Bioremediation of Hydrocarbon Contaminated Soils and Drill Cuttings Using Composting With Agricultural Wastes

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Declaration

I hereby certify that this work is my own, except where otherwise acknowledged, and that this work has not been submitted previously for a degree at this, or any other university.

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Davidson Dimabo Davis

Abstract

A compost-bioremediation approach was adopted in this study to explore more sustainable and economically viable methods of degrading pollutant hydrocarbons in oil-field drill cuttings and coal tar impacted soils (CTIS). The compost amendments used were agricultural waste products including grass cuttings, spent mushroom compost and straw. Laboratory-scale compost experiments were conducted to test the performance of different compost blends comprised of each contaminated medium and organic amendments in different mix ratios for 53 days. The compost mix type which produced the greatest reduction in pollutant hydrocarbon concentrations was further scaled-up and tested in an outdoor pilot scale compost treatment for 56 days. At the end of the lab-scale treatments, degradations in total petroleum hydrocarbon (TPH) concentrations of 85.1% and 90.6% were recorded for the drill cuttings and CTIS, compared to 36.7% and 28.4% that was achieved in the control experiments, respectively. The concentrations of total *n*-alkanes and polycyclic aromatic hydrocarbons (PAHs) were significantly decreased in the best performing compost mix types, however most of the 5 and 6-ring PAH compounds in the CTIS treatment compost mix exhibited recalcitrance to degradation and some even appeared to increase in concentration which is ascribed to increased PAH availability to solvent extraction and reduction in the compost mass during the composting-biodegradation process. The best performing compost mix type for treatment of CTIS was subsequently tested in outdoor tumbler compost bins after being scaled-up by a factor of 600; this was found to produce 78% degradation of TPH concentration at the end of the treatment period. Concentrations of total *n*-alkanes and PAHs were also significantly lowered by biodegradation. Low molecular weight (2 and 3-ring) PAHs were almost completely removed and 4-ring PAHs from the coal tar, including fluoranthene, pyrene, benzo[a]anthracene and chrysene were significantly degraded but not the 5 and 6-ring PAH compounds. Phytotoxicity assays showed that the seed germination in the treated matrix was 70% and 20% more, for corn and pea, respectively, 5 days after planting and 78% more for mustard 3 days after planting. Phosphatase enzyme activity was found to decrease in the treated matrices possibly due to the short time between end of composting and testing. The results generated from the chemical and toxicity assays of this study showed the efficacy of the composting treatment for hydrocarbon removal from these contaminated matrices and identified the best performing compost mix types ($DGMS_t3$ and SGS_t3) which can be further tested in field scale trials.

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Dedication

I dedicate this thesis to The Almighty God - The Sugmad, my loving mother and all who have the enthusiasm and desire to find solution to life’s challenges.

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List of Abbreviations

Sample Codes

D	Control sample of drill cuttings
DGS_t1	Compost treatment sample comprised of drill cuttings, grass clippings and straw in 1:1 drill cuttings/ organic amendments mix ratio
DGS_t2	Compost treatment sample comprised of drill cuttings, grass clippings and straw in 1:1.5 drill cuttings/ organic amendments mix ratio
DGS_t3	Compost treatment sample comprised of drill cuttings, grass clippings and straw in 1:2 drill cuttings/ organic amendments mix ratio
DMS_t1	Compost treatment sample comprised of drill cuttings, mushroom compost and straw in 1:1 drill cuttings/ organic amendments mix ratio
DMS_t2	Compost treatment sample comprised of drill cuttings, mushroom compost and straw in 1:1.5 drill cuttings/ organic amendments mix ratio
DMS_t3	Compost treatment sample comprised of drill cuttings, mushroom compost and straw in 1:2 drill cuttings/ organic amendments mix ratio
$DGMS_t1$	Compost treatment sample comprised of drill cuttings, grass clippings, mushroom compost and straw in 1:1 drill cuttings/ organic amendments mix ratio
$DGMS_t2$	Compost treatment sample comprised of drill cuttings, grass clippings, mushroom compost and straw in 1:1.5 drill cuttings/ organic amendments mix ratio
$DGMS_t3$	Compost treatment sample comprised of drill cuttings, grass clippings, mushroom compost and straw in 1:2 drill cuttings/ organic amendments mix ratio
CTIS	Control sample of coal tar impacted soil
SGS_t1	Compost treatment sample comprised of CTIS, grass clippings and straw in 1:1 CTIS/ organic amendments mix ratio
SGS_t2	Compost treatment sample comprised of CTIS, grass clippings and straw in 1:1.5 CTIS/ organic amendments mix ratio
SGS_t3	Compost treatment sample comprised of CTIS, grass clippings and straw in 1:2 CTIS/ organic amendments mix ratio
SMS_t1	Compost treatment sample comprised of CTIS, mushroom compost and straw in 1:1 CTIS/ organic amendments mix ratio
SMS_t2	Compost treatment sample comprised of CTIS, mushroom compost and straw in 1:1.5 CTIS/ organic amendments mix ratio

SMS_t3	Compost treatment sample comprised of CTIS, mushroom compost and straw in 1:2 CTIS/ organic amendments mix ratio
$SGMS_t1$	Compost treatment sample comprised of CTIS, grass clippings, mushroom compost and straw in 1:1 CTIS/ organic amendments mix ratio
$SGMS_t2$	Compost treatment sample comprised of CTIS, grass clippings, mushroom compost and straw in 1:1.5 CTIS/ organic amendments mix ratio
$SGMS_t3$	Compost treatment sample comprised of CTIS, grass clippings, mushroom compost and straw in 1:2 CTIS/ organic amendments mix ratio

Acronyms and other abbreviations

ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
DCM	Dichloromethane
EOM	Extractable Organic Carbon
GC-FID	Gas Chromatography with Flame Ionization Detector
GC-MS	Gas Chromatography-mass spectrometry
HMW	High Molecular weight
IARC	International Agency for Research on Cancer
ISO	International Organization of Standardization
LMW	Low Molecular Weight
PAH	Polycyclic Aromatic Hydrocarbons
TPH	Total Petroleum Hydrocarbon
USEPA	United States Environmental Protection Agency
WHO	World health Organisation
UNEP	United Nations Environment Program
MEF	Mutagenic Equivalent Factor
SMC	Spent Mushroom Compost
NDES	Niger Delta Environmental Survey
EU	European Union
EIA	Energy Information Administration
WEC	World Energy Resources
BTEX	Benzene, Toluene, Ethylbenzene and Xylene
EC	European Community
DETR	Department of Environment, Transport and regions

WBF	Water Based Fluid
SBF	Synthetic Based Fluid
OBF	Oil Based Fluid
EGASPIN	Environmental Guidelines and Standards for the Petroleum Industries in Nigeria

Chapter 1

INTRODUCTION

1.1 Impact of hydrocarbon contaminants in the environment

The self-regulatory capacity of the biosphere is increasingly threatened due to anthropogenic activities resulting in the production of toxic chemicals, including hydrocarbons and other organic and inorganic pollutants which may reach the air, water or soil (Sen and Chakrabarti, 2009; Beltrame *et al*., 2010; Prasad *et al*., 2010) Petroleum, which is predominantly composed of hydrocarbons, is the major source of energy that is the driving factor of modern industrialization and urbanization. However, the generation of hydrocarbon contamination is a side effect, as efforts to meet this demand have caused accidental spills, leakages and production of contaminated wastes during exploration, production, processing, transportation, storage, distribution and utilization. These have resulted in substantial hazards to humans and the ecosystem as well as significantly impacted negatively on the socio-economy of the operational areas, especially in developing countries like Nigeria (Ayotamuno *et al*., 2002; Ogbo and Okhuoya, 2008; Wang *et al*., 2011).

Sometimes the spillage from pipes is as a result of acts of vandalism, sabotage or terrorism (Okpokwasili and Amanchukwu, 1988; Omeje, 2005) and theft for artisanal refining, which typically involves the use of primitive illegal stills to refine crude oil into low grade fuel used locally for lighting, energy or transport. The distilleries are heated using open fires fed by crude oil that is tipped into pits in the ground. As part of the oil burns away, some seeps into the ground. (UNEP, 2011). Artisanal refining puts significant environmental pressure on operational area as shown in Figure 1.1.

Figure 1.1. Marine and terrestrial ecosystem heavily impacted by artisanal refining operations in Ogoniland, Niger Delta, Nigeria (Source: UNEP, 2011)

The exploration and extraction of crude oil and natural gas generates huge amounts of drill cuttings. During oil well drilling operations, drilling muds are used to help lubricate and cool the drilling bits and also to carry the drill cuttings to the surface for subsequent screening and disposal (Rojas-Avelizapa *et al*., 2007); they are also necessary to control internal pressure and stabilize the well, and constitute 5-25% of the discharged drill cuttings (Trannum *et al*., 2010). These drill cuttings, which are mixtures of drilling mud, rocks and particulate released from geological formations in the drill hole, pose waste management problems to the petroleum industry due to the large volume generated and their content of organic and inorganic contaminants such as petroleum hydrocarbons and heavy metals (Leonard and Stegemann, 2010). Crude oil drilling wastes are occasionally disposed at illegal oil waste dumpsites in places of weak regulatory influence as shown in Figure 4.2.

Figure 1.2. Fly tipping of oilfield waste in Ogoniland, Niger delta, Nigeria (Source: UNEP, 2011)

1.2 PAHs as major risk drivers

Polycyclic aromatic hydrocarbons (PAHs) are small but important class of hydrocarbon contaminants which are the most pertinent risk drivers. PAHs are composed of fused aromatic rings and most of them have low availability for microbial utilization due to their hydrophobic nature, low volatility and water solubility. As a result, they bind to sediments and soil particles, which promotes their accumulation in the polluted medium (Johnson *et al*., 2005; Sayara *et al*., 2010). They are highly persistent compounds in the environment, recalcitrant to microbial degradation and highly lipophilic, while some of them have carcinogenic and

mutagenic toxicity, especially the high molecular weight PAHs (Blanchard *et al*., 1999; Juhasz and Naidu, 2000). Some representative PAHs and their carcinogenic and mutagenic classifications are presented in Figure 1.3.

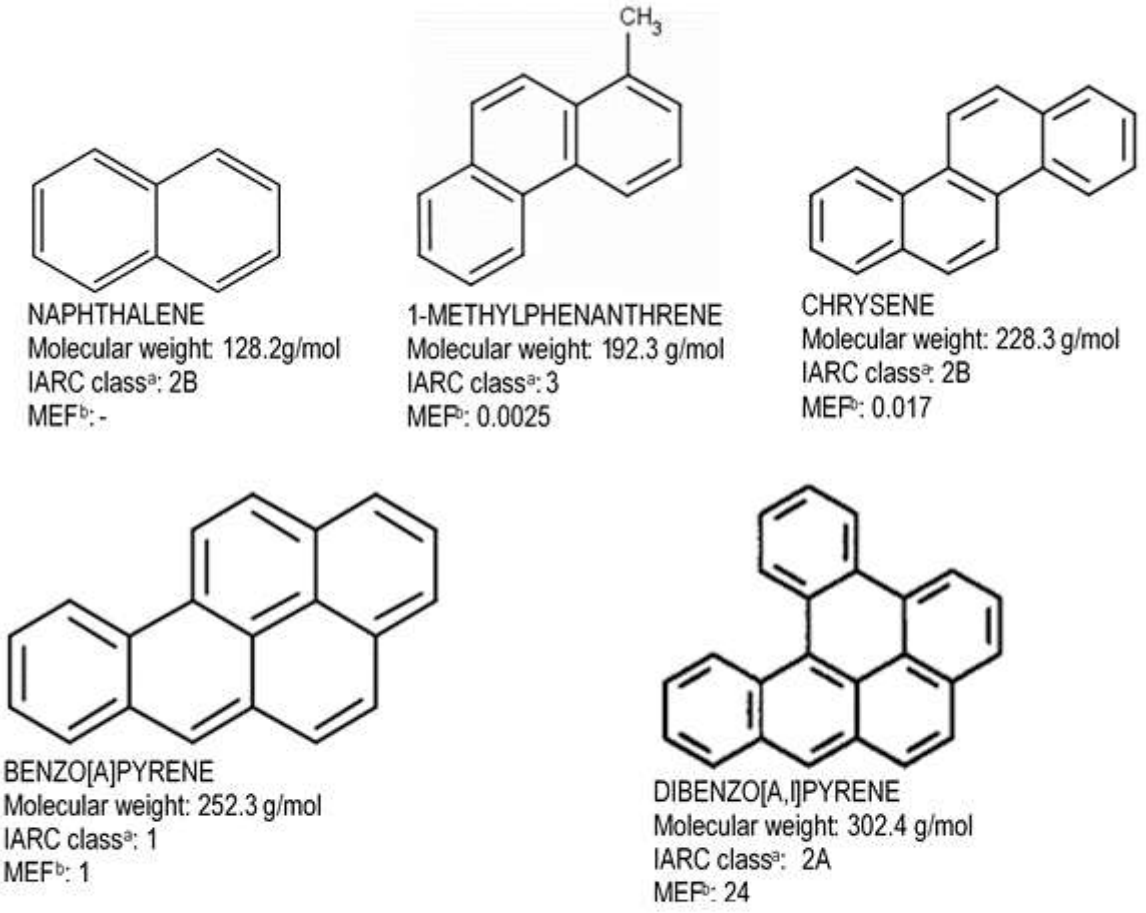


Figure 1.3. Carcinogenic and mutagenic classification of some representative PAHs
[a] Agents Classified by the IARC Monographs, Volumes 1–112 (IARC, 2012): 1 - Carcinogenic to humans; 2A - Probably carcinogenic to humans; 2B - Possibly carcinogenic to humans; 3 - Not classifiable as to its carcinogenicity to humans.
[b] Mutagenic Equivalency Factors from Durant et al. (1996)

Non-petroleum sources of PAH pollutants in the environment include: automobile exhausts; tobacco smoke; coal and wood combustion; spilling of minerals or tar oils; and use of pesticides and chemical fertilizers (Hafidi *et al*., 2008; Ahlawat *et al*., 2010). PAHs are one of the dominant contaminants in soils of industrial activities such as: former coal gasification sites; tar oil distillation plants and wood-preservation industries (Wischmann and Steinhart, 1997; Zhang, *et al*., 2011). Their presence in other soil environments are most often due to storage, consumption and disposal of fossil fuel, as well as leakage of industrial or sewage effluent (Cerniglia, 1992; Macleod and Semple, 2002).

PAHs are also widely distributed in ground water, marine sediments and the atmosphere. They have been detected at various concentrations in sediments from the North Sea, San Diego Bay in California, Red Sea, Central Pacific ocean and the Niger Delta region of Nigeria (Coates *et al*., 1997; Ohkouchi *et al*, 1999; Readman, *et al*., 2002, Breuer *et al*., 2004; Anyakora *et al*., 2005a). Atmospheric deposition of PAHs can often be the result of vehicular exhaust fumes (Lim *et al*., 1999; Hafidi *et al*., 2008).

The persistence of PAHs in the environment and their recalcitrance to remediation treatments in contaminated media increases in direct proportion with the increase in their numbers of fused aromatic rings (Cerniglia, 1992; Bamforth and Singleton, 2005). Because of their deleterious effects on terrestrial and aquatic inhabitants, 16 PAHs are classified as priority pollutants by the United States Environmental Protection Agency, (USEPA) among which benzo[a]pyrene is identified to be one of the most potential carcinogen (Kotterman *et al*., 1998; Bamforth and Singleton, 2005).

1.3 Strategies for degrading hydrocarbons in contaminated medium

The toxicity and carcinogenicity properties of hydrocarbon contaminants, especially polynuclear aromatic hydrocarbons (PAHs), have made their remediation a critical need. Several technologies have been advanced over the years to degrade these environmental contaminants in soils and drill cuttings (Khan *et al*., 2004; Ball *et al*., 2012). However, suitability of each technology is modulated by the physico-chemical, as well as biological properties of the contaminated medium (Sayara *et al*., 2011; Covino *et al*., 2010). Remediation and control of environmental damage by hydrocarbon contaminated oil-field drill cuttings and soil has been done by applying technologies using chemical processes - solidification and stabilisation (Leonard and Stegemann, 2010; Opete *et al*., 2010), physical processes - thermal desorption and microwave treatment (Robinson *et al*., 2008; Robinson *et al*., 2009; Li *et al*., 2009; Ball *et al*., 2011) and biological processes - bioremediation (Okparanma *et al*., 2009; Silva *et al*., 2009), among others.

Stabilization and solidification, microwave treatment and thermal desorption technologies have been applied to the treatment of drill cuttings waste. The process of stabilization and solidification involves the addition of chemical binders and subsequent solidification of the drill cuttings waste to achieve a durable dense

monolithic matrix that is expected to be more suitable for storage, landfilling or re-use. However the significant increase in volume of waste generation is one of the major disadvantages of this technology, even where the anticipated merits have not yet been fully achieved (Al-Ansary and Al-Tabba, 2007).

Microwave treatment and thermal desorption are thermal technologies which include a range of processes where heat at high temperatures of 820-1600 ^{o}C is applied to contaminated matrices to destroy the contaminants by incineration, gasification, volatilization and pyrolysis, among others (Ball *et al*., 2011). However huge capital equipment and demand for high cost of energy use, as well as exposure of personnel to resulting fugitive dust are major set-backs to these operations due to the high water and organic matter content of hydrocarbon contaminated drill cuttings and soils (Joo *et al*., 2007; Okparanma *et al*., 2009; Leonard and Stagemann, 2010; Ball *et al*., 2012).

In addition to the physical and chemical processes in use, biological processes have also been developed over the decades to degrade hydrocarbons in contaminated media. These processes are broadly called bioremediation which involves the utilization of the hydrocarbon degrading potential of organisms (bacteria, plants and fungi) or their enzymes to degrade contaminated medium into non-toxic residues (Gallego *et al*., 2001; Ball *et al*., 2012). Bioremediation is a natural process and has the advantage of being more environmentally friendly than the other physical and chemical processes in conversion of contaminated matrices into more stable and reusable products. During bioremediation, favourable conditions are created for the microorganisms to utilize and convert organic molecules, including hydrocarbons, to cell biomass and products such as carbon dioxide and water which do not adversely affect the environment (Atlas and Cerniglia, 1995; Ahlawat *et al*., 2010).

For effective bioremediation, the overall degradation rate of pollutants in contaminated matrix must be faster than natural attenuation processes - otherwise known as intrinsic bioremediation (Mitchel *et al.,* 2000; Mohan *et al*., 2008). To achieve this, bioremediation of contaminated medium is usually carried out either by biostimulation (provision of nutrients and favourable environment to stimulate microbial growth and activity), or by bioaugmentation (introduction of single strains or consortium of microorganisms with the desired contaminant degradative properties,

or by combination of both processes (Mancera-López *et al*., 2008; Sayara *et al*., 2011).

Bioremediation processes have been applied with varying degrees of success in degrading PAHs in contaminated matrices using microorganisms, especially under aerobic conditions (Breedveld and Sparrevik, 2000). Success is contingent on various factors that include: optimization of controlled parameters such as temperature, water content, nutrient availability, and also the concentrations and chemical structures of PAHs monitored and their bioavailability (Shuttleworth and Cerniglia, 1995; Sayara *et al*., 2011). Lower bioremediation successes have been recorded for high molecular weight PAHs (4-6 rings PAHs) due to low bioavailability which is mostly attributed to their hydrophobicity, which increases with the number of fused benzene rings. Hydrophobic PAH pollutants can be entrapped within the micropores of the soil by slow diffusion which results in their low bioavailability for microbial degradation (Shuttleworth and Cernigli, 1995; Semple *et al*., 2003). Optimization of the controlled parameters with agents capable of solubilising PAHs present in contaminated matrices for microbial catabolism is therefore a major requirement for effective bioremediation treatment (Semple *et al*., 2003).

1.4 Bioremediation by composting techniques

An interesting and inexpensive bioremediation technique for stimulating aerobic microbial metabolic treatment processes by mixing the contaminated medium with organic substrates under optimal conditions of oxygen, carbon to nitrogen to phosphorus ratios, moisture and temperature, is referred to as composting (Zhang *et al*., 2011; Sayara *et al*., 2009). Varieties of readily degradable agricultural wastes constitute useful organic substrates for composting which are added as: bulking agents - including sawdust, wood shavings, straw, spent mushroom compost and hay; and organic amendments - including food and food processing wastes, green plant materials and organic manure (Chiu *et al*., 2009; Rezaei *et al*., 2011). Bulking agents are low density materials which, when added to soils, could lower the soil bulk density, increase porosity, increase oxygen diffusion, and may help form after stable aggregates. These changes increase the soil aeration and microbial activity (Rastegarzadeh and Nelson 2006).

A remarkable agricultural waste product which has been found to be a useful amendment for compost-bioremediation of hydrocarbon contaminated media, is spent mushroom compost (SMC). Growing of mushrooms involves the preparation of compost, the medium on which mushrooms grow. The compost is a blend of natural ingredients like wheat straw, hay, corn cobs, cotton seed hulls, poultry manure, sawdust, rice bran, etc. SMC are the composted organic materials remaining after a crop of mushrooms is harvested. It is a by-product of mushroom production (Bayer, 1999). SMC harbours fungal biomass and large proportion of heterotrophic microbes and have the potential to chemically adsorb organic and inorganic pollutants. The diverse categories of microorganisms contained in SMC have the capacity to biologically breakdown the organic xenobiotic compounds present in soil and water (Ahlawat, *et al*., 2010). SMC has been reported to degrade various organopollutants when used as a compost amendment material during composting (Lau *et al*., 2003; Chiu *et al*., 2009).

The effectiveness of composting techniques on the bioremediation of PAHs polluted soils and drill cuttings with good percentage removals at comparatively lesser times than natural attenuation, have been shown (e.g. Al-Daher *et al*., 2001; Marin *et al*., 2006; Cai *et al*., 2007; Plaza *et al*., 2009). However, the use of composting to biotreat contaminated soils and oil-field drill cuttings is still an emerging in-situ and ex-situ bioremediation method because of the variable success rates reported, which are largely attributed to insufficient process control (Rastegarzadeh and Nelson, 2006; Williamson *et al*., 2009).

1.5 Motivation for this study

1.5.1 Impact of petroleum in the Niger Delta region of Nigeria

The Niger Delta region is vital to Nigeria's petroleum industry as most of the country's oil and gas fields are located in the area. Located in the southernmost part of Nigeria, the Niger Delta is Africa's largest delta covering the land between latitude 4^{o} 15' N and 4^{o} 50' N and longitude 5^{o} 25' E and 7^{o} 37' E with a total land area of 70,000 km^2 and characterized by extensive inter-connected creeks, deltaic tributaries, flood plains, mangrove swamps and other coastal features (NDES, 1995). The impact of spill of petroleum products and mismanagement of oil and gas production wastes have significant detrimental effects on the terrestrial and aquatic biota, which constitute the major sources of livelihood of the people of Niger Delta (Odokuma

and Dickson, 2003; Adedokun and Ataga, 2007). Pollution resulting from illegal activities such as sabotage, theft and recently, artisanal refining, also contribute to the overall impact on the ecosystem (UNEP, 2011).

Though substantial progress has been made to reduce chronic pollution, major accidents still occur, many polluted sites still exist, and many new ones are continuously discovered. These sites are most often not investigated to determine the extent of pollutants present and as a result are likely to be contaminating the food chain and being consumed in crops, water and fish whereby posing risk to humans and other terrestrial and aquatic biota (Anyakora *et al*., 2005b; UNEP, 2011).

To date, there is a lack of availability of systematic scientific information about the extent of PAH contamination in most of these petroleum spill sites in the Niger Delta region. Though many researchers have carried out independent remediation studies on hydrocarbon contaminated Niger Delta soils using different bioremediation techniques (e.g. Odokwuma and Dickson, 2003; Ebuehi *et al*., 2005; Adenipekun and Fasidi, 2005; Ayotamuno *et al*., 2006; Abu and Atu, 2008; Adoki and Orugbani, 2007), the use of composting techniques has not been common.

Research on the use of composting processes to biodegrade hydrocarbon pollutants in drill cuttings and soil is on-going, but the choice of composting ingredients studied is yet to cover the wide range of cheap agricultural wastes that are available, especially in some developing countries. Therefore, there is need to study the bioremediation potentials of such available agricultural waste materials to find possible mix ratios of compost ingredients for optimal performance. Similarly, existing reports have not exhaustively covered biodegrading potentials of blends with SMC and other agricultural waste materials for the remediation of oil contaminated drilling wastes and soils. Investigation into the combined effects of composting with SMC and other agricultural waste materials on degradation of hydrocarbon polluted media still constitute a major research gap.

Additionally, there is scarcity of information concerning the toxicity status of compost-treated soils and drill cuttings in most available reports. For prospects of potential economic value and re-use of these biotreated products, it is pertinent to test their toxicities. This is consistent with regulatory demands in some countries on the safety

of the composting processes and the end product after composting. The EU thematic strategy for soil protection demands that the treated product resulting from the composting process becomes valuable resource for tackling land degradation (E.C., 2006).

1.6 Research hypothesis

This work was done to test the key hypotheses that, (i) composting-bioremediation of hydrocarbon contaminated drill cuttings and soils with SMC and other agricultural wastes will substantially degrade pollutant hydrocarbons and (ii) toxicity of the treated biomass will reduce to level that would permit their reuse.

1.7 Aims and objectives

The overall aim of this research is to investigate a compost mix comprising SMC and other agricultural waste that would effectively degrade hydrocarbon pollutants in oil-field drill cuttings and coal tar impacted soils and reduce toxicity of the treated compost matrix to improve fertility for plant growth. The following are the specific objectives of this study:

I. To test the biodegradation of hydrocarbon pollutants in oil based drill cuttings and coal tar impacted soil by composting with different compositions (mix ratios) comprising grass clippings, SMC and straw in laboratory scale experiments.

II. To identify a compost composition that would produce the highest pollutant hydrocarbon reduction during the lab-scale experiments and then further test its performance in an up-scaled outdoor pilot scale compost-bioremediation experiment.

III. To test the toxicities of the products of lab-scale and outdoor pilot scale composting experiments, using microbial (phosphatase analysis) and plant (seed germination and growth) bioassays.

Chapter 2

LITERATURE REVIEW

2.1 Energy from fossil fuels

Human society depends on fossil fuels as an important source of energy which is likely continue to remain relevant for many decades to come (Brown *et al*., 2011; Maggio and Cacciola, 2012). According to the EIA International Energy Report (2012), fossil fuels account for 86% of the primary energy demand, which is made up of 35% crude oil, 28% coal, and 24% natural gas. Renewable sources such as solar, wind, geothermal, biomass and hydroelectricity account for about 9%, and nuclear power about 5%. Forecasts of peak years of world fossil fuel production has been estimated as being between 2009–2021 for crude oil, 2024–2046 for natural gas and 2042–2062 for coal (Maggio and Cacciola, 2012). The global reserves of oil are predominantly in the Middle East and North Africa but coal remains abundant and wide-spread around the world (Table 2.1) and is the second most largely consumed fossil fuel after crude oil. More than 75 countries have large coal deposits (WEC, 2013) including Nigeria, which has an estimated 4.0 billion tonnes in reserves (Adenikinju, 2003).

Table 2.1. Location of the world's main fossil fuel reserves in 2013

	Fossil fuel reserve			Global reserve (%)		
Region	Coal (billion tonnes)	Oil (billion tonnes)	Gas (trillion cubic meter)	Coal	Oil	Gas
North America	245	29	10	27	13	5
South America	15	44	8	2	20	4
Europe	274	14	53	31	6	25
West, East and South Africa	32	16	10	4	7	5
South and Central Asia	102	5	33	11	2	16
East Asia	116	2	3	13	1	1
Middle East and North Africa	0	110	85	0	49	40
Southeast Asia and Pacific	107	3	9	12	1	4
Total	891	223	211			

Data source: WEC 2013

2.2 Uses of fossil fuels' energy

Coal was amongst the first of the fossil fuels discovered and utilized for energy production. The discovery, mining and burning of coal for fuel followed a succession of uses for smelting of metals, the development of the steam engine, the steam ship, the locomotive and steam-electric power (Hubbert, 1949). The current share of coal in global power generation is over 40% with China alone using as much coal as the rest of the world (Asif and Muneer, 2007; WEC, 2013; Long *et al*., 2015). Coal was discovered in Nigeria in the early 1900s when it was produced in commercial quantity and was the most used fuel for railway transportation, electricity generation, and industrial heating operations. Currently there is insignificant use of coal for energy due to the conversion of most transportation, power generation and industrial heating systems to operate with diesel fuel and closure of coal power plants during the Nigeria civil war (1967–1970). As a result of the alternative source of energy, tar sands estimated as 31 billion barrels of oil equivalent, have remained untapped in Nigeria (Enibe and Odukwe, 1990; Ohimain, 2013).

The discovery and utilization of crude oil and natural gas, about 150 years ago, further augmented the developments arising from use of coal leading to the internal combustion engine, the automobile, the aeroplane and diesel-electric power (Hubbert, 1949). Figure 2.1 shows various uses of the three major fossil fuels as sources of energy for power, transportation and heat.

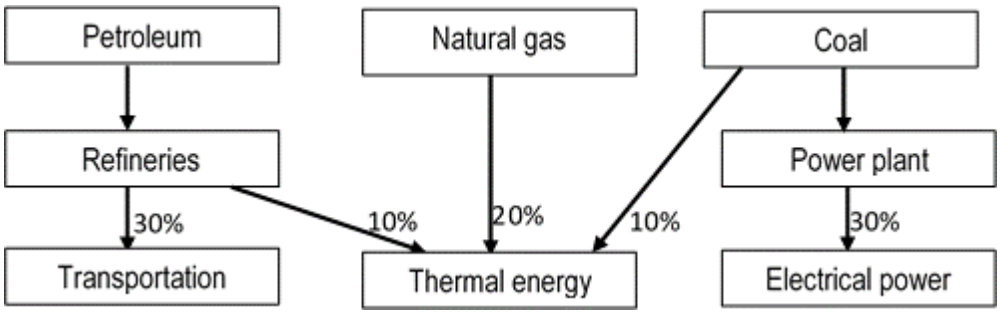


Figure 2.1. Fossil fuel system (source: Veziroğlu and Şahi˙n, 2008)

Apart from power generation, fossil fuels are also used significantly as direct or complementary raw materials for the manufacturing of other useful products. In coal chemical industry, coal is used as raw material to produce gasses, liquids and solids which are subsequently used to synthesize a series of chemicals. In coal gasification process, coal is reacted with steam, oxygen, air, hydrogen, carbon dioxide, or a

combination of these to produce gaseous products, which include hydrogen, carbon dioxide, carbon monoxide, methane, and other gases (Schobert and Song, 2002; Minchener, 2005; Xie *et al.,* 2010). Coal liquefaction technologies have been developed to commercial scales using processes which convert coal to methanol and the heavy fraction used for the production of diesel (Höök and Aleklett, 2010; Xie *et al.,* 2010). A coal-based polygeneration system, shown below (Figure 2.2), has been proposed for multi-production of electricity power, alcohol and ether fuel, coke and tar multi-components at low cost.

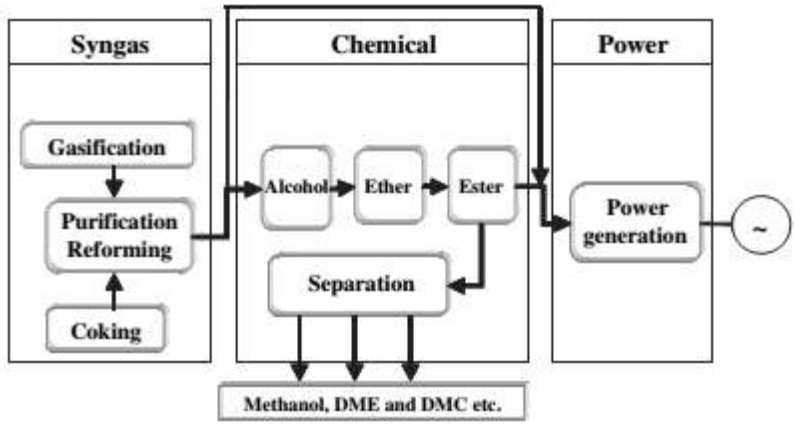


Figure 2.2. Proposed coal-based polygeneration system (Source: Xie et al., 2010)

Other uses of coal include: feedstock for production of aromatic and specialty chemicals; carbon-based materials; making of humic acid and calcium humates, which can be used as soil modifiers and fertilizers (Schobert and Song, 2002).

2.3 Formation and classification of coal

The fundamental knowledge behind the origin of coal is based on deposited plant debris in swampy environment which formed soft, spongy sediment called peat. Due to prolonged compaction in elevated temperatures at burial depth of several kilometres for several million years, the peat was changed into different classes of coal through physical and chemical processes referred to as coalification. The properties of the different class of coal are characterised by three independent geological parameters, namely: rank – defines the degree of coalification to which the original peat has been subjected during its burial history; type – describes the nature of plant debris from which the original peat was derived which include leaves, wood, algae; and grade – reflects the extent to which the original peat has been kept free of

contamination by inorganic minerals before and during peat formation and coalification. Based on these parameters, a high grade coal refers to a coal with high proportion of organic matter against low mineral matter content regardless of its rank or type (Teichmüller, 1989; Suárez-Ruiz and Crelling, 2008).

The classification of coal based on its rank describes the transformation from peat through lignite, subbituminous, bituminous, and anthracite coal in order of increasing percentage of carbon (Jones, 2010). Low rank coals refer to lignite and subbituminous coals which are classified as low grade fuels with low calorific value, high moisture content (25-65%) and low sulphur content. Low rank coals make up about half of the world's coal deposit and are mainly used to produce electricity, although there has been increasing interest in their processing to the production of a substitute to natural gas, liquid fuels and activated carbons in recent years (Varol and Ercanoglu, 2006; Bielowicz, 2012; Yu *et al*., 2013). Anthracite coal is classified as 'high rank coal' which has almost pure carbon content ranging from 80-100%. It burns with a tiny blue flame, almost smokeless, for longer duration than low rank coals and produce intense heat which make it an ideal blast furnace fuel for iron manufacturing (Chandler, 1972; Jones, 2010).

2.4 Coal tar

Coal tar is among the products of coal-to-gas processing (manufactured gas) plants formed by the pyrolysis of coal. The other products of coal pyrolysis include coke and coal oven gas (Hatheway, 1997; Krzesińska *et al*., 2006; He *et al*., 2014). During coal pyrolysis, reactions in the burning coal generate a complex mix of condensable volatile compounds including aromatic and aliphatic fragments which are collected as coal tar. (Ledesma *et al*., 2000). Generation of the volatile radical fragments results from thermal cleavage (cracking) of covalent (C-C) bonds in the coal feedstock (Krzesińska *et al*., 2006; Zhan *et al*., 2014; Liu *et al*., 2015). It is noted that the composition and physical properties of coal tar vary widely depending on the temperature gradient in the pyrolysis reactor, reactor condition and coal feedstock (anthracite or bituminous coal) as well as extent of weathering after release to the environment (Peters and Luthy, 1993; Liu *et al*., 2015).

Previous studies have shown that coal tars are nonaqueous phase liquids (NAPLs) primarily composed of hydrocarbons with predominant proportions of polycyclic

aromatic hydrocarbons (PAHs, 85%), and thus are used as primary feedstock for the production of aromatic and phenolic chemicals as well as contributing 10-15% of benzene, toluene, ethylbenzene and xylene (BTEX) production (Peters and Luthy, 1993; Haeseler *et al*., 1999; Schobert and Song, 2002). Other products that could be distilled from coal tar include pitch, motor fuel, creosote and heavy oils which were used for road paving as sealants (Hatheway, 1997).

2.5 Environmental impact of coal and its products

All the benefits derived from coal are essentially from the organic matter constituents, which also contribute substantially to environmental pollution, especially carbon dioxide emission during coal combustion and its effect on global warming (Ward, 2002; Milici, 2009). The inorganic fractions, comprising mainly mineral matters, typically contribute insignificantly to the value and utilisation of coal but are known to be source of sickness to coal mine workers as well as users of coal for domestic fuel (Ward, 2002; Finkelman *et al*., 2002). Among the severe health impacts associated with coal mine dust exposure suffered by coal miners include accelerated loss of lung function (Love *et al*., 1997; Beeckman *et al*., 2001); chronic bronchitis (Love *et al*., 1997) and pneumoconiosis and silicosis which are the most severe (Stephens and Ahern 2001). A high lung cancer mortality rate of five times the national average in China was cited to result from unventilated combustion of coal in homes and the carcinogenic agent was attributed to the PAHs in coal, which were released during the combustion (Mumford *et al*., 1995; Finkelman *et.al*., 2002). Other endemic diseases resulting from the inorganic fractions of coal released during domestic combustion of mineralized coal reported in China include: hyper-pigmentation, arsenism, hyperkeratosis, Bowen's disease and squamous cell carcinoma from arsenic poisoning; fluorosis from fluorine poisoning and selenosis from selenium poisoning (Zheng *et al*., 1999; Finkelman *et.al*., 2002).

The nonaqueous nature of coal tars and their composition of significant amount of PAHs derived from coal make them persistent pollutants in the environment and their subsequent classification as Group 1 carcinogens (IARC, 2010). The slow, continuous dissolution of constituent compounds from subsurface coal tars still persist in the environment and sights of manufacture gas plant several decades after the end of manufactured gas industry in the USA and Europe in the 1950s (Peters and Luthy, 1993; Hatheway, 1997; Taylor and Jones, 2001). The airborne toxicity

risks of coal-tar-based pavement sealants have been reported by Van Metre *et al*. (2012) stating that an estimated loss of one-quarter to one-half of PAHs in the seal coat product to the atmosphere occurred during the first 16 days after application. PAH concentrations in house dust of residential houses adjacent to play grounds, parking lots and driveways with coal-tar-based sealants have been reported to be 25 times higher than residents adjacent to parking lots built with unsealed asphalt, indicating high risk of non-dietary intake of PAHs in house dust by children (Mahler *et al*., 2010; Williams *et al*., 2012). Reapplication of coal-tar-based sealants every 3 to 5 years is recommended by manufacturers because they wear with traffic volume and time. As these materials wear off, abraded particulates from the surface of parking lots are washed into adjacent surface waters via storm water runoff (Scoggins *et al*., 2007). Studies have indicated that these washed sealants might contribute significantly to the PAH load of such adjacent surface waters (Mahler *et al*., 2005). Also, extensive studies on the direct and indirect biological effects of PAHs (including those from surfaces of coal-tar-based sealants) on aquatic invertebrates have been reported to include inhibition of reproduction, mortality, delayed emergence and sediment avoidance (Fleeger *et al*. 2003; USEPA, 2003; Bryer *et al*., 2010).

2.6 Uses and environmental impact of crude oil

Crude oil is the most utilized fossil fuel in the world having a wide variety of physical characteristic and chemical composition depending on the location of the oil field and the stage of production (Hocking, 2005; Wang *et al*., 2006). The chemical composition of crude oil is a complex mixture of both low and high molecular weight hydrocarbons including toxic PAHs, and polar compounds including asphaltenes containing nitrogen, sulphur and oxygen heteroatoms (Yamada *et al*., 2003; Wang *et al*. 2011). Refining of crude oil through varieties of processes such as distillation, cracking, alkylation and blending also produces useful non-fuel petroleum products which include lubricating oils, greases, hydraulic fluids, paraffin waxes, asphalts and skin care product like petrolatum - petroleum jelly (Wesołowski, 1981; Seymour and Henry, 2001; Wang *et al*., 2006).

The impacts of crude oil and petroleum products in the environment produce ecological disasters following their accidental or deliberate spills onto land or water which occur during extraction, processing/refining and transportation/marketing (Liu *et al*., 2011; Ogbo and Okhuoya 2008). Additionally, improper treatment and disposal

of crude oil production and processing wastes which include drill cuttings and spent catalysts and chemicals, also pose pollution threats to the environment (Curran, 1992; Leonard and Stegemann, 2010). Environmental pollution resulting from crude oil spills has been known to adversely affect terrestrial, marine and human lives (Wang and Fingas, 2003; Chima and Vure, 2014). For example, crude oil contamination can inhibit seed germination by acting as a physical barrier preventing or reducing access of the seeds to water and oxygen, prevent plant growth and clog the lenticels resulting in sublethal effects such as defoliation and subsequent mortality (Ogbo *et al*., 2009; Hoff *et al*., 2010). All of these constitute major concerns to the petroleum industry as enforcement of environmental regulations become stricter in oil producing countries of the world (Curran, 1992; Shah *et al*., 2011).

2.7 Hydrocarbons

Hydrocarbons are widespread components of the environmental carbon cycle which are produced from natural and anthropogenic sources. Natural hydrocarbons are produced by plants and are generally encountered at trace levels, while anthropogenic hydrocarbons are widely distributed; produced significantly from industrial and urban activities such as combustion of fossil fuels like crude oil and refined petroleum derivatives, coal, wood, etc. (Sicre *et al*., 1987). They are dominant constituents of liquid fossil fuels and products derived from them. Petroleum hydrocarbons exist in four major component fractions namely saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltenes. These fractions of hydrocarbons and their derivatives constitute environmental pollutants and pose health hazards to humans and plants when released to the environment (Atlas, 1981; Wang *et al*., 2006; Ogbo and Okhuoya, 2008).

2.7.1 Saturated hydrocarbons

Saturated hydrocarbons are composed of only single carbon and hydrogen bonds occurring either as straight chain or branched chain. They include normal alkanes, branched alkanes (also called paraffins) and cycloalkanes (also called naphthenes) and are predominant class of hydrocarbons in most crude oil (Atlas, 1981; Wang *et al*., 2006). The normal alkanes and their isomers from nC_1 to nC_{40} or more contain large numbers of compounds and often account for 20–50% of crude oil constituents depending on the source of the soil (Saeed *et al*., 1997; Van Beilen *et al*., 2003). The intermediate length normal alkanes (nC_{10} to nC_{20}) have low aqueous solubility, with

increasing melting and boiling points as the carbon numbers increase within the molecule. They tend to be more readily degradable compared to longer chain alkanes. The longer chain alkanes (*n*C_{20} to *n*C_{40}) are known as waxes and consequently are hydrophobic resulting in their poor bioavailability and difficult to degrade. Branched chain alkanes such as pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) are also less susceptible to degradation than the corresponding normal alkanes (Balba *et al*., 1998; Stroud *et al*., 2007).

2.7.2 Polycyclic aromatic hydrocarbons (PAHs)

PAHs comprise a wide class of organic compounds composed of two or more fused benzene rings and are natural constituents of fossil fuels. They are generated in the environment by natural and anthropogenic sources. Natural sources constitute a minor contribution of PAHs to the environment, which include incomplete combustion processes from forest and prairie fires, volcanic eruptions and oil seeps. Anthropogenic sources include oil spills, coal liquefaction and gasification processes, wood combustion, tobacco smoke, and particularly incomplete combustion of fossil fuels, industrial and automobile exhaust emissions, and waste incineration, which contribute significant levels of PAHs to the environment. (Cerniglia, 1992; Wilson and Jones, 1993; Grova *et al*., 2002; Anyakora *et al*., 2005b; Luan *et al*., 2006).

PAHs are generally lipophilic compounds that exhibit a high affinity for organic matter and possess properties such as low aqueous solubility and high solid-waste distribution ratios which prevent their straight microbial utilization which results to their accumulation in the polluted medium (Blumer, 1976; Johnson *et al*., 2005). As a result, they are highly persistent compounds in the environment, recalcitrant to microbial degradation, while some of them have been classified as hazardous organic compounds in the environment due to their known or suspected carcinogenicity and mutagenic toxicity, and are included in the European Community (EC) and United States Environmental Protection Agency (US EPA) priority pollutant list (Blanchard *et al*. 1999; Anyakora *et al*., 2005). Chemical structures of 16 priority PAHs pollutants are shown in Figure 2.3 among which benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene have been identified as potential human carcinogens (Guillén *et al*., 2000; Anyakora *et al*., 2005).

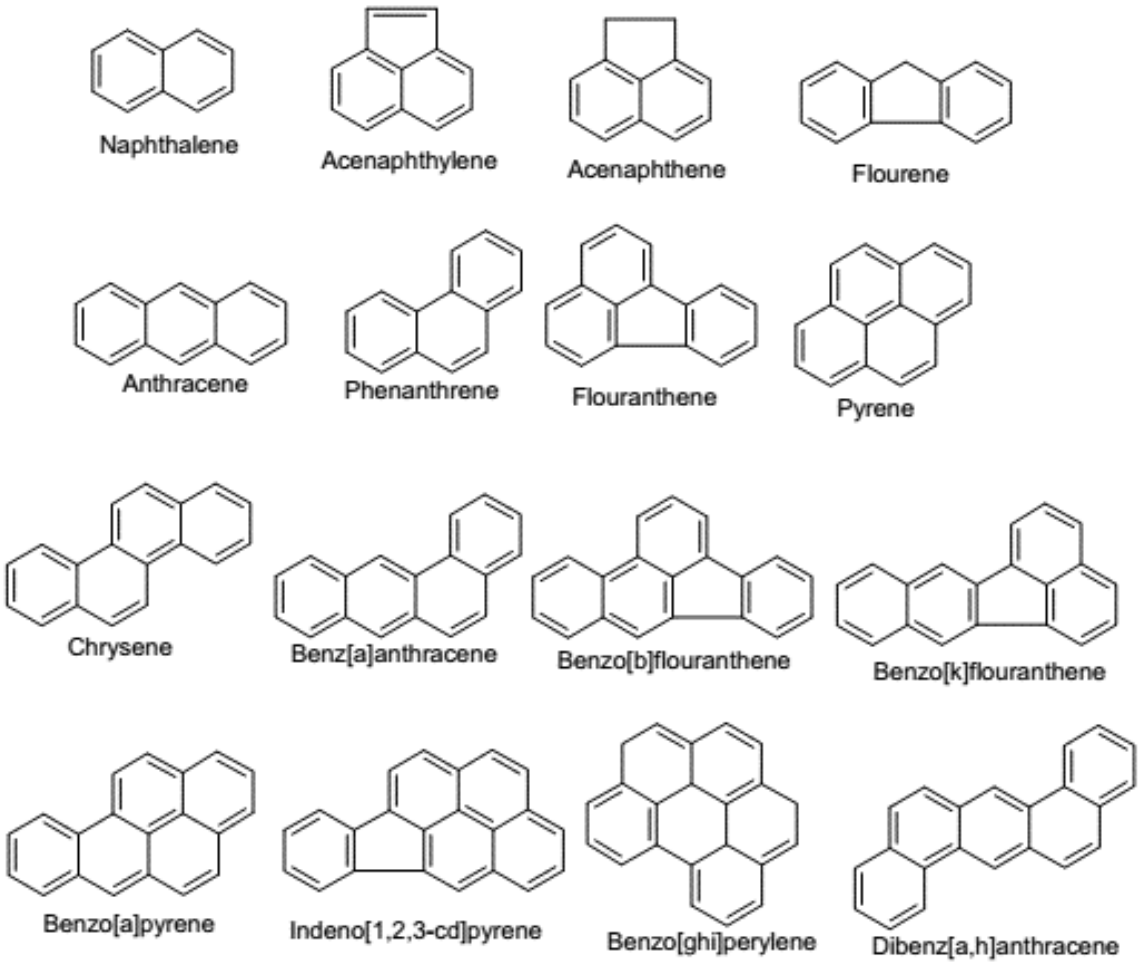


Figure 2.3. Chemical structures for the 16 US EPA priority PAHs pollutants (Source: Anyakora et al, 2005)

2.8 Risk assessment and management of contaminated land

About forty years ago the perception of land contamination was usually in terms of rare and poorly known incidents with possibly catastrophic consequences for humans and the environment. Major media attention was attracted by several incidents, e.g. those of Love Canal in New York State, Times Beach in Missouri, and Lekkerkerk in Netherlands, which resulted in responses by politicians to seek maximum risk control – pollution should be removed or contained completely (Ferguson, 1999). Today land contamination is perceived as a widespread infrastructural problem of varying intensity and significance in the industrialized and also many developing countries of the world, resulting in the enactment of several regulatory systems to control contaminated land. In the United Kingdom (UK), Part IIA of the Environmental Protection Act 1990 (Part 2A) (which became effective in 1999) and the land use planning are the two principal policy processes forming the core of the regulatory system (Ferguson, 1999; Lowe and Lowe, 2001).

The UK's policy on contaminated land, within the Part 2A regime, sets out legislation and regulation with objectives to (1) prevent the creation of new contamination; (2) promote the remediation of existing legacy contamination through the redevelopment of land; and (3) intervene through a regulatory process to deal with existing contamination where redevelopment is not likely but a threat exists (Luo *et al*., 2009). The fundamental concept of the UK contaminated land risk assessment and management is defined by the Source – Pathway – Receptor pollutant linkage model. Wherein, 'source' refers to a substance that is in, on or under the land which is potentially toxic and capable of causing pollution; the 'pathway' refers to the direct and indirect routes or means in the environment by which the potentially contaminating substance may be transferred to the receptor, and the 'receptor' is the entity that could be adversely affected by the contaminant. The receptor is comprised of various entities with different characteristics such as humans, aquatic ecosystems, or buildings. (DEFRA and Environment Agency, 2004; Rodrigues *et al*., 2009; Luo *et al*., 2009).

The fundamental point in the application of the Source – Pathway – Receptor pollutant risk assessment linkage model is that all three elements of the linkage must be present for a risk to exist. Thus, even where the concentrations of contaminant(s) present is above a background or an acceptable guideline value, the site will not be considered contaminated if there are neither pathways nor receptors to be affected. Where the presence, or likely presence, of all three pollutant linkages have been significantly established, the hazardous site may then be controlled by breaking the pollutant linkages by modifying the source (e.g. by bioremediation of the contaminated soil); managing or breaking the pathway (e.g. by pump and treat or use of a cover system); or by modifying the receptor (e.g. by limiting land use) (Luo *et al*., 2009).

2.9 Biodegradation of pollutant hydrocarbons in soil

Hydrocarbons are among the wide range of toxic organic chemicals that are commonly introduced inadvertently or deliberately into the environment (Balba *et al*., 1998). Established evidence suggest that microorganisms are the chief natural agents of degradation of toxic hydrocarbons from terrestrial and aquatic environments, due to their potential for utilizing organic substances as sources of nutrients and energy (Balba *et al*., 1998; Dua *et al*., 2002). The strategy of facilitating

and accelerating the natural processes of microbial degradation of hydrocarbon contaminants to less harmful substances is referred to as bioremediation (Bamforth and Singleton, 2005). During the biodegradation of hydrocarbon contaminants, bacteria, fungi and yeasts play prominent roles due to their wide distributions in marine and soil ecosystems. Whereas bacteria and yeasts appear to be the dominant degraders in aquatic environments, bacteria and fungi are the dominant degraders in soil ecosystems (Hanson *et al*., 1997; Balba *et al*., 1998).

2.9.1 Biodegradation of alkanes in soil

Many microbial genera and strains have been reported to grow on and degrade alkanes under aerobic condition (e.g. Atlas and Cerniglia, 1995; Van Beilen et al., 2003). As illustrated in Figure 2.4, the biodegradation of alkanes is usually initiated by an oxygenase enzyme which activates the alkanes by terminal oxidation to form primary alcohols. Sub-terminal oxidation results in secondary alcohols. The primary alcohols are further oxidised by dehydrogenase enzymes to aldehydes and fatty acids while the secondary alcohols are converted to corresponding ketones; the resulting fatty acids are further oxidised by cytoplasmic β-oxidation enzymes (Van Beilen *et al*., 2003; Stroud *et al*., 2007). In the absence of oxygen (anaerobic condition), the initial activation is achieved by the addition of a C_1 (CO_2) or a C_4 (fumarate) compound (Heider *et al*., 1999).

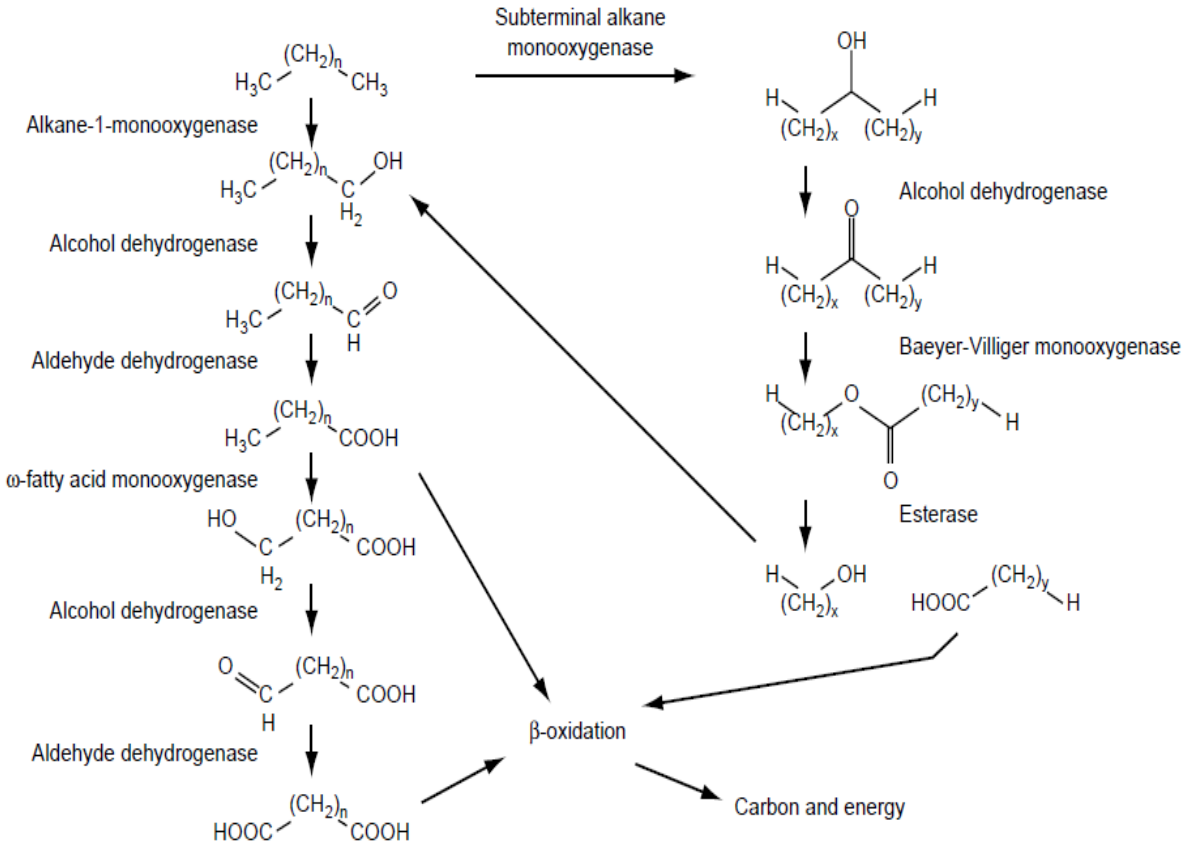


Figure 2.4. Pathway for the degradation of alkanes by terminal and sub and biterminal oxidation (Source: Van Beilen et al., 2003)

2.9.2 *Biodegradation of PAHs in soil*

A wide variety of bacteria and fungi strains have been identified with the ability to metabolize PAHs. Most of these microorganisms were isolated from soils and sediments that contain hydrocarbon contaminations. Some PAH-degrading bacteria that have been commonly studied include: *Pseudomonas aeruginosa, Pseudomons fluoresens, Mycobacterium spp., Haemophilus spp*., *Rhodococcus* spp. and *Paenibacillus* spp. Also, some lignolytic fungi which have been identified with the ability to degrade PAHs include *Phanerochaete chrysosporium, Bjerkandera adusta*, and *Pleurotus ostreatus.* Non-lignolytic fungi such as *Cunninghamella elegans, Chrysosporium pannorum* and *Aspergillus niger* have also been identified with PAH degrading ability (Johnson *et al*., 2005; Bamforth and Singleton, 2005; Haritash and Kaushik, 2009). Studies of biodegradation of PAHs have been reported to be effective mostly under aerobic condition, though the potential of microbial degradation of PAHs under anaerobic condition has been recognised recently with studies clearly demonstrating that PAH are capable of being degraded under both

denitrifying and sulfate-reducing anaerobic conditions (Rockne *et al*., 2000; Zhang *et al*., 2000).

Fundamentally, three different mechanisms of PAHs metabolism under aerobic conditions have been identified, with each representing degradative pathway by bacteria, lignolytic and non-lignolytic fungi, as illustrated in Figure 2.5. The general mechanisms of microbial degradation of PAH starts with the oxidation of the aromatic benzene ring catalysed by dioxygenase enzymes by bacteria (Antizar-Ladislao *et al*., 2004), lignolytic enzymes by lignolytic fungi (Mester and Tien, 2000) and cytochrome P_{450} monoxygenase enzyme by non-lignolytic fungi (Haritash and Kaushik, 2009). This is subsequent followed by the breakdown of the compound to PAH metabolites and/or carbon dioxide. The mechanism of anaerobic PAH degradation is still tentative, though, is thought to occur via the hydrogenation of the aromatic ring (Bamforth and Singleton, 2005). A proposed microbial anaerobic degradation of naphthalene under sulfate reducing conditions is shown in Figure 2.6.

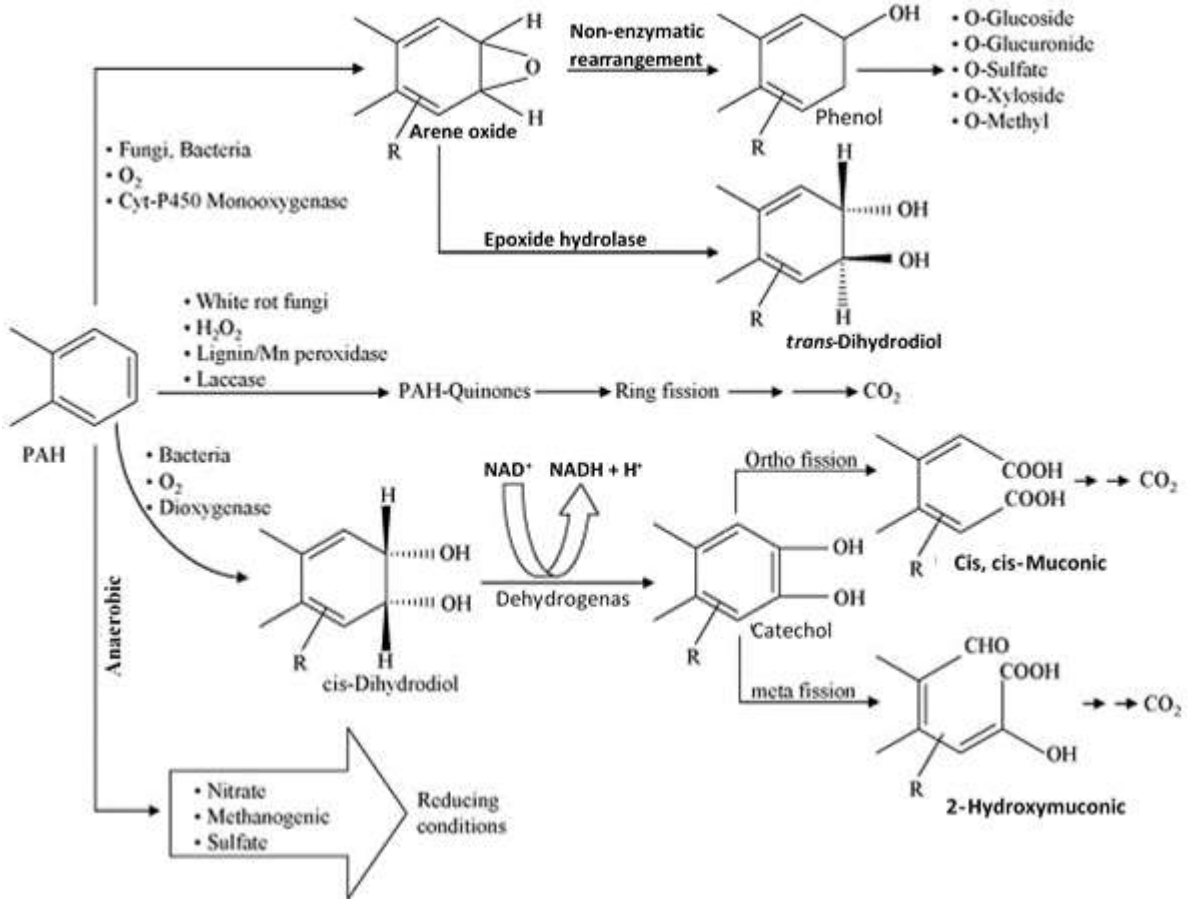


Figure 2.5. Proposed pathway for microbial catabolism of polycyclic aromatic hydrocarbons (Source: Haritash and Kaushik, 2009)

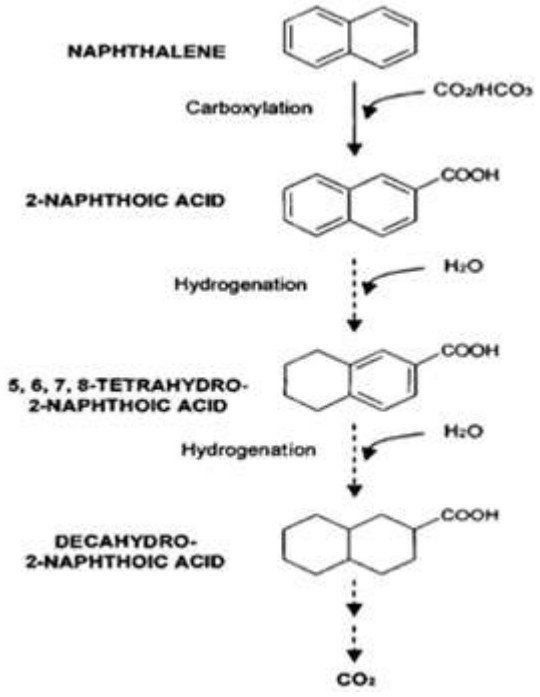


Figure 2.6. Proposed pathway for the anaerobic metabolism of naphthalene under sulfate-reducing conditions (Source: Bamforth and Singleton, 2005)

Some studies have shown that only 2 to 4-ring PAHs can be degraded by bacteria as sources of carbon and energy (Boonchan *et al*., 2000). Higher molecular weight PAHs composed of 5 to 7 fused aromatic rings are only partially oxidised by bacteria or metabolised by fungi. The recalcitrance to biodegradation by high molecular weight PAHs is largely attributed to hydrophobicity, which increases with molecular mass and affects the bioavailability and microbial utilization of these organic pollutants, whereby making them highly persistent in the environment (Juhasz and Naidu, 2000; Johnson *et al*., 2005).

2.9.3 Parameters for optimum biodegradation of PAHs in soil

The success of any bioremediation treatment depends mainly on the attainment of conditions for effective performance of the system. Studies have identified physicochemical conditions such as soil moisture, soil pH, redox potential, oxygen content, nutrient content and temperature as important parameters for optimum PAH degradation in soil (Wilson and Jones 1993). The appropriate control of the environmental conditions within the soil is therefore required during the remediation process (Sims, 1990). Table 2.2 presents some suggested optimum environmental conditions.

Table 2.2. Essential conditions for effective degradation of organic contaminants in soil

Parameter	**Conditions required for microbial activity**	**Optimum values for PAH degradation**
Soil moisture	25-85% of water-holding capacity	30-90%
Soil pH	5.5-8.5	7.0-7.8
Redox potentia	Aerobes and facultative anaerobes > 50mV Anaerobes < 50mV	
Oxygen content	Aerobic, minimum air Filled pore space of 10% Anaerobic <1% by volume	10-40% 0_2
Nutrient content	N and P for microbial growth C:N:P 120:10:1 (optimum value approximately)	P, C:N 60:1 C:P 800:1 Salt concentration <4%
Temperature (^oC)	15-54	20, 24-30, 27, 30

(*Source: Wilson and Jones, 1993*)

2.10 Compost-biodegradation of hydrocarbon contaminated soil

Traditionally, composting is the degradation of organic solid wastes such as agricultural and food waste materials to useful organic matrix which is used as soil amendment that provides nutrients to crops and enhance the tilt, fertility and productivity of soils. These waste materials are usually mixed with organic bulking agents such as wood chips, straw or some other organic waste materials to increase the porosity to allow both air and water to penetrate the matrix (Sasek *et al*., 2003; Ball *et al*., 2012). The use of composting technique has been successfully applied for the degradation of PAHs (Sasek *et al*., 2003; Yuan *et al*., 2009), petroleum hydrocarbons (Jørgensen *et al*., 2000; Marín *et al*., 2006), chlorophenols (Laine and Jørgensen, 1997), polychlorinated biphenyls (PCBs) (Michel *et al*., 2001) and explosives (Gunderson *et al*., 1997) at laboratory and field scales. Additionally, composting has been investigated and applied as a bioremediation strategy for the biodegradation of hazardous wastes in soil. Growing studies have been reported to show the effectiveness of composting in the remediation of a wide variety of organic wastes, such as municipal solid wastes, poultry litter, vegetable wastes, food processing residuals, sludge from waste treatment plants and other sludge generating processes (Juwarkar *et al*., 2010). Semple *et al*. (2001) has also reviewed the use of composted materials to remediate contaminated wastes.

During compost-bioremediation, favourable conditions such as oxygen availability, temperature, and moisture are created which enhance the proliferation of indigenous microorganisms that are capable of mineralization and humification of organic matter, increase the kinetics of enzymes responsible for the degradation of PAHs, and increase the solubility and mass transfer rates of contaminants (Sayara *et al*., 2010). The mineralization may degrade only a small fraction of pollutant, while the other prominent fraction may partially degrade to secondary compounds, volatilize, and adsorb to the compost matrix (Buyuksonmez *et al*., 1999). The physiochemical and biochemical processes of degrading organic pollutants during composting are similar to those that naturally occur in soils. However, composting may accelerate these processes since higher metabolic temperatures are generally developed than in the soil (Juwarkar *et al*., 2010). A variety of composting systems are used for compost-bioremediation of polluted soils. These include: open-air systems such as static aerated piles and mechanically turned windrows; and closed systems which include

modular containers and tunnels or buildings (Jørgensen *et al*., 2000; Sasek *et al*., 2003).

2.10.1 Regulatory considerations on composting

The composting of biodegradable wastes is controlled with regulations in many developed countries. In the United Kingdom, recycling and composting of wastes are vital components of the department of environment, transport and regions (DETR). The Waste Strategy 2000 (DETR 2000) targets for recycling and composting is set at 25% by 2005, 30% by 2010 and 33% by 2015 (Antizar-Ladislao *et al*., 2004). A similar regulatory directive in the EU, the European Community Landfill Directive 1999 (EC, 1999), also has established targets for the reduction of biodegradable wastes to landfills, set at 25% by 2010, 50% by 2013, and 65% by 2030 (Antizar-Ladislao *et al*., 2004). Regarding composting of catering wastes, the European Union (EU) regulation (EU Animal By-Product Regulation, 2003) stipulates that the composting of catering wastes containing meat must be done in a 'closed composting reactor' operated at least 70 ^{o}C for 1 hour. This implies that catering wastes containing meat cannot be composted in an open windrow, except as a second stage after treatment in the closed reactor. Composting of green wastes, kitchen wastes, and in general biodegradable organic wastes, which might include contaminated soil, will probably have an important role to play in the long term requirement of the EC landfill directive (Burnley, 2001; Antizar-Ladislao *et al*., 2004). In addition, there is increasing regulatory demand on the safety of the composting process and utility of the end product after composting. The EU thematic strategy for soil protection demands that the composting-treated product becomes valuable resource for tackling land degradation (European Commission, 2006). Figure 2.7 shows regulatory considerations for composting of PAH contaminated soil.

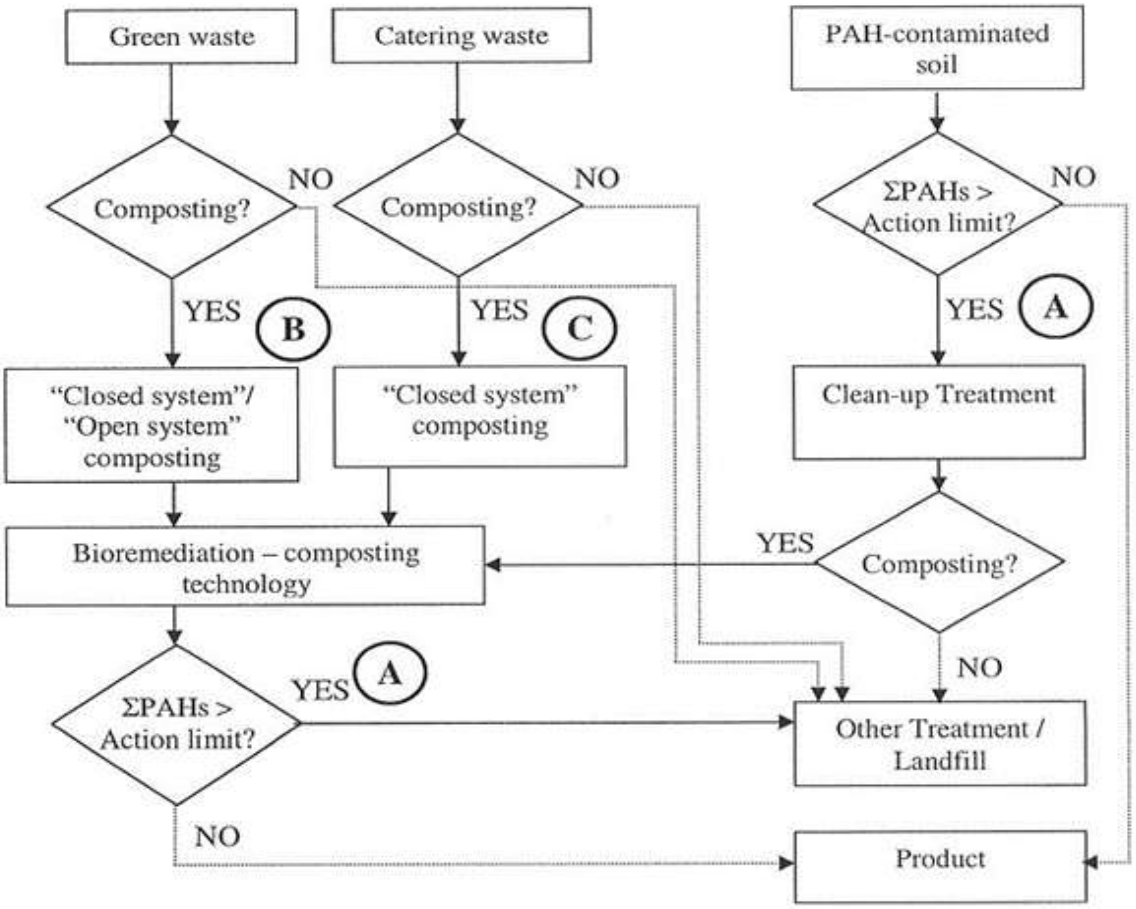


Figure 2.7. Regulatory considerations for bioremediation of PAH-contaminated soil using composting approaches (Source: Antizar-Ladislao et al., 2004)

2.10.2 *Essential factors for compost-bioremediation*

Studies have reported essential parameters for effective compost-bioremediation of hydrocarbon contaminated soils. They include appropriate moisture, temperature, oxygen, pH, conductivity and micronutrients and carbon to nitrogen to phosphorus ratios. The reported optimum range of moisture content for compost-bioremediation of hydrocarbons is reported as 60%, while the general moisture content for composting is ranging between 50-80%. (Richard *et al*., 2002). Previous studies have reported moisture content of 30-35% as the optimum range for TPH removal in drill cuttings during bioremediation treatment (Roldan *et al*., 2003; Rojas-Avelizapa *et al*., 2007).

The conventional composting temperature cycle passes through four major phases, each identified with microbiological conditions. The cycle begins with the mesophilic stage (30 – 45 ^{o}C) and progresses through the thermophilic (45–75 ^{o}C), cooling, to the maturation stage. The mesophilic stage is characterized with the greatest

microbial diversity, while in the thermophilic stage is associated with increased relative abundances of spore-forming bacteria (Nakasaki *et al*., 1985) and thermophilic fungi (Fogarty and Tuovinen, 1991). The occurrence of microbial decomposition and biomass formation happen mostly during the thermophilic stage of composting (Megharaj *et al*., 2011). The cooling stage is conducive for the reappearance of microbial colonization of mesophilic fungi whose spores survived the high temperatures of the thermophilic stage. The final stage of composting is referred to as maturation stage, during which most digestible organic matter is consumed by the microbial population, resulting in composted material that is considered stable.

Heterotrophic bacteria and fungi are most active near of neutral pH, as extreme pH values have been reported to have impede the ability of microbial population to degrade hydrocarbons (Leahy and Colwell, 1990). Similarly, compost mixtures having salinity of <1% have been reported as optimum for biodegradation of PAHs (Wilson and Jones, 1993). Carbon:nitrogen (C:N) ratios ranging between 9 – 200 have been widely reported for effective compost- bioremediation of hydrocarbon contaminated media (Huesemann, 1994; Roldan *et al*., 2003). However, a more narrow C:N range of 25-30 has been suggested by Fogarty and Tuovinen (1991) for optimum composting. Similarly, studies have reported a wide range of carbon: phosphorus (C:P) ratios (60-800) for hydrocarbon degradation (Huesemann, 1994). Additionally, consumption of oxygen and production of carbon dioxide are essential indicators of hydrocarbon mineralization by microorganisms during composting (Yerushalmi *et al*., 2003; Joo *et al*., 2007).

2.11 Drill cuttings waste

Drill cuttings are wastes which are produced in vast quantity during well drilling for crude oil and natural gas exploration and extraction. The well drilling process is facilitated by the use of drilling fluids, also known as ‘muds’, which are continuously pumped down the well to perform the following functions: lubricate and cool the drill bit; stabilize the well and maintain hydrostatic pressure; reduce friction between the drill pipe and wellbore; prevent inflow of fluid from the wellbore; non-damaging to the producing formation; and lift out rock cuttings from the well to the surface. The latter is considered the main function (Okpokwasili and Nnubia, 1995; Caenn and Chillingar, 1996; Hamed and Belhadri 2009). As the drill bit grinds and cuts the

geological formation in the well, these rock cuttings get entrapped within the drilling fluid and are carried to the surface where the cuttings are separated (Figure 2.8). The fluid can be modified by the addition of heavy minerals such as barite to sustain fluid density and rheological properties down-hole, before re-injection to continue the operation. Consequently, the drill cuttings that are discharged are comprised of rock cuttings with adhered drilling mud, oil and heavy metals. The drilling mud usually constitutes 5-25% of the discharged drill cuttings (Opete *et al*., 2010; Trannum *et al*., 2010; Ball *et al*., 2012).

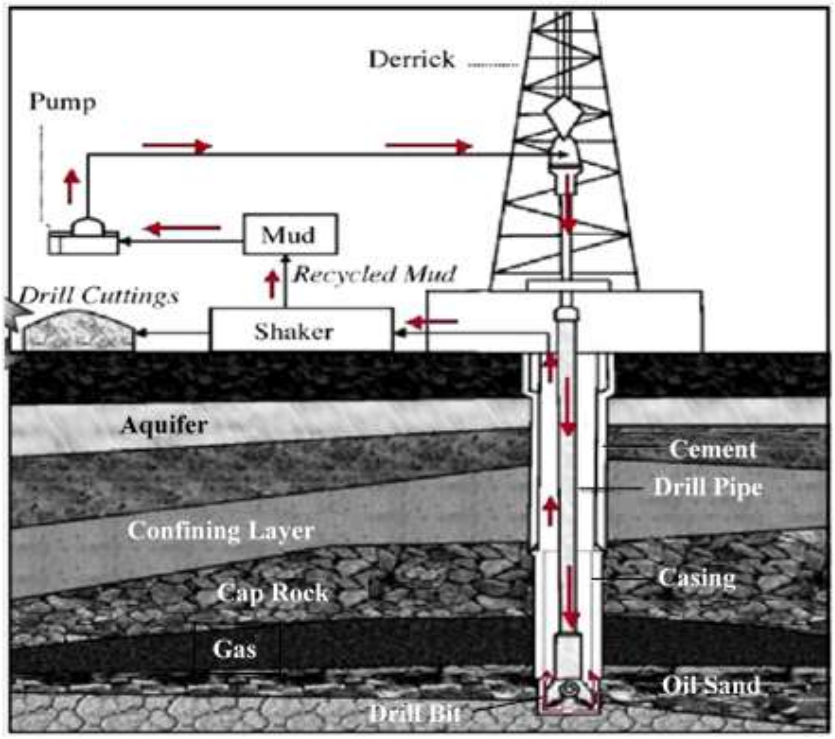


Fig. 2.8: Generation of drill cuttings from petroleum drilling operation (Adapted from Al-Ansary and Al-Tabbaa 2007)

2.11.1 Composition and environmental impact of drill cuttings

Generally, the physical characteristics and chemical composition of drill cuttings vary widely depending on factors, including drilling fluid used, geology of the drilled well, oil well location and disposal scenarios of the cuttings among others (Ji *et al*., 2004; Al-Ansary and Al-Tabbaa, 2007). Drilling fluids are categorised into three main groups depending on their base liquid. They include water based fluids (WBFs), synthetic based fluids (SGFs) and oil based fluids (OBFs). In WBFs the dominant fluid is fresh or salt water, in SBFs the dominant fluid is ester, ether, acetyl or olefin, and in OBFs mineral oils make up the dominant fluid. In addition, drilling muds also

contain weighting materials, clay or organic polymers, inorganic salts, inert solids and organic additives. See Table 2.3. (Leonard and Stegemann, 2010; Trannum *et al*., 2010; Ball *et al*., 2012).

Table 2.3. Comparison between water-based fluids, oil-based fluids and synthetic-based fluids

Water-based fluid	Oil-based fluid	Synthetic-based fluid
Solids are suspended in water and have at least one common ingredient – extremely hydrophilic clay that increases viscosity and prevents fluid loss from the borehole.	Solids are suspended in a hydrocarbon distillate (e.g. diesel or mineral oil) rather than water and may contain barite (BaSO4) used for controlling hydrostatic pressure on account of its high density.	Solids are suspended in a synthetic oil (such as vegetable esters, olefins, ethers and others), which provide drilling performance comparable to OBFs, but with far lower environmental and occupational health effects.

(Source: Ball et al., 2012)

When compared based on their technical performance, SBFs and OBFs are generally preferred over WBFs because they drill cleaner holes with less sloughing, are more effective in deeper well sections and high angle wells, have better performance in moist applications and poorly consolidated rock formations for their ability to drill gauge holes and minimise drilling problems, and they generate lesser volumes of drill cuttings (Shang *et al*., 2006; Robinson *et al*., 2010; Ball *et al*., 2012). However, there is increasing use of WBFs over OBFs as a result of stricter environmental legislation due to environmental impact and worker safety issues associated with the use of oil based mud. The diesel or mineral oils used for the production of OBFs can make them contain polynuclear aromatic hydrocarbons which are carcinogenic and less susceptible to biodegradation. Additionally, barite which has been the most frequently used weighting material in OBFs is usually associated with toxic trace metals such as mercury, lead and cadmium. On the other hand, SBFs have lower toxicity and bioaccumulation potential, faster biodegradability and are recyclable (US DOE, 2003; Shang *et al*., 2006; Trannum *et al*., 2010).

Until recently, drill cuttings were being discharged to sea bed without further treatment and this has resulted in significant negative impacts on marine ecology adjacent to oil platforms. In 2000, the accumulation of discharged drill cuttings on the bottom of the North and Central North Sea was estimated at 12 million m^3 (Breuer *et al*., 2004) with the shape of the pile ranging from 2 to 20 m in height and having orientation predominantly aligned with the prevailing tidal flow (Breuer *et al*., 2008).

The discharged drill cuttings have been reported to significantly disturb the benthic ecosystems through sedimentation (burial), toxic effects and oxygen depletion (Singsaas *et al*., 2008). Studies have reported that benthic effects from oil-based mud have been recorded several kilometres away from oil platforms, and up to 500 m for synthetic-based mud, but not more than 100 m for water-based muds which are less harmful (Gray *et al*., 1990; Olsgard and Gray, 1995; Currie and Isaacs, 2005; Trannum *et al*., 2010). Though water-based drilling muds are considered less toxic compared to oil-base and synthetic-based muds, they have been reported to contain an organic phase which is capable of increasing the risk of oxygen depletion and cause mortality of benthic organisms (Trannum *et al*., 2010). Results of toxicity study on drill cuttings contaminated by oil-based mud showed that after 180 days of discharge, less than 5% were biodegraded (Østgaard and Jensen, 1985).

2.11.2 Regulations guiding the management of drill cuttings

The potential environmental and health impacts of oil-based and synthetic-based, drilling mud and contaminated drill cuttings have attracted increasing community awareness and resulted in stringent environmental regulations to control the discharge of drill cuttings from offshore platforms. In 2000, environmental legislation for the UK and EU stipulated that the residual oil in drill cuttings must be less than 1% by weight before discharge into the North Sea (Oslo and Paris commission, 2000). This legislation is invariably viewed as a 'zero' discharge by operating companies due to limitation of current technologies in achieving this threshold (Robinson *et al*., 2008). Legislation in the Gulf of Mexico stipulated 5% oil concentration before discharge into the sea (US EPA, 2002). Furthermore, untreated oil based-fluid and synthetic based-fluid drill cuttings have been listed within the EU list of hazardous waste (CEC, 2007). Untreated water based-fluid drill cuttings are not listed as special waste except when they contain oil; however, they have to undergo some form of treatment to reduce their high liquid and salinity content before disposal to landfill (Ball *et al*., 2012). Al-Ansary and Al-Tabbaa (2007) reported that regulatory environmental laws in a developing country like Egypt have banned the discharge of any substance resulting from drilling and exploration in the territorial sea of exclusive economic zone without any prior treatment. They have indicated maximum limits for discharge into other marine environments. Also, in a developing country like Nigeria, the regulatory authority has stipulated guidelines for disposal of drilling mud and cuttings for the petroleum industry as shown in Table 2.4.

Table 2.4: Regulatory requirements for discharge of drilling mud and cuttings in Nigeria

Water Based Drilling Fluids and Cuttings	Oil Based Drilling Fluid Cuttings	Synthetic Based Drilling Fluid Cuttings	Environmental Monitoring Requirements
• To discharge, must submit proof that mud has low toxicity to Director of Petroleum Resources (DPR) with permit application. Discharges will be treated to DPR's satisfaction. • DPR will examine WBM to determine how hazardous and toxic it is. • Cuttings contaminated with WBM may be discharged offshore/deep water without treatment.	• To discharge, must submit proof that OBM has low toxicity to DPR with permit application. Discharges will be treated to DPR's satisfaction. • OBM must be recovered, reconditioned, and recycled. • Oil on cuttings, 1% with 0% goal. • On-site disposal if oil content does not cause sheen on the receiving water. • Cuttings samples shall be analyzed by Operator as specified by DPR once a day. • Point of discharge as designated on the installation by shunting to the bottom. • DPR to analyze samples at its own discretion for toxic/hazardous substances. • Operator to carry out first post drilling seabed survey 9 months after 5 wells have been drilled. Subsequent seabed surveys shall then be carried out after a further 18 months or further 10 wells • Operator must submit to DPR details of sampling and analysis records within 2 weeks of completion of any well. • Inspection of operations shall be allowed at all reasonable times.	• SBM must be recovered, reconditioned, and recycled. • SBM cuttings must Contain 5% drilling fluid or less for discharge. (10% for esters) • Special provision for higher retention limits have been granted for some deepwater wells	• Operator to carry out first post drilling seabed survey after 9 months or after 5 wells have been drilled, whichever is shorter. Subsequent seabed surveys shall then be carried out after a further 18 months or 10 wells.

(Source: Onwukwe and Nwakaudu 2012)

2.11.3 Treatment of drill cuttings

In view of recent restrictions on the discharge of drill cuttings at sea, petroleum companies have resorted to onshore treatment and disposal (Robinson *et al*., 2008). In oil producing countries, where there is weak enforcement of environmental regulations, drill cuttings are usually disposed on land without prior treatment which results in destruction of soil ecosystems. An example of this is in Nigeria, where despite regulation stipulating zero discharge of cuttings contaminated with water/oil based muds and /or esters in inland and near shore areas, oil and gas drilling wastes are occasionally disposed on land at illegal oil waste dumpsites in the Niger Delta region (EGASPIN, 2002; UNEP, 2011). Another example is in the Liaohe oilfield of northern China where yearly disposal of 213,000 tonnes of drill cuttings resulted in 250,000 m^2 of soil pollution. (Ji *et al*., 2004). Direct disposal to landfill is no longer attractive to petroleum companies in developed countries due to scarcity of hazardous landfill sites and expensive landfill taxes. Coupled with greater emphasis on sustainability, the management of drill cuttings now also includes identifying environmentally sustainable technologies that can treat them for re-use (Al-Ansary and Al-Tabbaa 2007; Ball *et al*., 2012). A wide range of physical, chemical and biological methods are being used to treat drill cuttings prior to re-use or disposal in order to remove the oil content and reduce the leachability of other contaminants.

2.11.4 Physical treatment methods

Common physical treatment methods include on-site and off-site burial and re-injection, and thermal treatment, which are disposal management methods for drill cuttings (Ball *et al*., 2012). Pit burial was the most common on-sight disposal technique, where the liquid contained in the drill cuttings are allowed to evaporate before burial in the same reserve pit used for collection and temporary storage of waste fluid. (Ball *et al*., 2012). However, on-site pit burial is considered unsuitable to the environment because of the presence of harmful components such as waste oil, salt, biologically available metals and other toxic components that could migrate from the pit and contaminate usable water resources. Also, the waste content usually results in anaerobic conditions that limit further degradation. A common off-site burial method is to send drill cuttings to a landfill that has bottom liner overlaid with a geological barrier to prevent soil contamination and a top liner which is drawn over the waste during non-active periods (Cripps *et al*., 1998). Landfills usually have two collection pits that collect rain water and subsequent leachate. Pit or landfill burial

methods are generally most suitable for WBF drill cuttings because of their low hydrocarbons and salt content (Morillon *et al*., 2002; Veil and Dusseault, 2003).

Re-injection method was one of the management techniques for drill cuttings developed by the oil and gas industry as a result of increasing tight regulations and environmental control. In this method, the drill cuttings are blended with waste drilling mud and water to create slurry which is then injected into the core of permeable sub-surface formations far below fresh water aquifers where it is likely to remain for the indefinite future (Figure 2.9). In offshore operation sea water is used for preparing the slurry. (Veil and Dusseault, 2003; Saasen, 2003; Sørheim *et al*., 2007; Onwukwe and Nwakaudu, 2012).

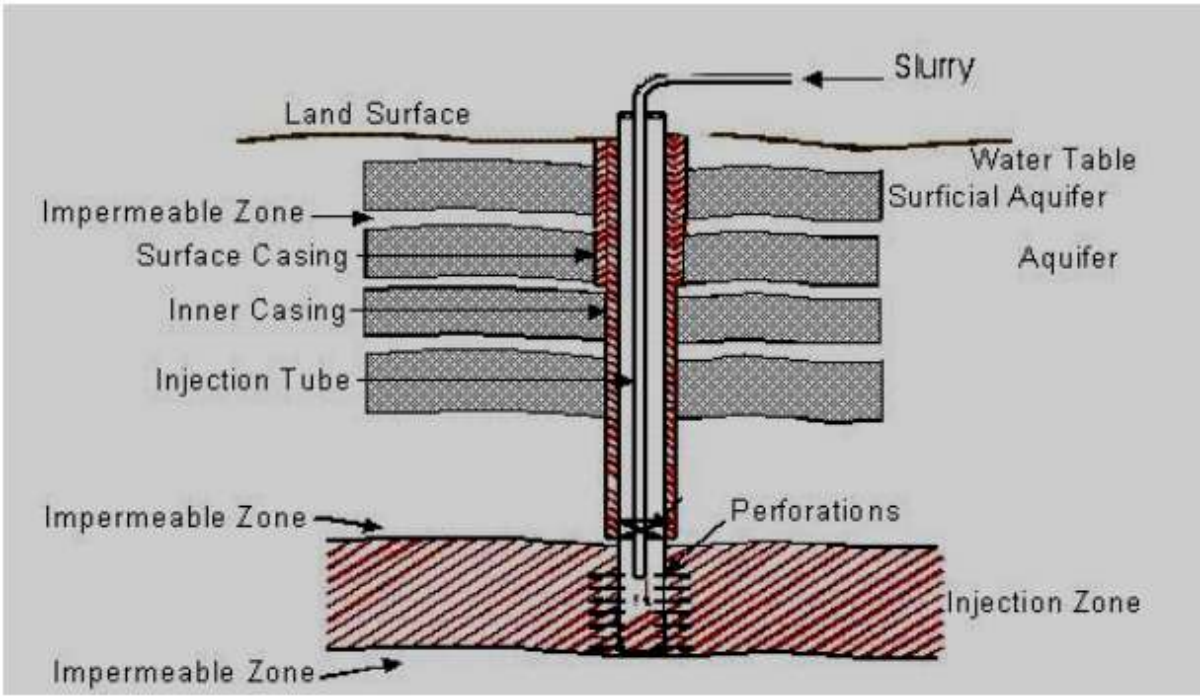


Fig. 2.9: Deep-well injection of drilling waste (Source:Onwukwe and Nwakaudu, 2012)

This method had been used successfully in Shell's Brent field in 1994, in the North Sea and in the Norwegian sector of the North Sea where leakage of five re-injection operations have been reported. In many cases, re-injection has not been practical due to limited appropriate geological setting (i.e. type of rock formation) and as a result the cuttings have to be treated onshore (Ball *et al*., 2012).

The use of thermal treatment for drill cuttings is usually done in combination with other treatment methods, either as a predisposal step or as a final treatment

following other pre-treatment (Ball *et al*., 2012). Incineration, microwaving and thermal desorption are the major thermal treatment techniques applied in the management of drill cuttings (Robinson *et al*., 2008; Ball *et al*., 2012). Most incineration of drill cuttings are carried out in rotary kilns in which the waste is tumbled to allow contact with hot burner gas to heat it up to temperatures of 820 to 1600 ^{o}C and reduce the contaminant to an inert residue. Some shortcomings of incineration treatment of drill cuttings have been, a possible increase in concentration of heavy metals in treated residue and exhaust gas may have to be treated to remove particulate and harmful combustion products such as sulfur dioxide, nitrogen oxide and hydrogen chloride (Ball *et al*., 2012; Onwukwe and Nwakaudu, 2012). In microwave treatment, instantaneous heat energy is delivered directly to all individual elements of the treated material through molecular interaction with electromagnetic fields whereby achieving heating time of less than 1% of those required using conventional heating methods.

Studies have reported that during microwave treatment of oil-based drill cuttings, the oil does not get heated directly because its dielectric loss factor is very low. It is the water that actually heats up and vaporizes due to its very high dielectric factor. The heat which evaporates the oil is supplied by the escaping steam which also poses entrained liquid-phase oil (Shang *et al*., 2005; Robinson *et al*., 2008). The major aim of microwave treatment of oil-based drill cutting is therefore to facilitate significant evaporation and subsequent recovery of the oil content such that the residual oil in the treated matrix could be less than 1%. When this is achieved, the microwave treatment process can be retrofitted to existing production platforms (Robinson *et al*., 2009). Generally, thermal treatment methods are associated with potential high cost and extensive energy dependence (Al-Ansary and Al-Tabbaa 2007).

2.11.5 Chemical treatment methods

Solidification and stabilization are two main treatment methods involving physical and chemical processes of immobilizing contaminants which are applied to drill cuttings with the objective of transforming them to less hazardous materials with possible reuse value. Solidification involves encapsulation of waste material into a durable monolithic solid with high structural integrity, while stabilization involves the techniques of reducing the hazard potential of a waste by converting the contaminants into least toxic and /or soluble form. Both techniques are frequently

applied together to change both the physical and chemical structure of the treated waste, to ensure containment of the contaminants in the matrix even if the monolith deteriorates (Al-Ansary and Al-Tabbaa 2007; Opete *et al*., 2010; Leonard and Stegemann, 2010; Ball *et al*., 2012). The most frequently used solidification/stabilization additives for treatment of drill cuttings include fly ash, cement, lime and calcium oxide (Ball *et al*., 2012), while testing of the resulting materials for use as sub-base material in road construction or as building material has been reported with varying degrees of successes (Tuncan *et al*., 2000; Morillon *et al*., 2002). However, the major objective of applying solidification/stabilization treatment to drill cuttings has not been fully realized due to incompatibility with organic compounds which inhibit binder hydration and are not chemically bound in binder hydration products (Trussell and Spence, 1994; Ball *et al*., 2012). Drill cuttings have been reported to significantly decrease the strength of solidification/stabilization products resulting in oil leachate concentration greater than 1% which is above the limit of the UK landfill acceptance criteria value for hazardous waste (Trussell and Spence, 1994; Al-Ansary and Al-Tabbaa 2007).

2.11.6 Biological treatment methods

Generally, drill cuttings are biologically impoverished, poorly sorted and weakly cohesive oil-rich silts with variable amounts of clay particles (Black *et al*., 2002). Several bioremediation strategies have been applied to optimize biological conditions that could promote the microbial degradation of toxic pollutants in drill cuttings. The most commonly used biological treating methods for drill cuttings include land farming, vermiculture, phytodegradation and composting (Vidali, 2001; Ji *et al*., 2004; Ball *et al*., 2012). In land farming techniques, the drill cuttings are spread on the land and then worked into the soil in order to allow the indigenous soil microbial community to break down the waste constituents by natural attenuation. Drill cuttings treatment by land farming usually involves controlled and repeated application to the top 10-30 cm of soil surface, periodically mixed by tilling to facilitate aeration and kept moist by irrigation. Soil nutrients may be supplemented by the addition of fertilizer, compost or manure to support the growth and activate the naturally occurring soil micro-organisms to mineralize the contaminating hydrocarbons as well as to promote dilution and potential attenuation of metals, and the transformation and assimilation of other constituents of drilling wastes (Juwarkar *et al*., 2010; Ball *et al*., 2012; Onwukwe and Nwakaudu, 2012).

Land farming has the advantage of being a relatively low-cost management approach for drilling wastes. However, the possible danger of ground water contamination by leachate percolating through the vadose zone or vaporize to dangerous levels in the atmosphere could pose environmental concerns, whereby requiring the implementation of additional controls. Additionally, repeated applications can result in the accumulation of high molecular weight hydrocarbons (Callahan *et al*., 2002; Ward *et al*., 2003). The biological processes in land spreading are similar to that of land farming, but the treatment methods differ in that, the drilling waste is spread on across the land surface in a single application in land spreading and the soil is tilled to a depth of 15–20 cm (Ball *et al*., 2012).

The use of vermiculture for active biodegradation of drill cuttings has not been extensively reported in literature. However, Ball *et al*. (2012) reported that earth worms were capable of facilitating the degradation of hydrocarbon contaminants in drilling waste following land farming. In that experiment, the authors reported a decrease in hydrocarbon concentrations from 4600 mg kg^{-1} to below 100 mg kg^{-1} in less than 28 days. Sørheim *et al*. (2007) also reported results of several laboratory and field experiments where two types of typical North Sea oil-based drill cuttings were prepared and then treated by vermiculture composting. After the treatment, decomposition of hydrocarbon components in the oily wet cuttings by vermicomposting was successfully accomplished. Treated composts were also found to impart considerable fertilizer effects on ryegrass and trees comparable to commercial NPK fertilizers.

Similar to vermiculture, the application of phytoremediation techniques to the treatment of drill cuttings has not been extensively reported. One report studied the effectiveness of a mature reed wetland in the biodegradation of extra heavy oil contaminated drill cuttings (Ji *et al*., 2004). After an in situ pilot treatment study for 2 years, only 4.2% of the initial hydrocarbons residual was retained in the surface soil while the soil property at deeper layer and reed quality indices were improved. The results showed that effective degradation of the extra heavy oil in drill cuttings is achievable with the reed wetland treatment system without extra fertilization.

Composting has been examined as a bioremediation strategy in the treatment of drilling muds and contaminated cuttings by Rojas-Avelizapa *et al*. (2007) and Okparanma *et al*. (2011). A field-scale composting of drilling mud and contaminated soil in biopiles was performed with organic bulking agent, urea and dipotassium phosphate to adjust the nutrient conditions for optimal microbial activity (Rojas-Avelizapa *et al*., 2007). After 180 days of treatment, total petroleum hydrocarbon (TPH) concentration decreased by 94% from 99300 ± 23000 mg $TPHkg^{-1}$ soil to 5500 ± 770 mg $TPHkg^{-1}$ for the amended biopiles while for the unamended biopile, 77% reduction of TPH to 22900 ± 7800 mg $TPHkg^{-1}$ was recorded. They therefore suggested that the addition of organic amendment, nutrient and water are essential for stimulation aerobic microbial activities for the degradation of TPH in the composting of drill cuttings.

A laboratory-scale composting treatment of oil-based drill cuttings was performed to examine the effects of varying masses of spent mushroom (*Pleurotus ostreatus)* substrate on PAHs in drill cuttings (Okparanma *et al*., 2011). The overall degradation of PAHs increased to between 80.25 and 92.38% with increasing substrate addition over a period of 56 days of treatment, while individual PAH degradation ranged from 97-98%. These results showed the suitability of spent white-rot fungi (*Pleurotus ostreatus*) substrate as compost amendment for biotreating PAH-contaminated oil-based drill cuttings.

In this present study, a commercial blend of several spent mushroom composts, straw and grass cuttings were used as organic compost amendments without the addition of nutrient. One of the objectives of this study is to explore and optimize a cheap composting technique for treating oil-based drill cuttings to a less hazardous and less toxic residue with possible reusable value as a planting medium.

Chapter 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Drill cuttings

The drill cuttings used were from a North Sea offshore well and comprised of dark grey, fine grained (<62µm) mudrock, coated in low toxicity, low aromatic mineral oil-based drilling mud (Baker-Hughes Inteq 'Carbosea'). The drill cutting samples were freeze-dried before being used for the relevant physico-chemical analyses.

3.1.2 Soil

The soils used for the experiments were from two different agricultural organic crop fields from farms managed by Newcastle University. The soil sample used for the laboratory-scale experiment was from Nafferton Farm, Stocksfield, Northumberland, while that used for the pilot-scale, outdoor compost experiment was from Cockle Park Farm, Ulgham, near Morpeth, Northumberland.

Geochemical analysis conducted on the respective soil samples indicated no history of major hydrocarbon contamination. Soil samples were sorted to remove leaves, roots and stones, ground down using a pestle and mortar, air-dried in a ventilated room and then passed through 2 mm aperture sieve before use for the experiments. The sub-samples used for relevant physico-chemical analysis were further crushed with mortar and pestle and passed through 1 mm aperture sieve.

3.1.3 Grass Clippings

The grass clippings used were obtained from domestic lawns and gardens within the Newcastle University after cutting with domestic lawnmower. Collected grass clippings were used while still fresh as part of the compost feedstock. The sample used for physico-chemical analysis was freeze-dried and then milled with freezer mill.

3.1.4 Coal Tar

The coal tar used as source of hydrocarbon pollutants for spiking the agricultural soil was a viscous un-weathered sample obtained from Monckton Coke & Chemical Co. Ltd, Barnsley, South Yorkshire.

3.1.5 Straw

The straw sample used for the laboratory-scale composting experiment was purchased from a pet shop, supplied by H. Eggleston Jnr & Son Ltd, Lanchester, Durham. It was milled to 2 mm particle size before use. The sample used for the field-scale experiment was obtained from the Cockle Park Farm in Ulgham Morpeth, Northumberland. Straw in bale was initially shredded with an electric garden shredder (BOSCH AXT 25 TC, HSS Hire) before grinding with CORONA Corn Mill (Landers, Medellin-Colombia) to achieve ~ 6 mm particle size. The straw sample was used as bulking agent in the compost mix which provides carbon required for effective microbial activity as well as stability and aeration to prevent collapse of the compost matrix. The straw sample used for physico-chemical analysis was taken from the milled-stock and then freeze-dried before milling to powder in a model 6750-230 SPEX Sample Prep freezer/mill.

3.1.6 Spent mushroom compost

As a part of the ingredients for the compost feedstock, the spent mushroom compost sample used for this experiment was supplied by Mr Muck's Garden Supplies Ltd (Doncaster, South Yorkshire). During preparation, the spent mushroom sample was sorted to remove lumps of gypsum particle which was added to serve as casing material during mushroom cultivation. Sorted sample was subsequently air-dried and passed through a 2 mm sieve before use for the laboratory-scale and outdoor pilot-scale composting experiments, respectively. The fraction of the sieved sample used for the physico-chemical analysis was freeze-dried and then milled with the freezer mill.

3.1.7 Garden compost

The garden compost used as control for the seed germination and growth experiment was Gro-Sure All-purpose Compost (Westland Horticulture Limited, UK) purchased from the garden section of a retail store (Wilkinson, Newcastle upon Tyne).

3.1.8 Solvents

Organic solvents supplied by Leading Solvent Supplies, Leeds, UK, were petroleum ether, methanol, and dichloromethane (DCM) of reagent grade. Each solvent was redistilled in a 30-plate Oldershaw distillation column into winchester bottles and then

stored in solvent storage cupboard until use. Analytical grade toluene was used for phosphatase enzyme assay was purchased from Fisher Scientific, UK.

3.1.9 Adsorbents and extraction thimbles and cotton wool

Aluminum oxide (alumina) and silica 60A (chromatography grade of particle size 70-200 micron) were supplied by Merck, UK, while cellulose extraction thimbles (Ø41 mm x 123 mm x 1 mm thickness) and cotton wool were purchased from VWR, BDH, UK.

They were pre-cleaned by Soxhlet extraction using 450 mL DCM: methanol (93:7, v/v) for a minimum of 16 hours and placed in fume cupboard overnight to dry. The dried alumina and silica were transferred into clean glass containers and activated in the oven at 120 ^{o}C for a minimum of 6 hours. The cotton wool was stored in clean glass bottles while the thimbles were wrapped in aluminium foil and kept in clean thimble boxes.

3.1.10 Copper tunings

The copper tunings used were of general purpose grade supplied by Fisher Scientific, UK. They were activated before use by adding 30 mL of 2M hydrochloric acid into a 100 mL conical flask containing the copper tunings. The flask was allowed to stand for 3 min before swirling for 2 min and the acid was poured into waste acid container. The copper tunings were then washed six times with deionised water to remove the acid, then methanol to remove water and finally DCM to remove methanol.

3.1.11 Analytical grade reagents

Sodium sulphate anhydrous (granular), calcium chloride dihydrate and 4-nitrophenol were supplied by Fisher Scientific, UK. Boric acid, DL-Malic acid, citric acid, sodium hydroxide, ammonium molybdate, ammonium metavanadate, potassium dihydrogen orthophosphate and standard buffer solutions (pH 4, 7 and 10) were purchased from VWR, BDH, UK. pNPP disodium salt hexahydrate (phosphatase substrate) was supplied by Sigma-Aldrich, UK.

Additional laboratory reagents used for artificially contaminating (spiking) soil for phytotoxicity experiments were: naphthalene (Sigma Chemical, USA); phenanthrene (VWR, BDH, UK); anthracene (Fluka Chemika); pyrene (Opkin and Williams, UK) and

perylene (Lancaster Synthesis, UK). The certified reference material (CRM) used for the phosphorus analysis was IPE sample 111, bought from WEPAL, Netherlands.

3.1.12 Standards

Surrogate standards used for analyzing the recovery of saturated and aromatic hydrocarbon analytes were squalane (Fluka Chemika) and 1,1'-binaphthyl (Kodak, UK), while *n*-heptadecylcyclohexane (ICN Ltd, UK) and *p*-terphenyl (Fisher Scientific, UK) were used as internal standards respectively. A semivolatile internal standard mix (deuterated standards) comprising acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12 and phenanthrene-10 in dichloromethane was bought from Supelco, Sigma-Aldrich, UK. It was used as internal standard for the quantification of aromatic hydrocarbon analytes. Sulfadiazine (minimum 99.0% purity) used as the calibration standard in CNS analysis was purchased from Sigma-Aldrich, UK.

3.1.13 Apparatus and instruments

The experimental apparatuses and analytical instruments used in the course of this project are listed as follows:

Apparatus	**Description**
Autoclave	SENTINAL by RODWELL scientific Instruments, UK
Centrifuge	SORVAL RC-5B Plus Superspeed Centrifuge by Kendro laboratory Products USA and Eppendorf centrifuge 5810, supplied by Scientific laboratory Supplies
CNS analyzer	Vario Max CNS Analyser, by Elementar Analysensytem GmbH, Germany
Compost thermometer	TFA Compost Thermometer by TFA Dostmann GmbH, Germany.
Compost tumbler	DRAPER 180L Compost Tumbler by Draper Tools Limited, UK
Conductivity meter	HANNA model HI9835 Microprocessor Conductivity/TDS meter
Digital Calliper	Model Z22855 Powerfix electronic digital calliper, Version 10/2013, by Owim GMbH and Co. KG
Electric oven	Model 400 by Memmert, Germany
Freeze dryer	Modulyod-230 from Thermo Electron Corporation, USA
Freezer mill	Model 6750-230 Freezer/mill by SPAX Sample Prep LLC, USA

Fume cupboard	Model FWF 20 by Waldner Laboreinrichtungen, Germany
Fume hood	Supplied by S+B UK Limited
Garden thermometer	Wilko Large Garden Thermometer, by Wilkinson, UK.
Heating mantle	Supplied by Thermo Scientific
Incubators	E-class Max Q400 shaker incubator, by Bamstead lab Line and Binder BF53 incubator with forced convection by Binder Germany
Laboratory dish washer	Miele G7783CD, supplied by Scientific Instrument Centre Limited, UK
Laboratory fridge and freezer	BioCold laboratory fridge and BioCold spark free laboratory freezer, supplied by Scientific laboratory Supplies
Mechanical shaker	Janke and Runkel model KS 500 orbital shaker, supplied by Sartorius Limited, UK
pH meter	JENWAY model 3020 pH meter supplied by S.H Scientific, UK
Rotary evaporator	Heidolph laboratory 4003, fitted with Heidolph Rotavac vario pump supplied by Scientific laboratory Supplies, UK
UV-spectrophotometer	Genesys 10S UV-VIS Spectrophotometer supplied by Thermo Fischer Scientific
Weighing balance	Salter HA-180M by A & D Company limited, Japan and Mettler PE11 by Mettler Instrument limited, Switzerland

Other apparatus and their respective methods of use are described in the appropriate sections below. All glassware was washed at 80 ^{o}C, rinsed with deionized water, oven dried and then rinsed with dichloromethane before use.

3.2 Methods: Determination of physico-chemical properties

3.2.1 Moisture content

The ISO (1993) method was adopted for determining the moisture content of the respective samples of each compost feedstock. In this gravimetric method, the tare weight (w_1) of the sample container was measured in a balance and recorded. The soil sample (~30 g) was then placed in the container, weighed and recorded as wet weight (w_2). The container with sample was placed in Memmert 400 electric oven set at 105 ^{o}C to dry for 24 hours. Dried soil sample with container was allowed to cool and equilibrate with room temperature before weighing to obtain the dry weight of the soil (w_3). The following formula was used to compute the moisture content of triplicate measurements of each sample:

$$Moisture\ content = \frac{(w_2 - w_3)}{w_3 - w_1} \times 100\ \% \qquad equation\ 3.1$$

The moisture content of compost mixtures was required to calculate the additional amount of water needed to be added to optimize moisture content. The mixture moisture content, which depends on the moisture content and weight of the constituent compost ingredients, was calculated using the following formula (Richard, 2000):

$$G = \frac{(Q_1 x M_1) + (Q_2 x M_2) + (Q_3 x M_3) + \cdots}{Q_1 + Q_2 + Q_3 + \cdots} \qquad equation\ 3.2$$

In which:

G = Mix moisture content (%)

Q_n = mass of material n ("as is", or "wet weight")

M_n = moisture content (%) of material n

3.2.2 Total organic carbon and nitrogen content

Every component of the different compost feedstocks were analyzed for total carbon and nitrogen content, respectively, using an Elementar Vario MAX CNS analyser. During the analysis, samples are combusted at 1145 ^{o}C in an oxygen atmosphere resulting in oxidation reaction which occurs in sequential order beginning with N → NO_2, then C → CO_2 and S → SO_2. Each reaction process product was adsorbed onto their respective sorbents and then desorbed sequentially by heating at 180-250 ^{o}C. The abundance of the different elements were recorded as peaks by the thermal conductivity detector. During the setup, sulfadiazine (N = 22.37%; C = 47.99%; S = 12.81%) which was used as calibration standard, was inserted in triplicate after 2 blanks to warm up the instrument. These were followed by inserting 2 blanks before the samples in triplicates. The calibration standard was inserted after every 5-10 samples and at the end of the samples. An aliquot dry sample of (100 mg) for soil and drill cuttings, and (10 g) for grass clippings, straw and spent mushroom compost, respectively, was weighed accurately (±1 mg) into ceramic crucibles for analysis. Raw data was corrected for analytical drift during the analysis using the Elementar software.

3.2.3 pH

The pH measurements of the respective compost ingredients were conducted according to the BS ISO (2005) protocol. In this procedure, an aliquot (5 mL) of sample was measured into a 60 mL plastic bottle to which 25 mL de-ionized water was added and stoppered. The bottle was placed in a horizontal position on Jankel and Runkel KS 500 orbital shaker and then shaken for 1 hour at a speed of 275 ± 10 revs per minute. The pH of the sample-water suspension was then measured after allowing to equilibrate for 2 hours using the probe of JENWAY Model 3020 pH meter which had been calibrated using standard buffer solutions. Triplicate measurements were performed for this analysis.

3.2.4 Salinity

The salinity of the respective compost ingredients was measures as specific electrical conductivity according to the protocol of BS ISO (1994) and then converted to salinity using relevant relationship table. In this procedure, an aliquot (5 g) of the sample was weighed into 60 mL plastic bottle to which was added 25 mL of de-ionized water. Blanks comprising 25 mL de-ionized water in 60 mL plastic bottles were also prepared. Bottles were stoppered, placed in a horizontal position on the Janke and Runkel KS 500 orbital shaker and then shaken for 30 minutes at a speed of 275 ± 10 revs per minute. The sample-water suspension was filtered through number 42 Whatman filter paper into universal tubes and then heated to 25 ^{o}C in a water bath for 1 hour. Electrical conductivity of the heated sample suspension was measured with a calibrated HANNA model HI 9835 Microprocessor Conductivity/TDS meter. Respective samples and blanks were prepared and measured in triplicate. The specific electrical conductivity was calculated as follows:

$$Specific\ electrical\ conductivity\ (\%) = \frac{(E_s - E_b)}{10} \qquad equation\ 3.3$$

Where,

E_s is the measured Conductivity of the sample, $\left(^{\mu S}/_{cm}\right)$

E_b is the measured Conductivity of the blank, $\left(^{\mu S}/_{cm}\right)$

3.2.5 Phosphorus content

The sample solution for phosphorus content measurement was prepared by dry combustion followed by acid digestion. Approximately 2 g of each sample and the certified reference material (International Plant-Analytical Exchange reference material, IPE sample 111) was measured into furnace crucibles in triplicate, and combusted in a furnace at 500 ^{o}C for 24 h with a blank control sample. Ten millilitres of 6M hydrochloric acid (HCl) was added to each sample after cooling and covered with watch glass, taking care that losses due to effervescence do not occur. The watch glass was then removed and rinsed with distilled water. The washings were collecting into respective crucibles. Crucibles were placed on a water bath where the solution was evaporated to dryness by heating at 102 ^{o}C for 1 h. The residue in crucibles were moistened with 2 mL of 36% *m/m* HCl, covered with respective watch glass and allowed to gently boil for 2 min. 10 mL distilled water was added and allowed to boil for another 2 min after which the contents of each crucible and washings of respective watch glass were quantitatively transferred and filtered (110 mm Whatman No. 541) into 50 mL volumetric flasks and then diluted to 50 mL.

Stock reagent solutions comprising 5% m/v ammonium molybdate and 0.25% m/v ammonium metavanadate, respectively, were prepared. 25 g ammonium molybdate and 1.25 g ammonium metavanadate were added to 300 ml distilled water in respective 500 ml volumetric flasks, warmed to dissolve and diluted to 500 ml. An approximately 1 mg/g phosphorus stock standard solution was also prepared. Potassium dihydrogen orthophosphate was dried at 102 ^{o}C for 1 h and allowed to cool in desiccator. The dried salt (0.879 g) was dissolved in deionized water in a 200 ml volumetric flask, to which was added with 1 mL HCl (36% m/m) before diluting to 200 ml. One drop of toluene was added to the solution. The reagents and phosphorus working standard solutions were prepared on the day of use.

Aliquots of 0, 5, 10, 15, 20 and 25 mL of the diluted standard solution were pipetted into separate 50 mL volumetric flasks. 2 mL of the sample solutions were each pipetted into respective 50 mL volumetric flask. To each of the flasks was added 5 mL of 5 M HCl, 5 mL of ammonium molybdate and 5 mL of ammonium metavanadate reagents. The content of the flasks were diluted to 50 mL and allowed to stand for 30 min before measuring the absorbance of the yellow phosphatase-vanado-molybdate

spectrophotometrically (Genesis 10S UV-VIS Spectrophotometer) at 400 nm in 10 mL Fisher brand cuvettes.

Absorbance measurements from the phosphorus standard solution were used to construct calibration graph relating absorbance corresponding to 0, 100, 200, 300, 400 and 500 µg of phosphorus within the range of 0 – 0.9 absorbance units, respectively. The standard graph was used to read the amount of phosphorus equivalent to absorbance of the samples and blank determinations. Actual phosphorus concentration was determined using Equations 3.4 – 3.5 (MAFF/ADAS Refer Book 427, 1986):

$$P = \frac{P_{sample} - P_{blank}}{40x2} x \frac{2}{W_s} \qquad Equation\ 3.4$$

$$P(\%) = \frac{P}{10} \qquad Equation\ 3.5$$

Where:

P = phosphorus concentration, $^{g}/_{kg}$

P_{sample} = amount of phosphorus in sample, μg

P_{blank} = amount of phosphorus in blank, μg

W_s = sample weight, g

$P(\%)$ = phosphorus concentration, %

3.3 Laboratory composting experiment

The objectives of this experiment were to ascertain the hydrocarbon degradation performance of different compost mixes for drill cuttings and coal tar impacted soil (CTIS) in a laboratory-scale composting experiment and perform geochemical analyses on the respective compost mixes to identify a compost mix type that would be most effective in petroleum hydrocarbon degradation. The compost amendments comprised of straw, grass clippings and spent mushroom compost (SMC). Different mix ratios were formulated based on the initial content of composting parameters in each of the constituent compost ingredients. The composting parameters were nitrogen, carbon, moisture and phosphorus content, as well as pH and salinity levels.

3.3.1 Experiment design

A total of 60 compost samples were prepared separately with the drill cuttings and CTIS, for pre-composting and post-composting geochemical and toxicity analysis, respectively. The experimental design for the respective contaminated media are presented in Figures 3.1 and 3.2, respectively. Triplicate samples of each mix type were prepared for 0 and 53 days of composting duration, respectively. Experimental controls were set up using the drill cuttings and CTIS, respectively, without compost amendments.

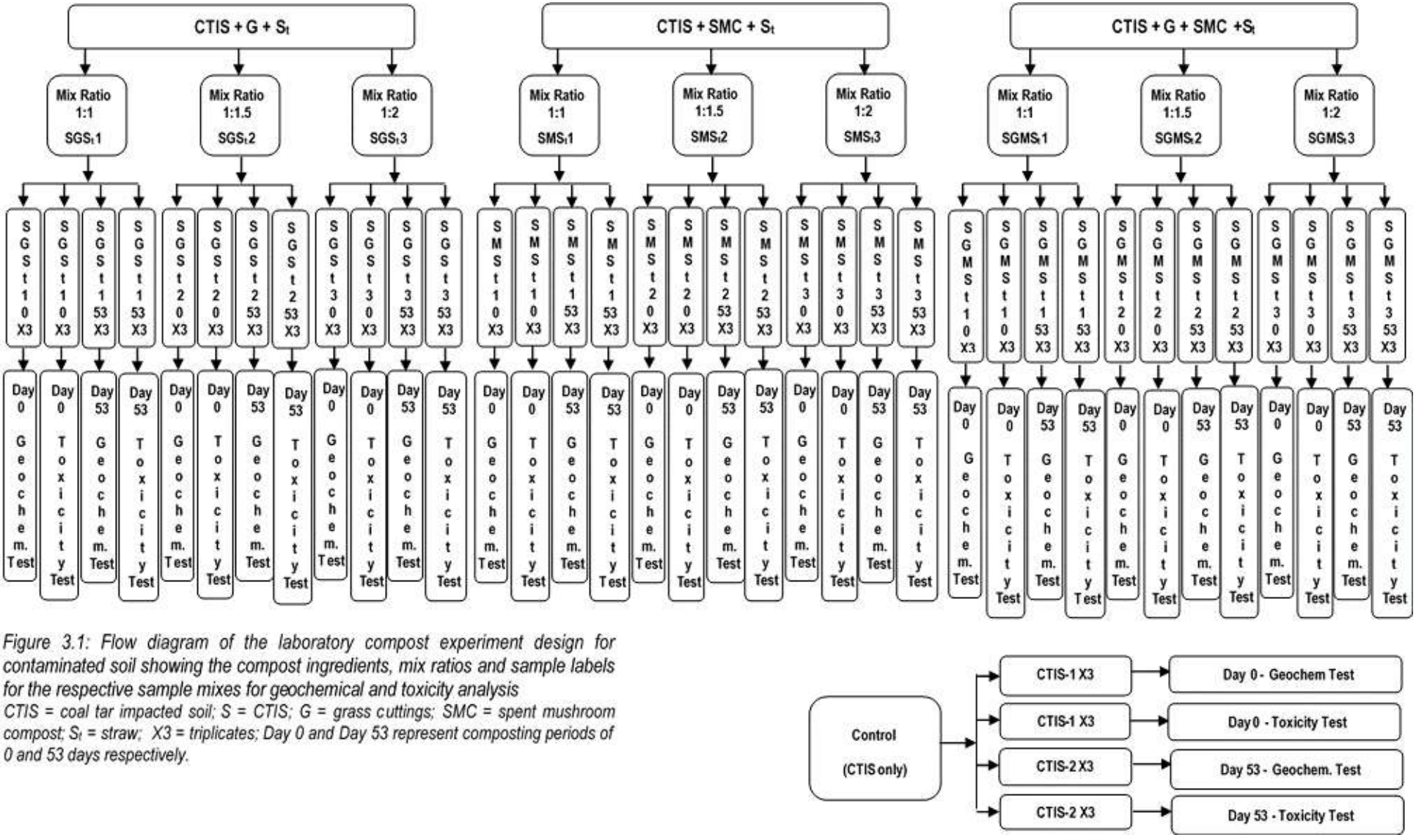
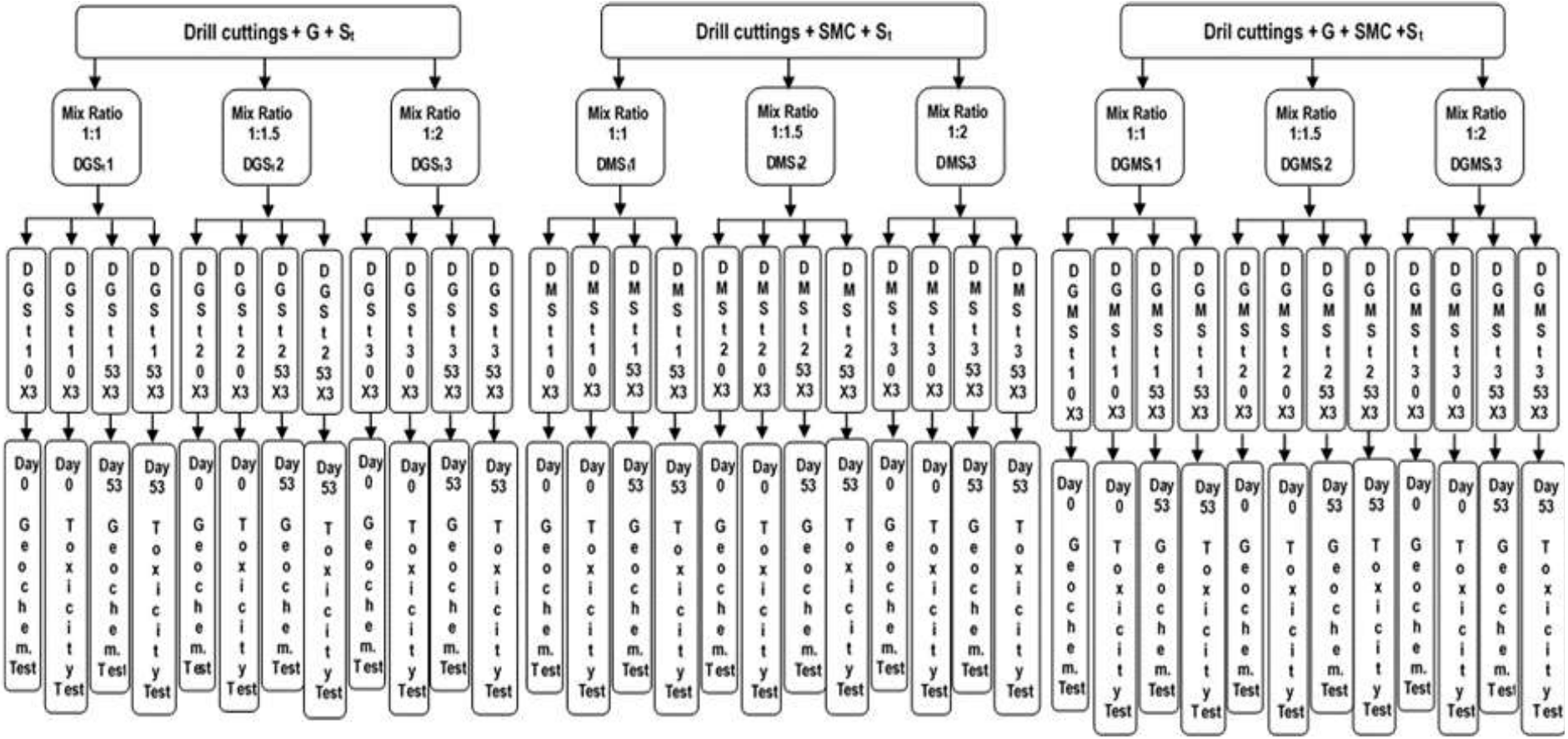


Figure 3.1: Flow diagram of the laboratory compost experiment design for contaminated soil showing the compost ingredients, mix ratios and sample labels for the respective sample mixes for geochemical and toxicity analysis

CTIS = coal tar impacted soil; S = CTIS; G = grass cuttings; SMC = spent mushroom compost; S_t = straw; X3 = triplicates; Day 0 and Day 53 represent composting periods of 0 and 53 days respectively.



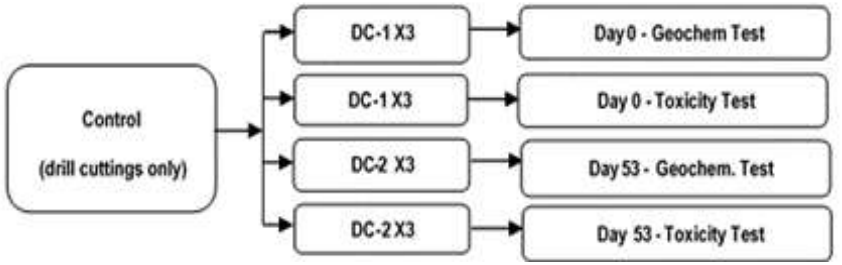


Figure 3.2: Flow diagram of the laboratory compost experiment design for drill cuttings showing the compost ingredients, mix ratios and sample labels for the respective sample mixes for geochemical and toxicity analysis.
D = drill cuttings; G = green waste; SMC= spent mushroom compost; S_t = straw; X3 = triplicates; Day0 and Day 53 represent composting duration of 0 and 53 days respectively.

3.3.2 Compost mix formulation

In the preparation of the compost mixes, the overall carbon - nitrogen (C:N) ratio was a major consideration and was based on the amount of carbon and nitrogen in the respective compost ingredients. A wide range of C:N ratios have been reported, e.g., 9-200, for effective compost-bioremediation of hydrocarbon contaminated media (Huesemann, 1994; Roldan et al., 2003). In this study, a C:N ratio of 28-31 was adopted. The compost mixtures were prepared for contaminant to amendment ratios of 1:1, 1:1.5 and 1:2, respectively. The number of compost ingredients in the different mixes varied from 3 to 4. The amount of the respective ingredients in the mixes required to achieve the target range of C:N ratio was calculated using the following formula (Richard, 2000):

$$R = \frac{Q_1C_1(100-M_1)+Q_2C_2(100-M_2)+Q_3C_3(100-M_3)+\cdots}{Q_1N_1(100-M_1)+Q_2N_2(100-M_2)+Q_3N_3(100-M_3)+\cdots} \qquad Equation\ 3.6$$

Where:

R = C:N ratio of compost mixture.

Q_n = weight of ingredient n.

$C_{n,}$= Carbon (%) of ingredient n.

N_n = nitrogen (%) of ingredient n.

M_n = moisture content (%) of material n.

3.3.3 Preparation of pollutant hydrocarbon-spiked soil

The CTIS sample was prepared by artificially spiking air-dried agricultural soil with coal tar. Approximately 0.02% coal tar contaminated soil samples were used for the lab-scale compost experiment. Gravimetric analysis was first carried out on the non-viscous coal tar to ascertain the mass density. Approximately 10.5 mg coal tar was diluted to 10 mL with dichloromethane. 1 mL solution was pipetted into a pre-weighed vial in triplicates and then evaporated under gentle stream of nitrogen gas to dryness. When constant weight of each vial was achieved, the dry weight of the 1 ml coal tar solution was quantified and found to be 1.0155 ±0.0002 mg. The coal tar sample was then estimated to contain 96.7% dry weight. Based on the gravimetric analysis, ~ 269 mg coal tar was dissolved to 20 mL in DCM before using it to spike 30 g of the

dry soil on a large watch glass (200 mm diameter). The wet coal tar-spiked soil was left in a fume cupboard overnight to allow for complete solvent evaporation. All the soil on the watch glass was then quantitatively transferred, made up to 1300 g with additional soil and homogenised in a stainless steel bowl.

3.3.4 Experimental procedure

Approximately 10 g of the CTIS sample was weighed into 120 x 250 mL amber bottles. The sample bottles were separated into two portions of 60, each representing pre-compost (Day 0) and post-compost (Day 53) samples. Compost ingredients were then added to the Day 0 and Day 53 samples to formulate different mix types in different ratios. A total of 9 different compost mix types comprising 3 mix ratios were prepared, for triplicate samples. Additional control samples comprising only the contaminated soil was also prepared in triplicate. A total of 30 compost mix samples were prepared separately for geochemical and toxicity analysis for Day 0 and Day 53 compost durations, respectively. For compost mixes with the drill cuttings sample, 10 g of the drill cuttings were weighed into 120 x 250 mL amber bottles and followed a similar procedure as described for CTIS samples above. Details of the constituent compost ingredients and their amount in each mix type for CTIS and drill cuttings are presented in Table 3.1 and Table 3.2, respectively.

All the Day 0 compost mix samples were stored in a freezer until needed for analysis. The control samples were sterilised at 120 ^{o}C for 1 h in an autoclave to inactivate microorganisms. Sterilised samples for Day 0 analysis were added to the stock in the freezer. The Day 53 samples, except for the Day 53 sterile control samples, were dosed with deionised water to optimise their moisture content. The moisture content was optimised to 60% and 35% for the mixes with CTIS and drill cuttings, respectively. All the day 53 samples including the controls were placed in a cupboard for incubation at room temperature (24 ^{o}C) for 10 days. The top of the sample bottles were tightly covered with aluminium foil with a few perforations to permit ventilation. The samples were re-weighed, water added to optimize moisture content and mixed thoroughly, except the sterile control samples, and then transferred to an incubator programmed to start at 26 ^{o}C.

Table 3.1: Composition of compost mixes for coal tar impacted soil experiments

Mix type	Sample codes for sub-mix types	Mix composition	Mix ratios	Mix moisture content %
1	SGS_t1	10 g soil + 3 g grass cuttings + 7 g straw	S/amendments = 1 : 1; G/S_t = 1 : 2.33	14.4
	SGS_t2	10 g soil + 5.5 g grass cuttings + 9.5 g straw	S/amendments = 1 : 1.5; G/S_t = 1 : 1.73	19.20
	SGS_t3	10 g soil + 8 g grass cuttings + 12 g straw	S/amendments = 1 : 2; G/S_t = 1 : 1.5	22.50
2	SMS_t1	10 g soil + 4 g mushroom compost + 6 g straw	S/amendments = 1 : 1; M/S_t = 1 : 1.5	16.9
	SMS_t2	10 g soil + 7 g mushroom compost + 8 g straw	S/amendments = 1 : 1.5; M/S_t = 1 : 1.14	22.20
	SMS_t3	10 g soil + 9.5g grass cuttings + 10.5 g straw	S/amendments = 1 : 2; M/S_t = 1 : 1.11	24.80
3	$SGMS_t1$	10 g soil + 1.7 g grass cuttings + 1.7 g mushroom compost + 6.6 g straw	S/amendments = 1 : 1; G/M/S_t = 1 : 1 : 3.88	10.6
	$SGMS_t2$	10 g soil + 3 g grass cuttings + 3 g mushroom compost + 9 g straw	S/amendments = 1 : 1.5; G/M/S_t = 1 : 1 : 3	13.90
	$SGMS_t3$	10 g soil + 4.5 g grass cuttings + 4.5 g mushroom compost + 11 g straw	S/amendments = 1 : 2; G/M/S_t = 1 : 1 : 2.44	16.70
4	Control	10g CTIS	-	1.7

*S = coal tar impacted soil (CTIS), G = grass cuttings, M = mushroom compost, S_t = straw, * = Control (soil without compost amendments)*

Table 3.2: Composition of compost mixes for drill cuttings experiments

Mix type	Sample codes for sub-mix types	Mix composition	Mix ratios	Mix moisture content %
1	DGS_t1	10 g drill cuttings + 4.5 g grass cuttings + 5.5 g straw	D/amendments = 1 : 1; G/S_t = 1 : 1.22	24
	DGS_t2	10 g drill cuttings + 7 g grass cuttings + 8 g straw	D/amendments = 1 : 1.5; G/S_t = 1 : 1.14	27.00
	DGS_t3	10 g drill cuttings + 9 g grass cuttings + 11 g straw	D/amendments = 1 : 2; G/S_t = 1 : 1.22	28.00
2	DMS_t1	10 g drill cuttings + 5.8 g mushroom compost + 4.2 g straw	D/amendments = 1 : 1; M/S_t = 1 : 0.72	22
	DMS_t2	10 g drill cuttings + 8.7 g mushroom compost + 6.3 g straw	D/amendments = 1 : 1.5; M/S_t = 1 : 0.72	26.00
	DMS_t3	10 g drill cuttings + 11.5 g mushroom compost + 8.5 g straw	D/amendments = 1 : 2; M/S_t = 1 : 0.74	28.50
3	$DGMS_t1$	10 g drill cuttings + 2.5 g grass cuttings + 2.5 g mushroom compost + 5 g straw	D/amendments = 1 : 1; $G/M/S_t$ = 1 : 1 : 2	13.4
	$DGMS_t2$	10 g drill cuttings + 4 g grass cuttings + 4 g mushroom compost + 7 g straw	D/amendments = 1 : 1.5; $G/M/S_t$ = 1 : 1 : 1.75	21.90
	$DGMS_t3$	10 g drill cuttings + 5 g grass cuttings + 5 g mushroom compost + 10 g straw	D/amendments = 1 : 2; $G/M/S_t$ = 1 : 1 : 2	23.70
4	Control	10 g drill cuttings	-	12.1

*D = drill cuttings, G = grass cuttings, M = mushroom compost, S_t = straw, * = Control (drill cuttings without compost amendments)*

The incubator temperature was increased at 2 ^{o}C /day until it reached 60 ^{0}C and kept there for 1 week before cooling down to 24 ^{0}C at the rate of 2 ^{o}C /day to end the experiment. Samples were re-weighed, water added to optimize moisture content and mixed twice a week during incubation except for the sterile control samples. Compost mixture samples with drill cuttings and coal tar contaminated soil were incubated in an E-class Max Q400 shaker incubator and a Binder BF53 incubator with forced convection, respectively (Fig 3.3).

3.4 Pilot-scale outdoor composting experiment

The outdoor composting experiment was setup with the objective of validate the best performance outcome from the lab-scale composting experiment for the contaminated soil. The compost mix which produced the best degradation of total petroleum hydrocarbon (TPH) during the lab-scale experiment was up-scaled and tested in a pilot-scale outdoor compost-bioremediation experiment in tumbler compost bins (DRAPER 180L Compost Tumbler, Draper Tools Limited, UK).

3.4.1 *Experimental procedure*

The SMS_t3 mix which produced the highest reduction of TPH during the lab-scale composting experiment was scaled-up by a factor of 600. The soil sample was spiked to achieve 0.5% coal tar contamination. 6 kg agricultural soil spiked with 30 g coal tar, 5.7 kg spent mushroom compost and 6.3 kg straw made up the outdoor compost mix. The mix was composted for 56 days in triplicates alongside 18 kg agricultural soil spiked with 90 g coal tar only, which was setup as the control (Fig. 3.4).

During the composting duration, the temperature in each compost bin was monitored using a TFA compost thermometer (TFA Dostmann, Germany) and aerated by tumbling 50 times, three times every week; subsampled one time and three times every week for geochemical and moisture content analysis, respectively, and watered when necessary. Temperature profiles were monitored from five points, including the centre of the composting stock in each bin. The compost bins were wrapped with bubble-wrap to optimize and conserve compost temperature. The ambient temperature was monitored by Wilko large garden thermometers (Wilkinson, UK) while the bins were each covered with umbrella to prevent excess watering due to rain and maintain aeration through ventilation holes.

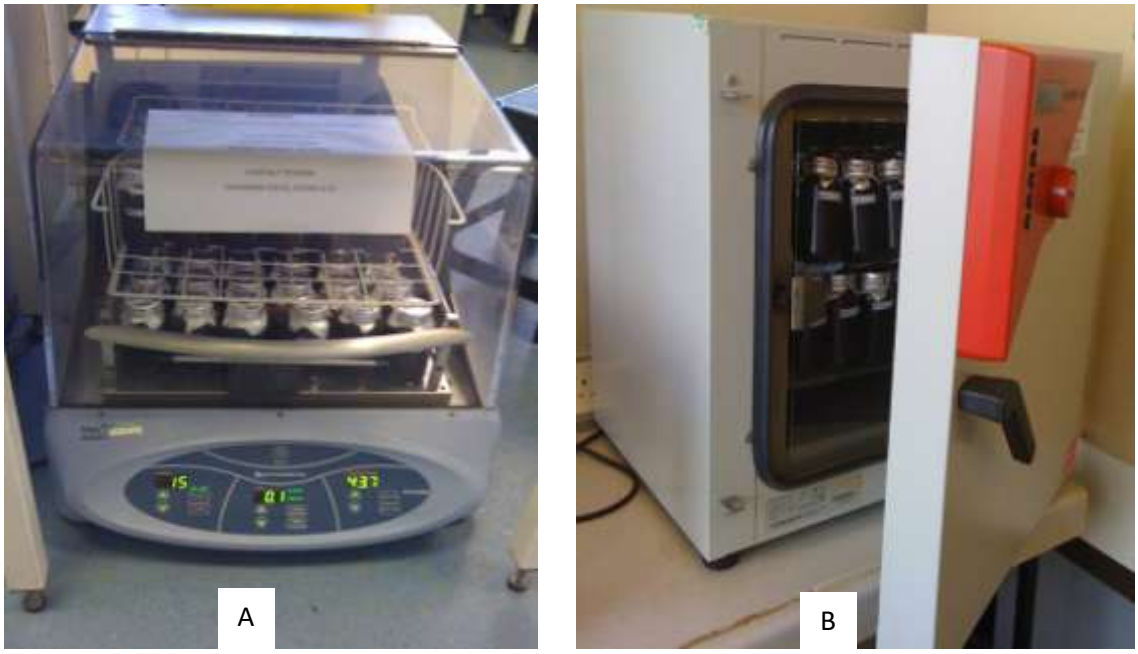


Figure 3.3: Sample bottles containing compost mixes in (A) E-class Max Q400 shaker incubator and (B) Binder BF53 incubator with forced convection

Figure 3.4: Pilot-scale outdoor compost-bioremediation experimental setup in tumbler compost bins.

3.5 Geochemical analysis

3.5.1 *Solvent extraction of organic matter using soxhlet apparatus*

Samples for geochemical analysis were freeze-dried (Modulyo d-230 freeze dryer) for a minimum of 24 hours after removal from the freezer (BioCold spark free laboratory freezer). Freeze-dried samples were transferred into pre-extracted cellulose thimbles and mixed with ~20% (w/w) anhydrous sodium sulphate and then spiked with known amounts of surrogate standards (squalane and 1,1'-binaphthyl). The tops of the thimbles were plugged with pre-cleaned cotton wool and placed in the Soxhlet extractors. The extraction solution, 450 mL of DCM:methanol (93:7, v/v), was prepared in a 500 mL round bottom flasks (RBF), to which was added ~5 g of activated copper tunings and 4 anti-bumping granules. The Soxhlet extraction apparatus was finally coupled to the condenser and extraction solvent flask and placed on the heating mantle and allowed to extract for a minimum of 16 hours.

After extraction, the extracts were concentrated to ~10 mL by rotary evaporation (Heidolph laboratory 4003 Rotavac). The sample extracts were initially concentrated to remove all solvents, 20 mL DCM added and further concentrated before making up to 10 mL in DCM. This was done in order to remove residual methanol which could interfere with the column chromatographic process. An aliquot (1 mL) of each extract was transferred to a pre-weighed 10 mL glass vial for gravimetric analysis. Based on the quantified weight of the 1 mL extractable organic matter (EOM), about 40 - 50 mg EOM weight was adsorbed onto ~5 g activated alumina for separation of total petroleum hydrocarbon (TPH) fractions by column chromatography. The 40-50 mg aliquot of EOM was been found to be an adequate amount to avoid overloading chromatography columns.

3.5.2 *Sample TPH extraction by column chromatography*

Aliquots (40 – 50 mg) of EOM of all sample extracts for geochemical analysis were eluted through silica/alumina columns to separate TPH fractions. The chromatographic columns (8 mm internal diameter by 245 mm long) were clamped vertically, plugged with small pieces of pre-cleaned cotton wool and then filled with petroleum ether. Activated silica gel (7 g) was weighed into a 20 ml glass beaker containing petroleum ether and stirred with a stainless steel spatula to form a slurry. The slurry was packed evenly in the columns with gentle tapping. Activated alumina (1 g) was subsequently added to the columns and the solvent (petroleum ether) was

reduced to just above the alumina layer. The EOM-alumina mix for each sample extract was allowed to dry and made to flow freely before adding to the columns. The TPH fraction of each sample extract was collected in a 250 mL RBF by first eluting with 70 mL petroleum ether to separate the saturated hydrocarbon fractions and then with 70 mL of 20% DCM/petroleum ether solution to separate the aromatic hydrocarbon fractions. Procedural blank columns was also run to check for contamination.

The TPH of the sample extracts were concentrated and made up to 10 mL in DCM before transferring a 1 mL aliquot into a glass autosampler vial. Known amounts of internal standards (*n*-Heptadecylcyclohexane, *p*-terphenyl and deuterated internal standard mix) were added to each GC sample vials, including the procedural blank, before analysis by GC-FID. The amount of internal standards added were same as the amount of surrogate standards present in the eluted fraction for GC analysis. The amounts of standards added are shown in Tables 3.3 – 3.5. Known amounts of each surrogate and internal standards were also added to a separate GC vial for relative response factor calculation for each batch of sample analysis.

Table 3.3: Amounts of surrogate and internal standards added to contaminated soil compost samples for Day 0 and Day 53 geochemical analysis

	Surrogate standards		Internal standards		
	Squalane	1,1'-binaphthyl	n-Heptadecylcyclohexane	p-terphenyl	Deuterated standard
Sample label	**(µg)**	**(µg)**	**(µg)**	**(µg)**	**(µg)**
SGS_t1 0A – 0C	498.84	78.46	39.80	39.80	14.39
SGS_t2 0A – 0C	483.5	78.46	29.94	28.94	14.39
SGS_t3 0A – 0C	483.5	78.46	21.71	21.71	14.39
SMS_t1 0A	496.66	78.46	47.03	22.82	14.39
SMS_t1 0B – 0C	493.51	78.46	47.03		14.39
SMS_t2 0A	493.51	78.46	39.8	19.02	14.39
SMS_t2 0B	493.51	78.46	50.79	22.84	14.39
SMS_t2 0C	493.51	78.46	39.8	19.02	14.39
SMS_t3 0Ai	493.51	78.46	25.55	11.41	14.39
SMS_t3 0B – 0C	286.03	78.46	14.47	5.70	14.39
$SGMS_t1$ 0A – 0C	286.03	78.46	21.71	9.51	14.39
$SGMS_t2$ 0A – 0C	286.03	78.46	18.09	18.09	14.39
$SGMS_t3$ 0A – 0C	287.95	78.46	14.47	5.70	14.39
S 0A – 0C (control)	290.21	79.06	86.83	36.13	10.076
SGS_t1 53A – 53C	115.18	79.06	50.10	24.12	10.076
SGS_t2 53A – 53C	112.64	79.06	50.10	24.12	10.076
SGS_t3 53A	112.64	79.06	36.74	18.55	10.076
SGS_t3 53B – 53C	110.72	79.06	36.74		10.076
SMS_t1 53A – 53C	110.72	79.06	50.1	25.97	10.076
SMS_t2 53A	110.72	79.06	46.76	24.12	10.076
SMS_t2 53B – 53C	115.96	79.06	46.76	20.41	10.076
SMS_t3 53A – 53C	115.96	79.06	43.42	20.41	10.076
$SGMS_t1$ 53A	115.96	79.06	50.1	25.97	10.076
$SGMS_t1$ 53B – 53C	114.48	79.06	50.1		10.076
$SGMS_t2$ 53A – 53C	114.48	79.06	50.1	25.97	10.076
$SGMS_t3$ 53A	114.40	79.06	46.76	25.97	10.076
$SGMS_t3$ 53B – 53C	113.02	79.06	46.76	22.26	10.076
S 53A – 53C (control)	113.02	79.06	50.1	24.12	10.076

S = soil, G = grass cuttings, M = mushroom compost, S_t = straw; 0, 7, 12, 28, 35, 42, 49 and 53 represent composting periods in days; A, B and C represent sample number

Table 3.4: Amounts of surrogate and internal standards added to drill cuttings compost samples for Day 0 and Day 53 geochemical analysis

	Surrogate standards		Internal standards	
Sample label	**Squalane (µg)**	**1,1'- binaphthyl (µg)**	**n-Heptadecylcyclohexane (µg)**	**p-terphenyl (µg)**
DGS_t1 0A – 0C	366.48	218.23	47.76	27.81
DGS_t2 0A – 0C	366.48	218.23	35.82	19.86
DGS_t3 0A – 0C	357.68	214.96	27.86	15.89
DMS_t1 0A – 0C	357.68	214.56	59.70	35.70
DMS_t2 0A – 0C	349.08	210.95	43.78	25.82
DMS_t3 0A – 0C	357.68	210.95	27.86	23.84
$DGMS_t1$ 0A – 0C	340.72	207.4	51.74	31.78
$DGMS_t2$ 0A – 0C	340.72	207.4	31.84	19.86
$DGMS_t3$ 0A – 0C	365.82	209.75	39.80	21.85
D 0A – 0C (control)	300.4	193.6	107.46	69.52
DGS_t1 53A	343.77	208.27	136.30	83.99
DGS_t1 53B – 53C	351.44	210.8	136.30	83.99
DGS_t2 53A – 53C	351.44	210.8	102.45	60.30
DGS_t3 53A – 53C	358.27	213.10	68.30	38.76
DMS_t1 53A – 53C	358.27	213.1	150.26	88.30
DMS_t2 53A – 53C	349.68	209.76	102.45	60.30
DMS_t3 53A – 53C	349.68	205.83	81.96	49.53
$DGMS_t1$ 53A – 53C	353.75	205.83	136.6	79.68
$DGMS_t2$ 53A	353.75	205.83	75.13	45.23
$DGMS_t2$ 53B – 53C	358.70	207.80	75.13	45.23
$DGMS_t3$ 53A – 53C	358.70	207.80	109.28	62.45
D 53A – 53C (control)	296.89	191.38	136.60	86.14

D = drill cuttings, G = grass cuttings, M = mushroom compost, S_t = straw; 0, 7, 12, 28, 35, 42, 49 and 53 represent composting periods in days; A, B and C represent sample number.

Table 3.5: Amounts of surrogate and internal standards added to outdoor soil compost subsamples for Day 0 and Day 56 geochemical analysis

	Surrogate standards		Internal standards		
	Squalane	1,1'-binaphthyl	n-Heptadecylcyclohexane	p-terphenyl	Deuterated standard
Sample label	**(µg)**	**(µg)**	**(µg)**	**(µg)**	**(µg)**
SMS_t3 0A – 0C	210.22	60.40	16.42	24.2	10.22
SMS_t3 7A – 7C	120.62	51.94	15.57	24.2	10.22
SMS_t3 14A – 14C	123.07	55.54	15.57	24.2	10.22
SMS_t3 21A – 21C	123.07	55.54	15.57	24.2	10.22
SMS_t3 28A	451.77	108	15.57	24.2	10.22
SMS_t3 28B – 28C	438.85		15.57		10.22
SMS_t3 35A – 35C	438.85	108	15.57	24.2	10.22
SMS_t3 42A – 42C	447.39	457.63	15.57	24.2	10.22
SMS_t3 49A – 49C	447.39	457.63	15.57	24.2	10.22
SMS_t3 56A – 56C	452.65	104.21	15.57	24.2	10.22
S 0A - 0C (control)	210.22	71.06	16.42	22.00	10.22
S 7A - 7C (control)	301.56	99.88	29.196	22.00	10.22
S 14A - 14C (control)	301.56	99.88	29.196	22.00	10.22
S 21A - 21C (control)	301.56	99.88	29.196	22.00	10.22
S 28A - 28C (control)	589.26	196.44	29.196	22.00	10.22
S 35A - 35C (control)	589.26	196.44	29.196	22.00	10.22
S 42A - 42C (control)	589.26	196.44	29.196	22.00	10.22
S 49A - 49C (control)	589.26	196.44	29.196	22.00	10.22
S 56A - 56C (control)	597.47	197.65	29.196	22.00	10.22

S = soil, G = grass cuttings, M = mushroom compost, S_t = straw; 0, 7, 12, 28, 35, 42, 49 and 56 represent composting periods in days; A, B and C represent sample number.

3.5.3 *Gas chromatography GC-FID analysis*

All the eluted sample TPH fractions were analysed by GC to check for chromatographic separation, resolution and possible contamination. Chromatogram peak areas and retention times were used for identification and quantification of aliphatic hydrocarbon fractions and TPH concentration values. Analyses were performed on a Hewlett-Packard 5890 II instrument coupled to a flame ionisation detector (FID) and a split/splitless injector. 1 µL of sample dissolved in DCM was injected by an HP7673 autosampler and separation was performed on a fused silica capillary column (30 m x 0.25 mm i.d) coated with 0.25 µm thick 5% phenylmethyl polysiloxane stationary phase, using hydrogen as the carrier gas (flows 1 mL/min, pressure of 50 kPa, slit at 30 mls/min). The GC oven temperature was programmed at 50 ^{o}C for 3 min and ramped at 4 ^{o}C /min to a final temperature of 300 ^{o}C held for 20 min. The acquired data was stored on a LabSystems Atlas laboratory data system for further processing, integration and printing.

3.5.4 *Gas Chromatography – Mass spectrometry (GC-MS) analysis*

The TPH fractions of the samples were further analysed using GC-MS to identify and quantify hydrocarbon peaks of interest. Analyses were carried out on a Hewlett-Packard (HP) 6890 instrument fitted with a split/split-less injector at 280 ^{o}C connected to a Hewlett-Packard 5973 mass selective detector (MSD) set with an electron voltage of 70ev, source temperature 230 ^{o}C, quadrupole temperature 150 ^{o}C, multiplier voltage 1800V and interface temperature 310 ^{o}C. Sample injection was performed by an HP7683 autosampler, while separation was performed on DB-35 column - fused silica capillary column (30 m x 0.25 mm i.d) coated with 0.25 µm thick 35% phenylmethyl polysiloxane stationary phase. The carrier gas used was helium and GC condition was same as described above. Fraction analysis was done in full scan mode while data acquisition was controlled using Chemstation software. Chromatogram peaks were identified and labelled after comparison of their mass spectra with those of the NIST05 library or from their relative retention times from geochemistry literature.

3.5.5 *Quantification of GC peaks (analytes) for TPH analysis*

Quantification of the GC peaks was based on the principle that the concentration of an analyte is directly proportional to its peak area and is measured in comparison to

the peak area of the internal standard. The GC peaks were quantified to estimate the TPH using equations 3.8 – 3.11:

$$RRF = \left(\frac{SS\ peak\ area}{IS\ peak\ area}\right) x \left(\frac{IS\ weight}{SS\ weight}\right) \qquad Equation\ 3.8$$

$$\%\ Recovery = \frac{SS\ peak\ area}{IS\ peak\ area} x \frac{IS\ weight}{SS\ weight} x \frac{1}{RRF} x \frac{1}{franction} \qquad Equation\ 3.9$$

$$TPH\ weight = \frac{Total\ GC\ area - Sum\ of\ standards\ peak\ areas - Total\ DCM\ area}{IS\ peak\ area} \qquad Equation\ 3.10$$

$$Corrected\ TPH\ weight = \frac{TPH\ weight}{\%\ recovery} x100 \qquad Equation\ 3.11$$

Where:

RRF = relative response factor; SS = surrogate standard; IS = internal standard

3.5.6 *Quantification of GC-MS peaks (analytes)*

The quantification of GC-MS peaks followed the same principle use for Equations 3.8 – 3.9 as stated above. Additional quantifications were done using Equations 3.12 – 3.13:

$$X\ (weight) = \frac{X\ peak\ area}{IS\ peak\ area} x \frac{IS\ weight}{fraction} \qquad Equation\ 3.12$$

$$X\ (corrected\ weight) = \frac{X\ peak\ area}{IS\ peak\ area} x \frac{IS\ weight}{fraction} x \frac{100}{\%\ recovery} \qquad Equation\ 3.13$$

Where:

X = analyte and IS = internal standard

3.6 Toxicity analysis

Compost samples were prepared separately with the contaminated soil and drill cuttings, for pre-composting and post-composting toxicity analysis, respectively. Microbial and plant bioassays were carried out to test the toxicity of the treated compost matrix with a view for prospects for its re-use. Soil phosphatase activity was measured in the laboratory-scale drill cuttings and coal tar contaminated soil composted samples. While seed germination and growth assays were carried out on the pilot-scale outdoor compost-bioremediated samples of the coal tar contaminated soil.

3.6.1 *Soil phosphatase activity assay*

In this assay, a colorimetric method was used wherein the intensity of the yellow colour formation in an alkaline solution of *p*-nitrophenol formed as a result phosphatase activity was measured at 400nm, following the method developed by Tabatabai and Bremner (1969).

3.6.2 *Preparation of reagents*

(1) Modified universal buffer (MUB)

12.1 g tris (hydroxymethyl) aminomethane, 11.6 g maleic (dicarboxylic) acid, 14.0 g citric, 6.3 g boric acid (hydrogen borate) and 19.5 g NaOH were dissolved in volumetric flask and made up to 1 L with deionised water. The stock solution was stored at 4 ^{o}C, diluted five times by volume and adjusted to pH 6.5 with 1.0 M HCl or 1.0 M NaOH before use.

(2) *p*-nitrophenyl phosphate (PNP) solution, 0.115 M

42.6811 g of disodium *p*-nitropenyl phosphate hexahydrate was dissolved in MUB in volumetric flask and solution was diluted to 1 L and stored at 4^{0} C before use.

(3) Standard *p*-nitrophenol solution

1.0 g p-nitrophenol was dissolved in deionised water and the solution was made up 1 L and stored at 4^{0} C.

(4) Sodium hydroxide, 0.5 M

20 g sodium hydroxide was dissolved in deionised water and solution was made up to 1 L in volumetric flask

(5) Calcium chloride, 0.5 M

73.5 g calcium chloride was dissolved in deionised water and the solution was diluted to 1 L in volumetric flask.

3.6.3 *Procedure*

The sample (1.0 g) was placed in a 50 mL Erlenmeyer flask, to which was added 4 mL MUB, 0.25 mL toluene and 1 mL PNP solution, and then swirled to mix the contents. The flask was stoppered and placed in an incubator at 37 ^{o}C for 1 hr. After incubation, the contents of the flask were transferred into a centrifuge tube, to which was added 0.5 M calcium chloride (1 mL) and 0.5 M sodium hydroxide (4 mL) and the mixture was centrifuged at 15000 rev/min for 30 min. The mixture supernatant was filtered through a Whatman No. 2 folded filter paper. The *p*-nitrophenol content was determined by measuring the yellow colour intensity of the filtrate with a spectrophotometer (Genesis 10S UV-VIS Spectrophotometer) at a wavelength of 400nm. Calculation of the *p*-nitrophenol concentration formed was done by reference to a calibration graph plotted with readings obtained from standards containing 0, 10, 20, 30, 40 and 50 µg of *p*-nitrophenol. Where colour intensity measurements exceeded the highest measurement from the calibration standards, an aliquot of the filtrate was diluted with deionised water until the colorimeter reading fell within the limits of the calibration graph.

To prepare the calibration graph, 1 mL of standard *p*-nitrophenol solution was dissolved in deionised water and made up to 100 mL in volumetric flask. Aliquots of 0, 1, 2, 3, 4 and 5 mL of the diluted standard solution were pippetted into respective centrifuge tubes and volumes were adjusted to 5 mL with deionised water. Each tube was dosed with 1 mL of 0.5 M calcium chloride and 4 mL of 0.5 M sodium hydroxide and then centrifuged at 4000 rev/min for 10 min. The yellow colour intensity was measured following the procedure as described above and readings were then plotted against the concentration of *p*-nitrophenol.

Controls were performed with each sample analysed to account for colour formation not resulting from *p*-nitrophenol released by phosphatase enzyme activity. The same procedure described above for assay of phosphatase activity was followed, but the addition of 1 mL of PNP solution was done after the incubation at 37 ^{o}C and the additions of the 0.5 M calcium chloride and 0.5 M sodium hydroxide.

3.7 Seed germination and growth assay

The toxicity of the outdoor compost-bioremediated samples was tested by using them as growing media for planted corn, pea and mustard seeds. The tests were carried out on the Day 0 and Day 56 compost samples. Sweet corn (*Zea saccharata var*), pea (*Pisum satirun*) and mustard (*Brassica sinapis alba*) species were used for the phytotoxicity assay.

3.7.1 *Procedure*

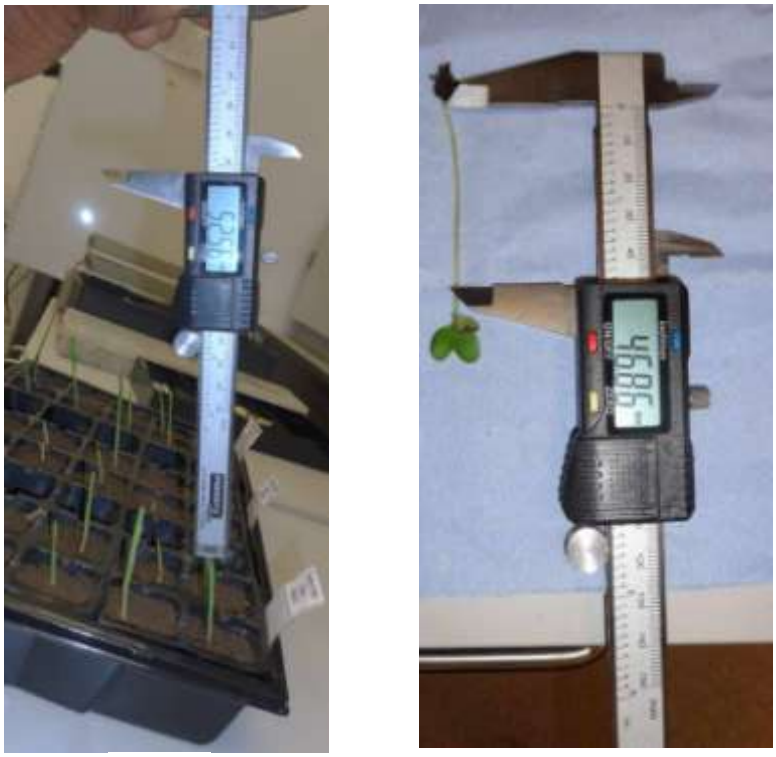
The compost sample (30 g), re-wetted to 80% moisture content, was filled into propagator cell inserts. Single seeds of corn and pea and 3 seeds of mustard were planted in respective cells in plots of 10 cells, in triplicates. The inserts were loosely covered with lids to reduce evaporation but allow for aeration and then placed on a windowsill to germinate and sprout. The experiment was set up during the summer when daylight was 14 h minimum and temperatures were 12 – 22 ^{o}C.

Controls were also set up using Grow-sure All-purpose compost (Westland Horticulture limited, Dungannon) as the growing medium. When 70% of seeds in the control germinated, the number of seeds germinated in all samples were recorded. Sprouting shoot height for corn and pea and stem length for mustard were additional parameters measured (fig. 3.5).

Additional seed germination experiments were set up following the procedure as described above, to assess the possible effect of nutrient and molecular weights of contaminant PAHs found in the Day 0 coal tar contaminated soil sample for the outdoor composting experiment. Aliquots (30 g) of the agricultural soil from Cockle Park farm were used as the planting medium. The parameters measured were number of seeds germinated, shoot height, stem length and root elongation. The shoot and root of germinated seeds were dried at 105 ^{o}C for 24 h and their mean dry weights calculated to assess sub-lethal effects (e.g. Dawson et al., 2007).

The soil was contaminated by spiking with representative PAH compounds among the 2 and 3-rings group and 4, 5 and 6-rings group, respectively, which are found in the day 0 coal tar spiked soil sample. Naphthalene, phenanthrene and anthracene were used as the representative compounds for the 2 and 3-rings PAH group while pyrene and perylene were used to represent the 4, 5 and 6-rings PAH group.The

amount of each PAH contaminant added to the soil was prepared to twice their concentrations in the day 0 CTIS. This was to augment for other unidentified hundreds of compounds present in the coal tar sample (Schobert and Song, 2002). Fresh soil samples without PAH contaminants were used for the control. The amounts of representative PAH compounds used for spiking the soil sample are shown in Table 3.6.



A B

Fig. 3.5. Measurement of: (A) shoot height; and (B) stem length of sprouting corn and mustard

Table 3.6: Amounts of representative PAH compounds used for spiking fresh soil sample for planting seeds.

2 and 3 - rings PAHs	Concentration (µg/g)	Total conc of representative PAHs (µg/g)	Proportion (%)	Total conc of available PAH contaminants (µg/g)	Amount required for spiking soil (µg/g)	Amount used for spiking soil (g)
Naphthalene (m/z: 128)*	235.7		39.53		342.6	1.55**
Acenaphthylene (m/z: 152)	211.4					
Fluorene (m/z: 166)	58.9	596.3		866.6		
Phenanthrene (m/z: 178)*	284.6		47.73		413.6	1.88**
Anthracene (m/z: 178)*	76.0		12.74		110.4	0.5**
4, 5 and 6-rings PAHs						
Fluoranthene (m/z: 202)*	275.7					
Pyrene (m/z: 202)*	208.9		87.48		1071.7	4.86**
Benz[a]anthracene (m/z: 228)	78.7					
Chrysene (m/z: 228)	66.8					
Benzo[b]fluoranthene (m/z: 252)	88.6					
Benzo[k]fluoranthene (m/z: 252)	58.1					
Benzo[j]fluoranthene (m/z: 252)	54.8					
Benzo[a]fluoranthene (m/z: 252)	31.0					
Benzo[e]pyrene (m/z: 252)	71.3	238.8		1225.1		
Benzo[a]pyrene (m/z: 252)	110.6					
Perylene (m/z: 252)*	29.9		12.52		153.4	0.7**
Indeno[1,2,3-cd]pyrene (m/z:276)	51.5					
Picene (m/z: 276)	5.8					
Benzo[g,h,i]perylene (m/z: 276)	53.6					
Anthanthrene (m/z: 276)	14.3					
Benzo[b]triphenyle (m/z:278)	6.4					
Dibenzo[a,h]anthracene (m/z: 278)	10.0					
Benzo[b]chrysene (m/z:278)	9.1					

** - PAH group representative compound used for spiking soil*

*** - Amount of PAH group representative compound used for spiking 2700 g soil at 16% MC_{db}*

3.8 *Statistical analysis*

A Two-Way Analysis of Variance (ANOVA) test was performed to compare different mean TPH, n-alkane and PAH values obtained for before and after composting treatment samples from triplicate experiments. Post Hoc analysis was performed to make pairwise comparisons between means of different samples using Scheffe test. The effect size of the magnitude of difference between two means was further estimated using Cohen's d analysis based on cohen's (1992) guidelines. If ANOVA test resulted in statistically significant differences, the independent samples t-test was used to compare two different means. Statistical tests were conducted with IBM SPSS statistics version 22 set at 95% confidence level.

Chapter 4

LABORATORY COMPOST-BIOREMEDIATION OF DRILL CUTTINGS AND COAL TAR IMPACTED SOIL

4.1 Introduction

The effects of laboratory-scale composting on the degradation of pollutant petroleum hydrocarbons in oil field drill cuttings and coal tar impacted soil (CTIS) are investigated in this chapter. During the experiments, organic amendments in different mix ratios with respective contaminated samples, were incubated following the natural composting temperature cycle for 53 days. Geochemical analysis was performed on the start and finished compost mixes to identify the mix types that resulted in the most hydrocarbon degradation. The drill cuttings, CTIS and compost amendments (comprised of grass cuttings, spent mushroom compost and straw) were tested for nitrogen, carbon, phosphorus and moisture contents, as well as for pH and salinity. These are considered the most important parameters in bioremediation composting of petroleum hydrocarbon contaminated soil studies (e.g. Richard, 2000; Bao *et al*., 2013; Gao *et al*., 2013) and those studies were used as guide for formulating the compost mixes.

In this chapter, the analytical results of initial compost parameters used are presented followed by depletion of extractable organic matter (EOM) data. These are followed by results of initial amount of total petroleum hydrocarbon (TPH) contained in the respective component of compost mixes as well as its degradation status in each compost mix type after treatment. Also, results of degradation of *n*-alkanes and PAHs, including the 16 EPA priority pollutants in the CTIS treatments, are reported for pre-composting and post-composting samples.

4.2 Results and Discussion

4.2.1 *Compost parameters analysis*

Table 4.1 presents the physiochemical and main compost parameters of the individual components of the compost matrix. The drill cuttings and soil substrate samples had carbon contents of 1.32% and 2.39%, nitrogen contents of 0.05% and 0.24%, giving carbon: nitrogen (C:N) ratios of 24:1 and 10:1 for them, respectively. All the compost ingredients used as amendments were characterized by high contents of carbon and nitrogen in comparison to the contaminated substrate

samples. The overall C:N ratios for each of the compost mixes prepared with drill cuttings and CTIS fell between 28 – 31. This is within the range of C:N ratios (9 – 200) which have been widely reported for effective compost- bioremediation of hydrocarbons contaminated medium (Huesemann, 1994; Roldan et al., 2003). However, a more narrow range of C:N ratios (25-30) has been suggested by Fogarty and Tuovinen (1991) for optimum composting. The carbon: phosphorus (C:P) ratios for each of the compost mixes range from 77.5 to 160.7, which is also within the wide range (60-800) reported for hydrocarbon degradation (Huesemann, 1994).

The pH values were found to vary between the ranges of 5.8 – 7.6 and 6.9 - 7.5 for the compost ingredients and compost mixes, respectively. Extreme pH values have been reported to impede the ability of microbial populations to degrade hydrocarbons, as heterotrophic bacteria and fungi are most active near pH of neutrality (Leahy and Colwell, 1990). Salinities of the compost mixes were found to be <1%, which is within the range for optimum biodegradation of PAHs reported by Wilson and Jones (1993).

Compost mixes with drill cuttings were adjusted to a moisture content of 35% during the experimentation period. Previous studies have reported moisture contents of 30-35% as the optimum range for TPH removal in drill cuttings during bioremediation treatment (Roldan *et al*., 2003; Rojas-Avelizapa *et al*., 2007). Similarly, the moisture content of the compost mixes with CTIS was optimized to 60%, which is within the general optimal moisture content range of 50-80% for composting (Richard et al., 2002). Following this, significant reduction of pollutants in compost-bioremediation of hydrocarbon contaminated soil at 60% moisture content have been reported (Lau *et al*., 2003; Antizar-Ladislao *et al*., 2005).

Table 4.1. Physiochemical and composting parameters of compost ingredients and mixes for drill cuttings and coal tar impacted soil (CTIS)

Ingredient	C %	N %	P %	Moist cont. %	pH	Salinity %	TPH µg/g	Mix types	Mix (contaminant/ Amendments) ratio	Mix C/N ratio	Mix C/P ratio	Mix pH	Mix salinity %
Drill cuttings	1.32	0.05	0.15	12.1	7.52	0.22	2560.0±35	DGS_t1	1 : 1	29.86	110.8	7.12	0.25
Soil	2.39	0.24	0.14	1.7	7.04	0.02	14.5±0.4	DGS_t2	1 : 1.5	29.35	130.0	7.02	0.26
Grass Cutting	30.41	2.78	0.47	68.30	5.82	0.44	279.8±14.3	DGSt3	1 : 2	29.95	148.6	6.98	0.26
SMC	32.7	2.1	0.73	66.1	7.57	0.34	498.6±6.5	DMS_t1	1 : 1	29.61	77.5	7.52	0.24
Straw	43.12	0.93	0.09	9.30	7.45	0.14	262.1±14.9	DMS_t2	1 : 1.5	29.67	88.93	7.52	0.24
								DMS_t3	1 : 2	29.85	97.1	7.52	0.24
								$DGMS_t1$	1 : 1	29.97	95.3	7.30	0.24
								$DGMS_t2$	1 : 1.5	28.96	106.0	7.30	0.23
								$DGMS_t3$	1 : 2	30.07	123.0	7.24	0.25
								SGS_t1	1 : 1	29.90	136.2	7.00	0.13
								SGS_t2	1 : 1.5	29.44	150.8	6.93	0.16
								SGSt3	1 : 2	29.18	160.7	6.88	0.18
								SMS_t1	1 : 1	29.46	105.9	7.27	0.12
								SMS_t2	1 : 1.5	29.38	110.1	7.32	0.15
								SMS_t3	1 : 2	30.00	117.7	7.35	0.16
								$SGMS_t1$	1 : 1	29.79	122.4	7.12	0.12
								$SGMS_t2$	1 : 1.5	29.71	132.6	7.10	0.15
								$SGMS_t3$	1 : 2	29.08	134.8	7.09	0.18

C = Carbon, N = nitrogen, P = phosphorus, D = soil, G = grass cuttings, SMC = M = spent mushroom compost, S_t = straw, S = coal tar impacted soil, TPH = total petroleum hydrocarbons
C, N, P, moist cont, pH and salinity values are mean of three replicates; TPH Values are mean (n=3) ± standard deviation.

4.2.2 Extractable organic matter

Concentrations of extractable organic matter (EOM) obtained from the Soxhlet solvent extractions of all start (day 0) and finished (day 53) compost mixes with drill cuttings and CTIS are presented in Table 4.2. The results showed significant ($p<0.05$) depletion of EOM concentrations in the compost mixes with drill cuttings and CTIS respectively. Reductions ranging from 52% - 70% and 71% - 82% were recorded in compost mixes with drill cuttings and CTIS, respectively. However, the decreases were not significantly ($P>0.05$) different within mix types with the respective contaminants. In the mixes with drill cuttings, the DMS_t3 and DGS_t1 mix types produced the least and most reduction of EOM concentration of 52.9% and 69.9%, respectively. For the mixes with CTIS, EOM concentration reductions of 71.6% and 80.7% were recorded as the minimum and maximum in the SMS_t1 and $SGMS_t3$ mix types, respectively.

Table 4.2. Extractable organic matter (EOM) for compost mixes with drill cuttings and coal tar impacted soil (CTIS)

Mixes with Drill cuttings				Mixes with CTIS			
Compost mix type	EOM (µg/g)		Reduction %	Compost mix type	EOM (µg/g)		Reduction %
	Day 0	Day 53			Day 0	Day 53	
Control	8100±500	4900±400	39.5	Control	1200±40	700±50	41.7
DGS_t1	13600±800	4100±500	69.9	SGS_t1	11600±400	2600±200	77.6
DGS_t2	15200±200	4700±400	69.1	SGS_t2	12800±500	2600±200	79.7
DGS_t3	16400±600	6200±300	62.2	SGS_t3	15000±600	3300±300	78.0
DMS_t1	10300±500	4100±400	60.2	SMS_t1	9500±500	2700±400	71.6
DMS_t2	11300±600	4900±700	56.6	SMS_t2	9700±200	3000±200	69.1
DMS_t3	10500±200	5000±300	52.9	SMS_t3	12400±600	2900±300	76.6
$DGMS_t1$	10500±500	4300±100	59.0	$SGMS_t1$	12100±700	2300±300	81.0
$DGMS_t2$	14600±800	6200±500	57.5	$SGMS_t2$	11600±500	2700±200	76.7
$DGMS_t3$	11700±700	3900±300	66.7	$SGMS_t3$	14000±800	2700±400	80.7

Values are mean (n=3) ± standard deviation; Control = drill cuttings and CTIS samples without compost amendments respectively; D = drill cuttings, G = grass cuttings, M = mushroom compost, S_t = straw, S = CTIS

Reduction of EOM concentration was significant (39.5%; $p<0.05$, *t*-test) and (41.7%; $p<0.05$, *t*-test) in the control sample for drill cuttings and CTIS, respectively, but was significantly ($p\leq0.001$) lower in relation to the organic amended compost mixes with drill cuttings and CTIS respectively. Generally, the compost mixes with CTIS

produced more reduction in EOM concentration than those with drill cuttings after composting for 53 days.

The reduction of contaminant hydrocarbons in compost mix types may not be predictable from the reduction of their respective EOM concentrations because of possible interference of biological, asphaltene and NSOs (polar nitrogen, sulphur and oxygen containing) compounds which are extractable along with the drill cuttings and coal tar hydrocarbons (Mills *et al*., 1999). For this reason, the observed levels of reduction in EOM concentrations may be attributed to the presence of biological compounds e.g. plant waxes, lipids, saccharides and pigments such as chlorophyll, among others, in the organic compost amendments which are easily consumable by microbes during the composting process. However, the method for determining EOM is a non-specific gravimetric technique which has been used as a screening tool for examining oil degradation (Wang and Fingas, 1997). Graphical representations of the EOM results are shown in Appendices A and B respectively.

4.2.3 *Degradation of total petroleum hydrocarbons (TPH) in compost mixes with drill cuttings*

In determining total petroleum hydrocarbons (TPH), the areas of resolved peaks and unresolved complex mixtures from the gas chromatography (GC-FID) chromatogram was analysed by subtracting areas of internal and surrogate standards, and solvent blank. The TPH analysis is considered a relatively improved assessment of hydrocarbon content than EOM method due to fewer interferences (Mills *et al*., 1999). However, shortcomings of both methods include possible significant loss of volatile fractions during analysis (Douglas *et al*., 1992). The concentration of total petroleum hydrocarbons (TPH) was analysed for in each of the compost mixes with drill cuttings before and after the bioremediation treatment and the result are shown in Table 4.3 and graphically in Appendix C. Each of the day 0 samples have equal amount of drill cuttings (10 g) which contributed most to the TPH concentrations. While the highest TPH concentration was found in DMS_t3 mix having 1679.2 µg/g, the least was 1199.3 µg/g found in $DGMS_t3$ mix.

The TPH concentration in each of the compost mixes at day 53 were significantly ($p \leq 0.05$, *t*-test) lower compared to their respective day 0 mixes. The most TPH reduction was found in the $DGMS_t3$ mix losing 85.1% of its initial TPH at 1199.3 µg/g.

It was closely followed by the DMS_t1 mix in which the TPH significantly reduced by 81.7% to 298.4 µg/g. The control sample recorded the least reduction of TPH (36.7%) at day 53. But unlike the trend observed in EOM, it was widely followed by the DGS_t3 mix in which TPH significantly reduced by 71.5% to 362.3 µg/g. The DGS_t3 mix recorded the least reduction of TPH compared to the other compost mixes with organic amendments. Pair wise comparison also revealed that reduction of TPH was significant (36.7%; $p<0.05$, *t*-test) in the control but was found to be significantly ($p\leq0.001$ ANOVA) lower compared to the mixes with organic amendments after day 53. Also, the Cohen's d value for the reduction of TPH in the control was estimated at 9.3, indicating a large effect, but was less than that of the DGS_t3 mix which had a Cohen's d value of 12.7. The $DGMS_t3$ mix had a Cohen's d value estimated at 18.4.

Table 4.3. Concentration and standard deviation (sd) of total petroleum hydrocarbon (TPH) in compost mixes with drill cuttings after composting for 53 days

	Concentration (µg/g of dry sample)				Reduction
Mix type	day 0	Std	day 53	Std	(%)
Control	2560.0	35.0	1619.4	110.8	36.7
DGS_t1	1679.2	66.8	325.4	26.9	80.6
DGS_t2	1376.6	48.3	391.1	43.2	71.6
DGS_t3	1269.6	75.8	362.3	32.6	71.5
DMS_t1	1633.3	75.6	298.4	23.8	81.7
DMS_t2	1456.9	38.5	331.0	32.1	77.3
DMS_t3	1313.9	105.4	280.8	24.9	78.6
$DGMS_t1$	1497.2	20.2	305.0	34.3	79.6
$DGMS_t2$	1368.8	38.5	284.8	36.4	79.2
$DGMS_t3$	1199.3	31.9	178.9	55.7	85.1

Values are mean (n=3); sd = standard deviation; Control = drill cuttings without compost amendments; D = drill cuttings; G = grass cuttings, M = mushroom compost, S_t = straw

GC-FID chromatograms showing the reduction of TPHs in each of the compost mixes with drill cuttings after composting for 53 days are presented in Figures 4.1, 4.2 and 4.3. Chromatograms for the day 0 samples have unresolved complex mixtures (UCM) or "humps", suggesting that the drill cuttings is comprised of fuel oil (Chaineau *et. al*., 1996) and has possibly commenced biodegradation (Skaare *et al*., 2009; Peters *et al*., 2005). However, the chromatograms for the day 53 samples show

heavily degraded humps barely showing diminished traces of those peaks, except for the control.

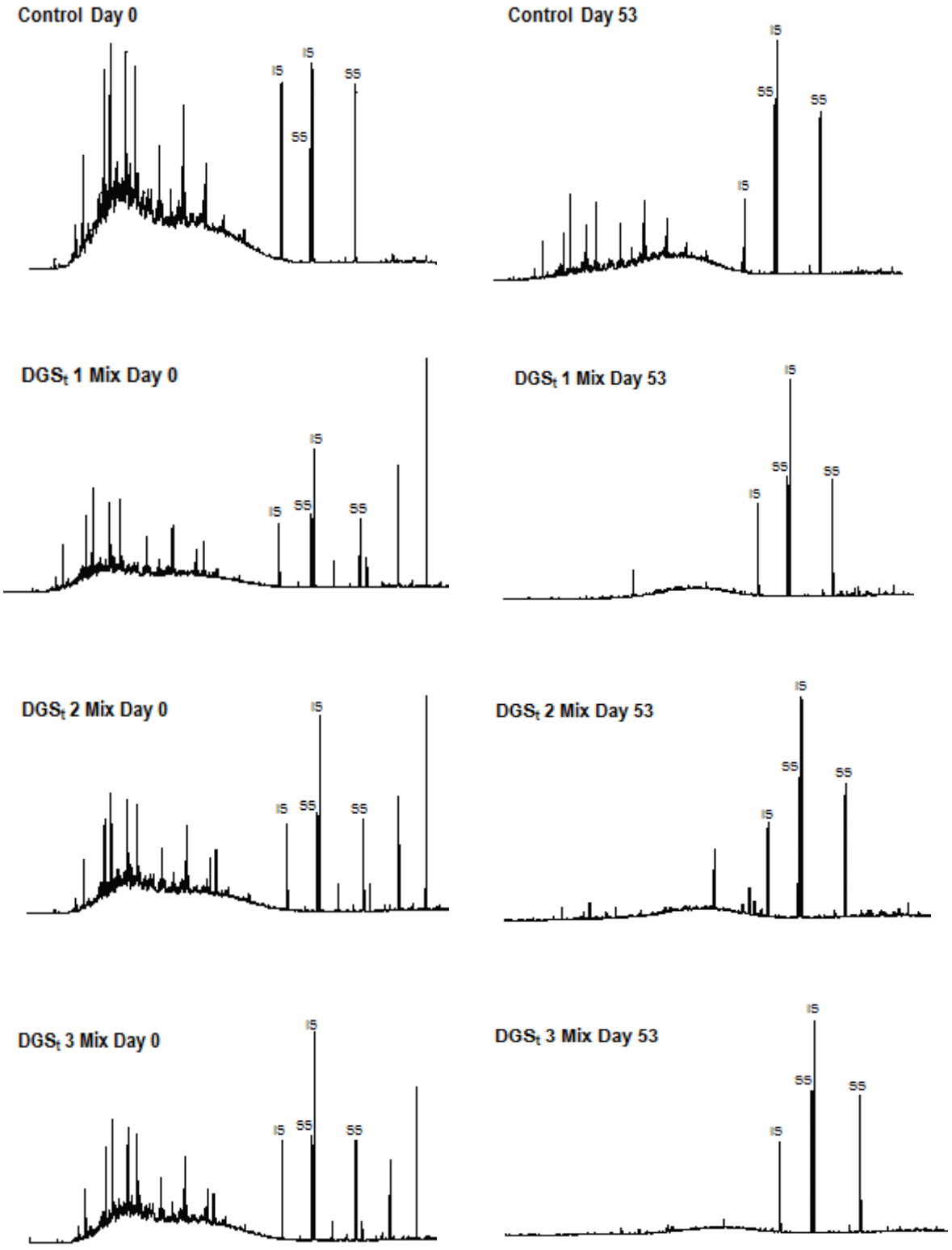


Figure 4.1. GC-FID chromatograms showing the reduction of total petroleum hydrocarbon (TPH) in the control and drill cuttings/grass cuttings/straw (DGS$_t$) mixes at day 0 and day 53 composting periods.
IS = internal standard, SS = surrogate standard

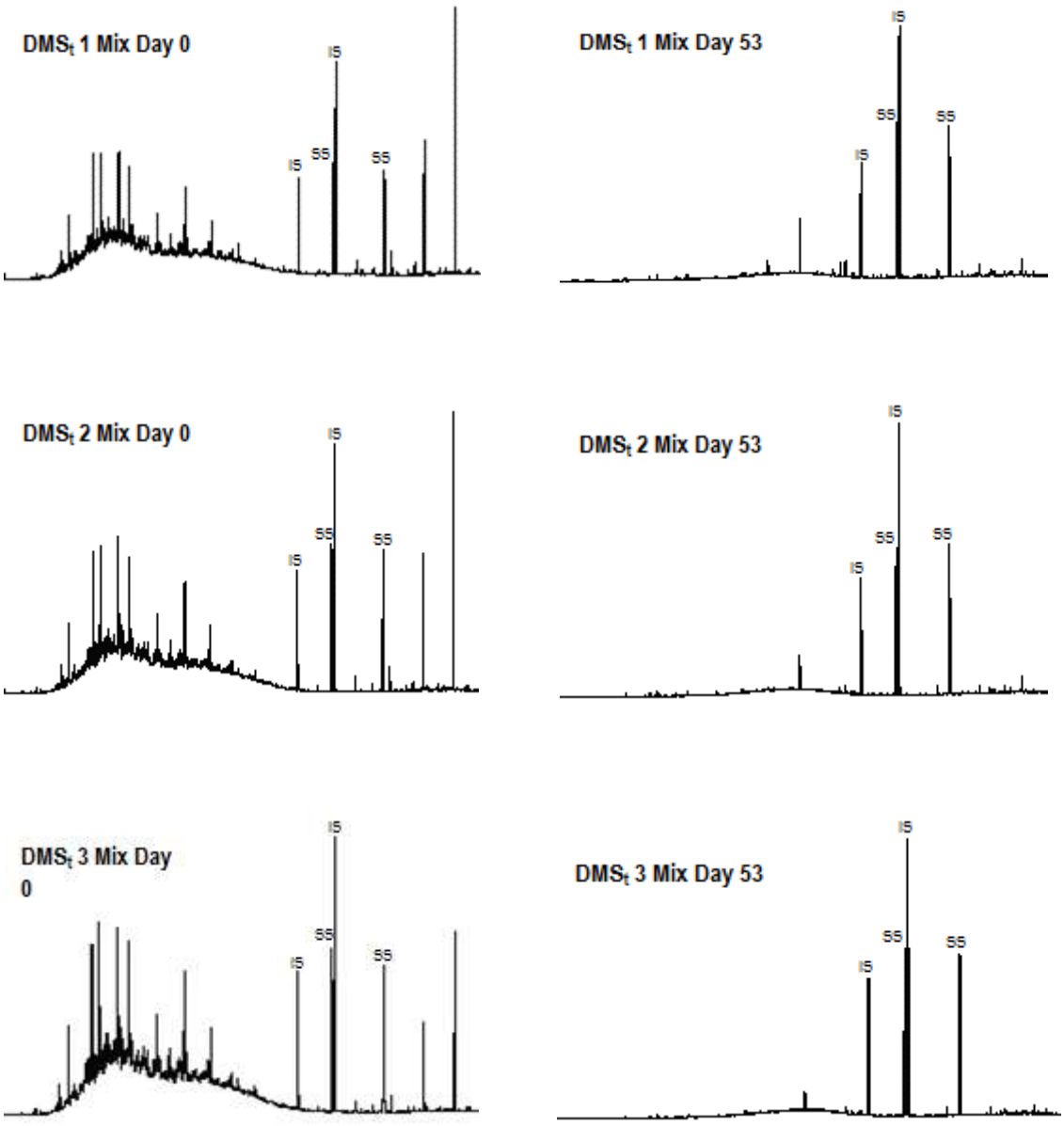


Figure 4.2. GC-FID chromatograms showing the reduction of total petroleum hydrocarbon (TPH) in the drill cuttings/ mushroom compost/straw (DMS$_t$) mixes at day 0 and day 53 composting periods.
IS = internal standard, SS = surrogate standard

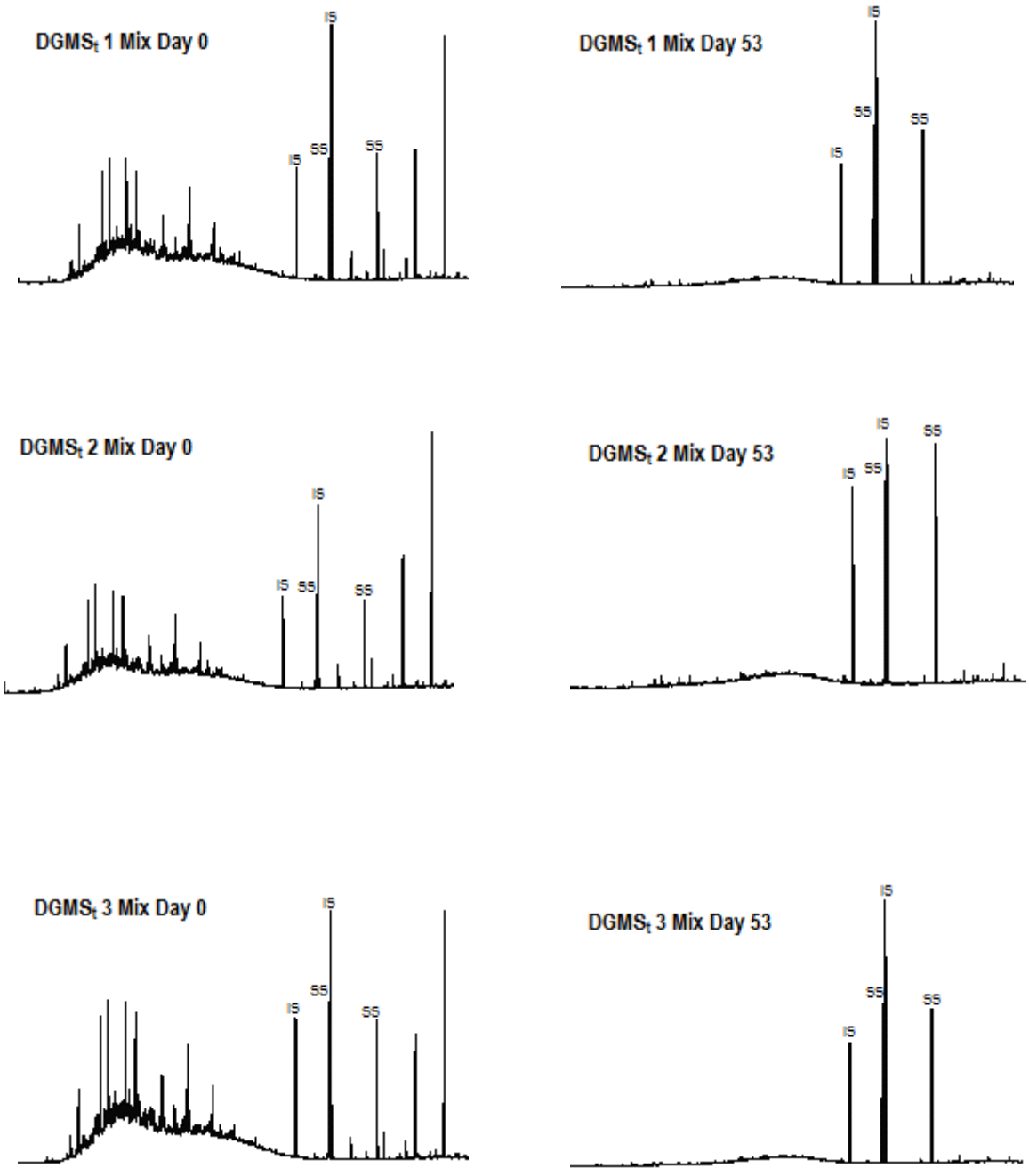


Figure 4.3. GC-FID chromatograms showing the reduction of total petroleum hydrocarbon (TPH) in the drill cuttings/grass cuttings/ mushroom compost/straw (DGMS$_t$) mixes at day 0 and day 53 composting periods.
IS = internal standard, SS = surrogate standard

4.2.4 *Degradation of total petroleum hydrocarbons (TPH) in compost mixes with CTIS*

The compost mixes with CTIS were given the same composting treatment applied to the mixes with drill cuttings. CTIS (10 g) having 106.9 µg/g TPH was used as the contaminant in each of the mixes. In the results showing concentrations of TPH in the compost mixes with CTIS (Table 4.4), the control recorded the least reduction of TPH (28.4%) by reducing to 76.5 µg/g at day 53. It was followed by the SGS_t3 mix type in which the TPH significantly reduced by 74% to 57.8 µg/g. However, the SGS_t3 mix recorded the least reduction of TPH compared to all the other mix types with organic amendments.

Table 4.4. Concentrations of total petroleum hydrocarbons (TPH) in compost mixes with coal tar impacted soil (CTIS) after composting for 53 days

	Concentration (µg/g of dry sample)				Reduction
Mix type	day 0	Std	day 53	Std	(%)
Control	106.9	1.8	76.5	11.0	28.4
SGS_t1	173.4	11.4	41.6	7.4	76.0
SGS_t2	209.3	10.9	41.5	8.5	80.2
SGS_t3	222.2	11.5	57.8	10.7	74.0
SMS_t1	248.6	12.1	48.2	5.4	80.6
SMS_t2	289.9	21.0	59.3	6.4	79.5
SMS_t3	334.0	23.4	31.4	4.4	90.6
$SGMS_t1$	193.3	10.9	47.8	11.1	75.3
$SGMS_t2$	212.0	6.6	39.3	6.4	81.5
$SGMS_t3$	239.5	12.9	53.4	11.1	77.7

Values are mean (n=3); sd = standard deviation; Control = CTIS without compost amendments; S = CTIS; G = grass cuttings, M = mushroom compost, S_t = straw

The most TPH reduction was recorded in the SMS_t3 mix, in which the TPH concentration significantly ($p<0.05$, *t*-test) reduced by 90.6% to 31.4 µg/g after composting for 53 days. The $SGMS_t2$ mix recorded the second highest reduction of TPH which reduced by 81.5% to 39.3 µg/g. Reduction of TPH in the control was significantly ($p<0.05$ ANOVA) lower compared to each of the compost mixes with organic amendments except for SGS_t1, SGS_t2, $SGMS_t1$ and $SGMS_t2$. Comparing the effect size of the difference between the day 0 and day 53 samples, the control, SGS_t3 and SMS_t3 mixes had Cohen's d values estimated at 3.2, 12.1 and 14.7,

respectively. This implies that though the reduction of TPH in control was significant, its effect is lesser than that of SGS_t3 mix which recorded the least TPH reduction among compost mixes with organic amendments.

The effects of the lab-scale compost-bioremediation treatment on TPH removal in the compost mixes with CTIS are shown in the GC-FID chromatograms (Figures 4.4, 4.5 and 4.6). Most of the prominent analyte peaks in the day 0 samples appears to be substantially degraded in their respective day 53 sample chromatograms.

Comparing the reduction of TPH among compost mixes with drill cuttings and CTIS, it was found that average TPH reductions of 74.6%, 79.2%, 81.3% and 76.7%, 83.6%, 78.2% were recorded for the DGS_t, DMS_t, $DGMS_t$ and SGS_t, SMS_t, $SGMS_t$ mixes, respectively, showing that the mixes with spent mushroom compost produced the most reduction of TPH. This could be attributed to the presence of residual enzymes, e.g. proteases, cellulases and hemicellulases, which have been reported to be present in spent mushroom compost (Chiu *et al*., 2009). They also contain lignolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase which act as Fenton reagents to produce reactive radicals for non-specific cleavage of a wide variety of highly recalcitrant organopollutants (Chiu *et al*., 1998; Hestbjerg *et al*., 2003; Gong *et al*., 2006).

Generally, the compost mix samples with organic amendments for the CTIS treatments had higher day 0 TPH values than their respective controls. This may be due to the presence of naturally occurring biogenic leaf wax *n*-alkanes in the organic amendments. Graphical presentation of Table 4.4 is shown in Appendix D.

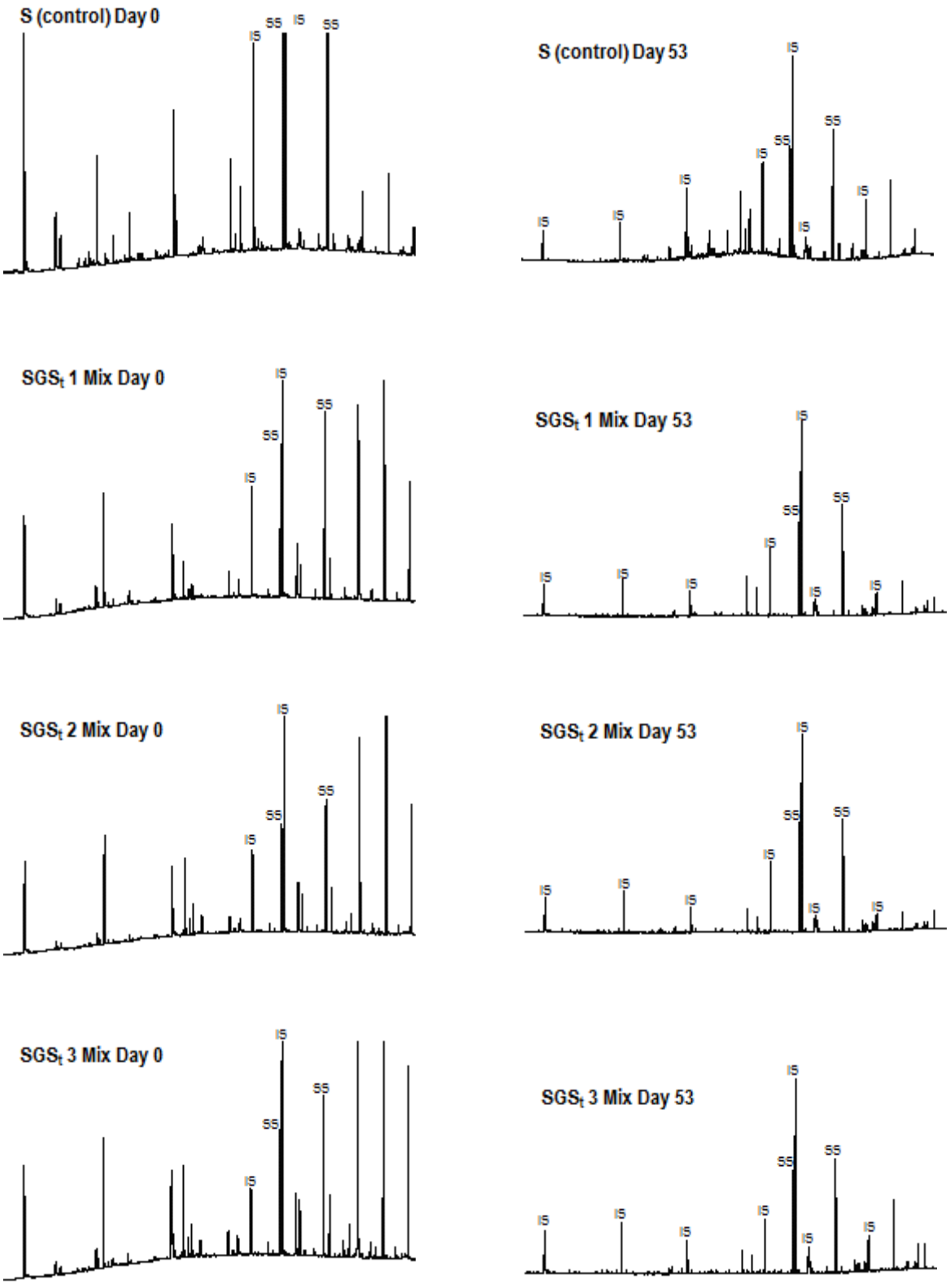


Figure 4.4. GC-FID chromatograms showing the reduction of total petroleum hydrocarbon (TPH) in the control and coal tar impacted soil/grass cuttings/straw (SGS_t) mixes at day 0 and day 53 composting periods.
IS = internal standard, SS = surrogate standard
NB: Semivolatile internal standards in the day 53 samples were not added to the day 0 samples before GC-FID analysis.

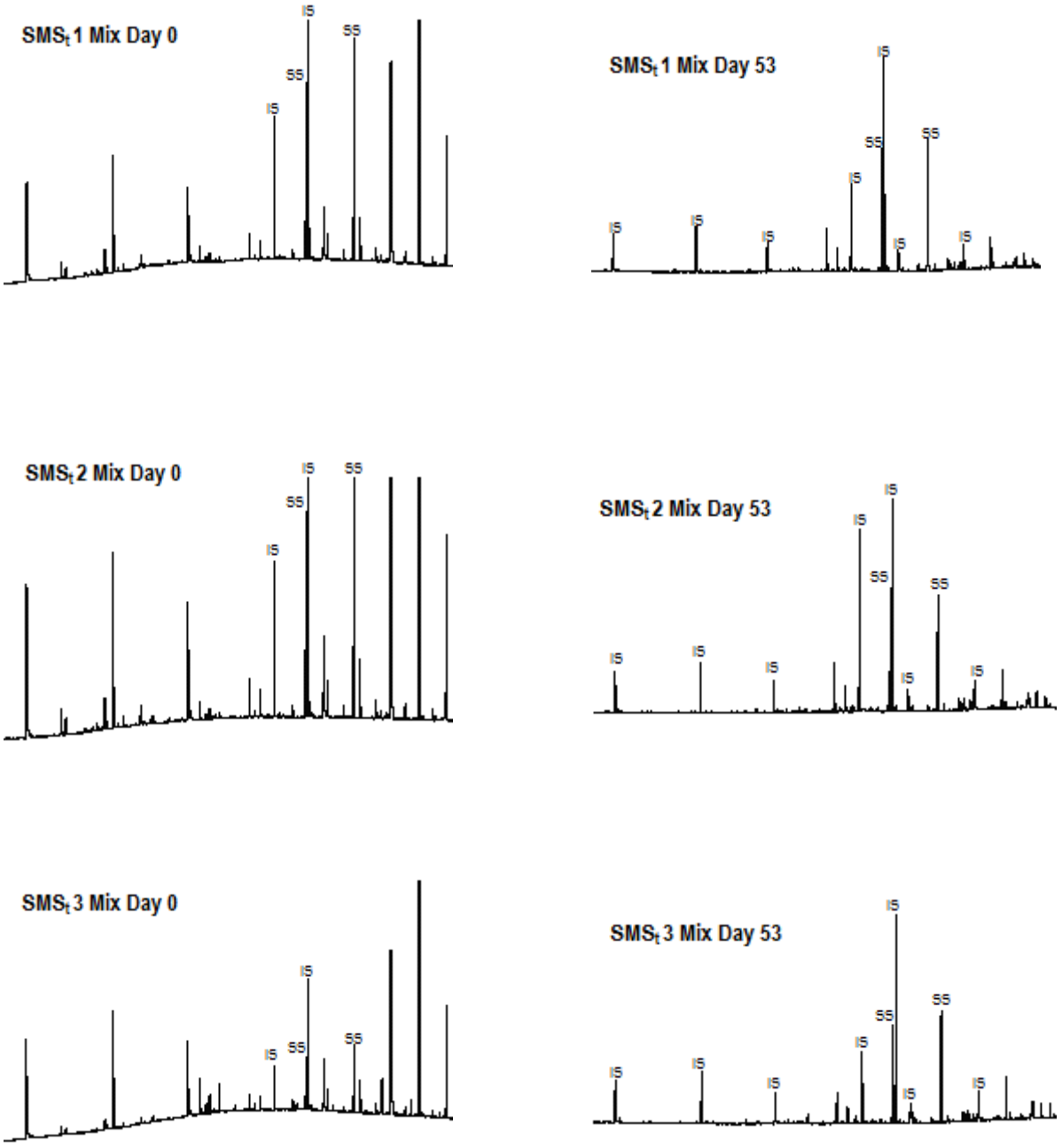


Figure 4.5. GC-FID chromatograms showing the reduction of total petroleum hydrocarbon (TPH) in the coal tar impacted soil/mushroom compost/straw (SMS$_t$) mixes at day 0 and day 53 composting periods.
IS = internal standard, SS = surrogate standard
NB: Semivolatile internal standards in the day 53 samples were not added to the day 0 samples before GC-FID analysis.

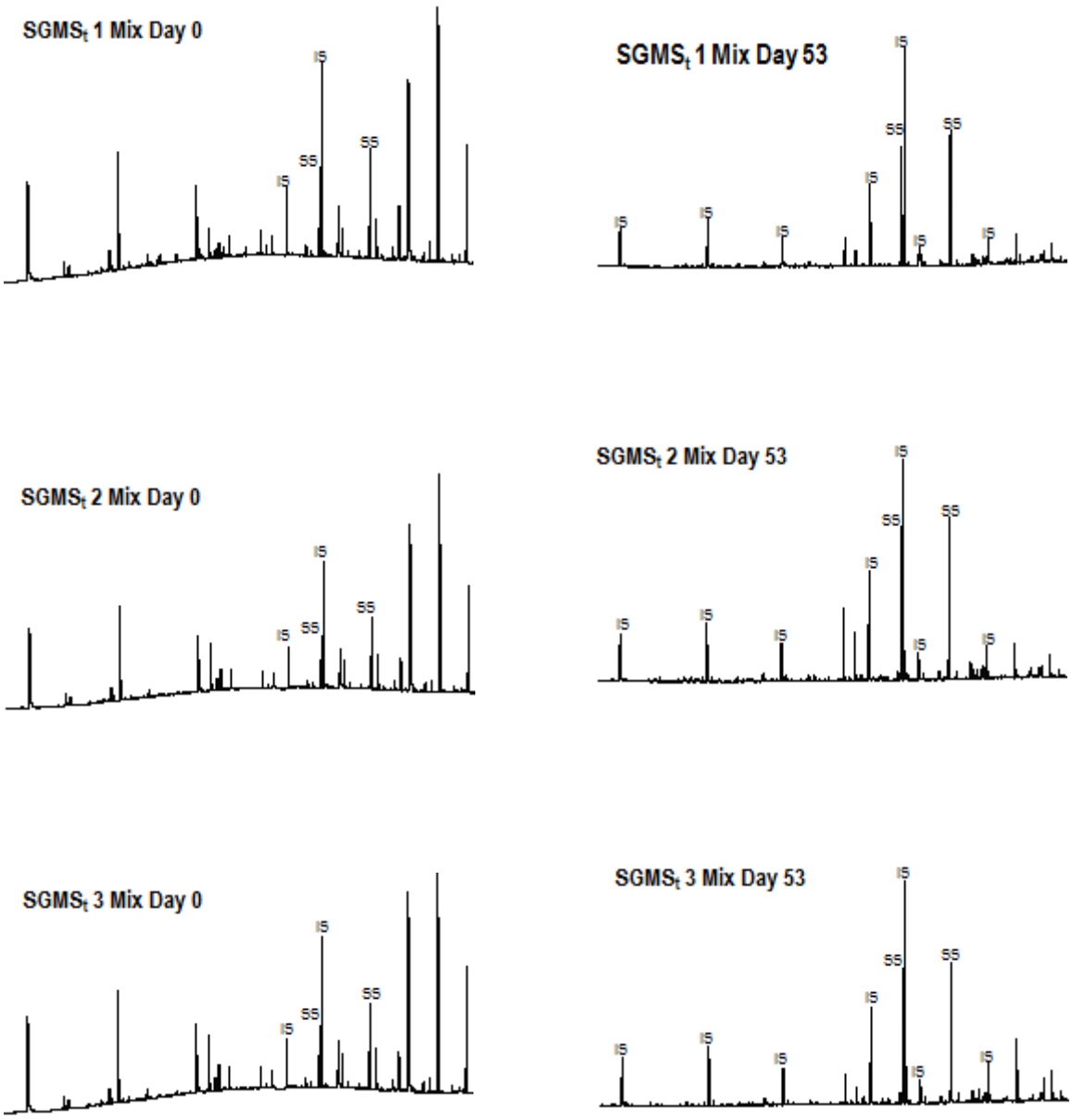


Figure 4.6. GC-FID chromatograms showing the reduction of total petroleum hydrocarbon (TPH) in the coal tar impacted soil/grass cuttings/mushroom compost/straw (SGMS$_t$) mixes at day 0 and day 53 composting periods.

IS = internal standard, SS = surrogate standard

NB: Semivolatile internal standards in the day 53 samples were not added to the day 0 samples before GC-FID analysis.

4.2.5 Compositions of n-alkanes and acyclic isoprenoid alkanes in compost amendments

The *n*-alkane compositions of the respective contaminated media and organic amendments used for formulating the different compost mixes, are presented in Table 4.5 and Figure 4.7. The distribution of *n*-alkanes ranged from nC_{13} to nC_{19} in a unimodal distribution and with a predominance of nC_{12} – nC_{16} with nC_{14} as the main peak in the drill cuttings (Fig.4.7a). The isoprenoid (pristane and phytane) peaks are seen to have substantial predominance over their paired nC_{17} and nC_{18} *n*-alkanes, respectively. This observation may be attributed to preferential degradation of the *n*-alkanes before the isoprenoids during storage due to exposure to oxygen through ventilation (Beškoski *et al*., 2011). Previous studies have reported that isoprenoids are more degradation resistant than *n*-alkanes (e.g. Barakat *et al*., 2002; Wang and Fingas, 2003; Wang *et al*., 2013a).

Long-chain *n*-alkanes (nC_{25} – nC_{33}) were found most abundant in the grass cuttings, spent mushroom compost and straw samples, (Figures 4.7b, 4.7c and 4.7d respectively), with strong odd over even carbon number predominance. This is consistent with earlier studies reported that land higher plants are generally characterised with nC_{25} - nC_{31} odd carbon number *n*-alkane predominance (Rieley *et al*., 1991; Collister *et al*., 1994, Duan and He, 2011). The carbon-number maxima (C_{max}) distribution is at nC_{31} for the grass cuttings and straw samples which is consistent with herbaceous plants (Cranwell, 1973; Cranwell *et al*., 1987). The spent mushroom compost sample has C_{max} distribution at nC_{33} (16.7 µg/g) which is, however, not significantly ($p<0.05$ *t*-test) higher than its nC_{31} concentration (14.7 µg/g).

Table 4.5. Concentration of n-alkanes in components of compost mixes

	µg/g of dry sample				
***n*-alkane**	**Drill cuttings**	**Soil**	**Grass clippings**	**SMC**	**Straw**
nC_{12}	1.0±0.08	-	-	-	-
nC_{13}	11.7±1.3	-	-	-	-
nC_{14}	17.8±1.7	-	-	-	-
nC_{15}	15.7±1.1	-	-	1.9±0.1	-
nC_{16}	8.4±0.6	-	-	-	-
nC_{17}	6.1±0.6	-	-	-	-
Pristane	16.6±0.9	-	-	-	-
nC_{18}	3.6±0.2	-	-	-	-
Phytane	8.6±0.5	-	-	-	-
nC_{19}	2.1±0.2	-	-	-	-
nC_{23}	-	-	-	1.2±0.05	-
nC_{25}	-	0.2±0.02	15.4±2.5	1.0±0.08	1.6±0.04
nC_{27}	-	0.5±0.04	9.0±0.8	1.3±0.07	3.8±0.08
nC_{29}	-	2.1±0.1	18.6±1.9	4.2±0.3	24.9±0.5
nC_{30}	-	-	-	-	2.4±0.07
nC_{31}	-	2.9±0.2	26.9±3.2	14.7±0.9	52.4±1.2
nC_{33}	-	1.0±0.1	17.7±2.6	16.7±1.0	10.8±0.3

Values are means (n=3) ± standard deviation; - = Not detected; SMC = spent mushroom compost; - = not detected.

The carbon numbers of *n*-alkanes found in the soil sample ranged from nC_{25} – nC_{33} with strong odd-to-even number carbon preferences and C_{max} at nC_{31} (Figure 4.7e). According to reports from previous studies, the typical distributions of *n*-alkanes found in the soil are often derived from terrestrial higher plants (Eglinton and Hamilton, 1967; Jones *et al*., 1983; Rieley *et al*., 1991 and Roa *et al*., 2010).

4.2.6 *Degradation of n-alkanes and acyclic isoprenoid alkanes in compost mixes with drill cuttings*

The *n*-alkane carbons number found in each mix type are derived from the individual components of the mix. Drill cuttings are the contaminant source being treated in each of the mixes. As a result, nC_{12} to nC_{19} *n*-alkanes are present in each mix type. Additionally, the nC_{25} to nC_{31} odd carbon alkanes found in the DGS_t, DMS_t and $DGMS_t$ mix types were derived from grass cuttings, mushroom compost and straw samples (Tables 4.6, 4.7 and 4.8). The nC_{23} and nC_{30} *n*-alkanes which were derived from the mushroom compost and straw samples, respectively, are only found in the mix types containing those samples.

The percentage removal of the *n*-alkanes was assessed for all mix types after the compost-bioremediation treatment for 53 days. The results showed that individual, as well as the sum of all *n*-alkanes present in each mix type were significantly ($p<0.05$ *t*-test) degraded when compared to their initial concentrations at day 0. Decreases in concentrations by 92, 93 and 93% were achieved for sum of *n*-alkanes ($\Sigma_{n\text{-alkanes}}$) in the DGS_t1, DGS_t2 and DGS_t3 mix types, respectively (Table 4.6). The DMS_t1, DMS_t2 and DMS_t3 mix types each produced $\Sigma_{n\text{-alkanes}}$ reduction rate of 91, 88 and 89%, respectively (Table 4.7), while the $DGMS_t$ 1, $DGMS_t$ 2 and $DGMS_t$ 3 mix types recorded $\Sigma_{n\text{-alkanes}}$ reductions of 92, 91 and 95%, respectively (Table 4.8). These significant removals of *n*-alkanes are consistent with the substantial degradation of chromatogram peaks shown in the TPH chromatograms for respective mix types, as well as in Tables 4.3, 4.4 and 4.5.

The control experiments, which exhibited the saturated hydrocarbon distribution of the drill cuttings at day 0, also recorded significant ($p<0.05$, *t*-test) $\Sigma_{n\text{-alkanes}}$ reduction at day 53. However, it recorded a significantly ($p<0.05$, ANOVA) lower $\Sigma_{n\text{-alkanes}}$ reduction compared to all the mixes with organic amendments, except for the DGS_t2, DGS_t 3 and DMS_t 3 samples after the incubation at day 53. Figure 4.8 shows m/z 85 chromatograms of saturated hydrocarbon fractions of representative treatment samples.

Ratios of isoprenoid to *n*-alkanes change under the influence of biodegradation have been used to evaluate the degree of microbial degradation in remediation studies such as from the conventional pristane/nC_{17} and phytane/nC_{18} ratios (Barakat *et al.*, 2002; Wang and Fingas, 2003; Stelga et al., 2009). Analysis of the chromatographic data revealed that all compost mixes with organic amendments exhibited increased pristane/nC_{17} and phytane/nC_{18} ratios after incubation for 53 days. The DGS_t, DMS_t, and $DGMS_t$ (Tables 4.6, 4.7 and 4.8) samples exhibited mean increase in pristane/nC_{17} and phytane/nC_{18} ratios of 1.44 and 1.19, 1.80 and 1.42 and, 1.50 and 1.31, respectively, indicating an occurrence of mild to moderate biodegradation according to reported scales (e.g. Peters and Moldowan, 1993; Head *et al*., 2003). This demonstrated that the hydrocarbon degradation conformed to the degradation pattern wherein the straight chain *n*-alkanes were preferentially degraded over the branched chained (pristane and phytane) compounds (e.g. Leahy and Colwell, 1990; Wenger *et al*., 2002; Garcia-Blanco *et al*., 2007). However, lower increases of

pristane/nC$_{17}$ and phytane/nC$_{18}$ ratios of 0.73 and 0.46 occurred in the control sample, suggesting very slight biodegradation or perhaps evaporation losses (e.g. Peters *et al*., 2005) which may have occurred during sterilisation in the autoclave and/or the incubation process.

Ressolved *n*-alkanes in each of the compost mix samples tested were significantly ($p<0.05$ *t*-test) degraded after the composting experiment except for nC$_{19}$ ($p=0.282$) in the sterile control sample. The pattern of preferential degradation of lower (nC$_{12}$-nC$_{19}$) over higher (nC$_{23}$- nC$_{33}$) molecular weight *n*-alkanes did not follow in the compost treatments with organic amendment contrary to finding in studies reported (Chandru *et al*., 2008; Wang *et al*., 2013b). This, however, was not the case in the control which showed decreasing percentage losses with increasing molecular weight *n*-alkanes. However, nC$_{23}$ recorded the smallest percentage reduction (35.2% average) in all the DGMS$_t$ mixes (Table 4.8). It may have been less accessible to microbial attack due to decrease solubility as a result of its very low concentration in the samples. Statistical plots of *n*-alkanes concentrations in each of the compost mixes with drill cuttings are presented in Appendices E – N.

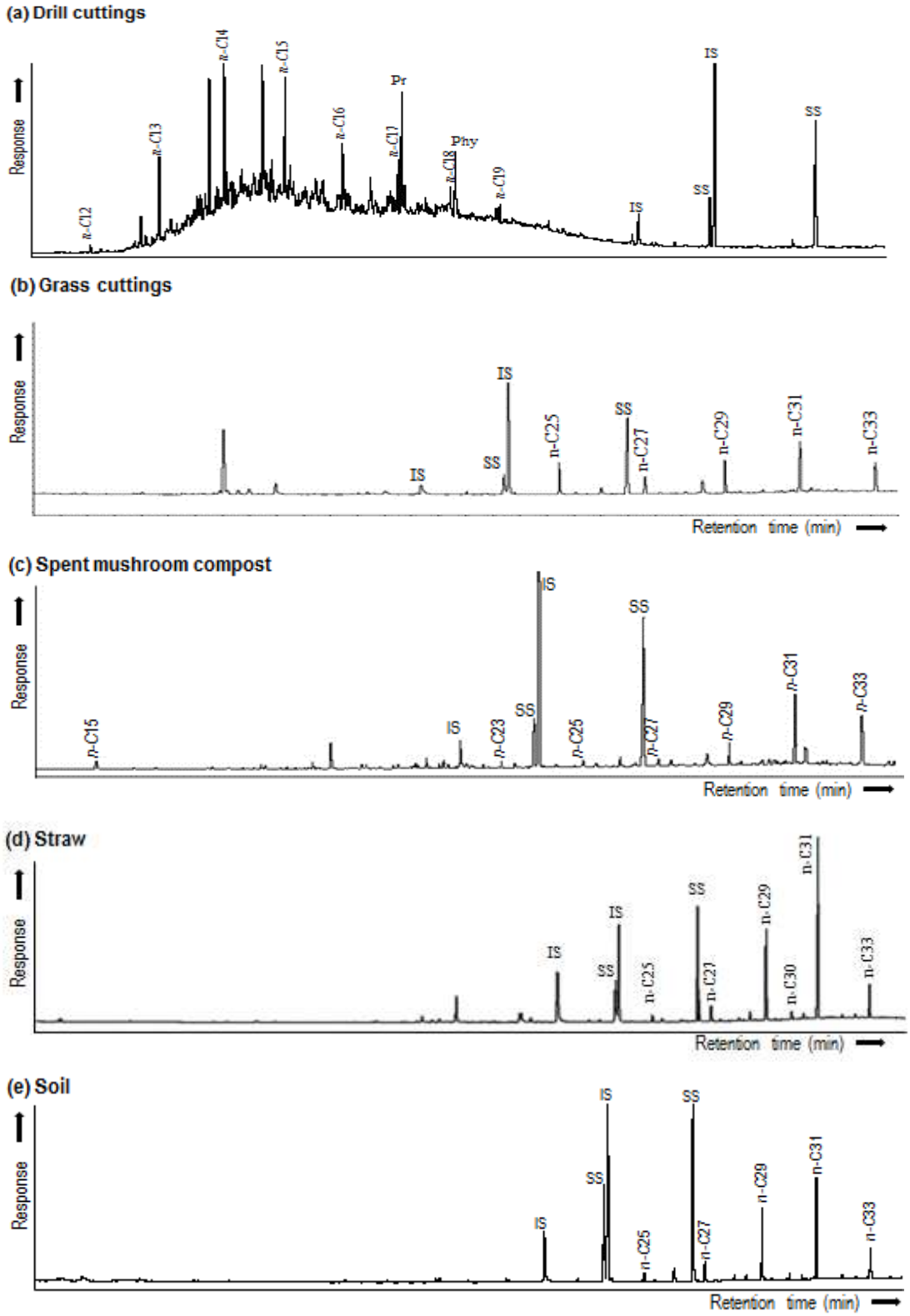


Figure 4.7. Chromatograms showing the distribution of n-alkanes in contaminated media and organic amendments used for formulating compost mixes.
IS = internal standard, SS = surrogate standard

Table 4.6. Changes of n-alkane and isoprenoid alkane concentrations in control and drill cuttings/grass cuttings/straw (DGS_t) mixes after composting for 53 days

	Control			DGS_t 1 mix			DGS_t 2 mix			DGS_t 3 mix		
	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate
Analyte	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**
nC_{12}	1.0±0.08	0.3±0.02	68.8	0.5±0.05	0.1±0.03	75.3	0.5±0.03	0.1±0.03	75.3	0.5±0.03	0.1±0.01	82.9
nC_{13}	11.7±1.3	4.7±0.7	59.7	6.9±0.4	0.5±0.1	90.8	5.0±0.3	0.5±0.1	90.8	4.7±0.3	0.2±0.02	96.4
nC_{14}	17.8±1.7	9.4±0.6	47.0	10.8±0.4	0.8±0.1	91.9	9.3±0.4	0.8±0.1	91.9	9.3±0.7	0.6±0.1	93.9
nC_{15}	15.7±1.1	8.2±0.2	47.5	8.8±0.2	0.5±0.1	94.1	7.9±0.3	0.5±0.1	94.1	7.1±0.5	0.4±0.05	94.4
nC_{16}	8.4±0.6	5.6±0.1	33.4	4.7±0.1	0.3±0.04	93.7	4.4±0.2	0.3±0.04	93.7	4.1±0.2	0.3±0.04	91.5
nC_{17}	6.1±0.6	4.6±0.1	45.1	3.9±0.1	0.4±0.01	89.7	3.5±0.1	0.3±0.03	92.5	3.2±0.2	0.4±0.03	88.8
Pristane	16.6±0.9	9.1±0.3	25.0	10.1±0.4	0.5±0.1	95.2	9.6±0.2	0.8±0.01	89.3	6.2±0.6	0.5±0.02	94.5
nC_{18}	3.9±0.2	3.4±0.1	31.3	2.6±0.05	0.3±0.05	86.8	2.2±0.1	0.2±0.06	90.8	1.9±0.2	0.3±0.02	86.6
Phytane	8.6±0.5	5.9±0.1	13.0	5.5±0.2	0.3±0.1	93.8	4.9±0.4	0.6±0.1	84.6	3.0±0.3	0.4±0.04	90.9
nC_{19}	2.1±0.2	1.9±0.04	7.1	1.2±0.1	0.1±0.01	91.7	1.1±0.08	0.1±0.01	91.7	1.0±0.1	0.1±0.01	90.8
nC_{25}	-	-	-	1.9±0.1	0.1±0.01	96.6	3.2±0.13	0.1±0.01	96.6	1.5±0.2	0.1±0.02	91.3
nC_{27}	-	-	-	2.0±0.1	0.1±0.02	96.9	3.3±0.26	0.1±0.02	96.9	2.2±0.2	0.2±0.02	93.1
nC_{29}	-	-	-	10.4±0.7	1.3±0.2	91.2	14.4±0.6	1.3±0.2	91.2	16.3±0.9	1.3±0.2	91.8
nC_{30}	-	-	-	0.6±0.05	0.1±0.03	80.8	0.7±0.1	0.1±0.03	80.8	1.0±0.04	0.1±0.01	92.8
nC_{31}	-	-	-	18.8±1.5	0.7±0.2	93.4	25.5±1.6	0.7±0.2	93.4	28.8±1.9	2.2±0.3	92.4
nC_{33}	-	-	-	6.3±0.6	0.3±0.04	95.8	7.1±.0.6	0.3±0.04	95.8	9.3±0.9	0.6±0.1	93.2
Σ (*n*-alkanes)	66.7	38.2	42.8	79.3	6.4	91.9	88.1	6.3	92.9	93.3	6.8	92.7
Phytane/*n*C17		0.73			1.58			1.45			1.30	
Pristane/*n*C18		0.46			1.36			1.56			0.64	

Values are mean (n=3) ± standard deviation; Control = drill cuttings sample without compost amendments; D = drill cuttings; G = grass cuttings; M = spent mushroom compost; S_t = straw; - = Not detected.

Table 4.7. Changes in n-alkane and isoprenoid alkane concentrations in drill cuttings/mushroom compost/straw (DMS_t) mixes after composting for 53 days

	DMS_t 1 mix			DMS_t 2 mix			DMS_t 3 mix		
	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate
Analyte	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**
nC_{12}	0.5±0.04	0.1±0.02	74.4	0.5±0.03	0.1±0.02	72.5	0.5±0.04	0.1±0.02	77.9
nC_{13}	6.8±0.2	0.5±0.1	92.5	5.2±0.2	0.5±0.1	90.2	4.7±0.3	0.5±0.1	88.8
nC_{14}	10.5±0.9	0.7±0.1	93.7	8.4±0.4	0.7±0.1	91.2	7.7±0.7	0.7±0.1	91.0
nC_{15}	9.3±0.4	0.5±0.1	94.3	7.3±0.4	0.5±0.1	93.3	7.0±0.4	0.6±0.1	91.9
nC_{16}	4.5±0.2	0.4±0.06	91.4	3.7±0.2	0.4±0.1	88.6	3.9±0.3	0.4±0.1	88.6
nC_{17}	3.8±0.1	0.2±0.02	94.9	2.8±0.1	0.3±0.01	96.8	2.4±0.2	0.2±0.01	90.6
Pristane	10.7±0.6	0.2±0.02	98.1	8.2±0.2	0.3±0.01	90.1	7.2±0.4	0.3±001	96.3
nC_{18}	2.4±0.1	0.2±0.01	90.3	1.8±0.1	0.3±0.01	94.9	1.6±0.1	0.3±0.01	84.0
Phytane	5.4±0.3	0.2±0.03	95.9	4.1±0.1	0.2±0.02	85.6	3.6±0.2	0.2±0.01	94.2
nC_{19}	1.0±0.1	0.1±0.02	88.9	1.0±0.1	0.1±0.003	88.6	1.0±0.1	0.1±0.02	89.0
nC_{23}	0.2±0.01	0.1±0.02	71.5	0.2±0.01	0.1±0.04	32.8	0.3±0.02	0.1±0.03	73.8
nC_{25}	0.6±0.03	0.1±0.02	87.7	0.8±0.03	0.1±0.01	87.4	0.9±0.05	0.1±0.02	88.3
nC_{27}	1.2±0.1	0.2±0.02	88.6	1.5±0.1	0.1±0.03	90.1	1.7±0.08	0.1±0.01	91.7
nC_{29}	6.8±0.5	0.3±0.03	95.2	8.2±0.6	0.9±0.3	88.5	9.3±0.7	1.1±0.01	87.7
nC_{30}	0.5±0.03	0.1±0.01	80.7	0.8±0.03	0.1±0.01	91.0	0.8±0.06	0.1±0.04	86.2
nC_{31}	14.5±1.0	0.9±0.05	93.8	19.5±0.6	1.4±0.03	93.0	21.7±2.3	1.1±0.1	94.9
nC_{33}	4.6±0.4	1.5±0.01	66.6	6.2±0.3	2.2±0.3	65.3	6.8±0.5	1.9±0.1	72.8
Σ (*n*-alkanes)	67.1	5.9	91.3	68.2	7.9	88.4	70.4	7.5	89.4
Phytane/*n*C17		1.78			1.79			1.83	
Pristane/*n*C18		1.31			1.46			1.48	

Values are mean (n=3) ± standard deviation; D = drill cuttings; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Table 4.8. Changes in n-alkane and isoprenoid alkane concentrations in drill cuttings/grass cuttings/mushroom compost/straw ($DGMS_t$) mixes after composting for 53 days

	$DGMS_t$ 1 mix			$DGMS_t$ 2 mix			$DGMS_t$ 3 mix		
	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate	Amount (µg/g of dry sample)		Removal rate
Analyte	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**
nC_{12}	0.4±0.03	0.1±0.01	66.8	0.5±0.04	0.1±0.02	73.1	0.4±0.03	0.1±0.02	75.5
nC_{13}	6.3±0.2	0.3±0.04	90.1	4.9±0.4	0.6±0.1	88.6	3.8±0.2	0.3±0.04	93.0
nC_{14}	9.6±0.6	0.5±0.01	92.9	8.2±0.7	0.7±0.1	91.8	7.7±0.6	0.8±0.1	89.8
nC_{15}	8.5±0.7	0.4±0.01	91.7	7.3±0.7	0.6±0.1	92.0	5.9±0.5	0.7±0.1	88.7
nC_{16}	4.9±0.3	0.3±0.04	94.8	4.2±0.3	0.4±0.05	90.3	3.4±0.3	0.2±0.03	95.6
nC_{17}	3.1±0.1	0.3±0.04	91.3	2.8±0.2	0.3±0.03	89.8	2.3±0.05	0.2±0.01	93.2
Pristane	8.6±0.5	0.3±0.05	96.0	7.5±0.6	0.4±0.1	95.2	6.8±0.3	0.2±0.02	96.8
nC_{18}	2.0±0.2	0.3±0.04	87.2	1.8±0.1	0.3±0.02	85.1	1.6±0.05	0.2±0.02	86.2
Phytane	4.4±0.3	0.3±0.03	94.3	3.9±0.2	0.2±0.03	94.9	3.5±0.1	0.2±0.01	94.6
nC_{19}	1.2±0.1	0.1±0.01	91.5	1.0±0.05	0.1±0.03	85.5	1.0±0.05	0.1±0.001	93.8
nC_{23}	0.2±0.01	0.1±0.03	33.1	0.2±0.02	0.1±0.01	36.8	0.1±0.01	0.1±0.02	35.5
nC_{25}	1.2±0.1	0.1±0.01	92.8	1.7±0.1	0.1±0.01	94.2	2.2±0.1	0.1±0.01	97.3
nC_{27}	1.3±0.1	0.1±0.02	91.3	2.2±0.1	0.1±0.02	93.5	2.3±0.1	0.1±0.01	97.0
nC_{29}	8.2±0.6	0.3±0.05	96.2	10.0±0.6	0.4±0.05	95.9	13.5±0.6	0.2±0.04	98.2
nC_{30}	0.5±0.02	0.01±0.02	84.6	0.7±0.05	0.2±0.04	70.3	0.8±0.1	0.02±0.004	98.0
nC_{31}	14.9±1.4	0.8±0.1	94.7	20.7±1.5	1.1±0.2	94.8	22.3±1.6	0.6±0.05	97.7
nC_{33}	4.8±0.3	0.9±0.1	82.1	6.2±0.3	1.1±0.05	82.7	7.8±0.3	0.7±0.1	90.5
Σ (*n*-alkanes)	67.2	5.5	91.8	72.5	6.2	91.4	77.4	4.2	94.6
Phytane/nC_{17}		1.53			1.45			1.51	
Pristane/nC_{18}		1.21			1.4			1.33	

Values are mean (n=3) ± standard deviation; D = drill cuttings, G = grass cuttings; M = spent mushroom compost; S_t = straw.

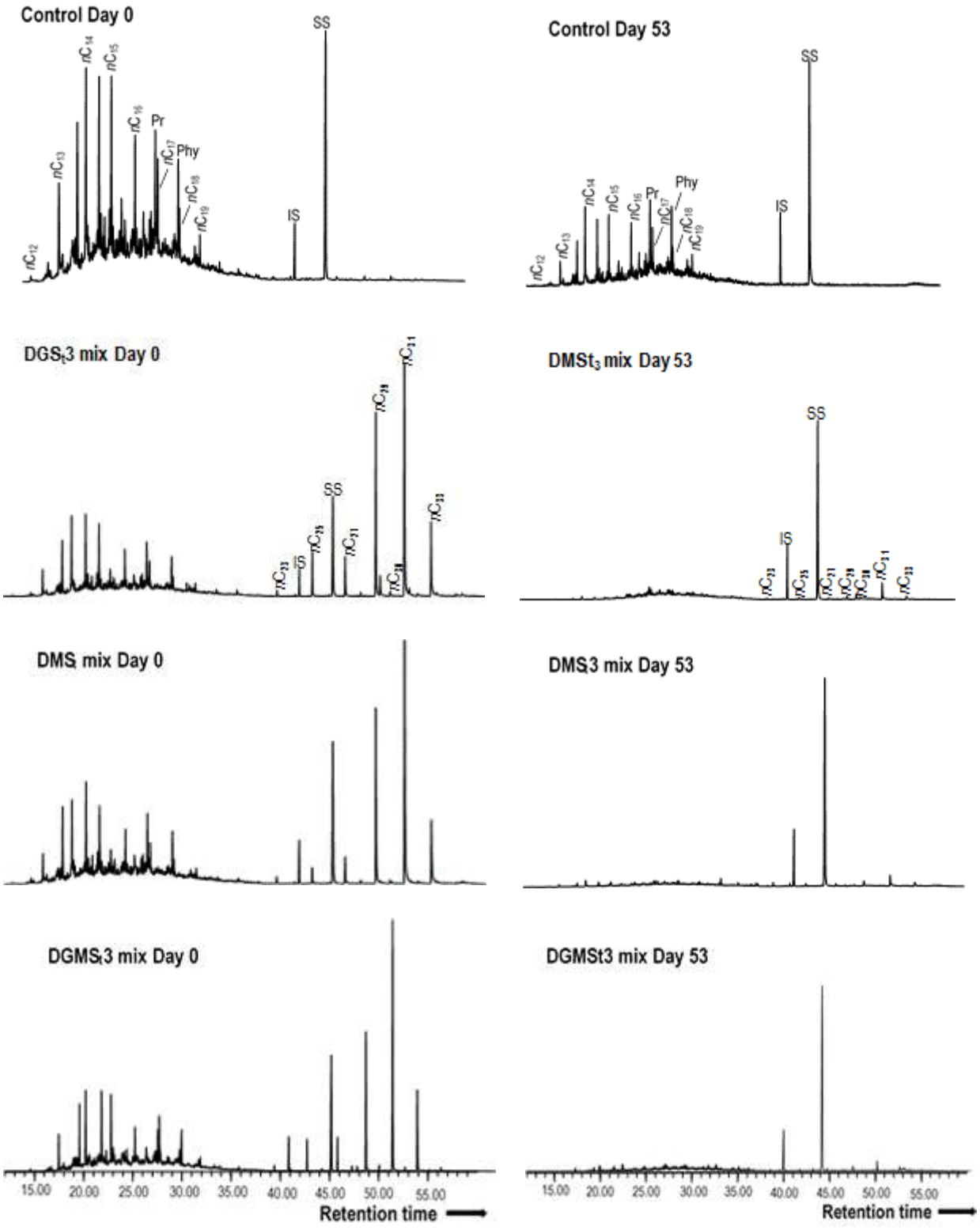


Figure 4.8. Mass chromatograms (m/z 85) of representative samples of compost mixes with drill cuttings saturated hydrocarbon fractions.
Control = drill cuttings without compost amendments; S = CTIS, G = grass cuttings, M = mushroom compost, S_t = straw, SS = surrogate standard, IS = internal standard.

4.2.7 *Degradation of n-alkanes and isoprenoid alkanes in compost mixes with CTIS*

The distribution of *n*-alkanes for the day 0 compost mixes with CTIS contain *n*-alkanes ranging from nC_{15} to nC_{33} with bimodal distributions and strong odd over even carbon number predominance as mentioned earlier (see Tables 4.9, 4.10 and 4.11). The *n*-alkane distributions in respective mix types are effectively derived solely from the individual organic amendments making up the mix as there were barely detectable *n*-alkanes in the coal tar sample used for spiking the soil. It can be observed that low molecular weight *n*-alkanes (nC_{12}- nC_{19}) are conspicuously absent in the distributions except for nC_{15} which is derived from the straw sample.

Analysis of the degradation of the alkanes in each compost mix revealed significant ($p<0.05$ *t*-test) removals of $\Sigma_{n\text{-alkanes}}$ after composting treatment for 53 days. The SGS_t1, SGS_t2 and SGS_t3 mix types achieved $\Sigma_{n\text{-alkanes}}$ removals of 93, 95 and 94% respectively (Table 4.9), while the SMS_t1, SMS_t2 and SMS_t3 mix types recorded $\Sigma_{n\text{-}}$alkanes removals of 92, 93 and 94% respectively (Table 4.10). Similar significant removals were also recorded in the $SGMS_t1$, $SGMS_t2$ and $SGMS_t3$ mix types in which $\Sigma_{n\text{-alkanes}}$ reductions of 89, 84 and 94%, respectively, were achieved (Table 4.11).

Concentrations of individual *n*-alkanes present in the respective compost mixes were also found to be significantly ($p<0.05$, *t*-test) lower at day 53. The average maximum and minimum *n*-alkanes removals in the SGS_t samples occurred in nC_{31} and nC_{30} which reduced by 97% and 87%, respectively (Table 4.9). The nC_{30} and nC_{23} *n*-alkanes recorded the average maximum and minimum removals of 96% and 40 % and, 95% and 46% in the SMS_t (Table 4.10) and $SGMS_t$ (Table 4.11) mix types, respectively. The degradation of nC_{23} *n*-alkane was found the least in the SMS_t and $SGMS_t$ mixes, respectively, which is possibly because it is less accessible to microbial utilisation due to lower abundance and solubility compared to the other *n*-alkanes present. Based on these results, degradation of the *n*-alkanes in the compost mixes with organic amendments was found to occur in the order of SMS_t>SGS_t> $SGMS_t$, after treatment for 53 days which is consistent with the TPH reduction trend for the CTIS. Graphical presentation of *n*-alkane concentrations are shown in Appendices O - W. Figure 4.9 shows m/z 85 mass chromatograms of the saturated hydrocarbon fractions of representative treatment samples with CTIS.

Table 4.9. Changes in n-alkane concentrations in coal tar impacted soil/grass cuttings/straw (SGS_t) mixes after composting for 53 days

	SGS_t 1 mix			**SGS_t 2 mix**			**SGS_t 3 mix**		
	Amount (µg/g of dry sample)		**Removal rate**	**Amount (µg/g of dry sample)**		**Removal rate**	**Amount (µg/g of dry sample)**		**Removal rate**
Analyte	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**
nC_{25}	1.7±0.1	0.2±0.04	88.5	2.6±0.1	0.2±0.03	93.0	2.4±0.1	0.2±0.04	92.2
nC_{27}	2.4±0.2	0.3±0.05	85.2	2.7±0.2	0.2±0.04	92.3	4.0±0.3	0.2±0.02	94.5
nC_{29}	11.1±0.7	1.4±0.2	87.1	13.2±1.3	1.2±0.1	90.8	14.9±0.5	1.6±0.3	89.0
nC_{30}	0.8±0.04	0.1±0.04	84.8	1.1±0.1	0.1±0.03	87.8	1.2±0.1	0.1±0.03	87.8
nC_{31}	23.1±2.2	0.8±0.1	96.3	26.8±0.2	0.7±0.1	97.3	30.0±1.9	0.8±0.1	97.4
nC_{33}	5.5±0.1	0.3±0.05	94.3	6.1±0.1	0.3±0.05	94.9	7.0±0.2	0.4±0.06	94.8
Σ (*n*-alkanes)	44.8	3.3	92.7	51.5	2.7	94.7	59.3	3.3	94.4

Values are means (n=3) ± standard deviation; S = CTIS, G = grass cuttings; M = spent mushroom compost; S_t = straw.

Table 4.10. Changes in n-alkane concentrations in coal tar impacted soil/mushroom compost/straw (SMS_t) mixes after composting for 53 days

	SMS_t 1 mix			**SMS_t 2 mix**			**SMS_t 3 mix**		
	Amount (µg/g of dry sample)		**Removal rate**	**Amount (µg/g of dry sample)**		**Removal rate**	**Amount (µg/g of dry sample)**		**Removal rate**
Analyte	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**
nC_{15}	0.3±0.02	0.1±0.01	81.0	0.2±0.03	0.1±0.01	78.2	0.4±0.03	0.03±0.004	88.2
nC_{23}	0.1±0.04	0.1±0.01	40.1	0.1±0.01	0.1±0.02	20.1	0.2±0.01	0.1±0.01	58.7
nC_{25}	0.7±0.04	0.09±0.01	88.9	0.7±0.05	0.1±0.01	88.3	1.1±0.04	0.1±0.02	93.2
nC_{27}	1.8±0.2	0.1±0.03	92.3	1.8±0.1	0.2±0.04	90.1	2.3±0.2	0.1±0.01	93.9
nC_{29}	10.1±0.8	1.5±0.2	84.9	11.2±1.4	1.4±0.1	87.9	12.4±1.0	1.2±0.2	90.1
nC_{30}	0.81±0.1	0.04±0.004	95.1	1.1±0.05	0.04±0.01	96.2	1.0±0.1	0.04±0.004	96.7
nC_{31}	21.2±1.9	0.9±0.1	95.9	22.7±3.5	0.9±0.1	95.8	26.4±1.4	1.1±0.2	95.8
nC_{33}	5.7±0.3	0.3±0.03	93.8	7.0±0.9	0.4±0.06	94.5	7.7±0.7	0.4±0.1	95.0
Σ (*n*-alkanes)	39.8	3.1	92.2	43.4	3.1	92.9	51.5	3.1	94.0

Values are means (n=3) ± standard deviation; S = CTIS, G = grass cuttings, M = spent mushroom compost, S_t = straw.

Table 4.11. Changes in in n-alkane concentrations in coal tar impacted soil/grass cuttings/mushroom compost/straw ($SGMS_t$) mixes after composting for 53 days

	$SGMS_t$ 1 mix			**$SGMS_t$ 2 mix**			**$SGMS_t$ 3 mix**		
	Amount (µg/g of dry sample)		**Removal rate**	**Amount (µg/g of dry sample)**		**Removal rate**	**Amount (µg/g of dry sample)**		**Removal rate**
Analyte	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**	**day 0**	**day 53**	**(%)**
nC_{15}	0.1±0.01	0.03±0.004	72.8	0.1±0.01	0.08±0.01	42.0	0.1±0.01	0.08±0.01	88.2
nC_{23}	0.04±0.003	0.03±0.01	19.0	0.1±0.01	0.05±0.01	58.8	0.1±0.004	0.06±0.01	58.7
nC_{25}	1.1±0.04	0.1±0.01	91.9	1.6±0.1	0.2±0.04	87.6	1.7±0.1	0.1±0.01	93.2
nC_{27}	2.0±0.1	0.2±0.06	88.8	2.0±0.2	0.2±0.04	89.2	2.2±0.3	0.1±0.03	93.9
nC_{29}	10.8±0.7	2.0±0.3	81.8	11.2±1.0	2.3±0.2	79.6	12.4±1.1	1.2±0.1	90.1
nC_{30}	0.8±0.02	0.4±0.004	95.3	1.0±0.1	0.1±0.01	94.3	0.5±0.03	0.04±0.01	96.7
nC_{31}	19.3±2.0	1.1±0.2	94.5	22.9±2.1	2.7±0.5	88.3	26.7±2.2	1.1±0.2	95.8
nC_{33}	4.7±0.4	0.9±0.1	81.5	6.3±0.6	1.3±0.3	80.3	7.1±0.6	0.4±0.1	95.0
Σ (*n*-alkanes)	38.8	4.3	88.9	45.3	6.8	84.9	50.7	3.2	93.7

Values are means (n=3) ± standard deviation; S = CTIS, G = grass cuttings, M = spent mushroom compost, S_t = straw.

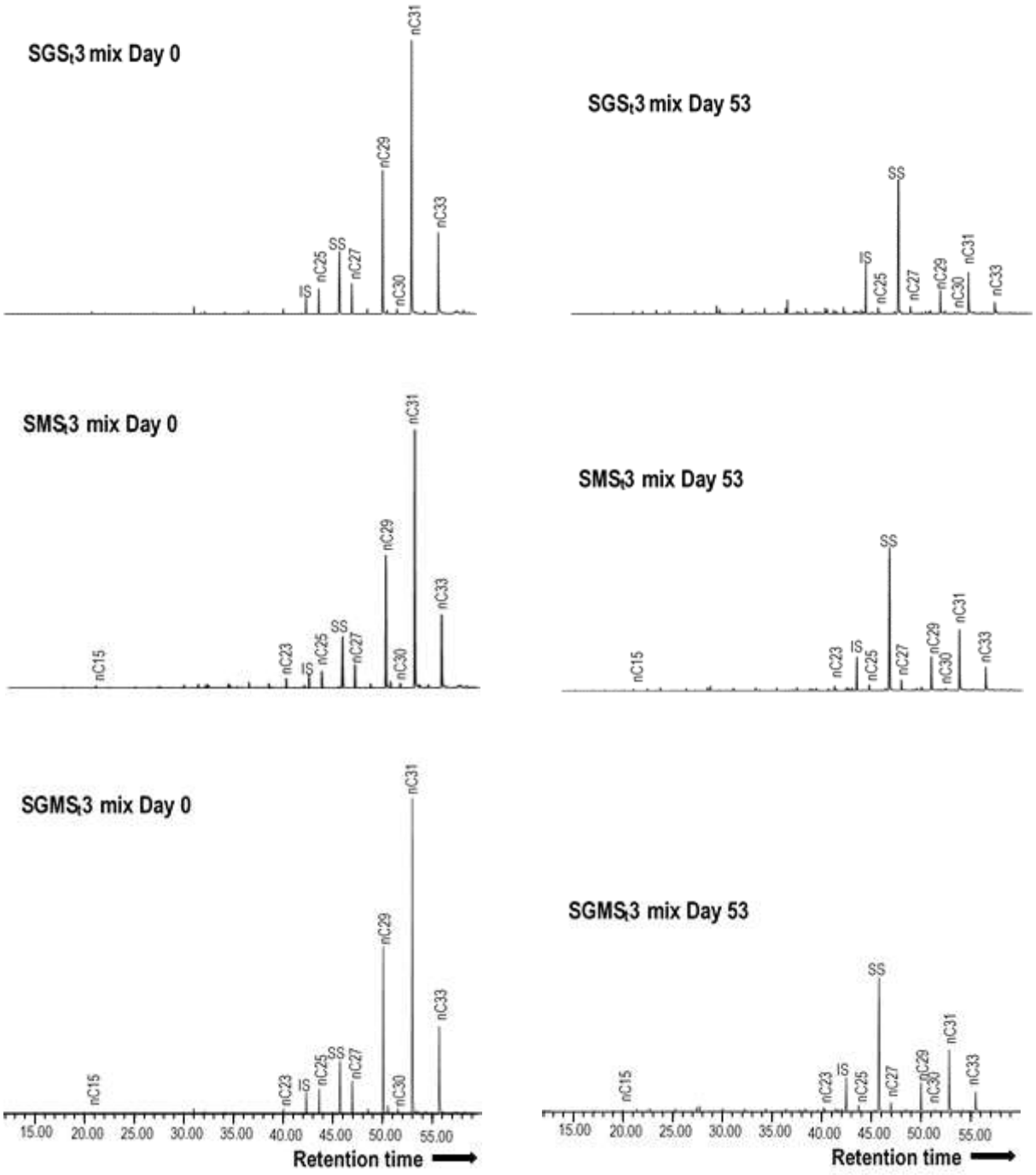


Figure 4.9. Mass chromatograms (m/z 85) of saturated hydrocarbon fractions in representative samples of compost mixes with coal tar impacted soil (CTIS).
Control = CTIS without compost amendments; S = CTIS, G = grass cuttings, M = mushroom compost, S_t = straw, SS = surrogate standard, IS = internal standard.

4.2.8 *Degradation of polycyclic aromatic hydrocarbons (PAHs) in compost mixes with CTIS*

Identification and quantification of the concentrations of coal tar PAHs in each of the compost mixes with CTIS was performed based on peak relative retention times compared with standards and mass spectral library searches. PAHs in the soil and each of the organic amendment samples were determined prior to use for preparing the compost mixes. A total of 21 PAHs (including 13 of the 16 priority EPA PAHs) were identified and measured in the agricultural soil sample with a total concentration of 0.6 µg/g (Table 4.12). The grass cuttings were found to contain 21 PAHs, including 13 of the 16 priority EPA PAHs, in concentrations totalling 4.5 µg/g. This high number of PAH traces in the grass cuttings may be attributed the high anthropogenic activities, e.g. vehicular traffic emissions, in the city environment of the Newcastle university campus where the grass cuttings sample were collected. PAHs from vehicular exhaust and tobacco smoke can accumulate on the soil where the grass grows (e.g. Menzie *et al*., 1992) and presumably onto the grass itself.

Analysis of the spent mushroom compost (SMC) sample revealed low quantities of 14 PAHs, including 10 of the 16 priority EPA PAHs, with total concentration of 0.2 µg/g. Wide varieties of aromatic compounds are found as natural products in SMC. Reported studies have suggested they are probably breakdown products of lignocellulose of the straw compost for cultivation of mushrooms and/or the metabolites of the mushroom (Lau *et al*., 2003; Chiu *et al*., 1998). The SMC sample used for this study (Mr. Mucks Mushroom Compost) is a mix of chicken and horse manure with wheat straw. The straw sample was found to contain the least amount of total PAH concentration (0.07 µg/g), with only 4 of the 16 priority EPA PAHs detectable at very low concentrations. This may be attributed to the environmental condition in the growing area, which is an organic agricultural farm field.

PAH concentrations at the beginning and end of the 53 day compost treatment and percent reduction for each treatment mix types are shown in Tables 4.13, 4.14, 4.15 and 4.16.

Table 4.12. Distributions of polycyclic aromatic hydrocarbons (PAHs) in coal tar impacted soil (CTIS) and compost amendments used for preparing compost mixes

	Soil	Grass cuttings	SMC	Straw
PAH analyte	**Amount (µg/g of dry weight)**			
Naphthalene (m/z: 128)	0.02±0.001	0.1±0.001	0.02±0.001	-
Acenaphthylene (m/z: 152)	-	-	-	-
Fluorene (m/z: 166)	-	-	-	-
Phenanthrene (m/z: 178)	0.1±0.004	0.3±0.02	0.01±0.001	0.03±0.001
Anthracene (m/z: 178)	0.01±0.001	0.04±0.002	0.003±0.0002	0.003±0.0003
Fluoranthene (m/z: 202)	0.1±0.001	0.6±0.02	0.02±0.001	0.02±0.002
Pyrene (m/z: 202)	0.1±0.001	0.5±0.002	0.03±0.002	0.01±0.001
Benz[a]anthracene (m/z: 228)	0.03±0.002	0.3±0.02	0.01±0.0003	-
Chrysene (m/z: 228)	0.1±0.001	0.4±0.02	0.02±0.0002	-
Benzo[b]fluoranthene (m/z: 252)	0.1±0.001	0.4±0.01	0.02±0.001	-
Benzo[k]fluoranthene (m/z: 252)	0.02±0.001	0.2±0.01	0.01±0.001	-
Benzo[j]fluoranthene (m/z: 252)	0.02±0.0003	0.2±0.01	0.01±0.0003	-
Benzo[a]fluoranthene (m/z: 252)	0.01±0.001	0.1±0.003	0.01±0.0003	-
Benzo[e]pyrene (m/z: 252)	0.1±0.001	0.3±0.02	0.01±0.001	-
Benzo[a]pyrene (m/z: 252)	0.03±0.001	0.3±0.02	0.01±0.001	-
Perylene (m/z: 252)	0.0±0.0004	0.1±0.003	0.003±0.0002	-
Benzo[b]triphenyle (m/z:278)	0.003±0.0004	0.04±0.003	-	-
Dibenzo[a,h]anthracene (m/z: 278)	0.003±0.0003	0.1±0.003	-	-
Benzo[b]chrysene (m/z:278)	0.004±0.0002	0.04±0.001	-	-
Indeno[1,2,3-cd]pyrene (m/z:276)	0.0±0.0002	0.3±0.02	-	-
Picene (m/z: 276)	0.002±0.0001	0.01±0.001	-	-
Benzo[g,h,i]perylene (m/z: 276)	0.04±0.002	0.3±0.01	-	-
Anthanthrene (m/z: 276)	0.003±0.0002	0.04±0.005	-	-
Total PAHs	0.6	4.5	0.2	0.07

Values are means (n=3) ± standard deviation; SMC = spent mushroom compost, - = not detected.

Percent total PAH was found to be significantly ($p<0.05$) reduced in each of the compost mix types, as well as the control, at the end of the composting period. The PAH totals were significantly reduced by 61.6, 60.9 and 63.8% in the SGS_t1, SGS_t2 and SGS_t3 mix types, respectively. In the SMS_t1, SMS_t2 and SMS_t3 mix types, total PAH reduction of 63.6, 67.3 and 66.1%, respectively, was achieved, while the $SGMS_t1$, $SGMS_t2$ and $SGMS_t3$ mix types produced total PAH reductions of 65.7, 47.4. and 59.1%, respectively. Total PAH was also significantly ($p=0.004$ *t*-test) reduced by 41.4% in the control, but the reduction was significantly ($p<0.001$ ANOVA) lower in relation to the reduction achieved in each of the compost mixes with organic amendments. This suggests that the compost amendments contribute significantly to

the depletion of total PAH during the treatment process. Comparing the removal of PAHs in all the treatment samples showed the mean reduction of total PAHs in the order of SMS_t> SGS_t> $SGMS_t$, suggesting the SMS_t compost mixes are the most effective.

The resolved PAHs under investigation were grouped as 2 and 3-ring PAHS, 4-ring PAHs and 5 and 6-ring PAHs and thus defined as small, medium and large molecular weight PAHs. The 2- and 3-ring PAHs comprising naphthalene, acenaphthylene, fluorine, phenanthrene and anthracene were the most abundant as well as the most degraded in all compost mix types. They made up 50.7% to 53.7% of the total PAH in the compost mixes and control samples treated (Tables 4.13, 4.14, 4.15 and 4.16). Statistically significant ($p<0.001$ *t*-test) decreases in sum of 2 and 3-ring ($\Sigma_{2 \text{ and } 3\text{-ring}}$) PAH of 95.7, 96.6 and 96.3% were achieved in the SGS_t1, SGS_t2 and SGS_t3 mix types, respectively (Table 4.14). The $\Sigma_{2 \text{ and } 3\text{-ring}}$ PAHs were also significantly ($p<0.001$ t-test) reduced by 97.9, 98.1 and 98.2% in the SMS_t1, SMS_t2 and SMS_t3 mix types (Table 4.15), respectively, and by 97.4, 94.8 and 96.5% in the $SGMS_t1$, $SGMS_t2$ and $SGMS_t3$ mix types, respectively (Table 4.16).

The 4-ring PAHs analysed comprised of fluoranthene, pyrene, benz[a]anthracene and chrysene. Comparing the sum of 4-ring ($\Sigma_{4\text{-ring}}$) PAHs at the start and end of the composting experiment showed significant reductions in each of the compost mixes with organic amendments (Tables 4.14, 4.15 and 4.16). The $\Sigma_{4\text{-ring}}$ PAHs were significantly reduced by 29.4% ($p=0.001$ *t*-test), 36.8% ($p=0.001$ *t*-test) and 46.2% ($p=0.001$ *t*-test) in the SGS_t1, SGS_t2 and SGS_t3 mix types, respectively (Table 4.14). Similar reductions of 30.4% ($p=0.003$), 42.8% ($p=0.001$) and 39.3% ($p=0.004$) were recorded in the SMS_t1, SMS_t2 and SMS_t3 mix types, respectively (Table 4.15). The $\Sigma_{4\text{-ring}}$ PAHs were also significantly reduced by 45.5% ($p=0.001$) and 33.3% ($p=0.015$) in the $SGMS_t1$ and $SGMS_t3$ mix types, respectively (Table 4.16). Reduction of $\Sigma_{4\text{-ring}}$ PAHs was, however, not significant ($p=0.283$) in the $SGMS_t2$ mix type (7.5%).

The measured resolved large PAHs (5 and 6-rings) are more in number than were measured for the other groups and were comprised of benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[a]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, benzo[b]triphenylene, dibenzo[a,h]anthracene, benzo[b]chrysene, indeno[1,2,3-cd]pyrene, picene, benzo[g,h,i]perylene, and

anthanthrene. Reduction in the summed concentrations of the 5 and 6-ring ($\Sigma_{5 \text{ and } 6\text{-ring}}$) PAHs varied from 1.8% to 24.1% and was significant ($p<0.05$) in all compost mixes with organic amendments except for $SGMS_t2$ (1.8%, p=0.779 *t*-test) and $SGMS_t$ 3 (11.7%, p=0.156 *t*-test) mix types at day 53 (Table 4.16). The $\Sigma_{2 \text{ and } 3\text{-ring}}$ PAHs were significant decreased by 70.2% (p=0.001 *t*-test) in the control after the composting period, but this was significantly lower compared to the compost mixes with organic amendments. Reduction of $\Sigma_{4\text{-ring}}$ and $\Sigma_{5 \text{ and } 6\text{-ring}}$ PAHs was not significant in the control (7.4%; p=0.512) and (6.9%; p=0.24), respectively.

Regarding the mean reductions of the PAH groups, the $\Sigma_{2 \text{ and } 3\text{-ring}}$ reduced by 96.2, 98 and 96.3%, $\Sigma_{4\text{-ring}}$ reduced by 37.4, 37.5 and 28.8%, and $\Sigma_{5 \text{ and } 6\text{-ring}}$ reduced by 16.4, 21 and 10.6% in the compost mix types comprising SGS_t, SMS_t, and $SGMS_t$, respectively (Tables 4.14, 4.15 and 4.16). This PAH group reduction trend is consistent with reports from earlier studies which showed that the ease of removals decreased in the order of $\Sigma_{2 \text{ and } 3\text{-ring}} > \Sigma_{4\text{-ring}} > \Sigma_{5 \text{ and } 6\text{-ring}}$ (Amir *et al*., 2005; Moretto *et al*., 2005; Antzar-Ladislao *et at*., 2006). Also, comparing the overall reduction of PAH groups among the different compost mix types comprising SGS_t, SMS_t, and $SGMS_t$, revealed reduction effectiveness in the order of $SMS_t > SGS_t, > SGMS_t$, which is consistent with total PAH removal trend.

Concentrations of the individual PAHs in the compost mix samples were not uniformly distributed, as some were degraded significantly while others posed resistance to degradation after treatment for 53 days. The coal tar impacted soil compost treatment experiment resulted in high percentage depletion of most resolved 2 and 3-ring PAH compounds, as shown in Figure 4.10. Increased concentrations of higher molecular weight PAH analytes are seen in the day 53 composted samples (Tables 4.13, 4.14, 4.15 and 4.16). The reasons for this are uncertain, but may be due to changes in the characteristics of the compost matrices due to microbial activities during the composting process, perhaps resulting in higher PAH availability for extraction during analyses.

At the end of the compost treatment, concentration reductions showed that naphthalene (98.1% to 99.5%), acenaphthylene (96.6% to 98.3%) and fluorene (79.4% to 99.3%) were the most degraded in the compost mixes and sterile control samples (Tables 4.13, 4.14, 4.15 and 4.16).. This indicates abiotic removal probably due to

volatilisation (e.g. Taylor and Jones, 2001). Nonetheless, naphthalene has been described as the most volatile and biodegradable PAH (Kriipsalu *et al*., 2008). However, naphthalene degradation is known to be enhanced by temperatures of 20-30 ^{o}C and aerobic conditions (Bauer and Capone, 1985) which were possibly created during the incubation process in this present work.

Phenanthrene and anthracene were significantly ($p<0.05$ *t*-test) degraded in the organic amended compost mixes. Mean reductions of phenanthrene by 92.4, 97.8 and 93.9% was recorded in the SGS_t, SMS_t and $SGMS_t$ compost mix types, respectively (Tables 4.14, 4.15 and 4.16). But this was not the case with the control which recorded 19.2% depletion of phenanthrene (Table 4.13). Similarly, 90.2, 89.6 and 87.3% mean reductions were recorded for anthracene in the SGS_t, SMS_t and $SGMS_t$ compost mix types, respectively, while a 43.3% reduction was recorded in the control.

Fluoranthene and pyrene (4-ring PAH compounds) were found to significantly ($p<0.05$) degraded in all the compost mixes with organic amendments. Mean percent reductions of 60.1, 51.6, and 50.8% for fluoranthene and 46.6, 53.4 and 41.5% for pyrene was recorded in the SGS_t, SMS_t and $SGMS_t$ compost mix types respectively (Tables 4.14, 4.15 and 4.16), but depletions of 18.9% ($p=0.123$) for fluoranthene and 3.4% ($p=0.537$) for pyrene was observed in the control (Table 4.13). Benz[a]anthracene and chrysene recorded varying degrees of increased concentrations in the organic amended compost mixes and control after the treatment period.

Most of the resolved 5 and 6-ring PAH compounds exhibited increased concentrations in the organic amended compost mixes except benzo[a]fluoranthene, benzo[a]pyrene and perylene which recorded varying degree of depletions ranging from 15% to 40.6%, 12% to 33.7% and 12.8% to 30.9%, respectively, in the control, SGS_t, SMS_t and $SGMS_t$ compost mix types respectively. Only four PAHs (benzo[k]fluoranthene, benzo[e]pyrene, dibenzo[a,h]anthracene and benzo[b]chrysene) out of the 14 resolved 5 and 6-ring PAHs were found to exhibit increased concentrations in the control (Table 4.13). This may be due to sample heterogeneity or perhaps their increased extractability in the day 53 samples whose organic matter content have been substantially reduced (Potter *et al*., 1999;

Ahtiainen *et al*., 2002; Cai *et al*., 2007). Additionally, a reported study has attributed increase in PAH concentration during composting to selective biodegradation in favour of the green waste component against soil organic matter, which may result to change in the ratio of soil to green waste in the mixture as well as calculation of the concentration of PAHs in the mixture (Antizar-Ladislao *et al*., 2005).

Difficulty with biodegradation of HMW PAHs under laboratory treatment conditions similar to results from this present work have been reported in other studies and have been attributed to their low water solubility and limited release from soil sequestration into aqueous media (Potter *et al*., 1999; Antizar-Ladislao *et al*., 2004) and toxicity to soil organisms (Sverdrup *et al*., 2002). Different strategies which have been developed to increase the bioavailability of HMW PAHs include high temperature (>70 ^{o}C) composting condition (Feitkenhauer *et al*., 2003), application of surfactant (Zheng and Obbard, 2002), or combined treatment with specialised PAH degrading microorganisms (Canet *et al*., 2001). Composting at high temperature is the current regulatory requirement (EC 2003) for pathogen control. However, such high temperatures have been reported to severely limit the microbial diversity, as well as, the enzymatic potential of composting systems (Antizar-Ladislao *et al*., 2006). Appendices X – AG present graphical variation of PAHs concentrations in the compost mixes with CTIS at start and end of the lab scale composting experiment.

Table 4.13. Variation of PAHs concentration in coal tar impacted soil (CTIS - control) at start and end of lab-scale compost-bioremediation treatment

PAH analyte (and measured ion)	Ring number	Control (µg/g of dry sample) day 0	Control (µg/g of dry sample) day 53	Reduction (%)
Naphthalene (M/Z: 128)	2	10.73±0.3	0.2±0.02	98.1
Acenaphthylene (M/Z: 152)	3	5.19±0.2	0.16±0.02	97.0
Fluorene (M/Z: 166)	3	1.88±0.1	0.39±0.05	79.4
Phenanthrene (M/Z: 178)	3	8.00±0.4	6.46±0.8	19.2
Anthracene (MZ: 178)	3	1.76±0.1	1.00±0.1	43.3
Fluoranthene (M/Z: 202)	4	5.87±0.3	4.76±0.5	18.9
Pyrene (MZ: 202)	4	4.81±0.2	4.64±0.6	3.4
Benz[a]anthracene (M/Z: 228)	4	1.66±0.1	1.81±0.3	-8.8
Chrysene (M/Z: 228)	4	1.61±0.1	1.71±0.3	-6.2
Benzo[b]fluoranthene (m/z: 252)	5	1.36±0.1	1.26±0.2	7.2
Benzo[k]fluoranthene (m/z: 252)	5	0.84±0.1	0.93±0.1	-11.6
Benzo[j]fluoranthene (m/z: 252)	5	0.83±0.04	0.83±0.1	0.1
Benzo[a]fluoranthene (m/z: 252)	5	0.46±0.03	0.38±0.04	18.2
Benzo[e]pyrene (m/z: 252)	5	1.24±0.04	1.27±0.2	-1.9
Benzo[a]pyrene (m/z: 252)	5	1.63±0.1	1.41±0.2	12.9
Perylene (m/z: 252)	5	0.48±0.02	0.38±0.02	21.2
Benzo[b]triphenyle (m/z:278)	5	0.12±0.01	0.12±0.02	4.4
Dibenzo[a,h]anthracene (m/z: 278)	5	0.17±0.01	0.18±0.03	-7.4
Benzo[b]chrysene (m/z:278)	5	0.15±0.01	0.16±0.02	-5.0
Indeno[1,2,3-cd]pyrene (m/z:276)	6	0.96±0.1	0.94±0.2	2.6
Picene (m/z: 276)	6	0.10±0.01	0.08±0.008	22.0
Benzo[g,h,i]perylene (m/z: 276)	6	1.23±0.04	0.99±0.1	19.5
Anthanthrene (m/z: 276)	6	0.20±0.01	0.17±0.02	16.6
Total PAHs		51.3	30.2	41.1

Values are means (n=3) ± standard deviation; Control = CTIS without compost amendments; Negative value mean increase in concentration.

Table 4.14. Variation of PAH concentrations in coal tar impacted soil/grass cuttings/straw (SGS_t) compost mix types after lab-scale compost-bioremediation for 53 days

PAH analyte	Ring number	SGS_t1 mix type (µg/g of dry sample) day 0	SGS_t1 mix type (µg/g of dry sample) day 53	SGS_t1 Reduction (%)	SGS_t2 mix type (µg/g of dry sample) day 0	SGS_t2 mix type (µg/g of dry sample) day 53	SGS_t2 Reduction (%)	SGS_t3 mix type (µg/g of dry sample) day 0	SGS_t3 mix type (µg/g of dry sample) day 53	SGS_t3 Reduction (%)
Naphthalene (M/Z: 128)	2	6.06±0.1	0.05±0.1	99.1	4.86±0.2	0.06±0.01	98.8	4.59±0.2	0.05±0.01	98.8
Acenaphthylene (M/Z: 152)	3	2.75±0.1	0.04±0.005	98.6	2.41±0.1	0.05±0.01	97.8	2.14±0.1	0.05±0.01	95.4
Fluorene (M/Z: 166)	3	1.03±0.04	0.01±0.002	98.6	0.89±0.05	0.01±0.001	98.9	0.81±0.1	0.01±0.001	98.7
Phenanthrene (M/Z: 178)	3	4.62±0.1	0.44±0.1	90.5	3.36±0.1	0.20±0.004	94.1	3.33±0.2	0.24±0.002	92.6
Anthracene (MZ: 178)	3	0.81±0.03	0.10±0.02	87.1	0.85±0.1	0.06±0.01	92.6	0.72±0.04	0.06±0.005	91.3
Fluoranthene (M/Z: 202)	4	3.29±0.2	1.63±0.2	50.6	2.83±0.9	0.95±0.1	66.2	2.41±0.1	0.88±0.2	63.5
Pyrene (MZ: 202)	4	2.47±0.1	1.64±0.1	33.8	1.87±0.1	0.91±0.1	51.4	2.04±0.1	0.93±0.2	54.6
Benz[a]anthracene (M/Z: 228)	4	0.94±0.1	0.99±0.1	-4.9	0.81±0.03	0.9±0.1	-8.1	0.69±0.04	0.77±0.1	-11.9
Chrysene (M/Z: 228)	4	0.89±0.1	1.11±0.1	-24.8	0.79±0.04	0.88±0.1	-12.3	0.62±0.03	0.85±0.1	-36.2
Benzo[b]fluoranthene (m/z: 252)	5	1.03±0.1	0.73±0.1	29.5	0.65±0.05	0.61±0.01	5.1	0.60±0.03	0.56±0.01	7.0
Benzo[k]fluoranthene (m/z: 252)	5	0.51±0.01	0.58±0.1	-12.8	0.42±0.04	0.51±0.01	-21.8	0.32±0.02	0.39±0.04	-22.8
Benzo[j]fluoranthene (m/z: 252)	5	0.48±0.01	0.53±0.05	-11.9	0.43±0.02	0.44±0.004	-3.0	0.38±0.02	0.45±0.1	-18.1
Benzo[a]fluoranthene (m/z: 252)	5	0.27±0.02	0.17±0.04	38.1	0.21±0.01	0.14±0.01	33.4	0.17±0.02	0.14±.01	15.1
Benzo[e]pyrene (m/z: 252)	5	0.77±.05	0.78±0.1	-1.9	0.65±0.04	0.66±0.1	-1.1	0.58±0.04	0.6±0.05	-5.5
Benzo[a]pyrene (m/z: 252)	5	1.04±0.03	0.66±0.1	36.5	0.81±0.05	0.60±0.05	25.9	0.71±0.03	0.62±0.1	11.8
Perylene (m/z: 252)	5	0.31±0.01	0.22±0.03	27.9	0.25±0.01	0.19±0.02	23.9	0.24±0.01	0.20±0.003	16.8
Benzo[b]triphenyle (m/z:278)	5	0.08±0.004	0.07±0.01	4.0	0.07±0.004	0.08±0.01	-19.4	0.06±0.001	0.07±0.02	-26.2
Dibenzo[a,h]anthracene (m/z: 278)	5	0.12±0.01	0.13±0.03	-9.1	0.08±0.01	0.1±0.02	-58.1	0.08±0.005	0.11±0.02	-37.3
Benzo[b]chrysene (m/z:278)	5	0.10±0.01	0.11±0.01	-5.3	0.08±0.01	0.10±0.01	-32.3	0.06±0.002	0.07±0.02	88.2
Indeno[1,2,3-cd]pyrene (m/z:276)	6	0.58±0.04	0.66±0.1	-13.4	0.49±0.03	0.58±0.004	-20.1	0.46±0.03	0.57±0.01	-23.0
Picene (m/z: 276)	6	0.05±0.02	0.04±0.01	19.2	0.05±0.003	0.03±0.004	41.0	0.05±0.004	0.04±0.004	16.3
Benzo[g,h,i]perylene (m/z: 276)	6	0.69±0.03	0.59±0.1	14.1	0.57±0.02	0.58±0.01	-1.9	0.49±0.01	0.58±0.07	-18.3
Anthanthrene (m/z: 276)	6	0.11±0.01	0.08±0.02	25.9	0.08±0.004	0.05±0.01	35.1	0.07±0.01	0.05±0.01	22.9
Total PAHs		29.0	11.4	60.8	22.5	8.7	62.9	21.1	8.3	60.6

Concentrations are mean (n=3) values ±standard deviation; S = coal tar impacted soil; G = grass cuttings; St = straw; Negative value mean increase in concentration.

Table 4.15. Variation of PAH concentrations in coal tar impacted soil/mushroom compost/straw (SMS_t) compost mix types after lab-scale compost-bioremediation for 53 days

PAH analyte	Ring number	SMS_t1 mix type (µg/g of dry sample) day 0	SMS_t1 mix type (µg/g of dry sample) day 53	Reduction (%)	SMS_t2 mix type (µg/g of dry sample) day 0	SMS_t2 mix type (µg/g of dry sample) day 53	Reduction (%)	SMS_t3 mix type (µg/g of dry sample) day 0	SMS_t3 mix type (µg/g of dry sample) day 53	Reduction (%)
Naphthalene (M/Z: 128)	2	6.11±0.2	0.04±0.005	99.4	5.47±0.1	0.03±0.004	99.4	4.28±0.4	0.02±0.003	99.5
Acenaphthylene (M/Z: 152)	3	2.88±0.1	0.06±0.02	97.9	2.40±0.1	0.06±0.01	97.6	2.02±0.1	0.03±0.004	98.2
Fluorene (M/Z: 166)	3	1.06±0.05	0.01±0.001	99.2	1.01±0.01	0.01±0.001	99.4	0.78±0.05	0.01±0.001	98.7
Phenanthrene (M/Z: 178)	3	4.76±0.2	0.12±0.01	97.6	4.12±0.3	0.09±0.01	97.8	3.46±0.1	0.07±0.01	98.0
Anthracene (MZ: 178)	3	0.89±0.04	0.11±0.02	87.9	0.78±0.05	0.08±0.01	89.3	0.72±0.04	0.07±0.01	89.6
Fluoranthene (M/Z: 202)	4	3.31±0.2	1.77±0.3	46.4	3.01±0.2	1.36±0.2	54.7	2.48±0.1	1.15±0.1	53.6
Pyrene (MZ: 202)	4	2.46±0.2	1.36±0.2	44.9	2.49±0.2	1.00±0.1	59.9	2.02±0.1	0.90±0.1	55.3
Benz[a]anthracene (M/Z: 228)	4	0.96±0.05	1.03±0.2	-6.9	0.90±0.06	0.85±0.1	6.1	0.69±0.04	0.62±0.1	10.3
Chrysene (M/Z: 228)	4	0.84±0.04	1.01±0.2	-20.8	0.88±0.07	0.98±0.1	-11.2	0.72±0.02	0.76±0.1	-5.6
Benzo[b]fluoranthene (m/z: 252)	5	0.88±0.03	0.66±0.05	24.3	0.71±0.02	0.56±0.1	21.3	0.63±0.03	0.43±0.04	31.1
Benzo[k]fluoranthene (m/z: 252)	5	0.52±0.02	0.55±0.06	-5.6	0.48±0.01	0.47±0.05	1.6	0.40±0.01	0.38±0.06	4.0
Benzo[j]fluoranthene (m/z: 252)	5	0.49±0.02	0.50±0.04	-1.5	0.43±0.02	0.43±0.03	0.9	0.38±0.02	0.36±0.04	6.1
Benzo[a]fluoranthene (m/z: 252)	5	0.32±0.01	0.19±0.02	40.6	0.25±0.02	0.16±0.02	38.6	0.21±0.02	0.13±0.03	37.8
Benzo[e]pyrene (m/z: 252)	5	0.76±0.03	0.70±0.07	8.5	0.66±0.03	0.58±0.1	12.0	0.58±0.03	0.47±0.03	19.2
Benzo[a]pyrene (m/z: 252)	5	1.04±0.04	0.75±0.1	28.2	0.81±0.04	0.62±0.1	23.9	0.71±0.1	0.51±0.06	28.2
Perylene (m/z: 252)	5	0.31±0.01	0.23±0.03	26.4	0.27±0.01	0.20±0.02	27.1	0.22±0.01	0.15±0.02	29.6
Benzo[b]triphenyle (m/z:278)	5	0.09±0.004	0.10±0.03	-13.5	0.06±0.004	0.07±0.01	-27.9	0.06±0.008	0.06±0.007	-6.6
Dibenzo[a,h]anthracene (m/z: 278)	5	0.12±0.01	0.12±0.02	0.2	0.10±0.008	0.11±0.01	-8.4	0.08±0.01	0.08±0.01	-3.1
Benzo[b]chrysene (m/z:278)	5	0.08±0.004	0.10±0.01	-42.7	0.09±0.008	0.10±0.01	-8.7	0.07±0.01	0.07±0.005	-1.5
Indeno[1,2,3-cd]pyrene (m/z:276)	6	0.59±0.01	0.61±0.07	-3.6	0.51±0.02	0.53±0.1	-5.6	0.44±0.01	0.45±0.05	-3.1
Picene (m/z: 276)	6	0.05±0.004	0.04±0.004	21.2	0.05±0.003	0.04±0.002	12.3	0.04±0.004	0.03±0.004	24.5
Benzo[g,h,i]perylene (m/z: 276)	6	0.67±0.05	0.61±0.07	8.8	0.59±0.04	0.52±0.1	11.7	0.47±0.003	0.39±0.03	17.4
Anthanthrene (m/z: 276)	6	0.08±0.005	0.05±0.01	29.2	0.08±0.004	0.05±0.003	33.8	0.08±0.01	0.04±0.004	48.1
Total PAHs		29.3	10.7	63.3	26.2	8.9	66.0	21.5	7.2	66.5

Concentrations are mean (n=3) values ±standard deviation; S = coal tar impacted soil; M = mushroom compost; St = straw; Negative value mean increase in concentration.

Table 4.16. Variation of PAH concentration in coal tar impacted soil/grass cuttings/mushroom compost/straw ($SGMS_t$) compost mix types after lab-scale compost-bioremediation for 53 days

PAH analyte	Ring number	$SGMS_t1$ mix type			$SGMS_t2$ mix type			$SGMS_t3$ mix type		
		(µg/g of dry sample)		Reduction	(µg/g of dry sample)		Reduction	(µg/g of dry sample)		Reduction
		day 0	day 53	(%)	day 0	day 53	(%)	day 0	day 53	(%)
Naphthalene (M/Z: 128)	2	5.18±0.1	0.04±0.004	99.3	4.29±0.03	0.05±0.01	98.8	4.50±0.1	0.03±0.004	99.2
Acenaphthylene (M/Z: 152)	3	2.56±0.1	0.06±0.007	97.7	2.10±0.1	0.06±0.01	97.3	1.85±0.1	0.04±0.004	97.9
Fluorene (M/Z: 166)	3	0.97±0.03	0.01±0.002	99.0	0.79±0.03	0.01±0.002	98.7	0.69±0.02	0.01±0.001	99.0
Phenanthrene (M/Z: 178)	3	4.63±0.2	0.15±0.02	96.7	3.45±0.2	0.23±0.05	93.2	1.97±0.1	0.16±0.03	91.8
Anthracene (MZ: 178)	3	0.89±0.03	0.10±0.01	88.5	0.83±0.02	0.14±0.02	83.8	0.65±0.02	0.07±0.01	89.7
Fluoranthene (M/Z: 202)	4	3.34±0.2	1.29±0.2	61.4	2.60±0.1	1.79±0.3	31.4	2.33±0.1	0.94±0.2	59.7
Pyrene (MZ: 202)	4	2.56±0.1	1.03±0.1	59.8	1.87±0.04	1.64±0.2	12.5	1.80±0.1	0.86±0.1	52.2
Benz[a]anthracene (M/Z: 228)	4	0.91±0.03	0.89±0.1	2.7	0.73±0.02	0.92±0.1	-26.1	0.69±0.05	0.63±0.1	6.0
Chrysene (M/Z: 228)	4	0.88±0.03	0.95±0.09	-8.1	0.75±0.03	0.94±0.1	-24.2	0.68±0.02	0.78±0.1	-15.0
Benzo[b]fluoranthene (m/z: 252)	5	0.81±0.1	0.65±0.07	20.2	0.66±0.1	0.59±0.1	10.6	0.57±0.02	0.47±0.1	18.4
Benzo[k]fluoranthene (m/z: 252)	5	0.44±0.04	0.46±0.05	-3.7	0.42±0.03	0.50±0.05	-18.8	0.38±0.01	0.40±0.05	-4.9
Benzo[j]fluoranthene (m/z: 252)	5	0.49±0.03	0.49±0.05	0.7	0.40±0.03	0.44±0.1	-9.5	0.32±0.01	0.35±0.05	-8.4
Benzo[a]fluoranthene (m/z: 252)	5	0.27±0.02	0.17±0.03	36.5	0.21±0.02	0.17±0.02	19.7	0.18±0.01	0.13±0.03	29.8
Benzo[e]pyrene (m/z: 252)	5	0.73±0.03	0.71±0.09	3.6	0.56±0.02	0.64±0.1	-14.5	0.51±0.01	0.51±0.1	-0.4
Benzo[a]pyrene (m/z: 252)	5	0.94±0.02	0.71±0.09	24.9	0.78±0.04	0.67±0.1	13.6	0.62±0.01	0.50±0.1	18.8
Perylene (m/z: 252)	5	0.29±0.02	0.22±0.03	24.4	0.23±0.01	0.20±0.03	13.4	0.21±0.002	0.16±0.02	20.9
Benzo[b]triphenyle (m/z:278)	5	0.08±0.01	0.08±0.01	-2.2	0.07±0.001	0.08±0.004	-12.9	0.05±0.003	0.06±0.01	-9.4
Dibenzo[a,h]anthracene (m/z: 278)	5	0.12±0.01	0.13±0.03	-11.6	0.09±0.01	0.10±0.01	-10.1	0.07±0.004	0.07±0.01	-0.2
Benzo[b]chrysene (m/z:278)	5	0.08±0.01	0.09±0.01	88.6	0.07±0.01	0.08±0.02	-9.5	0.07±0.004	0.07±0.01	0.4
Indeno[1,2,3-cd]pyrene (m/z:276)	6	0.56±0.04	0.67±0.08	-19.6	0.48±0.03	0.62±0.04	-28.6	0.38±0.02	0.50±0.1	-32.5
Picene (m/z: 276)	6	0.05±0.003	0.05±0.05	-4.9	0.04±0.01	0.04±0.005	-7.6	0.04±0.003	0.03±0.005	19.5
Benzo[g,h,i]perylene (m/z: 276)	6	0.62±0.03	0.67±0.08	-8.2	0.50±0.04	0.62±0.03	-23.9	0.42±0.03	0.48±0.1	-14.3
Anthanthrene (m/z: 276)	6	0.09±0.01	0.07±0.01	24.9	0.09±0.006	0.06±0.01	32.1	0.07±0.004	0.05±0.01	27.4
Total PAHs		28.0	9.7	65.7	22.1	10.6	51.9	19.1	7.3	61.6

Concentrations are mean (n=3) values ±standard deviation; S = coal tar impacted soil; G = grass cuttings; M = mushroom compost; St = straw; Negative value mean increase in concentration.

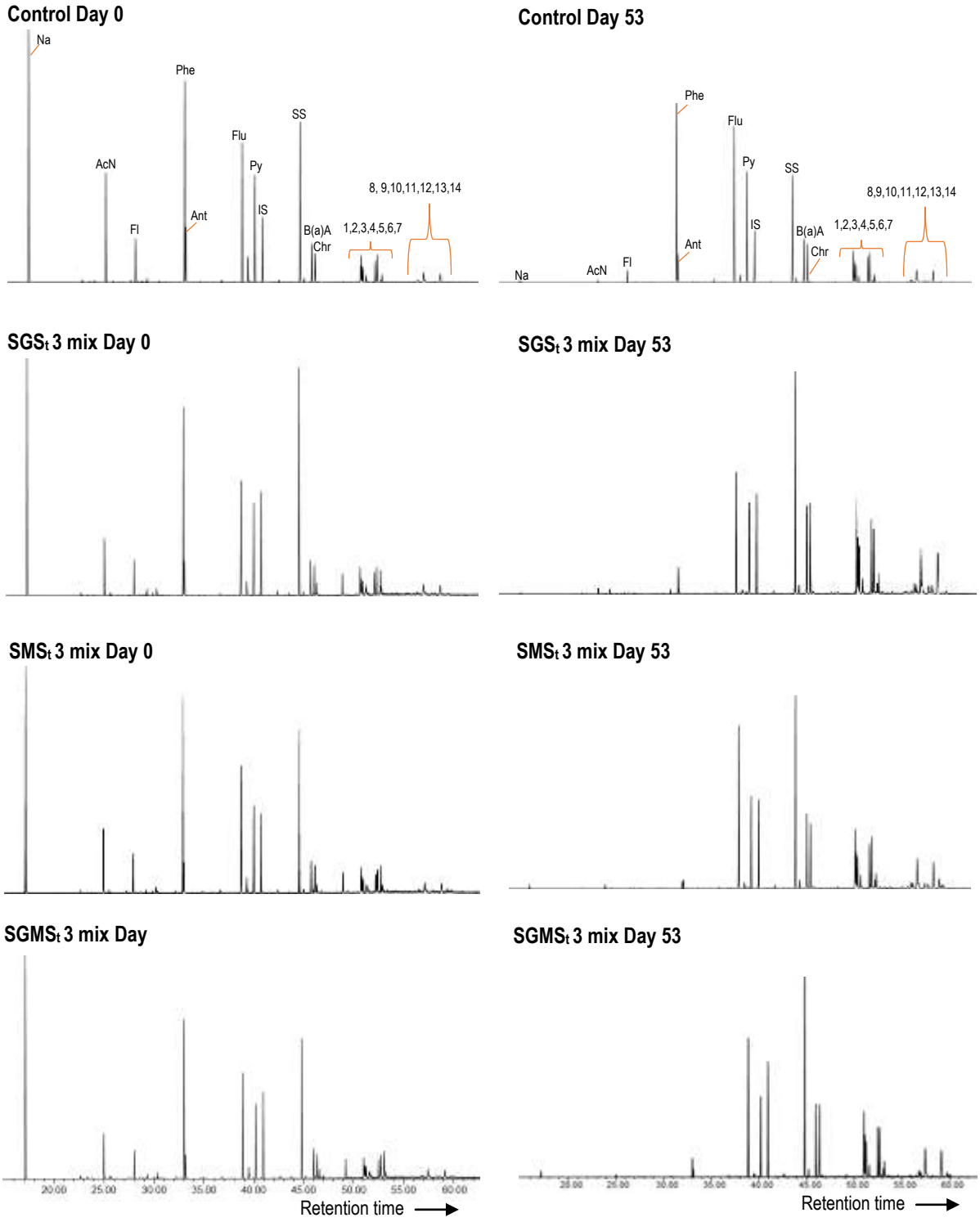


Figure 4.10. Summed mass chromatograms (m/z 28+152+166+178+202+228+252+276+278) of coal tar polycyclic aromatic hydrocarbons (PAHs) in compost mixes with coal tar impacted soil (CTIS) before and after composting treatment for 53 days.

Control = CTIS without compost amendments; S = CTIS; G = grass cuttings; M = mushroom compost; S_t = straw; Na – Naphthalene; AcN – Acenaphthylene; Fl – Fluorene; Phe – Phenanthrene; Ant – Anthracene; Flu – Fluoranthene; Py – Pyrene; IS = internal standard; SS = surrogate standard; B(a)A – Benz[a]anthracene; Chr – Chrysene; 1 = Benzo[b]fluoranthene; 2 = Benzo[k]fluoranthene; 3 = Benzo[j]fluoranthene; 4 = Benzo[a]fluoranthene; 5 = Benzo[e]pyrene; 6 = Benzo[a]pyrene; 7 = Perylene; 8 = Indeno91,1,3-cd]pyrene; 9 = Picene; 10 = Benzo[g,h,i]perylene; 11 = Anthanthrene; 12 = Benzo[b]triphenyle; 13 = Dibenzo[a,h]anthracene; 14 = Benzo[b]chrysene

4.3 Conclusions

Optimized conditions for biodegradation of hydrocarbon contaminants in drill cuttings and CTIS were achieved by lab-scale composting experiments with organic amendments. Grass cuttings, spent mushroom compost and straw were found to be effective organic amendments when blended in different mix ratios with the respective contaminated media. Contaminant/organic amendment mix ratios of 1:1, 1:1.5 and 1:2 were adopted in this study to prepare DGS_t, DMS_t and $DGMS_t$ compost mix types with drill cuttings and SGS_t, SMS_t and $SGMS_t$ compost mix types with CTIS, having C:N ratios ranging from 28.96 to 30.07. After 53 days of simulated composting treatment, TPH and total PAHs found were significantly reduced in all compost mix types treated.

The highest TPH reductions of 85.1% and 90.6% were achieved in the $DGMS_t3$ and SMS_t3 compost mix types, respectively, but these were not statistically significantly ($p>0.05$) higher compared to each of the other compost mix types. Σ(*n*-alkanes) concentrations were significantly reduced in all the organic amended compost mix types, with increases in pristane/nC_{17} and phytane/nC_{18} ratios indicating the occurrence of mild to moderate biodegradation. The concentrations of total PAHs were significantly reduced in all compost mix types containing CTIS and organic amendments. The concentrations of the summed 2 and 3-ring PAHs were found to be significantly ($p<0.002$) lower in the SGS_t, SMS_t and $SGMS_t$ compost mix types and control. This is partially attributed to abiotic factors (i.e. volatilisation) which may have prevailed during the composting process. Summed concentrations of 4-ring PAHs were also significantly reduced in all compost mix types containing CTIS and organic amendments, except for the $SGMS_t2$ mix type. There was decrease of summed 5 and 6-ring PAHs in all organic amended compost mix types. However, several PAH compounds in this category exhibited recalcitrance to degradation as well as increase in concentration after the composting period possibly due to sample heterogeneity or degradation of the compost matrix of the day 0 organic amended samples allowing enhanced PAH extractabilities.

Future work as a follow-up to this investigation would be to test the degree of hydrocarbon degradation of the $DGMS_t3$ and SMS_t3 compost mix types in an up-scaled out-door pilot scale compost-bioremediation treatment for drill cuttings and CTIS, respectively.

Chapter 5

PILOT-SCALE OUTDOOR COMPOST-BIOREMEDIATION OF COAL TAR IMPACTED SOIL

5.1 Introduction

This chapter examines the hydrocarbon degradation performance of scaled-up compost mixtures comprising organic amendments and coal tar impacted soil (CTIS) in an outdoor, pilot-scale, compost-bioremediation experiment. This investigation is a follow-up on the SMS_t3 compost mix type which produced the most degradation of hydrocarbon contaminants in soils during the lab-scale composting treatment described in Chapter 4. The SMS_t3 compost mix type was scaled-up by a factor of 600, amounting to 18 kg of compost mix, which comprised of 6 kg CTIS, 5.7 kg spent mushroom compost and 6.3 kg straw, to formulate a CTIS to amendments mix ratio of 1:2. The CTIS was prepared by spiking air-dried agricultural soil to 0.5% coal tar contamination (dry weight).

Composting was carried out in tumbler compost bins for 56 days, set up in triplicate with a control containing 18kg CTIS (Figure 3.4). The wall of the compost bins were covered with bubble wrap to provide some thermal insulation and preservation of the temperatures inside. During the composting process, the bins containing the compost mix were aerated 3 times weekly by giving equal numbers of tumbles in addition to manual breakdown of lumps using garden fork. Temperature measurements were also taken 3 times weekly at five different points in all compost bins to ensure accuracy of mean temperatures. Composting matrices were subsampled once and thrice weekly for geochemical and moisture content analysis, respectively. Moisture content of compost mix samples were adjusted to 60% with deionised water when necessary.

5.2 Results and Discussion

5.2.1 *Moisture and temperature changes during composting*

At two weeks into the composting process, it was observed that the organic amended compost matrices were becoming moister even without addition of water. Results of moisture content analysis were ranging from 62% to 70% whereas the moisture in the control bin was decreasing. Consequently no water was added while this trend continued until the seventh week of composting. This observation may be due to

increased microbial metabolic activities whereby readily available carbons are metabolised to carbon dioxide and water (Bamforth and Singleton, 2005; Said-Pullicino *et al*., 2007). An earlier study has reported similar observation during *ex-situ* bioremediation of PAH contaminated medium by composting wherein the moisture content in piles turned became higher than those in static (unturned) piles (Cai *et al*., 2007).

Temperatures measured were observed to vary at different location within the composting matrix in each bin. Temperatures taken at the middle of the matrices were generally higher than those at other points. The results of temperature measurements during the composting period are shown in Table 5.1. The temperature profile in the compost bins generally followed a similar trend to that of the ambient temperatures during most part of the composting period (Appendix AH) indicating strong influence by the ambient temperature. This may be an indication that the wall of the compost bins were poorly thermal insulated despite the outer covering with bubble wrap. In the treatment compost matrices, the temperature rose from 16 ^{o}C to 31 ^{o}C during the first 7 days, and then gradually decreased to 20 ^{o}C at day 14, indicating the end of the thermophilic phase. Thereafter the temperature fluctuated closely and reached constant level with the ambient temperature at day 49 through day 56, marking maturation phase. A similar trend was observed in the control, in which the temperature rose from 15 ^{o}C to 19 ^{o}C at day 12. It then dropped down to 17 ^{o}C at day 14 before fluctuating to constant level with the ambient temperature.

During the lab-scale composting experiments, the control and compost mix samples were incubated to 60 ^{o}C to simulate the typical thermophilic temperature range (40-60 ^{o}C) (Semple *et al*., 2001; Rogas-Avelizapa *et al*., 2007). However, neither samples managed to generate thermophilic temperature up to 60 ^{o}C in the compost bins during the outdoor composting treatment. This may be attributed to the regular turning and watering of the composting matrix which may have imparted a cooling effect (e.g. Atagana, 2004). The highest temperature recorded during the thermophilic phase in the compost mix was significantly ($P<0.05$ ANOVA) higher compared to the control, suggesting higher microbial activities. However, a reverse trend had been reported by Cai *et al.,* (2007) where the highest temperatures in the turned compost piles were considerably lower than those in a static pile due to

turning and mixing. Generally, temperatures recorded in the turned compost were considerably higher than those in the control during most part of the experimentation period.

Table 5.1. Variation of temperature in composting matrices

	Temperature (^{0}C)				
	Compost mix		Control		Ambient
Day	Mean	SD	Mean	SD	
0	16	0.9	15	0.3	15.0
3	21	1.0	16	0.3	18.5
5	28	1.3	18	0.8	20.0
7	31	1.4	19	0.4	21.5
10	28	1.0	20	0.4	20.0
12	26	1.7	19	0.4	21.0
14	20	0.7	17	1.3	17.5
17	20	0.9	18	1.6	18.0
19	19	1.4	15	0.4	17.0
21	19	0.8	16	0.7	15.0
24	20	1.5	17	2.0	17.0
26	20	1.0	16	0.4	18.5
28	20	0.6	19	0.7	20.0
31	21	0.6	21	0.3	19.0
33	22	0.7	19	0.8	21.5
35	22	0.8	19	0.5	18.5
38	20	0.6	20	0.6	18.5
40	24	1.6	20	0.7	20.5
42	23	1.2	19	0.3	19.0
45	16	0.7	20	0.8	18.0
47	20	0.4	18	0.5	19.0
49	16	0.7	18	0.6	18.0
52	15	0.7	14	0.4	16.0
54	15	1.3	16	0.4	15.0
56	15	0.8	15	0.4	15.0

Values are mean (n=5); SD = standard deviation; Control = CTIS without organic amendments

5.2.2 *Changes in total petroleum hydrocarbons (TPH) during composting*

During the composting treatment in the tumbler composting bins, residual TPH in the composting matrices was measured each week and the reduction in concentrations are shown in Table 5.2 and illustrated qualitatively by the gas chromatograms in Figure 5.1 and graphically in Appendix AI. As can be seen, the TPH decreased significantly ($p<0.05$ *t*-test) by 78% and 36% in the treatment compost mix and control, respectively, after composting for 56 days. The TPH reduction in the control was significantly ($p=0.001$ ANOVA) lower than those of the treatment compost mix. Analysis of the results revealed that 58% out of the 78% decrease of TPH in the treatment compost mix occurred after the thermophilic phase between days 14 and

42 at a temperature range of 20 and 23 ^{o}C. This may indicate that microbial activity at lower temperatures resulted in higher TPH removal during the in-vessel composting treatment. It has been previously been suggested that lower temperatures might allow some types of increased microbial activity during composting (Suler and Finstein, 1977; Liang *et al*., 2003).

Examination of the declining trend of TPH concentrations in the treatment compost mix (Appendix AI) revealed that the time frame between days 14 and 49 coincided with the period when the compost matrices were considered to generate excess moisture (>60%). Studies have reported 60% as the optimum moisture content for compost-bioremediation of hydrocarbon contaminated soil (Lau *et al*., 2003; Zhang *et al*., 2011). However, considering that most of the TPH degradation occurred within this period, this may be an indication that a moisture range of 62 to 70% is the optimum for TPH degradation using these particular tumbler bin composting systems. An earlier study has reported optimum microbial activities within moisture content range of 60 to 70% during composting of bio-solids blends (Liang *et al*., 2003). However, a similar claim may not be made in respect of temperature, as the compost temperature profile appears to be influenced largely by the ambient temperature after the thermophilic phase. Reduction of TPH after the outdoor composting (78%) was found to be significantly (p=0.006 *t*-test) lower compared to the lab-scale composting (90.6%) for the SMS_t3 compost mix. This may, at least in part, be due to higher volatilisation enabled by the higher incubation temperature (60 ^{o}C) during the lab-scale experiments. A similar comparison between the control samples revealed no significant (p=0.462) difference in TPH reduction between the outdoor (36%) and lab-scale (28%) conditions.

Table 5.2. Changes in total petroleum hydrocarbons (TPH) in composting matrices

Time (days)	TPH (µg/g of dry sample)			
	Compost mix		Control	
	Mean	SD	Mean	SD
0	1797	124	2474	72
7	1475	76	2250	57
14	1321	102	2207	68
21	1049	86	2143	96
28	840	109	1928	73
35	784	143	1842	64
42	557	72	1719	37
49	433	73	1686	55
56	387	39	1592	19
*Reduction (%)	78		36	

*Values are mean (n=3); SD = standard deviation; Control = CTIS without organic amendments; * = percent reduction at day 56.*

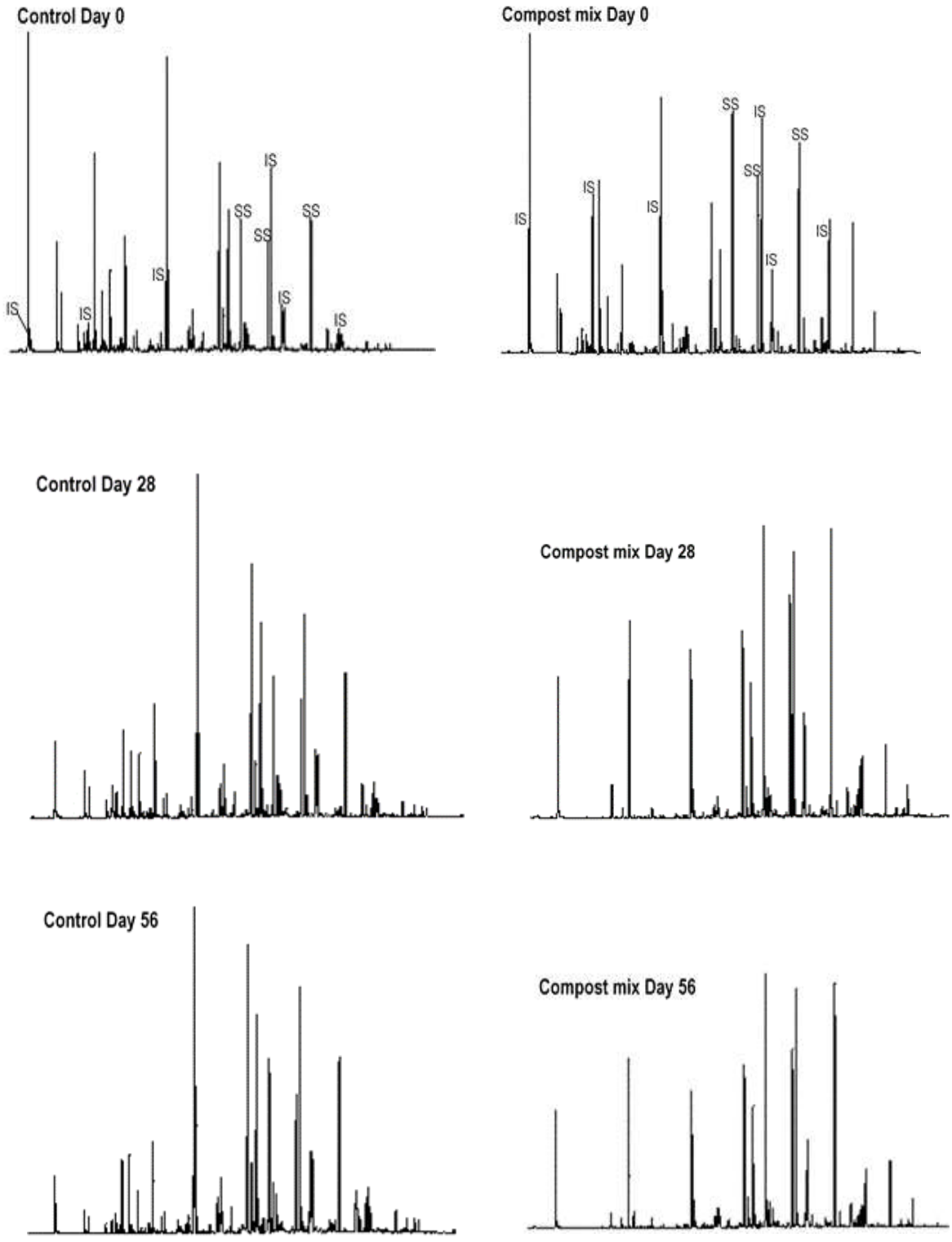


Figure 5.1. Total petroleum hydrocarbon (TPH) chromatograms of the control and compost mix during outdoor compost-bioremediation of coal tar impacted soil (CTIS)
Control = CTIS without organic amendments; IS = internal standard; SS = surrogate standard

5.2.3 *Degradation of saturated hydrocarbons in the CTIS-compost mix*

As noted in Chapter 4, all the resolved *n*-alkanes found in the compost mix samples used for the outdoor compost-bioremediation experiment were derived from the organic compost amendments (spent mushroom compost and straw). Changes in concentrations of *n*-alkanes during the composting period are as presented in Table 5.3 and gas chromatograms of saturated hydrocarbon fractions from the compost mixtures are shown in Figure 5.2. The result revealed that the total *n*-alkane concentration ($\Sigma_{n\text{-alkanes}}$) was significantly ($p<0.001$) degraded by 78% in the compost mix after treatment for 56 days. The $\Sigma_{n\text{-alkanes}}$ concentrations decreased by 69.5% to 43.9 µg/g at day 28 before decreasing to 31.6 µg/g at day 56. This is an indication that the $\Sigma_{n\text{-alkanes}}$ was significantly degraded during the first 28 days out of the 56 days of outdoor compost-bioremediation treatment. Similar to the trend observed in the TPH analysis, the decrease of $\Sigma_{n\text{-alkanes}}$ in the outdoor experiments was found to be significantly lower ($p=0.03$) compared to the lab-scale treatments which produced a 94% reduction.

Individual *n*-alkanes in the compost mix were also found to degrade significantly ($p<0.05$) at the end of the composting period. The $n\text{C}_{15}$ *n*-alkane was degraded the most, by 98.9%. This observation may be due to the fact that it has the lowest molecular weight compared to the other *n*-alkanes present, since lower molecular weight *n*-alkanes ($n\text{C}_{10}$ - *n*C19) are reported to be more readily degradable than higher molecular weight ($n\text{C}_{20}$ - $n\text{C}_{40}$) ones (Balba *et al*., 1998). However, the least degraded *n*-alkane was found to be $n\text{C}_{23}$, which decreased by 42.6% despite that it was not the highest molecular weight *n*-alkane in the compost matrix. This observation is consistent with the result obtained from the lab-scale experiment and may be attributed to its low concentration which may be below the threshold for effective microbial utilisation.

Table 5.3. Variation in compost mix n-alkane concentrations with time

	Concentration (µg/g of dry sample)					
	Day 0		Day 28		Day 56	
	Mean	SD	Mean	SD	Mean	SD
nC_{15}	18.8	4.3	0.3	0.1	0.2	0.03
nC_{23}	1.0	0.1	0.7	0.1	0.6	0.1
nC_{25}	4.3	0.8	1.3	0.2	0.8	0.2
nC_{27}	7.4	1.5	1.4	0.1	1.2	0.1
nC_{29}	44.8	8.6	17.5	1.8	12.4	1.0
nC_{30}	1.4	0.5	0.3	0.1	0.4	0.1
nC_{31}	42.0	9.5	10.8	0.8	8.0	1.3
nC_{33}	24.3	5.4	11.7	1.7	8.0	1.1
Σ(*n*-alkanes)	143.9		43.9		31.6	

Values are mean (n=3); SD = standard deviation.

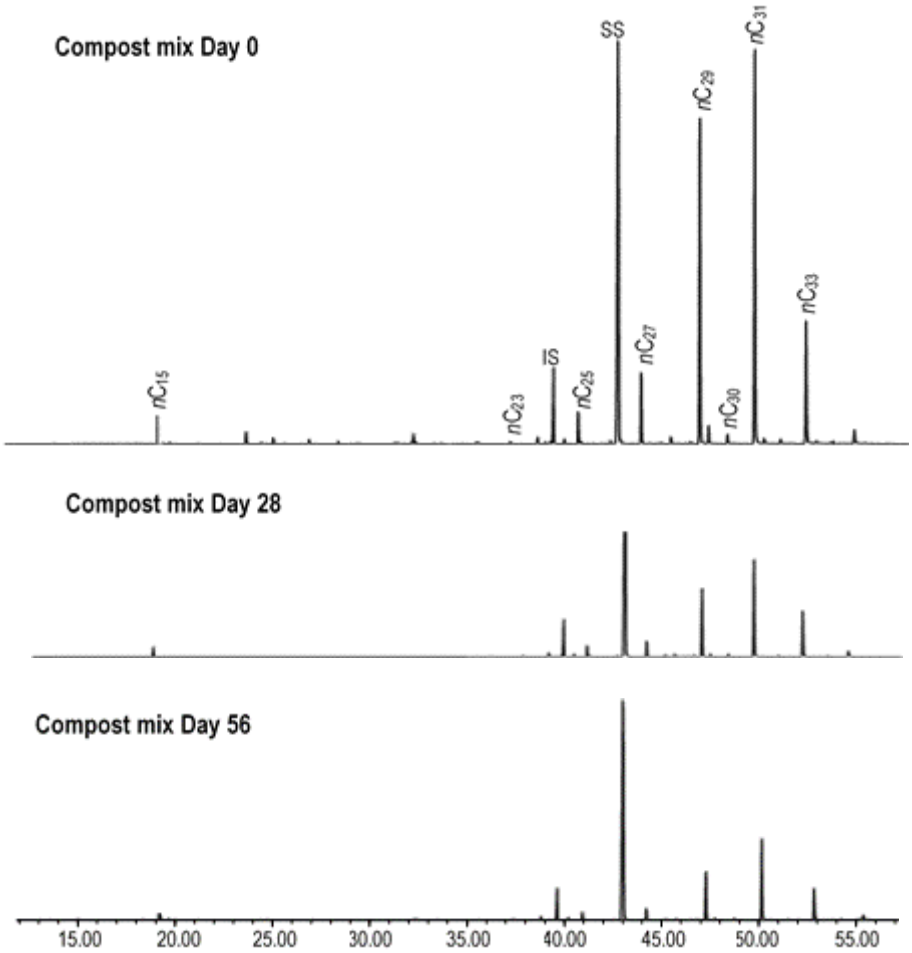


Figure 5.2. m/z 85 mass chromatograms of saturated hydrocarbons fractions showing n-alkane distributions from compost mixes at different durations of composting.
IS = Internal standard; SS = Surrogate standard

5.2.4 Degradation of polycyclic aromatic hydrocarbon (PAH) compounds

The total PAH concentrations in the compost mix at the start of treatment was comprised mainly of 23 coal tar derived PAHs, with insignificant contributions from the organic amendments as discussed earlier in Chapter 4. Examination of the results in Table 5.4 revealed that concentration of total PAHs (Σ_{PAHs}) degraded significantly ($p<0.05$) in the compost mix sample after treatment for 56 days. The Σ_{PAHs} decreased by 64.4% to 242.2 µg/g at day 56. Similar to the trend observed in *n*-alkanes degradation, the Σ_{PAHs} was already significantly ($p=0.001$) degraded by 62.1% to 257.8 µg/g at day 28, indicating that significant degradation of coal tar PAHs was achieved after outdoor compost-bioremediation treatment for 28 days in the tumbler compost bins. The reduction of Σ_{PAHs} at day 56 was slightly higher compared to the lab-scale composting treatment (63.8%). Degradation of Σ_{PAHs} by 29.5% and 38.2% were recorded in the control at 28 and 56 days of composting, respectively, which were significantly ($p<0.002$) lower compared to the treatment compost mix.

In the treatment compost mix, the total concentration of 2 and 3-ring ($\Sigma_{2 \text{ and } 3\text{-ring}}$) PAHs (naphthalene, acenaphthylene, fluorene, phenanthrene and anthracene) was significantly decreased at day 56. The $\Sigma_{2 \text{ and } 3\text{-ring}}$ PAHs was degraded by 91.4% ($p<0.001$ *t*-test) at day 28 before degrading further by 2.1% more at day 56 (Table 5.4). Similarly, concentration of 4-ring ($\Sigma_{4\text{-ring}}$) PAHs was significantly ($p=0.014$) degraded by 42.6% at day 28, before degrading further by 9.3% more at day 56. The degradation trend for the $\Sigma_{2 \text{ and } 3\text{-ring}}$ and $\Sigma_{4\text{-ring}}$ PAHs was consistent with the pattern observed in $\Sigma_{n\text{-alkanes}}$, whereby a significant amount of degradation was achieved during the first 28 days out of the 56 days of composting. However, there was no significant degradation in total concentration of 5 and 6-ring ($\Sigma_{5 \text{ and } 6\text{-ring}}$) PAHs during the composting treatment at day 28 and day 56. The $\Sigma_{5 \text{ and } 6\text{-ring}}$ PAHs decreased by 25.4% to 108.1 µg/g at day 28 but increased slightly to 119.0 µg/g at day 56 (Table 5.4). The increased concentration recorded at day 56 was most probably due to the fact that the 5 and 6-ring PAHs were temporarily less solvent extractable (Amir *et al*., 2005) as a result of their sequestration to the compost matrix (Semple *et al*., 2003; Kriipsalu *et al*., 2008).

Comparing the final removal rate of PAH groups in the treatment compost mix, the highest percentage removal occurred in the 2 and 3-ring group followed by the 4-ring

group (Table 5.4). The 5 and 6-ring group recorded the least percentage removal. This trend is consistent with the pattern observed during the lab-scale experiments in chapter 4 and established trend whereby the PAH groups degrade in decreasing order of $\Sigma_{2 \text{ and } 3\text{-ring}} > \Sigma_{4\text{-ring}} > \Sigma_{5 \text{ and } 6\text{-ring}}$ PAHs (e.g. Amir *et al*., 2005; Moretto *et al*., 2005; Antzar-Ladislao *et at*., 2006). The reduction in concentrations of PAH groups in the controls were found to be significant for the $\Sigma_{2 \text{ and } 3\text{-ring}}$ but not significant for the $\Sigma_{4\text{-ring}}$ and $\Sigma_{5 \text{ and } 6\text{-ring}}$ PAHs after the composting duration.

Reduction in concentrations of individual PAHs varied widely in the treatment compost mix and control samples after composting for 56 days (Table 5.4). Percent abundances of individual compounds within the 2 and 3-ring PAH group at days 28 and 56 were found to be significantly lower with respect to their day 0 concentrations in both samples. At day 28, naphthalene, acenaphthylene, fluorene, phenanthrene and anthracene decreased by 99.9, 96.5, 93.7, 83.2 and 73.3% in the treatment compost mix and 87.9, 76.6, 44.8, 28.7 and 43.3 in the control, respectively (Table 5.4). Only slight reduction occurred further at day 56. The high percentage reduction for naphthalene and acenaphthylene in the control may have been contributed by abiotic factors which may have prevailed when taking measurements and subsamples. Fluoranthene, pyrene, benz[a]anthracene and chrysene degraded significantly by 59.3, 56.1, 29.4 and 31.2%, respectively at day 56 in the treatment compost mix but were not significantly reduced in the control (Table 5.4).

Percentage reductions in concentrations of the individual 5 and 6-ring PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[a]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, benzo[b]triphenyle, dibenzo[a,h]anthracene, benzo[b]chrysene, indeno[1,2,3-cd]pyrene, picene, benzo[g,h,i]perylene and anthanthrene) were not significant in both samples after the composting duration (Table 5.4. Degradation of each PAH in the 5 and 6-ring group was in the range of 8.1 to 22.2% in the outdoor treatment compost mixtures, which was an improvement over the lab-scale experiment wherein some negative reduction in concentrations were recorded in the SMS_t3 sample. Variation of PAH compound distributions during the composting period are shown in Figures 5.3 and 5.4, and graphically in Appendix AJ.

Table 5.4. Changes in polycyclic aromatic hydrocarbon (PAH) concentrations during outdoor pilot scale compost-bioremediation treatment

PAH analyte	Ring number	Control				Compost mix			
		Concentration (µg/g of dry sample)			Total Reduction	Concentration (µg/g of dry sample)			Total Reduction
		Day 0	day 28	day 56	(%)	Day 0	day 28	day 56	(%)
Naphthalene (M/Z: 128)	2	235.7±37.6	28.6±3.5	8.9±0.9	96.2	99.1±7.7	0.1±0.02	0.1±0.01	99.9
Acenaphthylene (M/Z: 152)	3	211.4±35.8	49.6±5.2	32.9±4.3	84.4	77.6±7.2	2.7±0.31	1.9±0.11	97.6
Fluorene (M/Z: 166)	3	58.9±7.3	32.5±2.1	21.2±1.9	64.0	22.2±4.1	1.4±0.14	1.1±0.13	95.1
Phenanthrene (M/Z: 178)	3	284.6±24.0	202.8±33.3	141.1±16.7	50.4	95.2±5.8	16.0±1.92	12.0±2.12	87.4
Anthracene (MZ: 178)	3	76.0±8.2	43.0±4.4	46.3±58	39.2	28.1±4.5	7.4±0.86	5.8±0.63	79.5
Fluoranthene (M/Z: 202)	4	275.7±34.6	245.3±31.9	213.9±33.6	22.4	93.3±10.2	46.6±6.6	37.9±4.02	59.3
Pyrene (MZ: 202)	4	208.9±30.1	183.4±21.2	156.2±17.9	25.2	73.0±9.3	39.9±2.74	32.0±2.91	56.1
Benz[a]anthracene (M/Z: 228)	4	78.7±9.9	73.1±9.5	68.1±7.7	13.4	23.9±4.1	18.6±2.10	16.9±2.22	29.4
Chrysene (M/Z: 228)	4	66.8±5.9	62.9±5.4	59.5±7.5	10.9	22.5±3.1	16.9±2.0	15.5±1.78	31.2
Benzo[b]fluoranthene (m/z: 252)	5	88.6±7.4	82.9±6.5	80.4±13.8	9.2	25.8±3.1	19.3±2.13	20.7±2.06	19.7
Benzo[k]fluoranthene (m/z: 252)	5	58.1±7.3	53.5±5.9	51.7±6.1	11.0	14.8±1.7	11.5±1.03	12.4±3.63	16.1
Benzo[j]fluoranthene (m/z: 252)	5	54.8±4.2	49.4±6.5	47.0±5.5	14.3	15.4±1.8	11.7±1.21	12.3±1.47	20.3
Benzo[a]fluoranthene (m/z: 252)	5	31.0±14.3	27.8±4.6	26.5±2.3	14.4	8.1±0.9	6.1±0.73	6.3±0.55	22.2
Benzo[e]pyrene (m/z: 252)	5	71.3±5.5	65.5±6.1	67.1±5.7	5.9	20.6±1.9	15.3±2.08	17.1±1.87	17.4
Benzo[a]pyrene (m/z: 252)	5	110.6±13.8	102.2±11.8	101.1±12.1	8.6	26.4±3.0	19.7±2.57	21.5±3.01	18.3
Perylene (m/z: 252)	5	29.9±3.6	26.9±4.2	26.0±3.6	13.1	7.8±1.1	5.9±0.62	6.1±0.53	20.8
Benzo[b]triphenyle (m/z:278)	5	6.4±0.9	6.0±1.1	5.7±0.4	11.3	1.1±0.1	0.9±0.01	0.9±0.77	18.4
Dibenzo[a,h]anthracene (m/z: 278)	5	10.0±1.6	10.2±1.4	10.3±1.6	-2.7	1.8±0.3	1.2±0.11	1.4±0.12	20.2
Benzo[b]chrysene (m/z:278)	5	9.1±0.9	8.6±1.0	9.5±1.5	-4.4	1.4±0.2	1.0±0.10	1.2±0.10	13.0
Indeno[1,2,3-cd]pyrene (m/z:276)	6	51.5±4.4	51.±7.5	48.7±4.1	5.5	9.9±1.4	6.8±0.55	8.3±0.69	16.3
Picene (m/z: 276)	6	5.8±6.6	5.7±0.7	5.9±1.6	-1.8	0.9±0.02	0.7±0.05	0.8±0.09	8.1
Benzo[g,h,i]perylene (m/z: 276)	6	53.6±4.1	49.3±5.0	51.7±4.2	3.6	9.5±1.1	6.7±0.58	8.6±0.93	9.2
Anthanthrene (m/z: 276)	6	14.3±3.7	13.6±2.2	14.2±2.0	0.8	1.6±0.2	1.4±0.14	1.4±0.41	14.1
Total PAHs		2091.8	1474.2	1293.8	38.2	679.9	257.8	242.2	64.4

Values are mean (n=3) ± standard deviation; Control = CTIS sample without compost amendments; Negative value = increased concentration.

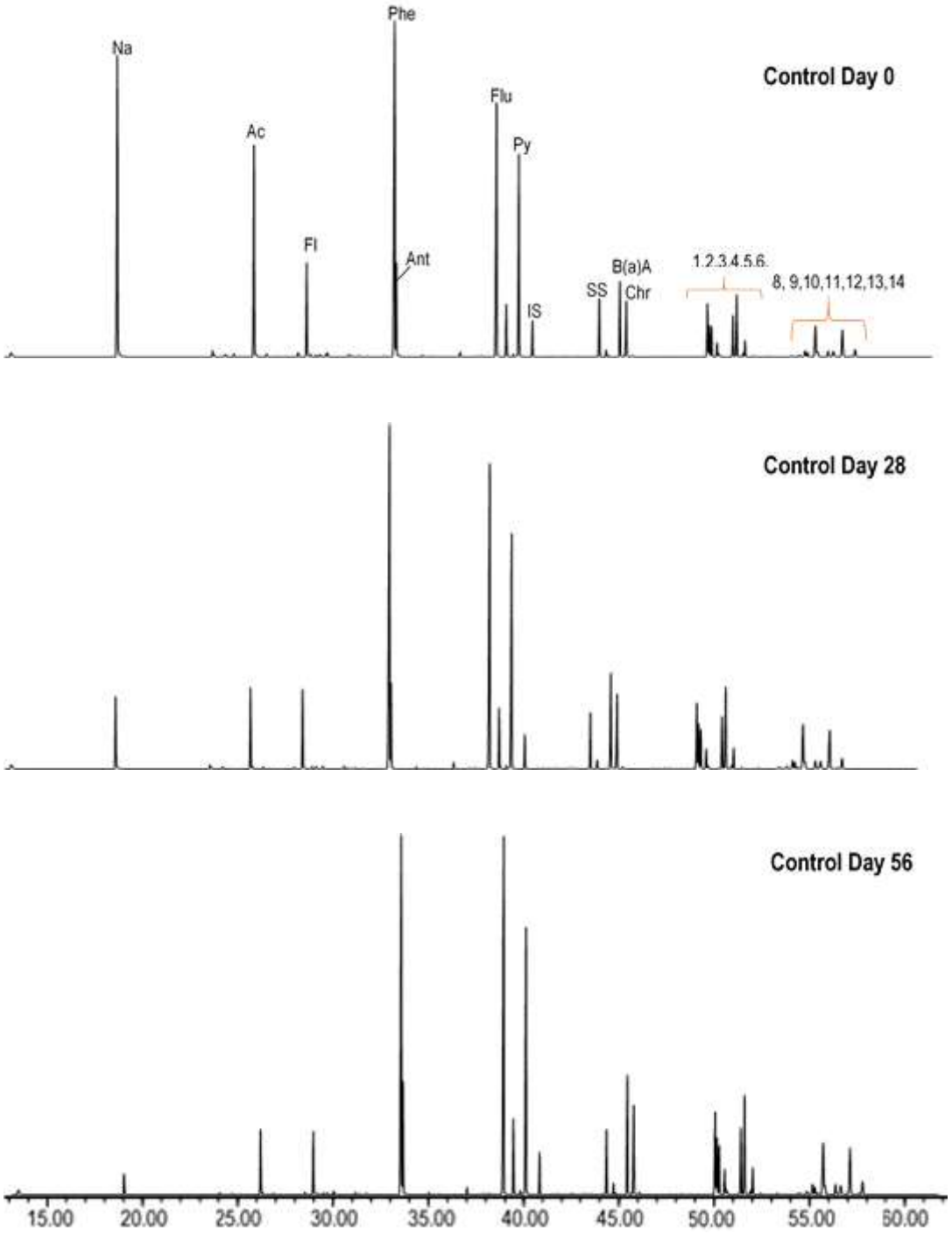


Figure 5.3. Summed mass chromatograms (m/z 128+152+166+178+202+228+252+276+278) of coal tar PAHs in the control, before and during composting treatment for 53 days.

Control = CTIS without compost amendments; IS = Internal standard; SS = Surrogate standard

Na – Naphthalene; AcN – Acenaphthylene; Fl – Fluorene; Phe – Phenanthrene; Ant – Anthracene; Flu – Fluoranthene; Py – Pyrene; IS = internal standard; SS = surrogate standard; B(a)A – Benz[a]anthracene; Chr – Chrysene; 1 = Benzo[b]fluoranthene; 2 = Benzo[k]fluoranthene; 3 = Benzo[j]fluoranthene; 4 = Benzo[a]fluoranthene; 5 = Benzo[e]pyrene; 6 = Benzo[a]pyrene; 7 = Perylene; 8 = Indeno91,1,3-cd]pyrene; 9 = Picene; 10 = Benzo[g,h,i]perylene; 11 = Anthanthrene; 12 = Benzo[b]triphenyle; 13 = Dibenzo[a,h]anthracene; 14 = Benzo[b]chrysene.

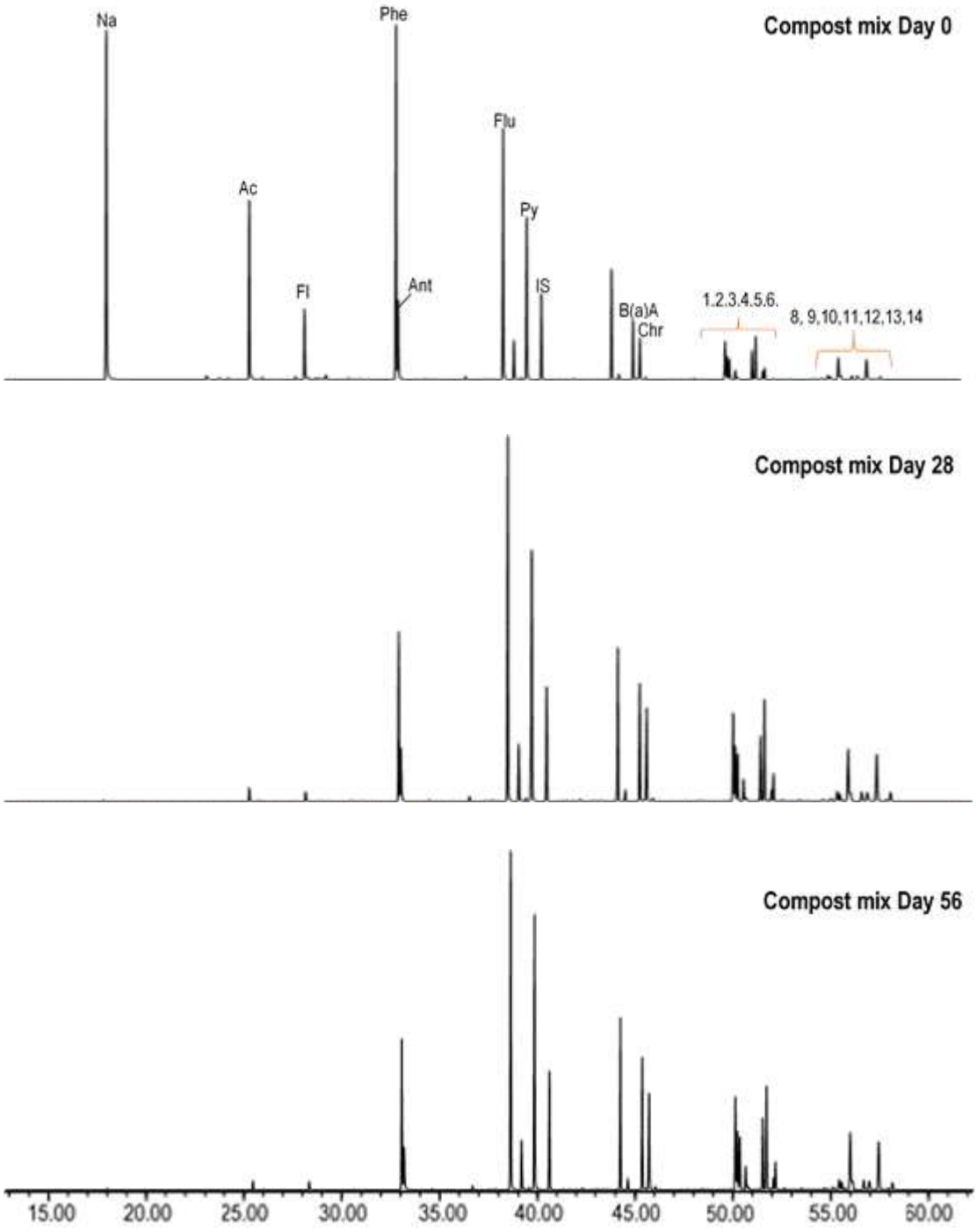


Figure 5.4. Summed mass chromatograms (m/z 128+152+166+178+202+228+252+276+278) of coal tar PAHs in the compost mix, before and during composting treatment for 53 days.
Control = CTIS without compost amendments; IS = Internal standard; SS = Surrogate standard
Na – Naphthalene; AcN – Acenaphthylene; Fl – Fluorene; Phe – Phenanthrene; Ant – Anthracene; Flu – Fluoranthene; Py – Pyrene; IS = internal standard; SS = surrogate standard; B(a)A – Benz[a]anthracene; Chr – Chrysene; 1 = Benzo[b]fluoranthene; 2 = Benzo[k]fluoranthene; 3 = Benzo[j]fluoranthene; 4 = Benzo[a]fluoranthene; 5 = Benzo[e]pyrene; 6 = Benzo[a]pyrene; 7 = Perylene; 8 = Indeno91,1,3-cd]pyrene; 9 = Picene; 10 = Benzo[g,h,i]perylene; 11 = Anthanthrene; 12 = Benzo[b]triphenyle; 13 = Dibenzo[a,h]anthracene; 14 = Benzo[b]chrysene.

5.3 Conclusions

Composting of CTIS with spent mushroom compost and straw in compost tumbler bins resulted in significant degradation of hydrocarbon contaminants during a scaled-up, outdoor, pilot-scale compost-bioremediation experiment. While composting was carried out for 56 days, concentrations of TPH, *n*-alkanes and PAHs detected in the treatment compost mix at the start of experiment, were found to have degraded significantly at 28 days into the treatment. The results showed that TPH was most degraded at lower temperatures after the thermophilic phase of the composting cycle, when the moisture content of the compost matrix was ranging between 62 and 70%. This may indicate the optimum moisture content range for TPH removal using tumbler bin composting systems. However, the TPH removal percent achieved was significant lower compared to the lab-scale experiments described in Chapter 4.

The degradation of individual *n*-alkanes was consistent with results from the lab scale experiments, but the $\Sigma_{n\text{-alkanes}}$ degradation was significantly lower than that from the lab-scale experiments. Degradation was significant for $\Sigma_{2 \text{ and } 3\text{-ring}}$ and $\Sigma_{4\text{-ring}}$ PAH groups. 2 and 3-ring PAHs were almost completely degraded, while 4-ring PAHs including fluoranthene, pyrene benzo[a]anthracene and chrysene were significantly degraded. The $\Sigma_{5 \text{ and } 6\text{-ring}}$ PAH group were not significantly degraded after the composting duration. It is suggested that the efficiency of the compost-treatment procedures used may improve if the tumbler compost bins are made thermally insulated, since the temperature profiles of the compost matrices were found to be strongly influenced by the ambient temperature.

Chapter 6

TOXICITY ANALYSIS OF HYDROCARBON CONTAMINATED DRILL CUTTINGS AND SOILS AFTER COMPOST BIOREMEDIATION

6.1 Introduction

In this chapter, the ecological health of compost bioremediated drill cuttings and coal tar impacted soil (CTIS) was assessed to determine the effectiveness of the remediation treatments. Microbial and plant bioassays were employed as biological indicators for assessing the quality of the compost-treated matrices resulting from the lab-scale and outdoor pilot-scale composting experiments discussed in chapters 4 and 5 respectively. Soil phosphatase activity assays were employed as the microbial indicator to assess the quality of the treated samples by evaluating the amount of *p*-nitrophenol released when the samples were incubated with sodium *p*-nitrophenyl phosphate (e.g. Tabatabai and Bremner, 1969). Phytotoxicity assays were applied as a biological indicator to further assess the ecological health of the outdoor compost-bioremediated samples, where the treated compost matrix was used as a growing medium for planted corn, pea and mustard seeds. The number of seeds germinated, sprouting shoot height for the corn and pea and stem length for mustard, were parameters measured relative to controls in which garden compost was used as the planting medium.

Additional seed germination assays were performed to assess the possible phytotoxicity effects of high and low molecular weight PAH compounds present in the CTIS sample (see 3.6.2.1 in chapter 3). Fresh agricultural soil was contaminated by spiking with naphthalene, phenanthrene and anthracene (representing the low molecular weight PAH group) at total concentration of 1455.4 µg/g and pyrene and perylene (representing the high molecular weight PAH group) at total concentration of 2059.3 µg/g and then used separately as a planting medium to grow corn, pea and mustard, as in the above. In addition to seed germination bioassays, the shoot and root of germinated seeds were dried at 105 ^{o}C for 24 h and their mean dry weights measured to assess sub-lethal effects (e.g. Dawson *et al.*, 2007).

6.2 Result and Discussion

6.2.1 *Phosphatase enzyme activity assays for toxicity assessment of compost matrices with drill cuttings and CTIS*

The compost mixtures comprising organic amendments with drill cuttings and CTIS, respectively, were biotreated by incubation following the natural composting cycle for 53 days as discussed in Chapter 4. Phosphatase enzyme activity assays were performed on the compost mixes and sterile control samples at the start (day 0) and end (day 53) of the incubation periods. Results of the measured phosphotase activity level in the lab-scale compost mixes with drill cuttings and CTIS are presented in Tables 6.1 and 6.2, respectively. The results show that the mean phosphatase activity in the organic amended compost mixes (18.6 and 12.2 µmole g^{-1} h^{-1}) were significantly higher ($p<0.05$ *t*-test) than the controls (0.49 and 0.53 µmole g^{-1} h^{-1}) at day 0 for the drill cuttings and CTIS respectively. This may be attributed to the organic amendment samples added. Organic amendments often stimulate microbial and enzymatic activities when added to soil because of their content of endo or exocellular enzymes (Goyal *et al*., 1993; Lee *et al*., 2008). Phosphatase activity may have been hampered in the control samples due to sterilisation. Steam sterilization has been reported to inactivate alkaline phosphatase (Eivazi and Tabatabai, 1977). Additionally, the control samples have lower carbon content than their respective organic amended samples, which may reduced their phosphatase activity (Debosz et al., 1999; Dawson et al., 2007).

Comparison of phosphatase activity between the organic amended compost mixes revealed that phosphatase activity at day 0 was higher in the mixes containing more easily degradable organic amendments, i.e., grass cuttings and straw e.g. in DGS_t and SGS_t mix types. This may have been induced by active microbial cellular enzymes in the compost humic matrix (Pascual *et al*., 1998). It is suspected that enzymatic activity in the compost matrices containing spent mushroom compost at day 0 may have been slowed due to the presence of gypsum and some other additives which were added as casing material to the mushroom compost. However, this opinion is contrary to the findings published by Pérez-Piquere *et al*. (2006) in which spent mushroom composts amendments enhanced development and activity of soil microflora better than green waste compost, though the authors also acknowledged that the green waste compost they used decomposed slowly and

acted as a long term source of nutrients, unlike the fresh garden grass cuttings used in this study.

Phosphatase activity reduced significantly ($p<0.05$ *t*-test) in the organic amended compost mixes with drill cuttings and CTIS after treatment for 53 days. Percent reduction of phosphotase activity ranged from 75.7 to 89.6% and 78.2 to 89.1% in the compost mixes with drill cuttings and CTIS respectively. These large reductions in phosphatase activity may be attributed to failures of microbial recolonisation in the compost matrices during the cooling phase under the experimental conditions used. After the incubation process at day 53, experimental samples were immediately stored by freezing until analysis for phospotase enzyme activity without allowing more time to observe the composting maturation phase. Also, the high temperature (60 ^{o}C) to which the samples were incubated might have inhibited the microbial diversity as well as the enzymatic potential of the compost matrix (e.g. Antizar-Ladislao *et al*., 2006). Co-contaminants such as heavy metals may be additional factors which may have influenced the significant decrease in phosphatase activity in the compost mixes after the incubation period. Heavy metal concentrations are usually higher in remediated soils and may have reduced the enzyme activities, especially when PAHs and heavy metals are found in combination (Dawson *et al*., 2007). Maliszewska-Kordybach and Smreczak (2003) reported that when heavy metals are found in combination with PAHs, toxicity increases. This was attributed to increase in permeability of bacteria membranes to heavy metals when they interact in the presence of lipophilic PAHs.

Phosphatase activity decreased by 34.5% and 30.4% in the sterile control for the drill cuttings and CTIS treatments, respectively (Tables 6.1 and 6.2), which were significantly ($p<0.05$ ANOVA) lower than their respective compost mixes with organic amendments. However, their relative reductions are less meaningful due to their very low amount at day 0. Graphical presentations of *p*-nitrophenol measurements during the lab-scale composting treatments for drill cuttings and CTIS are shown in Appendices AK and AL respectively, while calibration data and graph of standard of phosphorus analysis for each of the compost mix types are shown in Appendices AM - AW.

6.2.2 *Phosphatase enzyme activities in the outdoor compost bioremediation experiments*

Phosphatase enzyme activity reduced by 26.5% to 12.9 µmole g^{-1} hr^{-1} in the compost mix with organic amendments after the outdoor compost-bioremediation treatment in compost bins for 56 day (Table 6.3). This result is significantly (p=0.002 *t*-test) lower compared to the SMS_t3 sample during the lab-scale experiment (79.6%), indicating that there was much higher microbial activities in the outdoor treated compost matrix. The maximum temperature measured in the composting matrix was 31 ^oC which may have been more conducive for microbial enzymatic activities as opposed to the 60 ^oC incubation temperature applied to the sample during the lab-scale treatment as discussed earlier.

The phosphatase activity at day 0 in the compost mix for outdoor treatment was significantly (p=0.001 t-test) higher than that of the lab-scale treatment by 6.0 µmole g^{-1} hr^{-1}, whereas both samples contained the same type and proportion of organic amendments. This may be attributed to effect of storage temperature on the lab-scale samples which were stored -20 ^oC for 3 months before analysis whereas phosphatase activity analysis was performed on the outdoor samples the following day after preparing the compost mix. The impact of the cold temperature may have limited microbial activities and subsequently amount of *p*-nitrophenol released in the lab-scale samples. Some studies have reported opposing results when comparing 4 ^oC or -20 ^oC storage effects on microbial activity, such as when frozen temperatures (-20 ^oC) were reported to not significantly affect soil enzymatic properties by Lee *et al*., (2007) but a contrary result was published by Černohlávková *et al*. (2009). In general, during ice formation under freezing condition, salt concentrations are increased, cells are killed and cell structures are damaged resulting in decreased enzymatic activity (Stenberg *et al*., 1998; Černohlávková *et al*., 2009).

Unlike the trend observed in the compost mix sample, phosphatase enzyme activity in the controls increased by 16.4% to 10.4 µmole g^{-1} hr^{-1} at the end of the composting time. This observation may be because the outdoor control sample was not heat sterilised. As a result, ventilation in the bin might have sustained and increased microbial activities in the CTIS matrix. Results of *p*-nitrophenol measurements during the outdoor pilot-scale composting treatment are presented graphically in Appendix AX.

Table 6.1. Phosphatase enzyme activity data for lab-scale compost mixes with drill cuttings

	Day 0		Day 53		
	p-nitrophenol (µmole g^{-1} h^{-1})		*p*-nitrophenol (µmole g^{-1} h^{-1})		Reduction
Sample	**Mean**	**Std**	**Mean**	**Std**	**%**
Control	0.49	0.04	0.32	0.01	34.5
DGSt 1	20.04	2.39	2.08	0.32	89.6
DGSt 2	21.97	1.01	2.65	0.33	87.9
DGSt 3	28.71	0.82	3.46	0.53	87.9
DMSt 1	10.78	0.84	2.16	0.25	79.9
DMSt 2	16.32	0.65	3.94	0.20	75.9
DMSt 3	14.91	1.32	3.63	0.20	75.7
DGMSt 1	19.38	2.13	2.71	0.15	86.0
DGMSt 2	16.18	0.17	3.23	0.61	80.0
DGMSt 3	18.92	1.42	3.43	0.22	81.9

Values are mean (n=3); Std = standard deviation; Control = drill cuttings without organic amendments

Table 6.2. Phosphatase enzyme activity data for lab-scale compost mixes with coal tar impacted soil (CTIS)

	Day 0		Day 53		
	p-nitrophenol (µmole g^{-1} h^{-1})		*p*-nitrophenol (µmole g^{-1} h^{-1})		Reduction
Sample	**Mean**	**Std**	**Mean**	**Std**	**%**
Control	0.53	0.07	0.37	0.08	30.8
SGSt 1	11.83	0.17	1.29	0.06	89.1
SGSt 2	13.99	0.29	1.52	0.05	89.1
SGSt 3	19.97	1.16	2.37	0.29	88.1
SMSt 1	8.00	0.39	1.22	0.14	84.8
SMSt 2	9.50	0.40	2.07	0.23	78.2
SMSt 3	11.54	0.90	2.35	0.15	79.6
SGMSt 1	8.35	1.75	1.19	0.06	85.8
SGMSt 2	11.52	0.66	1.52	0.25	86.8
SGMSt 3	15.09	0.73	2.50	0.25	83.4

Values are mean (n=3); Std = standard deviation; Control = CTIS without organic amendments

Table 6.3. Phosphatase enzyme activity data for outdoor pilot-scale compost mixes with coal tar impacted soil (CTIS)

	Day 0		Day 53		
	p-nitrophenol (µmole g^{-1} h^{-1})		*p*-nitrophenol (µmole g^{-1} h^{-1})		Reduction
Sample	**Mean**	**Std**	**Mean**	**Std**	**%**
Control	8.94	0.99	10.40	0.33	-16.4
Compost mix	17.53	0.63	12.89	0.60	26.5

Values are mean (n=5); Std = standard deviation; Control = CTIS without organic amendments

6.2.3 Seed germination assays

The finished compost matrix from the outdoor composting treatment was used as a planting medium by filling ~30 g aliquots into propagator cells to form plots of 10 cells (in triplicate) for germinating corn, pea and mustard seeds. Single seeds of corn and pea and 3 seeds of mustard were planted in respective cells. Planted propagators were placed on a windowsill to germinate and sprout during the summer when daylight was 14 h minimum and temperatures were 12 – 22 ^{o}C. When 70% of a particular seed in the control plots germinated, the numbers of the seeds germinated in all sample plots was recorded. The setups of the seed germination and growth performance experiments in the different planting media are shown in Appendices AY - BM. Sprouting shoot height for corn and pea and stem length for mustard were additional parameters measured (Figure 3.4).

Results of the seed germination assay presented in Table 6.4 clearly show the effect of the outdoor compost-bioremediation treatment in decreasing phytotoxicity. The toxicity of the bioremediation control (CTIS only) was higher than that of the compost mix after treatment, while phytotoxicity responses were different for the different plant species used. At five days after planting, the amount of corn seeds germinated in the treated compost mix were 70% more than in the untreated mix (Table 6.4). Within the same time, 20% more pea seeds germinated in the treated mix compared to the untreated mix (Table 6.4). This observation may indicate that the peas were more sensitive to the residual toxicity in the treated compost mix, while the corn seeds showed more sensitivity to the toxicity of the untreated compost mix. Mustard seeds germination in the treated compost mix was 73% higher than that of the untreated mix (Table 6.4).

The amount of seeds germinated in the compost mix was significantly higher by 60% ($p<0.001$ *t*-test) and 50% ($p<0.001$ *t*-test), compared to the CTIS only after treatment for the corn and pea plants, respectively. However, there was no significant difference (13%, $p=0.092$) in the amount of mustard seeds germinated in the two substrates. This indicated that whereas phytotoxicity of the compost mix on corn and pea was significantly decreased after the treatment, the mustard seed appeared to have greater tolerance for the residual coal tar toxicity in the untreated CTIS. The implication of this observation could be that mustard growers may have to be very sensitive with the soil quality as growth performance in hydrocarbon polluted soil could be misleading. However, mustard seed germination assay has been reported as sensitive and robust enough to be used for assessing the quality of recovered soil from a former manufactured gas plant (Dawson *et al*., 2007). Furthermore, the increase of seed germination recorded for all test plants in the CTIS only experiments after the treatment period may be attributed to natural attenuation process which may have occurred within the 56 days treatment period (Kao, *et al*., 2001; Megharaj, *et al*., 2011). Variation in number of seeds germinated in the control was not significantly different from the treated compost mix for corn and mustard, except for pea ($p=0.013$ *t*-test). Graphical presentations of the result of the seed germination assay are shown in Appendices BN - BP.

Table 6.4. Result of seed germination toxicity assay for corn, pea and mustard

	Amount of corn seed germinated							
	Before compost-bioremediation				After compost-bioremediation			
	Day 5		Day 7		Day 5		Day 7	
Planting medium	Mean	Std	Mean	Std	Mean	Std	Mean	Std
Control	8	0.6	10	0.6	-	-	-	-
CTIS only	0	0.0	3	0.6	3	0.6	4	0.6
Compost mix	2	0.6	7	0.6	9	0.6	10	0.0
	Amount of pea seed germinated							
	Before compost-bioremediation				After compost-bioremediation			
	Day 5		Day 7		Day 5		Day 7	
Planting medium	Mean	Std	Mean	Std	Mean	Std	Mean	Std
Control	9	0.6	10	0.0	-	-	-	-
CTIS only	0	0.0	1	0.6	1	0.6	3	0.6
Compost mix	4	0.6	8	0.6	6	0.6	8	0.6
	Amount of mustard seed germinated							
	Before compost-bioremediation				After compost-bioremediation			
	Day 3		Day 5		Day 3		Day 5	
Planting medium	Mean	Std	Mean	Std	Mean	Std	Mean	Std
Control	28	0.6	29	0.6	-	-	-	-
CTIS only	2	0.6	21	2.1	20	0.6	28	0.6
Compost mix	2	0.6	7	0.6	24	1	26	1.5

Values are mean (n=10 for corn and pea, n=30 for mustard); Std = standard deviation; Control = organic compost; - = not measured.

6.2.4 *Plant growth assays*

The sprouting shoot height was measured for corn and pea seeds 7 days after planting while growing stem length of the mustard seed was measured 5 days after planting. This was to assess growth performance of the various seeds after germination in the remediated compost mixtures. All the plants tested showed progressive growth after germination in the different planting media. Percentage increases of mean shoot heights recorded in the CTIS-only were generally higher than those of compost mix for all seeds tested. The sprouting shoot lengths increased by 52%, 58% and 63% in the untreated CTIS-only as against 30%, 23% and 66% in the compost mix for corn, pea and mustard seeds respectively (Figures 6.1 – 6.3). These differences may be attributed to incomplete decomposition of the biomass in the compost mix after the composting period. As a result the inherent soil nutrients in the organic amendments may not have been fully released for the plants

to utilise, or alternatively the organic matter decomposition during the composting process may have produced inhibitory components.

Consistent with the observation during the seed germination assay (6.2.3), the mustard seed was not sensitive to the residual phytotoxicity of the untreated CTIS, as it recorded higher mean shoot height in the CTIS-only than the control after compost-bioremediation (Figure 6.3). There was no significant difference in shoot height of the sprouting pea in the compost mix compared to the control after compost-bioremediation (Figure 6.2). However, the shoot height of the sprouting corn in the compost mix was significantly lower compared to the control (Figure 6.1). This behaviour was the reverse of the trend observed during the seed germination assay and may be an indication that the corn seed was more susceptible to the residual phytotoxicity in the treated compost mix in the long term. A strong plant height inhibition has been reported for corn during 2 months growth test on gas work soils (Henner *et al*., 1999).

Generally, all the seeds tested exhibited higher growth rates in the day 56 treated compost mix compared to day 0 untreated compost mix, indicating that the compost-bioremediation treatment tested in this study reduced the coal tar toxicity as well as improved fertility of the CTIS. Furthermore, analysis of data resulting from this study revealed that pea and corn discriminated best between the planting media tested for seed germination and growth assays respectively.

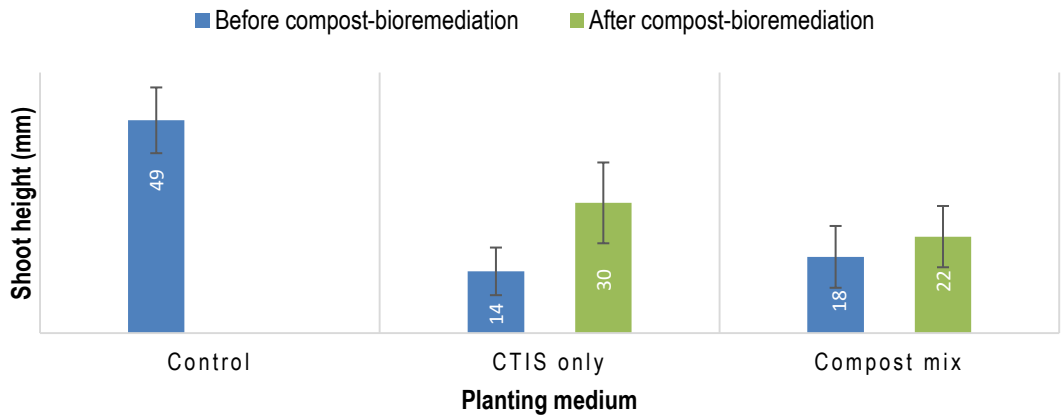


Figure 6.1. Corn shoots height 7 days after planting
Values are mean (n=10); Error bars indicate standard deviation; Control = Garden compost

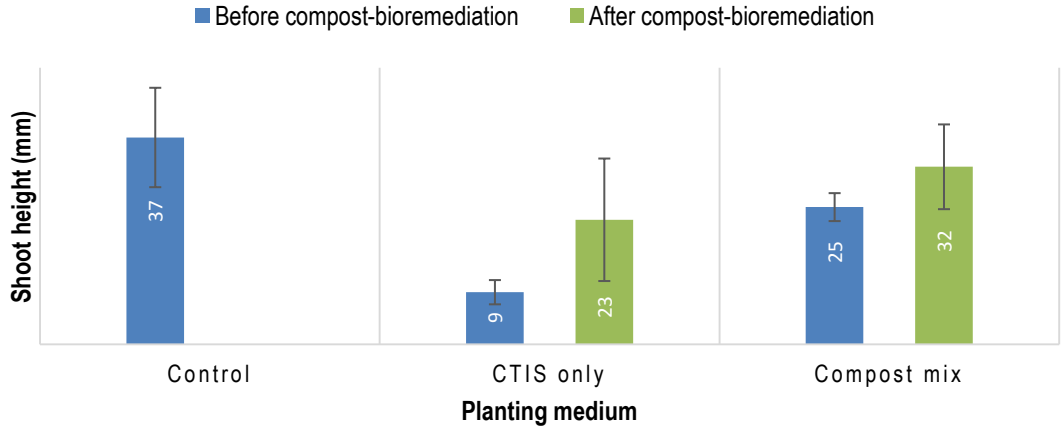


Figure 6.2. Pea shoots height 7 days after planting
Values are mean (n=10); Error bars indicate standard deviation; Control = Garden compost

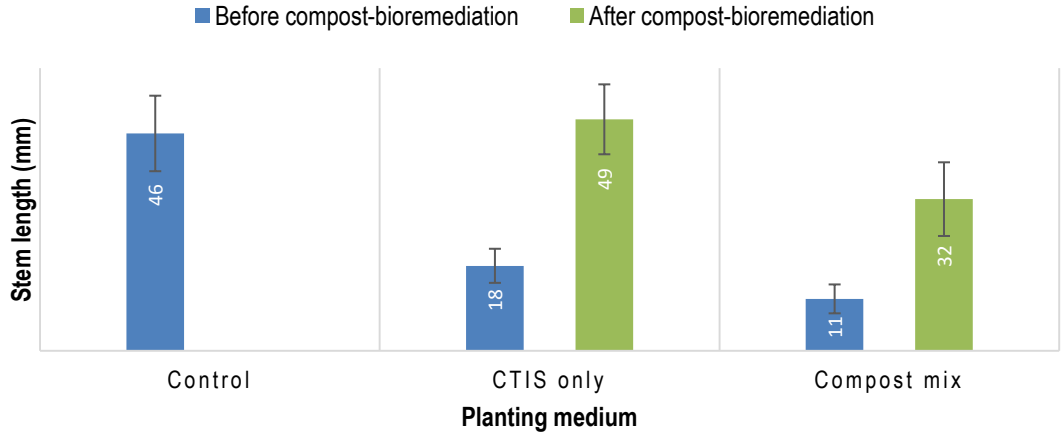


Figure 6.3. Mustard stem length 5 days after planting
Values are mean (n=10); Error bars indicate standard deviation; Control = Garden compost

6.2.5 *Phytotoxicity assays of PAH compounds in soil*

The seed germination and growth toxicity assays in the low molecular weight (LMW) and high molecular weight (HMW) PAH-spiked soils were performed at 9 days after planting for corn and pea and 7 days after planting for mustard. This longer experimentation duration was adopted due to decreasing day light time when the experiment was conducted. Analysis of the results in Table 6.4 revealed that pea and mustard seeds germination were not sensitive to the two soil conditions tested (Soil+LMW PAHs and Soil+HMW PAHs respectively). Also, their percentage germination in these planting media was not significantly different when compared to their respective controls (Appendix BQ). Only the corn seeds showed percentage germinations that were significantly ($p<0.05$) lower in relation to the control. Also, the percent corn germination in the two soil conditions was not significantly different. This may be an indication that corn germination was highly sensitive to the phytotoxicity of LMW as well as HMW PAH compounds under the conditions tested. Graphical plots of the results of the seed germination toxicity assay in PAH-spiked soil are shown in Appendices BR - BT.

Table 6.5. Results of seed germination toxicity assays for corn, pea and mustard planted in soils amended with different molecular weight PAHs

	Corn germinated		Pea germinated		Mustard germinated	
Growth medium	**Mean**	**Std**	**Mean**	**Std**	**Mean**	**Std**
Control	8	0.5	9	0.5	30	0.0
Soil + LMW PAHs	2	0.5	7	1.4	27	0.5
Soil + HMW PAHs	2	0.5	7	0.8	29	0.9

Values are mean (n=10 for corn and pea, n=30 for mustard); Std = standard deviation; Control = Fresh agricultural soil; LMW = Low molecular weight (1455.4 µg/g); HMW = High molecular weight (2059.3 µg/g)

Generally, corn and pea plants exhibited high phytotoxicity sensitivity to HMW and LMW PAHs based on shoot and root biomass assays (Table 6.6). The shoot biomass of the corn was not significantly ($p=0.061$ *t*-test) different in the LMW PAH-spiked soil but was found to be significantly ($p=0.019$ *t*-test) lower compared to the control in the HMW PAH-spiked soil. A similar comparison revealed that the root biomass was significantly ($p=0.005$ and $p=0.008$) lower in the LMW and HMW PAH-spiked soils, respectively. Root development at early stages of plant growth has been attributed to cell expansion which could be inhibited by toxicants present in the soil (Ren *et al*., 1996). For the pea, the shoot and root biomasses were found to be significantly ($p<0.04$) lower with respect to the control in both soil conditions. In contrast to the

trends observed for the corn and pea, root and shoot biomasses were not significantly different in both soil conditions in relation to the control for the mustard.

Based on the results from this study, corn was found to show most sensitivity to both LMW and HMW PAH toxicity in soil. This observation is consistent with findings from phytotoxicity study reported by Baek *et al*. (2004) but in contrast to some other reported studies which indicated that plant germination and growth are strongly inhibited by LMW aromatic compounds but not by HMW PAHs (Henner *et al*., 1999; Smreczak and Maliszewska-Kordybach, 2003) Benzene, toluene, xylene (BTX), styrene, and naphthalene have been identified as LMW PAHs responsible for plant germination and growth inhibition (Henner *et al*., 1999). The mustard consistently exhibited moderate to high tolerance to CTIS and PAH-spiked soils. This observation is also consistent with findings in previously reported studies where mustard was among plants used for phytotoxicity assays (e.g. Cajthaml *et al*., 2002; Smreczak and Maliszewska-Kordybach, 2003).

Table 6.6. Shoot and root biomass for sprouting peas planted in soils amended with different molecular weight PAHs

	Corn biomass (g)			
	Shoot		**Root**	
Planting medium	**Mean**	**Std**	**Mean**	**Std**
Control	0.064	0.013	0.757	0.106
Soil + LMW PAHs	0.033	0.007	0.112	0.054
Soil + HMW PAHs	0.013	0.006	0.163	0.126
	Pea biomass (g)			
	Shoot		**Root**	
	Mean	**Std**	**Mean**	**Std**
Control	0.209	0.020	0.646	0.021
Soil + LMW PAHs	0.037	0.003	0.147	0.091
Soil + HMW PAHs	0.058	0.051	0.104	0.012
	Mustard biomass (g)			
	Shoot		**Root**	
	Mean	**Std**	**Mean**	**Std**
Control	0.133	0.003	0.025	0.002
Soil + LMW PAHs	0.102	0.003	0.020	0.001
Soil + HMW PAHs	0.115	0.014	0.016	0.001

Values are mean (n=10 for corn and pea, n=30 for mustard); Std = standard deviation; Control = Fresh agricultural soil; LMW = Low molecular weight (1455.4 µg/g); HMW = High molecular weight (2059.3 µg/g)

6.3 Conclusions

The toxicity assays on the compost-bioremediated matrices showed that the bioremediation technique applied in this study was able to reduce the toxicity, as well as recover some functional capacity of the contaminated soils. Phosphatase activity significantly decreased in the organic amended compost matrices with drill cuttings and CTIS after 53 and 56 days of treatment in lab-scale and outdoor pilot scale, respectively, though the absolute phosphatase activities in the sterile controls were much less than in the organic amended compost mixes both before and after the composting period. The decrease of phosphatase activity in the outdoor CTIS remediated compost matrix was 53.1% lower than the equivalent lab-scale sample, indicating higher enzyme activity in the outdoor experiments possibly due to more favourable natural composting conditions. The outdoor control soil produced phosphatase activity 10.1% higher than its counterpart organic amended matrix at the end of the 56 days treatment duration which was attributed to more ventilation in the compost bins.

Results obtained from the phytotoxicity assays showed increases in seed germination and plant growth properties of the organic amended compost matrix after the outdoor compost-bioremediation. Pea and corn discriminated best between the planting media tested for seed germination and growth assays, respectively, while mustard consistently exhibited high tolerance to coal tar and PAH phytotoxicity. Pea germination was not sensitivity to low and high molecular weight PAH-spiked soil. Plant biomass sensitivity to low and high molecular weight PAH-spiked soil was significantly high for pea and corn but not for mustard. The observed high tolerance of mustard to phytotoxicity in this study, which is consistent with previously published reports, may indicate unsuitability of mustard for assessing the ecological health of coal tar and PAH contaminated soils after remediation. Additionally, reduction in toxicity of the compost-bioremediated matrices, as indicated by results of the phytotoxicity tests, may be due largely to the SMC amendment which has been indicated as the major contributing factor in TPH reduction in Chapter 4. This observation is consistent with reported studies in which significant reduction in soil toxicity was achieved after micoremediation (Cajtham *et al*., 2002).

Chapter 7

CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

The overall aim of this research was to investigate a compost mix type, comprising of cheap and readily available agricultural waste materials as organic amendments, that would effectively degrade hydrocarbon pollutants and reduce toxicity in oil-field drill cuttings and coal tar impacted soil (CTIS) using a compost-bioremediation treatment. To achieve this, compost mixes comprising of grass cuttings, spent mushroom compost and straw were blended with each of the contaminated media (drill cuttings and CTIS) in different mix ratios and then tested in laboratory scale composting treatment for 53 days in order to identify a compost mix type that would produce the most hydrocarbon degradation and to determine the effects of the degradation on the different types of hydrocarbons present. The best performed compost mix type was subsequently scaled-up and tested in an outdoor pilot scale compost-bioremediation treatment. The toxicity of the treated compost matrices was investigated by performing microbial and biological analyses on them using phosphatase enzyme activity and phytotoxicity assays respectively.

7.1.1 *Laboratory-scale biodegradation of pollutant hydrocarbons in drill cuttings and CTIS*

In the drill cuttings treatments, the highest reduction of total petroleum hydrocarbon (TPH) was 85.1% which was achieved in the compost mix type comprising drill cuttings, grass cuttings, spent mushroom compost and straw ($DGMS_t3$) having a contaminated medium to organic amendments mix ratio of 1:2. Although the percent reduction of TPH in this mix type was significantly higher compared to the unamended control, it was however not significantly different compared to the other compost mix types. This result indicated that each compost mix type has considerable potential for enhancing the biodegradation of hydrocarbon contaminants in oil field drill cuttings. Significant depletion of individual as well as total *n*-alkanes was also achieved in the $DGMS_t3$ mix type, with increases in pristane/*n*C17 and phytane/*n*C18 ratios, indicating the occurrence of mild to moderate petroleum hydrocarbon biodegradation.

The compost mix type comprising CTIS, spent mushroom compost and straw (SMS_t3), which also had a contaminated medium/organic amendments mix ratio of 1:2, was found to produce the most TPH reduction of 90.6% in the treatment of CTIS. Like the trend observed in the drill cuttings treatment, the TPH reduction in the SMS_t3 mix type was also found to be significantly higher compared to the no-amendment controls, but was not significantly different in relation to each of the other compost mix types with CTIS. Comparison of TPH removal performance in all the compost mix types with drill cuttings and CTIS revealed that all the mix types with spent mushroom compost amendment produced higher percent TPH reduction than the rest. In addition to the macro-and micronutrients present, this may have been due to the presence of ligninolytic fungi in the spent mushroom compost which are known to produce reactive radicals for non-specific cleavage of a wide variety of recalcitrant organopollutants (e.g. Gong *et al*., 2006). The individual as well as total *n*-alkane concentrations were also significantly degraded in the SMS_t3 compost mix types after the treatment periods.

In the CTIS treatment study, the concentration of total PAHs was significantly degraded. The total concentration of 2 and 3-ring PAHs was significantly degraded in all compost mix types and also in the controls. This was partially attributed to abiotic factors (mainly volatilisation) which may have prevailed during the treatment process. In the SMS_t3 compost mix type, the total concentration of 4-ring PAHs was significantly reduced but that of 5 and 6-ring PAHs was not reduced significantly. Several PAH compounds in the 5 and 6-ring category exhibited recalcitrance to degradation and even apparently increased in concentration after the treatment period. This is attributed to the fact that 5 and 6-ring PAHs are hydrophobic compounds which are more resistant to microbial degradation than lower ringed PAH, while their apparent increase in concentration was possibly due to heterogeneity effects and degradation of the compost matrices during the compost treatment period, perhaps making the PAH more easily extractable, but also increasing the relative concentrations of these recalcitrant pollutants in the remaining degraded compost mix.

7.1.2 *Biodegradation of pollutant hydrocarbons in CTIS during outdoor composting treatments*

In this study, the SMS_t3 compost mix type was scaled-up by a factor of 600 from the laboratory experiments and then tested in tumbler compost bins outdoors for 56 days. The concentrations of TPH, total *n*-alkanes and total PAHs were significantly degraded at 28 days into the treatment period. Concentrations of TPH were reduced by 53% at 28 days into the treatment before finally degrading by 78% at the end of the treatment period. The reduction in TPH concentration was 31.2% and 42.8% higher compared to the controls at 28 and 56 days of treatment respectively. The results also showed that TPH was most depleted at lower temperatures (20 – 23 ^{0}C) after the thermophilic phase of the composting cycle, when the moisture content of the compost matrix was ranging between 62 and 70%. This observation is considered a reason for concluding that 62% to 70% is a suitable moisture content range for TPH removal in the type of tumbler compost bins used for this study, which is higher than many reported optimal moisture contents for composting. Furthermore, the high TPH reduction achieved at lower temperatures supports reports from earlier studies that lower temperatures might allow increased microbial activity during composting (e.g. Liang *et al*., 2003). The achieved percentage TPH removal after the treatment duration was significantly lower compared to the lab-scale treatments. This is attributed to higher volatilisation enabled by the higher incubation temperature (60 ^{0}C) during the lab-scale experiments.

The total *n*-alkane concentrations were also significantly degraded (by 69.5%) at 28 days into the treatment before finally degrading by 78% after the treatment period. Similar to the trend observed in TPH removal, the reduction of total *n*-alkane concentrations was significantly lower relative to results obtained from the lab-scale experiments. Hydrocarbon degradation was significant for the sum of 2 and 3-ring and 4-ring PAH groups. The abundances of 2 and 3-ring PAHs were almost completely degraded while 4-ring PAHs including fluoranthene, pyrene benzo[a]anthracene and chrysene were significantly degraded. The sum of 5 and 6-ring PAH group were not significantly degraded after the composting duration. However, the concentrations of all the individual PAHs within the 5 and 6-ring PAH group were found to be lower after treatment unlike the lab-scale treatment where some apparent increases in concentrations were recorded. It is possible that higher hydrocarbon degradation performance may have been achieved if the tumbler

compost bins were made more thermally insulated as the temperature profiles of the compost matrices were found to be strongly influenced by the ambient temperature.

7.1.3 *Phosphatase enzyme activity assessment for compost treatment matrices with drill cuttings and CTIS*

Phosphatase enzyme activity significantly decreased in the organic amended compost matrices with drill cuttings and CTIS after the lab-scale treatment for 53 days. This low level of phosphorus mineralization may be an indication of the failure of full microbial recolonisation in the compost matrices during the cooling phase of the composting cycle. Additionally, the peak temperature (60 ^{0}C) at which the samples were incubated may have inhibited the enzymatic potential of the compost matrix as well as the microbial diversity (e.g. Antizar-Ladislao *et al*., 2006).

For the outdoor compost treatment experiments, phosphatase mineralisation was also found to decrease in the compost matrices after treatment for 56 days. Reduction in phosphatase enzyme activity was 26.5% which was significantly lower compared to the SMS_t3 sample (79.6%) during the lab-scale experiment, indicating higher microbial activities in the outdoor treated compost matrices. The mean maximum temperature measured in the outdoor composting matrix was 31 ^{0}C which may have been more conducive for microbial enzymatic activities as opposed to the 60 ^{0}C incubation temperature of the samples during the lab-scale treatments. Phosphatase enzyme activity was increased by 16.4% in the control at the end of treatment period. This observation is attributed to ventilation in the compost bins resulting in increased microbial activities compared to the lab-scale treatment.

Generally, the phosphatase enzyme activity assay applied in this study appeared not to correlate with the geochemical analysis and the other biological indicator (phytotoxicity). Whereas results where indicating reduction in hydrocarbon contamination and phytotoxicity, the enzyme activity assay was suggesting to the contrary. This may be due to the fact the phosphatase enzyme assay methodology used was developed specifically for soil samples and there was no indication of its generic application to other test samples such as the compost samples tested in this study. It is therefore concluded that phosphatase enzyme activity assay may not be ideal for testing hydrocarbon toxicity in compost matrices.

7.1.4 *Phytotoxicity assay on CTIS after outdoor compost-bioremediation*

Seed germination and plant growth properties of the organic amended compost matrix significantly increased after the outdoor compost-bioremediation treatment, indicating reduction in phytotoxicity of the CTIS. Pea and corn discriminated best between the planting media tested for seed germination and growth assays, respectively, while mustard consistently exhibited relatively high tolerance to coal tar and PAH phytotoxicity which is consistent with a previous report on the pollutant tolerance of mustard. The reasons for this are uncertain but may be related to the ability of mustard to utilize PAH compounds in soil at the concentration tested in this study. Pea germination was not sensitive to either low or high molecular weight PAH-spiked soil at the 1455.4 µg/g and 2059.3 µg/g level, respectively. Plant biomass of pea and corn but not mustard, showed increased sensitivity to low and high molecular weight PAH-spiked soil.

7.2 Future work

The outdoor pilot scale performance testing of the $DGMS_t3$ compost mix which produced the most degradation of contaminant hydrocarbons in oil field drill cuttings during the laboratory-based experiment was one of the objectives of this study which was not concluded as originally proposed. The inability to perform that experiment was due to disappointment with the supply of fresh oil-based drill cuttings sample. It is therefore recommended that an outdoor compost-bioremediation test of the $DGMS_t3$ compost mix be performed when oil-based drill cuttings sample is available.

The spent mushroom compost sample used for this study is a commercial grade blended for garden and agricultural use and comprised of spent mushroom composts obtained from cultivating different species of mushrooms. This is unlike the compost matrix obtained from growing specific mushroom species that is commonly used in previous bioremediation studies. Considering its effectiveness in contributing to the degradation of pollutant hydrocarbons in the compost mixes tested in this study, further studies to be conducted using material from well-defined sources and with well characterised compositions and microbiological properties. Investigation of the changes in the microbial populations and hydrocarbon degrading enzyme concentrations and the factors that control them would be useful for further optimising the composting conditions for hydrocarbon removal.

In this study, a possible limitation of performance efficiency of the compost bins due to poor thermal insulation was highlighted, especially with respect to enhancing the degradation of the 5 and 6-ring PAH compounds. It is therefore recommended that any future soil bioremediation work by composting in bins should consider ways of improving the thermal insulation of the bin walls.

Larger scale outdoor compost remediation trial experiments of sufficient sizes to sustain natural composting temperature cycles would be required before assessing whether this technology would be effective for full scale contaminated land site remediation.

The necessity and extent of remediation of hydrocarbon contaminated drill cuttings will depend on the concentration and composition of the hydrocarbons in them and the local regulations covering their disposal. In the UK these regulations include those enforced by the Environment Agency relating to hazardous waste and contaminated land and they may mean that some drill cuttings containing relatively low concentrations (e.g. <12,500ppm; Environment Agency, 2015) of low toxicity TPH, may not require remediation.

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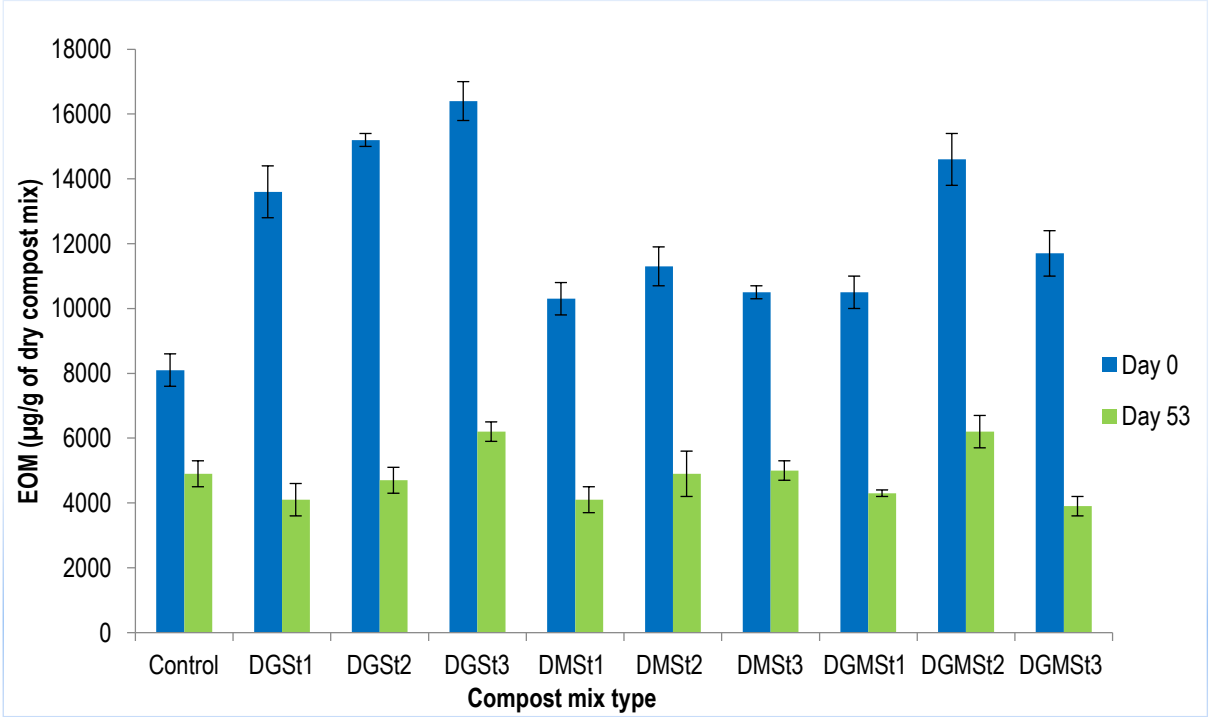
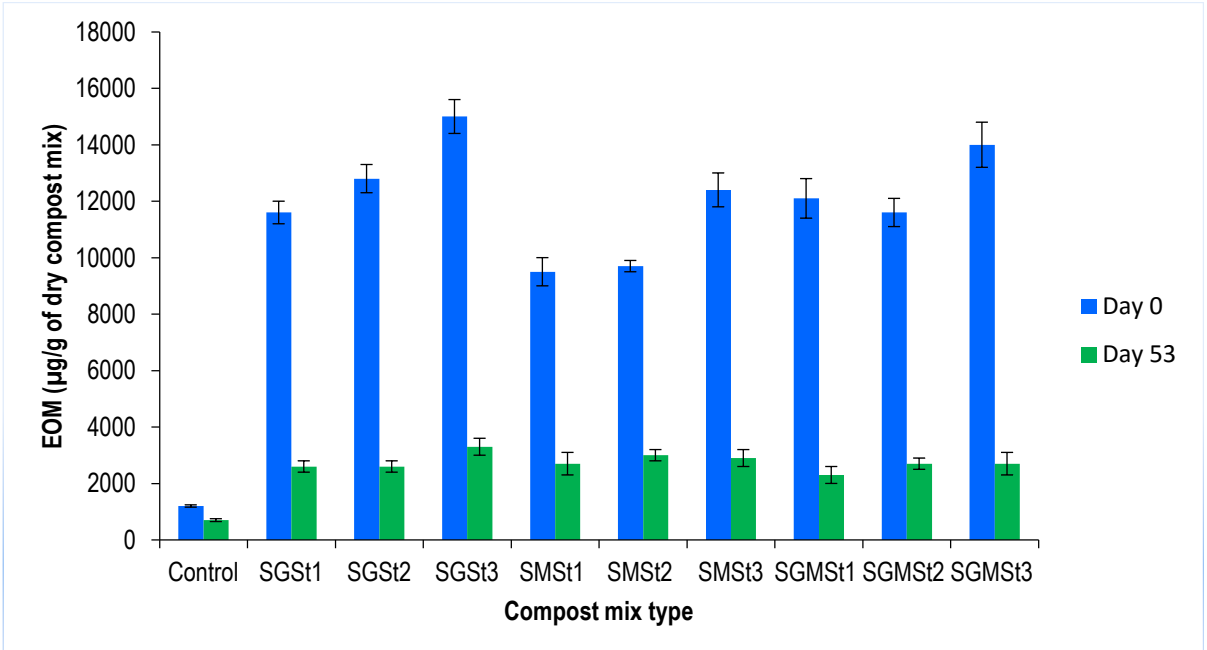
Appendices

Appendix A. Concentration of extractable organic matter (EOM) in compost mixes with drill cuttings after composting for 53 days.



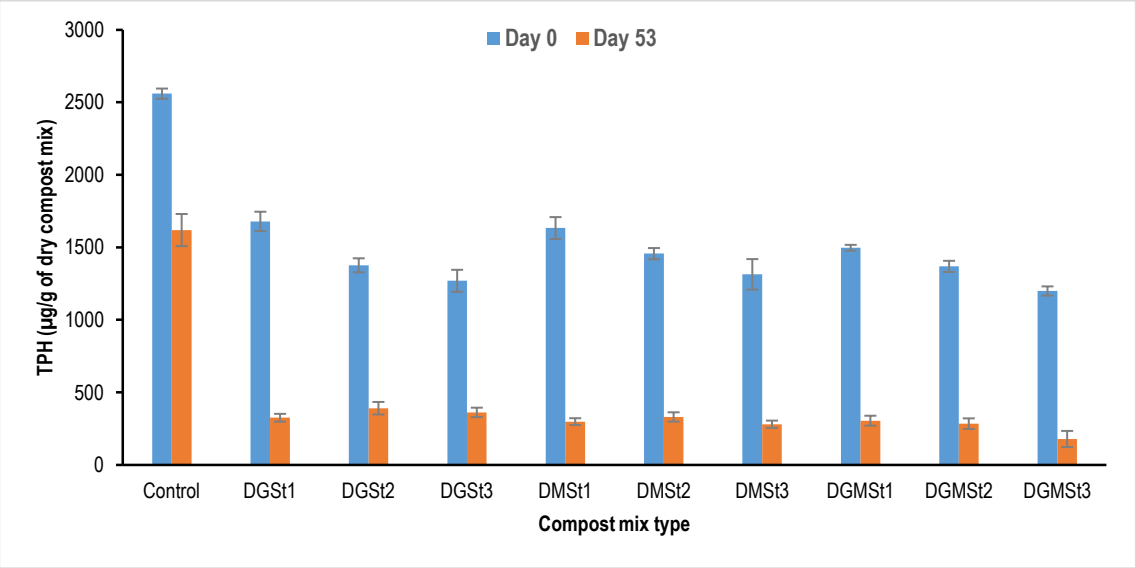
Error bars indicate ± one standard deviation from the mean (n=3); Control = drill cuttings samples without compost amendments; D = drill cuttings; G = grass cuttings; M = mushroom compost; S_t = straw.

Appendix B. Concentration of extractable organic matter (EOM) in compost mixes with coal tar impacted soil (CTIS) after composting for 53 days.



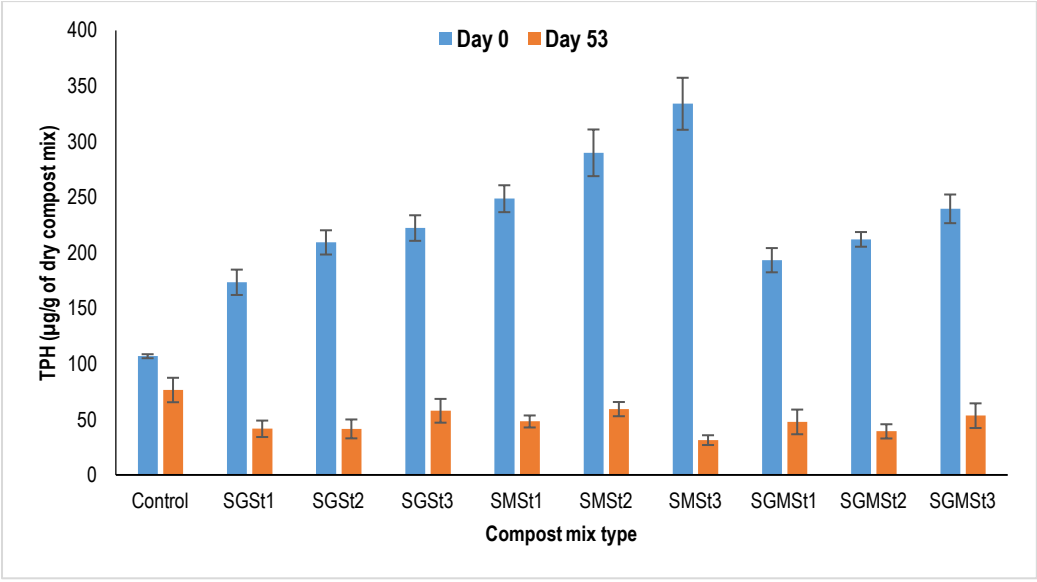
Error bars indicate ± one standard deviation from the mean (n=3); Control = CTIS samples without compost amendments; S = CTIS; G = grass cuttings, M = mushroom compost, S_t = straw.

Appendix C. Plot of total petroleum hydrocarbon (TPH) concentrations in compost mixes with drill cuttings at day 0 and day 53



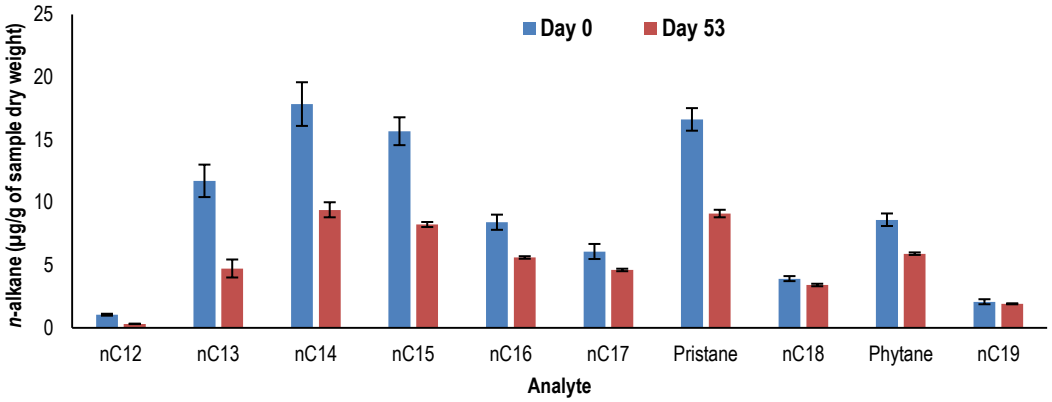
Error bars indicate ± one standard deviation from the mean (n=3); Control = drill cuttings samples without compost amendments; D = drill cuttings; G = grass cuttings; M = mushroom compost; S_t = straw.

Appendix D. Plot of total petroleum hydrocarbon (TPH) concentrations in compost mixes with coal tar impacted soil (CTIS) at day 0 and day 53



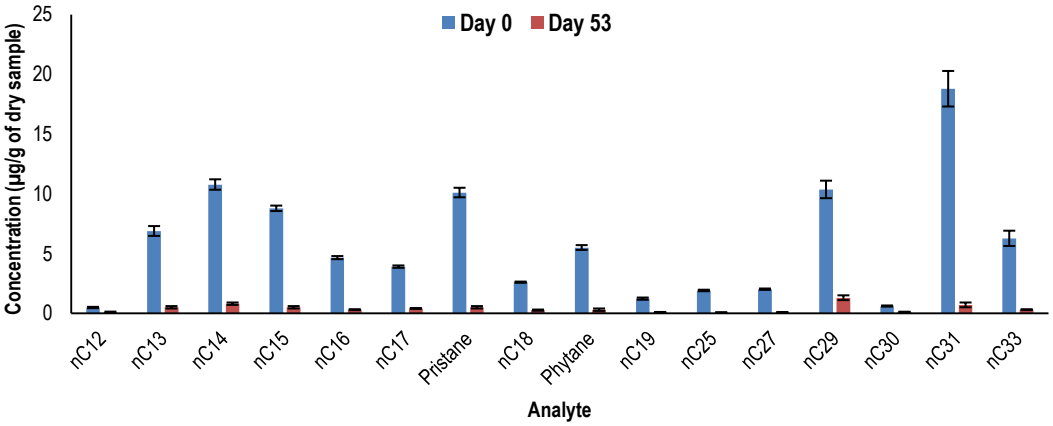
Error bars indicate ± one standard deviation from the mean (n=3); Control = CTIS samples without compost amendments; S = CTIS; G = grass cuttings; M = mushroom compost; S_t = straw.

Appendix E. Plot of n-alkane concentrations in the control for compost mixes with drill cuttings at day 0 and day 53



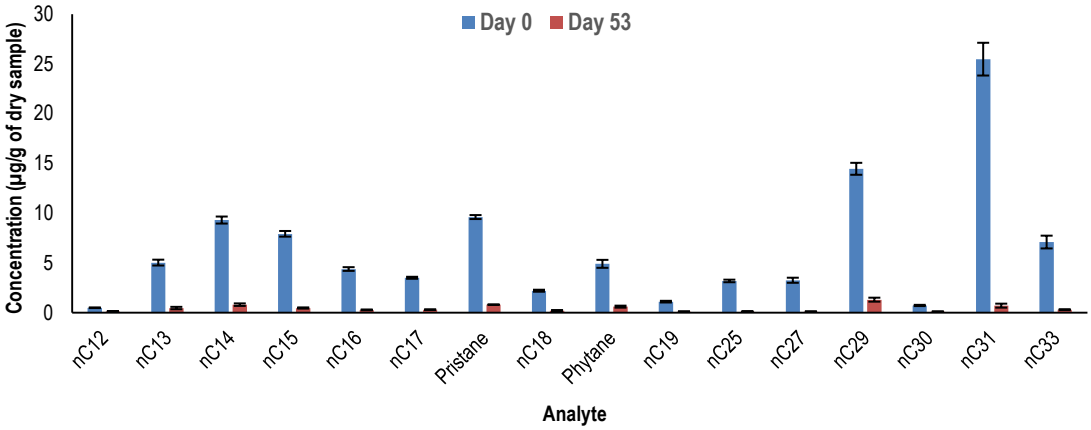
Error bars indicate ± one standard deviation from the mean (n=3); Control = drill cuttings samples without compost amendments.

Appendix F. Plot of n-alkane concentrations in drill cuttings/grass cuttings/straw 1 (DGS$_t$1) compost mix type at day 0 and day 53



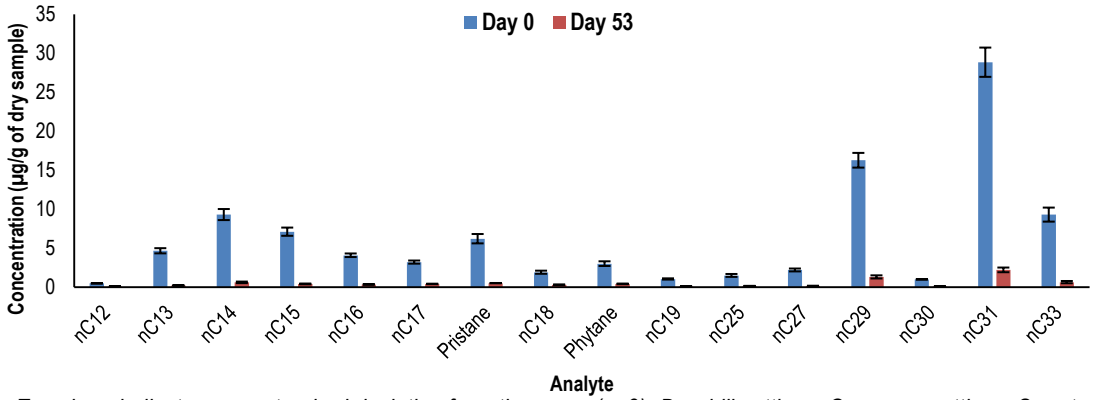
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; G = grass cuttings; S$_t$ = straw.

Appendix G. Plot of n-alkane concentrations in drill cuttings/grass cuttings/straw 2 (DGS$_t$2) compost mix type at day 0 and day 53



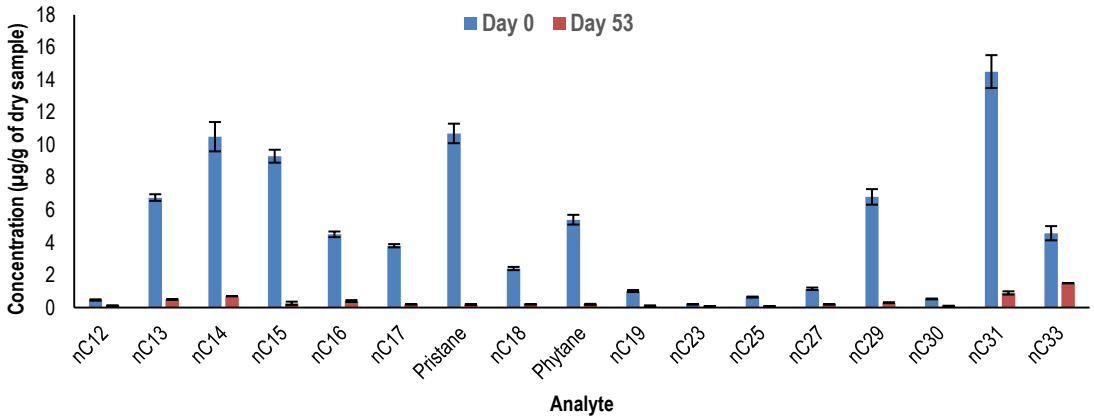
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; G = grass cuttings; S$_t$ = straw.

Appendix H. Plot of n-alkane concentrations in drill cuttings/grass cuttings/straw 3 (DGS_t3) compost mix type at day 0 and day 53



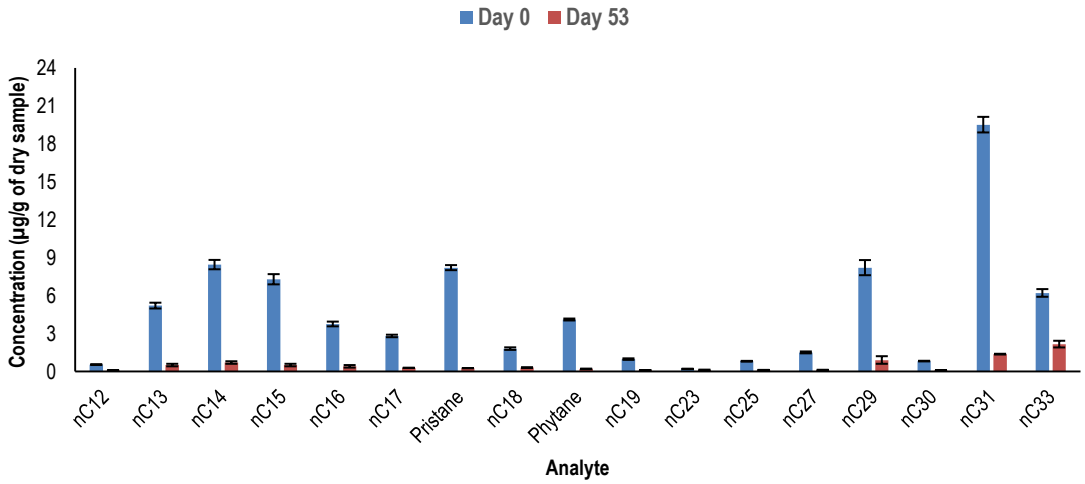
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; G = grass cuttings; S_t = straw.

Appendix I. Plot of n-alkane concentrations in drill cuttings/mushroom compost/straw 1 (DMS_t1) compost mix type at day 0 and day 53



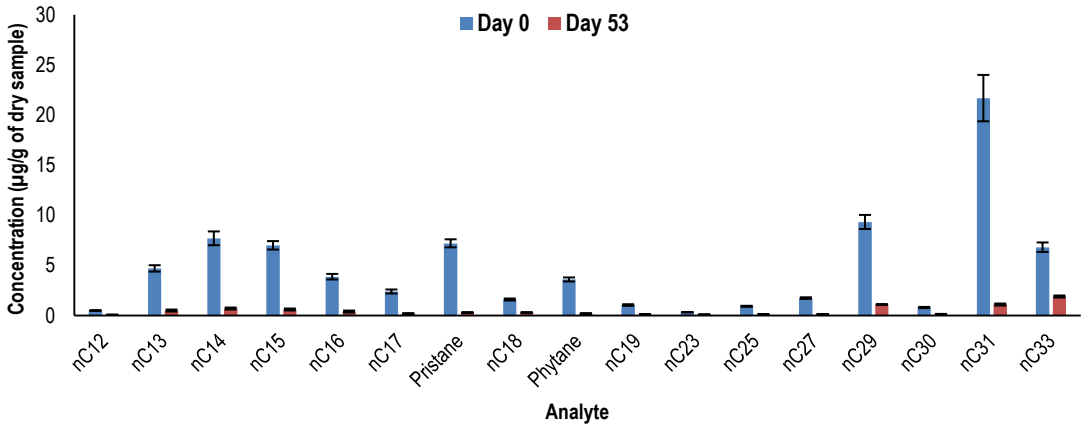
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; M = mushroom compost; S_t = straw.

Appendix J. Plot of n-alkane concentrations in drill cuttings/mushroom compost/straw 2 (DMS_t2) compost mix type at day 0 and day 53



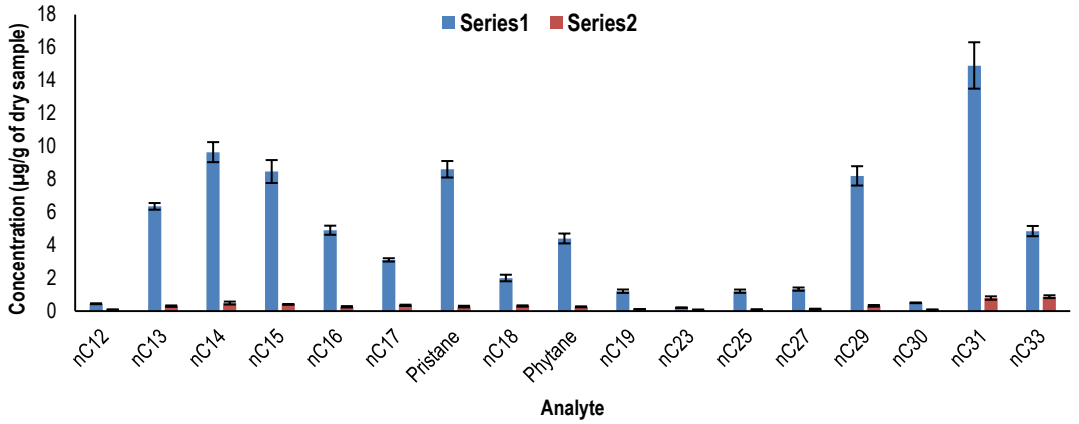
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; M = mushroom compost; S_t = straw.

Appendix K. Plot of n-alkane concentrations in drill cuttings/mushroom compost/straw 3 (DMS_t3) compost mix type at day 0 and day 53



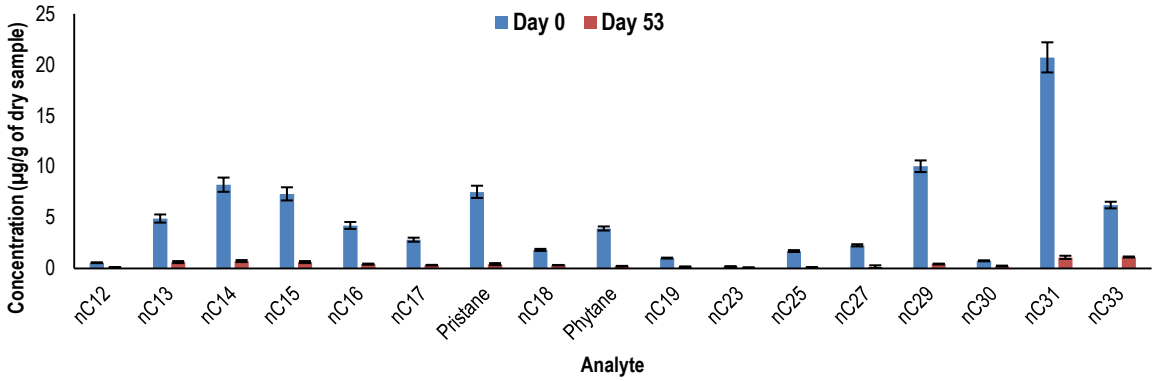
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; M = mushroom compost; S_t = straw.

Appendix L. Plot of n-alkane concentrations in drill cuttings/grass cuttings/mushroom compost/straw 1 ($DGMS_t1$) compost mix type at day 0 and day 53



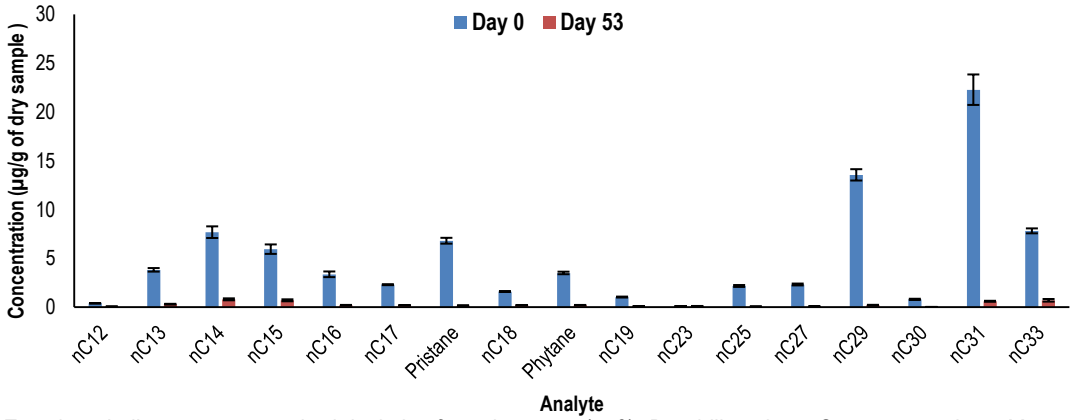
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; G = grass cuttings; M = mushroom compost; S_t = straw.

Appendix M. Plot of n-alkane concentrations in drill cuttings/grass cuttings/mushroom compost/straw 2 ($DGMS_t2$) $DGMS_t2$ compost mix type at day 0 and day 53



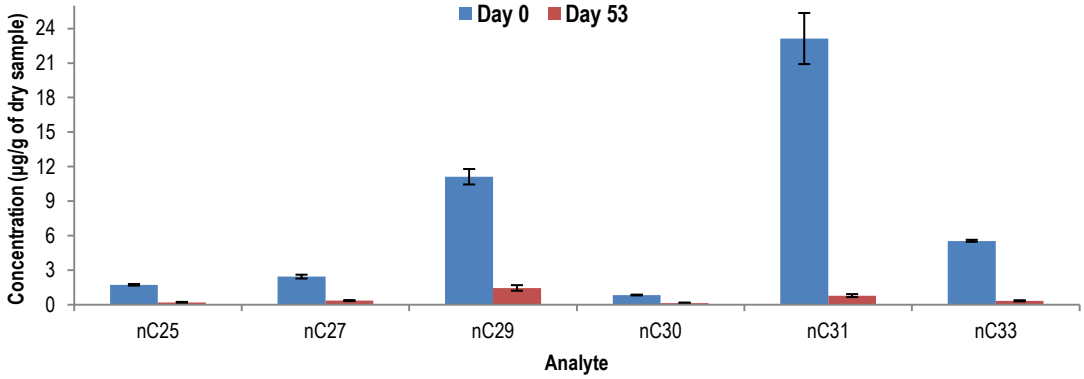
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; G = grass cuttings; M = mushroom compost; S_t = straw.

Appendix N. Plot of n-alkane concentrations in drill cuttings/grass cuttings/mushroom compost/straw 3 ($DGMS_t3$) compost mix type at day 0 and day 53



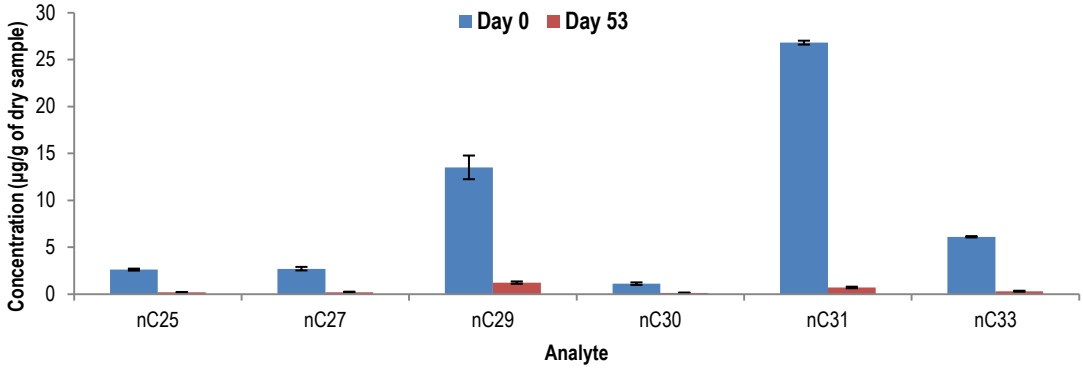
Error bars indicate ± one standard deviation from the mean (n=3); D = drill cuttings; G = grass cuttings; M = mushroom compost; S_t = straw.

Appendix O. Plot of n-alkane concentrations in coal tar impacted soil/grass cuttings/straw 1 (SGS_t1) compost mix type at day 0 and day 53



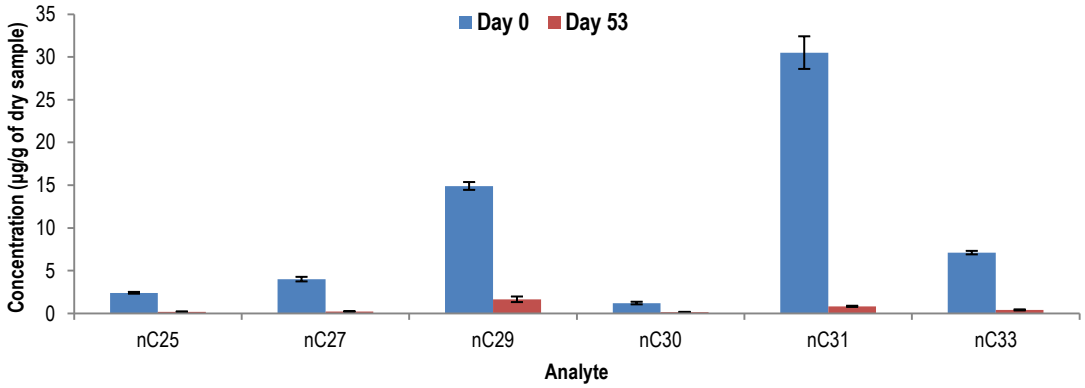
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; S_t = straw.

Appendix P. Plot of n-alkane concentrations in coal tar impacted soil/grass cuttings/straw 2 (SGS_t2) compost mix type at day 0 and day 53



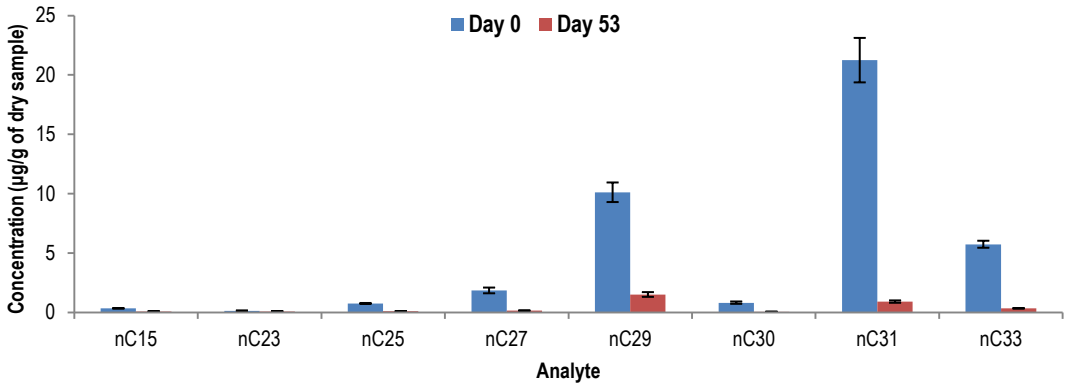
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; S_t = straw.

Appendix Q. Plot of n-alkane concentrations in coal tar impacted soil/grass cuttings/straw 3 (SGS_t3) compost mix type at day 0 and day 53



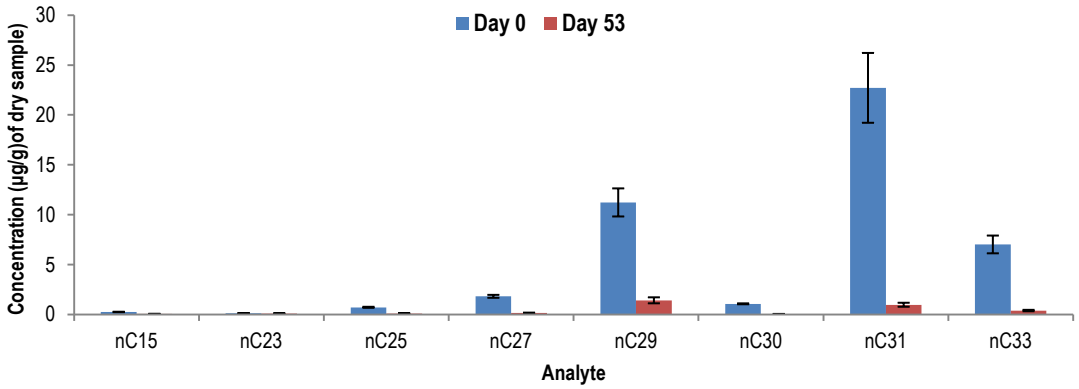
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; S_t = straw.

Appendix R. Plot of n-alkane concentrations in coal tar impacted soil/mushroom compost/straw 1 (SMS_t1) compost mix type at day 0 and day 53



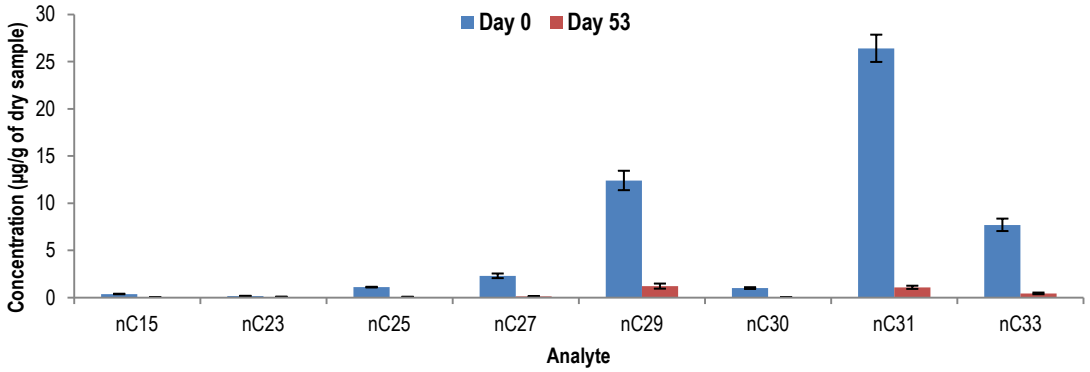
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; M = spent mushroom compost; S_t = straw.

Appendix S. Plot of n-alkane concentrations in coal tar impacted soil/mushroom compost/straw 2 (SMS_t2) compost mix type at day 0 and day 53



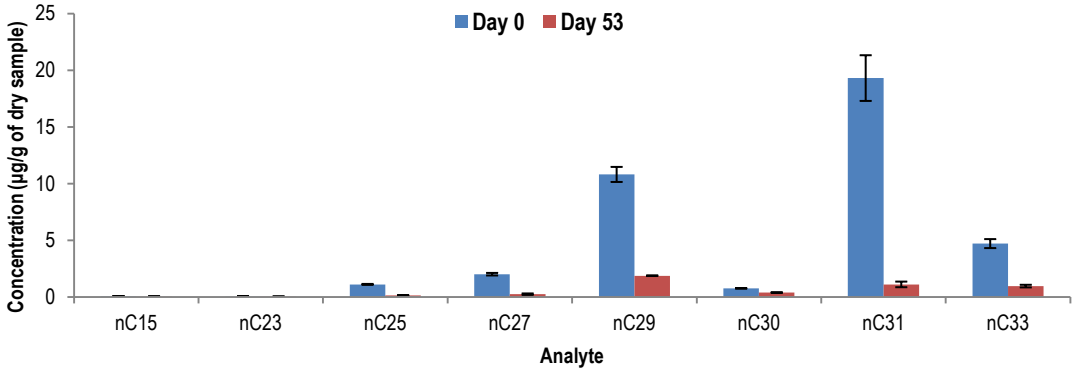
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; M = spent mushroom compost; S_t = straw.

Appendix T. Plot of n-alkane concentrations in coal tar impacted soil/mushroom compost/straw 3 (SMS_t3) compost mix type at day 0 and day 53



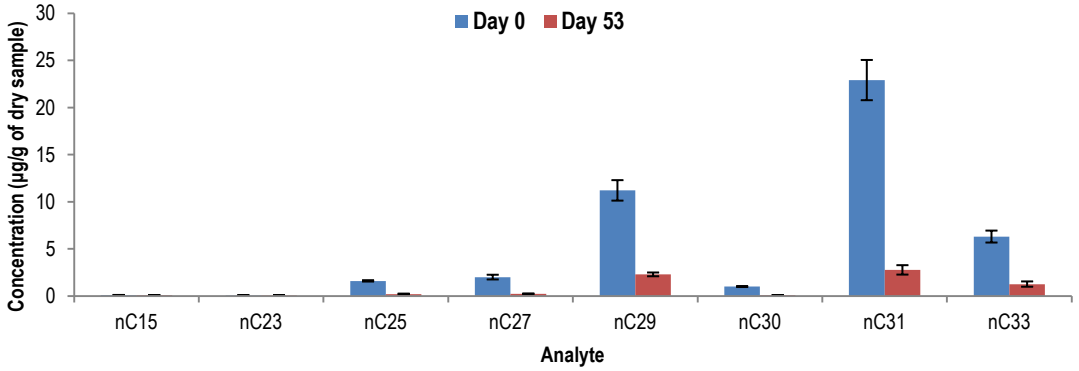
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; M = spent mushroom compost; S_t = straw.

Appendix U. Plot of n-alkane concentrations in coal tar impacted soil/grass cuttings/mushroom compost/straw 1 ($SGMS_t1$) compost mix type at day 0 and day 53



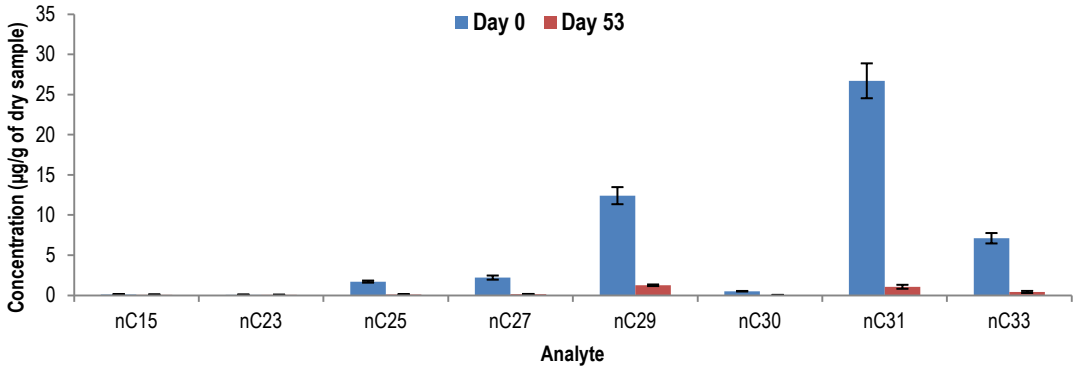
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix V. Plot of n-alkane concentrations in coal tar impacted soil/grass cuttings/mushroom compost/straw 2 ($SGMS_t2$) compost mix type at day 0 and day 53



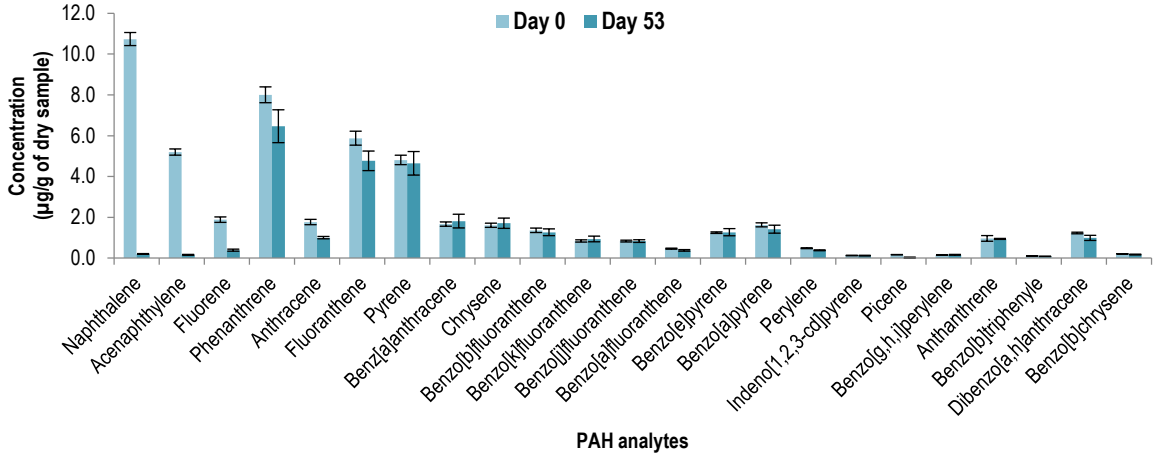
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix W. Plot of n-alkane concentrations in coal tar impacted soil/grass cuttings/mushroom compost/straw 3 ($SGMS_t3$) compost mix type at day 0 and day 53



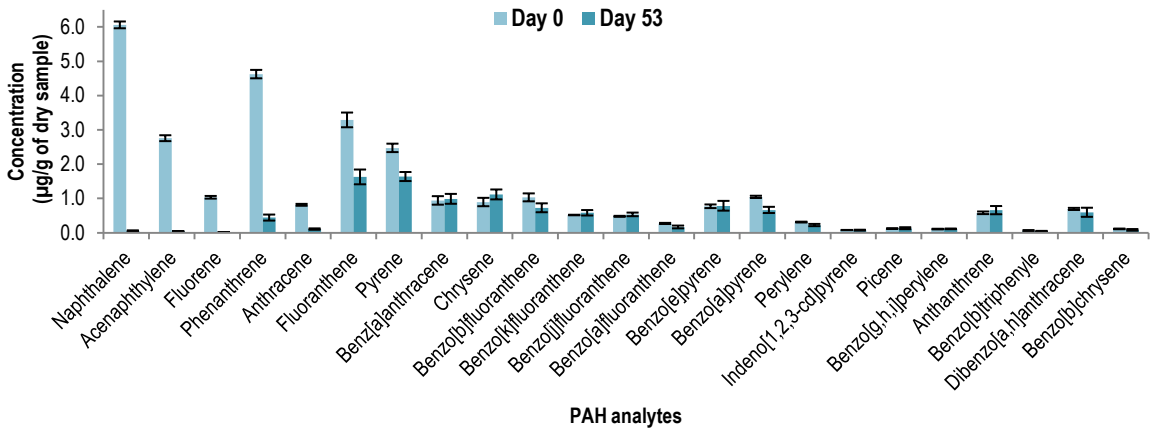
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix X. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in the control treatment sample for compost mixes with coal tar impacted soil (CTIS) at day 0 and day 53



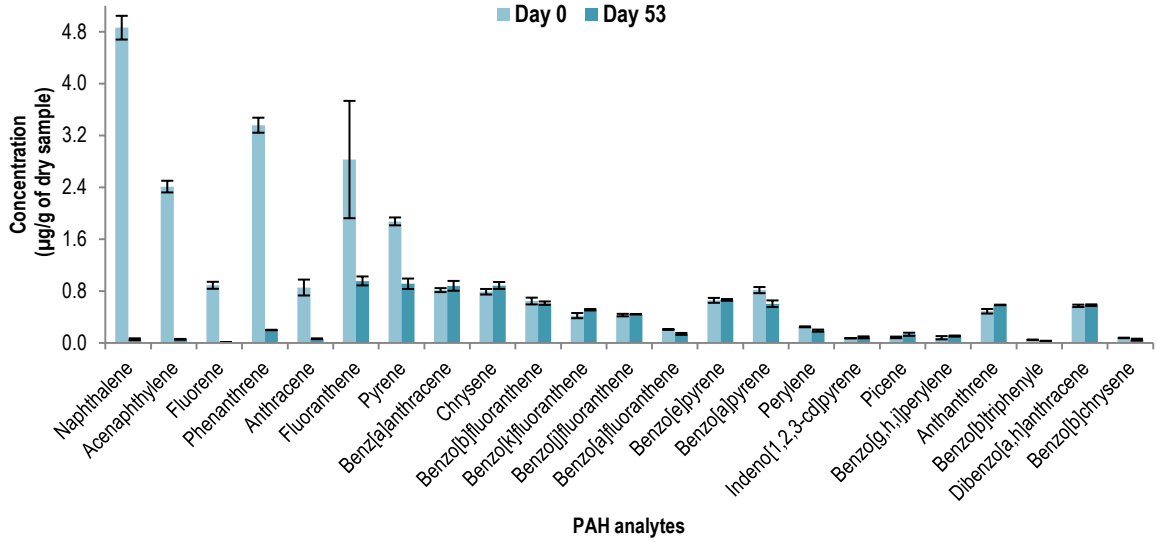
Error bars indicate ± one standard deviation from the mean (n=3); CTIS = coal tar impacted soil

Appendix Y. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/grass cuttings/straw 1 (SGS_t1) compost mix type at day 0 and day 53



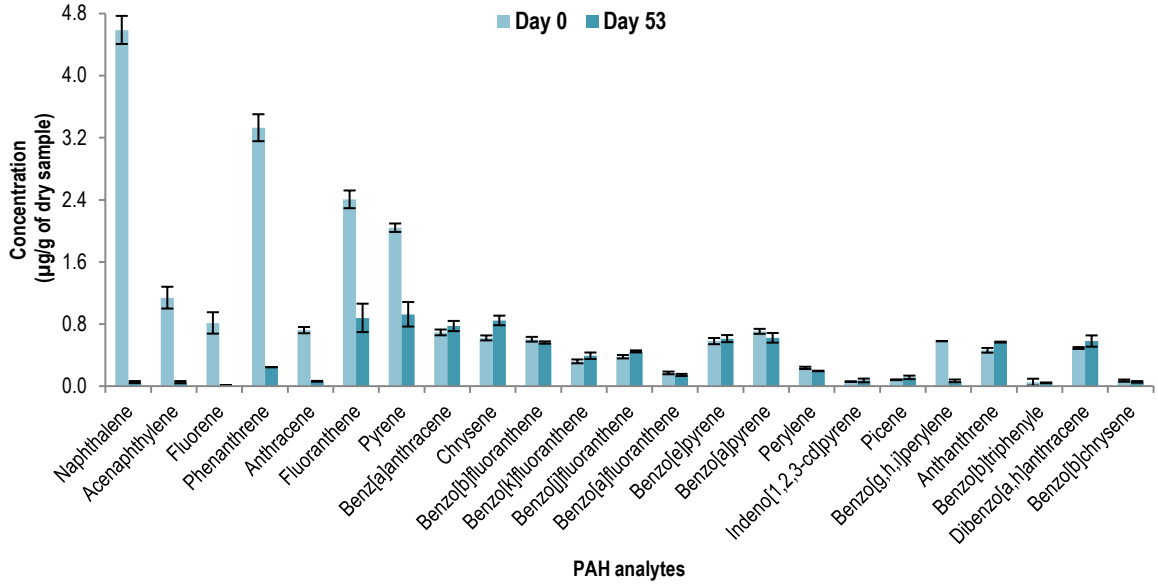
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; S_t = straw.

Appendix Z. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/grass cuttings/straw 2 (SGS_t2) compost mix type at day 0 and day 53



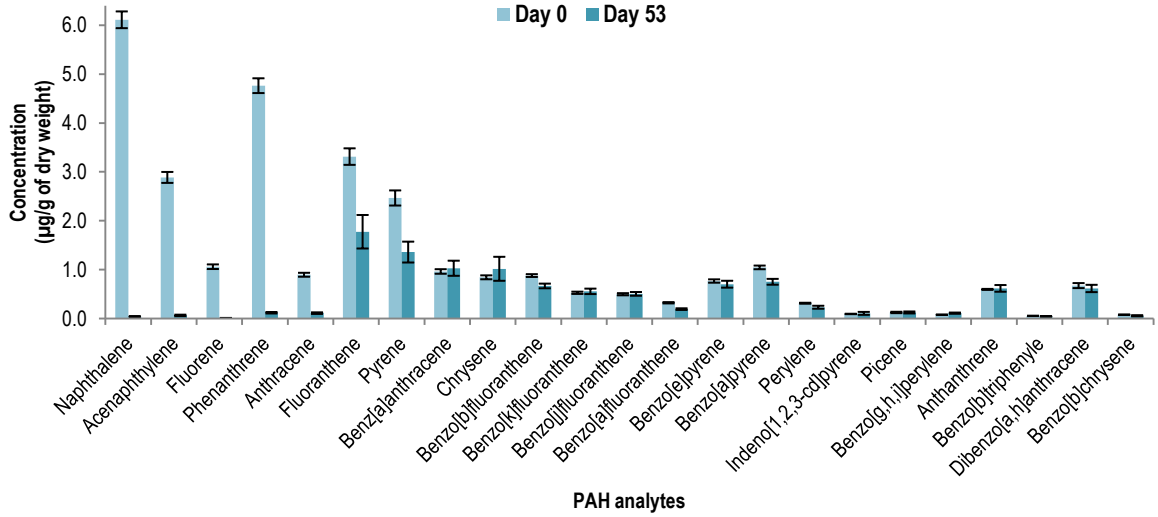
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; S_t = straw.

Appendix AA. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/grass cuttings/straw 3 (SGS_t3) compost mix type at day 0 and day 53



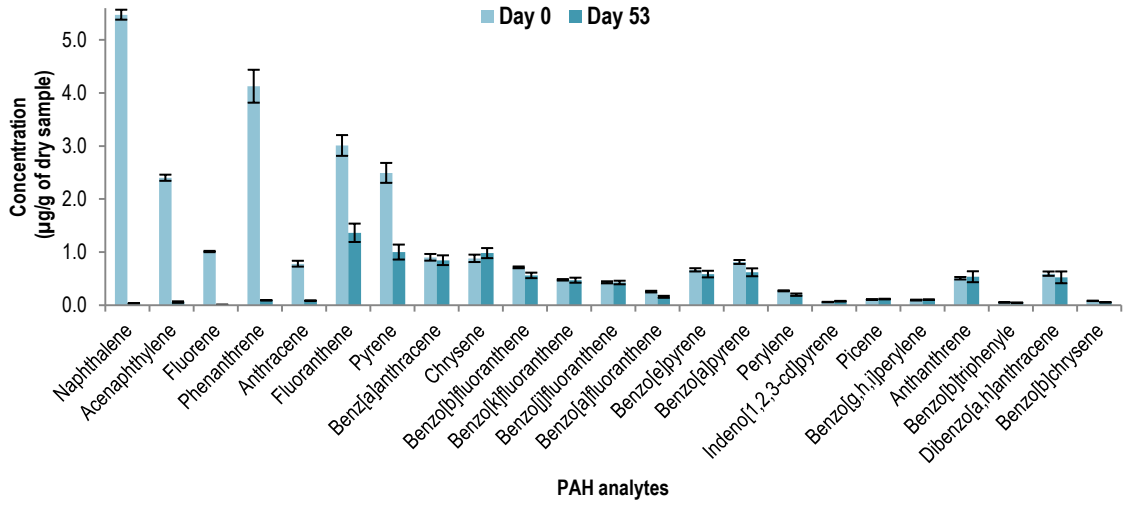
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; S_t = straw.

Appendix AB. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/mushroom compost/straw 1 (SMS_t1) compost mix type at day 0 and day 53



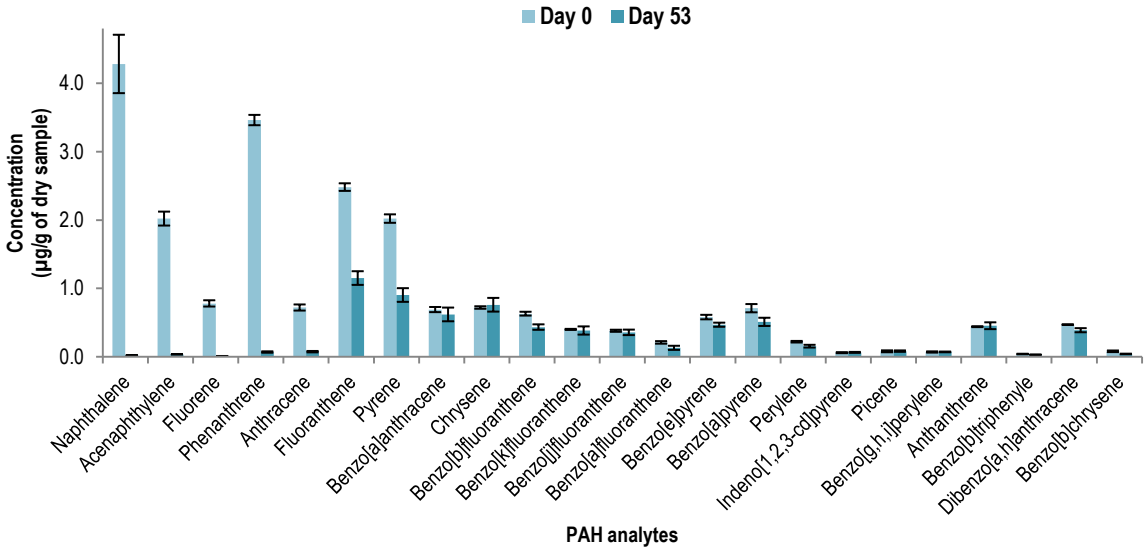
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix AC. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/mushroom compost/straw 2 (SMS_t2) compost mix type at day 0 and day 53



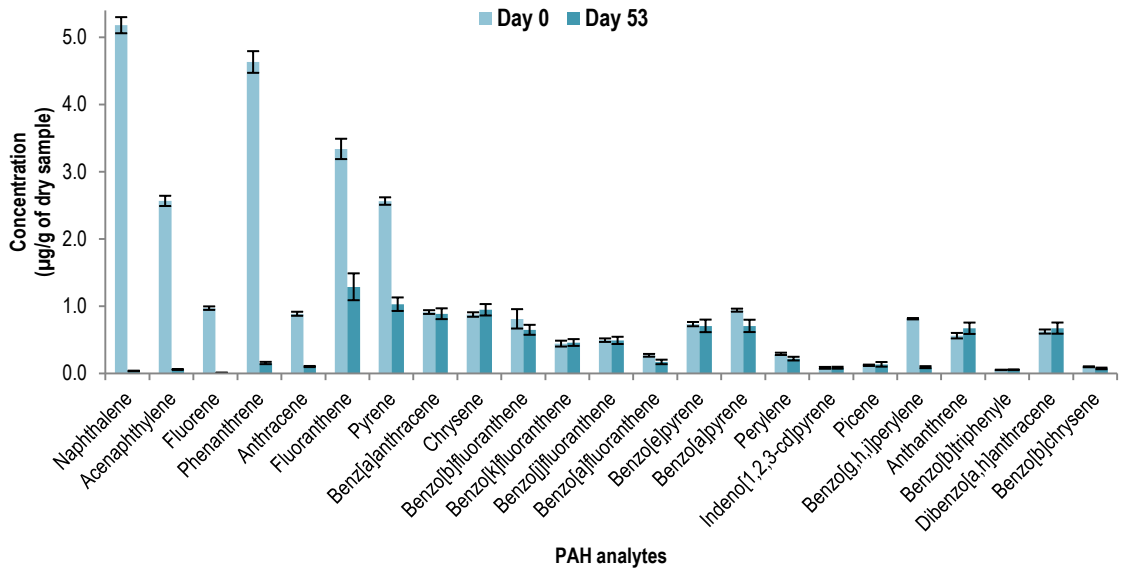
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix AD. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/mushroom compost/straw 3 (SMS_t3) compost mix type at day 0 and day 53



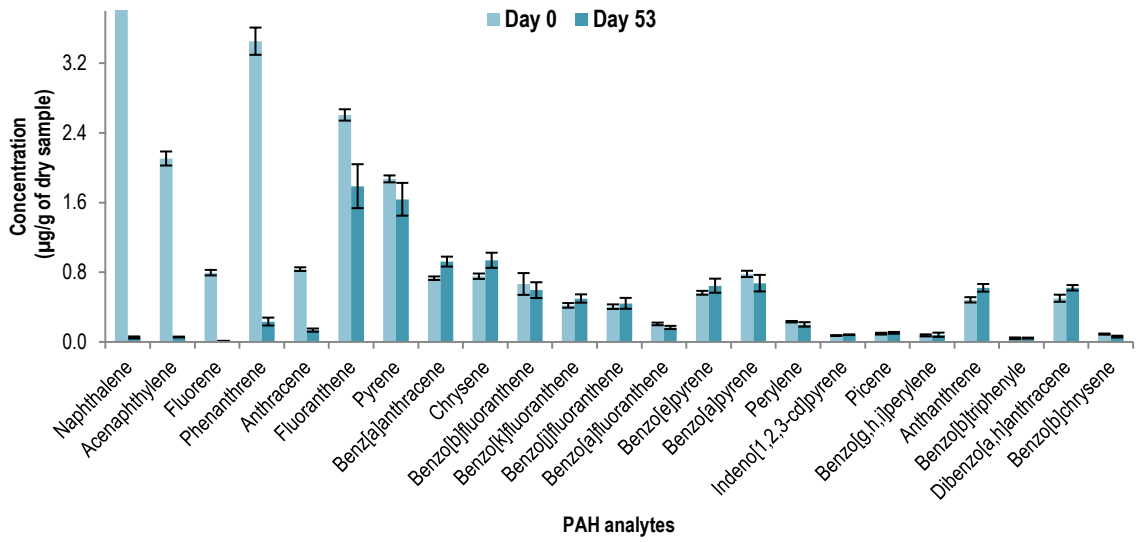
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix AE. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/grass cuttings/mushroom compost 1 ($SGMS_t1$) compost mix type at day 0 and day 53



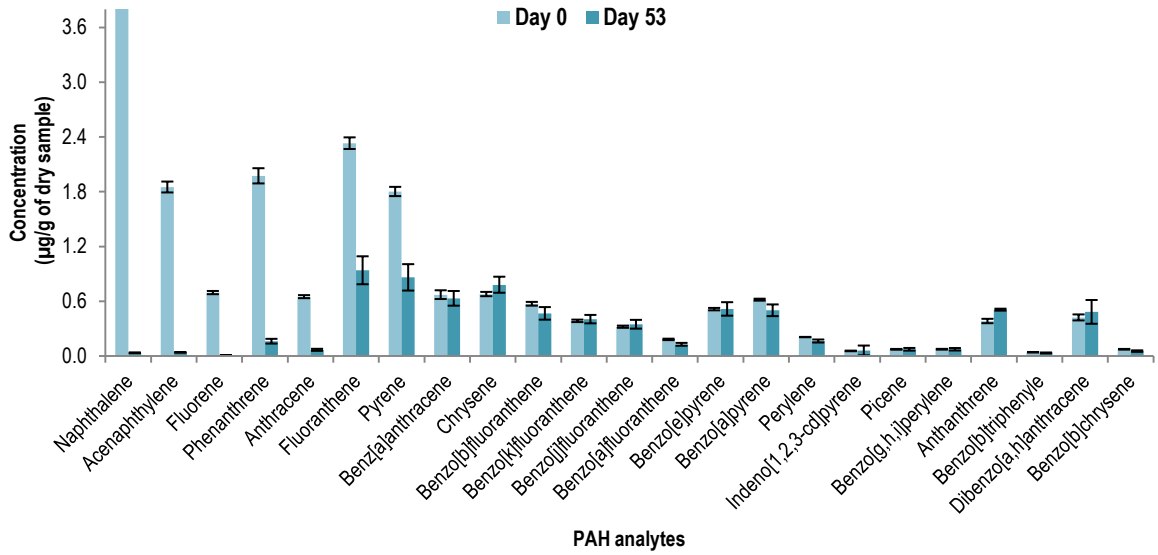
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix AF. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/grass cuttings/mushroom compost/straw 2 ($SGMS_t2$) compost mix type at day 0 and day 53



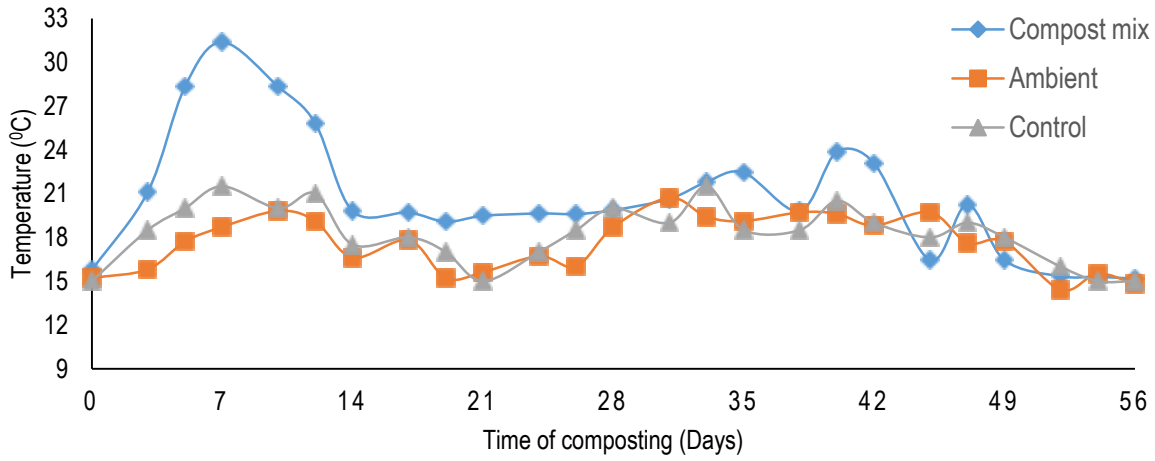
Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

Appendix AG. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in coal tar impacted soil/grass cuttings/mushroom compost/straw 3 ($SGMS_t3$) compost mix type at day 0 and day 53

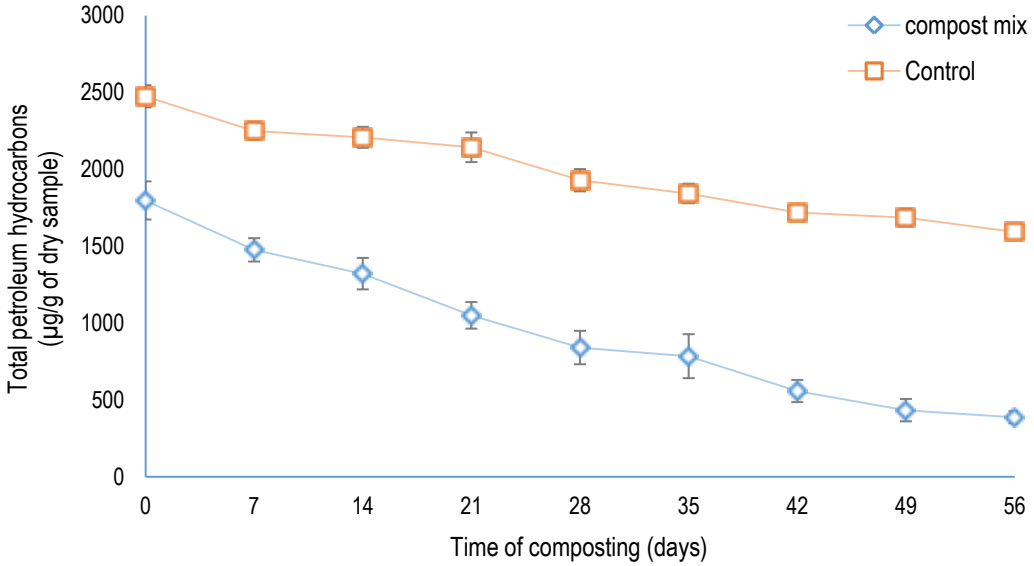


Error bars indicate ± one standard deviation from the mean (n=3); S = CTIS; G = grass cuttings; M = spent mushroom compost; S_t = straw.

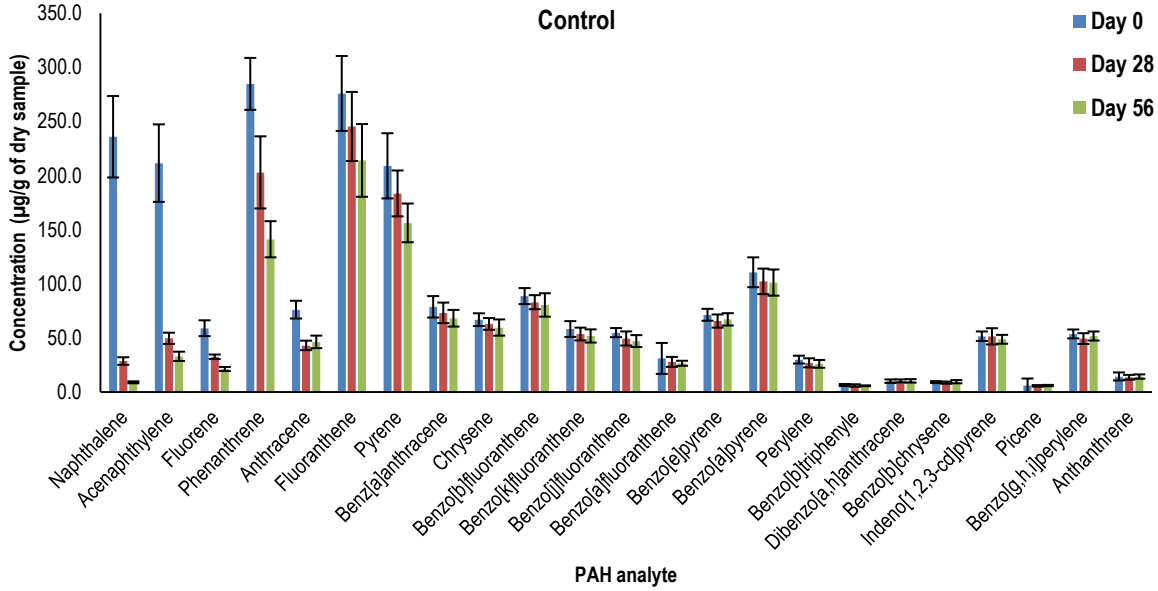
Appendix AH. Temperature variations during outdoor pilot-scale composting



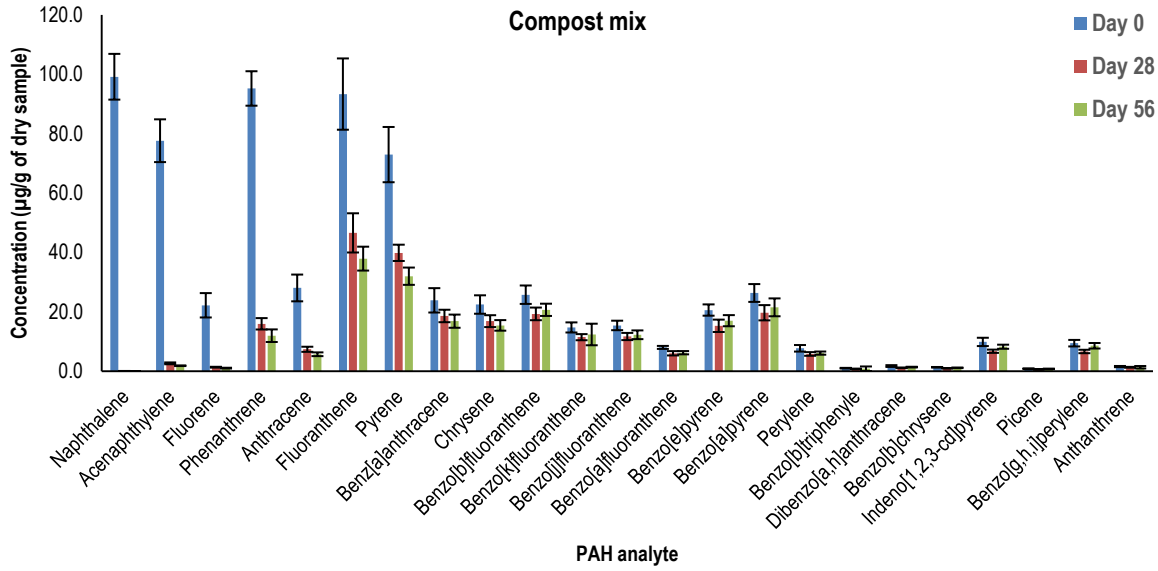
Appendix AI. Degradation of total petroleum hydrocarbon (TPH) during outdoor pilot-scale composting



Appendix AJ. Plot of polycyclic aromatic hydrocarbon (PAH) concentrations in the control and compost mix treatment samples for outdoor composting experiment at days 0, 28 and 56

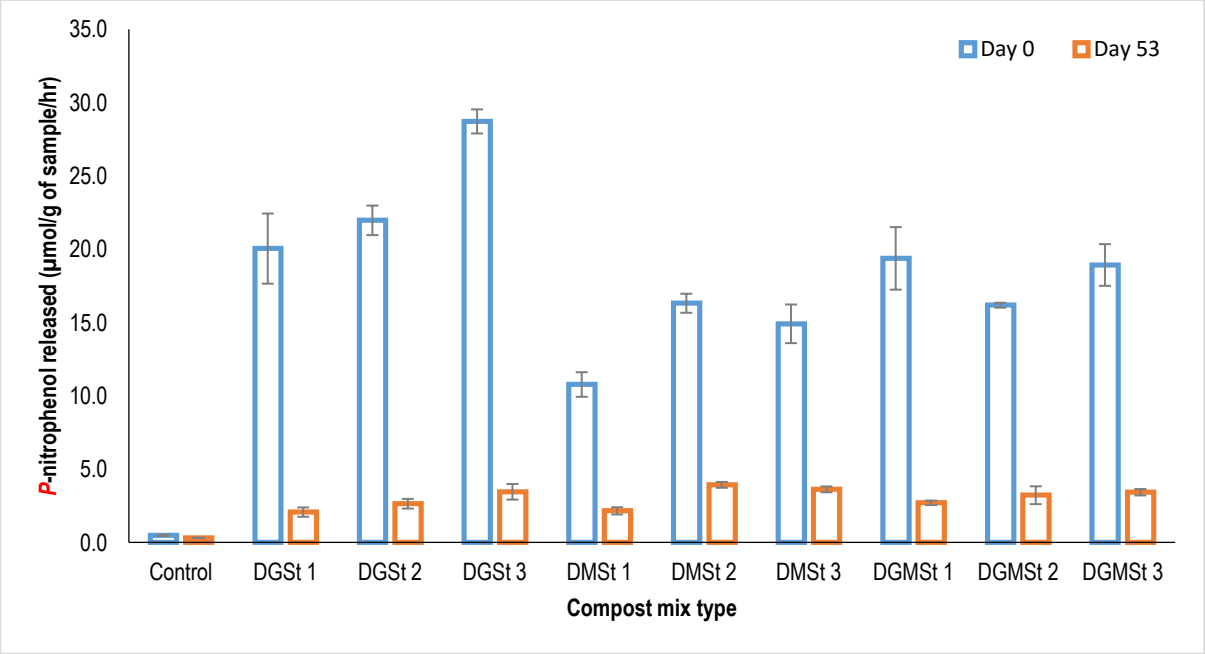


Error bars indicate ± one standard deviation from the mean (n=3)



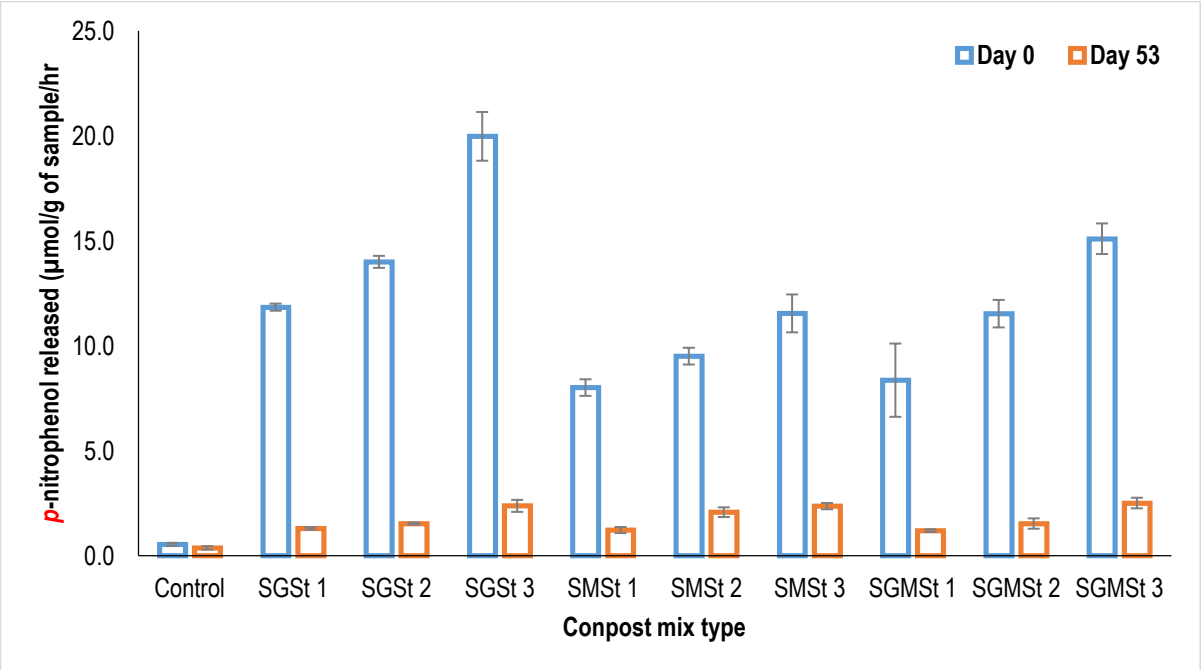
Error bars indicate ± one standard deviation from the mean (n=3)

Appendix AK. P-Nitrophenol released in compost mixes with drill cuttings.



Error bars indicate ± one standard deviation from the mean (n=3); Control = drill cuttings samples without compost amendments; D = drill cuttings; G = grass cuttings; M = mushroom compost; S_t = straw; Day 0 = before treatment; Day 53 = after treatment.

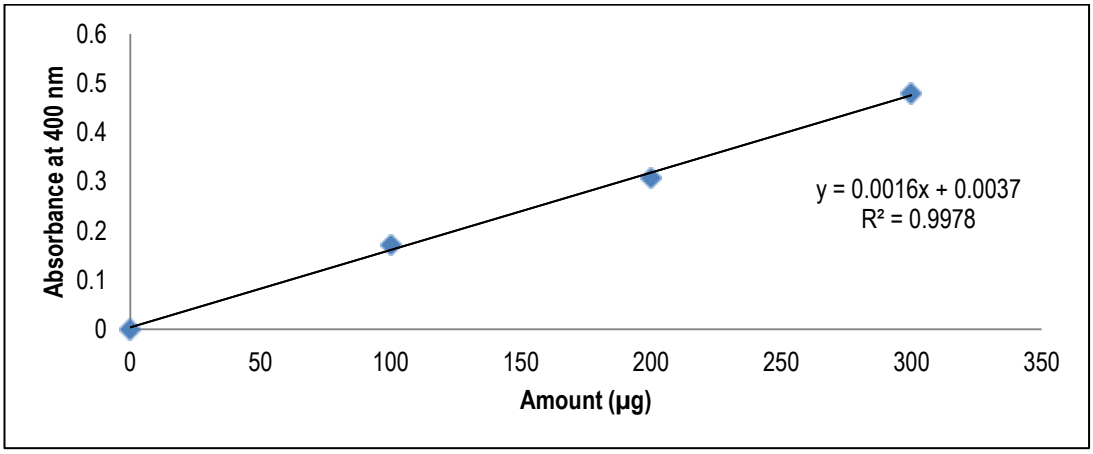
Appendix AL. P-*Nitrophenol released in lab-scale compost mix samples with coal tar impacted soil (CTIS).*



Error bars indicate ± one standard deviation from the mean (n=3); Control = CTIS samples without compost amendments; S = CTIS; G = grass cuttings; M = mushroom compost; S_t = straw; Day 0 = before treatment; Day 53 = after treatment.

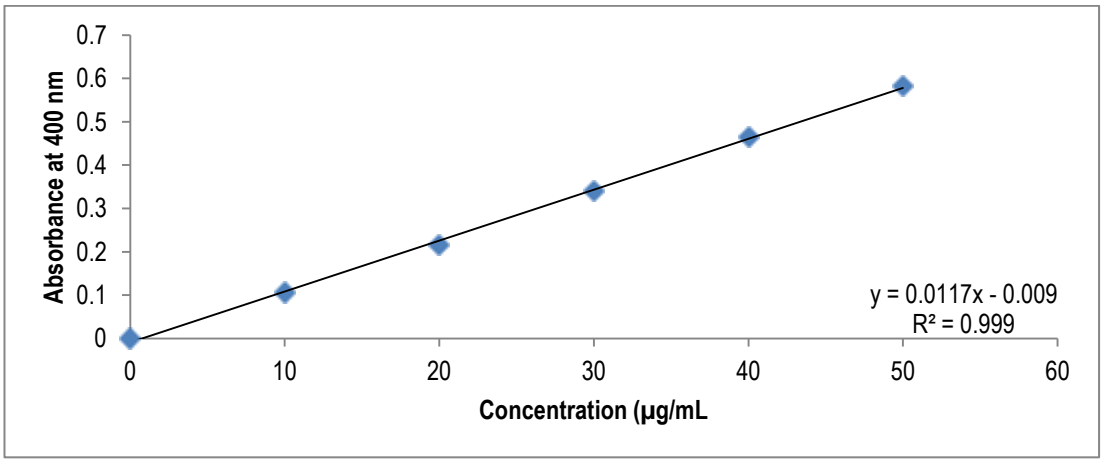
Appendix AM. Calibration data and graph of standard for phosphorus analysis.

Amount (µg)	Absorbance
0	0
100	0.172
200	0.307
300	0.479



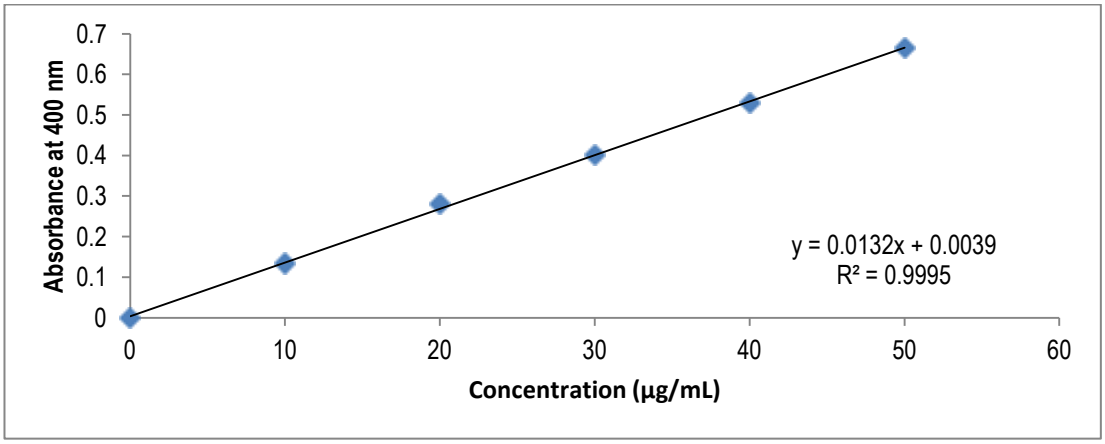
Appendix AN. Calibration data and graph of P*-Nitrophenol standard for phosphatase enzyme activity assay for drill cutting, drill cuttings/grass cuttings/straw 1 (DGS_t1) and drill cutting/mushroom compost/straw 2 (DMS_t2) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.106
20	0.215
30	0.34
40	0.465
50	0.582



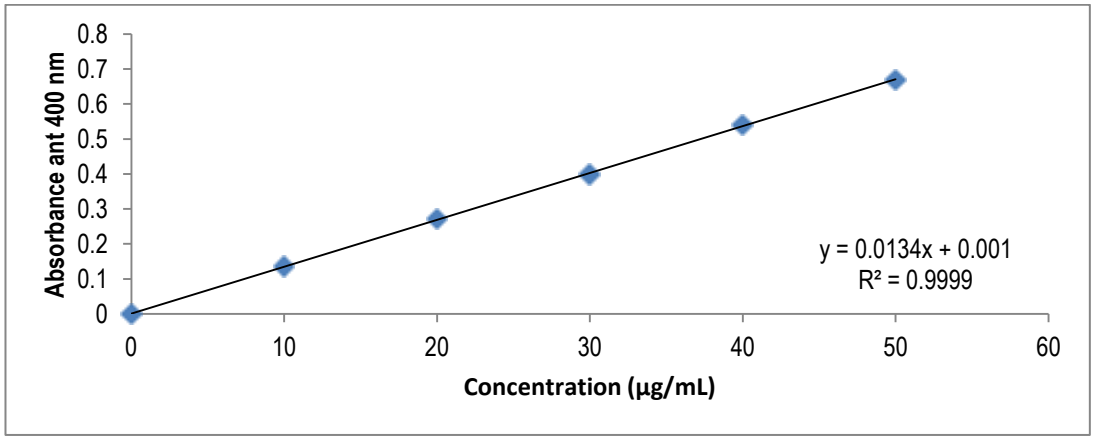
Appendix AO. Calibration data and graph of P-*Nitrophenol standard for phosphatase enzyme activity assay for drill cuttings/grass cuttings/ mushroom compost/straw 2 ($DGMS_t2$) sample.*

Concentration (µg/ml)	Absorbance
0	0
10	0.134
20	0.28
30	0.401
40	0.529
50	0.666



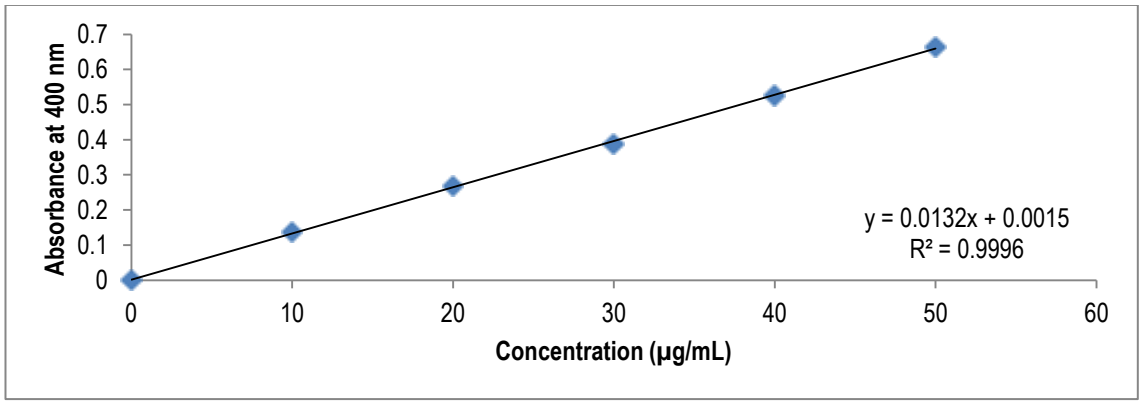
Appendix AP. Calibration data and graph of P-*Nitrophenol standard for phosphatase enzyme activity assay for drill cuttings/grass cuttings/straw 2 (DGS_t2) and drill cuttings/mushroom compost/straw 3 (DMS_t3) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.135
20	0.272
30	0.399
40	0.54
50	0.669



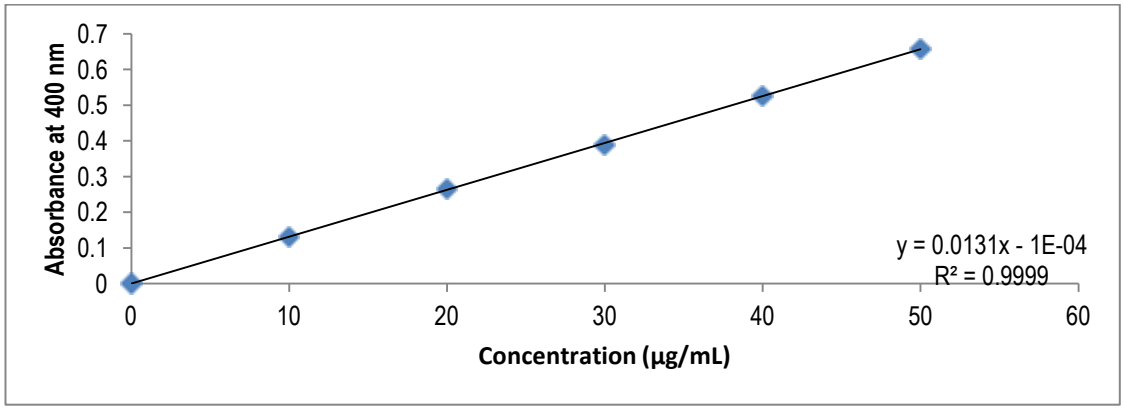
Appendix AQ. Calibration data and graph of P-Nitrophenol *standard for phosphatase enzyme activity assay for drill cuttings/grass cuttings/straw 3 (DGS_t3) and drill cuttings/grass cuttings/mushroom compost/straw 1 ($DGMS_t1$) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.136
20	0.269
30	0.388
40	0.526
50	0.663



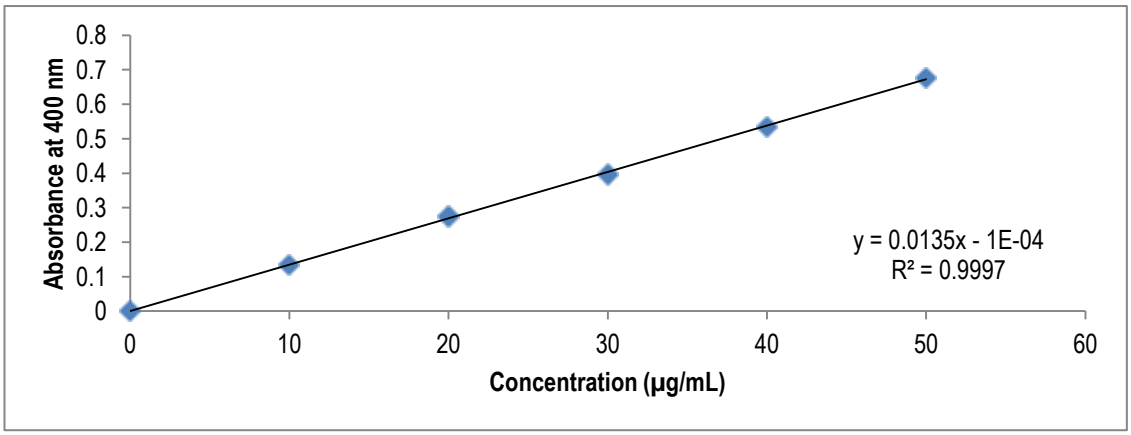
Appendix AR. Calibration data and graph of P-Nitrophenol *standard for phosphatase enzyme activity assay for drill cuttings/mushroom compost/straw 1 (DMS_t1) and drill cuttings/grass cuttings/mushroom compost/straw 3 ($DGMS_t3$) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.131
20	0.266
30	0.389
40	0.526
50	0.658



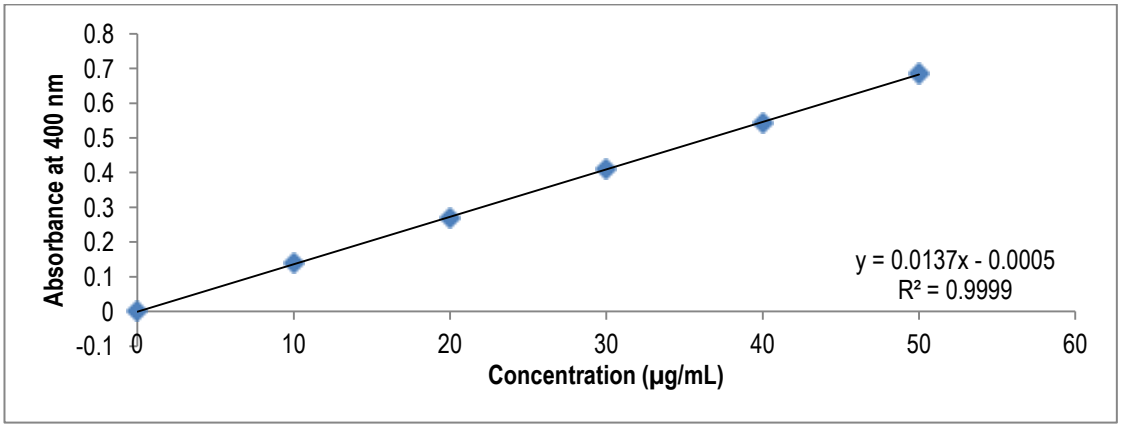
Appendix AS. Calibration data and graph of P-Nitrophenol *standard for phosphatase enzyme activity assay for CTIS (control) and coal tar impacted soil/mushroom compost/straw 2 (SMS_t2) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.134
20	0.275
30	0.397
40	0.535
50	0.677



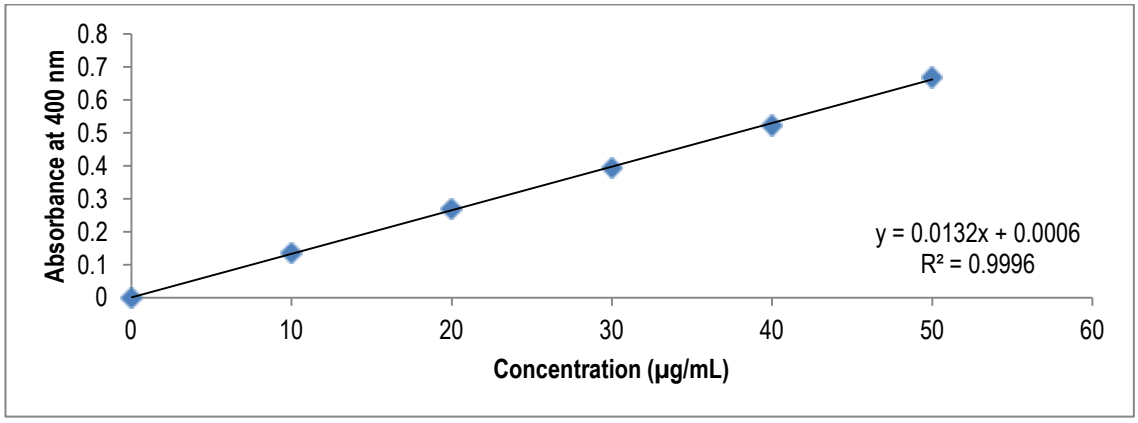
Appendix AT. Calibration data and graph of P-Nitrophenol *standard for phosphatase enzyme activity assay for coal tar impacted soil/mushroom compost/straw 3 (SMS_t3) sample.*

Concentration (µg/ml)	Absorbance
0	0
10	0.138
20	0.269
30	0.411
40	0.542
50	0.685



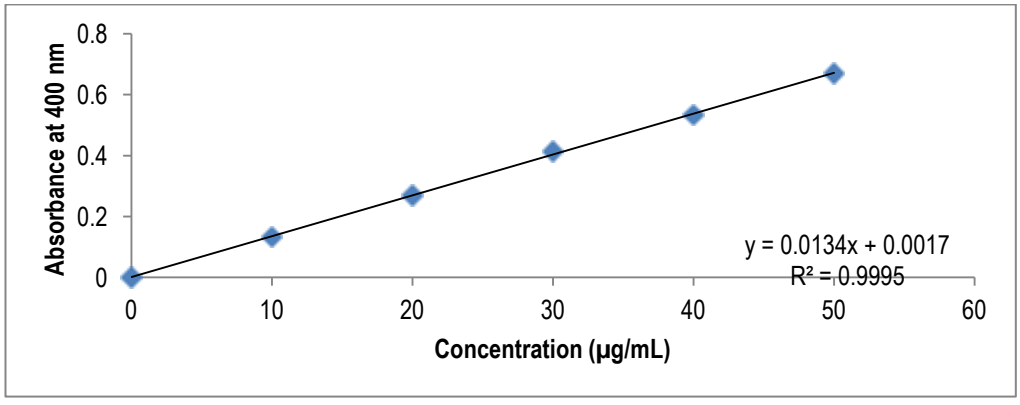
Appendix AU. Calibration data and graph of P-*Nitrophenol standard for phosphatase enzyme activity assay for coal tar impacted soil/grass cuttings/straw 1 (SGS_t1) and coal tar impacted soil/grass cuttings/mushroom compost/straw 1 ($SGMS_t1$) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.135
20	0.268
30	0.393
40	0.522
50	0.668



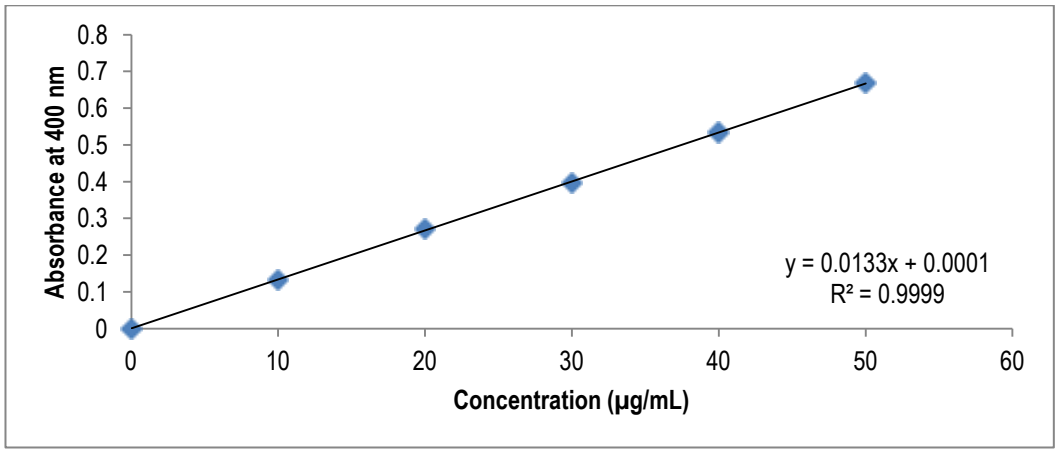
Appendix AV. Calibration data and graph of P-*Nitrophenol standard for phosphatase enzyme activity assay for coal tar impacted soil/grass cuttings/straw 2 (SGS_t2) and coal tar impacted soil/grass cuttings/mushroom compost/straw 2 ($SGMS_t2$) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.134
20	0.269
30	0.414
40	0.533
50	0.669

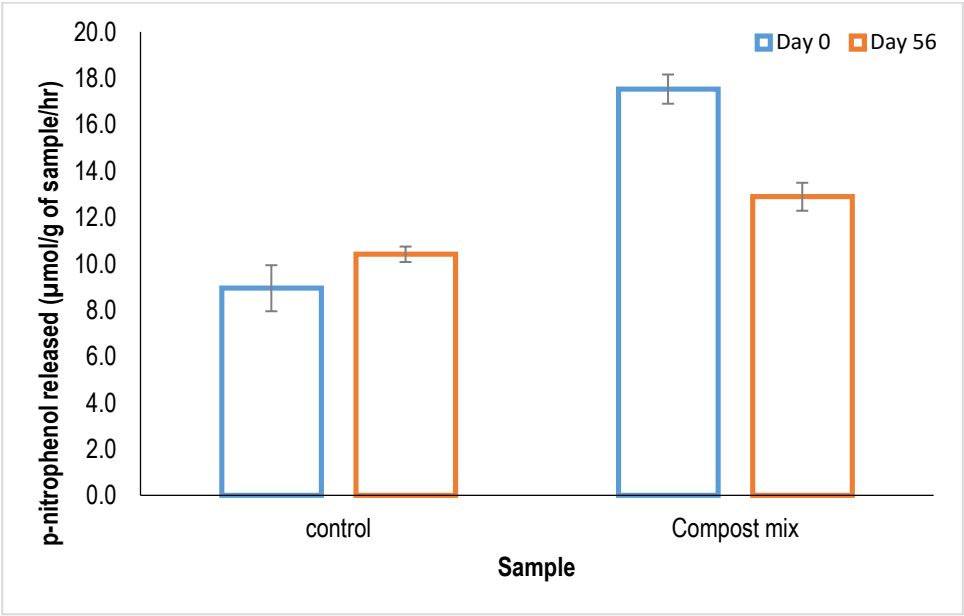


Appendix AW. Calibration data and graph of P-Nitrophenol *standard for phosphatase enzyme activity assay for coal tar impacted soil/grass cuttings/straw 3 (SGS_t3) and coal tar impacted soil/grass cuttings/mushroom compost/straw 3 ($SGMS_t3$) samples.*

Concentration (µg/ml)	Absorbance
0	0
10	0.133
20	0.271
30	0.395
40	0.534
50	0.668



Appendix AX. P-Nitrophenol released in outdoor compost mix samples with coal tar impacted soil (CTIS)



Error bars indicate ± one standard deviation from the mean (n=3); Control = CTIS sample without compost amendments; Day 0 = before treatment; Day 53 = after treatment

(a) 5 days after planting

(b) 7 days after planting

Appendix AY. Sprouting corn seeds at 5 and 7 days after planted in garden compost (control) .

(a) 5 days after planting

(b) 7 days after planting

Appendix AZ. Sprouting pea seeds at 5 and 7 days after planted in garden compost (control).

(a) 3 days after planting

(b) 5 days after planting

Appendix BA. Sprouting mustard seeds at 3 and 5 days after planted in garden compost (control).

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BB. Sprouting corn seeds 5 days after planting in coal tar impacted soil (CTIS).

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BC. Sprouting corn seeds 7 days after planting in coal tar impacted soil (CTIS).

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BD. Sprouting pea seeds 5 days after planting in coal tar impacted soil (CTIS).

(b) Before compost-bioremediation

(c) After compost-bioremediation

Appendix BE. Sprouting pea seeds *7 days after planting in coal tar impacted soil (CTIS*)

(a) Before compost-bioremediation (b) After compost-bioremediation

Appendix BF. Sprouting mustard seeds 3 days after planting in coal tar impacted soil (CTIS)

(a) Before compost-bioremediation (b) After compost-bioremediation

Appendix BG. Sprouting mustard seeds 7 days after planting in coal tar impacted soil (CTIS).

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BH. Sprouting corn seeds *5 days after planting in treatment compost mix.*

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BI. Sprouting corn seeds *7 days after planting in treatment compost mix.*

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BJ. Sprouting pea seeds *5 days after planting in treatment compost mix.*

(a) Before compost-bioremediation

(b) After compost-bioremediation

Appendix BK. Sprouting pea seeds *7 days after planting in treatment compost mix.*

(a) Before compost-bioremediation

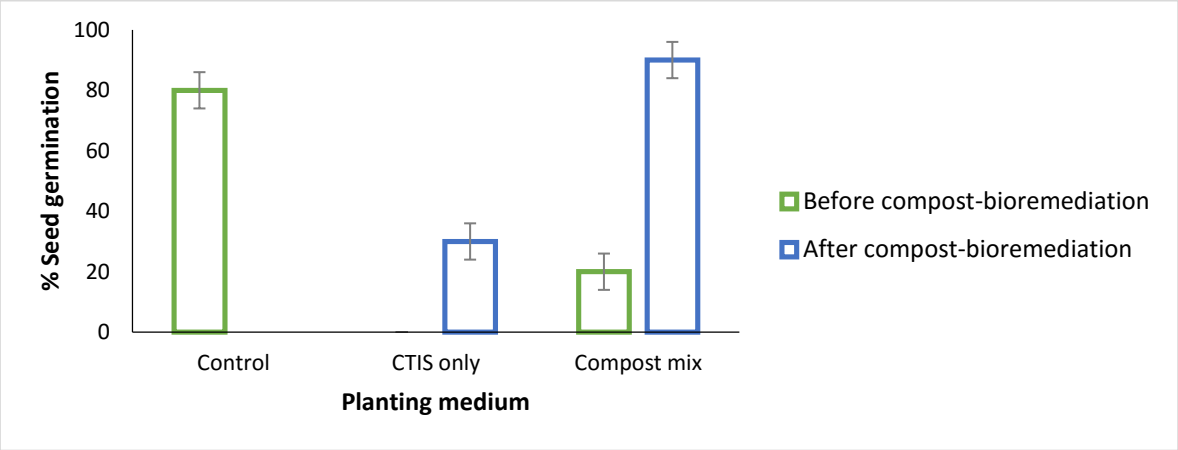
(b) After compost-bioremediation

Appendix BL. Sprouting mustard seeds *3 days after planting in treatment compost mix.*

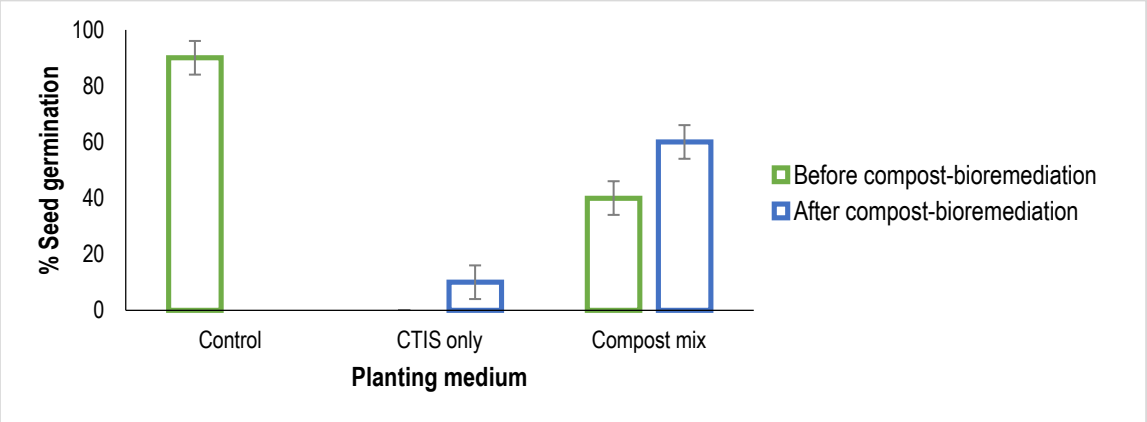
(a) Before compost-bioremediation

(b) After compost-bioremediation

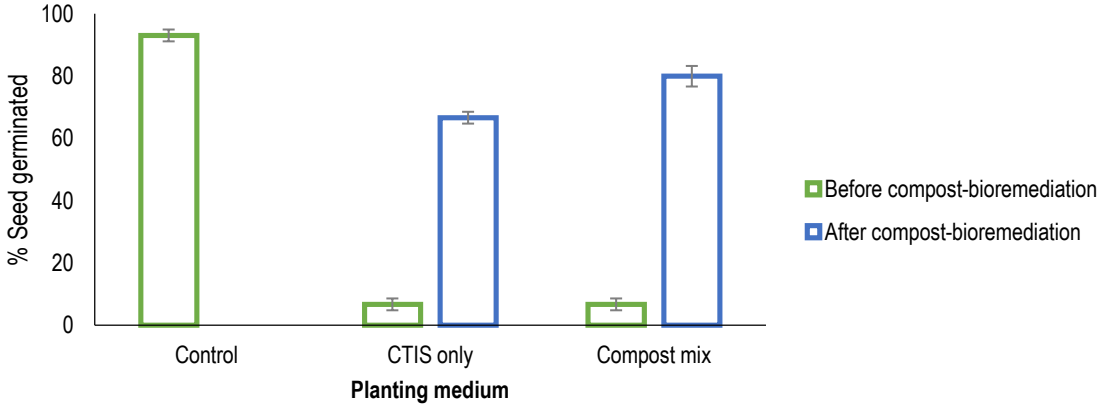
Appendix BM. Sprouting mustard seeds *5 days after planting in treatment compost mix.*



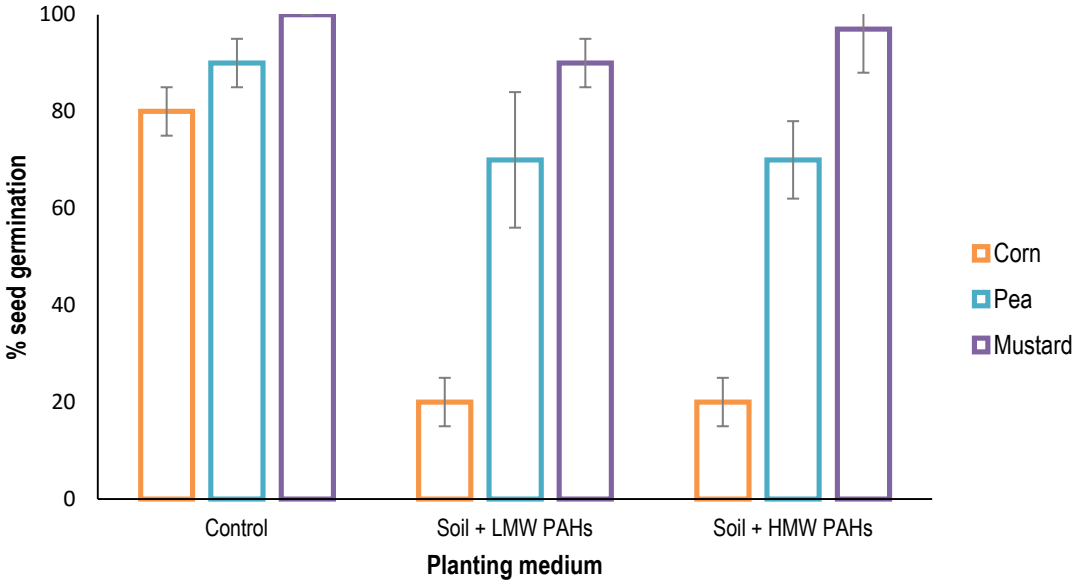
Appendix BN. Corn seed germination (%) 5 days after planting
Error bars indicate ± one standard deviation from the mean (n=10); Control = Garden compost



Appendix BO. Pea seed germination (%) 5 days after planting
Error bars indicate ± one standard deviation from the mean (n=10); Control = Garden compost

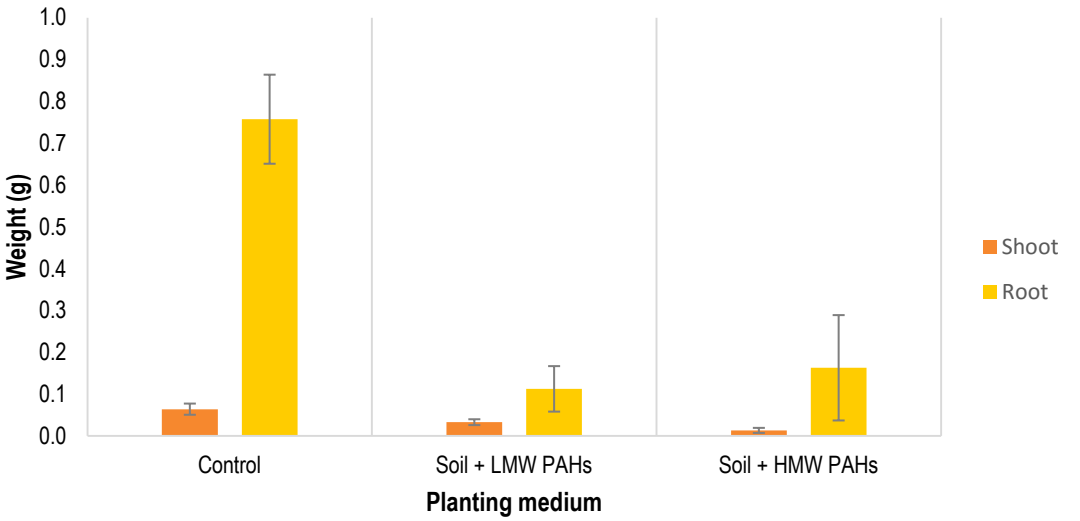


Appendix BP. Mustard seed germination (%) 3 days after planting
Error bars indicate ± one standard deviation from the mean (n=30); Control = Garden compost



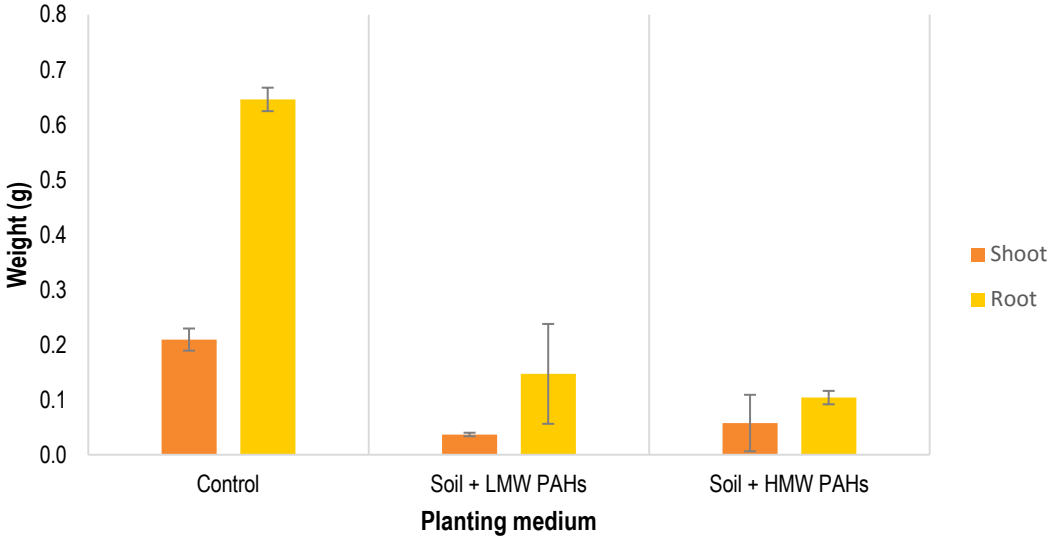
Appendix BQ. Variation of seeds germinated in soils amended with different molecular weight PAHs for corn, pea and mustard

Values are mean (n=10 for corn and pea, n=30 for mustard); Error bars indicate standard deviation; Control = Fresh agricultural soil; LMW = Low molecular weight (1455.4 µg/g); HMW = High molecular weight (2059.3 µg/g)



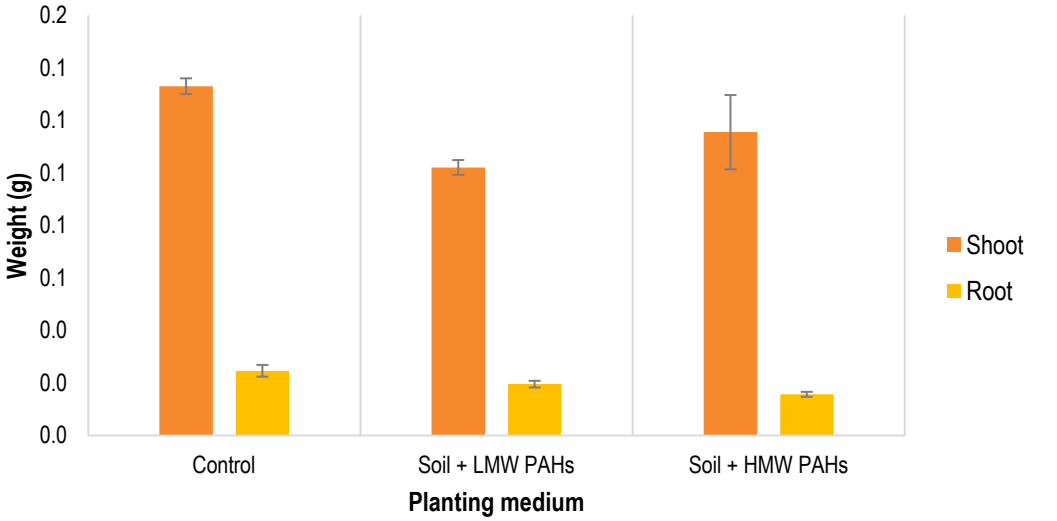
Appendix BR. Shoot and root biomass of sprouting corn in soils amended with different molecular weight PAHs after 9 days of planting

Values are mean (n=10); Error bars indicate standard deviation; Control = Fresh agricultural soil; LMW = Low molecular weight (1455.4 µg/g); HMW = High molecular weight (2059.3 µg/g)



Appendix BS. Shoot and root biomass of sprouting pea in soils amended with different molecular weight PAHs after 9 days of planting

Values are mean (n=10); Error bars indicate standard deviation; Control = Fresh agricultural soil; LMW = Low molecular weight (1455.4 µg/g); HMW = High molecular weight (2059.3 µg/g)



Appendix BT. Shoot and root biomass of sprouting mustard in soils amended with different molecular weight PAHs after 7 days of planting

Values are mean (n=30); Error bars indicate standard deviation; Control = Fresh agricultural soil; LMW = Low molecular weight (1455.4 µg/g); HMW = High molecular weight (2059.3 µg/g)