CRANFIELD UNIVERSITY

EMMANUEL ATAI

SOIL BIOENGINEERING FOR SUSTAINABLE BIOREMEDIATION OF OIL CONTAMINATED SOILS

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
ENVIRONMENTAL AND AGRIFOOD

PhD

Academic Year: 2022 –2023

Supervisor: Dr. Mark Pawlett
Associate Supervisor: Prof. Frédéric Coulon

November 2022

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ASSOCIATE SUPERVISOR: PROF. FRÉDÉRIC COULON

NOVEMBER 2022

This thesis is submitted in partial fulfilment of the requirements for the degree of PhD

Abstract

Contaminated soils arising from the petroleum industry remains a major problem globally, resulting in levels of petroleum hydrocarbons and metals that are dangerous to the environment. Modern remediation strategies focus on sustainability, thus maximizing environmental, social, and economic benefits. The use of materials derived from agricultural and industrial waste, for example biochar and spent mushroom compost (SMC), may provide a potential solution to sustainable remediation strategies. Biochar has numerous properties, e.g., high surface area and pore volume that may provide benefits to the remediation industry. SMC, a by-product of mushroom production, may contain diverse groups of microorganisms and extracellular enzymes important for the biotransformation of contaminants. Biochar and spent mushroom compost interactions in soil may induces diverse responses in microbial species leading to changes in soil enzyme activity, reshaping of microbial community structure and consequent enhancement of contaminants transformations. However, the mechanisms underlying these interactions are poorly understood, with unpredictable outcomes. There is a deficit of research designed to understand their collective response on soil fungi and the subsequent benefits to remediation success. Research needs to focus on the benefits of biochar towards affecting contaminant bioavailability of multiple rather than single contaminants. Combining biochar with SMC may facilitate the biodegradation of petroleum hydrocarbons in saline soils. The aim of the research was to develop a biotechnological approach for the best use of biochar and SMC to promote microbial remediation of soil contaminated with complex chemical mixture contaminants (hydrocarbons and heavy metals). It provides a mechanistic understanding of the physicochemical and biological parameters influencing the remediation approach. The study further sheds light into the influence of low carbon soil amendment on the behaviour and fate of heavy metal(loids) and petroleum hydrocarbons, and the underlying microbial community responses in a genuinely contaminated soil in a four-month microcosms study, and in crude oil and salt spiked soil in another four-month microcosms study.

Keywords: hydrocarbon, metal, biochar, Spent mushroom compost, bioamendment, microbial community

Acknowledgements

First and foremost, I would like to express my heartfelt appreciation to my supervisor, Dr Mark Pawlett, for his unwavering support throughout my PhD studies and research, as well as his availability, accessibility, patience, and vast knowledge. His advice was invaluable to me throughout the course of my research and thesis writing. In my PhD journey, I could not have asked for a better mentor and supervisor. I would also like to thank my associate supervisor, Prof. Prof. Frédéric Coulon, for his constant encouragement, insightful comments, and unwavering constructive criticism. I benefited immensely from his mentorship. His contribution to the team was extremely valuable.

I am grateful to all the PhD students and staff members of the Centre for Soil, Agrifood, and Biosciences, as well as the Environmental Analytical Facility technical team, particularly Nuannat Simmons, Tammy Cowley, Late Dawn Fowler, and Paul Barton; without their valuable support, this research would not have been possible. I specially recognise members of our community of practise, Bioprocesses and Environmental Technology.

I am grateful to the Petroleum Technology Development Fund in Nigeria for sponsoring my Ph.D. research. Special thanks to the UK Biochar Research Centre in Edinburgh for providing the biochar used in my research, Prof Magan Naresh for the spent mushroom compost, and RSK in the United Kingdom for the soil samples.

I am grateful to my lab colleagues, especially Raphael Jumbo, Ikeabiama Ndubuisi Azuazu, Okelani Aworabhi, and Rachel Hasler. The group has provided friendship as well as sound advice and collaboration. I am grateful to the Cranfield Christian Union members for the fellowship we have shared. The group has provided a sense of belonging and great encouragement.

Words cannot express my gratitude to Becky, my wife, who has been by my side every step of the way through this PhD. Thank you for always being there for me. This journey would not have been possible without you.

Danladi, Gaius, Instifanus, Sam, Maren, Esther, and Desmond, my friends who were always there for me no matter how far we were apart, I consider myself extremely fortunate to have you as friends.

Finally, I would like to thank my entire family: my parents, brothers, sisters, and in-laws, for your love and support over these years.

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List of abbreviations

ALK	Alkanes
ANOVA	Analysis of variance
BS	British standard
BSPT	Basic solid phase test procedure (Microtox assay®)
C/N	Carbon nitrogen ratio
CL:AIRE	Contaminated land: applications in real environments
DCM	Dichloromethane
EA	Environment agency
EC_{50}	Half maximal effective concentration
EU	European union
GC-FID	Gas chromatography with flame-ionisation detector
GC-MS	Gas chromatography-mass spectrometry
GC-TCD	Gas chromatography with thermal conductivity detector
HCl	Hydrochloric acid
HEX	Hexane
HM	Heavy metals (and metalloids)
HMW	High molecular weight
HPCD	Hydroxypropyl-cyclodextrin and hydroxypropyl-β -cyclodextrin
ICP-MS	Inductively coupled plasma mass spectrometry
ISTD	Internal standard
LMW	Low molecular weight
LOI	Loss of ignition
MeOH	Methanol
ML	Machine learning
MMW	Medium molecular weight
PAH	Polycyclic aromatic hydrocarbons
PCA	Principal components analysis
pH	"Power of hydrogen" negative log of hydrogen ion concentration
PHC	Petroleum hydrocarbons compounds comprising aliphatic and aromatic hydrocarbons
PLFA	Phospholipid fatty acids
r^2	Coefficient of determination
RA	Risk assessment
RHB	Rice husk biochar
RHB-SMC	Rice husk biochar + Spent mushroom compost
SGV	UK soil guideline values
SMC	Spent mushroom compost
SPE	Solid phase extraction
SuRF-UK	UK sustainable remediation forum
T0	Day 0
T10	Day 10
T120	Day 120
T60	Day 60
TC	Total carbon
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSA	Tryptone soya agar
UK	United Kingdom

USA	United States of America
WSB	Wheat straw biochar
WSB-SMC	Wheat straw biochar + Spent mushroom compost

1. Introduction

Environmental pollution, a major issue confronting scientists in the 21st century is a global problem common to both developed and developing countries (Gomes, Dias-Ferreira and Ribeiro, 2012; Meteku *et al.*, 2020). Urbanization, industrialisation, mining, and exploration are the four main anthropogenic activities contributing to worldwide environmental pollution (Ukaogo, Ewuzie and Onwuka, 2020). An important component of the earth greatly affected by pollution is soil environment, which has to do with instances when harmful compounds (also referred to as pollutants or contaminants) are present in soil in sufficient quantities to endanger human health and/or the ecosystem (Environmental Pollution Centers, 2022). The presence of xenobiotic chemicals such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons, toxic metals, solvents, and pesticides in the soil has resulted in global changes in the natural soil environment (George *et al.*, 2014). Hence, soil pollution endangers human health as well as natural habitats. It also has an impact on soil microorganism growth, morphology, and metabolism by causing functional disruptions, protein denaturation, and cell membrane integrity loss. Similarly, it has an impact on the soil's functioning and support of biological productivity as a vital system (Panneerselvam *et al.*, 2022). Contaminants enter the soil environment from a variety of sources, for example, oil spills into the environment because of crude oil production activities are among the most significant sources of soil contamination. Soils can become contaminated due to industrial activity, for example, soils from legacy sites like former gasworks. Similarly, rising temperatures owing to climate change cause soils to become saline due to evapotranspiration. Oil contamination, legacy site soil, and soil salinity are important aspects considered in this work and are briefly discussed here.

The oil and gas industry currently contributes the most to the global energy mix and since the turn of the century, crude oil supply has steadily increased to meet rising demand (Miller and Sorrell, 2014; Zou *et al.*, 2016). However, increased oil production is associated with increased risk of ecosystems contamination, which remains a global ecological issue (Lassalle *et al.*, 2020). Crude oil contamination is of enormous concern because of its impact on human health, causing intoxication, cancer, congenital disabilities, preterm birth, and cardiovascular diseases (Bruederle and Hodler, 2019). It also has diverse ecosystem damaging impact, affecting land devoted to agriculture, transforming useful lands into wastelands (Athar *et al.*, 2016; Ramirez *et al.*, 2017; Gaur, Narasimhulu and PydiSetty, 2018) causing serious threat to the ecosystem's rich biodiversity, as well as loss of drinking water (Albert, Amaratunga and Haigh, 2018).

Legacy sites like historic gasworks and gasholder are potential sites for soil and ground water contamination (Nicholson, Gould and Mallett, 2018). Gas was manufactured and produced for example, in the United Kingdom, from 1792 until 1981, when the last gasworks closed due to the conversion to natural gas, which began in 1967 and took ten years to complete (CL:AIRE, 2015). Coal gas production have resulted in sites contaminated not only with hydrocarbons but also with toxic metals, and as gasworks phase out, legacy sites become a source of contaminants whose leaching in the soil may lead to ground water contamination.

Climate change is now another important component that significantly affects soil conditions. Climate change and rising temperatures may cause an increase in evapotranspiration, which includes the evaporation of water from soils; and as water evaporates, salt accumulates in the soil, increasing salinity (Khamidov *et al.*, 2022)**.** Soil salinisation is one of the most severe land degradation problems, which results in poor plant development and impact soil microbial community activities because of build-up of osmotic stress and harmful ions (Yan *et al.*, 2015a)**.** Furthermore, in coastal areas, soils are frequently saline due to sea water intrusion on land, and while the microbial community may have already adapted to the saline condition, a crude oil spill in the soil may alter microbial function. Similarly, as ocean water recedes due to climate change, salinity in these areas rises (Tnay, 2019).

Soil remediation is often required due to the health risks that soil contamination poses to humans, animals, plants, microbes, and the entire ecosystem functions (EC, 2006). Though the remediation industry has grown over the last 50 years, there has been a shift from energy-intensive remediation technologies to green and sustainable remediation technologies, which has been supported by several initiatives such as SURF US and SURF UK, Green Remediation USEPA, and others (Ellis and Hadley, 2009). However, there are some drawbacks and pitfalls that must be overcome. Useful technologies like bioremediation, which detoxifies pollutants in soil and other habitats primarily by using microbes, plants, or microbial or plant enzymes (Gouma *et al.*, 2014) has cost-efficiency, environmental friendliness, and other advantages that allow for the incorporation of sustainable remediation features (Bwapwa, 2022; Xiong *et al.*, 2022).

Over time, efforts towards sustainable remediation have involved the use of soil amendments. An important example is the use of biochar, whose sustainability, efficiency, and lost-cost have been reported (Ahmad *et al.*, 2014a; Guo *et al.*, 2015; Oliveira *et al.*, 2017a; Cipullo *et al.*, 2019). Biochar, a carbon-rich product produced by the thermal decomposition of organic material possesses a number of remarkable properties, which has been exploited for various applications (Guo *et al.*, 2015). It has been used as a soil ameliorant, in environmental remediation, as well as mitigating climate change through high carbon sequestration (Oliveira et al., 2017). An equally important product which is a waste-product of agricultural industry is the spent mushroom compost (SMC), a by-product of mushroom production, which is likely to contain diverse groups of microorganisms and extracellular enzymes important for the biotransformation of contaminants.

Biochar and spent mushroom compost interactions in soil induces diverse responses in microbial species leading to changes in soil enzyme activity, reshaping of microbial community structure and consequent enhancement of contaminants transformations (Zhu et al., 2017). However, the mechanisms underlying these interactions have not been effectively clarified and standardised, as different biochar-microbe deployments has yielded variable results (Dike *et al.*, 2021). Hence, an understanding of biochar-microbe interactions is paramount to appreciating the link between biochar properties with diverse soil processes, especially contaminant degradation (Yuan *et al.*, 2019).

Equally, while biochar has enormous potential for bioremediation, its physical properties vary substantially depending on feedstock types and pyrolysis temperatures ((Yuan et al., 2019; Oliveira et al., 2017b). This variation is important as it is the physical properties that define its remediation potential. However, this has given rise to the challenge where, the inconsistency in the physicochemical and functional properties of different biochars makes match-making them with soil microbial degraders unreliable, and their subsequent use in soil remediation unpredictable.

Remediation success and some remediation frameworks frequently use reduction of total contaminant concentration to defined soil risk from the contaminant rather than use of bioavailable concentrations, which is the fraction to which receptors respond to, i.e. able to reach cellular membrane of organisms (Cipullo *et al.*, 2019). Furthermore, the focus of evaluating contaminant effects is frequently on a single contaminant rather than mixtures, despite the fact that contaminants in polluted soil frequently occur as a complex mixture of contaminants (Kienzler *et al.*, 2016). The important ability of biochar to sequester pollutants allows for a reduction in the bioavailability of organic and inorganic pollutants in contaminated soils (Yuan *et al.*, 2019). As a result, it is critical to further investigate the concept of bioavailability and how biochar and spent mushroom compost may affect it. At the same time evaluate the effects of the bioamendment on a complex chemical mixture contamination namely soil contaminated with hydrocarbons and metals.

While soil salinity conditions are increasing in some areas due to climate change, coastal area soils are already saline due to soil interactions with sea water. Hence, testing these bioamendments on saline soils to investigate how they may contribute to circumventing salinity-related limitations on the function of microbial crude oil biodegradation is a step in the right direction.

The use of biochar and spent mushroom compost may be a remediation strategy that provides opportunity to overcome soil nutrient limitation (via the SMC's rich organic matter and enzymes (Zhang and Sun, 2014)), increase sorption/decrease bioavailability of chemicals (via the sorption properties of biochar and SMC due to their surface properties (Guo, Song and Tian, 2020)), and increase surface contact of contaminants with the soil microbial community (as they often serve as surfaces for microbial growth from which the microbes feed on the contaminants adsorbed onto their surfaces; they also mediate microbial election transfers (Zhu *et al.*, 2017a))

1.1. **Aim and objectives**

The aim of the PhD research was to develop a comprehensive biotechnological approach for the best use of biochar and spent mushroom compost to promote microbial remediation of soil contaminated with complex chemical mixture contaminants. The PhD study provides a mechanistic understanding of the physicochemical and biological parameters influencing soil microbes in a biochar and spent mushroom compost bioengineered soils. The study further sheds new light into the influence of low carbon soil amendment on the behaviour and fate of metal(loids) and petroleum hydrocarbons, and the underlying microbial community responses.

The following specific objectives have been addressed to achieve the research aim:

- **Objective 1**: To critically review literature on the applications of biochar and spent mushroom compost for remediation
- **Objective 2**: To evaluate the effects of biochar and/or spent mushroom compost on the fate and behaviour of hydrocarbons and heavy metals(loids).

- **Objective 3**: To investigate how the amendments, influence the soil microbial degradation rates and dynamics.
- **Objective 4**: To link the biological activity, bioavailability of hydrocarbons and heavy metals(loids), and toxicity to define end-point remediation
- **Objective 5**: To investigate the influence of biochar and spent mushroom compost in promoting petroleum hydrocarbon degradation, increase in microbial community abundance and function in a saline soil.

1.2. Thesis structure and format

The PhD thesis is divided into five chapters, and three (2, 3, 4) of them were written in paper format (Figure 1.1). The following is a synopsis of each chapter:

Chapter 1: General introduction as well as the research context, background, aim, and objectives.

Chapter 2: Critical review of the current state of the art in bioremediation applications of biochar and spent mushroom compost; it aided in identifying gaps in the literature, narrowing the research focus, and organising the research plan.

Chapter 3: A four-month microcosm experiment was set up to study the effects of rice husk and wheat straw biochar, as well as spent mushroom compost amendments on/and (i) the fate and behaviour of hydrocarbons and metals(loids), (ii) how the amendments influence the soil microbial degradation rates and dynamics, (iii) to provide an opportunity to link the biological activity, bioavailability of hydrocarbons and metals(loids), and toxicity to define remediation end-point, in a genuinely contaminated soil obtained from former gasworks site having petroleum hydrocarbon (1493.34 mg/kg) and metals (642.8 mg/kg) contamination. Total exhaustive organic compound extraction was performed using dichloromethane: hexane, and pseudo-total element digestion was performed using aqua regia in accordance with ISO 11047. Furthermore, non-exhaustive methanol or hydroxypropyl—cyclodextrin (HP—CD) solutions were used (organics) as well as single solvent extractions (inorganics). Phospholipid fatty acid analysis (PLFA) (microbial community structure), and multi substrate induced respiration assay (microbial function), and ecotoxicity was determined by Microtox® basic solid phase test. This study's datasets were used to evaluate the behaviour and fate of metal and organic contaminants, as well as their effects on microbial communities.

Chapter 4: A second four-month microcosm experiment was set up to study the effects of rice husk and wheat straw biochar, as well as spent mushroom compost amendments on petroleum hydrocarbon, microbial community abundance and activity in saline soil. This was carried out by spiking soil with 10% crude oil and 1% salt giving rise to two soil types (non-saline and saline). Total exhaustive organic compound extraction was carried out with dichloromethane: hexane (TPH), Phospholipid fatty acid analysis (PLFA) (microbial community structure), and multi substrate induced respiration assay were done (microbial function). The datasets obtained in this study were used to confirm or not if salt is influencing TPH biodegradation, respiration, or microbial community.

Chapter 5: This chapter provides an overview and summary of each chapter's key findings. It also discusses how each chapter contributed to the research's overall aim and the study's overall impacts. This chapter summarised the research's novelty and made recommendations for future research.

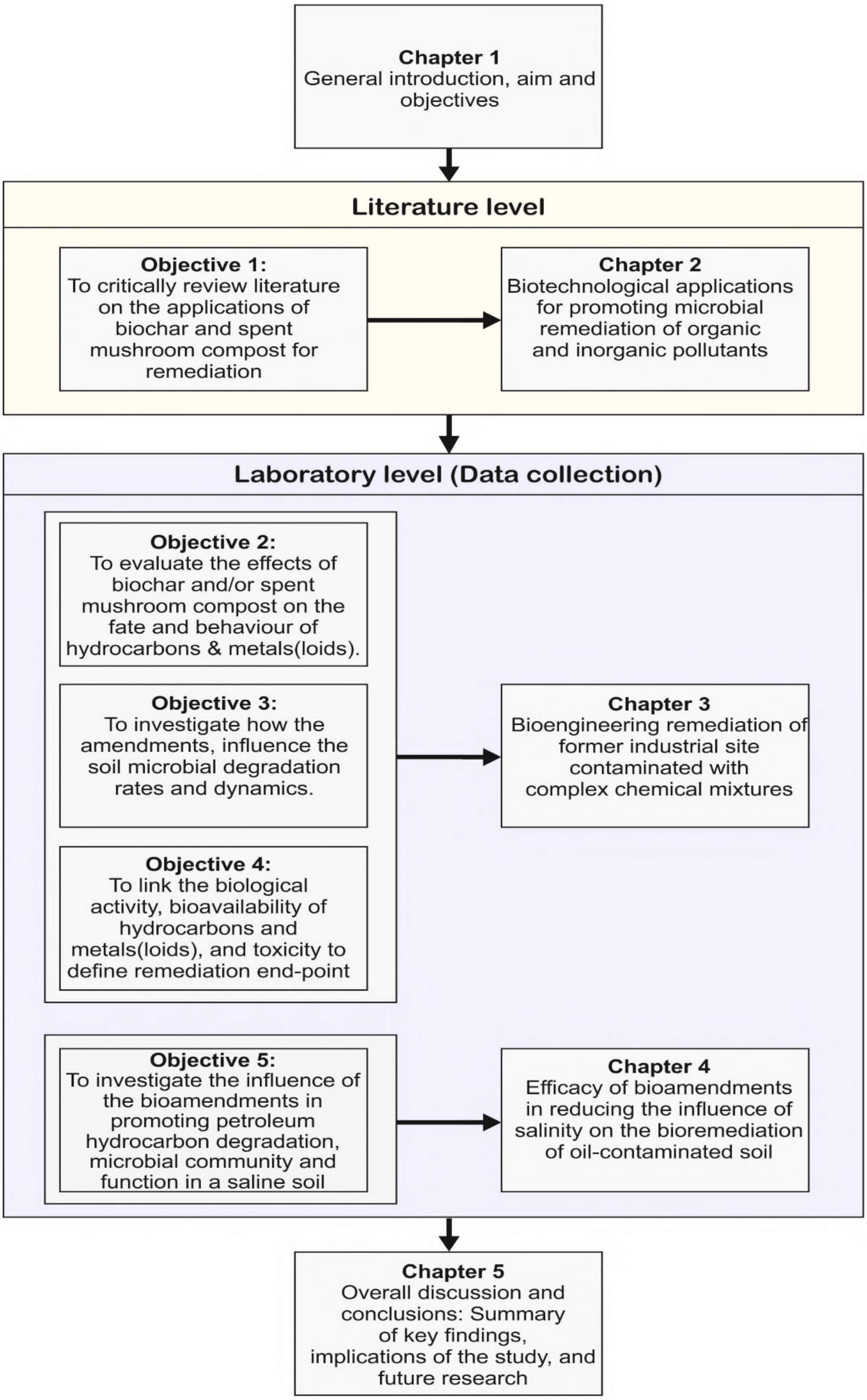

Figure 1.1. Chapters of the thesis and their alignment with each objective

1.3. Publications

Three manuscripts for publication are being prepared at the time of writing this thesis. In addition, one paper as a co-author has been published.

- Biotechnological applications for promoting microbial remediation of organic and inorganic pollutants **(in preparation)**
- Bioengineering remediation of former industrial site contaminated complex chemical mixtures **(submitted, under review)**
- Promoting soil petroleum hydrocarbons and microbial community recovery from crude oil and saline Environment **(in preparation)**

Other publications

I actively participated in laboratory investigations, specifically in chemical analysis, as part of a collaboration that led to a paper where I was listed as the 7th co-author. The paper:

Bourhane Z, Lanzén A, Cagnon C, Said OB, Mahmoudi E, Coulon F, **Atai E**, Borja A, Cravo-Laureau C & Duran R (2022) Microbial diversity alteration reveals biomarkers of contamination in soil-river-lake continuum, Journal of Hazardous Materials, 421: 126789. I contributed to the hydrocarbon analysis of samples.

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2. Biotechnological applications for promoting microbial remediation of organic and inorganic pollutants

Emmanuel Atai, Frederic Coulon, Mark Pawlett

School of Water, Energy and Environment, Cranfield University, Cranfield, MK430AL, UK

Abstract

Soil contamination is an important aspect of concern as environmental pollution continues to be a global problem. This is because soil provides diverse ecological functions important to humans, animals, microbes, and other life forms. Petroleum and its derivatives, as well as metals whose toxicity has been extensively studied, are the most common contaminants found in soil. Because of the ongoing persistence of these contaminants in soil as a result of anthropogenic activities, many studies in the field of environmental remediation have been conducted. However, in recent years, the environmental remediation field has paid increased attention to sustainability, which entails addressing risks in a safe and timely manner while also maximising the environmental, social, and economic benefits of the remediation work. The use of soil bioamendments like biochar and spent mushroom compost may be a sustainable strategy for the remediation of the hydrocarbons and metals in the soil. These materials have been shown to affects the bioavailability of organic and inorganic contaminants in soil, improve soil properties, making the soil environment more conducive to microbial growth and activity, improve soil nutrients, and directly improve the functions of microbes for the biotransformation of these contaminants. The efficiency and cost effectiveness of these materials since they are derived from readability available materials (agricultural and industrial waste), their physicochemical and functional properties which affects contaminants and promote microbial degradation function, makes them important bioresource materials for sustainable remediation of these organic and inorganic contaminants. Hence, this review focused on the effects of hydrocarbons and metals on the soil microbial community, as well as the use of biochar and spent mushroom compost in soil remediation. The review then goes on to examine the effects of biochar and spent mushroom compost on the environmental fate and behaviour of petroleum hydrocarbons and metals, as well as the effects of biochar and spent mushroom compost addition on the indigenous soil microbial community. It concluded by outlining the challenges and potential future research directions.

Keywords: biochar, Spent mushroom compost, hydrocarbons, metals, microbial community

2.1. Introduction

Soil contamination has to do with the presence of xenobiotic chemicals such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAH), solvents, pesticides and metals in levels causing alternation in the natural soil environment (George *et al.*, 2014). The US, EPA and its partners reported overseeing approximately 60,000 facilities that annually generate and manage over 30 to 40 million tonnes of hazardous waste (US Environmental Protection Agency, 2020). According to a government survey conducted in 2014, heavy metals, pesticides, chemical waste, and mining by-products contaminated up to 5% of China's cropland (Stanway, 2019). The European Environment Agency (EEA) estimates that soil contamination requiring cleanup exists at approximately 250,000 sites in EEA member countries, while potentially polluting activities occur at nearly 3 million sites (Onwubuya *et al.*, 2009; Gomes, Dias-Ferreira and Ribeiro, 2012). In Africa, for example, Nigeria, over five decades of oil exploration in the Niger Delta has resulted in the region being one of the most crude oil impacted deltas globally (Zabbey, Sam and Onyebuchi, 2017; Sam and Zabbey, 2018), affecting both the exposed human population and the environment. The issue of soil pollution has a domino effect that affects soil biodiversity, soil organic matter, and soil's ability to filter. Additionally, it depletes soil nutrients and contaminates groundwater and water held in the soil (FAO, 2021). The problem of soil contamination along with its enormous impacts has remain a challenge despite over 50 years of research in the remediation sector and petroleum derived products (hydrocarbons) and metals are still the most common soil pollutants posing significant risk to the environmental resources and to human health.

2.1.1. Soil contamination with hydrocarbon

Soil contamination with hydrocarbons has become common due to the world's reliance on petroleum as the major source of energy (Wu *et al.*, 2022). These hydrocarbons, whose primary source is crude oil, enter the environment either accidentally or through human activity, causing significant changes in the microbiological, chemical, and physical properties of soil (Abioye, 2011). Hydrocarbons are a diverse group of compounds that include linear, branched, cyclic, and aromatic compounds. The aliphatic group includes alkanes, iso-alkanes, and cyclo-alkanes. Other groups are the aromatic, naphtha aromatic, and polyaromatic compounds. Other non-hydrocarbon components of crude oil include resins containing other elements like nitrogen, sulphur, and oxygen, such as carbazoles, thiophenes, oxygenated hydrocarbons, and asphaltenes (Brown et al., 2017). While soil contamination with hydrocarbons mainly from oil spill, may occur in somewhat large areas of agricultural land near oil wells or pipelines, other major contamination sources include pesticides, automobile oils, urban storm water discharges, filling and distribution stations, and other petroleum related industrial, factory or workshop locations (Ellis and Adams, 1961; Srivastava et al., 2019).

When there is an oil spill, the behaviour of the oil in the soil varies; the rate and extent of infiltration will depend on soil properties (porosity, pore size, moisture levels), as well as physical properties (viscosity, density, surface tension), and the volume of spilled oil (Brown et al., 2017). Hence, the light non-aqueous liquid phase is retained in pore spaces or floats in groundwater, the more soluble component dissolves in groundwater, the volatile component partitions into soil gas, and the more viscous components sorb onto soil particles. These distributions are frequently not static and can change over time as a result of natural processes in the environment (Newell et al., 1995). Prominent among hydrocarbons of public concern are the PAHs. Polynuclear aromatic hydrocarbons, are a major subset of the aromatic hydrocarbons, consisting of multiple aromatic rings, uncharged, non-polar, and with delocalised electrons in their aromatic rings (Jones et al., 1989; Sun et al., 2021). PAHs are ubiquitous environmental pollutants whose primary source are because of partial combustion of organic materials, for example coal, petrol, and wood. Their presence in the environment through natural and anthropogenic causes, transport and fate, effects and removal are elucidated in Figure 2.1

2.1.2. Soil Contamination with Metals

Soil contamination with toxic metals which equally pose risks to human health and the ecological environment is a global problem of public health concern. As a result of anthropogenic activities like industrial, mining, and agricultural operations, these metals are now widely present in the environment. They are very poisonous, prevalent, and persistent in the environment, and have the ability to bioaccumulate along the food chain (Santos *et al.*, 2018). Generally speaking, metals having particularly high densities, atomic weights, or atomic numbers are considered potentially hazardous even though this is not always true. Different authors on separate context use criteria to include metalloids or not. It is viewed in terms of its properties or the characteristics it exhibits. For example, in metallurgy, physicists or chemists and biologists it is viewed in terms of density, atomic number, and chemical behaviour, respectively (Pourret, 2018). Potentially toxic metals are naturally occurring elements that comprise essential and nonessential metals. Examples include but limited to Fe, Sn, V, Se, Mn, Cu, Fe, Ni, Zn, Cd, Hg, As, and Pb. (Santos *et al.*, 2018). Some of the metals have been termed essential elements because they are required for certain biological processes like oxygen and electron transport, biosynthesis, metabolism, enzymes activities, and endocrine function (Kacar, Garcia and Anbar, 2021).

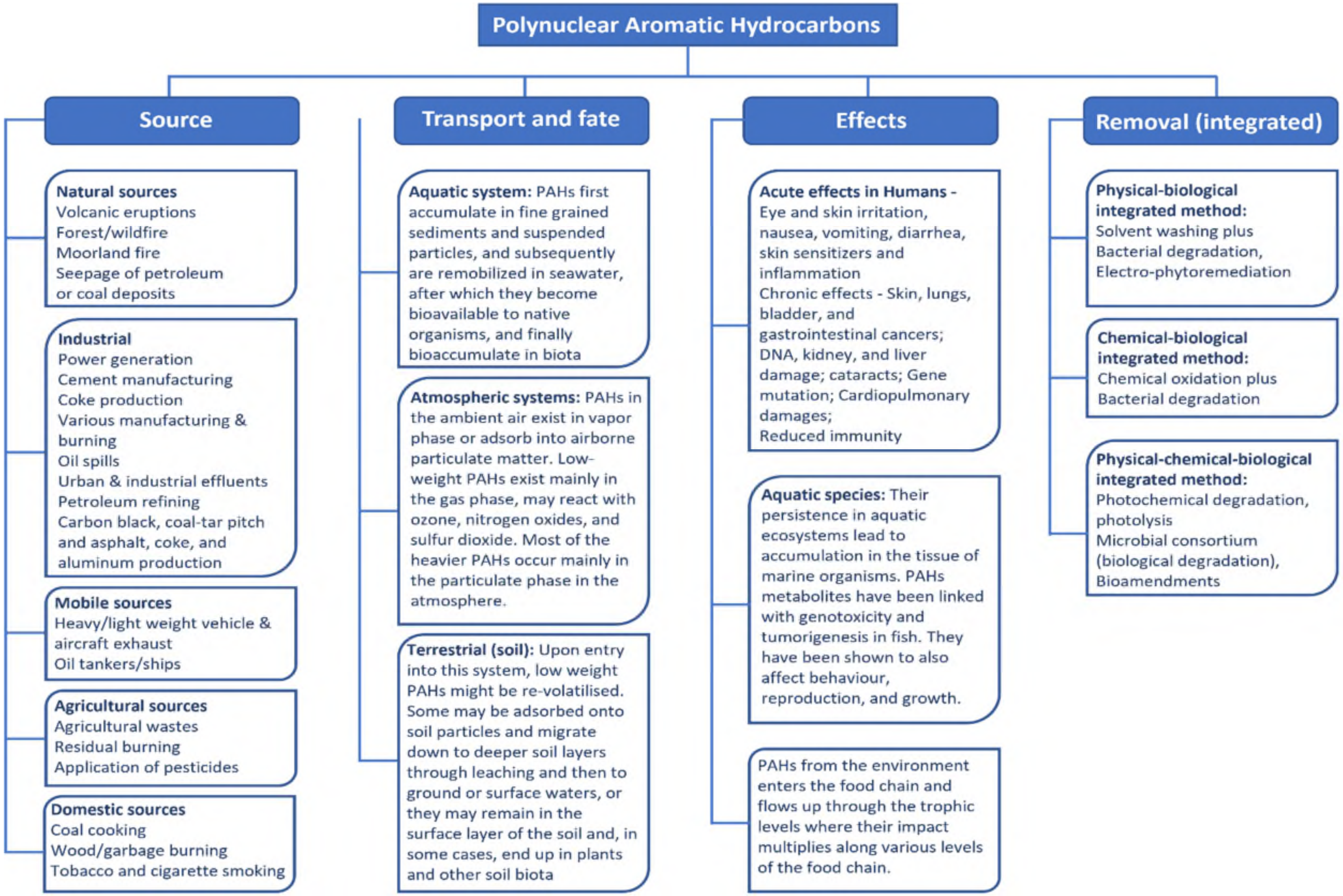

Figure 2. 1. PAH characteristics in the environment

An insufficiency of any of these essential metals may increase susceptibility to metal poisoning; an excess, on the other hand, may have negative biological effects (Venugopal and Luckey, 1978). A few non-essential metals however, have also been shown to have biological effects. Potentially toxic metals are said to be highly toxic or harmful to the environment, while others are toxic only in excess or in certain forms (Duffus, 2002). Metal sources, transport and effects in humans and animals are shown (Figure 2.2).

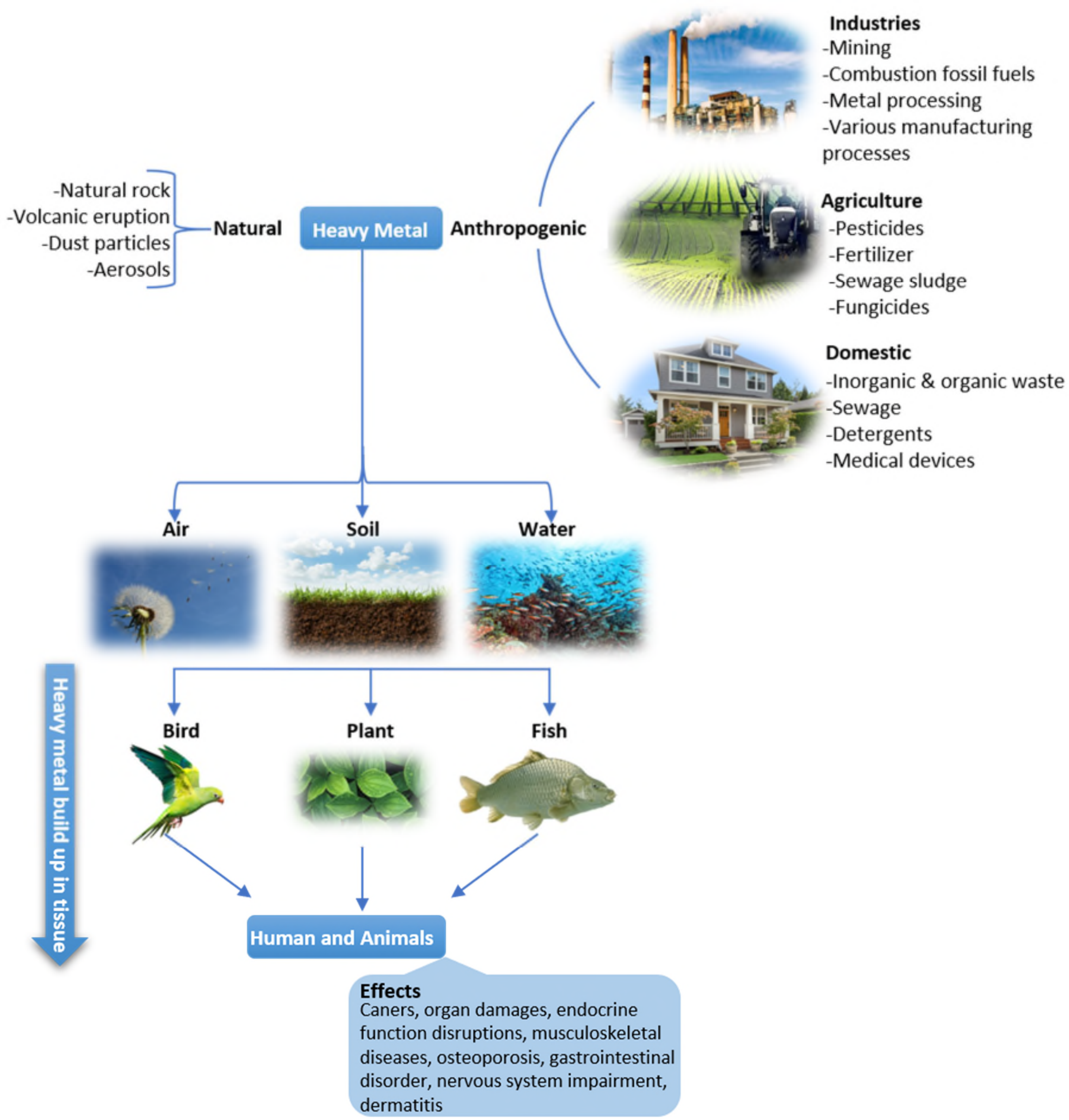

Figure 2. 2. Heavy metal sources, transport and effects in humans and animals.

2.1.3. Sustainable remediation of hydrocarbons and metals

In recent years, the environmental remediation field has paid increasing attention to sustainability (Ellis and Hadley, 2009), which entails addressing risks in a safe and timely manner while also maximizing the environmental, social, and economic benefits of the remediation work (SurF-UK, 2010). Bioremediation is one method that is both environmentally friendly and economically sustainable. Bioremediation has been deemed economically viable and socially acceptable due to the potential for job creation, access to farmland, and the creation of an environmentally safe legacy as a result of reduced greenhouse gas emissions (Nathanail *et al.*, 2017).

Efforts towards sustainable remediation over time have involved the use of soil amendments. An important example of such material is biochar, whose sustainability, efficiency, and cost-effectiveness has been reported (Ahmad *et al.*, 2014a; Guo *et al.*, 2015; Oliveira *et al.*, 2017a; Cipullo *et al.*, 2019). Biochar, a carbon-rich product produced by the thermal decomposition of organic material possesses a number of remarkable properties, which has been exploited for various applications (Guo *et al.*, 2015). It has been used as a soil ameliorant, in environmental remediation, as well as mitigating climate change through high carbon sequestration (Oliveira et al., 2017). An equally important product of agricultural waste industry is the spent mushroom compost (SMC), a by-product of mushroom production, which is likely to contain diverse groups of microorganisms and extracellular enzymes important for the biotransformation of contaminants (Zhang and Sun, 2014).

Biochar and spent mushroom compost induces diverse responses in soil microbial communities leading to changes in enzyme activities, reshaping of community structure, all of which has consequence on contaminants transformations (Zhu et al., 2017). However, the mechanisms underlying these interactions require great investigation. In exploring these interactions, attention is needed in understanding some drawbacks with biochar such as, the inconsistency in the physicochemical and functional properties of different biochars, which makes combining them with soil microbial degraders in remediation to give unpredictable outcomes. The effects of the biochar and spent mushroom on complex chemical mixtures (hydrocarbons and metals) and how effective biochar-SMC synergy could ensure contaminant remediation need to be examined. As a result, the interactive effects of hydrocarbons and metals on the soil microbial community are discussed, as well as avenues in which biochar and spent mushroom compost can be applied in soil to influence microbial activities toward metals and hydrocarbon remediation in soil.

2.2. Effects of hydrocarbons and metals on soil microbial community

The soil microbial communities, provides crucial ecosystem functions and services (Prescott *et al.*, 2019). They perform numerous important ecological and physiological functions such as biomass decomposition, biological element circulation, atmospheric nitrogen fixation, the formation of mycorrhiza and biologically active substances capable of stimulating growth, soil structure maintenance, and contaminant biodegradation (Sofo *et al.*, 2014; Furtak and Gajda, 2018). Given that ecosystem health is defined as stability and resilience in the face of disturbance, pollution by toxic compounds represents a chemical disturbance that will result in significant changes to soil microbial functions (Madrova *et al.*, 2018).

2.2.1. The impact of hydrocarbons on the soil microbial community

In general, hydrocarbon contamination of soil alters environmental conditions, causing shifts in microbial community structure and function (Margesin, Hämmerle and Tscherko, 2007), as evidenced by a rise or fall in groups and populations of soil microbial communities leading to changes in soil function mediated by the microorganisms. For example, petroleum pollution increased the abundance of proteobacterial proteins while decreasing the abundance of Rhizobiales (Aislabie, Novis and Ferrari, 2014; Bastida *et al.*, 2016), causing an interruption in soil nutrient balance leading to depletion of food reservoirs (Azari Moghaddam and Abu

Bakar, 2016). It has also been shown to induce an increase in microbial community function as evidenced by a 5-fold increase in metabolic quotient and cellulase activity in soil contaminated with petroleum (Galitskaya *et al.*, 2015) or an suppression of dehydrogenase and phosphatase enzymatic activities (Alrumman, Standing and Paton, 2015).

2.2.2. Metals' effects on the soil microbial community

Similarly, any decrease in microbial diversity or abundance caused by metal contamination may impair their ability to function in the soil. Metal concentrations in soil have a significant impact on the population size and overall activity of soil microbial communities. This is due to metal toxicity, which disrupts soil microorganism growth, morphology, and metabolism through functional disruption, protein denaturation, or the destruction of cell membrane integrity (Xie *et al.*, 2016). According to studies on the impact of metal concentration on microbial communities, richness and diversity of all soil microbial functional groups decline as metal concentration rises (Xie *et al.*, 2016; Hamidović *et al.*, 2020; Ma *et al.*, 2021). On the other hand, some metal concentrations can enhance the diversity of carbon utilisation and the structure of microbial communities (Ding *et al.*, 2017; Ma *et al.*, 2021). Microbial enzyme activities were found to decrease as metal pollution increased, but the amount of decrease varied between enzymes. In some instances, while enzymes involved in carbon cycling may be least affected, those involved in nitrogen, phosphorus, and sulphur cycling showed a significant decrease in activity (Kandeler, Kampichler and Horak, 1996). Bacterial communities such as *Acidobacteria*, *Chloroflexi*, and *Gemmatimonadetes* have been shown to be resistant to high levels of metal pollution (Li *et al.*, 2021). This resistance may be to a variety of metals or to a particular metal, such as a population that is resistant to cadmium (Pacwa-Płociniczak *et al.*, 2017) and others to lead (Sobolev and Begonia, 2008). Microbial redundancy may occur in metal-contaminated soil environments, and some microorganisms may exhibit some resistance or tolerance mechanisms. The change in the composition of the microbial community and the subsequent decline in their activity may cause the failure of fundamental soil functions (Moreira, Pereira and Castro, 2016).

2.3. The application of biochar in soil remediation

Biochars have received a lot of attention because of their significant environmental uses, such as removal of pollutants, carbon sequestration, and soil improvement (Oliveira *et al.*, 2017a; Zhu *et al.*, 2017a). It has been shown to have agronomic and environmental effects on soil, causing changes in nutrient dynamics, microbial functions, and soil contaminants and because of its ability to serve as a soil conditioner, applying biochar to soil has been reported to improve soil quality, leading to increased crop yield (Das *et al.*, 2021).

The International Biochar Initiative defines biochar as the solid product made when biomass is thermochemically converted in an oxygen-limited environment in a process referred to as pyrolysis (The International Biochar Initiative, 2015). Biochar is a stable solid material that is high in carbon and can last for thousands of years in soil (Guo *et al.*, 2015) making it an important carbon sink which is a means to mitigate global warning and climate change.

Biochar production and application for improving soil fertility is an ancient custom practiced by farmers in India, Europe, China, Japan, and America, where they make biochar by the burning agricultural waste in pits or trenches (Solomon *et al.*, 2007). In modern times, however, the production process (pyrolysis) is a direct thermal decomposition of biomass, such as wood, leaves and straw, and manure, in the absence of (or presence of limited) oxygen, yielding a mixture of solids (biochar itself), liquids (bio-oil), and gases (syngas) (Tripathi, Sahu and Ganesan, 2016). The relative yield of product formation in pyrolysis varies with temperature. Temperatures ranging from 400 to 500 °C (752-932 °F) produce more char, while temperatures above 700 °C (1292 F) increase the yield of liquid and gas parts. Pyrolysis with temperature above 700°C, called gasification, yields a relatively low amount of biochar (Turner *et al.*, 2008; Van Zwieten *et al.*, 2010; Guan *et al.*, 2016).

Biochar is distinguished by certain characteristics such as high adsorption capacity, specific surface area, microporosity, and ion exchange capacity (Ahmad *et al.*, 2014b). It has several remarkable physical and chemical properties (Figure 2.3), which give rise to its numerous potentials, which have been exploited for a variety of applications (Guo *et al.*, 2015). These properties are determined by the feedstocks and production conditions (pyrolysis temperature and heating rate) used during biochar production, and these two factors have a significant impact on physicochemical properties such as surface area, atomic ratio, polarity, pH, element composition, and thus the general surface property of the biochar (Oliveira *et al.*, 2017a).

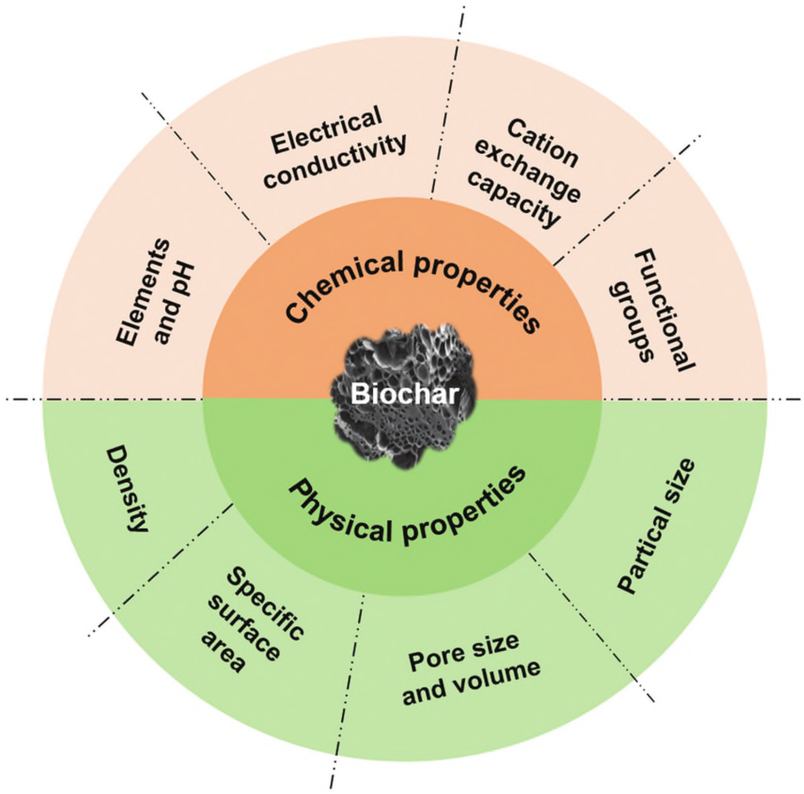

Figure 2. 3. The main physiochemical properties of biochar (Tang et al., 2020)

Biochar applications in soil, improve soil properties, which has a substantial influence on the various ecosystem functions (Figure 2.4). Furthermore, variations in biochar characteristics have significant implications for its suitability and efficacy in the remediation of both organic and inorganic pollutants (Oliveira *et al.*, 2017a).

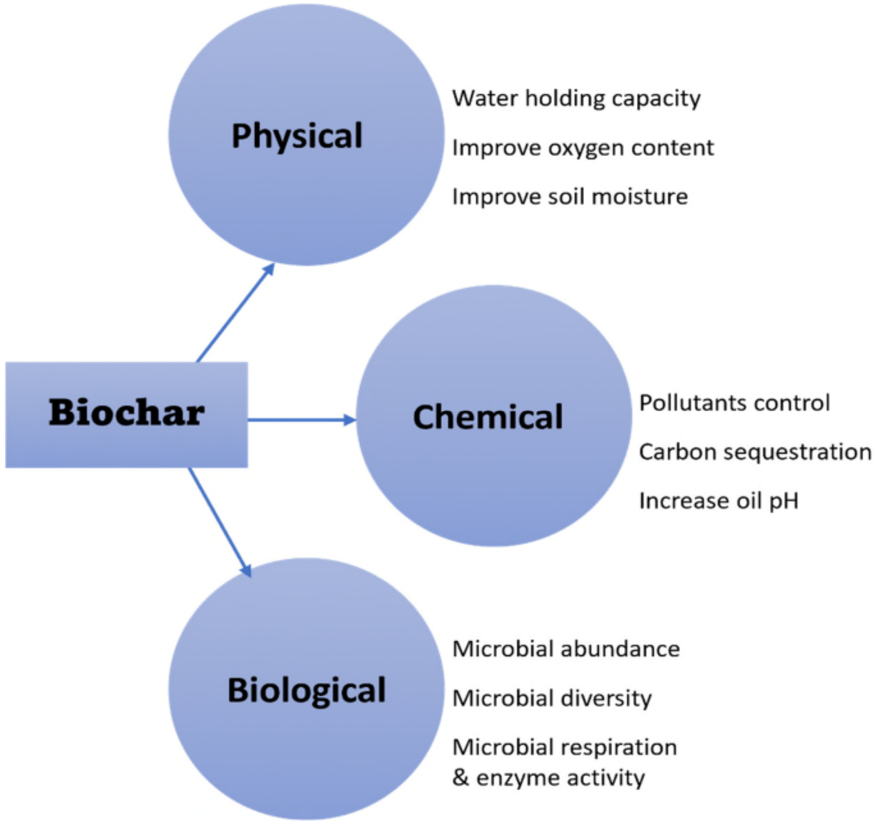

Figure 2. 4. Biochar applications for improvement of soil properties

One way to contribute to the efforts of sustainable remediation is the use of soil amendments like biochar and compost, whose raw materials e.g., agricultural wastes, municipal solid waste, , and forest residues, can easily be sourced (Cipullo *et al.*, 2019). These materials have received a lot of attention due to their sustainability, efficiency, and cost-effectiveness (Ahmad *et al.*, 2014a). Biochar is used to remediate organic and inorganic contaminated sites by limiting the mobility and fate of contaminants in the soil. Reduced mobility of contaminants reduces their bioavailability, lowering their risk and increasing the likelihood of transformation (degradation/accumulation) in the soil ecosystem (Ogbuagu, 2020). Several studies (Table 2.1) have shown that applying biochar to contaminated soils promotes hydrocarbon and heavy metal remediation (Guo, Song and Tian, 2020).

Table 2. 1. Biochar-facilitated soil organic/metal contamination remediation trials

			Organic pollutants			
Biochar source	**Soil type**	**Hydrocarbon**	**Experiment**	**Effect**	**Method**	**Reference**
Bamboo biochar pH 8.95 SSA 103.1m^2/g	Industrial site, 4520mg/kg TPH Sand pH 5.64	Diesel	Mesocosms to study the effects of various amendment	Reduced to 63.9%	Biochar as a soil amendment	(Chaudhary *et al.*, 2021) Chaudhary 2021
Wheat straw biochar 500 ºC	Agriculture soil pH 6.5 loam Forest soil pH 5.68 loam	PAH	PAH removal in 3 different petroleum contaminated soil	Biochar decreased the PAH content	Biochar-organic matter synergy and biochar sorption	(Kong *et al.*, 2021)
Pine biochar 350 ºC pH 7.2 OC 63.53%	Garden soil pH 6.99	Crude oil	Bio-stimulatory impact of biochar for remediation of crude oil soil at 10% and 15%	Biochar degraded 34% of the crude oil	Sorption, degradation, and phytoremediation	(Saeed *et al.*, 2021)
Pine wood pH 7.5 OC 85.2%	Washed sand pH 5.4 OC <0.1%	Phe	Batch adsorption and desorption experiments with 1 wt% biochar-amended soils	Phenanthrene sorption on wood-based biochar was less evident; sorption on biochar was more evident in low-OC soils and reduced mineralization	The source of biochar influenced its sorption performance	(Moreno Jiménez *et al.*, 2018)

Willow, coconut, wheat straw Non-activated (pH 8.0-9.9; SSA 3.1-26.3m^2/g) and steam activated (pH 7.2-8.8; SSA 246-841m^2/g)	Industrial site soils	PAHs	Solvent extraction of 5 Wt% biochar-amended, 60-day incubated soils and bioassays with garden cress. Springtail, and bacteria	Activated biochar further reduced bioaccessible PAHs in soil. Biochar reduced soil toxicity to springtail and bacteria but not phytotoxicity	Both SSA and surface interaction are important for biochar to immobilize PAHs	(Kołtowski *et al.*, 2016)
Sewage sludge pH 7.3 OC 27.1% N 3.4% S 4.6%	Texture unknown pH 6.8 OC 7.0%	PAHs	2, 5, and 10 mass% biochar mixing with soil; 8-week germination greenhouse pots with lettuce	Reduced PAHs bioaccumulation; enhanced plant growth	Likely strong sorption of PAHs by biochar through partition; stimulated soil microbial activity	(Khan *et al.*, 2013)
Soft wood 450ºC pyrolysis pH 10.0	Brownfield soil pH 7.7 CEC 9.5 cmol$_c$/kg	PCBs	Bioassay of 2.8 wt% biochar-amended soils in field plots and greenhouse pots	Pumpkin root decreased PCB content by >60%; mixing affected biochar effects	With thorough mixing with soil, biochar reduces PCB bioavailability by strong sorption	(Denyes, Rutter and Zeeb, 2013)
Rice straw 500ºC pyrolysis pH 8.9	Oil spill site Clay loam pH 6.5 OC 5.4%	Petroleum	180 –day lab incubation of 2 wt% biochar-amended soils	Soil microbial degradation of petro-hydrocarbon improved by 20%	Biochar as a biostimulant to furnish C, N, P, and other nutrients to microbes	(Qin, Gong and Fan, 2013)
Stinging nettle OC 52.1% N 2.1% SSA 3.5m^2/g	Mine spoil Sand pH 3.2 High As, Cu, Cd	Phe and As, Cu	Sunflower grown in PAHs-spiked, 1 wt% biochar-amended, 56-day lab-incubated soil pots	Biochar increased phenanthrene degradation by 44%; plant growth improved	Biochar supplies additional C, N, P and other nutrients	(Sneath, Hutchings and de Leij, 2013)

Bamboo pH 9.5 SSA 332 m^2/g	Loam pH 5.2 OM 4.7%	PCP	Column leaching of 14-day incubated, 2-5 wt% biochar amended, PCP-spiked soil	Residual PCP in and PCP leaching losses from soil columns were decreased	Sorption of PCP by biochar mainly via partition	(Xu *et al.*, 2012)
Bamboo biochar pH 8.95 SSA $103.1m^2/g$	Industrial site, 4520mg/kg TPH Sand pH 5.64	Diesel	Mesocosms to study the effects of various amendment	Reduced to 63.9%	Biochar as a soil amendment	(Chaudhary *et al.*, 2021) Chaudhary 2021
			Inorganic pollutants			
Biochar source	**Soil type**	**Metals**	**Experiment**	**Effect**	**Mechanisms**	**Reference**
Wheat straw pH 10.4 P 1.44% CEC 21.7 $cmol_c/kg$ SSA 8.9 m^2/g	Rice paddy soils Loam pH 4.9-6.1	Cd	1-5 years of rice and wheat grown in NPK-fertilized field pots with top 15-cm soil amended by biochar at 40 t/ha	Biochar elevated soil pH and reduced aoil 0.01 M $CaCl_2$-extractable Cd and crop grain Cd. The effect decreased over time	Precipitation; surface functional group complexation; Fe/Al/P mineral encapsulation on biochar surface and in pores.	(Cui *et al.*, 2011; Bian *et al.*, 2014; Sun *et al.*, 2016)
Rice husk 500ºC pyrolysis	Mining soil Loamy sand	Cd, Cu, Ni, Zn	16-day N_2 flushing of 5 wt% biochar-amended, 42-day incubated soil	Biochar increased dissolved Cu, Cd, Ni, and Zn under oxic conditions	Metal mobility was enhanced by biochar-introduced DOC	(El-Naggar *et al.*, 2018)
Grain husk pH 8.3 CEC 65.4 $cmol_c/kg$	Sand OM 0.78%	Zn	Spectroscopic examination of Zn-spiked, 1-5 wt% biochar-amended soils after 180-day plant growth	Aging increased biochar O, Si, Ca, Al, Mg, and Zn contents and reduced C, N, P, and K contents	Precipitation, surface sorption, and organo-mineral complexes on biochar surface and in pores	(Kumar *et al.*, 2018)

Rice straw pH 9.5 CEC 64.8cmol$_c$/kg	Greenhouse soil Loam pH 6.2-6.8 CEC 15-16.7 cmol$_c$/kg	Cd	Sequential extraction of soil from lettuce pots with 20 t/ha biochar amendment in top 15-cm soil	Lettuce Cd content reduced in lightly polluted but not in heavy polluted soil	Soil pH increased, exchangeable Cd decreased but Fe oxide- and OM-bound Cd increased	(Zhang *et al.*, 2017)
Soybean straw 300ºC pyrolysis pH 7.3 SSA 5.6m^2/g	Shooting rand soil Sandy loam pH 8.0 OM 5.2%	Cu, Pb, Sb	DTPA- and TCLP-extraction of 0.5-2.5 wt% biochar-amended, 30-day incubated soils.	Biochar-immobilized Pb and Cu but mobilized Sb	Stabilization of Pb and Cu *via* precipitation and electrostatic and π-π electron shift-adsorption	(Vithanage *et al.*, 2017)
Rice Straw pH 10.5 CEC 32.1 cmol$_c$/kg	Rice paddy soil pH 6.1 CEC 12.5 cmol$_c$/kg	As, Cd, Pb, Zn	One-season rice grown in field plots with 0-20 cm soil amended with 20 t/ha biochar and basal fertilization	Biochar reduced Cd, Zn and Pb but increased As in soil pore water and rice	Soil pH elevation and formation of iron plaque on rice surface	(Zheng *et al.*, 2015)
Rice straw Field trench smoldering with soil cover	Rice paddy soil pH 7.0	Cd, Pb, Zn	Leafy vegetables grown in field plots with 5 wt% biochar amendment of top 20-cm soil	Biochar reduced soil bioavailable and vegetable metals and increased soil biomass yield	Biochar reduces Cd, Pb, Zn solubility by elevating soil pH	(Niu *et al.*, 2015)
Orchard pruning	Mining soil OM 1.7%	As, Cd, Cu, Pb, Zn	Bioassay with bacteria and ryegrass of water extracts from 10 vol% biochar soil pots	Reduced free metals yet increased As and DOC-associated metals in soil pore water	Biochar enhanced soil As and metal mobility by altering soil pH, DOC, and P	(Beesley *et al.*, 2014)
Miscanthus straw pH 8.7	Former sewage field sandy loam pH 5.0 OC 2.6-4.9% P 0.3-0.5%	Cu, Pb, Zn, Cd	2-year orchard grass grown in field plots of top soil amended with 2.5-5.0 wt% biochar	Biochar reduced Cd and Zn but increased Cu and Pb in leachate	Soil pH elevation; biochar reduces soluble metals in soil but may increase colloidal transport in metal phosphates	(Schweiker *et al.*, 2014)

Sewage sludge pH 7.2 OC 28.0% N 2.6% P 5.8%	Loam sand pH 4.0 OC 0.24%	As, Cd, O, Cr, Cu, Ni, Pb, Zn	5 and 10 wt% biochar mixing up with NP fertilized soil; flooded greenhouse pots with growing rice	Decreased spoil EDTA extractable and bioaccumulated As, Cr, Co, Ni, and Pb but increased the portions of others	Not discussed; soil flooding may be considered	(Khan *et al.*, 2013)

SSA: specific surface area; OC: organic carbon; wt%: percentage by wight; CEC: cation exchange capacity; Phe: phenanthrene; PCP: pentachlorophenol; TPH: total petroleum hydrocarbon; $cmol_c$: centimoles of charge; OM: organic matter; DOC: dissolve organic carbon

2.4. Spent Mushroom Compost in remediation

Another important sustainable (economical and environmentally friendly) material useful for remediation are composting matrices. Compost are biological stabilised solid organic material that can be used as a source of nutrients and soil conditioner in agricultural applications formed in a process called composting (Lazcano, Gómez-Brandón and Domínguez, 2008), which has to do with accelerating the degradation of organic matter by microorganisms under controlled conditions, in which the organic material undergoes a characteristic thermophilic stage that allows sanitization of waste by elimination of pathogenic microorganisms (Mengistu *et al.*, 2017). Composts are use in remediation because they are rich in xenobiotic-degrading microorganisms such as bacteria, actinomycetes, and ligninolytic fungi, which can degrade pollutants or bio-transform them into less toxic compounds and/or immobilise pollutants within the organic matrix, in so doing reducing pollutant bioavailability (Gouma *et al.*, 2014). A very important compost type, which is an industrial waste, whose use may solve the industries disposal problem, is spent mushroom compost (SMC). Spent mushroom compost is a large byproduct of the mushroom industry. The commercial mushroom industry generates a massive amount of spent mushroom substrate as a waste byproduct as a mycelial unexploited leftover substrate after mushroom harvesting (Menaga, Rajakumar and Ayyasamy, 2021). For example, for every 1 kg edible mushroom produced, 5 kg of SMC is produced, resulting in millions of tonnes of SMC whose disposal becomes a major issue for mushroom farmers (Singleton, 2000; Law *et al.*, 2003). As a result, much emphasis has been placed on harnessing SMC's bioremediation potential. The use of SMC as a biostimulant in bioremediation can promote contaminant uptake in soil by enhancing extensive microbial actions in soil. SMC has reportedly been used as a soil amendment to effectively clean up hydrocarbon and metal contaminants (Asemoloye *et al.*, 2017).

Mushroom cultivation involves composting, spawning, casing, pinning, and cropping (The Mushroom Council, 2021). Compost provides the nutrients required for mushroom growth. Wheat straw and horse manure are two common materials used for mushroom compost. Inoculated mushroom compost is thoroughly mixed with mushroom spawn. The spawn is basically vegetatively propagated fungal mycelium. Casing is a top-dressing that is added to the spawn-run compost from which the mushrooms grow. Pins, the first recognisable mushroom formation from mycelium, are monitored to increase in number and size by adjusting temperature, humidity, and CO_2, a process known as pinning. The harvest of fully grown mushrooms comes next. The compost beddings are removed after 7-10 cycles of mushroom harvesting, hence the term "spent.". Some commercially cultivated mushroom includes the fungi, *Agaricus bisporus*, *Clitocybe nuda*, *Auricularia polytricha*, *Auricularia auricula-judae*, *Flammulina velutipes*, *Hypsizygus tessulatus*, *Pleurotus* species, *Rhizopus oligosporus*

Following mushroom harvest, spent mushroom compost is likely to contain a large and diverse group of microorganisms, as well as a diverse range of extracellular enzymes and a high organic matter content (Gouma *et al.*, 2014), giving rise to its potential ability to absorb and or degrade pollutants. An encouraging result was obtained when *P. pulmonarius* SMC was used to treat petroleum, oil and grease and di (2-ethylhexyl) phthalate (DEHP)-contaminated soil (Chiu *et al.*, 2009). It has been used as an organic substrate for the remediation of acid mine drainage

(Tran-Ly *et al.*, 2020). Mushroom compost can be a simple and cost-effective technology for treating metal-contaminated waste water (Newcombe and Brennan, 2010). A reported of SMC of *Pleurotus pulmonarius* removing 89.0±0.4% of 100mg PCP/L within 2 days at room temperature predominantly by biodegradation (Law *et al.*, 2003). It has been used to improve soil nutrient and reduce petroleum hydrocarbon concentration in soils (García-Delgado, Yunta and Eymar, 2013; Asemoloye *et al.*, 2017; Mohammadi-Sichani *et al.*, 2017, 2019). Hence, SMC is an indication of promising bioremediation adjuvant

2.5. Biochar and spent mushroom compost effect on the environmental fate and behaviour of petroleum hydrocarbons and metals

Biochar can reduce the risk of harm that organic and inorganic pollutants could cause in the soil ecosystem by breaking or reducing source-pathway-receptor linkages (Oni, Oziegbe and Olawole, 2019) (Figure 2.5). Contaminants typically migrate through soil from their source (source) to the receptor (biota) by dissolving in the soil solution (pathway). Biochar disrupts source-pathway connections by adsorbing contaminants on its surface (often irreversibly), thus, lowering the concentration of contaminants in soil solution (Beesley and Marmiroli, 2011). Biochar surface properties such as large surface area, cation exchange capacity (CEC), micro-porous structures, and active functional groups, enable it to bind to organic and inorganic pollutants on its surface in such a way that they no longer pose risk in the soil ecosystem.

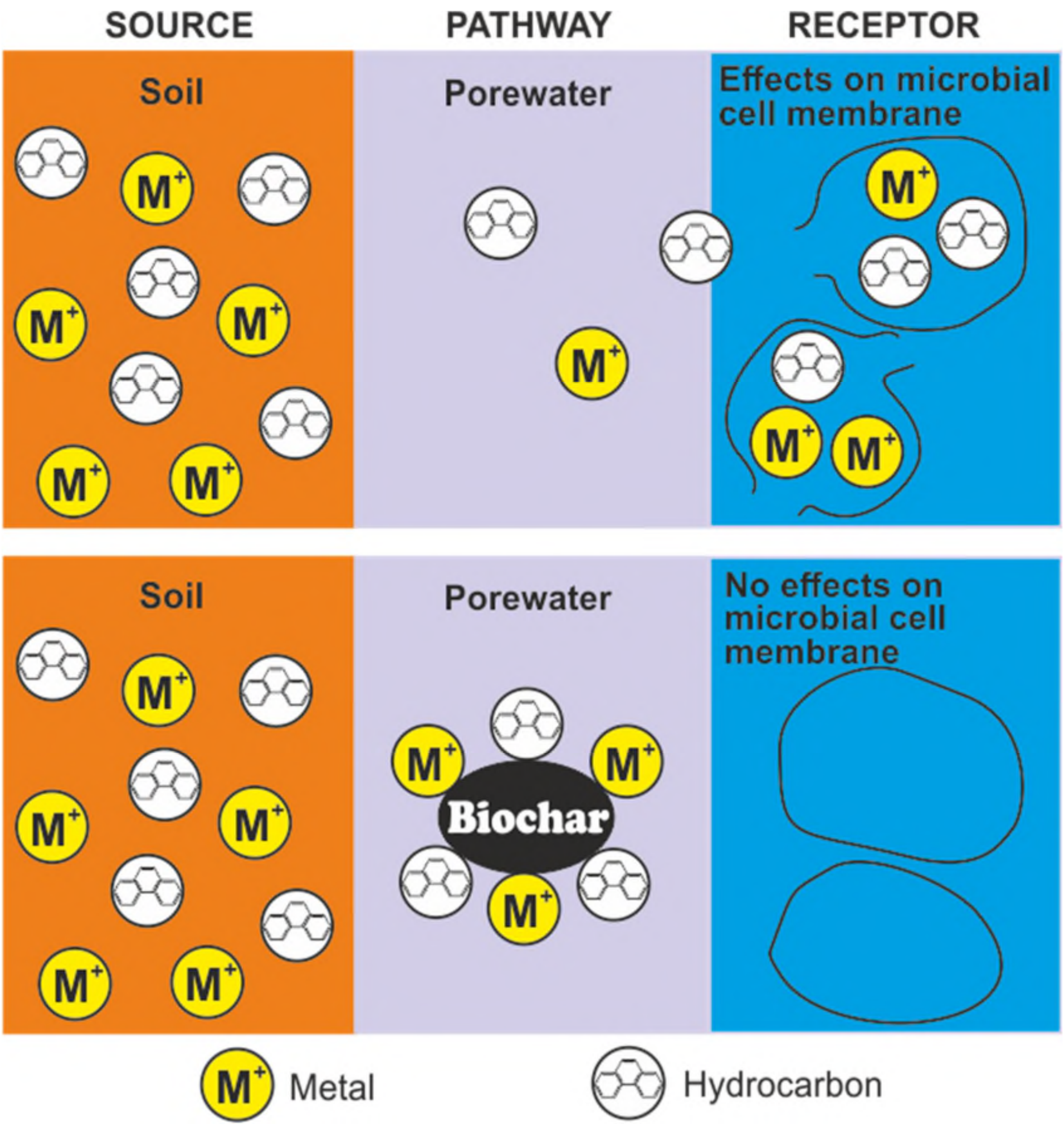

Figure 2. 5. Biochar remediation of organic and inorganic contaminants in soil through breaking a source-pathway-receptor linkage (Guo *et al*., 2015)**.** The source from which the contaminants are emanating from is the left side (orange section). The pathway is the mechanism that transport the contaminant from the source to the receptor (gold part). A receptor is the site where it can cause harm (for example, human tissue, other living organisms, or bodies of water) (the blue portion).

While hydrocarbons and metals concentrations exist in different partitions in soil, the most important fraction is the bioavailable portion of the contaminants. The bioavailability of a contaminant is the concentration or the fraction of the contaminant that becomes completely available to its intended/targeted biological destination. Biochar, by means of its sorption mechanism, has been demonstrated to reduce the bioavailability of both hydrocarbons and metals (Bian *et al.*, 2013; Abbas *et al.*, 2017; Cipullo *et al.*, 2019; Yang *et al.*, 2020) thereby reducing their uptake by the soil organisms. This action decreases the toxicity of soil contaminants to soil microbes leading to the elevation of microbial biomass because of enhanced microbial activity (Zhu *et al.*, 2017a)

Similarly, Spent Mushroom Compost can adsorb heavy metals and hydrocarbons, reducing their bioavailability and degrading them (Lau, Tsang and Chiu, 2003; Gouma *et al.*, 2014; Asemoloye, Chukwuka and Jonathan, 2020; Yu *et al.*, 2021). These could be possible because after mushroom harvest, SMC has been shown to contain fungal mycelia cell, diverse group of microorganisms and a range of extracellular enzymes like cellulase, hemicellulose, β-glucosidase, lignin peroxidases, and laccase (Singh, Abdullah and Vikineswary, 2003). It has been shown to contain a very high content of organic matter including cellulose, hemicellulose and lignin (Yu *et al.*, 2021). The nutritive, microbial communities as well as the enzymatic repertoire of SMC promotes the contaminants biotransformation in soil. Spent mushroom compost, like biochar, has a large surface area, a well-developed microporous structure, and an abundance of functional groups that are conducive to metallic ion capture via adsorption, coprecipitation, and ion exchange complexation (Li *et al.*, 2018; Corral-Bobadilla *et al.*, 2019; Wei *et al.*, 2020)

2.5.1 Chemical bioavailability in soil

The bioavailability of environmental organic and inorganic compounds is a requirement for the bioremediation of contaminated site as well as a risk factor for organisms exposed to the harmful chemicals. As a result, bioavailability is extremely organism specific and largely determined by biology (Harms, Schlosser and Wick, 2011). It is also a complex process that includes exposure dose, chemical release mass, and contaminant uptake into organisms, all of which are determined by substance qualities, compartment features, organism biology, and climatic effects (Anderson *et al.*, 2008).

Bioavailability, chemically speaking, refers to the proportion of contaminants present in the environment that are not sorbed or sequestered, but rather are mobile and therefore more likely to cause human exposure. It is the portion of the contaminant that is "freely available" in a particular medium and can reach an organism's cellular membrane within a specified period. In other words, a contaminant is considered bioavailable if it is mobile and can potentially come into contact with a biological membrane, leading to possible exposure (Cipullo *et al.*, 2018a).

When it comes to contaminated site assessment, bioavailability addresses the fundamental issue of contaminant exposure to a receptor. Exposure to pollutants may have adverse effects on biota. However, exposure is determined by the fraction of the total concentration of the contaminant that is biologically available not by the total concentration of the contaminant in the environmental medium. The interaction of the non-sequestered component of the contaminant with an organism during its life history influences exposure, which considers the route and length of exposure. As a result, exposure can occur only after the contaminant has been released (e.g., desorption) from the soil particle and has been delivered to the receptor (Anderson *et al.*, 2008).

Factors affecting bioavailability

Cipullo (2018b) collated the factors influencing the quantitative estimation of bioavailable fractions of chemicals (metals and oil derived compounds) based on physicochemical properties, receptors, and other additional factors. (Table 2).

Table 2.2. Physicochemical and biological properties, and other additional factors influencing bioavailability of chemicals in soil.

	Factors	**Metals**	**Oil derived compounds**
Physicochemical factors	Contaminants characteristics	Available in several elemental forms (metal speciation)	Molecular weight, polarity hydrophobicity, solubility octanol partitioning coefficient (K_{OW}), sorption coefficients (K_{OC}, K_{d}), acid dissociation constant (pK_a)
	Soil characteristic and sorption desorption	Influenced by both geochemical processes (e.g., redox/pH) and soil characteristics (e.g., particle size, organic content).	The bioavailability can be influenced by soil organic matter, clay concentration, organic matter (condensed humic material, soot particles), and quantity and type/quality of organic carbon.
	Transformation degradation (biological/chemical)	No degradation. Metals can only be bioaccumulated or sequestered	Organic compounds in soil can be changed and degraded by both biotic (microbial degradation) and abiotic processes (volatilization leaching, photodegradation).
	Oxidation/reduction cation exchange capacity, and soil pH	The presence of organo-mineral colloids has an impact. (adsorption). Precipitation in the presence of clay mineral and Fe, Mn, Al oxides, and carbonates after complexation with humus.	Changes in pH can influence primarily ionizable organic molecules, influencing sorption and removal of organic solutes from solution. Changes in redox potential and pH can hasten organic pollutant oxidation.
Biological factors	Uptake	Metals absorption is often based on bioassay exposures to a dissolved chemical, making metal solubility in solution and oxidation states extremely important.	Several factors involving concentration in soil, chemical form, soil pH, biological species, and individual species uptake pathways.
	Bioconcentration, bioaccumulation, and biotransformation	Metal bioaccumulation can occur in bacteria, fungi, and plants via biosorption or absorption and uptake.	There is strong correlations between the bio-concentration factor, bioaccumulation factor

			and the octanol: water partition coefficient (K_{OW})
Additional factors	Ageing	Rapid absorption via electrostatic adsorption is frequently followed by a subsequent transformation that results in the development of a more stable complex.	Several methods may occur, including assimilation into natural organic matter (absorption) and gradual diffusion into microscopic pores. (soil intraparticle).
	Co-contaminant interaction	Metal-metal interaction is largely competitive, influencing soil-surface affinity and sorption. Zn, for example, competes for Cd and Pb sorption sites. Heavy metal concentrations in shoots and roots can be greatly increased by metal-organic joint interactions such as Cu-pyrene.	Organic-metal interaction: high inorganic concentrations may alter PAH mobility. Competitive displacement and co-solvency are two examples of organic-organic interaction. Because of their interchangeability, molecules with comparable structures are very competitive for sorption sites.

Methods for estimating bioavailability

Different techniques are used for assessing the presence of metals in soils and sediments. These methods comprise exchange resins, diffusive gradient in thin films (DGT), conventional single-step extractions, and sequential extractions. In particular, exchange resins are considered passive samplers that capture ions and are employed to quantify the levels of free ions, solution fractions, and easily accessible metal pools in soils (Cipullo *et al.*, 2018a).

The method that can be used to perform fast screening analysis of the labile pool of elements in soils and sediments is Single extractions, which involves the use of specified solvent concentration to dissolve the bioavailable fractions out of the soil and sediment into the various solutions. Table 3 gives some examples of the protocols.

Table 2.3. Summary of the extraction procedures for single extractions

Type	Extractants	Soil/solution ration, time, temperature	reference
Water soluble	Water	1:5, 2h, room temp	(Neel *et al.*, 2007)
Exchangeable	$CaCl_2$ (0.01M)	1:5, 2h, room temp	(Gupta and Sinha, 2007)
Organically complexed	EDTA (0.05M)	1:5, 1h, room temp	(Ure, Davidson and Thomas, 1995)
Acid-extractable	HNO_3 (0.43M)	1:5, 4h, room temp	(Kim *et al.*, 2015)

Techniques for assessing organic contaminant bioavailability, on the other hand, required the design and deployment of partitioning-based methods and biomimetic extractions for quantifying organic contaminants bioavailability. Some of these techniques are aimed at "mimicking" uptake into diverse species and are hence referred to as "biomimetic" procedures, whereas others are aimed at assessing chemically determined exposure parameters. All these strategies can be divided into two categories. The first method involves removing some of the sorbed organic contaminants with a mild chemical extractant, a sorbent (e.g., Tenax, XAD resin), or a complexing agent (e.g., cyclodextrin). The second group of techniques that operate on the principle of equilibrium sampling include passive samplers like polyethylene devices (PEDs), semi-permeable membrane devices (SPMDs), polyoxymethylene (POM) samplers, thin ethylene vinyl acetate (EVA) or polydimethylsiloxane (PDMS) coatings, and fibre samplers, such as solid phase microextraction (SPME) (Cui, Mayer and Gan, 2013).

2.6. Effects of biochar and spent mushroom compost addition on the indigenous soil microbial community

An important ecologic and physiological function of soil microorganisms is the biotransformation of pollutants found within the soil ecosystem. This action can be aided and facilitated using biochar and spent mushroom compost (Figure 2.6). Hence, these adjuvants affect the soil microbial activity and biomass, influences soil enzyme activities, and reshape the microbial community structure (Mackie *et al.*, 2015; Ahmad *et al.*, 2016; Elaamer, 2020). Zhu et al. (2017a) compiled the mechanisms of how biochar affects soil microbial activities. These include: (1) biochar provides habitat for soil microbes with its pore structures and surfaces; (2) biochar supplies nutrients to soil microbes for growth with those nutrients and ions adsorbed on biochar particles; and (3) biochar modifies microbial habitats by improving soil qualities that are crucial for microbial growth (such as aeration, water retention, and pH); (4) it also modifies enzyme activities, which affect soil elemental cycles associated to microorganism; and (5) Biochar enhances soil pollutant sorption and breakdown while reducing their bioavailability and toxicity to microbes.

Spent mushroom compost like biochar also promotes microbial activities equally important for remediation of contaminants. The compost matrix serves as surfaces for microbial growth, their rich organic matter supplies nutrients to the microbes. It similarly affects soil properties like pH, aeration and water holding capacity. SMC has been shown to adsorb both organic and inorganic pollutants and in so doing decreasing their bioavailability and toxicity to microbes, thus allowing the microbes to thrive effectively (Lau, Tsang and Chiu, 2003; Singh, Abdullah and Vikineswary, 2003; Gouma *et al.*, 2014; Asemoloye, Chukwuka and Jonathan, 2020; Yu *et al.*, 2021).

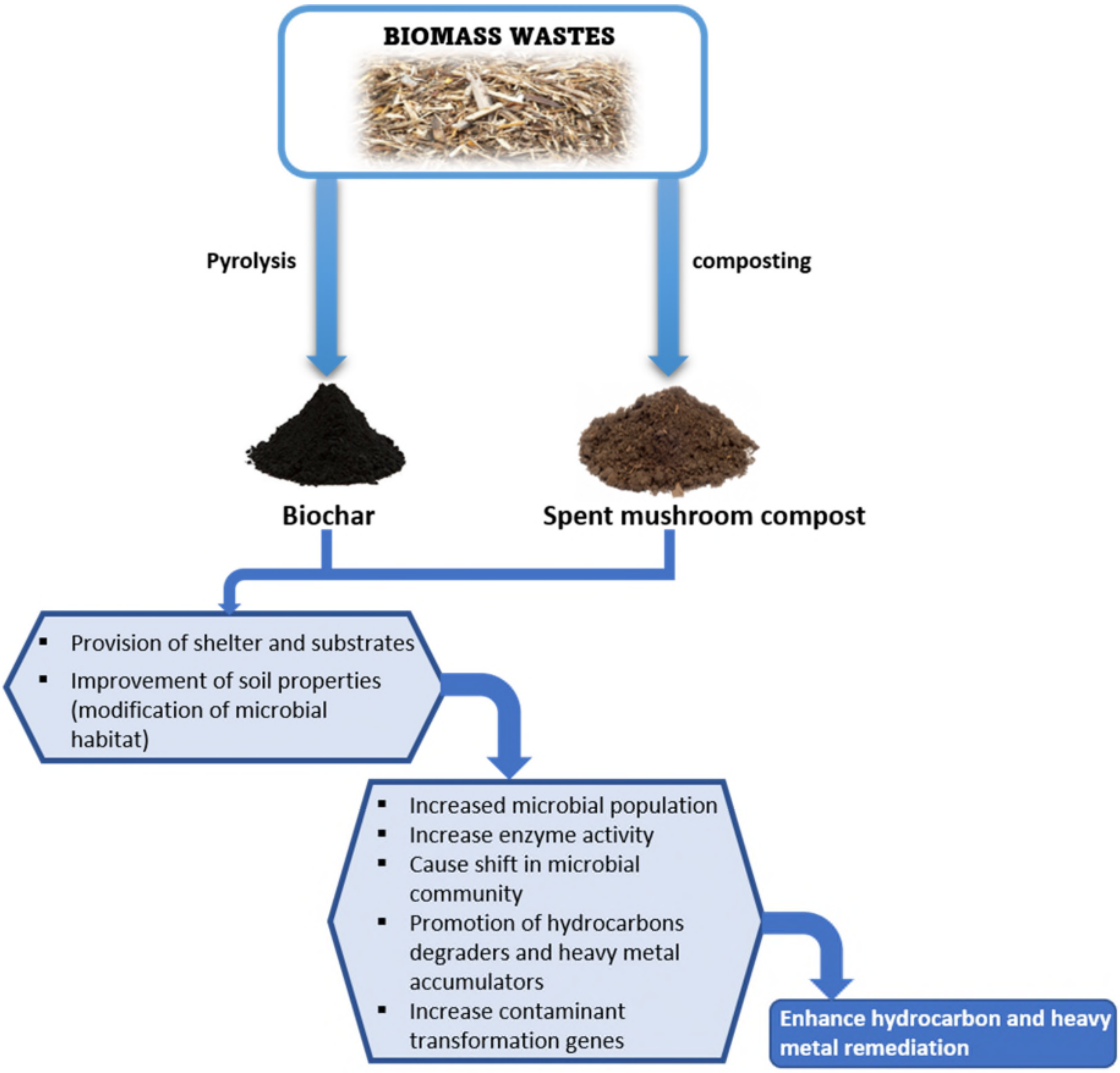

Figure 2. 6. How biochar and spent mushroom compost influences microbial remediation

2.7. Challenges

While the use of soil amendments, biochar and spent mushroom compost, to promote hydrocarbon and metals bioremediation offers enormous promises, there are however some drawbacks in the exercise of this technique. Some highlighted here in include:

- All techniques of bioremediation, including the bioengineering of soils using bioamendments as described here, require time.
- In some cases, using these materials may not be practical when the volume of contaminants is high.
- Reports of biochar toxicity in soil have been made.

- Given that most investigations on the materials have been lab-based and under controlled environmental settings, large-scale field trials may present a challenge.
- The production of high-quality biochar might require extensive of machinery, which would raise the projected cost.
- The sorption effects of biochar and SMC can cause the soil to lose vital nutrients.
- The abundance of organic matter in SMC may encourage the growth of unwelcome organisms like algae.

2.8. Conclusion and future research perspectives

As the realities of soil contamination with heavy metal and hydrocarbons persists, and the search continues for remediation strategies with minimal environmental inputs especially when the restoration of the soil ecosystem functions is in view, biochar and spent mushroom compost has good prospects. It has been shown how they impact positively the soil environment and in so doing promote microbial activities relevant in contaminants biotransformation. The detrimental effects of the presence of hydrocarbons especially PAH and heavy metals in soil have been highlighted going further in exposing how their effects may be circumvented. The promises of biochar and spent mushroom compost having a dual impact on contaminants; sequestering the contaminants while also creating a conducive habitat for soil microbial communities to thrive, which will in turn act on the contaminants to bio-transform them into harmless forms. There are still a lot of undeveloped features of the application of biochar and spent mushroom compost as a possible method for the remediation of organic and inorganic contaminated soil. There are some knowledge gaps that need to be filled, so further research is required.

- The properties of biochar vary depending on the biomass material and pyrolysis conditions. It is critical to select and compare different biochars, and explore different application rate to determine optimum performance conditions

- Characterise spent mushroom compost and examine the effect and contribution of its various components for remediation of complex chemical mixtures as well as on individual contaminants.
- The use of advance metagenomic techniques to identify species highly impacted due to the introduction of biochar and spent mushroom compost in soil to select them as important candidates for bioaugmentation studies.
- Explore the potential of biochar and spent mushroom compost to promote hydrocarbons and metals in an extreme environment like saline soils.
- It is necessary to track and assess throughout time the longstanding effects of biochar and spent mushroom compost on the physicochemical properties of soil.
- While the two materials have been
- Most studies investigating the use of biochar and spent mushroom compost separately for the remediation of polluted soils have been carried out in laboratories. Field-scale testing are required.

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3. Bioengineering remediation of former industrial sites contaminated with chemical mixtures

Emmanuel Atai, Frederic Coulon, Mark Pawlett

School of Water, Energy and Environment, Cranfield University, Cranfield, MK430AL, UK

Abstract

Former gasworks sites are contaminated with complex chemical mixtures, which necessitate remediation before such sites can be redeveloped. The use of bioamendments such as biochar and spent mushroom compost (SMC) provides opportunity to expand the use of green and sustainable remediation approach. Biochar has been reported to improve soil properties for microbial growth and sequester carbon in soil. But different biochar varies in properties and likely to affect the way in which the biochar interacts with the indigenous soil microbial community. Also, biochar's prospect maybe improved if applied with SMC. A full factorial microcosm experiment using biochar derived from rice husk (RHB) and wheat straw (WSB) (mixed with soil from former gasworks at 0, 2.5 and 5%), with and without spent mushroom compost was carried to evaluate fate of the contaminants (alkanes, PAH, and metals), the influence on the soil microbial community and its implication for remediation end points. After 120 days, the results showed that the bioamendments (RHB, WSB, SMC, RHB-SMC, WSB-SMC) had an average TPH reduction of 92%, with SMC and WSB-SMC having the highest degradation rates at 93%. While the bioamendments did not significantly affect the extent of TPH removal compared to the control, they did improve the degradation of high molecular weight (HMW) PAHs, particularly in RHB-SMC for EC17-20 (60%) and EC21-35 (62%) of total PAH concentration, and in WSB-SMC for HMW bioavailable PAH concentration (89%). The bioamendment affected the metals partitioning and distribution in the soils after 120 days of treatment leading to the decreased in the available phase fractions. The treatments increased microbial abundance in the soils, with Gram positives, Gram negatives, and fungi increasing by 4%, 8%, and 38%, respectively, after 120 days, particularly in SMC and mixed treatments (RHB-SMC and WSB-SMC). This was mirrored in increased microbial soil respiration. After 120 days, low metals (177.6±5 mg/kg) and TPH (21.2±7% mg/kg) bioavailability translated into higher EC50 (10624±710mg/L), indicating lower toxicity. There was a strong correlation between bioavailability and toxicity of TPH and metals with microbial relative abundance and activity. Overall, while engineered green and sustainable remediation may speed up the remediation process, it is not always necessary, and monitored natural attenuation may be

sufficient for site reclamation. Nonetheless, this strategy reduces metal bioavailability and degrades high molecular weight PAHs, as demonstrated here.

Key words: biochar, spent mushroom compost, legacy site, bioamendment, bioavailability, toxicity, hydrocarbon, metals, microbial community

3.2. Introduction

Former industrial sites are often contaminated with complex chemical mixtures, mainly hydrocarbons-derived products, polycyclic aromatic hydrocarbons (PAHs), metals and metalloids such as are lead, chromium, arsenic, zinc, cadmium, copper, mercury, and nickel. (CL:AIRE, 2015; Zhao *et al.*, 2022). Co-contamination of metals and PAHs increases toxicity in the environment and may make remediation of polluted soil more difficult (Li *et al.*, 2020). A high metal content in soil, for example, was reported to inhibit PAH degradation (Obuekwe and Semple, 2013). The most typical technique of remediating former gasworks sites has been excavation and transport to prescribed hazardous landfill locations, which has recently become less desirable due to rising costs (Baylis and Allenby, 2010; Haleyur *et al.*, 2018). The use of bioremediation has been proposed as a cost-effective and ecologically beneficial solution, even though the approach has shown less efficiency when co-contamination and high concentration level of contaminants are present (Zhang *et al.*, 2020). Since they can enhance soil's physical, chemical, and biochemical properties and reduce the need for inorganic fertilisation, organic residues like biowastes and composts are being used more and more in land remediation (Alvarenga *et al.*, 2009). Additionally, their use supports an integrated approach to waste management by encouraging nutrient recycling and reducing issues with final disposal (Wang, Yuan and Tang, 2021). Consequently, biochar and spent mushroom compost are two examples of organic residue products that can improve biodegradation of chemical pollutants. This could be a useful way to improve the bioremediation process.

Biochar, a low-cost carbon material, is emerging as a cost-effective alternative to activated carbon in the removal of organic and inorganic contaminants from the environment (Ahmad *et al.*, 2014a). Biochar has several remarkable properties, such as a high internal specific surface area, microporosity, surface negative charge, and durability against degradation, and has been used in a variety of applications (Guo *et al.*, 2015). For instance, in environmental remediation, where biochar can promote high contaminant complexation, immobilization, sorption and partitioning, as well as high carbon sequestration (Oliveira *et al.*, 2017a). Although the application of biochar has been demonstrated to be conceptually and experimentally successful, its success is dependent on the type of biomass feedstock material, carbonization process, pyrolysis conditions, and biochar dose (Bian *et al.*, 2013; Hassan *et al.*, 2020). The availability of low-cost, easily grown biochar biomass feedstock would increase biochar's sustainability and efficiency in remediation. Also, combining biochar with other low-cost adsorbents or degradation materials/organisms may also improve remediation performance (Anae *et al.*, 2021). Spent mushroom compost is an important material that, when combined with biochar, may help in the remediation process.

Spent mushroom compost (SMC) is a byproduct of mushroom production that is produced in large quantities and has also been reported to enhance the bioremediation of polluted soils (Asemoloye, Chukwuka and Jonathan, 2020). It also been reported to serve as soil conditioner thereby improving soil nutrient (Cai *et al.*, 2021) and binds and immobilise metals in soils thereby reducing their toxicity to the soil microbial community and plants (Wei *et al.*, 2020). It has these effects because, after mushroom harvesting, SMC is likely to contain not only a large and diverse group of microorganisms, but also a diverse range of extracellular enzymes, such as cellulase, hemicellulose, β-glucosidase, lignin peroxidases, and laccase. Additionally, it also has a high organic content, which includes cellulose, hemicellulose, and lignin (Gouma *et al.*, 2014). Equally, for every 1 kg of edible mushrooms produced, 5 kg of SMC is generated (Chang, Yang and Yang, 2021). This gives rise to millions of tonnes of SMC generated over time whose disposal becomes a major problem for mushroom farmers. Hence, the need for alternative use especially that SMC is considered a good biologically reactive material and has great potential for bioremediation of many toxic chemical contaminants (Sadiq *et al.*, 2018).

The recent global environmental consciousness, stringent legislation, and a shift in research toward the application of sustainable and circular processes has led to the scientific community's interest in innovative and environmentally friendly waste-stream utilisation systems (Ferronato and Torretta, 2019). Therefore, the use of both materials (biochar and SMC) enables a sustainable remediation technique that makes use of industrial and agricultural wastes, leading to a large decrease in environmental footprint (Hu *et al.*, 2021). Also as biochar is made from carbonaceous waste biomass and mushroom production is the world's largest solid-state fermentation industry (Letti *et al.*, 2018) which releases large amount of SMC as waste, the application of these materials for PAHs and metal (loids) bioremediation could be regarded as an efficient low-cost bioremediation method. Furthermore, due to the properties of these materials elucidated here, their use provides an opportunity to overcome soil nutrient limitation, increase sorption/decrease bioavailability of the chemicals, and increase surface contact of contaminants with the soil microbial community (Zhu *et al.*, 2017b), all of which have implications for improving soil microbial remediation of the hydrocarbons and metals.

However, while biochar can induce changes in soil microbial activities that lead to contaminants transformations, the mechanisms underlying these processes are still not yet fully understood (M. Zhang *et al.*, 2019). As a result, understanding biochar-microbe interactions is essential for recognising the link between biochar characteristics and a variety of soil processes, particularly contaminant degradation (Zhu *et al.*, 2017a; Yuan *et al.*, 2019). Also, as previously stated, because biochar success among other factors is dependent on the type of biomass feedstock material and application dose, it is important to compare biochar from different feedstock and application rates. In the context of using biochar and SMC for bioremediation, it is critical to evaluate the bioavailable fraction of pollutants, which is the fraction that has been reported to be able to permeate organisms' cellular membranes and cause toxicological impacts (Cipullo *et al.*, 2019; Yuan *et al.*, 2019). Similarly, while there have been studies on biochar with compost or bacteria sources, few have examined the use of biochar with a fungal bio-addition material, such as SMC, to study the fate and behaviour of mixed contaminants in bioremediation. As a result, the objective of this study is to examine how biochar and/or spent mushroom compost affect the fate and behaviour of hydrocarbons and metals(loids), promote biodegradation of hydrocarbons, as well as how the amendments affect the soil microbial community.

3.2. Materials and methods

3.2.1. Sample collection: Soil, biochar and spent mushroom compost

The soil was collected from a former gasworks site based in the UK. The site's background: Until 1896, the location was known as an "Old Freestone Quarry." Following the construction of the "Riverbank Gasworks" in May 1904, coal gas production commenced at the location. Between 1910 and 1938, various expansions were carried out. The gas works ceased in 1961, and the site was deemed suitable for conversion into a high-pressure gas reforming plant in 1962. Gas production began in 1966, with facilities being expanded in 1967. The factory discontinued operations in 1973. The soil samples were collected when the site was undergoing a supplementary investigation to further assess the significance of historic residual Non aqueous phase liquid (NAPL) impacts in some areas of the site. Soil samples were taken from three trial pits sunk between 1.0 and 2.0 metres below ground level. The soil was sieved with 5.60 mm in the lab to remove large particles and stones. It was then sieved through a 2 mm sieve and kept at 4 °C until soil characterization analyses and the setup of microcosms.

Rice Husk Biochar and Wheat Straw Biochar derived from rice husk and wheat straw pellets, both produced by the UK Biochar Research Centre, School of Geosciences, University of Edinburgh, were used in this study. The biochars production was done in a pilot-scale rotary kiln pyrolysis unit with a nominal peak temperature of 550°C, a pH of 9.94, and a total carbon content of 68.3 wt%. Both are biochars that have been thoroughly characterised (UK Biochar Research Centre, 2014)

Littleport Mushrooms LLP, which is owned by G's Fresh Ltd, UK, provided spent mushroom compost. and the major basidiomycete present was *Agaricus bisporus*, also known as the cultivated white button mushroom.

3.2.2. Microcosms experimental design

From the sieved stored soil, microcosms were set up in plant pots (sealed bottom), each containing 150 g of soil. Soils were amemended with either rice husk biochar, wheat straw biochar, or spent mushroom compost at 2.5% or 5% (Table 3.1).

Table 3. 1. Overview of the soil microcosm bioadmendments conditions

	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
Experiment Design	▪ 150g soil ▪ No Rice husk biochar (RHB) ▪ No Wheat straw biochar (WSB ▪ No Spent mushroom compost (SMC	▪ 150g soil ▪ Amended with 5% Rice husk biochar (RHB)	▪ 150g soil ▪ Amended with 5% Wheat straw biochar (WSB)	▪ 150g soil ▪ Amended with 5% Spent mushroom compost (SMC)	▪ 150g soil ▪ Amended with 2.5% Rice husk biochar (RHB) ▪ Amended with 2.5% Spent mushroom compost (SMC)	▪ 150g soil ▪ Amended with 2.5% Wheat straw biochar (WSB ▪ Amended with 2.5% Spent mushroom compost (SMC)
	((1 Control + 5 treatments) = 6)) × 3 replicates × 2 Sampling time = 6 × 3 × 2 = 36					
	36 microcosms					

The 5% biochar to soil ratio used in this work was chosen because it is frequently reported as the most effective application rate for reducing mobile contaminant concentrations in contaminated soils (Wang *et al.*, 2017; Novak *et al.*, 2018; Cipullo *et al.*, 2019)

All the microcosms were mixed manually to obtain homogenous samples and kept in 20 °C constant temperature room for the 120 days of the experiment. The soil moisture was adjusted twice a week by adding deionised water equivalent of the microcosms' weight loss within the range of the soil moisture content (29%). Samples were taken for chemical, microbiological, and toxicological analyses after 60 and 120 days to determine the effect of the treatment variables on the fate of contaminants present in oil contaminated soils (PAH and metal(loid)s), the influence on the soil microbial community and its implication for remediation end points. The triplicate treatments were sacrificed during each sampling point. There are no analytical replicates except for microbial respiration. The soils were allocated 40 g for soil characteristics, 20 g for metals, 15 g for hydrocarbons, PLFA 20 g, soil respiration 40 g, and microtox test 10 g. Except for PLFA, which was stored at -80 ºC and respiration, which was kept in a 4 ºC fridge, all samples were kept in a -20 ºC freezer.

3.2.3. Physico-chemical characterisation

Air-dried soil samples were analysed based on BS EN 13654-2:2001. Total nitrogen (TN) (0.001 mg), Total Carbon (TC) (0.001 mg) and Total Organic Carbon (TOC) (following the removal of carbonates with 4 mol/L hydrochloric acid dropwise until visible reaction stops) were based on BS 7755 Section 3.8:1995. They were analysed in vario EL 3 Element Analyzer (Elementar Analysensysteme GmbH, DE). Total phosphorous was determined by extracting with acid mixture (6ml 11.65mol/L hydrochloric and 2ml 15.8mol/L nitric) and determining the phosphorus content of the extract (ISO 11047, 1998) using a NexION ® 350 D ICP-MS (Perkin Elmer). A 0.5 mol/L sodium hydrogen carbonate solution at pH 8.5 was used to extract available phosphorous (AP) (5 g) from the soil. The extract was then analysed using spectrometry (ISO 11263, 1994). Soil pH was determined according to ISO 10390 (2005) using a soil:water ratio of 1:5 (Jenway 3540 pH Meter, Keison Products, UK). The organic content of the soil (%) was calculated using loss of ignition (LOI): (BS EN 13039, 2000). Based on BS ISO 11277:2009, the particle size distribution was determined using the sieve and sedimentation method, and the associated soil texture classes were identified using a soil texture calculator (Natural England Technical Information Note TIN037, 2008) and eventual sieving using 0.6mm, 0.212mm, 0.063mm sieves. Gravimetric soil moisture and dry matter (%) was determined by drying at 105 °C (ISO 11465, 1993).

3.2.4. Chemical analyses

3.2.4.1. Total and bioavailable hydrocarbons

A modified version of Risdon et al, (2008) was used to determine the total and bioavailable petroleum hydrocarbon, which included both aliphatic and aromatic compounds. A 2.5 g of soil were mixed with 15 mL of 1:1 dichloromethane:hexane solvent, and 50 mL of 50mM hydroxypropyl-β-cyclodextrin solution, respectively, to extract the total and bioavailable petroleum hydrocarbon content.

Total hydrocarbon content was determined by sonicating the samples (20 minutes) at room temperature (Ultrasonic Bath, U2500H, Ultrawave (UW), UK), shaken for 16 hours at 150 rpm (Multi Reax Shaker, Heidolph Instruments GmbH & CO. KG). On the second day, samples were sonicated for 20 minutes at room temperature before being centrifuged (2000g for 10 minutes) (Thermo ScientificTM, SorvallTM ST 40 Centrifuge Series). Following that, the supernatant was transferred to 6 mL SPE DSC-Si silica tubes for cleaning. A 0.5 mL sample of clean extract was combined with 0.5 mL of internal standards, including a deuterated alkane mix ($C10^{d22}$, $C19^{d40}$ and $C30^{d62}$) and deuterated PAH mix (naphthalene^{d8}, anthracene^{d10}, chrysene^{d12} and perylene^{d12}).

Samples were mixed with a 50 ml 50 mM cyclodextrin:water solution to determine the bioavailable hydrocarbon content. After 20 hours of shaking, the sample was centrifuged at 2000 g for 30 minutes. The supernatant was discarded, and the soil pellets were resuspended in a 1:1 dichloromethane:hexane solution and processed as described in the total hydrocarbon section above. Concentration of petroleum hydrocarbons present in the sample were detected and quantified by gas chromatography–mass spectrometry (GC–MS) using the Shimadzu GCMS-TQ8040 following the GC method described in Cipullo et al. (2019). The amount of compounds taken up by the cyclodextrin molecule (bioavailable concentration) was measured by subtracting the residual amount of organic compounds extracted by dichloromethane:hexane after the initial HP-β-CD (hydroxypropyl-β-cyclodextrin) wash, from the total amount extracted by dichloromethane:hexane.

3.2.4.2. Total and bioavailable metals

Total metal digestion was carried out using aqua regia and the ISO 11047 method (ISO 11047, 1998). In brief, 0.5 g of air-dried soil was extracted in a microwave digestion system by adding 6 ml of hydrochloric acid (11.65 mol/L) and 2 ml of nitric acid (15.8 mol/L) (Multiwave 3000 microwave oven, Anton Paar/Perkin Elmer, UK). After filtering through Whatman 542, the extract was diluted to 50 mL with deionized water.

For the determination of the bioavailable fractions of the metals, a single solvent extraction involving water soluble using: water; exchangeable: 0.01M $CaCl_2$; organically complexed: 0.05M EDTA; and acid extractible: 0.43M HNO_3 (Ure, Davidson and Thomas, 1995; Gupta and Sinha, 2007; Neel *et al.*, 2007; ISO 17586:2016(E), 2016). In brief, the extracting vessel and contents were shaken at 150 rpm (Multi Reax Shaker, Heidolph Instruments GmbH & CO. KG) for 4 h and centrifuged at 2000 *g* for 10 min (Thermo Scientific™, Sorvall™ ST 40 Centrifuge Series). After that, the extract was filtered through 0.45 m nylon syringe filters.

All total and single solvent extracts were diluted four times with 1% HNO3 before analysis with a NexION® 350D ICP-MS (Perkin Elmer) calibrated with a mixture of major (Ca, Fe, K, Mg, Mn, Na, S, Si, P) and trace (Al, As, Ba, Cd, Co, Cr, Cu, Hg, Li, Mo, Ni, Pb, Sb, Se, Sr, V, Zn) elements Working standards in matching sample matrix solutions (1% nitric acid) were created in both cases. A mixture of four internal standards was used to spike the calibration standards and sample extracts (Sc, Ge, Rh, and Bi). After each sample, the ICP-MS was calibrated, and the limit of detection was set at three times the variance of the acid blank. In each batch of seven samples, acid blanks (1% nitric acid), digestion blanks, and guidance materials (BGS102) were also analysed. A sufficient rinse time was programmed in between samples to assess the accuracy of the extraction and the sensitivity and contamination of the blanks.

3.2.5. Microbiological analysis

3.2.5.1. Respiration

MicroRespTM colorimetric microplate-based respiration system for measuring CO_2 evolved from soil which water or carbon substrates have been added is based on Campbell *et al.*(2003). The method gives responses to these substrates and reflects activity by measuring responses (CO_2 production) after 6 hours. Briefly: The detection plates – microplate plates with purified agar and indicator solution (cresol red, KCl, $NaHCO_3$) are added in a 1:2 ratio – were prepared and stored in sealed desiccator prior to use to avoid absorbing CO_2 from the environment. In the deepwell plates, 0.32g of soil samples and 93.6mg/ml substrates solution were added into it. Detection plate were read at 570 nm (Microplate readers, SpectraMax® Plus384, Molecular Devices) and assembled onto the deepwell plate with the MicroResp™ seal, secured in metal clamp and incubated at 25°C for 6 hours and re-reading the detection plate at 570 nm. Substrates (alanine, citric acid, glucose, gamma-aminobutyric acid, α-ketoglutaric acid, malic acid) were selected considering Creamer *et al.* (2016); lignin was added as a complex carbon source based on availability in the lab. The basal respiration rate was calculated using the CO_2 generated by the wells in which water other than substrates were added.

3.2.5.2. Phospholipid fatty acid analysis (PLFA)

Using Phospholipid fatty acid (PLFA) analysis based on Frostegard, Tunlid and Baath (1993), the microbial community structure was examined. In brief, from the freeze dried (Christ Alpha 1–2 LD plus −80 °C Freeze Dryer) soil samples, solid-phase soil extraction using 10g of each sample was performed using Bligh and Dyer solution (chloroform, methanol, and citrate buffer in 1:2:0.8 by volume). The extract was further derivatised by mild alkaline methanolysis. By using a GC-FID (Agilent Technologies 6890N) equipped with an HP-5 (Agilent Technologies) fused silica capillary column (30 m length, 0.32 mm ID, 0.25 m film), fatty acid methyl esters were analysed. GC conditions were as described by Pawlett et al (2013). The target responses of all discovered PLFA peaks were sum up to determine the relative abundance of each unique PLFA, which was reported as a percentage (mol%).

3.2.5.3. Ecotoxicological bioassay

Soil toxicity of biotreatments was evaluated using the Solid Phase Microtox® assay (Modern Water Monitoring Ltd). The assay was carried out as directed by the manufacturer (ModernWater Microtox Acute Toxicity). Briefly, 3.5 g soil was transferred into the diluent, shaken for 10 minutes (Multi Reax Shaker, Heidolph Instruments GmbH & CO. KG), centrifuged for 3 minutes at 1000g (Thermo ScientificTM, SorvallTM ST 40 Centrifuge Series), and the sample was transferred into SPT tubes in the incubator block (Microtox® Model 500 (M500) analyser) and serial dilutions made. The tubes were read after the Microtox Acute Toxicity Reagent was reconstituted and added. The performance of both the operator and the analytical system was checked using a 100 g/L zinc sulphate standard solution, and the 95 percent confidence range was kept below 15% variance throughout the investigation. For each sample, the soil dilution that inhibits 50% (EC_{50}) of the light output compared to the light output before soil addition was computed. As toxicity increases, Microtox® EC_{50} values decrease.

3.2.6. Data analysis

The significance and relationship between soil amendments (rice husk biochar (RHB), wheat straw biochar (WSB), spent mushroom compost (SMC), RHB+SMC, WSB+SMC, or un-amended) and incubation time on the alkanes, PAHS, metals, and microbial PLFA profiles triplicate datasets, were investigated using Repeated-measures ANOVA test

Principal component analysis was used for multi-variate datasets, to evaluate the variations between soil amendment and incubation time on microbial community dynamics and respiration profiles from multiple substrates induced respiration. Both Repeated-measures ANOVA and PCA were performed using Statistica (TIBCO Statistica® 13.3 June 2017).

Pearson correlation in SPSS (IBM SPSS Statistics for Windows, Version 21.0. Apr 2019) was used to establish correlation between the bioavailable fractions and the toxicity response of the Microtox®, PLFA profile and microbial soil activity dataset.

3.3. Results and Discussion

3.3.1. Soil sample and physicochemical properties

Total petroleum hydrocarbons (alkanes and PAHs) concentration was 1493 mg/kg at the onset and PAH accounting for 72% of the TPH. The total concentration of metal(loid)s was 642.8 mg/kg. The aliphatic fraction was characterised by the dominance of lower molecular weight fractions while the aromatic fraction was dominated by heavy molecular PAH compounds with 4 or more aromatic rings (Table 3.2)

The soil has a high moisture content (29%) and relatively moderate organic matter (11%) (Table 3.2). These levels have been reported to favour microbial activity (Griffiths et al., 2018). The alkaline pH of the soil (8.2) is higher than the values obtained from a previous study evaluating physical properties of nine UK soils (Mcgeough et al., 2016). This pH value also could be the reason for the low phosphorus level since pH >8.0 have been shown to have a potential for nutrient interaction issues (Griffiths et al., 2018).

The concentration of metals/metalloids was determined using Inductively coupled plasma mass spectrometry (*ICP-MS*). The Aqua Regia extraction allowed the pseudo-total metal(loids) to be quantified (Table 3.2). Metals/metalloids were almost entirely present in both the pseudo-total and bioavailable fractions, indicating soil contamination at the gasworks site. This contamination could be the result of residual spent oxides from gas purification, carbonisation byproducts, furnace residues, and residuals from batteries, pipelines, and paint (CL:AIRE, 2015). Some of the potentially toxic elements examined, such as Cr, Ni, Hg, and Se, were found to exceed soil guideline values (ALS Environment, 2009). Also, at pH less than 7, Zn and Cu values will be considered above limits. If the risk framework were based on the bioavailable fractions, this soil will be considered safe since all the elements here are below the guideline values. While moisture and organic properties are considerable, the pH level and the heavy metals/metalloids content of the soil might render it unsuitable for crops and other applications.

Table 3. 2. Physiochemical characteristics of the contaminated soil sample collected from former gasworks site in UK.

Characteristics	Analysis	Soil
Elements	Total C (%)	13.62
	Total N (%)	0.15
	Total P (%)	0.07
	C:N:P	100:1.1:0.5
	Total P (mg/kg)	718
	Available phosphorus (mg/kg)	5.39
Physical properties	Dry matter content (%)	77.72
	Water content (%)	28.67
	Water potential (MPa)	0.86
Chemical properties	pH	8.19
	Loss on ignition (%)	11.14
Average heavy metal(loids) (mg/kg)[a]	Cr	40.4±2.7
	Ni	136.0±5.9
	Cu	107.0±3.2
	Zn	248.5±1.4
	As	18.1±0.5
	Se	7.0±2.0
	Cd	0.1±0.0
	Pb	78.2±7.2
	Hg	7.5±7.5
Average petroleum hydrocarbon content (mg/kg)[b]	EC10-12	68.04
	EC12-16	111.48
	EC16-21	155.76
	EC21-35	73.14
	EC>35	1.56
	Σ Aliphatic	**409.98**
	EC10-12	15.24
	EC12-16	186.12
	EC16-21	710.82
	EC21-35	171.18
	Σ Aromatic	**1083.36**
	TPH	1493.34

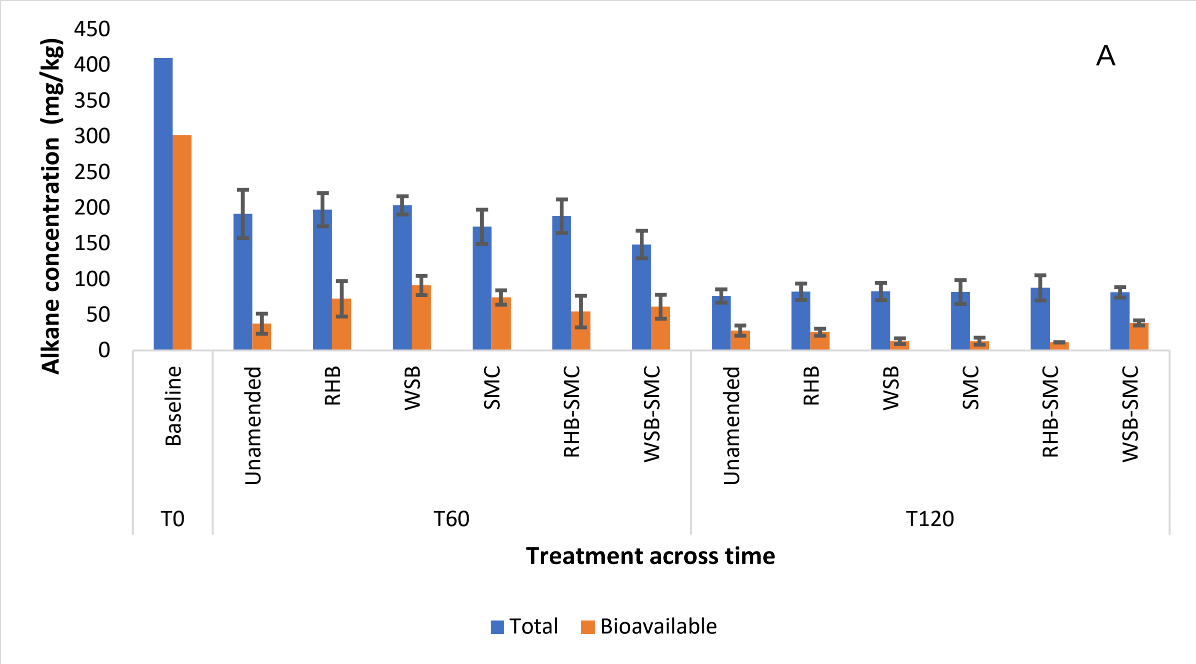
[a]These are the average of duplicate measurement ± standard deviation of the pseudo-total concentration (Aqua regia extraction). [b]There is no replication and so no standard deviation not available. C: carbon, N: nitrogen, P: phosphorus, Cr: chromium, Ni: nickel, Cu: cupper, Zn: zinc, As: arsenic, Se: selenium, Cd: cadmium, Pb: lead, Hg: mercury, EC: equivalent carbon number, TPH: total petroleum hydrocarbon

3.3.2. Behaviour and fate of chemical mixture fractions over time

3.3.2.1. Total and bioavailable hydrocarbon fractions concentrations

After 60 and 120 days, the total extractable aliphatic hydrocarbons fraction in all treatments was reduced to 53% and 80% of its initial concentration, respectively. The extent of degradation was mostly explained by the extent of degradation of the bioavailable fraction as shown in Figure 1a. No significant difference was observed between treatments (p=0.103), except temporal effects from day 0 to day 120 (p=0.001). With regards to aromatic hydrocarbons fraction, more than 98 % of the PAH were bioavailable, and their concentrations were reduced by 84 % and 97 % after 60 and 120 days, respectively (Figure 1b). Again, there was no significant difference between the treatments and control (p=0.6313). Only temporal effect was seen as significant on the loss of PAH (p=0.006). The absence of significant differences between the treatments and control group suggests that incorporating biologically active matrices did not provide any observable benefits in this study. This outcome may be attributed to the fact that the hydrocarbon level was below 15,000 mg/kg, most of the lighter fractions were already degraded, and the nutrient level was sufficiently high (as indicated in Table 2). Therefore, the use of biochar or SMC had minimal impact on the light fractions. However, both bioadmendment types had positive effect on the extent of degradation of the high molecular weight (HMW) PAHs such as chrysene, Benzo[a]pyrene, and Benzo[ghi]perylene (on average 37% for the EC17–20 and 39% for the EC21–35 compared to the control). For the bioavailable HMW PAHs ranging between EC17-20, the extent of degradation was 66% more effective than the control. This was observed for both the total and bioavailable PAH concentrations at 120 days (Figure 2) The RHB-SMC exhibited the most favourable results in terms of Total PAH concentration for both EC17-20 (60%) and EC21-35 (62 %). On the other hand, WSB-SMC performed better in terms of HMW bioavailable PAH concentration (89%). Typically, high molecular weight PAHs tend to be sorbed, leading to their presence in the total extraction phase (Total extractable TPH) but less prevalent in the bioavailable phase. Given that HMW PAHs are potent carcinogens and mutagens that pose a significant threat to human health, their elimination from the environment is vital (Pandey, Kapley and Brar, 2021). Thus, the study demonstrates that the bioamendments have enabled the degradation of these persistent PAHs to a certain extent, even if the overall concentration did not show a significant change.

Legacy sites that have a history of contamination often harbour microbial communities that have adapted to the presence of contaminants and have developed enzymatic and metabolic pathways for degradation (Li *et al.*, 2017), all of which may be activated once the soil condition changed. Also, laboratory studies are typically carried out at a small scale, and under stable conditions of temperature and moisture, which may not fully represent the complexity of interactions between organisms and environmental factors in field situations (Mazzocchi, 2008; Calisi and Bentley, 2009). For instance, Zhang et al. (2020) reported the abundance of microbial communities with functional genes related to xenobiotic biodegradation and metabolism in a long-term industrial contamination site. The application rate and timing of biochar and spent mushroom compost can also affect their effectiveness in reducing hydrocarbon concentration in soil. If the application rate was too low, 5% in this study, or if the timing (120 days) of the application was not optimal, it could have resulted in reduced efficacy of the treatments compared to the control. Soil characteristics can also influence it. For example, in the CNP ratio of the soil, it appeared to be low on nitrogen. While the use of biochar and compost has been shown to improve TPH biodegradation due to the functional properties of these materials, there have also been reports of potential drawbacks in using biochar, such as nutrient immobilization, sorption of contaminants rendering them unavailable for degradation, and biochar toxicity(Gouma *et al.*, 2014; Anasonye, 2017; Wang *et al.*, 2017; Zhu *et al.*, 2017a; Novak *et al.*, 2018). These factors could have influenced the results of this study.



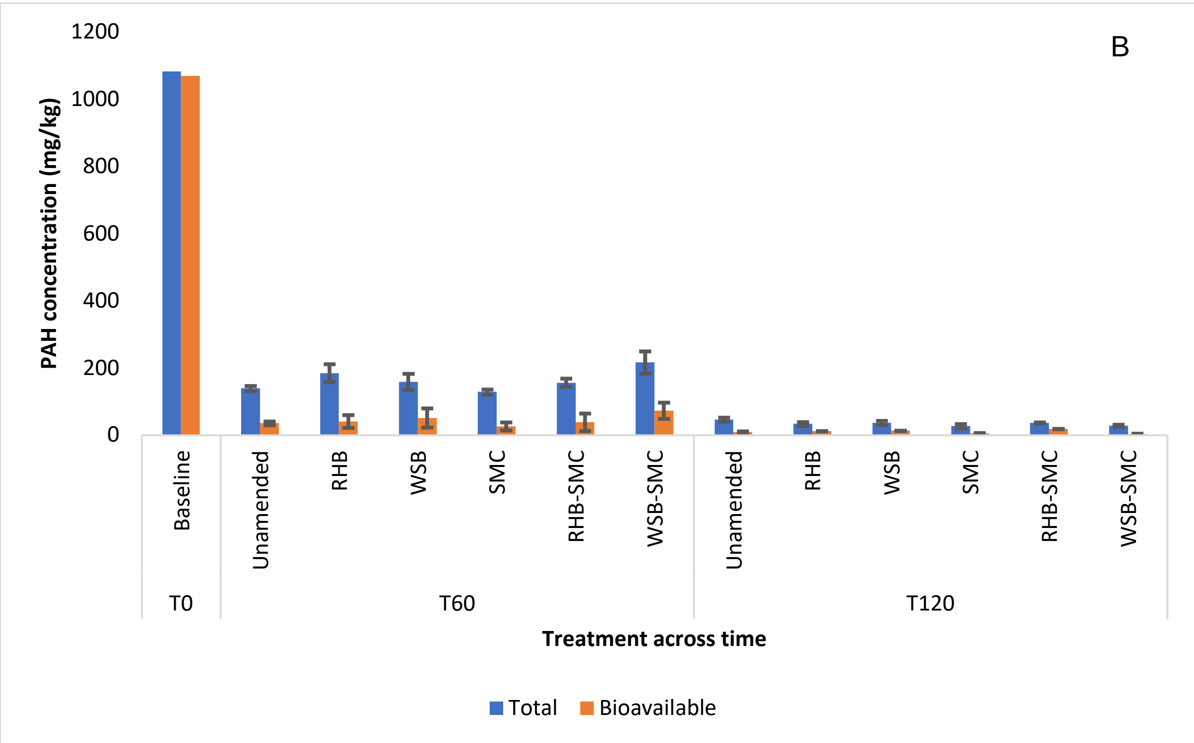


Figure 3. 1. Total and bioavailable aliphatic and aromatic hydrocarbons fractions change overtime. A: Aliphatic (alkanes); B: Aromatic (PAH). The error bars represent the standard error for each treatment's replicates.

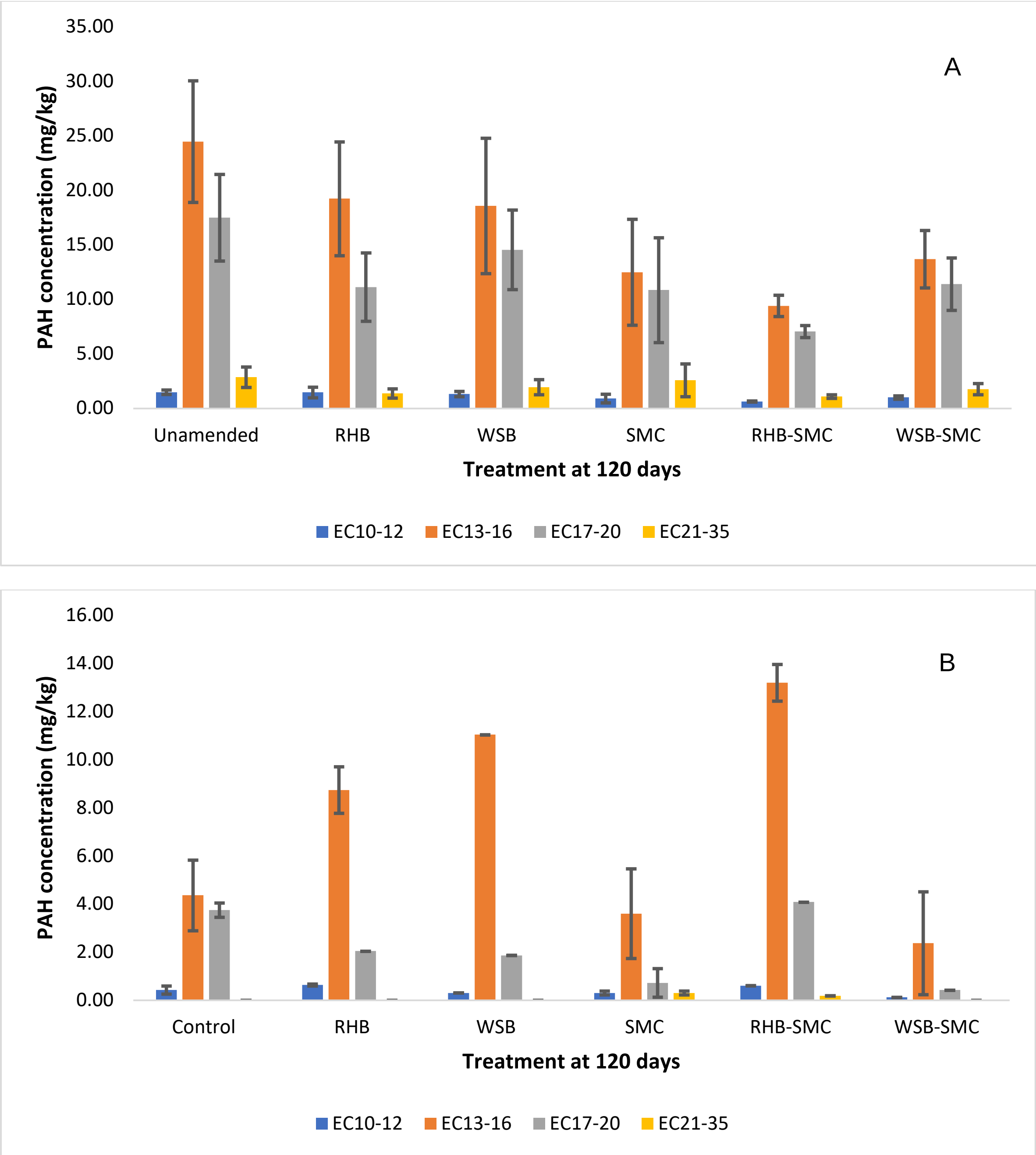


Figure 3. 2. Total and bioavailable aromatic (PAH) fractions at 120 days based on equivalent carbon (EC) number. A: Total; B: Bioavailable. The error bars represent the standard error for each treatment's replicates.

3.3.2.2. Metals and metalloids behaviour and fate during biotreatment

Metals including, Cr, Ni, Cu, Zn, As, Se, Cd, Pb, and Hg were among the key elements investigated (Figure 3.3). Upon careful observation, the sum of Total, Potentially available, and Highly mobile metals accounted for 100% at T0. Changes in these proportions, presumed to be caused by the effects of bioamendments, were observed at 60 and 120 days. Initially, most of the metals were found to be less potentially or readily available at day 0. However, as a result of the treatments, some metals became more available, while others decreased in occurrence during the course of the treatment at 60 and 120 days. It should be noted that the bioavailability of metals can have both positive and negative implications for ecosystem functioning, depending on whether they are essential for growth or toxic.

The bioamendments changed the behaviour of the metals over time, resulting in changes in distribution and partitioning, particularly in the bioavailable phase (potentially available and highly mobile) ($p=0.014$; 0.001). The most significant changes are observed in Cu, Zn, Cd, and Pb, and to a lesser extent Se, Ni, As, and the least Cr in all amendments. The highest changes were observed in WSB and SMC treatments. The bioavailable concentration of the metals at the end of the study were all below UK Environment Agency soil guideline values (ALS Environment, 2009). The amendment however, had no effect on how Hg was partitioned and distributed across all treatments.

Hence, the result indicated a demonstration of how bioamendment could reduce the bioavailability of toxic metals in soils. Studies have shown that biochar soil amendment at 4% and 5% stabilise Cd, Cu, Ni, Pb, and Zn and reduce their bioavailability due to biochar's ability to enhance sorption and cause chemical precipitation, which is heavily influenced by biochar cation exchange capacity, pH, and ash content (Guo, Song and Tian, 2020). Rice straw biochar has been shown to significantly reduced soil heavy metal solubility, with a maximum 35% reduction in root uptake of Cu and Pb (Wang *et al.*, 2019). Also, soil application of fine rice straw biochar resulted in 97.3 and 62.2 percent reductions in extractable Cu and Zn (Yang *et al.*, 2016).

Similarly, SMC mixed with 30% ochre, 40% steel slag, and 10% limestone was effective in removing metals with more than 90% removal efficiencies (Molahid, Mohd Kusin and Madzin, 2019). Furthermore, SMC amended soil has been demonstrated to reduce plant uptake of toxic metals. For example, soil amended with SMC caused decrease in Pb, Cd and Cu levels in *Atriplex halimus* shoot by 23.3%, 51.3% and 53%, respectively (Frutos, Gárate and Eymar, 2010).

All these studies highlighted all supported the ability of biochar and SMC to influence metal bioavailability in soil. This influence can manifest in either an enhancement or reduction of metal bioavailability, with both scenarios observed in the current study. Specifically, most metals showed increased availability at 60 days, followed by a decline at 120 days. However, notably, Arsenic exhibited sustained increased bioavailability even at the 120 days point.

When metals occur in contaminated soils in their pure or mixed solid forms in inert or slowly reactive phases, these phases are unlikely to control ion activity in soil solution (Degryse, Smolders and Parker, 2009). However, it has been demonstrated that the use of bioadmendment, such as biochar, can alter the distribution and partitioning of these metals in soil (Cipullo *et al.*, 2019). As a result, amending soils with biochar and spent mushroom compost may cause changes in the total extractible metals concentration in the soil resulting in the release or immobilisation of bioavailable concentrations of the metals.

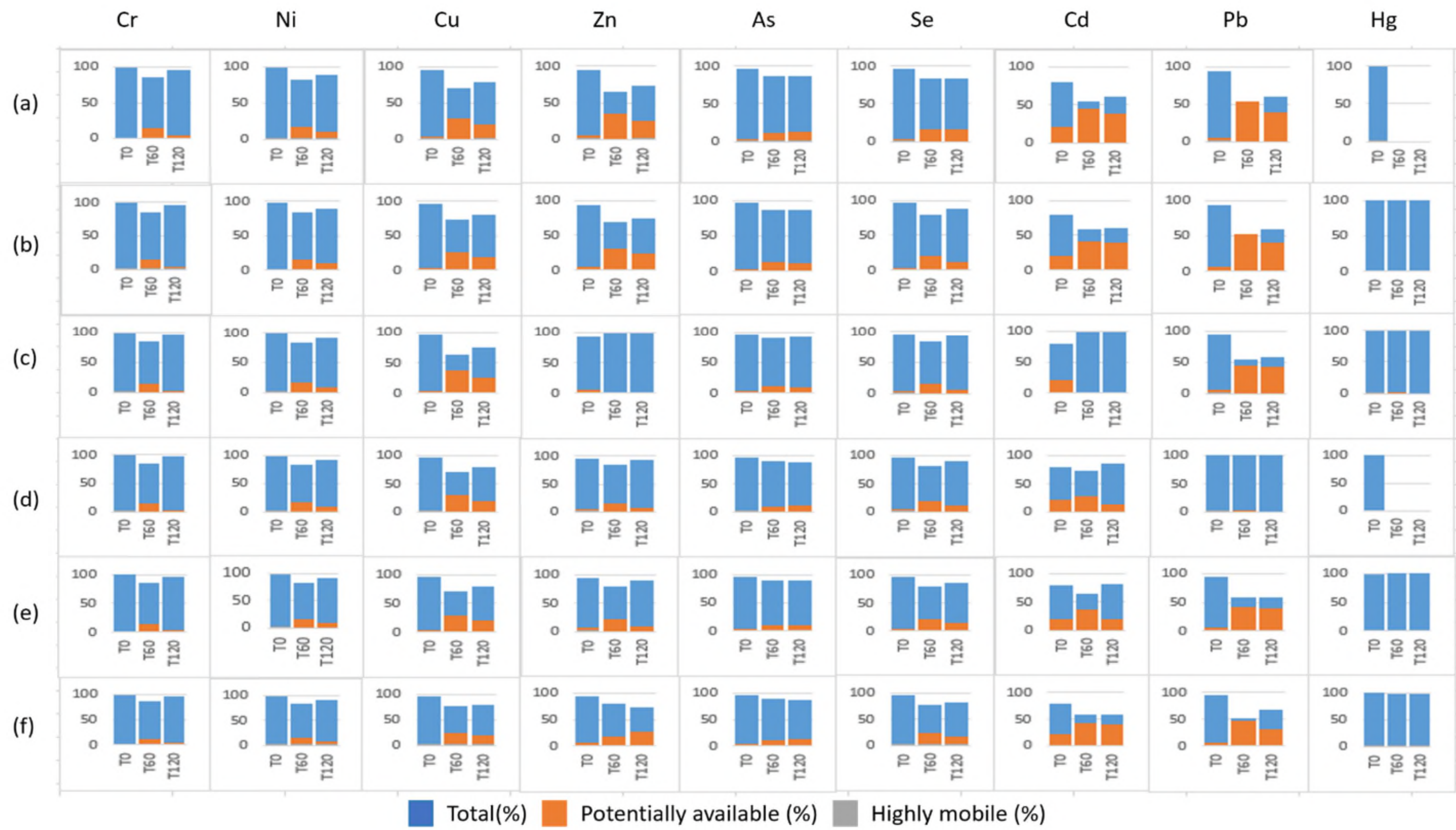


Figure 3. 3. Metals and metalloids total, solid phase distribution and available content in the soil of the treatments and un-amended soil from 60 to 120 days. Rice husk biochar RHB (a), Wheat straw biochar WSB (b), Spent mushroom compost SMC (c), Rice husk biochar+Spent mushroom compost RHB-SMC (d), Wheat straw biochar+ Spent mushroom compost WSB-SMC (e), unamended (f).

3.3.3. Hydrocarbon biodegradation indicators

3.3.3.1. Microbial community relative abundance and dynamics

When the bioamendments were applied, the relative abundance of microbial biomass increased, and a microbial community shift was observed (Figure 3.4). In general, except for actinomycetes, the microbial groups increased by an average of 34% and 12% at days 60 and 120, respectively.

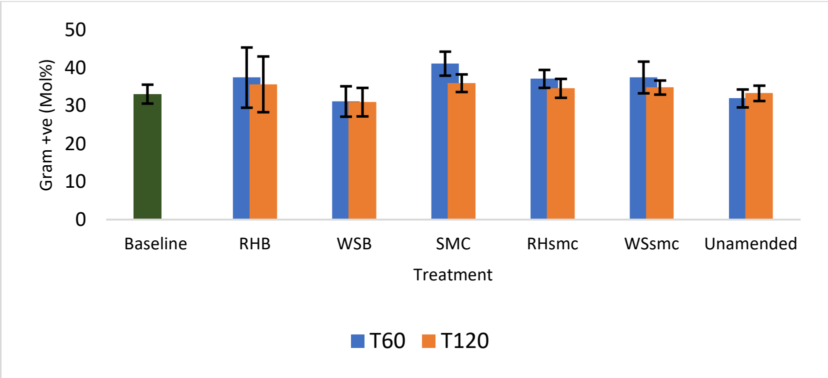
Specifically, for the Gram positives, all the treatments performed uniquely (p=0.0432) but there were no time effects (p=0.1064) (Figure 3.4a). On Gram negatives, the time impact was significant (p=0.0004) while the treatment effect was not (0.6653) (Figure 3.4b). In the fungi group (Figure 3.4d), there were significant differences in time and treatments (p=0.0001). Actinomycetes were significantly reduced in the SMC, RHB-SMC, and WSB-SMC (Figure 3.4c) with both time (p=0.03165) and treatment (p=0.0001) effects observed.

Increased in Gram negative populations are consistent with degradation activities occurring in petroleum impacted soils (Al-Hawash *et al.*, 2018; Cipullo *et al.*, 2019). The fungal population shows significant increase (p=0.0001) from the baseline at day 60, followed by a decline at day 120, which is typical of fungi, which thrive in complex environments and may have been succeeded by other microbial groups as the medium becomes less complex (Gouma *et al.*, 2014; Tesei, Sterflinger and Marzban, 2019; Dai *et al.*, 2022).

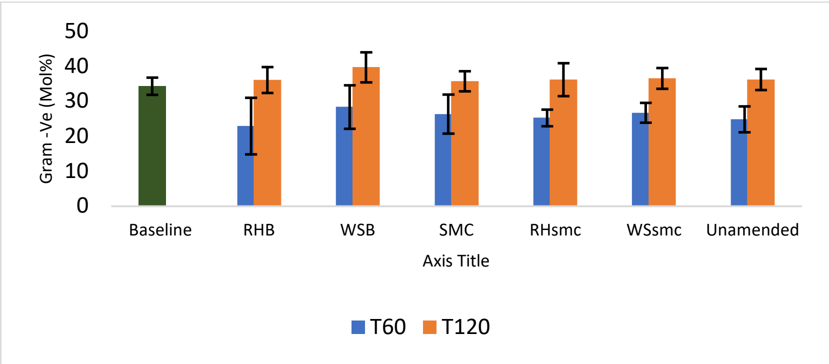
The bioadmendment influenced the microbial community and induced a community shift, as illustrated by cluster C, D and Cluster A, B at 60 and 120 days, respectively (Figure 3.5). The community spread decreased from 60 to 120 days, as evidenced by the formation of only two clusters at 120 days.

A decrease in the community spread from 60 to 120 days indicates that the microbial groups are merging, most likely due to the recovery of the contamination in the soils over time. The similarities in the behaviour of the microbial communities may explain the non-significant difference in the treatments' performance on the TPH degradation (Figure 3.3).

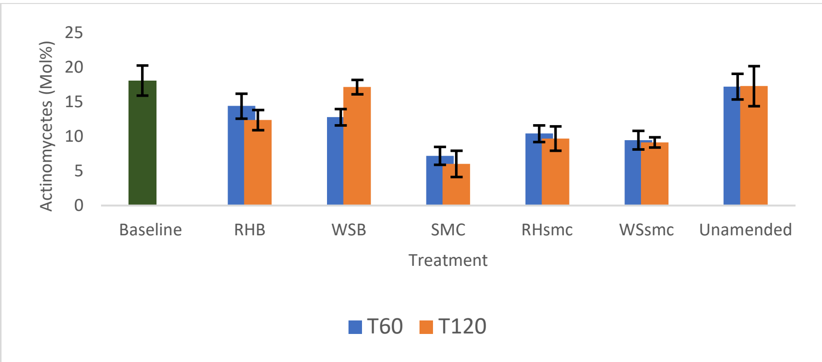
Incubation time has been shown to have a significant effect on the microbial community composition in the treatments (p=0.001). There is also a significant difference in the treatments, particularly in the SMC, RHB-SMC, and WSB-SMC, which differ from the unamended soil (p=0.001). Previously, soil amendment with biochar and spent mushroom compost has been shown to influence microbiological characteristics required for remediation (Zhang and Sun, 2014; Cipullo *et al.*, 2019; Dike *et al.*, 2021).



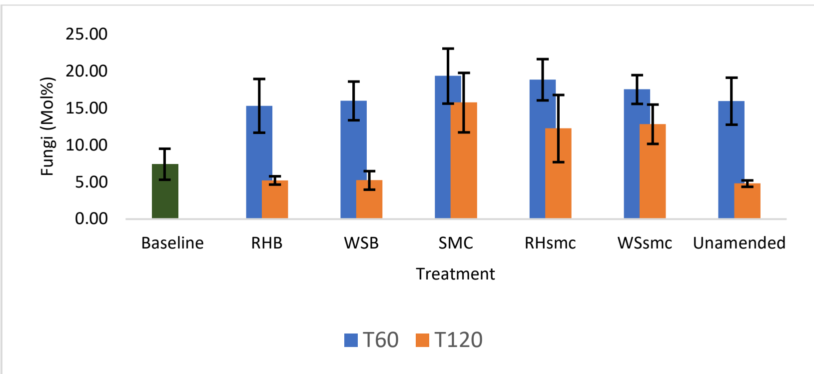
(a)



(b)



(c)



(d)

Figure 3. 4. Influence of bioamendments on the relative abundance of the different microbial groups at day 60 and 120. The error bars represent the standard error for each treatment's replicates. RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost, RHsmc: RHB+SMC, WSsmc: WSB+SMC

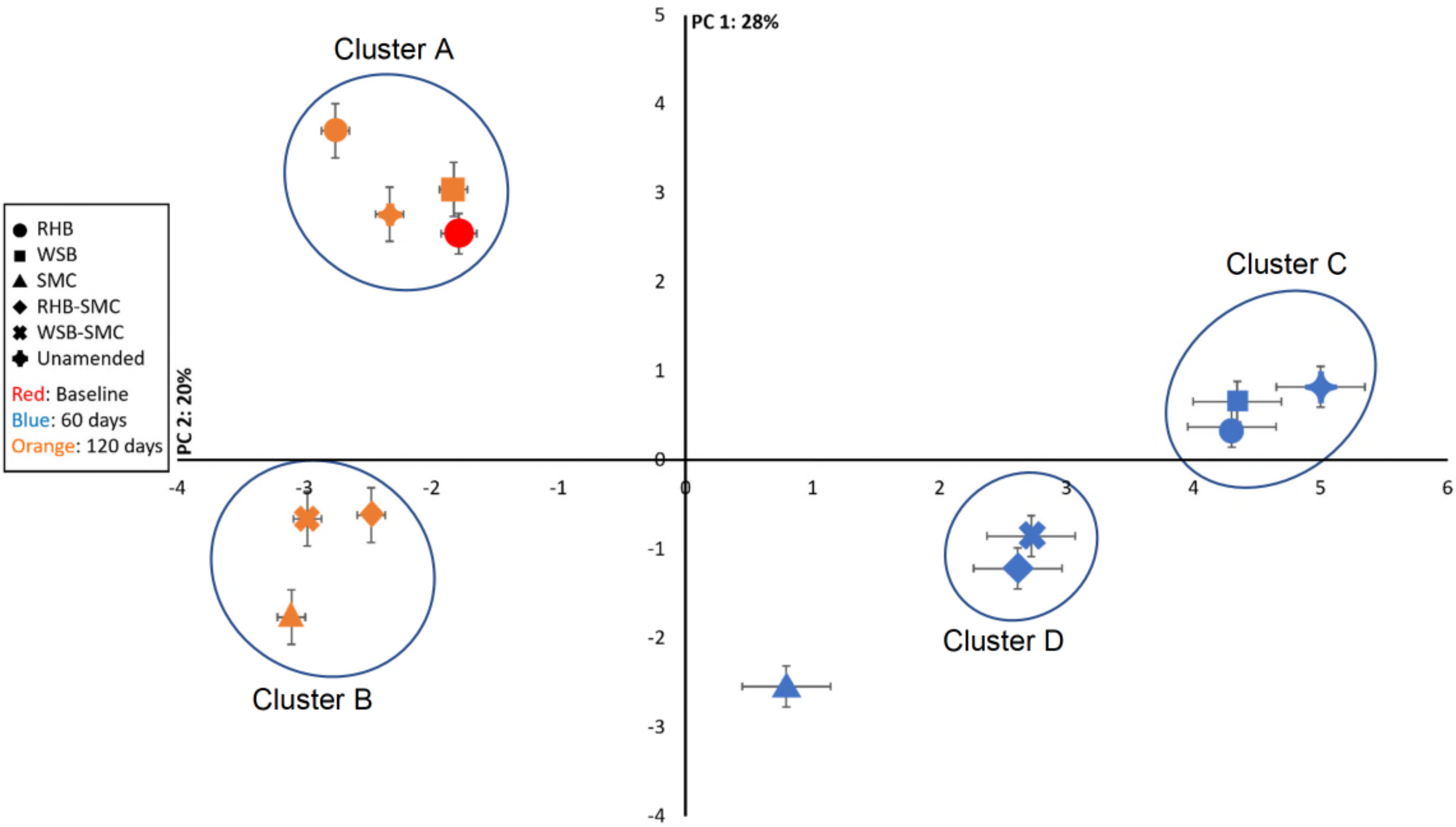


Figure 3. 5. Microbial community dynamics extracted from the treatments and unamended soil across incubation time from the onset (T0) to the end of incubation (T120). The error bars represent the standard error for each treatment's replicates. RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost

3.3.3.2. Microbial catabolic profile and activity

Preservation of soil function is one of the important components in sustainable remediation. Microbial decomposition and substrate conversion are important soil functions that are frequently observed using respiration proxy data (Kaurin and Lestan, 2018). The influence of the bioamendments on the microbial community function (CO2 production rate) was observed in all the treatments. The function was influenced by both biochar and, to a large extent, spent mushroom compost. Hence, in addition to nutrients, spent mushroom compost contains additional microbes that can improve the degradation process s (Gouma *et al.*, 2014). The amendments (p=0.0001) significantly influenced soil respiration, but time did not (p=0.2114).

In the RHB, WSB, and SMC, respectively, there was a 2-, 2.4-, and 5-fold increase in CO2 production at 60 days (Figure 3.6). Particularly when compared to the control, the biochar-SMC mixture produced the least effect. Similar trends were seen at 120 days, however this time the WSB-SMC increased by over 2.8 times. The factors responsible for the large effect reported for SMC-amended soil may consist of the high in nutrient content, improvement of the microbial community, and enhancement of important soil properties including aeration and pH adjustment toward a neutral (Kästner and Miltner, 2016). Also, biochar used in combination with SMC, a compost matrix can improve the quality of treatment as seen in WSB-SMC at 120 days, where biochar along with the effects of the SMC will increasing particle-size distribution-improving aeration, and improving cation exchange capacity (Cipullo *et al.*, 2019).

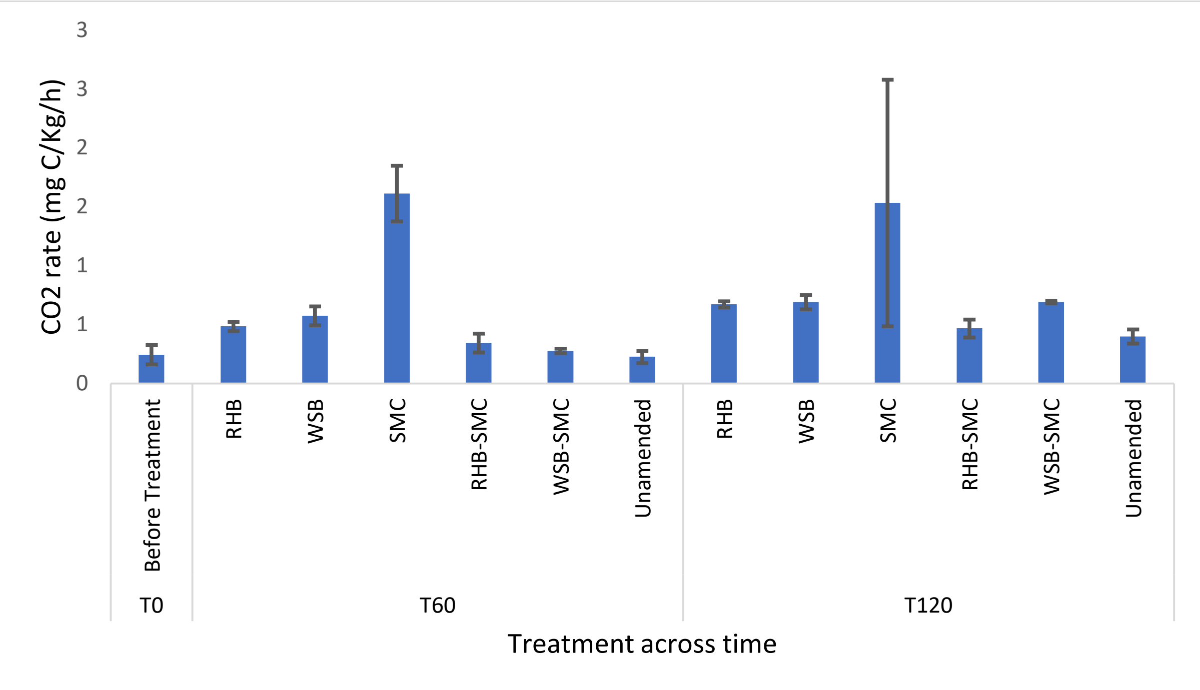


Figure 3. 6. Soil respiration expressed as CO_2 production (mg C/kg/h) for treatment with RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost; Unamended soil tested at 0, 60 and 120 days. The error bars represent the standard error for each treatment's replicates.

3.3.3.3. Ecotoxicity

It is critical to evaluate soil ecotoxicology before, during, and after any remediation treatment, because a decrease in contaminants does not always imply a decrease in toxicity (Coulon *et al.*, 2005). The simplest form of soil ecological organisms (bacteria cell) was used as the basis of the toxicity test, which was the Microtox® in vitro test (Morden Water, 2012). In Figure 3.7, TPH concentration (1493mg/kg) before treatment corresponded to high EC_{50} value of 2224mg/L. It should be noted that the higher the EC_{50} concentration, the lower the soil's toxicity. Hence, the continuous decrease in TPH observed from 60 to 120 days corresponded to a higher EC50 value, indicating decreasing toxicity. This implies that the amendment effects of decreasing contamination corresponded to lower soil ecotoxicity. Except for the RHB, all bioamendments showed decreased toxicity, with the greatest improvement seen in SMC<WSB-SMC<WSB<RHB-SMC. While this is expected as reported in Cipullo *et al.* (2019), other studies have revealed that toxicity increases as TPH decreases, with one of the most plausible causes being the generation of hazardous intermediates during biodegradation, such as reactive oxygen species, epoxides, certain aldehydes, and ketones (Xu and Lu, 2010; Mamindy-Pajany *et al.*, 2012; Jiang *et al.*, 2016).

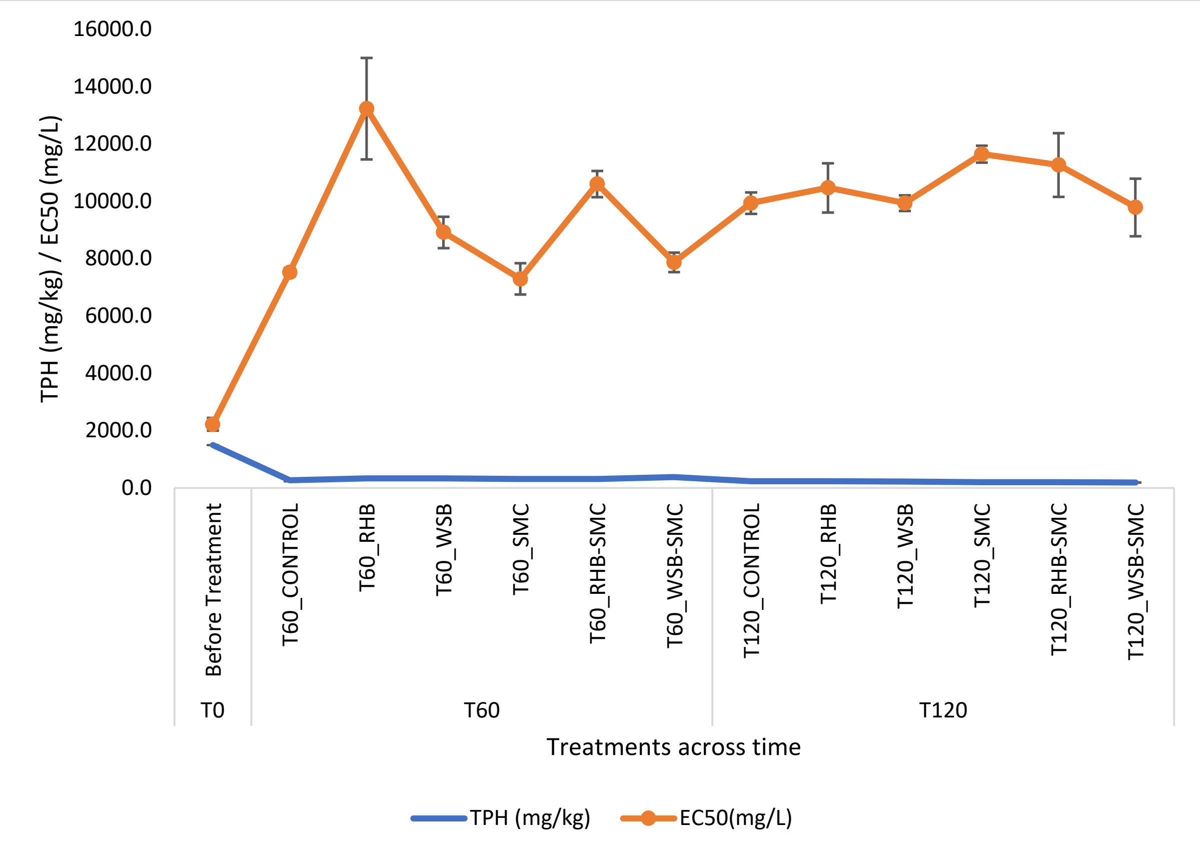
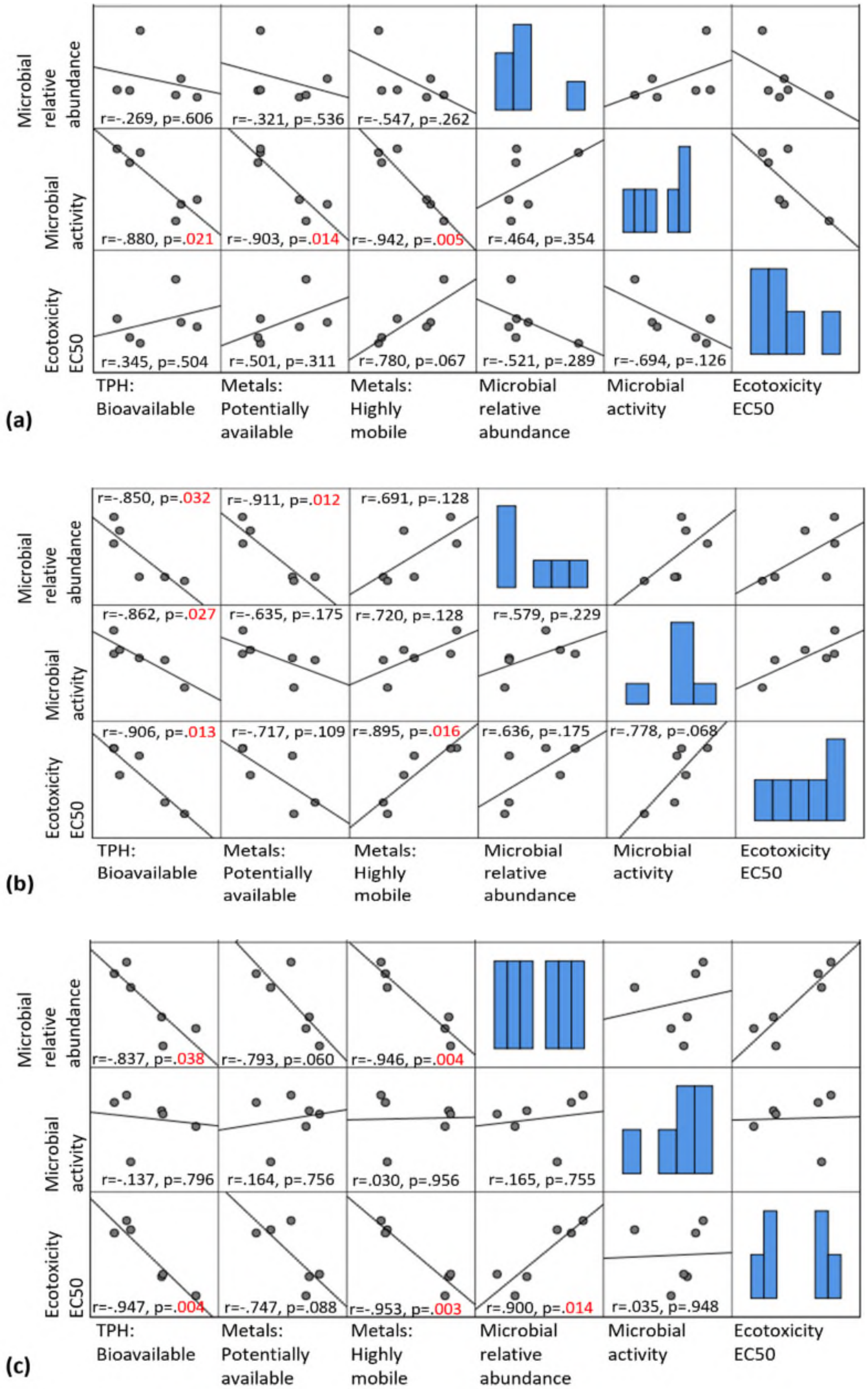


Figure 3. 7. Microtox Basic Solid Phase Test (BSPT) assay shown as EC_{50} concentration (mg/L) for light decrease values at the onset and at 60, 120 days. The error bars represent the standard error for each treatment's replicates.

3.3.4. Correlation of the bioindicators for determining remediation endpoints

The study findings demonstrate that the amendment used had an impact on the relationships between the bioavailability of TPH and metal(loids), soil toxicity, microbial abundance, and respiration, providing insights into the effects of chemical mixtures on microbial communities (Figure 8). The negative correlation observed for bioavailable data and the positive correlation for toxicity data showed that lower concentrations of bioavailable hydrocarbons and metals resulted in an increase in the microbial community and soil respiration function, most notably in the WSB and WSB-SMC treatments (Figure 8b and d). In contrast for the RHB (Figure 8a), a negative correlation was observed between soil toxicity (EC50) concentrations and microbial responses (relative abundance and activity). Similarly, in SMC and RHB-SMC, only microbial activity correlated negatively with soil toxicity (Figure 8c and d). This could be due to increased environmental stress increasing the demand for energy to thrive, causing microbes to increase activity to overcome the stress's limiting effect, or in some cases, the compounds are being utilised by the microbes for growth (Gouma *et al.*, 2014; Zhu *et al.*, 2017b; Rogiers *et al.*, 2021; Zhang and Guan, 2022).

This study demonstrated a correlation between bioavailability and toxicity and their impact on the soil microbial community. The findings suggested that lowering the bioavailable levels of contaminants led to a reduction in toxicity to the microbial community and its function. As a result, it indicated that if the only concern is the assessment of environmental risk receptors, then the remediation process could potentially be stopped once the bioavailable concentrations of contaminants have been reduced to a safe level. In the present study, nearly a 5-fold reduction in toxicity was observed, suggesting that a remediation endpoint below an EC50 value of 2225 mg/kg could be considered as appropriate.



(a)
Microbial relative abundance
Microbial activity
Ecotoxicity EC50
r=-.269, p=.606
r=-.321, p=.536
r=-.547, p=.262
r=-.880, p=.021
r=-.903, p=.014
r=-.942, p=.005
r=.464, p=.354
r=.345, p=.504
r=.501, p=.311
r=.780, p=.067
r=-.521, p=.289
r=-.694, p=.126
TPH: Bioavailable
Metals: Potentially available
Metals: Highly mobile
Microbial relative abundance
Microbial activity
Ecotoxicity EC50
(b)
Microbial relative abundance
Microbial activity
Ecotoxicity EC50
r=-.850, p=.032
r=-.911, p=.012
r=.691, p=.128
r=-.862, p=.027
r=-.635, p=.175
r=.720, p=.128
r=.579, p=.229
r=-.906, p=.013
r=-.717, p=.109
r=.895, p=.016
r=.636, p=.175
r=.778, p=.068
TPH: Bioavailable
Metals: Potentially available
Metals: Highly mobile
Microbial relative abundance
Microbial activity
Ecotoxicity EC50
(c)
Microbial relative abundance
Microbial activity
Ecotoxicity EC50
r=-.837, p=.038
r=-.793, p=.060
r=-.946, p=.004
r=-.137, p=.796
r=.164, p=.756
r=.030, p=.956
r=.165, p=.755
r=-.947, p=.004
r=-.747, p=.088
r=-.953, p=.003
r=.900, p=.014
r=.035, p=.948
TPH: Bioavailable
Metals: Potentially available
Metals: Highly mobile
Microbial relative abundance
Microbial activity
Ecotoxicity EC50

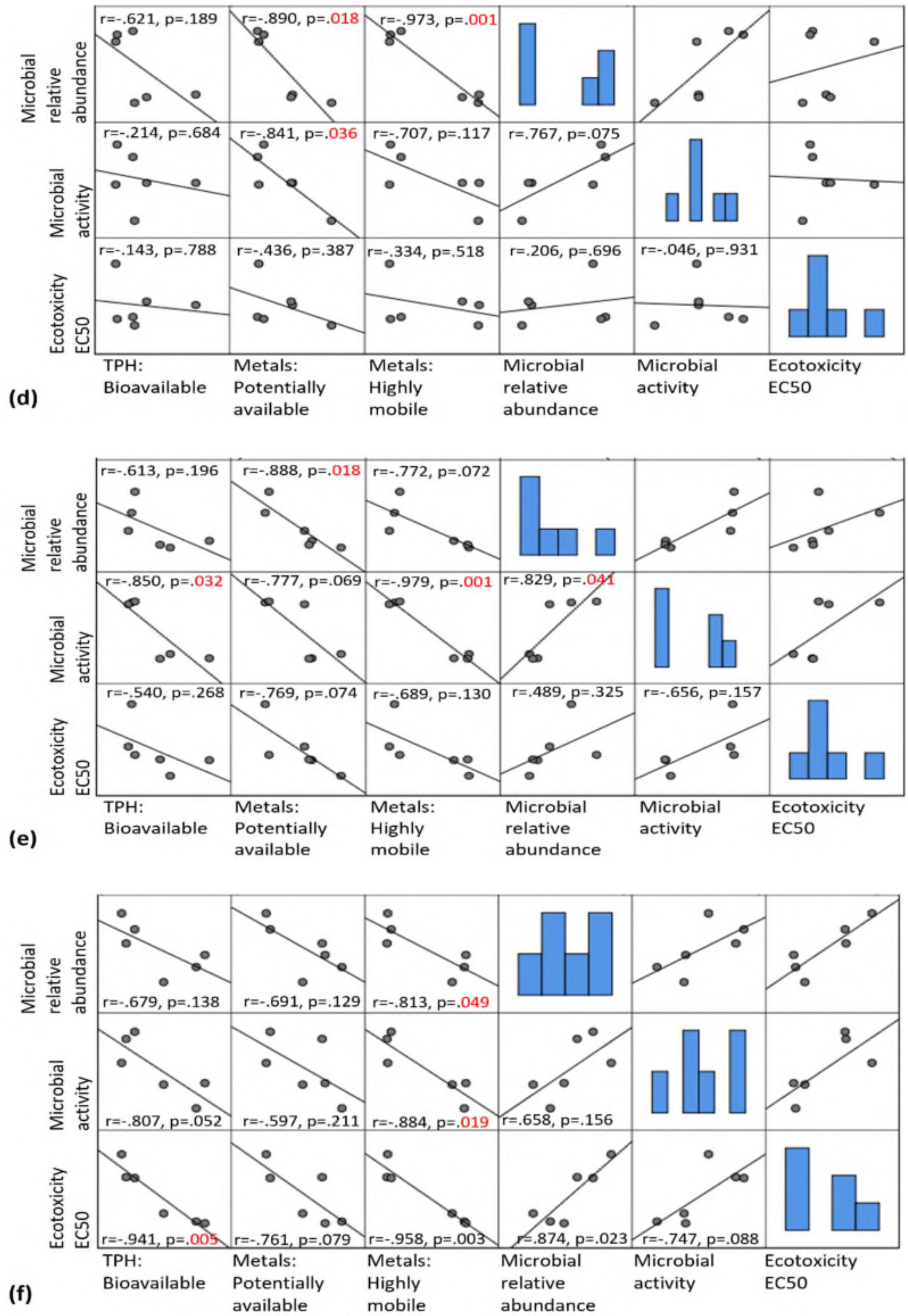


Figure 3. 8. Linear correlation (based on Pearson coefficient) between organic and inorganic bioavailable concentrations, toxicity data and the microbiological responses (Microbial relative abundance, and soil respiration). The various soil treatments, Rice husk biochar (a), Wheat straw biochar (b), Spent mushroom compost (c), Rice husk biochar+Spent mushroom compost (d), Wheat straw biochar+ Spent mushroom compost (e), unamended (f). g=Correlation is significant at the p<0.05

3.4. Conclusion

The study investigated the effectiveness of two types of biochar, namely rice husk biochar (RHB) and wheat straw biochar (WSB), as well as spent mushroom compost (SMC), in reducing the concentration of total petroleum hydrocarbons (TPH) and metals in soil. The results showed that all three bioamendments significantly increased the reduction of TPH by at least 92%, as evidenced by a decrease in the bioavailable concentration of TPH. Moreover, the bioamendments stabilised and lowered the toxicity of metals in the soil by changing their distribution and partitioning, leading to a drop in their bioavailable fractions below the UK CLEA soil guideline limits. In terms of the reduction of TPH containing both aliphatic and aromatic compounds, both RHB and WSB performed equally well, but WSB was more effective in reducing the bioavailable concentration of hydrocarbons. On the other hand, RHB was more effective in influencing the distribution and partitioning of metals. Interestingly, there was no significant difference in the effectiveness of the biochars and SMC in total and bioavailable compound recovery at the 2.5% and 5% rates applied, suggesting that SMC may be preferred for remediation due to its lower cost compared to biochar. However, combining SMC with biochar, such as RHB+SMC, can enhance their effectiveness in reducing metal available phase fractions. The study also found that the bioamendments, particularly SMC, positively influenced microbial abundance and activity, resulting in increased soil respiration function. Furthermore, the lower bioavailable and toxicity concentrations of the chemical mixtures because of the bioamendments reduced the level of contamination to below remediation endpoint for the soil. Overall, the study's findings provide valuable insights into the potential of bioamendments for soil remediation and the importance of considering soil microbial communities in soil remediation strategies. This knowledge can help advance the field by promoting the use of more sustainable and effective soil remediation approaches that consider the ecological implications of soil contamination in remediation.

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4. Efficacy of bioadmendments in reducing the influence of salinity on the bioremediation of oil-contaminated soil

Emmanuel Atai, Frederic Coulon, Mark Pawlett

School of Water, Energy and Environment, Cranfield University, Cranfield, Bedfordshire

Abstract

This study assessed how three bioamendments, rice husk biochar, wheat straw biochar and spent mushroom compost can stimulate microbial degradation of crude oil in a saline soil. A soil microcosm experiment was established to compare the response of soil microorganisms to crude oil (10% spike) in both saline (1% NaCl) and non-saline conditions. The soils were further amended with rice husk biochar (RHB), wheat straw biochar (WSB), or spent mushroom compost (SMC) at 2.5% or 5% and no amendment as control. The microcosms were incubated for 120 days at 20 °C. The initial TPH concentration were 5871±361mg/kg and 3471±138mg/kg, from which the average total degradation from the five treatments was 67% and 18% in the non-saline and saline soils, respectively. Biodegradation of TPH in non-saline soils was approximately four times that of saline soils. Compared to the control, biodegradation was most pronounced in the non-saline soil for alkanes in the RHB-SMC (15%) and polycyclic aromatic hydrocarbons (PAH) in the WSB-SMC (27%). The biggest decreases in saline soil were recorded for alkanes in the SMC (16%) and PAH in the RHB (49%). RHB and SMC had the greatest influence on biodegradation in saline soil. The bioamendment assisted in resolving the dispersal shift in the microbial communities, as evidenced by community clustering, particularly in the RHB and WSB treatments. Actinomycetes and fungi microbial groups demonstrated greater tolerance to soil salinity, especially in the RHB and WSB treatments. The production of CO_2, a measure of microbial activity, was highest in the RHB-SMC (56%) and WSB-SMC (60%) treatments in non-saline soil and in the RHB (50%) treatment in saline soil. WSB and RHB appeared to favour most of the microbial activity due to the substrates tested in non-saline and saline soils, respectively. It was established that increased microbial biomass and activity is associated with higher degradation rates. The bioamendments, to varying degrees, promoted the removal of salinity-related constraints on the function of microbial crude oil biodegradation, with RHB and WSB being the most efficient. Also, the beneficial properties of the biochars were further enhanced by the SMC, as demonstrated by the performance of the RHB-SMC and WSB-SMC treatments. This approach offers a green and sustainable solution

for soil pollution in the context of climate change-induced impacts on soils, coastal soils, and other high-salinity soils.

Key words: biochar, spent mushroom compost, bioamendment, hydrocarbon, microbial community,

4.1. Introduction

Although as the world moves towards energy transition, which involves replacing fossil fuels with low carbon energy sources, oil and gas continue to dominate the world's energy mix (Tian *et al.*, 2022). In 2020, coal, crude oil, and natural gas produced 68.4% of all energy in the EU - oil and petroleum products (34.5%), natural gas (23.7%), and solid fossil fuels (23.7%) (Eurostat, 2022). As a result, crude oil exploration will continue for some time, with the attendant environmental, ecological, economic, and other consequences. The potential of soil contamination from crude oil spills has increased with the activities related to crude oil exploration (Wu *et al.*, 2022). Petroleum contamination of soil damages its structure, function, and ecosystem service values (Wang *et al.*, 2013), necessitating soil clean-up in these environments.

Despite associative limitations such as high site specificity, longer treatment time, and limited application to moderately contaminated soils, bioremediation still holds great promise for petroleum-contaminated soils remediation due to its low cost and environmentally safe nature (Vasilyeva *et al.*, 2022). Climate change is now another important component that significantly affects bioremediation. Evapotranspiration, which includes the evaporation of water from soils, may rise because of climate change and rising temperatures. And, as water evaporates, salt accumulates in the soil, raising the salinity (Khamidov *et al.*, 2022)**.** Furthermore, in coastal areas, soils are frequently saline due to sea water intrusion on land, and while the microbial community may have already adapted to the saline condition, a crude oil spill in the soil may alter microbial function (Tnay, 2019). Growing salinity has a significant impact on the soil microbial community, which are responsible for driving soil bioremediation processes. This is because, salinity increases the effect of osmotic stress and the accumulation of toxic ions to soil microorganisms (Yan *et al.*, 2015a). Equally, salinity may affect the bioavailability of contaminants by altering their physicochemical properties such as solubility, sorption and desorption behaviour thereby impacting the ability of bioremediation organisms to to utilise them as substrates (Kumar *et al.*, 2022). High salinity levels can disrupt nutrient cycling and availability, limiting the resources needed for bioremediation organisms to degrade contaminants effectively; affecting the mobility and availability of contaminants in the environment, resulting in increased dispersal of contaminants and decreased bioremediation effectiveness because contaminants may become less accessible to bioremediation organisms (Mazhar *et al.*, 2022). Considering these concerns, a bioremediation strategy that promotes petroleum hydrocarbon remediation by reducing the effect of salinity on soil microbial degraders is required. For example, biochar and spent mushroom compost have contaminant adsorptive potential and are effective at increasing soil microbial activity (Alhujaily *et al.*, 2018; Guo, Song and Tian, 2020), making them good candidates for use in saline soil bioremediation.

Several studies have demonstrated the efficacy of using biochar, a low carbonaceous sorbent, for the bioremediation of petroleum-contaminated soils. The use of maise straw biochar yielded 60% TPH degradation after 4 months (Wang *et al.*, 2020). Biochar (5% w/w) in combination with nutrients (C:N:P:K), and biosurfactant (Rhamnolipid) removed 23% TPH after 110 days of landfarming (Okoro *et al.*, 2017). The content of TPH was reduced below 5 g/kg by 2% pine chips biochar in forest grey soil after two warm seasons (Vasilyeva *et al.*, 2022). Petroleum hydrocarbons degradation was accelerated (54.2%) after 10 weeks of incubation with microbial consortium immobilised on biochar (Li *et al.*, 2019). Woodchip Biochar improves the TPH phytoremediation of white clover plant by 68% after 62 days (Yousaf *et al.*, 2022). The ability of spent mushroom compost (SMC) to improve soil nutrient and reduced petroleum hydrocarbons contamination from soil has also been investigated (García-Delgado, Yunta and Eymar, 2013; Asemoloye, Ahmad and Jonathan, 2017; Mohammadi-Sichani *et al.*, 2017, 2019).

Biochar, a carbon-rich product produced by the thermal decomposition of organic material possesses a number of remarkable properties including high specific surface area, microporosity, and hydrophobicity, which has been exploited for various applications including environmental remediation (Guo *et al.*, 2015; Oliveira *et al.*, 2017b; Guo, Song and Tian, 2020). Spent mushroom compost (SMC), is a by-product of mushroom production, which contain high organic matter, diverse groups of microorganisms and extracellular enzymes important for the biotransformation of contaminants (Gouma *et al.*, 2014). These two materials are product of agricultural waste industry, hence their sustainability, efficiency, and cost-effectiveness (Ahmad *et al.*, 2014a; Cipullo *et al.*, 2019). The use of biochar and SMC may be a strategy for an opportunity to overcome soil nutrient limitation, increase sorption/decrease bioavailability of the chemicals (alkanes and PAHs, higher sodium ion concentration), and increase surface contact of contaminants with the soil microbial community.

Given that these materials have been studied and their potential for petroleum hydrocarbon soil remediation demonstrated (despite the fact that the exact reasons why their adsorptive effect leads to effective decrease of risk of petroleum soil contaminations remains less understood, and results vary depending on rate of application, type of feedstock used, and production conditions), it is equally important to investigate their effects on soil microbial communities during petroleum hydrocarbon remediation processes in saline soils. We hypothesised that rice husk and wheat straw biochar, as well as spent mushroom compost, would promote petroleum hydrocarbon reduction, increased microbial community abundance and function in a saline soil. Therefore, this study was designed to investigate the influence of rise husk biochar (RHB), wheat straw biochar (WSB) and spent mushroom compost (SMC) applied at two different rates on the fate and behaviour of hydrocarbons (alkanes and PAHs), as well as their influence on abundance and activity of microbial communities in non-saline versus saline soils.

4.2. Materials and methods

4.2.1. Sample collection: Soil, biochar and spent mushroom compost

Subsurface soil from soil heaps was collected from a construction site (52°04'03.2"N 0°37'40.1"W) at Cranfield University, United Kingdom and was air dried, homogenised, and sieved (2mm).

Rice Husk Biochar and Wheat Straw Biochar (UK Biochar Research Centre, UK) employed in this study were produced in a pilot-scale rotary kiln pyrolysis unit with a nominal peak temperature of 550°C, a pH of 9.94, and a total carbon content of 68.26 wt% (d.b). Both are biochars that have been thoroughly characterised (UK Biochar Research Centre, 2014). Spent mushroom compost (Littleport Mushrooms LLP-Gs Fresh Ltd, UK) used was produced following white button mushroom *Agaricus bisporus* production.

The crude oil used in this study was obtained from Shell Gas Direct Ltd, London, UK. It is a Crude Oil Sweet with <0.5% Sulphur. It is a raw petroleum extracted in its natural state from the ground and containing predominantly aliphatic, alicyclic, and aromatic hydrocarbons, as well as small amounts of nitrogen, oxygen, and sulphur compounds. specifically, n-hexane 0-5%, toluene 0-4%, cyclohexane 0-3%, benzene 0-2%, ethylbenzene 0-1%, cumene 0-1%, naphthalene 0-0.5%, hydrogen sulfide 0-0.01%

4.2.2. Microcosms experimental design

The sieved soil was spiked with crude oil at 87579mg/kg (10% w/w spike). Half of the soil was then spiked with 1% NaCl (w/w) to provide a salt stress. From the spiked soil, 250g was then placed in pots (8X10cm), and then amended with either rice husk biochar (RHB), wheat straw biochar (WSB), or spent mushroom compost (SMC) at rates of 2.5% or 5% with the following conditions: soil + 5% RHB, Soil + 5% WSB, Soil + 5% SMC, Soil + 2.5% RHB + 2.5% SMC, Soil + 2.5% WSB + 2.5% SMC, and Soil (unamended). All conditions were done in triplicate, The 5% biochar to soil ratio used in this work was chosen because it is frequently reported as the most effective application rate for reducing mobile contaminant concentrations in contaminated soils (Wang *et al.*, 2017; Novak *et al.*, 2018; Cipullo *et al.*, 2019)

All the microcosms were manually mixed to ensure homogeneity and incubated in 20 °C constant temperature room for the 120 days. The soil moisture was adjusted twice a week by adding deionised water equivalent of the microcosms' weight loss within the range of the soil moisture content at the onset of the experiment. Soil was sampled for chemical, and microbiological analyses after 10, 60 and 120 days.

4.2.3. Physico-chemical characterisations

Air-dried soil samples were analysed based on BS EN 13654-2:2001 and BS 7755 Section 3.8:1995 for Total nitrogen (TN) (0.001 mg), Total Carbon (TC) (0.001 mg) and Total Organic Carbon (TOC: following the removal of carbonates with 4 mol/L hydrochloric acid dropwise until visible reaction stops) with vario EL 3 Element Analyzer (Elementar Analysensysteme GmbH, DE). Total phosphorous was determined by extracting with acid mixture (6ml 11.65mol/L hydrochloric and 2ml 15.8mol/L nitric) and determining the phosphorus content of the extract (ISO 11047, 1998) using a NexION ® 350 D ICP-MS (Perkin Elmer). Available phosphorous (AP) (5 g) was extracted from the soil with a 0.5 mol/L sodium hydrogen carbonate solution at pH 8.5, the extract was then analysed by spectrometry (ISO 11263, 1994). Soil pH was determined according to ISO 10390 (2005) using a soil:water ratio of 1:5 (Jenway 3540 pH Meter, Keison Products, UK). The organic content of the soil (%) was calculated using loss of ignition (LOI): (BS EN 13039, 2000). The particle size distribution was determined using the sieving and sedimentation method according to BS ISO 11277:2009, and the following soil texture classes were calculated using a soil texture calculator (Natural England Technical Information Note TIN037, 2008) and eventual sieving using 0.6mm, 0.212mm, and 0.063mm sieves. Drying at 105 °C was used to determine gravimetric soil moisture and dry matter (%). (ISO 11465, 1993).

4.2.4. Chemical analyses – Total hydrocarbon

Total petroleum hydrocarbons, comprising both aliphatic and aromatic components, were determined using a variant of the method given by (Risdon et al., 2008). Soil extraction was done by taking 2.5 g of soil and combining it with 15 mL volume of 1:1 dichloromethane:hexane. The samples were thereafter sonicated for 20 minutes at room temperature (Ultrasonic Bath, U2500H, Ultrawave (UW), UK) then shaken for 16 hours at 150 r p m (Multi-Reax Shaker, Heidolph Instruments GmbH & CO. KG). On the second day, samples were sonicated for 20 minutes (room temperature) before centrifuging (2000g for 10 minutes) (Thermo ScientificTM, SorvallTM ST 40 Centrifuge Series). Following that, the supernatant was transferred to 6 mL SPE DSC-Si silica tubes for cleaning. A 0.5 mL sample was taken from the 10 mL and combined with 0.5 mL of internal standards, which included a deuterated alkane mix ($C10^{d22}$, $C19^{d40}$ and $C30^{d62}$) and deuterated polycyclic aromatic hydrocarbons (PAH) mix (naphthalene^{d8}, anthracene^{d10}, chrysene^{d12} and perylene^{d12}).

Gas chromatography-mass spectrometry (GC-MS) was used to identify and measure the concentration of petroleum hydrocarbons using an Agilent gas chromatograph connected to a Turbomass Gold mass spectrometer operating in positive ion mode at 70 eV. As described in Cipullo et al. (2019), a split-less injection was used with a sample volume of 1 µL. For a total run time of 38 minutes, the oven temperature was raised from 60 °C to 220 °C at a rate of 20 °C per minute, then to 310 °C at a rate of 6 °C per minute, and kept at this temperature for 15 min. For the quantitative measurement of the target aliphatic and aromatic hydrocarbons, the mass spectrometer was run in full scan mode (m/z range 50-500). Quantification for each compound was carried out by integrating the peak at a particular m/z. External multi-level calibrations were performed using alkane (C8-C40) and PAH (EPA 525 PAH Mix A) standard solutions (Sigma Aldrich, Dorset, UK) with concentrations ranging from 1 to 5 µg/mL. Blank controls were analysed every 18 samples for quality control.

4.2.5. Microbiological analysis

4.2.5.1 Respiration

MicroRespTM colourimetric microplate-based respiration system for measuring CO_2 evolved from soil which water or carbon substrates have been added is based on Campbell *et al.*(2003). This method gives responses of microbial decomposition and conversion of the substrates, and the activity are reflected by measuring CO_2 production after 6 hours. The method in brief: The detection plates – microplate plates with purified agar and indicator solution (cresol red, KCl,

$NaHCO_3$) are added in a 1:2 ratio – were prepared and stored in sealed desiccator prior to use to avoid absorbing CO_2 from the environment. In the deepwell plates, 0.32g of soil samples and 93.6mg/ml substrates solution were added into it. Detection plate were read at 570nm (Microplate readers, SpectraMax® Plus384, Molecular Devices) and assembled onto the Deepwell plate with the MicroResp™ seal, secured in metal clamp and incubate at 25°C for 6 hours and re-reading the Detection plate at 570nm. Substrates (alanine, citric acid, glucose, gamma-aminobutyric acid, α-ketoglutaric acid, malic acid) were selected considering the Creamer *et al.* (2016); lignin was added as a complex carbon source based on availability in the lab. The soils microbe's utilisation of these substrates was assessed as cumulative CO_2 production in a MicroResp^{TM} system (C.D. Campbell *et al.*, 2003). The basal respiration rate was calculated using the CO_2 generated by the wells in which water other than substrates were added.

4.2.5.2 Phospholipid fatty acid analysis (PLFA)

Using Phospholipid fatty acid (PLFA) analysis based on Frostegard, Tunlid and Baath (1993), the microbial community structure was examined. In brief, from the freeze dried (Christ Alpha 1–2 LD plus −80 °C Freeze Dryer) soil samples, solid-phase soil extraction using 10g of each sample was performed using Bligh and Dyer solution (chloroform, methanol, and citrate buffer in 1:2:0.8 by volume). The extract was further derivatised by mild alkaline methanolysis. By using a GC-FID (Agilent Technologies 6890N) equipped with an HP-5 (Agilent Technologies) fused silica capillary column (30 m length, 0.32 mm ID, 0.25 m film), fatty acid methyl esters were analysed. GC conditions were as described by Pawlett et al (2013). The target responses of all discovered PLFA peaks were sum up to determine the relative abundance of each unique PLFA, which was reported as a percentage (mol%).

4.2.6. Data analysis

Data analysis was done on each amendment at the 10, 60, and 120 day time points. This include ANOVA test (Repeated measures), which was used to determine the significance and relationship between soil amendment [rice husk biochar (RHB), wheat straw biochar (WSB), spent mushroom compost (SMC), RHB+SMC, WSB+SMC, or un-amended] and incubation time on the alkanes, PAHS, and microbial PLFA profiles and respirations datasets

Principal Component Analysis was used for multi-variate datasets, to evaluate the variations between soil amendment and incubation time on microbial community dynamics and respiration profiles from multiple substrates induced respiration. Both Repeated-measures ANOVA and PCA were performed using Statistica (TIBCO Statistica® 14.0.0.15 December 2020).

Pearson correlation in SPSS (IBM SPSS Statistics for Windows, Version 21.0. Apr 2019) was used to establish correlation between the Total petroleum hydrocarbon with PLFA profile and microbial soil activity dataset.

4.3. Result and Discussion

4.3.1. Soil sample and physicochemical properties

The soil analysis of the total petroleum hydrocarbon concentrations at 10 days (which is taken as the baseline for this study) yielded 5871mg/kg and 3471mg/kg, representing 7% and 4% recovery in non-saline and saline soils, respectively. The impact of the bioamendments on the fate and behaviour of the TPH, as well as the structure and function of the microbial community, were investigated for 120 days from this concentration. The lower molecular weight fractions were higher for the alkanes (decane being the least with 10 carbon number), as seen by the distribution of the different fractions (Figure 4.1a&b). In comparison to aromatics, the aliphatic were considerably greater in the TPH (96 % in both non-saline and saline soils). This has been typical for a recent crude oil spill site as the predominant hydrocarbon constituents are the paraffins (alkanes) (Truskewycz *et al.*, 2019) similar to the situation in this study where the crude oil spike was very recent. The aromatics, on the other hand, had a fairly even distribution of fractions (Figure 4.1c and d), though there were higher occurrence of phenanthrene, chrysene and indeno[1,2,3-cd]pyrene in the non-saline soils; benzo[k]fluoranthene, benzo[b]fluoranthene and chrysene in the saline soil.

In Table 4.1, the non-saline and saline soils had moisture contents of 29% and 30%, organic matter 93% and 92%, pH 8.5 and 8.2, respectively. These levels of the soils properties are similar to the ones reported to favour soil function (Griffiths et al., 2018). The 100:2:0.04 CNP ratio recorded did not indicate the usual agricultural soil nutrient proportion for optimal soil function, however low nutrients (CNP ratio) have previously been associated with increase in PAH microbial degraders (Singh *et al.*, 2014) probably due to increase microbial activity as a result of environmental stress induce by the contaminant. Additionally, the inorganic (available) phosphorus that Zheng et al. (2019) indicated to favour microbial activities appeared to be reduced (6.19 mg/kg in non-saline and 5.63 mg/kg in saline) in these soils. In soils with pH >8.0 are attributed to have low soil phosphorus (Griffiths *et al.*, 2018) because at higher pH phosphorus become complexed and less bioavailable. The high organic matter in the soil attributed to the carbon may confer certain advantages like enhancing water-holding capacity, cation exchange capacity, and improving clay soil structural stability by assisting in the consolidation of particles into aggregates (FAO, 2022), as such, providing a condition for improved microbial activities towards the hydrocarbon degradations.

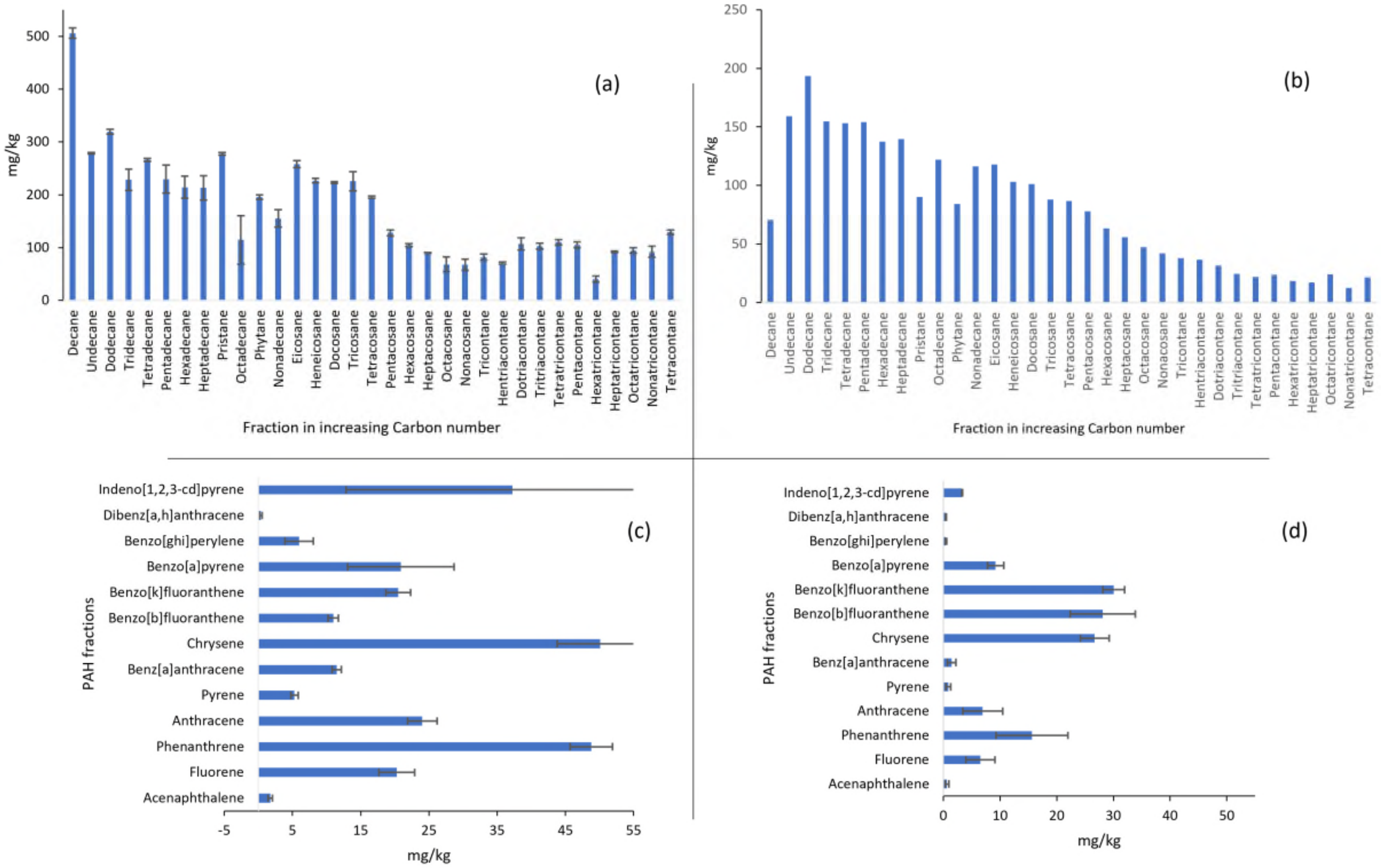


Figure 4. 1. The Total Petroleum Hydrocarbon profile of the non-saline and saline soils in order of increasing carbon number and molecular weight. (a) non-saline soil alkanes, (b) saline alkanes, (c) non-saline PAH, (b) saline PAH

Table 4. 1. Physiochemical characteristics of the crude oil spiked non-saline and saline soil.

Characteristics	Analysis	Non-saline soil	Saline soil
Elements	Total C (%)	6.11	6.39

	Total N (%)	0.11	0.14
	Total P (%)	0.07	0.07
	C:N:P	100:2:0.04	100:2:0.04
	C:N	54.54	44.64
	Total Organic C (%)	4	4
	Total P (mg/kg)	24.77	22.54
	Available phosphorus (mg/kg)	6.19	5.63
Physical properties	Dry matter content (%)	71	70
	Water content (%)	29	30
Chemical properties	pH	8.5	8.2
	Loss on ignition (%)	93	92

N: nitrogen, C: carbon, P: phosphorus

4.3.2 Fate of hydrocarbons – Alkanes and PAHs

The effect of the bioamendment on the crude oil contamination in the non-saline and saline soils was determined (Figure 4.2 and 4.3) and the hydrocarbons considered were the aliphatic (alkanes) and aromatic (PAHs). In the aliphatic (alkanes) (Figure 4.2), from the baseline of 5612.9±308 mg/kg and 3340.8±105 mg/kg, there was an average of 73% and 19% degradation in the non-saline and saline soils treatments respectively compared to the control after 120 days of incubation. The best performed treatment are the RHB-SMC (15%) in the non-saline soil and SMC (16%) in the saline soil. Though the degradation of alkanes induced by the bioamendment did not differ significantly (p=0.3331) between non-saline and saline soils, salinity in the saline soils was found to have a significant impact on alkane degradation. Salinity has been shown to affect soil pH, ion exchange capacity, soil organic matter, and the abundance of microbial biomass (W. Zhang *et al.*, 2019), all of which have an impact on the microbial community's ability to degrade hydrocarbons in the soil.

However, salinity had less effect on the degradation of the PAH (Figure 4.3), particularly in the two biochar (RHB and WSB) treatments. Compared to the control After 120 days of incubation, from there baseline of 257.68±53mg/kg and 130.33±78 there was an average of 61% and 39% degradation of PAHs in the non-saline and saline soils treatments, respectively. The best performed treatments are the WSB-SMC (27%) in the non-saline soil and RHB (49%) in the saline soil – where the higher percentage is been driven by the two biochars (RHB and WSB) treatments. The ability of biochars to stabilise ions and reduce their bioavailability through enhanced sorption may account for their ability to reduce the effect of salinity, and this is achieved through biochar electrostatic attraction, ion exchange capacity, and surface complexation (Guo, Song and Tian, 2020).

Combining biochar and SMC will allow you to take advantage of the beneficial properties of both materials, including biochar's high surface adsorption and chemical precipitation (Zhu *et al.*, 2017a), as well as Spent mushroom compost's high organic matter, enzymes, and diverse microbial community (Ntougias *et al.*, 2004; Sun *et al.*, 2021). As a result, combining biochar and SMC would have a synergistic effect on crude oil contamination remediation. This is demonstrated by the fact that the mixed treatments (RHB-SMC and WSB-SMC) performed best in non-saline soils for both alkanes and PAH.

While salinity causes reduced microbial activity in soil as a result of osmotic stress and toxic ions (Yan *et al.*, 2015b), several studies have used a bioaugmentation approach with halotolerant microbial degraders to address this issue (Ebadi *et al.*, 2017; Qu *et al.*, 2022; Wang *et al.*, 2022).This study's findings also indicated the possibility of mitigating the saline effect on crude oil degradation using bioamentments such as biochar and SMC.

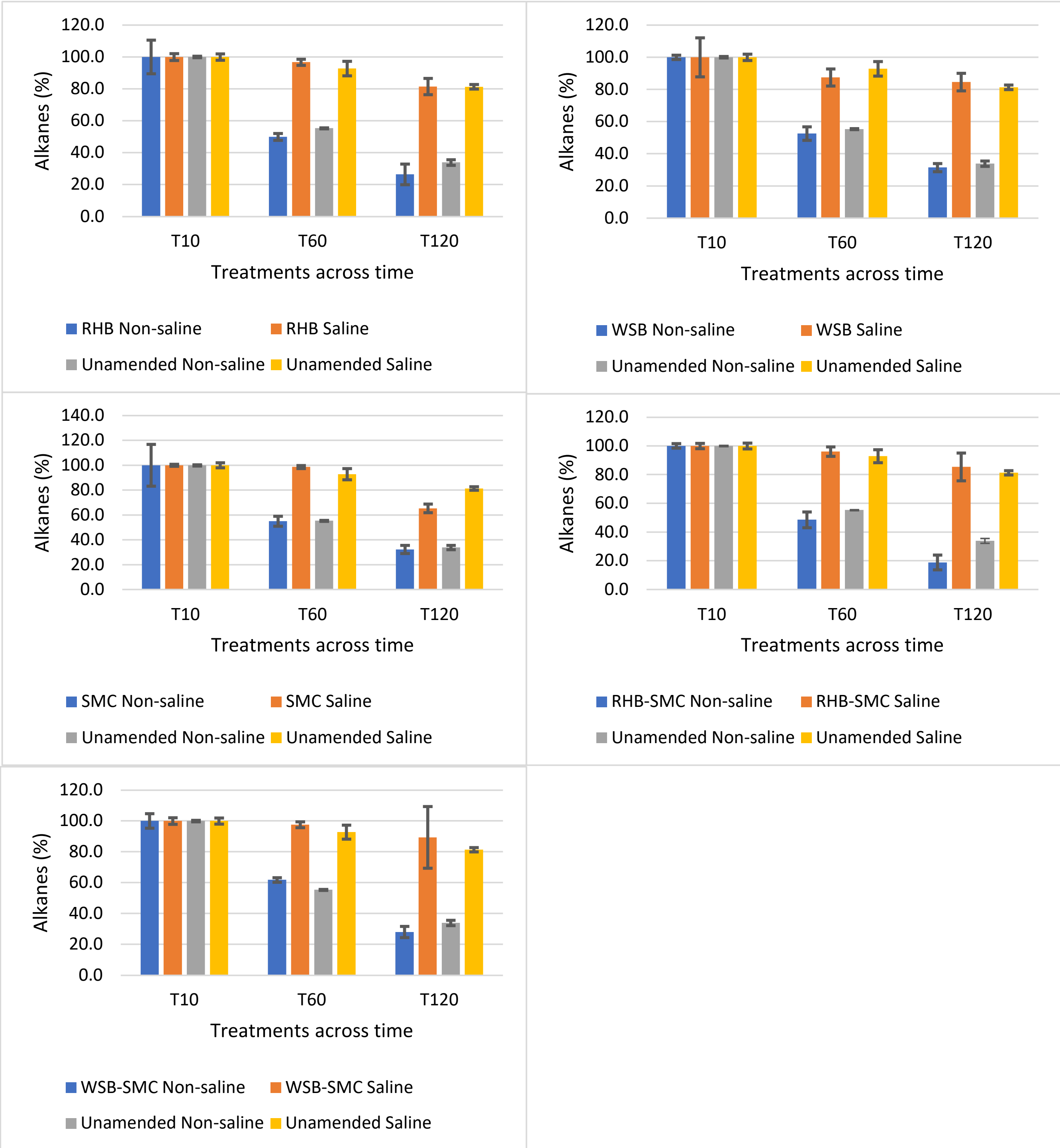


Figure 4. 2. The aliphatic (Alkanes) concentrations ± standard error and corresponding percentage degradation from 10 to 60 and 120 days in the various soil treatments for the non-saline and saline soils compared side by side with their respective controls

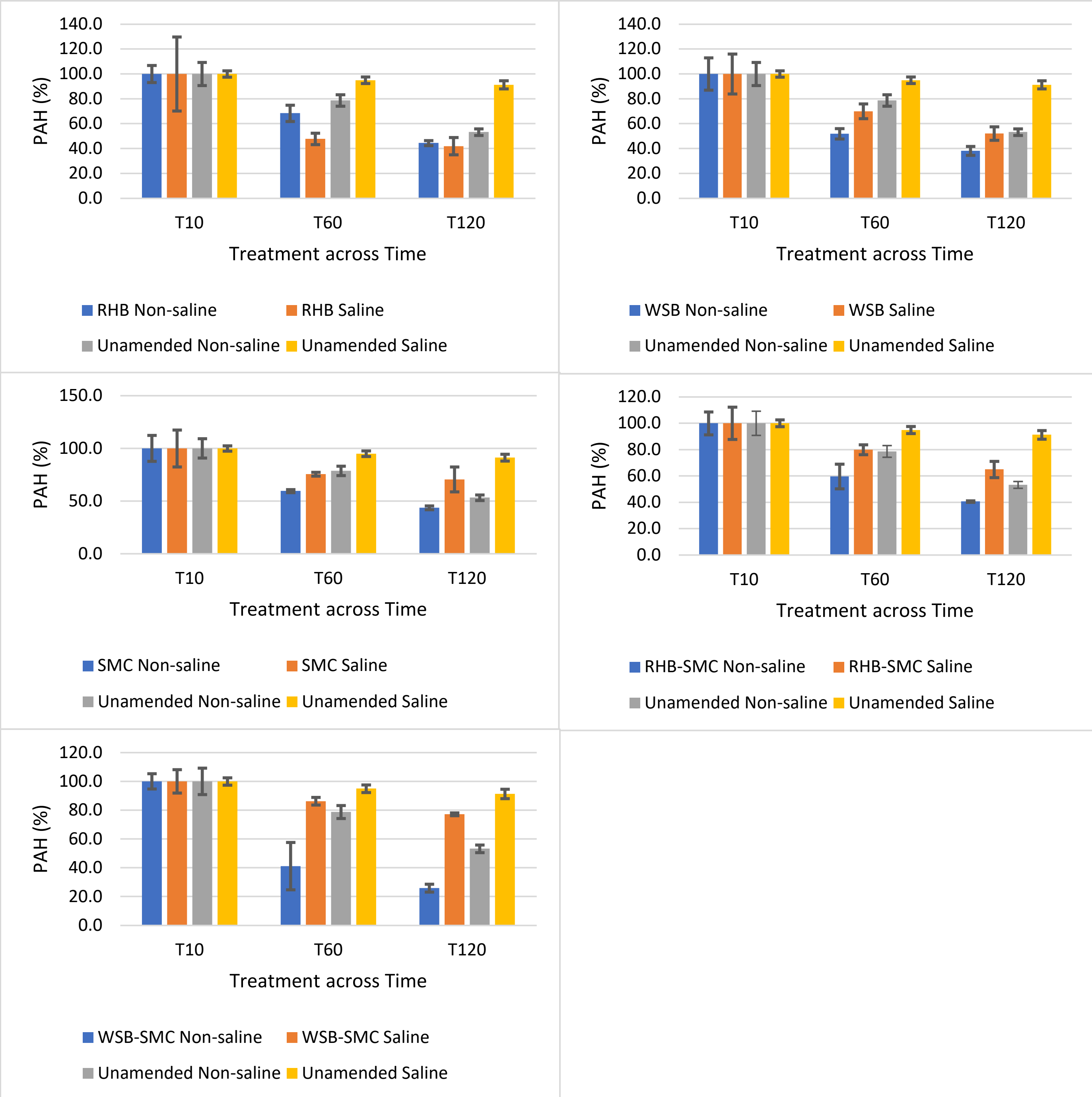


Figure 4. 3. The aromatic (PAHs) concentrations ± standard error and corresponding percentage degradation from 10 to 60 and 120 days in the various soil treatments for the non-saline and saline soils compared side by side with their respective controls

4.3.3. The influence of the bioamendments on the Microbial community in the soils

4.3.3.1. Effects on community dynamics

There was evidence of the impact of the crude oil and treatments at the start and throughout the period of treatment, as shown by what appeared to be a dispersal in the structure of the microbial communities in the treatments (Figure 4.4). The crude oil contamination effect on the microbial community is different in the non-saline and saline soils, resulting in shifts in the communities at different times (10 - 60 days) along the two soil types. Allison & Martiny (2008) reported a sensitivity of microbial community to population disturbance, where such change is often been associated with changes in ecosystem processes. In some cases, there may be a relative stability in the communities, which is likely to be as result of the microbial community resistance to the ecosystem disturbance (Shade *et al.*, 2012).

Treatment and time effects (p=0.0001; 0.0012) on the microbial community were observed, and these were expressed differently in non-saline and saline soils. In non-saline soils, there was increased variation in the communities at 10 days. The spread decreases over time with cluster formation at the end (120 days). In the saline soil, however, there was less spread at first, but an increase spread later in the communities. Such behaviour in the saline soil could be because, salinity causes low soil microbial activity due to osmotic stress and the possible toxicity of ions, and microbes can adapt to their environment by countering osmotic stress by synthesising osmolytes, which allows them to maintain cell turgor and metabolism (Yan *et al.*, 2015a).

The later reduction in the spread and formation of clusters by the microbial communities in the treatments indicates a reduction in the complexity of the soils, which in this case is primarily the hydrocarbon degradation engendered by the treatments administered. Hence the use the biochars and spent mushroom compost was able to influence the microbial communities in the treatments leading to remediation of the contaminants. And from other studies, the ways in which these materials could influence microbial communities in contaminated soil can be divided into three categories: effect on the soil environment, the contaminant, and the microbes. Biochar improve soil properties such as soil structure, water holder capacity, aeration, nutrient supply, soil pH, and electrical conductivity (Hu *et al.*, 2021). On the contaminant, biochar cause contaminant immobilisation via surface sorption and complex formation (Palansooriya *et al.*, 2019); and provide microbial shelter, induce changes in enzyme activity, and facilitate electron transfer (Zhu *et al.*, 2017a). Spent mushroom compost similarly exerts sorption on the contaminant, provides enzymes, serve as rich source of microorganisms to improve the microbial activities in the soil (Dabrowska *et al.*, 2021; Sun *et al.*, 2021).

Furthermore, at 60 and 120 days, the clustering of some of the saline soils with the non-saline soils is an indication of the effect of the treatments in mitigating the impact of soil salinity. This is especially true for the saline soil biochar (RHB and WSB) treatments. The salinity in the soil may have been impacted by biochar's capacity to improve soil characteristics, particularly through enhancing cation exchange capacity, balancing water holding capacity, and air porosity in soils (Vasconcelos, 2020); thus changing the soil salt ions. It has been demonstrated that adding 0.4% of biochar to soil reduces the soil's salt ion concentration by 26% (Xiao and Meng, 2020). Also, 5% biochar application reduced salt stress and oxidative damage (Huang *et al.*, 2019).

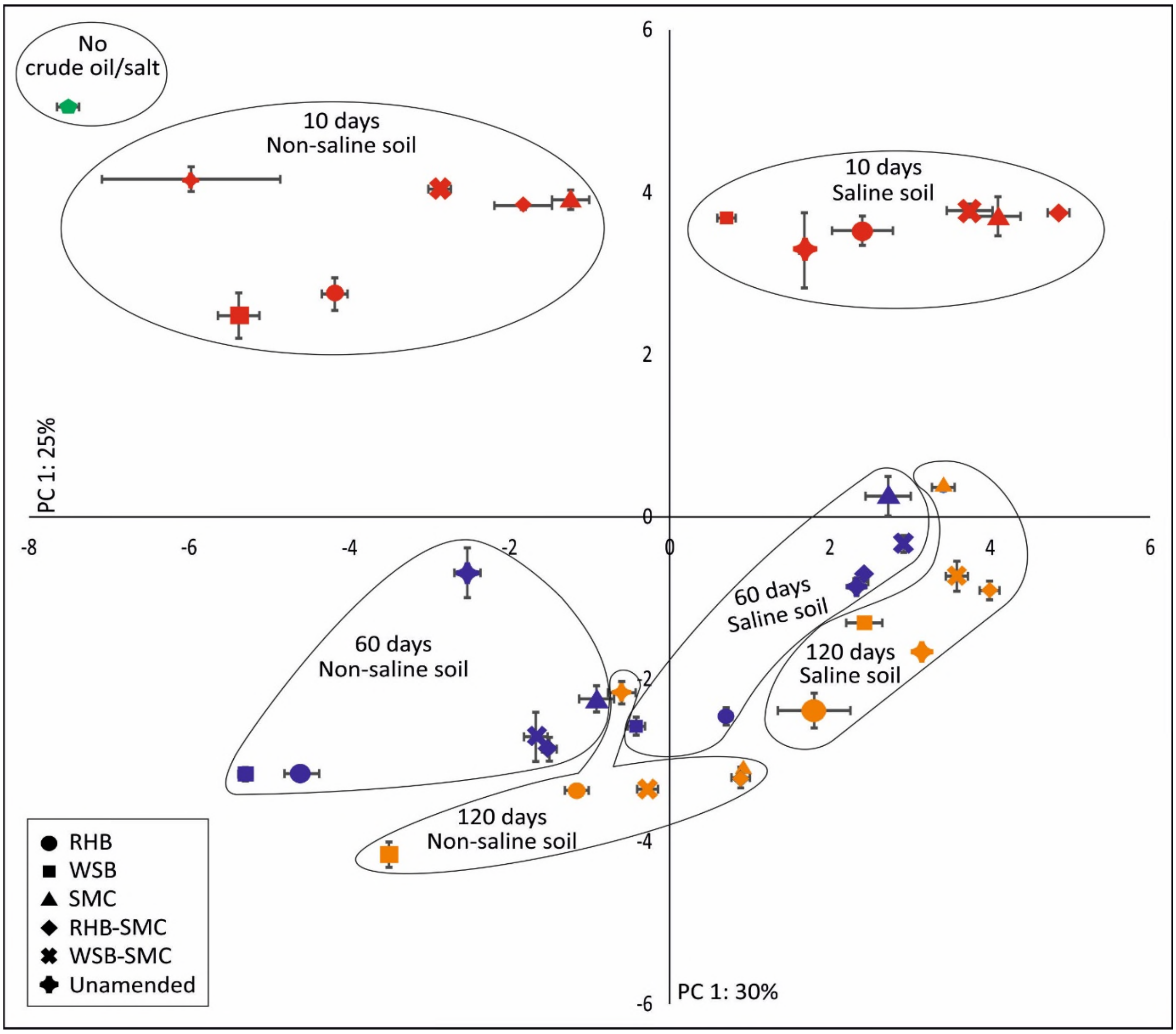


Figure 4. 4. Microbial community (PLFA) dynamics extracted from the treatments and unamended soils for the non-saline and saline soils across incubation time from the onset (10 days) to the end of incubation (120 days). The error bars represent the standard error for each treatment's replicates. RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost.

4.3.3.2. Effects on the Microbial groups' relative abundance

The introduction of salt and crude oil to the soil environment altered the microbial community composition and caused a shift in the microbial population (Figure 4.5).

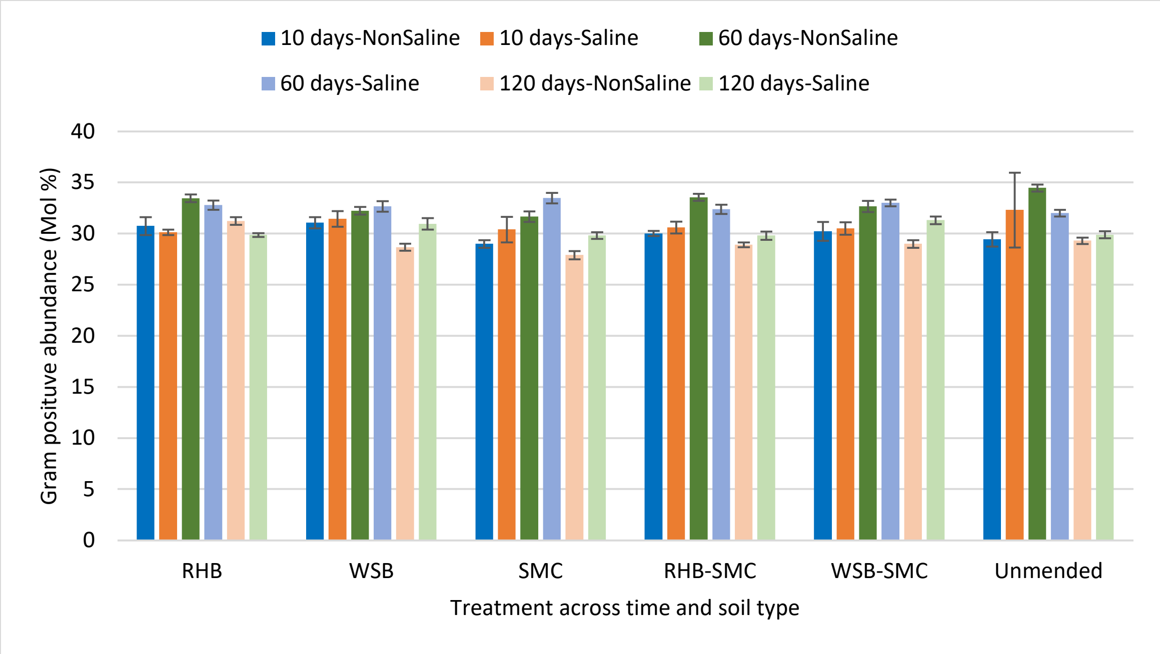
The abundance of the Gram positives bacteria group increased relatively less (0.5% and 3%) in both non-saline and saline soils, and there was no significant difference (p=0.4413) between the Gram-positive bacteria groups in non-saline and saline soils.

Microbial abundance in Gram negative bacteria decreased steadily to an average of 19% and 21% in non-saline and saline soils, respectively. For both soils, the biochar (RHB and WSB) treatments had the highest abundance. Salinity was a limitation factor for these microbial groups, and there was a significant difference (p=0.0001) between the Gram-negative groups in non-saline and saline soils. While there is a link between Gram-negative bacteria and petroleum hydrocarbon degradation (Cipullo *et al.*, 2019), their numbers may be decreasing due to the dominance of other groups after the Gram-negative catabolic step is completed (Xu *et al.*, 2018).

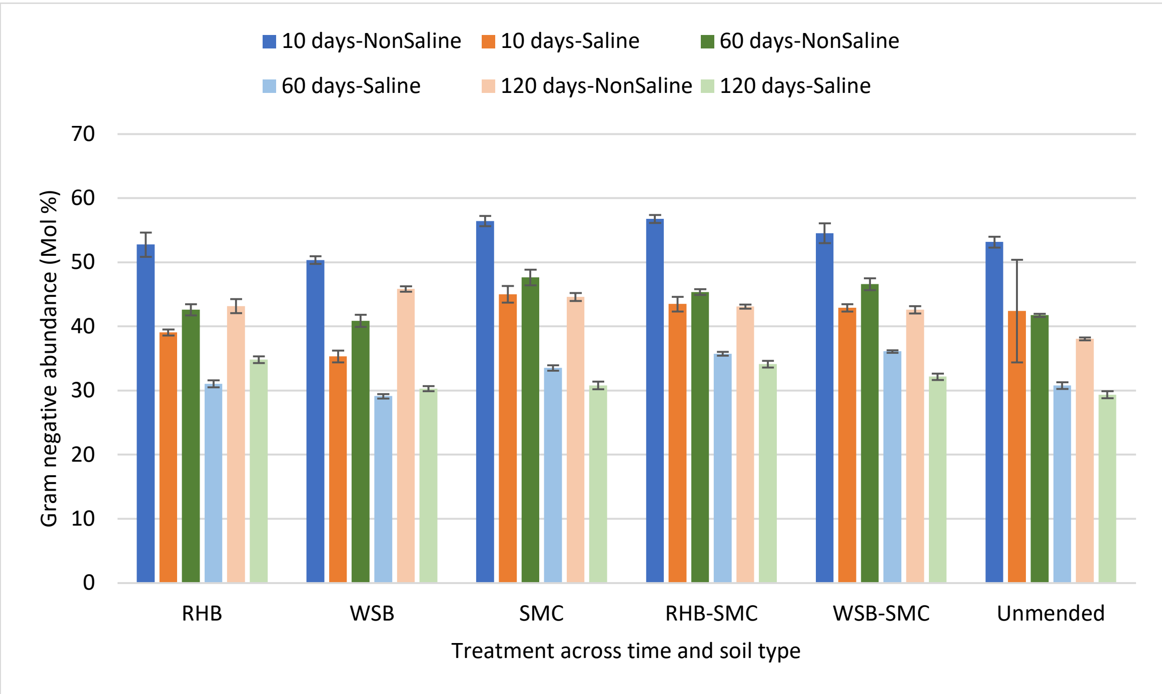
The microbial group actinomycetes was found to be more abundant in the saline soil treatments. However, the increase in number over time was greater in non-saline soils, particularly RHB (5%), SMC (30%), RHB-SMC (46), and WSB-SMC (8%). Only the WSB treatment increased (16%) in the saline soil. There was a significant difference (p=0.0001) between non-saline and saline soils, and salinity promoted Actinomycetes growth by an average of 57% when compared to non-saline soil. The greatest abundance was found in saline soils amended with RHB and WSB. As a result, biochar promoted the growth of actinomycetes more than other treatments.

In the fungal groups, the non-saline and saline soil treatments increased by 130% and 55%, respectively. For both soil types, the SMC, RHB-SMC, and WSB-SMC have the highest abundance. There were significant differences (p=0.00633) between the non-saline and saline soils once again. Salinity did not limit the fungal populations. The presence of a diverse fungal community in the spent mushroom compost increased the occurrence of a diverse fungal community in the SMC and other SMC-biochar treatments.

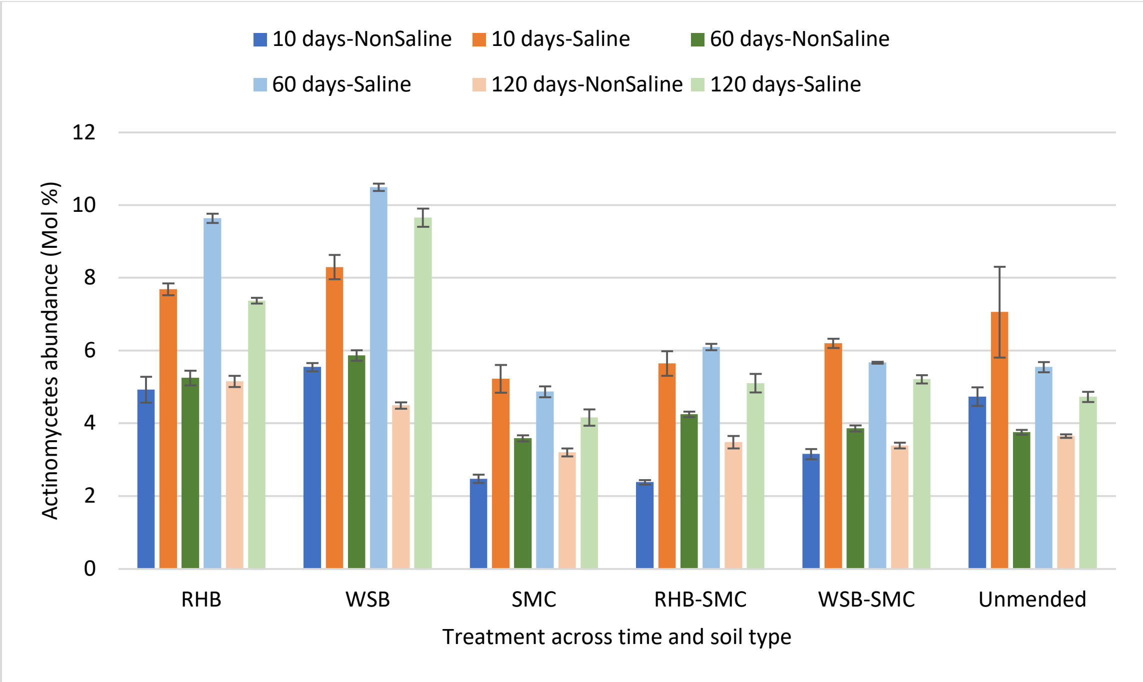
Although the test used in this study did not provide detailed information at the genus and species level of the observed hydrocarbon degraders, we did notice an increase in abundance of certain microbial groups, such as actinomycetes, Gram-negative bacteria, and fungi, which has been correlated with hydrocarbon reduction in previous studies (Al-Hawash *et al.*, 2018; Cipullo *et al.*, 2019; Wai, Yusop and Pahirulzaman, 2020). Furthermore, our findings indicated that the bioamendment treatment facilitated positive changes in the populations of these microbial groups. Notably, actinomycetes and fungi showed higher tolerance to soil salinity, and the addition of rice husk biochar (RHB) and wheat straw biochar (WSB) appeared to aid in their adaptability.



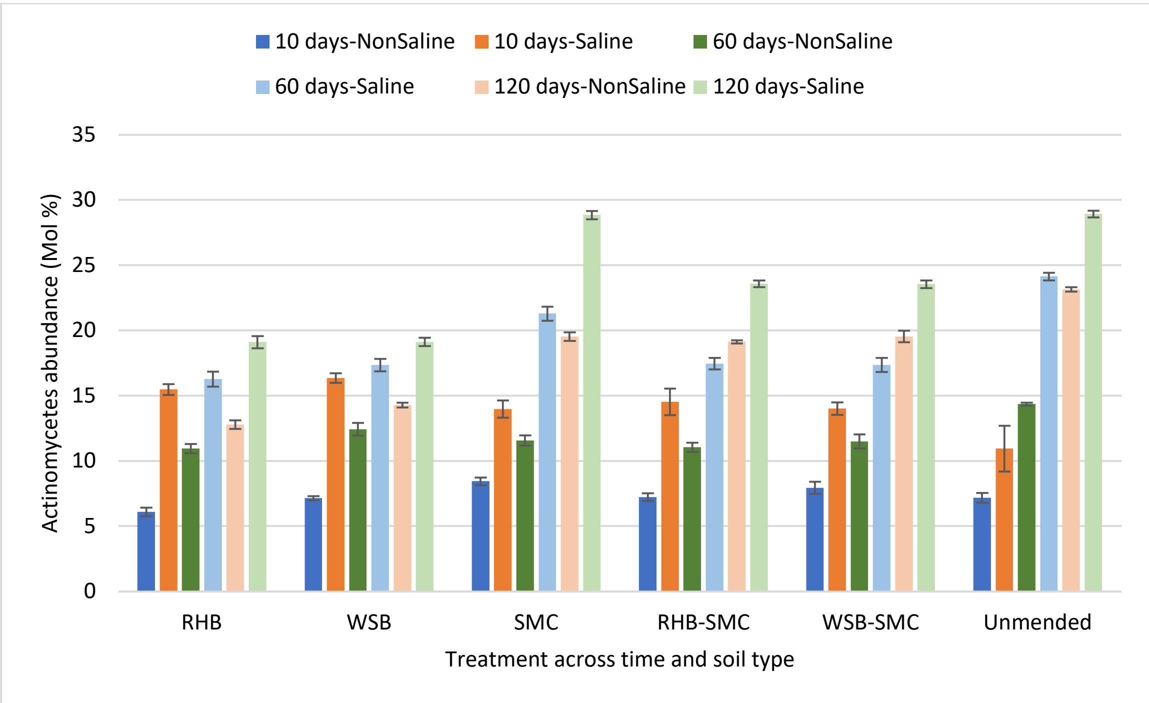
(a) Gram Positive bacteria



(b) Gram Negative bacteria



(c) Actinomycetes



(d) Fungi

Figure 4. 5. The influence of bioamendments on the microbial community demonstrated by the relative (Rel.) abundance of microbial groups in all treatments and their changes over 10, 60, and 120 days in the non-saline and saline soils. The error bars represent the standard error for each treatment's replicates.

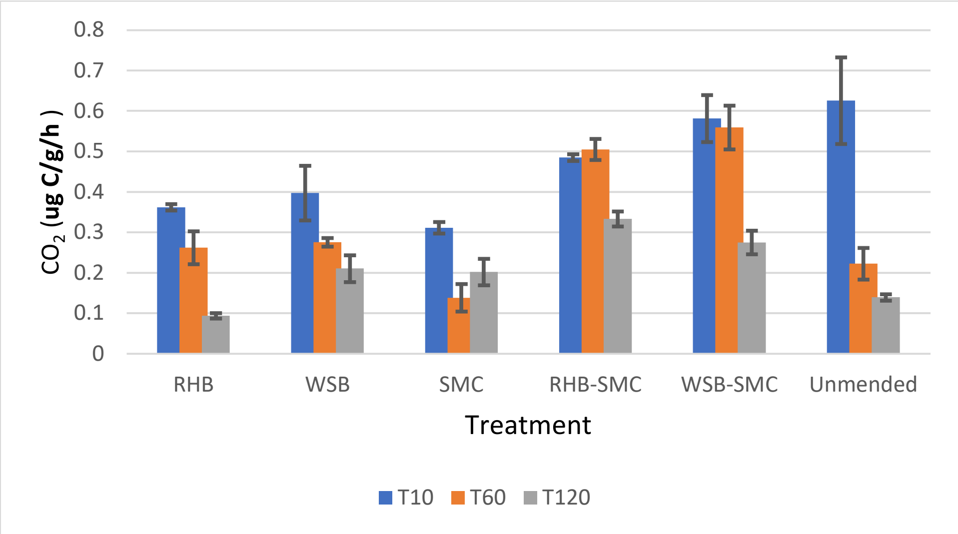
4.3.4. The influence of bioamendments on the non-saline and saline soil microbial activities

4. 3.4.1 Microbial function (basal respiration rate)

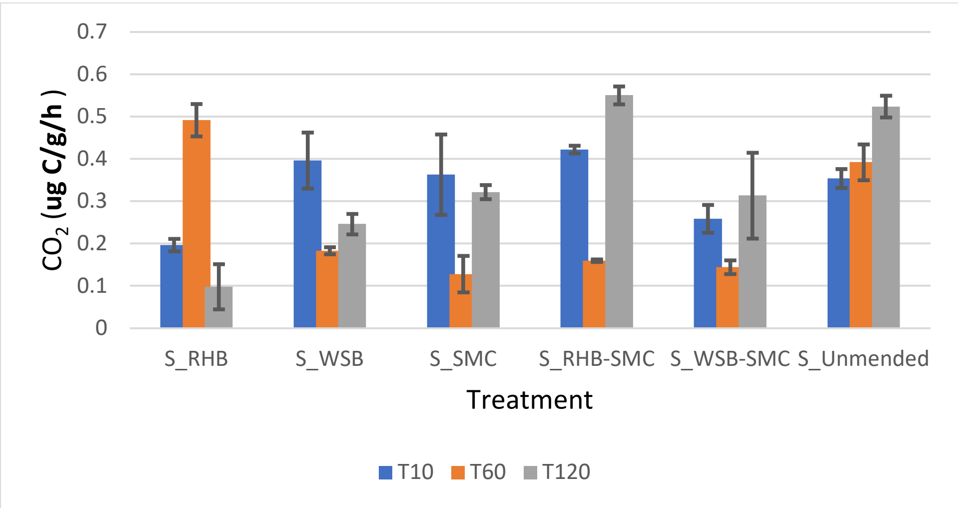
CO_2 production and microbial community changes can both be used as indicators of degradation rates (Chi and Hieu, 2017). The effects of the treatments were seen for both the non-saline and the saline soils in the measurable increase in CO_2 production (Figure 4.6), hence the characteristics of biochar and spent mushroom compost were able to influence the soil microbial activities in the soils amid the crude oil contamination and the salinity of the soil. Previous studies have reported that bioamendments, such as biochar and compost substrates, can improve soil function, including respiration (Yazdanpanah, Mahmoodabadi and Cerdà, 2016; Cipullo *et al.*, 2019).

In non-saline soil, the amount of CO_2 produced decreased over time from 10 to 120 days in most treatments, including RHB, WSB, and SMC, indicating a decline in microbial activity. However, the mixed treatments (RHB-SMC and WSB-SMC) showed 56% and 60% higher CO_2 production than the control at 60 days, followed by a decline towards 120 days. This suggests that the addition of SMC to RHB-SMC and WSB-SMC may have enhanced the microbial potential, leading to increased CO_2 production. The decrease in soil respiration in the other treatments could be attributed to a decrease in carbon availability as degradation progressed. Biochar is known for its ability to sequester carbon in soil for long periods of time, potentially reducing CO2 emissions to the atmosphere. Biochar can lock up carbon, preventing it from being released as CO2. In cases where biochar is added to soil and there is a net accumulation of carbon in the soil, the overall CO2 production may decrease as carbon is sequestered in the biochar-amended soil (Coxa *et al.*, 2017).

In saline soil, all treatments (except RHB) showed a decrease in CO_2 production at 60 days, followed by an eventual increase at 120 days. The RHB-SMC treatment showed the highest CO_2 production at 120 days, characterized by a sharp decline at the beginning then followed by an increase. This pattern may be attributed to the effects of the contaminants and their subsequent remediation promoted by the bioamendments. The toxicity of the crude oil and the salinity effect had an impact on microbial function, which was later seen to have been mitigated by the effects of the bioamendments. Previous studies have also observed that saline soils inhibit microbial activity, leading to reduced CO_2 production, and bioamendments can increase mineralizable carbon pools and enhance microbial activity by directly supplying carbon or mitigating chemical stress induced by contaminants (Kruger *et al.*, 2020). Biochar has also been shown to reduce oxidation and osmotic stress, promoting microbial activity in saline soils (Vasconcelos, 2020). Additionally, spent mushroom compost application can improve soil physicochemical properties, which in turn benefit soil microbial activities (Gumus and Seker, 2017).



(a) Non saline soil



(b) Saline soil

Figure 4. 6. Soil respiration expressed as CO_2 production (µg CO_2/g soil) for treatment with rice husk and wheat straw biochar, spent mushroom compost, or un-amended tested at 10, 60, and 120 days. (a) Non saline soil (b) Saline soil. The error bars represent the standard error for each treatment's replicates.

4.3.4.2 Microbial activities occasion by multiple induced substrate respiration

Sustainable remediation of contaminated soil requires efficient preservation of soil function and microbial decomposition and conversion of substrates is a crucial soil function (Kaurin and Lestan, 2018). The substrate-induced respiration (SIR) technique involves measuring the rate of microbial respiration in samples that have been amended with an excess of a readily available nutrition source, typically glucose (Aira and Domínguez, 2010). We carried out a substrate addition assay using sugars and organic acids, including oxalic acid, malic acid, gamma aminobutyric acid, alpha ketoglutaric acid, citric acid, glucose, and hydroxy propyl cyclodextrin, in that sequence of increasing complexity. The CO_2 production from the utilisation of the substrates were calculated and subjected to principal component analysis and the variations of the CO_2 responses of the treatments were plotted on graphs (Figure 4.7).

The result showed that from 10-120 days, the bioamendments caused changes in soil function (respiration), which were reflected in the pattern of all substrate utilisation. However, the differences between non-saline and saline soils were not significant ($p=0.8204$). At 60 and 120 days, the saline soil biochar treatments, particularly RHB, have a distinct appearance from other treatments, which could be an indication of the biochar's influence in mitigating the effect of salinity in the soils.

The two biochars, WSB for the non-saline soils, and RHB for the saline appeared to favour most of the microbial activity due to the substrates tested. Biochar properties may have promoted the soil microbial activity where as reported, are said to serve as a potential porous habitat for microbial growth and protection from predators, providing mineral nutrients, and improving soil basic properties such as increase in soil pH, aeration and water holding capacity (Dai *et al.*, 2021).

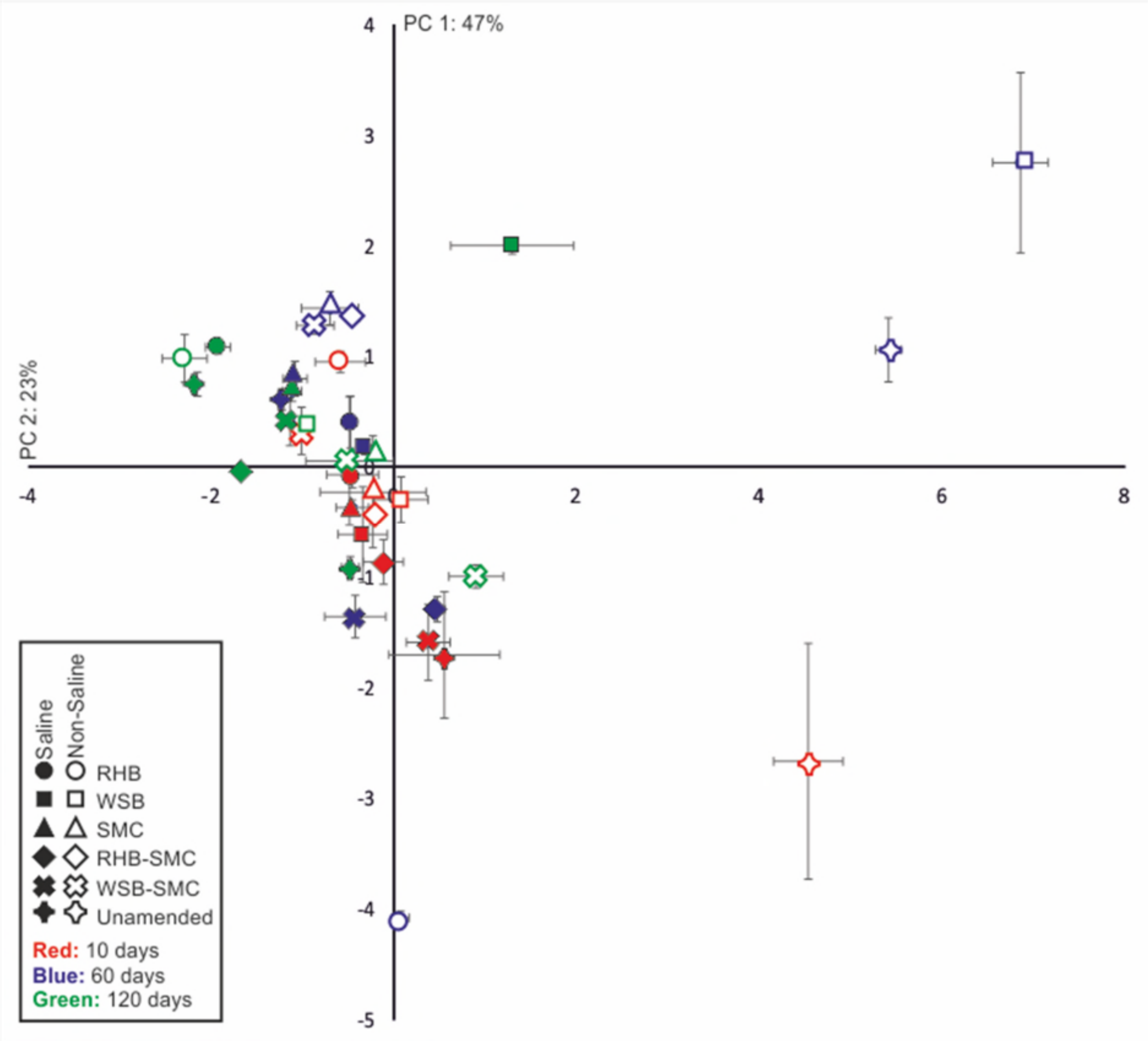


Figure 4. 7. Non-saline and saline soil microbial activity from the multiple substrates induced respiration of 7 substrates (oxalic acid, malic acid, gamma aminobutyric acid, alpha ketoglutaric acid, citric acid, glucose, and hydroxy propyl cyclodextrin) and water from the soil treatment involving RHB: rice husk biochar; WSB: Wheat straw biochar; SMC: Spent mushroom compost, and a mixture of the two biochar with Spent mushroom compost (RHB-SMC, WSB-SMC)

4.3.5. Correlation between total petroleum hydrocarbon and microbial community responses.

The total petroleum hydrocarbon, TPH (alkanes+PAHs) were plotted along with the microbial relative abundance (PLFA mol %) and microbial activity (respiration) (Figure 4.8a-f and 14a-f). The strength of the correlations between the TPH and the microbial responses data can provide an indication of which treatments promoted hydrocarbon degradation because of microbial abundance and their activity in the various soils. A strong negative correlation would favour this position as observed in our previous study and other reports (Ławniczak *et al.*, 2020). On the other hand, if there is a strong positive correlation between the relative abundance of microbes and their activity, it can indicate a prediction of high activity, which is expected in a healthy soil with a high biomass. Conversely, if there is high activity but low biomass, it may suggest the presence of environmental stress, as reported by Fließbach, Martens, and Reber (1994).

In the non-saline soil treated with WSB (Figure 4.8b), a strong negative correlation was observed between total petroleum hydrocarbon (TPH) levels and microbial relative abundance as well as microbial activity. This suggests that promoting microbial abundance could lead to enhanced activity, such as degradation of hydrocarbon substrates in the environment, as supported by previous studies (Chikere, Okpokwasili and Chikere, 2011; Ławniczak *et al.*, 2020; Pandolfo, Barra Caracciolo and Rolando, 2023). Similar positive correlations between microbial relative abundance and microbial activity were observed in WSB and SMC treatments (Figure 4.8b, c), indicating that higher biomass may indeed result in increased activity. The statistical significance of these correlations was established.

In the saline soil treatments (Figure 4.9a-f), there were correlations between TPH levels and microbial relative abundance as well as microbial activity, but the strength of these correlations varied among the treatments. Strong correlations were observed in RHB, SMC, RHB-SMC, and WSB-SMC treatments, while low correlation was observed in WSB treatment, and moderate correlation was observed in the Unamended treatment. Strong, moderate, and low correlations between microbial relative abundance and microbial activity were observed in RHB-SMC, WSB-SMC, and SMC treatments, respectively.

These findings confirmed the remediation strategy efficiency by identifying the presence of a relationship between the microbial population and hydrocarbon degradation, as well as the varied strengths of the associations. All other variables being equal, biochar, SMC, and a mixture of both can be administered at these rates and times to yield a predicted hydrocarbon degradation outcome. The method performance is seen particularly well in WSB and SMC for non-saline soils and RHB, SMC, RHB-SMC, and WSB-SMC for saline soils. This can aid decision-making in determining remedial measures and can serve as the foundation for retesting for validation and standardisation.

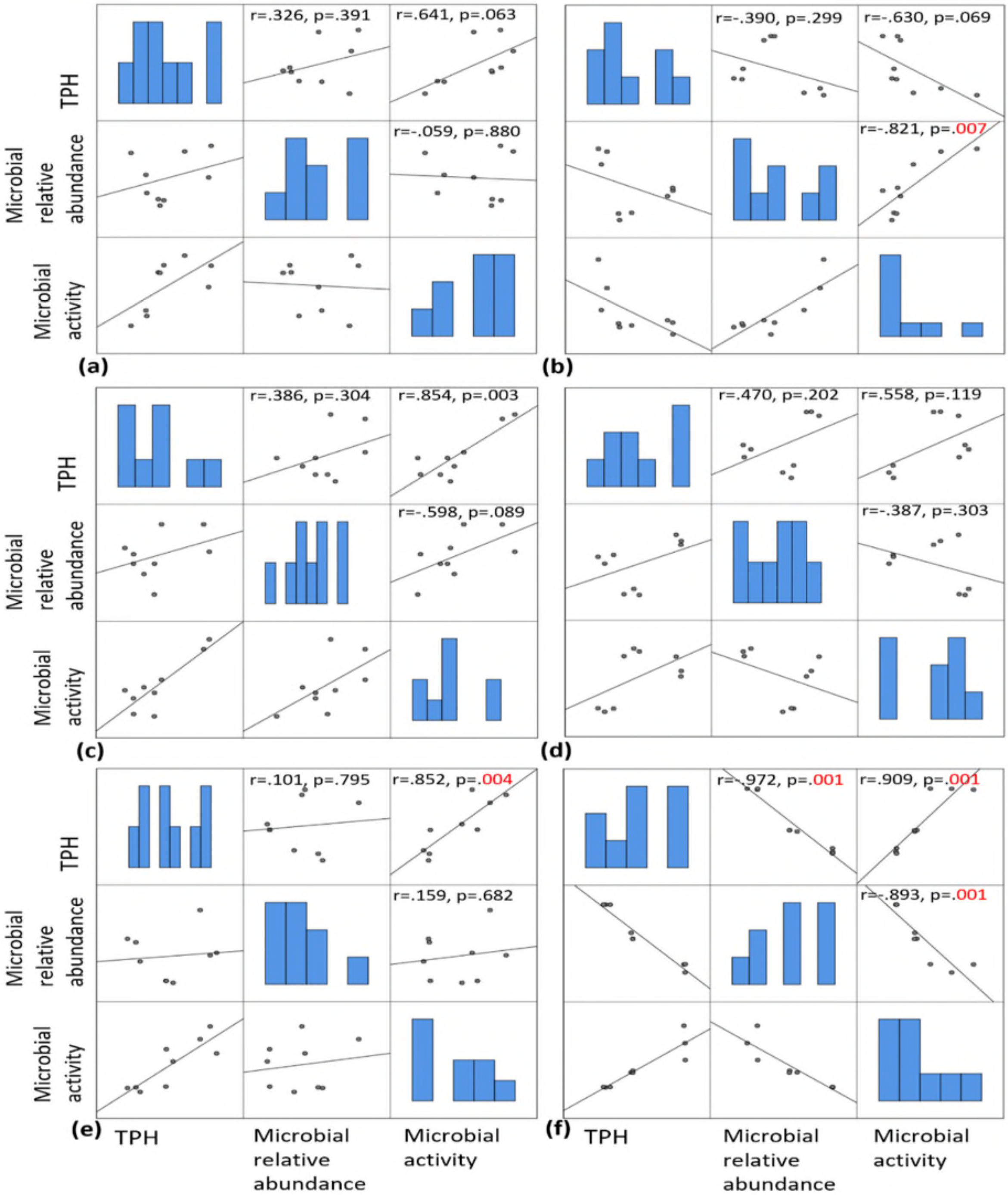


Figure 4. 8. Linear correlation (based on Pearson coefficient) between TPH concentrations and the microbiological responses (PLFA, soil respiration) in the various soil treatments of the **Non-Saline soil**, RHB (a), WSB (b), SMC (c), RHB-SMC (d), WSB-SMC (e), unamended (f).

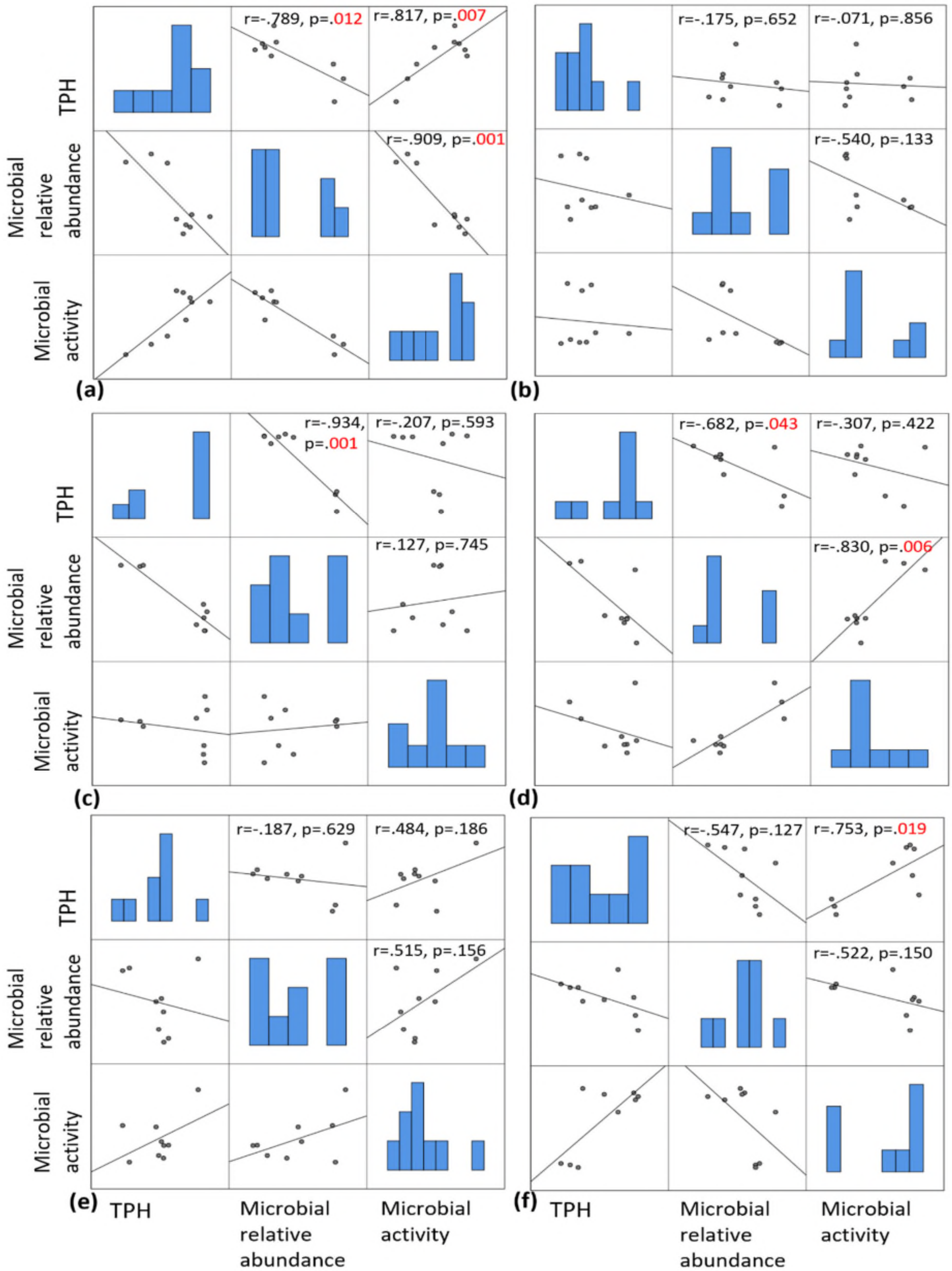


Figure 4. 9. Linear correlation (based on Pearson coefficient) between TPH concentrations and the microbiological responses (PLFA, soil respiration) in the various soil treatments of the **Saline soil**, RHB (a), WSB (b), SMC (c), RHB-SMC (d), WSB-SMC (e), unamended (f).

4.4. Conclusion

The impact of bioamendments on the hydrocarbon and microbial communities has been evaluated in both non-saline and saline soils indicating that from the initial TPH concentration, there was an average total degradation from the five treatments of 67% and 18% in the non-saline and saline soils, respectively. In non-saline soils, the biodegradation of total petroleum hydrocarbons (TPH) was approximately four times higher compared to saline soils, with the most pronounced degradation observed in the RHB-SMC (15%) and SMC (16%) treatments for alkanes, and in the WSB-SMC (27%) and RHB (49%) treatments for polycyclic aromatic hydrocarbons (PAHs), in non-saline and saline soils, respectively. The biochars, RHB and WSB, had the greatest impact on hydrocarbon biodegradation in saline soils, effectively mitigating the adverse effects of salinity on degradation, particularly for PAHs.

The shift in the microbial community was along the two soil types. The bioamendments reduced the spread of community variances, which suggests a decline in soil complexity, mostly due to hydrocarbon degradation. As a result, the introduction of biochars and spent mushroom compost in the treatments was able to impact the microbial communities in the treatments, resulting in contaminants remediation. The RHB and WSB had the most impact in reducing the effect of soil salinity on microbial populations.

Increased microbial biomass and activity were associated with higher degradation rates, with the WSB treatment showing the most significant correlation in non-saline soils, and RHB, SMC, RHB-SMC, and WSB-SMC treatments showing correlation in saline soils.

Overall, all the bioamendments had a significant positive impact on mitigating the effects of salinity on hydrocarbon degradation, microbial community abundance, and function. The RHB and WSB biochars were particularly most effective, and the addition of SMC further enhanced their performance.

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5. Overall discussion and implications of the PhD research findings

5.1. Introduction

This PhD research addressed the development of a biotechnological approach for the best use of biochar and spent mushroom compost to promote microbial remediation of soil contaminated with complex chemical mixture contaminants – primarily petroleum hydrocarbons and metals. Specifically, two types of soil: genuinely contaminated soil from legacy site and an artificially contaminated soil. Hence, the study explored a long term and simulated recent contamination.

To address this aim, two laboratory experiments were designed to (i) evaluate the effects of biochar and/or spent mushroom compost on the fate and behaviour of hydrocarbons and metals(loids), as well as (ii) their influences on the soil microbial changes and activities in the context of remediation; (iii) give an opportunity to link the biological activity, hydrocarbons and metal(loids) bioavailability, and toxicity to define remediation end-point; and to (iv) investigate the influence of biochar and spent mushroom compost in promoting petroleum hydrocarbon degradation, increase in microbial community abundance and function in a saline soil. This chapter presents the key findings as well as an overview of how the various objectives contributed to achieving the aim of the PhD study. (Figure 5.1).

Overall, this research discovered beneficial knowledge regarding the effects of biochar and spent mushroom compost on major physicochemical and biological factors influencing the biodegradation and detoxification of hydrocarbons and metals/metalloids in contaminated soils (Chapter 2). The study shed new light on the influence of low carbon input soil amendment on the behaviour and fate of metal(loids) and petroleum hydrocarbons, and the underlying microbial community responses, bringing the understanding that biochar combined with spent mushroom compost provide innovative solutions for bioengineering soil microbiology for recovery of hydrocarbon and metals from soil (Chapter 3). This research provides an opportunity to define end points of remediation that are safe for land reuse (i.e., agriculture, crop production, etc.) through the establishment of the link between microbial function, hydrocarbon and heavy metal bioavailability, and toxicity (Chapter 3). The study also provided understanding that the use of biochar and spent mushroom compost offers opportunity to alleviate the effect of salinity on microbial community and function during hydrocarbon biodegradation (Chapter 4). These findings addressed the sustainable remediation indicators of SURF-UK (see 5.3 research implication, Table 5.1).

Figure 5. 1. Schematic connections between the study's objectives (1–5) and its contribution to the land remediation industry.

Figure 5. 2. Schematic connections between the study's objectives (1–5) and its contribution to the land remediation industry.

5.2. Key findings and knowledge gaps addressed by this research

Global climate change necessitates the need to reduce the carbon footprint, with a focus on reducing, recycling, and reusing materials (Sizirici *et al.*, 2021). Biochar is a carbon sink because, after being thermally pyrolyzed, the carbonaceous materials remain inert and can remain in the soil for hundreds of years (Lehmann and Joseph, 2009). Spent mushroom compost, on the other hand, is a waste product of the mushroom production industry whose disposal has frequently been a problem (Zhang and Sun, 2014); thus, channelling this material for alternative environmental uses, such as soil bioengineering for remediation, is a worthwhile endeavour. Similarly, as evapotranspiration rises because of climate change, soil may become more saline, affecting soil functions (Yan *et al.*, 2015a). Coastal soils are another saline environment because of the constant interaction with sea water. Crude oil contamination of saline soils may impede remediation efforts. Therefore, it is crucial to research how well these materials work to understand interactions of salinity with microbial community development during hydrocarbon remediation.

Chapter One outlined the research knowledge gaps that drove this study's aim and objectives, specifically the need for: (1) identifying sustainable materials that could be deployed for soil bioengineering to improve microbial community structure and function in remediation; (2) improving appreciation of the science underlying the mechanistic effects of biochar and spent mushroom compost on soil properties, hydrocarbon and metals bioavailability, and microbial community structure and function; (3) determining the efficacy using these materials for remediation in the face of climate change effects such as increasing soil salinity.

Chapter Two involved the critical review of literature on soil pollution as a global issue, with petroleum hydrocarbons and derived products and metals being the two major contaminants frequently encountered on contaminated sites. And, despite over 50 years of environmental remediation research, there has not been complete agreement on the best approaches for remediation due to a lack of standardisation (Cipullo *et al.*, 2018b), and success has been based on case-by-case situations (Ortega-Calvo *et al.*, 2015). The current emphasis on sustainable remediation and the use of sustainable materials such as biochar and spent mushroom compost as soil bioengineering candidates for contaminant remediation was highlighted.

The review went on to provide a summary of the effects of hydrocarbons and metals on soil microbiology. Indicating the factors influencing the metabolism of oil degrading bacteria in soils, as well as how these factors can be mitigated using soil bioamendment to strengthen the effects of indigenous communities for remediation.

The use of biochar and spent mushroom compost was discussed, highlighting their relevant properties which are explored for soil bioamendments in hydrocarbon and metal soil remediation, also their pros and cons, and the research needs.

In Chapter Three, a four-month microcosm experiment was set up to study the effects of rice husk and wheat straw biochar, as well as spent mushroom compost amendments on/and (i) the fate and behaviour of hydrocarbons and metals(loids), (ii) how the amendments influence the soil microbial degradation rates and dynamics, (iii) to provide an opportunity to link the biological activity, bioavailability of hydrocarbons and metals(loids), and toxicity to define remediation end-point, in a genuinely contaminated soil obtained from former gasworks site having petroleum hydrocarbon (1493.34 mg/kg) and metals (642.8 mg/kg) contamination. Total exhaustive organic compound extraction was carried out with dichloromethane: hexane, and pseudo-total element digestion was carried out with aqua regia according to the ISO 11047 method. Furthermore, non-exhaustive hydroxypropyl-β-cyclodextrin (HP-β-CD) solutions (organics) extraction were used for organic bioavailable fractions. Phospholipid fatty acid analysis (PLFA) (microbial community structure), and multi substrate induced respiration assay (microbial activity), and ecotoxicity (Microtox® basic solid phase test) were determined.

The average effect of the five bioamendments on TPH (1493 mg/kg) reduction was 92%. SMC and WSB-SMC had the highest degradation rates, both at 93%. Compared to the control (91%), the bioamendments did not significantly affect the extend of TPH removal. However, at 120 days, the bioamendment reduced the high molecular weight (HMW) PAH better than the control. This is most noticeable in the RHB-SMC for both EC17-20 (59.67%) and EC21-35 (62.24%) of total PAH concentration. And in terms of HMW bioavailable PAH concentration, WSB-SMC outperformed EC17-20 (88.80%). The bioamendment affected the metals partitioning and distribution after 120 days of treatment leading to the decreased in the available phase fractions. The microbials groups Gram positives, Gram negatives, and fungi increasing by 4%, 8%, and 38%, respectively, particularly in SMC and mixed treatments (RHB-SMC and WSB-SMC). This was mirrored in increased microbial soil respiration. After 120 days, low metals (177.6±5 mg/kg) and TPH (21.2±7% mg/kg) bioavailability translated into higher EC50 (10624±710mg/L), indicating lower toxicity.

The following are the novelties derived from this study. The demonstrated association between TPH and metal bioavailability and toxicity, as well as microbial relative abundance and activity, raised confidence in adopting the bioavailability concept to characterise environmental contaminant risk. The study also found that, while engineered green and sustainable remediation might speed up the process, it is not always essential, and that monitoring natural attenuation may be adequate for site restoration. This method also reduces metal bioavailability while promoting the breakdown of high molecular weight PAHs. As a result, a practical green and sustainable technique for HMW PAH remediation from soil, as well as minimising metal toxicity by reducing available phase fractions, has been demonstrated. This approach offers the possibility of overcoming several important bioremediation limitations. In this case, using a biochar-SMC mix is a technique for overcoming soil nutrient constraint, increasing sorption/decrease bioavailability of chemicals (hydrocarbons and metals), and enhancing surface contact of contaminants with the soil microbial population. Finally, a bioamendment remediation technique that not only focuses on contaminants risk reduction but also includes a component to define the endpoint of the remediation.

Chapter Four had to do with a second four-month microcosm experiment was set up to study the effects of rice husk and wheat straw biochar, as well as spent mushroom compost amendments on petroleum hydrocarbon, microbial community abundance and activity in saline soil. This was carried out by spiking soil with 10% crude oil and 1% salt giving rise to two soil types (non-saline and saline). Total exhaustive organic compound extraction was carried out with dichloromethane: hexane (TPH), Phospholipid fatty acid analysis (PLFA) (microbial community structure), and multi substrate induced respiration assay were done (microbial activity). The datasets obtained in this study were used to confirm or not if salt is influencing TPH biodegradation, respiration, or microbial community and if the bioamendments helped to mitigate the salt effects and promoted degradation of the petroleum hydrocarbons.

The initial TPH concentrations were 5871±361mg/kg and 3471±138mg/kg, from which the average total degradation from the five treatments was 67% and 18% in the non-saline and saline soils, respectively. As against the control, biodegradation was most pronounced for alkanes in RHB-SMC (15%) and SMC (16%) treatments, and for PAH, WSB-SMC (27%) and RHB (49%) treatments in non-saline and saline soils. RHB and WSB were the treatments least affected by salinity as reflected by the shift in microbial communities. Microbial groups of actinomycetes and fungi demonstrated greater tolerance to soil salinity, particularly in the RHB and WSB treatments. At 60 days, microbial activity in RHB-SMC and WSB-SMC produced 56% and 60% more CO2 than the control in non-saline soil, respectively. At 10 and 60 days, the saline soil treatments produced 32% and 46% less CO_2 than non-saline soils, respectively, which was reversed at 120 days, particularly in the RHB-SMC treatment.

This study brings attention to the issue of soil salinity, which may be exacerbated by climate change, and highlights the potential complications it poses for petroleum hydrocarbon soil remediation and microbial degradation processes. The study developed a remediation strategy that utilises inexpensive and readily available low carbon and industrial waste materials to create a mixture to mitigate the effects of salinity, promote microbial growth and activity, culminating in the biodegradation of petroleum hydrocarbons. This approach offers a green solution for soil pollution in the context of climate change-induced impacts on soils, coastal soils, and other high-salinity soils.

5.3. Research implications

Sustainable remediation: Remediation strategies to mitigate against environmental pollution has evolved over a 50 year period (Ellis and Hadley, 2009; Petruzzelli *et al.*, 2016). However, sustainability has recently gained prominence in the field of environmental remediation (Ellis and Hadley, 2009), which entails addressing of risks in a safe and timely manner while also maximising the environmental, social, and economic benefits of the remediation work (SurF-UK, 2010).

This has prompted the development of remediation strategies that include sustainable remediation indicators in their remedial actions (Table 5.1). Efforts toward sustainable remediation may necessitate the use of soil amendments made from biological materials that are readily available or easily sourced, do not result in a scarcity of useful products, and contribute to a reduction in the carbon burden on the environment. The remediation strategy developed in this study took these criteria into account and meets them significantly. This is because the use of biochar for complex chemical mixtures soil remediation can be considered a sustainable, efficient, and cost-effective approach (Ahmad *et al.*, 2014a; Guo *et al.*, 2015; Oliveira *et al.*, 2017a; Cipullo *et al.*, 2019). Biochar, a carbon-rich substance created by the thermal breakdown of organic material possess exceptional properties and has been used in a variety of applications, including soil amelioration, environmental remediation, and climate change mitigating through high carbon sequestration (Guo *et al.*, 2015; Oliveira *et al.*, 2017b). Spent mushroom compost (SMC), an equally important product of agricultural waste industry, a by-product of mushroom production, which is likely to contain diverse groups of microorganisms and extracellular enzymes, fungal mycelia, all of which are important for the biotransformation of contaminants.

In general, the use of biochar is a low carbon input strategy because it is produced by pyrolyzing organic waste materials, and the resulting biochar, when applied to soil, remains inert for 100s of years (Guo *et al.*, 2015), making it a carbon sink approach. The alternative use of SMC, an industrial waste, for soil bioremediation solves the mushroom production industries' massive waste disposal problem.

Table 5. 1. Expansive categories of indicators for sustainability assessment of remediation options (SurF-UK, 2010). Light blue table represent research consideration of the indicators

	Environmental	**Social**	**Economic**
SURF-UK sustainable remediation indicators	1. Impacts on air (including climate change) 2. Impacts on soil 3. Impacts on water 4. Impacts on ecology 5. Use of natural resources and generation of wastes 6. Intrusiveness.	1. Impacts on human health and safety 2. Ethical and equity considerations 3. Impacts on neighbourhoods or regions 4. Community involvement and satisfaction 5. Compliance with policy objectives and strategies; 6. Uncertainty and evidence.	1. Direct economic costs and benefits 2. Indirect economic costs and benefits 3. Employment and capital gain 4. Gearing 5. Lifespan and 'project risks' 6. Project flexibility.

	Environmental	**Social**	**Economic**
Various ways the present work considered the SURF-UK sustainable remediation indicators	▪ The use of biochar is a carbon negative strategy because the pyrolised carbon remain inert in soils ▪ Hydrocarbons and metals are locked and prevented from leaching into ground water ▪ Biochar and SMC improve soil properties as well as microbial community and function ▪ Biochar and spent mushroom compost are all products from agricultural and industrial waste ▪ Strategy to promote remediation in saline soils where salinity may be caused by climate change	▪ Addressing the risk of hydrocarbons and metals which may bioaccumulate and negatively impact human health in the long run ▪ Since the materials used are waste, there will be no shortage of other useful products ▪ Secondary contamination is significantly reduced ▪ Agricultural waste generated may be sold to generate income ▪ These strategies were consistent with the principles of the circular economy, particularly the reuse of materials.	▪ This strategy included remediation endpoints, which detect when soils are no longer toxic, preventing costly continuous work. ▪ The use of waste indicates that materials are readily available or easily obtained.

Unclear biochar-microbe interactions: Biochar and spent mushroom compost interactions in soil induces diverse responses in microbial communities leading to changes in soil enzyme activity, reshaping of microbial community structure and consequent enhancement of contaminants transformations (Zhu et al., 2017). However, the mechanisms underlying these interactions require more investigation. Hence, an understanding of biochar-microbe interactions is paramount to appreciating the link between biochar properties with soil functions, especially contaminant degradation (Yuan *et al.*, 2019). Equally, biochar physical properties vary substantially depending on feedstock types and pyrolysis temperatures (Yuan et al., 2019; Oliveira et al., 2017b). This has given rise to the challenge where, the inconsistency in the physicochemical and functional properties of different biochars makes match-making them with soil microbial degraders unreliable, and their subsequent use in soil remediation unpredictable.

The use and comparison of different biochar (two in this case for the sake of time and resources) and different application rates (5% and 2.5), the determination of their efficacies on hydrocarbons and metals, as well as their influences on the microbial community, is how this study contributed to bridging this gap. Further research (see section 5.5) should focus on selecting the best performing biochar for a specific soil type and condition, application rate, and then standardising the method for that specific biochar based on its effects on contaminants and microbes.

Single contaminant and the use of total concentration: Remediation success and some remediation frameworks frequently use reduction of total contaminant concentration to defined soil risk from the contaminant rather than use of bioavailable concentrations, which is the fraction to which receptors respond to, i.e. able to reach cellular membrane of organisms (Cipullo *et al.*, 2019). Furthermore, the focus of evaluating contaminant effects is frequently on a single contaminant rather than mixtures, despite the fact that contaminants in polluted soil frequently occur as a complex mixture of contaminants (Kienzler *et al.*, 2016).

The important ability of biochar to sequester pollutants allows for a reduction in the bioavailability of both organic and inorganic pollutants in contaminated soil (Yuan *et al.*, 2019). Consequently, this work investigated the effects of bioamendments on soil from a site determined to be contaminated with complex chemical mixtures (petroleum and petroleum derived products and metals) and tracked bioavailability changes during treatment as well as corresponding changes in soil toxicity. We also discovered a link between TPH bioavailability and metal toxicity and soil microbial responses.

Climate change: As previously stated, using biochar is a carbon-negative approach because biochar is a carbon sink that, when added to soil, does not pose a carbon burden. And SMC are materials derived from agricultural and industrial waste, thereby encouraging material reuse, and contributing to the reduction of environmental carbon burden. Similarly, climate change is now an important factor that has a significant impact on bioremediation. Climate change and rising temperatures may cause an increase in evapotranspiration, which includes the evaporation of water from soils. Furthermore, as water evaporates, salt accumulates in the soil, increasing soil salinity (Khamidov *et al.*, 2022). Hence, testing these bioamendments on saline soils and the resultant knowledge of the fact that they contribute to circumventing salinity-related limitations on the function of microbial crude oil biodegradation is a step in the right direction.

Generally, the remediation strategy developed in this study provides an opportunity to overcome soil nutrient limitation (via the SMC's rich organic matter and enzymes), increase sorption/decrease bioavailability of chemicals (via the sorption properties of biochar and SMC due to their surface properties), and increase surface contact of contaminants with the soil microbial community (they often serve as surfaces for microbial growth from which the microbes feed on the contaminants adsorbed onto their surfaces; they also mediate microbial election transfers)

5.4. Limitations of the research

- The soil samples of Experiment one (Chapter 3) were obtained by proxy from a treatment site, with no additional information on the site, its geology, or reason for the specific sampling points; consequently, our appreciation of the impacts of anthropogenic and soil structural contribution on complex chemical mixture bioavailability, as well as the outlook of the contaminants from the different soils in the site, is limited.
- Due to limited laboratory access as a result of COVID 19, experiment time points were reduced from 5 to 2 in Chapter 3 and from 5 to 3 in Chapter 4. This has resulted in fewer time points affecting the amount of data required to study the effects of

bioamendments on contaminants' behaviours and microbial community responses to make better judgments and draw more effective conclusions.

- While the toxicity test used in this study (microtox basic solid test) focused on the simplest form of life (microbial), the toxicity study was based on a single ecotoxicity test rather than an array of bioassays that should have included seed germination and earthworm lethality tests. As a result, this result should be interpreted with caution because it cannot be generalised since it should have been supported by other bioassays.
- In-depth microbial community studies like the sequencing of of 16S and 18S rRNA genes to thoroughly understand the microbial dynamics, changes in the communities, whether species functional resistance, resilience, or redundancy, were not conducted due to a lack of available equipment, time constraints, and, most importantly, the ability to draw sufficient conclusions without that aspect because the emphasis was placed more on the efficacies of the treatments administered.

5.5. Further research recommendations

Future work should concentrate on:

- Choosing the best performing biochar for a specific soil type and condition, application rate, and standardising as a method for that specific biochar based on its repeated and validated effects on contaminants and microbes.
- The soil ecotoxicity analysis should be expanded to include other bioassays in order to produce more reliable and accurate information, reducing uncertainties. Consider the toxicity of complex chemical mixtures in comparison to single contaminants. For example, hydrocarbons and metals should be estimated separately for accurate estimation of environmental quality standards.
- In-depth microbial community studies to thoroughly understand the microbial dynamics and changes in the communities, including functional resistance, resilience, or redundancy, as well as specific microbial families and species driving the changes, in order to select the most effective species for use in bioaugmentation remedial actions.
- Decision support tools and predictive models based on more result data (expanded sample number), broader soil types, and concentration choices must be considered to aid decision making and save money and resources during this type of remediation.

5.6. References

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