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Composting of organic waste for enhanced bioremediation of PAHs contaminated soils

Borislava Lukic

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Borislava Lukic. Composting of organic waste for enhanced bioremediation of PAHs contaminated soils. Materials. Université Paris-Est, 2016. English. NNT : 2016PESC1134 . tel-01735701v2

HAL Id: tel-01735701

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**COMPOSTING OF ORGANIC WASTE
FOR ENHANCED BIOREMEDIATION
OF PAHS CONTAMINATED SOILS**

PhD Thesis Committee

Reviewers

Prof. Paolo Roccaro
University of Catania
Catania, Italy

Dr. Ana Paula de Campos Mucha
CIIMAR - Interdisciplinary Centre of Marine and Environmental Research
University of Porto
Porto, Portugal

Examiners

Dr. Hab. E.D. van Hullebusch
Université Paris-Est
Marne-la-Vallée, France

Dr. D. Huguenot
Université Paris-Est
Marne-la-Vallée, France

Promotor

Prof. G. Esposito
University of Cassino and Southern Lazio
Cassino, Italy

Co-Promotors

Prof. Michel Madon
Université Paris-Est
Marne-la-Vallée, France

Prof. P.N.L. Lens
UNESCO-IHE Institute for Water Education
Delft, The Netherlands

Co-Supervisor

Dr. A. Panico
Telematic University Pegaso
Naples, Italy

This research was conducted under the auspices of the Erasmus Mundus Joint Doctorate Environmental Technologies for Contaminated Solids, Soils, and Sediments (ETeCoS³).

Joint PhD degree in Environmental Technology



Docteur de l'Université Paris-Est
Spécialité : Science et Technique de l'Environnement



Dottore di Ricerca in Tecnologie Ambientali



UNESCO-IHE
Institute for Water Education

Degree of Doctor in Environmental Technology

Tesi di Dottorato – Thèse – PhD thesis

Borislava Lukić

Composting of organic waste for enhanced bioremediation of PAHs contaminated soils

To be defended December 15th, 2016

In front of the PhD committee

Prof. Paolo Roccaro	Reviewer
Dr. Ana Paula de Campos Mucha	Reviewer
Dr. Hab. Eric D. van Hullebusch	Examiner
Dr. David Huguenot	Examiner
Prof. Giovanni Esposito	Promotor
Prof. Michel Madon	Co-Promotor
Prof. Dr. ir. Piet Lens	Co-Promotor
Dr. Antonio Panico	Co-Supervisor



Erasmus Joint doctorate programme in Environmental Technology for Contaminated Solids, Soils and Sediments (ETeCoS³)

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- ADL: Acid determined lignin
ADS: Acid detergent solution
ANOVA: Analysis of variance
Anth: Anthracene
B(a)P: Benzo(a)pyrene
B(k)F: Benzo(k)fluoranthene
BAF: Bioaccumulation factor
BM: Buffalo manure
BOD: Biochemical oxygen demand
BTEX: Benzene, toluene, ethyl benzene, and xylenes
C: Carbon
CEC: Cation exchange capacity
CFU: Colony forming unit
CHC: Chlorinated hydrocarbons
Chry: Chrysene
CO₂: Carbon dioxide
COD: Chemical Oxygen Demand
d/w: Dry weight
DHA: Dehydrogenase activity
EEA: European Environment Agency
EPA: Environmental Protection Agency
ETeCoS³: Environmental Technologies for Contaminated Solids, Soils and Sediments
EU: European Union
FKW: Food and kitchen waste
foc: Mass fraction of soil organic carbon content
FVW: Fruit and vegetable waste
GC/MS: Gas chromatography/mass spectrometry
H: Hydrogen
HDPE: High density polyethylene
HMW: High molecular weight
HPCD: Hydroxypropyl- β -cyclodextrin
I₍₁₅₎: Inhibition at time 15 min

LIST OF ABBREVIATIONS

I₍₅₎: Inhibition at time 5 min
K: Potassium
KCl: Potassium chloride
K_d: Solid-water distribution coefficient
K_{oc}: Organic carbon partitioning coefficient
K_{ow}: Octanol-water partitioning coefficient
LMW: Low molecular weight
LOI: Loss on ignition
MPN: Most probable number
MS: Mass selective
MSW: Municipal solid waste
N: Nitrogen
NDS: Neutral detergent solution
OECD: Organisation for Economic Co-operation and Development
OM: Organic matter
P: Phosphorus
PAHs: Polycyclic Aromatic Hydrocarbons
PCA: Principal component analysis
Phen: Phenanthrene
Pyr: Pyrene
RBM: Reactor filled with soil and BM
RCF: Relative centrifugal force
RFKW: Reactor filled with soil and FKW
RFVW: Reactor filled with soil and FVW
RS0: Reactor filled with contaminated soil only without SS
RS05: Reactor filled with contaminated soil to SS mass ratio 1:0.5
RS1: Reactor filled with contaminated soil to SS mass ratio 1:1
RS2: Reactor filled with contaminated soil to SS mass ratio 1:2
RSS: Reactor filled with soil and SS
SS: Activated sewage sludge
T: Temperature
TEF: Toxicity equivalence factor
TKN: Total Kjeldahl nitrogen
TOC: Total organic carbon

LIST OF ABBREVIATIONS

TPF: 1,-3,-5- triphenylformazan

TPH: Total petroleum hydrocarbons

TTC: 2,-3,-5-triphenyltetrazolium chloride

US EPA: United States Environmental Protection Agency

v/v: Volume/volume

VOCs: Volatile organic compounds

w/w: Wet weight

WHO: World Health Organization

ρ_b : Bulk density

Acknowledgements

I would like to deeply thank the members of the Environmental Technologies for Contaminated Solids, Soils and Sediments (ETeCoS³) committee, Prof. Giovanni Esposito (University of Cassino and Southern Lazio, Italy), Prof. Eric D. van Hullebusch (Université Paris-Est, France) and Prof. Dr. ir. Piet Lens (UNESCO-IHE Institute for Water Education, The Netherlands) who gave me the opportunity to participate in this Erasmus Mundus joint doctorate programme.

I would like also to express my gratitude to the PhD thesis committee members, promotor Prof. Giovanni Esposito (University of Cassino and Southern Lazio, Italy), examiner Dr. Hab. Eric D. van Hullebusch (Université Paris-Est, France), co-promotor Prof. Dr. ir. Piet Lens (UNESCO-IHE Institute for Water Education, The Netherlands), examiner Dr. David Huguenot (Université Paris-Est, France), and co-supervisor Dr. Antonio Panico (Telematic University Pegaso, Italy). I would like to express my gratitude to reviewers of this thesis Prof. Paolo Roccaro (University of Catania, Italy) and Dr. Ana Paula de Campos Mucha (University of Porto, Portugal) for accepting to evaluate the present thesis work.

Afterwards, I would like to express my deep appreciation and gratitude to Prof. Massimiliano Fabbicino (University of Naples Federico II, Italy) and Dr. David Huguenot (Université Paris-Est, France) for scientific advices and organizational aid. This dissertation would not reach the final stage without their truly academic support to solve the scientific and technical aspects. I would like to express my sincere gratitude to Michele Rinaldi, Dr. Luigi Frunzo and Prof. Roberto Andreozzi by University of Naples Federico II (Italy), and to all personnel of Geomaterials and Environment Laboratory (LGE) by Université Paris-Est, (France) for providing me any unconditional support throughout this PhD work.

I sincerely appreciate the financial support provided by the European Commission in order to be performed this research through the Erasmus Mundus Joint Doctorate Programme ETeCoS³.

I would also like to thank my colleagues who made me feel very comfortable to work with them and to enjoy my time during this PhD work, in particular to Maria Rosaria, Nirakar, Carolina, Jimmy and all other PhD fellows of ETeCoS³ Programme.

Finally, I would like to extend my deepest gratitude to my family and friends for their infinite support and encouragement in the most difficult moments over the last three

ACKNOWLEDGEMENTS

years, and thus helping me get through the PhD program. Thanks to my mother and my deceased father to taught me about persistence, patience, diligence, modesty and how to be independent. You gave me the most precious tools for the craft that is called the life. Thanks to my brother Dejan, nephews Pavle and Đorđe, my Gianni, Ivana and all close friends for showing me always how proud you are of me and how much you love me. Your unconditional support showed me see the light even in the darkest points of the tunnel.

Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are organic pollutants widely distributed in the environment and very frequently detected in soils as they are mainly produced from an incomplete combustion of organic matter. They are toxic and even carcinogenic, therefore their removal from soils has been massively studied in the past years. Among all treatments suitable for treating soils contaminated by PAHs, biological ones are promising and challenging as they have a low or even no impact on the environment as well as their efficiency is tightly dependent on many factors not easy to control. The objective of this thesis has been to get more accurate knowledge on bioremediation of PAHs contaminated soils, by defining, through a series of experiments, the most suitable conditions for their biological remediation mainly in terms of physical and chemical soil characteristics, PAHs type and concentration, microbial density and composition, pH value, moisture content and availability of nutrients. The bioremediation experiments carried out in this thesis are based on landfarming as well as composting soil treatments and aimed at promoting the simultaneous biodegradation of PAHs and fresh organic wastes under controlled conditions. The expected result of this approach has been the conversion of organic pollutants into less harmful compounds, due to the activity of the microorganisms present in soil as well as in the organic waste added to soil. With the aim of deeply understanding the influence of the aforementioned factors on the bioremediation of PAHs contaminated soil, experiments were conducted on a synthetic soil, artificially contaminated, as well as on a real contaminated soil. In details, four different types of fresh organic wastes were selected to be added to PAHs artificially contaminated synthetic soil and the results showed that centrifuged activated sewage sludge (SS) was the most effective organic amendment compared to buffalo manure (BM), food and kitchen waste (FKW) and fruit and vegetable waste (FVW). The removal efficiency of total PAHs reached with SS was actually a little higher than 60%. Furthermore, this set of experiments proved that mesophilic conditions were more performing than thermophilic conditions, as well as the content of nitrogen, soluble fraction and proteins played an important role in the PAHs removal. Based on the previous results, the next set of experiments was conducted on a real contaminated soil amended with different amounts of centrifuged activated SS (e.g. contaminated soil to SS mass ratios were 1:2, 1:1, 1:0.5 and 1:0 as wet weight basis). In contrast to results obtained with synthetic soil, in a real

ABSTRACT

contaminated soil, SS amendment resulted in being not beneficial to PAHs removal. The best results were actually achieved where no SS was added (total PAHs removal efficiency of 32%, whereas with SS the best results showed a value of 14%), proving that the adaptation of microorganisms to PAHs is the key factor for the success of the bioremediation process coupled with setting favorable environmental conditions.

Keywords: Bioremediation, Composting, Organic waste, Polycyclic Aromatic Hydrocarbons (PAHs), Contaminated soil.

Résumé

Les hydrocarbures aromatiques polycycliques (HAP) sont des polluants organiques largement répandus dans l'environnement. Ils sont très fréquemment détectés dans les sols et sont principalement le produit d'une combustion incomplète de la matière organique. Etant toxiques et cancérigènes, de nombreuses études portant sur leur élimination des sols ont été massivement effectuées au cours des dernières années. Parmi tous les traitements disponibles pour traiter des sols contaminés par les HAP, les approches biologiques sont prometteuses car elles ont un impact limité, voire nul sur l'environnement. Cependant, leur efficacité est étroitement dépendante de nombreux facteurs difficiles à contrôler. L'objectif de cette thèse a été d'obtenir une connaissance plus précise concernant la dépollution biologique de sols contaminés par des HAP, en définissant à travers une série d'expériences, les conditions les plus appropriées pour leur élimination principalement en termes de caractéristiques physiques et chimiques du sol, de la structure des HAP et leurs concentrations, la densité et la composition microbienne, le pH et l'humidité du sol et la disponibilité des nutriments. Les expériences réalisées dans cette thèse, sont basées sur le compostage de déchets organiques avec des sols contaminés par des HAP. L'ajout de matière organique vise à promouvoir la dégradation biologique simultanée des HAP et des déchets organiques frais en conditions contrôlées. Les résultats attendus de cette approche sont la dégradation des polluants en composés moins nocifs, en raison de la stimulation de l'activité des micro-organismes présents dans le sol ainsi que dans les déchets organiques apportés. Dans le but de comprendre l'influence des facteurs précédemment mentionnés, les expériences ont été menées sur un sol synthétique, artificiellement contaminé, ainsi que sur un sol contaminé provenant d'un site industriel identifié comme pollué par des HAP. Quatre types de déchets organiques frais ont été sélectionnés pour être ajoutés au sol artificiellement contaminé par des HAP. Les résultats ont montré que les boues activées étaient l'amendement organique le plus efficace par rapport au fumier de bufflonnes, aux déchets de cuisine et aux déchets organiques à base de légumes. Un taux d'élimination des HAP totaux supérieur à 60% a été atteint avec les boues activées. En outre, cette série d'expériences a prouvé que les conditions mésophiles étaient plus favorables que les conditions thermophiles, mais également que la teneur en azote, l'importance de la fraction soluble et les teneurs en protéines sont très importantes pour l'élimination des HAP. Sur la base de ces résultats,

RÉSUMÉ

une série d'expériences a été menée sur un sol historiquement contaminé en apportant des quantités différentes de boues activées (ratio massique sol contaminé : boues activées variant de 1:2, 1:1 , 1:0,5 à 1:0). Contrairement aux résultats obtenus avec le sol artificiel, pour un sol naturellement contaminé, les amendements à base de boues activées n'ont pas stimulé l'élimination de HAP. Les meilleurs résultats ont été obtenu pour le sol non amendé par les boues activées (un rendement d'élimination des HAP totaux de 32% a été atteint en l'absence de boues activées, tandis qu'en présence d'un amendement à base de boues activées les meilleurs résultats ne dépassent pas 14% d'élimination des HAP totaux), cela prouve que la stimulation de micro-organismes pouvant dégrader les HAP est un facteur clé pour le succès du processus de dépollution biologique dans des conditions environnementales favorables.

Mots clés: Biodépollution, Compostage, Déchets organiques, Sols contaminés, Hydrocarbures aromatiques polycycliques (HAP).

Sintesi

Gli Idrocarburi Policiclici Aromatici (IPA) sono composti organici inquinanti molto diffusi nell'ambiente e soprattutto nel suolo perché prodotti da una combustione incompleta, ad alta temperatura, della sostanza organica. A causa della loro tossicità e della cancerogenità di alcuni di essi, la rimozione degli IPA dal suolo è stata oggetto negli anni recenti di numerosi studi. Fra tutti i possibili trattamenti di bonifica di suoli contaminati da IPA, quelli che ricorrono a processi biologici sono i più promettenti, anche se non semplici da gestire, dal momento che producono un impatto minimo sull'ambiente. Tuttavia la loro efficienza è strettamente legata a numerosi fattori, alcuni difficili da controllare. Questa tesi ha avuto come obiettivo l'accrescimento della conoscenza sul biorisanamento dei suoli contaminati da IPA, ricercando, attraverso una serie di studi sperimentali, le condizioni più idonee per una loro bonifica mediante processi biologici, in particolare in termini di caratteristiche fisiche e chimiche del suolo, natura e concentrazione degli IPA, densità e composizione dei microorganismi, valore del pH, contenuto di umidità e disponibilità dei nutrienti. Gli esperimenti di biorisanamento descritti in questa tesi sono basati sui principi del trattamento del suolo propri della tecnica del landfarming e del compostaggio e sono mirati a promuovere la biodegradazione simultanea degli IPA e dei rifiuti organici in ambiente controllato. Il risultato atteso dall'attività sperimentale è stata la trasformazione delle sostanze inquinanti organiche in composti meno dannosi, grazie all'attività dei microorganismi presenti tanto nel suolo quanto nei rifiuti organici che ad esso sono stati aggiunti. Allo scopo di acquisire maggiore conoscenza sull'influenza dei fattori sopra citati, sono stati condotti esperimenti sia su un suolo sintetico, contaminato artificialmente con IPA, sia su un suolo naturale, realmente contaminato da IPA. In particolare, quattro diverse tipologie di rifiuto organico sono state testate e aggiunte al suolo sintetico contaminato da IPA ed i risultati hanno dimostrato che il fango attivo centrifugato (SS) è stato l'ammendante organico più efficace rispetto al letame bufalino (BM), agli scarti di cucina (FKW) e ai residui di frutta e verdura (FVW). L'efficienza globale di rimozione degli IPA ottenuta con l'aggiunta di SS è stata di poco superiore al 60%. Inoltre, questa prima serie di esperimenti ha evidenziato che le condizioni di temperatura mesofile sono da preferirsi a quelle termofile ed inoltre che il contenuto di azoto, la frazione solubile e la quantità di proteine hanno svolto un importante ruolo nella rimozione degli IPA. Sulla base dei risultati ottenuti dalla prima serie di esperimenti, una successiva serie di

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test è stata condotta su un suolo naturale realmente contaminato al quale sono state aggiunte diverse quantità di SS (rapporto di massa tra suolo contaminato e SS pari a 1:2, 1:1, 1:0.5 e 1:0 su campione umido). Contrariamente ai risultati ottenuti con il suolo sintetico, nel suolo naturale, l'ammendante SS non si è dimostrato particolarmente efficace per la rimozione di IPA. I migliori risultati sono stati ottenuti nel test privo di SS (efficienza di rimozione degli IPA pari al 32%, mentre con SS il migliore risultato è stato il 14%), dimostrando che l'adattamento dei microorganismi agli IPA è il fattore chiave per il successo del biorisanamento di un suolo da essi contaminato, unitamente alla realizzazione di favorevoli condizioni ambientali.

Parole chiave: Biorisanamento, Compostaggio, Rifiuto organico, Idrocarburi Policiclici Aromatici (IPA), Suolo contaminato.

Samenvatting

Polycyclische aromatische koolwaterstoffen (PAK's) zijn organische verontreinigingen wijd verspreid in het milieu en zeer frequent gedetecteerd in grond, aangezien ze hoofdzakelijk geproduceerd zijn uit een onvolledige verbranding van organisch materiaal bij hoge temperatuur. Ze zijn toxisch zelfs kankerverwekkend, dus hun verwijdering uit bodems werd massaal onderzocht in de afgelopen jaren. Van alle beschikbare behandelingen, geschikt voor de behandeling van bodems verontreinigd met PAK's, is de meest belovend en uitagend de biologische behandeling omdat het een lage of geen invloed op het milieu heeft en hun efficiëntie hangt af van meerdere factoren die niet gemakkelijk te controleren zijn. Het doel van deze thesis is om nauwkeurigere kennis over bioremediatie van PAK's verontreinigde grond te krijgen, door te definiëren, door middel van een reeks experimenten, de meest geschikte voorwaarden voor de bioremediatie, vooral in termen van fysische en chemische eigenschappen van de bodem, PAK type en concentratie, de microbiële dichtheid en samenstelling, de pH-waarde, de vochtgehalte en beschikbaarheid van voedingsstoffen. De bioremediatie experimenten die in deze thesis werden uitgevoerd zijn gebaseerd op landfarming en compostering bodembehandelingen en ter bevordering van de gelijktijdige biodegradatie van PAK's en vers organisch afval onder gecontroleerde omstandigheden. Het verwachte resultaat van deze benadering is de omzetting van organische vervuulende stoffen in minder schadelijke verbindingen als gevolg van de activiteit van deze micro-organismen in de bodem in het organisch afval toegevoegd in de bodem. Met als doel de diepere inzicht in de invloed van genoemde factoren op de zuivering van PAK verontreinigde grond te krijgen, werden experimenten uitgevoerd op een kunstmatige bodem, kunstmatig verontreinigd, alsmede op een echte verontreinigde grond. Er werden vier verschillende soorten vers organisch afval gekozen te worden toegevoegd in PAK's kunstmatig verontreinigde bodem. De resultaten toonden aan dat gecentrifugeerd geactiveerd zuiveringsslib (ZS) was de meest effectieve organische wijziging in vergelijking met buffels mest (BM), levensmiddelen- en keukenafval (LKA) en groenten en fruit afval (GFA). De verwijdering efficiëntie van de totale PAK's bereikt met zuiveringsslib was eigenlijk een beetje hoger dan 60%. Deze reeks experimenten hebben verder bewezen dat mesofiele omstandigheden meer dan thermofiele omstandigheden presteren en dat het stikstofgehalte, oplosbare fractie en eiwit een belangrijke rol in het PAK's verwijdering spelen. Op basis van de vorige

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resultaten, werden de volgende reeks experimenten uitgevoerd op een echte verontreinigde grond gewijzigd met verschillende hoeveelheden geactiveerde zuiveringsslib (bijv. vervuilde grond tegenzuiveringsslib massaverhoudingen waren 1:2, 1:1, 1:0.5 and 1:0 als gewicht basis). In tegenstelling met de resultaten verkregen met kunstmatig bodem, in een echte verontreinigde grond, zuiveringsslib wijziging resulteerde als niet bevorderlijk voor PAK's verwijdering. De beste resultaten werden daadwerkelijk gerealiseerd waarbij geen zuiveringsslib werd toegevoegd (totaal PAK's verwijdering efficiëntie van 32%, waarbij met zuiveringsslib de beste resultaten een waarde van 14% vertoonden), waaruit blijkt dat de aanpassing van de micro-organismen aan PAK's de belangrijkste factor is voor het succes van bioremediatie proces in combinatie met het instellen van gunstige milieuomstandigheden.

Sleutelwoorden: Bioremediatie, Composteren, Organisch afval, Polycyclische aromatische koolwaterstoffen (PAK's), Verontreinigde grond.

Chapter 1

Introduction

Introduction

1.1. Soil contamination - global environmental concern

1.1.1. Contaminated site

Soil contamination could refer to the appearance of any chemical elements in the natural soil environment. It would occur if concentrations of such elements go beyond their concentrations naturally present in soil, or if concentrations of such elements do not exist naturally in certain environment (Environmental pollution centers, 2009). Commonly, the excessive presence of chemical elements relative to their natural content causes the soil contamination (Agnello, 2014) and results in a concern and risk to living organisms, human health and overall ecosystem. A site is called contaminated when the soil contamination is proved and it is different by a potentially contaminated site. This latter is a site where the soil contamination is suspected but not verified. Additionally, in contaminated sites a potential risk to humans and environment is present, while in potentially contaminated sites any risk of adverse impacts on ecosystem and environment should be investigated. Furthermore, remediation of contaminated sites might be requested depending on overall risk and use of the site, while remediation cannot be applied to potentially contaminated sites (van Liedekerke et al., 2014).

1.1.2. Causes of soil contamination

Soil contamination in environment could occur due to natural processes and anthropogenic activities. Nowadays, soil contamination occurs almost exclusively as human-made hazards. The most important anthropogenic sources of contamination in European countries refer to waste disposal, industrial and commercial activities, military due to performing specific military activities, storages and transport spills on land of different fuels or chemicals, nuclear due to an inappropriate disposal of nuclear waste or releases of radioactive material because of an accident and others (Antizar-Ladislao et al., 2004; Johnsen et al., 2005; Maila and Cloete, 2002; van Liedekerke et al., 2014). In particular, the recent report of van Liedekerke et al. (2014) indicated that the largest contamination in France and Italy has been caused by industrial and commercial

activities stating more than 70% and 50% respectively, compared to other sources. Percentage of soil contamination caused by waste disposal, both municipal and industrial, has been reported considerably higher in Italy (40%) compared to France which is approximately 12%. The percentage of soil contamination caused by storage, commonly due to leaking, has been slightly higher in France, i.e. 13%, compared to Italy, i.e. 5%. Contamination caused by transport spills on land, usually due to accidents, was lower than 2% in both countries. Similarly, the contamination caused by military, commonly due to disposal of military waste or performing specific activities, has been detected in very low amount in Italy (1%), while it has not been reported in France. Industrial and commercial activities include a large number of different activities from production to service sectors, thus their main classification and contribution, reported as percentage, to soil contamination are presented in Table 1.1.

Table 1.1. Production and service sectors' activities which contribute to soil contamination in France and Italy (adapted by van Liedekerke et al. (2014)).

Activities	France	Italy
Production Sector (%)		
Energy production	2	15
Oil industry	2	20
Chemical industry	20	30
Metal working industry	25	5
Electronic industry	0	0
Glass, ceramics, stone, soil industry	3	5
Textile, leather industry	5	0
Wood and paper industry	5	0
Food industry, processing of organic products	2	0
Others	27	0
Total of production sector	91	75
Service sector (%)		
Petrol stations	5	20
Car service stations	0	0
Dry cleaning	0	0
Printers	1	0
Mining sites	2	5

Others (service sector)	1	0
Total of service sector	9	25

1.1.3. Contaminated sites and main contaminants in Europe

According to a recent European Commission report (2014), the total number of 2,500,000 potentially contaminated sites and 342,000 contaminated sites were estimated in European countries. The assessment involved 32 EEA (European Environment Agency) member countries and the seven EEA cooperating countries in the West Balkan. Furthermore, an average estimated number of potentially contaminated sites is 4.2 per 1000 inhabitants, and an average number of contaminated sites is 5.7 per 10,000 inhabitants (van Liedekerke et al., 2014).

The most ubiquitous contaminants in soil are heavy metals (35%) and mineral oil (24%), while polycyclic aromatic hydrocarbons (PAHs) are the third ones common contaminants in Europe soil with a percentage of 11%. Aromatic hydrocarbons (BTEX) and chlorinated hydrocarbons (CHC) are present in slightly lower amount, i.e. 10% and 8% respectively. The contribution of phenols and cyanides is negligible considering that both amount to 1%. The contribution of PAHs to the total number of contaminants affecting the solid matrix in Italy amounts to 15%, thus making the PAHs the third most common contaminants, while in France to 9%, and PAHs are the fourth most common contaminants (Table 1.2).

Table 1.2. The most frequent contaminants in soils, sludge and sediments in Italy and France (adapted by van Liedekerke et al., 2014).

Contaminants	Frequency in Italy (%)	Frequency in France (%)
Chlorinated hydrocarbons (CHC)	10	12
Mineral oil	20	21
Polycyclic aromatic hydrocarbons (PAHs)	15	9
Heavy metals	40	50
Phenols	1	0
Cyanides	1	2
Aromatic hydrocarbons (BTEX)	10	1
Others	4	5

1.1.4. Polycyclic aromatic hydrocarbons – contaminants of concern

PAHs are released in the environment due to an incomplete combustion of organic substances, and thus distributed in the area where fossil fuels are largely used. Accordingly, heavy industries and transport could be considered as the main sources for PAHs contamination, and the urban areas as the most contaminated (Chien et al., 2010; Zhang et al., 2011). These organic compounds are composed of carbon (C) and hydrogen (H) atoms and consist of two or more fused benzene rings. Even if several hundreds of different compounds of PAHs exist, only 16 of them are listed as priority pollutants by U.S. Environmental Protection Agency (US EPA) (Gan et al., 2009; Li et al., 2008). The specific features of these hazardous contaminants are their high hydrophobicity which increases when their molecular weight increases, and low water solubility which decreases when their molecular weights increase. Such characteristics influence their stronger adsorption to the soil matrix and affect their availability and further degradation. Thus, an increase in PAHs molecular weights influences their persistence in environment (Cerniglia, 1992; Gan et al., 2009; Mohan et al., 2006).

PAHs can cause various adverse effects to humans and living organisms. Depending on exposure time, health effects could be acute after a single exposure or chronic after a repeated exposure. Additionally, contaminants' concentrations, specific toxicity of certain PAHs, the route of exposure and the characteristics of exposed individuals such as sex and ages, would influence significantly the overall adverse effect. The route of exposures to PAHs may be inhalation, ingestion or dermal (Bull, 2008; Mumtaz and George, 1995). The most common ways of general public exposure to PAHs include inhalation of both tobacco and wood smoke, vehicle exhausts as well as consumption of PAHs in food grown in contaminated soil. Occupational exposure of workers such as mechanics, street vendors and drivers, as well as people working in heavy industries such as mining, oil refining and metal working could occur due to inhalation of engine exhausts (Bull, 2008).

According to the carcinogenicity classifications verified by US EPA's (Carcinogenicity Risk Assessment Verification Endeavor Work Group, 1994), benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene have been reported as probable human carcinogens (Mumtaz and George, 1995). Additionally, chrysene may probably cause genetic defects, while benzo(a)pyrene may cause genetic defects, impair fertility,

and harm to the unborn child. Naphthalene may probably cause cancer and it is harmful if swallowed. Moreover, all of them are very toxic to aquatic organisms with long-term effects (UBA-Federal Environment Agency, 2012). Teratogenicity or embryotoxic effects of PAHs have been detected in animals exposed to naphthalene, benzo(a)anthracene and benzo(a)pyrene (Gilbert, 2013). The toxicity equivalence factor (TEF) calculated for PAHs is one of the most concerning factors considered at hazardous waste sites. The highest values of TEF have been reported for dibenzo(a,h)anthracene (i.e. 5) and benzo(a)pyrene (i.e. 1). Considerably lower value has been detected for benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-c,d)pyrene, i.e. 0.1. TEF value for anthracene, benzo(g,h,i)perylene and chrysene was even ten times lower (i.e. 0.01) compared to previous group of PAHs, while acenaphthene, acenaphthylene, fluoranthene, fluorine, phenanthrene and pyrene have been shown the lowest TEF value, i.e. 0.001 (Mumtaz and George, 1995). In this thesis a particular interest has been given to PAHs contaminants considering their adverse health effects, toxicity, distribution and persistence into environment.

1.2. Bioremediation treatment – Landfarming combined with composting of organic waste

Applications of innovative remediation techniques for soil treatments such as *in-situ* and *ex-situ* bioremediation technologies in France and Italy, in 2011, approximately amounted to 12% and 20%, respectively (van Liedekerke et al., 2014). The main advantages of bioremediation technologies are lower capital costs, a limited disruption of site activity and an environmentally friendly approach compared to alternative remediation technologies. Furthermore, they fit in the sustainable development strategy as a part of green engineering (Picado et al., 2001; Sayara, 2010). Within this thesis is developed an integrated system of landfarming treatment and biostimulation in order to overcome the limitations of landfarming treatment and provide an eco-friendly disposal of organic waste.

1.2.1. Landfarming treatment

Landfarming is a simply and favorable biological method used to treat the soil contaminated with organic contaminants, usually petroleum constituents. It does not

require a large use of mechanical equipment and it needs low capital costs which are the crucial advantages of this technology (Picado et al., 2001; Thassitou and Arvanitoyannis, 2001). Removal of organic contaminants from soil and their biodegradation are based on microbial activity of indigenous microorganisms. Indeed, biodegradation is a process influenced by the metabolic ability of microorganisms to transform or mineralize organic contaminants into less harmful, non-toxic substances, which are then integrated into natural biogeochemical cycles (Margesin and Schinner, 2001). Contaminated soils may be mixed with soil amendments such as bulking agents and nutrients in order to improve aeration and degradation ability of indigenous microbial community of the contaminated environment (Gan et al., 2009).

1.2.2. Composting of organic waste - biostimulation strategy

Composting is a process commonly used to degrade solid waste materials, but it can be considered as biostimulation strategy for landfarming of PAHs contaminated soils. Indeed, bioremediation of PAHs contaminated soils by indigenous microorganisms could be stimulated adding organic material (Álvarez-Bernal et al., 2006; Gan et al., 2009; Namkoong et al., 2002). Within such a process, the biodegradation of PAHs and organic wastes would be simultaneously conducted under controlled conditions, producing carbon dioxide, water and considerable amounts of heat (Yamada et al., 2007). Composting of organic waste as biostimulation strategy would improve microbial density, promote the activity of microorganisms in contaminated environment, adjust the moisture content and supply nutrients (Bamforth and Singleton, 2005). However, the success or failure of this integrated approach depend on many factors, but the most important are bioavailability of contaminants, and thus their biodegradability (Semple et al., 2001).

Within this PhD thesis, the effectiveness of next four organic waste amendments on PAHs removal from contaminated soil was tested using a synthetic soil artificially contaminated as well as a real contaminated soil.

1.2.2.1. Fruit and vegetable waste

Fruit and vegetable wastes consist of various organic fractions with an acidic pH and moisture content of 80% to 90% by weight. Generally, these wastes contain large

amounts of solid suspensions and a high biochemical oxygen demand (BOD). The chemical composition varies depending on the processed fruit or vegetable. In general, these wastes are consisted of carbohydrates and relatively small amounts of proteins and fat (Thassitou and Arvanitoyannis, 2001).

1.2.2.2. Food and kitchen waste

This type of waste is composed of by-products due to preparation of meals and any food that has not been consumed. It might include raw or cooked meat, bones, fish, bread and pastries, vegetables, fruit, plate scrappings, tea and coffee grounds, eggshells, dairy products, solidified fats, grease etc. Municipal solid waste (MSW) in the United States contains approximately 12% of food waste, while its range in Asian and European countries is among 20% to 45%. The main features of this waste are high content of moisture and fat, high ratio between organic matter and ash and a weak physical structure (Chang and Hsu, 2008).

1.2.2.3. Sewage sludge from wastewater treatment plants

Activated sewage sludge might be a rich source of nutrients such as nitrogen and phosphorus with a pH value nearby neutral, and a high microbial diversity (Ling and Isa, 2006). This waste contains very high moisture content which can be ranged commonly between 95% and 99% by weight. The biochemical composition of sewage sludge generally indicates a high content of protein and very high amount of carbohydrates.

1.2.2.4. Buffalo manure

Animal manures are good sources of macronutrients and micronutrients in environment. The nutrient value of buffalo manure depends on the quantity of nitrogen, phosphorus and potassium, while the quantity of these nutrients in manure depends on several factors such as type, age, breed and condition of animals and nature of food used to feed animals (Chandy, 2010; Infascelli et al., 2010). Organic matter and moisture content in buffalo manure are approximately 13% and 81% respectively, whilst content of mineral matter is about 5% (Chandy, 2010).

1.3. Objectives

1.3.1. General objectives

The general objectives of this study are:

- To improve the efficiency of landfarming treatment in PAHs removal from contaminated soil using a biostimulation strategy such as the composting of organic waste.
- To extend the knowledge about mechanisms which govern the contaminants' bioavailability and biodegradability in soil.
- To point out and contribute to general insight about the favorable operational conditions to conduct the bioremediation, in order to achieve a higher efficiency of PAHs removal from contaminated soil in a shorter removal time.
- To propose an integrated approach, i.e. application of landfarming enhanced by addition of certain organic amendment.

1.3.2. Specific objectives

The specific objectives of this study are:

- To evaluate the effectiveness of four types of organic wastes in stimulating PAHs removal from contaminated soil based on different removal of target contaminants in treatments.
- To perform the characterization of organic amendments including the biochemical organic compounds composition, the content of macronutrients like nitrogen (N) and phosphorus (P), and total organic carbon in order to better understand the process of PAHs biodegradation, since different physico-chemical features of organic amendments would affect the efficiency of the bioremediation treatment.
- To investigate PAHs removal efficiency in real contaminated soil simulating basic operational conditions of landfarming treatment, and amending that soil with sewage sludge. Since contaminated soil to sewage sludge amendment mass ratio has been considered as a critical factor because it is difficult to define the

ratio which will ensure the success of treatment, the specific objective was also to evaluate the optimum amount of sewage sludge for an effective bioremediation of PAHs contaminated soil.

- To monitor the evolution of pH values, temperature profiles and evaluate the effect of temperature increases and the soil toxicity in order to assess the most effective conditions for PAHs removal with focus on removal of high molecular weights PAHs.

1.3.3. PAHs indicators

The effectiveness of soil bioremediation was evaluated by monitoring the concentrations of four PAHs in experiments where was used a synthetic soil artificially spiked. Indicators of contaminants were anthracene (3 aromatic rings), chrysene (4 aromatic rings), benzo(k)fluoranthene (4 aromatic rings, but 5 rings in total) and benzo(a)pyrene (5 aromatic rings). Generally, the most studied PAHs are phenanthrene, pyrene and benzo(a)pyrene, but the objective of this research has been to investigate less studied PAHs with a high molecular weights PAHs, since concern about effectiveness of landfarming treatment remains in treating recalcitrant and high molecular weights PAHs (Antizar-Ladislao et al., 2005; Harmsen et al., 2007; Zhang et al., 2011). The selection criteria for these four PAHs indicators can be summarized in six points:

- The chosen compounds are those that show the highest concentrations at hazardous waste sites; exhibit the more harmful effects than other PAHs; exhibit the representative PAHs harmful effects; are the most common contaminants and thus the possibility that humans will be exposed to these PAHs is high; they are included in the list of 16 priority pollutants filled by U.S. EPA (Crane et al., 2010; Gehle, 2012; Gilbert, 2013; U.S. Environmental Protection Agency et al., 2013).
- Since PAHs with a different number of fused aromatic rings have different characteristics and thus demonstrate a different behavior under the same physico-chemical conditions, one PAH compound, representative of a group with the same number of fused aromatic rings, has been selected.

- PAHs with higher tendency to bioaccumulate, i.e. with higher octanol-water partitioning coefficient ($\log K_{ow}$) within the group with the same number of fused aromatic rings.
- The most toxic or that which exhibits the carcinogenic effect within the group with the same number of fused aromatic rings have been selected.
- The least explored within the group with the same number of fused aromatic rings.

Naphthalene was not considered as the indicator, even if it is the only representative of group with 2 fused aromatic rings among 16 PAHs listed by U.S. EPA as priority pollutants. The main reason has been its high volatility which would have disabled the spiking procedure. Indeed, spiking volatile organic contaminants is generally quite difficult, and often unsuccessfully (Sawada et al., 2004).

1.4. Novelty of the project

Landfarming treatment has been applied at field scale during the last five decades, and has resulted to be relatively successful to remove low molecular weights organic contaminants from soil (Maila and Cloete, 2004; Ward et al., 2003). However, its ability to remove high molecular weights organic contaminants such as PAHs has not proved to be very successful (Harmsen et al., 2007; Wick et al., 2011; Zhang et al., 2011). Therefore, an integrated approach using composting of organic waste as biostimulation strategy could overcome the possible limitations of landfarming treatment and achieve higher bioremediation efficiency. Such novel approach would be governed by sustainable development principles, since it is a completely biological and eco-friendly process able simultaneously to bioremediate contaminated soils and dispose organic waste. Even if various studies have already been conducted based on this bioremediation approach, there is no a systematic approach for bioremediation of contaminated sites, and applied conditions have to vary from case to case (Álvarez-Bernal et al., 2006; Mohan et al., 2006). Thus, the results of this study should improve and optimize the operating conditions for a successful PAH removal based on its own original contribution.

1.5. Structure of the thesis

The thesis manuscript is composed of six chapters and one Appendix.

- Chapter 1. This chapter is a general introduction to the present research work with a brief review on contaminated soils and sites in European countries. The bioremediation technology and biostimulation strategy for the removal of PAHs are proposed. Furthermore, the objectives and the original contributions of the thesis are specified.
- Chapter 2. Second chapter of this thesis presents a literature review focused on the efficiency and application conditions of landfarming technology as a suitable bioremediation treatment of PAHs contaminated soil. Moreover, the possibility of its improvement using the biostimulation strategy of composting process with organic wastes is described.

The following three chapters are related to the experimental activities conducted at laboratory scale.

- Chapter 3. The research work presented in this chapter evaluated the removal efficiency of 4 PAHs, i.e. anthracene, chrysene, benzo(k)fluoranthene and benzo(a)pyrene with an initial total concentration of 658 mg kg^{-1} soil dry weight (d/w) from a spiked OECD (Organisation for Economic Co-operation and Development) synthetic soil. The contaminants' removal was stimulated by adding to soil four different types of organic wastes.
- Chapter 4. The research work presented in this chapter is based on the characterization of biochemical features of organic wastes used as amendments in the experimental activities described in previous chapter. This work contributes to clarify the different efficiency of organic amendments in a bioremediation process of PAHs contaminated soil.
- Chapter 5. In this chapter is reported the study on the bioremediation process of a real co-contaminated soil by heavy metals and PAHs using landfarming treatment and centrifuged activated sewage sludge as organic amendment in different mass ratios. The removal efficiency of PAHs with an initial concentration of 620 mg of total PAHs kg^{-1} dry soil was evaluated.
- Chapter 6. In the last chapter are commented and discussed the results presented in chapters 3 to 5 in order to highlight the crucial points of this research work.

Accordingly, the future perspectives at laboratory scale and field scale are also discussed.

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CHAPTER 1

Chapter 2

**A review on the efficiency of
landfarming integrated with
composting as a soil remediation
treatment**

Abstract

Landfarming is an attractive bioremediation treatment approach for soils contaminated with polycyclic aromatic hydrocarbons (PAHs), since it is less expensive and more environmentally friendly than other soil remediation methods. PAHs are contaminants of concern due to their potentially harmful effects on human health. Therefore interest in developing removal methods has grown in the last few decades. These persistent and hydrophobic organic pollutants are commonly found at high concentrations in soils contaminated by industrial activities and nearby urban areas, and these hazardous situations make their removal even more urgent. This paper reviews the efficiency and application conditions of landfarming as a suitable bioremediation treatment. Moreover, this work discusses the feasibility of improving the bioremediation performance when landfarming is combined with biostimulation and bioaugmentation promoted by the composting of organic waste. This integration of landfarming and composting creates more favorable conditions for biological activity and has been shown to be both effective and economical in removing organic pollutants from contaminated soils.

Keywords

landfarming technology, polycyclic aromatic hydrocarbons (PAHs), bioremediation, composting, biostimulation.

A review on the efficiency of landfarming integrated with composting as a soil remediation treatment

2.1. Introduction

The pollution of soils with hazardous contaminants has been increasing recently as a consequence of intensive agricultural and industrial activities (Othman et al., 2011). Soil contamination can be defined as the presence of toxic chemicals in concentrations high enough to be of risk to human health and the surrounding ecosystem (Environmental pollution centers, 2009). Additionally, even when the concentrations of contaminants in soils are not very high, soil contamination is considered to have occurred if the concentrations exceed those that are naturally present (Environmental pollution centers, 2009). Natural background contaminants in soils refer to concentrations of hazardous materials attributable to geologic or ecological conditions and not to anthropogenic activities (MADEP Massachusetts Department of Environmental Protection, 2002). The natural background concentrations of PAHs in soils are listed in Table 2.1 (MADEP Massachusetts Department of Environmental Protection, 2002; Mumtaz and George, 1995).

Table 2.1. Natural background concentrations of PAHs in soil (adapted from Mumtaz and George (Mumtaz and George, 1995) and MADEP (MADEP Massachusetts Department of Environmental Protection, 2002)).

PAH compounds	Background soil concentrations ($\mu\text{g kg}^{-1}$)*	Background soil concentrations ($\mu\text{g kg}^{-1}$)**		
		Rural soil	Agricultural soil	Urban soil
Acenaphthene	500	1.7	6	/
Acenaphthylene	500	/	5	/
Anthracene	1000	/	11 - 13	/
Benzo(a)anthracene	2000	5 - 20	56 - 110	169 - 59 000
Benzo(a)pyrene	2000	2 - 1300	4.6 - 900	165 - 220
Benzo(b)fluoranthene	2000	20 - 30	58 - 220	15 000 - 62 000
Benzo(e)pyrene	/	/	53 - 130	60 - 14 000
Benzo(g,h,i)perylene	1000	10 - 70	66	900 - 47 000
Benzo(k)fluoranthene	1000	10 - 110	58 - 250	300 - 26 000
Chrysene	2000	38.3	78 - 120	251 - 640
Dibenzo(a,h)anthracene	500	/	/	/
Fluoranthene	4000	0.3 - 40	120 - 210	200 - 166 000
Fluorene	1000	/	9.7	/
Indeno(1,2,3-cd)pyrene	1000	10 - 15	63 - 100	8000 - 61 000
Naphthalene	500	/	/	/
Phenanthrene	3000	30	48 - 140	/
Pyrene	4000	1 - 19.7	99 - 150	145 - 147 000

*MADEP (MADEP Massachusetts Department of Environmental Protection, 2002).

**Mumtaz and George (Mumtaz and George, 1995).

The historic lack of specific regulations addressing soil contamination and insufficient enforcement by authorities have resulted in a drastic increase in the number and size of contaminated and potentially contaminated sites (Beškoski et al., 2012).

Serious environmental pollution has resulted from inadequate attention paid to sustainable development in the past. In the European Union (EU), there are around 3.5 million contaminated sites affecting 231 million people (Swartjes, 2011).

Approximately 250,000 sites in EU member states need remediation, and this number is even higher considering non-EU members. According to current pollution trends, there will be a 50% increase in the number of polluted sites that will need remediation by 2025 (Schippers et al., 2014; Swartjes, 2011). Furthermore, the remediation process is slow and expensive; in fact, in the last 30 years only 30% of sites that need remediation have received this treatment. Additionally, the number of suspected contaminated sites is expected to be similar to the number of known contaminated sites, making the actual situation worse than is currently perceived (Schippers et al., 2014; Swartjes, 2011).

According to the European Environment Agency (Prokop et al., 2000), the total estimated number of potentially contaminated sites in France includes 200,000 to 300,000 abandoned sites, approximately 68,000 authorized active industrial sites, and around 500,000 smaller active industrial sites. The situation in Italy appears to be less serious than that in France, with 8,873 potentially contaminated sites identified. These include abandoned as well as operating waste disposal and industrial sites (Panagos et al., 2013). It is important to note that the real number of potentially contaminated sites in Italy could exceed those currently identified, since regions covering one third of the country's land have not yet provided data (Panagos et al., 2013).

Concerning the above-mentioned data, it is important to precisely define "estimated" and "identified" sites. The estimated number of potentially contaminated sites is based on studies or experts' evaluations, while the identified number results from an analysis based on soil characteristics and hydrology data. If an estimated number has not been provided, then a ratio of identified to estimated contaminated sites equal to 0.7 can be applied (Panagos et al., 2013).

Furthermore, the high variability in the total number of potentially contaminated sites also depends on the criteria adopted by each country used to define site contamination. For example, in France, potentially contaminated sites include those where no evidence of contamination has been found but potentially polluting activities are conducted. In other countries, potentially contaminated sites only include those where potential contamination is determined through analysis. Some countries only consider sites with high national importance, while other countries also consider smaller sites such as those contaminated by leakage from storage tanks (Panagos et al., 2013).

Among the various contaminants, organic PAHs are present worldwide; thus, they are a global pollution problem (Harmsen et al., 2007; Kanaly and Harayama, 2000). According to the European Environment Agency, in the almost 90% of European sites

for which information on contaminants is available, PAHs represent about 13% of the total contaminants (Sayara, 2010). Indeed, they are the third major pollutant affecting soil in Europe (Table 2.2), after heavy metals and mineral oil. Aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes), phenols, and chlorinated hydrocarbons (CHC) occur slightly less frequently (Schippers et al., 2014).

Table 2.2. The main soil pollutants in Europe (adapted from Schippers et al. (Schippers et al., 2014)).

Pollutants	Frequency (%) ^a
Heavy metals	37.3
Mineral oil	33.7
Polycyclic aromatic hydrocarbons (PAHs)	13.3
Aromatic hydrocarbons (BTEX)	6
Phenols	3.6
Chlorinated hydrocarbons (CHC)	2.4
Others	3.6

^aCalculated on the basis of frequency of contaminants reported to be the most important in the investigated site.

Malawska and Wilkomirski (Malawska and Wilkomirski, 2001) provided a general soil assessment related to PAH content (Table 2.3) based on the total concentration of PAH in soil (Sayara, 2010).

Table 2.3. Standard limiting PAH content ($\mu\text{g kg}^{-1}$ dry weight [dw]) in the soil surface layer (adapted from Malawska and Wilkomirski (Malawska and Wilkomirski, 2001) and Sayara (Sayara, 2010)).

Total PAHs content ($\mu\text{g kg}^{-1}$ dw)	Soil assessment
< 200	Unpolluted (natural content)
200 - 600	Unpolluted (increased content)
600 - 1000	Slightly polluted
1000 - 5000	Polluted
5000 - 10000	Heavily polluted
>10000	Very heavily polluted

The presence of organic pollutants in the environment poses serious public, scientific, and regulatory issues because of their potential toxicity, mutagenicity, carcinogenicity, and tendency to bioconcentrate up the trophic ladder (Semple et al., 2001). Hence, interest in biodegradation mechanisms of PAHs and remediation of contaminated sites has increased in recent years and has led to the further development and practical use of remediation treatments (Johnsen et al., 2005; Othman et al., 2011; Semple et al., 2001). The choice of a remediation process is unique for each case and depends on many factors such as the type and concentration of contaminants, soil properties, legislated concentration thresholds, the available time to perform a remediation, and costs. All treatments are not suitable for all cases and the best method has to be selected through a cost-benefit analysis (Beškoski et al., 2012). Table 2.4 presents a brief overview of remediation treatments divided into seven general categories. Biological treatments are considered to be more attractive, environmentally friendly, and cost effective than alternative methods (Antizar-Ladislao et al., 2004; Gan et al., 2009; Sayara, 2010).

Table 2.4. Remediation technologies (adapted from Antizar-Ladislao et al. (Antizar-Ladislao et al., 2004), Gan et al. (Gan et al., 2009), and Sayara (Sayara, 2010)).

Remediation treatment	Main features
Bioremediation	Microbial activity completely mineralizes or transforms the contaminants to a less toxic, environmentally acceptable form.
Phytoremediation	Plants are used to extract, sequester, and detoxify environmental contaminants.
Chemical	Chemical reactions destroy, fix, or neutralize contaminants. Therefore, more recalcitrant organic contaminants can be easier destroyed or converted to less harmful ones.
Thermal	Heat is employed to destroy contaminants through incineration, gasification, and pyrolysis.
Physical	Contaminated soil is removed to a landfill or contained at the contaminated site.
Solidification/vitrification	Solidification is related to the encapsulation of contaminants within a monolithic solid of high structural integrity, with or without associated chemical fixation. This is termed “stabilization”. Vitrification is based on the use of high temperatures to fuse contaminated material.
Integrated remediation	Multiple remediation methods can be applied to the degradation of

techniques	contaminants if a single method is ineffective. This overcomes drawbacks to single methods and can lead to better removal results.
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Table 2.5 lists the costs of several remediation treatments using data from Juwarkar (Juwarkar et al., 2010). *In situ* bioremediation costs are lower than *ex situ* applications, usually ranging from \$30 to \$100 per cubic meter (\$20 to \$80 per cubic yard) of soil (van Cauwenberghe and Roote, 1998). The costs will vary depending on the conditions of the contaminated site. It may be approximately stated that slurry phase bioremediation costs are \$170 per ton (\$46 per cubic yard) of soil (U.S. Environmental Protection Agency (US EPA), 1995), while solid phase bioremediation costs, such as biopiling, range from \$130 to \$260 per cubic meter (\$100 to \$150 per cubic yard) (Remediation Technologies Screening Matrix and Reference Guide FRTR, n.d.). Composting treatment costs from \$50 to \$140 per ton (Michel Jr. et al., 2001). Landfarming costs are the lowest among these options, with a typical range from \$30 to \$60 per ton of contaminated soil (U.S. Environmental Protection Agency, 1994).

Once commonly used remediation techniques, such as land removal, incineration, or landfilling, have lost favor in recent years not only because of their higher costs, but also due to their less eco-friendly aspects. In contrast, bioremediation is considered a safe and efficient method for removing organic pollutants such as PAHs, pesticides, volatile organic compounds (VOCs), and organic solvents from contaminated soils. Further, this method has the benefit of removing contaminants without transferring them to another medium, which is done by most other methods (Beškoski et al., 2012; Mohan et al., 2006; Othman et al., 2011; Semple et al., 2001; Ward et al., 2003). Bioremediation treatments represent 25% of all remediation methods for the treatment of petroleum hydrocarbon pollutants (Beškoski et al., 2012). They are based on the capacity of microorganisms to interact with a huge range of anthropogenically and naturally occurring compounds, resulting in a structural change or complete degradation of their molecules (Mohan et al., 2006; Othman et al., 2011; Semple et al., 2001; Ward et al., 2003).

Table 2.5. Comparison of soil remediation treatment costs (adapted from Juwarkar (Juwarkar et al., 2010)).

Treatments	Costs (£/ton soil)
Biological	5 – 170
Chemical	12 – 600
Physical	20 – 170
Solidification/stabilization	17 – 170
Thermal	30 – 750

The microorganism activity depends on many factors, including the type and concentration of contaminants; their bioavailability, toxicity, and mobility; the presence and availability of macro and micronutrients; and the availability of activated enzymes (Mohan et al., 2006). One of the most important factors that can be improved to optimize and accelerate the degradation of organic pollutants is their bioaccessibility (Ward et al., 2003). Bioremediation is a very complex process that must account for site-specific conditions in order to minimize limitations and environmental effects. If bioremediation is not well managed and if the microbial processes are not correctly monitored, the results of the process could make the situation worse instead of better, for example to make more toxic environment (Juwarkar et al., 2010).

In developed countries, remediation standards have been tightened in terms of both the remediation treatments used and the quality of the remediated soil. Hence, modern standards include ecotoxicological tests to assess the results of bioremediation. These standards integrate the effects of all significant compounds, including those not chemically analyzed (Beškoski et al., 2012). For this reason, biological methods have an advantage compared with other methods, because the reduction in contaminant concentration achieved by biodegradation is related to contaminant bioavailability. Accordingly, residues of contaminants have no further impact on the environment, whereas non-biological methods might leave bioavailable contaminants in the soil at low concentrations (Beškoski et al., 2012).

One of the oldest and most attractive biological soil remediation treatments is landfarming, which can be performed either *in situ* or *ex situ*. According to the U.S. Environmental Protection Agency (US EPA) Ecology dictionary (U.S. Environmental

Protection Agency (US EPA), 2013), landfarming is a treatment in which hazardous waste is deposited on or in the soil, where it degrades naturally by the mechanism of microorganisms. Soil conditions are controlled by monitoring the moisture and nutrient content, frequency of aeration, and soil pH value in order to optimize the rate and efficiency of the contaminants' degradation. Occasionally, contaminated soil is spread over with waste material or mixed with soil amendments, such as bulking agents and nutrients, to improve the oxidation and degradation process with the existing microbial population (Gan et al., 2009).

Indeed, organic wastes have been reported to have a good potential for remediating even heavily contaminated soils (Atagana, 2004a). The aim of this work is to review the use and efficiency of the landfarming and composting processes as well as their combination in the removal of organic pollutants such as PAHs from contaminated soils. This approach has gained attention in the scientific community over the last 15 years, and further studies are required to improve the operating conditions used and the resulting performance. Compared to other soil remediation treatments, this process entails reduced risk of undesirable by products and it is easier controlled, in addition to being cost effective and efficient (Antizar-Ladislao et al., 2005).

2.2. Features of PAHs

2.2.1. Structure and physico-chemical characteristics of PAHs














Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds composed of two or more fused aromatic rings in a linear, angular, or clustered structure, usually containing only carbon (C) and hydrogen (H) atoms (Gan et al., 2009; Juhasz and Naidu, 2000). Several hundreds of different compounds of PAHs exist, but only 16 of them have been identified as "priority pollutants" by the US EPA, while 7 PAHs are classified as probable human carcinogens (Gan et al., 2009; Li et al., 2008).

Lower molecular weight (LMW) PAH compounds, containing 2 or 3 rings, have shown significant acute toxicity and other adverse effects to some organisms but are not carcinogenic, while higher molecular weight (HMW) PAHs, containing 4 to 7 rings, are significantly less toxic but may be carcinogenic, mutagenic, or teratogenic to a wide variety of organisms, including fish, amphibians, birds, and mammals (Gan et al., 2009; Juhasz and Naidu, 2000).

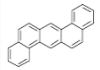
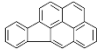

Table 2.6 reports on some of the chemical and physical properties of the 16 PAHs identified as “priority pollutants” by the US EPA, according to IPCS INCHEM (INCHEM), 1998) and Antizar-Ladislao et al. (Antizar-Ladislao et al., 2004). Among these properties, the most important characteristic that should be considered during the biodegradation process appears to be water solubility, which decreases with an increase in the number of fused benzene rings. This means that high molecular weight PAHs are more slowly desorbed from solids and dissolved into water than low molecular weight PAHs, and therefore are less available for microbial degradation (Cerniglia, 1992; Johnsen et al., 2005; Mohan et al., 2006). Furthermore, the octanol-water partition coefficient ($\log K_{ow}$) is proportional to the PAHs’ molecular weight, i.e. it decreases with a decrease in number of fused benzene rings. Actually, $\log K_{ow}$ is generally used to indicate the tendency of an organic pollutant to be adsorbed on soil particles (U.S. Geological Survey science for a changing world, 2014a). Higher $\log K_{ow}$ indicates a lower biodegradability of a compound and the higher potential for bioaccumulation (Baumard et al., 1998; González et al., 1992; Juhasz and Naidu, 2000). Similar to $\log K_{ow}$, the organic carbon partitioning coefficient ($\log K_{oc}$) shows a compound’s adsorbability and it is directly proportional to the PAHs’ molecular weight; i.e., as the number of fused benzene rings increases, $\log K_{oc}$ also increases. In more detail, $\log K_{oc}$ is defined as the solid-water distribution coefficient (K_d) divided by the mass fraction of the soil organic carbon content (f_{oc}) (Bathi, 2007; Chiou et al., 1998). A higher $\log K_{oc}$ value of PAHs indicates the greater tendency of sorption onto soil organic matter rather than solubilization in the aqueous phase (Corapcioglu, 1996). Conversely, a lower $\log K_{oc}$ value is characteristic of more mobile organic pollutants.

Differences in PAH properties influence their different behaviors under the same conditions and, consequently, the feasibility of their removal through bioremediation treatment.

Table 2.6. Structure and physico-chemical properties of 16 PAHs listed by the US EPA (adapted from IPCS INCHEM (INCHEM), 1998) and Antizar-Ladislao et al. (Antizar-Ladislao et al., 2004)).

PAH	Molecular weight ^a	Formula ^a	Structure ^a	Melting point (°C) ^a	Boiling point (°C) ^a	log K _{ow} ^a	Water solubility at 25°C (µg L ⁻¹) ^b	Vapor pressure (Pa at 25°C) ^a	Henry's low constant at 25°C (kPa) ^b
Naphthalene	128	C ₁₀ H ₈		81	218	3.00–4.00	3.17 × 10 ⁴	10.9	4.89 × 10 ⁻²
Acenaphthylene	152	C ₁₂ H ₈		95	270	3.70	/	5.96 × 10 ⁻¹	114 × 10 ⁻³
Acenaphthene	154	C ₁₂ H ₁₀		96.2	279	3.92–5.07	3.93 × 10 ³	5.96 × 10 ⁻¹	1.48 × 10 ⁻²
Fluorene	166	C ₁₃ H ₁₀		115–116	294	4.18	1.98 × 10 ³	8.86 × 10 ⁻²	1.01 × 10 ⁻²
Anthracene	178	C ₁₄ H ₁₀		218	342	4.46–4.76	73	2.0 × 10 ⁻⁴	7.3 × 10 ⁻²
Phenanthrene	178	C ₁₄ H ₁₀		100.5	338	4.45	1.29 × 10 ³	1.8 × 10 ⁻²	3.98 × 10 ⁻³
Fluoranthene	202	C ₁₆ H ₁₀		108.8	383	4.90	260	2.54 × 10 ⁻¹	6.5 × 10 ⁻⁴
Pyrene	202	C ₁₆ H ₁₀		150.4	393	4.90	135	8.86 × 10 ⁻⁴	1.1 × 10 ⁻³
Benzo[a]anthracene	228	C ₁₈ H ₂₀		160.7	425	5.61–5.70	14	7.3 × 10 ⁻⁶	1.2 × 10 ^{-6a, c}
Chrysene	228	C ₁₈ H ₂₀		253.8	431	5.61	2	5.7 × 10 ⁻⁷	6.7 × 10 ^{-7a, c}
Benzo[b]fluoranthene	252	C ₂₀ H ₁₂		168.3 ^b	481 ^b	6.57	1.2 ¹¹ (20°C)	/	5.1 × 10 ⁻⁵
Benzo[k]fluoranthene	252	C ₂₀ H ₁₂		215.7 ^b	480 ^b	6.84	0.76	/	4.4 × 10 ⁻⁵ (20°C)
Benzo[a]pyrene	252	C ₂₀ H ₁₂		178.1	496	6.04	3.8	8.4 × 10 ⁻⁷	3.4 × 10 ⁻⁵ (20°C)

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Dibenzo[a,h]anthracene	278	C ₂₂ H ₁₄		266.6	535	5.80–6.50	0.5 (27°C)	3.7 × 10 ⁻¹⁰	7 × 10 ⁻⁶
Indeno[1,2,3-cd]pyrene	276	C ₂₂ H ₁₂		163	536	7.66	62	/	2.9 × 10 ⁻⁵ (20°C)
Benzo[g,h,i]perylene	276	C ₂₂ H ₁₂		278.3	542	7.23	0.26	6 × 10 ⁻⁸	2.7 × 10 ⁻⁵ (20°C)

^aAntizar-Ladislao et al. (Antizar-Ladislao et al., 2004), except where noted.

^bIPCS INCHEM (INCHEM), 1998), except where noted.

^catm m³ mol⁻¹

2.2.2. Sources of PAHs

PAHs are widely distributed in the environment and have been detected in soils and sediments, groundwater, and in the atmosphere (Bamforth and Singleton, 2005; Okere and Semple, 2012). Their presence in the environment is only partly the result of natural processes such as forest fires and volcanic eruptions, and much more due to anthropogenic activities. The main activities responsible for the release of these pollutants are vehicular traffic, incomplete combustion of fossil fuels in home heating, accidental discharge of fuels during exploitation and transport, use and disposal of petroleum products, and waste incineration (Antizar-Ladislao et al., 2004; Beškoski et al., 2012; Johnsen et al., 2005; Kafilzadeh et al., 2011; Maila and Cloete, 2002). It is estimated that 0.1% of the annual productivity of crude oil reaches the environment as a result of anthropogenic activities (Beškoski et al., 2012). All major industries using fossil fuels in their productive cycles generate PAHs, and they are found in high concentrations near industrial sites involved in iron smelting, petroleum refining, coal gasification, thermal power generation, tar paper production, and wood preservation (Brown et al., n.d.; Zhang et al., 2011). Considering that PAHs in soils is directly related to transport and other industrial activities, their presence is also found in soils near urban areas (Chien et al., 2010), making the necessity for PAH monitoring and removal urgent.

The concentration of PAHs in contaminated soils depends on the nature of the industrial sites and sources of contamination (Antizar-Ladislao et al., 2004). Table 2.7 presents examples of typical PAH concentrations using data from Juhasz and Naidu (Juhasz and Naidu, 2000) and Antizar-Ladislao et al. (Antizar-Ladislao et al., 2004). Total PAH concentrations at wood-preserving industrial sites are generally 3 to 4 fold higher than those due to creosote production or gas works, and more than 20 fold higher than those at manufacturing gas plants. In contrast, the concentration of HMW PAHs at such sites is not that high (approximately 20% of the total PAH concentration), while their concentration at manufacturing gas plant sites is typically twice to even three times higher compared to that at wood preserving sites (40% to 60% of the total PAH concentration). On the other hand, data from gas work sites do not reflect a total PAH concentration as high as at wood preserving sites, but show an extremely high percentage of HMW PAHs at approximately 90%. Obviously, the concentration of a single PAH in polluted soil depends on the source of contamination. These differences

in PAH concentrations at specific sites need to be considered when a bioremediation treatment is planned in order to achieve successful removal (Antizar-Ladislao et al., 2004).

CHAPTER 2

Table 2.7. PAH concentrations (mg kg⁻¹) in soil contaminated by industrial activities (adapted from Juhasz and Naidu (Juhasz and Naidu, 2000), and Antizar-Ladislao et al. (Antizar-Ladislao et al., 2004)).

PAHs	CP ^a	WP ^a	WP ^b	SFMn ^a	GW ^a	PC ^b	MGP ^a	MGP ^b	SFMs ^a	COGEMA ^a
Naphthalene	1131	500	3925	6494	/	186	10	97	673	/
Acenaphthylene	33	/	49	3651	/	/	6	28	79	28
Acenaphthene	/	7100	1368	21 319	2	43	46	49	705	2
Fluorene	650	1900	1792	2497	225	87	16	14	32	4
Phenanthrene	1595	6400	4434	7902	379	156	84	26	266	51
Anthracene	334	2500	3307	1440	156	53	6	11	2	58
Fluoranthene	682	2200	1629	10053	2174	137	62	73	419	195
Pyrene	642	1000	1303	9481	491	99	51	47	/	173
Benzo[a]anthracene	/	300	171	1670	317	33	20	16	496	88
Chrysene	614	1000	481	2392	345	/	21	15	305	52
Benzo[b]fluoranthene/ benzo[k]fluoranthene	/	560	140	2271	498	/	48	21	513	99
Benzo[a]pyrene	/	60	82	536	92	15	10	14	224	106
Indeno[1,2,3-cd]pyrene	/		23	120	207	/	21	7	64	46
Dibenzo[a,h]anthracene	/	<30	/	192	2451	12	5	33	27	/
Benzo[g,h,i]perylene	/	<30	/	/	/	/	16	/	/	/
Total PAHs	5863	23600	18704	70633	7337	821	352	451	3815	974
% HMW PAHs	33	22	20	38	90	36	72	50	54	78

Note: CP - creosote production site; WP - wood preserving site; SFMn - Superfund site Minnesota; GW - gas works site; PC - petrochemical site; MGP - manufacturing gas plant site; SFMS - Superfund site Mississippi; COGEMA - French MGP site.

^aAntizar-Ladislao et al. (Antizar-Ladislao et al., 2004)

^bJuhasz and Naidu (Juhasz and Naidu, 2000).

2.2.3. PAHs in the environment and their bioavailability

PAHs released into the environment can adversely affect animal and human health through ingestion, inhalation, and dermal contact (Othman et al., 2011). According to a 2000 World Health Organization (WHO) Regional Office for Europe report, some PAHs may cause numerous adverse effects, even cancer (Antizar-Ladislao et al., 2004). Due to their hydrophobicity, PAHs are easily adsorbed onto the organic matter of solid particles, and they have the tendency to accumulate in soils, sediments, and animals, forming persisting micropollutants in the environment (Antizar-Ladislao et al., 2004; Cerniglia, 1992; Gan et al., 2009; Johnsen et al., 2005; Juhasz and Naidu, 2000).

The bioavailability of PAHs in soils is influenced by the contact time between soil and contaminants. The residence time in soils, also called ageing, facilitates the movement of contaminants into soil micropores, leading to their transformation and incorporation into stable soil solid phases and decreasing the chemical and biological availability of contaminants. The main mechanisms of ageing that limit the release of PAHs into the liquid phase are diffusion and sorption, collectively called sequestration. Sequestration makes pollutants inaccessible to microorganisms, limiting their bioavailability, and consequently decreases their biodegradation rate (Beškoski et al., 2012; Mohan et al., 2006; Semple et al., 2001; Wick et al., 2011). The ageing process can even make contaminants completely unavailable to biodegradation, even when fresh biodegradable compounds are added (Beškoski et al., 2012).

Due to the low solubility and hydrophobicity of PAH compounds, the sorption phenomenon is crucial for their transport and fate in the environment (Bathi, 2007; Chiou et al., 1998). Actually, the higher affinity of organic pollutants to adsorb onto soil organic matter may limit their bioavailability and thus affects bioremediation treatment. Bioaccumulation is the tendency of PAHs to accumulate in the tissue of organisms due to exposure to a contaminated medium, or by consumption of food containing the contaminants (Meador et al., 1995; U.S. Environmental Protection Agency (US EPA), 2010; U.S. Geological Survey science for a changing world, 2014b). The bioaccumulation factor (BAF), defined as the ratio of contaminant concentration in an organism to its concentration in the ambient environment (U.S. Geological Survey science for a changing world, 2014b), is usually used to measure the potential for bioaccumulation and is related to the contaminant hazard. This potential is correlated with the $\log K_{ow}$ and $\log K_{oc}$ values (Amorim et al., 2011), but according to Wei-chun et

al. (Wei-chun et al., 1995), it can be reduced depending on changes in the bioavailability associated with ageing of PAHs in contaminated soils. The contaminants' bioavailability is actually affected by its sorption on soil organic particles, if it is strong enough to limit diffusion and consequently reduce the potential for bioaccumulation (Wei-chun et al., 1995).

The characteristics of PAHs discussed in the previous subsections and their adverse health effects and toxicity have motivated the scientific community to conduct extensive studies on remediation treatments and to apply them at field scale (Gan et al., 2009; Okere and Semple, 2012). The most relevant results obtained using landfarming are presented and discussed in the following subsections.

2.3. Landfarming

The biological removal of PAHs using landfarming has been applied commercially at a large scale with relative success. This process actually has been widely used by the mineral oil processing industry, as it is a simple and cost-effective method to remediate soils accidentally contaminated by oil spills. It can also treat oil sludges settled in oil storage tanks that are produced by oil-water separators, dissolved air floatation units, or collected during drilling operations (ASTS Incorporated, 2010; Beškoski et al., 2012; Maila and Cloete, 2004; Ward et al., 2003). The U.S. oil industry began to use landfarming in the early 1970s to treat industrial oil waste by mixing it with soil in order to stimulate the biodegradation of mineral oil (Harmsen et al., 2007; Jørgensen et al., 2000; Wick et al., 2011). Based on this experience, landfarming was developed to account for different factors related to sustainable remediation of contaminated soils: cost, space requirements, time, energy demand, use of raw materials, and public acceptance (Harmsen et al., 2007).

The principle underlying this process is the use of microbial communities to remove organic contaminants mainly through their conversion into CO₂ and water (Maila and Cloete, 2004; Straube et al., 2003). Since bioremediation efficiency is affected by the release of VOCs into the atmosphere, as well by the adsorption of contaminants on soil particles, the main removal mechanisms involved in landfarming are volatilization of VOCs during the early stage of contamination or treatment, biodegradation, and adsorption (Maila and Cloete, 2004; U.S. Environmental Protection Agency (US EPA), 2012).

Maila and Cloete (Maila and Cloete, 2004) report that successful landfarming depends on specific conditions, such as well-drained soil, the biodegradability of pollutants by existing microorganisms, an abundant presence of microorganisms, and a closed greenhouse needed to minimize soil erosion and runoff from rain and to control air emissions. Appropriate environmental conditions include the pH value, availability of nutrients, and moisture content.

2.3.1. Process design

Based on the nature of its biochemical processes, landfarming is considered aerobic. Depending on location, the process is either *in situ* or *ex situ*, and on the basis of configuration, it can be *indoor* if the area is covered by a greenhouse or *outdoor* if the area is open (Beškoski et al., 2012; Gan et al., 2009; Othman et al., 2011). When *in situ* landfarming is performed, the remediation of contaminated soils occurs at the original pollution site, while in *ex situ* landfarming, the contaminated material is excavated and transported to the treatment facility (Bolton, 2012; Othman et al., 2011). *In situ* landfarming produces less dust since it does not require excavation and consequently releases fewer contaminants through volatilization into the environment compared to an *ex situ* system. *In situ* landfarming is usually applied to the soil surface layer at depths up to 50 cm, even though the effective oxygen diffusion needed for bioremediation in most soils can only be achieved at depths less than 30 cm. Bulking agents are commonly added to the soil in order to increase porosity, after which the contaminated soil is periodically mixed to ensure a proper air supply. Water is eventually added to enhance microbial activity. These processes all improve the biodegradation of organic pollutants. *In situ* landfarming is suitable when the contaminated soil is shallow and confined below a layer of clay or impermeable soil. The success of *in situ* landfarming largely depends on the soil characteristics (soil texture, moisture content, number of microorganisms, pH) and climate conditions (rainfall, wind, and temperature) (Beškoski et al., 2012; Joo et al., 2007). Critical disadvantages of this method are the infeasibility of rigorous process control, the long execution time, and low effectiveness in impermeable soil (Othman et al., 2011; Sayara, 2010).

Ex situ landfarming requires a shorter treatment time, is easier to control, and can treat a wider range of contaminants compared to *in situ* landfarming. However, *ex situ* landfarming requires additional costs for excavation and transport of the contaminated

material as well as capital costs for building and equipping treatment structures. Finally, there are additional labor and energy costs (Othman et al., 2011; Sayara, 2010). In *ex situ* landfarming, the excavated contaminated soil is mixed with nutrients and bulking agents (industrial wood waste is particularly useful) until a layer is formed that is not more than 0.5 m thick. The contaminated material is periodically tilled in order to ensure appropriate aeration and good homogeneity of the mixture, as well as improve the contact between contaminants and microorganisms (Beffa, 2002; Beškoski et al., 2012; Harmsen et al., 2007; Joseph, 2007; Juwarkar et al., 2010; Singh and Ward, 2004; Straube et al., 2003; Wick et al., 2011). Usually, the leachate produced by biological activity, the water supply used to maintain moisture content in the soil, and rainfall are collected using a leachate collection system (Beškoski et al., 2012; Wick et al., 2011) at the bottom of the treatment basin. Optimal moisture content is maintained through watering by irrigation or spraying, while the optimal pH value is adjusted by adding lime or ammonium sulfate and elemental sulfur, depending on soil acidity and alkalinity (Beškoski et al., 2012; Bolton, 2012; U.S. Environmental Protection Agency, 1994).

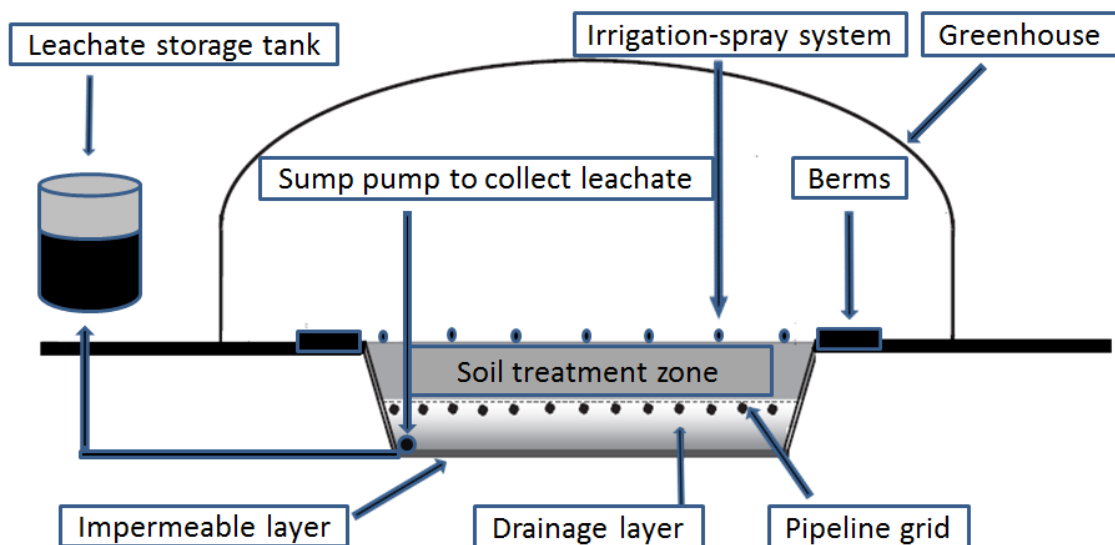


Figure 2.1. Landfarming treatment unit (Alken-Murray and Edwards Jr., 1999; Pavel and Gavrilescu, 2008; Picado et al., 2001).

The typical landfarming indoor treatment unit (Figure 2.1) is similar to a greenhouse. The base has a slight slope to enable the collection of leachate in a small well. The leachate is pumped from this well to a tank and stored prior to treatment. The bottom of

the unit is usually covered with an HDPE (high-density polyethylene) impermeable membrane sheet as a synthetic liner, or with clay as natural liner, in order to avoid leakage of the leachate and consequent groundwater contamination. The drained leachate is carried through a coarse sand or pea gravel layer followed by drainage to a tank, where it is stored or treated according to its contaminants. The contaminated soil is placed on top of the drainage layer. Prior to treatment, the contaminated soil can be subjected to pretreatment processes such as crushing, shredding, sieving, blending, etc., in order to improve its physical characteristics (i.e., porosity). Indoor landfarming treatment facilities can be equipped with a temperature control system composed of a pipeline grid placed below the soil treatment zone and connected to a hot water system. In outdoor landfarming, small berms are raised all along the perimeter of the treatment area to control water runoff and avoid cross contamination. A watering irrigation or spray system is placed above the contaminated soil layer to maintain adequate moisture content in the soil and to supply nutrients. The irrigation system is typically composed of pipes with holes to distribute a supply of water, nutrients, or bacteria necessary for bioremediation treatment. Soil aeration is performed either by periodic tilling that can be performed manually or with aeration equipment, or by insufflating air. When landfarming is conducted outdoors, soil erosion due to wind is controlled by terracing the contaminated soil into windrows or spraying water to minimize dust formation and consequent emission. Soil erosion due to rainfall is controlled by the construction of a water stream constraint system (i.e., perimetric berms) (Alken-Murray and Edwards Jr., 1999; ASTS Incorporated, 2010; Picado et al., 2001; Sayles et al., 1999; U.S. Environmental Protection Agency, 1994; U.S. Environmental Protection Agency (US EPA), 2012; Wick et al., 2011).

An outdoor landfarming configuration can be considered passive or intensive, based on management activities. To reduce costs and energy consumption, external interventions could be minimized in a passive operation, but this would lengthen the time of treatment. These negative aspects can be mitigated by using the contaminated area to cultivate biomass for energy and biofuel production during treatment, or by using the soil to cover waste in landfills. With intensive landfarming operations, processes are optimized using active management to reduce contaminants to residual concentrations over a shorter time (ASTS Incorporated, 2010; Harmsen et al., 2007).

Any landfarming system with any configuration needs a monitoring plan to control the biodegradation process and verify the reduction in contaminant concentrations.

Moreover, careful analyses of the groundwater and air quality are needed to show that the contaminants are contained within the treatment area and have not migrated into the environment (U.S. Environmental Protection Agency (US EPA), 2012; Wick et al., 2011). Landfarming processes must be performed with the aim of preventing adverse effects on the environment and avoiding the transfer of pollutants from the soil to other environmental media such as air or groundwater (Maila and Cloete, 2004).

Prior to initiating the landfarming process, it is important to study its feasibility by determining the types of indigenous microorganisms present in the soil and their metabolic activity, the presence of inhibitors, and the biodegradability and bioavailability of pollutants (Maila and Cloete, 2004).

2.3.2. Monitoring of operating parameters

The success of bioremediation in soils contaminated by PAHs actually depends on several factors. These include the abundant presence of microorganisms that excrete enzymes for degrading target pollutants that are bioavailable and accessible to the microorganisms (Othman et al., 2011; Wick et al., 2011). The bioavailability of contaminants is influenced by many factors, including their chemical structure and molecular weight as well as the properties of the soil, such as its texture, moisture, and organic matter content. Furthermore, physico-chemical interactions concerning PAHs, the soil, and environmental conditions can significantly affect the efficiency of the biodegradation process (Beškoski et al., 2012; Guerin, 2000; Johnsen et al., 2005; U.S. Environmental Protection Agency, 1994; Wick et al., 2011).

The main operational parameters that must be monitored before and during landfarming are briefly discussed in the following paragraphs.

- Types of microorganisms and their abundance

Landfarming to biodegrade PAHs and organic contaminants in general requires a consistent number of aerobic and heterotrophic microorganisms (U.S. Environmental Protection Agency, 1994; Wick et al., 2011). A wide range of diverse microorganisms, such as bacteria, algae, fungi, protozoa, and actinomycetes, naturally thrive in soil. Among these, bacteria form the most numerous and biochemically active group able to convert organic contaminants into harmless compounds, such as CO₂ and H₂O, when

oxygen is used as terminal electron acceptor (Maila and Cloete, 2005; U.S. Environmental Protection Agency, 1994).

The most common analysis to assess the microbial population of heterotrophic bacteria in soil samples is the total viable count, expressed in terms of colony-forming units (CFUs) per gram of soil. Typical microbial population densities range from 10^4 to 10^7 CFU per gram of dry soil. For an effective landfarming process, total heterotrophic bacteria should have a presence of at least 1,000 CFU per gram of dry soil (U.S. Environmental Protection Agency, 1994).

- Nutrient availability

The availability of inorganic nutrients such nitrogen and phosphorus is essential to support the growth of microorganisms and to stimulate their activity in biodegrading contaminants (Okere and Semple, 2012; Othman et al., 2011; Straube et al., 2003; U.S. Environmental Protection Agency, 1994; Wick et al., 2011). Usually, nutrients are not available in adequate amounts in contaminated soil and must be added. Since PAHs represent a source of carbon for microorganisms, the biodegradation process in contaminated soils is usually limited by nitrogen and phosphorus. Operating conditions where the C:N:P ratio is approximately 100:10:1 are considered the most optimal for PAH degradation (Chaîneau et al., 2003; Leys et al., 2005; Maila and Cloete, 2004; Okere and Semple, 2012; Straube et al., 2003; Wick et al., 2011). A ratio of 100:1:0.5 may still be considered effective (U.S. Environmental Protection Agency, 1994).

- pH value

Many sites contaminated by PAHs do not have optimal pH values for the bioremediation process, so it is common to adjust the pH before and during landfarming operation (Bamforth and Singleton, 2005; U.S. Environmental Protection Agency, 1994; Wick et al., 2011). The optimal pH range to support bacterial growth is from 6 to 8 (ASTS Incorporated, 2010; Haritash and Kaushik, 2009; U.S. Environmental Protection Agency, 1994). If the pH value increases to 9, the nitrogen will be converted into ammonia and becomes unavailable to microorganisms as a nutrient. This could cause the acrid-smelling release of ammonia into the environment (Sayara, 2010).

- Moisture content

An insufficient moisture content affects the appropriate growth of microorganisms, and excessive soil moisture affects soil aeration, reducing the availability of oxygen essential for aerobic metabolic processes (Beškoski et al., 2012; U.S. Environmental Protection Agency, 1994). Generally, the optimal moisture content for successful landfarming ranges between 30% and 85% of soil field capacity (water-holding capacity), or 12% to 30% in weight (Maila and Cloete, 2004; U.S. Environmental Protection Agency, 1994; Wick et al., 2011). Due to aeration, soil loses moisture and dries, thus reducing the activity of the microorganisms. This parameter must be carefully monitored during the process and adjusted when needed. In contrast, areas with a high rain precipitation rate can experience a too high moisture content if the drainage system is not correctly designed (U.S. Environmental Protection Agency, 1994). In areas where the average annual rainfall is more than 30 inches (approximately 750 mm), any bioremediation treatments should be conducted inside a greenhouse structure; otherwise, appropriate precautions are needed to avoid detrimental water accumulation in soils (U.S. Environmental Protection Agency (US EPA), 2012).

- Temperature

The growth, selection, and activity of microorganisms in soils depend on the soil temperature, which is influenced by ambient temperatures. The optimal temperature range for the growth of mesophilic microorganisms is between 10°C and 45°C. Usually, microbial activity doubles for each 10°C rise in temperature within this range. Since the ambient temperature varies seasonally, there are periods during the year when a decrease in microbial activity in the soil will reduce the effectiveness of the bioremediation treatment. Hence, the time when temperatures are optimal is usually called the “landfarming season”. In countries with a continental climate, the “landfarming season” could last from 7 to 9 months, while in warm regions it might last all 12 months. Countries with a colder climate could use an indoor configuration equipped with a greenhouse structure and adequate heating and covering systems. Alternatively, they could consider the use of special psychrophilic bacteria in landfarming (U.S. Environmental Protection Agency, 1994).

- Soil texture

Soil texture affects its porosity, permeability, moisture content, and density. Finely pulverized soils are less permeable compared to soils composed of larger particles. Soils

with low permeability are usually agglutinated and hinder the distribution and transport of water, nutrients, and air. Bulking agents are usually added to ensure an appropriate soil texture and porosity for the bioremediation process (Beškoski et al., 2012; U.S. Environmental Protection Agency, 1994). In contrast, clumpy soil need shredding or other similar pretreatments prior to the landfarming process (U.S. Environmental Protection Agency, 1994). The degradation time and efficiency depend on the type of contaminated soil, its clay and organic matter content, and the proportion of sand fractions (Beškoski et al., 2012). The distribution of contaminants is not homogenous in contaminated soils; therefore, the soil must be appropriately prepared and homogenized prior to treatment (Antizar-Ladislao et al., 2005).

- Features of contaminants

It is crucial to determine the characteristics and chemical structure of contaminants in a polluted site in order to evaluate the time required to achieve bioremediation and to complete feasibility studies. It is common for several contaminants to be present simultaneously in soils, and the biodegradation process and the relating monitoring system must be designed with the aim of removing the most recalcitrant of them. Even if almost all organic compounds present in sites contaminated by petroleum products are biodegradable, their biodegradation rate depends on the complexity of their molecular structure (U.S. Environmental Protection Agency, 1994; Wick et al., 2011). Actually, low molecular weight aliphatic and monoaromatic compounds have a much higher biodegradability rate than high molecular weight aliphatic and polycyclic aromatic compounds characterized by lower water solubility and bioavailability (Cerniglia, 1992; Juhasz and Naidu, 2000; U.S. Environmental Protection Agency, 1994; Wick et al., 2011).

Furthermore, all these compounds are characterized by a wide range of volatility levels that must be considered during landfarming. Tilling and mixing the soil enhance the release of VOCs into the atmosphere, and the emission of such volatile compounds needs to be strictly controlled. During indoor landfarming, vapors must be appropriately treated prior to venting them into the atmosphere. Among all petroleum products commonly found in a contaminated site, gasoline, diesel fuels, and kerosene contain a high volatile fraction, whereas heating and lubricating oils are mainly composed of non-volatile fractions (U.S. Environmental Protection Agency, 1994).

- Contaminants concentration

The type of organic pollutants and their concentrations both influence the growth of microorganisms responsible for biodegradation during bioremediation treatment. Specific pollutants could be toxic to microorganisms even at low concentrations, while others might inhibit microbial growth only if present at very high concentrations. Furthermore, bacteria show different resistances to different types of pollutants.

The next important factor that can influence the toxicity effect on bacterial growth is the volatile fraction (Margesin and Schinner, 2001; U.S. Environmental Protection Agency, 1994). Indeed, the presence of a very high volatile fraction from the total pollutant concentration would minimize the negative effect on microorganisms, considering it leads to the dissipation of pollutants into the atmosphere. Similarly, a high amount of recalcitrant organic pollutants within the total pollutant concentration can reduce the inhibitory effects on microorganisms. Actually, they would not be considered bioavailable and thus would not have any effect on the microorganisms. Therefore, an inhibiting effect on the bioremediation process is not exclusively due to the contaminant present in the soil and its concentration, but rather results from a specific combination of contaminants, microorganisms, and environmental conditions. Indeed, the volatile as well as bioavailable fraction of a compound can influence its toxicity level (Margesin and Schinner, 2001; U.S. Environmental Protection Agency, 1994). Toxic contaminants with a predominantly volatile or recalcitrant fraction have less of an effect than others on microorganism activity, because the amount of such compounds in contact with the microorganisms is limited by dissipation into the atmosphere (volatile fraction) and adsorption on soil particles (non-bioavailable fraction).

In addition, a contaminant present in a pretty low concentration in soils, although higher than the regulation threshold, can inhibit microorganisms because bacteria may not be able to obtain enough carbon necessary for its activity. This concentration limit depends on the specific microorganisms and contaminants present in the soil, and it is necessary to evaluate its value prior to treatment (U.S. Environmental Protection Agency, 1994).

2.3.3. Limitations of landfarming

Physical, chemical, and biological aspects can affect the feasibility and convenience of landfarming as a process to remove PAHs from contaminated soils. The physical aspects include the land requirements for treatment, the ability and limitations of

aeration equipment, the mobility of pollutants in the soil, and water requirements. Furthermore, the level of soil contamination in terms of type and concentration of pollutants could be so high that the bioremediation process would require extremely long times to achieve a satisfying result. Finally, the high toxicity and low bioavailability of contaminants and their lack of accessibility to the microorganisms could further extend time required to remove contaminants from soils (Harmsen et al., 2007; Johnsen et al., 2005; Maila and Cloete, 2004; Semple et al., 2001). These aspects are discussed in the following paragraphs.

- Recalcitrant contaminants removal

The effectiveness of landfarming in treating recalcitrant and high molecular weight organic pollutants is particularly critical (Antizar-Ladislao et al., 2005; Harmsen et al., 2007; Wick et al., 2011; Zhang et al., 2011). Such contaminants are characterized by a high hydrophobicity that significantly reduces their solubility in water and thus their bioavailability and accessibility to microorganisms (Antizar-Ladislao et al., 2005; Potter et al., 1999; Zhang et al., 2011). Considering that the metabolization of PAHs by microorganisms can occur only if contaminants are accessible to the microorganisms, their low bioavailability may limit biodegradation and result in their persistence in the environment (Antizar-Ladislao et al., 2005; Cui et al., 2013; Potter et al., 1999; Zhang et al., 2011). Landfarming is ineffective with low bioavailable organic compounds that remain adsorbed on soil. To overcome this limitation, surfactants can be added to the soil to enhance the bioavailability of organic pollutants, promoting their removal. The addition of surfactants decreases the interfacial tension between water and hydrophobic pollutants, and thus improves the release of pollutants into the water phase. Indeed, surfactants are molecules with a hydrophilic head and a lipophilic tail making hydrophobic compounds to be solubilized in the hydrophobic cores of the micelles, thus leading to PAH transfer into the water phase (Mohan et al., 2006). Otherwise, adsorbents can be added, resulting in the immobilization of pollutant residues and making them harmless in the environment (Maila and Cloete, 2004).

- Microbial population

An important biological factor that could limit the performance of landfarming in biodegrading organic pollutants is the lack of an adequate number of microorganisms (i.e., less than 1,000 CFU per gram dry soil). Furthermore, soil treatment can be

affected due to a lack of microorganisms able to degrade certain organic contaminants (U.S. Environmental Protection Agency, 1994; Wick et al., 2011). These aspects can be positively controlled by biostimulation and bioaugmentation practices, maintaining landfarming as a feasible treatment (U.S. Environmental Protection Agency, 1994).

- Toxicity of contaminants

High concentrations of organic pollutants and the presence of heavy metals in soils contaminated by PAHs can negatively affect the efficiency of landfarming (Beškoski et al., 2012; Sayara et al., 2010a; U.S. Environmental Protection Agency, 1994). High concentrations of contaminants which are toxic to the microbial community are related to the bioavailable fraction; otherwise, the toxicity to microorganisms will not be demonstrated (Loureiro et al., 2005; Saison et al., 2004). Concentrations of petroleum compounds that are more than 50,000 ppm and concentrations of total heavy metals that are more than 2,500 ppm are considered to be inhibitory for microbial growth and toxic to hydrocarbon-degrading bacteria (Beškoski et al., 2012; Khan et al., 2004; U.S. Environmental Protection Agency, 1994). When contaminants are at concentrations higher than these limits, the landfarming treatment is not recommended unless the soil is conditioned or amended (U.S. Environmental Protection Agency, 1994) so that the concentration of contaminants is reduced due to dilution.

- Volatilization issues

Volatilization of organic compounds, if not controlled, is a negative aspect of the landfarming process, since it has a significant impact on the environment. High rates of volatilization are to be expected in warm climate regions such as the southern United States, South America, Africa, and the Middle East (Ward et al., 2003). If a contaminated area treated by landfarming is not properly covered by a greenhouse (indoor configuration), then dust generation and the release of VOCs can worsen the air quality (Beškoski et al., 2012; Mohan et al., 2006; Sayara, 2010). Volatilization of PAHs is significant only for compounds with two aromatic rings, such as naphthalene and 1-methylnaphthalene (Mohan et al., 2006).

- Leaching

During landfarming treatment, the removal of organic contaminants can occur by transfer of contaminants from the solid phase to the liquid phase (leachate) (Smith et al.,

2011). The leachate is usually collected and further processed (Beškoski et al., 2012); otherwise, the removal of contaminants from the soil could result in water pollution and limit the use of landfarming.

- Degradation products

Another critical aspect of landfarming is the likely formation of metabolites that are even more toxic and more mobile than the initial organic contaminants (Antizar-Ladislao et al., 2005; Beškoski et al., 2012; Sayara, 2010; Singh, 2012; Zhang et al., 2011). Their accumulation in the environment can be induced by an incomplete degradation of organic contaminants (Beškoski et al., 2012; Saison et al., 2004; Sayara, 2010; Singh, 2012).

- Long-term treatment

A possible limitation in the use of landfarming is the long treatment time necessary to reduce contaminant concentrations to levels acceptable by local regulations (Beškoski et al., 2012; Maila and Cloete, 2004; Sayara, 2010). Landfarming requires longer times than other remediation methods, especially for *in situ* systems (Beškoski et al., 2012; Sayara, 2010). Hence, the time duration may not be in agreement with requirements by local legislation.

- Water requirements

Among all operating parameters, a low available water supply may be inadequate to achieving the necessary moisture content. The moisture percentage in the landfarming treatment should be kept between 30% and 85% of soil field capacity to support appropriate microbial growth and enable dust control (Maila and Cloete, 2004; U.S. Environmental Protection Agency, 1994; Wick et al., 2011). If the contaminated area is extremely vast, the amount of water required to maintain this favorable moisture level may not be easily or affordably supplied, especially for arid countries, which can significantly increase the treatment costs (Maila and Cloete, 2004).

- Treatment area and aeration equipment

The areal extent subject to remediation could be extremely large, and this aspect coupled with the traditionally long time required by landfarming could limit the use of this process because of an increased probability of human exposure to contaminants

(Maila and Cloete, 2004; U.S. Environmental Protection Agency, 1994). An additional constraint of landfarming treatment may be the capacity of tilling equipment used to aerate the soil, as it needs to be able to reach the “subsurface” contaminated soil (Maila and Cloete, 2004). Indeed, the soil depth that can be treated will depend on the capacity of conventional farm equipment used to reach this depth (Picado et al., 2001).

- Microbial activity conditions

Among solid phase treatment technologies, landfarming has a distinctive advantage in that it stimulates the activity of indigenous microorganisms in the soil that are already adapted to the presence of contaminants. Indigenous microbial communities are usually constrained in their activity and their capability to degrade contaminants, due to limiting factors such as inadequate aeration, poor contact of the microorganisms with the contaminants, and an insufficient supply of bacterial nutrients (N, P, and K) or carbon sources (Harmsen et al., 2007; Mohan et al., 2006; Wick et al., 2011). The most optimal C:N:P ratio to overcome this limitation and to reach a high hydrocarbon removal rate is 100:10:1 (Maila and Cloete, 2004; Okere and Semple, 2012; Wick et al., 2011).

2.4. Enhanced landfarming

Two main critical aspects of landfarming that limit the use of this soil bioremediation treatment are the potentially long times required to remove contaminants from soils and the difficulty in fully governing the biochemical reactions involved in the bioremediation process, as the performance is influenced by many factors that are not easily controlled (as discussed in the previous subsections). Both aspects may be less constraining if the conditions in which microorganisms thrive are improved by strategies such as biostimulation, bioaugmentation, and composting (Maila and Cloete, 2004; Mohan et al., 2006; Sayara et al., 2010b).

2.4.1. Bioaugmentation

Bioaugmentation consists of adding specific microorganisms to the soil that are harmless and particularly efficient at removing pollutants at a contaminated site (Joo et al., 2007; Juwarkar et al., 2010; Maila and Cloete, 2004). In soils where indigenous microorganisms are inadequate or absent, such as sites with high PAH concentrations,

bioaugmentation can promote and speed up the degradation process. This solution is frequently used in *ex situ* systems (Juwarkar et al., 2010; Maila and Cloete, 2004; Wick et al., 2011).

The success and high performance of bioaugmentation depends on many factors, including a sufficient amount of culture to facilitate the complete removal of target contaminants. Furthermore, the survival ability and catabolic activity of introduced microorganisms, their resistance to other co-contaminants present at the site which might affect biodegradation, and the bioavailability of contaminants should be considered (Beškoski et al., 2012; Juwarkar et al., 2010; Maila and Cloete, 2004). Indeed, the survival ability of added strains can be affected due to competition with indigenous microorganisms, or by co-contaminants present at the site that could be toxic to added strains. Accordingly, this process can be effective and rapid, but may also be unpredictable (Maila and Cloete, 2004). Thus, the most practical approach is to use microorganisms isolated from the soil that is being decontaminated (Beškoski et al., 2012).

Zhang et al. (Zhang et al., 2011) studied bioaugmentation in a soil contaminated by PAHs that was amended with a mixture of leaves, branches, and biowastes and inoculated with specific microorganisms (*Bacillus* sp. and *Fusarium* sp.).

The dissipation of the 16 U.S. EPA-listed PAHs was considerably enhanced and reached 64% in the inoculated treatment compared to soil with a waste-only treatment without inoculation. In the latter case, the PAH dissipation reached 50% after 60 days of incubation. A pilot scale landfarming study performed by Atagana (Atagana, 2003), in which the soil was bioaugmented with indigenous microorganisms, indicated high effectiveness in creosote removal. This study investigated the effects of biostimulation and bioaugmentation on creosote-contaminated soil over 16 weeks.

Little difference in removal efficiency was observed based on amendment type, e.g., sewage sludge, cow manure, or poultry manure; however, a difference was noticed in treatments amended with organic manures and bioaugmented with indigenous microorganisms. Actually, the highest removal efficiency of creosote, 89%, was reached during treatment with bioaugmentation (Atagana, 2003), while a creosote removal efficiency of 86% was reached during treatment amended with sewage sludge. Similarly, Chien et al. (Chien et al., 2010) designed an *ex situ* field-scale landfarming system for treating petroleum hydrocarbon-contaminated soils. Bioaugmentation was performed by adding a commercially available mixed microbial inoculum to the soil at a

mass ratio equal to 1:50. After 155 days of incubation, the removal efficiency of contaminants reached approximately 85%. Therefore, the addition of mixed microbial inoculums enhanced the efficiency of TPH removal, considering that the first-order TPH decay rate observed in the treatment with added microbial inoculums was 0.015 day^{-1} , while the TPH decay rate in the treatment under intrinsic conditions was one order of magnitude lower at 0.0069 day^{-1} . Additionally, the decay rate in treatment with added chicken manure was 0.0142 day^{-1} , showing a promising potential for chicken manure as a substitute for commercial microbial inoculum. On the other hand, Sayara et al. (Sayara et al., 2011) found that inoculation with the white-rot fungi *Trametesversicolor* was ineffective for PAH biodegradation during 30 days of treatment. The removal efficiency of PAHs was the same, 89%, with both the treatment amended with mature compost only, and the treatment amended with mature compost and inoculated with *Trametesversicolor*.

2.4.2. Biostimulation

Biostimulation of microbiological processes in contaminated soils is achieved by adjusting the pH value and adding nutrients to the soil such as nitrogen, phosphorus, and trace elements, with the aim of supplying microorganisms with a well-balanced feeding. Furthermore, in order to stimulate the growth of indigenous biodegrading microorganisms, it is essential to ensure an optimal moisture content and oxygen concentration in the soil. Microorganisms effectively metabolize contaminants under favorable environmental conditions for their growth (Joo et al., 2007; Maila and Cloete, 2004; Mohan et al., 2006; Wick et al., 2011). Biostimulation in landfarming has been used successfully to remove PAHs from soils (Juwarkar et al., 2010; Mohan et al., 2006; Zappi et al., 1996).

In more detail, McFarland and Qiu (McFarland and Qiu, 1995) stimulated a microbial community by adding corn cobs to improve soil porosity and thus the effectiveness of aeration in a silt loam soil spiked with benzo(a)pyrene. They reported an efficiency-bound residue formation of benzo(a)pyrene of 66% at 39°C after 95 days of incubation, with a soil-to-corn cob ratio equal to 2:1 in dry weight. Straube et al. (Straube et al., 2003) reported a total PAHs removal efficiency of 86% by performing a landfarming treatment with biostimulation. They added ground rice hulls as a bulking agent and dried blood as a slow-release nitrogen source. They achieved better results after a longer

incubation period, i.e., after 16 months, compared to the results of McFarland and Qiu (McFarland and Qiu, 1995), but the treatment was primarily limited to the removal of 3- and 4-benzene ring PAHs.

The study of Nduka et al. (Nduka et al., 2012) demonstrated that the use of fertilizer amendment, the type of microbe, and the type of organic pollutants present influenced the growth of the bacterial population and the efficiency of the contaminant removal. Actually, the amendment contributed to improving the microbial growth, as shown by a tripling in the population size compared to no amendment. Furthermore, at the same operating conditions, the hydrocarbons removal efficiency in the amended soil was more than 90%, while that in the unamended soil was less than 50%. An initial increase in the microbial population in the unamended soil was rapidly followed by a decrease, likely due to a lack of nutrients that could have been provided by fertilizer in the amended soil. Although no remarkable difference was observed in the biodegradation of hydrocarbons when the process was stimulated with synthetic fertilizer (i.e., NPK and urea) and natural organic fertilizer (i.e., cow dung and poultry litter), the latter is preferred as a biostimulating agent compared to a synthetic fertilizer because it is less expensive and more environmentally friendly.

2.4.2.1. *Composting of organic waste*

Composting is a process typically used to degrade organic solid waste materials and to convert them into compost, a soil amendment rich in humic acid and nutrients. Composting has also recently gained interest in the field of soil bioremediation as a means to remove PAHs (Gan et al., 2009; Sayara et al., 2010a). It is a biological process based on the activity of microorganisms in degrading organic materials, resulting in the release of heat, the production of thermogenesis, and the production of a solid and biologically stable material (i.e., compost) along with compounds such as carbon dioxide and water (Beškoski et al., 2012; Juwarkar et al., 2010; Semple et al., 2001).

The composting process can positively contribute to the removal of organic contaminants from soils due to an increase of temperature (Juwarkar et al., 2010), the addition of a carbon source and nutrients (biostimulation effect), and the addition of a wide number of microorganisms specializing in degrading organic compounds and already present in organic waste (bioaugmentation effect). The elevated temperature typical of a properly performing composting process can increase the biochemical

kinetics involved in the biodegradation process, namely the contaminants' solubility and mass transfer rate, thus making the contaminants more accessible and available to microorganisms for their metabolism (Antizar-Ladislao et al., 2005).

The performance of the composting process depends on operating parameters that are the same as those important to the success of landfarming: temperature, moisture content, pH value, aeration, nutrient content, C:N:P ratio, and composting material porosity (Juwarkar et al., 2010; Sayara et al., 2010a; Sayara, 2010). Thus, these parameters must be monitored. The optimal moisture content should be approximately 60%, the pH value should range between 6 and 7.5, the oxygen concentration should range from 5% to 15% of total volume, and the C:N:P ratio should be equal to 100:10:1 (Juwarkar et al., 2010; Park et al., 2001; Sayara et al., 2010a; Sayara, 2010).

2.4.3. Landfarming integrated with composting of organic waste

The biodegradation efficiency of PAHs in soils through landfarming integrated with the composting of organic waste depends on the physical, chemical, and biological properties of the soil, waste characteristics, and environmental conditions (Mohan et al., 2006). As soil contaminated with petroleum hydrocarbons usually has a high C:N ratio and relatively low organic matter content, the organic waste amendment supplies the indigenous microbial community with the appropriate nutrient content and carbon source (Joo et al., 2007; Okere and Semple, 2012; Wick et al., 2011). Additionally, the microbial density in the contaminated soil is enriched with strains of microorganisms naturally occurring in such waste (Joo et al., 2007; Okere and Semple, 2012; Soil Health, 2012), and the high water content of organic waste contributes to adequate soil moisture. Thus, landfarming integrated with the composting of organic waste can be an interesting option for improving the biodegradation process of organic pollutants. It even provides for the eco-friendly disposal of organic waste, since the waste is simultaneously biodegraded (Joo et al., 2007; Okere and Semple, 2012). An organic amendment can improve the soil texture and the oxygen transfer and provide an additional carbon source for the microorganisms. Studies report that indigenous microorganisms have increased capability for degrading PAHs when an organic substrate is added (Antizar-Ladislao et al., 2005; Semple et al., 2001).

Furthermore, the reduction of PAH concentrations in the soil will be more efficient with the addition of fresh organic waste and composting compared to the addition of mature

compost, according to Antizar-Ladislao et al. (Antizar-Ladislao et al., 2006) and Zhang et al. (Zhang et al., 2011). The advantage of this composting arises from the mixing of contaminated soil with waste material, and if the bioremediation treatment fails, it will not generate additional waste material above what already existed. Instead, if mature compost, considered a non-waste material, is used as an amendment, then if the bioremediation treatment fails it will generate more contaminated material than the starting amount (Antizar-Ladislao et al., 2006, 2005).

The main objectives of this eco-friendly and economical bioremediation strategy (Yamada et al., 2007) are to biotransform organic contaminants to less toxic substances or harmless compounds, such as carbon dioxide and water (a process called “mineralization”), and to adsorb contaminants to the organic matrix affecting their bioavailability, biodegradability, leachability, volatility, and persistence in the environment (Bamforth and Singleton, 2005; Semple et al., 2001; Ward et al., 2003; Wick et al., 2011). Furthermore, bioremediation enriches the indigenous microbial community of the contaminated soil with bacteria, actinomycetes, and lignolytic fungi (Bamforth and Singleton, 2005; Semple et al., 2001; Ward et al., 2003) that are already present in the organic waste (along with nutrients and moisture).

2.4.3.1. Fate of contaminants in bioremediation treatment of landfarming integrated with composting

Figure 2.2 summarizes the possible fate and behavior of organic contaminants and their intermediate metabolites during bioremediation. Processes which influence the fate of contaminants in soil include volatilization to air (loss), transfer to organisms (loss), biodegradation (loss), binding to soil mineral and organic matter fractions (persistence), and leaching into groundwater (loss) (Semple et al., 2003, 2001; Stokes et al., 2006). Mineralization is the most preferred mechanism and it may be enhanced by increasing the availability of contaminants to the microorganisms during the biodegradation process. The effectiveness of mineralization depends on the occurrence and kinetics of contaminant desorption from soil particles (Antizar-Ladislao et al., 2005; Semple et al., 2001). Actually, the high operational temperature during composting can enhance the contaminants’ desorption from soil due to the increased solubility of contaminants in water and their diffusion rate (Haderlein et al., 2006; Saison et al., 2004; Van Gestel et al., 2003). This results in a transfer of organic contaminants from the solid phase (soil

particles) to an aqueous phase where they are more accessible to microorganisms (Antizar-Ladislao et al., 2005; Semple et al., 2001).

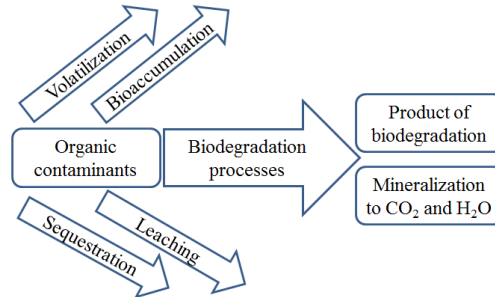


Figure 2.2. Fate of organic contaminants in soil (Semple et al., 2003, 2001; Stokes et al., 2006).

A factor inherent to the composting strategy is that a large amount of organic matter could be added to the system, which is considered as major factor in strongly binding organic contaminants to the soil matrix (Antizar-Ladislao et al., 2005). Once strongly bound to the soil matrix, recalcitrant fractions of contaminants are barely bioavailable and, thus, they do not influence the eventual toxicity of the soil (Stokes et al., 2006). The composting process only mineralizes the bioavailable fractions of organic compounds, while those that are not bioavailable remain bound to the soil matrix, reducing the risk of contamination of the environment. However, the fate of recalcitrant organic contaminants in the soil remains uncertain and it is a concern related to risk assessment that requires further studies (Antizar-Ladislao et al., 2005; Maila and Cloete, 2004).

2.4.3.2. Influence of different biomasses used on bioremediation of petroleum compounds

Readily available organic wastes, such as manure, sludge from municipal wastewater, municipal solid waste, and biosolids, have been studied as potential biomasses and they positively influence bioremediation efficiency. Nevertheless, the mechanisms of biostimulation induced by their addition require further studies to be completely understood and controlled (Álvarez-Bernal et al., 2006; Mohan et al., 2006). Most studies state the importance of optimizing the operating conditions during

bioremediation. High molecular weight PAHs are difficult to biodegrade and different studies have examined the best conditions to remove these contaminants (Antizar-Ladislao et al., 2004). Table 2.8 summarizes previous research on the addition of various organic waste products using different test conditions, and this is followed by a brief overview of the obtained results.

The majority of laboratory-scale composting studies on PAH-contaminated soils were performed in the mesophilic or low thermophilic temperature range in order to avoid the decrease in bioavailability that occurs when the process temperature is higher than 65 °C (Antizar-Ladislao et al., 2004; Potter et al., 1999). A preliminary study by Adenuga et al. (Adenuga et al., 1992) shows that pyrene can be successfully degraded by landfarming integrated with composting using a mixture of soil and sewage sludge under mesophilic and thermophilic temperature conditions (Antizar-Ladislao et al., 2004). Similarly, Civilini (Civilini, 1994) investigated PAH removal at a constant temperature of 45°C, mixing municipal solid waste and fertilizer with soil contaminated by creosote. The mixture was created at an optimal ratio for avoiding toxicity effects. The PAH removal efficiency was between 82% (on benzo(a)anthracene) and 99% (on fluorene) after 15 days of treatment. This study on the removal of contaminants was based on the total extractability of the PAHs and did not consider any fraction which was non-extractable (Antizar-Ladislao et al., 2004; Semple et al., 2001). Furthermore, Van Gestel et al. (Van Gestel et al., 2003) found that the composting temperature and level of microorganisms contributed to an efficiency of 82% in contaminant removal using a composting treatment, while only 17% of the contaminant removal efficiency could be attributed to additional composting-related factors such as nutrient supply and organic matter content. The first-order kinetic constant of diesel degradation in a soil mixed with biowaste was four times higher than that in a soil kept at room temperature, and 1.2 times higher than that in a soil at composting temperature (Van Gestel et al., 2003).

CHAPTER 2

Table 2.8. Previous research experiences related to soil bioremediation with the addition of organic wastes.

Organic waste	Soil	Process details	Contaminants	Reference
Composted sewage sludge	Spiked soil	Moisture content 40%, T was raised from 20 °C to 60 °C by 5 °C day ⁻¹ , 21 days	Pyrene	(Adenuga et al., 1992; Antizar-Ladislao et al., 2004)
Municipal solid wastes and fertilizer	Creosote-contaminated soil	Constant temperature at 45 °C, 15 days	Naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene	(Antizar-Ladislao et al., 2004; Civilini, 1994; Semple et al., 2001)
Biowaste – vegetable, fruit, and garden waste	Diesel oil-spiked soil	Soil-to-biowaste ratio 1:10, 12 weeks	Diesel oil	(Van Gestel et al., 2003)
Refinery sludge	Real contaminated soil	Semiarid climate, low rain (total rainfall ranges 200–250 mm year ⁻¹), annual average temperature 19°C, soil humidity 1% – 3%, aeration once a month, 11 months	Total petroleum hydrocarbons	(Juwarkar et al., 2010; Marin et al., 2005)
Green tree waste and manure	Tar residues contaminated soil	Field scale, green tree waste (< 5 days old), manure:soil ratio 15:5:80, moisture 60%– 80% field capacity, T reached maximum at 42 °C after 35 days, 224 days	16 PAHs	(Antizar-Ladislao et al., 2004; Guerin, 2000)
Sewage sludge, sterilized sludge, sludge to maintain pH, glucose plus N and P source	Soil of the former lake Texcoco	T 22 ± 2 °C, 112 days	Anthracene, phenanthrene	(Fernández-Luqueño et al., 2008)
Cow manure, modified	Reilly soil - creosote	C:N:P 100:5:1, corn cobs bulking agent, moisture 30%–	19 PAHs	(Antizar-Ladislao et

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OECD fertilizer, and activated sewage sludge	manufacturing and wood preserving	35%, T 41°C to 53 °C at the first 15 days and subsequently decreased to ambient T, 84 days		al., 2004; Potter et al., 1999)
Sewage sludge and compost	Diesel oil-spiked soil	Contaminated soil-to-organic amendments ratio 1:0.1, 1:0.3, 1:0.5, 1:1 (wet weight), 30 days	Total petroleum hydrocarbons	(Namkoong et al., 2002)
Green waste	Aged coal tar contaminated soil	Constant T at 38°C, 55°C, and 70°C separately, and comparative studies using a temperature profile, moisture content of 40%, 60%, and 80% field capacity, soil-to-green waste ratio 0.6:1, 0.7:1, 0.8:1, and 0.9:1, 8 weeks	16 PAHs	(Antizar-Ladislao et al., 2005; Sayara, 2010)
Sewage sludge	Petroleum refinery oil sludge-spiked soil	T 23°C to 25 °C, bulking agent grass, contaminated soil-to-sewage sludge ratio 1:0.1, 1:0.3, 1:0.5, 1:0.7, 1:1 (wet weight), tilled weekly, 9 weeks	Petroleum refinery oil sludge	(Ling and Isa, 2006)
Sewage sludge	Heavily contaminated soil with creosote (> 310,000 mg kg ⁻¹)	Field scale, moisture content 70% field capacity, aeration every 2 weeks, C:N:P ratio 25:1:1, 10 months	PAHs	(Atagana, 2004a)

Marin et al. (Marin et al., 2005) evaluated the effectiveness of landfarming in removing total petroleum hydrocarbon (TPH) content in a semiarid climate. They reported a TPH removal efficiency of 80% after 11 months, while an aliquot of 55% was reached during the first 3 months of treatment (Juwarkar et al., 2010). The degradation rate slowed sharply after 9 months due to drop in microbial activity, since the residues remaining in soil were recalcitrant hydrocarbons that were less accessible and bioavailable to further microbial metabolization. If the temperature conditions and soil moisture were higher, probably the treatment time would have been reduced with better results. A slightly higher removal rate was reached by Guerin (Guerin, 2000), and composting was demonstrated to be suitable for biostimulating PAH removal from soil. Operational parameters such as the appropriate biomass and the biomass-to-soil ratio, oxygen supply, and moisture content were critical factors in achieving effective bioremediation, and the long duration of the treatment was the main disadvantage. Low molecular weight PAH losses through volatilization were not detected. Removal of low molecular weight PAHs reached 100% efficiency, while the removal of medium and large PAHs achieved 90% and 70% efficiency, respectively, after 224 days of bioremediation treatment. Removal of the most bioresistant compounds, i.e., indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene, reached an approximate efficiency of 50% (Antizar-Ladislao et al., 2004).

Furthermore, Fernández-Luqueño et al. (Fernández-Luqueño et al., 2008) studied the effects of soil, contaminants, and biomass (i.e., sludge from industrial wastewater treatment plant) characteristics on PAH removal. After 112 days of treatment, the lowest concentrations of anthracene and phenanthrene were found in soil amended with sludge, while the highest were found in soil amended with glucose. The addition of glucose probably stimulated the growth of microorganisms that do not specialize in degrading PAHs. A higher removal of phenanthrene compared to anthracene was observed with the sludge treatment, and a pH adjustment of the soil did not have an effect. Since the removal of contaminants was significantly higher in soil amended with raw sludge compared to soil amended with sterilized sludge, it can be concluded that microorganisms in the sludge considerably contributed to the PAH removal. In contrast, Potter et al. (Potter et al., 1999) studied PAH removal using three types of biomass: cow manure, fertilizer from the Organisation for Economic Co-operation and Development (OECD), and sewage sludge. They did not observe any significant differences in the efficiency of PAH removal based on type of biomass. Actually, 2- to 3-ring PAHs were

removed by an average of 87% in all treatments after 84 days of incubation, and 4-ring PAHs were reduced by an average of 61%. However, the removal of 5- to 6-ring PAHs was not effective with any of these biomass conditions. Most of the decrease in the concentrations occurred within the first 28 days of treatment, with a peak on day 56 (Antizar-Ladislao et al., 2004). In related research, Lukić et al. (Lukić et al., 2016a; Lukić et al., 2016b) studied the influence of several organic wastes (i.e., buffalo manure, food and kitchen waste, fruit and vegetable waste, and activated sewage sludge) in the bioremediation of PAH-contaminated soil. After 140 days of incubation, the removal of low molecular weight PAHs in soil were significantly higher in treatments that displayed a mesophilic phase (i.e., 89% and 85% in treatments amended by sewage sludge and fruit and vegetable waste, respectively), compared to treatments that displayed a thermophilic phase (i.e., 71% and 69% in treatments amended by food and kitchen waste and buffalo manure, respectively). The most efficient removal of high molecular weight PAHs occurred with the treatment amended by sewage sludge, and this also led to the fastest removal of 3 out of 4 PAH indicators. Together, these studies show that the type of biomass used for bioremediation of PAH-contaminated soil is an important parameter that must be carefully considered.

Namkoong et al. (Namkoong et al., 2002) showed that, besides the type of biomass, the contaminated soil-to-organic waste mass ratio is a very important parameter for the biodegradation efficiency of TPHs. In this study, the highest TPH removal was reached at a ratio of 1:0.5 for both biomasses (i.e., sewage sludge and mature compost). In the experiments that included biomass, the TPH removal had an efficiency of 98% for both biomass types, while an efficiency of only 64% was reached without any added biomass. Fast removal was observed in the early stage of treatment during the first two weeks in all experiments, but slightly better results were obtained for the experiment with sewage sludge. The total volatilization loss was in the range of 1% to 3%, which indicated that volatilization was negligible compared to biodegradation. Furthermore, Antizar-Ladislao et al. (Antizar-Ladislao et al., 2005) reported that the operational temperature and the soil-to-biomass ratio are equally significant for PAH removal from aged coal tar-contaminated soil using a composting strategy. The highest removal efficiency of 16 U.S. EPA-listed PAHs (i.e., 75%) was observed at a constant treatment temperature of 38°C and a contaminated soil-to-green waste ratio equal to 0.8:1. In a comparative study with a temperature profile treatment, the removal of 16 U.S. EPA-listed PAHs was considerably lower, at 61%. In the study by Ling and Isa (Ling and Isa,

2006), the addition of sewage sludge also enhanced the biodegradation of oil and grease. The highest removal efficiency of contaminants was equal to 66% under low temperature conditions (i.e., 25°C), for a contaminated soil-to-sewage sludge ratio of 1:0.5. The residual concentration that remained in soil, 34%, was considered recalcitrant and adsorbed onto soil particles or trapped into soil micropores; thus, it was not available to the microorganisms. However, under higher temperature conditions, a higher removal efficiency could be expected due to higher bioavailability of organic pollutants as a consequence of enhanced thermal desorption. Atagana (Atagana, 2004a) reported the effectiveness of field-scale landfarming and showed high efficiency for contaminant removal. A creosote concentration was reduced from >310,000 to 1,762 mg kg⁻¹ during 10 months of treatment. In addition to a total creosote removal efficiency of more than 90%, the removal efficiency of 4- and 5-ring PAHs reached 76% to 87%, respectively, by the end of treatment.

2.5. Conclusions

The global market for remediation technologies in environmental engineering is growing steadily, with the current share of biological methods at about 10%. This is expected to grow considerably. This review paper provides insight into the efficiency and application conditions of landfarming treatment as a suitable biological method, and approaches to enhancing its efficiency through the composting of organic wastes in order to improve the biodegradation of organic contaminants in soil. Data from laboratory as well as pilot-scale studies on landfarming integrated with composting have shown this process to be ready for full-scale bioremediation of PAH-contaminated soil, since many of studies show promising and feasible results. The development of standardized procedures to study the effects of different conditions during landfarming is recommended, since the degradation efficiency of organic contaminants is influenced by different controlling factors. Certain natural conditions, the features of contaminated material, possible co-contaminations, and indigenous microbial communities are crucial parameters that affect the performance of this integrated treatment.

2.6. Acknowledgments

The authors would like to thank the financial support provided by the European Commission (Erasmus Mundus Joint Doctorate Programme ETeCoS³: Environmental Technologies for Contaminated Solids, Soils and Sediments, under the grant agreement FPA n° 2010-0009).

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A Review on the Efficiency of Landfarming Integrated with Composting as a Soil Remediation Treatment

Chapter 3

Evaluation of PAH removal efficiency in an artificial soil amended with different types of organic wastes

Abstract

The removal of polycyclic aromatic hydrocarbons (PAHs) from a spiked OECD (Organisation for Economic Co-operation and Development) artificial soil was investigated. Laboratory-scale thermally insulated bioremediation reactors were used to implement biostimulation strategy of composting. The selected PAHs included anthracene, chrysene, benzo(k)fluoranthene and benzo(a)pyrene with an initial concentration of 658 mg of USEPA 16 PAHs kg⁻¹ soil (d/w). The contaminants' removal was improved by amending the contaminated soil with four different types of fresh organic waste. After 140 days of incubation, the removal of three- and four-rings PAHs in all reactors were higher than five-rings PAHs. The reactor displaying a mesophilic phase during bioremediation ended with a removal of 89% and 59% for three- and four-rings PAHs, respectively. In contrast the reactor displaying a thermophilic phase ended with 71% and 41% removal for three- and four-rings PAHs, respectively. The highest five-rings PAH removal was obtained for reactors with buffalo manure and sewage sludge amendments (40 and 33%, respectively), while food and kitchen waste and fruit and vegetable waste amendments showed less efficiency (26 and 8%, respectively). Microtox[®] test data indicated lower toxicity in reactor amended with sewage sludge considering that this set-up reached the highest PAHs removal and DHA (dehydrogenase activity) compared to others.

Keywords

Polycyclic aromatic hydrocarbons, bioremediation, biostimulation strategy, composting of organic waste, OECD artificial soil.

Evaluation of PAH removal efficiency in an artificial soil amended with different types of organic wastes

3.1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment and are present in soils, sediments, groundwater and the atmosphere (Piskonen and Itävaara, 2004; Okere and Semple, 2012). The highest concentrations of these organic pollutants are found in soils located in the areas of industrial activities and nearby urban zones, that render the necessity for their removal even more urgent (Picado et al., 2001; Piskonen and Itävaara, 2004).

Generally, the main causes of their presence in soils are accidental forest fires as well as volcanic eruptions as natural phenomena, but most of the contamination originates from anthropogenic sources (Li et al., 2007; Sayara et al., 2011; Okere and Semple, 2012). PAHs are a global concern both for environment and human health. Moreover, their concentration in contaminated soils from industrial areas can be variable depending on the activity on the site (Juhasz and Naidu, 2000).

PAHs are chemical compounds composed of two or more fused aromatic rings containing carbon (C) and hydrogen (H) atoms (Piskonen and Itävaara, 2004; Sayara et al., 2011). Only 16 of them have been recognized as “priority pollutants” listed by U.S. Environmental Protection Agency (U.S. EPA) and the European Commission, and 7 of them are classified as probable human carcinogens (Li et al., 2007; Sayara et al., 2011). Lower molecular weight (LMW) PAH compounds, containing 2 or 3 rings, have shown significant acute toxicity and other adverse effects, whilst many higher molecular weight (HMW) PAHs, containing 4 to 7 rings are carcinogenic, mutagenic, or teratogenic to a large number of different living organisms (Li et al., 2007; Sayara et al., 2011; Smith et al., 2011). Their high hydrophobicity makes them easily adsorbed onto the soil organic matter and thus less available for biological uptake, forming persistent micro pollutants in soils (Li et al., 2007; Piskonen and Itävaara, 2004). PAHs hydrophobicity increases as the rings’ number of PAHs molecule increases (Meador et al., 1995).

Among several treatment technologies available to remove PAHs from soil, bioremediation is one of the most suitable to deal with these environmental contaminants (Harmsen et al., 2007; Sayara et al., 2011). Considering that PAHs biodegradation is a complex natural chemico-biological process, it can be influenced by limiting factors such as microorganism nature, water content, aeration, physico-chemical properties of contaminants, their concentration and bioavailability (Namkoong et al., 2002; Saison et al., 2004; Sayara et al., 2011).

Among the various bioremediation technologies, landfarming is the most favorable for remediation of PAHs contaminated soil due to the need for low capital costs and low technology to be implemented. This technique also allows the handling of large volumes of soil (Picado et al., 2001). In order to overcome possible limitations, it could be successfully applied when combined with composting of organic waste (Guerin, 2000; Straube et al., 2003; Zhang et al., 2011). Moreover, it could be a successful method for treating wastes such as animal manure, sewage sludge and municipal organic solid waste, if final residues of treatments would not be toxic. Due to high content of readily biodegradable organic matter and water in such wastes, incineration or landfill disposal is not economical (Joo et al., 2007). Thus, treatment of PAHs-contaminated soil combined with composting of organic waste could be an interesting option and a sustainable method. It would enable eco-friendly disposal of such waste and enhance the biodegradation rate of PAHs.

Even if the composting approach is found to have a potential for the remediation of PAHs contaminated soils (Antizar-Ladislao et al., 2005; Atagana, 2004a), there are not many studies related to its application with fresh organic waste, and it is still an emerging *ex-situ* bioremediation technology (Namkoong et al., 2002). Previous experiments dealing with PAHs contaminated soil composted with fresh green and bio-waste showed that about 50% of the PAHs were removed during 60 days of experimental activities (Zhang et al., 2011). Meanwhile Atagana (2004) managed to remove almost 100% of PAHs concentrations while composting contaminated soils with poultry manure, but for a much longer period of time (i.e. 19 months). Moreover, Guerin (2000) showed interesting results by applying cow manure and fresh green tree waste material, where removal of high molecular weight PAHs was at least 50% during the 7 months long treatment period.

Due to so many variations, there is a need to optimize the bioremediation process. Hence, the aim of this paper is to implement bioremediation by adding different fresh

organic waste to a PAHs-contaminated soil in order to enrich the soil with organic matter, nutrients and microorganisms. The focus of this research is to study the effectiveness of four types of fresh organic waste added to a LMW and HMW PAHs contaminated soil, and to monitor the removal of these compounds according to their properties. In addition, this study evaluates the microbial activity of the monitored processes as well as soil toxicity level due to the possible formation of even more toxic metabolites and intermediates as a result of incomplete PAHs degradation.

3.2. Materials and methods

3.2.1. Chemicals

Four PAHs listed by U.S. EPA as priority pollutants were purchased from Sigma-Aldrich: anthracene (Anth) (purity $\geq 99\%$), chrysene (Chry) (purity 98%), benzo(k)fluoranthene (B(k)F) (purity $\geq 99\%$) and benzo(a)pyrene (B(a)P) (purity $\geq 96\%$).

3.2.2. Soil and organic amendments

The soil used in these experiments was an artificial OECD (Organisation for Economic Co-operation and Development) soil prepared and stored according to OECD Guideline recommendations (OECD/OCDE 317, 2010). It consisted of 10% sphagnum-peat, 70% quartz sand (274739, Sigma-Aldrich), 20% kaolinite clay (03584 Fluka, Sigma-Aldrich) and 1% calcium carbonate (C4830, Sigma-Aldrich, purity $\geq 99\%$). The dry constituents of the soil were mixed thoroughly two weeks before starting the soil spiking procedure and stored at room temperature until use. The mixed dry soil was moistened with MilliQ water 48 h prior spiking in order to reach a stable soil pH and stored in the fridge.

Buffalo manure (BM) was obtained from a buffalo farm in Caserta area, Campania Region, Italy. Food and kitchen waste (FKW) was prepared using food chopper by mixing the following masses of ingredients expressed in weight percentages: fruit and vegetables 79%, meat 8%, dairy products 2%, bakery 6%, pasta and rice 5%. Fruit and vegetable waste (FVW) was prepared by mixing masses of fresh fruits (48%) and vegetables (52%), according to their average production in Italy (McDowell et al., 2007). The mixture was homogenized according to the same way as done for FKW.

Activated sewage sludge (SS) was collected from the wastewater treatment plant in Nola, Campania Region, Italy. Prior to its application, the sludge was centrifuged in order to reduce the initial moisture content (99%) until 88%. These organic co-substrates were selected to evaluate their effectiveness on the bioremediation processes of PAHs contaminated soil. The properties of the soil and organic amendments are presented in Table 3.1.

Table 3.1. Characteristics of soil and organic wastes.

Parameter/material	Soil	BM	FKW	FVW	SS
Moisture content (% w/w) ^a	6.2 ± 0.1 [*]	83.6 ± 0.2	81.2 ± 2.9	90.8 ± 0.2	88.0 ± 0.1
Volatile solids (% d/w) ^b	6.3 ± 0.1	63.0 ± 0.2	94.2 ± 0.7	93.8 ± 0.4	59.3 ± 0.6
Fixed solids (% d/w)	93.7 ± 0.1	37.0 ± 0.2	5.8 ± 0.7	6.2 ± 0.4	40.7 ± 0.6
Organic matter (OM) (% w/w) ^c	13.0 ± 3.4	12.9 ± 0.4	11.5 ± 1.2	10.5 ± 1.7	9.0 ± 4.8
Bulk density, ρ_b (g cm ⁻³)	0.9 ± 0.0	1.0 ± 0.0	0.8 ± 0.0	1.1 ± 0.0	0.9 ± 0.0

^aw/w: wet weight

^bd/w: dry weight

^cconversion factor 1.724 based on TOC content (Schumacher, 2002).

^{*}standard error of mean of three replicates.

3.2.3. Soil spiking

OECD soil was spiked at the initial concentration of both anthracene and chrysene of 235 mg kg⁻¹ soil (d/w), while initial concentration of 5-rings PAHs was 94 mg kg⁻¹ soil (d/w) for each of them. The soil was air-dried at room temperature for 24 h and sieve at 2-mm prior to homogenization. The soil was then mixed 2 times for 5-10 seconds with lab-blender, closed for 5 min, and again mixed on the same way. Afterward, the soil was air-dried for 3 days under a fume hood in darkness conditions until the total evaporation of solvent (Brinch et al., 2002; Sawada et al., 2004). Furthermore, the soil was stored at room temperature for 37 days in glass containers in the dark to avoid photolysis (Puglisi et al., 2007).

3.2.4. Composting reactors

Experiments were performed at laboratory scale in thermally insulated composting–bioremediation reactors made of Plexiglas[®] (Banks et al., 2000; Reddy and Ala, 2006), with an operational capacity of 1.1 L. Air supply was provided through air pump (Newair, Newa, Tecnoindustriasrl, Italy) and air distribution pipes were installed at the bottom of reactors. Prior to entering reactors, air was humidified with distilled water in order to decrease drying of soil/organic amendments mixture. The content of the reactor was placed on a Plexiglas[®] plate with small holes (\varnothing 1 mm), in order to provide uniform air distribution and in the same time to collect the leachates. Air outlet was placed on the top of reactors through air filter made of tubes of glass silk (HTS-AL-10, Distrelec, Italy) where volatile compounds were collected. The outlet tube was packed with 200 mg of resin (Amberlite XAD-2, Sigma Aldrich, Italy) and 300 mg of coconut activated carbon (Carbo-active granules, Newa, Italy). Air filter tubes were replaced at each sampling interval.

3.2.5. *Experimental conditions*

Experiments were performed in 4 reactors (1 per organic amendment) at room temperature and lasted 140 days. The spiked soil prepared as described in sections 3.2.3 was manually mixed with bulking agent at a ratio of 1:1.5 (v/v) in order to increase porosity and oxygen diffusion. As bulking agent, corn cobs chopped to 1-4 cm size, $\rho_b = 0.17$ (g cm⁻³) were used. The contaminated soil to organic waste ratio was 5:1 on dry weight basis for all reactors, i.e. for RBM reactor (soil amended with BM), RFKW (soil amended with FKW), RFVW (soil amended with FVW) and RSS (soil amended with SS). The quantity of soil used in reactors RBM and RFKW was 350 g per each, while in RFVW and RSS was 300 g per each of them. During the preparation of the mixtures, MilliQ water was added to set the moisture content at 60% as the best for PAH removal through the composting process (Antizar-Ladislao et al., 2005; Sayara, 2010; Zhang et al., 2011). The soil moisture level was maintained by spraying MilliQ water on daily basis.

Representative samples were collected in triplicate for each reactor immediately after filling reactors at day 0, and after 14, 28, 56, 84, 112 and 140 days of treatment. The content of approximately 150 g was collected from 3 different points within the matrix of each composting reactor, and sub-samples were extracted from them and used for carrying out the analyses.

The operating temperature, continuously monitored by a digital thermometer, showed different trends in each reactor according to the microbial activity, since the experiments were conducted at room temperature without any temperature control system. During the 140 days of treatment, temperatures reached thermophilic range in RBM and RFKW, while temperatures in RSS and RFVW were lower and limited to mesophilic range. Based on results of Lukić et al. (2016), the operating temperature in RBM reached 23, 56, 56, 26, 25, 26, and 24 °C at the day 0, 14, 28, 56, 84, 112, and 140 of experimental activities, respectively. A peak of temperature was reached after 29 days at 56.6 °C followed by the cooling and maturation stages of the process. Similarly, the thermophilic range of temperature was also reached in RFKW with the temperature of 23, 33, 57, 44, 39, 26 and 30 °C respectively at the same days of the experimental activities as mentioned above. Likewise, a peak of temperature was reached after 27 days at 57.6 °C followed by the cooling and maturation stages of the process. Unlike, the temperature trend in RFVW showed only mesophilic range, with temperatures of 21, 20, 21, 20, 21, 27, and 27 °C at the day 0, 14, 28, 56, 84, 112, and 140 of experimental activities, respectively. After 116 days a peak of temperature at 30 °C was reached. Finally, the temperature trend in RSS displayed mesophilic range, i.e. 20, 20, 22, 20, 22, 28, and 28 °C respectively at the same sampling days as already mentioned above, with a peak of temperature at 30 °C after 116 days.

3.2.6. *Physical and chemical analyses*

Moisture content was analyzed by weighing the samples extracted from the reactors before and after drying them for 24 h in an oven at 105 °C (ASTM, 1999), while volatile solids and fixed solids were determined according to the Standard Method 2540E (APHA, AWWA, WEF, 1998a). Organic matter content was calculated according to TOC values (Chemical Oxygen Demand (COD) Closed Reflux method) (APHA, AWWA, WEF, 1998b) (Table 3.2). All results are presented as average of triplicates with standard deviation. Since soil was mixed with organic amendments, PAH concentrations were calculated based on the inorganic ash content of the compost mixture, in order to avoid potential bias due to dilution by organic waste. Bulking agent was physically removed from samples, and was not considered within any analysis.

Table 3.2. Organic matter content in reactors on the beginning of bioremediation.

Parameter/reactors	RBM (Soil + BM)	RFKW (Soil + FKW)	RFVW (Soil + FVW)	RSS (Soil + SS)
OM concentration (g kg ⁻¹ w/w)	117.2 ± 29.2* ab**	141.2 ± 5.3 b	80.4 ± 6.0 a	108.1 ± 4.9 ab

* Standard error of mean of three replicates at 95% confidence level.

** Different letters in the row indicate on significantly different values between treatments (ANOVA, $p < 0.05$).

3.2.7. PAHs analyses

PAHs compounds were extracted using microwave extraction as operating procedure (U.S. Environmental Protection Agency, 2012a). In extraction vessel 1 g of sample was mixed with 40 ml of acetone and hexane in the same quantity. An internal standard of 320 µl (chrysene-d12, Supelco, Italy) was added to all samples to monitor extraction efficiency. The extraction vessels were properly closed in extracting frames and placed in a Start D Microwave Digestion System (Milestone). After the completion of extraction process for 35 min and cooling to the room temperature, 1 ml of each sample was stored in glass vial in freezer for the next analyses.

Concentrations of PAHs in the extracts were determined by gas chromatography/mass spectrometry (GC/MS) according to EPA Method 8270D (U.S. Environmental Protection Agency, 2012b). Analysis was performed on Agilent Technologies 6850 Network GC System coupled with a mass selective detector (Agilent Technologies 5973 Network) and a 30 m Zebron Phase ZB-5MS capillary column (0.25 mm inside diameter, 0.25 µm film thickness). The injection volume of samples was 2.0 µl, and run time was about 37 min for one sample. GC-MS system was calibrated prior to the analysis of samples using five calibration standards (5, 2.5, 1, 0.5, and 0.25 ppm). Standard curves were prepared by injecting a mixture of 14 PAHs (Mix A, Sigma Aldrich, Italy) diluted with acetone/hexane mixture (1:1). PAHs concentration was calculated by manual integration of the chromatograms.

3.2.8. Determination of microbial dehydrogenase activity

The determination of microbial dehydrogenase activity (DHA) on samples collected in triplicate from each reactor was performed at the end of bioremediation experiments.

Dehydrogenase activity was determined by colorimetric measurement of the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,3,5-triphenylformazan (TPF) according to the method of Casida Jr. (1977) and Kizilkaya (2008). Actually, 2.5 ml of reagent water and 30 mg glucose were added to six grams of solid sample. The enzymatic reaction started when 1 ml of 3% 2,3,5-triphenyltetrazolium chloride solution was added to the suspension. The samples were incubated for 24 h at 37 °C and subsequently extracted with methanol as a solvent. The red methanolic solutions of formazan were measured spectrophotometrically at 485 nm (Perkin Elmer UV/VIS Spectrometer Lambda 10), and results were expressed as $\mu\text{g TPF g}^{-1}$ dry sample.

3.2.9. Toxicity test

In order to evaluate the soil toxicity level, samples were collected prior to the initiation of the composting process as well as its termination. The aqueous extract was obtained by mixing 2 g homogenized soil with 20 ml distilled water for 16 h using an orbital shaker set at 200 rpm. After centrifugation at 4000 g (IEC Centra GP8R) for 20 min at 4 °C, 15 ml of the aqueous phase was stored in the freezer (ASTM, 2004). Bioassay was performed on a Microtox Model 500 Analyzer (Modern Water). Test was based on measurement of bioluminescence differences in the marine bacterium *Vibrio fischeri* (LUMISTox LCK 487, Hach Lange, France) by exposure to the 1 ml filtered aqueous extract of soil sample (Grange and Pescheux, 1985). Actually, when soil toxicity is high, bioluminescence in the marine bacterium *Vibrio fischeri* is low. Acute toxicity was measured as a function of decreased luminescence after 5 and 15 min exposure time at 15 °C. High inhibition at time 5 and 15 min, i.e. $I_{(5)}$ and $I_{(15)}$ respectively (%), indicates high soil toxicity.

3.2.10. Statistics

Data were analyzed by one-way analysis of variance (ANOVA) to test for significant differences of each contaminant removal within every treatment, and total contaminant reduction between treatments. All the experiments were done as triplicates. Statistical analysis was performed by using the R software (R i386, 3.1.1 version). The differences between individual means were tested using Tukey multiple comparison test, where significant p-values were obtained at the level of $p < 0.05$. On the figures and in the

tables, the letters (a, b, c, d) represent the homogenous groups obtained by the comparison of average values.

3.3. Results and discussion

3.3.1. PAH removal

3.3.1.1. Efficiency of different organic amendments in simulated composting treatment

According to the PAH concentrations commonly detected in real contaminated soil by industrial activities, the soil was spiked to reach a concentration of 658 mg total PAH kg⁻¹ (d/w) in all experiments. Selected concentration was considered according to the concentrations of each PAH as well as the concentration of total PAHs in real contaminated soil (Antizar-Ladislao et al., 2004; Juhasz and Naidu, 2000). Total PAH removal in each reactor after 140 days of bioremediation treatment is shown in Figure 3.1. In all treatments, removal of the LMW PAH, anthracene, was higher than HMW PAHs. Hence, the removal of Anth was much higher than that three pollutants regardless the applied organic amendments. Interestingly, the most favorable results were reached in RSS and RFVW reactors (89% and 85% for Anth, respectively). In contrast, reactors operated under thermophilic condition display lower anthracene removal, i.e. 71% in RFKW and 69% in RBM.

Similarly, the highest chrysene removal occurred in RSS (59%). The chrysene removal in RFVW was slightly lower than in RSS and equal to 50%, while in the other two reactors a chrysene removal lower than 50% was observed.

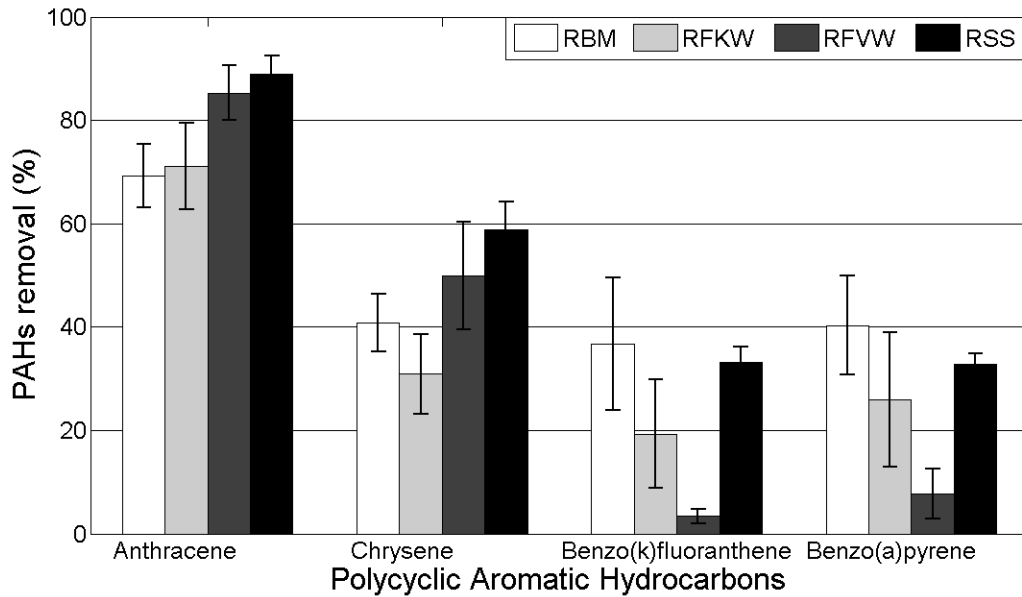


Figure 3.1. PAH removal yield (%) after 140 days of simulated composting treatments. Vertical bars represent the standard deviations of mean of three replicates at 95% confidence level.

Benzo(a)pyrene removal was slightly higher in all reactors compared to benzo(k)fluoranthene. Furthermore, considerably better removal has been reached in reactors filled with BM and SS compared to reactors filled with FKW and FVW amendments, but without being statistically significant compared to RFKW. Thus, B(a)P removal was 40% and 33%, and B(k)F removal was 37% and 33% in RBM and RSS, respectively. In other two reactors filled with food waste, the decrease of B(a)P and B(k)F concentrations did not exceed 30%. Moreover, concentrations of B(k)F and B(a)P in RFKW and RFVW on the end of treatment did not show significant differences compared to their initial concentrations (ANOVA, $p < 0.05$).

PAHs with larger number of fused aromatic rings and higher molecular weights showed more resistance to degradation due to lower bioavailability (Namkoong et al., 2002; Piskonen and Itävaara, 2004), resulting in a decrease in total removal that was less than 50% for both B(a)P and B(k)F, in all treatments. Moreover, even if they have the same molecular weights and the same number of 5-rings, B(a)P is characterized by $\log K_{ow} = 6.04$, while B(k)F by $\log K_{ow} = 6.84$. This difference in hydrophobic properties influences their behavior under the same operating conditions and consequently their degradation. Actually, the larger octanol-water partition coefficient is related to the

higher potential of bioaccumulation, which is the main responsible for the lower biodegradability of such compounds (Juhasz and Naidu, 2000).

Interestingly, treatments with activated SS and FVW amendments were found to be more successful for the removal of 3- and 4-rings PAHs. Moreover, both RSS and RFVW showed only mesophilic temperature range during the treatment. In the literature a mesophilic phase has been found to be more favorable in specific cases due to the richest microbial diversity able to degrade lower molecular weights organic pollutants with great success, but it was not found to be so efficient in degradation of recalcitrant PAHs (Antizar-Ladislao et al., 2005; Namkoong et al., 2002). In agreement with results of this research, Antizar-Ladislao et al. (2005) obtained a decrease of LMW PAHs concentration such as naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, and phenanthrene by an average of 89% at 38 °C which is twice compared to concentration reduced at 55 °C by an average of 45%. Otherwise, the reduced concentration for 4-rings PAHs such as fluoranthene, pyrene, benzo(a)anthracene, and chrysene was not so different and it was by an average of 67% at 38 °C compared to an average of 69% at 55 °C.

Treatments with BM and SS amendments have been showed to be more efficient for PAH removal than treatments with food waste amendments. Indeed, the removal of Anth in RBM was slightly lower compared to RFKW. Similarly, the removal of both Anth and Chry was lower in RBM compared to RFVW. In contrast, the removal of B(k)F and B(a)P in RBM was pretty higher compared to RFKW and RFVW. Since the removal of 5-rings PAHs is more difficult to reach compared to PAHs with lower molecular weights, the treatment with BM amendment could be consider as more efficient for PAH removal than treatments with food waste amendments. However, a clear distinction was also found between these two amendments firstly mentioned. Indeed, it was found higher total PAH removal in RSS compared to RBM, but removal efficiency of 5-rings PAHs was slightly better in RBM than in RSS. Those interesting results might be achieved due to the influence of high temperature occurring during the thermophilic phase, which contributed to higher removal efficiency of recalcitrant PAHs in RBM treatment. Actually, due to increased diffusion rate and contaminants solubility, PAHs bioavailability has been enhanced leading to a potential improvement of their biodegradation (Haderlein et al., 2006; Saison et al., 2004; Van Gestel et al., 2003). Otherwise, higher removal of total PAHs in RSS compared to RBM may be achieved due to very rich microbial diversity typical for activated sewage sludge (Ling

and Isa, 2006). As microorganisms are key player in the degradation of organic pollutants, greater extent of contaminants metabolization in RSS would be proportional to higher microbial diversity and density in activated sewage sludge compared to other waste products (Haderlein et al., 2006; Joo et al., 2007; Namkoong et al., 2002). Accordingly, in the study of Antizar-Ladislao et al. (2005) was not reached B(a)P removal through mineralization during composting at 38 °C in soil amended with green waste, while in this study B(a)P removal reached even 33% during composting at lower temperature in soil amended with SS. Nonetheless, temperature profile in RSS was in the range of mesophilic stage throughout the treatment with a peak at 30 °C (B. Lukić et al., 2016). Further, the equal amount of B(k)F removal, which is also recalcitrant organic pollutant and difficult to be removed from PAH contaminated soil, was reached in RSS, i.e. 33%. Thus, even if Namkoong et al. (2002) pointed out larger removal of contaminants from soil using SS amendment compared to compost, hereby stressing more significant contribution of SS in removing recalcitrant contaminants compared to other organic wastes. Moreover, the nutrient content of an organic waste has to be considered as an important parameter during the treatment of PAH removal. Actually, Lukić et al. (2016) have shown that SS contained the lowest C:N ratio and the highest content of soluble fraction and protein compared to BM, FKW and FVW. Accordingly, the treatment amended with SS was the most successful in PAH removal compared to other three treatments amended with organic wastes which contained a less favorable nutrient content.

Although being the most efficient, SS treatment has shown its benefits by providing the fastest removal for 3 out of 4 PAHs indicators, i.e. except for Chry. Indeed, in the early stage of experiment a significant difference between concentration values for Anth at day 0 and day 14 in treatment with SS amendment was found (Figure 3.2). It was considered as the most rapid removal, since the first significant differences of concentration values for Anth were observed between day 0 and day 56 in RBM treatment and between day 28 and day 56 in RFVW treatment (ANOVA, $p < 0.05$). Nevertheless, the slowest removal of 3-rings PAH indicator was observed in RFKW among the values at day 0 and 84. However, the first significant differences between concentration values for Chry at day 0 and day 28 in RFKW reactor was found. It was considered as the most rapid removal of 4-rings PAH indicator. Even if the most efficient removal of Chry was observed in RSS, the first significant differences between its concentration values was observed a little bit later compared to RFKW, i.e. between

values at day 84 and 112 (ANOVA, $p < 0.05$). Heavier molecular weights organic pollutants, i.e. 5-rings PAHs such as B(k)F and B(a)P, showed faster removal in RSS than in treatment with BM amendment. Actually, the first significant differences in RSS were observed among the values at day 0 and day 14, and at day 28 and 56, respectively (ANOVA, $p < 0.05$). For comparison, the first significant differences in RBM were observed among the values at day 28 and day 84 for both contaminants (ANOVA, $p < 0.05$).

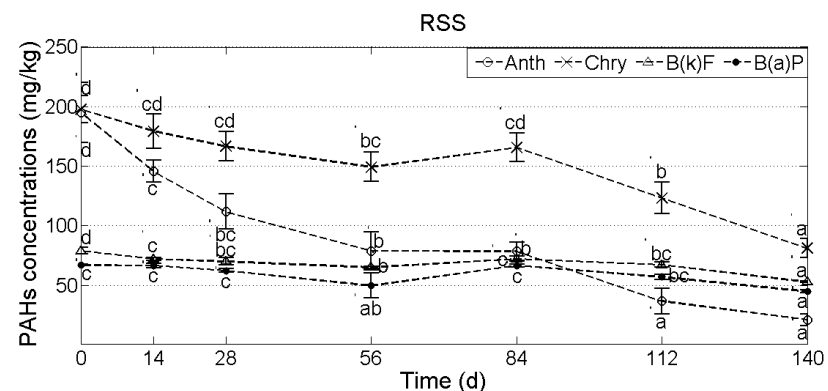
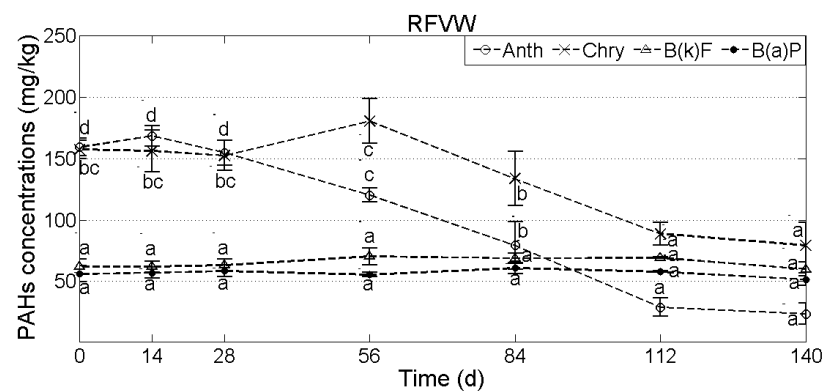
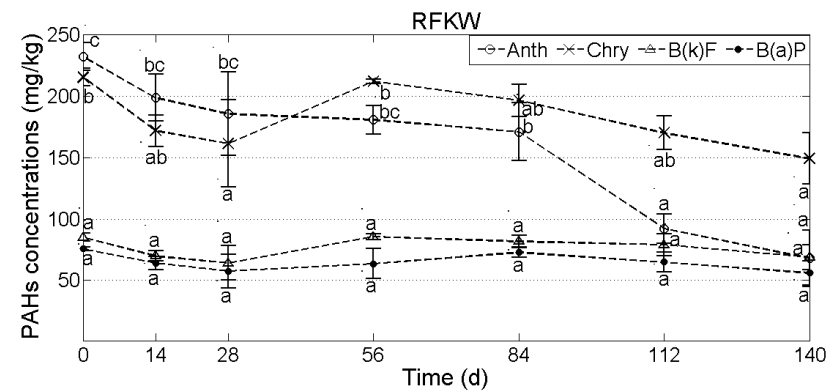
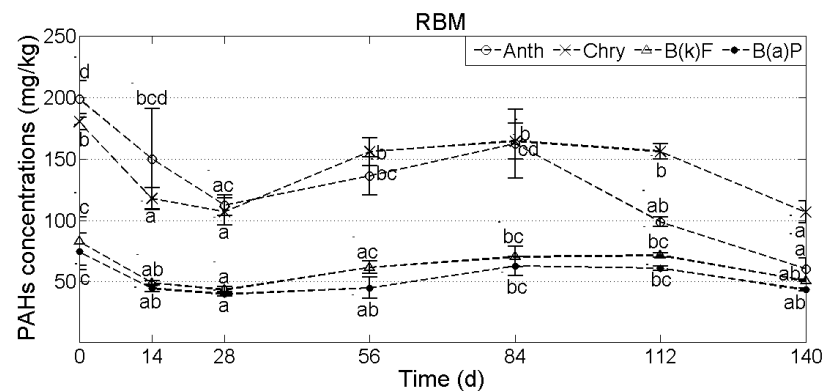


Figure 3.2. Dissipation of each contaminant among the treatments. Vertical bars represent the standard deviations of mean of three replicates at 95% confidence level, which could be covered with marker. Different letters indicate on significantly different values of PAH concentrations through incubation time for each contaminant separately (ANOVA, $p < 0.05$).

The delay in other treatments could be due to the lack of appropriate number of microorganisms able to degrade certain organic pollutants. It would take longer time to increase their number, therefore increasing the removal time. Another reason can be that microorganisms in other reactors needed to be adapted to the presence of organic pollutants and to activate appropriate enzymes. In contrast, RSS could avoid that phase considering that activated SS could easily contain PAHs in trace or other organic contaminants. Thus, SS could be expected to inoculate contaminated soils with microorganisms already adapted on such kind of pollutants and able to provide appropriate enzymes for their removal (Soil Health, 2012) compared to food wastes and buffalo manure where it might be not so common.

It is very interesting to compare the obtained results with data published by Straube et al. (2003) by performing landfarming technology with biostimulation (addition of bulking agent and dried blood as a slow-release nitrogen source) and bioaugmentation (addition of *Pseudomonas aeruginosa* strain 64). Even if they managed to achieve a total PAH removal of 86% and 87% respectively after 16 months, the removal was mainly related to 3- and 4-rings PAHs. Besides, the maximum Anth removal in their study was 72% after 16 months, while treatment with SS amendment performed in this research work resulted in 89% removal in less than 5 months. Moreover, treatments performed by Straube et al. (2003) did not manage to remove recalcitrant PAHs like B(k)F and B(a)P at all after 16 months, while all treatments with organic wastes described in this research study were able to remove them with different efficiency in about 5 months. Chry had a different pathway, actually landfarming with bioaugmentation showed the highest removal 78% after 16 months, but in the first 6 months of treatment the removal had not been detected yet (Straube et al., 2003). Conversely, RSS resulted in 59% of removal after only 140 days, and could be expected to reach similar level of removal as what was achieved by Straube et al. (2003) for a much shorter time.

Great potential of activated SS to enhance PAH removal from contaminated soils should be also investigated associated with thermophilic phase of composting process. It would ensure greater diffusion rate and enhanced bioavailability of contaminants that is supposed to facilitate their access to microorganisms (Ling and Isa, 2006). Actually, increased bioavailability enhances the biodegradation processes and ensures bioremediation as a feasible method (Piskonen and Itävaara, 2004).

3.3.1.2. *Volatilization and leaching*

Total volatilization loss and leaching were monitored in all reactors, since PAH removal can also be subjected to volatilization and leaching (Smith et al., 2011). As expected (Piskonen and Itävaara, 2004), only Anth was detected in its volatile form but in a very low concentration in all reactors. Indeed, Anth volatilization was below 0.5% on total removal in all treatments. Furthermore, the highest range of volatilization was observed after 3 weeks of treatment and decreased until the end of process (Fig. 3.3). Actually, the higher metabolism by microorganisms affects the lower volatilization throughout the treatment. Therefore, higher range of volatilization is more typical in the earlier stages of process when volatile contaminants have not been completely subjected to metabolism by microorganisms.

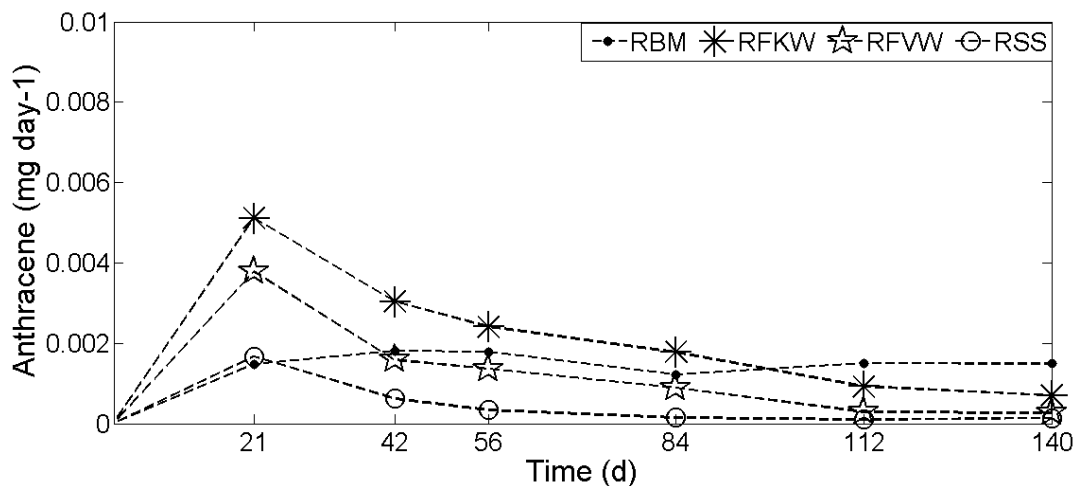


Figure 3.3. Anthracene volatilization during the bioremediation treatment

The leaching of PAHs from the soil was very low, and did not occur in all reactors. Actually, 2 out of 4 PAHs, i.e. Chry and B(k)F, were detected in the leachate of RSS, while in RFW only B(k)F was detected. All of them were present in extremely low quantity, i.e. 0.2% and 2.4% in relation to the initial concentrations of B(k)F in RFW and RSS respectively, and 1.3% of Chry in RSS leachate. There is no determined relationship among total PAH removal and their leaching. Possibly presence of persistent organic pollutants in leachate is expected, since they are resistant to other removal mechanisms such as microbial degradation and volatilization. Accordingly,

B(k)F has been found in leachate of treatments where some contaminants were detected, since it is considered as the most recalcitrant indicator in experiments.

3.3.2. Bioassays

3.3.2.1. Dehydrogenase activity (DHA) in bio-reactors

Metabolic activity of microorganisms measured as a biological activity indicator for PAH removal was dehydrogenase activity. Unfortunately, biological activities might be not consistent with contaminants removal, and often do not correspond to residual contaminants concentrations (Maila and Cloete, 2005). Nevertheless, monitored biological activities within this research work are related with PAHs concentrations at the end of bioremediation (Fig. 3.4).

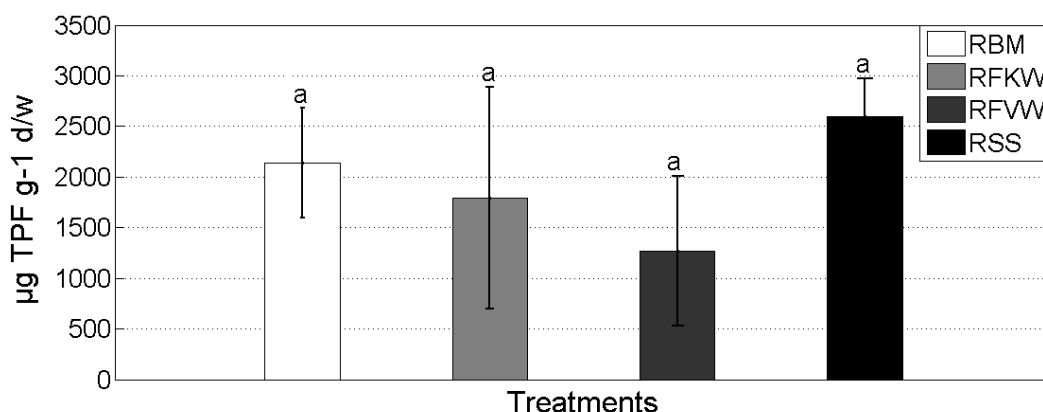


Figure 3.4. Dehydrogenase activities in each reactor after 140 days of bioremediation. Vertical bars are standard deviations of mean of three replicates at 95% confidence level. Different letters indicate on significantly different values between treatments (ANOVA, $p < 0.05$).

According to the statistical analysis, significant differences of dehydrogenase activities were not observed (ANOVA, $p < 0.05$), but different values of these biological indicators in $\mu\text{g TPF g}^{-1} \text{d/w}$ between reactors were obtained. Those values are positively correlated ($r = 0.8$) with removal of HMW PAHs, i.e. as values for DHA activity increase, values for total removal of Chry, B(k)F and B(a)P also increase in certain reactors. Accordingly, the highest activity has been measured in reactor with SS amendment ($2601 \mu\text{g TPF g}^{-1} \text{d/w}$) that is twice higher than the activity measured in

reactor amended with FVW (1269 $\mu\text{g TPF g}^{-1} \text{d/w}$). Consequently, DHA activity in RFKW was 40% higher (1792 $\mu\text{g TPF g}^{-1} \text{d/w}$), while RBM shown almost 70% higher microbial activity (2141 $\mu\text{g TPF g}^{-1} \text{d/w}$) than RFVW.

Dehydrogenase enzymes are the catalysts of metabolic process such as biological oxidation of organic compounds and subsequently the detoxification of xenobiotic (Balba et al., 1998; Casida Jr., 1977; Maila and Cloete, 2005). Considering that PAHs biodegradation is a complex metabolic process catalyzed by dehydrogenase enzymes, their activity is crucial for the overall success of bioremediation (Balba et al., 1998; Maila and Cloete, 2005). Further, for a successful utilization of DHA activities as bioindicator for PAH removal, it is useful to make them suitable and correlate the measurements with other bioindicators during bioremediation such as microbial biomass, soil enzymatic activities (urease, protease, β -glucosidase, phosphatase, arylsulphatase), seed germination, earthworm survival and microbial bioluminescence (Hinojosa et al., 2004; Maila and Cloete, 2005).

3.3.2.2. *Soil toxicity*

Microtox[®] test was used to perform an assessment of toxicity level, due to the fact that incomplete degradation of PAHs could lead to the formation of metabolites such oxy-PAHs that might be more toxic than their parent compounds (Saison et al., 2004). Therefore, prior implementing the bioremediation process, the contaminated samples demonstrated certain toxic effects due to the presence of organic pollutants. After composting treatment, the overall acute toxicity was considerably decreased, especially for RFKW treatment (69 to 62%) and then for RSS (65 to 56%) (Table 3.3). Decrease of acute toxicity for RFVW was pretty lower compared to RFKW and RSS treatments and amounted 48 to 35%. In contrast, for RBM, an increase of acute toxicity was detected.

Results of Microtox[®] test provided information on the bioavailable fraction of organic pollutants and confirmed the effectiveness of simulated composting treatments. Moreover, they demonstrated that toxicity level is not always in correlation with the contaminants concentration. Actually, the highest toxicity decrease was found in RFKW, while the residual PAHs concentrations were among the highest compared to other treatments. Indeed, the toxicity of the PAHs was reduced but not their extractability, and it confirms that the extraction with organic solvents is not correlated

with the bioavailability of the pollutants (Saison et al., 2004). The largest decrease in bioavailability shown in RFKW could occur due to the stronger sorption of pollutants on considerably higher amount of organic matter present on the beginning of process, than in the other three treatments (Table 3.2). Considered bioavailability is that one related to *Vibrio fischeri* used for the Microtox[®] test. This is a good example to show usefulness of bioassays to estimate the bioavailable fraction of organic pollutants (Loureiro et al., 2005). On the other hand, in RBM a significant removal of target contaminants was observed, but the decline toxicity was not detected. Even more, acute toxicity was higher on the end of composting treatment that could be the consequence of oxy-PAHs formation as a result of incomplete degradation (Singh, 2012), as explained above.

Within this set of experiments, microbial activities in reactors would be in accordance with Microtox[®] test data. Indeed, RFVW reactor with the lowest DHA activity showed also the lowest decline toxicity, while RSS reactor with the highest microbial activity, showed considerably higher decrease of toxicity compared to RFVW. In RSS the results indicated more than one third higher decrease of toxicity after inhibition at time 5 min and even more than 60% after inhibition at time 15 min. Results of Microtox[®] test for RFKW could not be related to DHA activity due to possible reduction of pollutant's bioavailability as it is explained in previous paragraph, neither for RBM since was detected an increase of toxicity mentioned above.

CHAPTER 3

Table 3.3. Decrease/increase of acute toxicity in soil at the end of composting treatment, measured with Microtox test at inhibition time of 5 and 15 minutes.

Reactors	Inhibition at time 5 min (%)		Inhibition at time 15 min (%)		Toxicity evolution (%)	
	0 day	140 days	0 day	140 days	Inhibition at time 5 min ($I_{(5)}$)	Inhibition at time 15 min ($I_{(15)}$)
RBM	19.7 ± 8.3	36.1 ± 7.8	17.3 ± 9.4	38.2 ± 6.3	+ 98.2 ± 37.9	+ 200.8 ± 170.4
RFKW	37.6 ± 0.8	11.6 ± 1.8	34.4 ± 2.3	13.1 ± 0.2	- 69.5 ± 4.3	- 61.7 ± 1.9
RFVW	40.6 ± 1.1	21.2 ± 6.9	34.8 ± 0.2	22.7 ± 8.4	- 48.2 ± 15.6	- 34.7 ± 24.6
RSS	20.3 ± 0.0	7.1 ± 0.0	20.3 ± 0.0	8.9 ± 1.8	- 64.8 ± 0.0	- 56.0 ± 8.8

3.4. Conclusions

This research work confirmed the potential success and high efficiency of bioremediation using composting of organic waste as biostimulation strategy to degrade persistent PAHs and to shorten their total removal time.

By considering all the data and results obtained, it can be concluded that activated SS is the most favorable organic amendment to be considered and used in composting treatments. Actually, RSS reactor was found to be the most efficient treatment in overall PAH removal considering that it showed the highest removal of Anth and Chry, and the fastest removal of Anth, B(k)F and B(a)P compared to other treatments. Even if B(k)F and B(a)P removal was a bit higher in RBM than in RSS, it is not considered as efficient due to the considerable increase of acute toxicity at the end of composting treatment compared to its initial value. Accordingly, this study highlights the importance of monitoring the changes in soil toxicity prior and after bioremediation. Ecotoxicity test should be used when biodegradation products are not monitored in addition to chemical analysis of contaminants. Furthermore, this research work showed that DHA enzymatic activities could be used successfully as bioindicator for PAH removal.

On the other hand, it could be interesting also to study PAH removal at constant temperature displaying a mesophilic phase only with BM and FKW amendments. It could provide information about time needed for microorganisms to adapt their physiological processes in response to present pollutants. Moreover, longer mesophilic phase could facilitate PAH removal due to the richest microbial diversity and possible increased microbial activity. This study confirmed the greatest success of treatment subjected to mesophilic phase. In this regard, there is a need for further studies to clarify all specificities and details in order to upgrade this approach and make use of all benefits of such complex process in the most efficient way.

3.5. Acknowledgments

The authors would like to thank the financial support provided by the European Commission (Erasmus Mundus Joint Doctorate Programme ETeCoS³: Environmental Technologies for Contaminated Solids, Soils and Sediments, under the grant agreement FPA n°2010-0009).

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Evaluation Of PAHs Removal Efficiency In An Artificial Soil Amended With Different Types Of Organic Waste

Chapter 4

Importance of organic amendment characteristics on bioremediation of PAH- contaminated soil

Abstract

This study investigates the importance of the organic matter characteristics of several organic amendments (i.e. buffalo manure, food and kitchen waste, fruit and vegetables waste and activated sewage sludge) and their influence in the bioremediation of a polycyclic aromatic hydrocarbons (PAH) contaminated soil. The removal of low molecular weights (LMW) and high molecular weights (HMW) PAHs was monitored in four bioremediation reactors and used as an indicator of the role of organic amendments in contaminant removal. The total initial concentration of LMW PAHs was 234 mg kg⁻¹ soil (dry weight), while the amount for HMW PAHs was 422 mg kg⁻¹ soil (dry weight). Monitoring of operational parameters and chemical analysis were performed during 20 weeks. The concentrations of LMW PAH residues in soil were significantly lower in reactors that displayed a mesophilic phase, i.e., 11% and 15%, compared to reactors that displayed a thermophilic phase, i.e., 29% and 31%. Residual HMW PAHs were up to five times higher compared to residual LMW PAHs, depending on the reactor. This demonstrated that the amount of added organic matter and macronutrients such as nitrogen and phosphorus, the biochemical organic compound classes (mostly soluble fraction and proteins), and the operational temperature are important factors affecting the overall efficiency of bioremediation. On that basis, this study shows that characterization of biochemical families could contribute to a better understanding of the effects of organic amendments and clarify their different efficiency during a bioremediation process of PAHs contaminated soil.

Keywords

Polycyclic aromatic hydrocarbons, bioremediation, co-composting, organic substrates, spiked soil, organic matter fractionation.

Importance of organic amendment characteristics on bioremediation of PAH-contaminated soil

4.1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread organic pollutants, which is to be expected considering that fossil fuels continue to be used as a principal source of energy (Dreyer et al., 2005; Nduka et al., 2012). Moreover, their global contamination has mainly resulted from industrial activities near urban areas (Dreyer et al., 2005). These chemicals are present in flue-gas condensates as well as in refinery and coal-gasification wastes. Furthermore, they are distributed because of transportation, disposal and accidental spills of petroleum products, making them important global environmental pollutants (Eom et al., 2007; Nduka et al., 2012; Okere and Semple, 2012). PAHs are present in the environment as a result of natural or anthropogenic activities, but commonly their contamination is associated with anthropogenic sources (Dreyer et al., 2005; Okere and Semple, 2012; Sayara et al., 2011). Soil is a major reservoir of PAHs among other environmental media, and stores more than 90% of the total PAHs found in the environment, because of the sorbing capacity of soil particles (Eom et al., 2007; Okere and Semple, 2012).

PAHs represent a significant ecological risk because they are toxic, carcinogenic, mutagenic, teratogenic, and resistant to biodegradation (Fernández-Luqueño et al., 2008; Sayara et al., 2011). Accordingly, they are of great environmental concern at many sites and are on the priority pollutants lists of the US Environmental Protection Agency (US EPA) and the European Commission (Fernández-Luqueño et al., 2008; Gan et al., 2009; Sayara et al., 2011).

In the last two decades, bioremediation has been the target of extensive studies as a clean-up technology for organic pollutant removal from contaminated soil (Picado et al., 2001). Its application is usually based on the addition of nutrients (biostimulation) or competent bacteria (bioaugmentation) that stimulate the biodegradation of PAHs (Sayara et al., 2011; Van Gestel et al., 2003). Moreover, biological processes benefit from a high public acceptance together with a growing interest and awareness (Picado et al., 2001). Considering the hydrophobic properties of PAHs, the success of soil

bioremediation could be limited by many physico-chemical and biological factors, but the most important is the bioavailability of contaminants (Sayara et al., 2011).

Co-composting is a promising bioremediation technique that consists of the supply of organic wastes instead of chemical fertilizers to contaminated soils. Organic wastes contain a high amount of organic matter and nitrogen, i.e., easily accessible nutrients that are essential for microbial growth and activity. The use of organic wastes as fertilizer can be an economical way to protect the environment and conserve natural resources. Furthermore, they improve soil structure, leading to a decreasing bulk density and a lower PAH volatilization rate (Ling and Isa, 2006; Van Gestel et al., 2003).

Antizar-Ladislao et al. (2005) showed that the removal of 16 USEPA-listed PAHs from coal tar-contaminated soil was enhanced by garden waste. Moreover, Atagana et al. (2003) developed a co-composting process with cattle manure and mixed vegetable waste of soil heavily contaminated with creosote and achieved a complete removal after 19 months. Regarding the biodegradation of hydrocarbons stimulated with synthetic (NPK and urea) or natural organic (cow dung and poultry litter) fertilizers, the work of Nduka et al. (2012) showed no remarkable difference. However, even if all of these contribute to enhance bacterial growth, use of organic amendments is recommended because they are more cost effective and environmentally friendly (Nduka et al., 2012).

The physico-chemical features and organic matter stability of all of these organic residues are heterogeneous, which could affect the efficiency of the bioremediation strategy. Therefore, biochemical characterization of the organic amendment is of interest for a better understanding of PAH biodegradation (Fernández-Luqueño et al., 2008).

The aim of this study was to evaluate the effectiveness of four different organic wastes in stimulating PAH removal. This work particularly focused on the characterization of organic amendments, such as biochemical organic compound composition (i.e., content of the soluble fraction, protein, hemicellulose, cellulose, and lignin) as well as the content of macronutrients like nitrogen (N) and phosphorus (P) and total organic carbon (TOC). In addition, the evolution of pH values and temperature profiles were monitored during the co-composting process as a result of diversities among these features of organic matter, and their influence on PAH degradation performance.

4.2. Materials and methods

4.2.1. Chemicals

Target contaminants were PAHs listed among the 16 USEPA priority pollutants and were obtained from Sigma Aldrich (Italy). Their total concentration was 657 mg of PAHs kg⁻¹ soil (dry weight) in all experiments and included anthracene (Anth) (purity ≥ 99%), chrysene (Chry) (purity 98%), benzo(k)fluoranthene (B(k)F) (purity ≥ 99%) and benzo(a)pyrene (B(a)P) (purity ≥ 96%). The concentrations of 3- and 4-ring PAHs accounted for 72% of the total contamination in equal amounts, while the contamination of B(k)F and B(a)P accounted for 28% of total contaminant concentrations.

4.2.2. Soil

The artificial OECD soil was prepared according to the recommendations and standard preparation protocol in the OECD guidelines (OECD/OCDE 317, 2010). The main constituents were sphagnum-peat (10%), quartz sand (70%) (274739, Sigma-Aldrich), kaolinite clay (20%) (03584 Fluka, Sigma-Aldrich) and calcium carbonate (1%) (C4830, Sigma-Aldrich, purity ≥ 99%).

4.2.3. Organic amendments

Buffalo manure (BM) and activated sewage sludge (SS) were obtained from a buffalo farm and wastewater treatment plant in the Campania region of Italy, respectively. Prior to analysis, the sludge was centrifuged at 4600 relative centrifugal force for 8 min to reduce excessive water content, while its volume was reduced by a factor of 8. Food and kitchen waste (FKW) was made by potato, tomato, egg plant, salad, broccoli, carrots, and apples at 9% (w/w) for each of them; mandarin and banana at 8% (w/w) for each; chicken, pork, bread, and biscuits at 3% (w/w) for each; pasta and rice at 2.5% (w/w) for each while fish by 2% (w/w), and finally cheese and milk by 1% (w/w) for each. Fruit and vegetable waste (FVW) was made with grapes, apples, oranges, melons, tomatoes, lettuce and cabbages, onion and carrots, in weight percentages of 11%, 15%, 12%, 9%, 24%, 11%, 9%, 8% respectively, in correspondence with fresh fruit and vegetables production in Italy (McDowell et al., 2007). All of these ingredients were

washed with distilled water and cut with a food chopper. The major properties of the organic substrates are presented in Table 4.1.

4.2.4. Soil spiking procedure

Before PAHs spiking, the soil was air-dried at room temperature for 24 h and passed through a 2-mm mesh to remove plant debris and other inert materials. The contaminants in powder form were dissolved in a solvent mixture of dichloromethane and acetone in a 1:1 volume ratio, and then added to OECD soil by mixing with a lab-blender two times for 5-10 s. The container was closed for 5 min to avoid any volatilization before the completion of the procedure, and the mixture was again blended in the same way. Between mixing intervals, soil was scraped from the container walls to contribute to better mixture homogenization. The spiked soil was placed under a fume hood and mixed manually for 3 days to evaporate the solvent (Brinch et al., 2002; Sawada et al., 2004). The ageing of the soil was performed in dark conditions at room temperature for 37 days. Soil properties are illustrated in Table 4.1.

Importance of Organic Amendment Characteristics on Bioremediation of PAH-Contaminated Soil

Table 4.1. Soil and organic wastes characteristics.

Parameter/material	Soil	Buffalo manure (BM)	Food and kitchen waste (FKW)	Fruit and vegetable waste (FVW)	Sewage sludge (SS)
Total solids content (% w/w) ^a	93.8 ± 0.1 ^b	16.4 ± 0.2	18.8 ± 2.9	9.2 ± 0.2	12.0 ± 0.1
Total Organic Carbon (% w/w)	7.5 ± 2.0	7.5 ± 0.2	6.7 ± 0.7	6.1 ± 1.0	5.2 ± 2.8
pH value	7.7 ± 0.1	7.7 ± 0.1	4.9 ± 0.1	4.6 ± 0.1	7.0 ± 0.1
Total Kjeldahl nitrogen (% w/w)	0.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	0.7 ± 0.0
Total phosphorus (% w/w)	0.0019 ± 0.0008	0.0032 ± 0.0005	0.0004 ± 0.0002	0.0009 ± 0.0002	0.0026 ± 0.0011
C:N ratio ^c	63:1	20:1	12:1	31:1	7:1
N:P ratio	63:1	115:1	1497:1	209:1	287:1

^aw/w: wet weight

^bstandard deviation of the average of three replicates

^cmass ratio

4.2.5. *Experimental set up*

Experimental activities were performed for 20 weeks in fully enclosed thermally insulated bioremediation reactors made of Plexiglas[®] (1.1 L). The duration was intended to include all four stages of the composting process (mesophilic, thermophilic, cooling, and maturation). The spiked soil was conditioned with buffalo manure, food and kitchen waste, fruit and vegetables waste and activated sewage sludge in four bioremediation reactors at a 5:1 ratio on a dry-weight basis, i.e. RBM, RFKW, RFVW and RSS respectively. The ratio of contaminated soil to bulking agent (corn cobs) was 1:1.5 (v/v). Corn cobs were chopped manually to 1-4 cm size, ρ_b 0.2 (g cm⁻³). Bulking agent is used to increase porosity and oxygen diffusion, and thus to ensure aerobic conditions (Joseph, 2007). Humidified air flow was provided by air distribution pipes connected to an air pump (Newair, NEWA, Tecno Industria Srl, Italy) from the bottom of the reactors, so that air could flow continuously up through the reactor mixture. Air filters packed with resin (Amberlite XAD-2, Sigma Aldrich, Italy) and coconut-activated carbon (Carbo-active granules, NEWA, Italy) were placed on top of the reactors. The approximate moisture content of the reactor mixtures was less than 60%, so additional MilliQ water was added to maintain it throughout the experimental activities.

4.2.6. *Sampling*

At the beginning of the experimental activities, sampling was performed in triplicate and then at 1, 2, 3, 4, 6, 8, 12, 16, and 20 weeks of bioremediation. The content of each reactor was collected from the middle of the mixture at three different points, and sub-samples were stored and used for further analysis. Bulking agent was considered as non-biodegradable under laboratory conditions and was not included in any analysis because it was large enough to be easily visible and physically removed from the samples.

4.2.7. *Soil and waste analysis*

Moisture content was analyzed by weighing the reduction in mass by drying the samples at 105 °C for 24 h (ASTM American Society for Testing and Materials, 1999). The pH value was measured in soil-water suspension according to Method 9045D (U.S. Environmental Protection Agency, 2012c).

Total organic carbon was determined by measuring the light absorbance at a wavelength of 600 nm on a spectrophotometer (Photolab Spektral) using the Chemical Oxygen Demand (COD) Closed Reflux method (APHA, AWWA, WEF, 1998).

Total nitrogen (N) was determined by the Kjeldahl method using concentrated H₂SO₄, K₂SO₄ and HgO to digest the sample on a Digestion Unit DK 6 Velp Scientifica and UDK 132 Automatic Distillation System for TKN (Janssen and Koopmann, 2005).

Samples for total phosphorus (P) analysis were subjected to perchloric and nitric acids, and boiled for 75 min, prior to being measured at a wavelength of 470 nm on a spectrophotometer (Photolab Spektral) (Pansu and Gautheyrou, 2006).

Protein content was evaluated colorimetrically using the Lowry assay (Dulekgurgen, 2004). The sample volume was 0.5 ml, and the absorbance was measured at a wavelength of 750 nm (Perkin Elmer UV/VIS Spectrometer Lambda 10).

Substrate fractionation was performed according to the Van Soest method based on successive physico-chemical extraction steps (ANKOM, 2015; "Ankom Protocol," n.d.; Van Soest, 1963). The samples were extracted with a neutral detergent solution (NDS), acid detergent solution (ADS), and 72% sulfuric acid solution (ADL-acid determined lignin) to remove a certain fraction of compounds. The samples were then dried in an oven (Binder) at 105 °C until total removal of moisture content and weighed after each extraction step to determine the content by weight of the soluble fraction, cellulose, hemicellulose, and lignin. The insoluble fraction of any solution that corresponded to lignin content was determined in a muffle at 550 °C for 3 h (Carbolite, AAF 1100). The presented results are average values of three samples with the corresponding standard deviation.

4.2.8. PAHs analysis

A microwave extraction method was used to extract target contaminants from solid samples according to US EPA (U.S. Environmental Protection Agency, 2012a). The procedure is based on the use of microwave energy to produce elevated temperature (100 to 115 °C) and pressure conditions (50 - 175 psi) in properly closed vessels filled with 1 g of sample and 40 ml of organic solvents (acetone:hexane volume ratio 1:1) which enables contaminant recovery. The extraction process was performed using the Start D Microwave Digestion System (Milestone) and chrysene-d12 (Supelco, Italy) as the internal standard during the procedure.

All analyses of PAH quantitation were performed on an Agilent Technologies 6850 Network GC System gas chromatograph coupled with a mass selective detector (Agilent Technologies 5973 Network) according to EPA Method 8270D (U.S. Environmental Protection Agency, 2012b). Separation was achieved using a Zebron Phase ZB-5MS capillary column with a length of 30 m, inner diameter of 0.25 mm and film thickness of 0.25 μm . Standard curves for each compound were prepared by injecting a mixture of 14 PAHs (Mix A, Sigma Aldrich, Italy).

Because of dilution of soil by organic waste, PAHs concentrations were calculated based on ash content to avoid possible bias. Samples were placed in a muffle furnace (RO – 8 Metrawatt GmbH) and held at 550 °C for 2h 45 min to burn the organic matter. Ash content was determined using a loss-on-ignition procedure and calculated from the ratio of pre- and post-ignition sample weights.

4.2.9. Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) with R software (R i386, 3.1.1 version) at the 95% confidence level. Comparison of means was tested by Tukey's multiple comparison test at the level of $p < 0.05$. Homogeneous groups given by the comparison of means are illustrated in the figures and in the tables by lower-case letters (a, b, c, d). Principal component analysis (PCA) was performed on the correlation matrix of the data set, to identify the correlation among the primary units and variables. It was performed using XLSTAT statistical software for Excel. All experiments were done in triplicates.

4.3. Results and discussion

4.3.1. Temperature profiles and pH values during bioremediation

The operating temperature of composting mixtures reached thermophilic conditions in RBM and RFW reactors with a peak at 56.6 °C after 29 days and 57.6 °C after 27 days, respectively (Figure 4.1). Increasing temperature trends began in the second week of treatment in both reactors, but a cooling phase started almost 3 weeks earlier in RBM reactor than in RFW reactor. The maturation phase started after 14 and 15 weeks of operation, respectively. In contrast, in RFW and RSS reactors, the measured temperatures (from 18.4

°C to 30.3 °C) allowed only mesophilic conditions. Increasing temperature trend observed in these reactors during the experimental activities was related to changes in ambient temperature.

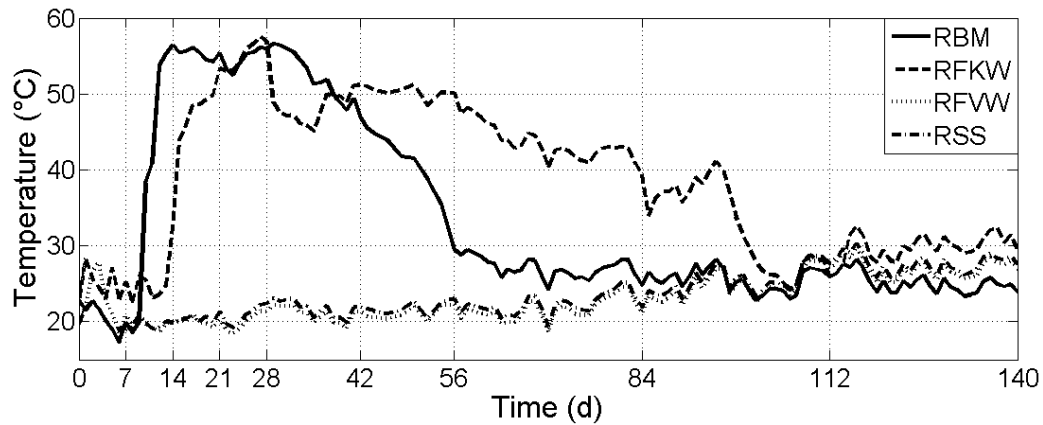


Figure 4.1. Temperature profiles during the co-composting process.

The increase in temperature and development of a thermophilic phase during the co-composting process in RBM and RFKW reactors is the result of a high content of easily biodegradable organic material (Guerin, 2000). Indeed, the bacteria could quickly metabolize these compounds and then release more energy. On the other hand, the temperature reached in RSS was lower. One hypothesis for the absence of a thermophilic phase could be related to the physical properties. SS turned into a highly jelly-like and sticky mass, in which an uniform distribution of air was difficult to achieve. This parameter is essential for extensive growth of aerobic microorganisms. Even if the thermophilic phase of the composting process was not displayed for the two RSS and RFW treatments, the occurrence of an aerobic process during the incubation could be confirmed by the occurrence of a typical pH value profile of the composting processes in all reactors.

At the beginning of the co-composting process, the pH value varied among the treatments as a consequence of composition differences among organic wastes: values were 8.4, 5.7, 6.5 and 7.3 in RBM, RFKW, RFW, and RSS, respectively (Figure 4.2). Major changes occurred within the first 3 weeks when massive variations were observed. The pH values continued to decrease to a range of around 6.5 in all reactors. Then, an increasing trend occurred in the first week. Afterwards, pH values gradually stabilized and became similar in

all treatments until the end of bioremediation, which is consistent with a classic composting process.

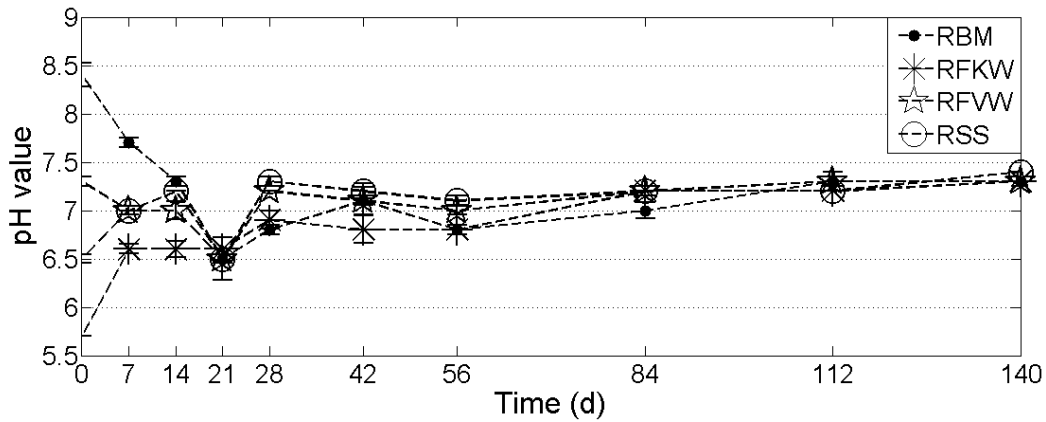


Figure 4.2. pH value in each reactor during the incubation. Vertical bars are standard deviations of mean of three replicates; some may not be clearly visible because they are covered with a marker.

There was no additional adjustment of pH during the remediation process because these values were close to those shown as optimal for PAH degradation (U.S. Environmental Protection Agency, 1994). Moreover, the decrease in pH value (RBM and RSS) was related to significant differences in TOC consumption (ANOVA, $p < 0.05$). In fact, acidification could be the result of a higher production of organic acids by microorganisms as a consequence of extensive degradation of readily available organic matter (Ling and Isa, 2006), which is consistent with the large content of soluble fraction in these two organic substrates.

4.3.2. TOC degradation and C:N:P ratio influence

Significantly higher TOC removal was observed in RBM and RFKW reactors compared to RFVW (Table 4.2) (ANOVA, $p < 0.05$). TOC removal in RBM was about one third higher compared to RSS.

Table 4.2. Monitoring of TOC degradation yield and operational parameters.

TOC concentration (g kg ⁻¹ , w/w ****)	Reactors			
	Time (weeks)	RBM	RFKW	RFVW
0	68.0 ± 16.9* **	81.9 ± 3.1 d	46.6 ± 3.5 ab	62.7 ± 2.8 e
1	55.3 ± 4.1 de	63.7 ± 10.3 c	48.9 ± 2.8 b	59.0 ± 3.9 de
2	48.8 ± 4.0 cd	54.5 ± 7.3 bc	47.7 ± 0.5 ab	56.4 ± 1.5 de
3	46.4 ± 1.2 bd	49.3 ± 1.5 ab	46.2 ± 5.2 ab	55.5 ± 8.5 cde
4	40.9 ± 2.7 ad	44.8 ± 1.7 ab	45.1 ± 1.2 ab	49.3 ± 1.3 ad
6	40.9 ± 3.6 ad	50.7 ± 3.6 ac	38.0 ± 2.4 a	53.5 ± 2.2 bcde
8	36.2 ± 4.4 abc	48.2 ± 3.0 ab	39.0 ± 6.9 ab	44.7 ± 0.9 ab
12	25.2 ± 4.7 a	43.0 ± 3.2 ab	38.1 ± 4.3 a	45.3 ± 3.8 ac
16	29.4 ± 2.1 ab	45.3 ± 3.2 ab	37.1 ± 3.6 a	42.7 ± 1.8 a
20	27.4 ± 2.0 a	40.3 ± 2.6 a	39.1 ± 1.7 ab	40.0 ± 2.2 a
Total TOC degradation yield (%)	57.6 ± 13.2 b***	50.8 ± 1.7 b	15.6 ± 9.6 a	36.0 ± 5.6 ab
C:N mass ratio	30:1	21:1	38:1	12:1
N:P mass ratio	97:1	300:1	137:1	216:1

*Standard deviation is the average of three replicates at the 95% confidence level.

**Different letters in the column indicate significant differences between values in a specific reactor through time (ANOVA, $p < 0.05$).

***Different letters in the row indicate the significantly different values among reactors (ANOVA, $p < 0.05$).

****w/w: wet weight.

This result is attributable to the more extensive microbial activity that led to the thermophilic phase (Sayara et al., 2011), which is typical of a composting process. The consumption of readily biodegradable organic compounds, i.e., organic matter, was faster in RBM than in RFKW.

Interestingly, in reactors where the thermophilic conditions were not reached, the RSS reactor displayed a TOC removal that was more than twice that reported for RFVW reactor. It is probable that the lower TOC degradation yield observed in RFVW compared to RSS was the result of a lower initial amount of easily biodegradable compounds, which affected the growth of microorganisms and consequently an increase in temperature. Indeed, at the beginning of bioremediation, RFVW contained two times less TOC (Table 4.2) compared to RFKW and considerably lower TOC content compared to the other two treatments. On the other hand, the residual PAHs decreased with the increase in the TOC consumption. This

behavior is similar in reactors with or without a thermophilic phase. Therefore, in RBM, higher TOC consumption was observed than in RFW. In reactors with unchanged temperature conditions, RSS resulted in rather higher TOC consumption than RFW.

Another parameter of importance, the C:N:P ratio, is the most optimal at 100:10:1 for growth of PAH-degrading species in soil (Leys et al., 2005; Okere and Semple, 2012). For example, although a thermophilic phase occurred in both reactors, significantly lower removal of HMW PAHs was observed in RFW compared to RBM. However, RFW exhibited the lowest total P content and had even a three times less favorable N:P ratio than RBM (Table 4.2), which would contribute to lower removal efficiency in this reactor. On the other hand, a much higher removal efficiency in RSS compared to RFW could be attributed to more favorable C:N ratios, i.e., 12:1 versus 38:1 respectively. Similarly, a less favorable C:N:P ratio detected in RFW contributed to its lower PAH removal efficiency compared to RSS, even if RFW was subjected to higher temperature. Actually, higher temperature would increase the diffusion rate of organic contaminants by decreasing their viscosity and increasing their solubility (Haderlein et al., 2006; Van Gestel et al., 2003). Thus, it would enhance the bioavailability of hydrophobic pollutants and improve their removal (Haderlein et al., 2006; Van Gestel et al., 2003). In contrast a less desirable C:N:P ratio in RFW probably inhibited the growth of certain microorganisms responsible for PAHs degradation (Leys et al., 2005; Nduka et al., 2012). Accordingly, bacteria contributed more to the removal of the easily biodegradable organic matter than to removal of PAHs.

4.3.3. Biochemical components of organic matter

4.3.3.1. Fractionation of organic residues

Different types of organic residues were evaluated with respect to the content of the soluble fraction, proteins, hemicellulose, cellulose, and lignin (Table 4.3). The soluble fraction is related to the most easily biodegradable organic fraction consisting of free sugars, lipids, pectin, starch, soluble proteins and non-protein nitrogen (ANKOM, 2015). The study showed that the contents of soluble fractions were significantly higher in BM and SS residues than in FKW (ANOVA, $p < 0.05$); these contents were even four times higher compared to FKW. Hence, it could be expected that organic residues with a high content of soluble fraction such as BM and SS could contribute to the growth of PAH-degrading microorganisms and thus be more favorable in degradation of organic pollutants. Indeed, this easily biodegradable organic

fraction is the main energy source for various organisms present in the soil and organic substrates (Bot and Benites, 2005; Haderlein et al., 2006). Therefore, a substantial content of soluble fraction in an organic substrate, such as BM and FKW, might influence temperature growth during bioremediation, allowing the destruction of dangerous pathogens.

Conversely, acid detergent soluble (ADS) extracts were significantly higher in FVW than in the other three residues (ANOVA, $p < 0.05$). ADS extract is made up of carbohydrate content such as hemicellulose, and proteins bound to the cell walls which are more complex compounds (ANKOM, 2015). Indeed, their content was not observed in SS, and was almost insignificant in FKW. Even if a slightly higher amount of hemicellulose fraction was determined in BM waste than in the other two residues, it was significantly lower than in FVW (12-times lower). Hemicellulose decomposes slowly compared to the soluble fraction and consists of mixed sugars and organic acids (ANKOM, 2015; “Ankom Protocol,” n.d.; Hood et al., 2011). Usually, hemicellulose is degraded by fungi, which have a slower metabolism than bacteria. Indeed, FVW contained a considerably higher content of hemicellulose compared to the soluble fraction and was thus more complex organic material and less biodegradable (Bot and Benites, 2005) than the other three organic wastes (Table 4.3). Furthermore, FVW displayed a three times lower quantity of organic soluble fraction compared to other organic residues, which could have affected the extensive growth of microorganisms at the beginning of bioremediation. An unsuitable content of soluble fraction could be a critical factor for the overall degradation process because there would be limited energy sources for microorganisms (Wick et al., 2011). In addition to the expected lower efficiency in PAHs removal compared to treatments with BM and SS residues, it was also observed the lowest TOC degradation yield during bioremediation (Table 2).

The extract of acid detergent lignin (ADL) solution or cellulose content was found to be significantly different among all four substrates (ANOVA, $p < 0.05$). Its content in FKW (39.7%) was almost six times higher than in SS waste (6.9%). Furthermore the cellulose content in SS was twice that of FVW (3.4%), and this fraction was more than eight times lower in BM waste (0.4%) compared to FVW. The fairly large amount of a stable polymer such as cellulose (Hood et al., 2011) in FKW (Table 4.3) led to slower decomposition of organic matter by bacteria, as already confirmed by the fact that the cooling phase started earlier in RBM than in RFKW (Section 4.3.1). This considerable amount of cellulose might have limited the fast release of some nutrients during the bioremediation process and thus affected the growth of various microorganisms responsible for PAH degradation. Nonetheless a slower decomposition of organic compounds performed in RFKW would allow for the

occurrence of stronger irreversible sorption of pollutants to the soil particles (Bamforth and Singleton, 2005).

Finally, the content of insoluble fraction (lignin) was found to be significantly higher in BM than in other organic residues (ANOVA, $p < 0.05$). Indeed, this content was three and six times higher compared to that in FVW and FKW, respectively, while SS contained no lignin fraction. RBM proved to be very effective in the removal of HMW PAHs, but not LMW PAHs. This inconsistency could be explained by the most probable presence of fungi in BM that had developed the required enzymes to degrade lignin, which has a structure similar to HMW PAHs (Haderlein et al., 2006).

Table 4.3. Organic matter fractionation of organic residues.

Organic residue/ parameter	Successive physico-chemical fractionation (%)				Protein content (% by volatile solids)
	Soluble fraction	Hemicellulose	Cellulose	Lignin	
BM	86.1 ± 2.2* c	6.0 ± 0.9 a	0.4 ± 0.1 a	7.4 ± 1.6 b	17.1 ± 13.8 a
FKW	57.6 ± 0.7 b**	1.4 ± 0.5 a	39.7 ± 0.9 c	1.2 ± 0.7 a	18.3 ± 4.7 a
FVW	22.3 ± 4.6 a	71.9 ± 6.7 b	3.4 ± 3.2 ab	2.4 ± 1.4 a	9.8 ± 4.9 a
SS	93.1 ± 3.5 c	0.0 ± 0.0 a	6.9 ± 3.5 b	0.0 ± 0.0 a	25.5 ± 4.5 a

*Standard deviation is the average of three replicates at the 95% confidence level.

**Different letters in each column indicate the significantly different values among organic residues (ANOVA, $p < 0.05$).

4.3.3.2. *Heterogeneity in protein content*

Content of protein in organic substrates and its influence on PAHs bioremediation could be an important operational parameter. Proteins consist of amino acid sequences whose main component is nitrogen (McKenzie L., 2015). The presence of nutrients such as nitrogen is widely recognized as a crucial factor that contributes to more extensive microbial activity and diversity that would lead to greater removal yield of organic pollutants (Leys et al., 2005; Wick et al., 2011).

In accordance with these findings, the present study confirmed that SS, with the highest content of proteins, showed the best efficiency in total PAH removal (see section 4.3.4). There were no significant differences in protein content among organic substrates (ANOVA, $p < 0.05$); however the numerical data indicated that the amount of protein in SS was twice that in FVW. BM and FKW residues had quite similar protein content in organic

matter, but these contents also were twice that of FVW and almost 50% lower compared to SS. Reactors filled with BM and FKW displayed a thermophilic phase and thus confirmed the presence of sufficiently extensive microbial activity. This extent can be related to the initial nitrogen content of the waste. The later differences that occurred and influenced the total PAH removal were probably conditioned by higher cellulose content in FKW, as explained in section 4.3.3.1.

In addition, the lower protein content in FVW compared to other organic residues resulted in insufficiently developed microbial activity in RFVW. This inadequacy could be due to the fact that microorganisms were limited by an inappropriate amount of nitrogen. Even more, because of the high amount of ADS extracts found in this waste, a significant part of the proteins would be those bound to the cell walls. They are more slowly decomposed and thus less available to microorganisms than soluble proteins which are present in the soluble fraction (ANKOM, 2015). Additionally, metabolization of hemicellulose by fungi would affect the rate of degradation because fungal metabolism is relatively slow compared to bacteria. Consequently, soil bioremediation stimulated with FVW as the organic substrate showed a lower efficiency in removal of HMW PAHs.

4.3.3.3. Efficiency of organic residues related to their biochemical components

A correlation biplot based on principal component analysis (PCA) is shown in Figure 4.3. The highest values of C:N ratio and content of hemicellulose were measured in RFVW and in samples of FVW, respectively. Because of the large representation of plants rich in carbon, results indicated the high content of hydrocarbons and relatively small amounts of proteins in this waste (Thassitou and Arvanitoyannis, 2001). Furthermore cellulose content and N:P ratio were highly correlated. Their highest values were defined in FKW samples and in RFKW, respectively. The highest value for cellulose detected in FKW was attributable to the presence of vegetables while the constituents of this waste were poor in phosphorus content. Furthermore, the highest values of lignin and proteins were measured in samples of BM and SS, respectively. The presence of lignin in BM would occur because of an incomplete degradation of all the branches eaten by certain buffalo during the digestion process. SS is generally considered a rich source of nitrogen, which is associated with protein content (Ling and Isa, 2006), and characterized by a high amount of carbohydrates. The data indicate that values for the soluble fraction were pretty similar in samples of BM and SS.

In fact, bioremediation efficiency can be related to a specific kind of biochemical compounds such as the soluble fraction and protein. The highest contents of these fractions were identified in SS amendment while the most efficient bioremediation treatment occurred in the reactor with SS amendment. Furthermore, the soluble fraction is the most easily biodegradable fraction composed by organic compounds with a simple chemical structure such as free sugars, starches and proteins (Bot and Benites, 2005). A higher content of soluble fraction enables faster decomposition of organic matter and release of nutrients, which contributes to further growth of bacteria responsible for PAH degradation.

According to these findings related to the nature of the waste and contaminant residues left in the soil (section 4.3.4), it can be emphasized that the biochemical components of organic matter and its transformation should be considered prior to amending soil with different organic wastes because the choice of the soil amendments could affect the degradation of PAH during a co-composting process.

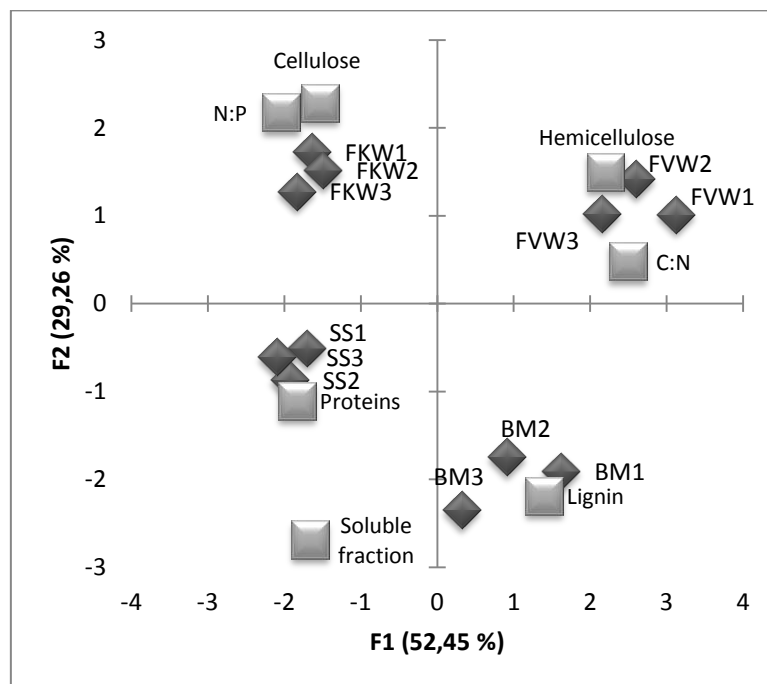


Figure 4.3. Principal components analysis of organic waste features: the content of the soluble fraction, protein, hemicellulose, cellulose, and lignin in samples of BM, FKW, FVW, and SS residues; and the C:N ratios and N:P ratios of their mixtures with soil in RBM, RFKW, RFVW, and RSS reactors. The tags of certain organic residues with different numbers are three replicates of that residue.

4.3.4. LMW and HMW PAH residues

The effect of organic amendments on bioremediation efficiency was assessed according to the decrease in concentrations of LMW, HMW and total PAHs in each reactor. After 20 weeks of long bioremediation, significant differences were observed in the overall decrease in contaminant concentrations relative to their initial concentrations (Table 4.4).

The dissipation of LMW PAH concentrations in each reactor was higher than for the other two selected groups of contaminants. Moreover, the amount of LMW PAH residues left in the soil after almost 5 months of treatment was the lowest in RSS (11%), and slightly higher in RFVW (15%) (Figure 4.4). Concentrations in RBM (31%) and RFKW (29%) were twice as high as in the reactor with FVW and even almost three times that of RSS.

CHAPTER 4

Table 4.4. Dissipation of PAHs concentrations during bioremediation treatment in each reactor.

Parameter	Reactors and concentrations of contaminants (mg kg ⁻¹ ash)					
	RBM			RFKW		
Time (week)	LMW PAHs	HMW PAHs	Total PAHs	LMW PAHs	HMW PAHs	Total PAHs
0	199.0 ± 14.8* d**	338.2 ± 36.4 d	537.2 ± 50.6 d	232.4 ± 11.3 c	376.4 ± 10.7 b	608.8 ± 19.9 c
2	150.0 ± 40.9 bd	212.0 ± 11.1 ab	362.0 ± 44.2 ac	198.9 ± 19.0 bc	306.0 ± 21.5 ab	504.9 ± 40.5 bc
4	112.6 ± 8.4 abc	190.8 ± 14.6 a	303.3 ± 22.2 ab	185.6 ± 34.0 bc	283.3 ± 62.4 a	468.8 ± 96.1 ac
8	136.4 ± 15.5 bc	263.0 ± 18.8 bc	399.4 ± 33.1 bc	180.8 ± 11.6 bc	361.4 ± 13.4 ab	542.2 ± 24.6 bc
12	162.7 ± 28.0 cd	298.1 ± 30.1 cd	460.8 ± 57.9 cd	171.2 ± 23.6 b	351.2 ± 19.9 ab	522.4 ± 43.1 bc
16	98.9 ± 4.1 ab	289.1 ± 4.4 cd	388.0 ± 8.5 bc	92.1 ± 11.8 a	314.1 ± 30.2 ab	406.1 ± 41.5 ab
20	60.7 ± 8.5 a	201.4 ± 7.7 a	262.1 ± 16.1 a	67.9 ± 23.1 a	274.1 ± 40.7 a	342.0 ± 63.2 a

Parameter	RFVW			RSS		
	LMW PAHs	HMW PAHs	Total PAHs	LMW PAHs	HMW PAHs	Total PAHs
0	159.4 ± 7.2 d	275.8 ± 19.0 bc	435.3 ± 25.2 c	195.0 ± 25.4 d	343.7 ± 14.4 e	538.7 ± 39.7 e
2	168.5 ± 8.1 d	275.4 ± 25.5 bc	444.0 ± 33.5 c	145.7 ± 9.0 c	318.0 ± 14.1 de	463.7 ± 21.9 d
4	154.9 ± 10.1 d	274.2 ± 22.1 bc	429.1 ± 31.1 bc	111.7 ± 14.7 bc	299.1 ± 16.5 cd	410.8 ± 17.0 cd
8	120.4 ± 5.6 c	306.7 ± 26.3 c	427.1 ± 31.7 bc	78.9 ± 16.0 b	264.3 ± 9.1 bc	343.2 ± 25.1 bc
12	79.5 ± 19.4 b	263.6 ± 29.4 bc	343.1 ± 47.6 b	78.6 ± 7.8 b	304.3 ± 14.5 d	382.8 ± 19.9 c
16	29.0 ± 7.4 a	215.8 ± 8.3 ab	244.8 ± 15.7 a	36.7 ± 10.5 a	247.6 ± 16.9 b	284.3 ± 27.4 b
20	23.5 ± 8.7 a	191.0 ± 19.5 a	214.5 ± 24.6 a	21.0 ± 4.9 a	178.9 ± 8.2 a	200.0 ± 13.1 a

*Standard deviation is for the average of three replicates at the 95% confidence level.

**Different letters in each column indicate the significantly different values of contaminant concentrations in each reactor (ANOVA, p < 0.05).

This study thus confirmed that temperature conditions related to the mesophilic phase can contribute to higher removal of LMW PAHs than conditions related to the thermophilic phase. Indeed, reactors with SS and FVW wastes achieved significantly higher removal compared to RFKW and RBM. This result could be attributable to the rich microbial diversity typical for the mesophilic phase and an unchanged microbial population, as no temperatures changes occurred. Temperature is indeed well known to affect the change in environment (Antizar-Ladislao et al., 2005). Therefore, the microbial population has more time to adapt metabolism according to existing contaminants. On the other hand, LMW PAH bioavailability and solubility in water is higher compared to HMW PAH, and thus the former can be more bioaccessible to the microorganisms (Antizar-Ladislao et al., 2005; Namkoong et al., 2002).

Because of the lower bioavailability of HMW PAHs, their residual concentrations in soil were larger and reached 52% in RSS. Significantly higher amounts of persistent PAH residues were found in reactors RBM (60%) and RFVW (69%). Finally, the lowest removal and the highest content of HMW PAHs remaining in soil were observed in RFKW (73%) (Figure 4.4). Therefore, different characteristics of these organic substrates conditioned differential removal of HMW and total PAHs in RFVW and RSS. Indeed, SS has been characterized by the greatest soluble fraction and protein content among all four organic substrates (Table 4.3). It contributed to the best nutrient supply for microorganisms and certainly enhanced their growth. In contrast, FVW substrate contains four times less of the soluble fraction compared to SS and an extensive amount of hemicellulose compared to the soluble fraction. In addition the content of protein was twice less than that of SS. Consequently, microbial activity in RFVW could be affected because of the lower supply of nutrients for microorganisms. The expected result would be a significantly higher final concentration of HMW PAHs and total PAHs left in soil compared to RSS (Figure 4.4). On the other hand, the greatest contaminant removal achieved in RSS would be expected to contribute to the high microbial diversity and density typical for SS (Haderlein et al., 2006; Namkoong et al., 2002).

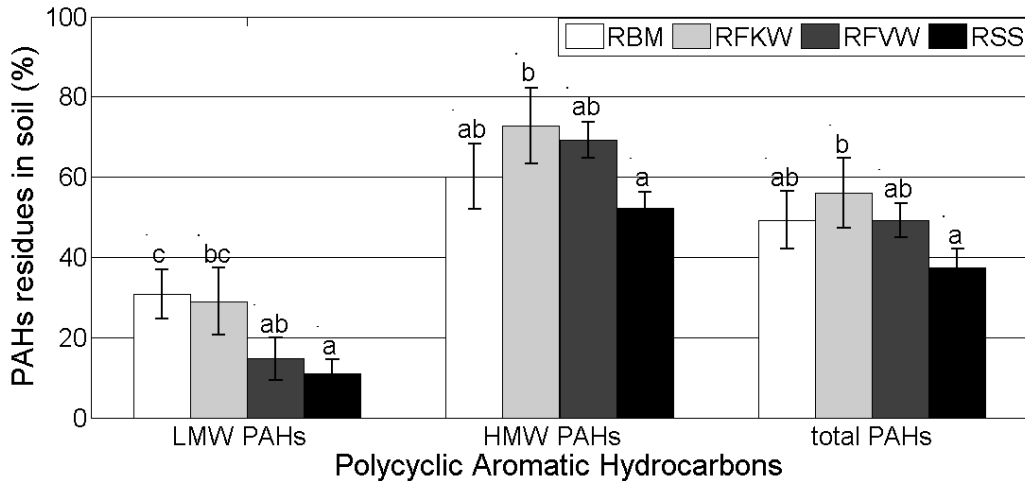


Figure 4.4. PAHs residue remaining in soil (%) at the end of bioremediation. Vertical bars indicate standard deviations of the mean of three replicates at the 95% confidence level. Different letters indicate the significantly different values among treatments for each group of contaminants (ANOVA, $p < 0.05$).

Regarding the higher removal of HMW PAHs in RSS, which displayed a mesophilic phase compared to RBM with typical thermophilic phase temperature conditions, it would be interesting to consider the C:N ratio. Actually, this bioremediation operational parameter was almost three times more favorable in RSS than in RBM. In addition, it can be concluded that C:N ratio has an important role in the complicated degradation of organic pollutants, among other crucial parameters. Although a C:N ratio that is even 10 times higher or lower than the optimal one 10:1 (Leys et al., 2005; Okere and Semple, 2012) will not necessarily affect the entire process, it will certainly influence the overall efficiency of the bioremediation. Accordingly, a good example can be the difference in C:N ratio between RSS (12:1) and RBM (30:1), as explained above.

The highest success in total PAHs removal was reached in the reactor prepared with SS. Indeed, the total contaminant residues left in soil (37%) were significantly lower than in other treatments. Furthermore, no difference was observed among RBM and RFVW related to total PAHs left in soil; it was 49% for both. The reactor amended with FKW was the only treatment in which a removal lower than 50% was achieved; consequently there was a significant difference in total PAH content left in soil (56%) compared to other treatments.

Reactors conditioned with BM and FVW displayed a similar performance regarding total PAH removal, while HMW PAH removal was higher in RBM than in RFVW. This result confirms once again the influence of high temperature conditions on recalcitrant PAH removal because RBM displayed a thermophilic phase. Thus, it increased PAH solubility and diffusion rate, which enabled higher bioavailability of HMW PAHs (Haderlein et al., 2006; Van Gestel et al., 2003). Enhanced bioavailability of HMW PAHs is only one of several parameters necessary to reach a higher removal threshold. Other aspects that can contribute to more successful removal of HMW PAHs in RBM than in RFVW would be a significantly higher content of soluble fraction and lignin in BM waste as explained in section 4.3.3.1.

Many studies have confirmed a significant influence of thermophilic phase conditions on PAH removal during the co-composting process (Haderlein et al., 2006; Mohan et al., 2006). This study demonstrated significantly higher removal of all three groups of contaminants achieved in RSS, in which there was a mesophilic phase, compared to RFKW, in which there was a termophilic phase. Indeed, SS contained a significantly higher amount of soluble fraction than FKW (ANOVA, $p < 0.05$), and almost a one third higher content of protein (Table 4.3). In contrast, the cellulose content was almost six times higher in FKW compared to SS. Considering the results of substrate fractionation, it is obvious that FKW is the organic amendment with a lower potential for bioremediation treatment compared to SS. Moreover, with FKW it was observed almost three times higher LMW PAH residues and approximately 30% higher HMW PAH and total PAH residues left in the soil than observed with RSS. Therefore these results confirm the importance of substrate fractionation for bioremediation efficiency of PAH-contaminated soil.

However, it is important to point out that it is difficult to extrapolate the results obtained using the artificial soil to real contaminated aged soils (Bergknut, 2006; Rhodes et al., 2008). Indeed, higher content of organic matter in an artificial soil strongly influences the sorption of the organic contaminants onto soil particles (Pu and Cutright, 2006; Rhodes et al., 2008). Furthermore, clay minerals present in an artificial soil may contribute to even stronger sorption capacity than organic matter (Pu and Cutright, 2006). Considering these two parameters, contaminants' degradation might be decreased in an artificial soil, since the bioaccessibility of contaminants is decreased (Sayara et al., 2010a). Another important parameter that distinguishes PAHs removal in an artificial and natural soil, is the presence of indigenous microorganisms in naturally

real contaminated aged soil, which is very important for successful bioremediation (U.S. Environmental Protection Agency, 1994) as the indigenous microbial communities are already adapted to the actual polluted environment.

4.4. Conclusions

The biochemical components of organic waste used to stimulate a bioremediation process is a very important factor that affects the overall efficiency of treatment. Hence, the content of organic matter, its biochemical origins, and the macronutrient content such as total nitrogen and phosphorus are essential to support microbial growth and catabolic activities responsible for partial or complete PAH degradation.

This study showed the importance of organic matter fractionation and proposed its consideration as an important parameter during the bioremediation process. Indeed, the high content of the soluble fraction and protein are the most important organic fractions of an organic waste. Furthermore, a higher protein content was related to a higher nitrogen content, i.e., as the content of protein increased, the content of nitrogen also increased. Based on that, a significant amount of protein would improve the C:N ratio. Hence, fresh organic wastes with such features should be considered as organic amendments for bioremediation of PAH-contaminated soil. Furthermore, this study confirmed that organic amendments with less favorable biochemical components, i.e., a lower content of the soluble fraction and protein, showed a lower potential for PAH removal during bioremediation.

Accordingly, the use of SS as amendment during bioremediation of PAH-contaminated soil was the most successful compared to the other organic residues. After 20 weeks of treatment, a removal of 89% was reached for LMW PAHs, while almost 50% removal was obtained for HMW PAHs. Thus, this substrate demonstrated its potential, especially considering the fact that two of the HMW PAHs are highly persistent organic pollutants. Accordingly, SS showed promise for use as an organic substrate in enhanced co-composting systems.

4.5. Acknowledgments

The authors would like to acknowledge the financial support provided by the European Commission (Erasmus Mundus Joint Doctorate Programme ETeCoS³: Environmental

Technologies for Contaminated Solids, Soils and Sediments, under the grant agreement FPA n° 2010-0009).

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Chapter 5

**Influence of activated sewage
sludge amendment on PAHs
removal efficiency from a real
contaminated soil: application of
the landfarming treatment**

Abstract

The removal of polycyclic aromatic hydrocarbons (PAHs) from a real co-contaminated soil by PAHs and heavy metals with an initial concentration of 620 mg of total PAHs kg^{-1} dry soil was investigated. The efficiency of landfarming in removing phenanthrene, pyrene, benzo(a)pyrene and the group of total 16 US EPA PAHs was evaluated. The process was biostimulated by adding centrifuged activated sewage sludge (SS) as an organic amendment. Experimental activities were conducted in four laboratory scale reactors filled with corn cobs as bulking agent to reach bulk density of 640 kg/m^3 . The tested ratios of contaminated soil to SS were 1:2, 1:1, 1:0.5 and 1:0 as wet weight basis. The process performance was monitored through chemical, microbiological and ecotoxicological analyses during 105 days of incubation. The results of analyses demonstrated that the treatment without centrifuged SS achieved a significantly higher total 16 US EPA PAHs removal efficiency (i.e. 32%) compared to treatments with amendment. In the same treatment, the removal efficiency of the PAHs bioavailable fraction was 100% for phenanthrene, benzo(a)pyrene and the group of total 16 US EPA PAHs, whereas 76% for pyrene. Ecotoxicity test performed with bioluminescent bacteria *Vibrio fischeri* confirmed the effectiveness of landfarming as bioremediation treatment since it was observed a decrease in soil toxicity at the end of experimental activities in all reactors. Finally, the results showed that indigenous microorganisms under certain and controlled operating conditions have greater potential for PAHs biodegradation compared to allochthonous microorganisms added with centrifuged SS.

Keywords

Polycyclic aromatic hydrocarbons, bioremediation, landfarming process, sewage sludge amendment, PAHs bioavailability.

Influence of activated sewage sludge amendment on PAHs removal efficiency from a real contaminated soil: application of the landfarming treatment

5.1. Introduction

Soil contamination by polycyclic aromatic hydrocarbons (PAHs) is an important global environmental problem considering that these organic contaminants are largely widespread in soils, water and wastewater (Fernández-Luqueño et al., 2008). Nowadays, fossil fuels are still the main source of energy and they contribute to such contamination generating serious damage to ecosystems (Nduka et al., 2012). The PAHs release in environment is mainly due to the productive and refinery cycles, transportations, extraction process, exploration and exploitation of crude oil and its accidental spills that occur quite frequently (Fernández-Luqueño et al., 2008; Nduka et al., 2012).

According to the U.S. Environmental Protection Agency, 16 PAHs have been listed as “priority pollutants” named 16 USEPA PAHs, even if several hundred different compounds of PAHs exist (Fernández-Luqueño et al., 2008; Gan et al., 2009; Li et al., 2008). Seven of them are classified as probable human carcinogens (Li et al., 2008). Due to their adverse health effects, toxicity, carcinogenicity, mutagenicity and environmental persistence, extensive studies on the remediation of soil contaminated by PAHs have been carried out in the last few decades (Gan et al., 2009).

Application of bioremediation to remove organic pollutants from soils is a promising treatment due to advances in molecular biology and process engineering (Cheng and Wong, 2006; Namkoong et al., 2002; Nduka et al., 2012; Sayara et al., 2011). Among all bioremediation processes, landfarming is commonly applied for removing PAHs from contaminated soils since it results in degrading or immobilizing organic contaminants rather than transferring them from one environmental matrix (i.e. soil) to another (i.e. water) (Straube et al., 2003; Thassitou and Arvanitoyannis, 2001). Furthermore, this process requires low capital costs since it is simple to operate and design (Thassitou and Arvanitoyannis, 2001). Landfarming treatment effectiveness is based on degradation capabilities of indigenous microbiological communities whose

activity is stimulated by supplying oxygen and nutrients, and mixing the soil in order to enhance the contact between microorganisms and contaminants (Straube et al., 2003). Furthermore, the growth of indigenous microorganisms is favored by an appropriate oxygen diffusion and an optimal moisture content (Sayara et al., 2011; U.S. Environmental Protection Agency, 1994). For instance, Saison et al. (2004) examined the effect of tillage and cropping only on PAHs removal efficiency from a contaminated soil. The highest PAHs removal efficiency obtained after 24 months under those semi-natural conditions was 35% in tilled soil, while in control soil reached 37%. Actually, they confirmed that basic operational conditions of landfarming should be processed in order to be expected an efficiency of such treatment.

Supplementing nutrients and readily degradable organic matter in PAHs contaminated soils by adding organic amendment such as centrifuged activated sewage sludge (SS) (Namkoong et al., 2002) can enhance the performance of landfarming. According to Oleszczuk (2007), about 8 million tons of sewage sludge as a residual product of wastewater treatment plants are produced every year in the EU. Due to so high production, disposal facilities could be limited to meet future requirements (Ling and Isa, 2006). Thus, a favourable method to stabilize and recycle SS can be soil fertilization and use as biostimulator within bioremediation process (Fernández-Luqueño et al., 2008; Ling and Isa, 2006; Oleszczuk, 2007).

The mass ratio of contaminated soil to SS is an important parameter and critical factor in PAHs biodegradation performance since it influences the microbial activity (Ling and Isa, 2006; Namkoong et al., 2002). Low application rate could affect nutrients availability and hinder the further growth of microorganisms, especially when the soil is heavily polluted; whereas high application rate could add a large amount of organic matter to soil, resulting in a greater sorption of PAHs and subsequent causing their retention in the soil (Okere and Semple, 2012).

Many studies confirmed the potential and beneficial use of SS to accelerate removal of organic pollutants from soil (Lukić et al., 2016a; Lukić et al., 2016b), but the overall effect depends on soil type and contaminants (Fernández-Luqueño et al., 2008; Ling and Isa, 2006; Okere and Semple, 2012). Contaminated soil often needs such kind of amendments due to deprivation in organic matter and nutrients and very low microbial population and activity (Ling and Isa, 2006). Otherwise, if a contaminated soil contains an appropriate amount of organic matter, nutrients and microbial population, it would

be interesting to study the feasibility and usefulness of SS amendment in such conditions.

Thus, the aim of this study has been to investigate the PAHs removal efficiency in a soil simulating basic operating conditions of landfarming technology on the one hand, and amending such soil with centrifuged SS on the other hand. Real co-contaminated soil by PAHs and heavy metals is characterized by suitable features necessary to be implemented an effective landfarming treatment, i.e. it contains an appropriate nutrients' ratio, content of organic matter and microbial population. A further objective of this study has been to evaluate the optimum amount of centrifuged SS to be added to soil for the most effective bioremediation of PAHs contaminated soil. In order to evaluate the most effective conditions for PAHs removal, this study monitored during the treatment the evolution of soil temperature, pH values, microbial activity, enumeration of heterotrophic bacteria, toxicity and concentrations of targeted contaminants. The possible presence of PAHs was also examined in the leachate collected from each reactor, in order to verify the PAHs transfer from soil to water.

5.2. Materials and methods

5.2.1 Soil

Soil was collected from a contaminated site located in North France, Paris area. Analysis conducted on soil have indicated a sandy-loam texture with the following granulometric composition: 20% clay, 23% fine silt, 8% coarse silt, 12% fine sand and 37% coarse sand. The soil was real co-contaminated by 16 US EPA PAHs and heavy metals; total concentration of 16 US EPA PAHs amounted to 620 mg kg⁻¹ (d/w), while concentrations of aluminium and iron amounted to 13,000 mg kg⁻¹ (d/w) and 20,000 mg kg⁻¹ (d/w) respectively; the concentrations of arsenic, cadmium, chromium, copper, mercury, lead, nickel, zinc and silver were 18, < 0.4, 21, 41, 2.4, 87, 18, 160 and < 5 mg kg⁻¹ (d/w), respectively. Prior the experiments the soil was air-dried, sieved through a 2 mm sieve and homogenized by mixing. Properties of the soil are listed in Table 5.1.

5.2.2 Activated sewage sludge

The choice of using centrifuged SS as amendment was based on findings of Lukić et al. (2016a, 2016b). Activated SS was collected from the wastewater treatment plant of a housing estate in Paris, France. Prior to its application, the sludge was centrifuged at 3260 g for 20 min in order to reduce the initial moisture content of 97%. Initial sewage sludge solid concentration was 30 g L⁻¹. The main properties of centrifuged SS are reported in Table 5.1.

Table 5.1. Soil and activated sewage sludge properties.

Parameter/material	Contaminated soil	Sewage sludge
Moisture content (% w/w) ^a	1.3 ± 0.0 ^b	93.6 ± 0.0
Fixed solids (% d/w) ^c	-	16.4 ± 0.4
Volatile solids (% d/w)	-	83.6 ± 0.4
Total Kjeldahl nitrogen, TKN (% w/w)	0.1	0.3 ± 0.1
Total phosphorus, P (% w/w)	0.01	-
Organic matter, OM (g kg ⁻¹ , w/w)	48.4	53.1 ± 0.2 ^d
Total Organic Carbon, TOC (g kg ⁻¹ , w/w) ^e	27.9	30.8 ± 0.1
pH	8.3	7.2 ± 0.0
Cation exchange capacity, CEC (meq/100 g)	203	-
C:N ratio ^f	21:1	12:1
N:P ratio	9:1	-
Bulk density, ρ _b (g cm ⁻³)	1.3 ± 0.0	1.1 ± 0.0

^aw/w: wet weight

^bstandard error is the average of three replicates

^cd/w: dry weight

^dresult based on volatile solids content

^econversion factor 1.724 based on OM content (Schumacher, 2002)

^fmass ratio

5.2.3 Experimental design

Experiments of landfarming treatment were carried out in four laboratory-scale bioremediation reactors with a working volume of 1.1 L. Reactors were hermetically closed and operated at room temperature. A manually mixing of their content was performed twice per week. Humidified air was insufflated from the bottom of reactors through a Plexiglas[®] plate with small holes (ø 1 mm) with the aim to provide an uniform air distribution. Plexiglas[®] plate was covered with pieces of corn cobs in order

to prevent clogging of plate by fine particles and enable air supply without channeling. Aeration rate of $200 \text{ L min}^{-1} \text{ m}^3$ was supplied using an air pump (Newair, Newa, Tecnoindustria srl, Italy) (Namkoong et al., 2002).

Experiments lasted 105 days and were conducted with four different mass ratios of contaminated soil to centrifuged SS on wet weight basis: 1:2 (reactor RS2), 1:1 (reactor RS1), 1:0.5 (reactor RS05) and 1:0 (reactor RS0), i.e. without mixing sewage sludge. Reactors RS2, RS1, RS05 and RS0 were filled with 297 g, 495 g, 567 g and 800 g of soil, and with 593 g, 495 g, 283 g and 0 g of sewage sludge, respectively. In order to ensure aerobic conditions and good aeration, corn cobs was used as bulking agent to reach bulk density of 640 kg/m^3 in each reactor. Moisture content in reactors RS2, RS1 and RS05 was maintained at 60%, while moisture content in reactor RS0 was maintained at 30%. Distilled water was added to keep constant the moisture content during the experimental activity. Phenanthrene (Phen), pyrene (Pyr), benzo(a)pyrene (B(a)P) and 16 US EPA PAHs (total PAHs) were used as targeted contaminants to evaluate the effectiveness of treatment.

5.2.4 *Sampling*

Sampling was carried out in triplicate for each reactor at the beginning of the experiment (day 0), and after 14, 35, 63 and 105 days of incubation time, due to the fact that the largest changes are expected at the beginning of the treatment which gradually become more uniform. Representative samples were collected from 3 different points in the middle of each reactor, and sub-samples were stored and used for further analyses. Bulking agent was physically removed from samples and excluded from any analysis, since it was considered as non-biodegradable during experiments.

5.2.5 *Chemical methods*

5.2.5.1 *Chemical and physical analyses*

Moisture content in samples was determined by mass loss after drying them in an oven at $105 \text{ }^\circ\text{C}$ for 24 h (ASTM American Society for Testing and Materials, 1999). Volatile solids and fixed solids were analyzed according to the Standard Method 2540E (APHA, AWWA, WEF, 1998), while pH value was determined in soil-water suspension

according to Method 9045D (U.S. Environmental Protection Agency, 2012). Organic matter (OM) content was calculated based on volatile solids values (Schumacher, 2002). According to Izhaki (1993), total Kjeldahl nitrogen (TKN) in SS was estimated based on protein content, i.e. protein content was divided by a factor 6.25. Lowry protocol was used for colorimetric evaluation of protein content (Dulekgurgen, 2004): the absorbance in samples of 0.5 mL was measured at a wavelength of 750 nm (Perkin Elmer UV/VIS Spectrometer Lambda 10). All results are shown in Table 5.1 as average of three replicates. Standard deviation is reported for all the values. PAHs analysis and analysis of soil related to TKN, total P, OM and total organic carbon (TOC) content, pH value, cation exchange capacity (CEC), texture and granulometric analysis were performed in an external laboratory.

5.2.5.2 Hydroxypropil- β -cyclodextrin (HPCD) extraction method

Hydroxypropil- β -cyclodextrin (HPCD) extraction method has been used to assess bioavailable fraction of PAHs according to Cui et al. (2013) and Cuypers et al. (2002). A volume of 150 mL of 50 mM HPCD solution were added to 7.5 g of soil sample. Erlenmeyers with samples in triplicate were sealed and set to an orbital shaker at 150 rpm for 20 h. After that, samples were centrifuged (Sigma, Fisher Bioblock Scientific 2-16 P) at 3260 g for 24 min to obtain the supernatant, which was collected and analyzed by an external laboratory to measure PAHs content.

5.2.6 Microbiological analysis

5.2.6.1 Dehydrogenase enzymatic activity (DHA)

Dehydrogenase enzymatic activity (DHA) on samples collected in triplicate was measured the day after sampling. Method is based on colorimetric measurement of 2,3,5-triphenyltetrazolium chloride (TTC) reduction to 1,3,5-triphenylformazan (TPF) according to Casida Jr. (1977) and Kizilkaya (2008). Six grams of solid sample were suspended in 2.5 mL of reagent water and 30 mg glucose. The enzymatic reaction took place by adding 1 mL of 3% 2,3,5-triphenyltetrazolium chloride solution to the suspension. After 24 h of incubation at 37 °C, the reaction was stopped by adding

methanol. The methanol suspension was filtered and washed with methanol until the reddish colour had disappeared from samples. The absorbance of red methanolic solutions of formazan was measured spectrophotometrically at a wavelength of 485 nm (Perkin Elmer UV/VIS Spectrometer Lambda 10). Results of enzymatic activities are reported in Figure 5.5 as $\mu\text{g TPF g}^{-1}$ dry sample.

5.2.6.2 *Most probable number (MPN) of total heterotrophs*

Total heterotrophic bacteria enumeration was performed using the dilution plate-counting technique in microwell plates filled with autoclaved Luria-Bertani broth for 5 days at 25 °C. An amount of 1 g was collected from each sample and mixed with 10 mL of autoclaved KCl solution (9 g L^{-1} , pH 7.0). Serial 7-fold dilutions were performed and microwell plates were inoculated with 20 μL of appropriate dilutions of the sample suspension. The number of total heterotrophs was calculated based on the number of positive microwells (visible turbidity) scored according to the method of Briones Jr. and Reichardt (1999). Results were expressed as MPN of total heterotrophs per gram of dry sample and reported in Figure 5.4.

5.2.6.3 *Toxicity assay*

Microtox tests were performed with Microtox Model 500 Analyzer (Modern Water) on samples of soil collected in triplicate at the beginning and at the end of the incubation time in order to evaluate any change of toxicity occurred during the experiments. The marine luminescent bacterium *Vibrio fischeri* (LUMIStox LCK 487, Hach Lange, France) was used as bioassay organism. The bacterium was exposed to 1 mL filtered aqueous extract of each sample and the different levels of toxicity were evaluated according to the bioluminescence differences (Grange and Pescheux, 1985). The extraction of aqueous phase was performed according to the standard test method (ASTM American Society for Testing and Materials, 2004), i.e. 20 mL MilliQ water was added to 2 g of homogenized sample and mixed with an orbital shaker at 200 rpm for 16 h. Samples were centrifuged (Sigma, Fisher Bioblock Scientific 2-16 P) at 3260 g for 24 min, and then aqueous extracts were collected and stored in the freeze until analysis were performed. Acute toxicity was determined based on the luminescence decrease after 5 and 15 min long exposure times at 15 °C.

5.2.7 *Statistical analysis*

Data obtained from chemical and microbiological analysis were evaluated by one-way analysis of variance (ANOVA) using R software (R i386, 3.1.1 version) at the 95% confidence level. All the analysis were done in triplicate. Comparison of means was performed using Tukey multiple test and significant p-values were obtained at the level of $p < 0.05$. Homogeneous groups obtained by the comparison of means are indicated in figures and tables with different letters (i.e. a, b, c and d).

5.3 **Results and discussion**

5.3.1 *Temperature changes and pH values in landfarming treatment*

Operational temperature in all four reactors displayed prevalent mesophilic conditions during the incubation period. The higher temperature range of mesophilic phase was reached only in RS1 with a maximum value of 39.5 °C after 21 days. Increase of temperature started after 3 days of incubation, while the equalization with ambient temperature and the beginning of maturation phase started after 77 days (Figure 5.1). Operational temperatures in RS2, RS05 and RS0 reactors were similar to ambient temperature ranging between 14.4 °C and 24.5 °C. An uneven temperature trend in the second half of the experimental activities was a consequence of ambient temperature changes in the laboratory.

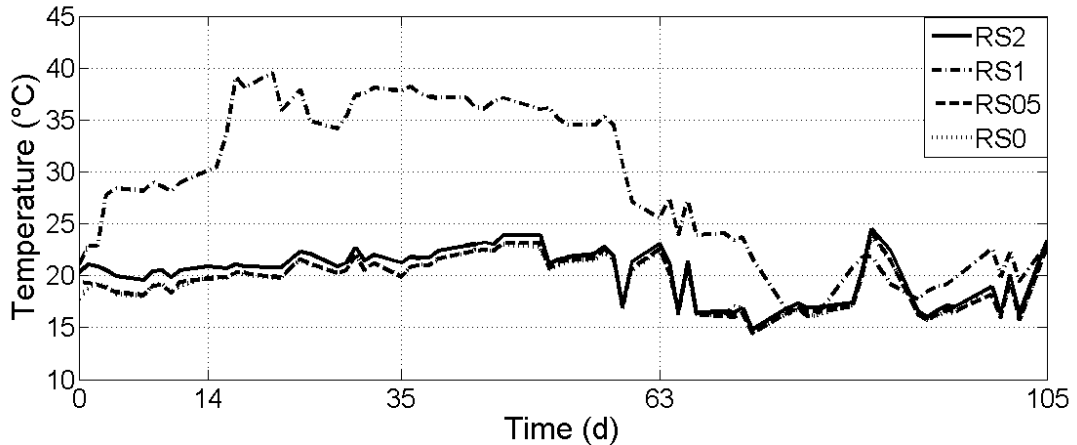


Figure 5.1. Temperature changes in landfarming treatments.

The C:N ratio at the beginning of treatment was pretty similar in all reactors, and therefore this parameter has likely not contributed to the differences in temperature profiles between RS1 and the other reactors shown in Figure 5.1. The increase of temperature, occurred exclusively in RS1, can be the result of the particular environmental conditions in terms of availability of OM and ratio between indigenous and allochthonous microorganisms in the reactor after the addition of 495 g of centrifuged SS. Actually, the amount of OM in RS05 and RS0 was significantly lower compared to RS1 and RS2 (ANOVA, $p < 0.05$) (Table 5.2), and therefore the insufficient amount of easily biodegradable OM in these reactors could limited the increase of temperature during landfarming treatment (Guerin, 2000). However, even if RS2 also contained a significantly higher content of organic matter compared to RS05 and RS0 at the beginning of experimental activities, an increase of temperature did not occur due to a decrease of the microbial activity as it is explained in section 5.3.4.

Table 5.2. Content of organic matter and C:N ratio in each reactor at the beginning of experimental activities.

Parameter/reactors	RS2	RS1	RS05	RS0
OM concentration (g kg^{-1} , w/w)	51.4 ± 1.0 * **	46.0 ± 2.2 b	38.0 ± 1.1 a	39.8 ± 1.0 a
C:N mass ratio	14:1	15:1	16:1	21:1

*Standard error is the average of three replicates at 95% confidence level.

**Different letters in the row indicate the significantly different values among reactors (ANOVA, $p < 0.05$).

At the beginning of the experiments, pH values in reactors amended with SS were pretty similar, i.e. 7.2, 7.4 and 7.5 in RS2, RS1 and RS05, respectively (Figure 5.2). Highest pH value was determined in reactor RS0, i.e. 8.2. However, after two weeks the pH value in RS0 started to decrease and after five weeks it reached a value of 7.6 that was similar to the pH range in other reactors.

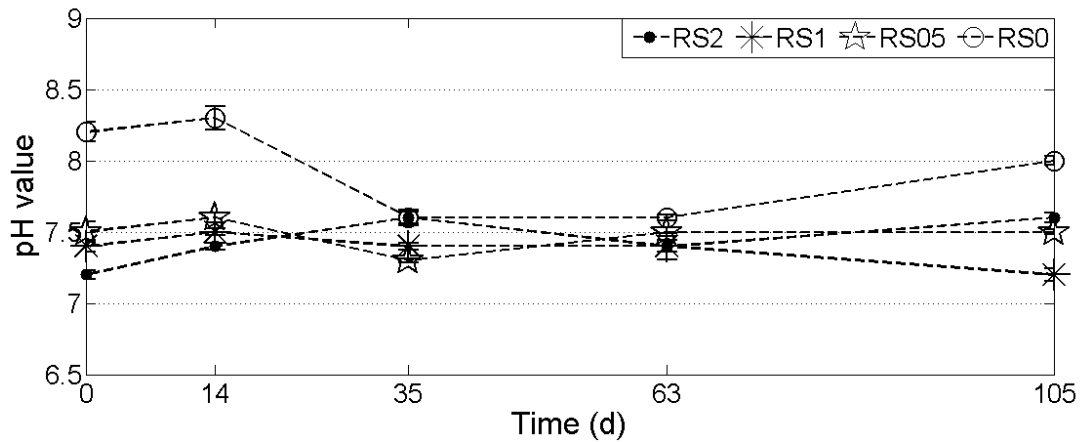


Figure 5.2. Changes of pH value in each reactor during landfarming treatment. Vertical bars are standard deviations of mean of triplicates, that could not be clearly visible as they are covered with marker.

At the end of the experiments, RS0 displayed an increase of pH value, i.e. 8.0, that could have affected a further PAHs removal, if pH value would continue to increase. Indeed, the optimal specific bacterial growth and PAHs oxidation are considered to occur when the pH is in the range of 6-8 (Haritash and Kaushik, 2009; U.S. Environmental Protection Agency, 1994).

5.3.2 PAHs removal and their bioavailability

5.3.2.1 PAHs removal related to total concentrations in soil

The effect of landfarming treatment on PAHs removal was evaluated by monitoring the concentrations of the targeted contaminants. After 105 days of treatment, the removal of Phen was higher in each reactor compared to Pyr and B(a)P (Figure 5.1). result

confirms that 3-rings PAHs are easier accessible to microorganisms responsible for their degradation compared to 4- and 5-rings PAHs (Mohan et al., 2006). Indeed, their less complex molecular structure and higher water solubility influence such property (Namkoong et al., 2002). However, the removal of Phen was significantly higher in RS0 (69%) compared to other three reactors amended with SS (ANOVA, $p < 0.05$). The highest Phen removal efficiency among reactors amended with SS was observed in RS2 (43%), while considerably lower efficiency was detected in RS1 (27%). Furthermore, significantly lower Phen removal efficiency was observed in RS05 (18%) compared to RS2 (ANOVA, $p < 0.05$).

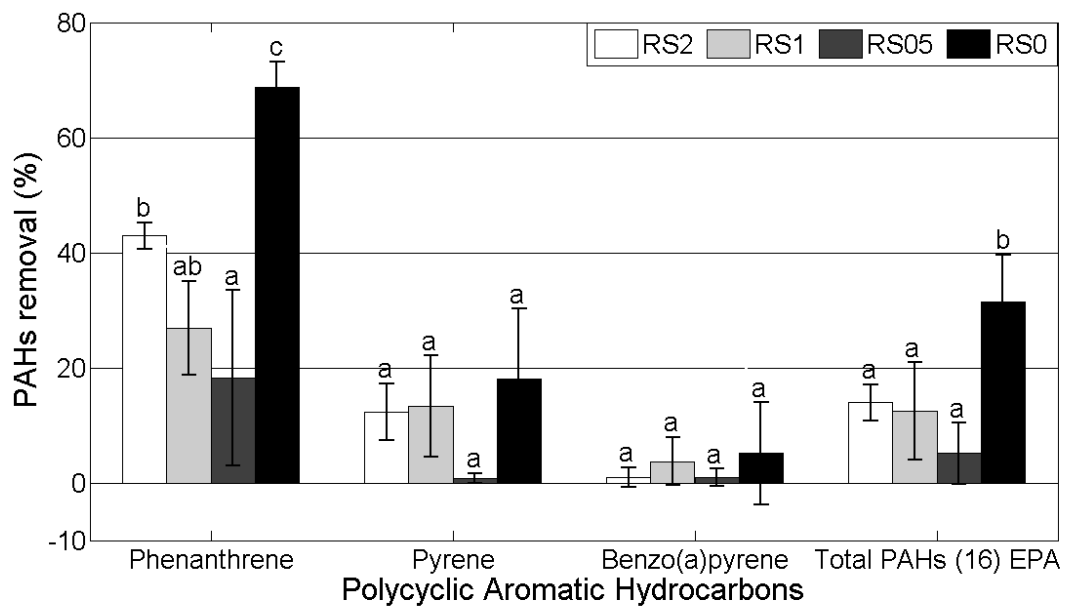


Figure 5.3. PAHs removal efficiency (%) in each reactor at the end of treatment. Vertical bars indicate the standard deviations of mean of three replicates at 95% confidence level. Different letters are used to distinguish the significantly different values among reactors for each contaminant (ANOVA, $p < 0.05$).

Pyr removal efficiency was pretty much lower in all reactors compared to Phen, due to more complex molecular structure and lower bioavailability. Considerably higher removal efficiency has been reached in RS0 (18%) compared to RS05 (0.8%), but without being statistically significant. Pyr removal efficiency detected in RS1 was equal to 13%, while slightly lower efficiency was detected in RS2, i.e.12%.

Similarly, the highest B(a)P removal efficiency occurred in RS0 (5%). The removal efficiency of this contaminant in RS1 was slightly lower compared to RS0 and equal to 4%, while results obtained in RS2 and RS05 showed a removal efficiency equal to 1% and 0.9%, respectively.

The best result in total PAHs removal and significantly different compared to other treatments was reached in RS0 (32%) (ANOVA, $p < 0.05$). Furthermore, slightly lower removal efficiency was observed in RS1 (13%) compared to RS2 (14%), while considerably lower efficiency of total PAHs removal was achieved in RS05 (5%). According to analysis performed on leachates collected from all reactors, the transfer of total PAHs from soil to liquid was completely negligible, since the amount detected in the liquid fraction was less than 0.1% compared to the total mass removed.

The results obtained confirm a considerable influence of temperature conditions on removal of PAHs with low molecular weights (LMW) and high molecular weights (HMW). Total PAHs removal efficiency in RS2 and RS1 was quite similar, i.e. in reactor displaying ambient temperature during all incubation period and in reactor displaying higher temperature conditions, respectively. Removal efficiency of 3-rings PAHs was considerably higher in RS2 than in RS1, unlike 4- and 5-rings PAHs. Moreover, removal efficiency of 5-rings PAH, i.e. B(a)P was even four fold higher in RS1 compared to RS2, while removal efficiency of 4-rings PAH, i.e. Pyr, was slightly higher in RS1 compared to RS2. Thus, these results prove that higher temperature conditions displayed in RS1 influenced higher water solubility and mass transfer of HMW PAHs and consequently contributed to their higher availability for further removal (Saison et al., 2004; Van Gestel et al., 2003). Additionally, all results achieved in RS05 showed the lowest efficiency of this treatment on PAHs removal. An inappropriate mass ratio of SS most likely has inhibited the biological degradation of PAHs because of their stronger sorption (Wilcke, 2000) compared to other reactors.

Furthermore, the highest removal efficiency achieved in RS0 likely occurred due to higher metabolic potential of indigenous microbial community to degrade targeted contaminants compared to strains added with SS and the absence of competition between them or any suppression effect (Maila and Cloete, 2004; Sudhir et al., 2014). Indeed, these results confirmed that indigenous microbial community is undoubtedly more able to remove PAHs during the incubation time of 105 days compared to allochthonous microorganisms, if the real contaminated soil has the favorable properties

to be treated by landfarming treatment. Soil used for the experiments contained a favorable C:N:P ratio (Table 5.1), similar to the most optimal of 100:10:1 (Maila and Cloete, 2004; Okere and Semple, 2012), and a number of total heterotrophic bacteria, i.e. > 1000 CFU g^{-1} dry soil, appropriate for the landfarming treatment (U.S. Environmental Protection Agency, 1994).

5.3.2.2 PAHs removal related to bioavailable fraction in RS0 reactor

Assessment of the bioavailable fraction (or bioaccessibility) of PAHs is an important factor since the metabolization of these compounds performed by microorganisms could occur only if contaminants are available to them and enter the water phase (Cui et al., 2013). Based on the results of HPCD test, a visible relationship between PAHs bioavailability and their chemical properties, such as number of aromatic rings and molecular weights, was noticed (i.e. higher molecular weights and number of rings lead to a lower bioavailability as it has been shown in Table 5.3). Moreover, it was found a strong negative correlation ($r = -1$) of Phen, Pyr and B(a)P bioavailability with their octanol-water partition coefficient ($\log K_{ow}$), i.e. as $\log K_{ow}$ values increase, the values for PAHs bioavailability decrease.

Table 5.3. Concentrations of PAHs bioavailable fraction and total removed fraction in each reactor.

Concentrations/Contaminants	Phen	Pyr	B(a)P	Total PAHs
Bioavailable fraction related to total concentration in soil (%)	$35.9 \pm 1.8^*$	24.1 ± 1.5	3.1 ± 0.1	27.3 ± 1.4
HPCD Bioavailable fraction in soil ($mg\ kg^{-1}$, d/w)	55.1 ± 2.8	18.5 ± 1.2	0.9 ± 0.0	168.6 ± 8.8
Total removed fraction in RS2 ($mg\ kg^{-1}$, d/w)	56.0 ± 10.5	7.6 ± 4.0	0.2 ± 0.4	67.6 ± 9.1
Total removed fraction in RS1 ($mg\ kg^{-1}$, d/w)	40.4 ± 17.8	9.9 ± 7.9	1.0 ± 1.1	74.8 ± 59.8
Total removed fraction in RS05 ($mg\ kg^{-1}$, d/w)	28.3 ± 24.5	0.6 ± 0.6	0.2 ± 0.4	31.7 ± 33.9
Total removed fraction in RS0 ($mg\ kg^{-1}$, d/w)	105.1 ± 8.3	13.6 ± 8.7	1.4 ± 2.4	193.3 ± 44.6

* Standard error is the average of three replicates.

A significant enhancement of PAHs bioavailability and consequently improvement of removal efficiency in RS0 was noticed on observed difference between bioavailable amount determined by HPCD test and total removed amount of contaminants. The total

removed amount of Phen was almost twice compared to its initial bioavailable fraction, while total removed fractions of B(a)P and total PAHs were approximately 60% and 15% higher compared to their initial bioavailable fractions (Table 5.3). Pyr was the only contaminant whose removed amount was a bit lower compared to its initial bioavailable fraction, i.e. 76%.

Those interesting results confirm the high efficiency of landfarming treatment considering that it was reached a value of 100% for the removal efficiency of the bioavailable fraction of Phen, B(a)P and total PAHs, and almost 80% of Pyr. Moreover, favorable treatments' conditions enhanced the PAHs bioavailability and consequently their access to microorganisms, and thus influenced their higher removal efficiency from contaminated soil compared to the other reactors.

5.3.3 Changes in most probable number (MPN) of total heterotrophic bacteria during landfarming treatment

Natural or anthropogenic changes in the functions of the soil ecosystem can cause negative impacts on indigenous microbial population and its activity. Accordingly, monitoring the total heterotrophs and soil enzyme activities as indicators for soil contamination could be a feasible and effective option (Hinojosa et al., 2004). In contrast, using microorganisms as bioindicators and their changes in bacterial numbers could be often not consistent with contaminants biodegradation (Maila and Cloete, 2005).

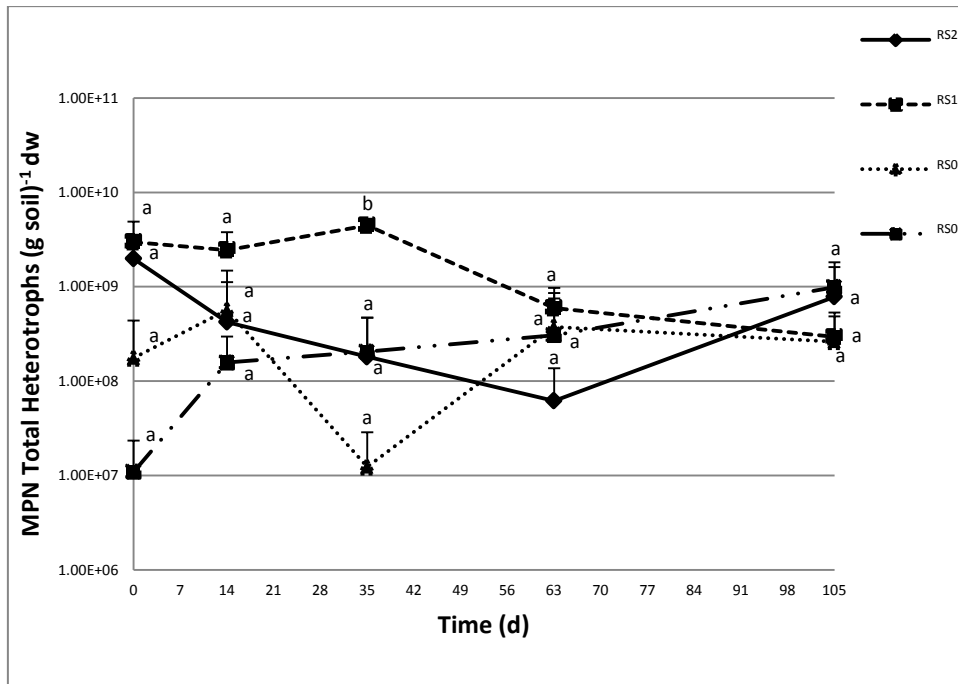


Figure 5.4. MPN of total heterotrophs during landfarming treatment in each reactor. Vertical bars indicate the standard deviations of mean of three replicates at 95% confidence level. Different letters are used to distinguish the significantly different values among reactors for each sampling day (ANOVA, $p < 0.05$).

At the beginning of the experimental activities, reactors amended with centrifuged SS showed considerably higher number of total heterotrophs, but without being statistically significant compared to RS0 (Figure 5.4). From the beginning of experimental activity, RS2 showed unfavorable conditions for microorganisms as their number decreased progressively up to day 63. Then, the trend turned in positive because microbial community added with SS was adapted to the new environment. The number of total heterotrophs in RS1 kept approximately constant during the first 35 days of incubation, afterward a decrease was observed and lasted until the end of the experimental activities. The beginning of decreasing trend of microbial population in this reactor is in relationship with the beginning of cooling phase of the treatment (subsection 5.3.1). However, microorganisms in RS05 showed a very variable trend during the 105 days of incubation, thus indicating the need of a longer time for their adaptation. This long lag phase consequently affected the PAHs removal efficiency (explained in section 5.3.2.1). In contrast, in RS0, a progressive increasing trend of total heterotrophs was observed during the first two weeks of treatment, since favorable conditions for their growth were

achieved such as an appropriate porosity, aeration and moisture content (Besalatpour et al., 2011). The increasing trend has been continued until the end of experimental activities when the highest number of total heterotrophs was reached compared to other three reactors.

5.3.4 Monitored dehydrogenase activity (DHA) during landfarming treatment

Dehydrogenase activity (DHA) can be considered as an indicator of microbial oxidative activities in soils (Doi and Ranamukhaarachchi, 2009; von Mersi and Schinner, 1991) and therefore, it can represent an important parameter for assessing the PAHs biodegradation, since the biological oxidation of organic contaminants is catalyzed by dehydrogenase enzymes (Balba et al., 1998; Maila and Cloete, 2005). In specific case, DHA has been turned out to be not fully correlated with biodegradation of contaminants (Hinojosa et al., 2004; Maila and Cloete, 2005).

This study confirms the findings of Von Mersi and Schinner (1991) showing that DHA activity is suitable to be an indirect parameter for microbial biomass determination, since trend line of DHA activity is pretty similar to the trend line shown by MPN of total heterotrophs in each reactor during landfarming treatment. Thus, both parameters can be used as bioindicators for the removal of contaminants. Furthermore, DHA activity did not show significant changes throughout the incubation time in RS2 (Figure 5.5) (ANOVA, $p < 0.05$). Actually, a decrease of DHA activity was observed during the first two months due to toxic effect of contaminants on added microbial community (explained in section 5.3.3). Its recovery and an increase of DHA activity was observed after 63 days, but without significant statistical and numerical changes at the end of treatment compared to its initial value, i.e. $1121 \mu\text{g TPF g}^{-1} \text{d/w}$ versus $1422 \mu\text{g TPF g}^{-1} \text{d/w}$, respectively. The initial decrease in microbial population and DHA activity in RS2 would be a reasonable explanation why an increase of temperature has been disabled compared to RS1, although both contained a significantly higher content of OM compared to other two reactors (section 5.3.1). A decreasing trend of DHA activity was observed in RS1 and ended with a significantly lower value on day 105 ($187 \mu\text{g TPF g}^{-1} \text{d/w}$) compared to its initial value ($931 \mu\text{g TPF g}^{-1} \text{d/w}$) (ANOVA, $p < 0.05$). Reactor RS05 displayed a significant decrease of DHA activity after two months of incubation. Consequently, its values at the beginning ($204 \mu\text{g TPF g}^{-1} \text{d/w}$) and at the end ($96 \mu\text{g TPF g}^{-1} \text{d/w}$)

TPF $\text{g}^{-1} \text{d/w}$) of treatment were not significantly different (ANOVA, $p < 0.05$). A progressive increase of DHA activity during the whole incubation period was observed only in reactor RS0. Indeed, DHA activity in this reactor was extremely higher at the end of experimental activities compared to the initial value, i.e. $279 \mu\text{g TPF g}^{-1} \text{d/w}$ and $2 \mu\text{g TPF g}^{-1} \text{d/w}$, respectively (ANOVA, $p < 0.05$).

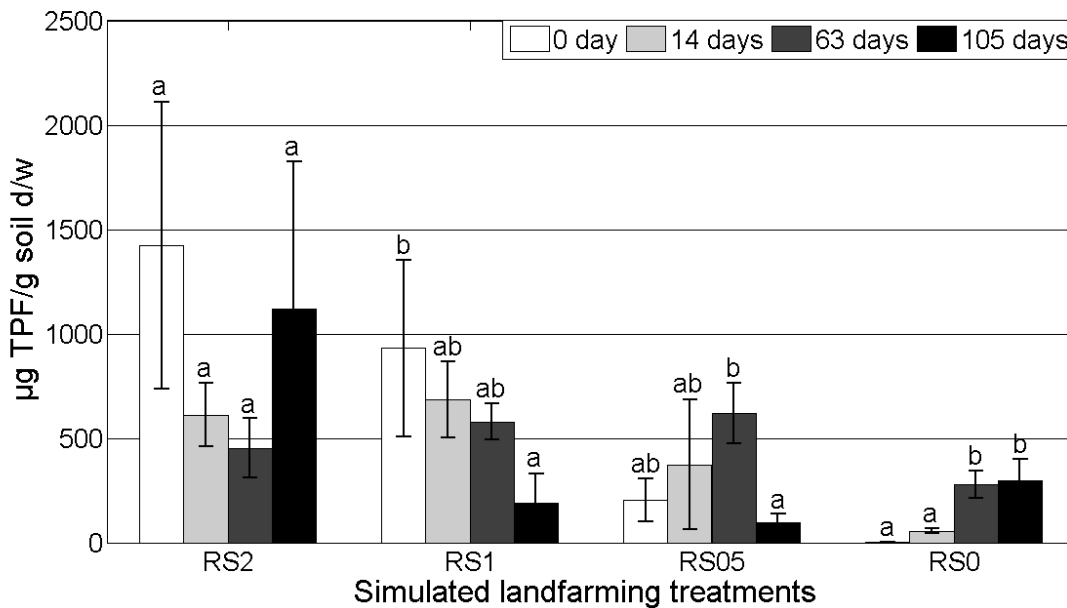


Figure 5.5. Dehydrogenase activities (DHA) during landfarming treatment. Vertical bars indicate the standard deviations of mean of three replicates at 95% confidence level. Different letters are used to distinguish the significantly different values of DHA activity on sampling days in each treatment (ANOVA, $p < 0.05$).

Thus, the favorable conditions of landfarming treatment undoubtedly stimulated the DHA activity of indigenous microbial community, which led to an effective PAHs removal. However, a decrease of DHA activities and microbial population in reactors amended with centrifuged SS indicates that PAHs had a toxic effect on allochthonous microorganisms (Serrano et al., 2009).

5.3.5 Evaluation of soil toxicity

Ecotoxicity tests are generally used to complement other analytical methods implemented to assess the results of bioremediation technologies. Actually, a decrease

of contaminants' concentrations in soil is not always related to the decrease of soil toxicity, since incomplete degradation of organic contaminants could cause the formation of metabolites that might be more toxic than initial contaminants (Saison et al., 2004; Singh, 2012). Based on these considerations, toxic effects of contaminated samples were compared at the beginning and at the end of landfarming treatment using Microtox[®] test. In accordance with the results discussed in previous subsections, the highest decrease of acute toxicity was observed in RS0, i.e. a reduction of 79% and 78% was observed at inhibition $I_{(5)}$ and $I_{(15)}$ respectively (Table 5.4). Indeed, RS0 was the unique reactor where was observed a significant increase of DHA activity during the incubation, as well as the highest MPN of total heterotrophs and PAHs removal at the end of experimental activities. A decrease of acute toxicity in RS2 and RS1 was considerably lower compared to RS0, showing a value of 38% and 6% for $I_{(5)}$ and 50% and 6% for $I_{(15)}$, respectively. Additionally, a considerable difference in the decrease of acute toxicity was observed for RS1 and RS2 although the total PAHs removal efficiency was pretty similar. The higher toxicity observed in RS1 compared to RS2 could be reasonably due to the higher temperature conditions displayed in this reactor which resulted in a higher PAHs bioavailability (explained in section 5.3.2.1). Although an increase of PAHs bioavailability could have favored their access to microorganisms, their biodegradation would be affected due to inhibited activity of microorganisms as proved by the decrease of DHA activity and MPN of total heterotrophs observed during incubation (explained in section 5.3.4 and 5.3.3). Consequently, it would affect contaminants' removal and further decrease of toxicity. Furthermore, reactor RS1 displayed the lowest decrease of toxicity, the unique significant decrease of DHA activity at the end of treatment compared to its initial value and the unique uniform decrease of total heterotrophs during incubation. In contrast, RS0 reactor displayed the highest decrease of toxicity, the unique significant increase of DHA activity at the end of treatment compared to its initial value and the unique uniform increase of total heterotrophs during incubation. Thus, results of ecotoxicity test that has shown a lower toxicity are in accordance with higher PAHs removal efficiency and higher microbial population growth as well as DHA activity. In contrast, decrease of acute toxicity was considerably higher in RS05 (49% and 47% for $I_{(5)}$ and $I_{(15)}$ respectively) compared to RS1, even if in RS05 was observed the lowest PAHs removal compared to the other reactors. These results indicate that soil toxicity is not always related to the

contaminants concentration. Moreover, it has been demonstrated that SS addition in certain amount causes stronger sorption of contaminants and thus affects their bioavailability and degradation (Wilcke, 2000). Consequently, it influences the decrease of toxicity in soil and the contaminants' removal efficiency.

However, the results of ecotoxicity test confirmed high effectiveness and success of landfarming treatment according to the operating conditions applied in reactor RS0.

Table 5.4. Decline of acute toxicity in soil at the end of landfarming treatment compared to its initial value, measured with Microtox test at inhibition time of 5 and 15 minutes.

Reactors / Decline toxicity (%)	RS2	RS1	RS05	RS0
Inhibition at time 5 min ($I_{(5)}$)	37.7 ± 12.7* ab**	6.0 ± 10.4 a	49.4 ± 12.4 bc	79.4 ± 15.7 c
Inhibition at time 15 min ($I_{(15)}$)	50.0 ± 25.1 ab	5.9 ± 10.2 a	47.1 ± 12.7 ab	78.3 ± 16.1 b

*Standard error is the average of three replicates at 95% confidence level.

**Different letters in the row indicate the significantly different values among reactors (ANOVA, $p < 0.05$).

5.4 Conclusions

Results presented and discussed in this paper confirm the effectiveness of landfarming treatment in removing the bioavailable fraction of PAHs from real co-contaminated soil by PAHs and heavy metal. Actually, LMW as well as HMW PAHs were successfully removed. Moreover, indigenous microbial community was more successful in degrading PAHs compared to mixture of indigenous and allochthonous microbial community which was added to the contaminated soil using centrifuged SS as organic amendment. Better metabolic potential of indigenous microbial community is based on the fact that those microorganisms were already adapted on such environment and their metabolic functions were developed in accordance to present contaminants. The success of such a system depended by favorable soil characteristics necessary to be achieved PAHs degradation, i.e. an appropriate nutrients' balance (C:N:P ratio) and microbial abundance, specifically total heterotrophs' number. Operating conditions are easier controlled in laboratory reactors than in the field and therefore, the success of landfarming treatment under the laboratory scale is difficult to compare with treatments performed in pilot and field scale which are usually less effective.

In this study, centrifuged SS amendment was not beneficial to substantially enhance PAHs removal in soil at the time scale of the experimental activities, as the best result

was obtained in treatment without organic amendment. Competition between indigenous and allochthonous microorganisms could be the cause of this result, since the first ones are already adapted to PAHs unlike the second ones. Observing the results of reactors amended with centrifuged SS, the PAHs removal was prevented in the treatment with the lowest amount of added SS most likely due to caused stronger sorption. In the treatments with the medium and the highest amount of added centrifuged SS was not achieved significant difference in PAHs removal. However, a difference in an overall efficiency between these two treatments was noticed as RS1 showed considerably lower decrease of acute toxicity compared to RS2.

Furthermore, results obtained during the research related to MPN of total heterotrophs and DHA activity can indicate the feasibility in using these parameters as bioindicators for monitoring of PAHs removal during bioremediation considering these results were consistent with removal of contaminants.

5.5 Acknowledgments

The authors wish to thank sincerely the European Commission for the financial support through Erasmus Mundus Joint Doctorate Programme ETeCoS³: Environmental Technologies for Contaminated Solids, Soils and Sediments, under the grant agreement FPA n°2010-0009.

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Chapter 6

**General overview and future
perspectives**

6 General overview and future perspectives

6.1. Overview of research studies

The present study was designed to evaluate the effects of an integrated approach of bioremediation treatment and biostimulation strategy. The study was based on the implementation of landfarming treatment and composting process by adding different types of fresh organic waste to soil contaminated by PAHs. According to this integrated system, the soil would be enriched with organic matter, nutrients and microorganisms naturally present in waste. Moreover, the soil structure would be improved due to the increased soil aggregate stability, moisture content, water infiltration and hydraulic conductivity. Since the bulk density of fresh organic waste is usually lower compared to soil, the addition of certain wastes would improve the bulk density of soil and its porosity. In addition, the amount of bulking agent necessary to perform the treatment would also be reduced, thus positively influencing the costs and finally, the increasing moisture content in soil will decrease the volatilization rate of organic pollutants. Thus, this promising approach based on a simultaneous biodegradation of PAHs and organic waste could improve the removal of organic contaminants and be a solution for an eco-friendly disposal of organic waste as well. In order to assess the effectiveness and feasibility of such system, two sets of experimental activities were performed. The design of experimental activities and process details are presented in Table 6.1.

6.1.1 *Study of the effectiveness of organic waste*

The first experimental stage of this study was focused on performing a bioremediation treatment adding different types of fresh organic waste to a PAHs contaminated soil in thermally insulated reactors (Chapter 3). The wastes used for the study were selected according to the principles of the environmental sustainability and in details buffalo manure, food and kitchen waste, fruit and vegetable waste and activated sewage sludge were chosen. The main objective was to evaluate the effectiveness of the selected wastes on the removal of PAHs with different properties from soil. The soil used in the first set of the experimental activities was an artificial OECD (Organisation for

Table 6.1. Resume of the researches activities.

Experimental research	Research article title and thesis chapter	Experimental design	Target contaminants	Objectives	Results	Conclusions
Biostimulation strategy – composting of organic waste	Evaluation of PAH removal efficiency in an artificial soil amended with different types of organic wastes Chapter 3	Treatment time: 140 days Setting: Four laboratory scale thermally insulated bioremediation reactors Soil: spiked OECD artificial soil Treatments: Soil + buffalo manure; Soil + food and kitchen waste; Soil + fruit and vegetable waste; Soil + activated sewage sludge.	Anthracene, chrysene, benzo(k)fluoranthene, benzo(a)pyrene	To evaluate the effectiveness of four types of fresh organic wastes in stimulating PAH removal from contaminated soil, and to monitor a different success rates in PAH removal based on their different properties.	The highest removal of three- and four-rings PAHs have been observed in reactor displaying a mesophilic conditions. In contrast, the highest removal of five-rings PAHs have been observed in reactor displaying a thermophilic phase, which was not considered as efficient due to increased toxicity.	Composting of organic waste used in soil bioremediation was considered as high efficiency biostimulation strategy for degradation of persistent PAHs and to shorten their total removal time. Activated sewage sludge is the most favorable organic amendment to be considered and used in composting treatments.

Table is continued on the next page

CHAPTER 6

Experimental research	Research article title and thesis chapter	Experimental design	Target contaminants	Objectives	Results	Conclusions
Biostimulation strategy – characterization of organic matter	Importance of organic amendments characteristics on bioremediation of PAH-contaminated soil Chapter 4	Extension of the first experimental set. Organic waste: Buffalo manure, food and kitchen waste, fruit and vegetable waste, activated sewage sludge Analysis: TOC, TKN, Total P, content of protein by Lowry assay, substrates fractionation by Van Soest method	LMW PAHs (anthracene), HMW PAHs (sum of chrysene, benzo(k)fluoranthene and benzo(a)pyrene), Total PAHs (sum of all four)	To perform the characterization of organic matter based on its biochemical composition, the content of macronutrients and total organic carbon, in order to clarify different efficiency of PAH removal related to different characteristics of organic amendments.	The concentrations of LMW PAH residues in soil were significantly lower in reactors which displayed a mesophilic phase, i.e. 11% and 15%, compared to reactors which displayed a thermophilic phase, i.e. 29% and 31%. The residual HMW PAHs were up to five times higher compared to residual LMW PAH, depending on the reactor.	The biochemical composition of organic waste used to stimulate bioremediation process is a very important factor which affects the overall efficiency of treatment. The high content of soluble fraction and protein are the most important organic fractions of an organic waste which should be considered for PAHs bioremediation.

Table is continued on the next page

General Overview and Future Perspectives

Experimental research	Research article title and thesis chapter	Experimental design	Target contaminants	Objectives	Results	Conclusions
Landfarming technology – assisted by activated sewage sludge amendment	Influence of activated sewage sludge amendment on PAHs removal efficiency from a real contaminated soil: application of the landfarming technology Chapter 5	Treatment time: 105 days Setting: Four laboratory scale thermally insulated bioremediation reactors Soil: Real co-contaminated soil Treatments: Soil to sewage sludge ratio 1:2; Soil to sewage sludge ratio 1:1; Soil to sewage sludge ratio 1:0.5; Only soil without sewage sludge amendment.	Phenanthrene, pyrene, benzo(a)pyrene, total 16 US EPA PAHs	To investigate PAH removal efficiency in real contaminated soil simulating basic operational conditions of landfarming technology, and amending that soil with sewage sludge. Furthermore, to evaluate an optimum amount of sewage sludge for an effective bioremediation of PAHs contaminated soil.	The treatment without sewage sludge amendment achieved significantly higher total 16 US EPA PAHs removal compared to other treatments. The removal efficiency related to bioavailable concentrations reached 100% for phenanthrene, benzo(a)pyrene and total 16 US EPA PAHs, while it reached 76% for pyrene.	The results showed that indigenous microorganisms under certain conditions have greater potential for PAHs biodegradation compared to microorganisms added with the organic amendment. Favorable landfarming conditions enhanced PAHs bioavailability and thus their removal.

Economic Co-operation and Development) soil spiked with four PAHs up to reach an initial concentration of 658 mg of PAHs kg⁻¹ soil (d/w). The initial PAHs concentrations simulated a real condition in a real contaminated soil by gas works site (Juhasz and Naidu, 2000). Accordingly, the spiked concentration of anthracene and chrysene was 235 mg kg⁻¹soil (d/w), while the spiked concentration of benzo(k)fluoranthene and benzo(a)pyrene was 94 mg kg⁻¹ soil (d/w) for each of them. Soil bioremediation using composting of organic waste was found a successful and highly efficient treatment, according to the obtained results.

Activated sewage sludge resulted in being the most effective organic amendment to degrade PAHs in terms of efficiency as well as time. The treatment performed using sewage sludge as amendment shows the highest dissipation of anthracene and chrysene, and the fastest dissipation of anthracene, benzo(k)fluoranthene and benzo(a)pyrene compared to other treatment. The emphasis of the first experimental stage are listed in the following lines:

1. the success in removing PAHs is closely related to their hydrophobic properties, i.e. PAHs with higher hydrophobicity are more resistant to degradation;
2. DHA enzymatic activities could be associated with the soil toxicity which will indicate lower toxicity in soil after higher biodegradation yield of contaminants related to higher enzymatic activity;
3. the occurrence of a thermophilic phase in the composting process of waste reduces the efficiency of the treatment the most likely because influenced the change in microbial diversity.

6.1.2 Study of the organic matter characterization

Based on the data obtained in the first experimental stage, a study aimed at investigating how the specific properties of the selected organic wastes could have influenced the experimental results was conducted (Chapter 4). The characterization of organic amendments was based on their biochemical compositions (i.e. content of soluble fraction, protein, hemicellulose, cellulose and lignin) and their content of macronutrients like nitrogen (N) and phosphorus (P), and total organic carbon. Substrates' fractionation by content of soluble fraction, hemicellulose, cellulose and lignin was obtained by successive physico-chemical extraction steps using the Van

Soest method. The obtained data were processed using statistical analyses such as one-way analysis of variance (ANOVA) and principal component analysis (PCA) in order to evaluate the interrelationships among the variables. According to the obtained results, it can be highlighted that the biochemical composition of certain organic waste should be considered carefully prior to implement soil bioremediation associated with composting of organic waste. Indeed, an unsuitable composition of organic matter and content of macronutrients would affect PAHs degradation, limiting the bacterial growth and consequently the catabolic activities responsible for the removal of organic contaminants. Therefore, the high content of soluble fraction and protein are the most important organic matter fractions of fresh organic waste to be considered in order to use a specific waste as organic amendment for the bioremediation of PAHs contaminated soil.

6.1.3 Study of the influence of activated sewage sludge amendment on PAHs removal efficiency by application of the landfarming treatment

The second set of experimental activities was focused on studying the influence and efficiency of different mass ratios contaminated soil to organic waste on PAHs removal. According to the results obtained from the previous experimental activity, activated sewage sludge was selected to be used as organic amendment, since the highest effectiveness of bioremediation was reached in treatment amended with this type of waste. Therefore, an experimental set was conducted in four laboratory scale reactors filled with a real co-contaminated (i.e. heavy metals and PAHs) soil and activated sewage sludge in the mass ratios of 1:2, 1:1, 1:0.5 and 1:0 as wet weight basis (Chapter 5). The soil was real co-contaminated with an initial concentration of 620 mg 16 US EPA PAHs kg⁻¹ soil (d/w) and total metals with the concentration of 33.353 mg kg⁻¹ soil (d/w) with the prevailing concentrations of aluminium and iron.

Treatments with the sewage sludge amendment have not been so effective in PAH removal compared to treatment with only soil based on elementary operational conditions of landfarming treatment. The lowest PAH removal has been detected in reactor with the contaminated soil to sewage sludge mass ratio of 1:0.5, since in this treatment have been detected the lowest microbial population and activity during the incubation compared to other two treatments amended with sewage sludge. Reactors

with the contaminated soil to sewage sludge mass ratio of 1:1 and 1:2 showed pretty similar total PAH removal, but the overall efficiency among treatments was considerably different. Indeed, the treatment with the contaminated soil to sewage sludge mass ratio of 1:1 showed a considerably lower decline of acute toxicity compared to treatment with the contaminated soil to sewage sludge mass ratio of 1:2, at the end of incubation. However, the reactor with medium amount of added centrifuged SS displayed higher temperature conditions of mesophilic phase which would have influenced a higher bioavailability of contaminants and thus affected the decrease of acute toxicity. Higher bioaccessibility of HMW PAHs to microorganisms might be specified based on their higher removal in treatment with medium amount of added centrifuged SS compared to treatment with the highest amount of added centrifuged SS. In contrast, removal of LMW PAH was higher in treatment with the highest amount of added centrifuged SS compared to treatment with medium amount of added centrifuged SS. Accordingly, such kind of PAHs are more easily accessible to the microorganisms thanks to their specific properties, thus without any influence of higher temperature conditions.

Furthermore, the treatment without sewage sludge as amendment reached significantly higher total 16 US EPA PAHs removal efficiency compared to other treatments. Accordingly, higher ability of the indigenous microbial community to degrade PAHs during the short treatment time has been proved compared to microorganisms added to the contaminated soil by the organic amendment. Naturally, the success of the indigenous microbial community to degrade PAHs depends on different operational parameters including C:N:P ratio and microbial abundance in soil. Actually, the indigenous microorganisms showed the best metabolic potential, since they were already adapted to PAHs present in the contaminated soil. Additionally, favorable landfarming conditions enhanced PAHs bioavailability, since the PAH removal efficiency was considerably higher compared to their bioavailable concentrations detected before the treatment.

Some of the results obtained at the end of the experiments are presented in Table 6.2. For further management and usage of remediated soil is important to know the soil conditions after the treatment.

Table 6.2. Final effects of biological treatments on some soil parameters.

Parameter/treatment	RBM	RFKW	RFVW	RSS	RS2	RS1	RS05	RS0
pH value	7.3 ± 0.0 ^a	7.3 ± 0.0	7.3 ± 0.0	7.4 ± 0.0	7.6 ± 0.0	7.2 ± 0.0	7.5 ± 0.0	8.0 ± 0.0
TOC (g kg ⁻¹ , w/w) ^b	27.4 ± 2.0	40.3 ± 2.6	39.1 ± 1.7	40.0 ± 2.2	25.2 ± 3.7	23.7 ± 0.8	17.9 ± 0.8	28.4 ± 1.7
MPN of total heterotrophs (MPN)	n.a. ^d	n.a.	n.a.	n.a.	7.8 × 10 ⁸ ± 1.0 × 10 ⁹	2.9 (± 2.4) × 10 ⁸	2.6 (± 2.2) × 10 ⁸	9.9 (± 6.3) × 10 ⁸
TH (g soil) ⁻¹ , d/w) ^c								
DHA activity (µg TPF g ⁻¹ ,d/w)	2140.9 ± 540.9	1792.1 ± 1098.1	1269.0 ± 739.2	2601.2 ± 366.8	1121.4 ± 704.4	186.9 ± 143.9	95.8 ± 41.4	297.2 ± 100.9
Acute toxicity at inhibition time of 5 min (%)	36.1 ± 7.8	11.6 ± 1.8	21.2 ± 6.9	7.1 ± 0.0	38.9 ± 2.0	68.9 ± 8.5	42.1 ± 9.1	14.8 ± 10.3
PAHs removal (%)								
Anthracene	69.2 ± 6.1	71.0 ± 8.3	85.3 ± 5.3	89.0 ± 3.5	n.a.	n.a.	n.a.	n.a.
Chrysene	40.7 ± 5.6	30.9 ± 7.8	49.8 ± 10.4	58.8 ± 5.5	n.a.	n.a.	n.a.	n.a.
Benzo(k)fluoranthene	36.7 ± 12.8	19.3 ± 10.4	3.4 ± 1.4	33.1 ± 3.0	n.a.	n.a.	n.a.	n.a.
Benzo(a)pyrene	40.3 ± 9.6	26.0 ± 13.0	7.7 ± 4.8	32.9 ± 1.9	1.0 ± 1.7	3.7 ± 4.1	0.9 ± 1.6	5.1 ± 8.8
Pyrene	n.a.	n.a.	n.a.	n.a.	12.3 ± 4.9	13.3 ± 8.8	0.8 ± 0.8	18.0 ± 12.3
Phenanthrene	n.a.	n.a.	n.a.	n.a.	42.9 ± 2.3	26.8 ± 8.1	18.2 ± 15.3	68.7 ± 4.4
Total 16 US EPA PAHs	n.a.	n.a.	n.a.	n.a.	13.9 ± 3.1	12.5 ± 8.5	5.1 ± 5.4	31.5 ± 8.0

Table is continued on the next page

CHAPTER 6

Parameter/treatment	RBM	RFKW	RFVW	RSS	RS2	RS1	RS05	RS0
PAH removal relates to bioavailable concentrations (%)								
Phenanthrene	n.a.	n.a.	n.a.	n.a.	102.2 ± 22.8 ^c	74.6 ± 35.5	53.2 ± 46.4	191.6 ± 24.5
Pyrene	n.a.	n.a.	n.a.	n.a.	42.3 ± 25.8	52.7 ± 41.0	3.3 ± 3.7	76.2 ± 53.6
Benzo(a)pyrene	n.a.	n.a.	n.a.	n.a.	27.4 ± 47.5	110.2 ± 126.5	30.2 ± 52.4	167.0 ± 289.2
Total 16 US EPA PAHs	n.a.	n.a.	n.a.	n.a.	40.0 ± 2.9	45.4 ± 37.2	19.5 ± 21.0	116.1 ± 32.8

^astandard error is the average of three replicates.

^bw/w: wet weight.

^cd/w: dry weight.

^dn.a.: data not available.

^eIf the stated value exceeds 100%, it indicates higher PAH removal for certain percentage that exceeded 100%, compared to their bioavailable concentrations at the beginning of treatments.

6.2 Future perspectives

The presented thesis has been based on literature overview and researches performed at laboratory scale in order to contribute in improving and clarifying the existing knowledge related to bioremediation of PAHs contaminated soil. However, biodegradation of PAHs is a complex process which depends on many parameters that have to be carefully taken into account before a landfarming treatment is conducted (U.S. Environmental Protection Agency, 1994). Moreover, differences in efficiency when a landfarming treatment is scaled from the laboratory scale up to pilot or field scale can occur. In spite of the well-known factors which may affect those differences like soil homogeneity or contact of nutrients with soil matrix, there are also other unknown variables which might be controlled differently in the field (Atagana, 2004). Accordingly, further researches are needed in order to overcome and highlight all possible challenges. Furthermore, prior to the implementation of landfarming treatment should be performed tests to ensure that there are not conditions which could disrupt further bioremediation (Atagana, 2004). Additionally, the monitoring of soil toxicity evolution should be recommended in order to complement the assessment of bioremediation efficiency. Since contaminants of concern could exhibit different chemical behavior in spiked soil compared to real contaminated soil, studies based on use of spiked soil might lead to inadequate expectations when applied in field scale. Consequently, studies related to landfarming technology and biostimulation strategy of composting with organic waste should be performed with soil sampled by contaminated site of concern (Semple et al., 2001).

Organic waste amendments can have the most significant impact on the effectiveness of bioremediation when added to soils with an inappropriate microbial density, pH value, and nutrients' content. Indeed, these parameters limit the application of landfarming treatment, but with an adequate management, i.e. providing optimal conditions for PAHs removal, the limitations could be successfully overcome using an eco-friendly and low cost approach by addition of organic waste. Therefore, further studies should be focused on implementing such integrated approach and demonstrating its success mainly under these conditions which limit application of landfarming technology.

On the other hand, this thesis showed that if contaminated soil already has all the features necessary to be performed an effectiveness landfarming, it does not mean that the addition of organic waste would be beneficial for such a process. Actually, in some

case organic waste amendments could even hinder the process and lead to less effective or unsuccessful bioremediation. According to the literature, indigenous microorganisms in contaminated environment are more able and successful in PAHs biodegradation compared to microorganisms which have been added to the contaminated environment by organic waste (Maila and Cloete, 2004; Sudhir et al., 2014). This fact is expected considering that they have been already adapted their functions and metabolisms on present contaminants. In contrast, addition of microbial community naturally occurred in the waste might affect the degradation capability of the indigenous microorganisms due to the competition. Indeed, the indigenous microorganisms can be suppressed by added species and have available less nutrients necessary for their growth. However, it does not mean that microorganisms naturally occurred in the waste are useless, they only need more time to adapt their functions and metabolism to the new environmental conditions, and after that period would better contribute to PAHs removal. Similarly, the PAHs degradation kinetics observed in the first experimental set were the most extensive in the fourth and fifth months of incubation. Therefore, the key points to study in order to justify the implementation of bioremediation using an organic waste are: to study the nutritional balance of C:N:P ratio in the soil as well in the organic waste; the way of establishing the balance and adaptation among indigenous and added microbial communities; ability to reach PAH removal efficiency higher than that one reached by indigenous microorganisms only, and if that removal efficiency will be enough higher to justify the use of organic waste; if the removal efficiency will be enough high to justify its implementation, it is important to know time needed to reach it. Other important benefit of composting process will be the stabilization of organic matter within maturation phase and formation of humic substance, which improves desorption of organic pollutants from soil matrix. Thus, it would improve the bioavailability of HMW PAHs (Sayara, 2010; Wick et al., 2011). Furthermore, the addition of organic waste would adjust and maintain soil pH value in favorable range from 6 to 8 for a long time. Therefore, there is a need to perform long experimental activities and to monitor a number of parameters in order to clarify and justify the correct use of this innovative approach.

6.3 References

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Appendix

Supplementary data: images

Bioremediation reactors



Sewage sludge inside the reactor



Pipes for exhaust air



Centrifuged activated sewage sludge



Experimental set.



Real co-contaminated soil.

pH meter



Oven



Muffle

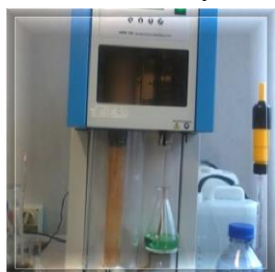


Desiccator



Laboratory equipment.

Automatic distillation system-TKN



Digestion unit-TKN and total P



Spectrophotometer-TOC



Chemical analysis.

Vessels with samples and solvents



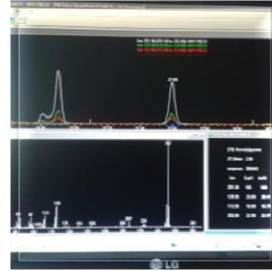
Microwave digestion system



Injection of samples in GC-MS

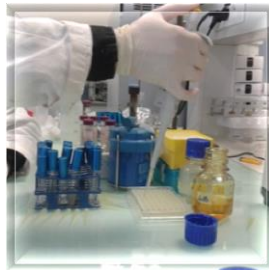


Chromatogram of GC-MS



PAHs analysis.

MPN of Total Heterotrophs



DHA activity



Microtox® test



Microbiological analysis.



Environmental Technologies for Contaminated Solids, Soils and Sediments (ETeCoS³)



Education, Audiovisual & Culture
Executive Agency



Education and Culture DG

ERASMUS MUNDUS



UNIVERSITÉ ———
— PARIS-EST

UNESCO-IHE
Institute for Water Education



UNIVERSITÀ DEGLI STUDI DI NAPOLI
FEDERICO II