by the end of the 20-week composting process across all treatments.

These end concentrations have been compared with data from the Massachusetts Energy and Environmental Affairs contingency plan that prescribes the use of Upper Concentration Limits (UCLs) to establish whether the levels of hydrocarbon pollutants in soil, surface water, and groundwater are within permissible limits for use. The UCLs specify the levels of contamination in soil and groundwater, which, if exceeded, may potentially pose a significant risk of harm to human health/public welfare, environmental resources, biota and habitats. While the UCL is a credible and verified guideline limit for evaluating TPH concentration, it is essential to be mindful that other agencies and governmental bodies alike have similar limits. Individual adoption depends on the end-use of the product and potential exposure to humans and animals.

Whilst the recorded decline in contaminant concentrations across treatments is encouraging and significant, further reductions may have been hindered by specific properties of contaminants being remediated in the soil. In this study, the hydrocarbon-contaminated soil was classified as weathered. It is believed that the light fractions of the hydrocarbon pollutants were possibly primarily degraded or transformed through biodegradation or other

physical processes. Suja *et al*. (2014) support this view and mention that weathering affects the biodegradation of petroleum hydrocarbons because of decreasing bioavailability. In addition, the GC-MS chromatographs of TPH analysis in Figure 5.3 showed that a significant proportion of the hydrocarbons were in the diesel fractions (middle range hydrocarbons from about C_{10} to C_{18}). According to Clark (1989), diesel does not readily evaporate like lighter fuels such as gasoline, thus rendering it persistent in the environment. It is also important to note that due to the lipophilic character of the hydrocarbon contaminants preferentially adsorb to particulate material and therefore accumulate in high concentrations in soils (Conell & Miller, 1984; Neff, 1979; Witt, 1995). The GC-MS chromatograph is also useful because of its ability to display the reduction of TPH concentration in a simple manner. This is reflected by the continuous size decrease of the area under the curve representing TPHs present in the tested sample.

It can be deduced from the TPH end concentrations that the composting process effectively broke hydrocarbon pollutants. That being said, the presence of amendments in the form of fungi and animal wastes all seem to have played a pivotal role in achieving this. Thomas *et al*. (1992) had earlier pointed out that the addition of organic amendments tended to increase the degradation rate of target contaminants. However, the authors caution that when added in excessively, these amendments have an inhibitory effect on the rate of pollutant degradation. Proceeding on this point, Cookson (1995) states that the carbon source in the amendments must not be a preferred carbon source that pre-empts the degradation of the target contaminants.

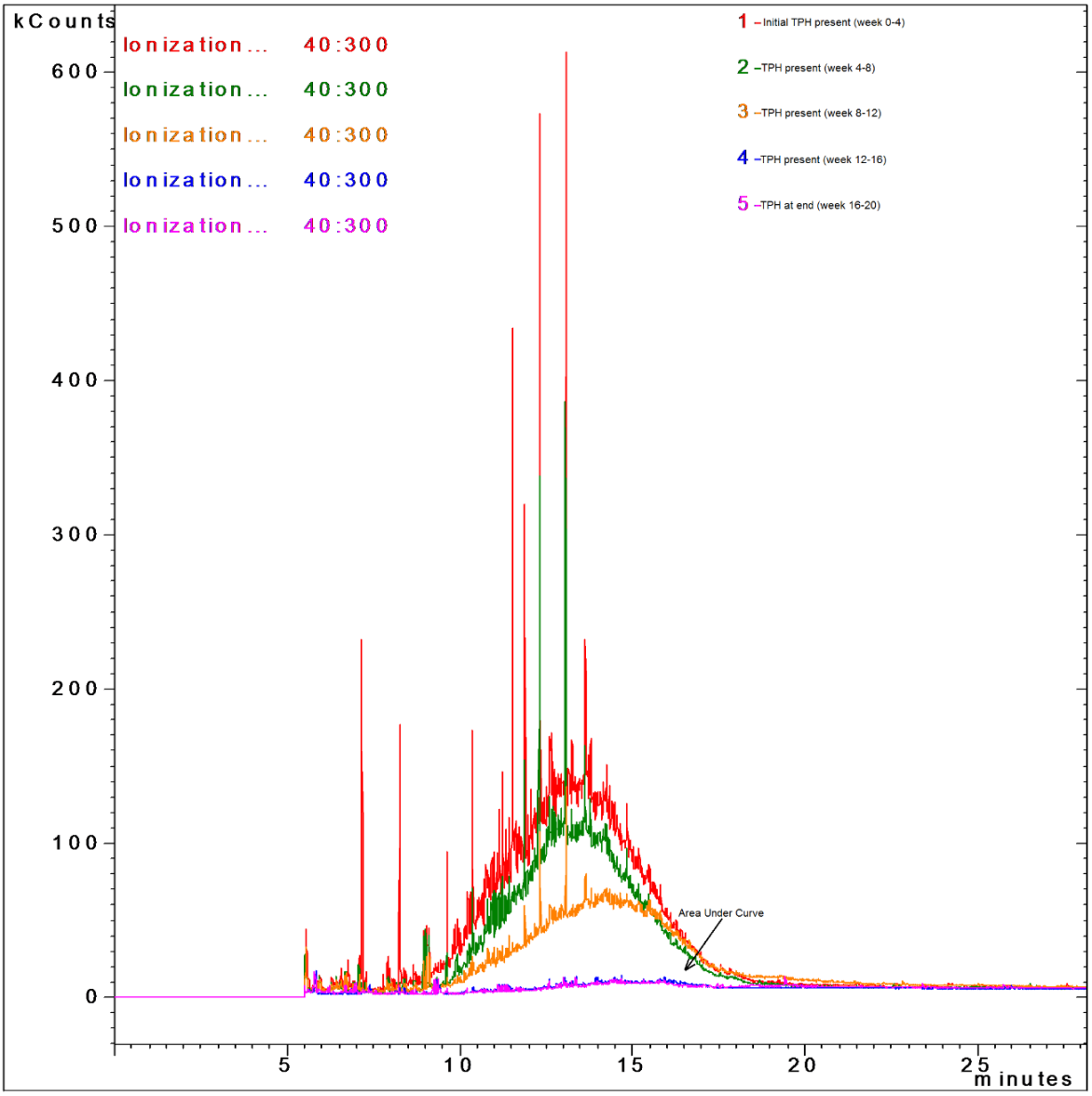


Figure 5.3: Chromatograph showing the diesel fraction (C_{10} to C_{18}) of the available TPH within the sample.

5.1.2 Changes in Polycyclic Aromatic Hydrocarbon

The effectiveness of composting as a remediation method for hydrocarbons was also assessed based on the reduction of PAH concentration. Thus, for monitoring the changes in pollutant levels, a grouping of PAHs into LMW, MMW, and HMW was undertaken. This categorisation provided a valuable system of summarising contaminant concentration during the process.

5.1.2.1 Low Molecular Weight (LMW) PAHs Percentage remaining

The concentration of LMW PAHs was significantly lower (one-way ANOVA F (9, 20) = 2.63, p = 0.034) in all the treatments by the end of the experimental period. However, it can be seen that the fungi in combination with animal manures performed comparatively poorer, degrading only 26.2% of the PAHs. On the other hand, the fungi treatment and animal manure treatments performed considerably better, degrading up to 49.8% and 68.5% of the PAHs respectively over the experimental period (Figure 5.4).

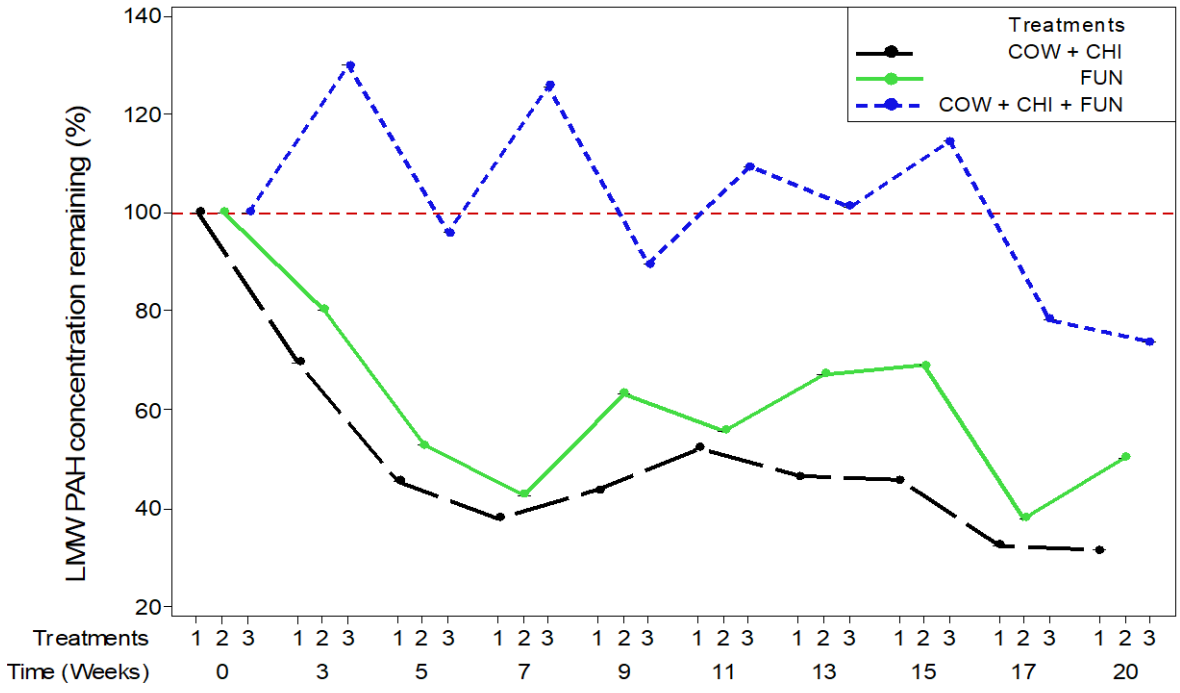


Figure 5.4: Mean percentage of LMW PAHs remaining in the different treatments of the 20-week experiment. Error Bars represent ±S.E. (n = 3).

Interestingly, the treatments that primarily consisted of single amendments, i.e. animal manure only and fungi, exhibited a similar pattern. Both treatments appeared to rapidly degrade the LMW PAHs within the first seven weeks. According to Alexander (1999), LMW PAHs are very soluble, volatile, and possess simple molecular structures, making them highly susceptible to use by many microorganisms as sole sources of carbon. It is also likely

that the forced aeration within the reactor vessel played a role in the losses of these PAHs. Williamson *et al*. (2009) opine that by virtue of their low K_{OW} values, LMW PAHs losses due to volatilisation are significantly enhanced by the forced aeration regimes utilised in composting reactor units.

In sharp contrast, the combination of animal manures and fungi yielded an irregular degradation pattern with several increases in PAHs noted at specific time points. A possible reason for this is sample heterogeneity. Mineralisation would probably have occurred because of the regular turning of the matrix or rainfall simulation. These factors could weaken the extent to which non-extractable LMW PAHs are bound to soil organic matter.

Whilst treatments 1 (cow and chicken manure) and 2 (fungi only) displayed similar trends in their percentage concentration remaining, treatment 3 appeared to vary significantly. The alteration of the chemical structures of MMW PAHs due to possible degradation may potentially result in the formation of metabolites that are LMW PAHs. This could possibly explain the inconsistency seen in treatment 3 (cow, chicken and fungi) vis-a-vis the intermittent hikes in the percentage concentration remaining observed. This notion is reinforced by the significant negative correlation (*rho* = -0.747, $p < 0.001$) between both PAHs categories, suggesting elevated levels of MMW PAHs would lead to increases in LMW PAHs.

5.1.2.2 Medium Molecular Weight (MMW) PAHs Percentage remaining

The MMW PAHs in all the treatments showed a significant decrease by the end of the experimental period (Kruskal-Wallis H (9) = 21.48, $p = 0.011$). The treatments displayed similar trends regarding the percentage remaining of these PAHs by week 20 (Figure 5.5). Further statistical analysis showed no significant difference between the treatments (Kruskal-Wallis H (2) = 1.43, $p = 0.488$) in terms of the MMW PAH percentage remaining at the end of the experimental period. However, it is worth pointing out that treatments one (cow and chicken) and two (fungi only) had marginally higher percentage removals at the end of the 20-week test period with 54.1% and 52.1% respectively, compared to the 41.9% for treatment three (cow, chicken and fungi). These MMW PAHs may have been lost due to leaching during the rainfall simulation. These compounds are less recalcitrant and can be transported via water added to the matrix.

The relatively similar losses in all three treatments may be attributed to the presence of the organic amendments. Williamson *et al.* (2009) explain that organic amendments directly influenced the microbial mineralisation of MMW PAHs through nutrient input and indirectly

via the enhancement of biomass which led to improved aeration suitable for contaminant degradation. Perhaps there is a possibility that the forced aeration stimulated the degradation of the pollutants.

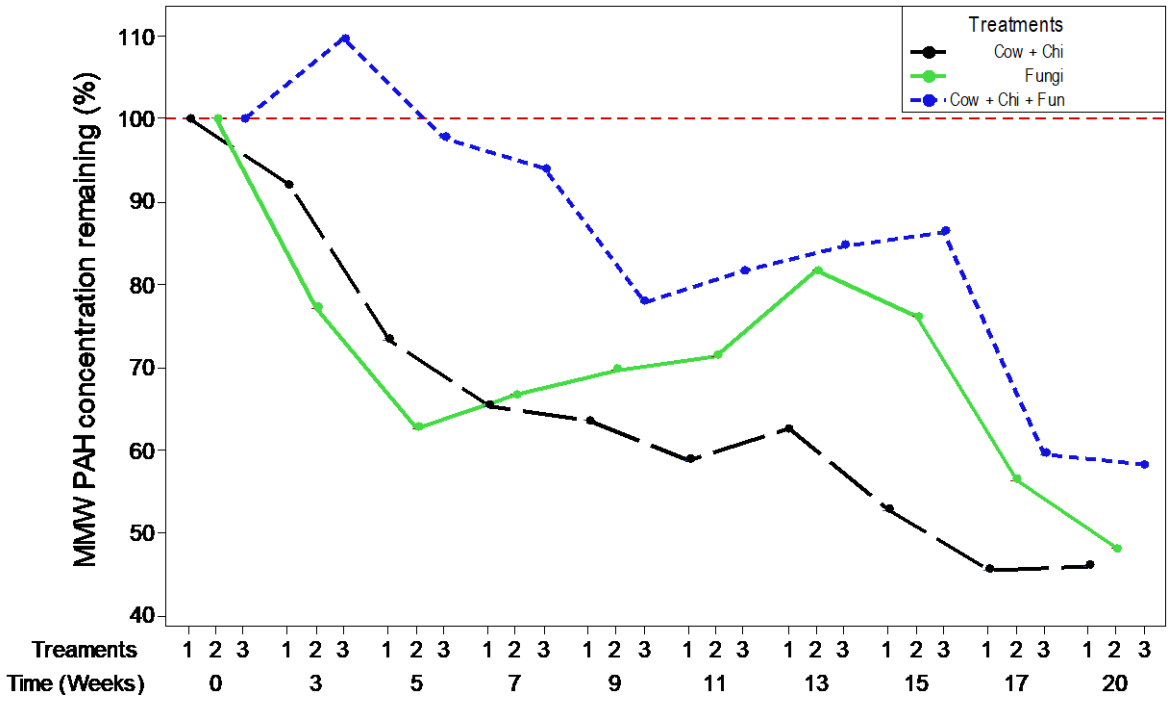


Figure 5.5: Mean percentage of MMW PAHs remaining in the different treatments of the 20-week experiment. Error Bars represent ±S.E. (n = 3).

It is also reasonable to assume temperature played a role in the loss of this category of pollutants. Bonten *et al*. (1999) indicated that temperatures as high as 120 °C enhanced the biodegradation of MMW PAHs in soils that possessed high organic matter while having the same temperature did not affect soils with high sand content. The authors explain that MMW PAHs in soils become more readily desorbed from the organic matter with higher temperatures, thus rendering them more bioaccessible for biodegradation. Francou *et al.* (2005) and Oleszczuk (2006) established that the greater biodegradation rate of MMW PAHs occurred mainly during the maturation phase rather than the composting stage. Thus, the authors indicate that mesophilic rather than thermophilic microflora were more capable of degrading this group of PAHs. The results of the current study appear to agree with this finding. Antizar-Ladislao *et al*. (2006) state that although temperatures above 70 °C is required by current legislation (BSI, 2011) to eliminate pathogens, such temperatures severely hamper microbial diversity and therefore biodegradation potential.

5.1.2.3 High Molecular Weight (HMW) PAHs Percentage remaining

The HMW PAHs remained virtually unchanged throughout the process, with a mean percentage remaining over 100% across all three treatments. it is important to note that there were marginal losses observed in treatments one and two by week 20; treatment three showed no reductions at any time point of the process (Figure 5.6). The concentration levels were likely beyond the detection limits of the GC-MS.

There was a significant difference amongst the treatments over time (Kruskal-Wallis H (9) = 21.61, p = 0.010). However, a Tukey post-hoc test indicated that there was no overall significant difference ($p < 0.05$, n = 3) between week 0 and 20 for all the treatments. Relative to the initial percentage concentration remaining, the losses in HMW PAHs by week 20 was generally seen to be minimal in treatment one (6.33%) and treatment two (7.84%). On the contrary, treatment three (cow, chicken, and fungi) had a 24.84% increase in HMW PAHs. The manure only treatment and fungi only treatment displayed a similar profile over time and perhaps gives an indication that these amendments perform better independently hence the slight reduction in HMW PAHs observed at the end of the experiment in both treatments. The slightly better performance of treatment 2 the (fungi only) may be due to white-rot fungi' ability to degrade humic-bound HMW PAHs despite the covalent binding of these PAHs to humic substances (Wunderwald *et al*., 2000). A similar pattern was observed with the cow and chicken treatment, it is possible that sample variability played a key role in the outcome. The recalcitrance of these HMW PAHs may also be a reason for their prevalence even after treatment. Huang *et al*. (2004) suggest that HMW PAHs remain problematic due to their strong hydrophobic properties and stability, particularly when dealing in aged contaminated soil. Perhaps, the weathering that the hydrocarbon contaminated samples used in this study have undergone may mean fractions of these compounds are less bioavailable and are more difficult to breakdown.

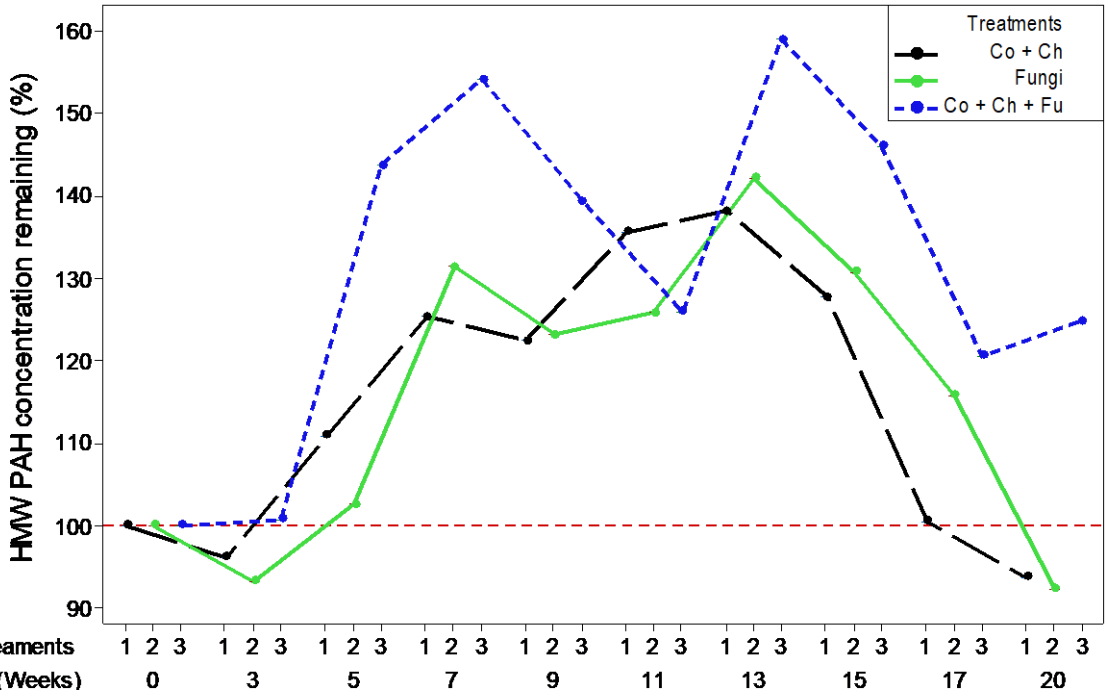


Figure 5.6: Mean percentage of HMW PAHs remaining in the different treatments of the 20-week experiment. Error Bars represent ±S.E. (n = 3).

The observed increase in HMW PAHs could be attributed to several factors. According to Tabak & Govind (1997), HMW PAHs are hydrophobic, tightly bound to soil particles, and are slow to desorb thus making their degradation difficult. It is, therefore, no surprise that bioavailability has been widely regarded to be vital in the breakdown of these contaminants. The releases of PAHs from sorptive sites due to temperature changes or substances acting as surfactants have been suggested to be responsible for the elevated concentration of HMW PAHs even after bioremediation (Gilot *et al*., 1997; Wong *et al*., 2004). However, experimental variability and errors could possibly explain the observed increases in the HMW PAHs during this study.

5.1.2.4 Changes in the total sum of PAHs Percentage remaining and Concentrations

There was an overall reduction in the percentage remaining of the total PAHs monitored. The treatments differed significantly (Kruskal-Wallis H (2) = 17.76, $p < 0.001$) with the lowest removal rate observed in treatment three after the 20-week testing period. Treatment one (cow and chicken manure) had the highest total PAH removal at 60.91%., followed by treatment two (fungi only) with a removal of 45.78%. The least effective was treatment three (animal manures combine with fungi) with a removal of just 20.23% of the total PAHs. The degradation of the total PAHs has displayed a biphasic pattern that consists of a preliminary short period of accelerated losses and a subsequent lengthier period of slower losses (Wilson & Jones, 1993). This phenomenon is evident in the results of this study (Figure 5.7).

It is important to note that there was an observed increase in the total sum of PAHs remaining for treatment 3 (cow, chicken and fungi) and this is a direct result of the low reduction rates recorded in the LMW and HMW PAHs of this treatment. This may have been a result of sampling error or equipment (GC-MS) defect at the time of sample analysis. It is possible that due to the heterogeneity of the matrix, the sample collected for analysis contained a comparatively higher concentration of PAHs which may have had inhibitory effect on the breakdown of these compounds due to their toxicity on the microbial population within the soil. Cerniglia (1992) states that the higher water solubility of LMW PAHs, often results in acute toxicity to microorganisms thereby rendering them incapable of biodegradation. Whereas, HMW PAHs have low water solubility and therefore tend to bind to organic matter within the soil matrix, thus making them less bioavailable for hydrocarbon utilising microorganisms. It is a possible reason for the overall increase in the total sum of PAHs observed for treatment 3 (cow, chicken, and fungi) in the current study.

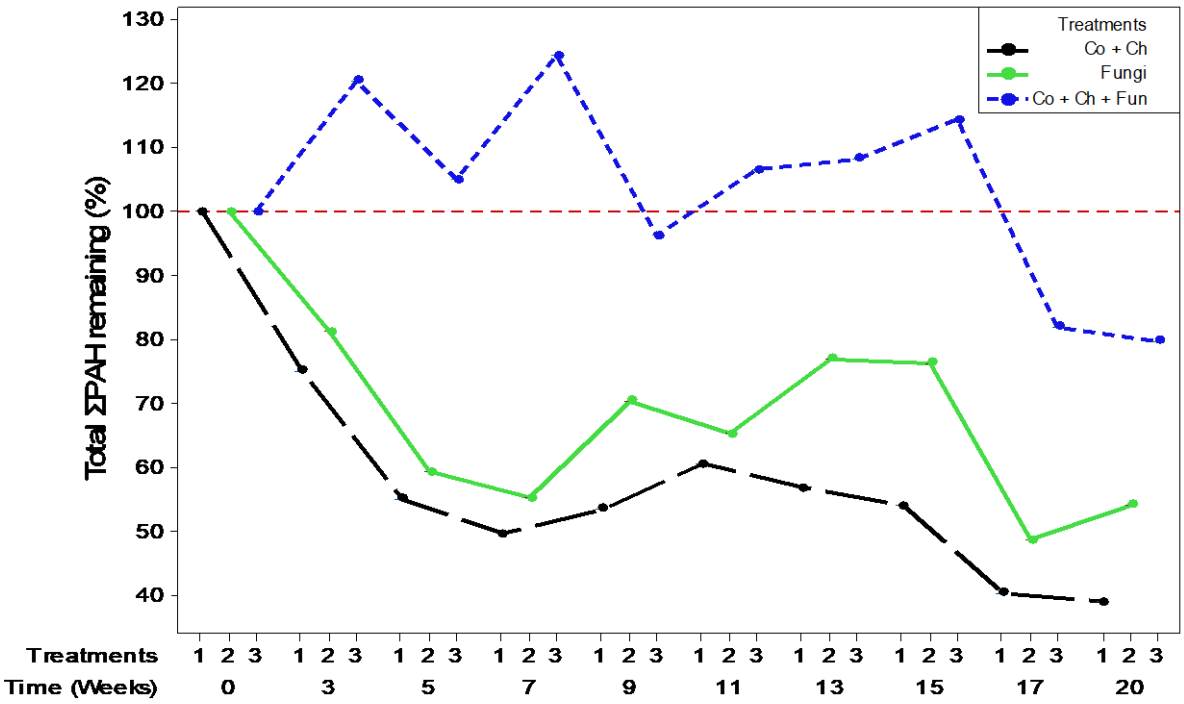


Figure 5.7: Mean percentage of the total sum of PAHs remaining in the different treatments of the 20-week experiment. Error Bars represent ±S.E. (n = 3).

The total percentage of PAH remaining can be seen to have dropped; however, the inclusion of the HMW PAHs data is likely to skew the results as considerably higher MMW and LMW PAHs degradation levels were achieved. Although there was a general decline in total PAHs, it is possible that by increasing the time for the process a greater level of reduction may be achieved particularly with the HMW PAHs.

Although, the method of grouping PAHs according to the molecular weights is a valuable tool in understanding and summarising contamination. The concentration of individual PAHs within these groups may distort the results obtained. This is reflected in subsequent statistical tests that revealed no significant difference (Kruskal-Wallis H (2) = 4.29, p = 0.117) in terms of the percentage remaining between weeks 0 and 20 for all the treatments.

It is important to note that there is considerable variability in the initial concentrations between treatments with notable outliers, particularly in anthracene, benzo(a)anthracene and acenaphthene. These inconsistencies are likely due to errors during sampling or the GC-MS analysis.

The percentage of the LMW PAHs were generally lower compared to the start. With the exceptions of fluorene which was significantly higher at the end of the animal manure treatment. There were no naphthalene reductions in the fungi only and animal manure combined with fungi treatments. Anthracene also showed no reduction in the fungi-manure combination treatment. All the MMW PAHs indicated a decrease in the end concentrations except for benzo(a)anthracene and chrysene in treatments two (fungi only) and three (cow, chicken and fungi). As expected, the HMW PAH percentage remaining did not differ between the treatments.

The overall trend in the results suggests that LMW PAHs and MMW PAHs mostly seemed to show a more significant reduction in their percentage remaining in the soil compared to the HMW PAHs. A host of studies have reported similar findings while investigating the use of bio-amendments to enhance hydrocarbon remediation in contaminated soils. As expected, the highly hydrophobic nature and electrochemical stability of HMW PAHs in contaminated soils make them persistent and their degradation quite problematic (Antizar-Ladislao *et al.*, 2005; Mueller *et al*., 1996; Weissenfels *et al*., 1992).

The end concentrations of individual PAHs for all the treatments have been compared to existing soil and sediment quality guidelines (Figures 5.8, 5.9, and 5.10). These concentrations will help establish the suitability of composting as a remedial method for hydrocarbon contaminated soils.

Comparing final concentrations of LMW and MMW PAHs with some of the existing soil and sediment quality guideline limits, composting has proven to be quite effective in reducing the concentration levels of these pollutants.

Naphthalene steadily decreased across all treatments, although the end concentration slightly exceeded the 2100 ng/g (ANZECC & NHMRC, 1992) Interim Soil Quality Guidelines

($ISQG_{high}$) upper threshold limit for the contaminant. A steep decline in acenaphthene was observed by the third week across all treatments, and its concentration remained consistently low and below the 500 ng/g $ISQG_{high}$ limit. Phenanthrene displayed a similar reduction pattern to naphthalene. It was seen to be slightly above the permissible concentration (1500 ng/g) for the animal manure only and fungi only treatments, which had end concentrations of 1962.4 ng/g and 2285.6 ng/g respectively. Remarkably, anthracene followed a similar trend as acenaphthene by dropping below the guideline $ISQG_{high}$ limit (1100 ng/g) by week three across all treatments, maintaining a concentration of 883.2 ng/g throughout the composting process.

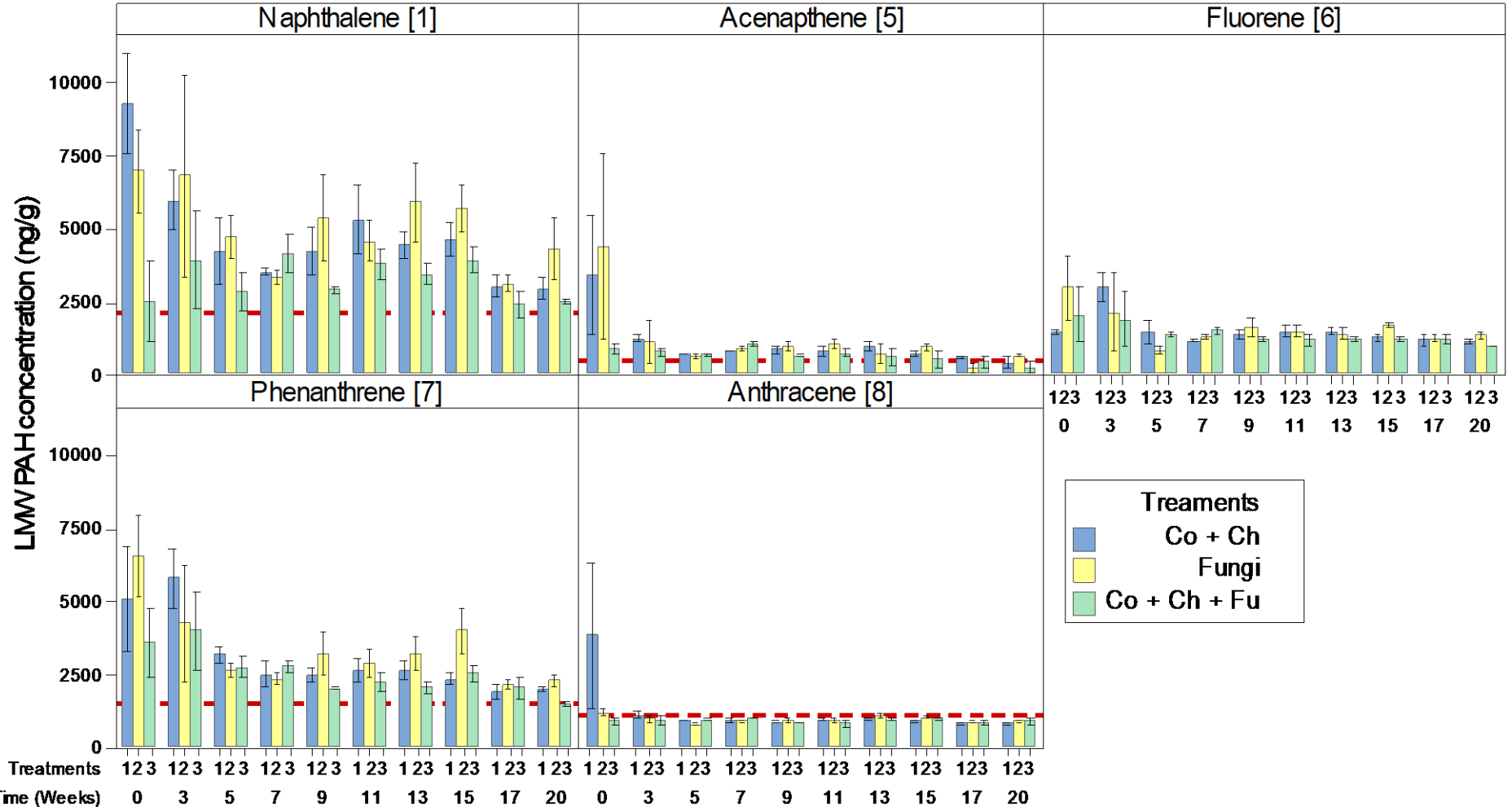


Figure 5.8: LMW PAH concentration changes over time across all the treatments. The red dotted lines indicate guideline threshold. Error Bars represent ±S.E. (n = 3).

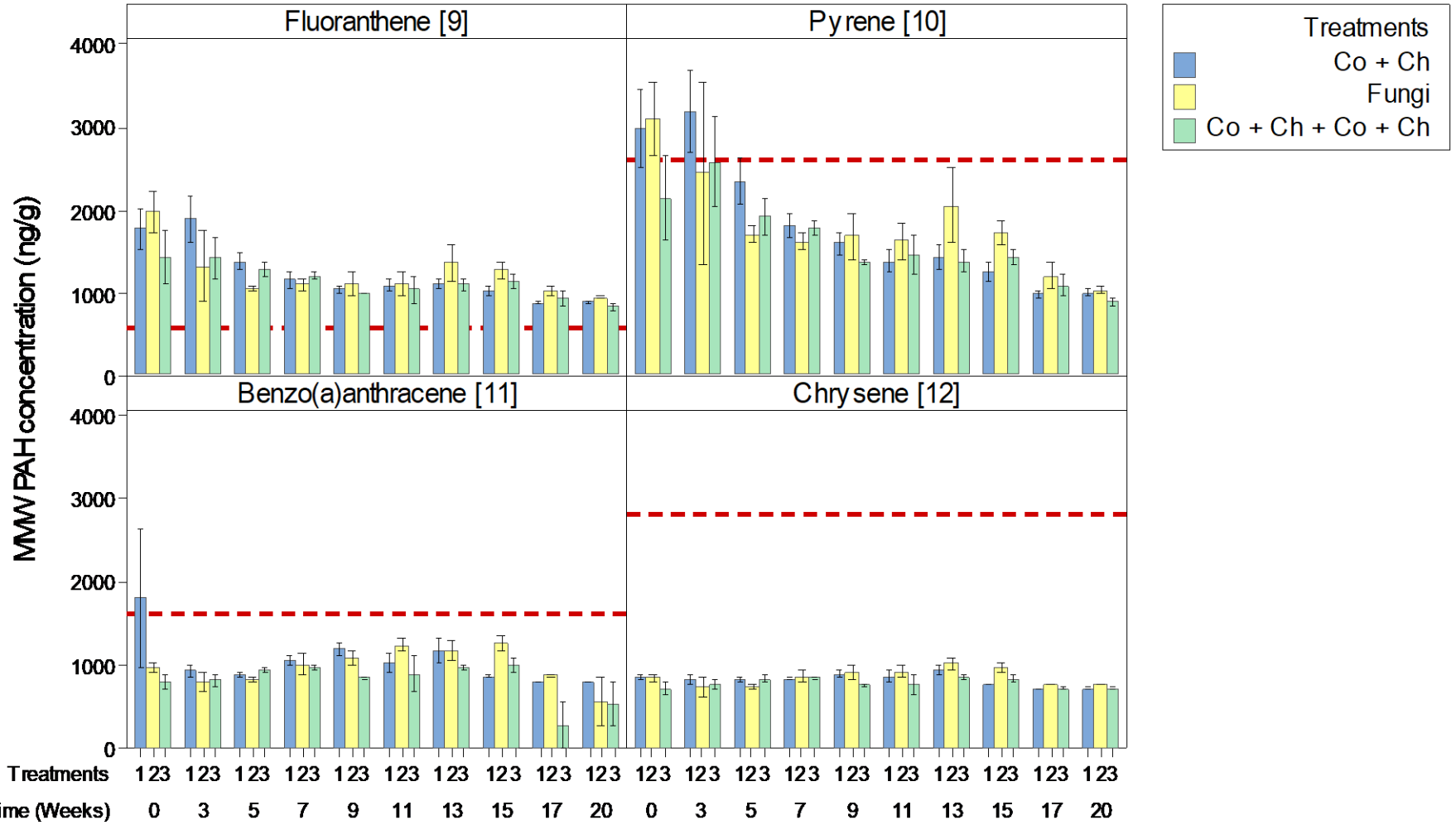


Figure 5.9: MMW PAH concentration changes over time across all the treatments. The red dotted lines indicate guideline threshold. Error Bars represent ±S.E. (n = 3).

In the MMW PAHs, benzo(a)anthracene and chrysene were both low throughout the composting process. Although there were continuous reductions in fluoranthene, the end concentration was still marginally higher than the lower $ISQG_{low}$ threshold limit of 600 ng/g but below the $ISQG_{high}$ threshold limit of 5100 ng/g. Pyrene on the other hand steadily declined with an end concentration of 967.9 ng/g, which is over 60% below the guideline value.

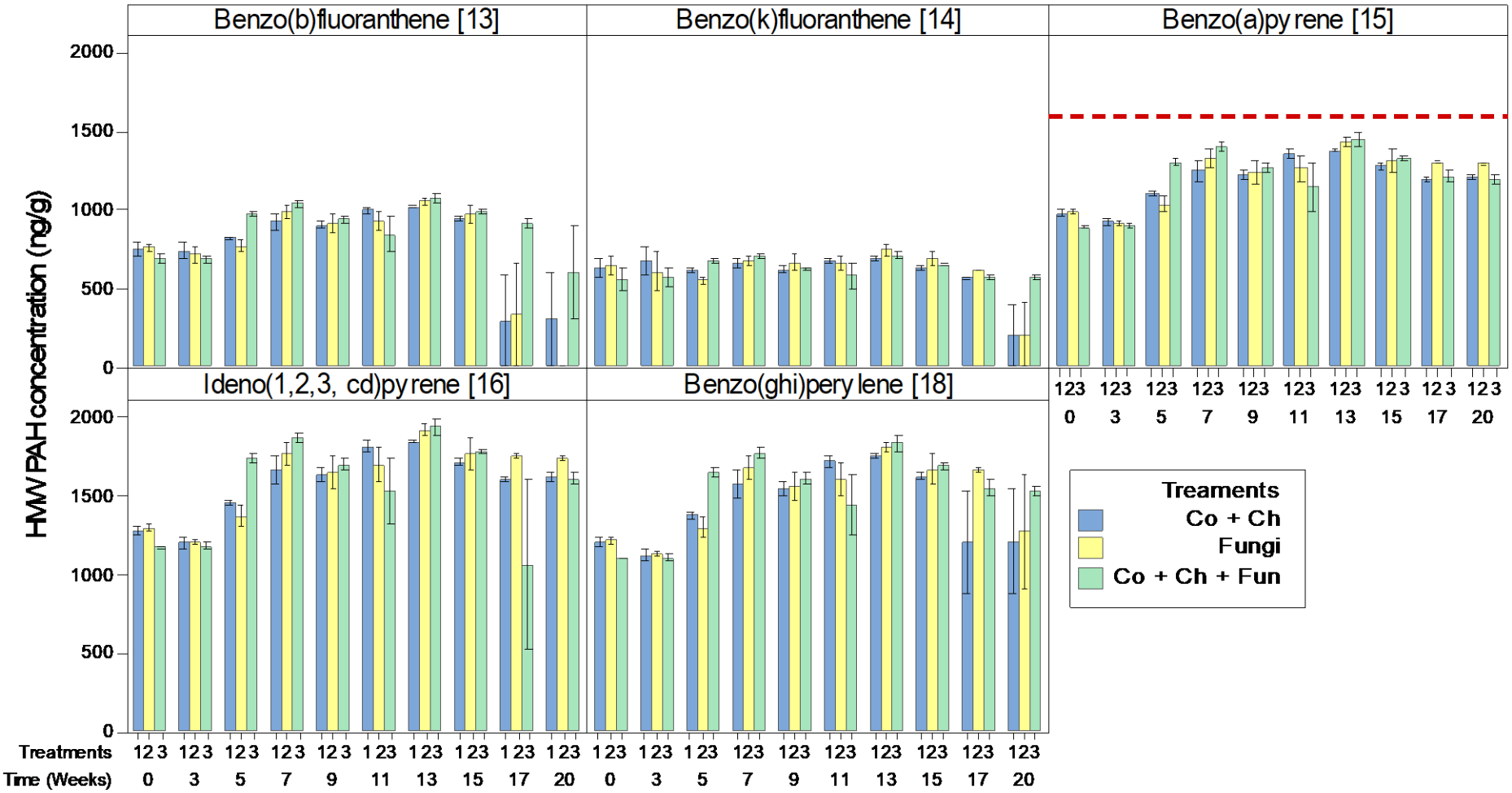


Figure 5.10: HMW PAH concentration changes over time across all the treatments. The red dotted lines indicate guideline threshold. Error Bars represent ±S.E. (n = 3).

The HMW PAHs concentrations were all found to be below proposed guideline values for all the treatments throughout the entirety of the process. It is worth pointing out that with the exceptions of benzo(b)fluoranthene and benzo(k)fluoranthene all the other PAHs in this group demonstrated either similar or higher end concentrations. This indeed is a testament to incredibly recalcitrant properties contaminants. Interestingly, benzo(b)fluoranthene was found to have been completely lost in the fungi only treatment. Elsewhere, the animal manure only and fungi only treatments recorded over 60% reductions in benzo(k)fluoranthene with an average mean concentration of 199.6 ng/g by week 20.

5.2 Soil Analysis

5.2.1Physico-Chemical Parameters

5.2.1.1 pH

The recorded pH values ranged from 6.51 (cow and chicken manure treatment, week 9) to 8.24 (cow, chicken, and fungi treatment, week 0) (Figure 5.11). There was a swift decline in pH values for both treatments containing animal manures, which went from slightly alkaline to a relatively neutral pH by the fifth week. However, the (cow and chicken) manure treatment further decreased to reach relatively mild acidic levels of 6.58 and 6.51 in the seventh and ninth weeks, respectively. It rose back and plateaued within a neutral pH range. Although many organisms can lower pH by CO_2 production, the results show that the fungi only treatment had a lower pH at the start than the other treatments and remained consistent by maintaining a relatively neutral pH throughout the experiment with the only notable decrease identified in week 17. Compared to other treatments, the animal manure with fungi substrate treatment had significantly different (higher) pH values (Kruskal-Wallis H (2) = 27.85, $p < 0.001$).

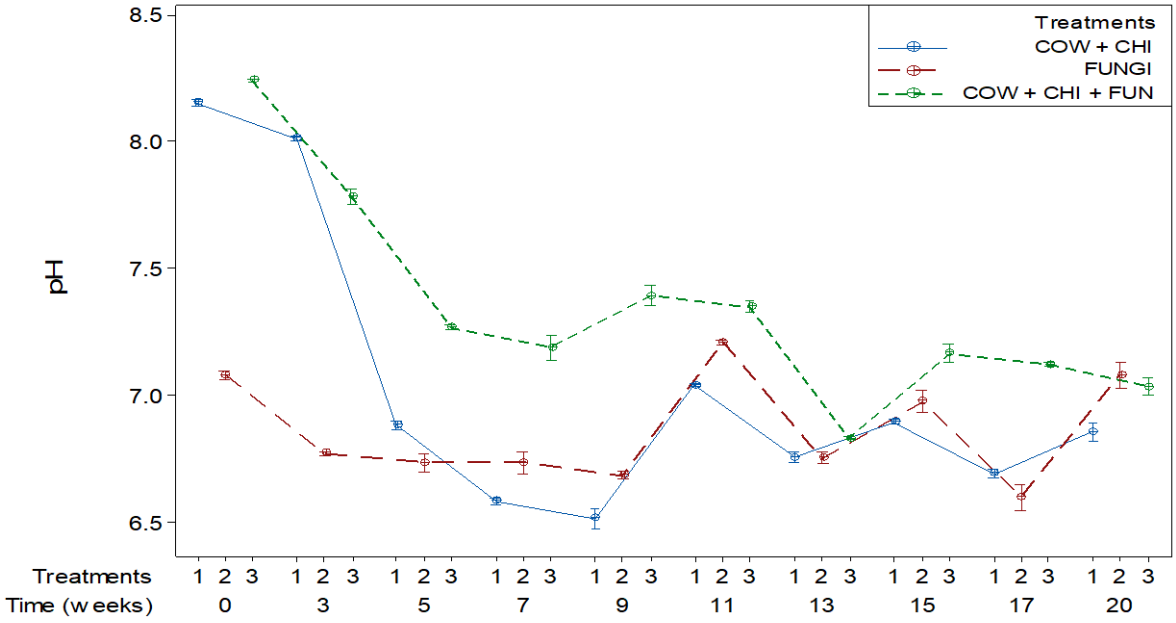


Figure 5.11: Mean pH values of the different treatments during the 20-week experimental period. Error bars represent ±S.E. (n = 3).

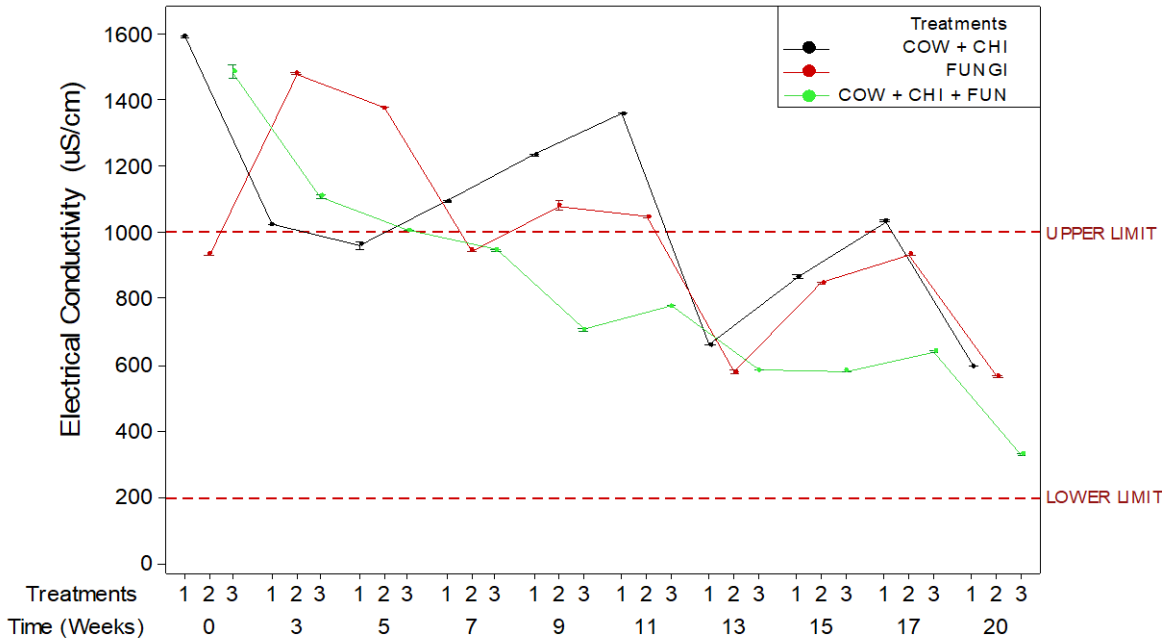
The apparent decrease in pH observed during the first five weeks in the treatments containing animal manures (chicken and cattle) may be due to the formation of organic acids during the initial stages of the composting process. Atagana (2008) explains that the decline in pH during composting may be related to manure and hydrocarbon degradation, leading to the release of acidic intermediates and final by-products that can lower pH. Another probable reason for the decrease in pH is the decomposition of organic matter and the production of organic and inorganic acids through the activities of indigenous microorganisms (Marthur *et al*., 1993). Huang *et al*. (2004) indicate that the vast quantities of CO_2 given off during composting may reduce pH. Eklind & Kirchmann (2000) state that the reduced pH seen at the later stages of composting is due to the volatilisation of ammoniacal nitrogen and the H^+ ions released as a result of the microbial nitrification process caused by nitrifying bacteria.

This behaviour (sharp decrease in pH) was not evident for the fungi only treatment which was recorded to be neutral at the initial stages of the experiment. However, the slight rises observed in the ninth and thirteenth weeks within all treatments could be due to metabolic activities possibly resulting from the production of metabolites in the compost systems. Nutrient presence inversely influenced the pH by causing an increase rather than a decrease in value as demonstrated in the cow and chicken manure treatment. Here, nitrate and chloride appeared to have a correlation with pH as indicated by a Spearman's test performed (*rho* = 0.529, p = 0.003) and (*rho* = 0.579, p = 0.001) respectively. Interestingly, however, in the treatment containing animal manure in combination with fungi substrate,

the pH was significantly influenced by phosphate (*rho* = 0.604, p < 0.001), as did chloride (*rho* = 0.713, p < 0.001) which seemed to affect pH regardless of the amendment combination used. Hanlon (2012) explains that as pH decreases so does phosphorus, and this is caused by its reaction with iron and aluminium. These results lead to the assumption that while the predominant microorganisms present in animal manures and fungi can combine to break down hydrocarbons, the nutrient required for these processes to take place as indicated through the facilitation of suitable pH conditions for composting may vary. In a later study, Hoitink *et al*. (1997) found that pH less than 5 is generally unfavourable for bacteria; the consequence of which is the prevention of various key processes including the colonisation of compost by bacterial biocontrol agents. Osuji & Ukale (2005) echoed this somewhat, indicating that acidic pH affects the solubility of minerals, thus enhancing the mobility of toxic elements and compounds. According to Manahan (1994), such low pH hinders N_2 fixation and decomposition activities and contains high levels of soluble aluminium and manganese, which are potentially toxic to plants. Generally, the drop in the pH would suggest the possible initiation of the composting process and this could be a consequence of hydrocarbon degradation by microorganisms present. Leaching as a result of the rainfall simulation may also play a role in the drop in pH displayed as ions are likely to have been transferred via the liquid medium.

5.2.1.2 Electrical Conductivity

Measurements of electrical conductivity (EC) varied significantly across all treatments (Kruskal-Wallis *H* (2) = 7.84, p = 0.020) over the experimental duration and ranged between 330 to 1594.33 µS/cm (Figure 5.12). The EC in treatments that contained animal manures (cow and chicken) and (cow, chicken, and fungi substrate) exhibited similar behavioural patterns up until the fifth week, where a spike was perceptible in the manure treatment that then sharply declined in week 11. The fungi only treatment, in contrast, had a significantly lower EC at the start before reaching a peak value in week three, upon which it consistently reduced over time. A higher concentration of water-soluble nutrients present in the animal manures may explain the initial high EC values recorded. These high values could be due to the release of mineral salts such as phosphates and ammonium ions by decomposing organic substances. Ali (2007) states that the increases in EC suggest an increase in soluble salts and continued mineralisation of organic matter, i.e. production of CO_2 and organic acids due to microbial activities.



'Limits'- United States Department for Agriculture maximum and minimum soil EC for crop production

Figure 5.12: Electrical Conductivity variation in the three treatments over the experimental duration. Error bars represent ±S.E. (n = 3).

The possible impact of leaching cannot be ruled out as a key factor behind the observed drop in EC across the treatments. However, as decomposition progresses the consumption of nutrients by microorganisms reduces, making nutrients accumulate in the soluble phase and a result increase EC (Raviv *et al*., 1987). This may also shed light on the general oscillating trend observed in the EC of all the treatments.

5.2.1.3 Moisture Content

The moisture content (MC) varied across treatments and indicated although not significantly different over time (Kruskal-Wallis H (2) = 8.23, $p < 0.05$) (Figure 5.13). The cumulative mean MC recorded for treatments one, two and three overtime were 33.08%, 31.66%, and 32.41% respectively. The decreases in MC observed at various points during the trials were partly due to rises in temperature and the forced aeration. The MC was maintained to a suitable level (30 - 55%) as suggested by Suler & Finstein (1977) through the application of water during the rainfall simulation tests (Section 3.4.3). Treatments containing animal manures had higher MC levels, and this could be attributed to the more significant water holding capacity of animal manure compared to fungi substrate.

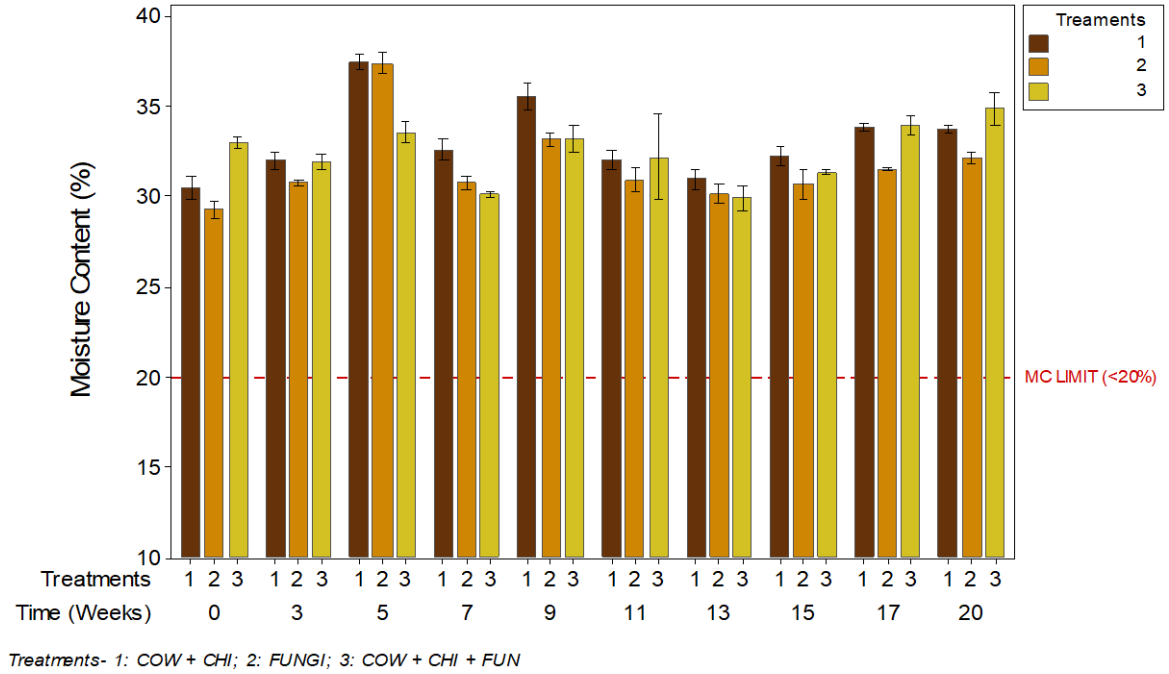


Figure 5.13: Moisture content variation across treatments over the experimental period. Error bars represent ±S.E. (n = 3).

According to Parr *et al*. (1983), the texture and density of the soil determine the water-holding capacity, which in turn affects the oxygen availability and microbial activity. It is likely that the optimum moisture conditions may very well vary with the different materials and soil types being investigated. It is worthy to note that the dependency on soil MC for biodegradation of petroleum hydrocarbons is compound-specific and soil specific (Holman & Tsang, 1995). The moisture content remained fairly consistent across all the treatments and the variability is likely due to the heterogeneity of the matrix or possible inconsistencies during sampling.

5.2.1.4 Organic Matter Content

The organic matter (OM) content was significantly higher from weeks 15 to 20 in the fungi only treatment (Kruskal-Wallis *H* (2) = 13.32, p = 0.001). The OM in the fungi only treatment was relatively similar to the other treatments from weeks (0-9). However, a notable increase was observed from the 11th week until the end of the experiment (Figure 5.14). Generally, the OM content saw a drop from week 7 on.

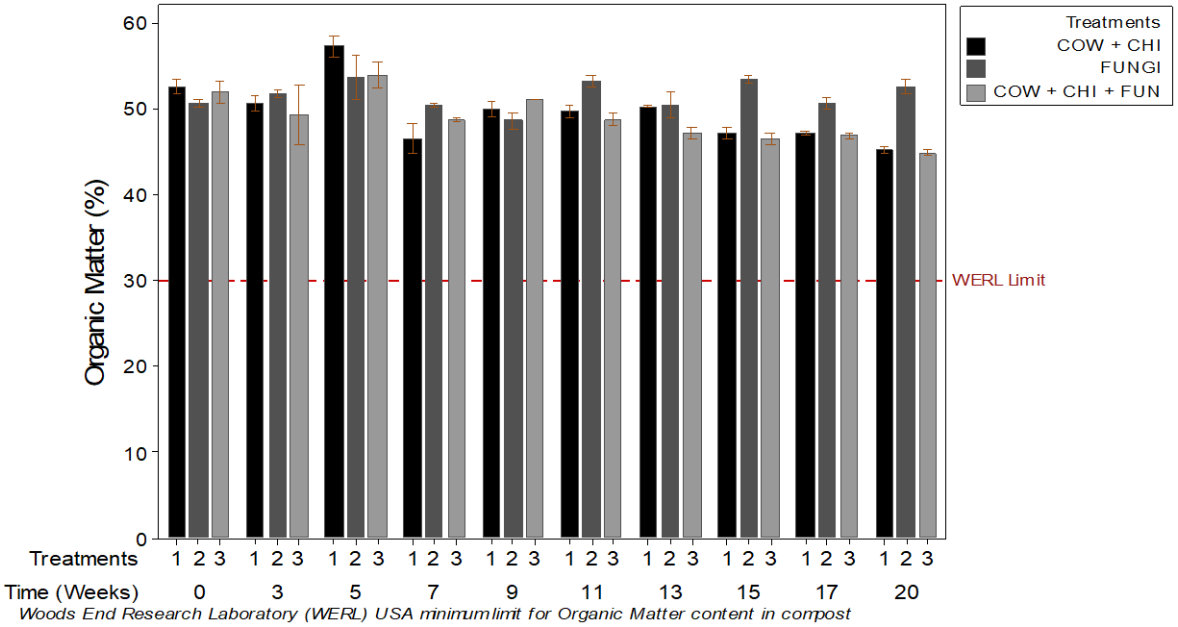


Figure 5.14: Organic matter content within each treatment over the 20-week experiment. Error bars represent ±S.E. (n = 3).

It is possible that the presence straw substrate in which the fungi mycelia was grown possessed higher contents of non-decomposable compounds, such as cellulose and lignin, which in turn may account for the lower degree of OM loss in the fungi only treatment. The decline noticed in the treatments with animal manures may be because of the decomposition of organic matter, which is often an indication of the composting process. The difference in changes for the manure containing treatments was similar throughout the entire study. There was a 13.9% and 13.5% loss in OM content in the (cow and chicken) and (cow, chicken and fungi substrate) treatments respectively. A correlation test performed between OM and composting time for the (cow and chicken) treatment (*rho* = -0.635, $p < 0.001$) and (cow, chicken with fungi) treatments (*rho* = -0.671, $p < 0.001$) and the fungi only treatment (*rho* = 0.118, $p = 0.533$). Sims & Bass (1984) state that organic matter is vital to microbial ecology and activity. Its high cation exchange capacity and high density of reactive functional groups help to bind both organic and inorganic compounds added to soils. This property can help microorganisms that can attack the bound compounds. Thus, the sorbents may immobilise the organic constituents and allow more time for biodegradation (Riser-Roberts, 1998).

5.2.1.5 Total Organic Carbon

The total organic carbon (TOC) content was calculated from the determined organic matter using a conversion factor of 1.724 (Equation 5.4) (Sahilemedihin & Taye, 2000).

$$\boldsymbol{TOC}\ (\%) = \frac{\boldsymbol{OM}\ (\%)}{\mathbf{1.724}} \qquad \textbf{(Equation 5.4)}$$

The total organic carbon (TOC) content showed no significant differences between sampling locations within each treatment (Kruskal-Wallis *H* (2) = 0.48, p = 0.788). However, it showed a significant difference between the overall mean TOC contents amongst the treatments (Kruskal-Wallis *H* (2) = 13.32, p = 0.001). There was also significant (Kruskal-Wallis *H* (9) = 30.72, p < 0.001) variations over time. Even though the TOC content remained relatively consistent throughout the process (Figure 15.5b), the minor mean loss of 7.95 ± 0.5% observed between all the treatments could result from mineralisation during the composting process (Lashermes *et al*., 2012). Earlier work by Adesodun *et al*. (2008) also points out that decreases in TOC result from the mineralisation of hydrocarbons with high carbon to nitrogen ratio, leading to increased CO2 evolution, which in turn reduces the TOC content. Similarly, Sims (1990) suggests that the carbon mineralising capacity of the soil is directly related to its TOC, meaning that the release of CO_2 is proportional to the organic matter level. This usually brings about a decrease in oxygen levels, thus affecting microbial metabolism (Osuji *et al*., 2006).

5.2.1.6 Total Nitrogen

The total nitrogen (TN) was calculated as the sum of measurements for ammonium, nitrate, and nitrite (Li *et al*., 2015) and its range was found to be considerably variable within the treatments over time (Figure 15.5a). The manure containing treatments generally had significantly higher overall TN contents (Kruskal-Wallis *H* (2) = 12.32, p = 0.002) than the fungi only treatments by week 15. The changes in the TN content in the manure containing treatments were similar. The peak values for animal manure-fungi treatment recorded at week zero (2.9%), followed by week 13 through to 20 (*M* = 1.04, *SD* = 0.38). In the manure only treatment, TN peaked at week 20 (2.1%), before then at weeks 17, 15, and 5 (*M* = 1.02, *SD* = 0.43). On the other hand, the highest TN for fungi only treatment was observed at weeks three (0.72%) and twenty (0.54%).

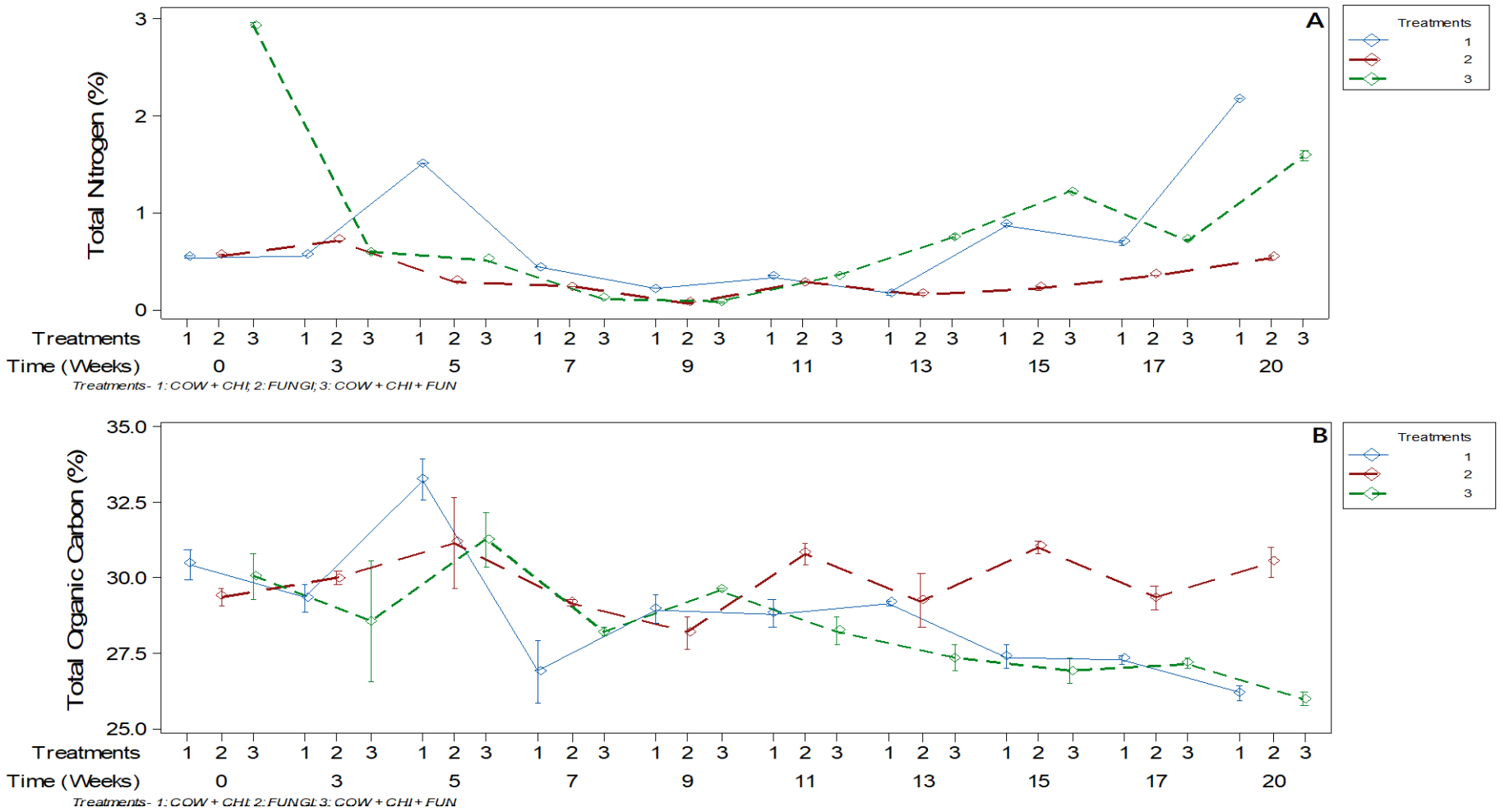


Figure 5.15: A) Changes in mean values of total nitrogen contents of different treatments during various stages of the experiment. B) Changes in mean values of total organic carbon contents of different treatments during various stages of the experiment. Error Bars represent ±S.E. (n = 3).

At the end of the composting process, the TN contents for all treatments was found to be within the desired range of 0.5 – 2.5% (Travis *et al*., 2003). Despite the drop in TN content seen from the start, a gradual rise in the pattern was displayed by week 13 to the end of the experiment and appears to be a function of increases of nitrate and ammonium. It is also possible that this may have been due to the binding of nitrogen to organic carbon which often prevents the loss of nitrogen through leaching (Alexander, 1977). According to de Bertoldi *et al*. (1983), nitrogen content tends to decrease during the process of composting via ammonia volatilisation. Despite this observed decrease, a partial recovery was seen to take place, and this would suggest the activities of nitrogen-fixing bacteria. These bacteria are often associated with the mesophilic phases of composting and may be present in the current study. As such, de Bertoldi *et al*. (1983) state that biological nitrogen fixation is inhibited by ammonia and thermophilic temperatures. Alexander (1980) indicates that at temperatures above 40 °C, the rate of nitrification is very low.

5.2.1.7 Carbon to Nitrogen ratio

The carbon-nitrogen ratio (C:N) was determined from the quotient of total organic carbon to total nitrogen (Equation 5.5) (Martin, 1991).

$$\boldsymbol{C}:\boldsymbol{N} = \frac{\boldsymbol{TOC}\ (\%)}{\boldsymbol{TN}\ (\%)} \qquad \textbf{(Equation 5.5)}$$

The C:N ratio for the treatments containing manures was significantly lower (Kruskal-Wallis H (2) = 13.85, p = 0.001) than the fungi only treatment by week 20. With no significant changes in the TOC content, the C:N ratio was predominantly influenced by the changes in TN (*rho* = - 0.562, p < 0.001). In weeks seven and nine, a spike in the C:N ratios of the animal manure with fungi treatment and the fungi only treatment was observed. Both possessed a ratio of as high as (413:1) (Figure 5.16). Although, hydrocarbon contamination could be responsible for the very high C:N ratios due to its various organic constituents, the spike observed is likely due to a sampling or analytical error. Alexander (1999) indicated that soil with TPH content as high as 380,000 mg/ kg showed C:N ratios of as high as (306:1).

Despite the high C:N ratio observed in week nine for treatments that contained fungi substrate, a steep decline followed in the subsequent weeks and this may have been as a consequence of higher temperatures within the treatments as correlation test results indicate a negative relationship exists between C:N and temperature (*rho* = -0.402, p < 0.001). Bitew (2008) suggests that temperature rises into mesophilic and thermophilic ranges favour decomposition leading to reductions in OM content, in turn, decreasing C:N

ratio. The changes in C:N ratios reflect the decomposition of organic matter and the level of stabilisation achieved during the composting process (Huang *et al*., 2006).

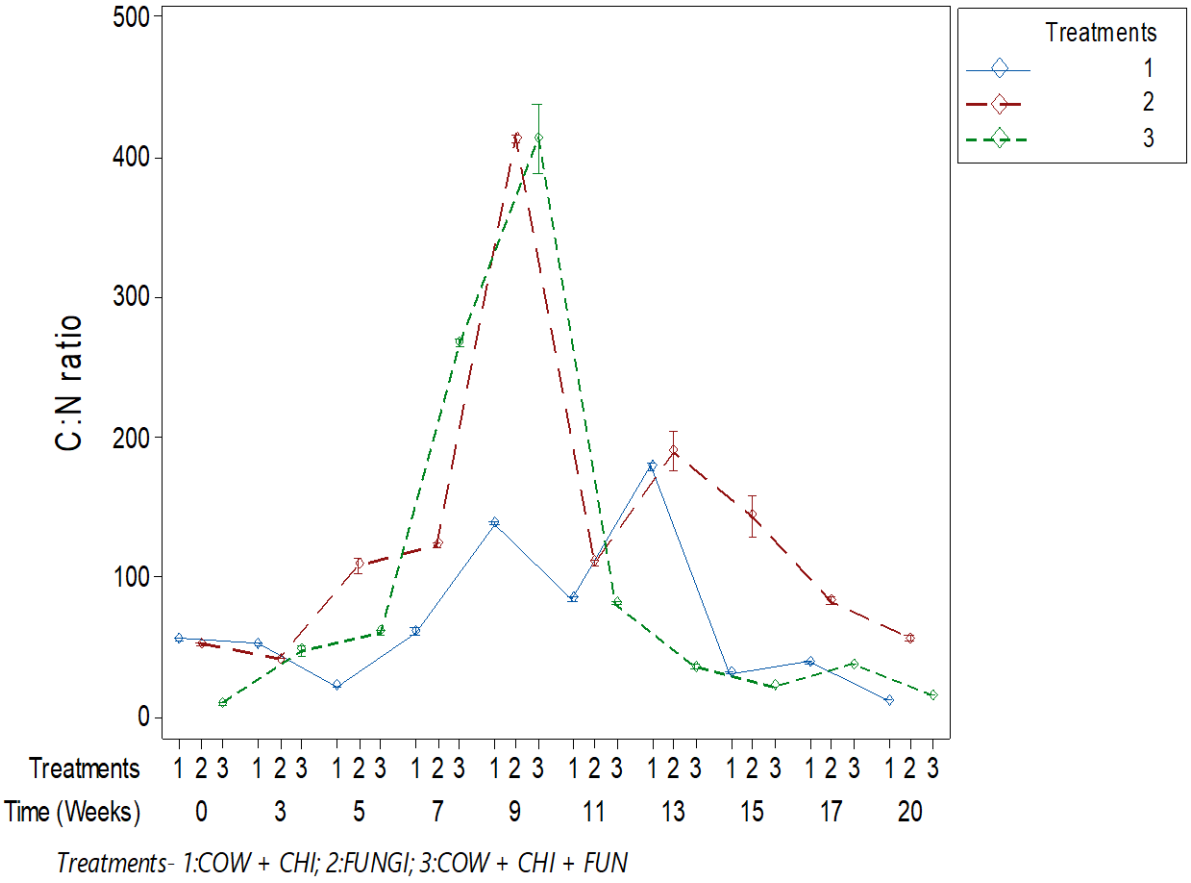


Figure 5.16: Changes in carbon to nitrogen ratio over the 20-week experimental period. Error Bars represent ±S.E. (n = 3).

Epstein (1997) and most experts recommend C:N ratios between 25:1 and 35:1 for effective compost bioremediation. By week 20, the treatments with animal manures, i.e. (cow and chicken) and (cow, chicken with fungi) were found to have C:N ratios below the recommended ratios at 12:1 and 16:1 respectively, except for the fungi only treatment that had a ratio of 57:1. However, similar proportions were found by Atagana (2008) while composting hydrocarbon contaminated soil with sewage sludge obtaining a ratio of 23:1 at the start before decreasing to 15:1 after the sixth month. Studies in the past have shown that in C:N ratios of as low as (11:1) have shown degradation of hydrocarbons in contrast to composts with high C:N ratios (54:1) (Eiland *et al*., 2001).

5.2.2 Nutrients

5.2.2.1 Nitrate-N and Ammonium-N

Soil concentrations of NO_3-N and NH_4-N are hugely dependent on biological activity and therefore fluctuate with physicochemical changes (Horneck *et al*., 2011). The changes in the concentration of NO_3-N and NH_4-N in this study followed typical trends of these two forms of nitrogen during aerobic composting (Figures 5.17 & 5.18).

Nitrate-N (NO_3-N) levels varied significantly (Kruskal-Wallis H (9) = 69.30, $p < 0.001$) within all treatments over time. Interestingly, however, the overall difference in the mean levels present in each individual treatment was found not to be significant (Kruskal-Wallis H (2) = 2.68, $p = 0.262$). As expected, treatments with animal manures had the highest nitrate-N contents due to the extra levels of nitrogen present, particularly in the chicken manure. A pattern consistent with a convex shaped function is evident in NO_3-N levels, which ranged between 0.56 to 17.15 mg/kg (Figure 5.17).

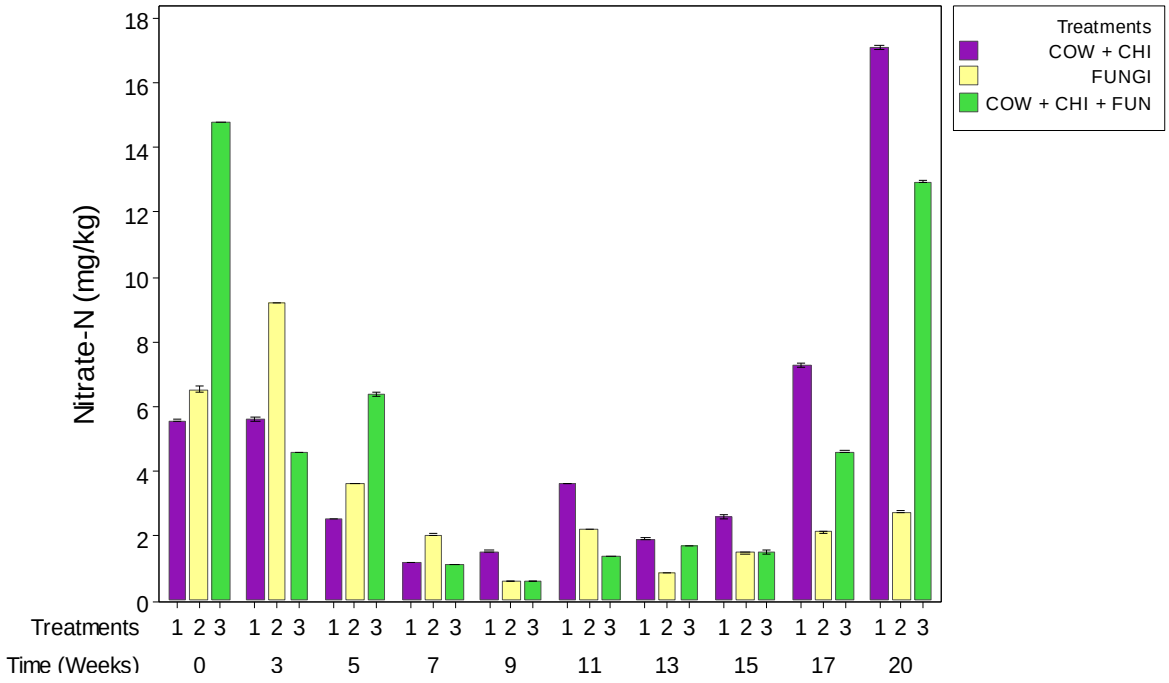


Figure 5.17: Changes in Nitrate-N concentrations over a 20-week period. Error bars represent ±S.E. (n = 3).

Although the NO_3-N steadily decreased in all treatments between weeks zero and nine, the results surprisingly revealed the fungi only treatment to have similar concentrations of nitrate-N as with the treatments containing animal manures. Then again, this trend changes from week 11 to the end of the trials at week 20. At this point, an increase in nitrates can be

seen in manure containing treatments whereas the fungi only treatment showed only a minor rise.

Hydrocarbon degradation thought to be an aerobic process, at least the initial oxidation of the terminal-end methyl group. The by-products of hydrocarbons and monoaromatic hydrocarbons (BTEX) can also be degraded under anaerobic conditions in the presence of nitrate via the denitrification process (Schocher *et al*., 1991).

It is possible that biological denitrification which is also described as dissimilatory nitrate reduction may explain the initial decrease in the amounts of NO_3-N in the first nine weeks. Here nitrate serves as the terminal electron acceptor in the oxidation of organic substances (Kaplan & Kaplan, 1982a). Nitrogen gas is often the end product in dissimilatory denitrification for the production of energy via the respiratory transport chain (Riser-Roberts, 1998). Similarly, Kaplan *et al*. (1984) suggest assimilatory denitrification as a probable reason for nitrate reduction within a composting process, often due to the reduction of nitrate to ammonia used during cellular synthesis. However, the increments may result from the net loss of dry mass in terms of carbon dioxide and water loss by evaporation due to heat evolved during oxidation of organic matter (Fang & Wong, 1999; Inoko *et al*., 1979). It is possible that the observed increase in nitrates concentrations could be influenced by nitrogen-fixing bacteria that remain active even towards the latter stages of the process. The substantial reduction observed within all treatments in the 7^{th} and 9^{th} week may well be a result of temperature rise, which causes excess ammonia production, thus inhibiting the activity and growth of nitrifying bacteria (Morisaki *et al*., 1989). It is also possible that the decline in nitrate (NO_3) content was due to the oxidation of organic matter by the bacteria into gaseous (N_2) (Tiedje, 1982), implying that nitrate can be utilised as an alternative electron acceptor instead of oxygen in the bacterial respiration. Jørgensen *et al*. (2000) suggest that denitrification only occurs when oxygen availability is limited or entirely absent and affects microbial activity during the composting process.

The ammonium-N (NH_4-N) contents in the animal manure containing treatments differed significantly from the fungi only treatment (Kruskal-Wallis H (2) = 14.58, p = 0.001) and showed consistently higher quantities of ammonium over the experimental duration (Figure 5.18). In the first five weeks, the peak level of 73.33 and 53.67 mg/kg observed in the manure-fungi and manure only treatments respectively may be due to ammonification or the mineralisation of organic-N compound. However, it should be noted that the fungi only treatment remained comparatively low over time and recorded the least amount of NH_4-N at 0.28 mg/kg in week five. After the initial spikes in NH_4-N concentrations in treatments

containing animal manures within the third and fifth weeks, before being trailed subsequently by a decline.

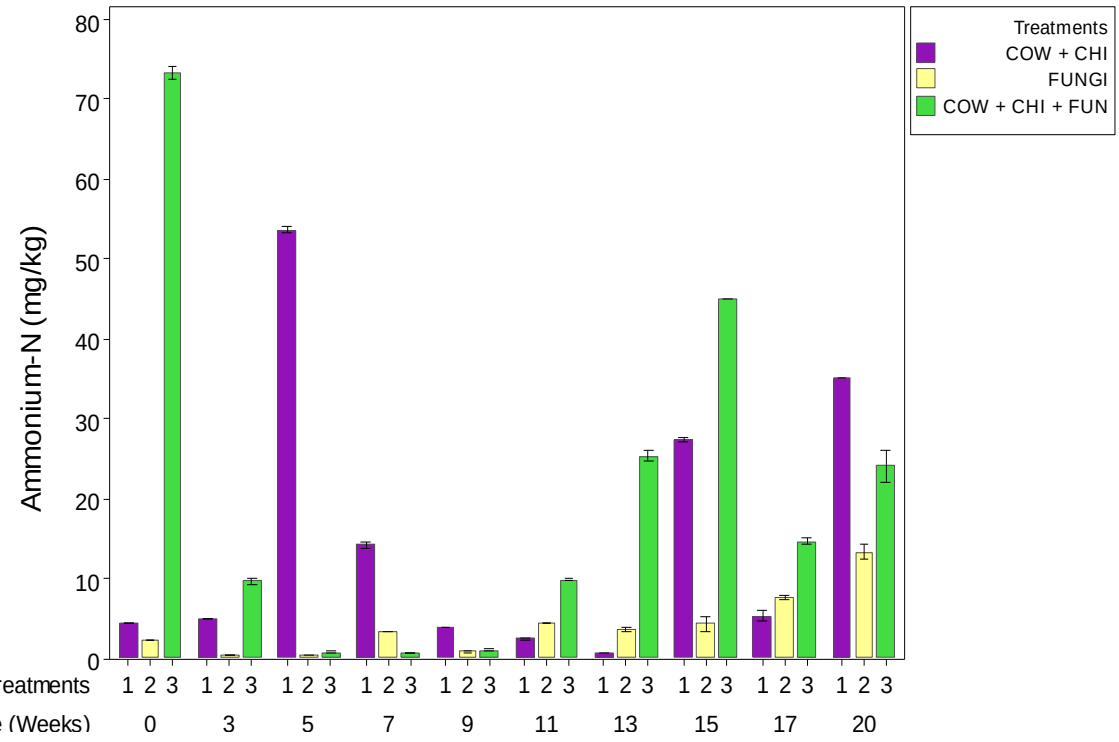


Figure 5.18: Changes in Ammonium-N concentrations over a 20-week period. Error bars represent ±S.E. (n = 3).

Generally, in hydrocarbon contaminated soils, the ammonia-N level is not consistent as and this may be due to its volatility while nitrite is unstable and readily converted to nitrate (John *et al*., 2011). An ammonium-N content is an essential indicator of compost stability and Zucconi *et al*. (1981) recommend that for compost to be stabilised entirely, the maximum concentration threshold should be no more than 400 mg/kg.

5.2.2.2 Phosphate

The phosphate (PO_4-P) concentrations in all treatments significantly differed over the entire experimental period (Kruskal-Wallis *H* (9) = 28.63, p = 0.001) with peak levels of 160 mg/kg (cow, chicken with fungi), 56 mg/kg (cow and chicken), and 32 mg/kg (fungi only) all observed between weeks zero and three. The concentration levels dropped below 25 mg/kg in all the treatments by week five and remained so for subsequent weeks until the conclusion of the experiment. A significant difference was present between treatments (Kruskal-Wallis *H* (2) = 10.35, p = 0.006) indicating the higher availability of phosphates in the animal manure treatments compared to the fungi only treatment (Figure 5.19).

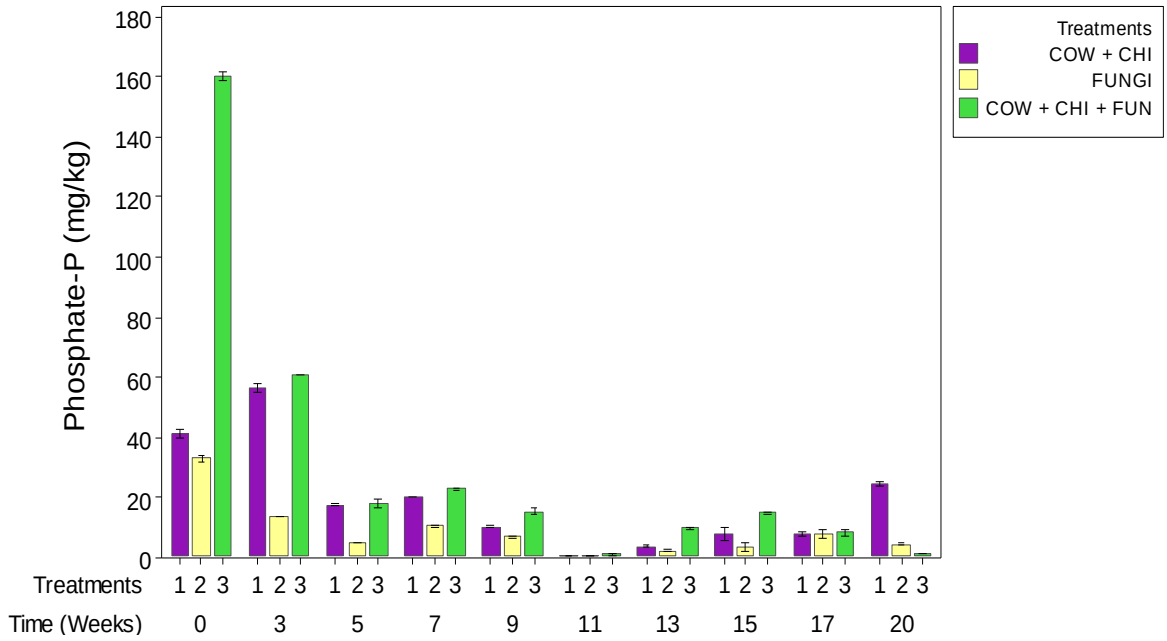


Figure 5.19: Changes in Phosphate-P concentrations in individual treatments during the 20-week composting process. Error bars represent ±S.E. (n = 3).

The presence of hydrocarbon contamination in soil sample is likely responsible for the precipitous decline in phosphates-P concentration. Alexander (1987) explains that decomposing microorganisms utilise minor amounts of phosphorus to synthesise protoplasm with the remainder forming soluble phosphates that can be lost through leaching. The mineralisation of organic phosphorus and its subsequent consumption by microorganisms cannot be ruled out as a possible reason behind the decline of phosphate-P (Huang *et al*., 2004). The observed correlation (*rho* = 0.715, $p < 0.001$) of pH and phosphate levels might further explain the decline. In alkaline soils (soil pH greater than 7) as is the case with the current study, Ca^{2+} is the dominant cation (positive ion) that will react with phosphate. Leaching that occurred during the rainfall simulation is also a probable cause for decreased phosphate levels across the treatments over time.

5.2.2.3 Chloride

Chlorine occurs predominantly as chloride (Cl^-) in soil (White & Broadley, 2001). High concentrations of chloride were observed at the start in all treatments ranging from 810 to 1160 mg/kg with the animal manure containing treatments possessing the peak levels. By week three, an 80.5% decrease in concentration was seen across all treatments (Figure 5.20). The trend remained until the ninth and eleventh weeks where notable increases were witnessed in the animal manure combined with fungi substrate treatment and fungi substrate only treatment. However, this increment was to follow a steep decline by week 13, a pattern that lasted through the entirety of the process. It is worthy to note that no

overall significant difference existed between the sum of the mean concentrations of each treatment (Kruskal-Wallis *H* (2) = 3.36, p = 0.187); however, the mean concentration within the treatments varied significantly over time (Kruskal-Wallis *H* (9) = 69.25, p < 0.001).

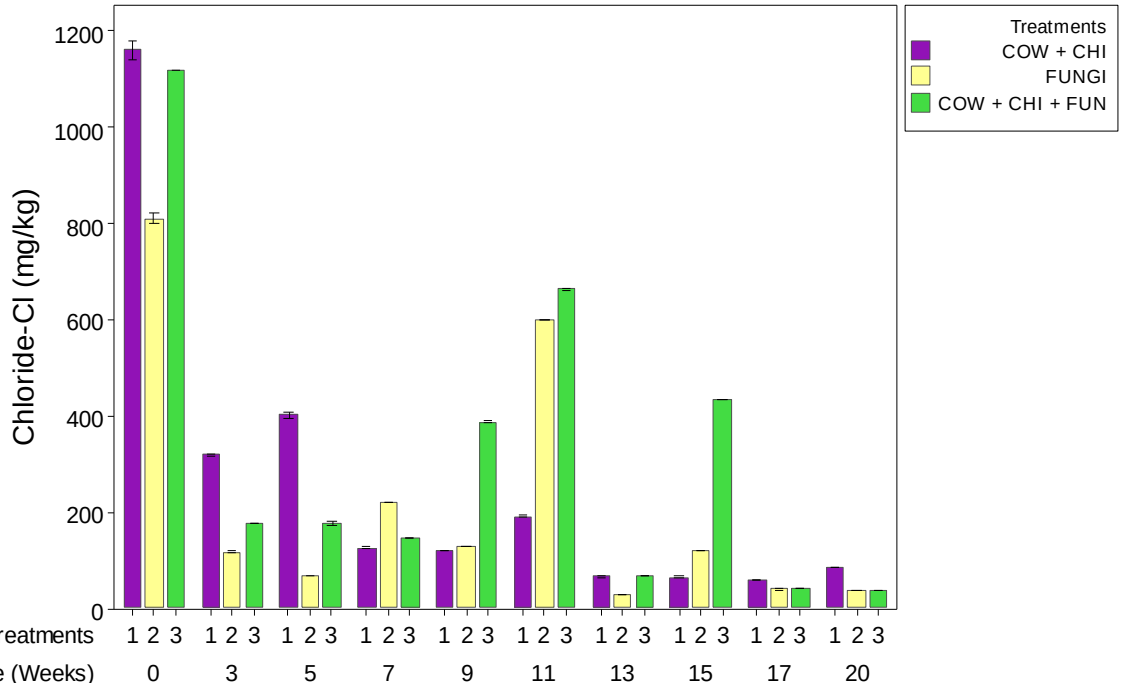


Figure 5.20: Changes in Chloride-Cl concentrations over the 20-week experimental period. Error Bars represent ±S.E. (n = 3).

Organic manure input in soil substantially increases the chloride (Cl^-) levels within it may likely be the reason for the elevated levels observed in this study at the start of the process. White & Broadley (2001) state that since Cl^- shows little adsorption to soil components unlike other soil anions, it is not often chemically altered by soil organisms and thus used as a tracer for soil water movement which would support the idea that leaching is likely to expound the reduction in Cl^- concentration over time during this study. This idea is supported by Tisdale *et al*. (1985) who opined that movement of Cl^- within the soil is determined by water fluxes and, in particular, the relationship between precipitation and evapotranspiration. It is possible that the rainfall simulation could have a similar effect on the soil matrix by transferring Cl^- ions into the leachate. By the end of the composting process, the chloride levels in each treatment were well below the critical concentration for toxicity of about 4000 – 7000 and 15000 – 10000 mg/kg for Cl^- sensitive and tolerant plant species respectively (White & Broadley, 2001).

5.2.3 Temperature

The temperatures within the treatment reactors (Figure 5.21b) and ambient temperatures (Figure 5.21a) were obtained using automated data loggers that continuously recorded and stored data through the entire process. The ambient temperature fluctuated within a range between 10 °C and 17 °C during the composting period. All the treatments were found to be within the mesophilic ranges (20 – 40 °C), and Sayara *et al*. (2011) suggest this to be a reflection of the availability of readily biodegradable materials within the treatments. Peak temperatures in the manure containing treatments were recorded in week 17 at 26.7 °C for the (cow and chicken) and 24.3 °C for the (cow and chicken with fungi). The fungi only treatment's temperature peaked at the 20th week with a value of 23.1 °C. There was a significant difference (Kruskal-Wallis H (2) = 25.29, $p < 0.001$) between mean temperatures within each reactor over time. The animal manure treatments generally had higher overall mean temperatures. As expected during composting, the initial temperature was comparatively average. This was followed by a decline in week 11 and a gradual increase there on, thus displaying common temperature patterns often associated with aerobic activities during composting. Although it would be expected for temperatures to reach and sometimes surpass 60 °C during composting, it is still possible that composting may occur even at lower temperatures. Indeed, the effects of temperature on the degradation rates of hydrocarbon contamination in soils have divided opinions amongst experts. However, several researchers agree that lower temperatures, i.e. within the mesophilic range are more favourable for hydrocarbon degradation (Baraniecki *et al*., 2002; Eriksson *et al*., 2001).

The temperature within the cow and chicken treatment reactor remained consistently higher than the other two throughout the process. It is thought that the presence of fungi substrate (chopped straw) may account for the lower temperatures experienced in the fungi only and animal manure with fungi treatments as it heightens ventilation rate because of the lower resistance to airflow. This, in turn, causes greater evaporation of moisture and therefore lower temperature (Ali, 2007). Ruggieri *et al*. (2008) point out that the temperature during composting increases in the presence of readily biodegradable organic matter in the composted matrix. This is in agreement with earlier suggestion by Eiland *et al*. (2001) who indicate that materials such as straw which have considerable proportion of lignocellulose components are more challenging for microorganisms to break down. This also sheds light on a possible reason the treatments containing fungi substrate possessed a lower temperature in the current study. Operating temperatures must remain between 20 to 35

°C, below 20 °C, microbial activity is relatively low, and the ability of organisms to degrade hydrocarbon contaminants is reduced (Diaz, 2003).

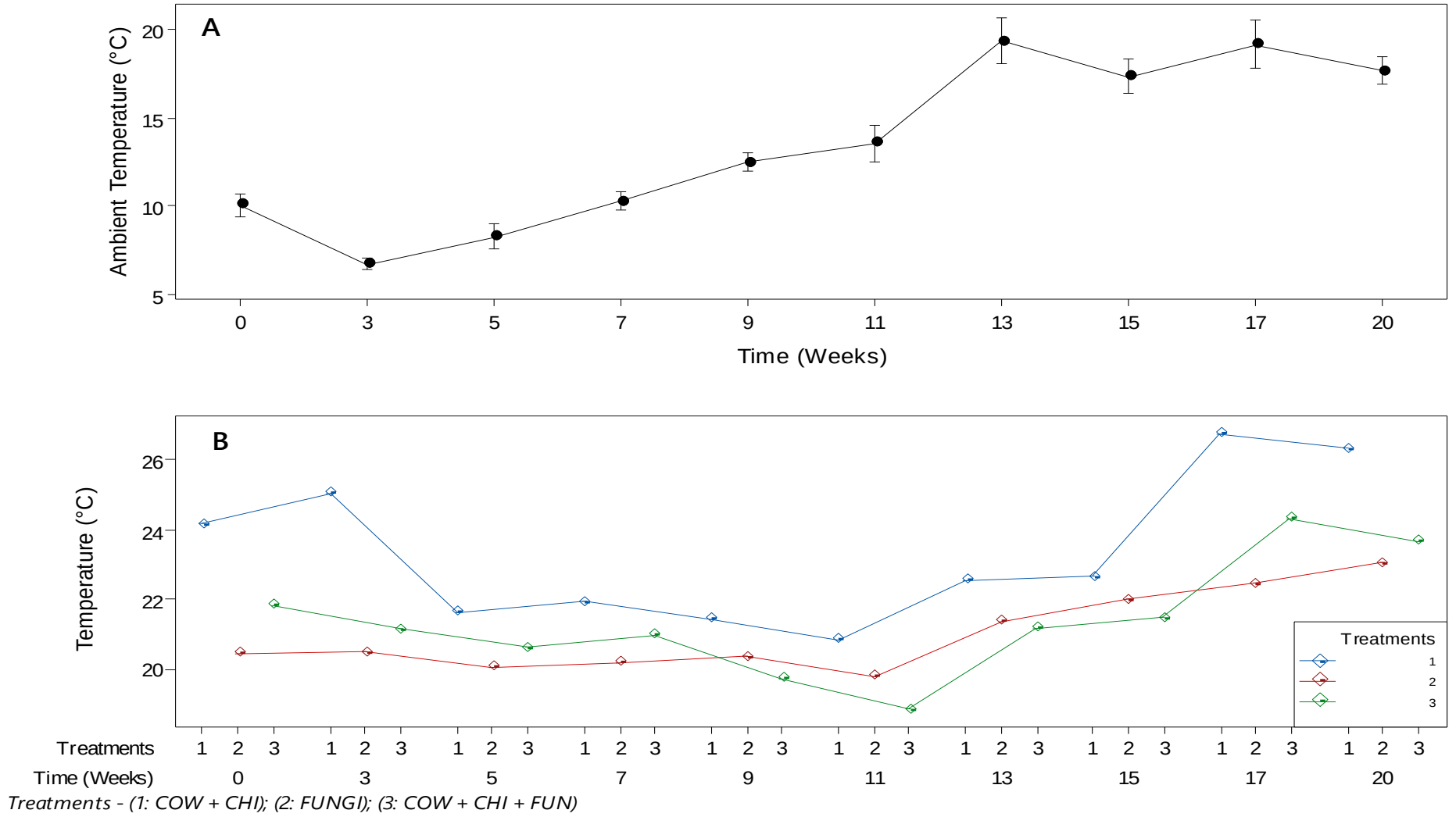


Figure 5.21: A) Variation in ambient temperature during the experimental period. B) Time-course variation of temperature profiles for individual treatments. Error Bars represent ±S.E. (n = 3).

Temperature plays a vital role in controlling the nature and extent of microbial hydrocarbon metabolism (Sayara, 2010). Temperature rises often lead to increased diffusion rates of organic compounds by reducing their viscosity, resulting in increased bioavailability, solubility and diffusion rate (Mohan *et al*., 2006). However, Leahy & Colwell (1990) suggests that increases in solubility due to elevated temperatures may enhance their toxicity which limits or inhibits microbial activity. Thus, studies have demonstrated that mesophilic temperatures are more favourable for the degradation of a vast array of hydrocarbon contaminants as compared with fewer degradation rates observed within thermophilic temperature ranges (Sayara, 2010).

5.2.4 Changes in Metal Concentration

Heavy metals have been described as to be non-degradable and persistent in the environment, and most have been found to be toxic to living organisms (Hares & Ward, 2004). According to Abha & Singh, (2012) the bioremediation of hydrocarbon-contaminated soils is inhibited by heavy metal presence because they restrict the ability of microorganisms thereby reducing the overall efficiency of the remediation process. With the aforementioned in mind, the influence of the composting process on the changes in metal concentrations was assessed. Metals classified as potentially toxic elements (PTEs) were the focus of this study. These include Copper, Arsenic, Zinc, Nickel, Lead, Cadmium, and Chromium. It is worth mentioning that the concentrations found in these metals were all within permissible limits for agricultural purposes in all the treatments by the end of the composting process.

5.2.4.1 Copper and Arsenic

The effect of microorganisms on the changes in metal concentrations during this study cannot be overlooked. Ron *et al*. (1992) point out that biotransformation often results in the changes to arsenic, with the production of less toxic or volatile compounds, such as the oxidation of arsenite [As(iii)] to arsenate [As(v)]. This process is often stimulated by aerobic heterotrophic microorganisms in the contaminated soil (Alexander, 1977). The introduction of organic amendments and aeration as is the case in this study is said to stimulate the oxidation of arsenite to arsenate (Sims & Bass, 1984). It is also possible that the changes in arsenic concentration will be a consequence of methylation which Woolson (1977) states is a vital process in soils containing metal; with trimethylarsine a prominent by-product of the process. In the case of this study, arsenic concentrations were found to differ significantly amongst the various treatments (one-way ANOVA F (2, 27) = 3.39, p = 0.049). Even though the initial test indicated a significant difference in the arsenic concentrations

over time, Tukey post-hoc test revealed there to be no significant difference ($p < 0.05$) between the initial (0.48 mg/kg) and end (0.62 mg/kg) concentrations of the metal (Figure 5.22).

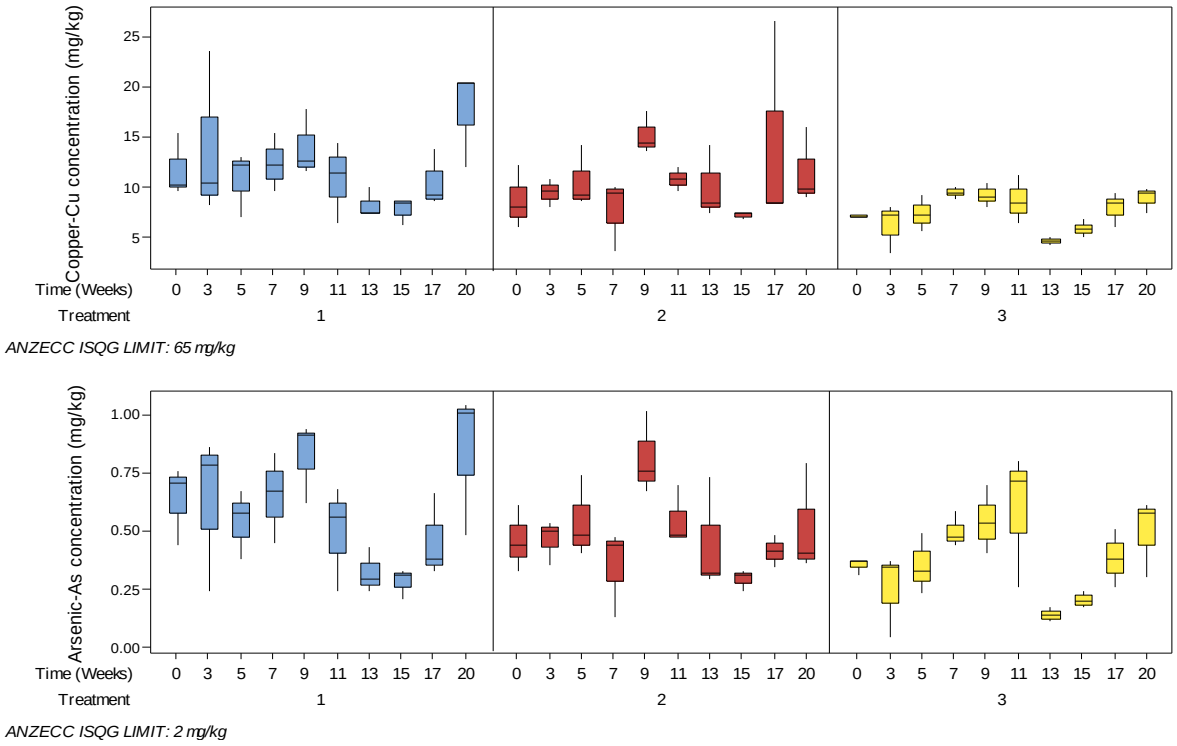


Figure 5.22: Changes in concentrations of metals in the soil; top: Copper and bottom: Arsenic. Treatment 1: Cow and Chicken; Treatment 2: Fungi only; Treatment 3: Cow, Chicken, and Fungi.

According to Wuana & Okieimen (2011), the adsorption of arsenic increases as iron (Fe) content increases and that iron and aluminium hydrous oxides specifically adsorb the metal. Results of the current study seem to reflect these characteristics as displayed in very strong significant positive correlation ($r = 0.960$, $p < 0.001$) between arsenic and iron.

The concentration of copper was relatively the same before and after treatment with no significant difference observed (one-way ANOVA F (9, 20) = 1.30, $p = 0.299$). The average initial concentration was 9.16 mg/kg while at the end of the process was 12.63 mg/kg (Figure 5.22). Copper has been found to rapidly stabilise once released into the environment and this could explain the insignificant change in its concentration as reflected in the results of the current study.

5.2.4.2 Zinc and Nickel

The zinc concentrations were found not to be significantly different over time (one-way ANOVA F (9, 20) = 1.30, $p = 0.295$). The animal manure only and fungi only treatments significantly differed (one-way ANOVA F (2, 27) = 7.97, $p = 0.002$) with the animal manure-

fungi combination treatment. In the manure-fungi treatment, zinc concentration was 41.9 mg/kg while the manure only and fungi only treatments were 64.12 mg/kg and 57.31 mg/kg respectively. Figure 5.23 shows the changes in zinc and nickel over the composting period.

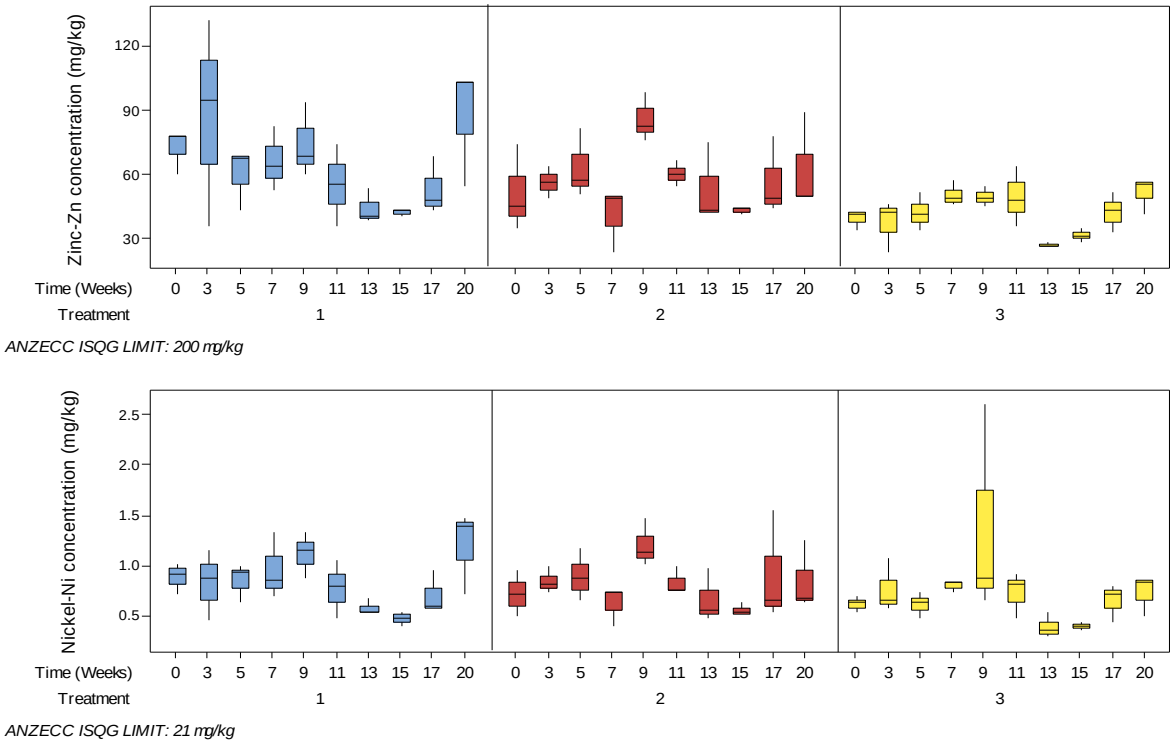


Figure 5.23: Changes in metal concentrations metals in the soil; top: Zinc and bottom: Nickel. Treatment 1: Cow and Chicken; Treatment 2: Fungi only; Treatment 3: Cow, Chicken, and Fungi.

The concentration of nickel was not significantly different amongst the treatments (one-way ANOVA F (2, 27) = 0.87, p = 0.431). A slight increase was recorded in the overall mean concentration of this metal in the treatments from 0.73 mg/kg to 0.92 mg/kg. The persistence of this metal in this study can be attributed to its high adsorption to soil particles, which results in its immobility and low bioavailability. With that being said, a drop in pH to within acidic levels enhances nickel mobility, which often results in it being leached into groundwater (Riser-Roberts, 1998). According to JRB Associates Inc (1984), the presence of clay within a soil matrix and an increase in pH to near nutrient levels reduces the toxic effect of these metals on microbial populations. Microorganisms usually tend to be adversely affected by nickel and its compounds but in most cases develop a resistance to the metal after a while (Wuana & Okieimen, 2011).

5.2.4.3 Cadmium and Lead

The minimal changes seen in cadmium concentration might have been due to its inhibitory effects on soil bacteria(JRB Associates Inc, 1984)(JRB Associates Inc, 1984)(JRB

Associates Inc, 1984)(JRB Associates Inc, 1984)(JRB Associates Inc, 1984)(JRB Associates Inc, 1984). The concentrations of the metal in this study had an average range of 0.03 mg/kg to 0.07 mg/kg for all the treatments (Figure 5.24). Despite this, microbial populations in polluted soils have been found to adapt to heavy metal contamination (Lu *et al.,* 2013). It is possible that the microbial biomass present in the study contributed to the soil cadmium-binding capacity, thus affecting the bioavailability of the metals; with dead cells sorbing more cadmium than live cells (Kurek *et al*., 1982; Luo *et al.*, 2010).

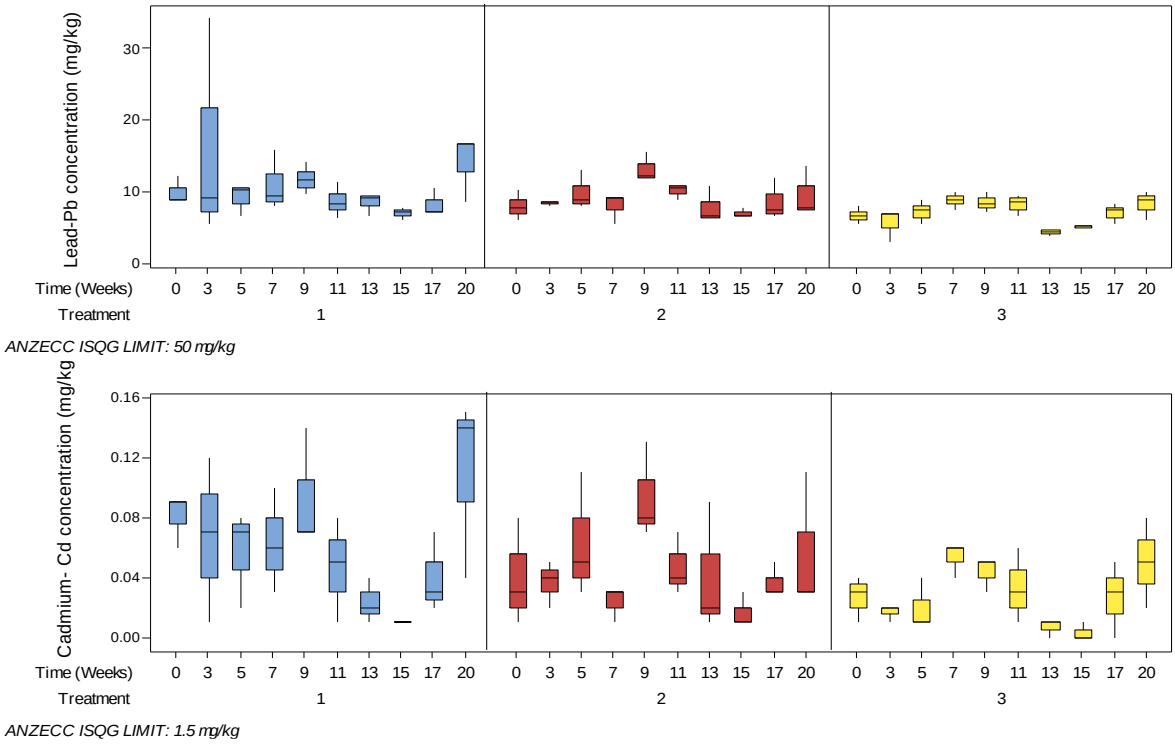


Figure 5.24: Changes in metal concentrations metals in the soil; top: Lead and bottom: Cadmium. Treatment 1: Cow and Chicken; Treatment 2: Fungi only; Treatment 3: Cow, Chicken, and Fungi.

Riser-Roberts (1998) states that in nature, cadmium exists in the valence state of (+2) therefore making its oxidation or reduction highly unlikely. In light of this, Ron *et al*. (1992) suggest that soils with high sand contents and relatively alkaline pH as the current study may lose cadmium via precipitation. Riser-Roberts (1998) points out that soil pH is a principal factor governing the solubility of cadmium and its resultant availability; these properties increase with decreasing pH. Mugo & Rusin (2014) identify fungal biomass as a natural, inexpensive material with a high cadmium-adsorption capacity. The current study corroborates this finding as the results show treatments that contained fungi amendments had a lower total mean concentration of cadmium at 0.03 mg/kg compared to the manure only treatment at 0.06 mg/kg. In a review, Wuana & Okieimen (2011) explains that the acidification of soils and surface waters due to acid rain resulted in the increased geochemical mobility of cadmium.

Consequently, its surface-water concentration tends to increase as lake water pH decreases. The authors also indicate that cadmium is produced as an inevitable by-product of zinc. This may likely be the case in the current study based on the correlation result of the two metals ($r = 0.932$, $p < 0.001$).

The lead concentration between the treatments varied significantly (one-way ANOVA F (2, 27) = 6.70, $p = 0.004$). The highest quantity of this metal was recorded in treatment one at 10.4 mg/kg. Treatments two and three had concentrations of 8.9 mg/kg and 6.9 mg/kg respectively. However, the concentrations of this metal did not change significantly (one-way ANOVA F (9, 20) = 1.18, $p = 0.357$) amongst the treatments apropos to time (Figure 5.24). Lead and its compounds tend to influence soil microbial activities, e.g. inhibition of nitrogen mineralisation, stimulation of nitrification, and the synthesis of soil enzymes (JRB Associates Inc, 1984). Soil pH plays a prominent role in removing lead as it increases the solubility and mobility of the metal thereby making it more bioavailable for biodegradation (Peter, 2011). The author further states that this will lead to a decrease with an increased concentration of phosphate.

5.2.4.4 Chromium

The average range of chromium concentration was from 0.65 mg/kg to 1.8 mg/kg amongst the treatments over the composting period. A decline in its concentration was observed in all treatments by week nine of the process as shown in Figure 5.25. Statistical test showed there was no significant difference between the three treatments (one-way ANOVA F (2, 27) = 0.87, $p = 0.430$). This metal is described as being amenable to oxidation and reduction by microorganisms since it commonly exists as several oxidation states, as Cr(iii) (trivalent state) and Cr(vi) (hexavalent state) (Wuana & Okieimen, 2011). Changes may likely have occurred in this metal due to the forced aeration adopted for the experimental process. Hornick *et al.* (1983) state that this metal is most soluble and mobile in soils when in its hexavalent state and the aerobic conditions rapidly reduce it to its trivalent form. Consequently, forming insoluble hydroxides and oxides that cannot leach.

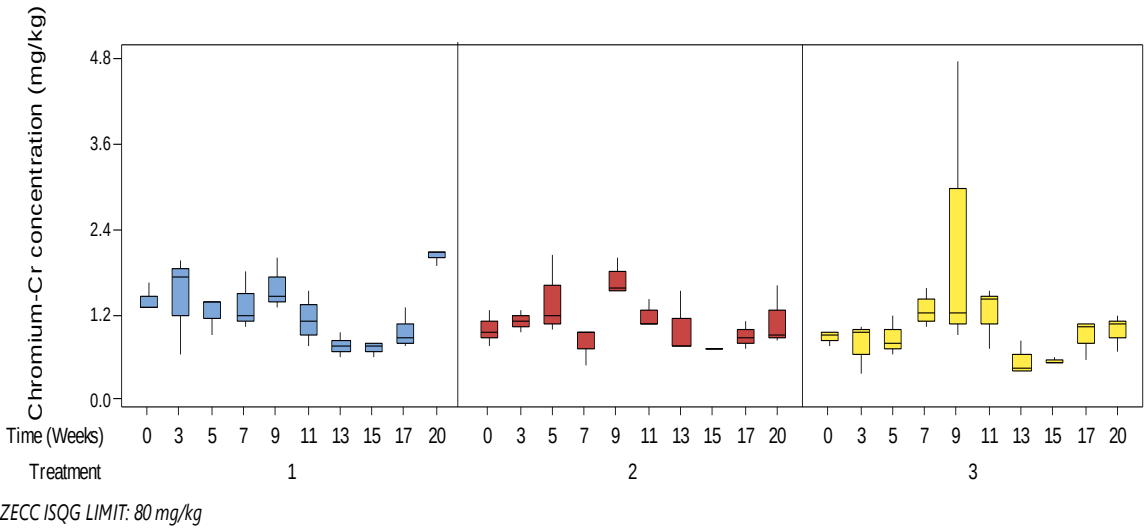


Figure 5.25: Changes in chromium concentration metals in the soil. Treatment 1: Cow and Chicken; Treatment 2: Fungi only; Treatment 3: Cow, Chicken, and Fungi.

It is also possible that the almost neutral pH in this study inhibited potential changes of trichromates to dichromates (Hornick *et al*., 1983). The rate of chromium mobility is decreased based in soils with high clay content and low pH. Chromium was found to positively, correlate significantly with aluminium ($r = 0.823$, $p < 0.001$) and iron ($r = 0.785$, $p < 0.001$). chromates and dichromates are also adsorbed to soil surfaces, especially iron and aluminium oxides (Chrostowski *et al.*, 1991).

5.3 Microbial Community Changes

5.3.1 Hydrocarbon Tolerant Bacteria

The total counts of hydrocarbon tolerant bacteria (HTB) varied over the test period across all treatment combinations ranging from 2.00 x 10^3 to 1.63 x 10^5 CFU/g (Figure 5.26). Total amounts of HTB colonies recorded was significantly different over the five-month duration of the experiment (Kruskal-Wallis *H* (4) = 20.48, p < 0.001) and between all three treatments (Kruskal-Wallis *H* (2) = 15.23, p < 0.001).

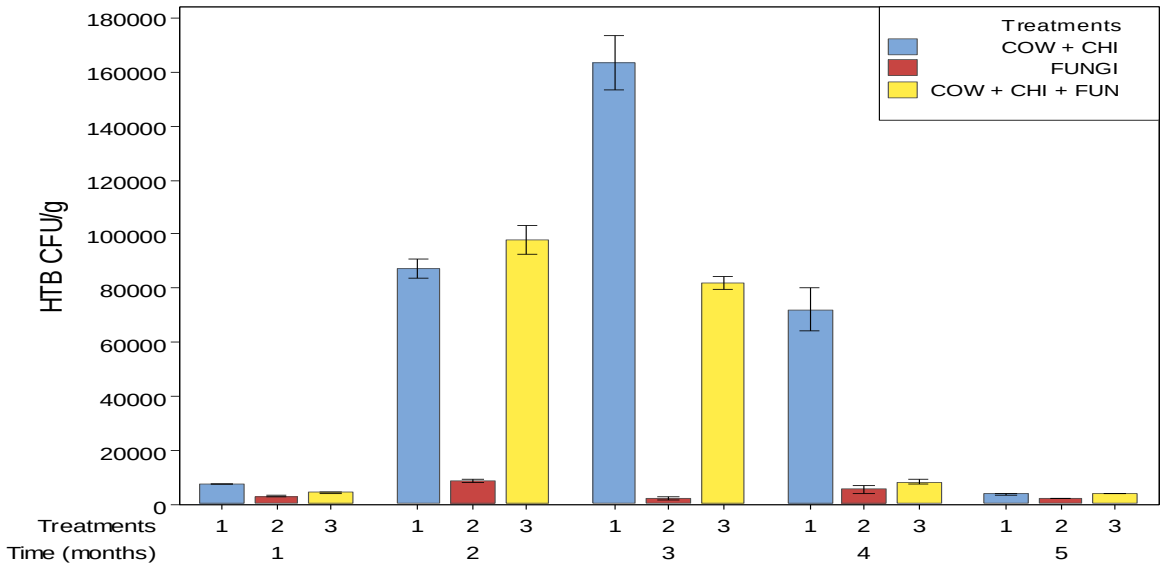


Figure 5.26: Mean HTB counts in CFU/g of the sample in the different treatments over different time points of the 150-day experimental period. Error bars represent mean ±S.E. (n = 3).

The results showed an increase in HTB population sizes in the treatments with organic amendments, most notably treatment one (cow and chicken manures) which had the highest number of these microorganisms, followed by treatment three (fungi substrate in combination with cow and chicken manures). Comparatively, treatment two (fungi inoculated substrate only) had very low HTB counts through the entirety of the experiment as anticipated. The higher number of HTB in treatment one may be due to commensalism between the indigenous microorganisms present in the animal manures; this effectively keeps competition for nutrients at a minimum.

A symbiotic relationship may exist between the various microorganisms found within the animal manures and fungi inoculated substrate. This would likely lead to competition for available nutrients, in turn, leading to lower growth rates of HTB in treatment two. Possible explanations could be hypothesised that the microorganisms present in treatments using animal manures, and fungi inoculated substrates combined with animal manures utilised

weathered hydrocarbons within the contaminated samples as additional carbon sources for biodegradation. A notably low HTB count was seen in all treatments within the first month. It is possible that these microorganisms were still acclimatising to the conditions, and once fully established, a surge in the microbial population was observed by the second month. An earlier study by Atlas (1984) explains that this period may be attributed to the selective inhibition of members of microbial communities as a result of the toxic components of hydrocarbons present in the soil that may have caused reduced organic/inorganic nutrient balance for the indigenous bacterial population. However, this conflicts with Ibekwe *et al*. (2006) who suggest bacteria with the ability to utilise hydrocarbons respond quite rapidly and as early as seven days after the addition of organic manures. Zhou & Crawford (1995) suggest that low temperatures can lengthen the acclimation period and delay onset biodegradation. Another possible reason for this may be attributed to the very low levels of contaminant used to artificially spike the soil may have also had a vital role in the quicker response of HTB. Atlas (1995) states that population levels of HTB and their proportions within the microbial community appear to be a sensitivity index of environmental exposure to hydrocarbons. In unpolluted soils, HTB generally constitutes less than 0.1% of the microbial community; in hydrocarbon-polluted soils, they can constitute up to 100% of the viable microorganisms.

It is apparent from a Spearman's correlation test performed; that a common key factor that affected HTB counts in all treatments was temperature. The results show a strong negative correlation exists, indicating an inverse relationship between both variables except for treatment two that had a much weaker negative correlation with temperature as the scatterplot in Figure 5.27 shows. Generally, temperature decreases often lead to increases in HTB counts. These relationships are significant for treatment one ($rho = -0.895$, $p < 0.001$) and treatment three ($rho = -0.868$, $p < 0.001$) but not significant for treatment three ($rho = -0.142$, $p = 0.614$). Sims & Bass (1984) describe temperature as one of the key factors influencing microbiological activity and the rate of organic matter decomposition.

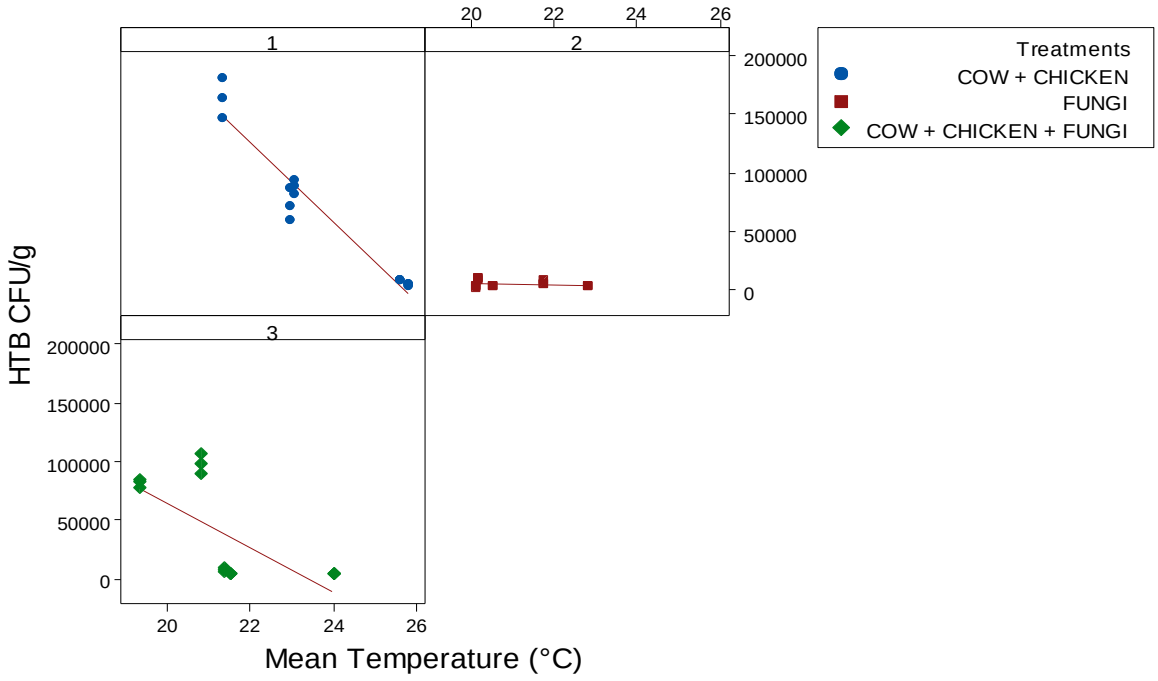


Figure 5.27: Scatterplots showing the relationship between temperature and HTB counts.

It is worthy to note that the greatest HTB counts were recorded at a temperature range of between 20.8 °C – 21.3 °C signifying a predominantly mesophilic microbial population within the treatment (Figure 5.29). A possible explanation for this might be that most soils, particularly those in cold climates, tend to contain psychrophilic microorganisms that grow best at temperatures below 20 °C (JRB Associates Inc, 1984) and are also effective at sub-zero temperatures. According to the Texas Research Institute Inc (1984), microbial utilisation of hydrocarbons can occur at temperatures between -2 to 70 °C. Atlas (1995) indicates an increased evaporation rate of short-chain alkanes and other low-molecular-weight hydrocarbons at higher temperatures, usually cause solvent-type membrane toxicity to microorganisms and is not too conducive for microbial growth activities. However, JRB Associates Inc (1984) points out that raising the temperature increases the degradation rate of organic compounds in soil due to a decrease in adsorption, making more organics available for microorganisms to degrade.

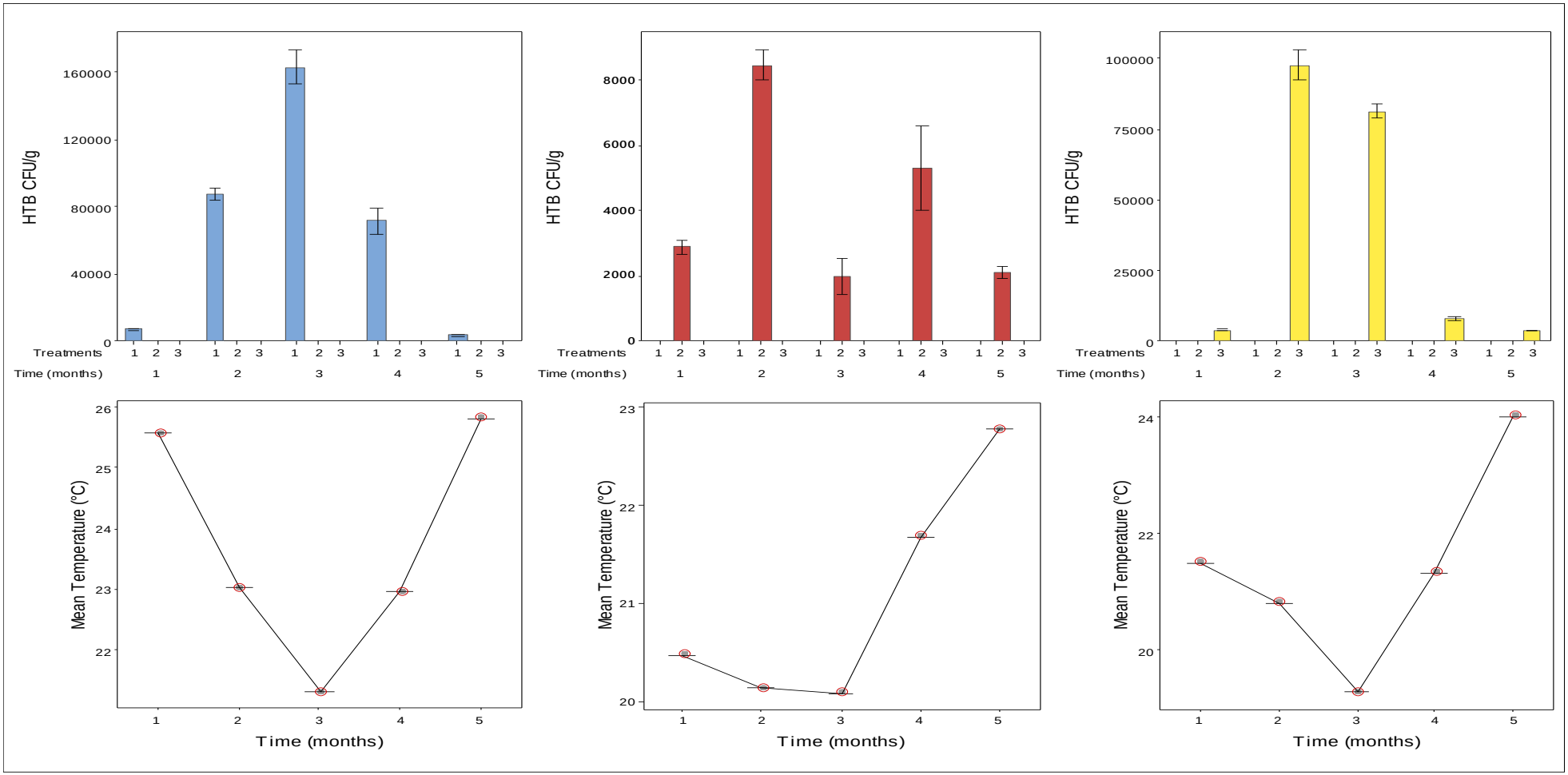


Figure 5.28: Interval plots with corresponding line plots below, showing HTB response to temperature variations in each treatment. Error bars within the interval plots represent mean ±S.E. (n = 3). Blue: Cow + Chicken; Red: Fungi; Yellow: Cow + Chicken + Fungi.

5.3.2 Hydrocarbon Tolerant Fungi

The total hydrocarbon tolerant fungi (HTF) ranged from between 1.56 x 10^4 to 1.35 x 10^6 CFU/g over the five-month experiment. The results showed a significant difference existed (Kruskal-Wallis *H* (4) = 37.60, p < 0.001) amongst the treatment combinations over time but surprisingly, there was no significant difference between overall counts in individual treatments (Kruskal-Wallis *H* (2) = 0.53, p = 0.769). Notably, there seemed to be a consistent pattern of fluctuation in the colony numbers monthly within all treatments with the most substantial rises observed in the second and fourth month (Figure 5.29).

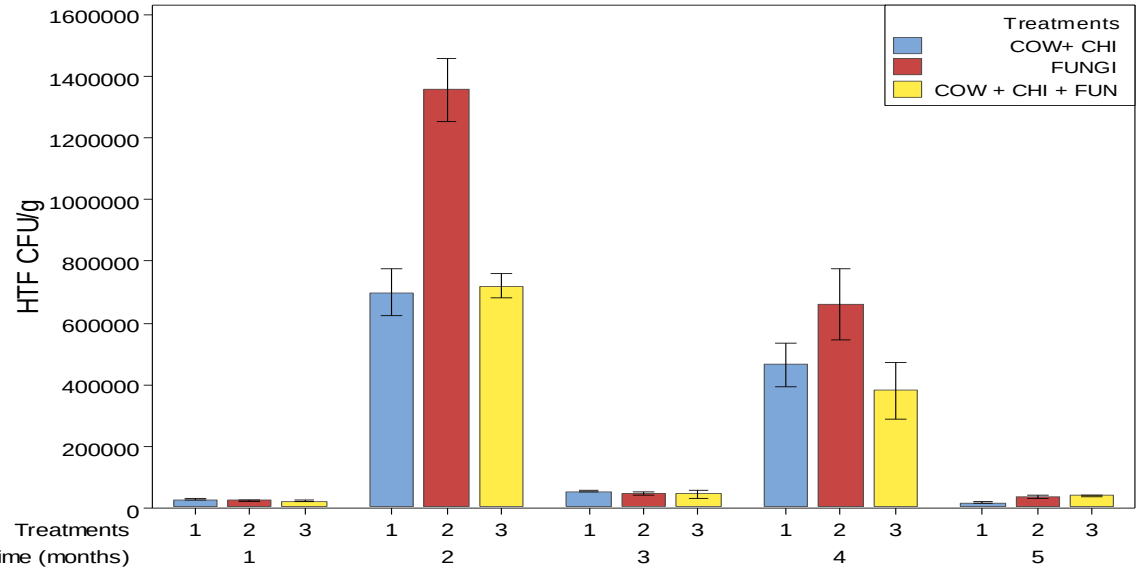


Figure 5.29: Mean HTF counts in CFU per g sample in the different treatments over different time points of the 150-day experimental period. Error bars represent mean ±S.E. (n = 3).

The monthly fluctuations in the HTF numbers may be due to the changing physicochemical conditions and the presence of contaminants. This bimodal growth pattern displayed may be a lag-like phase that allows the HTF to acclimatise before showing exponential growth.

It could also be that the lighter fractions of hydrocarbons i.e. aliphatics are initially readily available and by month 4 the heavier and more recalcitrant compounds are left and these may not be as easy to utilise by the HTF. The results indicate similar ranges of HTF populations in treatment one (cow and chicken) and treatment three (cow, chicken, and fungi) with the highest recorded average in treatment two (fungi only) as expected. The presence of HTF signifies the ability of fungi to survive in hydrocarbon-polluted environments. These organisms play an essential role in hydrocarbon-oxidising activities of the soil and seem to be at least as versatile as bacteria in metabolising aromatics (Fewson, 1981; Jones & Edington, 1968). The effect of temperature was prominent across all treatments as revealed by the correlation test performed. Treatments one and three showed

moderate negative correlation with temperature (*rho* = -0.590, p = 0.021) and (*rho* = -0.469, p = 0.078) respectively with treatment two showing no significant correlation (*rho* = -0.207, p= 0.458) with temperature. The mean temperatures varied significantly amongst all treatments (Kruskal-Wallis *H* (2) = 15.78, p < 0.001) with the highest temperature observed in treatment one (*M* = 23.73, *SD* = 1.78) followed by treatments three (*M* = 21.37, *SD* = 1.58) and two (*M* = 21.03, *SD* = 1.08). The slightly higher temperatures in treatments one and three could probably be attributed to animal manures that contain high bacterial loads as opposed to treatment two that contains no animal manure. These wastes are likely to have significantly diverse microbial populations particularly thermophiles which often generate heat during composting. The interference of animal manure on the microbial community profiles cannot be ruled out, as bacteria often require higher temperatures for growth as stated in Section 5.3.1.

The presence of fungi inoculated material in treatments two and three as a possible reason for the slightly lower temperature cannot be ruled out particularly as the treatment with just fungi inoculated material had the lowest mean temperature. It is possible that the fungi microbial communities present within the various treatments thrived fairly better at lower temperature ranges. Cooke & Rayner (1984) point out that most soil fungi are mesophiles with a temperature optimum between 20 and 35 °C, but with an ability to grow from about 10 to 45 °C. Interestingly, there was no correlation between HTF with time (*rho* = 0.015, p = 0.925), indicating that time played an insignificant role in the population growth of HTF. The results also showed that the peak levels of HTF population was in the second month at pH values 6.7 and 6.8 for treatments one and two, and 7.2 for treatment three (Figure 5.30). The optimum pH was found to be between the ranges of 6.72 to 7.22.

Suppose population dynamics seen in fungal communities could potentially be explained by r selection (population ecology) as these can potentially lead to boom and crash cycles. This could explain the significant shifts observed in the number of fungal communities present. The fungi first exploit either the straw substrate or readily available hydrocarbons, enabling large increases in number, the populations decreasing severely when the initial resource is used up. The use of an alternative food source, such as less readily available hydrocarbons or lignins could account for the 2nd peak in abundance.

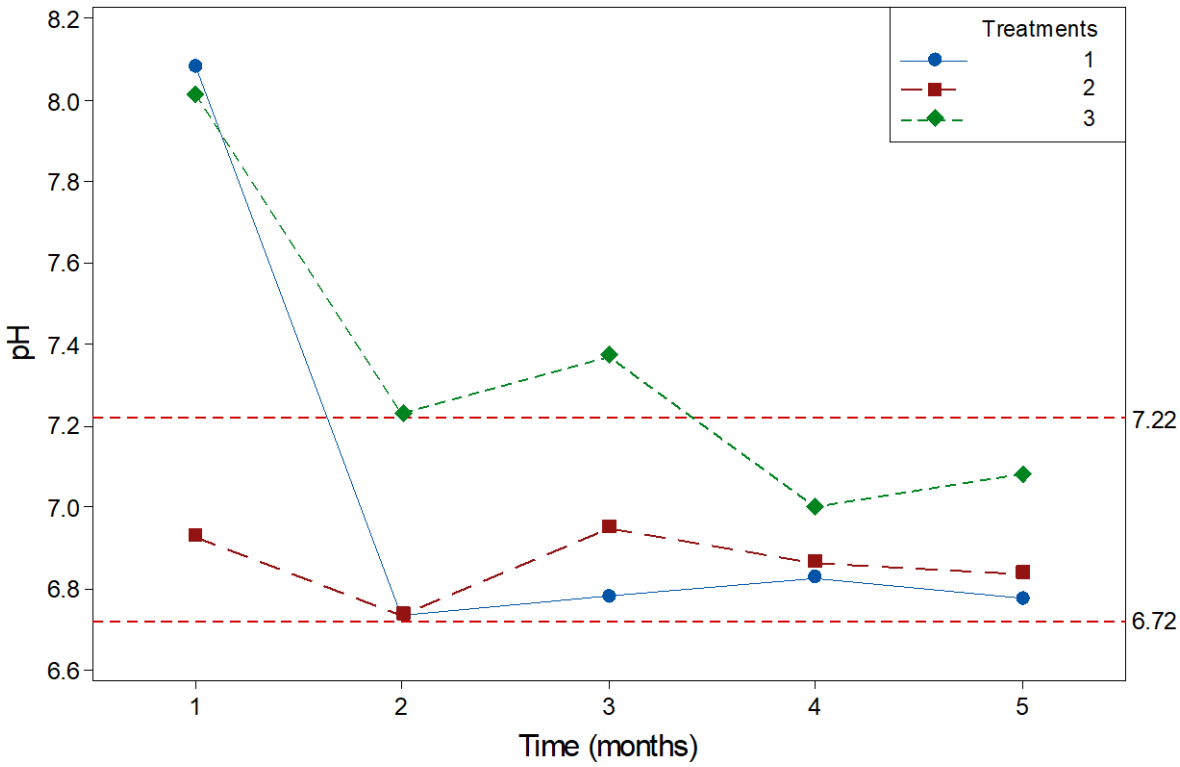


Figure 5.30: Line plot showing pH ranges for optimum HTF colony growth in all treatments over the five-month experiment phase. 1: Cow + Chicken; 2: Fungi; 3: Cow + Chicken + Fungi.

The pH appeared to significantly affect the amounts of HTF present in the treatments collectively. The Spearman correlation test showed a modest negative correlation (*rho* = -0.385, p = 0.009) indicating decreases in pH possibly influence HTF populations. it is possible that slightly acidic environments provided better growth conditions. This is evidenced further by the fungi only treatment which showed a strong negative correlation with pH (*rho* = -0.643, p = 0.010). This relatively acidic pH is within the optimal zone identified as suitable for fungi. Cooke & Rayner (1984) suggest that the role of soil acidity on fungal growth may be difficult to assess due to the ability of fungi to alter the pH values of their environment. However, the results are similar with findings of Pawar (2012) who established that the greater fungal population in hydrocarbon contaminated soils were found at low soil pH conditions within a range of 5.5 to 6.5. This agrees with Riser-Roberts (1998) who describes fungi as a significant component of microbial biomass in the soil that thrive in acidic conditions as they contribute to most decomposition processes.

5.3.3 Human Pathogen Indicators (*Escherichia coli* and *Salmonella* spp)

There is no consensus on which indicator is most suitable for use as a process validation for pathogen survival during composting. Nevertheless, some researchers have proposed *Salmonella* spp to be the best indicator for determining compost quality (Sidhu *et al*., 1999), while others (Shepherd Jr. *et al*., 2007) indicated that generic *Escherichia coli* (*E. coli*)

would be a better indicator for predicting pathogen survival in a composting system. So, following the compost quality guidelines described in the PAS 100:2011 the presence of two dominant indicator species (*E. coli* and *Salmonella* spp) were assessed in this study. The results indicated no prevalence of *E. coli* in the samples, therefore, meeting the stipulated PAS 100:2011 requirement of less than 1000 CFU/g upper limit and remained below the detectable threshold throughout the experimental process. Attachment of bacteria to the soil is an essential aspect of the bacterial fate and transport, and this may explain the absence of *E. coli* in the sample. Guber *et al*. (2005) suggest that maximum *E. coli* attachment occurred in the absence of manure, stating that increasing manure content generally resulted in the decreased attachment that resulted in lower levels of *E. coli*. This aligns with the current study's findings, which employs animal manure as a key component for remediating hydrocarbon contamination in soils through composting. *Salmonella* spp was detected and found to be marginally higher than the recommended permissible limit (Figure 5.31). Generally, there was no significant difference (Kruskal-Wallis H (2) = 2.11, p = 0.348) observed in the mean indicator organism levels between all treatments. Despite the apparent yet uncharacteristic elevated level of *Salmonella* spp found within treatment three in the third month of 6.10×10^2 CFU/g, the average levels in all the treatments remained relatively consistent and ranged from 0.33×10^1 to 2.39×10^2 CFU/g throughout the 150-day project duration. It seems possible that the spike in colony numbers in month three is due to pathogen regrowth or experimental error. This regrowth shows the survival of pathogens through the composting process or possible recontamination during turning of the sample in the reactor. Several studies have demonstrated *Salmonella* spp regrowth from below detectable levels both during and after high-temperature periods meeting or exceeding the regulatory requirements (Erickson, 2009; Shepherd Jr. *et al*., 2007). Previous work carried out by Salter & Cuyler (2003) demonstrated that *Salmonella* spp numbers initially decreased before noticeable regrowth during periods of temperature higher than 55 °C.

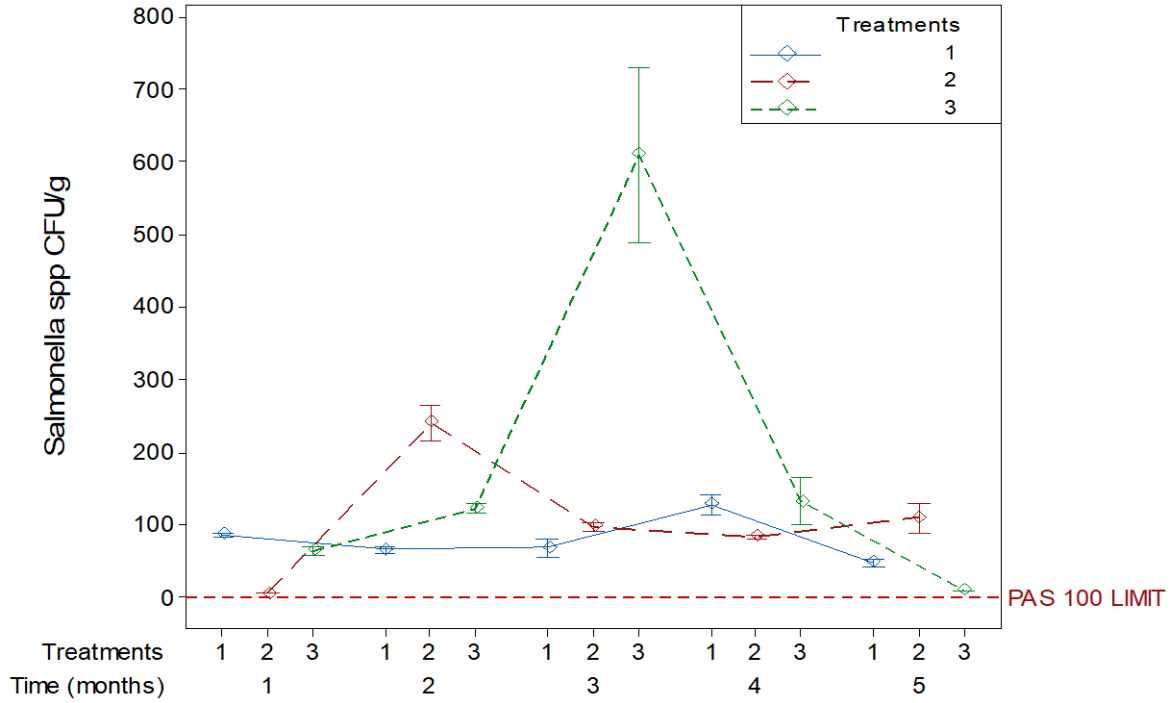


Figure 5.31: Interval plot showing changes in Salmonella spp over the experimental duration. Error bars represent mean ±S.E. (n = 3). 1: Cow + Chicken; 2: Fungi; 3: Cow + Chicken + Fungi.

According to Carr *et al*. (2015) and Islam *et al*. (2004), a significant amount of poultry and cattle manures contain *Salmonella* spp. The prevalence of these organisms throughout the experimental process even in the fungi only treatment of this study would suggest that these organisms are present in the contaminated soil sample. Earlier findings of Scott *et al*. (2006) and Diepeningen *et al*. (2005) showed that *Salmonella* spp could survive for extended periods (up to 12 months) in hydrocarbon-polluted sediment, with an observed range of between $<10^2$ to 10^6 CFU/g. Prior studies have noted the key role of temperature on the growth of *Salmonella* spp. Williams & Benson (1978) state that *Salmonella* spp survived for at least 18 months in animal manures at mesophilic temperature ranges of 15 to 25 °C. However, the authors point out that the inactivation of the microorganisms was much quicker when the animal waste was exposed to elevated temperatures of over 40 °C. This may explain the significant negative correlation (*rho* = -0.473 p = 0.001) between temperature and *Salmonella* spp.; indicating the greater presence of *Salmonella* spp at the relatively lower temperatures of the current study. A key observation from the results is the inability of the compost to reach thermophilic temperatures (>55 °C) which ideally would be required for pathogen destruction based on U.S. Environmental Protection Agency (US-EPA) standards, known as Process to Further Reduce Pathogens (PFRPs). This drawback might be due to the high hydrocarbon contamination levels that have most likely suppressed the growth of thermophilic microorganisms needed to enable the process to reach and maintain high temperatures. Indications that hydrocarbon presence played a significant role

as a limiting factor with respect to achieving thermophilic temperatures within the composting process in this study becomes apparent when compared with similar studies. Fernandes *et al*. (1994) found that an aerated static pile system containing mixtures of animal manures, straw, and peat moss was able to reach temperatures that comply with PFRPs requirements. Importantly, it is worthy to note that even when all USEPA stipulated requirements are achieved, one or more pathogens still remained viable for all types of composting systems (Wichuk & McCartney, 2007). Previous work by Hay (1996) corroborates these findings by suggesting that extended survival of low numbers of *Salmonella* spp produced was observed in animal manure amended with sawdust and held at 55 °C, indicating that despite meeting time-temperature criteria, the product still contained the pathogen. For compost to be freely utilised, the end-product must possess low pathogen concentration; it is also of utmost importance to ensure against pathogen regrowth (de Bertoldi et al., 1983). Based on the overall results, the persistence of *Salmonella* spp may be related to its presence in the feedstock, which in the case of this study would be the various animal manures, mature green waste and contaminated soil. There is also a possibility that some degree of recontamination may have occurred by tainted turning equipment.

5.4 Leachate Analysis

A significantly small number of studies have studied the leachate released during the composting process. The few that have studied this suggest leachate from both green and non-green feedstock composting operations contain high organic compounds, nutrients, and/or metals that can adversely impact water quality (ODEQ, 2001).

The principal aim of this section of the study was to monitor the concentrations of contaminants in the leachate generated following a rainfall simulation test to compare the recorded concentrations against existing water quality guidelines for Livestock Feeding (LSF) and Agricultural Irrigation Water (AIW). It is important to note that the leachate investigated was not subjected to any physical, chemical or biological type of treatment. This segment of phase two in the current study will establish possible migration of contaminants from the soil to the leachate. The concentration of pollutants in the leachate will be measured against thresholds to determine the suitability of the leachate for the desired end-use. With that knowledge, suggestions will be made on the need to implement management practices to minimise leachate transfer and recommend if protective

mechanisms should be constructed or simple remedial treatment applied to reduce contaminants.

5.4.1 Physico-Chemical Parameters

5.4.1.1 pH and Electrical Conductivity

The pH change amongst the treatments was marginal as they displayed a similar trend over the entire period ranging from 7.2 to 8.4 (Figure 5.32). It was seen to be slightly alkaline initially before a gradual decline into the neutral pH zone was observed.

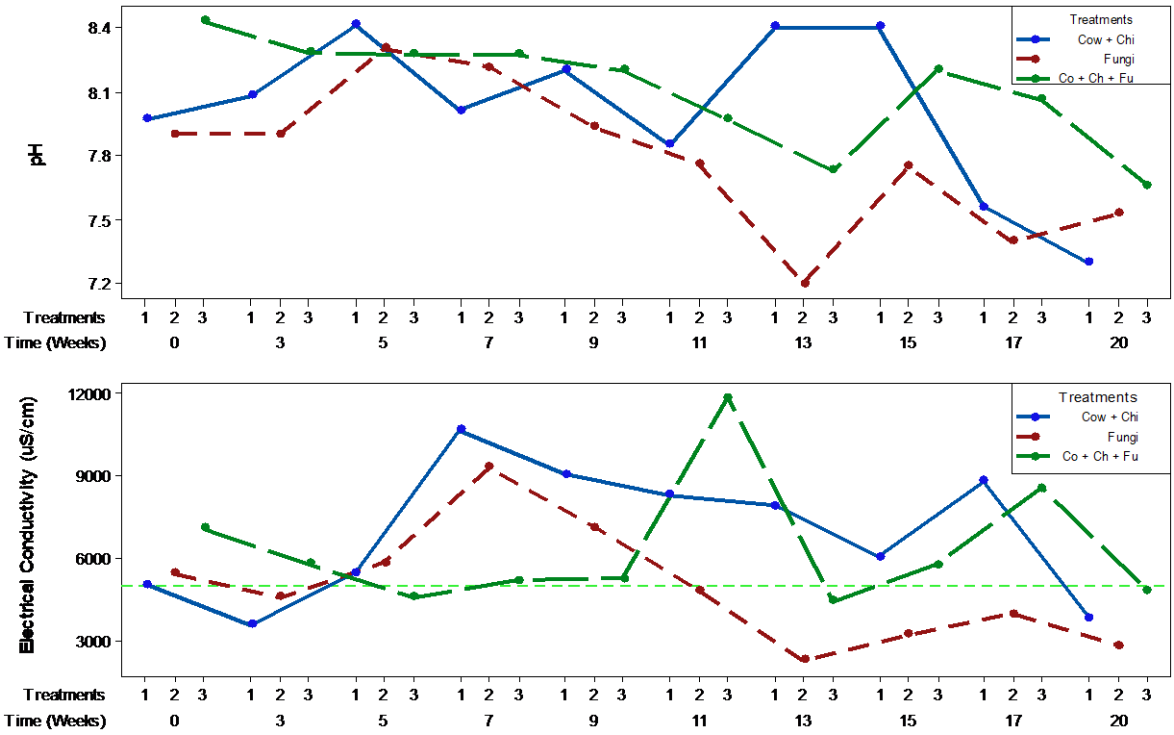


Figure 5.32: pH and Electrical conductivity values of the leachate.

There was no significant difference between the treatments (one-way ANOVA *F* (2, 27) = 2.56, p = 0.096). The end pH values of treatments one, two, and three were 7.3, 7.5, and 7.6 respectively. Perhaps the decline in pH might have occurred due to a dilution effect as continued water application on the matrix would result in more ions being transferred into leachate over time. It is also possible that the highly aerobic conditions enhanced the oxidation of organic acids in the reactors, possibly causing a reduction of CO_2 in the composted matrix's pore spaces, leading to the pH decline. The relative drop observed may be attributed to the stimulation of hydrolytic and fermentative bacteria present with the soil, thereby leading to carboxylic acids forming (Adams *et al.*, 2014).

The EC was found not to differ significantly amongst the treatments (one-way ANOVA *F* (2, 27) = 2.56, p = 0.096) and ranged from 2260 to 11780 µS/cm (Figure 5.32). The EC concentration was generally high and no significant changes over time were observed (one-way ANOVA *F* (9, 20) = 1.68, p = 0.160). The elevated EC may be due to a considerable release of the inorganic constituents from the composted soil within the first 11 weeks, after which it decreased. This is an opinion shared by Amadi (2014), who reports that high EC values signify the abundant existence of major cations and anions within a water sample. The highest end-value for EC was recorded in the manure-fungi treatment at 4800 µS/cm while the manure only and fungi only treatments had values of 3790 µS/cm and 2790 µS/cm respectively.

The end value of the pH in all the treatments was within permissible limits for livestock feeding and agricultural irrigation use. Regarding the final EC values, the leachate from the manure only and manure-fungi treatments was slightly above the threshold for agrarian irrigation, although it would suffice for livestock feeding. On the other hand, the leachate from the fungi only treatment is within permissible limits for agricultural irrigation and livestock feeding.

5.4.1.2 Nutrients

5.4.1.2.1 Nitrate and Ammonia

The nitrate concentration over the composting period was in a range of 4 to 428 mg/L (Figure 6.29). Although, the individual averages for treatments one, two, and three were 80.4, 6.76, and 34.05 mg/L. The higher levels displayed by treatments one and three is expected as these are manure containing reactors indicating the higher presence of particulates. However, the treatments did not differ significantly as demonstrated by statistical results (one-way ANOVA *F* (2, 27) = 2.15, p = 0.136). The nitrate concentration was consistent amongst the treatments throughout the process except for treatment one in week 17, which saw a considerably elevated concentration.

Ammonium had a relatively higher concentration throughout the process compared to nitrate. Its concentration was within a range of 1.2 to 214 mg/L. It is possible that bacteria present in the leachate were unable to break down ammonia into nitrate fully. This possibly is because ammonia usually varies with pH and is more prevalent in its molecular state NH_3 when conditions are more alkaline as observed with the leachate. Despite the variation displayed in ammonia concentrations by the treatments as shown in Figure 5.33, no significant difference (one-way ANOVA F (2, 27) = 2.20, p = 0.130) was found between

them. The presence of phosphate may have also contributed to the variation in ammonia concentration as evidenced in significant positive correlation results (r = 0.570, p = 0.001). This is because phosphates are often by-products of manure degradation and readily react with ammonia.

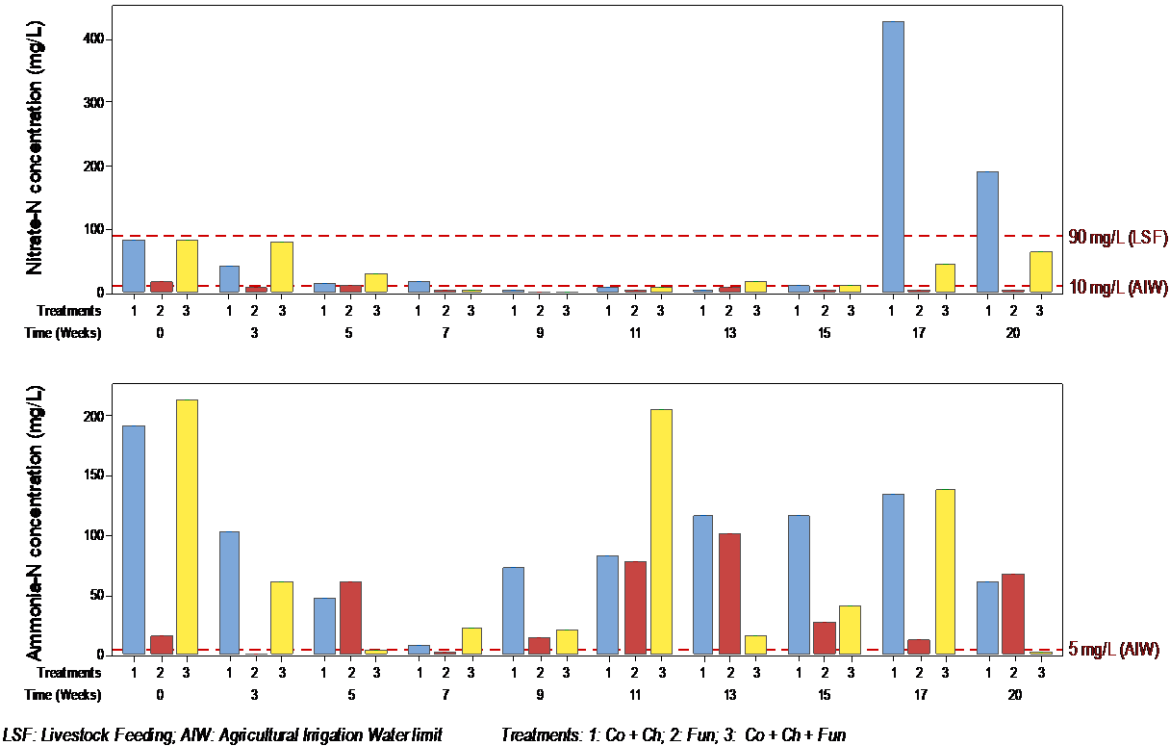


Figure 5.33: Nitrate and Ammonia content in the leachate of the various treatments.

The disparity in concentrations between ammonia and nitrates can be attributed to microbial utilisation within the solid matrix resulting in low levels in the leachate. Lower nitrate levels seen could possibly be due to its volatile behaviour and potential denitrification which reduces it to N_2 so that it can serve as an electron acceptor within the nitrogen cycle. However, John *et al*. (2011) suggest that ammonium salts are more stable compounds and preferentially required at higher rates than molecular nitrogen. Hence, ammonium might obstruct nitrogen fixation because most microorganisms use ammonium rather than nitrates, thereby making it more bioavailable.

In terms of applicability of the leachate, the end concentration of nitrate in the manure containing treatments was fractionally higher than the threshold value for irrigation while the leachate of the fungi only treatment was within acceptable limits for use.

5.4.1.2.2 Phosphate and Chloride

The phosphate concentration was seen to significantly drop by the fifth week of the process and remain low until the completion of the composting experiment. It ranged between 8.25 to 199.5 mg/L. The changes in phosphate concentration were similar for all treatments as reflected in Figure 5.34. The phosphate concentration differed significantly over the composting period (one-way ANOVA F (2, 27) = 2.20, p = 0.130) with a mean concentration declining from 126.2 mg/L at week zero to 21.25 mg/L by the end of the process.

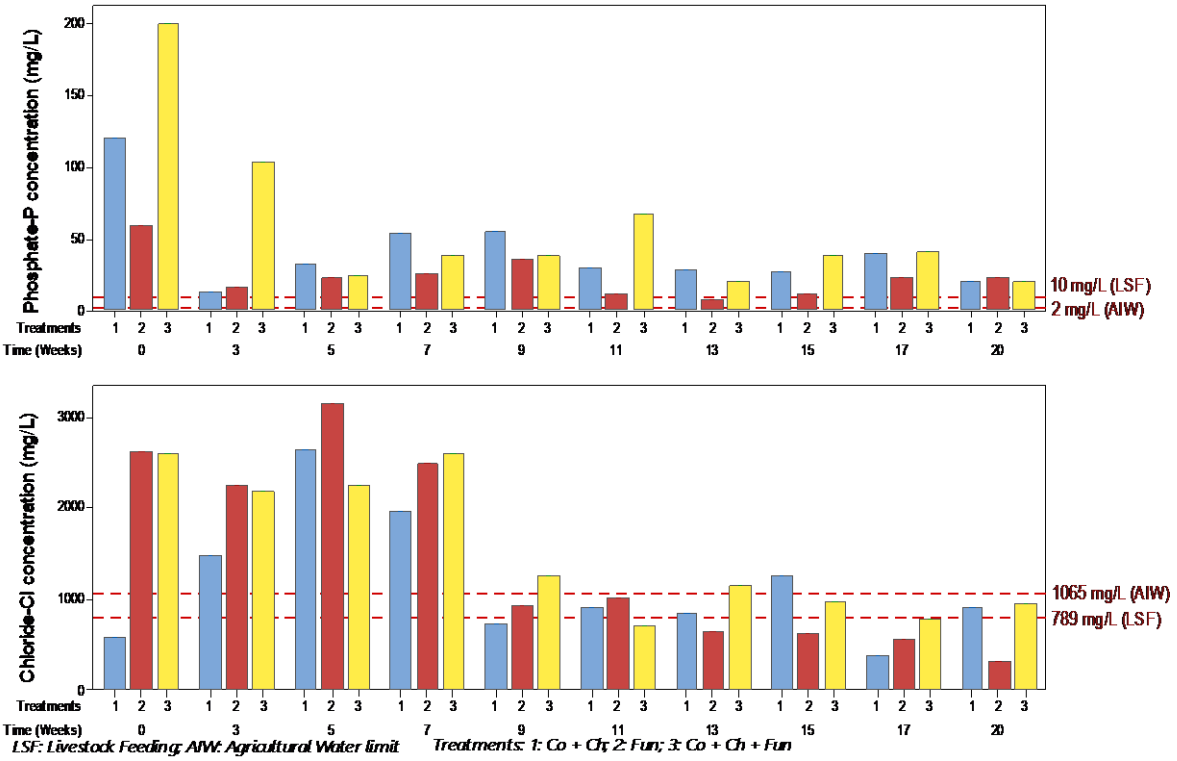


Figure 5.34: Phosphate and Chloride content in the leachate of the various treatments.

The chloride concentration was quite high initially until it significantly declined by week nine. It ranged between 3150 to 312 mg/L. Bennett *et al*. (1993) suggest that the increased presence of chlorides in waters can mainly be attributed to the dissolution of salt deposits in the form of ions (Cl^-). Although, the total mean concentration of chloride did not differ significantly between treatments (one-way ANOVA F (2, 27) = 0.54, p = 0.591), it differed significantly over time (one-way ANOVA F (9, 20) = 7.50, p < 0.001). Kirsch (2009) describes waters with a chloride content over the range of 150 to 10,000 mg/L as brackish water.

The phosphate concentrations in all three treatments were only minimally above the permissible limit for LSF and AIW. However, the chloride was suitable for AIW in all treatments, but only treatment two had leachate suitable for LSF.

5.4.1.3 Chemical Oxygen Demand (COD)

The Chemical oxygen demand (COD) test is widely used to measure the quantity of dissolved particulate organic matter in water (Sawyer *et al*., 2003). It has been used in this study to measure the number of oxidisable/organic contaminants in the leachate collected from each treatment during this study. The CODs ranged between 300 to 2800 mg/L. The highest mean values for this parameter was noted in the manure only treatment (1300 mg/L) and manure-fungi combination treatment (1010 mg/L). The fungi manure had a lower mean COD of 710 mg/L.

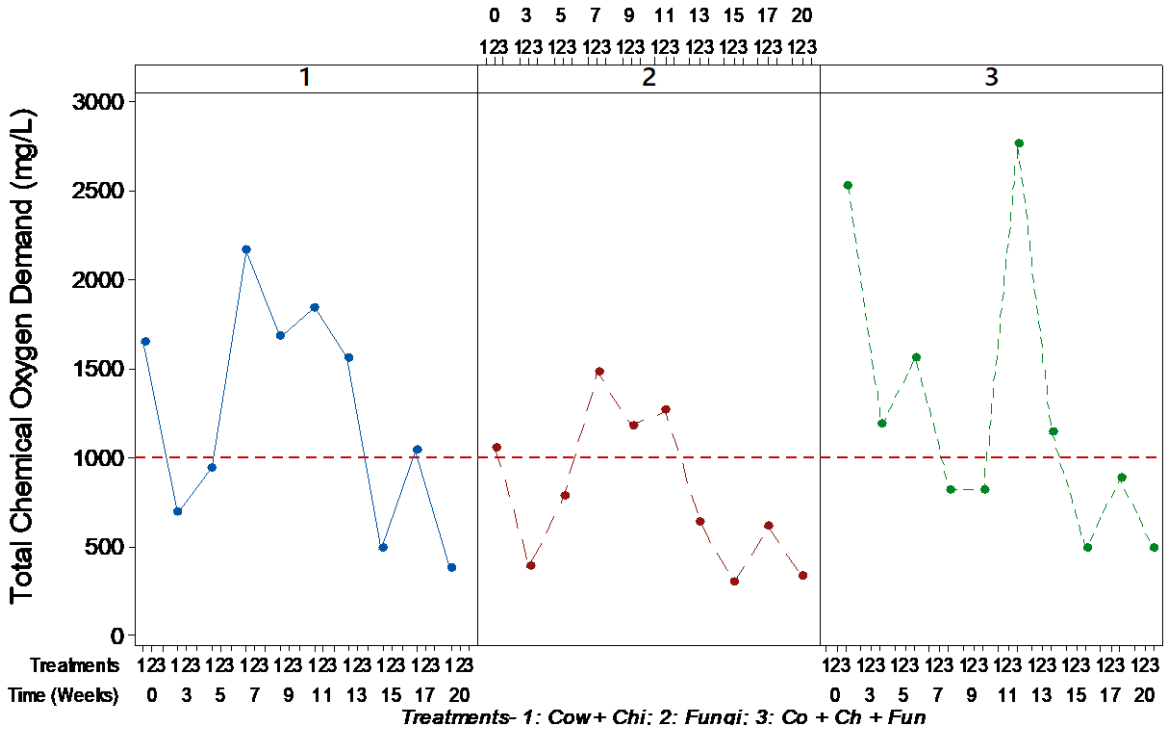


Figure 5.35: Chemical Oxygen Demand of the leachate of the various treatments.

An almost zigzag trend was observed in all the treatments (Figure 5.35). The initial CODs were relatively high before a steep decrease that was followed by a sharp rise. The samples were not filtered, and thus total COD was obtained. This might be responsible for the initial spikes observed. It is possible that the leachate released during the initial stages of composting is enriched with organics and this tends to be accompanied by high COD values. Medjor *et al*. (2018) found that COD drop in hydrocarbon-contaminated groundwater samples is due to various aromatic, straight-chain aliphatics, and nitrogenous compounds

in hydrocarbons that are not readily oxidisable. Depending on the desired end-use, Vaidya *et al.* (1997) report that the COD values of water must be within a range of 200 to 1000 mg/L before it can be released for any use in the environment. By the end of the composting process, all treatments were recorded to have COD values below 500 mg/L.

5.4.2 Total Petroleum Hydrocarbon Concentrations

The TPH was seen to decline continuously, and by week 13 no TPH could be detected in the leachate of any of the treatments (Figure 5.36). All the treatments had a similar pattern in terms of TPH loss from the leachate thus no significant difference (one-way ANOVA F (2, 27) = 0.05, p = 0.956). The level of TPHs found was in the range of 2.70 to 105.28 µg/ml.

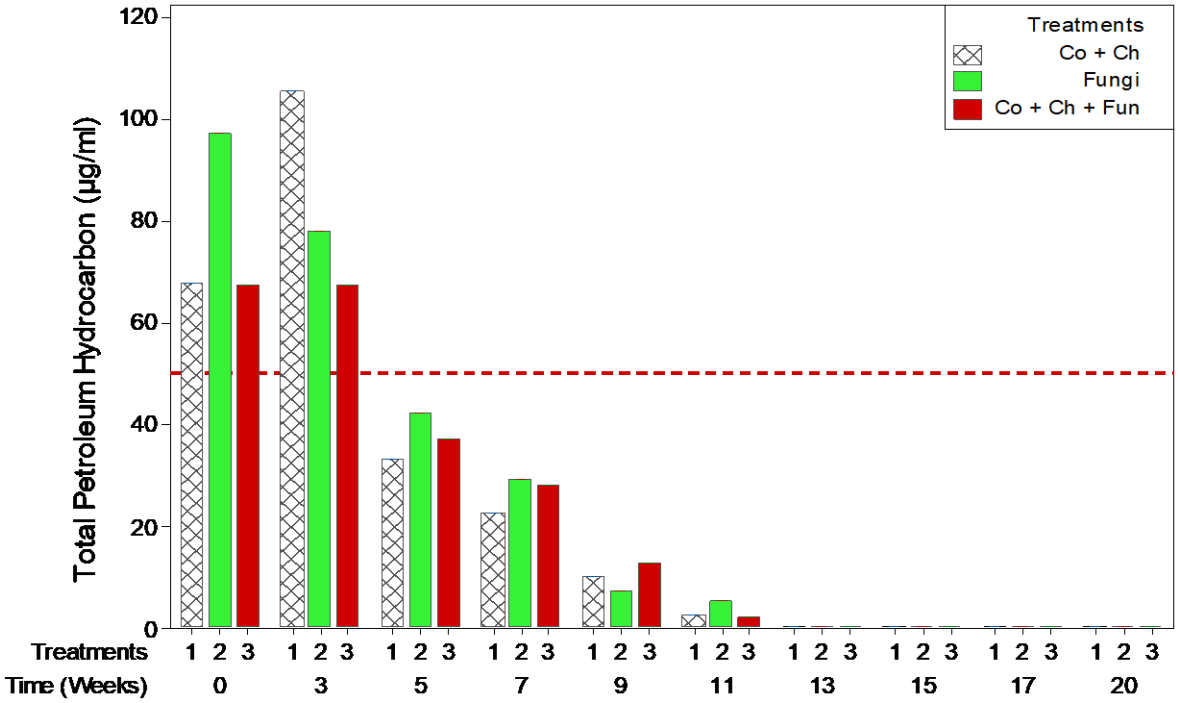


Figure 5.36: TPH concentration in the leachate of the various treatments.

The correlation results (r = 0.87, p < 0.001) generally suggest that the TPH concentration of the soil significantly influences the changes observed in leachate TPH concentrations. Possible losses due to volatilisation and microbial degradation within the leachate cannot be ruled out. The end TPH concentration suggests composting soils will contain very low concentrations of hydrocarbons that are biodegradable. The results of this study are in agreement with those of Huddleston *et al*. (1986), which found out that water-soluble organics leaching was less than 1% of the total organic content of refinery oily waste while conducting rainfall simulation tests.

5.4.3 Polycyclic Aromatic Hydrocarbon Concentrations

Most PAHs found were generally low in concentration in a range of 0 to 2.6 ng/ml in all treatments. Figure 5.37 indicates PAHs were consistently lost over time in all the treatments. Interestingly only LMW PAHs and a few MMW PAHs were detected in the leachate. These groups of PAHs have been described as the most predominantly leachable components from hydrocarbon-contaminated matrixes that are highly soluble in water (Salanitro *et al*., 1997). The final PAH concentrations are below the acceptable limits for use in LSF and AIW.

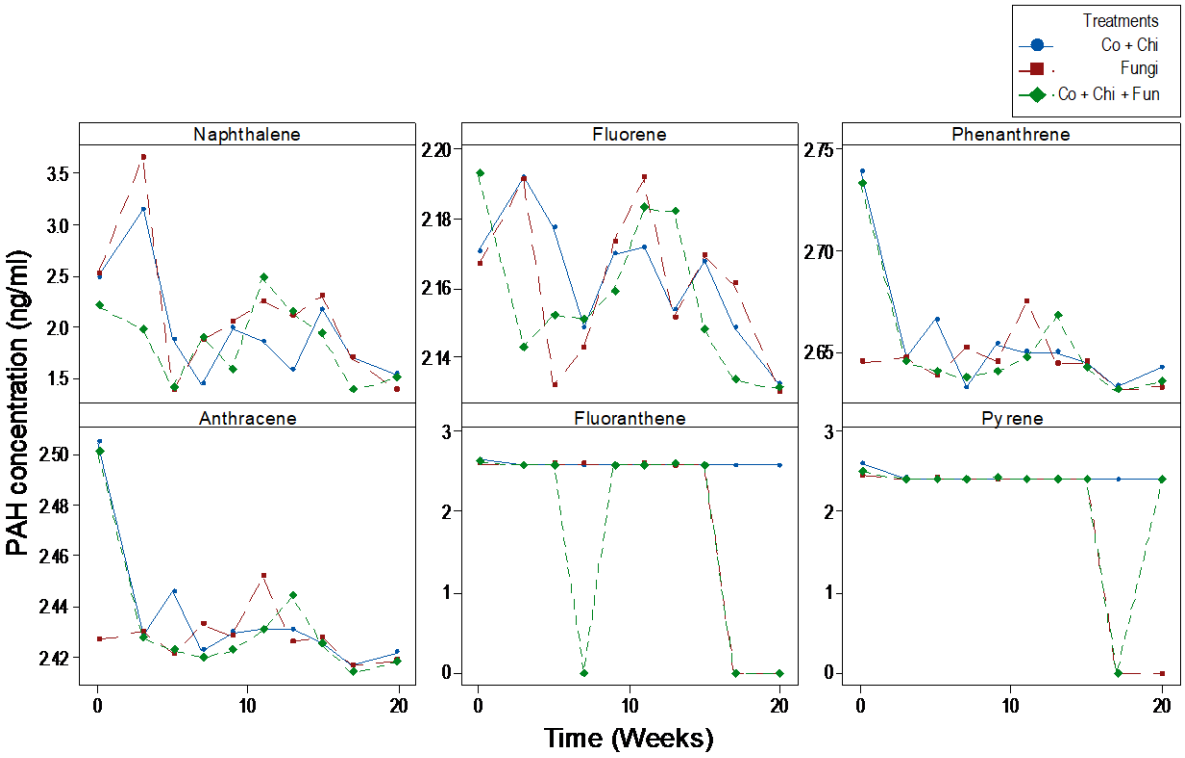


Figure 5.37: PAH concentration levels in the leachate of the various treatments.

5.4.4 Metal Concentrations

Metal concentrations between treatments generally showed a similar trend in terms of the changes observed over time (Figure 5.38). Although most concentrations seemed to decline relative to their initial concentrations, nickel and zinc were found to be the opposite. The elevated levels of zinc in leachate may be due to water-soluble fractions of zinc in the soil matrix (Wuana & Okieimen, 2011). In general, these contaminants are within permissible limits for application in AIW and LSF.

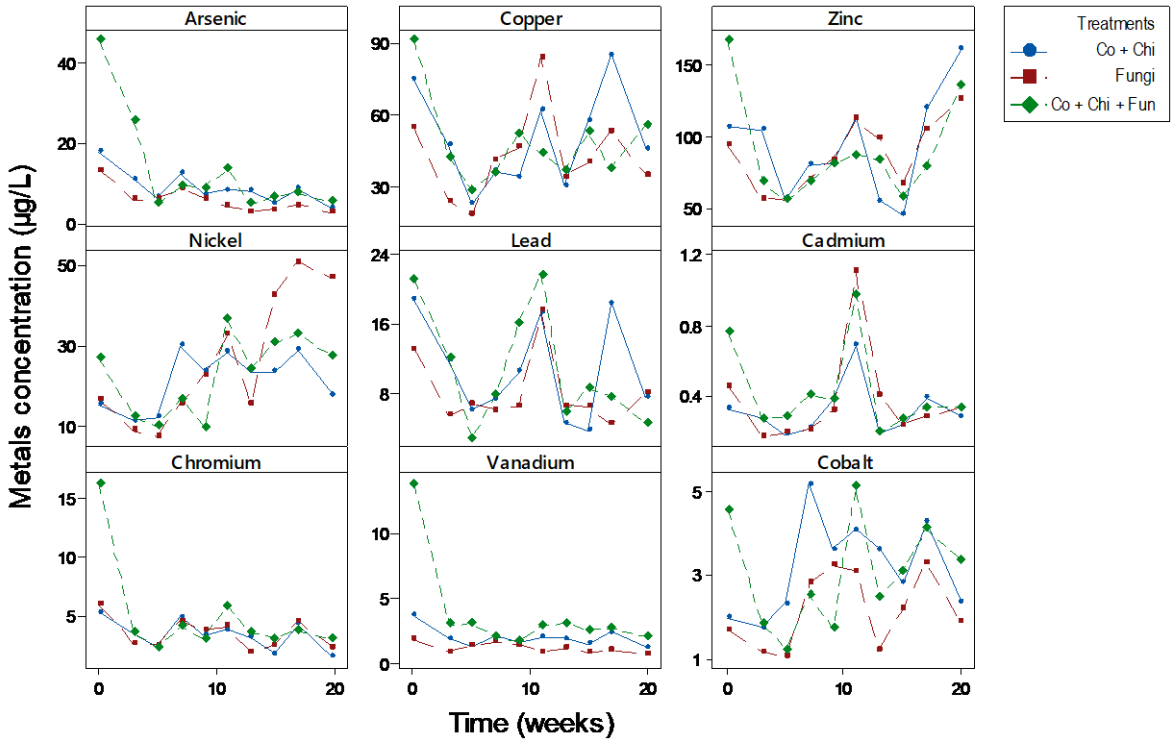


Figure 5.38: Metal concentration levels in the leachate of the various treatments.

5.5 Summary

This chapter assessed the effects of the three most effective treatment combinations from the previous bench-scale experiments. In this phase, a pilot-scale study was conducted using modified 100 L reactors with forced aeration and a leachate collection component. The amendments used were animal (cow and chicken) manures, and fungi (*Pleurotus ostreatus*) inoculated substrates. Mature green waste compost was used as a bulking agent. During the composting process, various physicochemical parameters and the concentration levels of key contaminants were measured at specific time intervals to determine the effectiveness of the composting remediation. A high-intensity rainfall simulation was conducted to investigate the potential formation of leachate and to determine if the pollutant concentration levels allow for its use in livestock feeding and agricultural irrigation.

TPH concentration reduced across all the treatment and the cow and chicken treatment had the highest reduction at 96%, closely followed by the fungi treatment which had a 95% reduction. The manure-fungi combination treatment managed a reduction of 87%. Specific nutrients monitored seemingly influenced the rates of TPH degradation. Chloride was a common factor in all three treatments. After the process, the end concentrations of the TPHs were found to be below the guideline values.

There was an occurrence of PAH degradation in all the treatments. Although, LMW and MMW PAHs seem to have been degraded considerably more than HHMW PAHs. Minimal loss of HMW PAHs took place after 20 weeks, thus suggesting this category of PAHs takes much longer to degrade due to their recalcitrant behaviour. The animal manure only and fungi only treatment showed some capability of degrading HMW PAHs. The animal manure-fungi combination treatment performed unexpectedly less than expected in HMW PAH degradation. Even though HMW PAHs were not significantly degraded, the end concentrations of all the groups of PAHs monitored did not exceed the permissible threshold.

The Microbial analysis confirmed the presence of microorganisms able to tolerate hydrocarbon pollution. It also revealed that a mesophilic temperature range of between 21 °C and 21.3°C was conducive for Hydrocarbon-tolerant bacteria (HTB) and Hydrocarbon-tolerant fungi (HTF) as well. The fungi-only treatments contained significantly higher quantities of HTF compared to the animal waste treatments which were dominated by HTB.

The physicochemical properties observed in the three treatments generally varied in comparable patterns. The pH and EC end values did not differ significantly amongst the treatments. Moisture content remained relatively consistent at an average of 32.8 %. A moderate drop of 4% in the OM was observed across treatments by the end of the process. It is possible that similar processes possibly occurred independently of the amendments utilised for each treatment.

The overall metal concentrations in the soils were more or less unchanged and were within acceptable limits. Slight concentration reductions are likely the result of adsorbance to soil particles or leaching during rainfall simulations.

The intense rainfall simulation generated leachate as expected and contaminant transfer was relatively low and in most cases below threshold values. Physicochemical properties of the leachates in all treatments exhibited similar changes over time.

By week 13, TPHs were below detection in the leachates. It was found that LMW and two MMW PAHs were transferred from the solid matrix to the leachate while no HMW PAHs were found. The concentrations of these hydrocarbons in the leachate were significantly lower than the soil, and their final concentrations were generally below the threshold values. Metal content was also very low, and like hydrocarbons, it was within safe levels for use in LSF and AIW.

CHAPTER 6

6.0 CONCLUSIONS

This study aimed at establishing the suitability of integrated-composting as a remediation method for treating hydrocarbon-contaminated soils and determine whether leachate from the process can be utilised for agricultural irrigation and livestock feeding. This was tested with three hypotheses, namely:

- The combined use of bacterial and fungal dominated inocula promotes the breakdown of TPHs and PAHs in weathered hydrocarbon-contaminated soils.
- Intense rainfall during the remediation process leads to the formation of leachate that will contain contaminants.
- The leachate generated from the process, if any, can be used for livestock feeding and agricultural irrigation without requiring further treatment, provided that the concentration levels of the contaminants are within guideline limits.

The results from the microcosm and mesocosm tests suggest the first hypothesis should be accepted, as the combination of both microorganisms was proven to be effective in the degradation of TPH and PAHs. Perhaps changes to the mix ratio and operating conditions, particularly temperature, may improve the efficiency of the treatments. However, it should be noted that the animal manure only and fungi only treatments individually showed an ability to degrade TPHs and PAHs. The second hypothesis was accepted because the rainfall simulation test showed that leachate was generated and contained contaminants. The third and final hypothesis was also accepted as the contaminants in the leachate were within safe levels for agricultural irrigation and livestock feeding.

The initial screening phase using the dynamic respiration index tests to simulate composting under controlled laboratory conditions demonstrated to be a helpful protocol in measuring the rate of aerobic activities during composting. However, evidence from the study found that aerobic activities do not often indicate hydrocarbon degradation. Results from the hydrocarbon analysis revealed that treatments containing animal manures in combination with fungi, fungi only, and animal manures only had the most significant hydrocarbon degradation rates despite not possessing high respiration indexes. The outcomes from the microcosm study formed the basis for developing the second phase mesocosm that was essentially a scaled-up version of the testing rigs used for the initial screening.

The presence of hydrocarbon-tolerant bacteria and fungi is an indicator that microorganisms are stimulating the biodegradation process. The rates of aerobic activities exhibited by these

microorganisms are evident in the respirometric tests. The microbial populations present in the animal manure-fungi combination treatment show that both consortia can simultaneously degrade hydrocarbons as far as optimal conditions are established. Results revealed that the amount of hydrocarbon-tolerant fungi (HTF) was significantly higher than hydrocarbon-tolerant bacteria (HTB). This is likely an indication that fungi may have superior resistance to hydrocarbon than bacteria. Perhaps, the hydrocarbon contaminants in the soil are highly recalcitrant and more structurally complex, making fungal degradation more effective. Different mix ratios of bacterial and fungal inoculated substrates should be considered to provide crucial information on their combined use as the current study has only considered one ratio.

Results from the microcosm study and mesocosm study were found to be comparable and differed mainly in magnitude. In the DR-4 tests, the cow and chicken manure, fungi only and the animal manure and fungi treatments had overall TPH removals of 58.9 %, 73.2 % and 56.9 % respectively. These removal rates were significantly higher in the mesocosm study, where the same treatments had 96 %, 87.3 % and 95 % respectively. Despite its lower removal rate in phase one, the combination of animal waste and fungi proved to be very effective on a larger scale. These results show that the fungi only treatment had a better rate of TPH removal at the higher temperature of 50 °C as seen in the DR-4 test. In contrast, fungi combined with animal manures was more effective at TPH reduction when the average temperature was 21.8 °C. This implies that a higher temperature would be more suitable for a large-scale application of fungi only treatment for TPH contaminated soil. On the other hand, lower temperature conditions would be preferable when fungi is combined with animal manures, as reflected in the results of the TPH reduction in the mesocosm study. These findings further indicate that temperature plays a vital role in the composting process of hydrocarbons.

The breakdown of HMW PAHs was evident from the DR4 results whereas, very minimal degradation was observed for these compounds in the mesocosm study with less than 5 % removal seen across all the treatments. Considering the composting process was limited to just 20 weeks due to time constraints, it will be useful to conduct these tests for an extended period of at least six months for significant HMW PAH degradation to be seen. The effect of prolonged weathering the soil has undergone may influence the biodegradation rate of the pollutants as more recalcitrant and less bioavailable components within the polluted soil are left. It may be worth considering altering essential operational conditions such as; the temperature, aeration and turning regime, and complemented with nutrients to optimise the effectiveness of the process.

The changes observed in the organic matter content is often an indication of composting taking place. As such, composting is likely to have occurred during the DR-4 and large-scale reactor tests. Results show an average organic matter loss of 4 % for the chosen treatments during the DR-4 and 6 % loss during the mesocosm reactor test. The moisture content was similar, although slightly higher in the microcosm with an average of 40 % compared to 32 % of the mesocosm. It was sufficient to stimulate microbial populations responsible for hydrocarbon degradation. At the start of the microcosm and mesocosm tests, the mean pH was similar at 7.84 and 7.67 and the end at 6.73 and 6.87 respectively. However, there were significant changes in the electrical conductivity of the mesocosm study with an average initial value of 1336 µS/cm to 397.3 µS/cm. The effect of the high-intensity rainfall simulation is likely the cause for this decline. Ion transfer from the soil to the leachate occurred as EC values greater than 7000 µS/cm was seen. In the DR-4 tests, the EC value saw a slight increase from 1724 µS/cm initially to 1862 µS/cm at the end.

Nutrient availability and physicochemical parameters were found to play crucial roles in TPH degradation. Chloride concentrations and temperature particularly seem to be common variables influencing hydrocarbon degradation across all the treatments. The animal manure only treatment was particularly susceptible to changes in the moisture content, nitrates, phosphates. The fungi only treatment was influenced by changes in moisture, organic matter and phosphates. The animal manures with fungi combination treatment, variations in organic matter, electrical conductivity and nitrates influenced TPH degradation.

There was a considerable quantity of leachate generated following the rainfall simulation at each period. The levels of contaminants found were within acceptable prescribed guideline limits. The leachate from the process does not pose a significant threat to crops or livestock. However, the overall quality can be improved with further treatments such as phytoremediation using reed bed systems, activated sludge systems, biologically activated carbon systems, trickling filters and a host of options available. The chloride and EC levels are relatively higher than would be liked for livestock feeding. Thus, suitable methods of desalination should be considered.

The discrepancy between the respiration data and the hydrocarbon degradation is a significant finding of this study as it suggests that the environment most conducive to composting is not necessarily the best suited to hydrocarbon degradation. This has major implications regarding the applicability of the respiration index as an indicator of hydrocarbon degradation and, as such, would presumably be of major import for both academics and practitioners. This will undoubtedly serve as a base for future studies.

The study further enhances our understanding of composting as it has shown that the process is an effective method for hydrocarbon degradation under high-intensity rainfall. This would be particularly useful in tropical regions around the globe. It also suggests that contrary to numerous previous researches, composting can occur even at temperatures as low as 20 °C as seen in the current study.

This study has shown that on-site remediation can be a feasible option when considering hydrocarbon contamination in small areas instead of large spill sites. This was achieved by piling contaminated soil into a simple bioreactor system with forced aeration, animal wastes, and fungi inoculated substrate. This system is easily assembled and used by individuals impacted by oil spills, e.g. local subsistence farmers in the Niger Delta region of Nigeria who often low crop production due to oil contamination of their farmlands. However, one must be mindful that practical considerations must be made when adopting a remedial strategy where large-scale contamination is involved. Therefore, it is essential for the chemical and biological processes of composting to be thoroughly assessed at laboratory and pilot scales before commencing field scale ventures.

The study has found that composting if fully understood, as a remediation technology will provide an environmentally friendly and potentially less expensive option for the remediation of hydrocarbon contamination.

6.1 FURTHER WORK

Whilst valuable findings were obtained from this project, some limitations could also benefit from further study.

- It would be useful to conduct the microcosm study with a wider range of mix ratios and perhaps consider the use of other readily available animal manures with different properties and other species of fungi. Contaminated soil to amendment ratios of 1:0.5, 1:0.7, 1:1, 1:5, 1:2 by weight should be trialled as these combinations may yield higher pollutant removal and also better explain the relationship between respiration rates and hydrocarbon degradation. Hydrocarbon losses through volatilisation process should be monitored in future studies alike to assess how much degradation is actually as a result of this process.

- The results for the leachate are presented over discreet periods with water guidelines exceeded at different points in time. This does not allow for a spill partway through the process, nor does it determine if the final accumulated water will exceed guidelines. It would

be useful when conducting similar research in the future to calculate the concentration and quantity of water produced within each time period, then determine the total volume and the associated concentration. Alternatively, a constant rate of leaching could be assumed and the concentrations evaluated.

- Temperature plays a vital role in the composting process and as such, it would be useful to conduct investigations on the relationships between temperature profile characteristics and the process performance indicators in the microcosm- and mesocosm-scale composting experiments. Understanding the temperature profiles during the experiments could benefit from the development of more standardised procedures for the temperature monitoring, specifically with regards to location and frequency. In the current study, effort was made to place temperature data loggers in the same position within the different reactors. However, it is likely that the locations where the data loggers were placed within the samples in the reactors differed, leading to potentially variable temperature records between them. Therefore, to obtain less variability and more uniform temperature data across all the reactors, locations along the vertical axis in the reactors may be expressed in a dimensionless form, perhaps as a ratio of height to the total height of the composted matrix and standard positions chosen. With regards to temperature data logging frequency, as data loggers can now record at intervals of seconds or minutes, appropriate intervals and corresponding data smoothing techniques should be properly understood.

- Molecular biology could be studied further to understand the interaction of the different microbial communities and potentially disaggregate them using microbiological and chemical mapping to determine the best way to manipulate these microorganisms during the degradation process and classify the specific microbial population involved in the breakdown of hydrocarbons. This study only considered in totality the microorganisms that are tolerant to hydrocarbons. Therefore, further isolation and identification of specific hydrocarbon degrading microorganisms would be useful in better understanding actual microbiological processes that stimulate the breakdown of pollutants during the composting process.

- Investigating the impact of weathering on hydrocarbon contamination degradation in the soil will be beneficial in future studies and this can be done by conducting similar experiments simultaneously with soils artificially spiked with the same contaminants. This would establish the rate at which contaminants can be potentially transferred, transformed, and degraded. In the current study, it was difficult to fully establish if higher levels of hydrocarbon concentrations for particular compounds that remained even after the experiment were influenced by earlier weathering the sample had undergone.

Understanding if pollutant transfer may be more prevalent in fresh contamination compared to weathered contaminations would inform engineers on the applicability of composting as a suitable remediation technique especially in areas that experience high intensity rainfall.

- Despite the notable degradation of hydrocarbons observed, it must be stated that the removal of HMW PAHs remained incomplete and perhaps the experimental process could benefit from and extended time. However, where rapid removal of these contaminants is required, composting bioremediation may not be the most efficient treatment method for the complete removal of HMW PAHs in weathered hydrocarbon contaminated soils and perhaps further treatment may be required. These other treatment options may be less environmentally friendly and possess considerable carbon footprint. Therefore, it would be useful to conduct a life cycle analysis (LCA) of various remediation options whilst also performing a risk assessment of the contaminated matrix requiring treatment. By doing so, an economic-based remediation-efficiency against a risk matrix could be established and can potentially provide a supplementary decision support tool for environmental practitioners. In a similar light, the research would hugely benefit from the use of both physical and mathematical modelling of the composting process as the results would be a useful predictive tool when scaling up laboratory-based microcosm tests into mesocosm tests and actual full-scale field investigations.

CHAPTER 7

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CHAPTER 8

8.0 APPENDICES

APPENDIX 1

Table 8.1: Combined review of similar studies on composting hydrocarbon contaminated soils. The "Remediation Strategy"-column describes the study and experimental paramters as far as they provided; the "Observation/Result"-column summarises the key outcomes; the "Ref"-column refers to the literature reference (Loick et al., 2009)**;** *reviewed by (Antizar-Ladislao et al., 2004) **reviewed by (Wilson & Jones, 1993)**.**

Remediation Strategy	Observation/Result	Ref.
Soil type: municipal sludge **Contamination**: radiolabelled phenanthrene (1.3-1.6 mg per kg dry sludge) **Amendment**: benchtop laboratory compost apparatus simulating aerated-pile composting conditions **Conditions**: **Time**: 10 -20 days	10-11% of phenanthrene degraded 15-17% of not extractable phenanthrene metabolites remained in the compost bound to organic matter or incorporated by mkicro-organisms	(Racke and Frink 1989)*
Soil type: sandy loam, un-acclimatised **Contamination**: wood-preserving and petroleum-refining wastes added to soil (total PAH 490-6646 mg per kg soil) **Amendment**: **Conditions**: batch reactors, soil columns **Time**: 354 days	Generally greater decrease of LMW PAHs than HMW, no detectable degradation of 5-rings PAHs; decrease in HMW correlated with oil and grease contents of the wastes and therefore attributed priamrily to co-oxidation processes	(April *et al.*1990)

Soil type: sandy soil **Contamination**: gasoline, lubricating oil (lower ring PAHs), crude oil (highest concentration of HMW PAHs) **Amendment**: nutrient addition (fertiliser, KNO_3, NH_4NO_3 NaH_2PO_4, urea) **Conditions**: batch cultures (soil slurry), 20 ^{0}C, no pH control **Time**:	Bioremediation by in situ organisms would appear to be most effective for low and medium distillate hydrocarbons, such as lubricating oil, which consists of lower ring PAHs, volatile aromatics, and aliphatic compounds, although provision of an adequate oxygen supply and nutrients is important to enhance degradation	(Morgan and Watkinson 1990)**
Soil type: loam sand clay **Contamination**: fuel products, low to high distillate fuels added to soil **Amendment**: fertiliser (NH_2NO_3, K_2HPO_4) **Conditions**: treatability study, soil columns, weekly tilling, 50% moisture, pH 7.5-7.6 (adjusted with lime), 17-37 ^{0}C **Time**: 4 weeks	Biodegradation played a relatively minor role in reduction of lower-distillate fuels compared with vaporisation and was ineffective in removal of the heavier fuels; Bioremediation of soil contaminated with medium distillate fuels was effective	(Song *et al.* 1990)**

Soil type: 68% sand, 16% silt, 22% org matter, 16% clay **Contamination**: jet fuel, heating oil, disel oil (all are medium distillate fuels), 2.3 ml per cm2 applied to soil (hydrocarbon content 50-70 mg per g soil) **Amendment**: fertiliser-urea 10mg per cm^2 superphosphate 4.3mg per cm^2, C:N (200:1), C:P (1000:1) **Conditions**: treatability study, outdoor lysimeter, watering, weekly tilling to 15cm, pH 7.4 (adjusted with lime), 16-15 ^0C **Time**: 20 weeks	≥90% reduction of hydrocarbons in soil for all fuels (to <5 mg per g soil) treatment was effective in increasing the rate of biodegradation	(Wang and Bartha 1990; Wang *et al.* 1990)**
Soil type: sandy surbsurface and surface soil **Contamination**: creosote **Amendment**: nutrient solution 50 mg per 3 kg soil tilling **Conditions**: treatability study, landfarming chamber, pH 7.1, moisture 8-12% 23 ^0C, no inoculation **Time**: 12 weeks	In surface soil: enhanced LMW PAH degradation >50% after 12 weeks, loss of HMW PAHS considerably lower In subsurface soil: PAH concentrations remained high although actual carbon turnover was greater than in surface soils. Once creosote phelonics were extensively degraded (8-12 weeks) there was significant increase in activity towards 2- and 3-ring PAHs. However, still little loss of HMW PAHs; nutrient addition appeared to have a positive effect on the extent of biodegradation of HMW PAHs but no significant effect on the LMW PAHs; general rate of biodegradation of the containment material: phelonics> heterocyclics>LMW PAHs>HMW PAHs>pentachlorophenol	(Mueller *et al.*1991)**

Soil type: **Contamination**: PAH contaminated sewage sludge applied to different soils **Amendment**: **Conditions**: glass microcosms **Time**:	different behaviour of PAHs in each soil, probably due to different soil characteristics and history of PAH exposure half-lives for phenanthrene 83-193 days and for Benzo(g.h.i)perylene 282-535 days lowest half-life values for most PAH compounds in spiked soil suggesting s higher susceptibility os spiked PAHs to both abiotic and biological degradation PAH compounds with less than four benzene rings are susceptible to abiotic loss processes; losses by these mechanisms were insignificant for compounds with four or more benzene rings	(Wild and Jones 1993)
Soil type: **Contamination**: 2- to 4-ring PAHs (100 mg per kg soil) and other semi-volatile compounds <10 mg per kg soil **Amendment**: composting with leaves **Conditions**: windrows (~19 m^3) **Time**: 150 days	Complete breakdown after 150 days with most loss during the first 63 days. Amendment ratio did not affect the extent of degradation of PAHs during the study it was observedthat temperature, moisture and C/N were deficient for optimal composting conditions	(Crawford *et al.* 1993)*
Soil type: **Contamination**: pyrene **Amendment**: soil and soil-mature compost mixtures **Conditions**: **Time**: 100 days	>80% pyrene degradation (significantly enhanced) after 20 days when mature compost was added (5% removal without compost addition)	(Mahro and Kastner 1993)*

Soil type: Ah horizon of para-brown soil from an uncontaminated, rural area near Hamburg, Germany **Contamination**:[^{14}C]Anthracene, [^{14}C]Hexadecane **Amendment**: mature compost (1:4) compost wt.: soil dry wt.) **Conditions**: Co-composting contaminated soil with mature compost **Time**: 103 days	23% of [^{14}C] Anthracene transformed into $^{14}CO_2$ and 42% was irreversibly fixed to the soil matrix. In the un-supplemented control reactor more than 88% of the optional [^{14}C]Anthracene could be recovered 21% of the labelled carbon of [^{14}C] Hexadecane could not be extracted. The compost could stimulate the depletion of hydrocarbons by either mineralization or the formation not extractable bound residues (humification)	(Kastner *et al.* 1995)
Soil type: slit loam **Contamination**: spiked with Benzo[a]pyrene (150 mg per kg soil) **Amendment**: laboratory scale (125 ml) in-vessel composting with and without *Phanerochaete chrysosporium*; soil amended with corncobs (soil:corncobs (soil:corncobs 2:1) **Conditions**: periodically aerated **Time**: 95 days	No difference between inoculated (62.8% removal and un-inoculated (65.6% removal) systems although initial removal rates were faster in the inoculated incybations. During poison tests with 4% $HgCl_2$, Benzo[a]pyrene removal was observed suggesting irreversable adsorption to compost matarial A substantial concentration of P. chrysosporium was found in both (inoculated and un-inoculated) systems after 95 days suggesting that amending soil with suitable fungal substrates may be sufficient	(Mcfarland and Qiu 1995)*
Soil type: **Contamination**: Benzo(a)pyrene (BaP) **Amendment**: Inoculation with *Phanerochaete chrysosporium* **Conditions**: **Time**: 95 days	Fungi increased the formation of bound residue in the first 30 days from 0.73 mg per kg soil per day to 1.58 mg per kg soil per day Despite this after the 95 days fungal inoculation was found to be ineffective with 62% (inoculated), 65 % (un-inoculated) and 49% (poisoned compost) BaP-removal No loss through volatilisation and mineralisation, nearly all of the BaP removal was attributed to bound residue information	(McFarland and Qui 1995)*

Soil type: **Contamination**: [^{14}C]anthracene **Amendment**: mature compost **Conditions**: 3L compost reactors, temp 21± 2 ^{0}C, continuously aerated with humudified air, 60% moisture **Time**: 103 days	23% of [^{14}C]anthracene was mineralised to $^{14}CO_2$ and 42% was irreversibly bound to the soil-composit matrix In soil-only incubations ~88% [^{14}C]anthracene was recoverable by solvent extraction with the formation of bound residue less significant	(Kastner *et al.* 1995)*
Soil type: non contaminated sterilised and non-sterilised agricultural top soil **Contamination**: spike with [14C]pyrene **Amendment**: fungi Kuehneromyces mutabilis and Agrocybe aegerita **Conditions**: soil mixed with sawdust, 70% moisture **Time**: 63 days	5.1% [14C]pyrene degraded by K. mutabilis, 1.5% degraded by A. aegerita, when soil was terilised and inoculated 27% [14C]pyrene degradation by indeginous micro-organisms in no sterilised and non inoculated soil 47.7% [14C]pyrene degradation by indigenous micro-organisms and K.mutabilis in non sterilised and inoculated soil 38.5% [14C]pyrene degradation by indigenous micro-organisms and A. aegerita in non sterilised and inoculated soil	(Sack and Fritsche 1996)

Soil type: precontaminated and uncontaminated soil **Contamination**: total PAH 0.082 to 1571 mg per kg soil (pre-contaminated soil) 14C-PAHs were added to a final concentration of : [14C]-Phenanthrene 500 ng per g soil, [14C]-Pyrene 100 ng per g soil, [14C]-Benz[a]anthracene 214 ng per g soil, [14C]-Chrysene 237 ng per g soil, [14C]-Benz[a]pyrene (BaP) 136 ng per g soil **Amendment**: **Conditions**: 1g soil mixed with 10 mi H_2O and incubate in the dark at 20 ^{O}C **Time**: 2 months	[14C]PAHs (except BaP) readily mineralised in pre-contaminated soils with 30-60% phenanthrene, 10-55% pyrene, 5-40% Benz[a]anthracene, 10-50% chrysene, 2-9% BaP <5% [14C]PAHs mineralised in uncontaminated soils <10% loss by metabolite production and cellular incorporation No relation between microbial-comminuty siz and the fate of [14C]PAHs Negative correlation between the fraction os silt and clay to the extend of [14C]PAH mineralisation and positvely correlated to the amount of [14C]PAHs remaining in the soil after extraction	(Carnichael and Pfaender 1997)
Soil type: simulated municipal solid waste **Contamination**: spiked with a mixture of 3- and 4-ring PAHs **Amendment**: **Conditions**: laboratory scale, batch-type, in-vessel composter, 50-60% moisture during active composting and 30% during curing; aerated and/or stirred; 50 ^{O}C during active composting; nutrients (0.8% ammonia nitrogen, 2.3% nitrate, 32.9% urea) during active composting **Time**: 30 days active composting + 30 days compost curing	Removal of anthracene, Phenanthrene, and pyrene mainly through biotic processess Fluorene lost with abiotic processes accounting for 75% Benz[a]anthracene was resistant to biodegradation throughout both phases, but 40-50% was lost abiotically Most biodegradation occurred during active compposting	(Joyce *et al.* 1998)*

Soil type: weathered-hydrocarbon contaminated soil from imperial oil, Sarnia, ON, and uncontaminated soil **Contamination**: mineral oil and grease including aliphatic-, polar-, and aromatic hydrocarbons **Amendment**: alfalfa and maple-leaves adding either 4.2% $CaCO_3$ and PO_4^{3-} **Conditions**: 1 litre jars, aerated, 50-60% moisture, temperature profile; altering C/N ratio (17-49 mol/mol), addition, temperature, length of thermophilic phase **Time**: 30 days	Increased degradation with: - low C:N ration (~17~18) - 23 ^{0}C, than 5 days at 50 ^{0}C, but - maintaining 50 ^{0}C for 30 days much better than 50 ^{0}C for 6 days and 23 ^{0}C for the remaining time - adding $CaCO_3$ or PO_4, but adding both togetther had no effect - increasing the leaves/alfalfa amendment	(Beauding *et al.*1999)
Soil type: from abandoned wood preservation site in southern Norway **Contamination**: creosote (including 16 USEPA PAHs) **Amendment**: fertiliser pre-treated and not pre-treated, straw inoculated with *Pleurotus osetreatus* **Conditions**: soil was mixed and layered with straw inoculated with *Pleurotus ostreatus* at 8 ^{O}C or 22 ^{O}C **Time**: 2 months	*P.ostreatus* had an overall postive effect on the degradation of aged creosote contaminated soil enhanced degradation by increased temperature and ferilizer pre-treatment which had a good effect on the microbial community at low temperatures even without addition of fungi	(Eggen and Sveum 1999)

Soil type: real contaminated soil and spiked sterile soil **Contamination**: PAH **Amendment**: fungal (*Bjerkandera sp.*) pre-colonised hemp stem wood **Conditions**: **Time**: 90 days	Up to 70% PAH removal in real aged soil in real soil the *Bjerkandera sp*. Inoculated soil showed lowest residual PAH concentrations compared with inoculated soil after 56 days in spiked soil ver broad optimal growth ranges for fungi and high residual concentrations after *Bjerkandera sp.* treatment which could be partly overcome by miscible solvent or surfactant addition The limited bioavailability of PAHs seems to be the most important bottleneck for implenetation of a white-rot-fungus-based remediation technique for polluted soils	(Grotenhuis *et al.* 1999)
Soil type: contaminated sewage sludge **Contamination**: PAHs **Amendment**: Ligneous waste **Conditions**: co-composting **Time**: 90 days	31% decrease of total PAHs during the first 20 days followed by an 8% increase up to day 90	(Lazzari *et al.* 1999)
Soil type: **Contamination**: [14C]anthracene (1000 mg per kg soil) **Amendment**: pure soil and soil-compost mixture (4:1) **Conditions**: bioreactor in laborartory scale continuously aerated, 60% moisture **Time**: 176 days	Complete transformation of [14C]anthracene less formation of bound residues (20.7%) and a higher mineralisation (67.2%) in the compost mixture than in pure soil (43.8% mineralised, 45.4% transformed into bound residue)	(Kastner *et al.* 1999)*

Soil type: silty soil **Contamination**: TPH 40g per kg soil and PAH 630 mg per kg soil **Amendment**: 640 g soil + 250 g maple leaves + 750g alfalfa + 80g $CaCO_3$ **Conditions**: moisture content 50%, incubated at 55 ^{0}C, aerated continuously or intermittently **Time**: 35 days active composting + 90 days maturing at ambient temp; after that mixing the resulting compost with more PAH contaminated soil (1:4) and coompost for 100 days	>50% mineralisation of pyrene by day 15 <3% mineralisation of pyrene by day 15 in unamended 0.7 % maximum total mineralisation in abiotic controls Enhanced pyrene mineralisation by addition of humic acid to the soil compost mixture inhibitited pyrene mineralisation with fulvic acid addition	(Haderlein *et al.* 1999; Haderlein *et al.* 2001)*
Soil type: silty clay **Contamination**: tar residues, total PAH 4.3-6915 mg per kg soil of this 180-300 mg naphthalene per kg soil, 70-230 mg phenanthrene per kg soil, 58-71 mg Benzo(a)pyrene per kg soil **Amendment**: green tree waste (Eucalyptus sp. leaf and stem waste):manure:soil (3:1:16) **Conditions**: 130 m^3 windrow, regular mixed, 60-80% moisture, max temp (42 ^{0}C) reached after 35 days **Time 244 days**:	Complete removal of LMW PAHs 90% removal of MWH PAHs 70% HMW PAHs;~50% of each of the most resistant PAHs (Indeno[1,2,3-cd]pyrene, Benzo[g,h,i]perylene) was lost with 120mg/kg the total PAH concentration was lower than with land treatment No volatisation	(Guenin 2000)*

Soil type: from manufactured gas plant site commissioned in 1838 at Clitheroe, Lancashire, UK **Contamination**: coal-tar (including 16 USEPA PAHs) **Amendment**: fungi (*Phanerochaete crysosporium, Pleurotus ostreatus, Coriolus versicolor*, and Wye isolate #7) inoculated wheat-straw (single species and any combination) in a ratio of 5:2 (soil:fungi) **Conditions**: 50% moisture, 22 ^{o}C **Time**: 32 days	Greatest PAH losses in biotic control small or negligible differences in microcosms inoculated with one or more fungi Results suggest that the use of the autochthonous micro-flora, with no introduction of foreign micro-organisms, offers the greatest potential for PAH degradation	(Canet *et al.* 2001)
Soil type: spiked loamy sand **Contamination**: petroleum hydrocarbons (5000 mg HC per kg soil) by mixing diesel fuel and motor oil (1:1, v:v) **Amendment**: steer manure and inorganic fertiliser [$(NH_4)2SO_4$] **Conditions**: **Time**: 41 days	32% reduction in hydrocarbon-concentrations in the control treatment ~54% reduction in hydrocarbon-concentrations with the $(NH_4)2SO_4$ fertilizer-treatment up to 81% reduction in hydrocarbon-concentrations with 20% manure amendment	(Wellman *et al.* 2001)

Soil type: from a former tar-contaminated site **Contamination**: 11 individual three-to six-ring unsubstituted aromatic hydrocarbons (PAH) **Amendment**: **Conditions**: composit pile aerated **Time**: 42 days active composting + 100 days maturation	42-68% removal of 3-4 ring PAHs 33-57% removal of higher-molar mass PAHs No further decrease of PAHs in additional 100 d of compost matuaration in open-air field	(Cajthaml *et al.* 2002)
Soil type: **Contamination**: phenanthrene, anthracene, pyrene (100 mg per kg soil each) **Amendment**: pig manure at three different ratios (12.5%, 25% and 50% w/w dry weight basis) **Conditions**: co-composting **Time**:	90% removal of PAHs 25% pig manure application rate showed the most efficient removal of 3ring PAHs (Phenanthrene and anthracene) No significant difference in pyrene removal for the treatment with 25 or 50 % pig manure	(Wong *et al.* 2002)
Soil type: soil form a sawmill area **Contamination**: creosote (23.6 g PAH per kg fresh weight soil) about 78% were small PAHs; also highly contaminated with arsenic , chromium, and copper **Amendment**: Mycobacterium sp., nutrients, spruce bark, chips **Conditions**: windows ($5m^3$), some of the soil was pre-treated with 50% hydrogen peroxide **Time**: 163 days	Almost 90% reduction of 2- and some 3-ring PAHs PAH reduction in all windrows PAH concentrations in inoculated piles temporarily increased (after 4 months) but decrease to the same amount than the control pile (~80%) after 5 months Inoculation did not speed up the process markedly pre-treating soil with hydrogen peroxide achieved similar removal rates (96% small PAHs and 57% medium and large PAHs) in shorter periods of time	(Ahtiainen *et al.* 2002)*

Soil type: wood preservation site **Contamination**: creosote (total PAH 6473 mg per kg soil) **Amendment**: inoculation with spent mushroom substrate **Conditions**: **Time**: 12 weeks + 3 weeks	50% (anthracene and acenaphthene) to 87% (phenanthrene, fluorene) reduction of 3-ring PAHs after 12 weeks 87% (anthracene) and 97-99% (fluorene, phenanthrene, acenapthrene) PAH-removal after additional re-inoculation and 3 weeks incubation 43% (fluorene) and 34% (pyrene) reduction of 4-ring PAHs after 12 weeks 59% (fluoranthene) and 51% (pyrene) after additional re-inoculation and 3 weeks incubation	(Eggen and SaSek 2002)
Soil type: former tar-producing plant **Contamination**: 16 USEPA PAHs (total conc. 2832 mg per kg soil) **Amendment**: adding mushroom compost (consisting of wheat straw, chicken manure, and gypsum) in and mid phase; thermally insulated composting chamber (~1000kg volume) **Conditions**: 57:53 soil:compost; 64% moisture **Time**: 42 days in composting chamber + 100 days in windrow	42-68% removal of 3- to 4-ring PAHs after 42 days 35-57% removal of 5- and 6- PAHs after 42 days No further decrease after additional 100 days	(Cajthaml *et al.* 2002)*

Soil type:two contaminated soils **Contamination**: PAHs **Amendment**: *Pleurotus ostreatus* in the form of homogenised refuse from the commercial production of fungi **Conditions**: concrete cylinders (height, 50cm; diameter, 60cm), treatments (control, soil mixed with autoclaved sawdust medium, and soil mixed with P.ostreatus refuse) **Time**: 9 weeks	78 % reduction of 3-ring PAHs 41 % redution of 4-rinh PAHs 4 % reduction of 5- and 6-ring PAHs	(Hestberg *et al.* 2003)
Soil type: spiked garden soil (sandy loam soil) **Contamination**: napthalene, phenantharene, Benzo[a]pyrene and Benzo[g.h.i]perylene (200 mg per kg soil) **Amendment**: spent mushroom compost of *Pleurotus pulmonarius* (mixture of wheat straw, dried blood, horse manure, ground chalk) (19:1 soil:compost) **Conditions**: 60% moisture 4 to 80 ^{0}C at 200 rpm continuous shaking **Time**: 2 days	82% (naphthalene) to 59% (phenanthrene) PAH removal due to biodegradation and sorption when traeting 100 mg PAH per litre at room temp with 1% spent mushroom compost Highest sorption removal with phenanthrene (46%) increase in PAH removal with temperature; complete removal of three PAHs (not phenanthrene) at 50 ^{0}C; complete removal of all 4 PAHs at 80 ^{0}C with 5% mushroom compost	(Lau *et al.* 2003)*

Soil type: interior thermophilic and exterior pesophilic zone of yard waste compost between 3 and 6 months old **Contamination**: phenanthrene (100 mg per kg soil) **Amendment**: **Conditions**: inubated in the dark at 22±2 or 60±2 ^{O}C in biometers **Time**: 90 days	Dominant effect due to heterogeneity of compost waste from thermophilic zone: - 1-2% mineralisation when incubated at 60 ^{O}C - 17% mineralisation when incubated at 22 ^{O}C waste from mesophilic zone: negligible - mineralisation when incubated at 60 ^{O}C - 8% mineralisation when incubated at 22 ^{O}C	(Carlstrom and Tuovinen 2003)*
Soil type: manufactured-gas plant soil **Contamination**: 12 USEPA PAHs (total conc. 610 mg per kg dry soil) **Amendment**: adding mushroom compost (consisting of wheat straw, chicken manure, and gypsum) in mid phase; thermally insulated composting chmaber (~1000kg volume) **Conditions**: 64:36 soil:compost; 64% moisture **Time**: 54 days in composting chmaber + 100 days in windrow	20-60% of indivodual PAHs were degraded until day 54 37-80 % of individual PAHs were degraded until day 154 No visual isation Temp increased to 70 ^{O}C up to day 12 then it progressivley decreased	(SaSek *et al.* 2003)*
Soil type: soil from manufacture-gas-plant-area **Contamination**: petroleum hydrocarbons **Amendment**: inoculating soil with white rot fungi *Irpx lacteus*, *Pleurotus ostreatus*, and bacterium *Pseudomonas putida* **Conditions**: **Time**: 10 weeks	Up to 66% decrease in phenanthrene, anthracene, fluoranthene and pyrene concentrations	(SaSak *et al.* 2003)*

Soil type: mispah form (FAO:lithosol) from creosote treatment plant **Contamination**: total PAHs >3g per kg soil **Amendment**: sawdust with poultry manure **Conditions**:soil:sawdust:poultry manure (2:2:1), 350 kg as static pile **Time**: 19 months	Removal below the remediation target (1 mg per kg soil) of all PAHs except chrysene after 11 months removal of chrysene below 1 mg per kg soil after 17 months After 19 months only traces were left (0.1-0.5 mg per kg)	(Atagana 2004)
Soil type: lagooning sewage sludge **Contamination**: PAHs (total 0.24 mg per kg sludge) **Amendment**: straw (8:25:1, sludge:straw) **Conditions**: co-composting pile, turned every 25 days **Time**: 180 days	75 % decrease until day 30 ~50 % increase until day 60 Slight decrease until day 90 88 % further decrease until day 180 Each single PAH showing similar behaviour to the total PAH profile Decrease during stabilisation (more notable for larger PAHs (≥4-rings) Maximum desorption after day 60 for naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, Benzo[a]anthracene, chrysene	(Amir *et al.* 2005)

Soil type: manufactured gas plant site commissioned in 1838 at Clitheroe, Lancashire, UK **Contamination**: 16 USEPA PAHs (total PAHs 100.3 mg per kg dry soil) **Amendment**: silve-sand, green-waste ((3:3:10); soil:silver-sand:green-waste) **Conditions**: incubated at different temperatures (38 ^{0}C, 55 ^{0}C, 70 ^{0}C) **Time**: 98 days	80.9 % highest PAH-removal after 111d at 38 ^{0}C generally more removal of PAHs with fewer aromatic rings	(Antizar-Ladislao *et al.* 2005)
Soil type: manufactured gas plant site commissioned in 1838 at Clitheroe, Lancashire, UK **Contamination**: 16 USEPA PAHs (total PAHs 100.3 mg per kg dry soil) **Amendment**: silve-sand, green-waste ((3:3:10); (35:35:10); (40:40:10), (45:45:10) soil:silver-sand:green-waste) **Conditions**: incubated at different temperatures (38 ^{0}C, 55 ^{0}C, 70 ^{0}C) **Time**: 98 days	75.1 % highest PAH-removal after 8 weeks at 38 ^{0}C with a mixing ratio of (0.4:0.4:1)	(Antizar-Ladislao *et al.* 2005

Soil type: **Contamination**: soot waste (total PAHs ~200 mg per kg soil) **Amendment**: sewage sludge and yard waste (high alkalinity pH 12.8) **Conditions**: co-composting in closed tank with forced aeration **Time**: 60 days in closed tank followed by 70 days with natural aeration	68 % PAH removal Significant sel-drop in waste pH and increase in mass temperature after 30 days, progressive drop in the PAH concentration following this degradation more effective on lower molecular weight PAHs (2-4 rings)	(Moretto *et al.*. 2005)
Soil type: manufactured gas plant site commissioned in 1838 at Clitheroe, Lancashire, UK **Contamination**: 16 USEPA PAHs (total PAHs 100.3 mg per kg dry soil) **Amendment**: silve-sand, green-waste ((3:3:10); (35:35:10); (40:40:10), (45:45:10) soil:silver-sand:green-waste) **Conditions**: incubated at different temperatures (38 ^{0}C, 55 ^{0}C, 70 ^{0}C) and with different moisture contents (40%, 60%, 80%) **Time**: 98 days	82.0% highest PAH-removal rate at 38 ^{0}C with a mixing ration of (0.35:0.35:1) and a moisture content of 60% More removal of small PAHs than large except for 70 ^{0}C there the other way round	(Antizer-Ladislao *et al.* 2006)

APPENDIX 2

Table 8.2: Hydrocarbon-degrading bacteria. The "organisms"-column identifies organisms able to degrade PAHs; "identified metabolites"- column highlights metabolites found; the "references"-column refers to the literature sources

Organisms	Identified Metabolites	References
Naphthalene	1,2-dihydroxynapthalene	

Acinetobacter calcoaceticus		(Sutherland *et al*. 1995; Hamann *et al*. 1999; Annweiler *et al*. 2000; Li *et al*. 2000; Samantha *et al*. 2002; Mrozik *et al*. 2003)
Alcaligenes denitrificans	1-naphthol	
Bacillus thermoleovorans	2,3- dihydroxynapthalene	
Comamonas testosteroni	2-carboxycinnamic acid	
Corynebacterium renale	2-hydroxybenzoic-2-carboxylic acid	
Mycobacterium sp.	3-(2-carboxyphenyl)-2-propenoic acid (2-carboxycinnamic acid)	
Pseudomonas sp.	Benzene-1,2-doil (catechol)	
Pseudomonas stutzeri	gentisate	
Pseudomonas vesicularis	Naphthalene1,2-oxide	
Acenaphthylene Beijerinckia sp.	1,2-acenaphthenedione	(Grifoll *et al*. 1995; Sutherland *et al*. 1995; Pinyakong *et al*. 2004)
Pseudomonas sp.	1,2-dihydroxyacenaphthylene (possibly)	
Pseudomonas aeruginosa	1,8-naphthalenedicarboxylic acid acenaphthoquinone	
Sphingomonas sp	Cis-1,2-acenaphthylenedihydrodiol cis-acenaphthalene-1,2-diol	
Acenaphthene *Alicaligenes eutrophus*	1,2-acenaphthenedione	(Grifoll *et al*. 1995; Sutherland *et al*. 1995; Pickard *et al*. 1999; Pinyakong *et al*. 2004)
Beijerinckia sp.	1,8-naphthalenedicarboxylic acid	
Pseudomonas sp.	3-hydroxyphthalic acid	
Pseudomonas aeruginosa	7,8-diketonaphthyl-1-acetic acid acenaphtoquinone	

Sphigomonas sp.	Cis1,2-acenaphthalenedihydrodiol trans-acenaphthene diols	
Fluorene *Arthrobacter sp.*	1,10-dihydro-1,10-dihydroxyfluorene-9-one	(Boldrin *et al*. 1993; Trenz *et al*. 1994; Grifoll *et al*. 1995; Bogan *et al*. 2003; Mrozik *et al*. 2003)
Brevibacterium sp.	1,1a-dihydroxy-1-hydrofluorene-9-one	
Cycloclasticus sp.	1,1-dyhdroxy-1-hydro-9-fluorenone	
Mycobacterium sp.	1-hydroxy-9-fluorenone	
Austroafricanum	2-formyl-1-indanone	
Pseudomonas aeruginosa	2-hydroxy-6-(2-carboxyphenyl)-6-oxo-2,4-hexadienoic acid	
Phenanthrene *Aeromonas sp.*	1,2-dihydroxynaphthalene-> mineralised via naphthalene pathway	(Grifoll *et al*. 1995; Sutherland *et al*. 1995; Geiselbrecht *et al*. 1998; Hamann *et al*. 1999; Pickard *et al*. 1999; Samanta et al. 2002; Bogan *et al*. 2003; Mrozik *et al*. 2003; Jacques *et al*. 2005; Keum *et al*. 2006)
Arthrobacter	1-napthol	
Bacillus sp.	2-carboxybenzaldehyde acid	
Beijerinckia sp.	2-formylbenzoic acid	
Flavobacterium sp.	3,4-dihydroxyphenanthrene	
Mycobacterium sp.	4,5-dihydroxyphthalic acid	
Pseudomonas putida	Cis-3,4-dihydroxy-3,4-dihydrophenanthrene	
Sinorhizobium sp.	Cis-4-(1-hydroxynaph-2-yl)-2-oxobut-3-enoic acid	
Paucimobilis	Coumarin salicyclic acid	
Vibrio sp.	Naphthalene-1,2-diol	

Anthracene *Beijerinckia sp.*	1,2-dihydroanthracene	(Grifoll *et al*. 1995; Sutherland *et al*. 1995; Goyal and Zylstra 1996; Kastner *et al.* 1998; Hamann *et al*. 1999; Pickard *et al*. 1999; Dean-Ross et al. 2001; Mrozik *et al*. 2003; Jacques *et al.* 2005)
Flavobacterium sp.	2-hydroxy-3-naphthaldehyde, 2-hydroxy-3-naphthoic acid	
Rhodococcus sp.	Cis-4-(2-hydroxynaphth-3-yl)-2-oxobut-3-enoic acid	
Xanthomonas maltophila	Salicylic acid Simple aliphatic compounds	
Fluoranthene *Alcaligenes dentrificans*	2-carboxybenzaldehyde	(Mueller *et al*. 1990; Sutherland *et al*. 1995; Harayama *et al*. 1997; Sepic *et al*. 1999; Hamann *et al*. 1999; Samanta et al. 2002; Mrozik *et al*. 2003)
Gordonia sp.	3-hydroxymenthyl-4,5-benzocoumarin	
Pasteurella sp	9-carboxymenthylene-fluorene-1-carboxylic acid, 9-fluorenone	
Rhodococcus sp.	9-hydroxy-1-fuorene-carboxylic acid	
Pyrene *Coriolopsis galica*	1,2-and 4,5-dihydroxypyrene	(Heitkamp *et al*. 1988; Sutherland *et al*. 1995; Sack and Fritsche 1996; Kastner *et al*. 1998; Pickard *et al*. 1999; Samanta et al. 2002; Bogan *et al*. 2003; Mrozik *et al*. 2003; Jacques *et al*. 2005)
Cycloclasticus sp.	2-hydroxy-2-(phenanthren-5-one-4-enyl)acetic acid	
Pseudomonas citronellolis	Cis-4,5-dihydroxy-4,5-dihydroxypyrene	
Sphingomonas yanoikuyae	Phenanthrene 4-carboxyl acid Phthalic acid Pyrenol	

	Trans-4,5-dihydroxy-4,5-dihydropyrene Trans-4,5-pyrenedihydrodiol Trans-dihydrodiol	
Benz[a]anthracene *Beijerinckia sp.*	1-hydroxy-2-carboxyanthracene or the corresponding phenanthrenes	(Gibson *et al*. 1975; Sutherland *et al*. 1995; Mrozik *et al*. 2003)
Mycobacterium sp.	1-hydroxy-2-carboxyanthracene or the corresponding phenanthrenes	
Chrysene *Gordonia sp.*		(Sutherland *et al*. 1995; Harayama *et al*. 1997; Samanta *et al*. 2002)
Rhodococcus sp.		
Sphingomonas		
Paucimobilis		
Benzo[b]fluoranthene *Sphingomonas*		(Samanta *et al*. 2002)
Paucimobilis		
Benzo[a]pyrene *Coriolopsis gallica*	4,5-dihydroxy[a]pyrene	(Gibson *et al*. 1975; Sutherland *et al*. 1995; Juhasz *et al*. 1996; Harayama *et al*. 1997; Pickard et al. 1999; Bogan *et al*. 2003; Mrozik *et al*. 2003)
Pseudomonas cepacia	Cis-4-(hydroxypyren-7-yl)-2-oxobut-3-enoic acid	
Sphingomonas	Cis-7,8-benzo[a]pyrenedihydrodiol	
Dibenz[a,h]anthracene *Pseudomonas cepacia*		(Juhasz *et al*. 1996)

Sphingomonas		
Paucimobilis		

Table 8.3: Hydrocarbon-degrading fungi. The "organisms"-column identifies organisms able to degrade PAHs; "identified metabolites"- column highlights metabolites found; the "references"-column refers to the literature sources.

Organisms	Identified Metabolites	References
Naphthalene A variety of fungi	1-naphthol 2-naphthol 4-hydroxy-1-tetralone Glucuronide Sulphate conjugates Trans-1,2-naphthalenedihydrodiol	(Sutherland *et al*. 1995)
Acenaphthene *Coriolopsis gallica*	1,2-acenaphthenedione	(Sutherland *et al*. 1995; Pickard *et al*. 1999)
Cunninghamella elegans	1,2-acenaphthenedioneol 1,5-dihydroxyacenaphthrene 1-acenaphthenol 1-acenaphthenone	
Fluorene *Cunninghamelia elegans*	2-hydroxy-9-fluorenone	(Sutherland *et al*. 1995; Cerniglia *et al*. 1999; Boonchan *et al*. 2000)
Chrysosporium		
Pleurotus ostreatus		
Trametes versicolor		

Phenanthrene *Aspergillus niger*	1-methoxyphenanthrene	(Sutherland *et al*. 1995; Cerniglia *et al*. 1999; Pickard *et al*. 1999; Boonchan *et al*. 2000)
Penicillum sp.	Glucoside conjugate	
Chrysosporium	Trans-3,4-phenanthrenedihydrodiol	
Pleurotus ostreatus	Trans-9,10-phenanthrenedihydrodiol	
Anthracene *Bjerkandera sp.*	1-anthrol	(Field *et al*. 1992; Sutherland *et al*. 1995; Bezalel *et al*. 1996; Cerniglia *et al*. 1997; Katsner *et al*. 1998; Pickard *et al*. 1999)
Coriolopsis gallica	9,10-anthraquinone	
Phanerochaete laevis	Trans-1,2-anthracenedihydrodiol	
Ramaria sp.		
Trametes sp.		
Fluoranthene *Penicillium sp.*	9-hydroxyfluorene trans-2,3-dihydrodiol	(Sutherland *et al*. 1995; Cerniglia *et al*. 1997; Pickard *et al*. 1999; Boonchan *et al*. 2000)
Trametes versicolor		
Pleurotus ostreatus		
Pyrene *Aspergillus niger*	1,6-dihydroxypyrene	(Lambert *et al*. 1994; Lange *et al*. 1994; Sutherland *et al*. 1995; Bezalel *et al*. 1996; Cerniglia 1997; Pickard *et al*. 1999; Boonchan *et al*. 2000)
Coriolopsis gallica	1,8-dihydroxypyrene	
Crinipellis maxima	1,8-pyrenequinone	
Penicillum janthinellum		
Pleurotus ostreatus		
Benzo[a]anthracene	Glucuronide conjugates	

Candida krusei		(Sutherland *et al*. 1995; Cerniglia 1997; Boonchan *et al*. 2000)
Rhodotorula minuta		
Racemosum		
Chrysene *Cunninghamella elegans*	2-chrysenyl sulfate	(Cerniglia 1997)
Penicillium janthinellum	2-hydroxy-8-chrysenylsulfate	
Syncephalastrum	Trans-1,2-chrysenedihydrodiol	
Benzo[a]pyrene *Aspergillus ochraceus*	1,6-quinone	(Haemmerli *et al*. 1986; Sanglard *et al*. 1986; Lange *et al*. 1994; Field *et al*. 1992; Sutherland *et al*. 1995; Bezalel *et al*. 1996; Cerniglia 1997; Grotenhuis *et al*. 1999; Boonchan *et al*. 2000)
Bjerkandera sp.	3,6-quinone	
Candida tropicalis	6-hydroxybenzo[a]pyrene	
Chrysosporium pannorum	7,8-dihydrodiol-9-10-expoxide	
Mortierelia verrucosa	7,8,9,10tetrahydrobenzo[a]pyrene	
Neurospora crassa	Benzo[a]pyrene (+)-7,8-diol-9, 10-epoxide-2 (highly carcinogenic – with C.elegans trace amounts detected (Sutherland *et al*. 1995))	
Benzo[a]pyrene *Cunninghamella elegans*	10-hydroxy-3-benzo[e]pyrenyl sulfate 3-benzo[e]pyrenyl sulfate Benzo[e]pyrene-3-0-β-glucopyranoside	(Cerniglia 1997)

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UNIVERSITY OF PORTSMOUTH

Postgraduate Research Student (PGRS) Information				Student ID:	407633		
PGRS Name:	Aliyu Shuaibu						
Department:	SCES	First Supervisor:	Professor John Williams				
Start Date: (or progression date for Prof Doc students)							
Study Mode and Route:	Part-time	☐	MPhil	☐	MD	☐	
	Full-time	☒	PhD	☒	Professional Doctorate	☐	

Title of Thesis:	A Study of the Feasibility of Integrated-Composting as a Method for the Remediation of Hydrocarbon-Contaminated Soil Under Intense Rainfall Conditions
Thesis Word Count: (excluding ancillary data)	60,697

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e)	Does your research comply with all legal, ethical, and contractual requirements?	YES ☒ NO ☐

Candidate Statement:	
I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)	
Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	6223-71BE-E2D0-1607-F93D-434B-B5BC-8659
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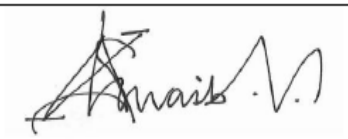
Signed *(PGRS)*:		**Date:** 23rd September 2022